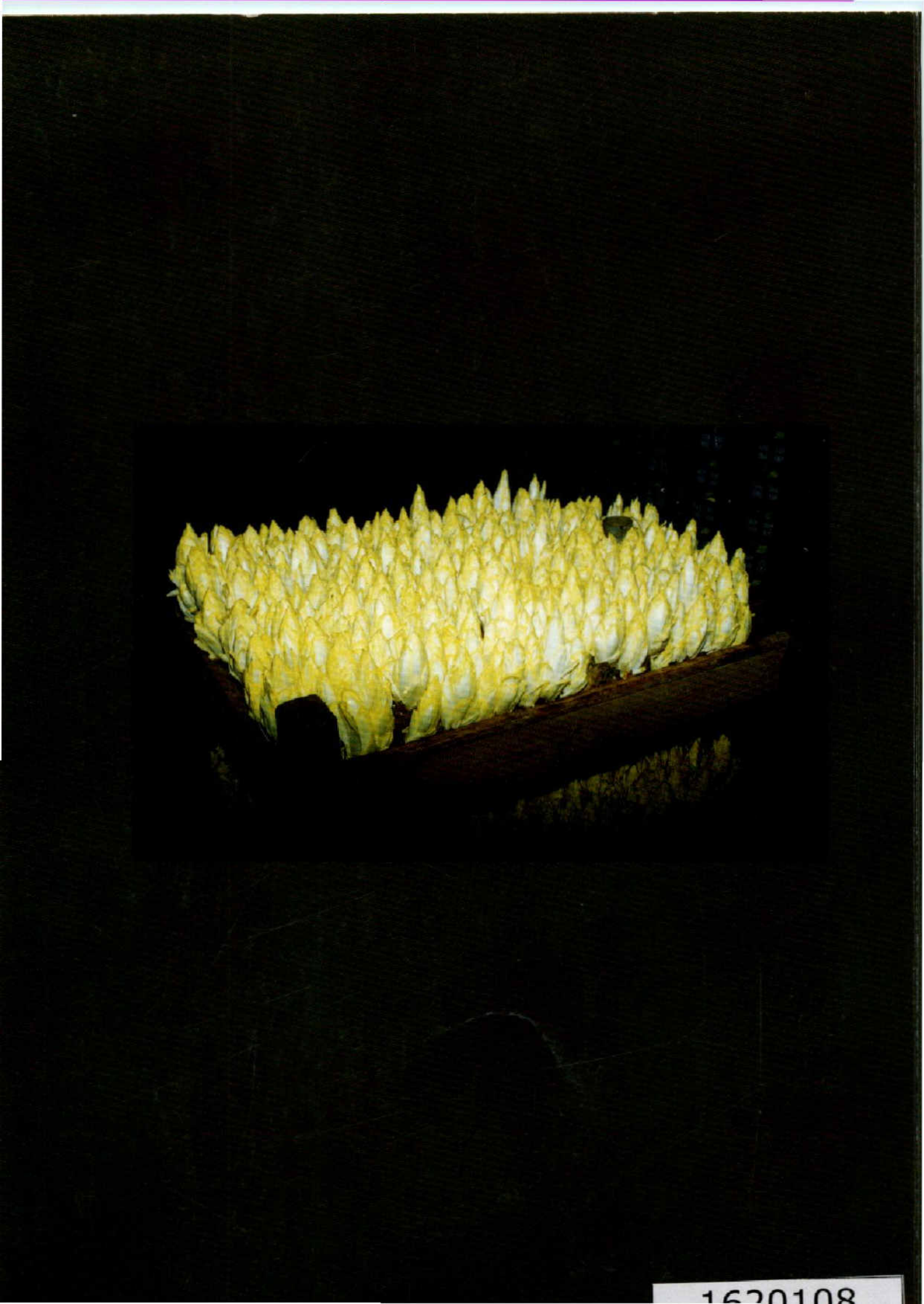
Transannular Cyclisation Reactions of the Germacrane System Mediated by Enzymes from *Cichorium intybus*

Dennis P. Piet



A 350

Promotor: dr. Æ. de Groot, hoogleraar Bio-Organische Chemie

Co-promotor: dr. M.C.R. Franssen, universitair docent Organische Chemie

A 350

Transannular Cyclisation Reactions of the Germacrane System Mediated by Enzymes from *Cichorium intybus*

Dennis P. Piet

Dennis P. Piet

Transannular Cyclisation Reactions of the Germacrane System Mediated by Enzymes from *Cichorium intybus*

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
dr. C.M. Karssen
in het openbaar te verdedigen
op maandag 30 september, 1996
des namiddags te 16.00 uur in de Aula
van de Landbouwuniversiteit te Wageningen.

ISBN 90-5485-588-6

Contents

Voorwoord

Het schrijven van een proefschrift is een eenzame bezigheid. Het werken in een chemisch laboratorium daarentegen niet. Het zal U dan ook niet verbazen dat het werk, beschreven in dit proefschrift, niet helemaal door mij alleen is uitgevoerd. Zo'n beetje 60% van de vakgroep Organische Chemie heeft, in meer of mindere mate, bijgedragen tot het tot stand komen van dit epistel, een lofzang op de witlof. Om deze mensen nu allemaal te noemen gaat te ver maar sommigen wil ik niet onvermeld laten.

Aede de Groot, oppergod van de vakgroep Organische Chemie, druk als je bent, sta je toch altijd voor je mensen klaar, als een rots in de woelige ambtenarenbranding.

Maurice Franssen, mijn directe, zeer professorabele begeleider, vastgelijmd aan zijn computer, innig vergroeid met zijn muis en alleen op zaal om mijn radiootje zachter te zetten. De term: "op 10 Piet!" had niets met een commerciële radiozender te maken maar meer met het volume. Je snelheid waarmee je mijn literatuurvervuilende artikelen en de eerste zes hoofdstukken van dit proefschrift nakeek verbaast mij nog steeds.

Hugo Jongejan, het schijnt dat jij zaalhoofd was maar volgens mij weet je alleen nog niet van welke zaal. Echt vaak ben ik je in ieder geval niet op mijn werkplek tegengekomen. Toch bedankt voor het 'draaien' van de weinige 'exacte massa's'.

Beb van Veldhuizen, onze NMR-man, zonder jouw werk was er van dit boekje weinig terechtgekomen.

Maarten Posthumus, Hoofd massaspectroscopie, ondanks dat je 'bhhuitengewoon' druk was (en nog steeds bent), kon ik steeds bij je terecht voor je expertise en een snelle co-injectie op je nieuwe, nog steeds niet goed werkende, speeltje.

Hans Wijnberg, de synthese-goeroe, voor je adviezen tijdens de synthese van de substraten en voor de opheldering van menig NMR-spectrum.

De secretaresses, zonder hun arbeid ligt de vakgroep natuurlijk stil.

Natuurlijk mijn legertje 'tutjes', mijn doctoraal-studenten: Andre Pots, Rimko ten Have, Margo Claassens, Ingrid Elbers, Gilda de Sousa Carvalho (ERASMUS) en Mark Dignum, jullie werk is helaas niet in dit boekje terug te vinden. Enzymzuivering en cyclisatie experimenten zijn dus toch niet zo simpel als jullie hadden gedacht.

Hendra Willemen, jij zag als enige student je inspanningen wel bekroond in de hoofdstukken 5 en 6.

Jan-Willem de Kraker, mijn 'nopper', mijn KIP-per bij het AB-DLO. Je dreigt, net als ik en vele van mijn collega-leeftijds- en opleidingsgenoten, te gaan behoren tot de 'Verloren Generatie'. Afgestudeerd op het hoogtepunt of in de nasleep van de recessie, in een tijd waarin de chemische sector de winst liever terug in het bedrijf laat

vloeien dan te spenderen aan het creëren van nieuwe arbeidsplaatsen. Gelukkig kom je met het huidige onderwijsbeleid binnen vijf jaar geheid aan een baan. Je werk aan het zuiveren van het FPP-cyclase is dan toch nog, zij het gedeeltelijk, gelukt. De resultaten staan beschreven in hoofdstuk 7. Ook Harro Bouwmeester, jouw directe chef, bedankt voor je commentaar op dit hoofdstuk.
Natuurlijk de kroeg, 'Loburg', een goede plaats om af te pilsen na een week hard werken.
Mijn collega-AIO's/OIO's/analisten die het leven op de vakgroep en in de kroeg dragelijk maakten.
In het bijzonder Cindy Nieuwkerk, mijn collega borrelcommissaresse, met wie ik geruime tijd de 'evenementencommissie' van de vakgroep heb mogen runnen. Onze barbeque heeft toch maar mooi de (kabel)-krant gehaald.
En als laatste Henk Swarts, gewillig slachtoffer van het eerste uur in 'Super Maze Wars'. De pauzes zullen nooit meer hetzelfde zijn.

Dennis

'I smile in remembrance'

aan mijn ouders,
grootouders en Ada

1. General introduction

1.1. Historical background

Chicory (*Cichorium intybus* L.) and endive (*Cichorium endiva* L.) are two of the many species of the Compositae family of which the leaves have been used as vegetables throughout the ages. In classical antiquity, the leaves and the roots of the chicory plant were not only used as a food supplement but also for medicinal purposes. The juice of the chicory was used against eye disease and poisoning[1]. At first the wild plant was used but since approximately 300 BC chicory has been cultivated[2]. Several authors in ancient times, like Aristophanes (450-380 BC) and Theophrastus (372-287 BC), mention the chicory plant and its use. Dioscorides discussed the healing power of chicory in his famous book on medicine, Plinius in *Naturalis Historiae* stated that the infusion of chicory is good for the liver, kidneys and stomach and Horatius mentioned chicory in one of his odes[2,3]. The ancient Germans made magic potions of the chicory plant and both the Arabs and the Europeans in the middle ages knew chicory as a nutritional source and as a remedy for several diseases[4].

Figure 1.1.

In full bloom, wild chicory can reach about 1.5 m in height (Figure 1.1.). Its flowers have a characteristic blue, sometimes slightly pink colour. Cultivated chicory is one of the earliest known and most widely used raw material for the manufacture of coffee substitutes. It has been used for many years in European countries where production and consumption of coffee substitutes have a long tradition e.g. The Netherlands, Belgium, France, Germany and Russia. Chicory is responsible for a specific bitter-sweet, sour flavour of the coffee-like beverages and also gives 'body' to the brews. Brews from roasted chicory roots were first prepared in the 16th century[5,6]. In 1770, the use of roasted chicory roots was mentioned in Holland as an additive to coffee and around 1800 coffee was completely replaced by chicory brews[7]. The first factory for roasting chicory for coffee substitutes was founded in Holzminden, Germany in 1760. During the 19th and 20th century, the use of chicory

as a substitute for coffee changed to that of an additive. The importance of chicory gradually decreased in favour of other raw materials, especially roasted cereals. Chicory slowly lost its significance as a complete coffee substitute after the Second World War and its use as a coffee substitute is now limited to some vegetarians. In Japan, chicory extracts are also used for tabacco flavouring[8]. Due to its hygroscopicity and high content of fructose, it plays a role as a tobacco humidifier. Nowadays in western Europe, chicory leaves are cultivated for vegetable consumption only and the roots are considered to be a waste.

1.2. Production of chicory

Chicory sprouts are appreciated for their bitter taste. The raw leaves can be used as a salad additive or (boiled or blanched) as a vegetable. The production of the chicory can be comprised into two phases, the growing of the roots in the field and the forcing of the well-known white sprout under controlled conditions. In the Netherlands the seed is sown in April-May and the carrot-like root is harvested in September-October. After removing the foliage, the roots are embedded close together in wooden pits and provided with an ample supply of liquid nutrition. The roots are left to sprout in the dark and after approximately three weeks the characteristic white, cone-like heads can be harvested. Some 100.000 tons of chicory roots are produced annually in the Netherlands and they are regarded a waste product. Currently the roots are being used as constituents of cattle feed.

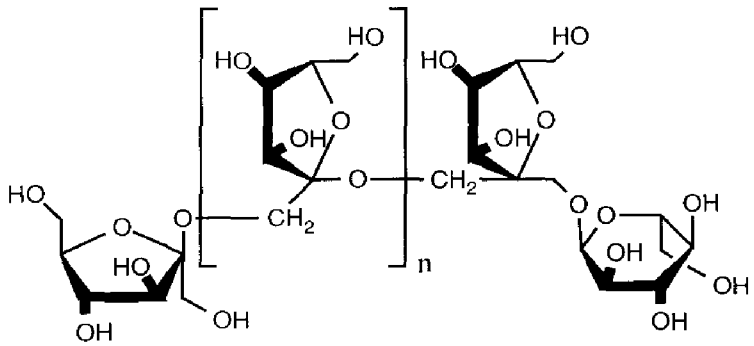
1.3. Constituents of the chicory root

1.3.1. Primary metabolites

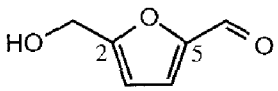
One of the most distinctive features in the biochemistry of the Compositae is the production of storage carbohydrates based on fructose instead of glucose. The main carbohydrate in the roots of the chicory is inulin (**1**). Its name is derived from *Inula helenium*, the roots of which are a particularly rich source of inulin[9]. Two other members of the Compositae family, which are also rich in inulin, are Jerusalem artichoke (*Heliantus tuberosus*)[10] and dahlia (*Dahlia variabilis*)[11]. Inulin is a fructose polymer, terminated by a glucose unit with a polymerisation degree of approximately 35, which means that the average molecular weigth lies around 6000 D. The inulin content of chicory roots is approximately 16% (w/w) but is heavily dependent on the

storage conditions[12]. Inulin cannot be digested by humans because of the lack of the appropriate enzymes, but it can be used as a raw material for the production of fructose, an interesting sweetener, which is 1.3-1.6 times sweeter than saccharose. Fructose can be obtained from inulin by hydrolysis under acidic conditions: pH 1-2 for 1-2 hours at 80-100°C. Since fructose is easily degraded by acid through enolisation and dehydration, a number of side reactions can occur during this hydrolysis. A more gentle way to produce fructose from inulin is by enzymatic degradation. Complete enzymatic hydrolysis of inulin was obtained in 3 hours at 50°C using an inulinase from the microbe *Kluyveromyces fragilis*[13]. Inulin has some medicinal value in diabetic diets, since diabetics tolerate fructose much better than glucose[14].

Figure 1.2.



1



2

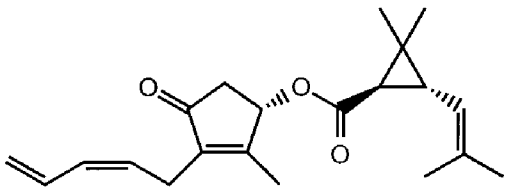
From a synthetic point of view, inulin can be used as a starting material for the production of 5-hydroxymethylfurfural (**2**, 5-HMF), a building block for the synthesis of a number of products[15]. Particulary the products formed by oxidation of 5-HMF at the 2- and the 5-position could find applications in the field of polymer chemistry[16]. Other carbohydrates in chicory roots are cellulose and pectin[17]. These components provide the structural skeleton of both the individual plant cells and the whole plant. Proteins and free amino acids are not very abundant in the roots as was determined by nitrogen determinations[4]. The lipid fraction of the root is rather small. A crude fat content of 1.6% was reported which was especially rich in linoleic acid[18].

1.3.2. Secondary metabolites

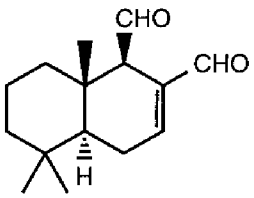
Secondary metabolites are often characteristic for a narrow range of species and fulfil an important function in the chemical defence of plants. Apart from a chemical, biogenetic and taxonomic point of view, secondary metabolites have become of

interest because of the ecological advantage they impart to the species that produce and accumulate them[19]. A good example in this respect is pyrethrin I (**3**), the pyrethrolone ester of chrysanthemic acid, isolated from *Chrysanthemum cinerariae-folium*, which has a strong insecticidal activity[20]. The dried and powdered flowers have long been used as a fly powder. Pyrethrin I (**3**) is active against *Musca domestica*, non-toxic towards warm blooded animals and rapidly degraded by oxidative metabolisms.

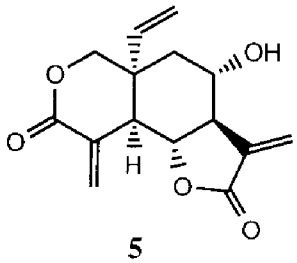
Figure 1.3.



3



4



5

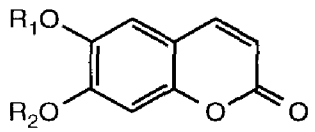
Another good example of a plant-insect interaction is given by polygodial (**4**), a potent insect antifeedant isolated from the bark of the East African *Warburgia* species[21]. A third example of a biologically active secondary metabolite is vernolepin (**5**), isolated from *Vernonia hymenolepis*[22] possessing spasmolytic[23] as well as antitumoral activity, both *in vivo* and *in vitro*[24].

Both the wild and the cultivated chicory are noticeably free from herbivory and insect attacks. The leaves of the chicory are completely unpalatable for three Coleoptera and one lepidopteran species tested[25]. The entire chicory plant, especially the fresh root, is extremely bitter-tasting to humans. Cultivation has had little effect on taste and the secondary metabolism of chicory. Two major groups of secondary metabolites have been isolated which play an important role in the chemical defence of chicory: phenolics and sesquiterpene lactones.

1.3.2.1. Phenolics

The phenolic compounds found in the chicory can be divided into two groups: coumarins and caffeic acid derivates. Coumarins are claimed to be partly responsible for the bitter taste of chicory[26].

Figure 1.4.

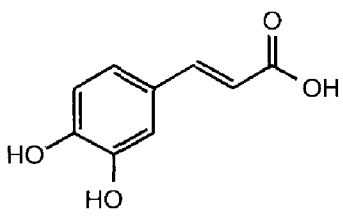


6: $R_1 = -H$; $R_2 = -H$
7: $R_1 = -H$; $R_2 = -Glc$
8: $R_1 = -Glc$; $R_2 = -H$

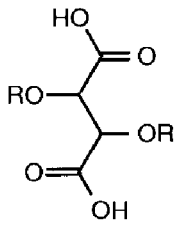
Three coumarins are reported in the leaves of chicory: esculetin (**6**), cichoriin (**7**) and esculin (**8**). Only cichoriin (**7**) is also found in the roots[27]. All three coumarins were investigated as insect antifeedants for desert locusts (*Schistocerca gregaria*)[27]. Cichoriin (**7**) was found to possess a significant antifeeding activity from $6x10^{-3}$% (w/w) while esculetin (**6**) and esculin (**8**) showed no activity even at a concentration of 0.1%. Several hydroxycoumarins as a class were tested but they rarely showed any effects on insects. The average content of cichoriin (**7**) in the chicory leaves was reported to be 0.04%. Coumarin itself inhibits feeding of some insect species only in high concentrations[28].

Caffeic acid (**9**) is one of the major phenolics in the *Compositae* family[29]. It is also a prime constituent of green and roasted coffee beans[30]. Two derivatives of caffeic acid are reported in the leaves and in the roots of chicory: cichoric acid (**10**, dicaffeoyltartaric acid)[31] and chlorogenic acid (**11**)[32].

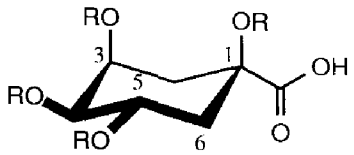
Figure 1.5.



9



10



11

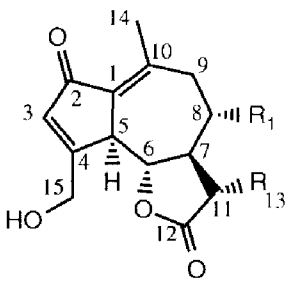
R = –caffeoyl (**9**)

The term chlorogenic acid is, in fact, used for a mixture of caffeoylquinic acids. These caffeoylquinic acids are polyphenolic compounds which act as a substrate for phenolases. The brown pigment that develops at the surface of freshly cut fruits and vegetables is due to the activity of this enzyme. In the roots of chicory 3-, 4-, 5-, 3,4-, 3,5- and 4,5-(di)caffeoylquinic acid are present[32]. No anti-feedant or other insecticidal activity has been reported for any of the chlorogenic acids.

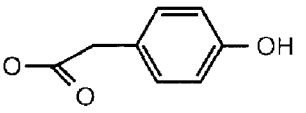
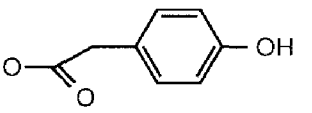
1.3.2.2. Sesquiterpene lactones

The presence of sesquiterpene lactones in chicory is associated with its bitter taste and it is likely that this repellent taste acts as a deterrent to mammalian herbivores. These bitter substances often contain an α,β-unsaturated lactone, which has been shown to be associated with anti-tumor, cytotoxic, anti-microbial and phytotoxic activity[33]. They are known to poison livestock[34-37], to act as insect antifeedants[38] and to cause allergic contact dermatitis in humans[39-44]. The bulk of the bitter principles isolated from chicory possess a guaiane framework. The three major sesquiterpene lactones found in both the leaves and the roots of chicory are the guaianolides lactucin (**12**), 8-deoxylactucin (**13**) and lactucopicrin (**14**). The 11,13-dihydro derivatives **15-17** were also isolated from the fresh roots[45]. The group of Seto reported the two eudesmanolides sonchuside C (**18**) and cichoriolide A (**19**) together with the germacranolides sonchuside A (**20**) and cichorioside C (**21**)[46].

Figure 1.6.



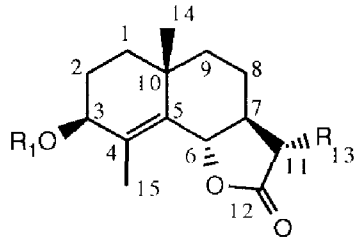
Guaianolide

Name	R	R_1
lactucin (**12**)	$=CH_2$	OH
8-deoxylactucin (**13**)	$=CH_2$	H
lactucopicrin (**14**)	$=CH_2$	
dihydrolactucin (**15**)	CH_3	OH
dihydro-8-deoxylactucin (**16**)	CH_3	H
dihydrolactucopicrin (**17**)	CH_3	

Lactucin (**12**) and lactucopicrin (**14**) were known to possess bitter tasting properties ever since their isolation from lactucarium, the dried latex of *Lactuca virosa*[47]. Lactucarium had the interest of scientists because of its potential pharmaceutical uses as a hypnotic due to its sedative properties. It was proposed in the early 19th century that the bitter compound of lactucarium was responsible for the sedative properties[47]. Much attention was therefore given to the identification of the sedative compound(s). Until now, no publications are known in which the relationship between the sedative properties and the bitter principles has been established.

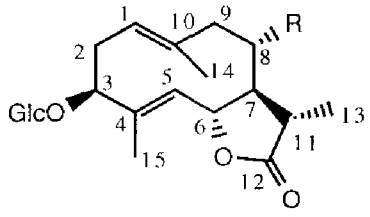
kalmerende werking

Figure 1.7.



Eudesmanolide

Name	R	R_1
sonchuside C (**18**)	CH_3	Glc
cichoriolide A (**19**)	$=CH_2$	H



Germacranolide

Name	R
sonchuside A (**20**)	H
cichorioside C (**21**)	OH

The six guaianolides **12-17** were isolated from fresh chicory roots and their bitterness was determined in sensory analysis studies[45]. Reduction of the exocyclic methylene group of the α,β-unsaturated lactone ring enhances the bitterness slightly while esterification of the hydroxyl function of lactucin (**12**) at C_8, especially with 4-hydroxyphenylacetic acid, lowers the threshold value for bitterness significantly.

Table 1.

Compound	Threshold	Compound	Threshold
12	1.7 ppm	**15**	1.4 ppm
13	0.5 ppm	**16**	0.2 ppm
14	1.1 ppm	**17**	1.1 ppm

The two guaianolides 8-deoxylactucin (**13**) and lactucopicrin (**14**) were tested as insect antifeedants for desert locusts (*Schistocerca gregaria*)[27]. Both sesquiterpene lactones exerted a similar effect on feeding. A fairly sharp threshold value at a concentration of 0.2% dry weight was reported, leveling off at a higher concentration to a deterrency of about 70%. Lactucin was not tested due to insufficient availability of the compound. The average value of the sesquiterpene lactone content was determined at 0.42% in the root and 0.26% in the leaves of chicory[27]. The average concentration of sesquiterpene lactones in both the roots and the leaves exceeds the level at which feeding

deterrance is reported. This indicates that the major chemical defence of chicory is due to the presence of both cichoriin (**7**) in the leaves and of lactucin (**12**), 8-deoxylactucin (**13**) and lactucopicrin (**14**) in the leaves and the root of the plant. All four compounds occur in the plant in a sufficient concentration to be effective. The general biosynthesis of sesquiterpenes will be discussed in Chapter 2.

1.4. References and notes

1. Hegi, G., *Illustrierte Flora von Mitteleuropa*, J.F. Lehmann, Munich (1906-1931).
2. Springer W., Die blaue Blume, Bartholdy und Klein, Berlin (1940).
3. Beitter H. and Schröder, C.-H., in *Handbuch der Lebensmittelchemie*, (Schormüller, J. ed.), Springer, Berlin, Vol. **VI**, 96-138 (1970).
4. Maier H.G., in *Coffee Volume 5: Related Beverages*, (Pazola Z., ed.), Elsevier Applied Science, London and New York, 19-57 (1987).
5. Ciupka P., *Kaffee, Kaffee-Ersatz und Kaffee-Zusatz*, Otto Meissner Verlag, Schloss Bleckede bei Hamburg, **1** (1949), **4** (1958).
6. Maier H.G., *Kaffee*, Parey, Berlin (1981).
7. Ukers W.H. *All about Coffee*, 2nd ed., The Tea and Coffee Trade Journal Company, New York (1935).
8. Sannai A., Fujimori T. and Kato K., *Agric. Biol. Chem.*, **46**, 429 (1982).
9. Heywood V.H. and Harborne J.B., *An overture to the Compositae*. in *The Biology and Chemistry of the Compositae*, (Heywood V.H., Harborne J.B and Turner B.L., eds.), Academic Press, London, 1-20 (1977).
10. Beck R.H.F. and Praznik W., *Starch/Staerke*, **38**, 391 (1986).
11. Fleming S.E. and Groot Wassink J.W.D., *CRC Cit. Rev. Food Sci. Nutr.*, **12**, 1 (1975).
12. Täufel K., in *Handbuch der Lebensmittelchemie*, (Tillemans J., ed.), Springer, Berlin (1934).
13. Zittan L., *Starch/Staerke*, **33**, 373 (1981).
14. Wagner H., *Pharmaceutical and economic uses of the Compositae*, in *The Biology and Chemistry of the Compositae*, (Heywood V.H., Harborne J.B and Turner B.L., eds.), Academic Press, London, 411-433 (1977).
15. Nakamura Y. and Morikawa S., *Bull. Chem. Soc. Jpn.*, **53**, 3705 (1980).
16. Vinke P., *Oxidation of Carbohydrates and Derivates using Carbon Supported Noble Metal Catalysts*, PhD-thesis, Delft University (1991).
17. Maier H.G., in *Coffee Volume 5: Related Beverages*, (Clarcke R.J. and Macrae R. ed.), Elsevier Applied Science, London and New York, 193-205 (1987).

18. Kim, T.-Y., Yoon K.-W. and Lee K.-W., *Korean J. Food Sci. Technol.*, **10**, 258 (1978).
19. Croteau R. and Johnson M.A., in *Biosynthesis and Biodegradation of Wood Compounds*, Academic Press, New York, 379-439 (1985).
20. Crombie L. and Elliott M., in *Fortschritte der Chemie Organischer Naturstoffe*, (Zechmeister L., ed), Springer-Verlag, Wien, Vol. **XIX**, 120 (1961).
21. Harborne J.B., *Introduction to Ecological Biochemistry*, 2nd ed., Academic Press, London (1982).
22. Kupchan S.M., Hemingway R.J., Werner D. and Karim A., *J. Org. Chem.*, **34**, 3903 (1969).
23. Vlietinck A.J., in *Biologically Active Natural Products*, (Hostettmann R. and Led P.J., eds), Clarendon Press, Oxford, 42 (1987).
24. Kupchan S.M., Eakin M.A. and Thomas A.M., *J. Med. Chem.*, **14**, 1147 (1971).
25. Jermy T., *Ent. Exp. Appl.*, **9**, 1 (1966).
26. van Hee L., *Ann. Gembloux*, **71**, 211 (1965).
27. Rees S.B. and Harborne J.B., *Phytochemistry*, **24**, 2225 (1985).
28. Adams C.M. and Bernays E.A., *Ent. Exp. Appl.*, **23**, 101 (1978).
29. Hegnauer R., *The chemistry of the* Compositae, in *The Biology and Chemistry of the Compositae*, (Heywood V.H., Harborne J.B and Turner B.L., eds.), Academic Press, London, 283-335 (1977).
30. Wolfrom M.L., Plunkett R.A. and Laver M.L., *J. Agr. Food Chem.*, **8**, 58 (1960).
31. Scarpati M.L. and Oriente G., *Tetrahedron*, **4**, 43 (1958).
32. Clifford M.N., Shutler S, Thomas G.A. and Ohiopehai O., *Food Chem.*, **24**, 99 (1987).
33. Rodriguez E., Towers G.H.N. and Mitchell J.C., *Phytochemistry*, **15**, 1573 (1976).
34. Sperry O.E., Dollahite J.W., Hoffman G.O. and Camp B.J., *Texas Plants Poisonous to Livestock*, Texas A&M University, College Station (1964).
35. Schmutz E.M., Freeman B.N. and Reed R.E., *Livestockpoisoning Plants of Arizona*, University of Arizona Press, Tucson (1968).
36. Ivie G.W., Witzel D.A., Herz W., Kanna R., Norman J.O., Rushing D.D., Johnson J.H., Rowe L.D. and Veech J.A., *J. Agric. Food Chem.*, **23**, 841 (1975).
37. Ivie G.W., Witzel D.A. and Rushing D.D., *J. Agric. Food Chem.*, **23**, 845 (1975).
38. Burnett W.C., Jones S.B., Mabry T.J. and Padolina W., *Biochem. System. Ecol.*, **2**, 25 (1974).
39. Mitchell J.C., *Trans. St. John's Hosp. Derm. Soc.* (London), **55**, 174 (1969).
40. Mitchell J.C., Fritig B., Singh B and Towers G.H.N., *J. Invest. Derm.*, **54**, 233 (1970).
41. Mitchell J.C., Dupuis G. and Towers G.H.N., *Br. J. Derm.*, **87**, 235 (1972).

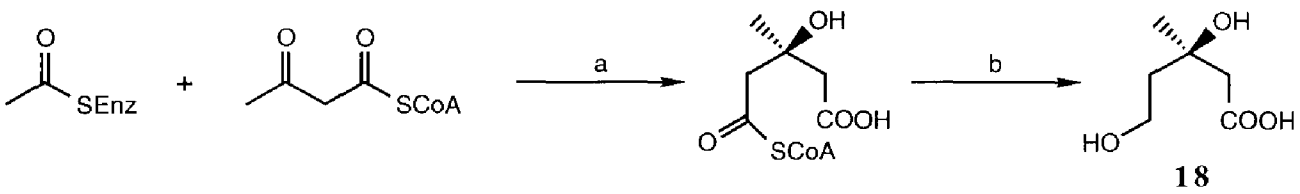
42. Mitchell J.C., Roy A.X. and Dupuis G.H.N., *Arch. Derm.*, **104**, 73 (1971).
43. Bleumink E., Mitchell J.C. and Nater J.P., *Arch. Dermatol.*, **108**, 220 (1973).
44. Lonkar A., Mitchell J.C., Calnan C.D., *Trans. St. John's Hosp. Derm. Soc.* (London), **60**, 43 (1974).
45. van Beek, T.A., Maas, P, King B.M., Leclercq E., Voragen A.G.J. and de Groot Ae., *J. Agric. Food Chem.*, **38**, 1035 (1990).
46. Seto M., Miyase T., Umehara K., Ueno A, Hirano Y. and Otani N., *Chem. Pharm. Bull.*, **36**, 2423 (1988).
47 Schenk G. and Graf H., *Arch. Pharm.*, **274**, 537 (1936).

2. Germacrane sesquiterpenes: (bio)synthesis and cyclisation reactions

2.1. Biosynthesis of farnesyl pyrophosphate

It is widely recognised that terpenoid biosynthesis starts from mevalonic acid (**18**)[1]. The biosynthesis of mevalonic acid (**18**) proceeds *via* the condensation of enzyme bound acetate with acetoacetate coenzyme A (CoA) and is catalysed by two enzymes: 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMG-CoA synthase) and HMG-CoA reductase[2]. The genes encoding these enzymes have been well characterised in plants[3,4], mammals[5] and bacteria[6-8].

Scheme 2.1.[a]

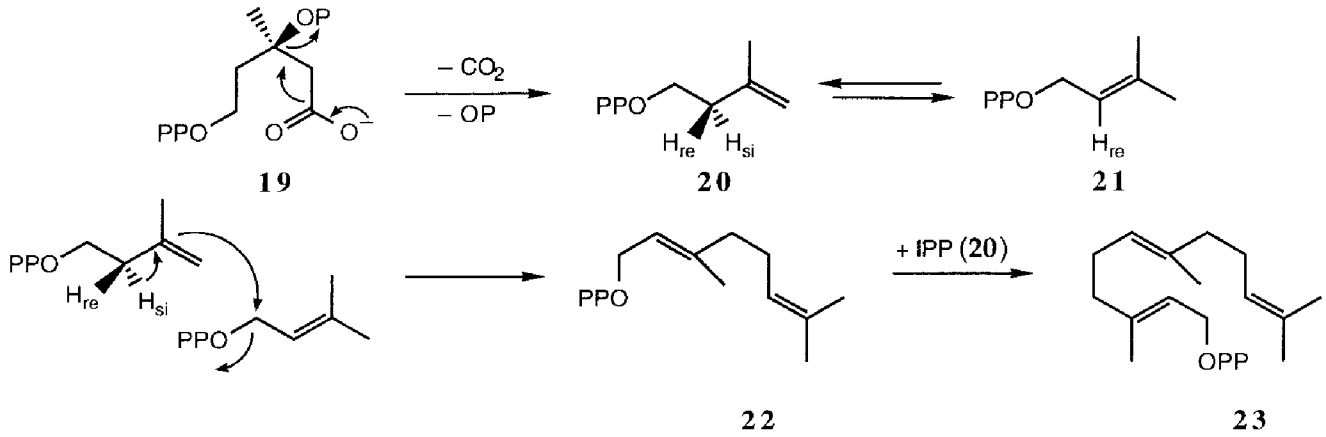


18

[a] a) HMG-CoA synthase; b) HMG-CoA reductase.

Mevalonic acid (**18**) is converted by the action of mevalonic kinase and ATP to 3-phosphoromevalonate which, in turn, undergoes phosphorylation mediated by a second kinase to 5-pyrophosphoromevalonate. Mevalonic kinase has been purified from a number of organisms and its mode of action is well documented[9-15]. Decarboxylation and dephosphorylation of the intermediate 3-phosphoro-5-pyrophosphoromevalonate (**19**) yields isopentenyl pyrophosphate (IPP, **20**), the 'Biological Isoprene Unit'[16,17] which serves as the fundamental building block for all terpenoids. Enzymatic isomerisation of IPP, catalysed by isopentenyl pyrophosphate isomerase, gives the corresponding allylic isomer, dimethylallyl pyrophosphate (DMAPP, **21**)[18]. Condensation of DMAPP with IPP generates a new C_{10} allylic pyrophosphate, geranyl pyrophosphate (**22**), which may undergo further condensation with a second IPP to yield the C_{15} homologue farnesyl pyrophosphate (**23**, Scheme 2.2.). As a consequence, terpenes exhibit a carbon content in multiples of five carbons which leads to a simple classification based on their number of carbon atoms. This classification divides the terpenes into *hemiterpenes* (C_5), *monoterpenes* (C_{10}), *sesquiterpenes* (C_{15}), *diterpenes* (C_{20}), *sesterpenes* (C_{25}) etc.[19].

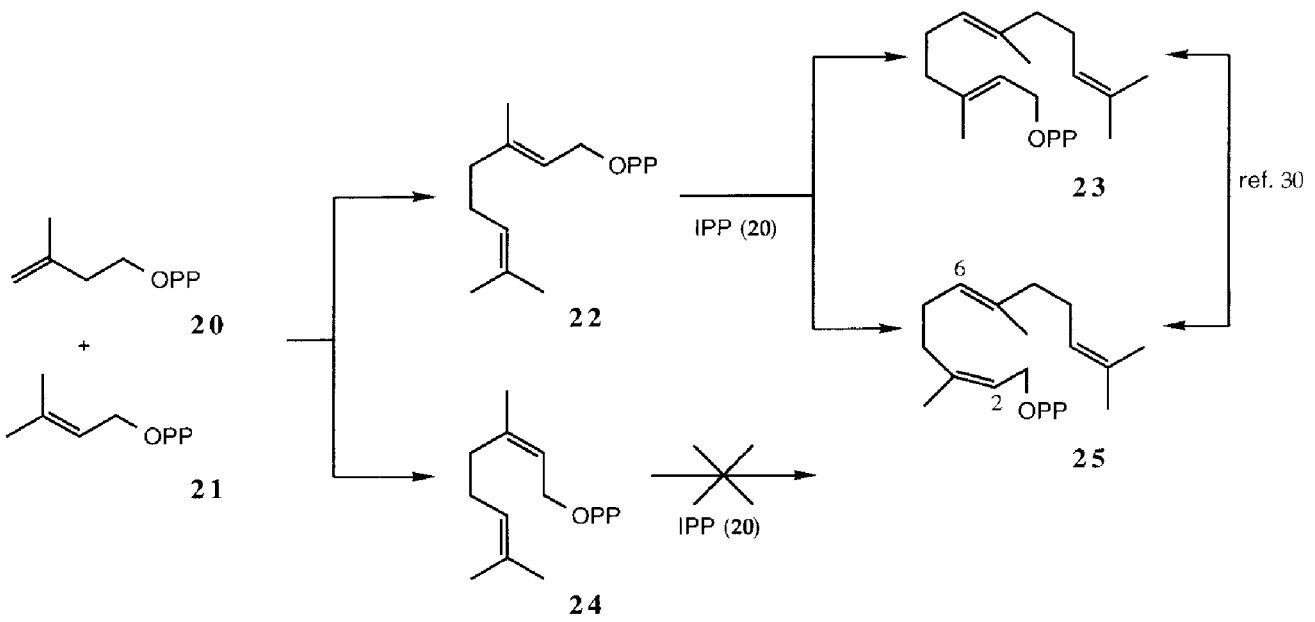
Scheme 2.2.



Biosynthetic tracer studies revealed that the formation of the trans-double bond of geranyl pyrophosphate (**22**) and (E,E)-farnesyl pyrophosphate (**23**) involves retention of H_{4re} and loss of H_{4si} of IPP (**20**) whereas a cis-double bond requires the loss of the corresponding H_{4re} and retention of the H_{4si} atoms[20-25]. The condensation of IPP (**20**) with DMAPP (**21**) gives geranyl pyrophosphate (**22**) or neryl pyrophosphate (**24**) depending on the enzyme involved[26-28].

Cori demonstrated that only the trans isomer **22** was directly transformed into both (E,E)- and (Z,E)-farnesyl pyrophosphate (**23** and **25** respectively) by a cell-free extract of *Citrus sinensis*[23-25]. Neryl pyrophosphate (**24**) was found to be unreactive in further condensation reactions with IPP in this system[29].

Scheme 2.3.

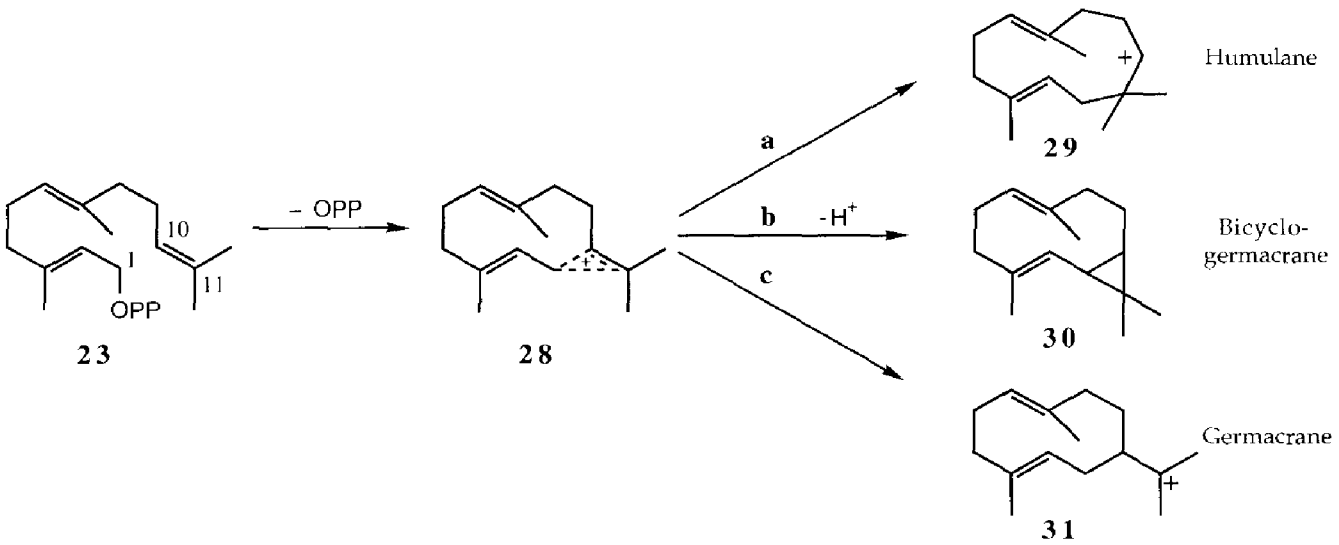


It is, therefore, unlikely that a 6-cis double bond can be obtained at this stage of the biogenesis. The 2-cis double bond of (Z,E)-farnesyl pyrophosphate (**25**) can be constructed either by direct formation from geranyl pyrophosphate (**22**) or *via* an allylic isomerisation of (E,E)-farnesyl pyrophosphate (**23**). The stereochemical details of these condensation reactions, backed by substantial literature on both subjects, were brilliantly summarised by Cane[30].

2.2. Cyclisation reactions of farnesyl pyrophosphate

The precursor of all sesquiterpenes is farnesyl pyrophosphate (**23**); its biosynthesis has been outlined in scheme 2.2. Head (C_1) to tail (C_{10}-C_{11}) cyclisation of **23**, as outlined in scheme 2.4., leads to a number of macrocyclic compounds. This cyclisation is initiated by an enzyme-mediated dissociation of the pyrophosphate group[31], resulting in the formation of an incipient carbocation at the head position of the farnesyl chain. Participation of the terminal double bond, under formation of a 'bridged' carbocation, paves the way for the formation of these macrocyclic ring structures[32-34].

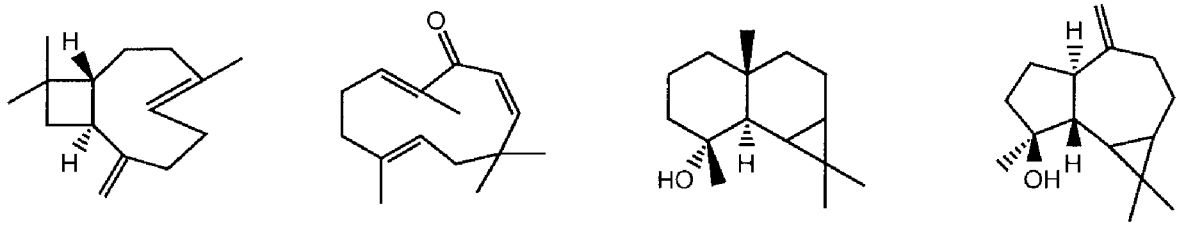
Scheme 2.4.



Pathway **a** depicts the formation of the 11-membered monocyclic sesquiterpenes of the humulane class (**29**)[35,36], which are likely precursors for a number of other naturally occurring sesquiterpenes like caryophyllene (**32**)[37,38] and zarumbone (**33**)[39]. Pathway **b** involves a 1,3-deprotonation of **28** which accounts for the formation of the *gem*-dimethylcyclopropane ring to form the bicyclogermacrane framework[40]. This 10-membered bicyclic sesquiterpene is the corresponding biosynthetic precursor of

maaliane- and aromadendrane sesquiterpenes[32,33,41], for example (+)-maaliol (**34**)[42,43] and (+)-spathulenol (**35**), the latter possessing repellant properties against the leaf cutter ant (*Atta cephalotes*)[44].

Figure 2.1.



32 **33** **34** **35**

Cyclisation of cation **28**, as depicted in pathway **c**, yields the germacra-1(10),4(5)-diene skeleton which is believed to be the precursor of numerous eudesmanes and guaianes[32-34].

2.3. Stereochemical aspects of germacrane cyclisation

The literature on the isolation and characterisation of natural sesquiterpenes is overwhelming. Excellent overviews have been presented by Fraga[45-47]. Annually, the isolation of some 200 new germacrane, eudesmane and guaiane sesquiterpenes, mainly from higher plants, is reported. The elucidation of the ring fusion of a number of sesquiterpenes, particularly in newly isolated guaianes, is still a major challenge. Up to now it is generally accepted that (E,E)-1,5-germacranes and their 1,10-epoxides cyclise into trans-fused eudesmanes while 4,5-epoxides of (E,E)-1,5-germacranes cyclise into cis-fused guaianes[32,33]. In the following paragraphs, the attention is focused on transannular cyclisation reactions of germacranes, as reported in the literature. The possible biogenetic relationship between the double bond geometry of configurationally isomeric germacranes and the stereochemistry of the cyclised products is discussed.

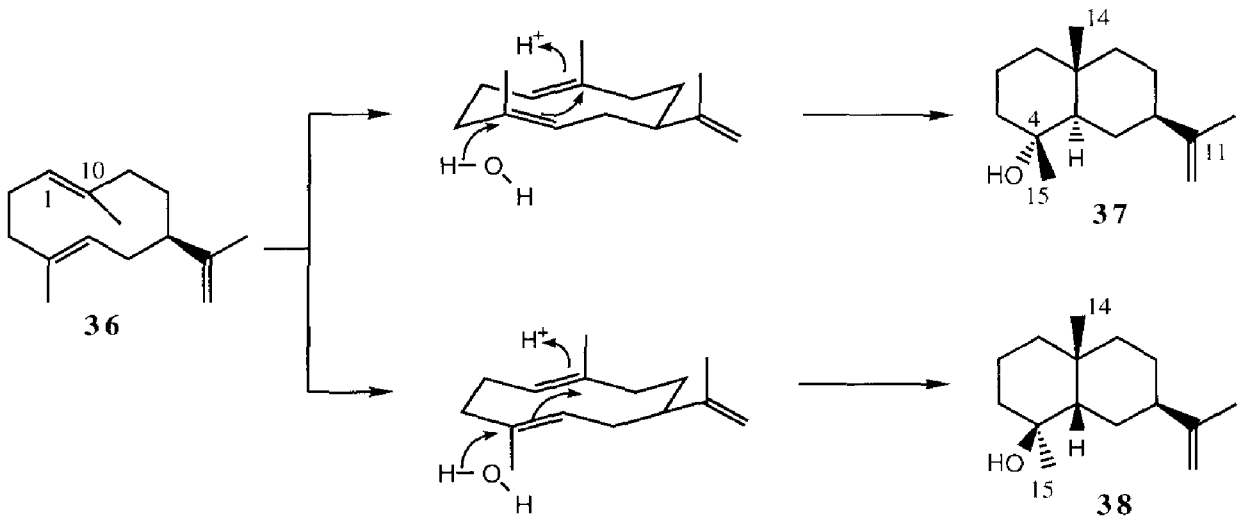
2.3.1. Transannular cyclisation reactions of (E,E)-1,5-germacranes

The flexibility of the 10-membered ring system has frequently been abused to explain the observed stereochemistry of the cyclisation products. However, the classical chemical concepts of strain and steric interactions were thought to be not necessarily

the dominant factors in the biosynthesis of natural products, since the substrates must concur with the conformational requirements of the enzymes involved in the cyclisation process.

The cyclisation of (E,E)-1,5-germacranes into eudesmanes, possessing a trans-fused decalin framework, is believed to proceed via a transannular cyclisation through the chair-chair conformation of the germacrane, which involves protonation of the 1,10-double bond, as illustrated by the cyclisation of germacrane A (**36**)[48] into selen-11-en-4α-ol (**37**)[49]. Eudesmanes with a cis-fused decalin framework like amiteol (**38**)[50] were postulated to be formed through the energetically unfavourable boat-boat conformation[33].

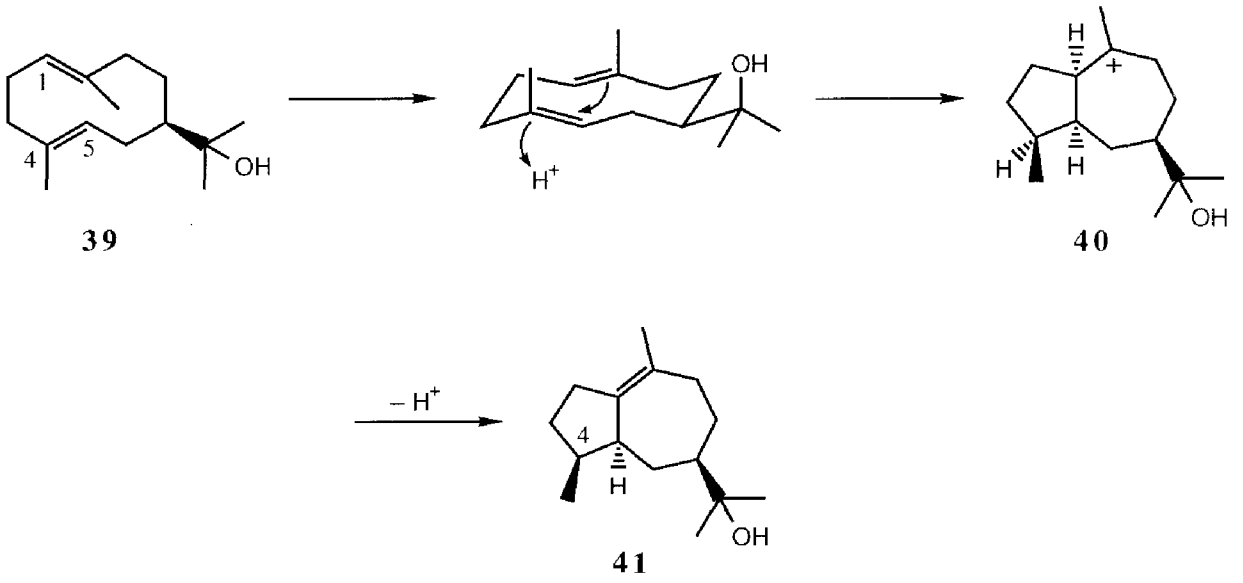
Scheme 2.5.



It is relatively easy to elucidate the stereochemistry of the ring fusion of eudesmane sesquiterpenes using (2D) ^{1}H- and ^{13}C-NMR techniques. In systems like selen-11-en-4α-ol (**37**), both methyl groups, Me_{14} and Me_{15}, are in an axial position and will therefore display a Nuclear Overhauser Effect (NOE). This effect will not be observed in cis-fused eudesmanes like amiteol (**38**). Cis-fused eudesmanes also differ significantly from their trans-fused counterparts in their ^{13}C-NMR spectrum. The carbon atom of the bridgehead methyl group, C_{14}, resonates at approximately 30 ppm while in trans-fused eudesmanes C_{14} resonates between 15-20 ppm[51] depending on its chemical environment. The work of Kesselmans, describing the total synthesis of all stereoisomers of eudesm-11-en-4-ols, substantiates the above mentioned observations[52].

The biosynthesis of guaiane sesquiterpenes was thought to proceed via the same type of cyclisation procedure which is responsible for the formation of eudesmane sesquiterpenes, this time involving protonation of the 4,5-double bond. The biosynthesis of bulnesol (**41**)[53] was postulated to start from hedycaryol (**39**)[54,55]. Cyclisation into the cis-fused guaiane cation **40**, followed by selective deprotonation of the bridgehead methine, should give bulnesol (**41**)[32].

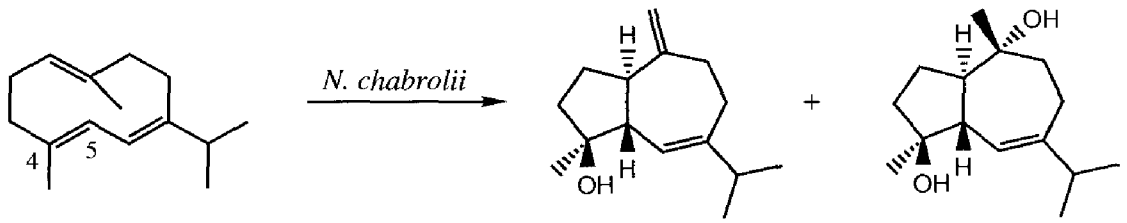
Scheme 2.6.



Unlike bulnesol (**41**), the majority of guaiane sesquiterpenes possesses a hydroxyl function at C_4 or another functional group derived from, e.g., dehydration. This hydroxyl function is the result of the epoxidation of the C_4-C_5 double bond of a germacrane intermediate, followed by cyclisation and bond formation between C_1 and C_5. Biomimetic cyclisations of (E,E)-1,5-germacrane-4,5-epoxides have confirmed that these epoxides are indeed precursors for guaianes while (E,E)-1,5-germacranes and their 1,10-epoxides cyclise into eudesmanes[53,56-59]. This subject will be discussed in detail in chapter 5.

The absolute stereochemistry of the guaiane framework, obtained through cyclisation of germacrane-4,5-epoxides derived from (E,E)-1,5-germacranes, still causes confusion in the literature. For instance, three different configurations were postulated for alismol (**43**), one of the cyclisation products of germacrene C (**42**)[60-63]. An X-ray structure of the diol **44**, isolated together with **42** and **43** from the soft coral *Nephthea chabrolii*[62], confirmed the absolute stereochemistry of alismol (**43**), according to the authors. Unfortunately, this X-ray structure of **44** has never been published.

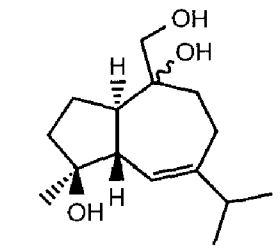
Scheme 2.7.



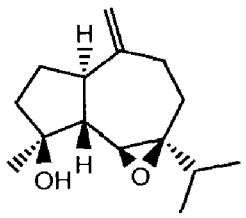
42 **43** **44**

Germacrene C (**42**) is extremely sensitive to oxygen and, as a consequence, is readily converted into **43** by air epoxidation of the C_4-C_5 double bond followed by spontaneous cyclisation and deprotonation. In an aqueous acetone solution, **42** is converted into **44**. Both cyclisations proceed in high yields[62]. Only recently, additional NOE-experiments on three new sesquiterpenes (**45-47**) isolated together with **43** and **44** from the fresh rhizomes of *Alisma orientale*[64,65], and detailed reinvestigation of the physiochemical properties of **43** assigned the absolute stereochemistry of **43**.

Figure 2.2.



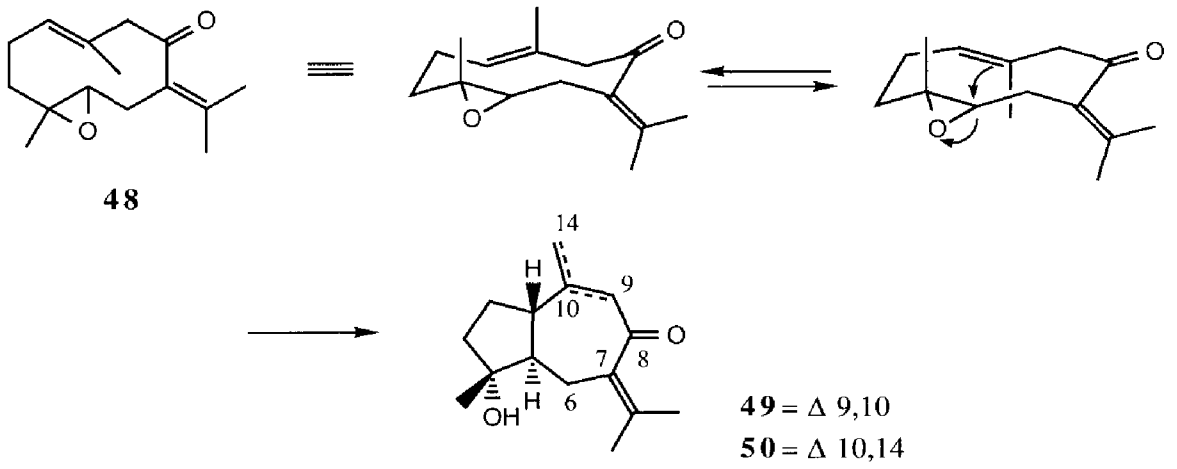
45 = α - OH; β - CH_2OH
46 = α - CH_2OH; β - OH



47

The observation that (E,E)-1,5-germacranes and their epoxides cyclise into guaianes and eudesmanes possessing a trans-fused skeleton has recently been confirmed by several *in vivo* cyclisation reactions[66,67]. For instance, procurcumenol (**49**), one of the cyclisation products of germacrone-4,5-epoxide (**48**) has a trans-fused guaiane framework, which was established by the chemical isomerisation of its isomer isoprocurcumenol (**50**). The absolute stereochemistry of **50** was ascertained by means of its X-ray structure[68]. The trans-fusion of the guaiane framework might implicate that, in general, the cyclisation of simple germacrane-4,5-epoxides, derived from (E,E)-1,5-germacranes, does not occur through the energetically more favourable chair-chair conformation but through the more twisted boat-boat conformation.

Scheme 2.8.



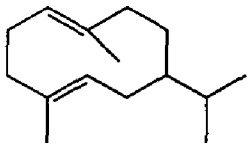
These recent findings are in contrast to earlier postulated cyclisation mechanisms[32,33]. Although claims have been made that 4,5-epoxides derived from (E,E)-1,5-germacranes cyclise into guaianes with a cis-fused framework[69,70], the establishment of the stereochemistry frequently originates from earlier postulated cyclisation methods, insufficient spectral data and the lack of an X-ray structure of the cyclised product. Nowadays, these earlier stereochemical assignments are considered doubtful, so some caution must be exercised in interpreting these results.

This does not exclude, however, the existence of cis-fused guaianes. A number of guaiane sesquiterpenes with a cis-fused framework have actually been identified by means of their X-ray structure[71-74]. Especially the tribes Eupatorieae and Anthemideae of the Compositae family are a rich source of cis-fused guaianes[75]. These cis-fused guaianes all possess a lactone moiety, either at C_6-C_7 or at C_7-C_8. Some aromadendrenes, bearing a *gem*-dimethylcyclopropane ring at C_6-C_7 also have a cis-fused guaiane framework[41]. The presence of these annulated rings near the 1,5-diene system of the germacrane might exert a directing effect on the transannular cyclisation. Whether this influence on the conformational mobility of the 10-membered ring is electronic or steric in nature, is not known and therefore remains a matter of speculation.

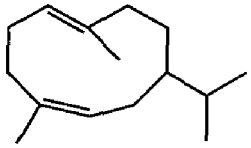
2.3.2. Transannular cyclisation reactions of other 1,5-germacranes

The discovery of configurationally isomeric germacranes, i.e. heliangolides[76,77] or (Z,E)-1,5-germacranes, melampolides[77,78] or (E,Z)-1,5-germacranes and (Z,Z)-1,5-germacranes[79,80] has led to a reclassification of the 1,5-germacranes into four sub-groups which are characterised by the geometry of the C_1-C_{10} and C_4-C_5 double bonds in the cyclodecadiene framework[77,80].

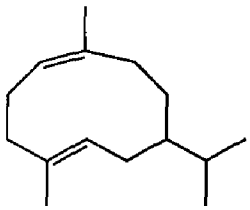
Figure 2.3.



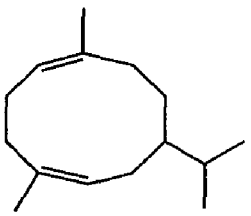
(E,E)-1,5-germacrane



Heliangolide
(Z,E)-1,5-germacrane



Melampolide
(E,Z)-1,5-germacrane



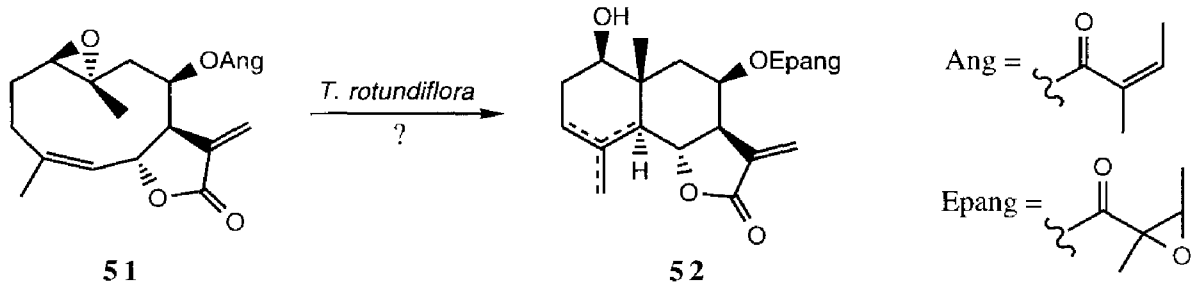
(Z,Z)-1,5-germacrane

The isolation of these (Z,E)-, (E,Z)- and (Z,Z)-1,5-germacranes together with eudesmanes and guaianes from the same natural source might implicate that these germacranes play an important role as biogenetic precursors in the biosynthesis of these cyclic sesquiterpenes. The following paragraphs present an attempt to establish a biogenetic relationship between the configuration of the double bond of the germacrane skeleton and the ring fusion of the cyclisation products. However, due to the scarcity of *in vitro* and the lack of *in vivo* cyclisation studies, this relationship is only based on the co-occurrence of configurationally isomeric germacranes and cyclised sesquiterpenes with an identical substitution pattern and is, therefore, rather speculative.

2.3.2.1. Heliangolides

The isolation of cis-fused guaianes and trans-fused eudesmanes together with heliangolides might indicate a possible biogenetic relationship[81-83] (Scheme 2.9.).

Scheme 2.9.



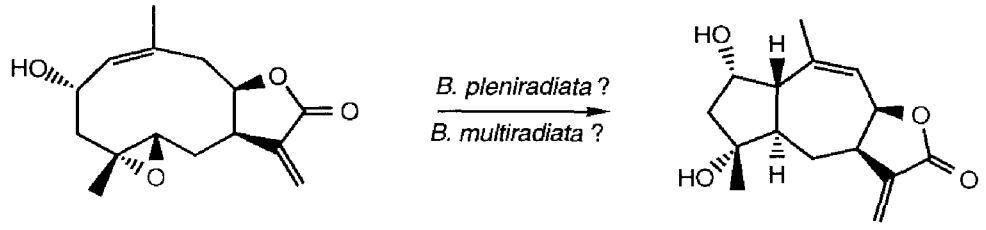
51 **52**

The relationship between the transannular cyclisation products of (E,E)-1,5-germacrane-1,10-epoxides and trans-fused eudesmanes bearing a hydroxyl function at C_1 has been firmly established by *in vitro* and *in vivo* cyclisations[66,67,84]. If this observation is extrapolated to the heliangolide-1,10-epoxides, eptocarpinolide **51** is the likely precursor of trans-fused eudesmanes like **52**. Both compounds were isolated from *Tithonia rotundiflora* (Scheme 2.9.)[85]. However, no *in vivo* or *in vitro* cyclisations of heliangolides or their epoxides have ever been reported.

2.3.2.2. Melampolides

A possible biogenetic relationship between melampolides and the ring stereochemistry of guaiane sesquiterpenes has been postulated by Herz *et al*[86] (Scheme 2.10.).

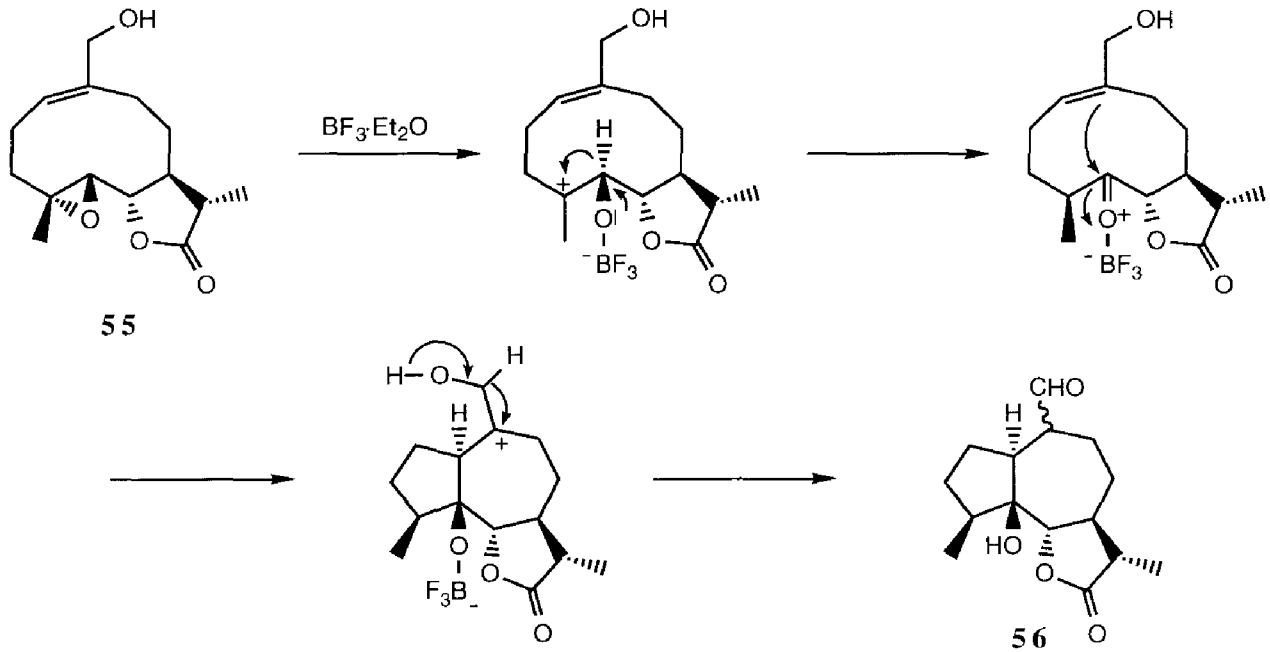
Scheme 2.10.



53 **54**

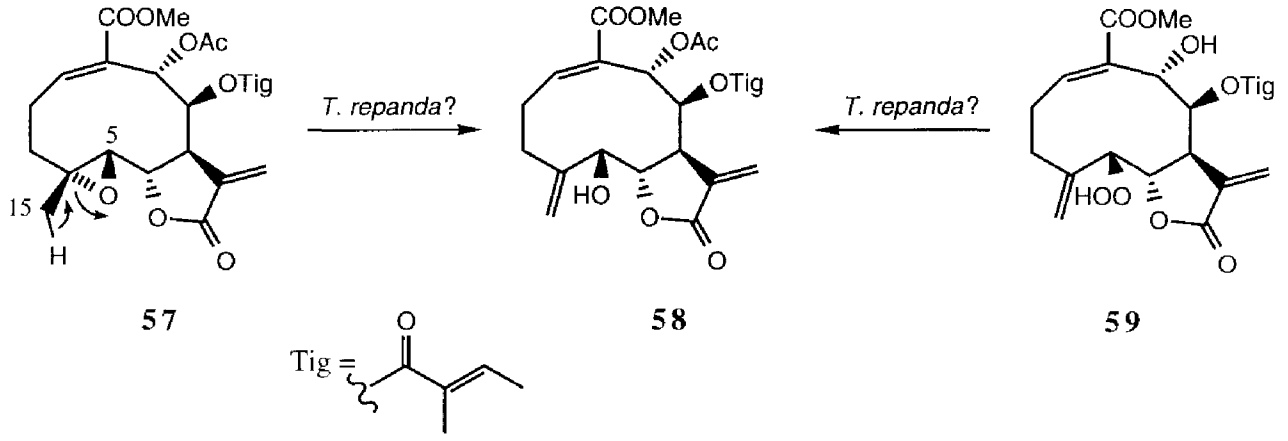
The melampolide-4,5-epoxide baileyin (**53**) was isolated together with the trans-fused guaianolide pleniradin (**54**) from *Baileya pleniradiata* and *B. multiradiata*. The stereochemistry of pleniradin (**54**) was confirmed by X-ray analysis. A biomimetic conversion of the melampolide-4,5-epoxide melampomagnolide B (**55**) into the trans-fused epimeric guaianolide aldehyde **56** using $BF_3{\cdot}Et_2O$ was reported by Gonzalez *et al*[87]. Two hydride shifts were postulated to account for the observed regio- and stereochemistry of the cyclised products, as outlined in scheme 2.11.

Scheme 2.11.



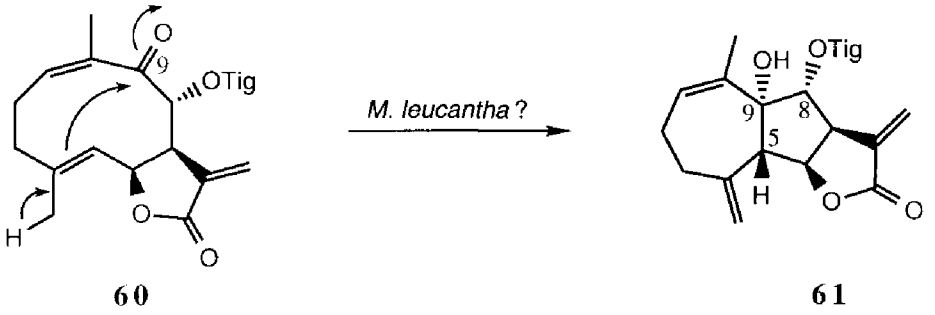
Melampolides are thought to be the biogenetic precursors of a number of compounds. Several trans-fused eudesmanes have been isolated together with melampolides[88,89]. Sesquiterpene lactones of the repandolide type like **58** are thought to be derived from melampolide-4,5-epoxides like **57**; both compounds were isolated from the aerial parts of *Tetragonotheca repanda*[90]. Proton abstraction from Me_{15}, followed by subsequent epoxide ring opening, or, alternatively, a singlet oxygen process via the C_5 hydroperoxide followed by bioreduction of the hydroperoxide group to give the C_5 alcohol function, was postulated as a possible mechanism (Scheme 2.12.). The isolation of hydroperoxide **59** from the aerial parts of *Smallanthus macvaughii* supports these findings[91]. The bioreduction of hydroperoxides like **59** into 2-hydroxy-germacradienes like **58** was also described by Doskotch *et al.*[92,93].

Scheme 2.12.



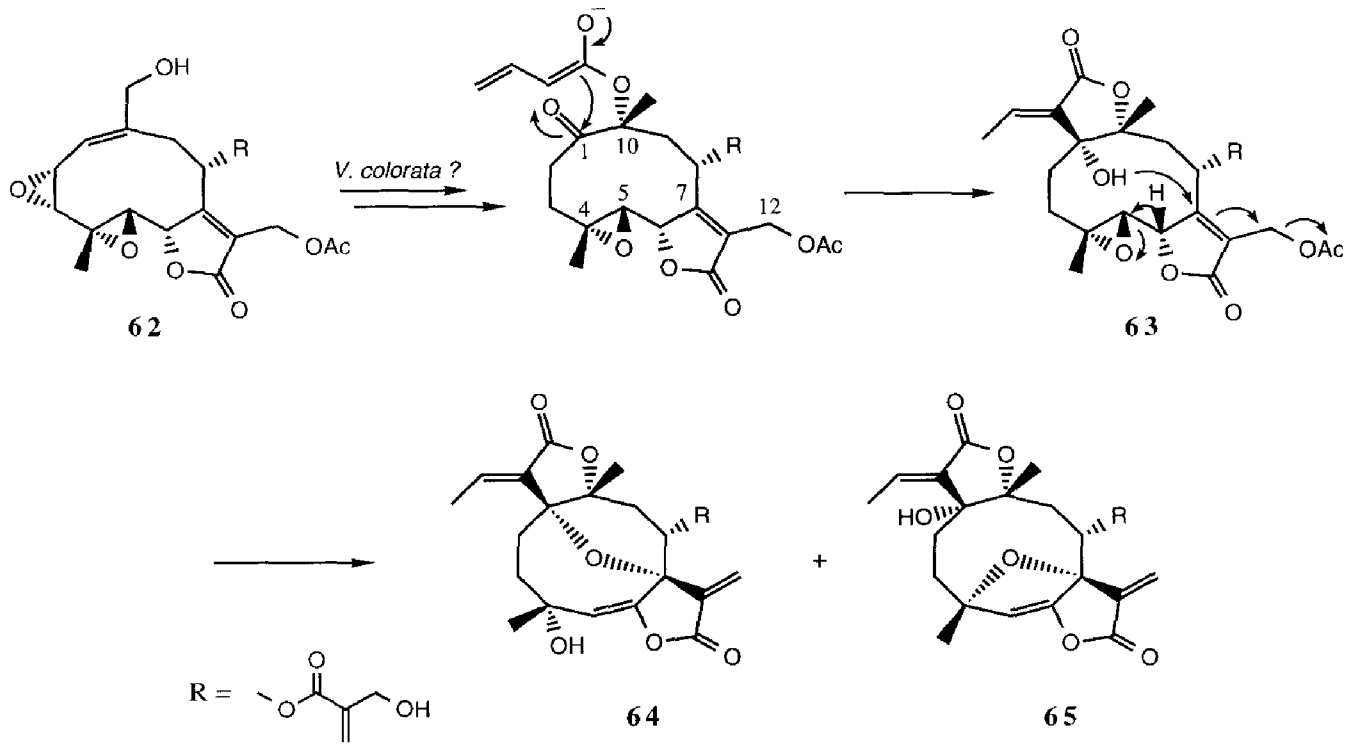
The sesquiterpene lactones of the montahibisciolide type are also thought to be biogenetically derived from a melampolide precursor[94]. Both compounds were isolated from the aerial parts of *Montanoa leucantha*. Instead of a 'regular' trans-annular cyclisation, the keto function at C_9 of melampolide **60** participates in the cyclisation reaction to give montahibisciolide **61** (Scheme 2.13.). X-ray analysis confirmed the α-orientation of the hydroxyl at C_9 and the tiglate function at C_8 as well as the β-orientation of H_5.

Scheme 2.13.



The cyclic ether 16E-isobrachycalyxolide **64** is probably derived from vernocistifolide **62**, a highly oxidised melampolide. Both compounds were isolated from *Vernonia colorata*[95,96]. The biosynthetic route to **64** presumably starts with modifications of **62** followed by an intramolecular aldol condensation from the butenoate ester at C_{10}, giving the alcohol **63** (Scheme 2.14.).

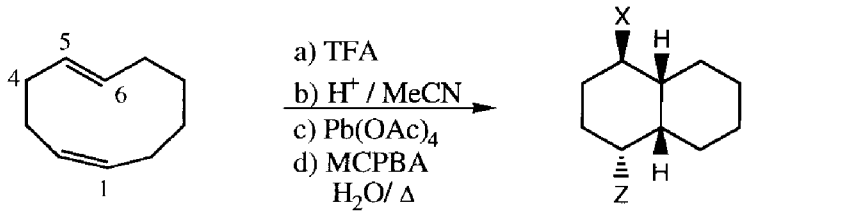
Scheme 2.14.



Subsequent ether formation between C_1 and C_7 concomitant with deacetylation at C_{12} followed by opening of the 4,5-epoxide ring should yield **64** (Scheme 2.14). The fact that not only **64** but also **65** is a natural product suggests that the ether formation does not necessarily has to preceed the epoxide ring opening.

The scarcity of 'regular' transannular cyclisation reactions of melampolides and their epoxides is blamed on the considerably greater centre to centre distance of the double bonds in the (E,Z)-cyclodeca-1,5-diene system[97]. Instead, oxidative modifications of the 10-membered ring become a competitive biosynthetic process. The cyclisation reaction of (Z,E)-cyclodeca-1,5-diene **66** gives more insight into the transannular cyclisation reaction of heliangolides and melampolides. Due to the lack of ring substituents, **66** is a compound possessing both an heliangolide and a melampolide framework. Cyclisation of **66** using trifluoroacetic acid or sulphuric acid in acetonitril gave cis-fused decalins (**67a, b**)[98]. The reaction of **66** with $Pb(OAc)_4$ produced a trans-1,4-diacetoxy-cis-decalin (**67c**)[99]. These findings indicate that cyclisation was initiated by addition of the electrophile on C_5, the carbon in the double bond with the trans configuration, concertedly followed by bond formation between C_1 and C_6 and incorporation of the nucleophilic counterpart of the reagent or the solvent.

Scheme 2.15.



66

67a: X = H; Z = $OCOCF_3$
67b: X = H; Z = NHCOMe
67c: X = Z = OAc
67d: X = Z = OH

The preferential reaction of such reagents with the trans double bond implies that the conversion of the cyclodecadiene to a substituted cis-decalin is accompanied by a substantial strain relief. The energy profit gained by a nucleophilic attack at the trans double bond therefore must find its origin in relieving the strain imposed by the C_4-C_5-C_6 bond angle of the 10-membered ring into an sp^3-hybridisation which resembles the normal tetrahedral angle[100]. Mono-epoxidation as well as methylenation also occurs principally on the trans double bond[98]. Cyclisation of the E-epoxide of **66** in boiling water renders a trans-substituted cis-fused decalin (**67d**) similar to the acid-induced cyclisations.

These results are in sharp contrast to the trans-fused eudesmanes which have been isolated together with heliangolides and melampolides from natural sources. However, as stated earlier, the postulated formation of these trans-fused eudesmanes from (Z,E)- and (E,Z)-germacranolides is highly speculative since their occurrence can also be due to the cyclisation of unidentified (E,E)-1,5-germacranolides or their 1,10-epoxides. No cyclisation products with a guaiane framework were reported in the cyclisation reaction of **66**. It is, however, not unlikely that cis-fused guaianes will be formed after cyclisation of the Z-mono-epoxides derived from heliangolides. Several Z-epoxides, for instance **62**, have been isolated from nature.

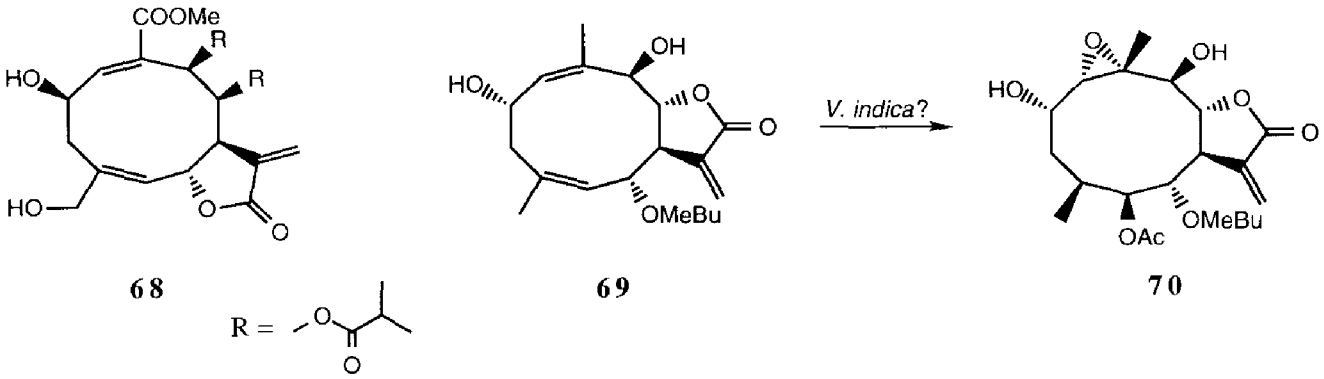
2.3.2.3. (Z,Z)-1,5-germacranes

The (Z,Z)-1,5-germacranes are relatively few in number. The first (Z,Z)-1,5-germacrane was isolated by Bohlmann *et al.* from *Chrysanthemum poteriifolium*. Its stereochemistry was established by spectroscopic methods[101]. Several years later the first X-ray structure of a (Z,Z)-1,5-germacrane, longicornin A (**68**), isolated from *Melampodium longicorne*[80], unambiguously established the existence of this long sought

class of germacranes. Just like the melampolides, oxidative modifications of (Z,Z)-1,5-germacranes play an important role in their biogenesis. The diol acetate **70** is probably derived from (Z,Z)-1,5-germacranolide **69**. Both compounds were isolated from the aerial parts of *Vicoa indica*[102] (Scheme 2.16.)

It is not unlikely that (Z,Z)-1,5-germacranes undergo a transannular cyclisation reaction. The flexibility of the 10-membered ring is considerably larger as compared to the (E,E)-, (Z,E)- and (E,Z)-1,5-germacranes. This diminishes the centre to centre distance of the double bonds which might hamper transannular cyclisation of the melampolides and heliangolides[101,103,104].

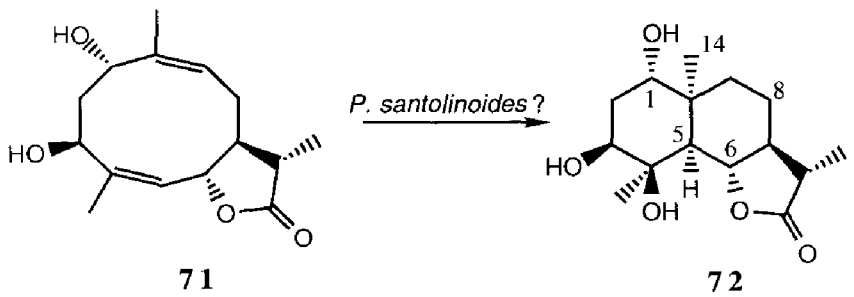
Scheme 2.16.



2.3.2.4. Cyclisation reactions of miscellaneous germacranes

Not only (E,E)-1,5-germacranes can exert transannular cyclisation reactions. Cyclisations of germacranes with double bonds located at positions other than C_1 and C_5 could also play a significant role in the biosynthetic pathway of plant secondary metabolites (Scheme 2.17.).

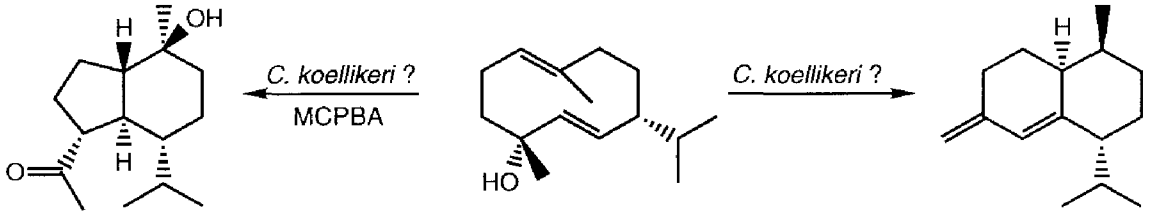
Scheme 2.17.



The occurrence of the cis-fused eudesmane sesquiterpene **72** together with the (Z,Z)-1,6-germacranolide **71**, both isolated from the aerial parts of *Pyrethrum santolinoides*, is an indirect evidence for a transannular cyclisation reaction of this (Z,Z)-1,6-germacranolide[105]. Positive NOE's between Me_{14} and H_5, H_1 and H_6 and H_1 and H_8 ascertained the cis-fused framework of the eudesmane.

The (E,E)-1,6-germacrane **73** can be isolated from several organisms which make use of this alcohol for defence purposes[106-108]. Especially the soft coral *Clavularia koellikeri* is rich in secondary metabolites whose biological pathway may find their origin in **73**.

Scheme 2.18.

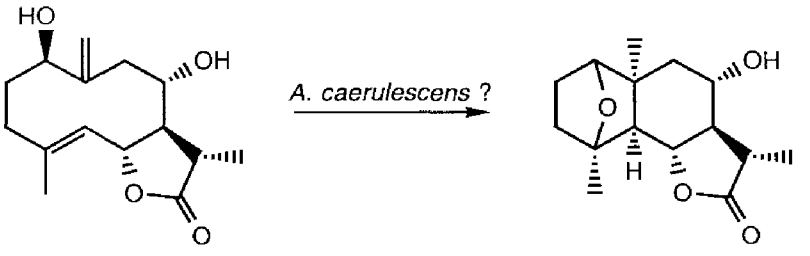


74 **73** **75**

Two major metabolites were isolated from *C. koellikeri*, the oplopane *ent*-oplopanone (**74**) and the cadinane nephthene (**75**). Chemical epoxidation of **73** gave **74** thus supporting the role of (E,E)-1,6-germacranes in the biosynthesis of oplopanes, as outlined in scheme 2.18.

The isolation of shonachalin A (**76**), bearing an exocyclic double bond, together with the cis-fused eudesmane **77** from *Artemisia caerulescens* might indicate a possible biogenetic relationship. The absolute stereochemistry of **77** was established by X-ray analysis[109].

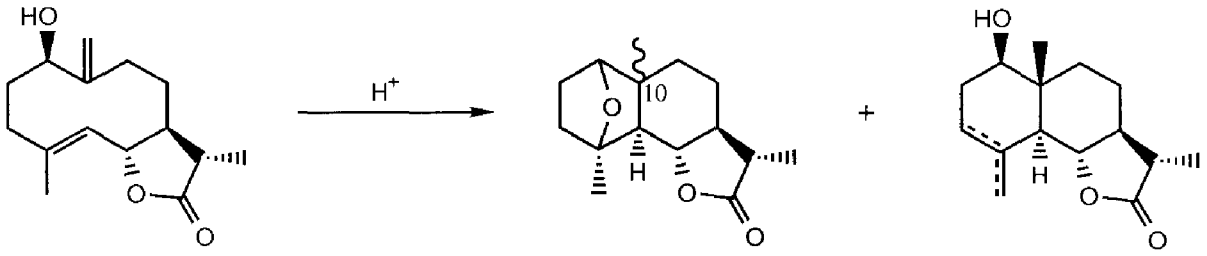
Scheme 2.19.



76 **77**

On the other hand, *in vitro* cyclisation reactions of the 8α-dehydroxy derivative of shonachalin A (**76**), gallicin (**78**), gave a 4 : 1 mixture of the trans- and cis-fused eudesmanes **79** and **80** together with dehydrated compounds (Scheme 2.20.). The absolute configuration of **79** was established by means of X-ray crystallography. An identical reaction of shonachalin A (**76**) only gave the cis-fused eudesmane **77** accompanied by dehydration products. An explanation for the discrepancy in these *in vitro* reactions could not be given.

Scheme 2.20.



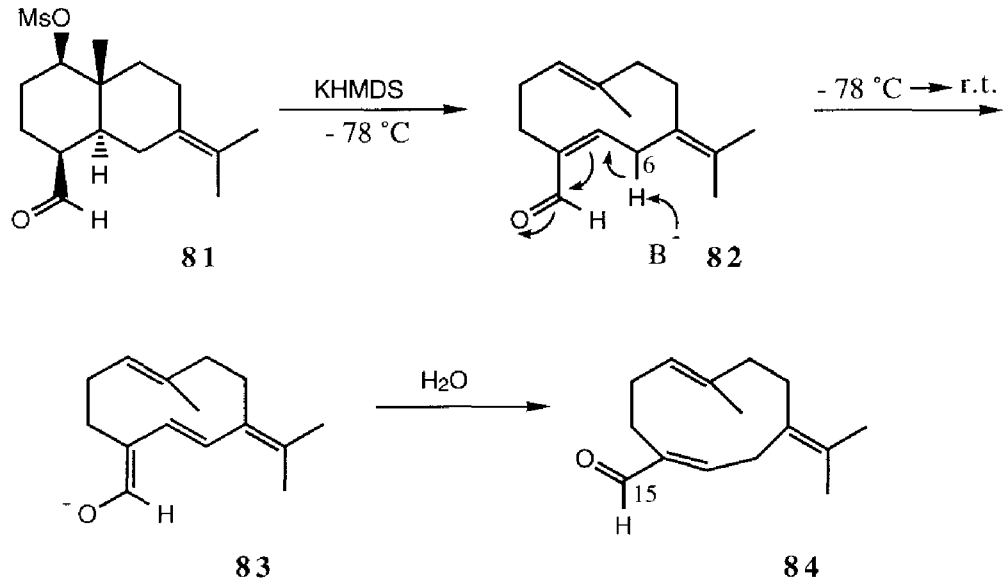
78

79: 10β-Me
80: 10α-Me

2.3.3. Cis - trans isomerisation of germacranes

In principle, the biogenetic precursor of melampolides could be (E,Z)-farnesyl pyrophosphate, having a 6-cis double bond. However, as became clear from paragraph 2.1., this route can be ruled out since neryl pyrophosphate (**24**) is unreactive towards IPP (**20**). The fact that the configurationally isomeric germacranes are present in nature might suggest an isomerisation reaction at a later stage in the biosynthesis of these germacranes. The C_{10} methyl group (Me_{14}) of nearly all melampolides is oxidised either as an aldehyde, a carbomethoxy or an hydroxymethyl group[78,89,93,110-119]. This oxidised Me_{14} may be involved in an, either or not enzyme-mediated, keto-enol tautomerisation leading to a substantial strain relief when the E-double bond is isomerised into a Z-double bond. Such a spontaneous isomerisation was reported by Minnaard in his attempts to synthesise germacrene B aldehyde (**82**)[120], as outlined in scheme 2.21. The base-induced fragmentation of aldehyde **81** proceeded at low temperature to give **82** *in situ*. Upon warming the mixture to room temperature, γ-deprotonation of C_6 occurred under the alkaline reaction conditions, giving enolate **83**. Quenching of the enolate yielded (E,Z)-germacrene B aldehyde **84**.

Scheme 2.21.



(Z,Z)-1,5-germacranes are frequently isolated together with melampolides, suggesting a close biogenetic relationship[93,111,121]. The biogenesis of (Z,Z)-1,5-germacranes, which are oxidised on Me_{14} and Me_{15}, can be explained by cis-trans isomerisation of a melampolide precursor, as described above. However, several (Z,Z)-1,5-germacranes lack the oxidised Me_{15} group. Therefore, it is not unlikely that (Z,Z)-1,5-germacranes can also be formed from heliangolides by an isomerisation of the C_1-C_{10} trans double bond.

Judging from the previous paragraphs it becomes clear that (Z,E)-, (E,Z)- and (Z,Z)-1,5-germacranes seem to play an important role in the biosynthesis of eudesmane-, guaiane- and other sesquiterpenes but conclusive evidence is lacking in many cases. The scarcity of *in vitro* and the complete absence of *in vivo* cyclisation studies of these germacranes emphasises that much work still needs to be done in order to get a clear understanding of the biosynthesis of these secondary metabolites.

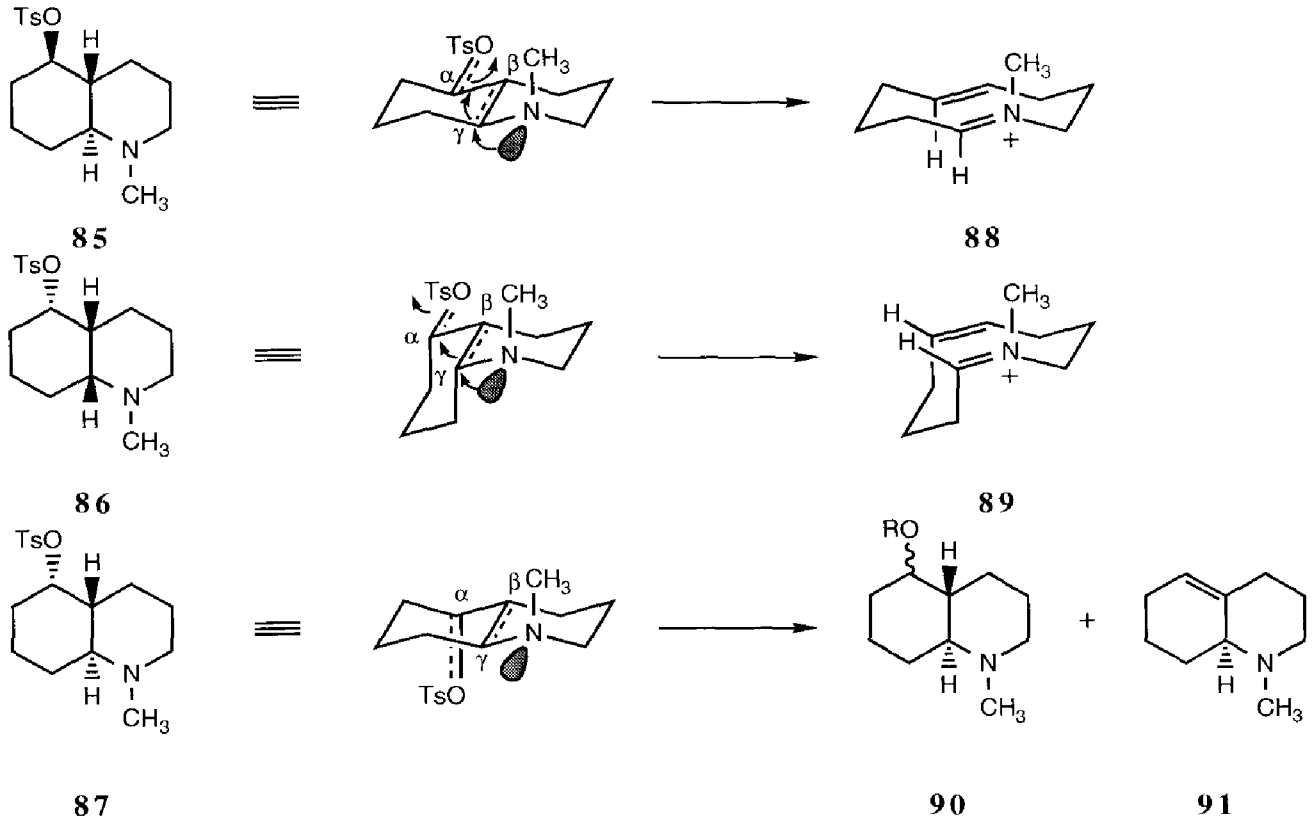
2.4. Synthesis of the cyclodecadiene ring system

Ever since the discovery of the class of germacrane sesquiterpenes and their role as precursors in the formation of several active natural compounds, chemists are intrigued with the synthesis of these facile intermediates. Over the years, a number of techniques have been developed for the construction of medium sized ring compounds. Attempts have been made to adapt the classical Dieckmann diester

condensation but even highly diluted solutions did not promote intramolecular cyclisation in synthetically useful yields[122]. The first and for a long time the only convenient method for synthesising medium sized ring compounds was the acyloin condensation of α,ω-diesters using sodium in refluxing xylene[123-125].

Numerous examples of germacrane synthesis have been reported in the literature throughout the years. These methods included the use of photochemistry[126-128], Cope rearrangements of 1,2-divinyl cyclohexyl compounds[129-131] and Wittig-[2,3]-rearrangements of 13-membered, monocyclic and acyclic ring compounds[132-136]. By far the most convenient way to prepare compounds possessing a 10-membered ring structure is *via* a heterolytic fragmentation reaction of a properly functionalised decalin ring system, utilised by Grob *et al.*[137]. The high stereospecificity of this 'Grob fragmentation' is clearly demonstrated by the solvolysis of the three N-methyl-decahydroquinolin-5-ol tosylates **85**, **86** and **87**. Grob concluded that a concerted process should operate if both the C_α-OTs bond and the orbital of the nitrogen lone pair are anti and parallel oriented (= antiperiplanar) to the C_β-C_γ bond, and that the lone pair of the nitrogen atom accelerates the dissociation of the C_α-OTs bond.

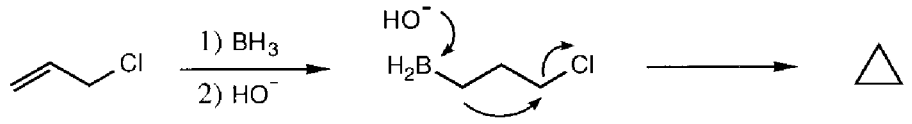
Scheme 2.22.



Only the isomers **85** and **86**, where the equatorial tosylate groups exhibit the necessary antiperiplanarity of the orbitals involved are found to undergo exclusive fragmentation to give the iminium ions **88** and **89**. The concerted character of this fragmentation process is emphasized by a much higher reaction rate when compared to their 1-decalol tosylate homologues. The axial tosylate **87** reacts more slowly than **85** and **86** and only substitution and elimination products (**90** and **91**) are formed. This implicates a stepwise mechanism in which the rate-determining step is the loss of the tosylate group giving a cationic intermediate followed by typical cationic reactions like interceptions of nucleophiles and elimination reactions.

The ability of a boron-carbon bond to supply electrons to the carbon is well precedented in the literature[138-144]. In 1958, Hawthorne and Dupont described the synthesis of cyclopropane (**93**) via hydroboration of 3-chloropropene (**92**) followed by hydrolysis of the resulting organoborane intermediate with an aqueous base[145] (Scheme 2.23.).

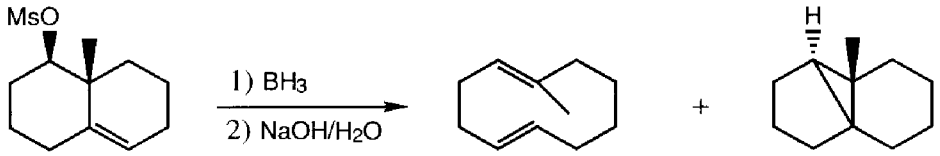
Scheme 2.23.



92 **93**

With this knowledge in hand, Marshall utilised this hydroboration-fragmentation reaction in order to synthesise the (E,E)-1,5- and 1,6-cyclodecadiene framework starting from an appropriately functionalised octahydronaphthalene derivate[146,147]. Hydroboration-fragmentation of the mesylate **94** afforded the (E,E)-cyclodeca-1,5-diene **95** and the tricyclic hydrocarbon **96** in a 93 : 7 ratio. The tricyclic hydrocarbon **96** results from a 1,3-elimination from the tertiary alkane boronate arising from hydroboration at the bridgehead carbon (Scheme 2.24.).

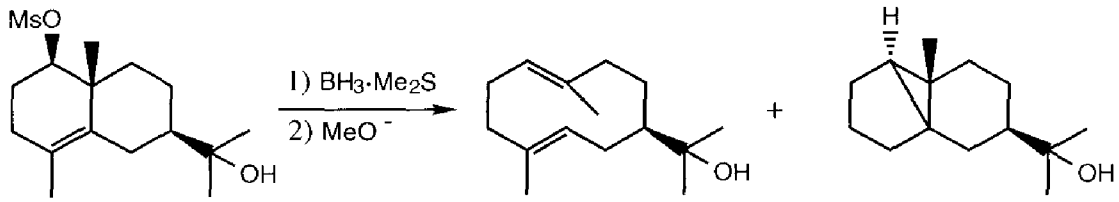
Scheme 2.24.



94 **95** **96**

Hydroboration generally places the boron at the less substituted carbon of the double bond. In the case of a tetrasubstituted double bond, discrimination of the two positions of the internal olefin is determined by both steric and electronic factors[138]. In the total synthesis of (+)-hedycaryol (**39**), described by Minnaard *et al.*[144] the 'Marshall fragmentation' of mesylate **97** was utilised to produce both **39** and the tricyclic compound **98** in a 85 : 15 ratio[149], as outlined in scheme 2.25.

Scheme 2.25.



97 **39** **98**

2.5. Scope of this thesis

Chicory roots are an agricultural waste product. This makes the root a cheap and interesting starting material for the elucidation of the biosynthesis of the bitter principles in chicory. A number of configurationally isomeric cyclodeca-1,5- and -1,6-dienes and germacranes, as well as their epoxides, have been synthesised in order to study the substrate specificity of the cyclising enzyme(s) in chicory. These enzyme mediated cyclisations are compared to chemically induced cyclisation reactions. From these cyclisation studies, a better understanding of the transannular cyclisation reaction mechanism of the germacrane system has to emerge, which can aid in the elucidation of the biosynthesis of sesquiterpene bitter principles in chicory and eudesmanes and guaianes in general.

In Chapter 3, the synthesis of two (E,E)-cyclodeca-1,5-dienes and the cyclisation behaviour of these compounds, together with the natural (E,E)-1,5-germacrane (+)-hedycaryol (**32**), towards a chicory root homogenate was investigated. The role of substituents at C_4 and C_7 on the conformation of the 10-membered ring and on its mode of cyclisation is discussed. In Chapter 4, the synthesis and biotransformation of three (E,E)-cyclodeca-1,6-dienes is described. Also, a detailed reinvestigation of the stereochemical features of the natural trinor-guaiane dictamnol, possessing a framework similar to that of the cyclisation products described in this chapter, is

presented. In Chapter 5, the biotransformation of derivatives of the readily available natural sesquiterpene germacrone (**93**) is discussed while chapter 6 describes the biotransformation of (E,Z)-cyclodeca-1,5-dienes and the role of the 1,5-double bond geometry on the ring fusion of the cyclised products. Finally, in chapter 7, the cyclisation of farnesyl pyrophosphate (**23**), together with the partial purification of the FPP-cyclising enzyme from chicory, is described. In conclusion, the substrate specificity of the germacrane cyclasing enzyme of *C. intybus* is discussed and an active site model for the germacrane cyclase in proposed together with two tentative biosyntheses of the sesquiterpene lactones in chicory.

2.6. References and notes

1. Clayton R.B., *Quart. Rev.*, **19**, 168 (1965).
2. Beale M.H., *Nat. Prod. Rep.*, **7**, 387 (1990).
3. Caelles C., Ferrer A., Balcells L., Hegardt F.G. and Boronat A., *Plant Mol. Biol.*, **13**, 627 (1989).
4. Learned R.M. and Fink G.R., *Proc. Natl. Acad. Sci. USA.*, **86**, 2779 (1989).
5. Roitelman J. and Schechter I., *J. Lipid Res.*, **30**, 97 (1989).
6. Beach M.J. and Rodwell V.W., *J. Bacteriol.*, **171**, 2994 (1989).
7. Jordan-Starck T.C. and Rodwell V.W., *J. Biol. Chem.*, **264**, 17913 (1989).
8. Jordan-Starck T.C. and Rodwell V.W., *J. Biol. Chem.*, **264**, 17919 (1989).
9. Qureshi N. and Porter J.W., *Conversion of Acetyl-Coenzyme A to Isopentenyl Pyrophosphate*, in *Biosynthesis of Isoprenoid Compounds*, Vol. 1 (Porter J.W. and Spurgeon S.L., eds.), John Wiley & sons, New York, 47-94 (1981).
10. Tchen T.T., *J. Biol. Chem.*, **233**, 1100 (1972).
11. Levy H.R. and Popjak G., *Biochem. J.*, **72**, 35 (1959).
12. Levy H.R. and Popjak G., *Biochem. J.*, **75**, 417 (1960).
13. Markely K. and Smallman E., *Biochim. Biophys. Acta*, **47**, 327 (1961).
14. Rogers J.L., Shah S.P.J. and Goodwin T.W., *Biochem. J.*, **99**, 381 (1966).
15. Goodfellow R.D. and Barnes F.J., *Insect Biochem.*, **1**, 271 (1971).
16. Ruzicka L., Eschenmoser A. and Heuser H., *Experientia*, **9**, 357 (1953).
17. Rilling H., Tchen T.T. and Bloch K., *Proc. Natl. Acad. Sci. USA.*, **44**, 167 (1958).
18. Clifford K., Cornforth J.W., Mallaby R. and Phillips G.T., *Chem. Commun.*, 1599 (1971).
19. Manitto P., *Biosynthesis of Natural Products*; Ellis Horwood Ltd: Chichester, 213 (1981).
20. Popjak G. and Cornforth J.W., *Biochem. J.*, **101**, 533 (1966).

21. Cornforth J.W., Cornforth R.R., Popjak G., and Yengoyan L., *J. Biol. Chem.*, **241**, 3970 (1966).
22. Gough D.P. and Hemming F., *Biochem. J.*, **117**, 309 (1970).
23. George-Nascimento C. and Cori O., *Phytochemistry*, **10**, 1803 (1971).
24. Chayet L., Pont-Lezica R., George-Nascimento C. and Cori O., *Phytochemistry*, **12**, 95 (1973)
25. Jedlicki E., Jacob G., Faini F., Cori O. and Bunton C.A., *Arch. Biochem. Biophys.*, **152**, 590 (1972).
26. Banthorpe D.V., Bucknall G.A., Doonan H.J., Doonan S. and Rowan M.G., *Phytochemistry*, **15**, 91 (1976).
27. Banthorpe D.V., Ekundayo O. and Rowan M.G., *Phytochemistry*, **17**, 1111 (1978).
28. Banthorpe D.V., Modawi B.M., Poots I. and Rowan M.G., *Phytochemistry*, **17**, 1115 (1978).
29. Jacob G., Cardemil E., Chayet L., Telez R., Pont-Lezica R. and Cori O., *Phytochemistry*, **11**, 1683 (1972).
30. Cane D.E., *Tetrahedron*, **36**, 1109 (1980) and references cited therein.
31. Cane D.E., *Biosynthesis of Sesquiterpenes*, in *Biosynthesis of Isoprenoid Compounds*, Vol. 1 (Porter J.W. and Spurgeon S.L., eds.), John Wiley & Sons, New York, 283-374 (1981).
32. Hendrickson J.B., *Tetrahedron*, **7**, 82 (1959).
33. Parker W, Roberts J.S. and Ramage R., *Quart. Rev*, **21**, 311 (1967).
34. Devon T.K. and Scott A.I., *Handbook of Naturally Occurring Compounds Vol. II Terpenes*, Academic Press, New York, 56 (1972).
35. Chapman A.C., *J. Chem. Soc.*, **67**, 54 (1895).
36. Corey E.J. and Hamanaka E., *J. Am. Chem. Soc.*, **89**, 2758 (1967).
37. Corey E.J., Mitra R.B. and Uda H., *J. Am. Chem. Soc.*, **86**, 485 (1964) and references cited therein.
38. Ohtsuka Y., Niitsuma S., Tadokoro H., Hayashi T. and Oishi T., *J. Org. Chem.*, **49**, 2326 (1984).
39. Dev S., *Tetrahedron*, **8**, 171 (1960).
40. Nishimura K., Shinoda N. and Hirose Y., *Tetrahedron Letters*, 3097 (1969).
41. Gijsen H.J.M., *(+)-Aromadendrene as Chiral Starting Material for the Synthesis of Sesquiterpenes*, PhD-thesis, Wageningen (1993).
42. De Pascual Teresa J., Urones J.G. and Fernandez A., *Phytochemistry*, **22**, 2753 (1983).
43. McMurry J.E. and Bosch G.K., *J. Org. Chem.*, **52**, 4885 (1987).
44. Hubert T.D. and Wiemer D.F., *Phytochemistry*, **24**, 1197 (1985).
45. Fraga B.M., *Nat. Prod. Rep.*, **9**, 217 (1992).

46. Fraga B.M., *Nat. Prod. Rep.*, **10**, 397 (1993).
47. Fraga B.M., *Nat. Prod. Rep.*, **11**, 533 (1994).
48. Weinheimer A.J., Youngblood W.W., Washecheck P.H., Karns T.K.B. and Ciereszko L.S., *Tetrahedron Letters*, 497 (1970).
49. Corbett R.E. and Smith R.A.J., *Tetrahedron Lett.*, 1009 (1976).
50. Naya Y., Prestwich G.D. and Spanton S.G., *Tetrahedron Letters*, **23**, 3047 (1982).
51. Atta-ur-Rahman and Ahmad V.U., *^{13}C-NMR of Natural Products*, Vol. 1, 480-494 (1992).
52. Kesselmans R.P.W., *Total Synthesis of All Stereoisomers of Eudesm-11-en-4-ol*, PhD thesis, Agricultural University Wageningen (1992).
53. Kato M., Kosugi H. and Yoshikoshi A., *Chem. Commun.*, 185 (1970).
54. Jones R.V.H. and Sutherland M.D., *J. Chem. Soc., Chem. Commun.*, 1229 (1968).
55. Southwell I.A., *Phytochemistry*, **9**, 2243 (1970).
56. Brown E.D., Sutherland J.K. and Sam T.W., *J. Chem. Soc., Perkin I*, 2332 (1975).
57. Brown E.D., Sutherland J.K., *J. Chem. Soc., Chem. Commun.*, 1060 (1968).
58. El-Feraly F.S. and Benigni D.A., *J. Chem. Soc., Perkin Trans I*, 355 (1983).
59. Parodi F.J., Fronczek F.R and Fischer N.H., *J. Nat. Prod.*, **52**, 554 (1989).
60. Bohlmann F. and Knoll K.-H., *Phytochemistry*, **18**, 995 (1979).
61 Bohlmann F, Gupta R.K., King R.M. and Robinson H., *Phytochemistry*, **20**, 331 (1981).
62. Bowden B.F., Coll J.C. and Mitchell S. J., *Aust. J. Chem.*, **33**, 1833 (1980).
63. Oshima Y., Iwakawa T. and Hikino H., *Phytochemistry*, **22**, 183 (1983).
64. Yoshikawa M., Hatakeyama S., Tanaka N., Fukuda Y., Murakami N. and Yamahara J., *Chem. Pharm. Bull.*, **40**, 2582 (1992).
65. Yoshikawa M., Yamaguchi S., Matsuda H., Kohda Y., Ishikawa H., Tanaka N., Yamahara J. and Murakami N., *Chem. Pharm. Bull.*, **42**, 1813 (1994).
66. Sakui N., Kuroyanagi M., Ishitobi Y., Sato M. and Ueno A., *Phytochemistry*, **31**, 143 (1992).
67. Sakamoto S., Tsuchiya N., Kuroyanagi M. and Ueno A., *Phytochemistry*, **35**, 1215 (1994).
68. Yoshihara M., Yang C., Zheng C., Shibuya H., Hamamoto Y., Tanaka N. and Kitagawa I., *Chem. Pharm. Bull.*, **34**, 434 (1986).
69. Garcia-Granados A., Molina A. and Cabrera E., *Tetrahedron*, **42**, 81 (1986).
70. Maldonado E., Mancera C., Fiallega G. and Ortega A., *Phytochemistry*, **38**, 1265 (1995).
71. Kupchan S.M., *J. Am. Chem. Soc.*, **88**, 3674 (1966).
72. Kupchan S.M., Hemingway J.C., Cassady, J.M., Knox J.R., McPhail A.T. and Sim G.A., *J. Am. Chem. Soc.*, **89**, 465 (1967).

73. Kupchan S.M., Kelsey J.E., Maruyama M., Cassaday J.M., Hemingway J.C. and Knox J.R., *J. Org. Chem.*, **34**, 3876 (1969).
74. Sosa V.E., Oberti J.C., Gil R.R., Ruveda E.A., Goedken V.L., Gutierrez A.B. and Herz W., *Phytochemistry*, **28**, 1925 (1989).
75. Daniewski W.M., Danikiewicz W., Gumulka M., Pankowska E., Krajewski J., Grabarczyk H. and Wichlacz M., *Phytochemistry*, **34**, 1639 (1993).
76. Nishikawa M., Kamiya K., Takabatake A. and Oshio H., *Tetrahedron*, **22**, 3601 (1966).
77. Neidle S. and Rogers D., *J. Chem. Soc., Chem. Commun.*, 140 (1972).
78. Fischer N.H., Wiley R. and Wander J.D., *J. Chem. Soc., Chem. Commun.*, 137 (1972).
79. Bohlmann F. and Zedro C., *Chem. Ber.*, **108**, 1902 (1975).
80. Malcolm A.J., Carpenter J.F., Fronczek F.R. and Fischer N.H., *Phytochemistry*, **22**, 2759 (1983).
81. Bohlmann F., Ang W., Trinks C., Jakupovic J. and Huneck S., *Phytochemistry*, **24**, 1009 (1985).
82. Fang N. and Mabry T.J., *Phytochemistry*, **27**, 283 (1988).
83. De Heluani C.S., De Lampasona M.P., Catalan C.A.N., Goedken V.L., Gutierrez A.B. and Herz W., *Phytochemistry*, **28**, 1931 (1989).
84. El-Feraly F.S., Benigni D.A. and McPhail A.T., *J. Chem. Soc. Perkin Trans I*, 355 (1983).
85. Perez A.L., Guerrero C., De la Luz Cruz M. and Romo de Vivar A., *Phytochemistry*, **23**, 823 (1984).
86. Herz W., Murari R. and Blount J.F., *J. Org. Chem.*, **44**, 1873 (1979).
87. Gonzalez A.G., Galindo A., Mar Alfonso R., Mansilla H. and Lopez M., *Tetrahedron*, **44**, 4585 (1988).
88. Maldonado E., Vaughan J. and Ortega A., *Phytochemistry*, **23**, 813 (1984).
89. Nishimura K., Miyase T., Ueno A., Nori T., Kuroyanagi M. and Fukushima S., *Phytochemistry*, **25**, 2375 (1986).
90. Seaman F.C., Juneau G.P., DiFeo D.R., Jungk S. and Fischer N.H., *J. Org. Chem.*, **44**, 3400 (1979).
91. Castro V., Jakupovic J. and Dominguez X.A., *Phytochemistry*, **28**, 2727 (1989).
92. Doskotch R.W., El-Feraly F.S. and Hufford C.D., *J. Chem. Soc., Chem. Commun.*, 402 (1976).
93. El-Feraly F.S., Chan Y.M., Fairchild E.H. and Doskotch R.W., *Tetrahedron Lett.*, 1973 (1977).
94. Quijana L., Trejo R.I., Collera O. and Rios T., *Phytochemistry*, **36**, 1443 (1994).

95. Jakupovic J., Zdero C., Boeker R., Warning U., Bohlmann F. and Jones S.B., *Lieb. Ann. Chem.*, 111 (1987).
96. Zdero C., Bohlmann F., Wasshausen D.C. and Mungai M.G., *Phytochemistry*, **30**, 4025 (1991).
97. Fischer N.H., Wiley R.A., and Perry D.L., *Rev. Lat. Quim.*, **7**, 87 (1976).
98. Traynham J.G. and Hsieh H.H., *J. Org. Chem.*, **38**, 868 (1973).
99. Traynham J.G., Franzen G.R., Knesel G.A. and Northington Jr., D.J., *J. Org. Chem.*, **32**, 3285 (1967).
100. Sicher J., Svoboda M., Zavada J., Turner R.B., and Goebel P., *Tetrahedron*, **22**, 659 (1966).
101. Bohlmann F. and Ehlers D., *Phytochemistry*, **16**, 137 (1977).
102. Sawaikar D.D., Rojatkar S.R. and Nagasampagi B.A., *Phytochemistry*, **37**, 585 (1994).
103. Bohlmann F. and Zdero C., *Phytochemistry*, **16**, 1243 (1977).
104. Bohlmann F. and Zdero C., *Chem. Ber.*, **108**, 1902 (1975).
105. Abdel-Mogib M., Jakupovic J., Dawidar E.M., Metwally M.E. and Aboe-Elzahab M., *Phytochemistry*, **28**, 268 (1989).
106. Kitagawa I., Cui Z., Son B.W., Kobayashi M. and Kyogoku Y., *Chem. Pharm. Bull.*, **35**, 124, (1987).
107. Nagahama S., Tazaki M., Kobayashi H. and Sumimoto M., *Phytochemistry*, **33**, 879 (1993).
108. Bergström G., Wassgren A-B. and Birgersson G., *Acta Chim. Scan.*, **48**, 187 (1994).
109. Marco J.A., Sanz-Cervera J.F., Garcia-Lliso V., Domingo L.R., Carda M., Rodriguez S., Lopez-Ortiz F. and Lex J., *Liebigs Ann.*, 1837 (1995).
110. Seaman F.C., Fischer N.H. and Stuessy T.F., *Biochem. Syst. Ecol.*, **8**, 263 (1980).
111. Stokes S., Castro V., Poveda L., Papastergiou F. and Jakupovic J., *Phytochemistry*, **31**, 2894 (1992).
112. Kisiel W. and Gromek D., *Phytochemistry*, **34**, 1644 (1993).
113. Van N.L., and Fischer N.H., *Phytochemistry*, **18**, 851 (1979).
114. Maldonado E., Vaughan J. and Ortega A., *Phytochemistry*, **23**, 813 (1984).
115. Olivier E.J., Perry D.L. and Fischer N.H., *J. Org. Chem.*, **45**, 4028 (1980).
116. El-Feraly F.S., *Phytochemistry*, **22**, 2239 (1983).
117. Herz W. and Kalyanaraman P.S., *J. Org. Chem.*, **40**, 3486 (1975).
118. Asada H., Miyase T. and Fukushima S., *Chem. Pharm. Bull.*, **32**, 1724 (1984).
119. Asada H., Miyase T. and Fukushima S., *Chem. Pharm. Bull.*, **32**, 3403 (1984).
120. Minnaard A.J., Forthcomming PhD-thesis, Wageningen (1996).
121. Fischer N.K., Seaman F.C. and Russell A.W., *J. Org. Chem.*, **43**, 4984 (1978).

122. Leonard N.J. and Schimelpfenig Jr. C.W., *J. Org. Chem.*, **23**, 1708 (1958).
123. Stoll M. and Hulstkamp J., *Helv. Chim. Acta*, **30**, 1815 (1947).
124. Stoll M. and Rorré A., *Helv. Chim. Acta*, **30**, 1823 (1947).
125. Brannock K.C., Burpitt R.D., Goodlett V.W. and Thweatt J.C., *J. Org. Chem.*, **28**, 1464 (1963).
126. Corey E.J. and Hortmann A.C., *J. Am. Chem. Soc.*, **87**, 5736 (1965).
127. Williams J.R. and Callahan J.F., *J. Org. Chem.*, **45**, 4475 (1980).
128. Williams J.R. and Callahan J.F., *J. Chem. Soc., Chem. Commun.*, 404 (1979).
129. For a review of the Cope rearrangement see Rhoads S.J. and Raulins N.R., *Org. React.*, **22**, 1 (1975).
130. Grieco P.A. and Nishizawa M., *J. Org. Chem.*, **42**, 1717 (1977).
131. Rao A.S., Kelkar G.R. and Bhattacharyya S.C., *Tetrahedron*, **9**, 275 (1960).
132. Gopalan A. and Magnus P., *J. Am. Chem. Soc.*, **102**, 1756 (1980).
133. Takahashi T., Nemoto H., Kanda Y., Tsuji J. and Fujise Y., *J. Org. Chem.*, **51**, 4315 (1986).
134. Marshall J.A., Lebreton J., DeHoff B.S. and Jenson T.M., *Tetrahedron Lett.*, **28**, 723 (1987).
135. Kodama M., Matsuki Y. and Ito S., *Tetrahedron Lett.*, **17**, 1121 (1976).
136. Takahashi T., Kitamura K., Nemoto H., Tsuji J., *Tetrahedron Lett.*, **24**, 3489 (1983).
137. Grob C.A., Kiefer H.R., Lutz H.J. and Wilkens H.J., *Helv. Chim. Acta*, **50**, 416 (1967).
138. Brown H.C. and Zweifel G., *J. Am. Chem. Soc.*, **82**, 4708 (1960).
139. Brown H.C. and Keblys K.A., *J. Am. Chem. Soc.*, **86**, 1791 (1964).
140. Brown H.C. and Keblys K.A., *J. Am. Chem. Soc.*, **86**, 1795 (1964).
141. Brown H.C. and Cope O.J., *J. Am. Chem. Soc.*, **86**, 1801 (1964).
142. Pasto D.J. and Snyder R., J. Org. Chem., 31, 2777 (1966).
143. Brown H.C. and Gallivan Jr. R.M., *J. Am. Chem. Soc.*, **22**, 2906 (1968).
144. Brown H.C. and Unni M.K., *J. Am. Chem. Soc.*, **90**, 2902 (1968).
145. Hawthorne M.F. and Dupont J.A., *J. Am. Chem. Soc.*, **80**, 5830 (1958).
146. Marshall J.A. and Bundy G.L., *J. Am. Chem. Soc.*, 88, 4291 (1966).
147. Marshall J.A. and Bundy G.L. *J. Chem. Soc., Chem. Commun.*, 854 (1967).
148. Minnaard A.J., Wijnberg J.B.P.A. and de Groot Ae., *Tetrahedron*, **50**, 4755 (1994).
149. van der Heijden K.A., *De Synthese en Cyclisatie van (+)-Hedycaryol*, MSc-Thesis, Wageningen (1994).

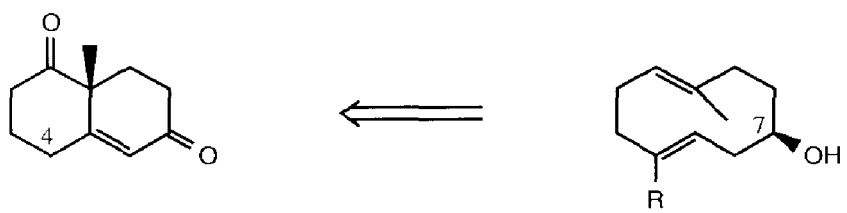
3. Synthesis and biotransformation of (E,E)-cyclodeca-1,5-dienes

3.1. Introduction

As described in Chapter 2, the hydroboration-fragmentation reaction, or 'Marshall fragmentation' of suitably functionalised hydronaphthalene precursors into (E,E)-cyclodeca-1,5-dienes is an attractive strategy. Application of this strategy has two advantages over all other approaches. In the first place, simple and reliable methods have been developed for the synthesis of the hydronaphthalene framework. The second and more important factor is the highly developed understanding of the stereochemistry and conformational analysis in hydronaphthalene systems. Since successful methods for the preparation of appropriately functionalised hydronaphthalenes in the synthesis of eudesmanes have been developed in our laboratory[1,2], it was obvious to investigate the utility of these compounds as intermediates for the synthesis of (E,E)-cyclodeca-1,5-dienes.

The need for a short, simple, general and efficient synthesis of compounds possessing the (E,E)-cyclodeca-1,5-diene framework is evident in order to screen possible cyclisation activity in chicory roots. Our aim was to synthesise (E,E)-cyclodeca-1,5-dienes **100** and **101**, which were expected to be obtained through a small number of transformations from the well-known Wieland-Miescher ketone (**99**)[3]. Straightforward chemistry is expected to lead to the (E,E)-cyclodeca-1,5-diene framework of **100**, lacking the methyl group at C_4 which is characteristic for natural germacranes. The introduction of this methyl group in the eudesmane skeleton is well documented in literature[4] and offers an opportunity to investigate the influence of that methyl group on the conformation and the transannular cyclisation reaction of the (E,E)-cyclodeca-1,5-diene framework. The hydroxyl function at C_7 is expected to serve as a handle to facilitate solubilisation in the aqueous incubation medium.

Scheme 3.1.



99

100: R = -H
101: R = -CH_3

The total synthesis of (+)-hedycaryol (**39**)[5], conducted at our laboratory, made it also possible to investigate the role of a bulky substituent like an isopropanol moiety at C_7 on the transannular cyclisation reaction. In this chapter[6,7], the synthesis of (E,E)-cyclodeca-1,5-dienes **100** and **101** is described, followed by the biotransformation of **100**, **101** and **39** by a chicory root homogenate. The substrate specificity of this homogenate is tested by comparing the transformations with a boiled root sample (enzyme blank), the incubation medium (solvent blank) and chemically induced cyclisations. The role of the methyl group at C_4 and different substituents at C_7 on the conformation and the cyclisation pattern of the germacrane ring system is discussed.

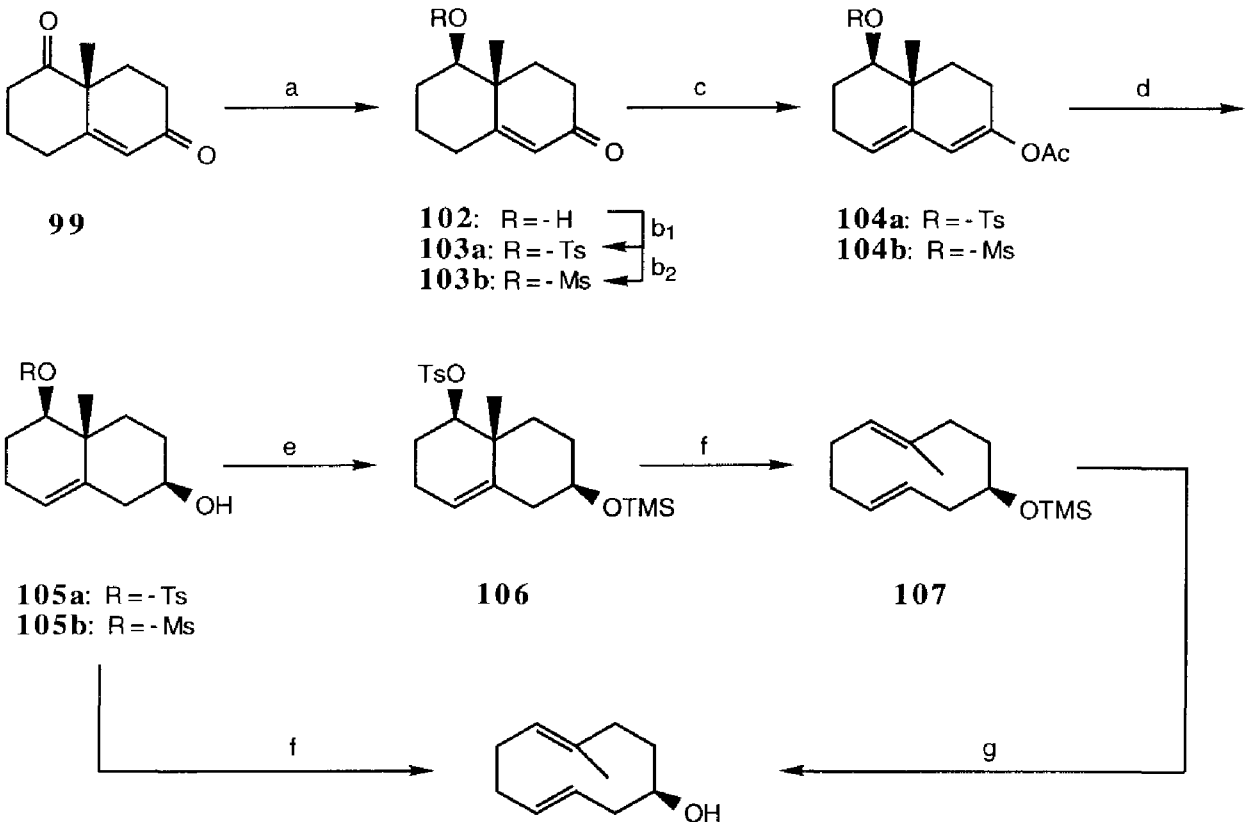
3.2. Synthesis of the (E,E)-cyclodeca-1,5-dienes 100 and 101*

The starting material for the synthesis of the (E,E)-cyclodeca-1,5-dienes **100** and **101** was the readily available Wieland-Miescher ketone (**99**). The ketone was obtained *via* a Robinson annulation of the Michael adduct, obtained from 2-methyl-cyclohexa-1,3-dione and methyl vinyl ketone[8] with a catalytic amount of pyrrolidine[9]. The non-conjugated carbonyl of **99** was stereoselectively reduced (Scheme 3.2.) to give the α-alcohol **102** which was tosylated using tosyl chloride (TsCl) in pyridine to give tosylate **103a**. The tosylate was converted into dienol acetate **104a** by trimethylsilyl chloride (TMSCl) and sodium iodide in acetic anhydride in good yield[10]. The enol acetate moiety was reduced with excess $NaBH_4$ in EtOH to give the tosyl alcohol **105a** in almost quantitative yield. The alcohol function of **105a** was protected as its trimethylsilyl ether (**106**) using TMSCl and hexamethyl disilazane (HMDS) in pyridine and subjected to the 'Marshall fragmentation' to give the protected (E,E)-cyclodeca-1,5-diene TMS-ether **107**. Desilylation of **107** using tetrabutyl ammonium fluoride (TBAF) in THF proceeded at room temperature almost quantitatively to yield (E,E)-cyclodeca-1,5-diene **100**. Additional experiments showed that replacing the tosyl group for a mesyl group and leaving the hydroxyl function unprotected during the Marshall fragmentation, did not alter the course, speed and yield of the reaction significantly. The first observation is in agreement with literature reports[11].

For the synthesis of (E,E)-cyclodeca-1,5-diene **101**, alcohol **102** had to be methylated at C_4 (Scheme 3.3.). Following the procedure of Kim *et al.*[12], **102** was silylated using tertiary butyl dimethylsilyl chloride (TBDMSCl) and imidazole to give **108** followed by ketalisation with concomitant double bond isomerisation to yield **109**. Desilylation of

* Compounds **100** and **101** are also known as 'Pietol-1' and 'Pietol-2'

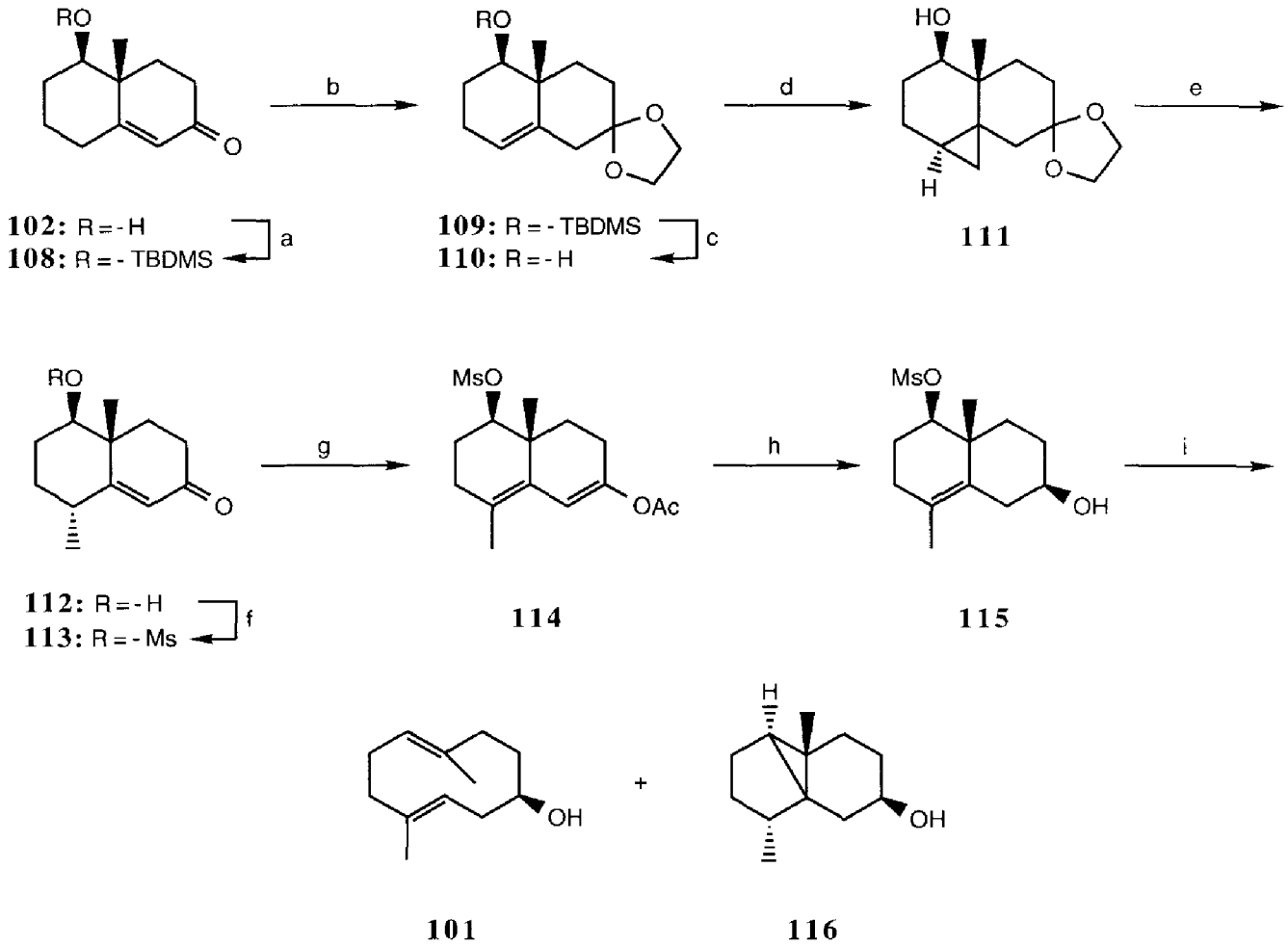
Scheme 3.2.[a]



[a] a) $NaBH_4$; b_1) TsCl; b_2) MsCl; c) TMSCl/NaI/Ac_2O; d) $NaBH_4$; e) TMSCl/HMDS; f_1) BH_3·Me_2S; f_2) 4 N NaOH; g) TBAF.

109 with TBAF at slightly elevated temperatures gave alcohol **110** which could not be obtained directly through ketalisation of **102** due to a retro-aldol reaction[13]. Cyclopropanation of **110**, employing the Simmons-Smith reaction[14], gave the 4α,5α-methanodecalol **111**. Exposure of **111** to 70% perchloric acid in CH_2Cl_2 resulted in cleavage of the cyclopropane ring and equilibration of the methyl group into the more stable equatorial position giving **112**. This reaction was accompanied by an acid-catalysed, retro-aldol reaction limiting the yield to only 56% after chromatographic separation. Similar to the synthesis of **100**, the secondary alcohol function of **112** was mesylated using MsCl in pyridine followed by acetylation of the α,β-ketone **113** to give dienol acetate **114**. Reduction of **114** with excess $NaBH_4$ gave the mesyl alcohol **115** which was subjected to the Marshall fragmentation to give, almost quantitatively, a 9 : 1 mixture of (E,E)-cyclodeca-1,5-diene **101** and tricyclo[5.3.$0^{1,7}$.$0^{2,7}$]decanol **116**. The desired (E,E)-cyclodeca-1,5-diene was purified by an aqueous $AgNO_3$-extraction[15].

Scheme 3.3.[a]



[a] a) TBDMSCl/Imidazole; b) EG/p-TSA/Δ; c) TBAF; d) Zn-Cu/CH_2I_2; e) 70% $HClO_4$; f) MsCl; g) TMSCl/NaI/Ac_2O; h) $NaBH_4$; i_1) $BH_3 \cdot Me_2S$; i_2) 4 N NaOH.

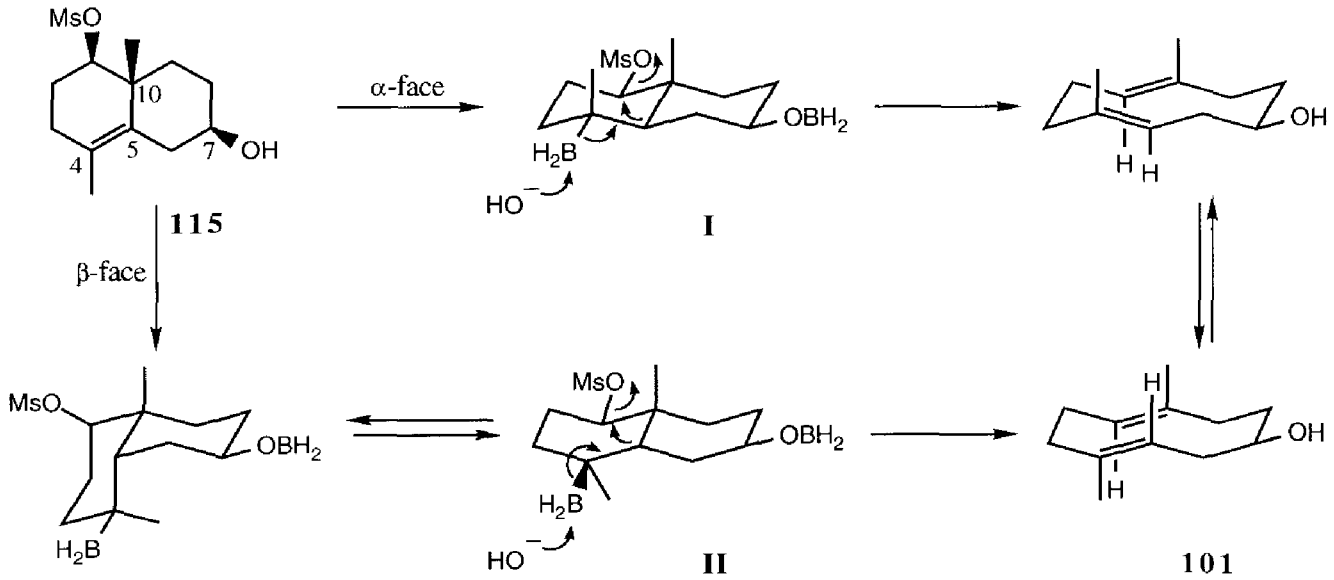
Hydroboration generally places the boron at the less substituted carbon atom of the double bond. Therefore, hydroboration of **105b** and **106** will occur predominantly at C_4. In the case of the tetra-substituted unsaturated mesyl alcohol **115**, discrimination between the two positions of the olefinic bond is determined by steric and electronic factors[16]. A four-centre transition state is suggested in the reversible hydroboration addition step[17]. Hydroboration of **115** at C_4 from the bottom-side of the molecule (called α-face in this context) will result in an energetically favourable all-chair conformation with the alkoxyborane substituent at C_7 in an equatorial position (**I**, Scheme 3.4.)[18]. This conformation possesses the necessary antiperiplanar geometry for a concerted fragmentation (Scheme 3.4.). In the case of hydroboration at C_5 from the bottom-side of the molecule, γ-elimination, leading to the tricyclodecane framework,

can not occur because this conformation does not possess the necessary geometry for backside displacement of the mesylate[20,21].

It is not unlikely that the formation of alkoxyboranes during the hydroboration of **115** is responsible for the predominating α-face attack of the BH_3·Me_2S complex. In a study towards the Marshall fragmentation of functionalised hydronaphthalene systems in which the C_7 hydroxyl group was replaced by an ether function, which can therefore not react with the BH_3·Me_2S complex, the formation of the tricyclodecane ring system was found to predominate[22].

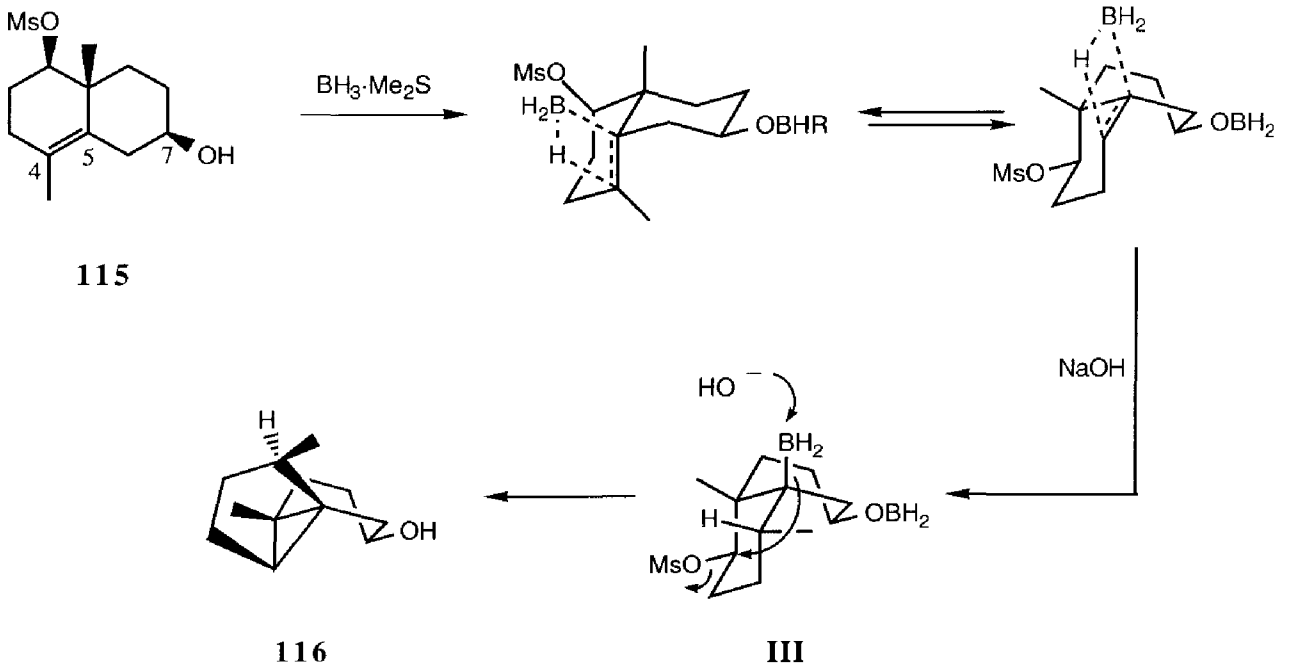
Hydroboration at C_4 from the β-face of the molecule followed by base induced fragmentation can also lead to the germacrane ring system. The intermediate borane then has to react through the energetically unfavourable boat-boat conformation (**II**) to give **101** with the methyl groups at C_4 and C_{10} oriented anti towards each other (Scheme 3.4.). Although this mode of hydroboration cannot be excluded, α-face hydroboration seems the preferred pathway.

Scheme 3.4.



The formation of **116** can be explained by the approach of the BH_3·Me_2S complex from the β-face of the molecule towards C_5 which will result in electrostatical repulsion between the boron and the pseudo-axial alkoxyborane at C_7. Only this conformation (**III**) possesses the necessary geometry for backside displacement of the mesylate to yield **116** as outlined in scheme 3.5.

Scheme 3.5.



115 **116** **III**

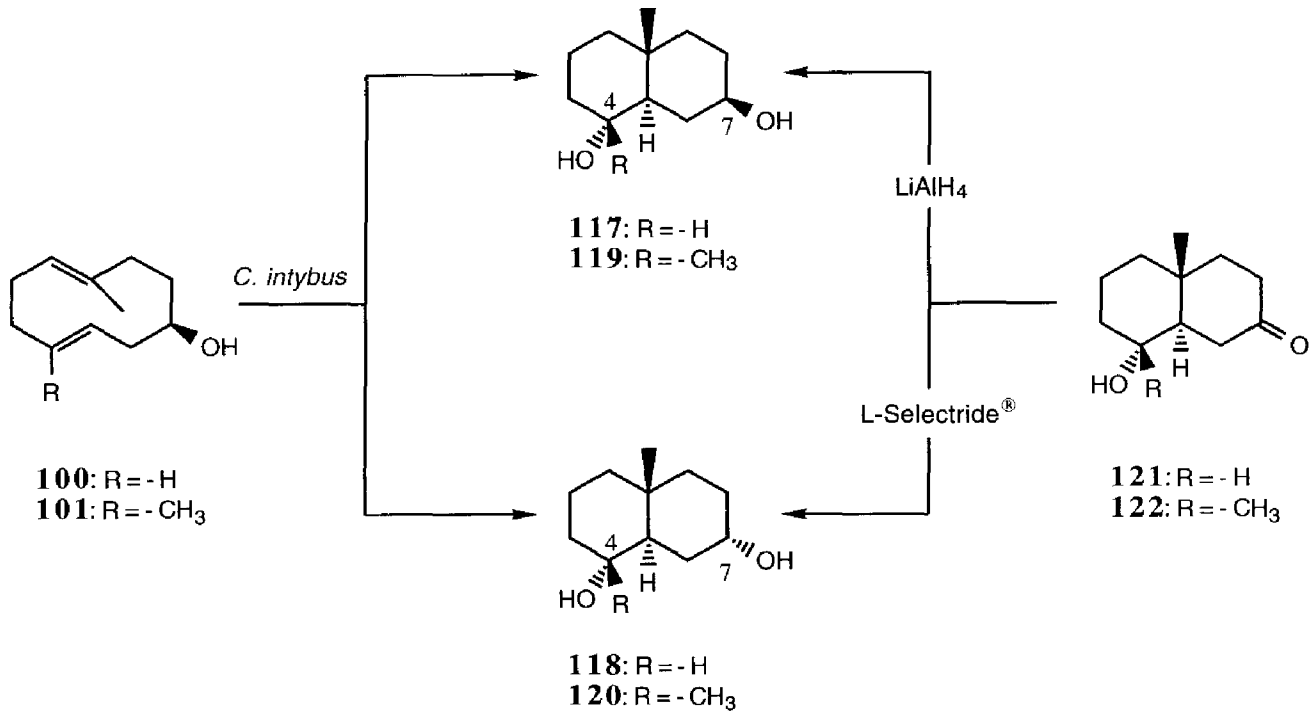
3.3. Biotransformation reactions

3.3.1. (E,E)-cyclodeca-1,5-dienes 100 and 101

When (E,E)-cyclodeca-1,5-diene **100** was administered to a suspension of mortared chicory root and incubated for 10 days, a 2 : 1 mixture of the epimeric alcohols **117** and **118**, with the epimeric centre at C_7, was obtained. The same trend was observed when (E,E)-cyclodeca-1,5-diene **101** was administered to a suspension of mortared chicory root. A 2 : 1 mixture of the epimeric alcohols **119** and **120** was obtained after a 10 days incubation period. The boiled root sample as well as the substrate blank showed no conversion. The epimeric mixtures were separated using preparative capillary GC. Their identity was ascertained upon comparison with authentic samples which were obtained through reduction of the known ketones **121**[23] and **122**[24]. Both retention times and fragmentation spectra of **109 - 112**, obtained through the biotransformation of **100** and **101**, were identical on GC-MS to the products obtained through the reduction of **121** and **122** (Scheme 3.6.).

The epimeric alcohols **117 - 120** originate from the two syn conformations of **100** and **101**, with the substituents at C_4 and C_{10} located in the same plane, either 'above' or 'below' the 10-membered ring. As these substituents invert, the position of the

Scheme 3.6.



100: R = - H
101: R = - CH_3

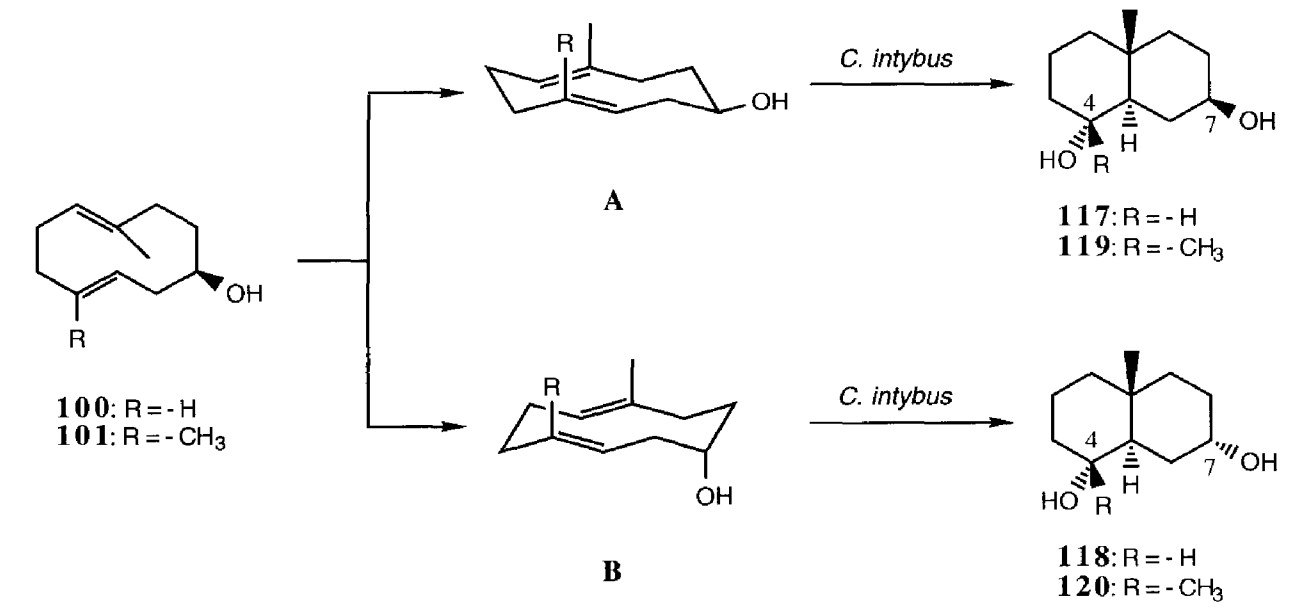
117: R = - H
119: R = - CH_3

118: R = - H
120: R = - CH_3

121: R = - H
122: R = - CH_3

hydroxyl group at C_7 changes from equatorial to axial. Cyclisation through conformer **A** leads to **117** and **119**, while conformer **B** is responsible for the formation of **118** and **120** (Scheme 3.7.).

Scheme 3.7.



100: R = - H
101: R = - CH_3

A

B

117: R = - H
119: R = - CH_3

118: R = - H
120: R = - CH_3

Cyclisation products with a cis-fused eudesmane framework, or any other cyclisation products, were not detected. Although the heat of formation values[25] (ΔH_f) of the syn- (**A** ,**B**) and the anti-conformation (**C**, **D**) of both **100** and **101** only differ marginally, cyclisation through the anti-conformation, with the substituents at C_4 and C_{10} located in the two different planes with respect to the 10-membered ring, does not occur. Table 3.1. showes that these ΔH_f-values of the four possible conformations of (E,E)-cyclodeca-1,5-dienes **100** and **101** are only marginally different. Therefore the fact that cyclisation through conformations **C** and **D** does not occur probably means that the activation barrier towards the formation of cis-eudesmanes is significantly higher than for trans-eudesmanes. Acid induced cyclisation of **100** and **101** in an aqueous medium gave **117-120** in the same ratio as the enzyme mediated cyclisation.

Figure 3.1.

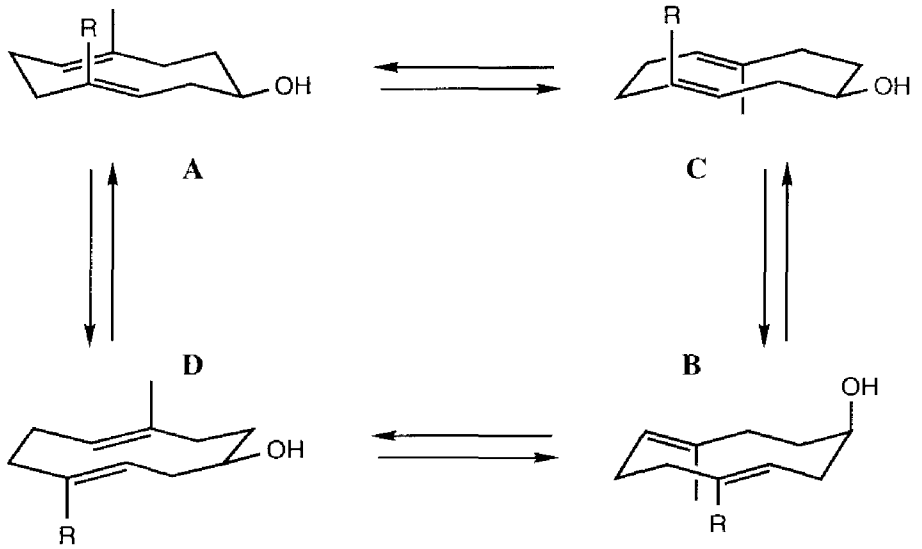


Table 3.1.

	A (Kcal/mol)	**B** (Kcal/mol)	**C** (Kcal/mol)	**D** (Kcal/mol)
100	– 39.12	– 37.49	– 38.31	– 38.63
101	– 48.83	– 47.19	– 48.01	– 47.91

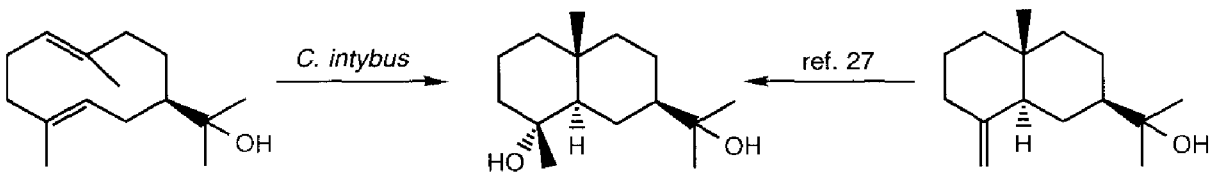
As additional proof, a large scale incubation of **100** with a root suspension of fresh chicory was performed. After 7 days, the products were isolated to give the di-TMS derivatives **123** and **124** after silylation and flash chromatography. Separation of the epimeric mixture was performed by preparative capillary GC. Analytical GC experiments on the cyclisation products of **100** (both **117**, **118** and **123**, **124**) using a

chiral column revealed the racemic nature of the products. The chicory root suspension is therefore not capable of inducing an enantiomeric excess.

3.3.2. (+)-Hedycaryol

When (+)-hedycaryol (**39**)[26] was administered to a mortared root suspension of fresh chicory and incubated for 4 days, cryptomeridiol (**125**)[27,28] was obtained as the sole product (Scheme 3.8.). The identity of **125** was confirmed by direct comparison with an authentic sample synthesised from natural β-eudesmol (**127**)[29] by epoxidation and subsequent reduction of the 4,14-epoxide[27]. The ^{1}H- and ^{13}C NMR values of **125** are identical to those reported in the literature[30].

Scheme 3.8.

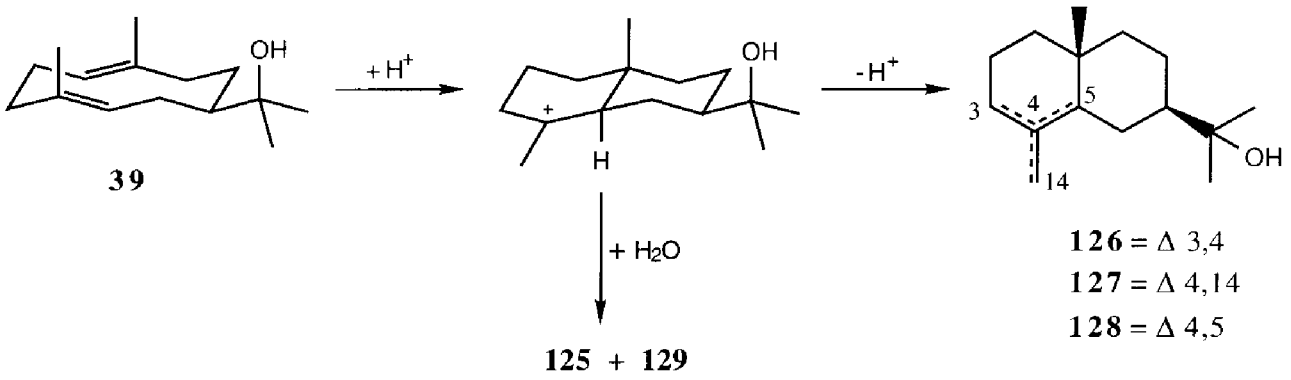


39 **125** **127**

In contrast to the selective biotransformation of **39**, its chemically induced cyclisation always gives rise to mixtures of eudesmanes. If water is present in the reaction medium, this mixture usually consists of **125** and α-, β- and γ-eudesmol (**126**, **127** and **128** respectively, Scheme 3.9.). The ratio in which these products are formed depends on the reaction conditions. For instance, treatment of **39** with sulfuric acid in aqueous acetone gives a mixture of predominantly **125** and **128** accompanied by small amounts of **126**, **127** and another eudesmane **129**, judging from its GC retention time and mass spectrum to be the C_4 epimeric alcohol of **125**. These findings indicate that probably a carbocation intermediate is involved in the acid induced cyclisation of **39** (Scheme 3.9.). Processes in which the capture of a nucleophile is accompanied by extensive elimination are believed to proceed through a step-wise formation involving an intimate ion pair[31].

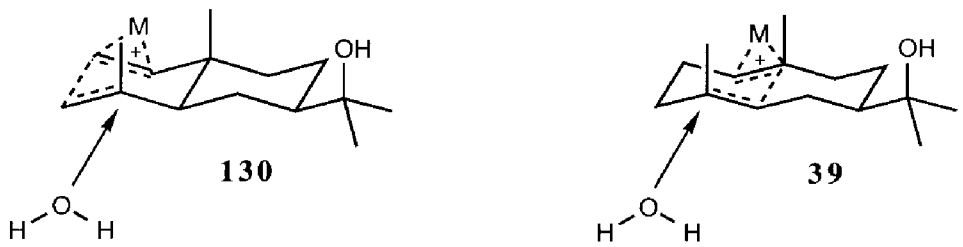
An olefinic cyclisation reaction which is thought to proceed more synchronously is the oxymetallation reaction. Renold *et al.* reported the oxymetallation of elemol (**130**) Acid induced cyclisation of **100** and **101** in an aqueous medium gave **117-120** in the same ratio as the enzyme mediated cyclisation. using $Hg(OAc)_2$ in aqueous THF

Scheme 3.9.



followed by reductive demercuration yielding 69% of **125** and 7% of a mixture of **126-128**[32]. A product-like transition state followed by capture of the nucleophile from a pseudo equatorial direction, as indicated in scheme 3.10, has been proposed to explain the product outcome. Treatment of hedycaryol (**39**) with $Hg(OAc)_2$ under identical reaction conditions gave almost the same ratio of **125** (60%) and **126-128** (15%). This indicates that the oxymercuration - deoxymercuration reaction of elemol (**130**) and hedycaryol (**39**) probably proceeds via a similar type of transition state (Scheme 3.10.). The more concerted nature of this reaction is reflected by the formation of a larger amount of **125**, as compared to the acid induced cyclisation of **39**.

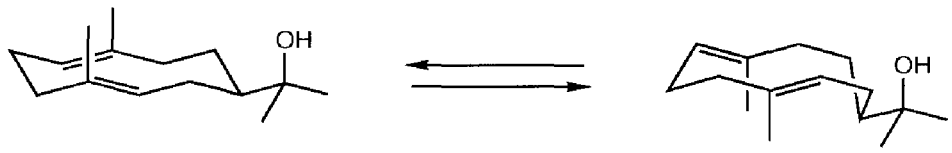
Scheme 3.10.



M = Hg(OAc)

When **39** was incubated with a root suspension of fresh chicory, no eudesmols were detected after completion of the enzymatic reaction. Dehydration of **125** was not observed in both the reaction- and incubation media, even after prolonged exposure to the experimental conditions. Therefore, the exclusive formation of **125** in the biotransformation of **39** by *C. intybus* must have occurred through syn conformer **A** as postulated in figure 3.2. Inversion of the methyl groups (**B**) will place the large isopropanol group at C_7 in an unfavourable pseudo-axial position and cyclisation through this conformation is not likely to occur.

Figure 3.2.



A **B**

3.4. Concluding remarks

The results presented in this chapter show that the biotransformation of **100**, **101** and **39** is induced by enzymatic protonation of the 1,10-double bond followed by cyclisation and subsequent incorporation of a water molecule. The water molecule is stereoselectively incorporated from the α-side. This indicates that the syn conformation of the (E,E)-cyclodeca-1,5-diene framework is preferred during cyclisation and that the transannular cyclisation reaction, initiated by *C. intybus*, is of a concerted nature. Some decades ago, cyclisation through different conformations was postulated to be responsible for the wide variety of naturally occurring guaianes and eudesmanes[33-35]. Our findings confirm the generally accepted idea that compounds possessing an (E,E)-cyclodeca-1,5-diene framework cyclise through the all-chair or syn conformation. Since there are two all-chair conformations possible for the 10-membered ring system, additional ring substituents determine the ratio in which these conformers exist. Judging from the identical ratio in which the cyclisation products of **100** and **101** are formed it can be concluded that the methyl group at the C_4 position has no effect on the mode of cyclisation. The formation of two epimeric diols from **100** and **101** and only one from **39** indicates that the nature of the substituent at C_7 has a major influence on the conformation of the germacrane ring system and thus on the mode of cyclisation.

3.5. Experimental

Melting points are uncorrected. Chemical shifts are reported relative to TMS (δ 0.00) at 90, 200 and 500 MHz for ^{1}H-NMR and at 25.3 and 125.7 MHz for ^{13}C-NMR. All spectra were recorded in $CDCl_3$ unless noted otherwise. Mass spectral data were obtained with a Hewlett Packard 5890 GC-MS equipped with a Hewlett Packard 5970 series mass selective detector using a capillary DB-17 column (30 m x 0.25 mm, d_f 0.25 μm) and helium as the carrier gas. Accurate mass measurements were obtained with a MS 902

equipped with a VG-ZAB console. Preparative GC was performed with a Gerstel preparative DCS system using a Hewlett Packard methyl silicone pre-column (5 m x 0.53 cm; d_f = 2.65 mm) and a Quadrex methyl silicone main column (25 m x 0.53 cm; d_f = 5.0 mm) in two separate Hewlett Packard 5890 II gas chromatographs using hydrogen as the carrier gas. GC analyses were carried out on a Fisons GC 8000 equipped with a FID and a DB-17 fused silica capillary column (30m x 0.25 mm, d_f 0.25 µm). Peak areas were integrated electronically with a Fisons integrator DP700. Column chromatography was performed on Merck silicagel 60 and deactivated Al_2O_3 (grade III) using petroleum ether (bp. 40-60, PE)- EtOAc as the solvent system.

A suspension of fresh chicory root (20% w/v) was produced by mortaring the peeled root in a solution of 0.25 M sucrose, 3 mM Tris·HCl, 10 mM $MgCl_2$ and 0.2 % (w/v) Bovine Serum Albumin (BSA). The pH of this sucrose / Tris / $MgCl_2$/ BSA-solution (STMB) was set at 7.0 using 2-morpholino-ethanesulfonic acid (MES). The stability of **100**, **101** and **39** towards the buffer and an inactivated chicory root sample (obtained by boiling the root suspension for 30 min) was investigated as a control to test the possibility of non-enzymatic reactions. Neither showed any reaction. Incubations were performed in sealed 4 ml vials at room temperature in a KS 500 shaker at 260 rpm containing 200 µl root suspension, 790 µl STMB-solution and 10 µl 0.1 M substrate in EtOH. The incubation medium was extracted with 0.5 ml EtOAc and its contents were analysed by GC-MS. All solvents were distilled before use.

(4aα,5α)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methyl-2(3H)-naphtalenone (102) To a stirred suspension of 20.04 g of 2-methylcyclohexa-1,3-dione in 100 ml of water was added 20 ml of freshly distilled MVK. The reaction mixture was stirred overnight at 60°C, cooled and extracted with 3 x 75 ml of EtOAc. The combined organic layers were washed with 150 ml of brine, dried over $MgSO_4$ and evaporated to give 31.09 g (100%) of the Michael-adduct as a golden oil. ^{1}H-NMR (90 MHz): δ 2.80-2.60 (m, 4H); δ 2.50-1.95 (m, 6H); δ 2.10 (s, 3H); δ 1.25 (s 3H). The crude Michael-adduct was taken up in 150 ml of toluene, 1.85 ml of pyrrolidine was added and the reaction mixture was stirred for 2h at 80°C. After addition of 3 ml of conc. HCl, the solvent was evaporated and the tarry, dark-brown residue was flash chromatographed (silica; EtOAc). Evaporation of the solvent gave 24.46 g (87%) of **99** as a dark brown oil. ^{1}H-NMR of **99** (90 MHz): δ 5.85 (bs, 1H); δ 3.00-1.50 (m, 11H); δ 1.45 (s, 3H). The crude **99** was dissolved in 200 ml of EtOH and cooled to 0°C. After adding carefully 1.72 g of $NaBH_4$, the reaction mixture was stirred for 1h, 5 ml of AcOH was added and the solvent was evaporated. The residue was taken up in 150 ml of CH_2Cl_2 and extracted with 3 x 100 ml of saturated $NaHCO_3$. The aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were washed with 100 ml of brine, dried over $MgSO_4$ and evaporated to give 24.20 g (98%) of **102** as a light-brown oil. ^{1}H-NMR of **102**: δ 5.75 (d, 1H, J = 1.7 Hz); δ 4.05 (dd, 1H, J = 11.2, 4.5 Hz); δ 2.40-1.30 (m, 11H); δ 1.15 (s, 3H).

(4aα,5α)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methyl-2(3H)-naphthalenone 5-(4'-methylbenzene -sulfonate) (103a). To a stirred solution of 12.41 g of **102** in 60 ml of dry pyridine was added 14.50 g of TsCl. The reaction mixture was stirred for 3 days, 75 ml of $CHCl_3$ was added and the mixture was washed with 2 x 75 ml of saturated $NaHCO_3$. The aqueous layers were extracted with 2 x 50 ml of $CHCl_3$. The combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 20.33 g (88%) of **103a** as a solid. mp. 98-99°C (from PE/EtOAc 1:1); ^{1}H-NMR of **103a**: δ 7.76 (dd, 2H, J = 6.8 Hz, 1.6 Hz); δ 7.54 (d, 2H, J = 8.2 Hz); δ 5.74 (d, 1H, J = 1.6 Hz); δ 4.24 (dd, 1H, J = 10.9 Hz, 5.1 Hz); δ 2.42 (s, 3H); δ 2.30-2.10 (m, 4H); δ 1.95-1.25 (m, 6H); δ 1.19 (s, 3H); ^{13}C-NMR of **103a**: δ 198.34 (s), δ 165.20 (s), δ 144.70 (s), δ 133.78 (s), δ 129.63 (d), δ 127.483 (d), δ 125.97 (d), δ 87.47 (d), δ 40.72 (s), δ 33.59 (t), δ 33.13 (t), δ 31.18 (t), δ 27.74 (t), δ 22.62 (t), δ 21.44 (q), δ 16.28 (q).

(4aα,5α)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a-methyl-2(3H)-naphtalenone 5-methanesulfonate (103b). To a stirred solution of 9.28 g of **102** in 50 ml of dry pyridine was added 4.20 ml of MsCl. The reaction mixture was stirred for 1.5h, the solvent was evaporated, the residue was taken up in 75 ml of $CHCl_3$ and extracted with 3 x 25 ml of 10% HCl solution. The combined aqueous layers were extracted with 2 x 25 ml of $CHCl_3$. The combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 12.53 g (94%) of **103b** as a solid. mp. 127-129°C. ^{1}H-NMR of **103b**: δ 5.81 (d, 1H, J = 1.7 Hz); δ 4.44 (dd, 1H, J = 11.4 Hz, 4.9 Hz); δ 3.03 (s, 3H); δ 2.50-1.85 (m, 9H); δ 1.50 (m, 1H); δ 1.26 (s, 3H); ^{13}C-NMR of **103b**: δ 198.34 (s), δ 165.08 (s), δ 126.36 (d), δ 87.25 (d), δ 40.81 (s), δ 38.91 (q), δ 34.20 (t), δ 33.33 (t), δ 31.33 (t), δ 28.33 (t), δ 22.73 (t), δ 16.38 (q).

(4aα,5α)-2-acetoxy-3,4,4a,5,6,7-hexahydro-5-hydroxy-4a-methylnaphthalene 5-(4'-methylbenzenesul fonate) (104a). To a stirred mixture of 16.80 g of **103a** and 27.84 g of NaI in 200 ml of acetic anhydride, cooled to 0°C, was added dropwise 16.50 ml of TMSCl. The reaction mixture was stirred at 0°C for 2h, after which the solvents were evaporated. The residue was taken up in 150 ml of CH_2Cl_2 and washed with 2 x 100 ml of saturated $NaHCO_3$ and 2 x 100 ml of 2M $Na_2S_2O_3$. The combined aqueous layers were extracted with 2 x 150 ml of CH_2Cl_2. The combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated yielding 16.70 g of **104a** (88%) as a solid. mp. 109-110.5°C. (from EtOH). ^{1}H-NMR **104a**: δ 7.78 (dd, 2H, J = 6.8 Hz, 1.6 Hz); δ 7.29 (d, 2H, J = 8.2 Hz); δ 5.76 (d, 1H, J = 2.1 Hz); δ 5.30 (t, 1H, J = 3.6 Hz); δ 4.42 (dd, 1H, J = 11.3 Hz, 4.7 Hz); δ 2.42 (s, 3H); δ 2.40-2.15 (m, 3H); δ 2.25-1.85 (m, 3H); δ 2.09 (s, 3H); δ 1.71 (ddd, 1H, J = 12.5 Hz, 5.6 Hz, 1.4 Hz); δ 1.18 (dt, 1H, J = 12.5 Hz, 6.1 Hz); δ 1.04 (s, 3H); ^{13}C-NMR of **104a**: δ 168.90 (s), δ 148.11 (s), δ 144.30 (s), δ 136.56 (s), δ 134.13 (s), δ 129.50 (d), δ 127. 50 (d), δ 122.71 (d), δ 115.60 (d), δ 86.90 (d), δ 36.43 (s), δ 32.02 (t), δ 24.49 (t), δ 24.04 (t), δ 21.40 (q), δ 20.78 (q), δ 17.39 (q).

(4aα,5α)-2-acetoxy-3,4,4a,5,6,7-hexahydro-5-hydroxy-4a-methylnaphthalene 5-methanesulfonate (104b). To a stirred mixture of 6.85 g **103b** and 14.5 g of NaI in 130 ml of acetic acid, cooled to 0°C, was added dropwise 9 ml of TMSCl. The mixture was stirred at 0°C for 1h after which the solvents were

evaporated. The residue was taken up in 100 ml of CH_2Cl_2 and washed with 2 x 50 ml of saturated $NaHCO_3$ and 2 x 50 ml of 2M $Na_2S_2O_3$. The aqueous layers were extracted with 2 x 100 ml of CH_2Cl_2. The combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated to give 7.60 g of an oil which was chromatographed (silica; PE/EtOAc 1:2) to give 7.26 g (91%) of **104b** as an oil. ^{1}H-NMR of **104b**: δ 5.69 (d, 1H, J = 2.4 Hz); δ 5.32 (dd, 1H, J = 3.6 Hz, 3.6 Hz); δ 4.55 (dd, 1H, J = 8.6 Hz, 7.1 Hz); δ 2.99 (s, 3H); δ 2.40 (m, 2H); δ 2.28 (m, 2H); δ 2.15 (m, 1H); δ 2.08 (s, 3H); δ 2.04 (m, 1H); δ 1.93 (ddd, 1H, J = 12.7 Hz, 5.6 Hz, 1.5 Hz); δ 1.46 (dddd, 1H, J = 12.4 Hz, 12.4 Hz, 5.6 Hz, 0.4 Hz); δ 1.06 (s, 3H); ^{13}C-NMR of **104b**: δ 169.11 (s), δ 147.63 (s), δ 136.64 (s), δ 122.78 (d), δ 115.69 (d), δ 86.65 (d), δ 38.63 (q), δ 32.58 (t), d 36.47 (s), δ 24.49 (2 x t), δ 24.22 (t), δ 20.87 (q), δ 17.51 (q).

(2α,4aα,5α)-1,2,3,4,4a,5,6,7-octahydro-4a-methyl-2,5-naphthalenediol 5-(4'-methylbenzenesulfonate) (105a). To a stirred suspension of 15.35 g of **104a** in 300 ml of EtOH, cooled to 0°C, 14.0 g of $NaBH_4$ was added and the reaction was stirred for 16h at room temperature. After adding carefully 10 ml of acetic acid, the reaction mixture was evaporated. The residue was taken up in 150 ml of CH_2Cl_2 and washed with 100 ml of saturated $NaHCO_3$. The aqueous layer was extracted with 2 x 100 ml of CH_2Cl_2. The combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated to give 13.44 g of **105a** (98%) as a solid. mp. 117-119 °C (dec.). (from PE/EtOAc 1:1) ^{1}H-NMR of **105a**: δ 7.71 (dd, 2H, J = 8.4 Hz, 1.9 Hz); δ 7.26 (d, 2H, J = 8.1 Hz); δ 5.19 (broad s, 1H); δ 4.32 (dd, 1H, J = 11.2 Hz, 4.6 Hz); δ 3.37 (m, 1H); δ 2.51 (broad s, 1H); δ 2.36 (s, 3H); δ 2.24 (ddd, 1H, J = 13.7 Hz, 4.8 Hz, 1.8 Hz); δ 2.20-1.90 (m, 3H); δ 1.80-1.65 (m, 5H); δ 1.36 (ddt, 1H, J = 12.6 Hz, 12.0 Hz, 3.9 Hz); δ 1.00 (s, 3H); ^{13}C-NMR of **105a**: δ 144.30 (s), δ 138.61 (s), δ 134.19 (s), δ 129.49 (d), δ 127.36 (d), δ 120.71 (d), δ 89.01 (d), δ 70.59 (d), δ 40.76 (t), δ 38.26 (s), δ 35.41 (t), δ 30.53 (t), δ 24.43 (t), δ 24.15 (t), δ 21.36 (q), δ 17.73 (q).

(2α,4aα,5α)-1,2,3,4,4a,5,6,7-octahydro-4a-methyl-2,5-naphthalenediol 5-methanesulfonate (105b). To a stirred solution of 7.26 g of **104b** in 150 ml of EtOH, cooled to 0°C, was added 7.0 g of $NaBH_4$ and the resuting mixture was stirred overnight at room temperature. After careful addition of 10 ml of acetic acid, the solvent was evaporated, the resulting residue was taken up in 150 ml of CH_2Cl_2 and washed with 2 x 100 ml of saturated $NaHCO_3$. The combined aqueous layers were extracted with 4 x 50 ml of CH_2Cl_2, the combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated to give 6.26 g (99.5%) of **105b** as an oil. ^{1}H-NMR of **105b** δ 5.30 (dd, 1H, J = 4.1 Hz, 2.4 Hz); δ 4.52 (dd, 1H, J = 10.1 Hz, 6.1 Hz); δ 3.50 (m, 1H); δ 3.00 (s, 3H); δ 2.35 (m, 1H); δ 2.15-1.85 (m, 8H); δ 1.51 (m, 1H); δ 1.25 (m, 1H); δ 1.11 (s,3H); ^{13}C-NMR of **105b**: δ 138.61 (s), δ 121.18 (d), δ 88.80 (d), δ 71.03 (d), δ 40.04 (t), δ 38.77 (q), δ 38.52 (s), δ 36.08 (t), δ 30.94 (t), δ 25.20 (t), δ 23.99 (t), δ 17.97 (q).

(2α,4aα,5α)-1,2,3,4,4a,5,6,7-octahydro-5-hydroxy-2-[(trimethylsilyl)oxyl-4a-methylnaphthalene 5-(4'-methanesulfonate) (106). To a stirred solution of 13.00 g of **105a** and 8.2 ml of HMDS in 50 ml of dry pyridine was added dropwise 4.9 ml of TMSCl. The reaction mixture was stirred for 45 minutes after which 3 ml of water was added. The solution was taken up in 75 ml of $CHCl_3$ and washed with 100 ml of

5% HCl and 100 ml of saturated $NaHCO_3$. The combined aqueous layers were extracted with 2 x 75 ml of $CHCl_3$. The combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated to yield 15.43 g of **106** (97%) as a solid. An analytical sample was crystallized mp. 82-84.5°C (from PE/EtOAc 1:1); ^{1}H-NMR of **106:** δ 7.77 (d, 2H, J = 8.4 Hz); δ 7.31 (d, 2H, J = 8.2 Hz); δ 5.23 (broad s, 1H); δ 4.40 (dd, 1H, J = 11.2 Hz, 4,5 Hz); δ 3.40 (m, 1H); δ 2.42 (s, 3H); δ 2.16 (d, 2H, J = 7.3 Hz); δ 2.01 (m, 2H); δ 1.85-1.30 (m, 5H); δ 1.05 (s, 3H); δ 1.00 (m, 1H); δ 0.10 (m, 9H); ^{13}C-NMR of **106:** δ 144.25 (s), δ 138.51 (s), δ 134.45 (s), δ 129.48 (d), δ 127.42 (d), δ 120.89 (d), δ 88.94 (d), δ 70.82 (d), δ 40.84 (t), δ 38.29 (s), δ 35.41 (t), δ 30.67 (t), δ 27.47 (t), δ 24.20 (t), δ 21.41 (q), δ 17.76 (q), δ 2.20 (s), δ -0.32 (q).

(E,E)-8-methyl-1-[(trimethylsilyl)oxy]-cyclodeca-3,7-diene (107). To a stirred solution of 14.30 g of **106** in 100 ml of dry THF, cooled to 0°C, under a nitrogen atmosphere was added dropwise 45 ml of 2M $BH_3.Me_2S$ complex. The reaction mixture was stirred for 1h at 0°C and was allowed to warm slowly to room temperature. After 2.5 days, 3 ml of water was added followed by 30 ml of 2M NaOH. The mixture was stirred for 3h and extracted with ether. The organic layer was washed with 2 x 75 ml of brine and the combined aqueous layers were extracted with 2 x 75 ml of ether. The combined organic layers were washed with 75 ml brine, dried on $MgSO_4$ and evaporated to yield an oil. Column chromatograph (Al_2O_3; CH_2Cl_2) yielded 5.40 g of **107** (65%) as an oil. ^{1}H-NMR of **107:** δ 5.25-4.65 (m, 3H); δ 3.52 (m, 1H); δ 2.46 (broad d, 1H J = 14.1 Hz); δ 2.40-0.70 (m, 9H); δ 1.30 (s, 3H); δ 0.12-0.07 (m, 9H); ^{13}C-NMR of **107:** δ 137.40 (s), δ 132.39 (d), δ 125.16 (d), δ 124.72 (d), δ 75.75 (d), δ 43.24 (t), δ 38.22 (t), δ 37.23 (t), δ 32.69 (t), δ 26.89 (t), δ 16.58 (q), δ -0.18 (q).

(E,E)-8-methyl-cyclodeca-3,7-dien-1-ol (100). (from **107**) To a stirred solution of 5.40 g of **107** in 60 ml of THF, 11 ml of 1M TBAF in THF was added dropwise. After 3h, the reaction mixture was washed with 3 x 50 ml of water. The combined aqueous layers were extracted with 3 x 50 ml of ether. The combined organic layers were washed with 50 ml of brine, dried on $MgSO_4$ and evaporated to yield an oil. Column chromatography over (Al_2O_3; CH_2Cl_2) yielded 3.74 g of **100** (99%) as a colourless oil which solidified upon standing. (from **105b**) To a stirred solution of 7.60 g of **105b** in 100 ml of dry ether, cooled to 0°C, was added dropwise 15 ml of 2M $BH_3.Me_2S$ complex. The resulting mixture was stirred overnight at room temperature, 5 ml of water was added followed by careful additon of 30 ml of cold 4M NaOH. The mixture was stirred overnight and extracted with ether. The organic layer was washed with 2 x 75 ml of brine and the combined aqueous layers were extracted with 2 x 75 ml of ether. The combined organic layers were washed with 75 ml brine, dried on $MgSO_4$ and evaporated to yield 4.28 g of an oil. Column chromatography (silica; PE/EtOAc 1:1) yielded 2.61 g (54%) of **100** as a colourless oil which solidified upon standing. mp 24.5-28°C. ^{1}H-NMR of **100:** δ 5.04 (dddd, 1H, J = 15.4 Hz, 10.6 Hz, 4.8 Hz, 2.4 Hz); δ 4.81 (ddd, 1H, J = 15.2 Hz, 11.5 Hz, 3.2 Hz), δ 4.77 (dd, 1H, J = 11.1 Hz, 5.6 Hz); δ 3.62 (dt, 1H, J = 9.9 Hz, 3.5 Hz); δ 2.56 (dd, 1H, J = 13.4 Hz, 2.1 Hz); δ 2.37 (dd, 1H, J = 12.9 Hz, 6.5 Hz); δ 2.13 (dt, 1H, J = 12.7 Hz, 2.2 Hz); δ 2.05-1.95 (m, 4H); δ 1.90-1.75 (m, 3H); δ 1.33 (s, 3H); ^{13}C-NMR of **100:** δ 136.97 (s), δ 131.96 (d), δ

125.37 (d), δ 124.96 (d), δ 75.21 (d), δ 42.72 (t), δ 38.10 (t), δ 36.79 (t), δ 32.63 (t), δ 26.86 (t), δ 16.57 (q); Calc. for (M^+): 166.1357; Found: 166.1357.

(4aα,5α,8β)-4,4a,5,6,7,8-hexahydro-5-hydroxy-4a,8-dimethyl-2(3H)-naphthalenone 5-methanesulfonate (113). To a stirred solution of 1.44 g of **112** in 20 ml of dry pyridine, cooled to 0°C, was added 0.8 ml of MsCl. The reaction mixture was stirred at 0°C for 1h, after which cold saturated $NaHCO_3$ was added. The resulting mixture was taken up in 50 ml of CH_2Cl_2 and extracted with 2 x 50 ml of saturated $NaHCO_3$. The combined aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2. The combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to yield 1.81 g (90%) of **113** as a white solid. mp: 118.5-120°C (dec.). ^{1}H-NMR of **113**: δ 5.82 (d, 1H, J = 1.9 Hz); δ 4.44 (dd, 1H, J = 11.2 Hz, 5.3 Hz); δ 3.02 (s, 3H); δ 2.45-2.35 (m, 3H); δ 2.15-2.05 (m, 3H); δ 2.00–1.88 (m, 3H); δ 1.24 (s, 3H); δ 1.05 (d, 3H); ^{13}C-NMR of **113**: δ 198.71 (s), δ 168,25 (s), δ 123.96 (d), δ 87.05 (d), δ 40.91 (s), δ 38.94 (q), δ 34.35 (t), δ 33.12 (d), δ 33.03 (t), δ 31.42 (t), δ 28.19 (t), 17.46 (2 x q).

(4aα,5α)-2-acetoxy-3,4,4a,5,6,7-hexahydro-5-hydroxy-4a,8-dimethylnaphthalene 5-methanesulfonate (114). To a stirred mixture of 1.76 g of **113** and 3.50 g of NaI in 40 ml of acetic anhydride, cooled to 0°C, was added dropwise 2.15 ml of TMSCl. The reaction mixture was stirred for 1h at 0°C after which the solvents were evaporated. The residu was taken up in 50 ml of CH_2Cl_2 and washed with 2 x 25 ml of saturated $NaHCO_3$ and 2 x 25 ml of 2M $Na_2S_2O_3$. The aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2, washed with brine, dried on $MgSO_4$ and evaporated. The oily residue was chromatographed (silica; EtOAc) to yield 2.01 g (99%) of **114** as a colourless oil. ^{1}H-NMR of **114**: δ 6.01 (d, 1H, J = 2.0 Hz); δ 4.55 (dd, 1H, J = 10.0 Hz, 6.5 Hz); δ 3.02 (s, 3H); δ 2.60-2.45 (m, 1H); δ 2.40-2.10 (m, 5H); δ 2.13 (s, 3H); δ 1.94 (ddd, 1H, J = 12.7 Hz, ;5.8 Hz, 1.6 Hz); δ 1.62 (s, 3H); δ 1.55-1.40 (m, 1H); δ 1.06 (s, 3H); ^{13}C-NMR of **114**: δ 169.43 (s), δ 147.73 (s), δ 129.12 (s), δ 127.98 (s), δ 112.54 (d), δ 87.04 (d), δ 38.71 (q), δ 36.57 (s), δ 32.81 (t), δ 31.47 (t), δ 24.69 (t), δ 23.95 (t), δ 21.02 (q), δ 18.27 (q), δ 17.35 (q).

(2α,4aα,5α)-1,2,3,4,4a,5,6,7-octahydro-4a,8-dimethyl-2,5-naphthalenediol 5-methanesulfonate (115). To a stirred solution of 1.97 g of **114** in 40 ml of EtOH, cooled to 0°C, 1.98 g of $NaBH_4$ was added and the reaction was stirred overnight at room temperature. After carefully adding 3 ml of acetic acid, the reaction mixture was evaporated. The residue was taken up in in 50 ml of CH_2Cl_2 and washed with 2 x 50 ml saturated $NaHCO_3$. The combined aqueous layers were extracted with 2 x 25 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated under exclusion of light to yield 1.62 g (94%) of **115** as a colourless oil. ^{1}H-NMR of **115**: δ 4.49 (dd, 1H, J = 8.6 Hz, 7.0 Hz), δ 3.55-3.37 (m, 1H); δ 3.00 (s, 3H); δ 2.74 (ddd, 1H, J = 13.6 Hz, 4.7 Hz, 2.1 Hz); δ 2.20-1.80 (m, 8H); δ 1.60-1.40 (m, 1H); δ 1.59 (s, 3H); δ 1.30-1.10 (m, 1H); δ 1.09 (s,3H); ^{13}C-NMR of **115**: δ 130.81 (s), δ 126.01 (s), δ 89.02 (d), δ 70.67 (d), δ 38.76 (q), δ 38.36 (s), δ 36.24 (t), δ 35.15 (t), δ 31.42 (t), δ 30.82 (t), δ 25.15 (t), δ 18.99 (q), δ 18.11 (q).

(E,E)-4,8-dimethyl-cyclodeca-3,7-dien-1-ol (101). To a stirred solution of 1.54 g of **115** in 40 ml of dry ether under a nitrogen atmosphere, cooled to 0°C, was added dropwise 15.5 ml of 2M BH_3·Me_2S complex in THF which reacted vigorously as indicated by the evolution of hydrogen gas. The reaction was stirred for 8h at room temperature after which 3 ml of water followed by 25 ml of 4M NaOH was carefully added. The resulting two-phase mixture was stirred overnight after which the organic phase was separated and washed with 2 x 50 ml of 2M NaOH. The combined aqueous layers were extracted with 2 x 50 ml of ether and the combined organic phases were washed with 50 ml of brine, dried on $MgSO_4$ and evaporated to yield 950 mg (94%) of an oil containing **101** and **116** in a 9 : 1 ratio according to GC. The oil was taken up in 50 ml of tert-butylmethylether (TBME) and extracted with 4 x 25 ml of 20% $AgNO_3$. The combined $AgNO_3$-layers were extracted with 25 ml of TBME and then cooled to 0°C. The combined organic phase was washed with brine, dried on $MgSO_4$, concentrated and purified by column chromatography (silica; PE/EtOAC 1:1) to yield 16 mg (3%) of **116** as an oil. After addition of 70 ml of 25% ammonia, the $AgNO_3$-layers were extracted with 4 x 25 ml of TBME. The combined organic layers were washed with 50 ml of brine, dried on $MgSO_4$ and evaporated to yield 479 mg (47%) of **101** as a solid. mp: 71-72.5°C (from diisopropylether). ^{1}H-NMR of **101**: Due to extensive coalescence of the ^{1}H-NMR-spectrum of **101**, no additional information could be obtained other than **101** existing in multiple conformations in a $CDCl_3$ solution at room temperature. ^{13}C-NMR of **101**: δ 137.76 (s), δ 131.01 (s), δ 126.70 (d), δ 126.48 (d), δ 76.15 (d), δ 38.68 (2 x t), δ 38.49 (t), δ 37.29 (t), δ 26.21 (t), δ 16.81 (q), δ 15.99 (q). Mass spectrum of **101** (m/e): 41 (85), 43 (38), 53 (44), 55 (55), 67 (100), 68 (60), 79 (50), 81 (50), 91 (31), 93 (35), 105 (49), 119 (20), 120 (20), 121 (18), 123 (12), 147 (27), 162 (28), 180 (4); Calc. for: 180.1514, Found: 180.1512; Anal. calc. for $C_{12}H_{20}O$: C 79.94%, H 11.18%; Found: C 79.20%, H 11.33%. ^{1}H-NMR of **116**: δ 3.50-3.40 (m, 1H); δ 2.12 (dd, 1H, J = 12.9 Hz, 4.9 Hz); δ 2.00-0.80 (m, 12 H); δ 0.97 (d, 3H, J = 6.3 Hz); d 0.93 (s, 3H); ^{13}C-NMR of **116**: δ 68.47 (d), δ 37.39 (d), δ 36.70 (t), δ 34.74 (d), δ 34.46 (t), δ 33.51 (t), δ 31.24 (t), δ 24.06 (t), δ 20.05 (q), δ 17.06 (q)[36].

(1α,4aβ,7β,8aα)-decahydro-4a-methyl-naphthalene-1,7-diol (117). To a stirred solution of 70 mg of **121** in 5 ml of ether, cooled to 0°C, was added 100 mg of $NaBH_4$. The resulting mixture was stirred for 2h after which 3 ml of saturated NH_4Cl was added. The layers were separated, the aqueous layer was extracted with 2 x 10 ml of ether , the combined organic layers were washed with 15 ml of brine, dried on $MgSO_4$ and evaporated to yield 56 mg (79%) of **117** as a solid. mp: 122-124.5°C. ^{1}H-NMR of **117**: δ 3.58 (m, 1H, J = 5.1 Hz); δ 3.39 (dt, 1H, J = 10.3 Hz, 4.4 Hz); δ 2.16 (m, 1H, J = 9.4 Hz, 4.5 Hz, 2.4 Hz); δ 2.00 (m, 1H, J = 12.4 Hz, 3.2 Hz); δ 1.78 (m, 1H); δ 1.56 (m, 4H); δ 1.39 (m, 4H); δ 1.09 (m, 4H); δ 0.89 (s, 3H); ^{13}C-NMR of **117**: δ 71.28 (d), δ 69.71 (d), δ 49.84 (d), δ 40.00 (t), δ 36.99 (t), δ 36.10 (t), δ 32.15 (t), δ 34.60 (s), δ 30.70 (t), δ 20.08 (t), δ 16.71 (q). Mass spectrum of **117** (m/e): 41 (100), 43 (61), 55 (86), 57 (46), 67 (82), 79 (55), 81 (60), 95 (70), 96 (51), 107 (36), 109 (64), 122 (21), 123 (32), 133 (32), 137 (19), 141 (15), 148 (18), 151 (20), 166 (30), 184 (17).

(1α,4aβ,7α,8aα)-decahydro-4a-methyl-naphthalene-1,7-diol (118). To a stirred solution of 70 mg of **121** in 5 ml of ether, cooled to 0°C, was added 0.25 ml of L-selectride®. The resulting mixture was stirred for 2h after which 5 ml of EtOH and 3 ml of 35% of H_2O_2 was added. After an additional hour of stirring, 10 ml

of water was added and the mixture was extracted with 3 x 10 ml of ether. The combined organic layers were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 36 mg (51%) of **118** as an oil. ^{1}H-NMR of **118**: δ 4.12 (t, 1H, J = 2.6 Hz); δ 3.31 (dt, 1H, J = 10.4 Hz, 4.4 Hz); δ 2.74 (bs, 2H); δ 2.00 (m, 2H); δ 1.80-1.05 (m, 11H); δ 0.80 (s, 3H); ^{13}C-NMR of **118**: δ 70.08 (d), δ 66.36 (d), δ 44.70 (d), δ 40.70 (t), δ 36,79 (t), δ 35.42 (t), δ 34.72 (s), δ 30.07 (t), δ 28.15 (t), δ 20.30 (t), δ 15.90 (q); Mass spectrum of **118**(m/e): 41 (100), 43 (63), 55 (86), 67 (88), 79 (67), 81 (63), 95 (61), 107 (45), 109 (63), 133 (57), 148 (37), 151 (20), 166 (20), 184 (22).

(1α,4aβ,7β,8aα)-decahydro-1,7-[di(trimethylsilyl)oxy]-4a-methylnaphthalene (123). ^{1}H-NMR of **123**: δ 4.03 (m, 1H, J = 2.8 Hz); δ 3.31 (dt, 1H, J = 10.6 Hz, 4.5 Hz); δ 1.85 (m, 1H); δ 1.81 (ddd, 1H, J = 13.6 Hz, 5.7 Hz, 2.9 Hz); δ 1.57 (m, 5H); δ 1.45 (dt, 1H, J = 12.6 Hz, 2.7 Hz); δ 1.29 (m, 2H); δ 1.12 (m, 3H); δ 0.78 (s, 3H); δ 0.06 (s, 18 H); ^{13}C-NMR of **123**: δ 70.94 (d), δ 66.89 (d), δ 44.23 (d), δ 40.64 (t), δ 36.90 (t), δ 35.67 (t), δ 34.64 (s), δ 31.10 (t), δ 29.33 (t), δ 20.48 (t), δ 16,12 (q), δ 0.37 (q), δ 0.18 (q). Mass spectrum of **123** (m/e): 73 (100), 75 (78), 149 (75), 183 (21), 195 (20), 238 (21), 285 (4), 328 (11).

(1α,4aβ,7α,8aα)-decahydro-1,7-[di(trimethylsilyl)oxy]-4a-methylnaphthalene (124). ^{1}H-NMR of **124**: δ 3.52 (m, 1H, J = 5.2 Hz); δ 3.34 (dt, 1H, J = 10.2 Hz, 4.5 Hz); δ 2.01 (m, 1H); δ 1.86 (m, 1H, J = 12.5 Hz, 3.4 Hz); δ 1.63 (m, 1H); δ 1.53 (m, 3H); δ 1.35 (dt, 1H, J = 13,5 Hz, 3.5 Hz); δ 1.32 (m, 1H); δ 1.22 (m, 1H); δ 1.11 (dt, 1H, J = 14.1 Hz, 5.0 Hz); δ 0.99 (m, 3H); δ 0.82 (s, 3H); δ 0.10 (s, 9H); δ 0.08 (s, 9H); ^{13}C-NMR of **124**: δ 72.20 (d), δ 70.67 (d), δ 49.94 (d), δ 40.31 (2 x t), δ 36.63 (t), δ 33.08 (t), δ 33.08 (s), δ 31.52(t), δ 20.42 (t), δ 16.92 (q), δ 0.27 (2 x q); Mass spectrum of **124** (m/e): 73 (100), 75 (98), 149 (56), 191 (17), 195 (17), 204 (16), 285 (5), 299 (6), 328 (14).

(1α,4aβ,7β,8aα)-decahydro-1,4a-dimethylnaphthalene-1,7-diol (119). To a stirred solution of 50 mg of **122** in 5 ml of ether, cooled to 0°C, was added 100 mg of $LiAlH_4$. The resulting mixture was stirred for 2h after which 300 mg of Glauber's salt was added. Stirring was continued for 30 minutes followed by addition of $MgSO_4$, 15 minutes of stirring, filtering off the solids and evaporation of the solvent to yield 46 mg (91%) of **119** as a solid. mp: 159-161°C; ^{1}H-NMR of **119**: δ 3.64 (m, 1H, $W_{1/2}$ = 20.3 Hz); δ 2.11-2.04 (m, 1H); δ 1.81-1.72 (m, 2H); δ 1.52 (s, 2H); δ 1.59-1.27 (m, 7H); δ 1.22-0.95 (m, 3H); δ 1.01 (s, 3H); δ 0.89 (s, 3H); ^{13}C-NMR of **119**: δ 72.01 (d), δ 71.82 (s), δ 52.41 (d), δ 42.99 (t), δ 42.52 (t), δ 40.53 (t), δ 34.10 (s), δ 31.09 (t), δ 30.49 (t), δ 22.74 (q), δ 20.10 (t), δ 18.57 (q). Mass spectrum of **119** (m/e): 39 (16), 41 (39), 43 (100), 55 (28), 67 (28), 71 (38), 79 (17), 81 (25), 95 (41), 109 (16), 110 (26), 137 (12), 165 (5), 180 (4), 183 (2), 198 (6).

(1α,4aβ,7α,8aα)-decahydro-1,4a-dimethylnaphthalene-1,7-diol (120). To a stirred solution of 2.39 g of **122** in 100 ml of THF, cooled to -78°C under a nitrogen atmosphere, was added dropwise 22 ml of L-Selectride®. The resulting mixture was stirred for 30 minutes at -78°C after which the temperature was raised to room temperature in 30 minutes. A solution of 80% of EtOH was added followed by an additional 2h of stirring. The resulting solution was cooled to 0°C and 200 ml of 35% of H_2O_2 was added. After 3h, the organic solvents were evaporated and the aqueous phase extracted with 10 x 80 ml of CH_2Cl_2. The

combined organic layers were washed with 3 x 80 ml of water, 100 ml of brine, dried on $MgSO_4$ and evaporated to yield 2.06 g (85%) of **120** as a solid. mp: 117-119°C; ^{1}H-NMR of **120**; δ 4.20 (m, 1H,$W_{1/2}$ = 7.2 Hz); δ 1.92-1.30 (m, 12 H); δ 1.56 (s, 2H); δ 1.21-1.14 (m, 1H); δ 1.07 (s, 3H); δ 0.87 (s, 3H); ^{13}C-NMR of **120**: δ 71.71 (s), δ 66.84 (d), δ 47.33 (d), δ 43.72 (t), δ 41.01 (t), δ 38.42 (t), δ 34.95 (s), δ 28.48 (t), δ 28.21 (t), δ 22.19 (q), δ 20.12 (t), δ 17.59 (q). Mass spectrum of **120** (m/e): 41 (38), 43 (100), 55 (26), 67 (27), 71 (25), 79 (17), 81 (25), 93 (20), 95 (60), 122 (26), 138 (11), 148 (10), 162 (9), 165 (18), 183 (17).

Cryptomeridiol (125). To a solution of 425 mg of **127**[29] in 20 ml of MeOH was added magnesium monoperoxyphthalate (MMPP; 1.2 equivalents) and the mixture was stirred overnight at room temperature. After addition of 10 ml of saturated $Na_2S_2O_3$ and 10 ml of saturated $NaHCO_3$ the mixture was extracted with 6 x 20 ml of CH_2Cl_2, washed with 50 ml of brine, dried on $MgSO_4$ and evaporated to yield a colourless oil. The crude product was purified by column chromatography to give 335 mg (74%) of eudesmol-4,14-epoxide as an oil which solidified upon standing. mp 58-59.5°C (from diisopropyl ether); ^{1}H-NMR: δ 2.65 (dd, 1H, J = 4.7 Hz, 1.8 Hz); δ 2.46 (d, 1H, J = 4.7 Hz); δ 1.98 (s, 1H); δ 1.65-1.13 (m, 14H); δ 1.09 (s, 3H); δ 1.08 (s, 3H); δ 0.78 (s, 3H); ^{13}C-NMR: δ 72.55 (s), δ 59.38 (s), δ 50.88 (t), δ 48.95 (d), δ 47.23 (d), δ 41.47 (t), δ 40.99 (t), δ 35.76 (s), δ 35.50 (t), δ 27.39 (q), δ 26.90 (q), δ 22.33 (t), δ 21.04 (t), δ 20.56 (t), δ 16.99 (q); Mass spectrum (m/e): 59 (100), 79 (32), 81 (27), 91 (30), 93 (31), 147 (34), 149 (37), 165 (26), 205 (15), 220 (6), 238 (0.1). To a stirred suspension of 120 mg of $LiAlH_4$ in 10 ml of dry THF, cooled to -10°C, was added dropwise a solution of 300 mg of β-eudesmol-4,14-epoxide in dry THF. The mixture was stirred overnight at room temperature, cooled to 0°C and the excess $LiAlH_4$ was destroyed with Glauber's salt. After stirring for 30 minutes at room temperature, $MgSO_4$ was added and the solids were filtered off after an additional 15 minutes of stirring. Evaporation of the solvent yielded 292 mg (90%) of **125**. mp 135.5-137°C (from diisopropylether), $[a]_D$: -24.8° ($CHCl_3$; c 0.83); ^{1}H-NMR of **125**: δ 1.92 (ddd, 1H, J = 12.7 Hz, 5.5, Hz, 2.5 Hz); δ 1.79 (ddt, 1H, J = 12.5 Hz, 3.2 Hz, 1.6 Hz); δ 1.20 (s, 6H); δ 1.11 (d, 3H, J = 0.7 Hz); δ 0.86 (s, 3H); ^{13}C-NMR and MS data of **125** correspond with those reported in the literature[27,30,37].

Acid induced cyclisation of 39. Typical reaction: A 10 µl solution of 0.1 M of **39** in EtOH was added to a stirred solution of 390 µl acetone and 600 µl of 1M H_2SO_4, stirred overnight at room temperature, extracted with 1 ml of water / EtOAc (1:1) and analysed by GC. The resulting solution contained cryptomeridiol (**125**, 31%), α-eudesmol (**126**, 14%), β-eudesmol (**127**, 11%), γ-eudesmol (**128**, 42%) and probably *epi*-cryptomeridiol (**129**, 4%). Mass spectrum of **129** (m/e): 41 (44), 43 (100), 59 (92), 67 (27), 71 (31), 81 (37), 108 (33), 109 (37), 123 (31), 149 (92), 164 (40), 189 (10), 207 (8), 225 (1).

Oxymercuration-deoxymercuration of 39. To a stirred solution of 19 mg of **39** in 1 ml of THF was added dropwise a suspension of 30 mg of $Hg(OAc)_2$ in 1 ml of water and 1 ml of THF. After stirring for 5 minutes at room temperature, 1 ml of 3 M NaOH was added immediately followed by a solution of 10 mg of $NaBH_4$ in 1 ml of 3M NaOH. Mercury settled and the mixture was extracted with 4 x 10 ml of EtOAc. The organic layers were washed with 20 ml of saturated NH_4Cl and 20 ml of brine, dried on Na_2SO_4 and

evaporated to give 26 mg of a colourless oil which contained cryptomeridiol (**125**, 60%), α-eudesmol (**126**, 2%), β-eudesmol (**127**, 9%), γ-eudesmol (**128**, 4%) and two unidentified compounds (7% of a compound with $M^+ = 220$ and 6% of a compound with $M^+ = 204$).

3.6. References and notes

1. Wijnberg J.B.P.A., Vader J. and de Groot Ae., *J. Org. Chem.*, **48**, 4380 (1983).
2. Wijnberg J.B.P.A., Jongedijk G. and de Groot Ae., *J. Org. Chem.*, **50**, 2650 (1985).
3. Wieland P. and Mischer K., *Helv. Chim. Acta*, **33**, 2215 (1950).
4. Grieco P.A., Oguri T., Gilman S. and DeTitta G.T., *J. Am. Chem. Soc.*, **100**, 1616 (1978).
5. Minnaard A.J., Wijnberg J.B.P.A. and de Groot Ae., *Tetrahedron*, **50**, 4755 (1994).
6. Piet D.P., Franssen M.C.R. and de Groot Ae., *Tetrahedron*, **50**, 3169 (1994).
7. Piet D.P., Minnaard A.J. van der Heijden K.A., Franssen M.C.R., Wijnberg J.B.P.A. and de Groot Ae., *Tetrahedron*, **51**, 243 (1995).
8. Tietze L.F. and Utecht J., *Synthesis*, 957 (1993).
9. Marshall J.A., Seitz D.E., Snyder W.R. and Goldberg B., *Synth. Commun.*, **4**, 79 (1974).
10. Chowdhury, P.K., Sharma R.P. and Barua J.N., *Tetrahedron Lett.*, **24**, 3383 (1983).
11. Bentley T.W. and Llenwellyn G., *Prog. Phys. Org. Chem.*, **17**, 121 (1990).
12. Kim M., Kawada K. and Watt D.S., *Synth. Commun.*, **19**, 2017 (1989).
13. Los M. and Mighell A.D., *Tetrahedron*, **21**, 2297 (1965).
14. Simmons H.E. and Smith R.D., *J. Am. Chem. Soc.*, **81**, 4256 (1959).
15. Southwell, I.A., *Phytochemistry*, **9**, 2243 (1970).
16. Brown H.C. and Zweifel G., *J. Am. Chem. Soc.*, **82**, 4708 (1960).
17. Brown H.C. and Keblys K.A., *J. Am. Chem. Soc.*, **86**, 1795 (1964).
18. Hydroboration of **127** gave an initial vigorous reaction indicating the formation of mono- and possible di-alkoxyboranes[19].
19. Brown H.C. and Unni M.K., *J. Am. Chem. Soc.*, **90**, 2902 (1968).
20. Grob C.A. and Schiess P.W., *Angew. Chem.*, **6**, 1 (1967).
21. Grob C.A. *Angew. Chem., Int. Ed. Engl.*, **8**, 543 (1969).
22. Sabol J.S., *Studies on the applicability of boronate fragmentation to germacranolide systems*, PhD.-thesis, Northwestern University (1981).
23. Wijnberg J.B.P.A., Vader J. and de Groot Ae., *J. Org. Chem.*, **48**, 4380 (1983).
24. Kesselmans R.P.W., Wijnberg J.B.P.A. and de Groot Ae., *J. Org. Chem.*, **56**, 7232 (1991).

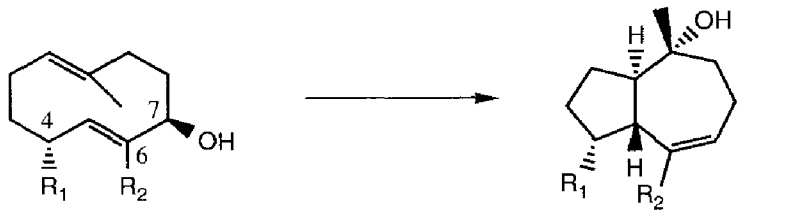
25. The heat of formation energy values represent the heat involved on formation of the compound from its constituent elements under standard conditions. All calculations were performed using MOPAC 6.0 with the PM 3 parameter set (Molecular Simulations Inc.) running on a Silicon Graphics 4D25 computer; Steward J.J.P., *J. Comp. Chem.*, **10**, 209 (1989).
26. Minnaard A.J., Wijnberg J.B.P.A. and de Groot Ae., *Tetrahedron*, **50**, 4755 (1994).
27. Sumimoto M., Ito H., Hirai H. and Wada K., *Chem. Ind.*, 780 (1963).
28. Abdel-Moneim F.B., Ahmed Z.F., Fayez M.B.E. and Ghaleb H., *Planta Med.*, **13**, 209 (1969).
29. Isolated from the natural oil of *Amyris balsamifera*.
30. Evans F.E., Miller D.W., Cairns T., Baddeley G.V. and Wenkert. E., *Phytochemistry*, **21**, 937 (1982).
31. Lambert J.B. and Putz G.J., *J. Am. Chem. Soc.*, **95**, 6313 (1973).
32. Renold R. and Ohloff G., *Helv. Chim. Acta*, **62**, 985 (1979)
33. Hendrickson, J.B., *Phytochemistry*, **7**, 82 (1959).
34. Parker W. and Roberts J.S., *Quart. Rev.*, **21**, 331 (1967).
35. Sutherland J.K., *Tetrahedron*, **30**, 1651 (1974).
36. Quaternary carbons could not be assigned because the compound was not entirely pure.
37. Irwin, M.A.and Geissman, T.A., *Phytochemistry*, **12**, 849 (1973).

4. Biotransformation of allylic (E,E)-cyclodeca-1,6-dienols

4.1. Introduction

In chapter 3 it has become clear that the biotransformation of (E,E)-cyclodeca-1,5-dienes, initiated by *C. intybus*, is induced by an enzymatic protonation of the C_1-C_{10} double bond followed by transannular cyclisation and stereoselective incorporation of a water molecule. In this chapter[1], the attention is focused on the biotransformation of (E,E)-cyclodeca-1,6-dienes in which one of the double bonds is flanked by an alcohol group. The group of Marshall[2-4] has demonstrated that solvolysis of the *p*-nitro-benzoate derivative of the allylic alcohol in the (E,E)-cyclodeca-1,6-diene **131** resulted in a stereoselective cyclisation to give hydroazulene **134** possessing a trans-fused framework (Scheme 4.1.).

Scheme 4.1.



131: R_1 = -H; R_2 = -H
132: R_1 = -H; R_2 = -CH_3
133: R_1 = -CH_3; R_2 = -H

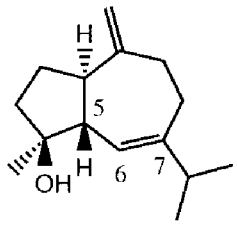
134: R_1 = -H; R_2 = -H

To further investigate the action of cyclising enzymes in *C. intybus*, (E,E)-cyclodeca-1,6-dienols **131-133** were synthesised and their behaviour towards a chicory root homogenate was studied. If the initial cyclisation starts with protonation of the 1,10-double bond (like in **39**, **100** and **101**, see chapter 3), trans-fused decalins are to be expected. If cyclisation is initiated by the protonation of the allylic alcohol group, an internal nucleophilic reaction, leading to trans-fused hydroazulenes like **134**, is likely to be the preferred pathway. Analysis of the reaction products of **131-133** will give more information concerning the role of methyl groups at C_4 and C_6 on the conformation and the cyclisation pattern of these (E,E)-cyclodeca-1,6-dienols.

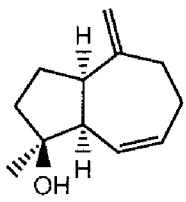
Hydroazulenes possessing a C_6-C_7 double bond like **134** resemble natural products like alismol (**43**)[5]. The ring fusion of **43** was established as being trans, as discussed in chapter 2. Recently, the structure of a trinor-guaiane similar to alismol, was published[6]. The stereochemistry of this compound, named dictamnol (**140**), was

assigned as depicted in figure 4.1.; i.e. possessing a cis-fused guaiane framework. Since **140** was already synthesised at our laboratory and major discrepancies were found between our NMR spectral data of **140** and those reported in the literature, natural dictamnol was isolated and its stereochemistry was reinvestigated. In section 4.3., a revised structure for natural dictamnol is proposed[7].

Figure 4.1.



43

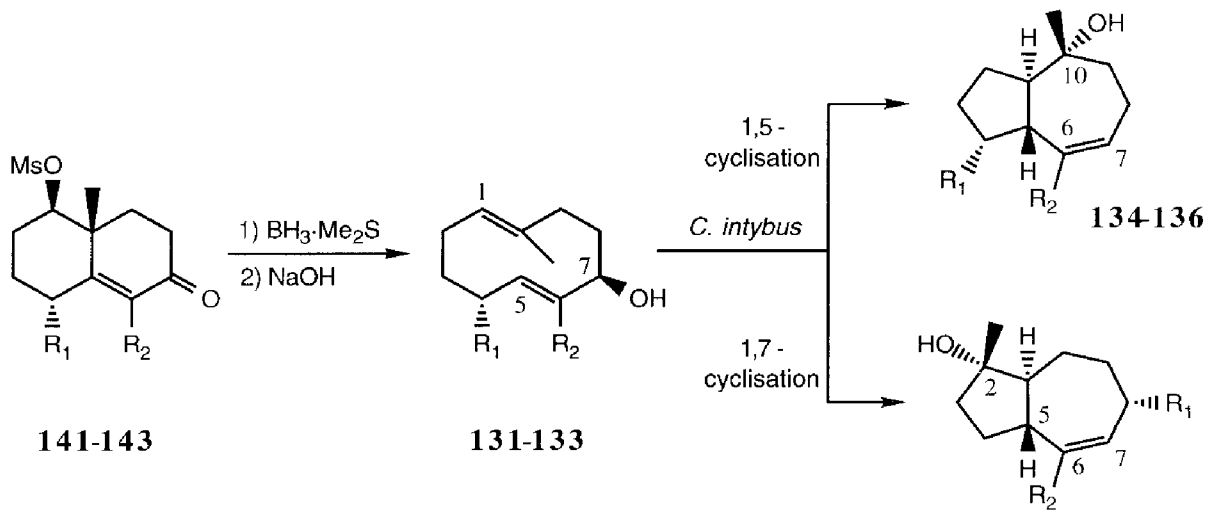


140

4.2. Synthesis and biotransformation of (E,E)-cyclodeca-1,6-dienols 131-133

The substrates for the biotransformation studies were prepared from the readily available mesylates **141-143** obtained from their Wieland-Miescher ketone derivatives[8-10].

Scheme 4.2.



141-143

131-133

134-136

137-139

131, **141**: R_1 = -H; R_2 = -H
132, **142**: R_1 = -H; R_2 = -CH_3
133, **143**: R_1 = -CH_3; R_2 = -H

134, **137**: R_1 = -H; R_2 = -H
135, **138**: R_1 = -H; R_2 = -CH_3
136, **139**: R_1 = -CH_3; R_2 = -H

Table 4.1.

Precursor	Product ratio 1,5- vs 1,7-products	
131	**134 : 137**	4 : 1
132	**135 : 138**	8 : 1
133	**136 : 139**	3 : 1

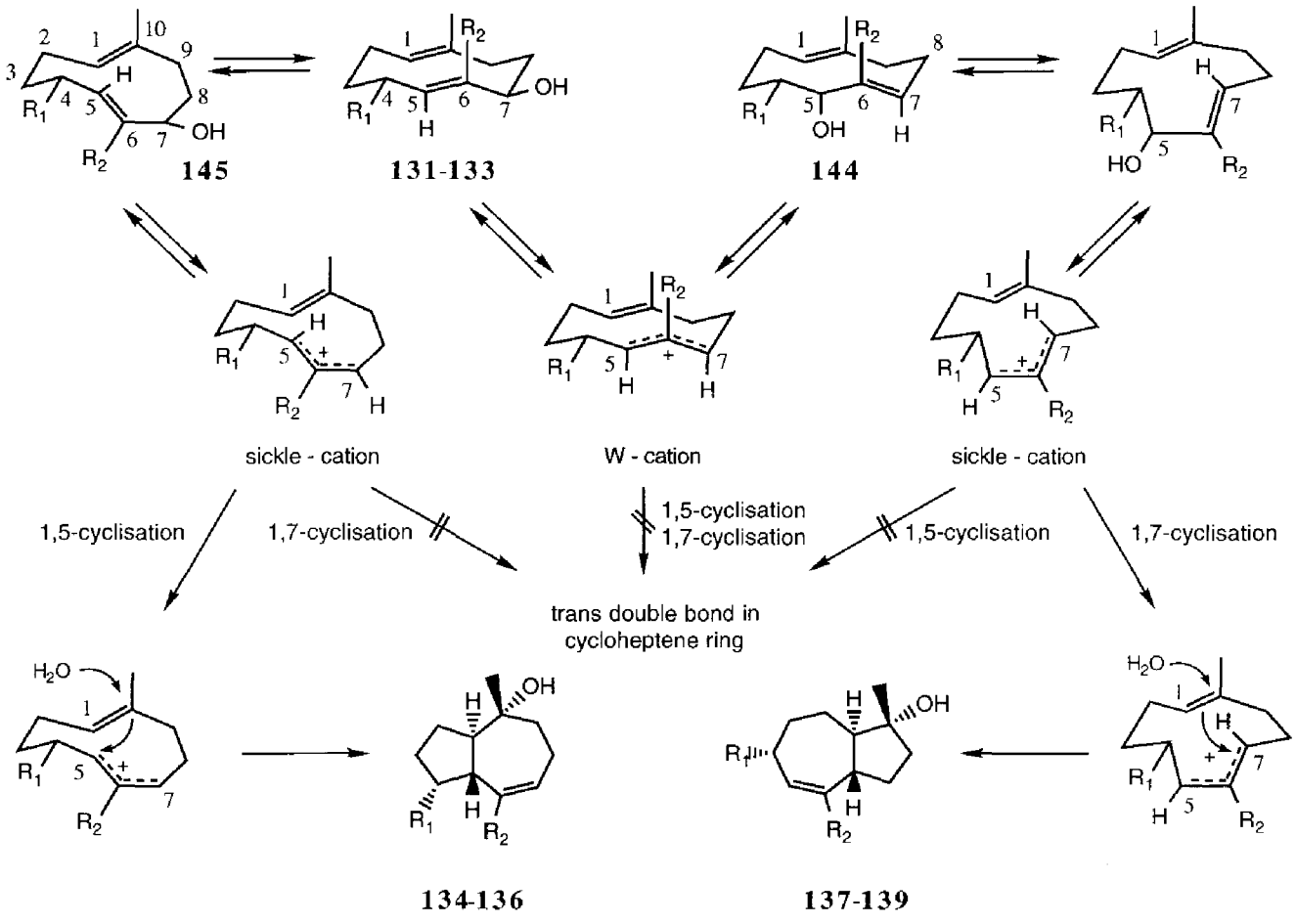
Hydroboration followed by base-induced fragmentation gave the (E,E)-cyclodeca-1,6-dienols **131-133** in excellent yields. Cyclisation of **131-133** was mediated by an enzymatic activation of the allylic alcohol followed by transannular cyclisation to give mixtures of 1,5- and 1,7-cyclisation products (**134-136** and **137-139**, respectively), as outlined in scheme 4.2. and table 4.1. The control reactions of **131-133** showed no conversion after a 10 days incubation period. The formation of the 1,7-cyclisation products was predicted in the literature but such hydroazulenes were never actually found in the solvolysis of the *p*-nitrobenzoate derivatives of **131** and **133**[2-4].

A rationalisation of the product formation in presented in scheme 4.3. Ionisation of the allylic alcohol would lead to the formation of an allylic cation. This cation is either sickle- or W-shaped, since the 10-membered ring system is flexible and can occur in multiple conformations through rotations around the C_1-C_2, C_9-C_{10} and the C_4-C_5, C_6-C_7 bond. Both the 1,5- and the 1,7-cyclisation pathway through the W-cation would give a highly strained trans double bond in the cycloheptene ring. 1,7-Cyclisation through the sickle-cation would also lead to a highly strained trans double bond whereas 1,5-cyclisation would give an energetically more favourable cis double bond in the cycloheptene ring.

The formation of the 1,7-cyclisation products can only be achieved via an enzyme mediated allylic isomerisation reaction of an (E,E)-cyclodeca-1,6-dienol into an allylic (E,E)-cyclodeca-1,5-dienol (**144**), as outlined in scheme 4.3. Rotation around the C_5-C_6, C_7-C_8 bond, followed by ionisation of the intermediate allylic (E,E)-cyclodeca-1,5-dienol (**144**) generates a sickle-cation wich would lead, after a 1,7-cyclisation, to the energetically more favourable cis cycloheptene ring.

The discrepancy between these findings and those reported in the literature[2-4] may lie in the different reaction conditions. Solvolysis of the *p*-nitrobenzoate ester of **131** and **133** in a refluxing dioxane-water-$NaHCO_3$ mixture[2-4] will result in a concerted cyclisation in which the C_1-C_{10} double bond is actively involved the cyclisation process. The developing positive charge at C_{10} is neutralised either by regioselective incorporation of a water molecule leading to **134-136** or deprotonated products[11] as reported by Marshall *et al.*[4].

Scheme 4.3.



In the biotransformation reaction of **131-133**, initiated by *C. intybus*, the first step will be dissociation of the protonated allylic leaving group into an intimate ion pair[12]. In the Johnson model for oxidosqualene cyclising enzymes[13], cyclisation is initiated by proton donation from a specific amino acid residue which acts as a general acid catalyst. The ring fusion and the stereochemistry of the product(s) are directed by electron-rich, aromatic amino acid residues (e.g. tryptophan or tyrosine) which stabilise carbon centers that become cationic in transition states and/or high energy intermediates. This directing effect is known as 'the aromatic hypothesis'[14]. If this model is extrapolated from squalene cyclase to the germacrane cyclase from chicory roots, the π-systems of an aromatic side chain residue could interact with the positive charge of the allylic cation. The prolonged lifespan of the allylic cation makes an *in situ* formation of an allylic (E,E)-cyclodeca-1,5-dienol (**144**, Scheme 4.3.) possible. This allylic (E,E)-cyclodeca-1,5-dienol is, after a rotation around the C_5-C_6, C_7-C_8 bond, the precursor of the 1,7-cyclisation products (**137-139**) which are found in all three biotransformation reactions. The somewhat higher yield of the 1,5-cyclisation product obtained from **132** is probably caused by steric factors. Due to steric hindrance between

the methyl groups at C_6 and C_{10}, rotamer **145** is preferred with respect to **132**. This increased population of the sickle-cation leads to an increase of 1,5-cyclisation products at the expense of the 1,7-cyclisation products. The introduction of a methyl group at C_4 seems to have little influence on the product ratio.

The stereochemistry of the 1,5-cyclisation product **134** was established through an independent synthesis of its dihydro derivative[3]. Additional NOE-difference experiments on **134** confirmed these findings. A detailed NMR study of the 1,7-cyclisation product **137** was undertaken in order to establish the stereochemistry of this product. Since the 1,7-cyclisation products **138** and **139** are expected to be formed through the same cyclisation pathway, their stereochemistry must be identical to that of **137**. It is known from NMR studies on alismol (**43**) and structurally related compounds that the coupling constant, or J-value, between H_6 and the bridgehead proton H_5 is relatively small: 2-3 Hz[15-17]. In a cis-fused hydroazulene ring system like that of cis-dictamnol (**140**, see section 4.3.), $J_{6,5}$ was found to be 5.7 Hz[7]. This observation makes the coupling constant between the bridgehead proton and the olefinic proton adjacent to the bridgehead proton a useful tool in establishing the ring fusion in these hydroazulene ring systems. $J_{6,5}$ in **137** was found to be 2.7 Hz implying a trans fusion of the hydroazulene ring. Additional NOE-difference experiments on H_5 (δ 2.86) showed a small effect on the methyl group at C_2 and no effect on H_1 thus ascertaining the trans fusion of the ring and establishing the anti orientation between the methyl group at C_2 and the bridgehead proton, H_1.

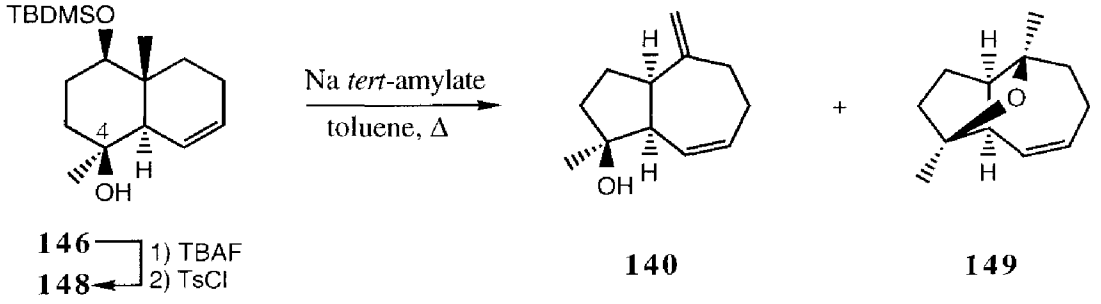
4.3. Total synthesis of 5-*epi*-dictamnol; structure revision of natural dictamnol

The position of the C_6-C_7 double bond in the guaiane framework is characteristic for natural compounds like alismol (**43**)[5]. Recently, the isolation of a trinor-guaiane sesquiterpene from the roots of *Dictamnus dasycarpus* Turcz. has been reported with a framework identical to **43**[6]. Based on information obtained by spectroscopic techniques, it has been proposed that this natural compound, named dictamnol, possesses a cis-fused hydroazulene framework with a double bond at C_6-C_7 (identical to the 1,5-cyclisation products of **131-133**), an exocyclic methylene unit at C_{10} and a tertiary hydroxyl group at C_4 as depicted in **140** (figure 4.1.). Since **140** was already synthesised in our laboratory and major discrepancies were found upon comparison of the NMR spectral data of **140** to that of natural dictamnol, a detailed reinvestigation of the stereochemistry of natural dictamnol was undertaken.

From previous work on the total synthesis of sesquiterpenes[18,19] performed at our laboratory, it is known that stereochemically rigid trans perhydronaphthalene-1,4-diol monosulfonate esters rearrange smoothly upon treatment with sodium *tert*-amylate in refluxing apolar solvents to give cis-fused perhydroazulenes with an exocyclic methylene unit at C_{10}. This base-induced and -directed rearrangement reaction was utilised in the total synthesis of trinor-guaiane **140**.

The starting material for the synthesis of **140** was the readily available monoprotected olefinic 1,4-diol **146**[19]. Desilylation of the TBDMS ether using 40% of aqueous hydrogen fluoride followed by tosylation of the secondary alcohol (**147**) gave the tosylate **148** in 67% yield. A short treatment of **148** (15 minutes) with 5 equivalents of sodium *tert*-amylate in refluxing toluene gave, after chromatographic separation, 60% of **140** together with 21% of the cyclic ether **149**, as outlined in scheme 4.4.

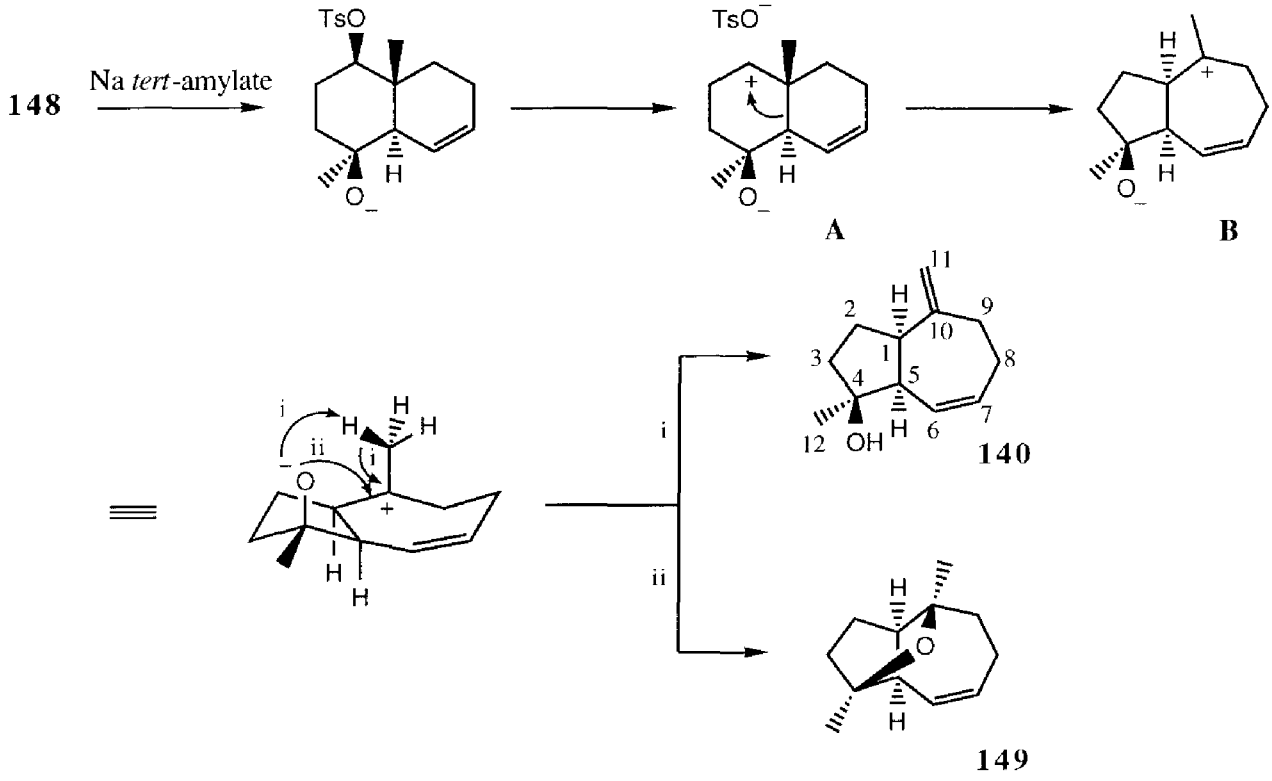
Scheme 4.4.



The formation of **140** and **149** proceeds via internal heterolysis of the tosylate ester bond, intramolecularly induced by the deprotonated hydroxyl group[18-22]. The resulting dipolar intermediate **A** rapidly rearranges to the thermodynamically more stable cis-fused tertairy carbocation **B**, as outlined in scheme 4.5. In **B**, the original angular methyl group and the alkoxide substituent are close together thus explaining the formation of **140** by selective deprotonation (route i) and of **149** by direct trapping of the positive charge by the alkoxide group (route ii).

The cis fusion of **140** and its stereochemistry around C_4 were ascertained by NOE difference experiments[23]. Irradiation of **140** at H_1 (δ 3.05) resulted in a clear NOE with H_5 (δ 2.66). Irradiation of the methyl group at C_4 (Me_{12}) gave a clear NOE with H_5, indicating that these protons are located at the same side of the molecule. In addition, the formation of the cyclic ether **149** next to **140**, also indicates a cis-fused hydroazulene framework.

Scheme 4.5.



As the NMR spectral data of **140** were clearly different from those of a pure sample of natural dictamnol isolated from the roots of *D. dasycarpus* (Table 4.2.), it was assumed that the natural compound has a different stereochemistry as proposed in the literature[6].

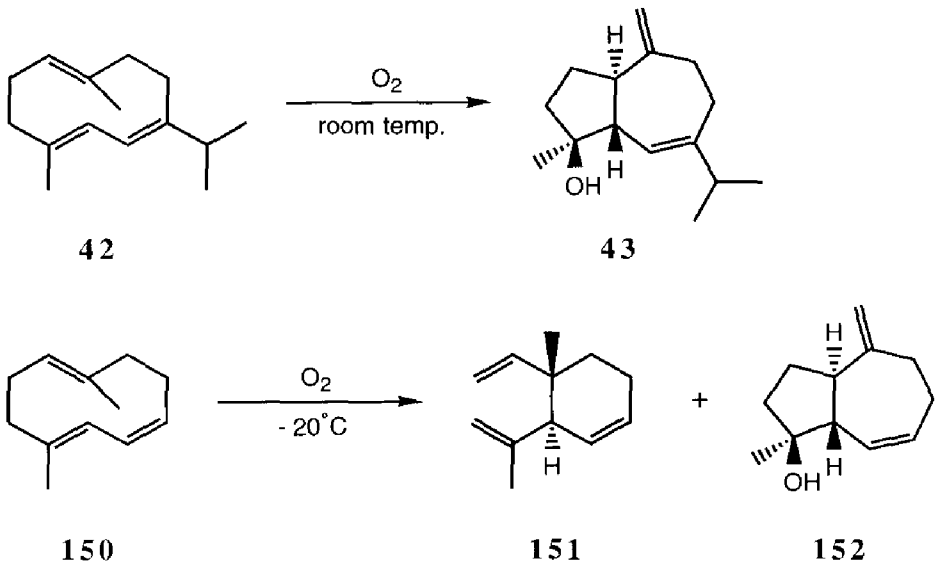
NOE difference measurements on natural dictamnol revealed the presence of a trans-fused ring hydroazulene framework. By irradiation of the methyl group Me_{12} (δ 1.25), no NOE with H_5 was observed, while a NOE was present between this methyl group and H_1. It was therefore clear that H_1 and Me_{12} are situated at one side of the molecule and H_5 on the opposite side. Detailed analysis of the ^{1}H-NMR spectral data for **140** and natural dictamnol also revealed different ring junctions in both compounds. As outlined in table 4.2., the coupling constant between H_6 and H_5 ($J_{6,5}$) amounts to 2.4 Hz for natural dictamnol and 5.7 Hz for **140**. The $J_{6,5}$-values for alismol (**43**) and structural related trans-fused guaianes range from 2.5-3.1 Hz[16,24-26]. These values are similar to the $J_{6,5}$-value found for natural dictamnol. From this similarity it is therefore concluded that these compounds all possess a common structural characteristic, *i.e.*, a trans-fused hydroazulene framework.

Table 4.2.

	Natural Dictamnol			**140**		
Atom	δ_C	δ_H	J (Hz)	δ_C	δ_H	J (Hz)
1	47.46	2.46 (m)		48.99	3.05 (ddd)	10.6, 7.7, 7.7
2	25.71	1.92 (m)		29.27	1.98 (m)	
		1.80 (m)			1.79 (m)	
3	40.50	1.73-1.78		40.33	1.90 (ddd)	12.6, 7.4, 2.1
					1.65 (ddd)	12.6, 11.0, 8.0
4	80.78			80.44		
5	55.86	2.38 (ddq)	11.8, 2.4, 2.4	53.21	2.66 (ddq)	10.6, 5.7, 1.7
6	130.16	5.80 (d)	11.2, 2.4, 1.2	125.68	5.55 (ddt)	12.0, 5.7, 1.5
7	132.06	5.89 (dddd)	11.2, 5.3, 5.3, 2.4	132.32	5.83 (dddd)	12.0, 5.2, 5.2, 1.7
8	29.17	2.11 (m)		30.38	2.29-2.25	
		2.31 (m)				
9	37.17	2.22 (dd)	13.6, 8.9	32.68	2.51 (m)	
		2.58 (t)	13.6		2.22 (m)	
10	153.80			152.56		
11	107.57	4.84 (s)		109.99	4.79 (s)	
		4.76(s)			4.73 (s)	
12	24.56	1.25 (s)		27.04	1.27 (s)	

Additional support for the trans-fused framework of natural dictamnol was obtained by comparison of the chemical behaviour of germacrene C (**42**)[27] and pregeijerene (**150**)[28]. The latter compound is the major constituent of the essential oil of the leaves of *Amyris diatrypa* Sprengel[29]. It is known from the literature that germacrene C (**42**) cyclises into alismol (**43**) under the influence of air at room temperature[15]. A similar cyclisation was detected for pregeijerene (**150**). After being in contact with air for one week at -20°C, a sample of **150**[30] was completely converted into a mixture of two major and several minor compounds According to GC-MS analysis, the main products of the mixture were geijerene (**151**, 45%) and compound **152** (20%) which exhibits exactly the same retention time and mass spectrum as natural dictamnol, isolated from *D. dasycarpus*.

Scheme 4.6.

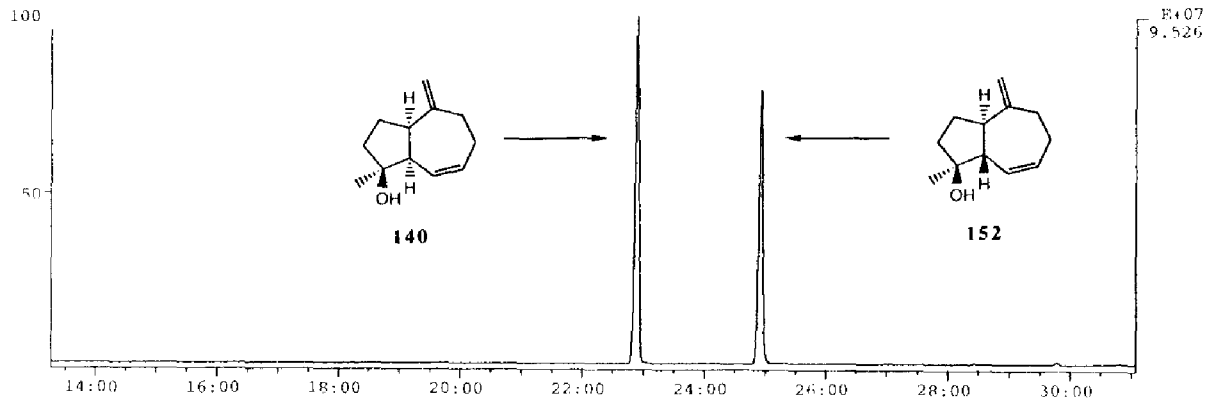


42 **43**

150 **151** **152**

A Cope rearrangement of **150** explains the formation of **151**, whereas air-oxidation must be responsible for the formation of **152**. Because, as mentioned earlier, air-oxidation of **42** exclusively gives the trans-fused guaiane **43**, it is to be expected that air-oxidation of the structurally related **150** will also result in a trans-fused hydroazulene framework as illustrated in structure **152**. It is therefore most likely that natural dictamnol possesses the stereochemistry as postulated in formula **152** (Scheme 4.6.)[31]. To further exclude the possibility that **140** and **152** are identical, a mixture of the two products was co-injected on GC. As can be seen from figure 4.3., **140** and **152** are clearly two different compounds.

Figure 4.3.



Based on the results described above, it is concluded that dictamnol (**152**), isolated from *D. dasycarpus*, possesses a trans-fused hydroazulene framework as shown in scheme 4.6. and not the cis-fused structure **140**, as depicted in figure 4.1. In addition, it seems likely that pregeijerene (**150**) is the natural precursor of dictamnol (**152**), although **150** could not be detected in *D. dasycarpus*[32].

4.4. Experimental

Melting points are uncorrected. Chemical shifts are reported relative to TMS (δ 0.00) at 200 and 400 MHz for ^{1}H-NMR and at 25.3 and 50.6 MHz for ^{13}C-NMR. All spectra were recorded in $CDCl_3$ unless noted otherwise. Mass spectral data were obtained with a Finnigan EI-MAT95 spectrometer and a Hewlett Packard 5890 GC-MS equipped with a Hewlett Packard 5970 series mass selective detector using a capillary DB-5 and DB-17 columns (30 m x 0.25 mm, d_f 0.25 μm) and helium as the carrier gas. Preparative GC was performed on a Varian 3700 using a carbowax HP-column (10% on chromosorb 100-120; 2 m x 0.63 cm) equiped with TSD (150 mA) and hydrogen as the carrier gas. Elemental analyses were determined on a Carlo Erba elemental analyzer 1106. Column chromatography was performed on Merck silicagel 60 and deactivated Al_2O_3 (grade III) using petroleum ether (bp. 40-60, PE) - EtOAc as the solvent system. All solvents were distilled before use. The (E,E)-cyclodeca-1,6-dienols **131** and **133** were prepared according to literature prescriptions[2,4]. From 1 kg of commercially available *D. dasycarpus* Turcz. was isolated 96 mg of dictamnol (**152**) and 775 mg of fraxinellone (**153**)[33] as described[6].

A suspension of fresh chicory root (20% w/v) was produced by mortaring the root in a solution of 0.25 M sucrose, 3 mM Tris·HCl, 10 mM $MgCl_2$ and 0.2 % (w/v) Bovine Serum Albumin (BSA). The pH of this sucrose / Tris / $MgCl_2$/ BSA-solution (STMB) was set at 6.5 using 2-morpholino-ethanesulfonic acid (MES). The stability of **131**-**133** towards the buffer and an inactivated chicory root sample (obtained by boiling the root suspension for 30 min) was investigated as a control to test the possibility of non-enzymatic reactions. Neither showed any reaction. Incubations were performed in sealed 4 ml vials at room temperature in a KS 500 shaker at 260 rpm containing 200 μl root suspension, 790 μl STMB-solution and 10 μl 0.1 M substrate in EtOH. The incubation medium was extracted with 0.5 ml EtOAc and its contents were analysed by GC-MS.

(E,E)-8-methylcyclodeca-2,7-dien-1-ol (131). ^{1}H-NMR of **131**: δ 5.47 (ddd, 1H, H_5, J = 15.5 Hz, 10.7 Hz, 3.1 Hz); δ 5.19 (ddd, 1H, H_6, J = 15.5 Hz, 9.2 Hz, 1.6 Hz); δ 4.88 (ddd, 1H, H_1, J = 11.2 Hz, 1.3 Hz, 1.3 Hz); δ 4.06 (ddd, 1H, H_7, J = 10.7 Hz, 9.1 Hz, 4.1 Hz); δ 2.35-1.93 (m, 6H); δ 1.70-1.40 (m, 5H); δ 1.50 (d, 3H, J = 1.3 Hz). ^{13}C-NMR of **131**: δ 137.21 (d), δ 130.82 (d), δ 130.70 (d), δ 130.15 (s), δ 75.72 (d), δ 37.44 (t), δ 33.43 (t), δ 29.18 (t), δ 29.00 (t), δ 27.33 (t), δ 16.69 (q). Mass spectrum of **131** (m/e): [M^+] 166 (16), 148 (18), 133 (19), 124 (39),

109 (31), 107 (37), 105 (29), 97 (26), 95 (30), 93 (38), 91 (53), 83 (88), 81 (100), 80 (31), 79 (71), 77 (29), 67 (77), 55 (62), 53 (41), 43 (55), 41 (99), 39 (70).

(E,E)-4,8-dimethylcyclodeca-2,7-dien-1-ol (133). ^{1}H-NMR of **133** (C_6D_6): δ 5.19 (dd, 1H, H_6, J = 15.4 Hz, 8.7 Hz); d 5.01 (dd, 1H, H_5, J = 15.4 Hz, 9.2 Hz); δ 4.87 (broad d, 1H, H_1, J = 9.5 Hz), δ 3.98 (ddd, 1H, H_7, J = 10.6 Hz, 8.8 Hz, 4.1 Hz); δ 2.25-1.90 (m, 4H); δ 1.80-1.35 (m, 3H); δ 1.42 (s, 3H, C_2-Me); δ 1.20-0.95 (m, 2H); δ 1.28 (s, 1H, -OH); δ 0.92 (d, 3H, C_4-Me, J = 6.6 Hz). ^{13}C-NMR of **133** ($CDCl_3$): δ 142.49 (d), δ 130.40 (s), δ 130.33 (d), δ 129.42 (d), δ 75.75 (d), δ 39.51 (d), δ 37.39 (t), δ 36.37 (t), δ 29.87 (t), δ 28.17 (t), δ 22.18 (q), δ 16.68 (q). Mass spectrum of **133** (m/e): [M^+] 180 (4), 162 (3), 147 (5), 138 (9), 123 (8), 121 (10), 95 (30), 93 (21), 91 (21), 83 (50), 81 (61), 79 (34), 67 (55), 55 (57), 53 (34), 43 (45), 41 (100), 39 (66).

(E,E)-2,8-dimethylcyclodeca-2,7-dien-1-ol (132). To a solution of 3.85 g of mesylate **141**[10] in 150 ml of dry ether under a nitrogen atmosphere, cooled to 0°C, was added dropwise 25 ml of 2M BH_3·Me_2S complex in THF. The reaction was stirred overnight at room temperature after which 10 ml of water was carefully added followed by 50 ml of 4N NaOH. The resulting mixture was stirred for 1h, the organic phase was separated, the aqueous phase was extracted with 2 x 30 ml of ether, the combined organic phases were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 2.60 g of a yellow oil. GC analysis showed the presence of **132** and the tricyclic compound **154**[34] in a 7:3 ratio, respectively. Purification of **132** from the reaction mixture was performed by $AgNO_3$-extraction as described[35] to yield 880 mg of **132** and 7 mg of **154**. m.p. of **132**: 59.5-61°C. ^{1}H-NMR of **132**: δ 5.25 (dd, 1H, H_5, J = 10.9 Hz, 1.6 Hz); δ 4.83 (broad d, 1H, H_1, J = 10.7 Hz); δ 4.22 (dd, 1H, H_7, J = 11.0 Hz, 3.4 Hz); δ 2.30-2.05 (m, 6H); δ 1.80-1.45 (m, 5H); δ 1.57 (t, 3H, J = 1.4 Hz); δ 1.55 (t, 3H, J = 1.4 Hz). ^{13}C-NMR of **132**: δ 134.78 (s), δ 133.78 (d), δ 131.31 (d), δ 129.72 (s), δ 80.40 (d), δ 38.32 (t), δ 28.99 (t), δ 28.27 (t), δ 28.16 (t), δ 25.81 (t), δ 15.60 (q), δ 10.74 (q). Mass spectrum of **132** (m/e): [M^+] 180 (9), 162 (23), 147 (29), 133 (28), 123 (19), 119 (20), 105 (35), 97 (100), 95 (40), 93 (45), 91 (34), 81 (37), 79 (46), 77 (24), 67 (66), 55 (59), 53 (40), 43 (64), 41 (99), 39 (59).

Acid induced cyclisation of 131-133. Typical reaction: To a solution of 75 mg of **131** in 10 ml of a 1:1 acetone/water mixture was added 5 drops of concentrated H_2SO_4. The reaction mixture was stirred overnight at room temperature and extracted with 2 x 10 ml of EtOAc. The combined organic layers were washed with 10 ml of saturated $NaHCO_3$ and 10 ml of brine, dried over $MgSO_4$, evaporated and subjected to column chromatography (PE/EtOAc 4:1) to give 15 mg of **134** and 3 mg of **137**. In a similar way, 50 mg of **135** and 10 mg of **138** was obtained from 110 mg of **132** and 7 mg of **136** from **133**. The small amount of **139** formed in the acid induced cyclisation could not be separated from **136** due to identical r_f-values. ^{1}H-NMR of **134** (C_6D_6): δ 5.69 (m, 2H, H_6, H_7); δ 2.35-1.40 (m, 13H); δ 1.05 (s, 3H, C_{10}-Me). ^{13}C-NMR of **134** (C_6D_6): δ 136.04 (d), δ 129 94 (d), δ 74.40 (s), δ 54.73 (d), δ 42.82 (t), δ 41.09 (d), δ 34.24 (t), δ 27.18 (t), δ 24.06, (t), δ 23.76 (t), δ 21.72 (q). Mass spectrum of **134** (m/e): [M^+] 166 (0.3), 148 (26), 133 (26), 119 (17), 108 (31), 93 (46), 91 (33), 81 (26), 80 (18), 79 (46), 77 (18), 67 (46), 43 (100), 41 (41), 39 (31).

^{1}H-NMR of **137**: δ 5.68 (dddd, 1H, H_7, J = 11.6 Hz, 7.4 Hz, 4.3 Hz, 2.9 Hz); δ 5.33 (dt, 1H, H_6, J = 11.6 Hz, 2.7 Hz, 2.7 Hz); δ 2.85 (dddd, 1H, H_5, J = 6.1 Hz, 5.8 Hz, 3.0 Hz, 2.5 Hz); δ 2.41 (m, 1H, H_8); δ 2.08-1.98 (m, 2H); δ 1.85 (ddd, 1H, H_8, J = 14.3 Hz, 11.1 Hz, 3.3 Hz); δ 1.75-1.50 (m, 7H); δ 1.28 (s, 1H, -OH); δ 1.23 (s, 3H, C_2-Me). ^{13}C-NMR of **137**: δ 134.84 (d), δ 130.29 (d), δ 74.53 (s), δ 54.46 (d), δ 39.72 (d), δ 35.59 (t), δ 35.10 (t), δ 31.20 (q), δ 25.80 (t), δ 23.25 (t), δ 22.04 (t). Mass spectrum of **137** (m/e): [M^+] 166 (0.7), 148 (26), 133 (36), 119 (25), 108 (31), 106 (15), 105 (27), 93 (52), 92 (19), 91 (48), 81 (25), 80 (26), 79 (52), 76 (23), 67 (48), 55 (17), 53 (17), 43 (100), 41 (48), 39 (36).

^{1}H-NMR of **135**: δ 5.48 (m, 1H, H_7); δ 2.20 (m, 2H); δ 2.00-1.30 (m, 11H); δ 1.66 (s, 3H, C_6-Me, J = 1.2 Hz); δ 1.16 (s, 3H, C_{10}-Me). ^{13}C-NMR of **135**: δ 141.09 (s), δ 125.30 (d), δ 75.33 (s), δ 52.50 (d), δ 44.79 (d), δ 42.55 (t), δ 31 94 (t), δ 26.35 (t), δ 25.06 (t), δ 22.96 (t), δ 22.57 (q), δ 21.81 (q). Mass spectrum of **135** (m/e): [M^+] 180 (0.3), 162 (30), 147 (51), 133 (60), 120 (19), 119 (22), 107 (27), 106 (18), 105 (38), 95 (22), 93 (31), 81 (22), 79 (45), 77 (23), 67 (36), 55 (24), 53 (19), 43 (100), 41 (47), 39 (30).

^{1}H-NMR of **138**: δ 5.54 (ddd, 1H, H_7, J = 7.5 Hz, 5.8 Hz, 1.6 Hz); δ 2.52 (m, 1H); δ 2.28 (m, 2H); δ 2.05 (m, 2H); δ 1.95-1.40 (m, 8H); δ 1.78 (s, 3H, C_6-Me); δ 1.12 (s, 3H, C_2-Me). ^{13}C-NMR of **138**: δ 143.35 (s), δ 123.03 (d), δ 75.00 (s), δ 51.83 (d), δ 44.72 (d), δ 35.63 (t), δ 33.24 (t), δ 30.31 (q), δ 29.93 (t), δ 26.54 (q), δ 26.05 (t), δ 22.60 (t). Mass spectrum of **138** (m/e): [M^+] 180 (0.2), 162 (31), 147 (42), 133 (44), 122 (28), 120 (17), 119 (30), 107 (38), 105 (51), 94 (50), 93 (38), 91 (38), 79 (52), 76 (26), 67 (38), 55 (28), 43 (100), 41 (53), 39 (33).

^{1}H-NMR of **136**: δ 5.71 (m, 2H), δ 2.35-1.30 (m, 12H), δ 1.20 (s, 3H, C_{10}-Me), δ 0.85 (d, 3H, C_4-Me, J = 6.9 Hz). ^{13}C-NMR of **136**: δ 132.87 (d), δ 130.50 (d), δ 75.25 (s), δ 51.01 (d), δ 44.51 (d), δ 42.63 (t), δ 37.31 (d), δ 32.86 (t), δ 24.14 (t), δ 23.61 (t), δ 21.72 (q), δ 15.45 (q). Mass spectrum of **136** (m/e): [M^+] 180 (0.1), 162 (8), 147 (10), 122 (11), 107 (15), 81 (27), 79 (29), 67 (19), 55 (16), 53 (15), 43 (100), 41 (38), 39 (28). Mass spectrum of **139** (m/e): [M^+] 180 (4), 165 (8), 163 (9), 149 (15), 123 (26), 109 (50), 95 (42), 91 (18), 81 (57), 67 (54), 55 (54), 53 (28), 43 (24), 41 (100), 39 (56).

(1α,4α,4aα,8aβ)-1,2,3,4,4a,5,6,8a-octahydro-1,4a-dimethylnaphthalene-1,4-diol (147). To a solution of 2.00 g of **146**[19] in 50 ml of acetonitrile was added 2 ml of 40% aqueous hydrogen fluoride and the resulting mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into 150 ml of saturated aqueous $NaHCO_3$ and extracted with 100 ml of EtOAc. The organic layer was washed with brine, dried on $MgSO_4$ and evaporated to give 1.28 g of **147**. m.p. of **147**: 122-122.5°C (from PE/EtOAc). ^{1}H-NMR of **147**: δ: 5.77-5.62 (m, 2H, H_6, H_7); δ 3.26 (dd, 1H, H_1, J = 11.4, 4.1 Hz); δ 2.07-1.19 (m, 11H); δ 1.16 (s, 3H, Me_{12}); δ 0.97 (s, 3H, Me_{11}). ^{13}C-NMR of **147**: δ 129.32 (d), δ 123.96 (d), δ 78.20 (d), δ 70.95 (s), δ 49.92 (d), δ 38.79 (t), δ 37.95 (s), δ 34.68 (t), δ 29.32 (q), δ 27.02 (t), δ 22.89 (t), δ 11.68 (q). Mass spectrum of **147** (m/e); [M^+] 196 (0.1), 181 (1), 178 (16), 163 (12), 160 (9), 145 (20), 120 (100), 118 (33), 107 (69), 105 (39). Anal. Calcd. for **147**: $C_{12}H_{20}O_2$: C 73.43, H 10.27. Found: C 72.84, H 10.30.

(1α,4α,4aα,8aβ)-1,2,3,4,4a,5,6,8a-octahydro-1,4a-dimethylnaphthalene-1,4-diol 4-(4'-methylbenzene sulfonate) (**148**) To a solution of 0.90 g of **147** in 50 ml of pyridine was added 1.75 g of TsCl. Upon completion of the reaction, the solvent was evaporated and the residue was taken up in 75 ml of EtOAc. The organic phase was washed with 50 ml of 10% aqueous H_2SO_4, 50 ml of saturated $NaHCO_3$, brine, dried on $MgSO_4$ and evaporated to give 1.70 g of **148**. m.p. of **148**: 120-121°C (from PE/EtOAc). ^{1}H-NMR of **148**: δ 7.76 (d, 2H, J = 8.2 Hz); δ 7.30 (d, 2H, J = 8.2 Hz); δ 5.76-5.60 (m, 2H, H_6, H_7); δ 4.27 (dd, 1H, H_1, J = 11.9, 4.3 Hz); δ 2.41 (s, 3H, TsMe); δ 2.36-1.09 (m, 10H); δ 1.17 (s, 3H, Me_{12}); δ 1.04 (s, 3H, Me_{11}). ^{13}C-NMR of **148**: δ 144.40 (s), δ 134.59 (s), δ 126.66 (3d), δ 127.60 (2d), δ 123.08 (d), δ 89.17 (d), δ 70.33 (s), δ 50.12 (d), δ 38.52 (t), δ 37.57 (s), δ 34.25 (t), δ 29.06 (q), δ 24.48 (t), δ 22.66 (t), δ 21.61 (q), δ 12.35 (q). Anal. Calcd. for **148**: $C_{19}H_{26}O_4S$: C 65.11, H 7.48. Found: C 64.80, H 7.46.

(1α,7α,8β)-8-methyl-2-methylenebicyclo[5.3.0]dec-5-en-8-ol (**140**). A solution of 0.35 g of **148** in 11 ml of degassed dry toluene was refluxed under an argon atmosphere. To the refluxing solution was added 2.5 ml of 2.2 M sodium *tert*-amylate in toluene[36] at once. The reaction mixture was refluxed for an additional 10 minutes, quenched with 15 ml of precooled saturated aqueous NH_4Cl and then quickly cooled to 0°C. The mixture was vigorously stirred for 20 minutes and extracted with 25 ml of PE. The organic phase was washed with brine, dried on $MgSO_4$ and evaporated. The remaining product mixture was separated by column chromatography (silica; PE/EtOAc 25:1) to afford, in order of elution, 26 mg of cyclic ether **149** and 107 mg of **140** . ^{1}H-NMR and ^{13}C-NMR of **140**: see Table 4.2. Mass spectrum of **140** (m/e): [M^+] 178 (2), 163 (12), 160 (17), 145 (38), 131 (15), 120 (60), 105 (79), 93 (33), 92 (47), 91 (79), 79 (61), 78 (26), 77 (33), 71 (28), 65 (18), 53 (16), 43 (100), 41 (34), 39 (37).

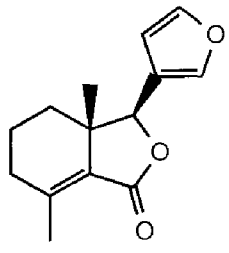
(1α,2β,7α,8β)-2,8-dimethyl-2,8-epoxybicyclo[5.3.0]dec-5-ene (**149**). ^{1}H-NMR of **149**: δ 5.88 (dt, 1H, H_6, J = 11.5, 5.4 Hz); δ 5.55 (ddt, 1H, H_7, 11.5, 7.1, 1.5 Hz); δ 2.41 (broad d, 1H, H_1, J = 3.3 Hz); δ 2.29-2.13 (m, 3H); δ 1.92-1.83 (m, 2H); δ 1.73-1.45 (m, 4H); δ 1.26 and δ 1.22 (2s, 2 x 3H, Me_{11} and Me_{12}). ^{13}C-NMR of **149**: δ 131.85 (d), δ 127.45 (d), δ 87.82 (s), δ 82.18 (s), δ 55.23 (d), δ 49.99 (d), δ 41.87 (t), δ 35.64 (t), δ 28.33 (q), δ 25.16 (t), δ 25.02 (t), δ 18.16 (q). Mass spectrum of **149** (m/e): [M^+] 178 (16), 163 (15), 160 (11), 145 (29), 120 (88), 118 (27), 107 (31), 105 (93), 93 (41), 92 (55), 91 (84), 85 (20), 79 (69), 78 (27), 77 (33), 43 (100).

4.5. References and notes

1. Piet D.P., Franssen M.C.R. and de Groot Ae., *Phytochemistry*, submitted.
2. Marshall J.A. and Huffman W.F., *J. Am. Chem. Soc.*, **92**, 6358 (1970).
3. Marshall J.A., Huffman W.F. and Ruth J.A., *J. Am. Chem. Soc.*, **94**, 4691 (1972).
4. Marshall J.A., *Synthesis*, 517 (1972).
5. Yoshikawa M., Hatakeyama S., Tanaka N., Fukada Y., Murakami N. and Yamahara J., *Chem. Pharm. Bull.*, **40**, 2582 (1992).

6. Takeuchi N., Fujita T., Goto K., Morisaki N., Osone N. and Tobinaga S., *Chem. Pharm. Bull.*, **41**, 923 (1993).
7. Piet D.P., Orru R.V.A., Jenniskens L.H.D., van de Haar C., van Beek T.A., Franssen M.C.R., Wijnberg J.B.P.A. and de Groot Ae, *Chem. Pharm. Bull.*, submitted.
8. Buchsacher P. and Furst A., *Org. Synth.*, **63**, 37 (1985).
9. Dutcher J.S., Macmillan J.G. and Heathcock C.H., *J. Org. Chem.*, **41**, 2663 (1976);
10. Marshall J.A. and Ruth J.A., *J. Org. Chem.*, **39**, 1971 (1974).
11. This form of anchimeric assistance is very common in norbornene systems. For examples see: Winstein S. and Shatvatsy M., *J. Am. Chem. Soc.*, **78**, 592 (1956) and Goering H.L. and Closson W.D., *J. Am. Chem. Soc.*, **83**, 3511 (1961).
12. Lee C.C., Clayton J.W., Lee D.G. and Finlayson A.J., *Tetrahedron*, **18**, 1395 (1962).
13. Johnson W.S., *Tetrahedron*, **47**, xi-1 (1991).
14. Buntel C.J. and Griffin J.H., *Evolution of Sterol and Triterpene Cyclases.* in: *Isopentenoids and Other Natural Products: Evolution and Function*, (Nes W.D., ed.), ACS Symposium Series, **562**, 52 (1994).
15. Kitagawa I., Kobayashi M., Cui Z., Kiyota Y. and Ohnishi M., *Chem. Parm. Bull.*, **34**, 4590 (1986).
16. Jurek J. and Scheuer P.J., *J. Nat. Prod.*, **56**, 508 (1993).
17. Yoshikawa M., Fukuda Y., Hatakeyama S., Tanaka N., Matsuda H., Yamahara J. and Murakami N., *Chem. Pharm. Bull.*, **41**, 1194 (1993).
18. Wijnberg J.B.P.A., Jenniskens L.H.D., Brunekreef G.A. and de Groot Ae., *J. Org. Chem.*, **55**, 941 (1990).
19. Jenniskens L.H.D., Wijnberg J.B.P.A. and de Groot Ae., *J. Org. Chem.*, **56**, 6585 (1991).
20. Orru R.V.A., Wijnberg J.B.P.A., Jenniskens L.H.D. and de Groot Ae., *J. Org. Chem.*, **58**, 1199 (1993).
21. Orru R.V.A., Wijnberg J.B.P.A., Bouwman C.T. and de Groot Ae., *J. Org. Chem.*, **59**, 374 (1994).
22. Orru R.V.A., Wijnberg J.B.P.A.and de Groot Ae., *J. Org. Chem.*, **60**, 4233 (1995).
23. The small positive NOE's found in most small and medium-sized molecules are difficult to detect by nuclear Overhauser and exchange spectroscopy (NOESY), partly because of sensitivity problems exacerbated by long relaxation delays needed between experiments: Morris G.A., *Magn. Reson. Chem.*, **24**, 371 (1986). Therefore, NOE-difference spectroscopy instead of NOESY was used to determine NOE effects in **140** and natural dictamnol.
24. Bohlmann F. and Jakupovic J., *Phytochemistry*, **18**, 1987 (1979).

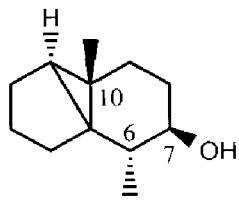
25. Yoshikawa M., Yamaguchi S., Matsuda H., Kohda Y., Ishikawa H., Tanaka N., Yamahara J. and Murakami N., *Chem. Pharm. Bull.*, **42**, 1813 (1994).
26. Yoshikawa M., Yamaguchi S., Matsuda H., Tanaka N., Yamahara J. and Murakami N., *Chem. Pharm. Bull.*, **42**, 2430 (1994).
27. Morikawa K. and Hirose Y., *Tetrahedron Lett.*, 1799 (1969).
28. Jones R.V.H. and Sutherland M.D., *Aust. J. Chem.*, **21**, 2255 (1968).
29. Adams R.P., Zanoni T., van de Haar C., van Beek T.A. and Posthumus M.A.P., *Essent. Oil Res.*, submitted.
30. Preparative GC at 120°C of the essential oil of the leaves of *A. diatrypa* gave an almost pure sample of **150**. At temperatures higher than 120°C, Cope rearrangement of **150** into **151** started to occur: Kubeczka K.-H., *Phytochemistry*, **13**, 2017 (1974).
31. Because air-oxidation of **42** resulted in the formation of (±)-**43**[31], it was assumed that air-induced cyclisation of **150** also led to a racemic mixture, in this case (±)-dictamnol. Following the isolation procedure in the literature[6], 1 kg of commercially available *D. dasycarpus* (Uchida Wakanyaku Company, Ltd.) provided 96 mg of natural dictamnol. Natural dictamnol was reported to have a specific optical rotation of $[\alpha_D]$ = + 55° (*c* = 0.1, MeOH). However, natural dictamnol isolated from *D. dasycarpus* showed no significant optical activity $[\alpha_D]$ ± 0° (*c* = 0.1, MeOH; *c* = 0.9, $CHCl_3$).
32. However, GC-MS analysis of the essential oil of the leaves of *A. diatrypa* revealed the presence of both pregeijerene (**150**) and dictamnol (**152**).
33.



153

^{1}H-NMR of **153**: δ 7.43 (m, 2H); δ 6.33 (broad s, 1H); δ 4.86 (broad s, 1H); δ 2.31 (m, 1H); δ 2.11 (s, 3H); δ 1.90-1.65 (m, 2H); δ 1.55-1.20 (m, 3H); δ 0.84 (s, 3H). ^{13}C-NMR of **153**: δ 169.90 (s), δ 148.54 (s), δ 143.38 (d), δ 139.72 (d), δ 127.31 (s), δ 120.56 (s), δ 109 50 (d), δ 83.36 (d), δ 42.93 (s), δ 32.03 (t), δ 31.58 (t), δ 20.27 (q), δ 18.40 (q), δ 18.18 (t). Mass spectrum of **153** (m/e): [M^+] 232 (1), 136 (56), 108 (37), 93 (100), 91 (20), 79 (21), 77 (21), 41 (21), 39 (38). Also see: Tokoroyama T., Fukuyama Y., Kubota T. and Yokotani K., *J. Chem. Soc., Perkin Trans I*, 1557 (1981).

34.



154

^{1}H-NMR of **154**: δ 3.14 (dd, 1H, H_7, J = 10.6 Hz, 4.6 Hz); δ 2.00-1.30 (m, 12 H); δ 0.98 (d, 3H, J = 6.8 Hz, C_6-Me); δ 0.96 (s, 3H, C_{10}-Me). ^{13}C-NMR of **152**: δ 73.12 (d), δ 39.57 (s), δ 39.28 (d), δ 32.12 (t), δ 30.91 (t), δ 30.41 (d), δ 28.12 (t), δ 26.94 (t), δ 26.64 (t), δ 24.45 (s), δ 16.76 (q), δ 15.93 (q). Mass spectrum of **154** (m/e): [M^+] 180 (7), 162 (5), 147 (82), 134 (22), 133 (88), 123 (29), 121 (42), 120

(23), 119 (45), 111 (47), 108 (27), 107 (61), 106 (25), 105 (74), 95 (52), 93 (99), 90 (84), 81 (52), 79 (90), 77 (54), 67 (75),

35. Piet D.P., Minnaard A.J., van der Heijden K.A., Franssen M.C.R., Wijnberg J.B.P.A. and de Groot Ae., *Tetrahedron*, **51**, 243 (1995).
36. Sodium *tert*-amylate (2.2 M in toluene) was prepared as described: Conia M.J.-M., *Bull. Soc. Chim.*, **17**, 537 (1950).

5. Biotransformation of sesquiterpene germacrane derivatives

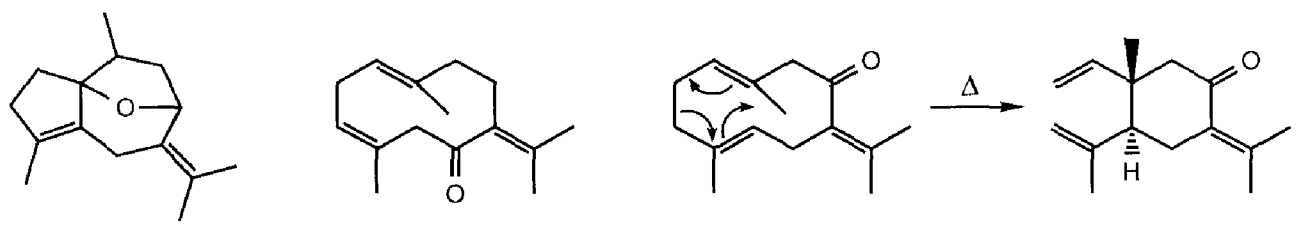
5.1. Introduction

In chapters 3 and 4, the synthesis of (E,E)-1,5- and (E,E)-1,6-cyclodecadienols and their biotransformation by a chicory root homogenate is described. From these preceding chapters it has become clear that the mode of the cyclisation reaction initiated by *C. intybus* starts by protonation of the substrate followed by a transannular cyclisation and stereoselective incorporation of a water molecule. The relative ease with which these (E,E)-cyclodecadienols can be synthesised, especially **100** and **131**, makes these germacrane synthons attractive substrates in order to detect cyclisation activity in *C. intybus* and possibly other organisms. In this chapter[1], the attention is focussed on the biotransformation of sesquiterpene germacranes, derived from germacrone (**155**), a natural germacrane.

5.1.1. Germacrone

Germacrone (**155**) is the major constituent of the essential oil of *Geranium macrorrhizum* L., belonging to the family of Geraniaceae. This commercially available oil, also known as Bulgarian geranium oil or 'zdravets'-oil (zdrave: Bulgarian for 'health'), possesses a typical, pleasant and refreshing odour[2]. The zdravets is a grass-like perennial plant growing in the Bulgarian highlands at altitudes of 800 to 1700 meters above sea level. The natural oil is obtained by simple steam distillation of the green parts of the wild zdravets.

Figure 5.1.



156 **157** **155** **158**

Since attempts towards industrial cultivation of zdravets were unsuccessful, the total annual amount of oil, produced from wild-growing plants, is limited to several

hundreds of kilograms. At room temperature, zdravets oil is a mixture of crystals and liquid. Germacrone (**155**), the solid part of the natural oil, is obtained by simple crystallisation from EtOH and comprises roughly 50% of the total oil. From its discovery in 1929[3] until 1959, germacrone was assigned an incorrect structure twice[4,5] (**156, 157**; Figure 5.1.). The correct structure was determined after the discovery that germacrone undergoes a [3,3]-sigmatropic or Cope-rearrangement[6] into β-elemenone (**158**) at elevated temperatures[7]. An X-ray structure of the silver nitrate adduct[8] as well as the total synthesis of germacrone[9] further substantiated the proposed α,β-unsaturated (E,E)-1,5-cyclodecadienone structure.

Germacrone is a readily accessible and stable germacrane and can be used as a synthon in the synthesis of a number of other natural and unnatural germacranes. Biotransformation of these germacranes by a chicory root homogenate may aid in determining the substrate specificity of germacrane cyclising enzymes in *C. intybus*. In the following paragraphs, the synthesis of germacrone derivatives and their biotransformation by a chicory root homogenate is described.

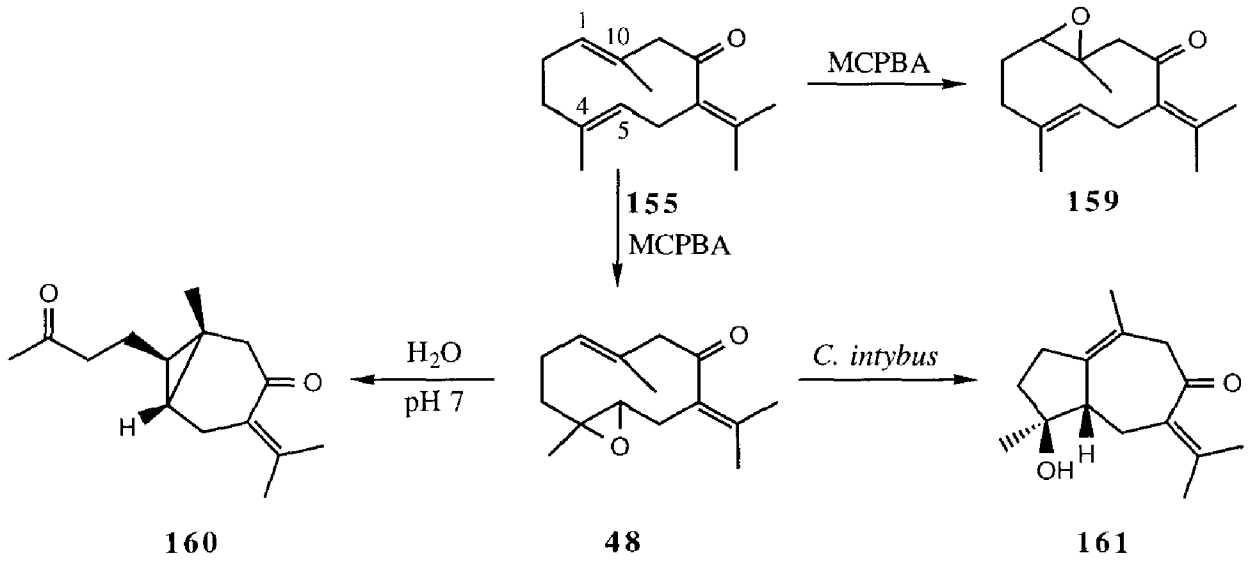
5.2. Synthesis and biotransformation of germacrone derivatives

5.2.1. Germacrone epoxides

A number of biotransformation studies of germacrone by suspension cultured plant cells are reported[10-12]. These plant cells are able to transform germacrone into a number of guaiane, eudesmane and other sesquiterpenes, mainly after *in vivo* epoxidation of either the C_4-C_5 or the C_1-C_{10} double bond. Apart from transannular cyclisation reactions, some rearrangements reactions were also reported. When germacrone (**155**) was administered to a suspension of mortared chicory roots, no transformation was observed after a 10 days incubation period. Therefore, germacrone was epoxidised chemically to study the biotransformation of these epoxides. Treatment of germacrone (**155**) with an equimolar amount of MCPBA results in a mixture of the two mono-epoxides germacrone-4,5-epoxide (**48**) and germacrone-1,10-epoxide (**159**) of which **48** is the major component. When **48** was administered to a suspension of mortared chicory roots and incubated for 8 days, a 4 : 1 mixture of curcumenone (**160**)[13] and neoprocurcumenol (**161**)[14] was obtained. Their ^{1}H- and ^{13}C-NMR spectra are identical to those reported in the literature[13,15,16]. Both controls clearly showed that the conversion of **48** into **160** is an uncatalysed process. After 8 days, **48** was completely converted into **160**; no **161** was detected in the controls. Thus,

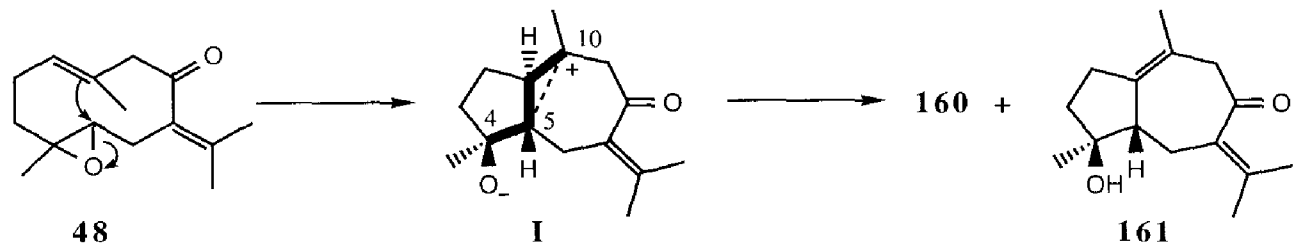
the formation of **161** appeared to be enzyme-mediated. The sp^2-bridgehead carbon in the guaiane system of **161** is characteristic for the bitter compounds in *C. intybus* [17-20]. Analytical HPLC-experiments using a chiral column revealed the racemic nature of **161**.

Scheme 5.1.



The spontaneous formation of **160** *via* a homofragmentation reaction can only proceed through the *trans* guaiane intermediate **I** as depicted in scheme 5.2. Homofragmentation reactions are fast and favoured reactions when orbital interaction through the three intervening C-C single bonds (through-bond interaction) is accompanied by 1,3-bridged through-space interaction[21]. Both conditions are met in **I**[22]. A bridging between the cationic centre at C_{10} and the back lobe of the O^-- C_4-C_5 orbital is believed to be involved in this reaction.

Scheme 5.2.

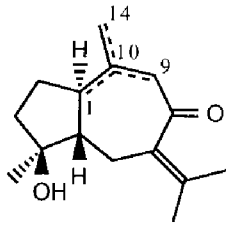


The enzymatically formed product **161** is the most stable of all *trans* guaiane double bond isomers as is determined from the calculation of their Heat-of-Formation (ΔH_f) using semi-empirical methods[23]. The ΔH_f values of **49**, **50**, **160** and **161** are given in

table 5.1. Although the ΔH_f-value of **160** is 14.4 kcal/mol higher than **161**, spontaneous homo-fragmentation predominates the enzyme-mediated deprotonation.

Tabel 5.1.

Compound	ΔHf (kcal/mol)
49	– 90.81
50	– 87.03
160	– 80.49
161	– 94.86

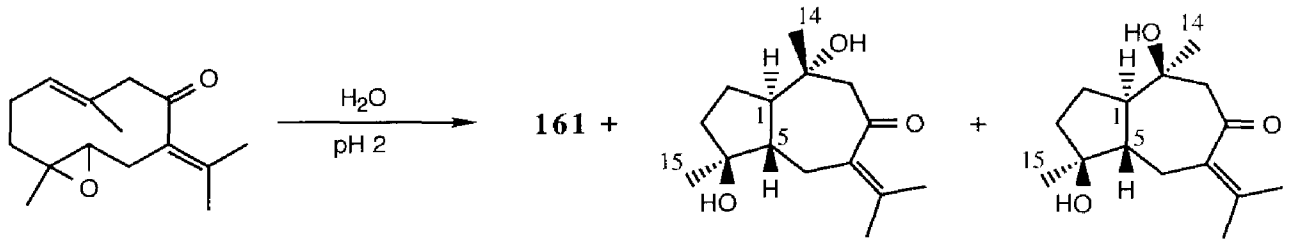


49 = Δ 9,10
50 = Δ 10,14
161 = Δ 1,10

The formation of neoprocurcumenol (**161**) is consistent with the Johnson model for oxidosqualene cyclising enzymes[24] as postulated in chapter 4. Cyclisation is initiated by proton donation from a specific amino acid residue and the subsequent cationic intermediate is stabilised according to 'the aromatic hypothesis'[25]. Contrary to the (E,E)-cyclodecadienes from chapters 3 and 4, a proton is abstracted to give the higher substituted alkene. There are two possible explanations for the product outcome. Firstly, the interaction can reduce the charge density at C_{10} in intermediate **I** which will reduce the dipolar character of **I**. This will lead to reduced bridging between C_5 and C_{10} necessary for homofragmentation reactions. Secondly, the interaction will distort the ideal conformation needed for through bond interaction. Even small deviations from this conformation influence the rate in which homofragmentation reactions occur[26]. This distortion gives way to other reactions of which selective deprotonation towards the thermodynamically most stable alkene is favoured.

Acid-induced cyclisation of **48** in an anhydrous medium gave mixtures of the three guaiane double bond isomers neoprocurcumenol (**161**), procurcumenol (**49**) and isoprocurcumenol (**50**). The ring fusion of **50** has recently been established as being *trans* by means of NOE and X-ray crystallography[16]. Several groups have also reported the isolation of cis-fused guaianes originating from **48**, but no solid spectral or crystallographic evidence was presented[12,27,28]. Acid-induced cyclisation of **48** in an aqueous medium gave **161** and two guaiane diols **162** and **163** (Scheme 5.3.). The ^{13}C-NMR spectra of **162** and **163** were identical to those of zedoarondiol and isozedoarondiol, respectively[15,16,28].

Scheme 5.3.



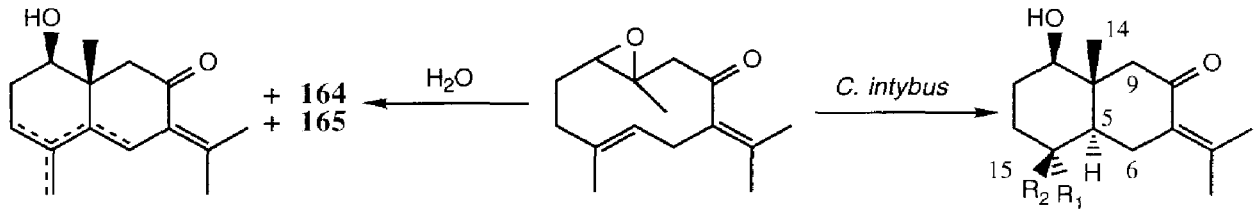
48 **162** **163**

C-14: δ 20.52 C-14: δ 32.15

The ring fusion of **162** has unambiguously been established as trans by means of X-ray analysis[28]. According to Kuroyanagi *et al.*, isozedoarondiol (**163**) is a cis-fused guaiane with Me_{14} and Me_{15} in an anti relationship towards the bridgehead protons, H_1 and H_5. In this relationship, the distance between the protons at Me_{14} and Me_{15} is calculated to be approximately 2.12 Å. This means that a NOE between Me_{14} and Me_{15} must be present. However, when Me_{14} (δ 1.04) was irradiated, no NOE with Me_{15} (δ 1.23) was observed. In fact, a clear NOE was present between Me_{14} and H_1 (δ 2.64) indicating that Me_{14} and H_1 are located at the same side of the molecule. No NOE was observed between H_1 and H_5, confirming the trans-fused ring structure. The syn relationship between H_1 and Me_{14} was further ascertained from the ^{13}C-NMR chemical shift of C_{14}. Posner *et al.* reported a significant downfield shift for C_{14} in guaianes possessing a syn relationship between H_1 and C_{14} compared to their anti counterparts[29]. The C_{14} of isozedoarondiol (**163**) in the ^{13}C-NMR spectrum resonates at δ 32.15. Compared to zedoarondiol (**162**), an 11.6 ppm downfield shift was observed for C_{14} (δ 20.52). Based on these observations it was concluded that isozedoarondiol (**163**) possesses a trans-fused guaiane skeleton and is, in fact, a C_{10} epimer of zedoarondiol (**162**) as outlined in scheme 5.3.

The minor epoxide formed upon treatment of germacrone (**155**) with one equivalent of MCPBA is germacrone-1,10-epoxide (**159**). When **159** was administered to a root suspension of mortared chicory and incubated for 8 days, a 2 : 1 mixture of epimeric diols **164** and **165** was obtained. Both controls gave a complex mixture of dehydrated products (total 52%) and both diols **164** (34%) and **165** (14%, Scheme 5.4.). The products could be separated column chromatography. Separation of the dehydrated products was unsuccessful but judging from the ^1H-NMR the mixture consisted of the α-, β- and γ-alkene as well as the conjugated isomer in a 1 : 1 : 2 : 1 ratio.

Scheme 5.4.



159

164: $R_1 = -OH$; $R_2 = -CH_3$
165: $R_1 = -CH_3$; $R_2 = -OH$

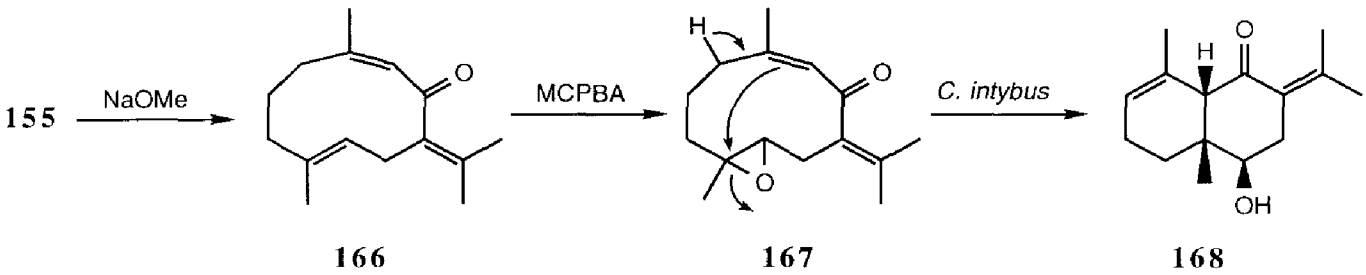
Sakamoto *et al.*[12] reported the biotransformation of **159** by suspension cultured cells of *Curcuma zedoaria* into a cis and a trans-fused eudesmane diol, thus proposing that cyclisation had occurred through different germacrane conformers as was postulated by Parker and Roberts[30]. However, NOE-experiments on both diols **164** and **165** clearly indicated a trans-fusion of both decalin ring systems. Irradiation of **164** at Me_{15} (δ 1.05) gave a positive NOE effect with Me_{14} (δ 0.74). When Me_{14} was irradiated a clear NOE was present between Me_{14} and Me_{15} and the equatorial H_9 proton (δ 2.46). A NOE between Me_{14} and Me_{15} in the spectrum of **165** was absent. In fact, a clear NOE was present between Me_{15} (δ 1.25), H_6 (δ 2.70) and the H_5 bridgehead proton (δ 1.46) indicating the methyl group at C_4 to be in an equatorial position. Irradiation of Me_{14} (δ 1.04) gave a positive NOE with the equatorial H_9 proton (δ 2.58). The ^1H- and ^{13}C-NMR data of **165**, obtained through cyclisation in an aqueous acidic medium, are identical to those of the cis eudesmane as postulated by Sakamoto *et al.*[12]; hence, the structure of **165** has to be revised to the trans-fused eudesmane with the stereochemistry as depicted in scheme 5.4.

The 1,10-germacrone epoxide (**159**) appears to follow the same cyclisation pathway as the (E,E)-cyclodeca-1,5-dienes as discussed in chapter 3. In that chapter it became clear that (E,E)-cyclodeca-1,5-dienes cyclise through their all-chair conformation into decalines with a trans-fused ring structure. However, the cyclisation process of germacrone-1,10-epoxide (**159**) does not appear to be as concerted as that of the (E,E)-cyclodeca-1,5-dienes. The incorporation of a water molecule during cyclisation of (E,E)-cyclodeca-1,5-dienes, initiated by chicory root enzymes, takes places from a pseudo-equatorial direction resulting in only one relative configuration at C_4. Cyclisation of **159**, on the other hand, does not show such a neat incorporation but a more or less random uptake of a water molecule from the surrounding medium.

5.2.2. Isogermacrone 4,5-epoxide

Isogermacrone (**166**), an (E,Z)-1,6-germacrene[31,32], was prepared by base-catalysed isomerisation of germacrone (**155**) and appeared to be unreactive towards a chicory root homogenate. Isogermacrone 4,5-epoxide (**167**) is the sole product formed upon treatment of **166** with one equivalent of MCPBA in the presence of solid Na_2CO_3. When **167** was administered to a suspension of freshly mortared chicory roots and incubated for 3 days, a complete conversion into the cis-fused eudesmane **168** was observed (Scheme 5.5.). Both controls showed a 50% conversion into the same product. The ^1H- and ^{13}C-NMR data were identical to those reported in the literature[32].

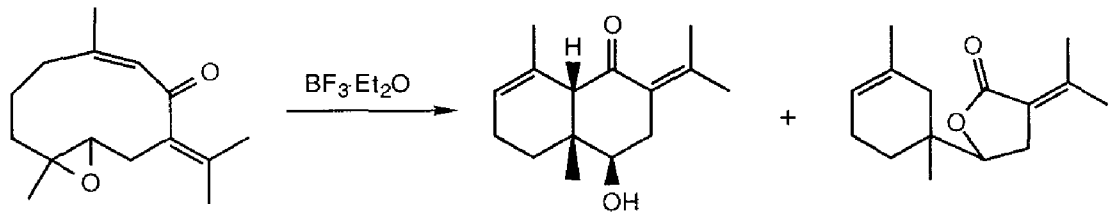
Scheme 5.5.



166 **167** **168**

The ring fusion of eudesmane **168** has unambiguously been established as cis by means of X-ray crystallography[32]. Chemically induced cyclisation of **167** using $BF_3{\cdot}Et_2O$ gave **168** and the lactone **169**[33] in 86% and 14% respectively (Scheme 5.6.). No lactone formation was observed in either controls.

Scheme 5.6.



167 **168** **169**

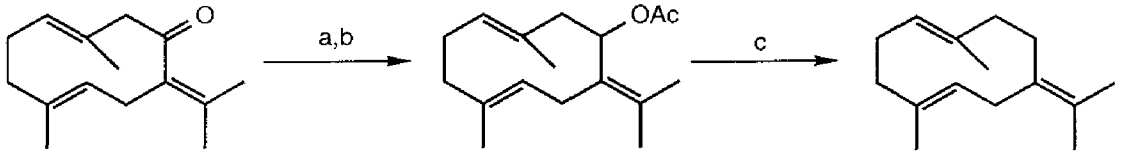
The non-enzymatic transformation of **167** into **168** reveals the instability of the epoxide towards the incubation medium. The catalytic role of the chicory root cyclising enzymes seems to be rather limited in this reaction, since they increase the rate of cyclisation only by a factor of 2.

This very poor catalytic activity may also suggest that epoxides derived from (E,Z)-cyclodeca-1,6-dienes are no intermediates in the biosynthesis of the bitter compounds in chicory. No cis-fused eudesmanes have been reported as secondary metabolites in *C. intybus* or other members of the Compositae family up to now.

5.2.3. Germacrene B epoxides

Germacrene B (**170**), a naturally occurring and relatively stable germacrene, was obtained in three steps from germacrone (**155**, scheme 5.7.)[34-36].

Scheme 5.7.[a]

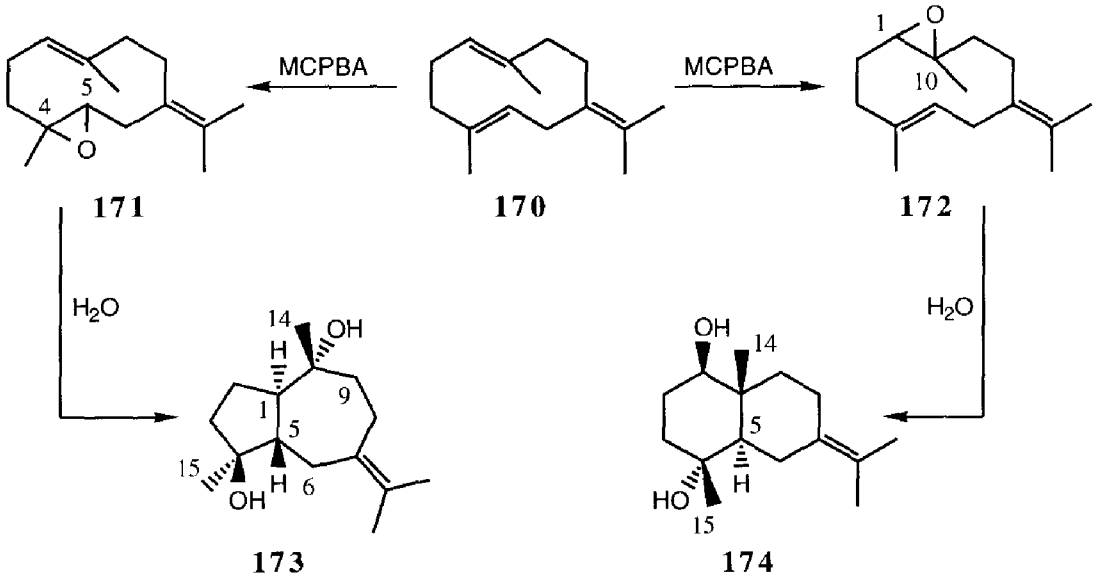


155 **170**

[a] a) $LiAlH_4$; b) Ac_2O/pyr.; c) Li/NH_3.

Homogenous epoxidation using one equivalent of MCPBA led to over-epoxidised products, starting material and only minute amounts of mono-epoxides. Using a two-phase-system[37], the desired mono-epoxides could be obtained in moderate yield. Column chromatography gave the 4,5-epoxide (**171**) and the 1,10-epoxide (**172**) as an inseparable mixture. Contrary to the work of Brown *et al.*, **172** did not disintegrate during chromatographic workup procedures[38] Also, **171** could not be crystallised from the mono-epoxide mixture as reported. Consequently the mono-epoxide mixture was used for the biosynthetic studies (Scheme 5.8.). Unfortunately, **171** and **172** cyclise spontaneously under the incubation conditions. After 1 day, the chicory root suspension as well as both controls showed complete conversion. The 4,5-epoxide **171** was transformed into the guaiane-diol **173** and the 1,10-epoxide **172** into the eudesmane diol **174**. Both products have a trans-fused ring system as was clearly established by means of 2D-NMR experiments. The position of the bridgehead protons H_1 and H_5 in **173** were ascertained by ^1H-^{13}C correlated NMR to be δ 2.89 and δ 1.85 respectively (C_6D_6). Irradiation of **173** at Me_{14} (δ 1.28) displayed a NOE with H_9 (δ 2.78) and H_5 (δ 1.85). Irradiation at Me_{15} (δ 1.33) gave a very clear NOE with H_6 (δ 2.37) but not with H_1.

Scheme 5.8.



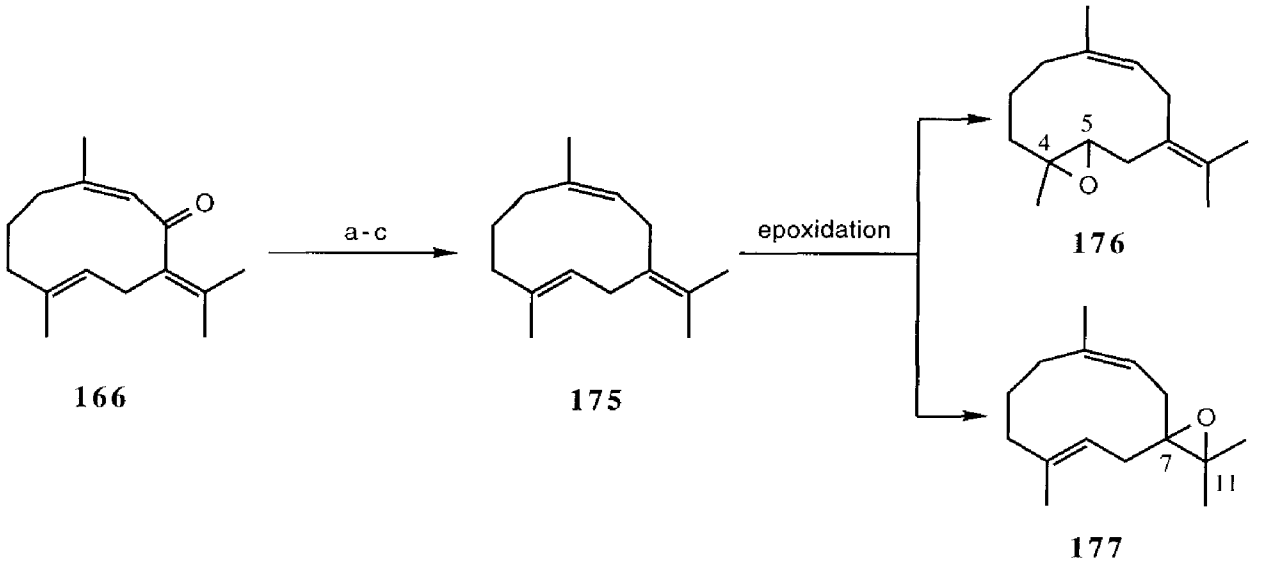
The lack of a NOE between Me_{15} and H_1 does not implicate that the ring-fusion of **173** is cis, but it indicates that the distance between Me_{15} and H_1 is to big to display a NOE. This was confirmed by semi-empirical calculations on **173** where the distance between the protons at Me_{15} and H_1 was calculated to be 3.64 Å. No NOE was observed between H_1 and H_5. Irradiation of **174** at Me_{14} (δ 0.96) gave a NOE with Me_{15} (δ 1.12) and *vice versa*. Furthermore, no NOE was observed between Me_{15} and H_5 ascertaining the trans fusion of the decalin system of **174**.

5.2.4. Isogermacrene B epoxides

Isogermacrene B (**175**) was obtained in three steps from isogermacrone (**166**), similar to the synthesis of germacrane B (**170**). Isogermacrene B (**175**) also appeared to be unreactive towards a chicory root homogenate. Epoxidation of **175** with MCPBA, as well as with *in situ* generated dimethyldioxirane[39], gave a 1 : 1 mixture of isogermacrene B 4,5-epoxide (**176**) and isogermacrene B 7,11-epoxide (**177**, Scheme 5.9.)

When **176** was administered to a chicory root suspension and incubated for 6 days, three products (**178-180**) were obtained in a 5 : 3 : 2 ratio, respectively. Both controls showed no conversion. The ^{1}H-NMR spectrum of the major cyclisation product (**178**) displayed a characteristic steroid-nonsteroid conformation equilibrium[40-42] i.e., coalescence effects due to the existence of an equilibrium mixture of two conformers.

Scheme 5.9.[a]



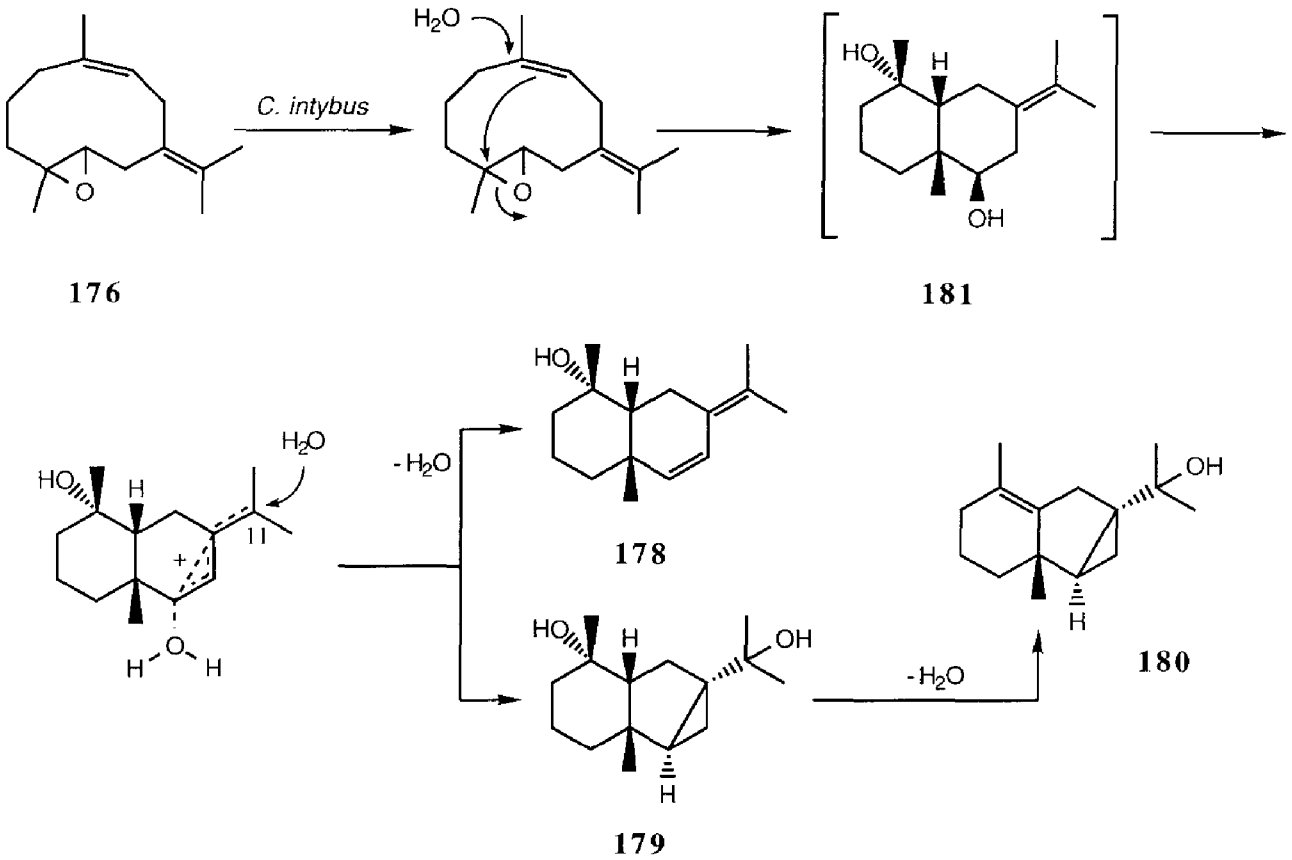
166 **175** **176** **177**

[a] a) $LiAlH_4$; b) Ac_2O/pyr.; c) Li/NH_3.

The presence of a broad olefinic singlet at δ 5.26 of two protons in **178** suggested a different mode of cyclisation for **176** as compared to its 9-oxo-counterpart, iso-germacrone 4,5-epoxide (**167**), since **168** has only one olefinic proton. Therefore cyclisation must have led to the *in situ* formation of diol **181** followed by dehydration under the incubation conditions to give diene **178** (Scheme 5.10.). A multiplet at δ 0.66 in the ^{1}H-NMR spectrum as well as a triplet resonance at δ 7.94 in the ^{13}C-NMR spectrum of **179** suggested the presence of a cyclopropane ring.

Since it is known that cyclases from *C. intybus* are able to stabilise charged intermediates, it is quite likely that both **178** and **179** were formed through the same, positively charged intermediate. The formation of a bridged carbocation and the subsequent distribution of that positive charge, as depicted in scheme 5.10. by the dotted lines, followed by incorporation of a water molecule at C_{11} accounts for the formation of **179**. A distinct NOE between the protons of the bridgehead methyl group (δ 0.92) and one of the cyclopropane protons at C_7 (δ 0.66) revealed their close proximity thus ascertaining the relative position of the three-membered ring in **179**. The ^{13}C-NMR spectrum of **180** also showed the triplet resonance at high field for C_7 (δ 10.24) and an additional two quaternary carbons at δ 136.36 and δ 126.08, respectively. The ^{1}H-NMR spectrum of **180** revealed the presence of a methyl group at a double bond and the absence of olefinic protons. It was therefore concluded that **180** is a dehydration product of **179** as shown in scheme 5.10.

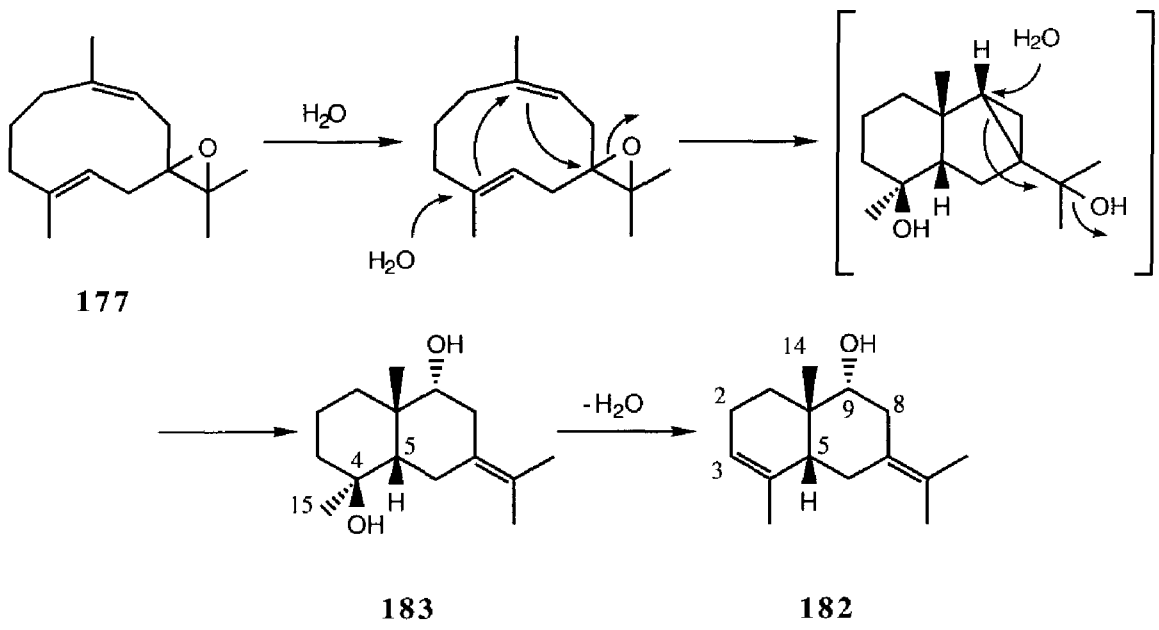
Scheme 5.10.



When **177** was administered to a chicory root suspension and incubated for 10 days, three main products were obtained in a 2 : 3 : 2 ratio of which only the latter two could be isolated (**182** and **183**, respectively). However, both controls also showed complete conversion after 10 days. The major cyclisation product (**182**) displayed the presence of three methyl groups attached to double bonds, one methyl group bound to a quaternary carbon atom, an olefinic proton and a proton on an oxygen-bearing carbon.

The identity of all protons and carbon atoms of **182** were assigned using COSY and ^{13}C-^{1}H correlated NMR techniques. Irradiation of the α-hydroxyl proton at δ 3.48 (H_9) resulted in a clear NOE between H_9 and the tertiary methyl group at δ 0.98 (Me_{14}) indicating that these groups protrude at the same side of the molecule. It was known from NOE-difference experiments on the cis-fused eudesmane **168**, obtained through cyclisation of isogermacrone 4,5-epoxide (**167**), that a clear NOE is present between the tertiary methyl group Me_{14} and the bridgehead proton H_5. Unfortunately, the position of H_5 in the ^{1}H-NMR spectrum of **182** was obscured because its chemical shift was identical to that of the three methyl groups located on the double bonds.

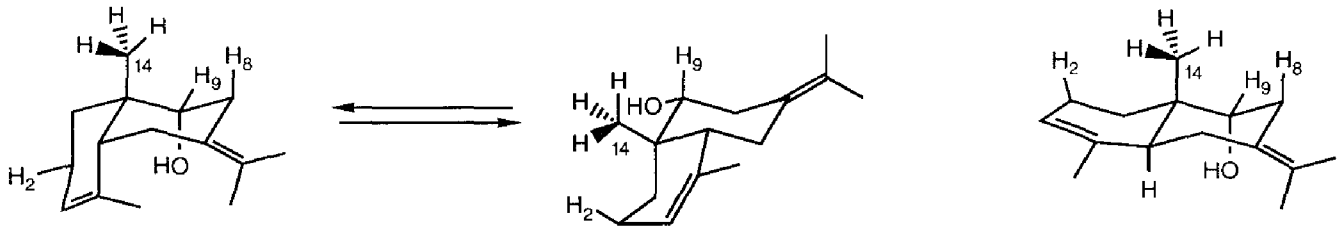
Scheme 5.11.



183 **182**

However, a careful examination of molecular models of **182** led to the conclusion that the ring fusion of **182** has to be cis. As mentioned earlier, cis-fused eudesmanes can occur in the steroid conformation, the nonsteroid conformation, or as an equilibrium mixture of these conformations. In this equilibrium, the axial substituents shift to equatorial positions and *vice versa*. Since the ^{1}H-NMR spectrum of **182** is relatively sharp, one conformation seems to predominate. Conformational analysis on both conformations[23] indeed showed a clear preference for one conformation; the non-steroid conformation as illustrated in scheme 5.12. (steroid vs nonsteroid = 0.4% : 99.6%). In the nonsteroid conformation the distance between the protons at Me_{14} and both H_2 and H_9 is calculated to be 2.10 Å and 2.21 Å, respectively. A very clear NOE of approximately equal magnitude was indeed present on H_2 and H_9 when Me_{14} was irradiated. In the steroid conformation, the distance between the protons of Me_{14} and H_9 was calculated to be 2.22 Å but a NOE between Me_{14} and H_2 is not to be expected as can be seen in scheme 5.12. Also, a clear NOE must be present between Me_{14} and H_8 since the distance between these two groups was calculated to be 1.80 Å. However, the NOE on H_8 was very small as compared to the effect on H_2 and H_9. If the ring fusion of **182** is trans (**182'**), the distance between the protons at Me_{14} and both H_2 and H_8 is calculated to be 2.03 Å and 1.81 Å, respectively. Irradiation of Me_{14} will therefore result in a clear NOE between Me_{14} and H_8 and a somewhat smaller effect between Me_{14} and H_2. As already mentioned, irradiation of Me_{14} resulted in a NOE of approximately equal magnitude on H_2 and H_9. It was therefore concluded that **182** possesses a cis-fused eudesmane framework with a distinctive preference for the nonsteroid conformation.

Scheme 5.12.



182 (steroid)
ΔH_f = – 64.39 KJ/mol

182 (nonsteroid)
ΔH_f = – 67.41 KJ/mol

182' (trans fused)

The ^{1}H-NMR spectrum of the minor cyclisation product (**183**) showed the presence of two methyl groups attached to double bonds and two tertiary methyl groups. In addition, the ^{13}C-NMR spectrum of **183** showed the presence of a tertiary oxygen-bearing carbon. It was therefore concluded that **183** bears an extra hydroxyl group, placed at C_4, and is the precursor of **182**. From a mechanistic point of view, the orientation of the methyl group at C_4 (Me_{15}) and the bridgehead proton (H_5) has to be anti since their relative stereochemistry was already fixed in the trans double bond of **177**.

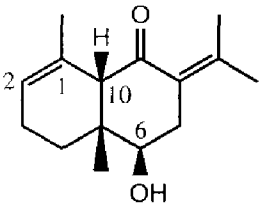
5.3. Concluding remarks

The mode of the chemically induced cyclisation of the germacrone epoxides **48** and **159** is profoundly influenced by the carbonyl function at C_8 as compared to the cyclisation of the germacrene B epoxides **171** and **172**. This influence is best illustrated by the formation of the homofragmentation product **160** and the mixture of the epimeric guaiane diols **162** and **163** from **48**, whereas only one cyclisation product (**173**) was obtained from **171**. The nature of the directing effect of the carbonyl at C_8 on homofragmentation reactions in systems like **I** (Scheme 5.2.) is not yet fully understood. The observation that **159** cyclises into two epimeric diols **164** and **165** whereas **172** gave only one product (**174**) can hardly be ascribed to an electronic influence of the carbonyl function in **159**. Probably, the introduction of a sp^2-centre at C_8 influences the configuration around the developing cationic centre at C_4 to such an extend that water incorporation can occur from either side of the molecule.

The epoxides of the (E,Z)-1,6-germacranes cyclise into cis-fused eudesmanes. The mode of cyclisation between isogermacrone 4,5-epoxide (**167**) and isogermacrane B 4,5-epoxide (**176**) differs. The keto-function at C_8 in the germacrane ring system must

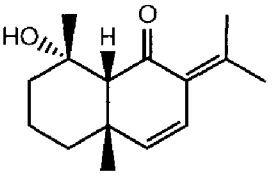
therefore have a profound influence on the transannular cyclisation. Semi-empirical calculations[23] revealed a preference for the elimination of the hydroxyl group at C_1, as outlined in figure 5.2. Two explanations for the preferred elimination of the hydroxyl function at C_1 can be adduced; i) the introduction of two additional sp^2-centres in the right-hand ring, although leading to a conjugated system, leads to an increase in ring strain and ii) dehydration at C_1 reliefs the steric hindrance of the methyl groups at C_1 and C_5.

Figure 5.2.



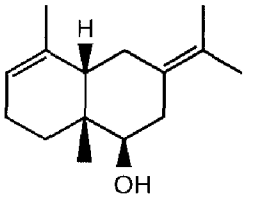
168

ΔH_f = - 91.30 Kcal/mol



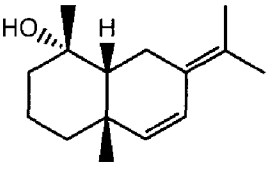
168'

ΔH_f = - 88.34 Kcal/mol



178'

ΔH_f = - 67.09 Kcal/mol



178

ΔH_f = - 62.09 Kcal/mol

The fact that **178** is the major product in the cyclisation of **176** and no C_1-dehydration products are formed suggests that the transition state leading towards the conjugated system is lowered to such an extent, that the cyclisation *via* this pathway is favoured. This is in contrast to the cyclisation of **167** which solely yielded the C_1-dehydrated product. The driving force behind this mode of cyclisation must therefore lie in the delocalisation of the developing positive charge in **181** over multiple carbon centres, as outlined in scheme 5.10., thus effectively lowering its transition state energy. If cyclisation of **167** would proceed through a diol intermediate like **181**, dehydration at C_1 would probably lead to mixtures of dehydrated products since an anti elimination is only possible if a proton is abstracted from the methyl group at C_1 since the tetrahedral angles of the alcohol group with H_2 and H_{10} are unfavourable ($\angle H_2$ - OH_{C1} = 52°; $\angle H_{10}$ - OH_{C1} = 69°). Apparently, the C_1-C_2 double bond of **168** is not formed

by dehydration of a diol intermediate but in an earlier stage of the reaction, as outlined in Scheme 5.5.

5.4. Experimental

Melting points are uncorrected. Chemical shifts are reported relative to TMS (δ 0.00) at 90, 200 and 500 MHz for ^{1}H-NMR and at 25.3 and 125.7 MHz for ^{13}C NMR. All spectra were recorded in $CDCl_3$ unless noted otherwise. Mass spectral data were obtained with a Hewlett Packard 5890 GC-MS equipped with a Hewlett Packard 5970 series mass selective detector using a capillary DB-17 column (30 m x 0.25 mm, d_f 0.25 μm) and helium as the carrier gas. Accurate mass measurements were obtained with a MS 902 equipped with a VG-ZAB console. Column chromatography was performed on Merck silicagel 60 and deactivated Al_2O_3 (grade III) using petroleum ether (bp. 40-60, PE)- EtOAc as the solvent system. Chiral analytical HPLC was performed on a Varian 5000 HPLC equipped with an HPLC-chiral II ET 250/8/4 NUCLEOSIL® with 5% of *iso*-PrOH in hexane as the solvent system and a Spectraflow 773 absorbance detector set at 254 nm. All solvents were distilled before use. A suspension of fresh chicory root (20% w/v) was produced by mortaring the root in a solution of 0.25 M sucrose, 3 mM Tris·HCl, 10 mM $MgCl_2$ and 0.2 % (w/v) Bovine Serum Albumin (BSA). The pH of this sucrose / Tris / $MgCl_2$/ BSA-solution (STMB) was set at 6.5 using 2-morpholino-ethanesulfonic acid (MES). The stability of the substrates towards the buffer and an inactivated chicory root sample (obtained by boiling the supernatant for 30 min) was investigated as a control to test the possibility of non-enzymatic reactions. Incubations were performed in sealed 4 ml vials at room temperature in a KS 500 shaker at 260 rpm containing 200 μl root suspension, 790 μl STMB-solution and 10 μl 0.1 M substrate in EtOH. The incubation medium was extracted with 0.5 ml EtOAc and its contents were analysed by GC-MS.

Germacrone (155): Isolated from the natural oil of *Geranium macrorrhizum*[36,43]. mp. 55-56°C. ^{1}H- and ^{13}C-NMR data were identical to those reported in the literature[31]. Mass spectrum of **155** (m/e): 41 (55), 67 (70), 91 (40), 107 (100), 135 (64), 136 (55), 175 (23), 218 (14); Calcd. for **155** [M^+]: 218.1670; Found 218.1671.

Epoxidation of Germacrone: To a stirred solution of 1.02 g of **155** (4.68 mmol) in 40 ml of CH_2Cl_2, cooled to 0°C, 1.04 g of MCPBA (85-90%) was added and the reaction was stirred for 30 minutes. The reaction mixture was washed with 2 x 50 ml of saturated $NaHCO_3$ solution and the combined aqueous layers were extracted with 50 ml of CH_2Cl_2, the combined organic layers dried were on $MgSO_4$ and evaporated to yield 1.08 g of a white solid. The epoxides were purified by column chromatography (Al_2O_3; 10% EtOAc in PE) to give 657 mg of **48** (mp 79-79.5°C) and 116 mg of **159** (mp 63-64.5°C). The ^{1}H- and ^{13}C-NMR were identical to those reported in the literature[15]. Calcd. for **48** [M^+]: 234.1620; Found: 234.1619; Calcd. for **159** [M^+]: 234.1620; Found: 234.1619.

Cyclisation reactions of 48: To a stirred solution of 155 mg of **48** in 4 ml of EtOH was added to 75 ml of water buffered at pH 2.0 and the resulting mixture was shaken for 24h at room temperature. The reaction mixture was extracted with 2 x 25 ml of CH_2Cl_2 and 2 x 25 ml of EtOAc, the combined organic layers were dried on $MgSO_4$ and evaporated to give 100 mg of a yellow oil. Column chromatography (Al_2O_3; EtOAc/PE 2 : 1) gave 30 mg of neoprocurcumenol (**161**), 32 mg of zedoarondiol (**162**) and 10 mg of isozedoarondiol (**163**). The ^{1}H- and ^{13}C-NMR of **161** and the ^{13}C-NMR of **162** and **163** were identical to those reported in the literature[15,16,28]. Mass spectrum of **161** (m/e): 43 (100), 67 (51), 68 (47), 77 (29), 79 (27), 91 (38), 93 (25), 105 (51), 121 (58), 163 (53), 173 (15), 176 (14), 177 (15), 201 (10), 219 (8), 234 (38).

^{1}H-NMR of **162**: δ 2.92 (d, 1H, H_9, J = 12.6 Hz); δ 2.78 (d, 1H, H_6, J = 14.7 Hz); δ 2.55 (d, 1H, H_9, J = 12.6 Hz); δ 1.98-1.92 (m, 2H); δ 1.89 (d, 3H, Me_{12}, J = 1.5 Hz); δ 1.79 (d, 3H, Me_{13}, J = 1.0 Hz); δ 1.75-1.63 (m, 4H); δ 1.37 (m, 1H); δ 1.17 (s, 3H, Me_{15}); δ 1.14 (s, 3H, Me_{14}). Mass spectrum of **162** (m/e): 43 (100), 81 (20), 109 (7), 149 (8), 173 (4), 191 (7), 234 (6), 252 (0).

^{1}H-NMR of **163**[44]: δ 3.06 (d, 1H, H_9, J = 16.1 Hz); δ 2.64 (dt, 1H, H_1, J = 10.0 Hz, 5.8 Hz); δ 2.34 (d, 1H, H_6, J = 14.2 Hz); δ 2.21 (dd, 1H, H_9, J = 16.1 Hz, 1.2 Hz); δ 1.84 (s, 3H, Me_{12}); δ 1.82 (dd, 1H, H_5, J = 5.6 Hz, 1.9 Hz); δ 1.75 (m, 1H, H_2); δ 1.71 (s, 3H, Me_{13}); δ 1.70-1.57 (m, 3H); δ 1.36 (m, 1H, H_2); δ 1.23 (s, 3H, Me_{15}); δ 1.04 (s, 3H, Me_{14}). Mass spectrum of **163** (m/e): 43 (100), 121 (9), 149 (8), 173 (5), 174 (5), 191 (10), 234 (5), 252 (0).

To solution of 153 mg of **48** in 4 ml of EtOH was added 75 ml of water buffered at pH 7 and the resulting mixture was shaken for 7 days at room temperature. The reaction mixture was extracted with 25 ml of EtOAc and 50 ml of CH_2Cl_2. The combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 85 mg of an oil. Column chromatography (Al_2O_3; 10% EtOAc in PE) gave 58 mg of curcumenone (**160**). The ^{1}H- and ^{13}C-NMR of **160** were identical to those reported in the literature[13]. Mass spectrum of **160** (m/e): 43 (100), 67 (44), 68 (54), 77 (18), 79 (21), 91 (22), 105 (16), 107 (20), 133 (18), 149 (28), 161 (30), 163 (18), 176 (47), 191 (7), 219 (5), 234 (9).

To a stirred solution of 329 mg of **48** in 10 ml of a 1 : 1 benzene / toluene mixture, cooled to 0°C, was added 24 mg of pTSA. The reaction mixture was stirred for 4h, washed with saturated $NaHCO_3$ solution, the aqueous layer was extracted with 25 ml of EtOAc, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 330 mg of a dark yellow oil. Column chromatograpy (silica; EtOAc/PE 1 : 1) gave 163 mg of a 3 : 1 mixture of **161** and isoprocurcumenol (**50**) and 30 mg of procurcumenol (**49**). The ^{1}H- and ^{13}C-NMR of **49** were identical to those reported in the literature[15,16]. Mass spectrum of **49** (m/e): 43 (100), 55 (18), 57 (20), 67 (21), 69 (20), 77 (21), 79 (22), 91 (32), 105 (34), 123 (41), 133 (27), 145 (25), 147 (34), 165 (18), 173 (24), 191 (9), 201 (16), 216 (44), 234 (8). Mass spectrum of **50** (m/e): 43 (100), 67 (55), 77 (30), 79 (39), 105 (72), 107 (44), 119 (23), 121 (57), 131 (16) 133 (27), 145 (29), 158 (30), 173 (19), 191 (22), 234 (9).

Cyclisation reaction of 159: To a solution of 99 mg of **159** in 7.5 ml of acetone and 5 ml of water was added 5 drops of conc. H_2SO_4. After stirring for 75 min at room temperature, solid $NaHCO_3$ and 25 ml of CH_2Cl_2 was added. The mixture was extracted with 3 x 25 ml of CH_2Cl_2, the combined organic layers were

washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 97 mg of a colourless oil. Column chromatography (silica; EtOAc) yielded 35 mg of eudesmane double bond isomers, judging from the ^{1}H-NMR to be the α-, β- and γ-alkene as well as the isopropylidene conjugated isomer in a 1 : 1 : 2 : 1 ratio, 10 mg of **165** as a colourless oil and 25 mg of **164** (mp 197-198.5°C) as a crystalline solid.
^{1}H-NMR of **164**: δ 3.32 (dd, 1H, H_1, J = 11.4 Hz, 4.0 Hz); δ 2.70 (dd, 1H, H_6, J = 16.0 Hz, 5.3 Hz); δ 2.58 (d, 1H, H_9, J = 16.0 Hz); δ 2.53 (m, 1H, H_6); δ 2.06 (dd, 1H, H_9, J = 15.0 Hz, 1.1 Hz); δ 2.06 (dd, 3H, Me_{12}, J = 2.1 Hz, 1.3 Hz); δ 1.90-1.55 (m, 5H); δ 1.82 (d, 3H, Me_{13}, J = 0.6 Hz); δ 1.46 (dd, 1H, H_5, J = 12.8 Hz, 5.5 Hz); δ 1.30-1.18 (m, 1H); δ 1.23 (d, 3H, Me_{15}, J = 1.4 Hz); δ 1.03 (d, 3H, Me_{14}, J = 1.0 Hz). ^{13}C-NMR of **164**: δ 202.12 (s), δ 146.49 (s), δ 129.80 (s), δ 78.60 (d), δ 70.88 (s), δ 55.29 (s), δ 47.49 (d), δ 40.26 (t), δ 39.59 (t), δ 29.94 (q), δ 26.68 (t), δ 25.80 (t), δ 23.81 (q), δ 23.07 (q), δ 12.61 (q). Mass spectrum of **164** (m/e): 39 (34), 41 (63), 43 (47), 55 (32), 67 (47), 79 (35), 83 (31), 91 (26), 105 (21), 115 (15), 119 (27), 133 (25), 147 (31), 148 (27), 163 (17), 173 (31), 201 (7), 219 (10), 234 (100), 252 (0); Calcd. for **164** [M^+]: 252.1725; Found: 252.1728.
^{1}H-NMR of **165**: δ 3.32 (m, 1H, H_1, J = 11.1 Hz, 4.0 Hz); δ 2.86 (dd, 1H, H_6, J = 15.4 Hz, 4.3 Hz); δ 2.56 (bs, 2H, -OH); δ 2.47 (d, 1H, H_9, J = 15.1 Hz); δ 2.10 (m, 1H, H_6); δ 1.97 (dd, 1H, H_9, J = 15.1 Hz, 1.0 Hz); δ 1.85 (d, 3H, Me_{12}, J = 2.0 Hz); δ 1.69 (d, 3H, Me_{13}, J = 1.1 Hz); δ 1.65-1.35 (m, 5H); δ 1.04 (s, 3H, Me_{15}); δ 0.75 (s, 3H, Me_{14}). ^{13}C-NMR of **165**: δ 202.72 (s), δ 143.47 (s), δ 130.70 (s), δ 77.62 (d), δ 70.91 (s), δ 56.73 (s), δ 50.34 (d), δ 40.99 (t), δ 40.72 (t), δ 28.56 (t), δ 25.76 (t), δ 23.05 (q), δ 24.39 (q), δ 24.25 (q), δ 12.49 (q). Mass spectrum of **165** (m/e): 39 (47), 41 (100), 43 (58), 53 (40), 55 (36), 67 (42), 69 (31), 77 (40), 79 (35), 81 (22), 83 (19), 91 (36), 93 (26), 95 (20), 105 (26), 106 (18), 107 (33), 109 (27), 119 (29), 121 (56), 136 (34), 163 (35), 201 (8), 219 (5), 234 (98), 252 (0). Calcd. for **165** [M^+]: 252.1725; Found: 252.1728.

Isogermacrone (166): To a solution of 2.30 g of sodium in 100 ml of EtOH, cooled to 0°C, was added dropwise 4.00 g of **155** in 30 ml of EtOH. After stirring for 3 days at room temperature, 2 ml of water was added and the solvent was evaporated. The residue was taken up in 50 ml of ether, washed with 50 ml of water, the combined aqueous layers were extracted with 2 x 50 ml of ether, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 3.65 g of **166** (mp 52-53.5°C). The ^{1}H- and ^{13}C-NMR of **166** were identical to that reported in the literature[32]. Mass spectrum of **166** (m/e): 41 (86), 53 (41), 67 (66), 68 (100), 77 (33), 79 (37), 81 (28), 91 (42), 93 (34), 96 (34), 109 (32), 121 (25), 147 (16), 161 (11), 175 (9), 203 (15), 218 (17).

Isogermacrone 4,5-epoxide (167): To a solution of 3.65 g of **166** and 5 g of solid Na_2CO_3 in 30 ml of CH_2Cl_2 was carefully added 3.80 g of MCPBA. The mixture was stirred for 45 min at room temperature, 50 ml of water was added, the organic layer was separated, the aqueous layer was extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to yield 3.84 g of **167** (mp 67-69.5°C). The ^{1}H-NMR of **167** was identical to that reported in the literature[31]. ^{13}C-NMR of **167**: δ 202.72 (s), δ 152.74 (s), δ 133.23 (s), δ 130.50 (d), δ 130.46 (s), δ 64.14 (d), δ 60.07 (s), δ 36.76 (t), δ 28.83 (t), δ 28.35 (t), δ 23.53 (q), δ 21.94 (t), δ 21.47 (q), δ 19.81 (q), δ 15.92 (q). Mass spectrum of **167** (m/e):

39 (55), 41 (88), 43 (100), 53 (33), 55 (33), 67 (53), 79 (37), 95 (34), 105 (21), 107 (19), 109 (22), 121 (26), 149 (23), 161 (9), 163 (10), 191 (6), 219 (5), 234 (7).

Cyclisation of 167: To a solution of 304 mg of **167** in 10 ml of dry ether, cooled to 0°C, was added 0.8 ml of $BF_3 \cdot Et_2O$. The reddish mixture was stirred for 1h at 0°C, 10 ml of water was added, the organic layer was separated, the aqueous layer was extracted with 20 ml of ether, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated to give 265 mg of a red oil. Column chromatography (silica; EtOAc/PE 1 : 2) gave 12 mg of **169** and 135 mg of **168** (mp 97-99°C). The ^{1}H- and ^{13}C-NMR of **168** and **169** were identical to those reported in the literature[31]. Mass spectrum of **168** (m/e): 41 (55), 67 (48), 91 (35), 93 (100), 109 (56), 121 (91), 125 (24), 173 (5), 234 (4); Mass spectrum of **169** (m/e): 41 (45), 67 (43), 79 (48), 93 (76), 109 (35), 121 (100), 219 (5), 234 (6).

Germacrene B (170): To a solution of 5.08 g of **155** in 60 ml of dry ether, cooled to 0 °C, was carefully added 1.5 g of $LiAlH_4$. The grey suspension was stirred for 3h, Glauber's salt was added in small portions and the resulting mixture was stirred for 30 minutes. After addition of $MgSO_4$ the mixture was stirred for an additional 30 minutes, the solids were filtered off and the solvent was evaporated to give 5.01 g of germacrol as a colourless oil. Both the ^{1}H- and ^{13}C-NMR of germacrol were uninterpretable due to coalescence effects. To a solution of 1.99 g of germacrol in 35 ml of dry pyridine was added 6 ml of acetic anhydride. The mixture was stirred overnight, the solvent was evaporated and the residue was chromatographed (silica; 10% EtOAc in PE) to give 2.27 g (96%) of germacrol acetate. Both the ^{1}H- and ^{13}C-NMR of germacrol acetate were uninterpretable due to coalescence effects. To a deep-blue mixture of 1.05 g of lithium metal in 125 ml of liquid NH_3, cooled to -78°C and stirred for 1h, was added dropwise a solution of 2.27 g of germacrol acetate in 20 ml of ether. The resulting mixture was stirred for an additional 1.5 h after which solid NH_4Cl was added until the deep-blue colour disappeared. The NH_3 was allowed to evaporate overnight after which 100 ml of water was added. The aqueous layer was extracted with 3 x 75 ml of ether, the combined organic layers were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 1.54 g of an oil (87%). Column chromatography (silica; PE) yielded 1.32 g (75%) of **170** as a colourless oil. ^{1}H-NMR of **170**: δ 4.71 (bd, 1H, H_1, J = 12.4 Hz); δ 4.55 (bd, 1H, H_5, J = 10.5 Hz); δ 2.91 (bd, 1H, H_6, J = 13.1 Hz); δ 2.49 (m, 2H); δ 2.35-1.85 (m, 7H); δ 1.70, 1.68 (2s, 2 x 3H, Me_{12}, Me_{13}); δ 1.53, 1.50 (2s, 2 x 3H, Me_{14}, Me_{15}). ^{13}C-NMR of **170**: δ 137.07 (s), δ 133.19 (s), δ 131.63 (s), δ 128.15 (d), δ 126.31 (d), δ 124.47 (s), δ 40.42 (t), δ 38.83 (t), δ 33.53 (t), δ 32.46 (t), δ 25.74 (t), δ 20.71 (q), δ 20.40 (q), δ 17.02 (q), δ 16.02 (q). Mass spectrum of **170** (m/e): 41 (100), 53 (57), 55 (46), 67 (73), 79 (51), 81 (50), 91 (65), 93 (85), 121 (94), 133 (30), 147 (18), 161 (27), 189 (13), 204 (16).

Epoxidation of Germacrene B: To a solution of 2.29 g of **170** in 40 ml of CH_2Cl_2, 40 ml of water and 15 g of solid $NaHCO_3$, cooled to -10°C, was added in small portions 1.95 g of MCPBA. The resulting suspension was stirred for 30 minutes at -10°C. The organic layer was separated, washed with 4 x 25 ml of saturated $NaHCO_3$ solution, the combined aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2, the combined

organic layers were dried on $MgSO_4$ and evaporated to give 2.32 g of a yellow viscous oil. Column chromatography (Al_2O_3; 5% of EtOAc in PE) yielded 735 mg of a mixture of **171** and **172** as a white solid. Extensive chromatography gave an analytical sample of **171**. ^{1}H-NMR of **171** : δ 4.99 (dd, 1H, H_1, J = 10.5 Hz, 5.7 Hz); δ 2.69 (bd, 1H, H_6, J = 14.1 Hz); δ 2.53 (dd, 1H, H_5, J = 9.3 Hz, 1.3 Hz); δ 2.51 (m, 1H); δ 2.30-1.85 (m, 8H); δ 1.71, 1.70, 1.67 (3s, 3 x 3H, Me_{12}, Me_{13}, Me_{14}); δ 1.17 (s, 3H, Me_{15}). ^{13}C-NMR of **171**: δ 135.33 (s), δ 129.83 (s), δ 129.24 (s), δ 124.27 (d), δ 66.35 (d), δ 61.19 (s), δ 40.28 (t), δ 37.49 (t), δ 36.24 (t), δ 36.12 (t), δ 23.93 (t), δ 20.77 (q), δ 20.69 (q), δ 17.18 (q), δ 16.56 (q).

Cyclisation of 171 and 172 A solution of 100 mg of the mono-epoxide mixture in 1.5 ml of EtOH was added to 75 ml of water buffered at pH 7.0 and shaken for 24h at room temperature. The reaction mixture was extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were dried on $MgSO_4$ and evaporated to give 89 mg of a colourless oil. Column chromatography (Al_2O_3; EtOAc/PE 2:1) gave 13 mg of eudesmanediol **174** (mp 155-157°C) and 26 mg of guaianediol **173** (mp 93-94.5°C). ^{1}H-NMR of **173** (C_6D_6): δ 2.89 (dt, 1H, H_1, J = 10.0 Hz, 6.2 Hz); δ 2.78 (m, 1H, H_9); δ 2.41 (bs, 1H, -OH); δ 2.37 (dd, 1H, H_6, J = 13.2 Hz, 2.4 Hz); δ 2.28 (bs, 1H, -OH); δ 1.96-1.90 (m, 2H, H_8, H_9); δ 1.86-1.82 (m, 2H, H_3, H_5); δ 1.74 (m, 1H, H_3); δ 1.71 (s, 3H, Me_{12} or Me_{13}); δ 1.69 (s, 3H, Me_{13} or Me_{12}); δ 1.58 (ddd, 1H, H_8, J = 14.1 Hz, 12.6 Hz, 5.5 Hz); δ 1.51 (m, 1H, H_2); δ 1.38 (m, 1H, H_2); δ 1.37 (bd, 1H, H_6, J = 12.3 Hz); δ 1.33 (s, 3H, Me_{15}); δ 1.28 (s, 3H, Me_{14}). ^{13}C-NMR of **173** ($CDCl_3$): δ 131.22 (s), δ 126.02 (s), δ 82.36 (s), δ 74.69 (s), δ 53.34 (d), δ 52.56 (d), δ 37.21 (t), δ 30.82 (q), δ 30.50 (t), δ 28.87 (t), δ 26.77 (t), δ 25.09 (t), δ 24.91 (q), δ 20.16 (q), δ 20.04 (q). Mass spectrum of **173** (m/e): 41 (35), 43 (100), 55 (21), 67 (19), 79 (7), 81 (15), 91 (17), 107 (31), 119 (17), 122 (37), 159 (10), 162 (10), 187 (8), 220 (12), 238 (0.3). Calcd. for **173** [M^+ - H_2O]: 220.1827; Found: 220.1828.
^{1}H-NMR of **174**: δ 3.26 (dd, 1H, H_1, J = 10.5 Hz, 4.6 Hz); δ 2.76 (dt, 1H, H_6, J = 13.5 Hz, 2.5 Hz); δ 2.53 (ddt, 1H, H_9, J = 13.7 Hz, 6.3 Hz, 2.1 Hz); δ 1.90-1.55 (m, 7H); δ 1.66 (d, 3H, Me_{12} or Me_{13}, J = 0.9 Hz); δ 1.63 (s 3H, Me_{13} or Me_{12}); δ 1.45 (dd, 1H, J = 11.5 Hz, 4.1 Hz); δ 1.12 (s, 3H, Me_{15}); δ 1.08 (m, 1H); δ 0.94 (d, 3H, Me_{14}, J = 0.6 Hz). ^{13}C-NMR of **174**: δ 130.34 (s), δ 121.30 (s), δ 79.38 (d), δ 71.65 (s), δ 53.72 (d), δ 41.19 (t), δ 41.03 (t), δ 39.14 (s), δ 28.52 (t), δ 24.88 (t), δ 24.33 (t), δ 22.10 (q), δ 20.00 (q), δ 19.94 (q), δ 12.40 (q). Mass spectrum of **174** (m/e): 41 (45), 43 (100), 55 (34), 67 (24), 93 (29), 107 (27), 119 (22), 121 (19), 135 (21), 159 (20), 163 (17), 176 (21), 187 (16), 203 (14), 220 (9), 238 (24). Calcd. for **174** [M^+]; 238.1933; Found 238.1934.

Isogermacrene B (**175**): Isogermacrene B (**175**) was prepared according to the synthesis of germacrene B (**170**). Starting from 3.46 g of **166**, 1.75 g of **175** was obtained. Similar to the synthesis of germacrane B, the ^{1}H- and the ^{13}C-NMR of isogermacrol[45] and isogermacrol acetate could not be interpreted due to coalescence effects. ^{1}H-NMR of **175**: δ 5.00 (m, 2H); δ 2.90-2.40 (2 broad s, 5H); δ 2.25-1.90 (broad s, 5H); δ 1.73 and δ 1.69 (3s, 6H, Me_{12} and Me_{13}); δ 1.67 and δ 1.59 (2d, 3H, Me_{14} and Me_{15}, J = 1.2 Hz and 1.0 Hz, respectively). ^{13}C-NMR of **175**: δ 133.10 (s), δ 132.09 (s), δ 126.06 (s), δ 125.94 (d), δ 125.54 (d), δ 124.89 (s), δ 38.70 (t), δ 32.12 (t), δ 31.25 (t), δ 28.53 (t), δ 22.64 (q), δ 20.71 (q), δ 20.32 (q), δ 19.64 (t), δ 14.75 (q). Mass spectrum of **175** (m/e): 41 (100), 53 (40), 55 (46), 67 (40), 77 (40), 79 (43), 81 (43), 91 (77), 93 (71), 105 (78), 107 (59), 108 (27), 119 (51), 133 (51), 147 (32), 161 (20), 189 (26), 204 (40).

Epoxidation of isogermacrene B (175): To a stirred solution of 155 mg of **175**, cooled to 0°C, was added 168 mg of MCPBA. The resulting mixture was stirred for 30 minutes, washed with 2 x 10 ml of saturated $NaHCO_3$, the combined aqueous layers were extracted with 2 x 20 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated to dryness to give 167 mg of an oil which was chromatographed (silica; 5% EtOAc in PE) yield 41 mg of isogermacrene B 4,5-epoxide (**176**) and 43 mg of isogermacrene B 7,11-epoxide (**177**).
^{1}H-NMR of **176**: δ 4.99 (dd, 1H, H_9, J = 10.3 Hz, 5.1 Hz); δ 2.87 (broad d 2H, J = 14.5 Hz); δ 2.67 (dd, 1H, J = 10.7 Hz, 1.4 Hz); δ 2.20-1.70 (m, 5H); δ 1.68 (d, 3H, J = 1.9 Hz, Me_{12}, Me_{13} or Me_{14}); δ 1.65 and δ 1.60 (2s, 6H, Me_{12}, Me_{13} or Me_{14}); δ 1.27 (s, 3H, Me_{15}); δ 0.98 (m, 1H). ^{13}C-NMR of **176**: δ 133.88 (s), δ 127.14 (s), δ 126.90 (s), δ 125.36 (d), δ 66.79 (d), δ 60.72 (s), δ 36.22 (t), δ 31.99 (t), δ 31.24 (t), δ 28.06 (t), δ 22.39 (q), δ 22.23 (t), δ 20.61 (q), δ 20.32 (q), δ 16.33 (q). The ^{1}H- and the ^{13}C-NMR of **177** could not be interpreted due to coalescence effects.

Cyclisation of 176: To a stirred solution of 100 mg of **176** in 10 ml of acetone was added 190 ml of water buffered at pH 2. The resulting mixture was stirred overnight, extracted with 4 x 25 ml of CH_2Cl_2, the combined organic layers were washed brine, dried on $MgSO_4$ and evaporated. The resulting residue was chromatographed (silica; EtOAc/PE 1:1) to give 49 mg of **178**, 12 mg of **179** and 13 mg of **180**.
^{1}H-NMR of **178** (C_6D_6): δ 5.35 (m, 2H); δ 2.50-2.05 (m, 6H); δ 1.70 (s, 3H, Me_{14}); δ 1.70-1.40 (m, 4H); δ 1.53 (s, 3H, Me_{15}); δ 1.18 and δ 1.16 (2s, 6H, Me_{12} and Me_{13}). The ^{13}C -NMR of **178** was uninterpretable due to coalescence effects. Mass spectrum of **178** (m/e): 41 (26), 43 (100), 91 (13), 105 (12), 106 (11), 107 (10), 119 (6), 147 (10), 162 (9), 205 (3), 220 (0.1).
^{1}H-NMR of **179** (C_6D_6): δ 1.80-1.66 (m, 3H); δ 1.64-1.44 (m, 3H); δ 1.27 (s, 3H, Me_{14}); δ 1.26 (m, 1H); δ 1.18 and δ 1.17 (2s, 6H, Me_{12} and Me_{13}); δ 1.17 (m, 1H); δ 0.97 (dd, 1H, H_6, J = 7.6 Hz, 4.4 Hz); δ 0.89 (s, 3H, Me_{15}); δ 0.84 (broad s, 2H, -OH); δ 0.66 (m, 2H). ^{13}C-NMR of **179** (C_6D_6): δ 72.07 (s), δ 70.18 (s), δ 51.82 (d), δ 43.38 (t), δ 41.38 (s), δ 35.42 (t), δ 34.01 (s), δ 33.04 (d), δ 28.44 (q), δ 28.10 (q), δ 25.32 (t), δ 23.34 (q), δ 22.69 (q), δ 22.03 (t), δ 8.42 (t). Mass spectrum of **179** (m/e): 41 (46), 43 (100), 55 (16), 79 (31), 91 (21), 93 (18), 107 (39), 135 (16), 162 (5), 187 (4), 220 (1).
^{1}H-NMR of **180** (C_6D_6): δ 2.51 (m, 1H, H_9, J = 14.4 Hz); δ 2.38 (broad d, 1H, H_9, J = 14.3 Hz); δ 1.90-1.60 (m, 4H); δ 1.54 (s, 3H, Me_{14}); δ 1.50-1.30 (m, 3H); δ 1.22 and δ 1.13 (2s, 9H, Me_{12}, Me_{13} and Me_{15}); δ 1.08 (dd, 1H, H_6, J = 7.9 Hz, 4.0 Hz); δ 0.68 (ddd, 1H, H_7, J = 7.9 Hz, 4.5 Hz, 1,5 Hz); δ 0.04 (t, 1H, H_7, J = 4.2 Hz). ^{13}C-NMR of **180** (C_6D_6): δ 136.36 (s), δ 126.08 (s), δ 69.51 (s), δ 41.74 (s), δ 34.18 (s), δ 32.70 (t), δ 32.00 (t), δ 31.69 (d), δ 30.55 (t), δ 27.81 (q), δ 27.58 (q), δ 25.93 (q), δ 19.49 (q), δ 12.28 (t), δ 10.23 (t). Mass spectrum of **180** (m/e): 41 (52), 43 (97), 59 (100), 91 (47), 105 (28), 119 (12), 147 (26), 159 (10), 163 (10), 187 (10), 202 (16).

Cyclisation of 177: To a stirred solution of 55 mg of **177** in 5.5 ml of acetone was added 5.5 ml of 0.1 M H_2SO_4. The resulting mixture was stirred for 30 minutes, extracted with 4 x 10 ml of CH_2Cl_2, the combined organic layers were washed with 50 ml of brine, dried on $MgSO_4$ and evaporated. The resulting residue was chromatographed to give 7.5 mg of **182** and 8.5 mg of **183**.

^{1}H-NMR of **182**: δ 5.32 (m, 1H, H_3); δ 3.48 (broad s, 1H, H_9); δ 2.73 (m, 1H, H_6); δ 2.59 (ddd, 1H, H_8, J = 13.9 Hz, 5.1 Hz, 1.8 Hz); δ 2.38 (broad d, 1H, H_8, J = 13.9 Hz); δ 2.08 (m, 2H, H_2); δ 1.84 (m, 1H, H_1); δ 1.76 (d, 3H, Me_{12} or Me_{13}, J = 1.2 Hz); δ 1.74 (s, 3H, Me_{13} or Me_{12}); δ 1.70 (dt, 3H, Me_{15}); δ 1.75-1.70 (m, 2H); δ 1.09 (dt, 1H, H_1, J = 12.9 Hz, 4.8 Hz); δ 0.98 (s, 3H, Me_{14}). ^{13}C-NMR of **182**: δ 136.54 (s), δ 126.83 (s), δ 126.24 (s), δ 120.40 (d), δ 76.18 (d), δ 44.90 (d), δ 37.23 (s), δ 33.75 (t), δ 32.26 (t), δ 27.21 (t), δ 23.17 (t), δ 22.90 (q), δ 20.68 (q), δ 20.54 (q), δ 20.52 (q). Mass spectrum of **182** (m/e): 41 (100), 43 (55), 55 (54), 67 (47), 83 (41), 91 (39), 93 (55), 107 (46), 108 (33), 109 (47), 119 (13), 121 (14), 145 (10), 159 (10), 187 (16), 202 (9), 220 (18).

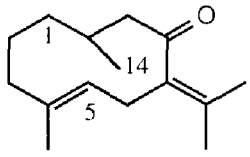
^{1}H-NMR of **183**: δ 3.39 (broad s, 1H, H_9); δ 2.50-2.35 (m, 3H); δ 1.71 (s, 3H, Me_{12} or Me_{13}), δ 1.70 (s, 3H, Me_{13} or Me_{12}); δ 2.20-1.40 (m, 10H); δ 1.23 (s, 3H, Me_{15}); δ 1.17 (s, 3H, Me_{14}). ^{13}C-NMR of **183**: δ 126.66 (s), δ 125.75 (s), δ 76.63 (d), δ 73.51 (s), δ 49.95 (d), δ 38.51 (s), δ 36.68 (t), δ 33.24 (t), δ 30.78 (t), δ 29.84 (q), δ 28.12 (t), δ 24.79 (q), δ 20.24 (q), δ 20.09 (q), δ 18.39 (t). Mass spectrum of **183** (m/e): 41 (47), 43 (100), 55 (26), 93 (21), 107 (19), 119 (10), 135 (17), 159 (12), 160 (15), 187 (8), 220 (4), 238 (0.6).

5.5. References and notes

1. Piet D.P., Schrijvers R., Franssen M.C.R. and de Groot Ae., *Tetrahedron*, **51**, 6303 (1995).
2. Ognyanov I. and Ivanov D., *Perfumery Essent. Oil Record*, **49**, 617 (1958).
3. Weinhaus H. and Scholtz H., *Ber. Schimmel & Co., Jubiläums Ausgabe*, 280 (1929).
4. Ognyanov I., Ivanov D., Herout V., Horák M., Plíva J. and Sorm F., *Chem. Ind. (London)*, 820 (1957).
5. Herout V., Horák M., Schneider B. and Sorm F., *Chem. Ind. (London)*, 1089 (1959).
6. For a review of these rearrangement reactions see: Bennett G.B., *Synthesis*, 589 (1977).
7. Ohloff G., *Angew. Chem.*, **71**, 162 (1959).
8. Hikono H, Konno C., Nagashima T., Kohama T. and Takemoto T., *Tetrahedron Lett.*, **12**, 337 (1971).
9. Takahashi T., Kitamura K., Nemoto H. and Tjusi J., *Tetrahedron Lett.*, **24**, 3489 (1983).
10. Asakawa Y., Takahashi H. and Toyota M., *Phytochemistry*, **30**, 3993 (1991).
11. Sakui N., Kuroyanagi M., Ishitobi Y., Sato M. and Ueno A., *Phytochemistry*, **31**, 143 (1992).
12. Sakamoto S., Tsuchiya N., Kuroyanagi M. and Ueno A., *Phytochemistry*, **35**, 1215 (1994).

13. Shiobara Y., Asakawa S. Y., Kodama M., Yasuda J. and Takemoto T., *Phytochemistry*, **24**, 2629 (1985).
14. Hikino H., Konno C., Nagashima T., Kohama T. and Takemoto T., *Chem. Pharm. Bull.*, **25**, 6 (1977).
15. Kuroyanagi M., Ueno A., Koyama K. and Natori S., *Chem. Pharm. Bull.*, **38**, 55 (1990).
16. Yoshihara M., Yang C., Zheng C., Shibuya H., Hamamoto Y., Tanaka N. and Kitagawa I., *Chem. Pharm. Bull.*, **34**, 434 (1986).
17. Pyrek S.J., *Phytochemistry*, **24**, 186 (1985).
18. Rees S.B. and Harborne J.B., *Phytochemistry*, **24**, 2225 (1985).
19. Seto M., Miyase T., Umehara K., Ueno A., Hirano Y. and Otani N., *Chem. Pharm. Bull.*, **36**, 2423 (1988).
20. van Beek T.A., Maas P., King B.M., Leclercq E., Voragen A.G.J. and de Groot Ae., *J. Agr. Food Chem.*, **38**, 1035 (1990).
21. Orru R.V.A., Wijnberg J.B.P.A., Bouwman C.T. and de Groot Ae., *J. Org. Chem.*, **59**, 374 (1994).
22. Paddon-Row M.N., *Acc. Chem. Res.*, **15**, 245 (1982).
23. All calculations were performed using MOPAC 6.0 with the PM 3 parameter set (Molecular Simulations Inc.) running on a Silicon Graphics 4D25 computer; Steward J.J.P., *J. Comp. Chem.*, **10**, 209 (1989).
24. Johnson W.S., *Tetrahedron*, **47**, xi-1 (1991).
25. Buntel C.J. and Griffin J.H., *Evolution of Sterol and Triterpene Cyclases*. in: *Isopentenoids and Other Natural Products: Evolution and Function*, (Nes W.D., ed.), ACS Symposium Series, **562**, 52 (1994).
26. Orru R.V.A., Wijnberg J.B.P.A. and de Groot Ae., *J. Org. Chem.*, **26**, 4233 (1995).
27. Garcia-Granados A., Molina A. and Cabrera E., *Tetrahedron*, **42**, 81 (1986).
28. Kuroyanagi M., Ueno A., Ujiie K. and Sato S., *Chem. Pharm. Bull.*, **35**, 53 (1987).
29. Posner G.H., Kevin A.B., Loomis G.L., Frazee W.J., Ramsharan D.M. and Karle I.L., *J. Am. Chem. Soc.*, **102**, 7498 (1980) and references cited therein.
30. Parker W. and Roberts J.S., *Quart. Rev.*, **21**, 311 (1967).
31. Tsankova E., Cerrini S. and Lamba D., *Tetrahedron*, **39**, 3397 (1983).
32. Simova S.D., Bozhkova N.V. and Orahovats A.S., *Org. Magn. Res.*, **22**, 431 (1984).
33. Tsankova E., *Tetrahedron Lett.*, **23**, 771 (1982).
34. Takeda K., Horibe I. and Minato H., *J. Chem. Soc., Perkin I.*, 2212 (1973).
35. Ognyanov I., Ivanov D., Herout V., Horak M., Pliva J. and Sorm F., *Coll. Czech. Chem. Comm.*, **23**, 2033 (1958).

36. Clark Jr. B.C., Chamblee T.S. and Iacobucci G.A., *J. Agr. Food Chem.*, **35**, 514 (1987).
37. Imuta M. and Ziffer H., *J. Org. Chem.*, **44**, 1351 (1979).
38. Brown E.D., Sutherland J.K. and Sam T.W. *J. Chem. Soc. Perkin I*, 2332 (1975).
39. Murray R.W. and Jeyaraman R., *J. Org. Chem.*, **50**, 2847 (1985).
40. Browne L.M., Klinck R.E. and Stothers J.B., *Can. J. Chem.*, **57**, 803 (1979).
41. Browne L.M., Klinck R.E. and Stothers J.B., *Org. Magn. Reson.*, **12**, 561 (1979).
42. Dalling D.K., Grant D.M. and Johnson L.F., *J. Am. Chem. Soc.*, **93**, 3678 (1971).
43. 'Zdravets' oil was purchased from Prof. Dr. Gantchev, Bulgarska Rosa Plovdiv Ltd, Bulgaria.
44. DMSO-d_6 and CD_3OD were added to enhance solubility.
45. The $LiAlH_4$ reduction of **166** also gave 8% of 1,4-reduction.



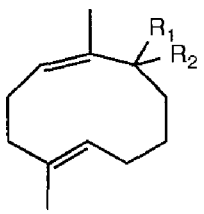
^{1}H-NMR: δ 5.16 (t, 1H, H_5, J = 8.1 Hz); δ 2.93 (d, 2H, H_6, J = 8.0 Hz); δ 2.20 (m, 2H); δ 1.85 (m, 3H); δ 1.65, 1.60, 1.56 (3 x s, 3 x 3H, Me_{12}, Me_{13}, Me_{15}); δ 1.33 (m, 3H); δ 1.15 (m, 1H); δ 0.82 (d, 3H, Me_{14}, J = 6.8 Hz). The ^{13}C-NMR could not be completely interpreted due to coalescence effects.

6. Synthesis and biotransformation of (E,Z)-cyclodeca-1,5-dienes

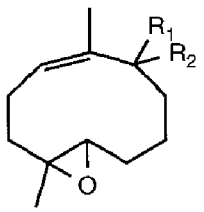
6.1. Introduction

As already discussed in chapter 2, the isolation of configurationally isomeric germacranes together with various cyclisation products might suggest a possible biogenetic relationship between these compounds. In chapter 5, it became clear that transannular cyclisation reactions are not restricted to the (E,E)-cyclodecadiene framework, since epoxides of (E,Z)-1,6-germacranes could also be cyclised, either or not mediated by *C. intybus*. It also became clear from chapter 2, that oxidative modifications of melampolides and their epoxides are favoured over a transannular cyclisation reaction from one double bond to another or to the epoxide. Ring substituents were involved in the cyclisation process, or the monocyclic framework remained intact. In this chapter, the role of the Z-double bond and functional groups in a (E,Z)-cyclodeca-1,5-diene system on the stereochemistry of the cyclised products is discussed to further study the substrate specificity of the chicory root cyclases. For that purpose, a number of substrates possessing the (E,Z)-cyclodeca-1,5-diene framework and their epoxides are synthesised (Figure 6.1.) and their biotransformation by a chicory root homogenate is discussed.

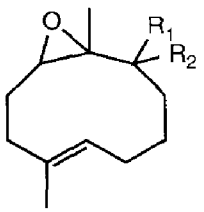
Figure 6.1.



184: $R_1, R_2 = O$
189: $R_1 = -OH, R_2 = -H$



191: $R_1, R_2 = O$
195: $R_1 = -OH, R_2 = -H$
201: $R_1, R_2 = -H$



211: $R_1 = -OH, R_2 = -H$
214: $R_1, R_2 = O$

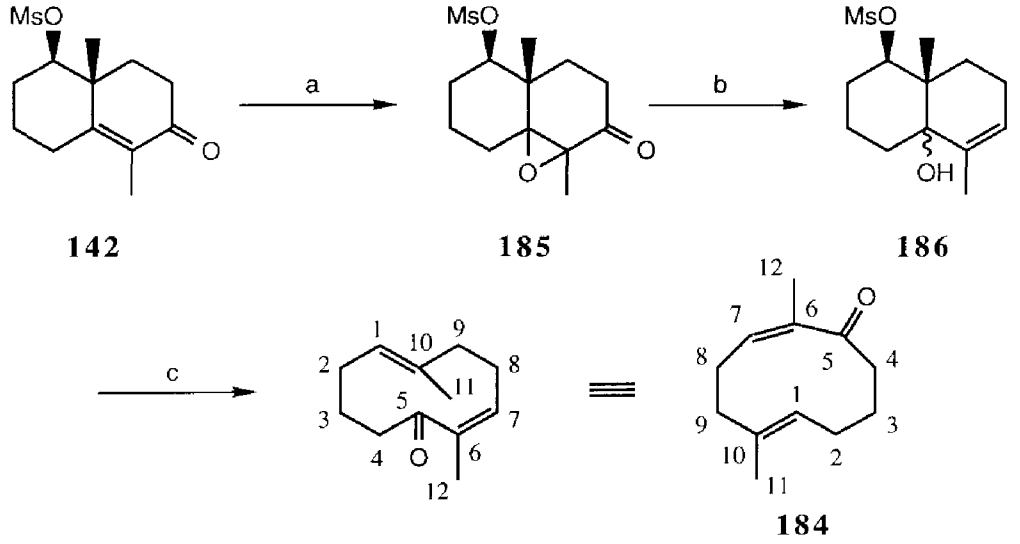
6.2. Synthesis and biotransformation of (E,Z)-cyclodeca-1,5-dienes

6.2.1. (Z,E)-2,6-dimethyl-cyclodeca-2,6-dien-1-one (184)

The starting material for the synthesis of **184** was the readily available mesylate **142**[2]. The α,β-unsaturated double bond of **142** was epoxidised using H_2O_2 to give **185**.

Subsequently, the epoxide was opened by a hydrazine treatment to give alcohol **186**. (E,Z)-cyclodeca-1,5-dienone **184** was prepared by fragmentation of the alcohol **186** with potassium *tert*-butoxide in *tert*-butyl alcohol (Scheme 6.1.)[3].

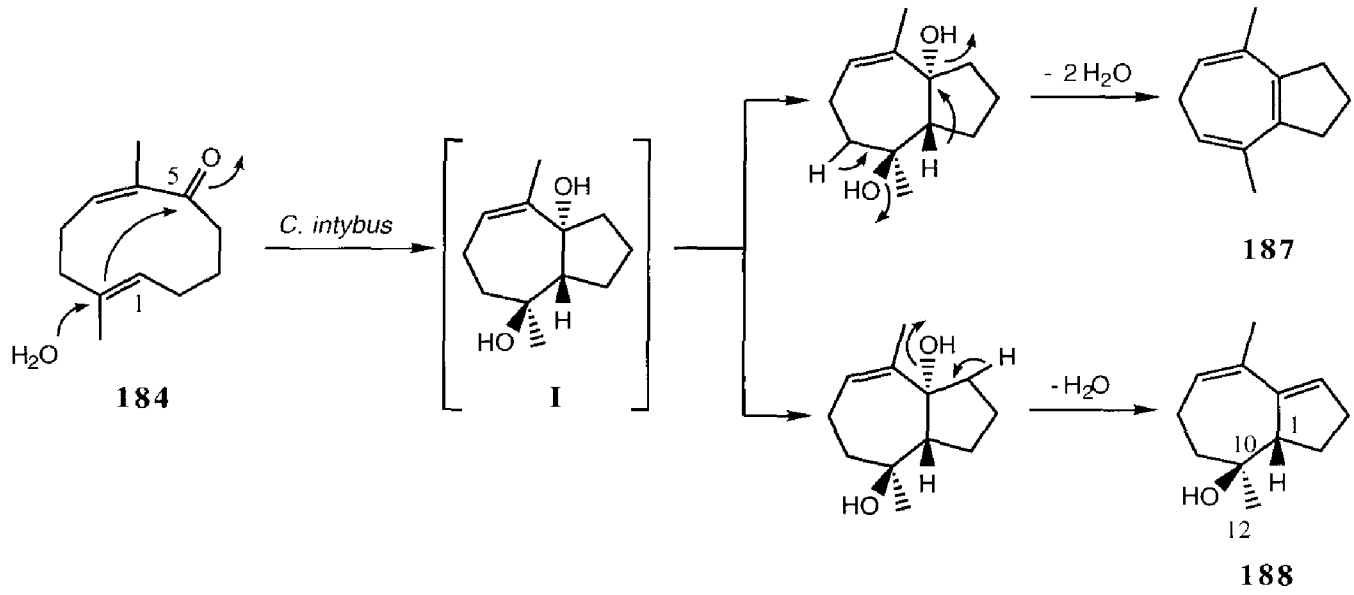
Scheme 6.1.[a]



[a] a) H_2O_2/NaOH; b) NH_2NH_2/NaOAc; c) *t*-BuOK/*t*-BuOH.

When (E,Z)-cyclodeca-1,5-dienone **184** was administered to a root suspension of fresh chicory and incubated for 10 days, a 3 : 7 mixture of triene **187** and hydroazulene **188** was obtained, as outlined in scheme 6.2. Both controls showed no conversion. Both the ^{1}H- and ^{13}C-NMR spectra of the minor cyclisation product (**187**) were surprisingly simple, indicating a plane of symmetry in the molecule. The only way for such a symmetrical molecule to be formed is if cyclisation is initiated by a carbon-carbon bond formation between C_1 and C_5 to give intermediate **I**, as outlined in scheme 6.2., followed by the successive elimination of two molecules of water. Elimination of the bridgehead hydroxyl function to give the double bond in the five-membered ring, as outlined in scheme 6.2., has to account for the formation of the major cyclisation product (**188**). Irradiation of the bridgehead proton (δ 3.02) did not show a NOE-effect on the methyl group at C_{10} (δ 1.10) and *vice versa*. This means that both groups are situated at different sides of the molecule. The ^{13}C-NMR spectrum of **188** showed a quaternary oxygen-bearing carbon together with two quaternary and two tertiary olefinic carbons. The ^{1}H-NMR spectrum of **188** showed two olefinic protons with a different multiplicity and a methyl group attached to a oxygen-bearing tertairy carbon atom. These observations show that a hydroxyl group is present in **188**.

Scheme 6.2.



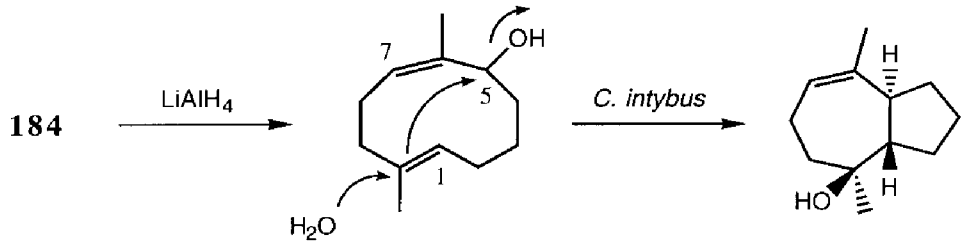
This mode of cyclisation confirms, in part, the tentative biosynthesis of montahibisciolide **61** from melampolide **60** in *M. leucantha*, as outlined in section 2.3.2.2. and scheme 2.13. The cyclisation of **60** was believed to start with a carbon-carbon bond formation between C_1 and the keto function at C_5, identical to the cyclisation observed for **184**. Probably, the presence of a lactone moiety and a tiglate group in **60** are responsible for a different mode of proton abstraction and prevent the elimination of a water molecule from **61**.

6.2.2. (Z,E)-2,6-dimethyl-cyclodeca-2,6-dien-1-ol (189)

Reduction of **184** using $LiAlH_4$ gave (E,Z)-cyclodeca-1,5-dienol **189** in almost quantitative yield. When **189** was administered to a root suspension of fresh chicory and incubated for 42 hours, hydroazulene **135** was obtained as the sole product. Compound **135** has been observed before as the major cyclisation product of (E,E)-cyclodeca-1,6-dienol **132** (Scheme 4.2.). Both controls showed no conversion (Scheme 6.3.).

Contrary to the cyclisation of **132** (Scheme 4.3.), ionisation of the allylic (E,Z)-alcohol **189** will lead to either a U-shaped cation (not a W-shaped cation as is formed by **132**) or a sickle-shaped cation, as outlined in scheme 6.4. In chapter 4 it was postulated that the W-cation is necessary in order to permit isomerisation of an allylic (E,E)-cyclodeca-1,6-dienol (**132**) into an allylic (E,E)-cyclodeca-1,5-dienol (**144**).

Scheme 6.3.

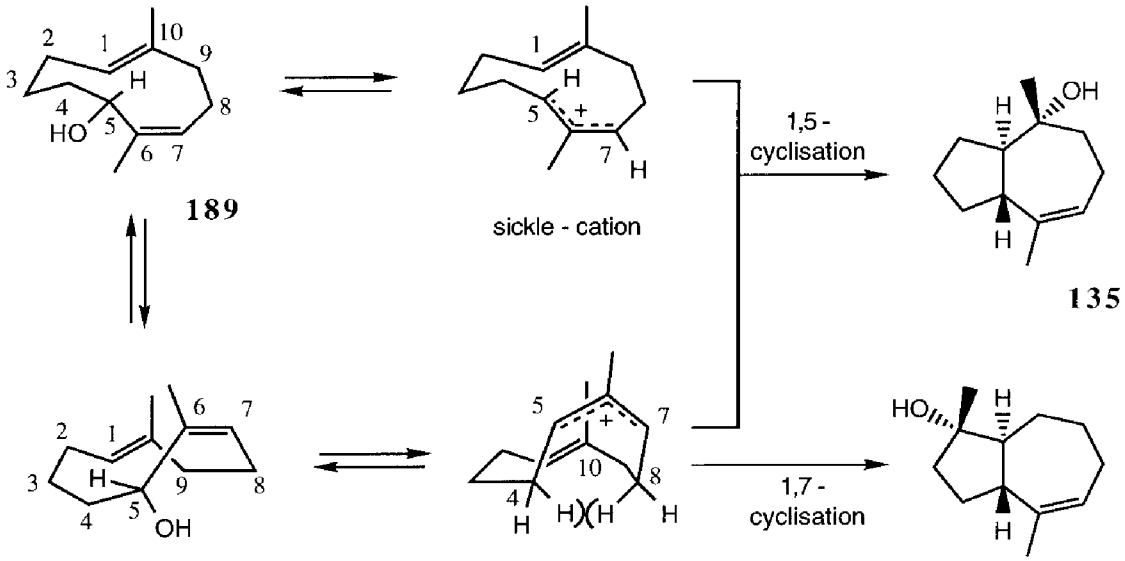


189 **135**

This allylic (E,E)-cyclodeca-1,5-dienol is, after a rotation around the C_5-C_6, C_7-C_8 bond, followed by isomerisation and 1,7-cyclisation, the precursor of the minor cyclisation product **138** (section 4.2., scheme 4.3.). This 1,7-cyclisation product, however, was not detected in the cyclisation reaction of **189**.

Ionisation of **189** would yield the sickle-shaped cation as postulated in scheme 6.4. 1,7-Cyclisation through this intermediate would lead to a highly strained trans double bond in the cycloheptane ring, whereas 1,5-cyclisation would give the energetically more favourable cis double bond. Rotation around the C_5-C_6,C_7-C_8 bond yields rotamer **190** which, after ionisation, gives the U-shaped cation.

Scheme 6.4.



In principle, both the 1,5- and the 1,7-cyclisation pathway through the U-cation can occur, giving the corresponding cyclisation products. However, studies of molecular

models of the U-cation revealed that this intermediate is highly strained and that there is a severe hindrance between the protons at C_4 and C_8, as outlined in scheme 6.4. The fact that no 1,7-cyclisation product is formed probably means that the energy barrier towards the formation of the U-shaped cation is either to high or, if the cation is formed, the 1,5-cyclisation predominates the 1,7-cyclisation pathway.

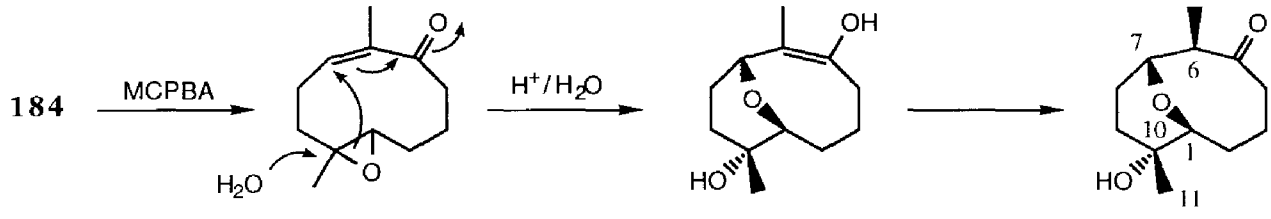
6.3. Biotransformation of (E,Z)-cyclodeca-1,5-diene epoxides

6.3.1. The E-epoxides

6.3.1.1. Z-2,6-dimethyl-6,7-epoxycyclodec-2-enone (191)

Epoxidation of **184** using MCPBA gave the E-epoxide **191** as the sole product. Unfortunately, after a 10 days incubation period with a root suspension of fresh chicory, no reaction was observed. Acid-induced cyclisation of **191** in an aqueous medium, however, gave the cyclic ether **192** as the main product (50%) and three minor compounds which could not be identified (Scheme 6.5.).

Scheme 6.5.



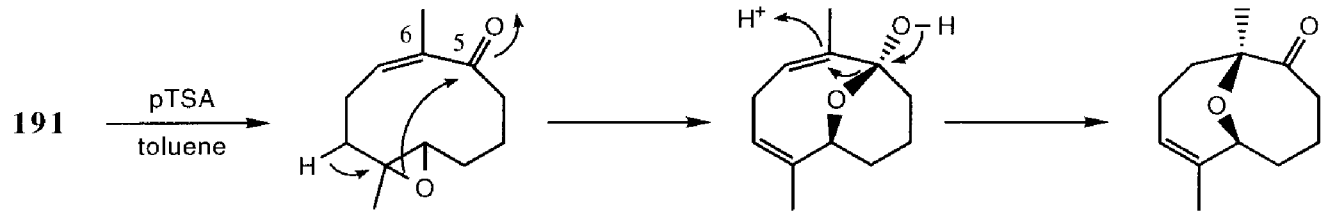
191 **192**

Using COSY and ^1H-^{13}C correlated NMR techniques, the relevant protons and carbon atoms of **192** could be assigned. The cyclic ether **192** is the product of an internal Michael addition of the epoxide followed by incorporation of a water molecule at C_{10}. The relative stereochemistry at the carbon atoms C_1 and C_{10} was already fixed in the E-epoxide **191**. Therefore, the relative stereochemistry of C_7 is known since the attack of the epoxide on C_7 must have occurred from the same side of the molecule at which Me_{11} is situated. The stereochemistry of the methyl group at C_6 was deduced from molecular models and semi-empirical calculations. The ΔH_f-value of **192**, as depicted in scheme 6.5., was calculated to be 3.4 Kcal/mol lower than its C_6-epimer. Since cyclisation was performed in an acidic environment, the secondary methyl group,

situated next to the keto function, will equilibrate to the energetically most favourable configuration.

Chemically induced cyclisation of **191** in an anhydrous medium gave the cyclic ether **193** as the main product (59%) and several minor compounds which could not be identified (Scheme 6.6.).

Scheme 6.6.



194 **193**

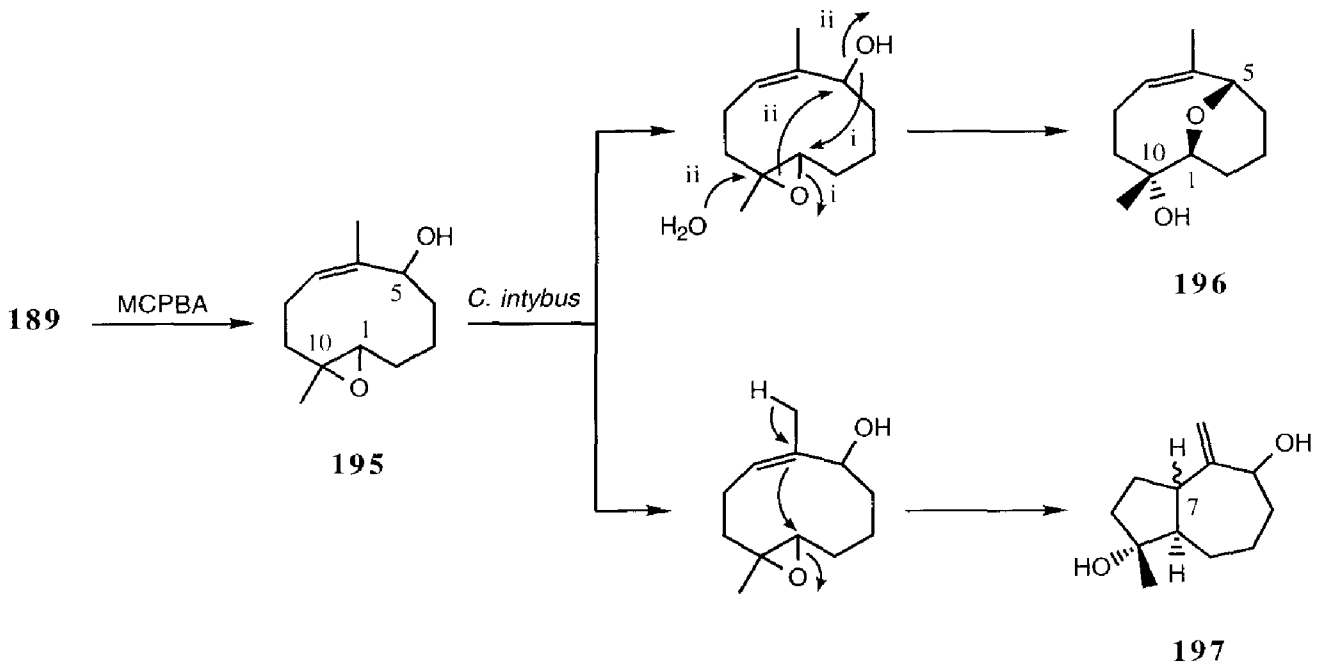
A direct nucleophilic 1,3-addition of the epoxide at C_6 to give **193** seems very unlikely since C_6 is the carbon atom which has the highest electron density within the enone system. A possible cyclisation mechanism might start with a nucleophilic attack of the epoxide at C_5 to give the intermediate lactol **194**. Protonation of the C_6-C_7 double bond followed by a 'pinacol-like' rearrangement[4] would account for the formation of the cyclic ether **193**.

The difference in product formation obtained through cyclisation of **191**, i.e. **192** *vs.* **193**, must be due to a solvent effect. The solvent plays an important role in the (de)stabilisation of the charge distribution in the enone system. The dielectric constant of a solvent is a good indicator of the ability of that solvent to accommodate charge separation. Water, a polar solvent with a high dielectric constant, is one of the best-known ionising solvents[5]. This means that in an aqueous environment the α,β-unsaturated moiety of **191** is highly polarised, i.e. the δ^+-value of C_7 is increased compared to C_5. The nucleophile, in this case the epoxide, therefore will attack at C_7 rather than C_5, giving the cyclic ether **192** as the main product. Non-polar solvents like toluene are not effective in stabilising the development of charge separation. This means that the δ^+-value at C_5 in **191** is enhanced in non-polar solvents compared to C_7. Nucleophilic attack of the epoxide at C_5 gives, *in situ*, the lactol **194**, which rearranges *via* a 'pinacol-like' mechanism under the acidic reaction conditions into the cyclic ether **193**.

6.3.1.2. Z-2,6-dimethyl-6,7-epoxycyclodec-2-enol (195)

Epoxidation of **189** using an equimolar amount of MCPBA gave the E-epoxide **195**. When **195** was incubated for 10 days with a root suspension of fresh chicory, 8% of starting material was detected together with a 3 : 1 mixture of cyclic ether **196** and hydroazulene diol **197** (total 38%) together with several minor products which could not be identified (Scheme 6.7.) In both controls an identical conversion was observed but instead of numerous minor products, ca. 50% of starting material was still present.

Scheme 6.7.



It became clear from the MS-spectrum that the molecular weight of **196** was identical to that of **195**. Since three oxygen-bearing carbons were present in the ^{13}C-NMR spectrum, one quaternary and two tertiary, a transannular ether formation has to account for the formation of the major cyclisation product. The conclusion that the ether bridge was formed between C_1 and C_5 and not between C_{10} and C_5 was based on two observations. Firstly, the characteristic double doublet multiplicity of a $-CR_2\underline{H}(OH)CH_2R-$ moiety was not present in the ^{1}H-NMR spectrum. Secondly, the distance between C_1 and C_5 is shorter as compared to C_1 and C_{10}.

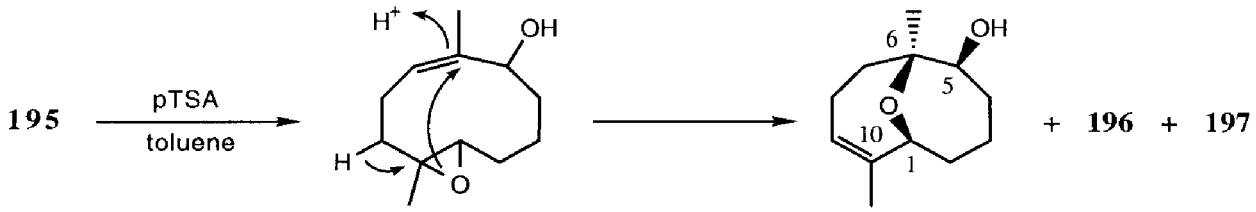
There are two possible mechanisms that might explain the formation of **196**. In the first mechanism, cyclisation is initiated by protonation of the epoxide followed by an intramolecular attack of the C_5-hydroxyl group at C_1 and subsequent opening of the epoxide (route i). A second mechanism starts with protonation of the allylic alcohol

group at C_5 followed by an intramolecular attack of the epoxide and subsequent incorporation of a water molecule at C_{10} (route ii). It is not yet clear which mechanism is preferred.

The exocyclic methylene unit of **197** was clearly present in both the ^{1}H- and ^{13}C-NMR spectra. Also present in the ^{13}C-NMR spectrum of **197** were one quaternary and one tertiary oxygen-bearing carbon atom as well as two tertiary carbon atoms in the aliphatic region. The latter observation, combined with the fact that a methyl group bound to an oxygen-bearing quaternary carbon was present in the ^{1}H-NMR spectrum indicated a hydroazulene framework. The relative stereochemistry at C_1 and C_{10} was derived from the E-epoxide **195**. Using 2D NMR techniques it was possible to assign the relevant protons and carbon atoms. Unfortunately, it was not possible to deduce the ring fusion of the hydroazulene framework by means of NOE-difference spectroscopy. However, the position of the bridgehead proton H_7 is found at rather low field (δ 3.43) in the ^{1}H-NMR spectrum of **197**. From the structure elucidation of natural and synthetic dictamnol (**140** and **152**, respectively, see section 4.3.) and from the work of Gijsen[6] it is known that bridgehead protons adjacent to an exocyclic methylene unit in a cis-fused hydroazulene framework resonate at low field in the ^{1}H-NMR spectrum as compared to their trans-fused counterparts. However, due to the absence of a C_7 epimer of **197**, assigning the ring fusion of **197** as cis is rather speculative.

Acid-induced cyclisation of **195** in an anhydrous environment gave a 1 : 2 : 3 mixture of cyclic ether **198**, cyclic ether **196** and hydroazulene diol **197** (total 62%) and several minor compounds which could not be identified (Scheme 6.8.).

Scheme 6.8.



198

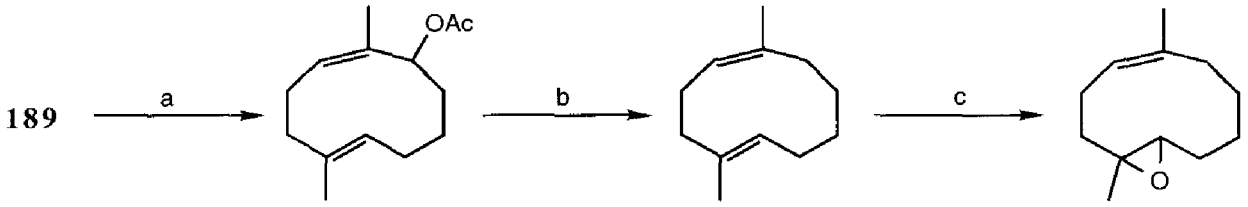
One of the protons in the ^{1}H-NMR spectrum of **198** bound to an oxygen-bearing carbon showed the characteristic double doublet multiplicity of a $-CR_2\underline{H}(OH)-CH_2R-$ moiety with a large J-value, indicating an axial position of H_5 and thus an equatorial

position for the hydroxyl group at C_5. Formation of the ether bridge between C_5 and C_{10} may also lead to a cyclisation product which matches the NMR data. However, analogous to the formation of **193** from **191** (Scheme 6.6.), ether bridge formation between C_1 and C_6 is most likely to be the preferred pathway. Due to the absence of the keto function at C_5, the C_6-C_7 double bond is likely to be protonated in a similar fashion to that of the intermediate lactol **194**, as outlined in scheme 6.6. Also, the distance between C_5 and C_{10} is considerably larger as compared to C_5 and C_1. An attack of the C_5-hydroxyl group at the epoxide will therefore take place at C_1 rather than at C_{10} leading to the formation of a product with two protons attached to an ether bridge. This structure is not compatible with the observed signals and is therefore rejected.

6.3.1.3. Z-2,6-dimethyl-6,7-epoxycyclodec-2-ene (201)

From the preceding paragraphs it became clear that the E-epoxides of (E,Z)-cyclodeca-1,5-dienes preferably cyclise by means of a transannular ether-bridge formation instead of transannular carbon-carbon bond formation, as observed in chapters 3–5. To exclude the participation of the 'interfering' C_5-substituents in the cyclisation reaction, (E,Z)-cyclodeca-1,5-diene **200** was synthesised (Scheme 6.9.). Epoxidation of **200**, obtained in two steps from **189**, with one equivalent of MCPBA gave the E-epoxide **201** in good yield.

Scheme 6.9.[a]

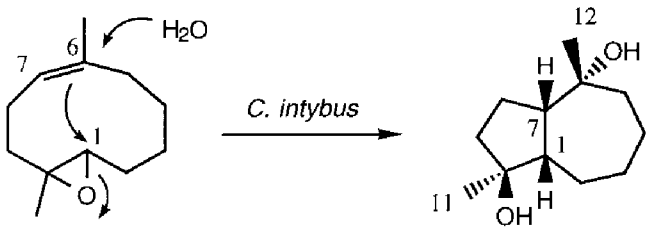


199 **200** **201**

[a] a) Ac_2O/pyr.; b) Li/NH_3; c) MCPBA.

When **201** was incubated with a chicory root suspension for 3 days, hydroazulene diol **202** was obtained as the sole product. Both controls showed an identical conversion, only in a 40% yield (Scheme 6.10.). The ^{13}C-NMR showed the characteristic downfield shift for the methyl group attached to C_6 indicating the syn relationship between Me_{12} and H_7[7] which was locked in the E-epoxide **201**.

Scheme 6.10.

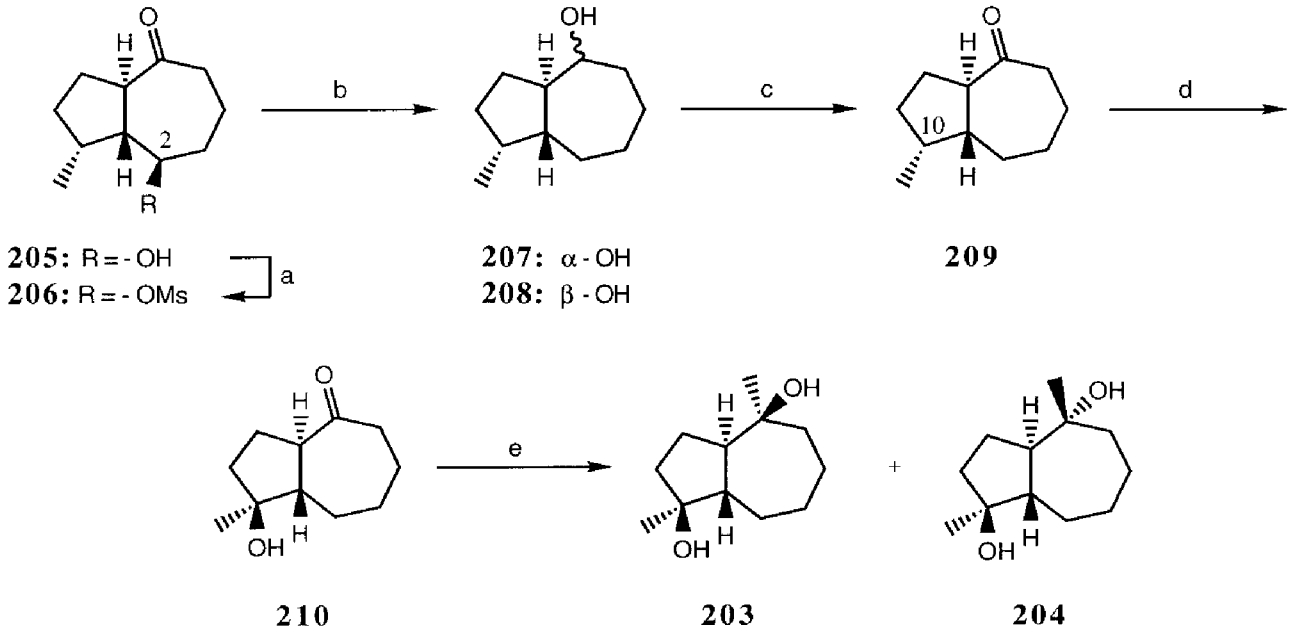


201 **202**

Since the relative stereochemistry at C_1 and C_{10} was also locked in the E-epoxide **201**, the only question that had to be resolved is the ring fusion of the hydroazulene framework. This ring fusion could not be established by means of 2D NMR spectroscopy because the ring protons as well as the methyl groups overlap each other in the ^{1}H-NMR spectrum.

The answer was found in the total synthesis of the two epimeric hydroazulene diols **203** and **204** from the known keto alcohol **205**[6] (Scheme 6.11.). The C_2-hydroxyl group of **205** was treated with MsCl to give mesylate **206** which was reduced with $LiAlH_4$ to give a 1 : 1 mixture of the epimeric alcohols **207** and **208**.

Scheme 6.11.[a]

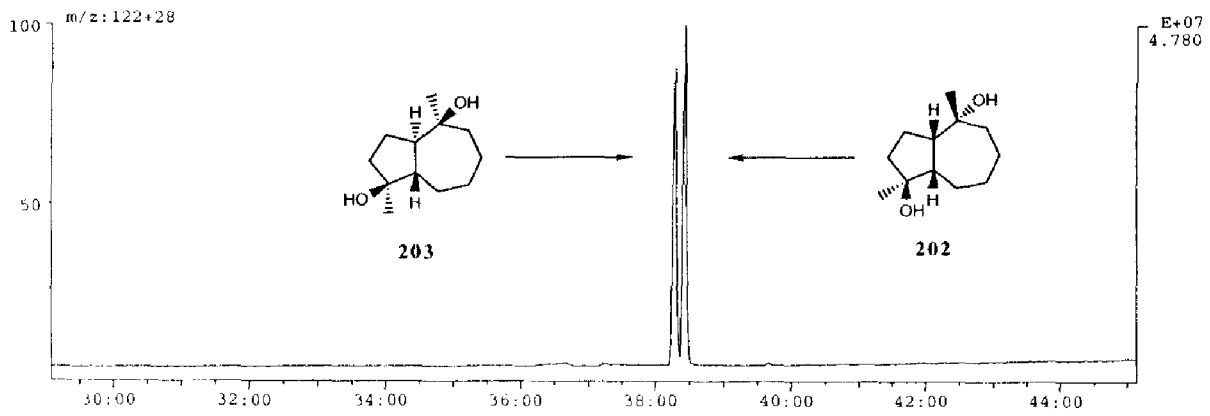


[a] a) MsCl; b) $LiAlH_4$; c) PDC/DMF; d) $RuO_2/NaIO_4$; e) MeLi.

The crude mixture was oxidised using PDC in DMF to yield the trans-fused hydroazulene **209**. The crucial step in the synthesis of **203** and **204** is the hydroxylation of the five membered ring at C_{10}. This was achieved using ruthenium(IV)oxide (RuO_2) and sodium periodate ($NaIO_4$) in a mixture of CCl_4, MeCN and H_2O at 50°C[8]. Unfortunately, forcing the reaction to completion led to a very poor yield as a result of over-oxidation. Treatment of the keto alcohol **210** with MeLi at 0°C gave a 8 : 1 mixture of the desired diols **203** and **204**, as outlined in scheme 6.11. The crude mixture of **203** and **204** was co-injected with **202** on the GC-MS. From this co-injection it became clear that the hydroazulene diols **202**, **203** and **204** are three different compounds on the basis of their different retention time.

Figure 6.2. showes the co-injection of a mixture of purified **203** and **202**. It is therefore concluded that transannular cyclisation of **201** yields the cis-fused hydroazulene diol **202** with the stereochemistry as depicted in scheme 6.10.

Figure 6.2.



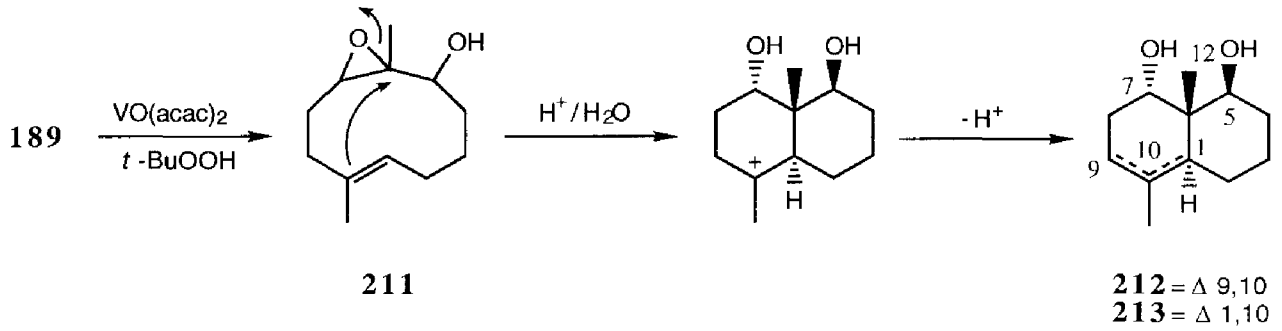
6.3.2. The Z-epoxides

6.3.2.1. E-2,6-dimethyl-2,3-epoxycyclodec-2-enol (211)

(E,Z)-cyclodeca-1,5-dienol **189** was epoxidised using the Sharpless epoxidation reaction[9] to give the Z-epoxide **211** in good yield. When **211** was incubated for 10 days with a root suspension of fresh chicory, no product formation could be detected. Acid-induced cyclisation of **211** in an aqueous medium gave a 4 : 7 mixture of eudesmane

diols **212** and **213** (total 48%) and several minor compounds which could not be identified (Scheme 6.12.).

Scheme 6.12.



211

212 = Δ 9,10
213 = Δ 1,10

The ^{13}C-NMR spectrum of **212** showed a quaternary and a tertiary carbon atom in both the olefinic and the aliphatic region as well as two tertiary oxygen-bearing carbon atoms. By means of NOE-difference spectroscopy, the relative stereochemistry at C_5, C_6 and C_7 could be determined. From a mechanistic point of view, the position of the alcohol group at C_7 and Me_{12} has to be anti, since their stereochemistry was already locked in the Z-epoxide **211**. Irradiation of Me_{12} (δ 1.21) resulted in a clear NOE on H_7 (δ 3.62) but not on H_5. This indicates that H_7 and Me_{12} are located at the same side of the molecule and that H_5 is situated at the opposite side.

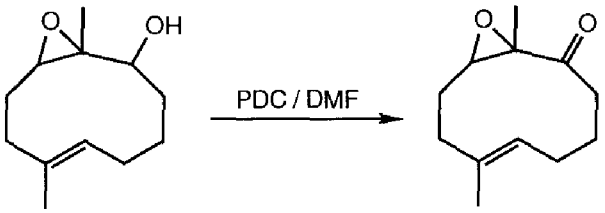
The ring fusion of **212** could not be established with NOE-difference experiments since the position of the bridgehead proton H_1 was obscured by other signals. However, it is known from the work of Kesselmans that cis-fused decalins differ significantly from their trans-fused counterparts in their ^{13}C-NMR spectrum[10]. The bridgehead methyl group of trans-fused decalins resonates at approximately 15-20 ppm while for cis-fused decalines this value is approximately 30 ppm. In the ^{13}C-NMR spectrum of **212**, the resonance of Me_{12} was found to be 16.00 ppm. It was therefore concluded that **212** possesses a trans-fused decalin framework.

6.3.2.2. E-2,6-dimethyl-2,3-epoxycyclodec-2-enone (214)

Direct epoxidation of the α,β-unsaturated double bond of **184** with H_2O_2 was unsuccesful. Therefore, Z-epoxide **211** was oxidised using PDC in DMF to give the desired Z-epoxide ketone **214** in good yield (Scheme 6.13.). When **214** was incubated for 10 days with a root suspension of fresh chicory, 77% of starting material was still

present together with 3 products in a 1 : 2 : 2 ratio which could not be identified. Both controls showed an identical conversion. Also chemically induced cyclisation reactions in both aqueous and anhydrous media gave no unambiguous product formation. No further cyclisation studies on **214** were undertaken.

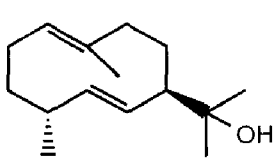
Scheme 6.13.



211 **214**

6.4. Concluding remarks

The cyclisation reactions of the (E,Z)-cyclodeca-1,5-dienes as presented in this chapter have shed some light on the role of heliangolides and melampolides as intermediates in the biosynthesis of sesquiterpene secondary metabolites. The reluctance of the (E,Z)-cyclodeca-1,5-diene framework to undergo a 'regular' transannular cyclisation reaction, i.e. a carbon-carbon bond formation between both double bonds of the germacrane skeleton, is blamed on the considerably greater centre to centre distance between those double bonds[11]. An additional explanation can be found in the UV-absorption spectra of germacranes and cyclodecadienes. One of the striking properties of both 1,5- and 1,6-(E,E)-germacranes is an anomalous UV-absorption at approximately 210-215 nm due to a spatial overlap of the π-lobes of the double bonds in the cyclodecadiene ring system[12]. This overlap is the driving force behind the C-C bond formation in transannular cyclisation reactions of (E,E)-1,5-germacranes and, possibly, the (E,E)-1,6-germacranes. The UV-spectra of the (E,E)-cyclodeca-1,5-dienes **100**, **101**, **132** and **170**, as well as the (E,E)-1,6-germacrane allohedycaryol (**215**), indeed showed an anomalous absorption at approximately 213 nm. This absorption was not found in the UV-spectra of the (E,Z)-cyclodecadienes **175**, **189** and **200** which means that a spatial overlap between both double bonds in the (E,Z)-cyclodeca-1,5- and 1,6-dienes is not present and, therefore, a transannular C-C bond formation is not favoured. Instead, ring substituents are involved in the cyclisation process to relief ring strain which often leads to unusual products.



215

One of the intentions of this chapter was to investigate the role of the double bond stereochemistry on the ring fusion of the cyclised products. The cyclisation reaction of two compounds fulfilled that intention, the E-epoxide **201** and the Z-epoxide **211**. Cyclisation of **201** gave a cis-fused hydroazulene while the latter yielded a trans-fused decalin. In all other compounds, the functional groups present on the molecule interferred with the cyclisation process to yield a variety of products.

The co-occurrence of the melampolide-4,5-epoxide baileyin (**53**, see section 2.3.2.2.) together with the trans-fused guaianolide pleniradin (**54**) in *Baileya pleniradiata* and *B. multiradiata*[13] is in contrast with the outcome of the cyclisation experiments on **201**. However, baileyin (**53**) possesses a lactone moiety at C_7-C_8 which might exert a directing effect on the mode of cyclisation.

In general, cyclisation reactions of (E,Z)-cyclodeca-1,5-dienes and their epoxides, initiated by *C. intybus*, starts with protonation of the substrate, a mode of action also observed in chapters 3-5. A transannular cyclisation reaction followed by stereo-selective incorporation of a water molecule is observed in all cyclisation reactions mediated by a chicory root homogenate, except in the formation of the symmetrical triene **187** from **184**. This competing deprotonation process is probably favoured on sterical grounds.

Much work still needs to be done in order to obtain more insight in the role of the (E,Z)-cyclodeca-1,5-diene framework in the biosynthesis of sesquiterpenes. Cyclisation experiments on (to be) isolated configurationally isomeric germacranes, followed by simple comparison of the cyclised product(s) with the contents of the host organism, will contribute to the process of understanding the biosynthesis of sesquiterpenes in nature.

6.5. Experimental

Chemical shifts are reported relative to TMS (δ 0.00) at 90, 200 and 400 MHz for ^{1}H-NMR and at 25.3 and 50.6 MHz for ^{13}C-NMR. All spectra were recorded in $CDCl_3$. Mass spectral data were obtained with a Hewlett Packard 5890 GC-MS equipped with a Hewlett Packard 5970 series mass selective detector using capillary DB-5 and DB-17 columns (30 m x 0.25 mm, d_f 0.25 μm) and helium as the carrier gas. co-injection of **202** and **203** was performed on Supelcowax 10 (60 m x 0.25 mm, d_f 0.25 μm) on a Finnigan MAT 95 mass spectrometer using nitrogen as the carrier gas. Column chromatography was performed on Merck silicagel 60 and deactivated Al_2O_3 (grade III) using petroleum ether (bp. 40-60, PE) - EtOAc as the solvent system.

All solvents were distilled before use. (E,Z)-cyclodeca-1,5-dienone **184** was synthesised according to the literature[1]. In the hydrazine reduction of **185**, NaOAc was used instead of HOAc which increased the yield of the reaction significantly.

A suspension of fresh chicory root (20% w/v) was produced by mortaring the root in a solution of 0.25 M sucrose, 3 mM Tris·HCl, 10 mM $MgCl_2$ and 0.2 % (w/v) Bovine Serum Albumin (BSA). The pH of this sucrose / Tris / $MgCl_2$/ BSA-solution (STMB) was set at 6.5 using 2-morpholino-ethanesulfonic acid (MES). The stability of the substrates towards the buffer and an inactivated chicory root sample (obtained by boiling the root suspension for 30 min) was investigated as a control to test the possibility of non-enzymatic reactions. Incubations were performed in sealed 4 ml vials at room temperature in a KS 500 shaker at 260 rpm containing 200 µl root suspension, 790 µl STMB-solution and 10 µl 0.1 M substrate in EtOH. The incubation medium was extracted with 0.5 ml of EtOAc and its contents were analysed by GC-MS.

(Z,E)-2,6-Dimethyl-cyclodec-2,6-dienone (184). ^{1}H-NMR of **184**: δ 5.38 (broad t, 1H, H_7, J = 8.5 Hz); δ 4.78 (t, 1H, H_1, J = 7.4 Hz); δ 2.35-2.20 (m, 4H); δ 2.15-1.80 (m, 6H); δ 1.89 (m, 3H, Me_{12}); δ 1.45 (d, 3H, Me_{11}, J = 1.4 Hz). ^{13}C-NMR of **184**: δ 207.55 (s), δ 140.76 (s), δ 134.03 (s), δ 130.25 (d), δ 127.75 (d), δ 40.67 (t), δ 36.98 (t), δ 27.97 (t), δ 26.49 (t), δ 25.15 (t), δ 20.14 (q), δ 17.64 (q). Mass spectrum of **184** (m/e): [M^+] 178 (6), 163 (8), 160 (6), 145 (8), 135 (10), 107 (28), 93 (23), 82 (54), 67 (33), 55 (40), 53 (57), 43 (39), 41 (85), 39 (100).

Acid-induced cyclisation of 184: To a solution of 425 mg of **184** in 15 ml of acetone and 10 ml of water was added 25 drops of H_2SO_4. The resulting mixture was stirred overnight after which solid $NaHCO_3$ was carefully added. The solvent was concentrated under reduced pressure and extracted with 2 x 20 ml of EtOAc. The combined organic layers were dried on $MgSO_4$ and evaporated to give 367 mg of an oil which was chromatographed (silica; 15% of EtOAc in PE) to give 73 mg of **187** and 142 mg of **188**. ^{1}H-NMR of **187**: δ 5.08 (dt, 2H, H_7, H_9, J = 6.8 Hz, 1.2 Hz); δ 2.77 (t, 4H, H_2, H_4, J = 7.5 Hz); δ 2.01 (t, 2H, H_8, J = 6.8 Hz); δ 1.78 (t, 2H, H_3, J = 7.3 Hz); δ 1.78 (s, 6H, Me_{11}, Me_{12}). ^{13}C-NMR of **187**: δ 142.28 (2 x s), δ 133.54 (2 x s), δ 116.63 (2 x d), δ 35.55 (2 x t), δ 27.15 (t), δ 22.18 (t), δ 20.29 (2 x q). Mass spectrum of **187** (m/e): [M^+] 160 (26), 145 (100), 130 (20), 129 (18), 128 (19), 117 (23), 115 (26), 91 (26), 77 (21), 65 (18), 51 (23), 41 (23), 39 (51). ^{1}H-NMR of **188**: δ 5.70 (broad s, 1H, H4); δ 5.58 (t, 1H, H_7, J = 5.6 Hz); δ 3.02 (t, 1H, H_1, J = 5.7 Hz); δ 2.50-2.30 (m, 3H); δ 2.10-1.90 (m, 3H); δ 1.81 (broad s, 3H, Me_{12}); δ 1.78 (m, 2H); δ 1.47 (broad s, 1H, -OH); δ 1.10 (s, 3H, Me_{11}). ^{13}C-NMR of **188**: δ 143.78 (s), δ 132.63 (s), δ 128.84 (d), δ 128.77 (d), δ 75.63 (s), δ 56.64 (d), δ 44.27 (t), δ 31.27 (t), δ 26.31 (t), δ 24.06 (t), δ 23.35 (q), δ 22.47 (q). Mass spectrum of **188** (m/e): [M^+] 178 (4), 160 (10), 145 (10), 135 (28), 120 (17), 105 (50), 93 (37), 92 (19), 91 (43), 79 (37), 77 (28), 65 (18), 55 (17), 53 (16), 43 (100), 41 (41), 39 (41).

(Z,E)-2,6-Dimethyl-cyclodec-2,6-dienol (189). To a solution of 285 mg of **184** in 15 ml of dry ether, cooled to 0°C, was carefully added 190 mg of $LiAlH_4$. The grey suspension was stirred for 2.5 h, Glauber's salt

was added in small portions and the resulting mixture was stirred for 30 minutes. After addition of $MgSO_4$ the mixture was stirred for an additional 30 minutes, the solids were filtered off and the solvent was evaporated to give 282 mg of **189** as a gum. ^{1}H-NMR of **189**: δ 5.14 (t, 1H, H_7, J = 8.3 Hz); δ 4.79 (t, 1H, H_1, J = 7.3 Hz); δ 4.21 (t, 1H, H_5, J = 6.9 Hz); δ 2.20-1.95 (m, 7H); δ 1.66 (s, 3H, Me_{11} or Me_{12}); δ 1.55 (s, 3H, Me_{12} or Me_{11}); δ 1.65-1.55 (m, 3H); δ 1.40-1.20 (m, 1H). ^{13}C-NMR of **189**: δ 139.52 (s), δ 129.88 (s), δ 128,73 (d), δ 125.60 (d), δ 68.75 (d), δ 37.25 (t), δ 39.94 (t), δ 28.28 (t), δ 26.79 (t), δ 25.48 (t), δ 17.26 (q), δ 16.87 (q). Mass spectrum of **189** (m/e): [M^+] 180 (0), 162 (42), 147 (63), 133 (73), 119 (29), 107 (31), 106 (21), 105 (44), 95 (25), 94 (20), 93 (37), 79 (51), 67 (39), 55 (26), 53 (21), 43 (100), 41 (47), 39 (30).

Acid induced cyclisation of 189. To a solution of 282 mg of **189** in 10 ml of acetone and 10 ml of water was added 10 drops of concentrated H_2SO_4. The reaction mixture was stirred overnight at room temperature, 0.5 g of solid $NaHCO_3$ was added and the resulting mixture was concentrated. The aqueous phase was extracted with 2 x 20 ml of EtOAc, the combined organic layers were washed with 25 ml of brine, dried on $MgSO_4$ and evaporated to give 213 mg of an oil which was chromatographed (silica; 20% of EtOAc in PE) to give 110 mg of **135**.

Z-2,6-Dimethyl-6,7-epoxycyclodec-2-enone (191). To a stirred suspension of 42 mg of **184** in 3 ml of CH_2Cl_2, 3 ml of water and 0.5 g of solid $NaHCO_3$, cooled to 0°C, was added 60 mg of MCPBA. The reaction mixture was stirred for 30 minutes, the layers were separated and the organic layer was washed with 5 ml of brine. The combined organic layers were washed with 2 x 5 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated to give 50 mg of an oil which was chromatographed (silica; 65% of EtOAc in PE) to give 34 mg of **191**. ^{1}H NMR of **191**: δ 5.54 (broad t, 1H, H_7, J = 7.9 Hz); δ 2.63 (dd, 1H, H_1, J = 5.7 Hz, 2.5 Hz); δ 2.55-1.90 (m, 8H); δ 1.93 (broad s, 3H, Me_{12}); δ 1.30-1.10 (m, 2H); δ 1.19 (s, 3H, Me_{11}). ^{13}C NMR of **191**: δ 207.95 (s), δ 139.36 (s), δ 132.79 (d), δ 62.21 (d), δ 60.56 (s), δ 41.91 (t), δ 28.24 (t), δ 34.66 (t), δ 23.17 (t), δ 21.15 (t), δ 19.87 (q), δ 17.87 (q). Mass spectrum of **191** (m/e): [M^+]194 (0.2), 151 (2), 133 (4), 122 (12), 109 (22), 95 (18), 79 (16), 67 (20), 55 (30), 53 (20), 43 (100), 41 (52), 39 (48).

Acid induced cyclisation of 191. (aqueous) A solution of 140 mg of **191** in 5 ml of acetone was added to 100 ml of water buffered at pH 2. The resulting milky suspension was stirred at room temperature for 72h. The aqueous phase was extracted with 2 x 20 ml of CH_2Cl_2, the combined organic layers were washed with 25 ml of brine, dried on $MgSO_4$ and evaporated. The residue was purified by column chromatography (silica; EtOAc/PE 7 : 3) to give 32 mg of **192**. ^{1}H-NMR of **192**: δ 4.32 (dt, 1H, H_7, J = 9.2 Hz, 7.4 Hz); δ 3.47 (quintet, 1H, H_6, J = 6.7 Hz); δ 2.81 (m, 1H); δ 2.10-1.50 (m, 10H); δ 1.20 (s, 3H, Me_{11}); δ 0.86 (d, 3H, Me_{12}). ^{13}C-NMR of **192**: δ 218.24 (s), δ 85.30 (s), δ 82.66 (d), δ 73.88 (d), δ 49.55 (d), δ 45.64 (t), δ 35.87 (t), δ 35.10 (t), δ 25.02 (t), δ 24.55 (t), δ 19.53 (q), δ 11.34 (q). Mass spectrum of **192** (m/e): [M^+] 212 (4), 194 (9), 155 (12), 130 (17), 113 (21), 112 (16), 99 (29), 95 (16), 57 (21), 55 (33), 44 (43), 43 (100), 41 (27).

(anhydrous) To a solution of 49 mg of **191** in 50 ml of toluene was added 5 mg of pTSA and the resulting mixture was stirred for 20h at room temperature. The reaction mixture was washed with 2 x 20 ml of saturated $NaHCO_3$ solution, the combined aqueous layers were extracted with 2 x 25 ml of ether, dried on $MgSO_4$ and evaporated to give 56 mg of an oil which was chromatographed (silica; 20% EtOAc in PE) to give 16 mg of **193**. ^{1}H-NMR of **193**: δ 5.57 (dt, 1H, H_9, J = 8.3 Hz, 1.5 Hz); δ 4.45 (broad s, 1H, H_1); δ 2.28 (m, 1H); δ 2.19 (s, 3H, Me_{12}); δ 2.10-1.20 (m, 9H); δ 1.50 (broad s, 3H, Me_{11}). ^{13}C-NMR of **193**: δ 212.43 (s), δ 136.93 (s), δ 127.19 (d), δ 81.11 (s), δ 77.52 (d), δ 36.58 (t), δ 31.83 (t), δ 25.25 (t), δ 25.59 (q), δ 22.92 (t), δ 21.14 (q), δ 15.04 (t). Mass spectrum of **193** (m/e): [M^+] 194 (0.9), 176 (27), 151 (23), 133 (54), 109 (14), 107 (23), 105 (31), 93 (28), 91 (27), 81 (40), 67 (32), 55 (39), 53 (19), 43 (100), 41 (38), 39 (27).

Z-2,6-Dimethyl-6,7-epoxycyclodec-2-enol (195). To a stirred suspension of 98 mg of **189** in 10 ml of CH_2Cl_2, 10 ml of water and 1 g of solid $NaHCO_3$, cooled on ice, was added 109 mg of MCPBA. The reaction mixture was stirred for 30 minutes, the layers were separated and the organic layer was washed with 10 ml of brine. The combined organic layers were washed with 2 x 10 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated to give 95 mg of an oil which was chromatographed (silica; EtOAc/PE 1 : 1) to give 64 mg of **195**. ^{1}H-NMR of **195**: δ 5.33 (m, 1H, H_7); δ 4.44 (t, 1H, H_5, J = 8.1 Hz); δ 2.58 (dd, 1H, H_1, J = 9.9 Hz, 1.4 Hz); δ 2.27 (m, 1H); δ 2.10-1.65 (m, 7H); δ 1.64 (broad s, 3H, Me_{12}); δ 1.32 (broad s, 1H, -OH); δ 1.20 (m, 1H); δ 1.31 (s, 3H, Me11); δ 0.90 (m, 1H). ^{13}C-NMR of **195**: δ 138.59 (s), δ 128.10 (d), δ 67.29 (d), δ 65.45 (d), δ 60.44 (s), δ 37.30 (t), δ 34.98 (t), δ 26.67 (t), δ 23.73 (t), δ 22.07 (t), δ 16.69 (q), δ 16.66 (q). Mass spectrum of **195** (m/e): [M^+] 196 (0), 163 (5), 145 (4), 135 (6), 109 (23), 97 (19), 95 (22), 84 (19), 83 (18), 82 (16), 81 (22), 79 (33), 69 (17), 67 (23), 57 (22), 55 (47), 53 (16), 43 (100), 41 (54), 39 (33).

Acid induced cyclisation of 195. To a solution of 55 mg of **195** in 50 ml of toluene was added 6 mg of pTSA and the resulting mixture was stirred for 20h at room temperature. The reaction mixture was washed with 2 x 20 ml of saturated $NaHCO_3$ solution, the combined aqueous layers were extracted with 2 x 25 ml of ether, dried on $MgSO_4$ and evaporated to give 46 mg of an oil which was chromatographed (silica; EtOAc/PE 7 : 3) to give 4 mg of **198**, 15 mg of **196** and 5 mg of **197**. ^{1}H-NMR of **196**: δ 5.18 (broad d, 1H, H_7, J = 6.1 Hz); δ 4.05 (t, 1H, H_5, J = 8.3 Hz); δ 3.54 (broad d, 1H, H_1, J = 9.7 Hz); δ 2.60-1.30 (m, 11H); δ 1.57 (broad s, 3H, Me_{12}); δ 1.26 (s, 3H, Me_{11}). ^{13}C-NMR of **196**: δ 141.53 (s), δ 122.26 (d), δ 81.41 (s), δ 79.50 (d), δ 75.24 (d), δ 38.19 (t), δ 34.34 (t), δ 33.56 (t), δ 24.63 (t), δ 24.40 (q), δ 23.10 (q), δ 18.52 (t). Mass spectrum of **196** (m/e): [M^+] 197 (0.7), 178 (6), 152 (8), 136 (12), 134 (11), 109 (26), 95 (22), 94 (79), 93 (19), 81 (20), 79 (73), 67 (19), 55 (27), 53 (17), 43 (100), 41 (42), 39 (25). ^{1}H-NMR of **197**: δ 5.09 (broad s, 1H, H_{12}); δ 4.89 (broad s, 1H, $H_{12'}$); δ 4.48 (dd, 1H, H_5, J = 3.5 Hz, 1.7 Hz); δ 3.43 (ddt, 1H, H_7, J = 8.8 Hz, 1.4 Hz); δ 1.99 (ddd, 1H, H_1, J = 12.4 Hz, 10.2 Hz, 2.2 Hz); δ 1.92 (m, 2H); δ 1.80-1.50 (m, 8H); δ 1.17 (s, 3H, Me_{11}); δ 1.05 (m, 1H). ^{13}C-NMR of **197**: δ 154.14 (s), δ 111.43 (t), δ 81.82 (s), δ 75.74 (d), δ 53.41 (d), δ 41.00 (d), δ 40.95 (t), δ 36.23 (t), δ 26.82 (t), δ 25.76 (t), δ 24.51 (q), δ 23.04 (t). Mass spectrum of **197** (m/e): [M^+] 196 (0), 178 (2), 163 (10), 160 (25), 145 (19), 135 (15), 120 (17), 107 (16), 105 (21), 93 (30), 92 (17), 91 (30), 81 (18), 79 (37), 77 (15), 71 (18), 67 (23), 55 (27), 53 (17), 43 (100), 41 (38), 39 (22). ^{1}H-NMR of **198**: δ 5.35 (t, 1H, H_9, J = 7.8 Hz); δ 4.28 (broad s, 1H, H_1);

δ 3.69 (dd, 1H, H_5, J = 13.1 Hz, 4.1 Hz); δ 3.40 (broad s, 1H, -OH); δ 2.71 (m, 1H); δ 2.10-1.60 (m, 7H); δ 1.68 (broad s, 3H, Me_{11}); δ 1.50-1.10 (m, 2H); δ 0.95 (s, 3H, Me_{12}). ^{13}C-NMR of **198**: δ 140.60 (s), δ 125.08 (d), δ 81.07 (d), δ 77.67 (s), δ 72.91 (d), δ 36.92 (t), δ 24.91 (q), δ 23.99 (t), δ 22.01 (q), δ 21.74 (t), δ 20.66 (t), δ 15.73 (t). Mass spectrum of **198** (m/e): [M^+] 196 (1), 178 (6), 152 (6), 135 (12), 134 (11), 109 (28), 94 (89), 81 (26), 79 (100), 67 (22), 55 (26), 53 (21), 43 (78), 41 (43), 39 (23).

(Z,E)-1-Acetoxy-2,6-dimethyl-cyclodeca-2,6-diene (199). To a solution of 1.12 g of **189** in 20 ml of dry pyridine was added 50 mg of DMAP and 3.5 ml of Ac_2O. The resulting mixture was stirred for 1.5 h at room temperature after which the solvent was evaporated. The residue was taken up in 30 ml of CH_2Cl_2, washed with 25 ml of saturated $NaHCO_3$-solution and 25 ml of brine. The combined aqueous layers were extracted with 2 x 20 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated to give 1.38 g of **199** as an oil. ^{1}H-NMR of **199**: δ 5.66 (t, 1H, H7, J = 7.6 Hz); δ 5.18 (broad t, 1H, H_1, J = 6.7 Hz); δ 4.87 (m, 1H, H_5); δ 2.59 (m, 1H); δ 2.20-1.90 (m, 6H); δ 1.75 (broad s, 3H, Me_{11} or Me_{12}); δ 1.74 (broad s, 3H, Me_{12} or Me_{11}); δ 1.68 (s, 3H, -OOCMe); δ 1.65-1.55 (m, 2H); δ 1.35 (m, 1H). ^{13}C-NMR of **199**: δ 169.09 (s), δ 135.69 (s), δ 130.93 (s), δ 128.06 (2 x d), δ 71.70 (d), δ 37.42 (t), δ 32.49 (t), δ 28.28 (t), δ 26.64 (t), δ 26.01 (t), δ 20.73 (q), δ 17.74 (q), δ 17.02 (q). Mass spectrum of **199** (m/e): [M^+] 222 (0.8), 180 (4), 162 (41), 147 (61), 133 (29), 119 (20), 107 (17), 105 (33), 93 (18), 91 (27), 81 (27), 79 (38), 77 (16), 67 (24), 55 (30), 53 (23), 43 (100), 41 (39), 39 (24).

(Z,E)-2,6-Dimethyl-cyclodeca-2,6-diene (200). To a deep-blue mixture of 1.0 g of lithium metal in 125 ml of liquid NH_3, cooled to -78°C and stirred for 1h, was added dropwise a solution of 1.28 g of **199** in 20 ml of ether. The resulting mixture was stirred for an additional 1.5 h after which solid NH_4Cl was added until the deep-blue colour disappeared. The NH_3 was allowed to evaporate overnight after which 100 ml of water was added. The aqueous layer was extracted with 3 x 75 ml of ether, the combined organic layers were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 890 mg of an oil which was chromatograped (silica; PE) to yield 634 mg of **200** as a colourless oil. ^{1}H-NMR of **200**: δ 5.05 (m, 2H, H_1, H_7); δ 2.10-1.80 (m, 8H); δ 1.66 (d, 3H, Me_{11} or Me_{12}, J = 1.1 Hz); δ 1.62 (d, 3H, Me_{12} or Me_{11}, J = 0.8 Hz); δ 1.60 (m, 2H); δ 1.42 (m, 2H). ^{13}C-NMR of **200**: δ 138.48 (s), δ 127.77 (s), δ 122.80 (2 x d), δ 37.82 (t), δ 29.49 (t), δ 28.28 (t), δ 26.28 (t), δ 26.16 (t), δ 25.87 (t), δ 22.23 (q), δ 17.61 (q). Mass spectrum of **200** (m/e): [M^+] 164 (26), 149 (43), 135 (17), 121 (21), 108 (17), 107 (64), 95 (33), 94 (32), 93 (74), 91 (26), 81 (78), 80 (16), 79 (79), 77 (26), 68 (54), 67 (100), 65 (18), 55 (46), 53 (58), 41 (95), 40 (22), 39 (74).

Z-2,6-Dimethyl-6,7-epoxycyclodec-2-ene (201). To a stirred suspension of 148 mg of **200** in 10 ml of CH_2Cl_2, 10 ml of water and 750 mg of solid $NaHCO_3$, cooled on ice, was added 195 mg of MCPBA. The reaction mixture was stirred for 30 minutes, the layers were separated and the organic layer was washed with 10 ml of brine. The combined organic layers were washed with 2 x 10 ml of CH_2Cl_2, dried on $MgSO_4$ and evaporated to give 145 mg of an oil which was chromatographed (silica; 10% of EtOAc in PE) to give 116 mg of **201**. ^{1}H-NMR of **201**: δ 5.21 (broad t, 1H, H_7, J = 7.7 Hz); δ 2.82 (d, 1H, H_1, J = 9.3 Hz); δ 2.50-1.00 (m, 12H); δ 1.64 (s, 3H, Me_{12}); δ 1.33 (s, 3H, Me_{11}). ^{13}C-NMR of **201**: δ 136.96 (s), δ 124.45 (d), δ 64.53 (d), δ

60.93 (s), δ 37.77 (t), δ 28.45 (t), δ 27.41 (t), δ 26.39 (t), δ 23.96 (t), δ 23.37 (t), δ 21.66 (q), δ 17.66 (q). Mass spectrum of **201** (m/e): [M^+] 180 (0.8), 165 (4), 147 (3), 122 (23), 111 (18), 109 (21), 107 (36), 96 (19), 95 (41), 94 (15), 93 (43), 81 (66), 79 (48), 69 (23), 68 (20), 67 (68), 55 (55), 53 (31), 43 (100), 41 (95), 39 (59).

Acid induced cyclisation of 201. A solution of 29 mg of **201** in 3 ml of acetone was added to 60 ml of water buffered at pH 4. The milky suspension was stirred for 7 days at room temperature and extracted with 2 x 20 ml of CH_2Cl_2. The combined organic layers were washed with 25 ml of brine, dried on $MgSO_4$ and evaporated to give 28 mg of an oil which was chromatographed (silica; EtOAc/PE 3 : 1) to give 19 mg of **202**. ^{1}H-NMR of **202**: δ 2.30-2.10 (m, 2H); δ 1.90-1.45 (m, 14H); δ 1.18 (s, 3H, Me_{11} or Me_{12}); δ 1.14 (s, 3H, Me_{12} or Me_{11}). ^{13}C-NMR of **202**: δ 81.13 (s), δ 74.24 (s), δ 54.40 (d), δ 49.95 (d), δ 43.25 (t), δ 40.69 (t), δ 31.54 (q), δ 28.78 (t), δ 26.98 (t), δ 25.23 (t), δ 25.15 (t), δ 23.54 (q). Mass spectrum of **202** (m/e): [M^+] 180 (1), 165 (10), 162 (12), 122 (45), 107 (15), 95 (17), 93 (18), 81 (32), 71 (21), 67 (19), 55 (19), 43 (100), 41 (25).

(1α,3aα,8β,8aβ)-Octahydro-8-hydroxy-1-methyl-4(2H)-azulenone 8-methylsulfonate (206). To a solution of 441 mg of **205** in 5 ml of dry pyridine was added 1.0 ml of MsCl. The reaction mixture was stirred for 1.5h at room temperature, the solvent was evaporated, the residue was taken up in 30 ml of CH_2Cl_2 and extracted with 2 x 20 ml of brine. The combined aqueous layers were extracted with 3 x 20 ml of CH_2Cl_2, dried on $MgSO_4$, concentrated and the residue was purified by colomn chromatography (silica; EtOAc/PE 1 :1) to give 525 mg of **206** as a solid. mp. 99.5-100.5°C. ^{1}H-NMR of **206**: δ 4.82 (dt, 1H, H_2, J = 10.5 Hz, 4.4 Hz); δ 3.01 (s, 3H, -OMs); δ 2.95 (m, 1H); δ 2.60-2.30 (m, 5H); δ 1.90-1.40 (m, 7H); δ 0.94 (d, 3H, Me_{11}, J = 7.0 Hz). ^{13}C-NMR of **206**: δ 211.63 (s), δ 83.76 (d), δ 52.26 (d), δ 47.32 (d), δ 42.77 (t), δ 39.70 (q), δ 37.00 (d), δ 35.36 (t), δ 31.94 (t), δ 22.65 (t), δ 18.63 (t), δ 13.51 (q).

(1α,3aα,8β)-Octahydro-1-methyl-4(2H)-azulenone (209). To a solution of 460 mg of **206** in 20 ml of dry ether, cooled to 0°C, was carefully added 1.0 g of $LiAlH_4$. The grey suspension was stirred for 2h, Glauber's salt was added in small portions and the resulting mixture was stirred for 30 minutes. After addition of $MgSO_4$ the mixture was stirred for an additional 30 minutes, the solids were filtered off and the solvent was evaporated to give 297 mg of a 1 : 1 mixture of **207** and **208** as a colourless oil. Mass spectrum of **207** (m/e): [M^+] 168 (4), 150 (21), 135 (70), 121 (18), 112 (18), 111 (100), 108 (30), 107 (21), 95 (41), 94 (39), 93 (34), 83 (21), 82 (30), 81 (75), 67 (54), 57 (52), 55 (73), 43 (35), 41 (74), 39 (47). Mass spectrum of **208** (m/e): [M^+] 168 (4), 150 (12), 135 (100), 121 (19), 111 (34), 107 (40), 95 (36), 94 (42), 93 (44), 81 (55), 79 (46), 67 (55), 55 (53), 41 (73), 39 (40). The crude mixture of **207** and **208** was dissolved in 5 ml of DMF and added to a solution of 2.00 g of PDC in 3 ml of DMF. The reaction mixture was stirred for 1h at room temperature, poured into 50 ml of water and extracted with 4 x 30 ml of PE. The combined organic layers were washed with 40 ml of brine, dried over $MgSO_4$ and evaporated to give 252 mg of an oil which was purified by column chromatography (silica; 5% of EtOAc in PE) to yield 138 mg of **209** as an oil. ^{1}H-NMR of **209**: δ 2.88 (m, 1H); δ 2.46 (m, 1H); δ 2.38 (dd, 1H, J = 11.8 Hz, 3.9 Hz); δ 2.30-1.15 (m, 12H); δ 0.78 (d, 3H, Me_{11}, J = 7.0 Hz). ^{13}C-NMR of **209**: δ 214.13 (s), δ 54.32 (d), δ 47.93 (d), δ 43.61 (t), δ 39.09 (d), δ 32.97 (t), δ

32.66 (t), δ 29.18 (t), δ 23.58 (t), δ 23.51 (t), δ 14.42 (q). Mass spectrum of **209** (m/e): [M^+] 166 (42), 151 (23), 138 (18), 137 (88), 124 (38), 111 (40), 109 (76), 95 (50), 81 (97), 79 (37), 67 (100), 55 (69), 54 (35), 53 (38), 41 (95), 39 (88).

(1β,3aα,8β)-Octahydro-1-hydroxy-1-methyl-4(2H)-azulenone (210). To a bottle containing 3 ml of CCl_4, 3 ml of MeCN, 3.5 ml of water and 750 mg of $NaIO_4$ was added 135 mg of **209** and 20 mg of $RuO_2 \cdot xH_2O$. The bottle was closed air-tight and rotated around its axis in a waterbad of 50°C until the colour of the mixture had turned from yellow to black (16h). GC-analysis revealed the presence of 23% of **210** and 45% of starting material. An additional portion of 470 mg of $NaIO_4$ was added and the reaction was continued. Two additional batches of 1.0 g of $NaIO_4$ were needed to deminish the amount of starting material to approximately 10%. The organic phase was separated, reduced, taken up in 20 ml of CH_2Cl_2 and washed with 2 x 15 ml of 10% $Na_2S_2O_3$-solution. The combined water layers were extracted with 3 x 15 ml of CH_2Cl_2 dried on $MgSO_4$, concentrated and purified by column chromatography (EtOAc/PE 1 : 1) to yield 6 mg of **210** as an oil. ^{1}H-NMR of **210**: δ 2.81 (ddd, 1H, H_7, J = 11.0 Hz, 9.7 Hz, 5.3 Hz); δ 2.52 (broad t, 1H, J = 4.2 Hz); δ 2.45 (dd, 1H, J = 8.6 Hz, 3.4 Hz); δ 2.40-2.15 (m, 2H); δ 2.15-1.80 (m, 3H); δ 1.70-1.20 (m, 7H); δ 1.15 (s, 3H, Me_{11}). ^{13}C-NMR of **210**: δ 213.03 (s), δ 80.78 (s), δ 53.93 (d), δ 53.45 (d), δ 43.45 (t), δ 40.29 (t), δ 30.35 (t), δ 29.10 (t), δ 23.58 (t), δ 21.63 (q), δ 20.57 (t). Mass spectrum of **210** (m/e): [M^+] 182 (8), 125 (77), 124 (44), 111(24), 98 (23), 97 (33), 83 (16), 81 (29), 79 (21), 67 (34), 58 (23), 55 (58), 43 (100), 41 (43), 39 (29).

(1α,3aβ,4α,8α)-Octahydro-1,4-dimethyl-1,4-azulenediol (203) and its 4α epimer (204). To a solution of 6 mg of **210** in 10 ml of dry ether, cooled on ice, was added 0.5 ml of 1.6M MeLi in ether. The reaction mixture was stirred for 30 minutes after which it was quenched with 10 ml of brine. The layers were separated, the organic phase was washed with 10 ml of brine, the combined organic layers were extracted with 3 x 15 ml of ether, dried on $MgSO_4$ and evaporated to give 6 mg of a solid which consisted of a 8 : 1 mixture of the diols **203** and **204** according to GC. Column chromatography (silica; EtOAc/PE 3 : 1) yielded 3 mg of **203** as a solid. ^{1}H-NMR of **203**: δ 1.90-1.20 (m, 16H); δ 1.17 (s, 3H, Me_{11} or Me_{12}): δ 1.12 (s, 3H, Me_{12} or Me_{11}). ^{13}C-NMR of **203**: δ 80.83 (s), δ 73.24 (s), δ 52.68 (d), δ 49.09 (d), δ 44.31 (t), δ 41.16 (t), δ 29.52 (t), δ 29.31 (q), δ 29.06 (t), δ 22.76 (t), δ 22.37 (q), δ 22.27 (t). Mass spectrum of **203** (m/e): [M^+] 180 (3), 165 (6), 162 (8), 147 (7), 137 (10), 122 (39), 107 (17), 95 (19), 81 (34), 71 (17), 67 (16), 55 (18), 43 (100), 41 (27). The mass spectrum of **204** was identical to that of **203**.

E-2,6-Dimethyl-2,3-epoxycyclodec-2-enol (211). To a solution of 484 mg of **189** in 15 ml of benzene was added 50 mg of $VO(acac)_2$ and 0.9 ml of 5-6M *t*-BuOOH in decanes. The reaction mixture was stirred for 1.5 h and washed with 2 x 25 ml of 10% $Na_2S_2O_3$ solution The combined aqueous layers were extracted with 2 x 25 ml of EtOAc, dried on $MgSO_4$ and evaporated to give 660 mg of a brownish oil which was purified by column chromatography (silica; 20% EtOAc in PE) to give 346 mg of **211** as a colourless oil. ^{1}H-NMR of **211**: δ 5.13 (m, 1H, H_1); δ 3.24 (dd, 1H, H_5, J = 5.3 Hz, 2.8 Hz); δ 2.82 (dd, 1H, H_7, J = 10.7 Hz, 2.7 Hz); δ 2.28 (broad dt, 1H, J = 13.8 Hz); δ 2.30-2.00 (m, 4H); δ 1.90-1.65 (m, 2H); δ 1.63 (d, 3H, Me_{11}, J = 1.0

Hz); δ 1.50-1.20 (m, 4H); δ 1.22 (s, 3H, Me_{12}). ^{13}C-NMR of **211**: δ 134.46 (s), δ 125.30 (d) δ 74.36 (d), δ 66.31 (d), δ 64.46 (s), δ 32.76 (t), δ 31.88 (t), δ 30.50 (t), δ 28.48 (t), δ 27.45 (t), δ 19.81 (q), δ 16.71 (q). Mass spectrum of **211** (m/e): [M^+] 196 (0), 178 (1), 163 (2), 139 (25), 112 (36), 96 (24), 94 (23), 93 (21), 81 (30), 71 (59), 67 (35), 55 (73), 53 (26), 43 (100), 41 (65), 39 (36).

Acid induced cyclisation of 211. A solution of 54 mg of **211** in 5 ml of acetone was added to 100 ml of water buffered at pH 1. The milky suspension was stirred for 20h at room temperature and extracted with 2 x 20 ml of CH_2Cl_2. The combined organic layers were washed with 25 ml of brine, dried on $MgSO_4$ and evaporated to give 58 mg of an oil which was chromatographed (silica; EtOAc/PE 3 : 2) to give 8 mg of **212** and 24 mg of **213**. ^{1}H-NMR of **212**: δ 5.37 (broad s, 1H, H_9); δ 4.04 (dd, 1H, H_5, J = 11.1 Hz, 4.8 Hz); δ 3.64 (m, 1H, H_7); δ 2.94 (broad s, 1H, -OH); δ 2.81 (broad s, 1H, -OH); δ 2.50-2.20 (m, 2H); δ 2.08 (broad s, 1H); δ 1.70-1.30 (m, 6H); δ 1.58 (s, 3H, Me_{11}); δ 1.21 (s, 3H, Me_{12}). ^{13}C-NMR of **212**: δ 134.62 (s), δ 120.97 (d), δ 77.57 (d), δ 70.00 (d), δ 46.17 (d), δ 40.72 (s), δ 32.04 (t), δ 30.28 (t), δ 22.75 (t), δ 21.31 (q), δ 20.26 (t), δ 16.00 (q). Mass spectrum of **212** (m/e) [M^+] 196 (15), 178(12), 163 (17), 145 (24), 135 (29), 107 (100), 91 (43), 79 (35), 77 (23), 67 (21), 55 (29), 53 (21), 43 (40), 41 (47), 39 (27). ^{1}H-NMR of **213**: δ 3.93 (dd, 1H, H_5, J = 11.0 Hz, 4.8 Hz); δ 3.76 (dd, 1H, H_7, J = 6.6 Hz, 4.0 Hz); δ 3.36 (broad s, 1H, -OH); δ 2.99 (broad s, 1H, -OH); δ 2.40 (m, 1H); δ 2.15-1.90 (m, 2H); δ 1.85-1.55 (m, 6H); δ 1.59 (s, 3H, Me_{11}); δ 1.20 (m, 1H), δ 1.09 (s, 3H, Me_{12}). ^{13}C-NMR of **213**: δ 133.02 (s), δ 125.35 (s), δ 74.97 (d), δ 72.47 (d), δ 44.31 (s), δ 30.09 (t), δ 29.09 (t), δ 26.43 (t), δ 24.69 (t), δ 24.39 (t), δ 19.60 (q), δ 17.69 (q). Mass spectrum of **213** (m/e) [M^+] 196 (0), 178 (39), 145 (100), 121 (48), 199 (62), 107 (96), 93 (41), 91 (44), 77 (30), 67 (22), 55 (35), 53 (24), 43 (64), 41 (57), 39 (35).

E-2,6-Dimethyl-2,3-epoxycyclodec-2-enone (214). To a solution of 2.0 g of PDC in 5 ml of DMF was added 346 mg of **211** in 3 ml of DMF and the resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with 80 ml of water and extracted with 6 x 30 ml of ether. The combined organic layers were washed with 100 ml of brine, dried on $MgSO_4$ and evaporated to give 290 mg of **214** as a colourless oil. ^{1}H-NMR of **214**: δ 4.82 (broad t, 1H, H_1, J = 8.1 Hz); δ 2.88 (dd, 1H, H_7, J = 10.3 Hz, 3.1 Hz); δ 2.50-2.00 (m, 7H); δ 1.85-1.70 (m, 2H); δ 1.61 (broad s, 3H, Me_{11}); δ 1.57 (s, 3H, Me_{12}); δ 1.30 (m, 1H). ^{13}C-NMR of **214**: δ 205.55 (s), δ 134.44 (s), δ 126.58 (d), δ 64.77 (d), δ 63.50 (s), δ 39.05 (t), δ 31.82 (t), δ 27.90 (t), δ 27.81 (t), δ 25.25 (t), δ 19.73 (q), δ 19.26 (q). Mass spectrum of **214** (m/e): [M^+] 194 (0), 179 (1), 137 (3), 133 (5), 95 (17), 81 (23), 79 (15), 68 (20), 67 (35), 55 (43), 53 (23), 43 (100), 41 (51), 39 (39).

6.6. References and notes

1. Wharton P.S. and Baird M.D., *J. Org. Chem.*, **36**, 2932 (1971).
2. Dutcher J.S., Macmillan J.G. and Heathcock C.H., *J. Org. Chem.*, **41**, 2663 (1976).
3. The representation of **184** is chosen in order to display the analogy of the cyclisation reactions of (E,Z)- cyclodeca-1,5-dienes in this chapter and other

cyclodecadienes in the previous chapter. The numbering of the carbon atoms in the (E,Z)-cyclodeca-1,5-dienes has been changed accordingly.

4. Schubert W.M. and LeFevre P.H., *J. Am. Chem. Soc.*, **94** 1639 (1972).
5. Fainberg A.H. and Winstein S., *J. Am. Chem. Soc.*, **78**, 2770 (1956).
6. Gijsen H.J.M., *(+)-Aromadendrene as Chiral Starting Material for the Synthesis of Sesquiterpenes*, PhD-thesis, Wageningen (1993).
7. Posner G.H., Kevin A.B., Loomis G.L., Frazee W.J., Ramsharan D.M. and Karle I.L., *J. Am. Chem. Soc.*, **102**, 7498 (1980) and references cited therein.
8. Tenaglia A., Terranova E. and Waegell B., *Tetrahedron Lett.*, **30**, 5271 (1989).
9. Sharpless K.B. and Michaelson R.C., *J. Am. Chem. Soc.*, **95**, 6136 (1973).
10. Kesselmans R.P.W., *Total Synthesis of All Stereoisomers of Eudesm-11-en-4-ol*, PhD thesis, Agricultural University Wageningen (1992).
11. Fisher N.H., Wiley R.A. and Perry D.L., *Rev. Lat. Quim.*, **7**, 87 (1976).
12. Jain T.C. and Banks C.M., *Can. J. Chem.*, **58**, 447 (1980) and references cited theirein.
13. Herz W., Murari R. and Blount J.F., *J. Org. Chem.*, **44**, 1873 (1979).

7. Studies towards the biosynthesis of sesquiterpenes in chicory

The mode of cyclisation of several germacranes and germacrane analogues, induced by *C. intybus*, has been discussed in chapters 3-6. By studying these cyclisation reactions, parallels can be drawn to the biosynthesis of the sesquiterpene lactones in chicory, which are believed to be formed through cyclisation of germacrane intermediates. In this chapter, the attention is focussed on an earlier stage of the sesquiterpene biogenesis in the chicory, i.e. the cyclisation of farnesyl pyrophosphate (**23**) into the germacrane framework. Furthermore, a summary and discussion of the cyclisation reactions which were unambiguously accelerated by the germacrane cyclase from chicory is presented. Based on these biotransformation reactions, an active site model for the enzyme is proposed. Finally, two tentative biosynthetic routes of guaiane-, eudesmane- and germacrane lactones in *C. intybus* are proposed.

7.1. Partial purification of the FPP-cyclase from chicory*

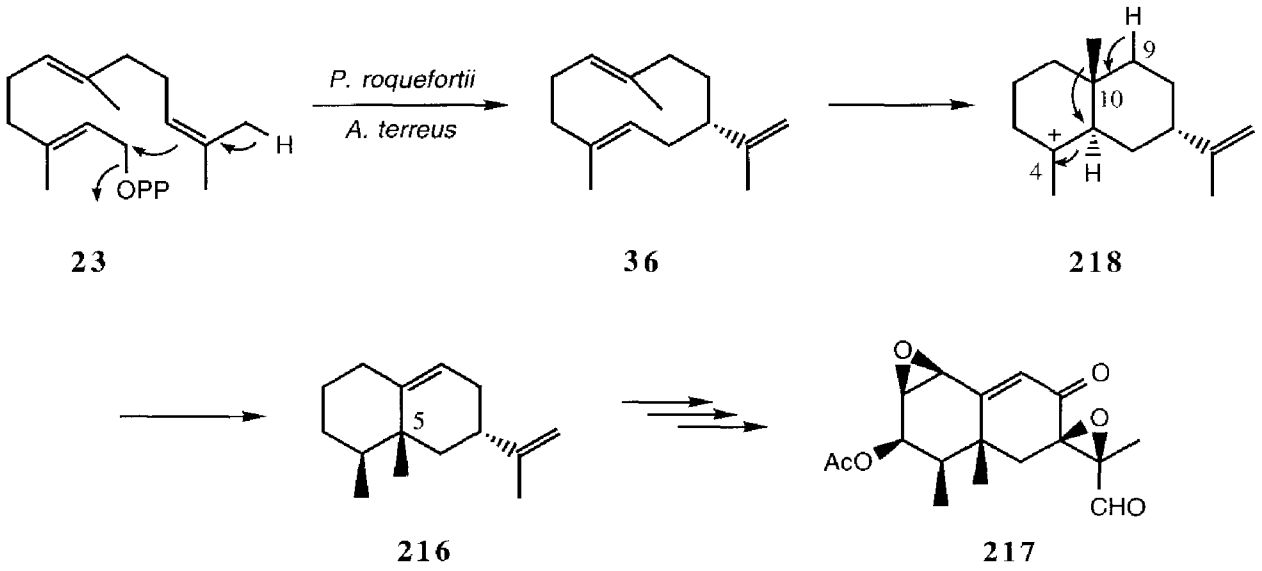
7.1.1. Introduction

Only in the last 15 years, information has become available on the cyclase catalysed conversion of farnesyl pyrophosphate (**23**) into cyclic sesquiterpenes. One of the sesquiterpene cyclases that has been thoroughly investigated is aristolochene synthase, an enzyme which catalyses the conversion of **23** into aristolochene (**216**). Aristolochene (**216**), an eremophilane sesquiterpene, has been isolated from a variety of species. The (-)-enantiomer is found in *Aristolochia indica*[1], the leaf oil of *Bixa orellana*[2] and in the defensive secretions of the *Syntermes* soldier termites[3] while the (+)-enantiomer has been found in *Aspergillus terreus*[4,5]. Aristolochene (**216**) is most likely the precursor of the powerful mycotoxin PR-toxin (**217**)[6-9] (Scheme 7.1.). The corresponding aristolochene synthases have been isolated from *Penicillium roquefortii*[6] and *A. terreus*[9]. According to the proposed mechanism, **23** cyclises by a nucleophilic attack of the terminal double bond on the carbon atom bearing the allylic pyrophosphate group, followed by deprotonation of one of the adjacent methyl groups to give germacrene A (**36**), as outlined in scheme 7.1. Germacrene A (**36**) cyclises into the eudesmane cation **218** followed by a 1,2-hydride shift from C_5 to C_4

* The partial purification of the FPP-cyclase from chicory has been performed in collaboration with J.-W. de Kraker and Dr. H.J. Bouwmeester from AB-DLO, Wageningen.

and a methyl shift from C_{10} to C_5. Subsequent deprotonation yields the C_9-C_{10} double bond to give the eremophilane type sesquiterpene aristolochene (**216**).

Scheme 7.1.

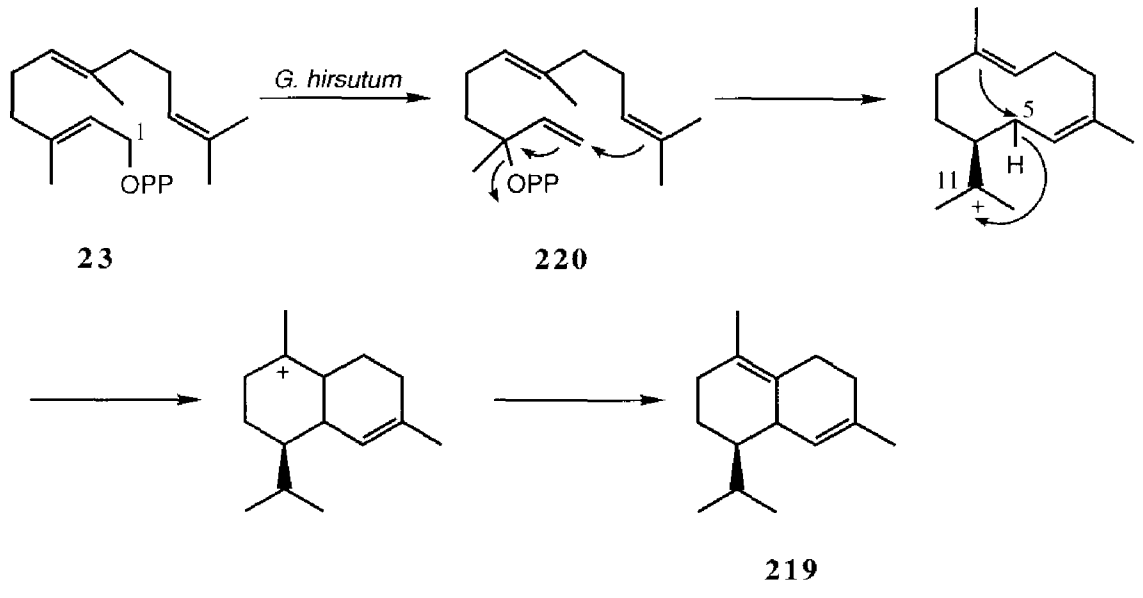


23 **36** **218**

216 **217**

More recently, the biosynthesis of δ-cadinene (**219**) from **23** was reported. δ-Cadinene cyclase in cotton (*Gossypium hirsutum*) was induced by infecting the stems with *Verticillium dahliae*[10] or *Xanthomonas campestris* pv. *malvacearum*[11,12]. The proposed pathway for the formation of δ-cadinene starts with an isomerisation of farnesyl pyrophosphate (**23**) into nerolidyl pyrophosphate (**220**), as shown in scheme 7.2.

Scheme 7.2.



23 **220**

219

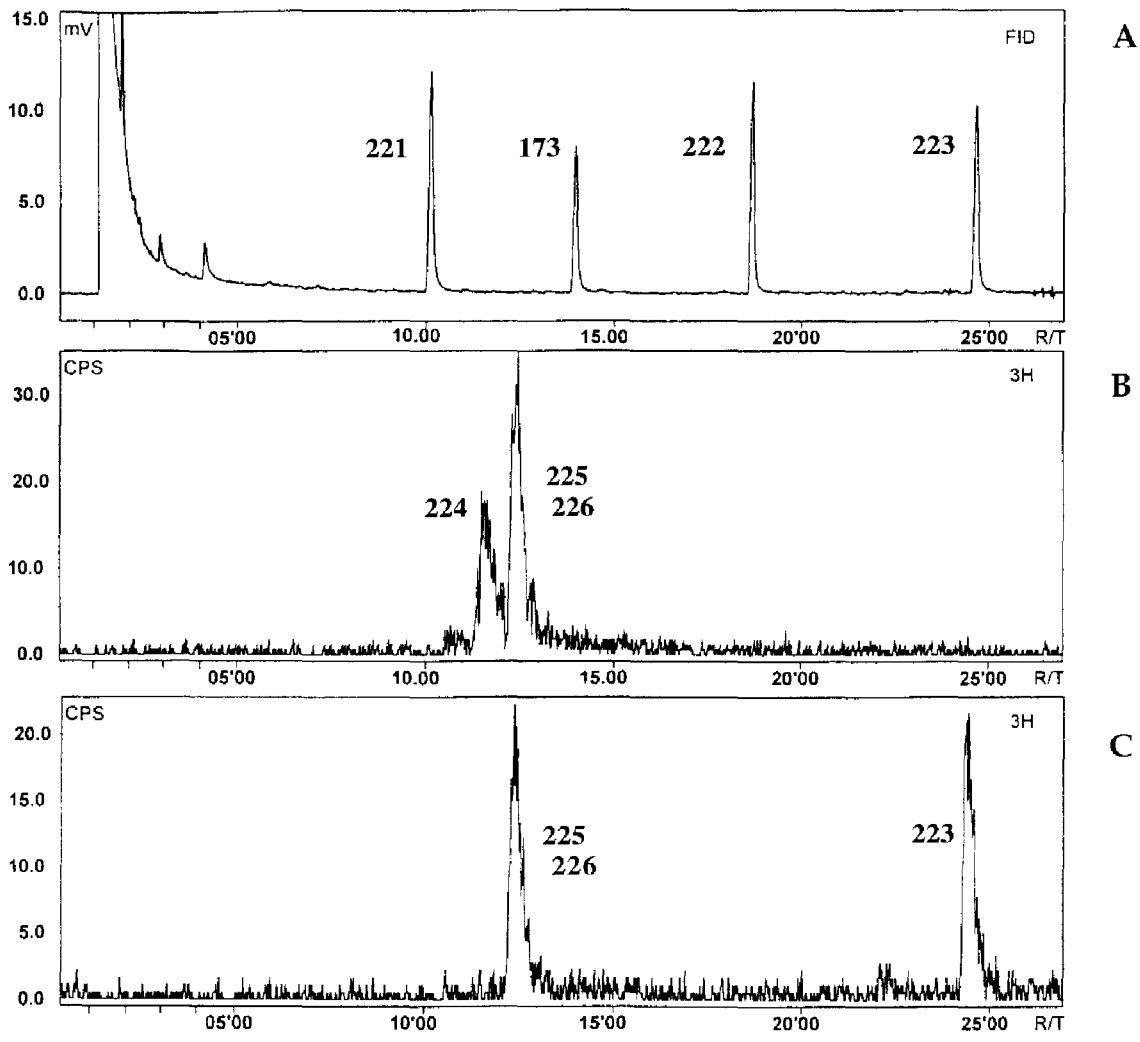
Nerolidyl pyrophosphate (**220**) cyclises by a nucleophilic attack of the terminal double bond on C_1 followed by a 1,3-hydride shift from C_5 to C_{11}, transannular cyclisation from C_1 to C_5 and deprotonation of the bridgehead carbon to give **219**.

As stated earlier, the knowledge of sesquiterpene cyclases is still limited. Only a few of these sesquiterpene cyclases have been isolated and studied, but from these studies several generalisations have already begun to emerge. All sesquiterpene cyclases examined up to date, from both plant and microbial sources, are soluble proteins with a molecular mass of 40.000-100.000[13]. Most of these proteins are monomers with a mass of 40.000-60.000 but at least two were reported to be homo-dimers with a subunit mass of 40.000-44.000[14,15]. The only cofactor required is a divalent metal ion, preferably Mg^{2+} or, to a lesser extent, Mn^{2+}. The turnover numbers are modest, falling in the range of 0.02-0.3 s^{-1} [13]. The apparent K_m-values for FPP (**23**) lie in the range of 1-20 μM, the pI-value is relatively low (4-5) and the pH optimum is generally within one unit of neutrality. The isolation and study of these proteins is complicated by their relatively low abundance in the host organism. The sesquiterpene cyclases are often located in tissues containing high levels of compounds with a low molecular mass, interfering substances (oils, pigments, phenolics, waxes and resins) and other enzymes (proteases, phenol oxidases and dephosphorylases) that may hinder the isolation and preliminary activity studies[16]. Especially the presence of oils, resins, phenolic substances and phenol oxidases inactivate cyclases through covalent and non-covalent interactions[17,18]. This means that measures must be taken to minimise these destructive interactions, for instance by working at low temperature and adding several chelating, reducing and adsorbing agents to the extraction buffers. Several protocols for composing extraction and assay buffers are available and they serve as a valuable tool in the purification of sesquiterpene cyclases[13,16].

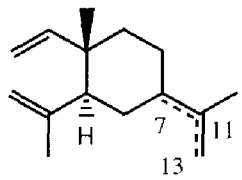
7.1.2. Partial purification of FPP-cyclase from *C. intybus*

The generalisations as discussed in the previous paragraph were used as a guideline for the partial purification of the sesquiterpene cyclase from *C. intybus*, i.e. it was assumed that the cyclase is a soluble protein with physical properties comparable to those already isolated and investigated. A 100.000 g supernatant was prepared from 50 g of fresh chicory roots in 80 ml of a suitable extraction buffer. A 3 ml sample was desalted using an Econo-Pac 10DG desalting column (Bio-Rad) which was equilibrated with the assay buffer. The proteins were eluted with 4 ml of assay buffer and aliquots of 1 ml were incubated with a mixture of 20 μM of unlabeled and [1-^{3}H]-FPP (± 1 μCi /

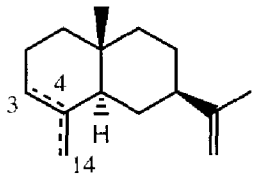
Figure 7.1.



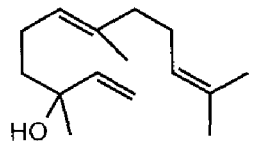
A: GC-trace of the carrier containing **173, 221-223**; **B**: pentane fraction; **C**: ether fraction



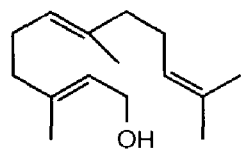
221: Δ 7,11
224: Δ 11,13



225: Δ 3,4
226: Δ 4,14



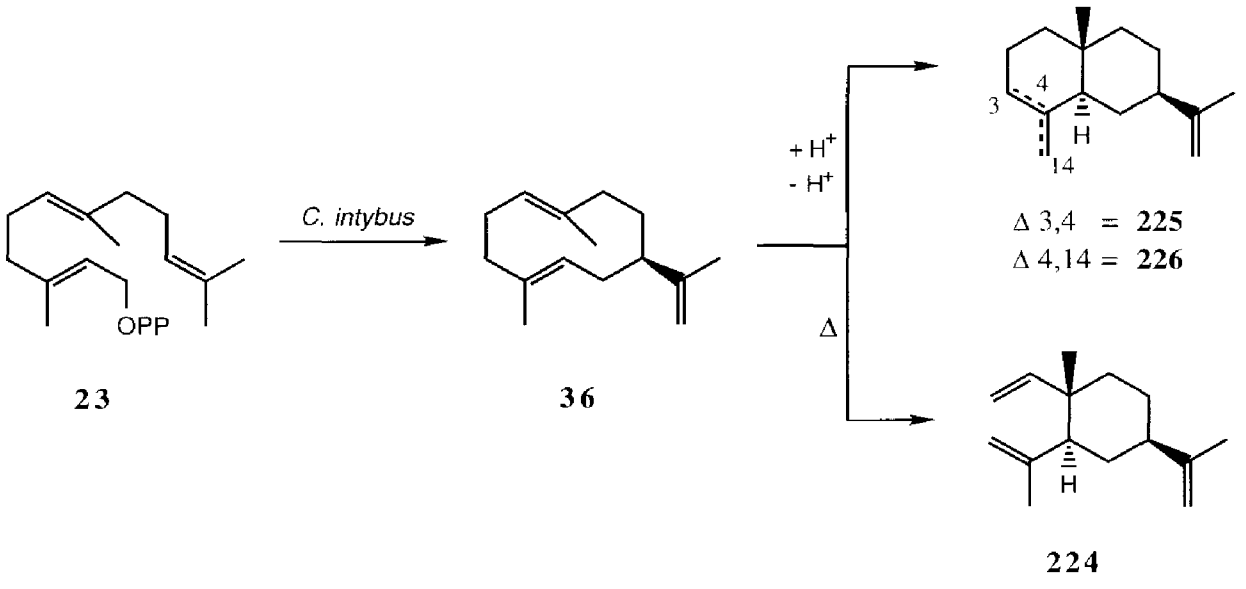
222



223

assay). Following the incubation at 30°C for 1 hour, the assays were cooled on ice, extracted with 1 ml of pentane and filtered through Al_2O_3 covered with $MgSO_4$. The extraction-filtration procedure was repeated with 1 ml of 20% of ether in pentane and 2 x 1 ml of ether. The pentane and ether phases were collected separately. After addition of a carrier solution containing γ-elemene (**221**), germacrane B (**173**), trans-nerolidol (**222**) and (E,E)-farnesol (**223**), the samples were analysed using radio-GC. Two labeled compounds were detected, as outlined in figure 7.1. However, the retention time of the labeled compounds did not coincide with any of the carrier compounds. An additional experiment of the desalted supernatant with 20 μM of unlabeled FPP (**23**) followed by a 1 hour incubation period at 30°C and subsequent analysis of the products by GC-MS revealed that not two but three products were formed: β-elemene (**224**), α-selinene (**225**) and β-selinene (**226**). The identification of these compounds was performed using essential oils as reference mixtures. Compound **224** was present in the natural oil of *Mentha mirennae*[19] and both **225** and **226** were present in the natural oil of *Cyperus rotundus*[20]. Both the retention time and fragmentation specta of **224-226**, obtained through cyclisation of **23**, were identical to the corresponding compounds in *M. mirennae* and *C. rotundus*. Radio-GC analysis of a co-injection of the ether fraction with the carrier solution indicated the presence of α- and β-selinene (**225** and **226**, respectively) and (E,E)-farnesol (**223**), released from the substrate by dephosphorylase activity. Typical total conversions amounted to 35% of **224**, 10% of **225** and **226** and 20% of **223**. Boiled enzyme controls did not produce significant levels of sesquiterpenes.

Scheme 7.3.



23 **36** **224**

The formation of β-elemene (**224**), α- and β-selinene (**225** and **226**) by the sesquiterpene cyclase of *C. intybus* indicates that farnesyl pyrophosphate (**23**) is transformed into germacrene A (**36**, Scheme 7.3.). However, germacrene A (**36**) is exceptionally labile towards elevated temperatures and acidic conditions[21]. A Cope rearrangement of **36** explains the formation of **224** while a protonation-cyclisation-deprotonation sequence accounts for the formation of **225** and **226** (Scheme 7.3.). No oxidised equivalents of **36** and **224-226** were detected, which might indicate that the oxidising enzymes in *C. intybus* are not located in the 100.000 g supernatant and are, probably, membrane bound. In general, this is the case for cytochrome P_{450} enzymes which are involved in the oxidation of terpenes[22].

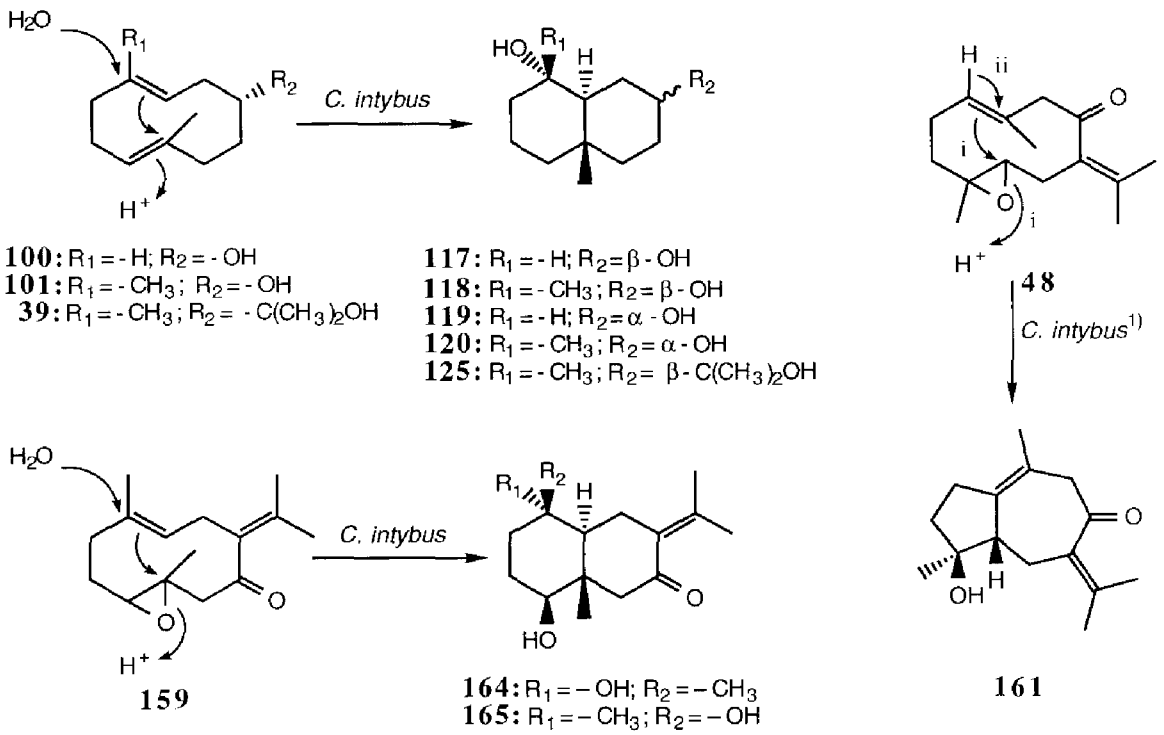
7.2. Substrate specificity of germacrane cyclising enzymes from chicory

A summary of the cyclisation reactions which were unambiguously accelerated by the cyclase from *C. intybus* is presented in scheme 7.4. The substrates are displayed in an identical manner and are divided according to their geometry in order to show the analogy in the cyclisation reactions. From the cyclisation studies described in scheme 7.4., several generalisations can be made concerning the substrate specificity of the germacrane cyclising enzymes in the chicory. Cyclisation is initiated by enzyme-mediated protonation of the most nucleophilic site of the substrate followed by a transannular cyclisation and stereoselective water incorporation or deprotonation. Apparently, the active site of the cyclase is able to accommodate various configurationally isomeric cyclodecadienes[23].

In chapter 4, the germacrane cyclase from *C. intybus* was compared with the oxidosqualene cyclase as postulated by Johnson[24]. In this model, cyclisation is initiated by protonation from a specific amino acid residue which acts as a general acid catalyst. An identical kind of initiation can be envisaged for the germacrane cyclase from chicory. When cyclisation proceeds, electron-rich aromatic side chains, which are essential features of cyclase active sites[25], seem to direct the folding of the substrate and stabilise positively charged transition states and/or high energy intermediates. While in the oxidosqualene model the reaction is terminated by deprotonation, the germacrane cyclase often showes incorporation of a water molecule. Only two substrates displayed selective deprotonation (**48** and **167**, respectively) which means that, apparently, specific positioning of the substrate in the active site is a prerequisite for deprotonation.

Scheme 7.4.

(E,E)-cyclodeca-1,5-dienes and epoxides thereof:



100: R_1 = - H; R_2 = - OH
101: R_1 = - CH_3; R_2 = - OH
39: R_1 = - CH_3; R_2 = - $C(CH_3)_2OH$

117: R_1 = - H; R_2 = β - OH
118: R_1 = - CH_3; R_2 = β - OH
119: R_1 = - H; R_2 = α - OH
120: R_1 = - CH_3; R_2 = α - OH
125: R_1 = - CH_3; R_2 = β - $C(CH_3)_2OH$

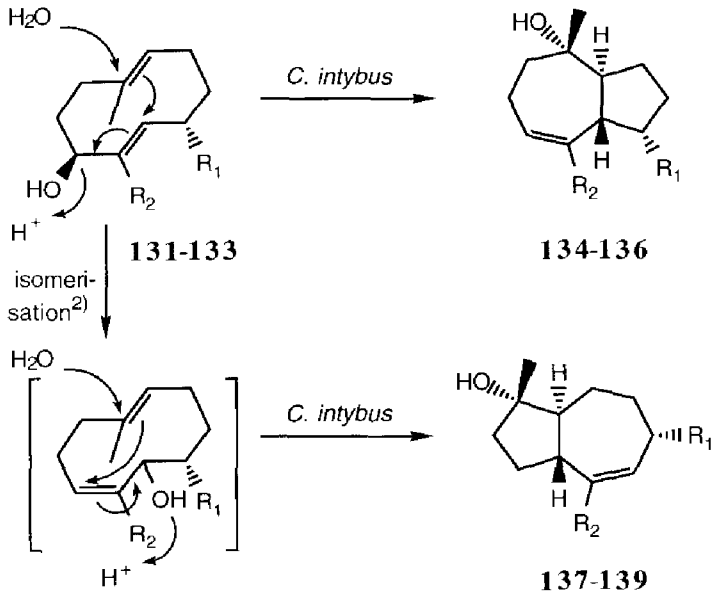
48

159

164: R_1 = – OH; R_2 = – CH_3
165: R_1 = – CH_3; R_2 = – OH

161

(E,E)-cyclodeca-1,6-dienes:



131-133

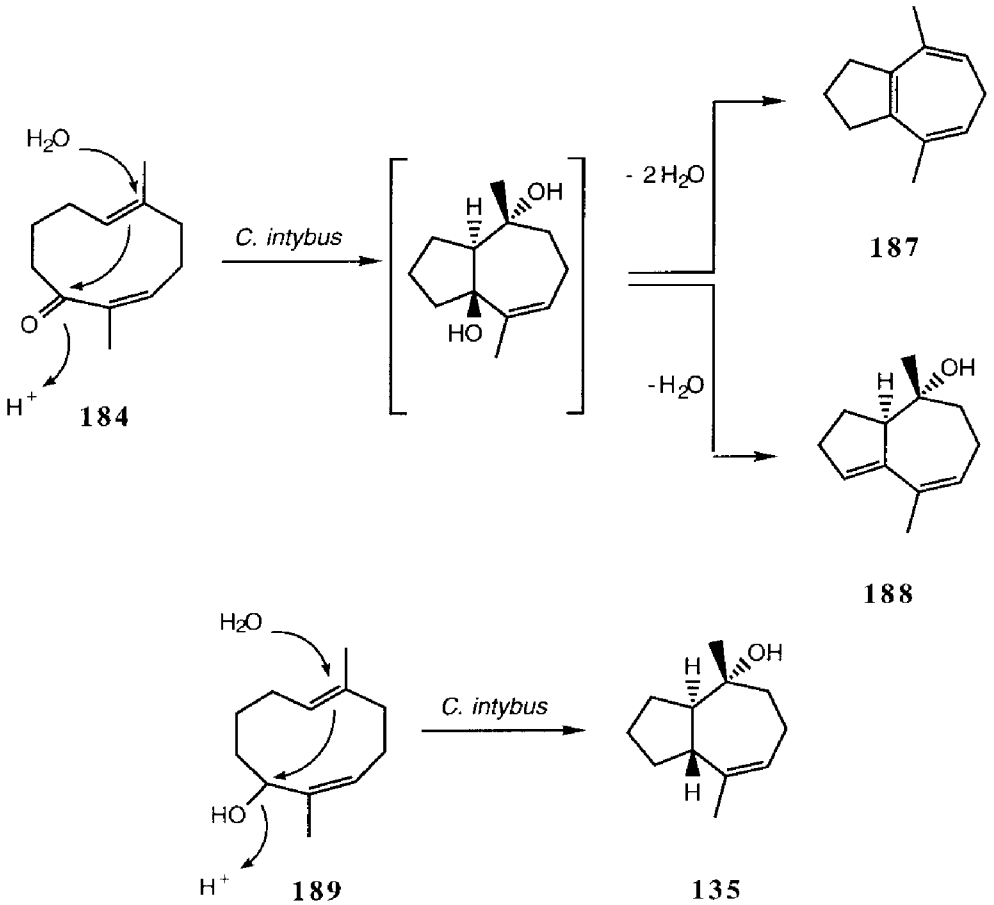
134-136

137-139

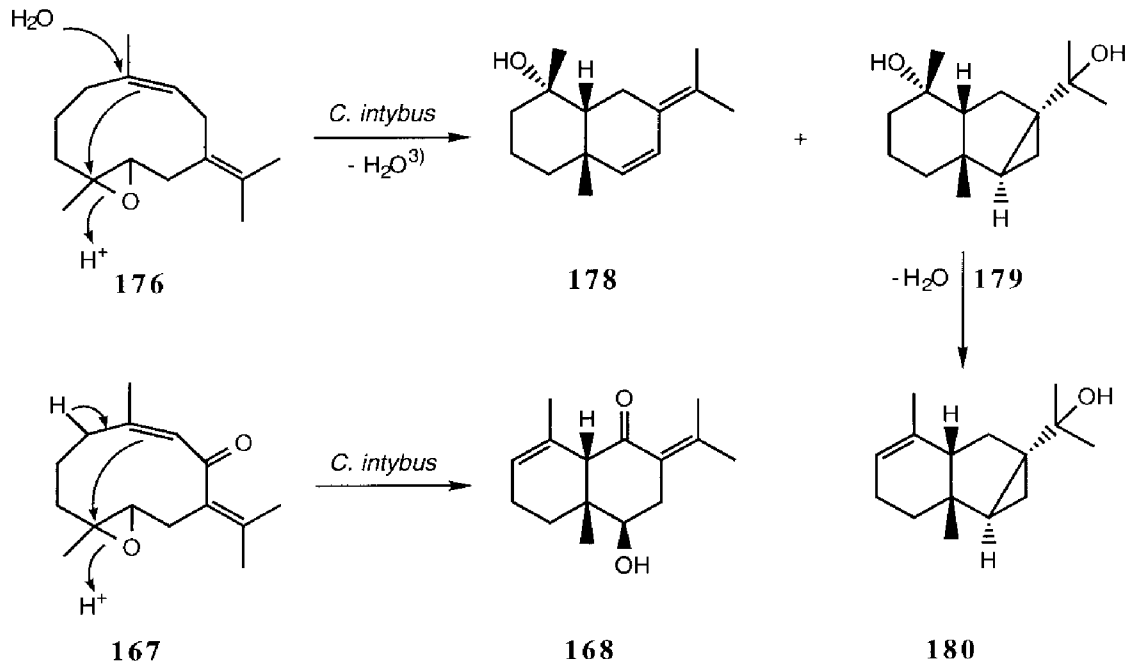
131,134,137: R_1 = - H; R_2 = - H
132,135,138: R_1 = - H; R_2 = - CH_3
133,136,139: R_1 = - CH_3; R_2 = - H

1) The annotations i and ii represent two consecutive reaction steps
2) For the isomerisation reaction, see scheme 4.3.

(E,Z)-cyclodeca-1,5-dienes:

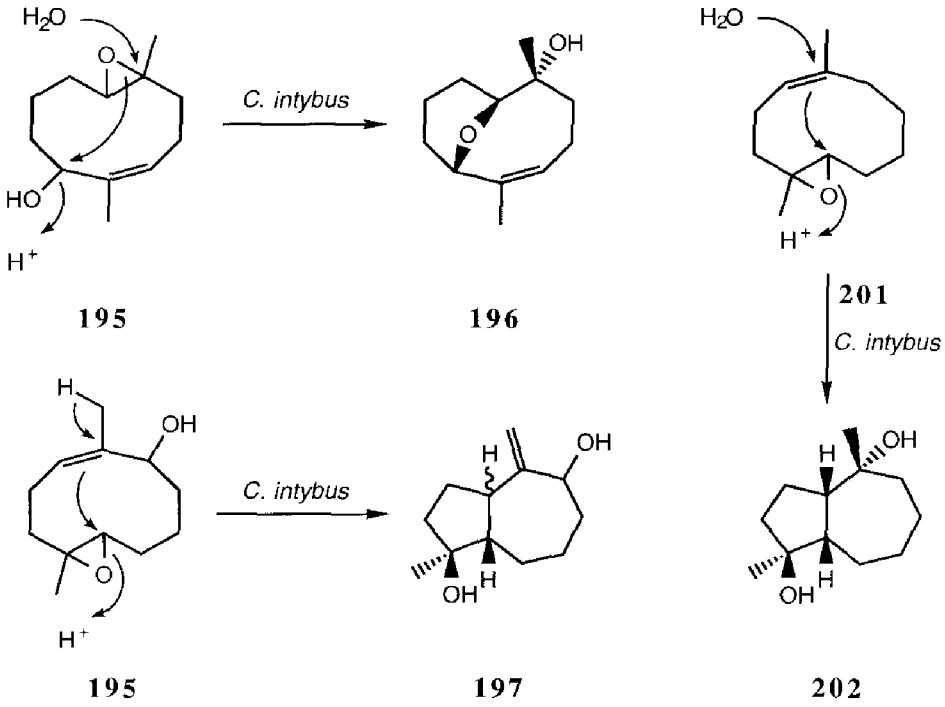


(E,Z)-cyclodeca-1,6-diene epoxides:



3) The formation of **178-180** proceeded through a diol intermediate, see scheme 5.10.

(E,Z)-cyclodeca-1,5-diene epoxides:



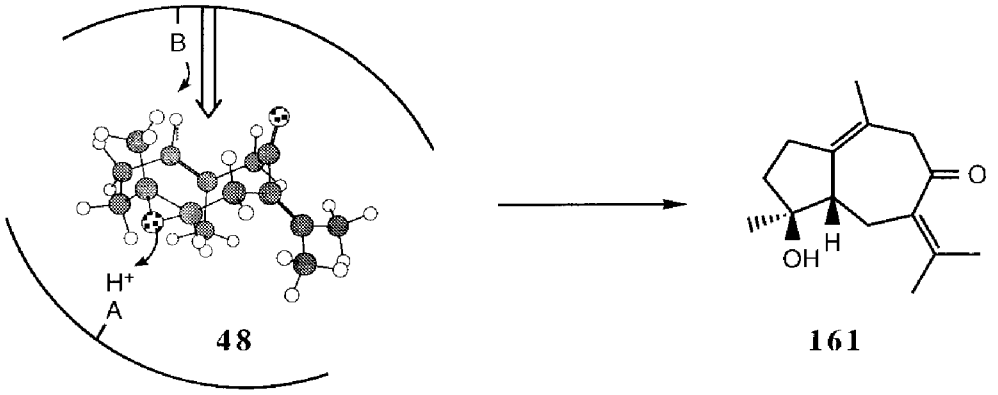
195 **196** **195** **197** **202**

In order to postulate an active site model for the germacrane cyclase from *C. intybus*, the biotransformation of germacrone-4,5-epoxide (**48**) into neoprocurcumenol (**161**) was taken as a starting point. Following the model of Johnson and Ourisson[26-28], three crucial elements have to be present in the active site: an acidic side (AH^+), a basic side (B) and (a) nucleophilic side(s) (arrow). These nucleophilic sides represent the electron-rich side chains. Upon incorporation of **48** into the active site, cyclisation is initiated by the protonation of the epoxide by AH^+ followed by transannular cyclisation and stabilisation of the developing positive charge by the electron-rich side chains. Subsequently, B abstracts H_1 to form **161**, as shown in scheme 7.5.

In this postulated model, the incorporation of a water molecule in the cyclisation product can be rationalised by the action of the basic group B in the active site. Since the model is based on the deprotonation process of specifically suited substrates, deviations of the specific shape of the substrate may lead to either a distortion of the active site, or to a drastic change in the distance between the reaction sites of the substrate and the active site of the enzyme, thus changing the outcome of the product. It is to be expected that the distance between the acidic group AH^+ and the most nucleophilic site of the substrate is more or less identical in every enzyme mediated cyclisation reaction. Therefore, structural changes in the substrate are likely to have repercussions on the distance between the basic group B, the stabilising nucleophilic group and the developing positive charge at the opposite side of the ring when

cyclisation is initiated. If the specific distance between B and the positively charged carbon centre of the substrate, which is required for deprotonation, is not met, B is most likely to abstract a proton from a water molecule than from the substrate. This leads to stereoselective incorporation of a water molecule in the cyclisation products.

Scheme 7.5.



Thus the mode of action of the germacrane cyclase from *C. intybus* can be characterised as follows:

-) protonation of the substrate takes place at the most nucleophilic site of the substrate.
-) depending on the conformation and configuration of the substrate, the reaction is terminated either by deprotonation or water incorporation.

Acid-induced cyclisation studies on the substrates discussed in chapters 3-6 revealed an almost identical product formation as compared to the enzyme-induced cyclisation reactions. Therefore, the ring fusion of the cyclisation products appears to be dependent on the geometry and position of the double bond in the cyclodecadiene framework rather than being directed by the enzyme. Only three substrates showed an anomalous cyclisation pattern i.e., germacrone-4,5-epoxide (**48**), hedycaryol (**39**) and germacrone-1,10-epoxide (**159**). All other substrates, which cyclisation was unambiguously accelerated by the germacrane cyclase, showed an identical product formation in both the biotransformation and the acid-induced cyclisation reaction. It is noteworthy that **39**, **48** and **159** are (derivatives of) (E,E)-1,5-germacranes, a framework which is identical to germacranes endogenous to chicory. The fact that configurationally isomeric germacranes and cyclodecadienes are also accepted indicates that the cyclase has a broad substrate specificity. The accepted substrates can be grouped into (derivatives of) (E,E)-cyclodeca-1,5-dienes (**39**, **48**, **100**, **101**, **159**), (E,Z)-cyclodeca-1,5-

dienes (**184, 189, 201**), (E,E)-cyclodeca-1,6-dienes (**131-133**) and (E,Z)-cyclodeca-1,6-dienes (**167, 176**). The broad substrate specificity is nicely illustrated by the conversion of compound **195**. This substrate can appearently be bound in two different orientations, leading to **196** and **197**, as outlined in scheme 7.4.

During this research it also became clear that additional ring substituents play an important role in the conformational aspects of the substrate. This was especially observed in the cyclisation reaction of (+)-hedycaryol (**39**) into cryptomeridiol (**125**), as discussed in chapter 3. The large isopropanol group of **39** prohibited inversion of the germacrane ring, as was observed for the (E,E)-cyclodeca-1,5-dienols **100** and **101**. The role of the C_8-keto function of germacrone-1,10-epoxide (**159**) in the formation of the epimeric mixture of the diols **164** and **165** still remains unsolved. The presence of a sp^2-centre at C_8 probably influences the configuration of the substrate to such an extend, that water incorporation can occur from either side of the molecule. The unambiguous product formation of the (E,E)-1,5-germacranes and the (E,E)-cyclodeca-1,5-dienes, together with the fact that no (E,Z)- 1,5- and (E,Z)-1,6-germacranes have (yet) been isolated from chicory, indicates that the active site of the germacrane cyclase from chicory is specifically suited for (E,E)-1,5-germacranes and epoxides thereof. However, endogenous germacranes from chicory also possess an additional lactone moiety. Since no endogenous germacranes were (or could be) tested, the role of the lactone moiety on the cyclisation process remains unclarified.

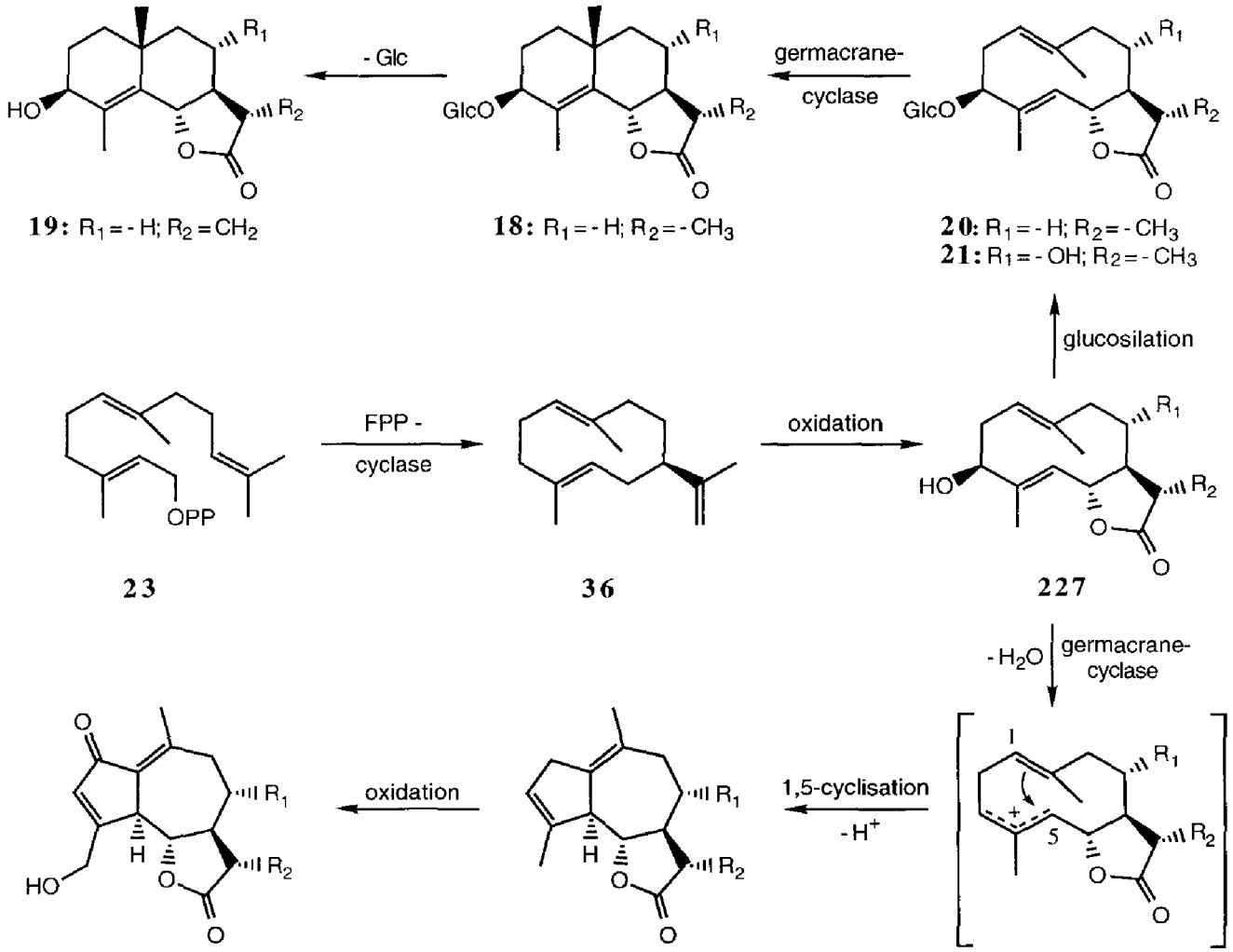
7.3. Proposed biosynthesis of sesquiterpenes in chicory

Chicory is a rich source of guaiane lactones, i.e. lactucin (**12**), 8-deoxylactucin (**13**) and lactucopicrin (**14**) as well as their 11,13-dihydro-derivatives (**15-17**). The group of Seto[29] also reported the presence of the eudesmanolides sonchuside C (**18**) and cichoriolide A (**19**) together with the germacranolides sonchuside A (**20**) and cichoriolide C (**21**). These eudesmanolides and germacranolides possess a hydroxyl function at C_3, which is sometimes glucosilated. The guaianolides lack this function and have a carbon-carbon double bond instead. It might be possible that this hydroxyl group is originally present in the biogenetic precursors of the guaianolides. Based on the co-occurrence of germacranes and various cyclisation products with an identical substitution pattern (see section 2.3.2.), it is believed that oxidative modifications of the germacrane framework preceed the transannular cyclisation process.

With the results of the chapters 3-5 and the previous paragraphs in this chapter, it is now possible to postulate a biosynthesis of the endogenous sesquiterpene lactones in chicory.

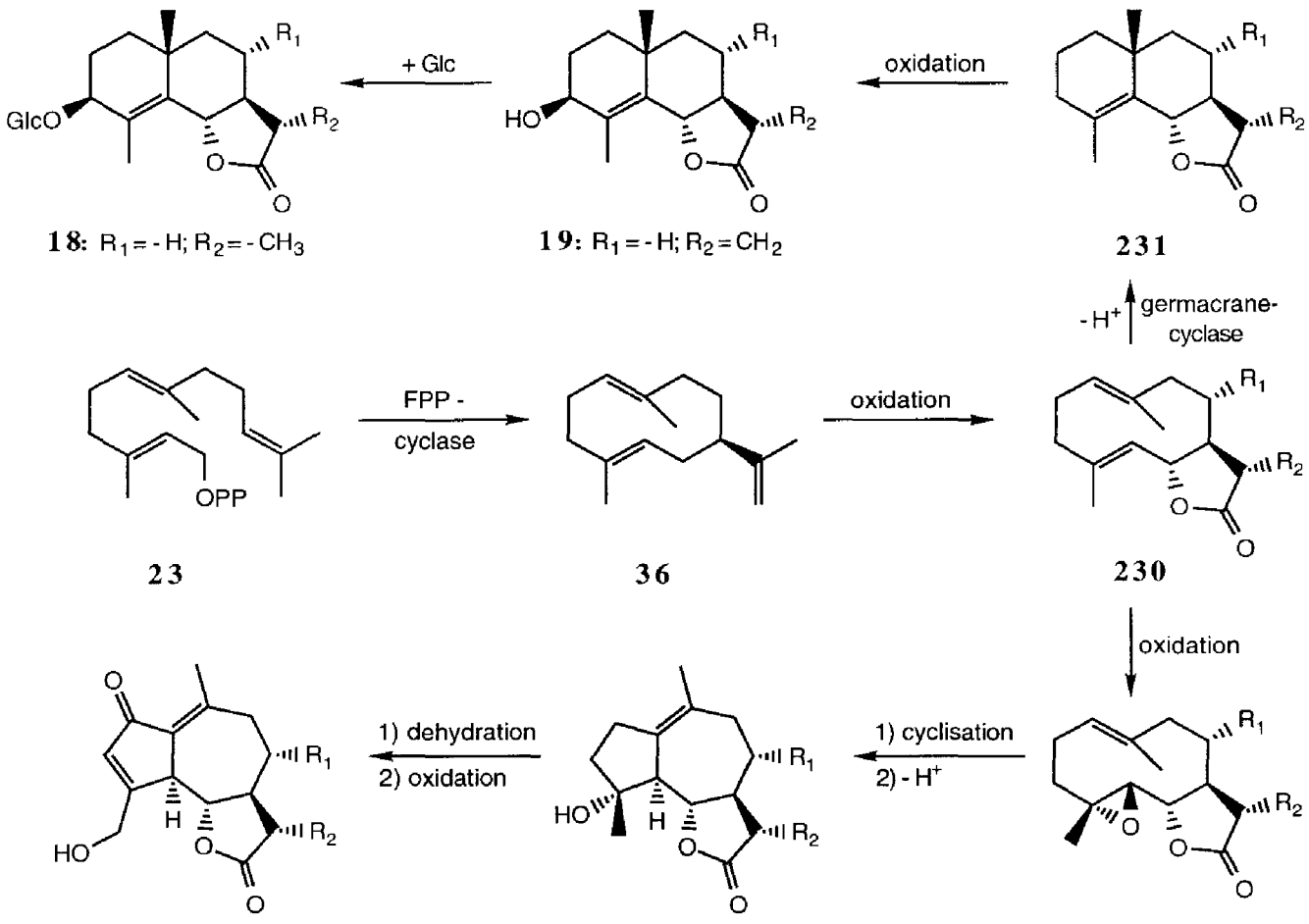
Cyclisation of farnesyl pyrophosphate (**23**) by the FPP-cyclase of *C. intybus* gives germacrene A (**36**) which is transformed, after several oxidation steps, into intermediate **227** (Scheme 7.6.). This intermediate might be the branching point in the biosynthesis of guaianolides, eudesmanolides and germacranolides. Enzyme-mediated cyclisation of **227** could start with the protonation of the C_3-hydroxyl group giving allylic cation **228**.

Scheme 7.6.



This cation then gives **229** after a 1,5-cyclisation, as was observed for the (E,E)-cyclodeca-1,5-dienes **131-133** in chapter 4, followed by a selective deprotonation towards the bridgehead carbon atom of the cyclised cationic intermediate. This selective deprotonation was also observed in the formation of neoprocurcumenol (**161**) from germacrone 4,5-epoxide (**48**, see chapter 5). Compound **229**, which has not been detected in *C. intybus* thus far, is further oxidised into the guaianolides **12-17**. Glucosilation of the C_3-hydroxyl function of **227** gives sonchuside A (**20**) and cichorioside C (**21**) which can be cyclised by germacrane cyclasing enzymes into the corresponding eudesmanolides, e.g. **18**. The ability of germacrane cyclases to transform (E,E)-cyclodeca-1,5-dienes into compounds with a decalin framework has been described in chapter 3.

Scheme 7.7.



18: R_1 = -H; R_2 = -CH_3
19: R_1 = -H; R_2 = CH_2
231
23
36
230
233
232
12: R_1 = -OH; R_2 = CH_2
15: R_1 = -OH; R_2 = -CH_3
13: R_1 = -H; R_2 = CH_2
16: R_1 = -H; R_2 = -CH_3
14: R_1 = -*p*-$OOCCH_2C_6H_4OH$; R_2 = CH_2
17: R_1 = -*p*-$OOCCH_2C_6H_4OH$; R_2 = -CH_3

Presumably, glucosilation of the C_3-hydroxyl group prevents the 1,5-cyclisation process towards the guaianolides, although an unglucosilated eudesmanolide (**19**) has been isolated from *C. intybus*. However, **19** may be the result of glucosidase activity which has been reported in the chicory[30].

An alternative biosynthetic route may proceed through intermediate **230** (Scheme 7.7.), also a likely oxidation product of germacrene A (**36**). Enzyme mediated cyclisation of **230** gives eudesmanolide **231** which can be hydroxylated to give cichoriolide A (**19**). Glucosilation of the 11,13-dihydroderivative of **19** will yield sonchuside C (**18**). Enzyme mediated epoxidation of **230** may give the corresponding epoxide **232**. Cyclisation followed by selective deprotonation towards the bridgehead carbon atom would yield guaianolide **233**. The ability of germacrane cyclases to perform this selective cyclisation-deprotonation reaction has been demonstrated in chapter 5. Compound **233** has to be further oxidised in order to give the guaianolides **12-17**.

7.4. Concluding remarks

The scope of this thesis, to detect and study the substrate specificity of cyclising enzymes in chicory, and postulate a biogenetic route for the sesquiterpene lactones in *C. intybus*, has been fulfilled for the greater part for the cyclisation reactions that are involved. A large number of germacrane synthons have been synthesised and the majority of these compounds have been cyclised by a root homogenate of fresh chicory into a wide variety of bicyclic products. With this information, two biosynthetic routes starting from farnesyl pyrophosphate (**23**) towards the sesquiterpene lactones, present in chicory, could be presented. The latter route, represented by scheme 7.7., seems less likely because it involves several additional steps.

Unfortunately, nothing can be said with respect to the oxidising enzymes in chicory. It is also not clear if the cyclase, which catalyses the cyclisation of germacranes into guaianes and eudesmanes and the cyclase that catalyses the transformation of farnesyl pyrophosphate into the germacrane framework, are identical or are, in fact, two different enzymes. Consequently, a lot of work still needs to be done before a complete insight in the biosynthesis of the sesquiterpene lactones from *C. intybus* can be obtained.

7.5. Experimental

Mass spectral data were obtained with a Hewlett Packard 5890 Series II GC-MS equipped with a Hewlett Packard 5972A series mass selective detector using a capillary HP-5MS column (30 m x 0.25 mm, d_f 0.25 μm) and helium as the carrier gas. Radio-GC was performed on a Carlo-Erba HRGC 4160 with a Raytest RAGA 93 radioactivity detector using a CP wax 52 CB column (25 m x 0.32 mm) and helium as the carrier gas. The Econo-Pac 10DG (Bio-Gel P-6) desalting column was obtained from BioRad, (E,E)-nerolidol (**222**) was obtained from Roth and (E,E)-farnesol (**223**) was obtained from Aldrich. [1-^{3}H]-FPP (**23**, Specific activity: 15-20 Ci/mol) was obtained from Amersham and is dissolved in ethanol / 0.1 M NH_4HCO_3 1:1. The unlabeled (trilithium salt of) FPP was synthesised according to the procedure of Popják *et al*[31].

Enzyme purification: The cleaned, dehaired chicory roots (50 g) were chopped in a blender at 4°C in 80 ml of extraction buffer consisting of glycerol (20% w/v), 50 mM of 3-morpholino-2-hydroxypropanesulfonic acid (MOPSO), 50 mM of $Na_2S_2O_5$, 50 mM of ascorbic acid, 10 mM of $MgCl_2$, 5 mM of dithiothreitol (DTT) and 5.0 g of insoluble polyvinyl-polypyrrolidone at pH 7.0. The blender was rinsed with 40 ml of buffer and the obtained 120 ml of homogenate was stirred with 25 g of XAD-4 for 10 min. and filtered through a cheesecloth moisturised with extraction buffer. The filtrate was centrifuged at 20.000 g (20 min., 4°C), the pellet was discarded and the supernatant was centrifuged at 100.000 g (90 min., 4°C). This supernatant was used as the crude enzyme source.

Enzyme assay: The desalting column was washed with 10 ml of demineralised water and equilibrated with 15 ml of assay buffer consisting of glycerol (10% w/v), 5 mM of MOPSO, 10 mM of $MgCl_2$, 1 mM of ascorbic acid and 2 mM of DTT at pH 7.0. The 100.000 g supernatant (3 ml) was adsorbed on the column and eluted with 4 ml of assay buffer. A 1.0 ml aliquot of the eluent was incubated with 20 μM of a mixture of unlabeled and labeled FPP (**23**, approximately 1 μCi/aliquot) for 1h at 30°C. The incubation was stopped by shaking the sample with 1.5 ml of pentane and cooling the assay on ice. The pentane phase was filtered through a Pasteur pipette filled with Al_2O_3 (grade III), covered with $MgSO_4$. The extraction-filtration procedure was repeated with 1 ml of 20% of ether in pentane and 2 x 1 ml of ether. The pentane and ether phases were collected separately. Both aliquots were counted with scintillation spectrometry, a carrier solution of **173**, **221-223** was added and the mixture, after concentrating under N_2, was analysed by radio-GC. A second incubation using only unlabeled FPP (**23**) was performed in an identical way as described above. The contents of the incubation mixture was analysed on GC-MS.

γ-Elemene (221): A pure sample of 250 mg of **173**[32] was quantatively converted into **221** by heating germacrene B at 160°C under an argon atmosphere for 16h. ^{1}H-NMR of **221**: δ 5.77 (dd, 1H, H_1, J = 17.4 Hz, 10.9 Hz); δ 4.95-4.80 (m, 3H); δ 4.60 (broad s, 1H); δ 2.55-2.35 (m, 2H); δ 2.10-1.85 (m, 2H); δ 1.93 (dd, 1H, J = 12.7 Hz, 1.4 Hz); δ 1.65 (d, 3H, Me_{15}, J = 0.8 Hz); δ 1.60 (2 x s, 6H, Me_{12}, Me_{13}); δ 1.40 (m, 2H); δ 1.00 (s, 3H,

Me_{14}). ^{13}C-NMR of **221**: δ 149.95 (d), δ 147.96 (s), δ 130.95 (s), δ 121.01 (s), δ 111.85 (t), δ 109.92 (t), δ 53.05 (d), δ 40.10 (t), δ 39.93 (s), δ 31.32 (t), δ 25.46 (t), δ 24.77 (q), δ 20.05 (q), δ 19.94 (q), δ 16.77 (q). Mass spectrum of **221** (m/e): [M^+] 204 (8), 189 (10), 161 (21), 136 (17), 131 (23), 122 (20), 121 (100), 108 (24), 107 (51), 105 (44), 93 (77), 91 (49), 81 (28), 79 (42), 77 (35), 69 (15), 67 (49), 65 (16), 55 (35), 53 (41), 43 (21), 41 (82), 39 (45).

7.6. References and notes

1. Birnbaum G.I., Huber C.P., Post M.L., Stothers G.B., Robinson J.R., Stoessl A. and Ward E.W.B., *J. Chem. Soc., Chem. Commun.*, 330 (1976).
2. Lawrence B.M. and Hogg J.W., *Phytochemistry*, **12**, 2995 (1973).
3. Baker R., Cole H.R., Edwards M., Evans D.A., Howse P.E. and Walmsley S., *J. Chem. Ecol.*, **7**, 135 (1981).
4. Cane D.E., Rawlings B.J. and Yang C.-C., *J. Antibiot.*, **40**, 1331 (1987).
5. Cane D.E., Salaski E.J. and Prabhakaran P.C., *Tetrahedron Lett.*, **31**, 1943 (1990).
6. Hohn T.M. and Plattner R.D., *Arch. Biochem. Biophys.*, **272**, 137 (1989).
7. Moreau S., Lablanche-Combier A. and Biguet J., *Phytochemistry*, **20**, 2339 (1981).
8. Gorst-Allman C.P. and Steyn P.S., *Tetrahedron Lett.*, **23**, 5359 (1982).
9. Chalmers A.A., DeJesus A.E., Gorst-Allman C.P. and Steyn P.S., *J. Chem. Soc., Perkin Trans I*, 2899 (1981).
10. Benedict C.R., Alchanati I., Harvey P.J., Liu J., Stipanovic R.D. and Bell A.A., *Phytochemistry*, **39**, 327 (1995).
11. Davis G.D. and Essenberg M., *Phytochemistry*, **39**, 553 (1995).
12. Davis E.M., Tsuji J., Davis G.D., Pierce M.L. and Essenberg M., *Phytochemistry*, **41**, 1047 (1996).
13. Croteau R. and Cane D.E., *Meth. Enzymol.*, **110**, 383 (1985).
14. Hohn T.M. and vanMiddlesworth F., *Arch. Biochem. Biophys.*, **251**, 756 (1986).
15. Munck S.L. and Croteau R., *J. Biol. Chem.*, unpublished results.
16. Alonso W.R and Croteau R., *Prenyltransferases and Cyclases* in *Methods in Plant Biochemistry* (Dey P.M. and Harborne J.B., series eds.), Vol. 9 (Lea P.J., ed.), Academic Press, London, 239-260 (1993).
17. Loomis W.D. and Battaile J., *Phytochemistry*, **5**, 423 (1966).
18. Loomis W.D., Lile J.D., Sandstorm R.P. and Burbott A.J., *Phytochemistry*, **18**, 1049 (1979).
19. Maat L., Straver E.J.M., van Beek T.A., Posthumus M.A. and Piozzi F., *J. Essent. Oil Res.*, **4**, 615 (1992).
20. Posthumus M.A., unpublished results.

21. Weinheimer A.J., Youngblood W.W., Washecheck P.H., Karns T.K.B. and Cierszko L.S., *Tetrahedron Lett.*, 497 (1970).
22. Durst, F., *Biochemistry and physiology of plant cytochrome P-450.* In: *Frontiers in Biotransformation* Vol. 4. *Microbial and plant cytochromes P-450: Biochemical characteristics genetic engineering and practical implications* (K. Ruckpaul and H. Rein, Eds), Taylor & Francis, London, pp 191-232 (1991).
23. Humulane derivatives (eleven-membered rings) were not accepted as substrates; Dignum M.J.W., *Zuur- en witlofwortel gekatalyseerde omzettingen van 1,3-humuladion epoxiden*, MSc-thesis, Wageningen Agricultural University (1996).
24. Johnson W.S., *Tetrahedron*, **47**, xi-1 (1991).
25. Buntel C.J. and Griffin J.H., *Evolution of Sterol and Triterpene Cyclases.* in: *Isopentenoids and Other Natural Products: Evolution and Function*, (Nes W.D., ed.), ACS Symposium Series, **562**, 52 (1994).
26. Rohmer M., Bouvier P. and Ourisson G., *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 847 (1979).
27. Ourisson G., Rohmer M. and Poralla K., *Ann. Rev. Microbiol.*, **41**, 301 (1987).
28. Ourisson G., *Pure Appl. Chem.*, **61**, 345 (1989).
29. Seto M., Miyase T., Umehara K., Ueno A, Hirano Y. and Otani N., *Chem. Pharm. Bull.*, **36**, 2423 (1988).
30. Leclercq E., *Sesquiterpene Lactones and Inulin from Chicory Roots: Extraction, Identification, Enzymatic Release and Sensory Analysis*, PhD.-thesis, Wageningen Agricultural University (1992).
31. Popják G., Cornforth J.W., Cornforth R.H., Ryhage R. and Goodman D.S., *J. Biol. Chem.*, **237** (1962); A 10 mM stock-solution was prepared in ethanol / 0.2 M NH_4HCO_3.
32. Germacrene B (**173**) was prepared as earlier described; D.P. Piet, R. Schrijvers, M.C.R. Franssen and Ae. de Groot, *Tetrahedron*, **51**, 6303 (1995).

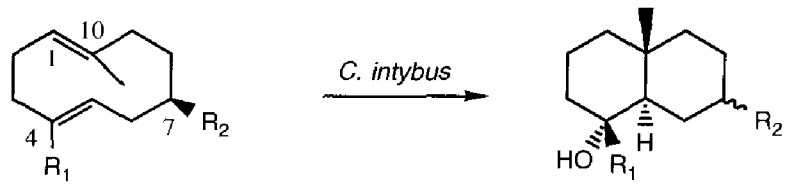
8. Summary

Chicory (*Cichorium intybus* L.), one of the many species of the Compositae family, has been cultivated for the production of the leaves since 300 BC as a food supplement and since the 16th century as a substitute for coffee. The sprouts of the chicory are appreciated for their bitter taste. This bitter taste is associated with the presence of sesquiterpene lactones. The majority of these sesquiterpene lactones possess a guaiane framework, a small number possesses a eudesmane- or a germacrane framework. The abundance of these sesquiterpene lactones is not limited to the leaves of the plant. Considerable amounts of are also present in the root, currently an agricultural waste product. Not only is the root a rich source of sesquiterpene lactones, it also contains a large amount of inulin (**1**), a storage carbohydrate which is based on fructose instead of glucose. Fructose, an interesting sweetener, is a versatile building block in the synthesis of several polymers and natural products. The bitter principles in the chicory may find their application as a bitter tasting additive in consumer goods.

The biosynthesis of the sesquiterpene lactones in the chicory is believed to start from a head to tail cyclisation of farnesyl pyrophosphate (**23**) into a germacrane, followed by cyclisation into eudesmanes and guaianes. This thesis deals with the cyclisation of germacrane synthons and natural germacranes, induced by a root homogenate of fresh chicory. The goal is to determine the substrate specificity of the germacrane cyclase of chicory and to obtain more insight in the biosynthesis of the sesquiterpene bitter principles in *C. intybus*.

A general introduction on the history, use and contents of the chicory is given in chapter 1. In chapter 2, an overview of the literature on the (bio)synthesis of germacrane sesquiterpenes and their possible biotransformation into a variety of cyclised products, is presented.
In chapter 3, the synthesis of two (E,E)-cyclodeca-1,5-dienols possessing the germacrane framework (**100** and **101**), is described. The cyclisation behaviour of these compounds and the natural germacrane (+)-hedycaryol (**39**) towards a chicory root homogenate is discussed. The cyclising enzymes in this homogenate transform the 10-membered ring compounds into products with a eudesmane skeleton by protonation of the C_1-C_{10} double bond followed by transannular cyclisation and subsequent stereoselective incorporation of a water molecule at C_4. The flexibility of the 10-membered ring system was demonstrated by the formation of the epimeric diols **117-120** from **100** and **101**. The relatively small hydroxyl function at C_7 permitted

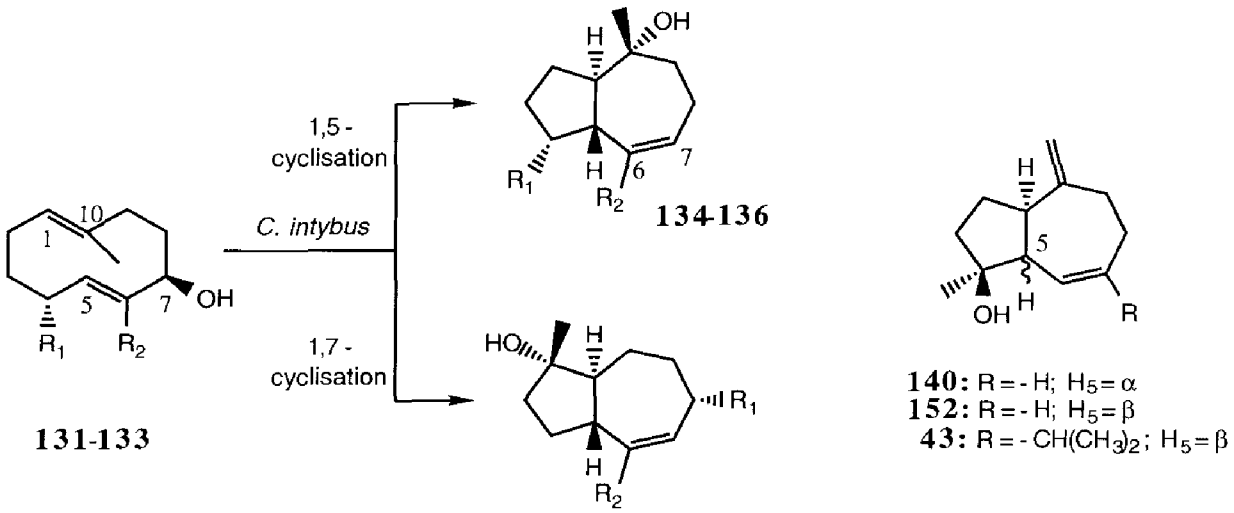
inversion of the germacrane framework, enabling cyclisation through two different syn-conformations. The large isopropanol group of **39** prohibits this inversion to such an extent that cyclisation takes place only through one conformation to give crypto-meridiol (**125**).



100: R_1 = - H; R_2 = - OH
101: R_1 = - CH_3; R_2 = - OH
39: R_1 = - CH_3; R_2 = - $C(CH_3)_2OH$

117: R_1 = - H; R_2 = β - OH
118: R_1 = - CH_3; R_2 = β - OH
119: R_1 = - H; R_2 = α - OH
120: R_1 = - CH_3; R_2 = α - OH
125: R_1 = - CH_3; R_2 = β - $C(CH_3)_2OH$

In chapter 4, the synthesis of the three (E,E)-cyclodeca-1,6-dienols **131-133** and their cyclisation by a chicory root homogenate is described. Two kinds of hydroazulene alcohols were obtained in these reactions arising from 1,5- and 1,7-cyclisation. The 1,5-cyclisation products (**134-136**) are formed through an internal nucleophilic displacement of the allylic alcohol moiety by the C_1-C_{10} double bond, while in the formation of the 1,7-cyclisation products (**137-139**), an allylic isomerisation reaction of the (E,E)-cyclodeca-1,6-dienol skeleton into an allylic (E,E)-cyclodeca-1,5-dienol skeleton preceded the internal nucleophilic displacement reaction. Hydroazulenes possessing a C_6-C_7 double bond like **134** resemble natural products like alismol (**43**).



131-133

134-136

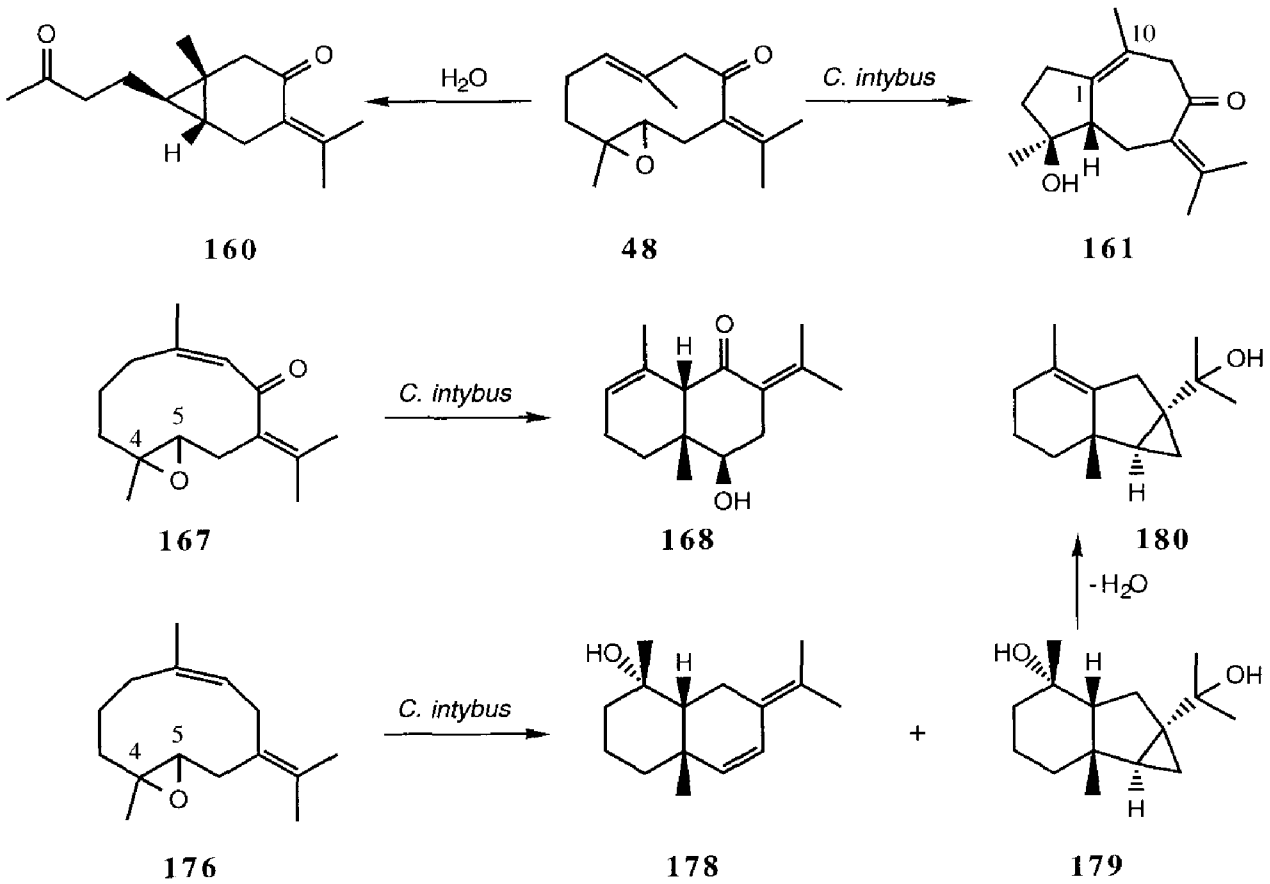
137-139

131,134,137: R_1 = - H; R_2 = - H
132,135,138: R_1 = - H; R_2 = - CH_3
133,136,139: R_1 = - CH_3; R_2 = - H

140: R = - H; H_5 = α
152: R = - H; H_5 = β
43: R = - $CH(CH_3)_2$; H_5 = β

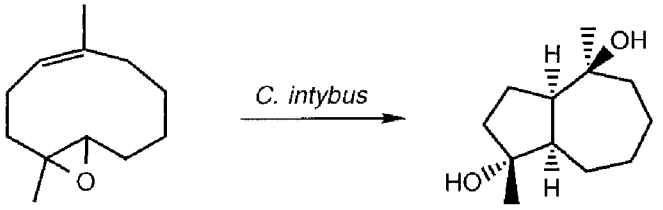
Recently, the structure of a trinor-guaiane, dictamnol (**140**), similar to alismol, was published. The ring fusion of dictamnol (**140**) was postulated as cis. Since **140** was already synthesised at our laboratory and major discrepancies were found between our NMR spectral data of **140** and those reported in the literature, serious doubt about the stereochemistry and the ring junction arose. Therefore, natural dictamnol was isolated, its stereochemistry was reinvestigated and a structural revision into a trans-fused hydroazulene (**152**) is proposed.

In chapter 5, the biotransformation of derivatives of germacrone, a readily available sesquiterpene germacrane, is described. In a number of cases, enzyme mediated cyclisation of the chemically epoxidised germacrone derivatives, had to compete with spontaneous cyclisation reactions. However, some selectivity was observed, especially in the biotransformation of germacrone-4,5-epoxide (**48**) into neoprocurcumenol (**161**). Compound **161** is the only product obtained through an enzyme-mediated cyclisation of **48**. The C_1-C_{10} double bond in **161** is characteristic for guaiane bitter principles in the chicory. In boiled root samples, the only conversion that was observed was a homofragmentation reaction of **48** into curcumenone (**160**).



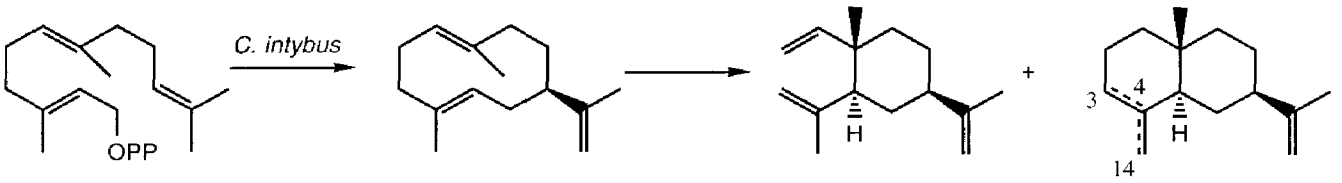
The synthesis of isogermacrone (**166**) paved the way for studying the influence of the position and the stereochemistry of the double bond on the ring fusion of the cyclisation products. The 4,5-epoxides of isogermacrone (**167**) and isogermacrene B (**176**) were transformed by a chicory root homogenate into a cis-fused eudesmane and two tricyclo[4.4.0.0]sesquiterpenes.

Chapter 6 deals with the synthesis of (E,Z)-cyclodeca-1,5-dienone **184** and the biotransformation of **184** and structurally related compounds. Transannular cyclisation reactions of (E,Z)-cyclodeca-1,5-dienes appear to proceed in a different way as compared to the (E,E)-cyclodeca-1,5-dienes in chapter 3-5. Instead of a carbon-carbon bond formation between both double bonds of the germacrane skeleton, ring substituents are involved in the cyclisation process to relieve ring strain. If no additional ring substituents are present, e.g. in E-epoxide **201**, a cis-fused hydroazulene diol (**202**) is obtained. The Z-epoxides **211** and **214** were not or not unambiguously transformed by a chicory root homogenate.



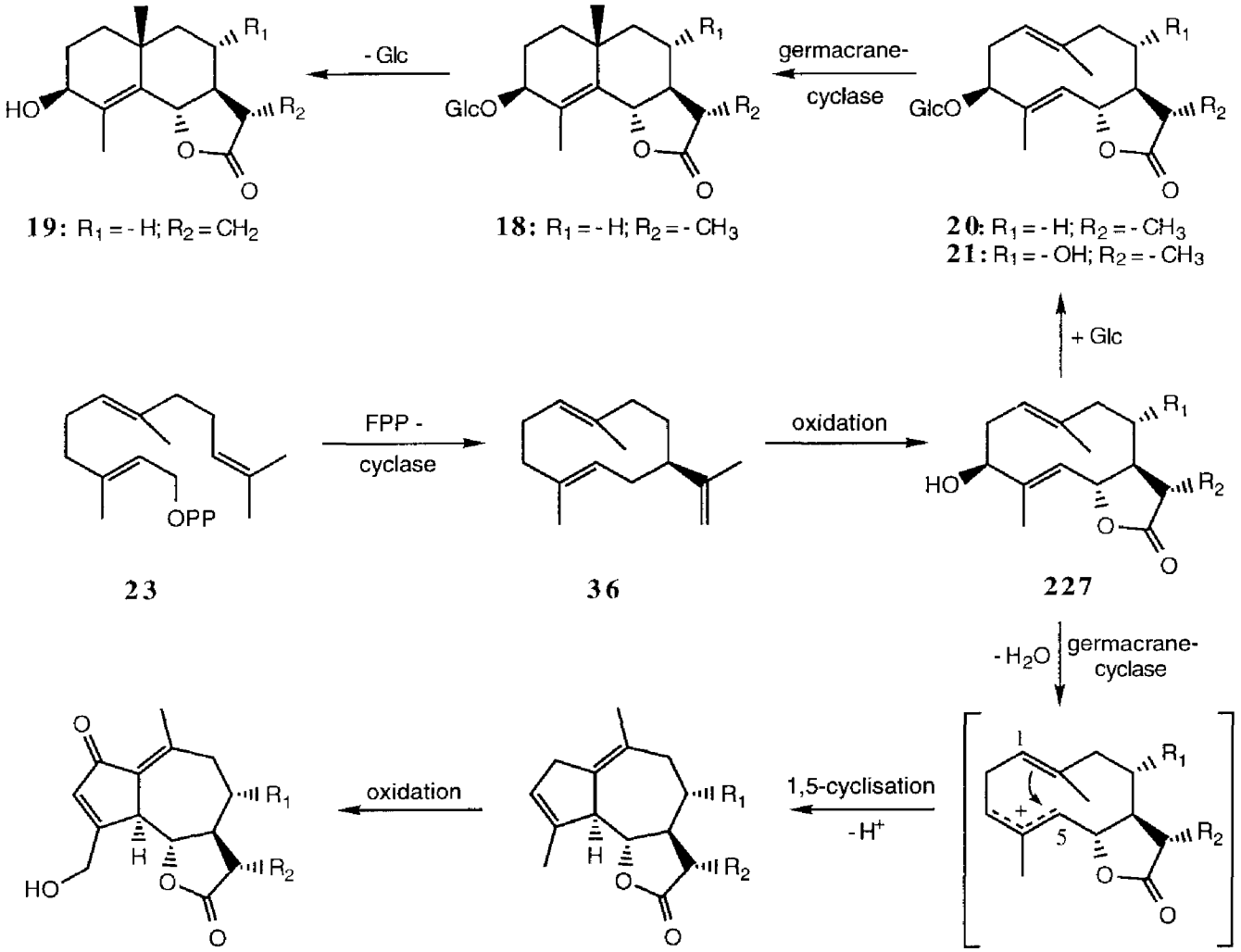
201 **202** **211:** R_1 = -OH; R_2 = -H; **214:** R_1, R_2 = O

In chapter 7, the biotransformation of farnesyl pyrophosphate (**23**) by a partially purified chicory root homogenate is described. Radio-GC and GC-MS analysis of the incubation products obtained from [1-^{3}H]-farnesyl pyrophosphate revealed that **23** was initially transformed into germacrene A (**36**). However, the Cope rearrangement product, (β-elemene, **222**) and two cyclisation products of **36**, α- and β-selinene (**223** and **224**) were the only products that were detected in the assay, since **36** is sensitive towards acid and elevated temperatures.



23 **36** **222** **223:** Δ 3,4; **224:** Δ 4,14

In conclusion, the substrate specificity of the germacrane cyclase is discussed and an active site model for the germacrane cyclase in proposed together with two tentative biosyntheses of the sesquiterpene lactones in chicory. The most likely biosynthesis starts with cyclisation of farnesyl pyrophosphate (**23**) in germacrene A (**36**) followed by several oxidation steps to give intermediate **227**. Enzyme mediated cyclisation of **227** would start with the protonation and subsequent dehydration of the C_3-hydroxyl group giving allylic cation **228**. This cation then would give **229** after a 1,5-cyclisation, followed by a selective deprotonation towards the bridgehead carbon atom. Further oxidation of **229** would give the guaianolides **12-17**.



19: R_1 = - H; R_2 = CH_2

18: R_1 = - H; R_2 = - CH_3

20: R_1 = - H; R_2 = - CH_3
21: R_1 = - OH; R_2 = - CH_3

23

36

227

228

229

12: R_1 = - OH; R_2 = CH_2
15: R_1 = - OH; R_2 = - CH_3
13: R_1 = - H; R_2 = CH_2
16: R_1 = - H; R_2 = - CH_3
14: R_1 = - *p*-$OOCCH_2C_6H_4OH$; R_2 = CH_2
17: R_1 = - *p*-$OOCCH_2C_6H_4OH$; R_2 = - CH_3

Glucosidation of the C_3-hydroxyl function of **227** gives sonchuside A (**20**) and cichorioside C (**21**) which may be cyclised by germacrane cyclasing enzymes into the corresponding eudesmanolides, e.g. **18**. Presumably, glucosidation of the C_3-hydroxyl group prevents the 1,5-cyclisation process towards the guaianolides.

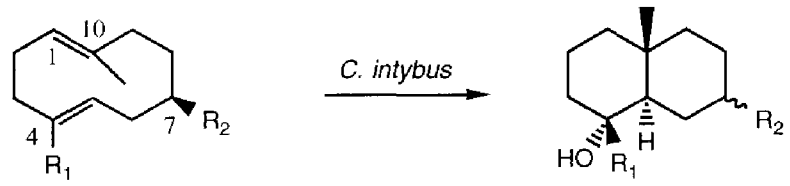
9. Samenvatting

Witlof (*Cichorium intybus* L.) is één van de vele soorten in de Compositae familie en wordt al sinds 300 voor Christus verbouwd als groente. Vanaf de 16de eeuw worden zowel de bladeren als de wortel, in geroosterde vorm, gebruikt als koffie surrogaat. De witlof staat vooral bekend om zijn bittere smaak. Deze bittere smaak wordt veroorzaakt door de aanwezigheid van sesquiterpeen lactonen. De meerderheid van deze sesquiterpeen lactonen bezit een guaiaan skelet, slechts een klein percentage bezit een eudesmaan- of een germacraan skelet. Deze sesquiterpeen bitterstoffen komen niet alleen voor in de bladeren van de plant. Ook in de witlofwortel, tot op heden een afvalstof, komen zij ruimschoots voor, wat een toepassing als veevoer op grote schaal, in de weg staat. De witlofwortel is ook rijk aan inuline (**1**), een fructose polymeer. Fructose, een interessante zoetstof, kan worden gebruikt als uitgangsstof in de synthese van polymeren en natuurproducten. De bitterstoffen uit de witlofwortel kunnen mogelijk worden toegepast als smaakadditieven in voedsel en als vervangers voor kinine, een anti-malaria middel, in bittere dranken zoals tonic en bitter lemon.

Aangenomen wordt dat de biosynthese van de sesquiterpeen lactonen start door een 'kop-staart' cyclisatie van farnesyl pyrofosfaat (**23**) tot een germacraanskelet, gevolgd door cyclisatie van het germacraan in eudesmanen en guaianen. In dit proefschrift is het cyclisatie gedrag beschreven van germacraan synthonen en natuurlijke germacranen, geïnduceerd door een witlofwortel homogenaat. Het doel was om de substraatspecificiteit te bepalen van het germacraan cyclase in witlof en om meer inzicht te kunnen krijgen in de biosynthese van de sesquiterpeen bitterstoffen in witlof.

Een algemene introductie over de historie, het gebruik en de inhoudsstoffen van witlof wordt besproken in hoofdstuk 1. In hoofdstuk 2 wordt een literatuuroverzicht gegeven van de (bio)synthese van germacraan sesquiterpenen en hun mogelijke biotransformatie in verschillende gecycliseerde producten.
In hoofdstuk 3 wordt de synthese van twee (E,E)-cyclodeca-1,5-dienolen (**100** en **101**), besproken. Het cyclisatiegedrag van deze substraten en het natuurlijke germacraan (+)-hedycaryol (**39**), ten opzichte van een witlofwortel homogenaat, wordt beschreven. De cycliserende enzymen in dit homogenaat transformeren deze tien-ring systemen in verbindingen met een eudesmaanskelet door protonering van de C_1-C_{10}, dubbele band gevolgd door een transannulaire cyclisatie en het stereoselectief inbouwen van een watermolecuul op C_4. De flexibiliteit van het tien-ring systeem komt tot uitdrukking door de vorming van de epimere diolen **117-120** uit **100** en **101**. De relatief kleine hydroxylgroep op C_7 laat inversie toe van het germacraan systeem

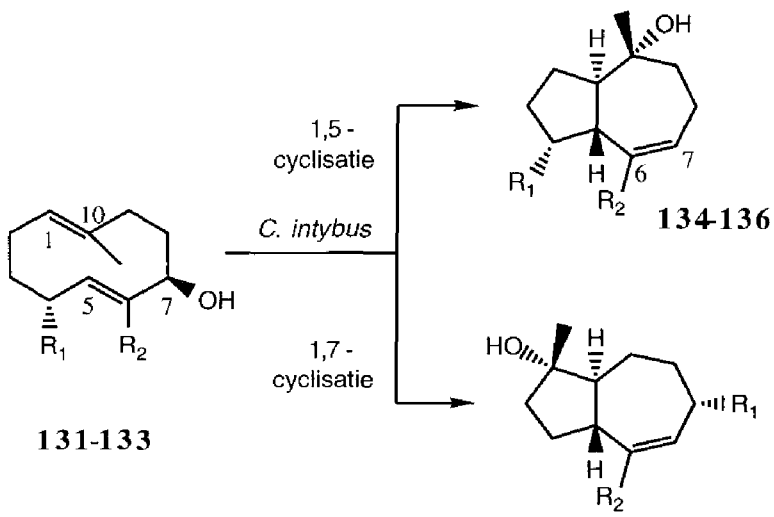
waardoor cyclisatie vanuit twee verschillende syn-conformaties mogelijk wordt. De relatief grote isopropanolgroep van **39** verhindert deze inversie in een dusdanige mate dat cyclisatie slechts vanuit één conformatie kan plaatsvinden, wat aanleiding geeft tot de vorming van slechts één product, cryptomeridiol (**125**).



100: R_1 = - H; R_2 = - OH
101: R_1 = - CH_3; R_2 = - OH
39: R_1 = - CH_3; R_2 = - $C(CH_3)_2OH$

117: R_1 = - H; R_2 = β - OH
118: R_1 = - CH_3; R_2 = β - OH
119: R_1 = - H; R_2 = α - OH
120: R_1 = - CH_3; R_2 = α - OH
125: R_1 = - CH_3; R_2 = β - $C(CH_3)_2OH$

In hoofdstuk 4 wordt de synthese van de drie (E,E)-cyclodeca-1,6-dienolen **131-133** en hun cyclisatie door een witlofwortel homogenaat besproken. Twee verschillende hydroazuleen alcoholen worden verkregen door deze reakties via 1,5- en 1,7-cyclisatie. De 1,5-cyclisatie producten (**134-136**) worden gevormd door een intramoleculaire nucleofiele substitutie van de allylische alcohol groep door de C_1-C_{10} dubbele band. Aan de vorming van de 1,7-cyclisatie producten (**137-139**) gaat een allylische isomerisatie reaktie vooraf van het allylische (E,E)-cyclodeca-1,6-dienol systeem in een allylisch (E,E)-cyclodeca-1,5-dienol systeem.

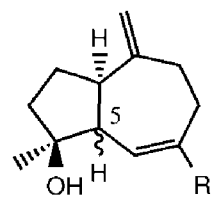


131-133

134-136

137-139

131,134,137: R_1 = - H; R_2 = - H
132,135,138: R_1 = - H; R_2 = - CH_3
133,136,139: R_1 = - CH_3; R_2 = - H

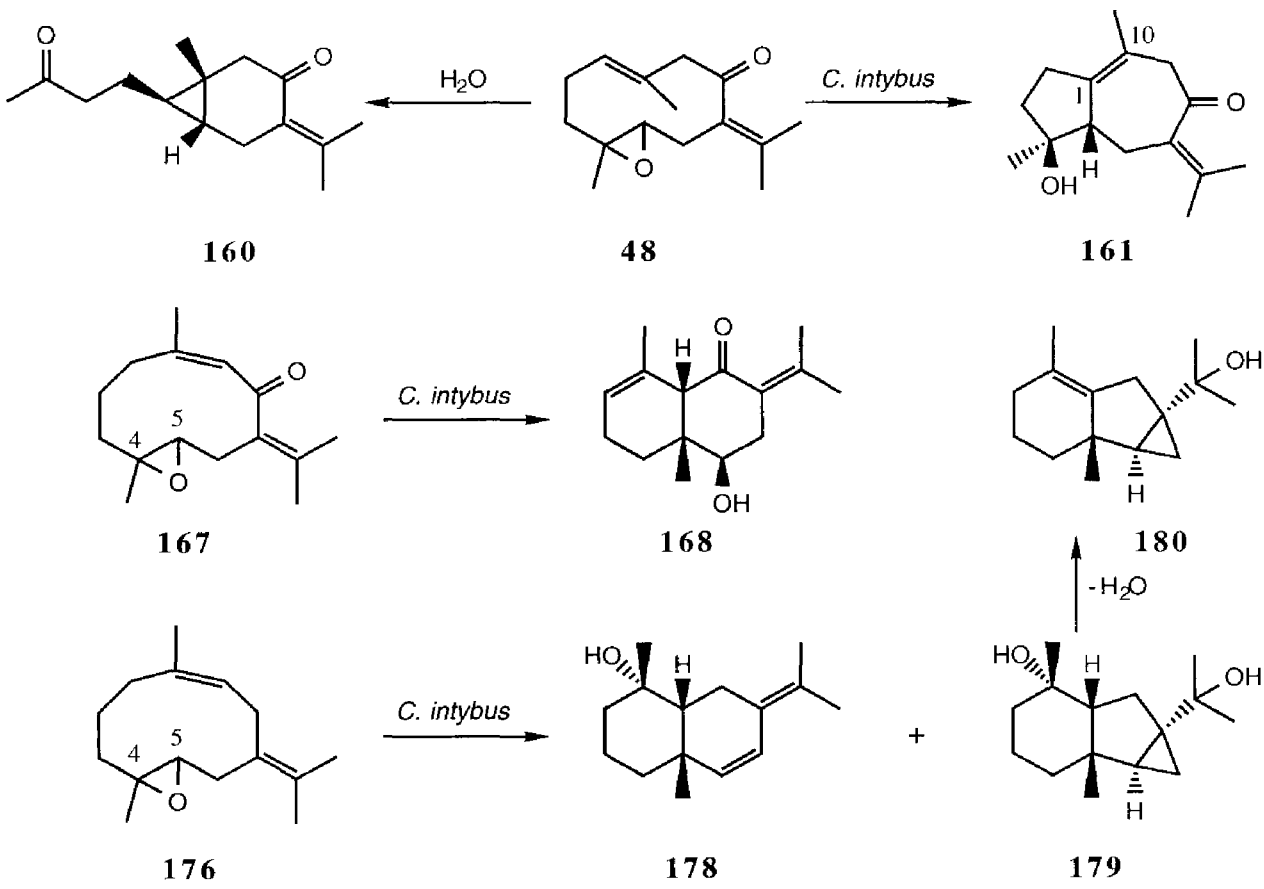


140: R = - H; H_5 = α
152: R = - H; H_5 = β
43: R = - $CH(CH_3)_2$; H_5 = β

Hydroazulenen met een dubbele band op de C_6-C_7 plaats, zoals **134**, lijken veel op natuurstoffen zoals alismol (**43**). Recentelijk is de structuur van dictamnol (**140**), een

trinor-guaiaan dat veel op alismol (**43**) lijkt, gepubliceerd. De ringverknoping van dictamnol (**140**), werd als cis gepostuleerd. Omdat **140** reeds eerder gesynthetiseerd was op de vakgroep en er grote verschillen werden aangetroffen tussen de NMR data van **140** en de natuurstof zoals die in de literatuur werd weergegeven, is natuurlijk dictamnol geïsoleerd en is de stereochemie onderzocht. Een revisie van de structuur van natuurlijk dictamnol van cis-verknoopt **140** naar het trans-verknoopte hydroazuleen **152**, wordt voorgesteld.

In hoofdstuk 5 wordt de biotransformatie van derivaten van germacron, een op grote schaal verkrijgbaar sesquiterpeen germacraan, beschreven. In een aantal gevallen ondervond de enzymatische cyclisatie reaktie competitie van spontane cyclisatiereakties. Een hoge mate van selectiviteit werd vooral verkregen tijdens de biotransformatie van germacron 4,5-epoxide (**48**) tot neoprocurcumenol (**161**). Neoprocurcumenol (**161**) is het enige product wat verkregen wordt door enzymatische cyclisatie. De C_1-C_{10} dubbele band van **161** is karakteristiek voor guaiaan sesquiterpeen lactonen in witlof. In een reaktie met een gekookt witlofwortel homogenaat werd **48** volledig omgezet in curcumenon (**160**) door een homofragmentatie reaktie.



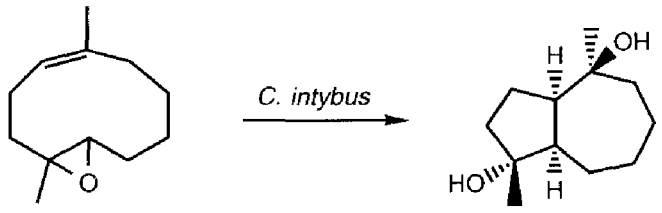
160 **48** **161**

167 **168** **180**

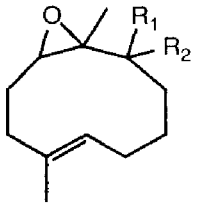
176 **178** **179**

De synthese van isogermacron (**166**) maakte het mogelijk om de invloed van de positie en de stereochemie van de dubbele band op de ringverknoping van de cyclisatie producten te onderzoeken. De 4,5-epoxides van isogermacron (**167**) en isogermacreen B (**176**) werden omgezet door een witlofwortel homogenaat in een cis-verknoopt eudesmaan en twee tricyclo[4.4.0.0]sesquiterpenen.

Hoofdstuk 6 behandelt de synthese van (E,Z)-cyclodeca-1,5-dienon (**184**) en de biotransformatie van **184** en gerelateerde verbindingen. De transannulaire cyclisatie reaktie van (E,Z)-cyclodeca-1,5-dienen blijkt anders te verlopen dan die van de (E,E)-cyclodeca-1,5-dienen die in de hoofdstukken 3 t/m 5 besproken worden. In plaats van een koolstof-koolstof band vorming tussen beide dubbele banden in het germacraan systeem spelen hier de ringsubstituenten een belangrijke rol in de cyclisatie reaktie en de daarmee gepaard gaande opheffing van de ringspanning. Als er geen additionele ringsubstuenten aanwezig zijn, zoals in E-epoxide **201**, wordt een cis-verknoopt hydroazuleendiol (**202**) verkregen. De Z-epoxides **211** en **214** werden niet, of niet éénduidig, omgezet door een witlofwortel homogenaat.

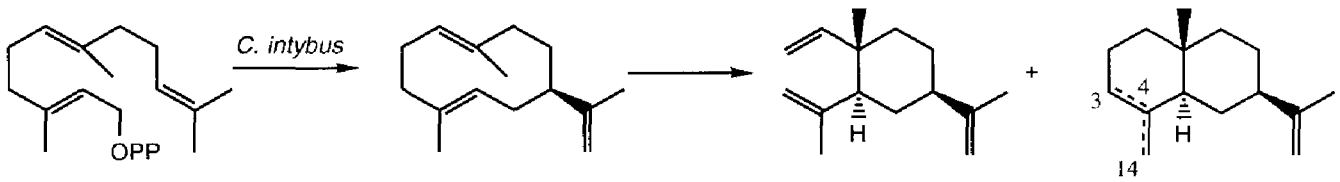


201 **202**



211: R_1 = -OH; R_2 = -H
214: R_1, R_2 = O

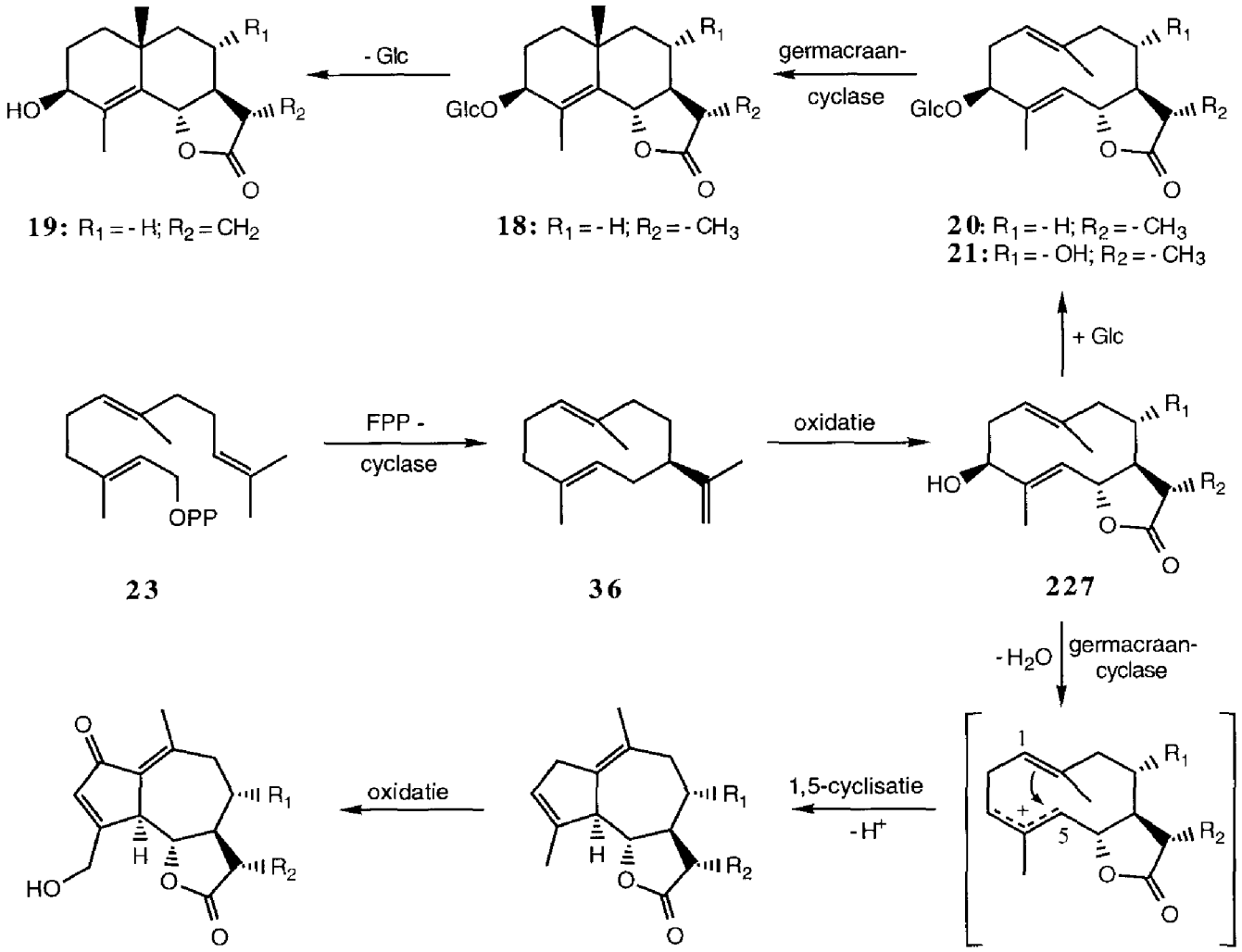
In hoofdstuk 7 wordt de biotransformatie van farnesyl pyrofosfaat (**23**) door een gedeeltelijk gezuiverd witlofwortel homogenaat besproken. Radio-GC en GC-MS analyse van de incubatie producten van [1-^{3}H]-farnesyl pyrofosfaat lieten zien dat **23** werd omgezet in germacreen A (**36**). Het Cope omleggings product van **36** (β-elemeen, **222**) en twee cyclisatie producten (**223** en **224**) werden geïdentificeerd als enige producten in deze reaktie omdat **36** gevoelig is voor zuur en warmte.



23 **36** **222**

223: Δ 3,4
224: Δ 4,14

Voorts wordt in dit hoofstuk de substraat specificiteit van het germacraan cyclase besproken. Aan de hand van de verkregen resultaten kon een active site model voor germacraan cyclases in witlof worden gepostuleerd alsmede twee mogelijke biosynthese routes van sesquiterpeen lactonen in witlof. De meest waarschijnlijke biosynthese begint met de cyclisatie van farnesyl pyrofosfaat (**23**) in germacreen A (**36**) welke vervolgens wordt omgezet, door verschillende oxidatie stappen, in het intermediair **227**. Een enzymgekatalyseerde cyclisatie van **227** zou dan starten met de protonering en afsplitsing van de C_3-hydroxyl groep om zo het allylisch kation **228** te geven. Dit kation gaat dan over in verbinding **229** door een 1,5-cyclisatie gevolgd door een selectieve deprotonering naar het bruggehoofd koolstof atoom. Verdere oxidatie van verbinding **229** geeft de guaianolides **12-17**.



19: R_1 = - H; R_2 = CH_2

18: R_1 = - H; R_2 = - CH_3

20: R_1 = - H; R_2 = - CH_3
21: R_1 = - OH; R_2 = - CH_3

23 **36** **227**

228 **229**

12: R_1 = - OH; R_2 = CH_2
15: R_1 = - OH; R_2 = - CH_3
13: R_1 = - H; R_2 = CH_2
16: R_1 = - H; R_2 = - CH_3
14: R_1 = - *p*-$OOCCH_2C_6H_4OH$; R_2 = CH_2
17: R_1 = - *p*-$OOCCH_2C_6H_4OH$; R_2 = - CH_3

Glucosidering van de C_3-hydroxyl groep van **227** geeft sonchuside A (**20**) en cichoriolide C (**21**) welke door germacraan cyclases uit witlof in de overeenkomstige eudesmanolides, bijvoorbeeld **18**, kan worden omgezet. Vermoedelijk verhindert de geglucosideerde C_3-hydroxyl groep het 1,5-cyclisatie proces naar de guaianolides.

10. Curriculum vitae

Dennis Patrick Piet werd op 17 mei 1968 geboren te 's-Gravenhage. In 1986 behaalde hij het HAVO diploma aan het Segbroek College te 's-Gravenhage. In hetzelfde jaar begon hij aan zijn HBO studie aan het toenmalige Van Leeuwenhoek Instituut, de tegenwoordige Hogeschool Rotterdam en Omstreken, te Delft, met als specialisatierichting Organische Chemie. Zijn stageperiode bracht hij door aan de Technische Universiteit Delft onder leiding van Dr. ir. L. Maat en Dr. ir. R.H. Woudenberg. In 1990 studeerde hij met lof af en begon aan een verkorte doctoraal opleiding aan de Rijks Universiteit Leiden. Na 22 maanden, inclusief een stageperiode aan de vakgroep Organische Fotochemie onder leiding van Prof. J. Cornelisse en Dr. ir. H.M. Barentsen, studeerde hij in 1992 af. Van augustus 1992 tot augustus 1996 was hij als assistent in opleiding (AIO) verbonden aan de vakgroep Organische Chemie aan de Landbouw Universiteit Wageningen. Daar werd het in dit proefschrift beschreven onderzoek verricht onder leiding van Prof. Æ. de Groot en Dr. M.C.R. Franssen.

11. List of publications

1) Synthesis of 5β-methyl-6-demethoxythebaine and its Diels-Alder Reaction to 6α,14α-ethenoiso-morphinans and 6β,14β-ethenomorphinans (Chemistry of Opium Alkaloids, Part XXXV).
R.H. Woudenberg, D.P. Piet, A. Sinnema, T.S. Lie and L. Maat, *Recl. Trav. Chim. Pays-Bas*, **110**, 405 (1991)

2) Synthesis of 8-methyl-*trans,trans*-cyclodeca-3,7-dien-1-ol and Biotransformation into 4aβ-methyl-1,2,3,4a,5,6,7,8,8aα-decahydronaphthalene-1,7β-diol by *Cichorium intybus*.
D.P. Piet, M.C.R. Franssen and Ae. de Groot., *Tetrahedron*, **50**, 3169 (1994).

3) Biotransformation of (±)-4,8-dimethyl-cyclodeca-3(E),7(E)-dien-1-ol and (+)-Hedycaryol by *Cichorium intybus*.
D.P. Piet, A.J. Minnaard, K.A. van der Heijden, M.C.R. Franssen, J.B.P.A. Wijnberg and Ae. Groot., *Tetrahedron*, **51**, 243 (1995).

4) Biotransformations of Germacrane Epoxides by *Cichorium intybus*.
D.P. Piet, R. Schrijvers, M.C.R. Franssen and Ae. de Groot, *Tetrahedron*, **51**, 6303 (1995).

5) Intramolecular *Meta* Photocycloaddition of Conformationally Restrained 5-Phenylpent-1-enes. Part I: Bichromophoric Cyclohexane Derivatives.
H.M. Barentsen, E.G. Talman, D.P. Piet and J. Cornelisse, *Tetrahedron*, **51**, 7469 (1995).

6) Synthesis of (1α,7α,8β)-(±)-8-methyl-2-methylenebicyclo[5.3.0]dec-5-en-8-ol. Structure Revision of Natural Dictamnol.
D.P. Piet, R.V.A. Orru, L.H.D. Jenniskens, C. van de Haar, M.C.R. Franssen, J.B.P.A. Wijnberg and Ae. de Groot, *Chem. Pharm Bull.*, accepted.

7) Biotransformation of allylically activated (E,E)-cyclodeca-1,6-dienes by *Cichorium intybus*.
D.P. Piet, M.C.R. Franssen and Ae. de Groot, *Tetrahedron*, submitted.

8) Biotransformation of E,Z-1,5- and E,Z-1,6-cyclodecadienes by *Cichorium intybus*.
D.P. Piet, H.M. Willemen, M.C.R. Franssen, J.B.P.A. Wijnberg and Ae. de Groot, *Tetrahedron*, in preparation.