Potato glycoalkaloids as starting material for the synthesis of steroid hormones

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Chapter 1

Potato glycoalkaloids

1.1 General introduction

One of the most important food crops today is the potato. The origin of the potato derives from a wild species domesticated in the Andes and transported to Europe by Spanish explorers between 1565 and 1570. It was not until the nineteenth century that it was appreciated as a food plant, except in Ireland where it already had become the most important food. With a world-wide production of 350 million ton per annum[1], potatoes are now a major food crop grown throughout the world[2-5]. Europe (including the former Soviet Union) accounts for 90% of the world's production. Although potatoes are considered commonly as a carbohydrate source, they are an equally good source for high-quality proteins[6,7]. Potatoes contain approximately 2% protein on a fresh basis, but this usually increases to 10% on a dry-weight basis, which is equal to that of most cereals.

With the increased interest in potatoes and potato products, scientists are continually trying to improve the pest resistance, yield, quality and processing properties of commercial cultivars. This can be accomplished by crossing them with wild-type potato species or by altering their genetic pattern through molecular biological techniques. Secondary metabolites such as glycoalkaloids could perhaps improve the pest resistance but the exact role of glycoalkaloids in potato plants against fungi, insects, bacteria, nematodes and slugs is still not clear. Most researchers have concluded that glycoalkaloids play only a minor role[8]. Protection against insects is suggested because higher glycoalkaloid levels are found after insect damage[9-11].

Members of the *Solanaceae* family, the *Solanum* genus in particular, biosynthesize a variety of alkaloidal compounds, mostly in the form of glycosides and are therefore called glycoalkaloids.

In the first half of the 19th century, one of the first glycoalkaloids isolated from the potato was solanine[12-14]. Almost 100 year later, it was discovered that solanine actually was a mixture of two compounds, α-solanine and α-chaconine[15-17], both are glycosides of the same steroidal alkaloid solanidine. Today, at least 90 structurally different steroidal alkaloids have been isolated from more than 300 *Solanum* species[18-25]. In commercial cultivars, the most common glycoalkaloids are α-solanine and α-chaconine; these and similar compounds have been shown to have toxic effects in humans[26-33]. Therefore, care must be taken that in the production of new potato varieties glycoalkaloids do not raise to an unsafe level.

1.2 Structure and analysis of glycoalkaloids

1.2.1 Chemical structure

The major *Solanum* alkaloids are steroidal alkamines. They all possess the C27 cholestane skeleton with a nitrogen atom incorporated, and belong to one of the five groups representing different types of structure as described by Schreiber[22]: solanidanes, spirosolanes, 22,26-epiminocholestanes, α-epiminocyclohemiketals, and 3-aminospirostanes (Figure 1.1).

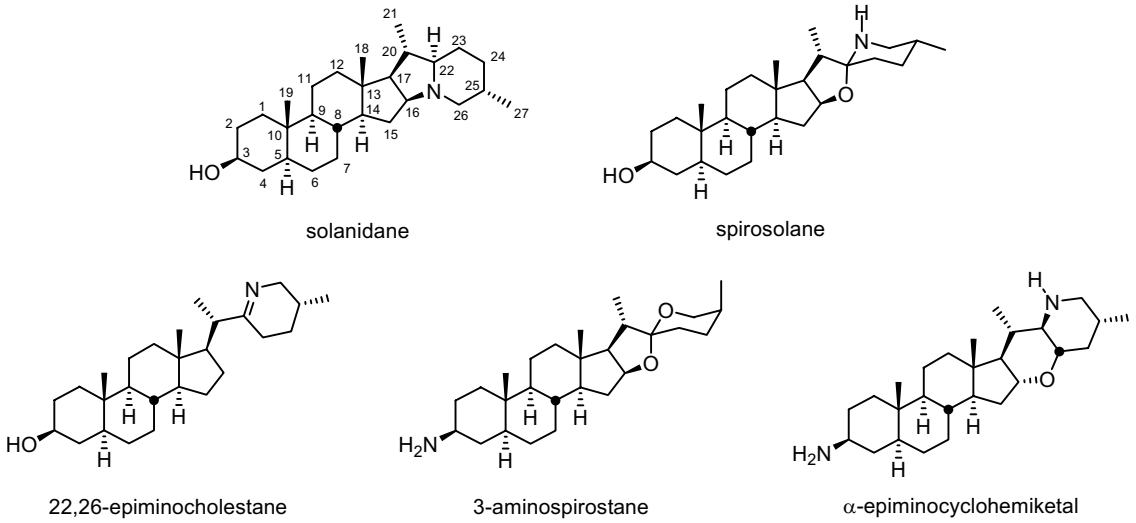


Figure 1.1

The major glycoalkaloids found in the various potato species are of the solanidane and spirostane type. The major glycoalkaloids in commercial *Solanum tuberosum* cultivars are α-solanine and α-chaconine, both containing the aglycone solanidine (**1**) (Figure 1.2). Demissidine (**2**), identified as 5,6-dihydrosolanidine, is another major solanidane, which was first isolated from the Mexican wild species *S. demissum* Lindl. and later, also from *S. Chacoense* and from some tomato varieties[34]. X-ray analysis of the hydroiodide of demissidine has permitted the complete stereochemical assignment of all atoms of **1** and **2**. Both natural solanidanes possess the 20*S*:25*S*:N*S* configuration. The H-atom attached to C22 has the α-orientation. The six-membered, N-containing F-ring exists in the chair-conformation and C25 has the *S* configuration, which means that the methyl group is equatorially positioned.

Two spirosolanes, solasodine (**3**) and tomatidenol (**4**), were found in wild potato species such as *S. berthaultii* and *S. vernei*, and in cultivars that have been crossed with these wild species[35,36]. Both compounds are almost identical and only differ in the position of the N-atom in the F-ring. Tomatidine (**5**) (5,6-dihydrotomatidenol) has been isolated from some *Solanum*

species[37]. Tomatidine (**5**) and tomatidenol (**4**) belong to the 25*S*-stereochemical series, just like solanidine (**1**) and demissidine (**2**), whereas solasodine (**3**) belongs to the 25*R*-stereochemical series.

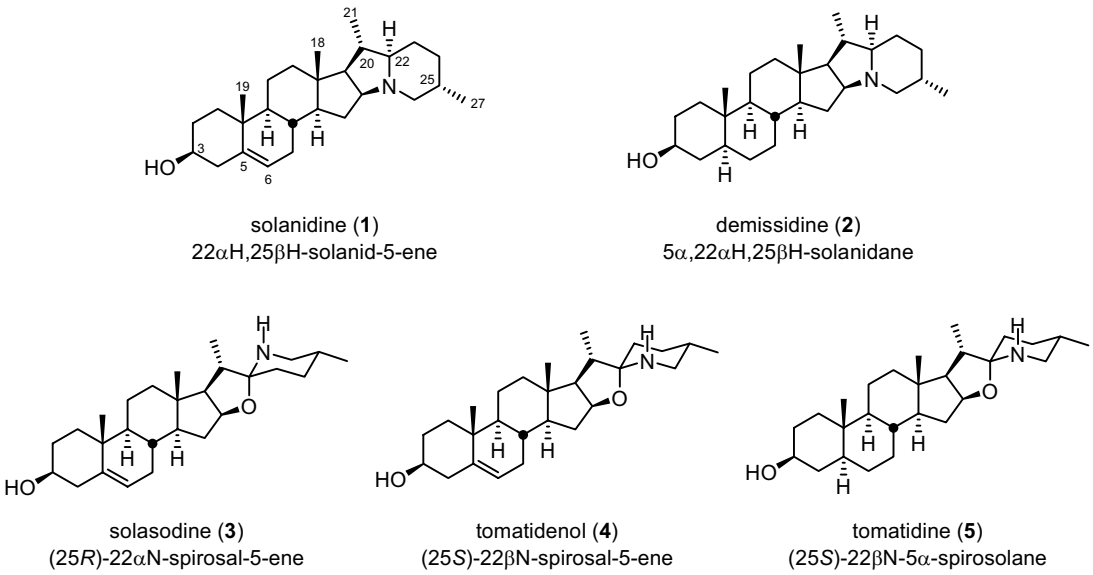


Figure 1.2

The free aglycones are rarely found in potato. Only traces are observed probably due to artifacts formed by hydrolysis in the analytical procedure. The steroid glycoalkaloids generally exist as glycosides with various sugar groups attached to the 3-hydroxy group. Both α-chaconine (**6**) and α-solanine (**7**) have a trisaccharide attached to solanidine (**1**)[17,38]. In α-chaconine (**6**) the trisaccharide is a branched bis-α-L-rhamnopyranosyl-β-D-glucopyranose (chacotriose) unit whereas α-solanine (**7**) possesses a branched α-L-rhamnopyranosyl-β-D-glucopyranosyl-β-galactopyranose (solatriose) sidechain (Figure 1.3a and b).

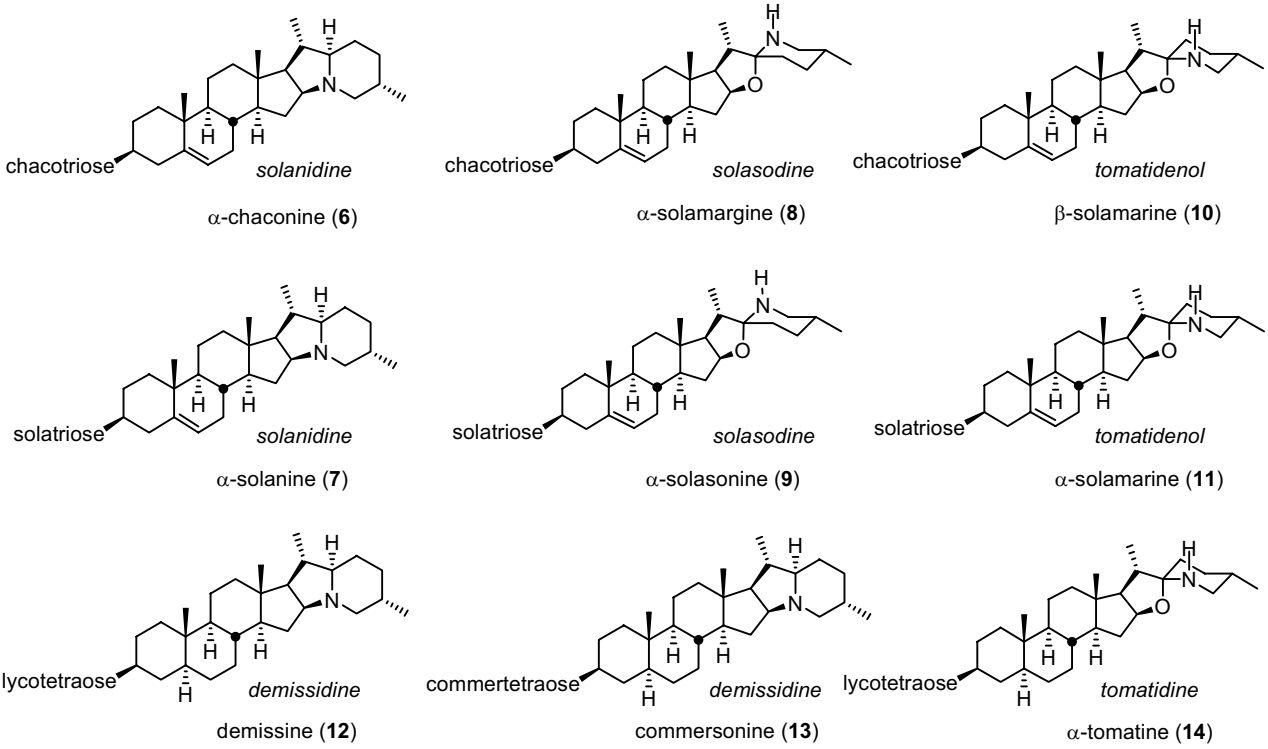


Figure 1.3a: Glycoalkaloids

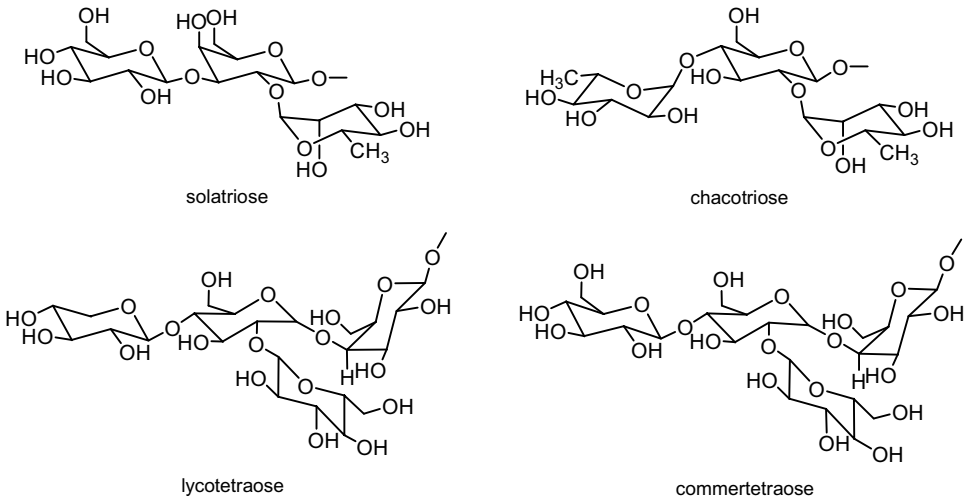


Figure 1.3b: Carbohydrate sidechains

The major solasodine glycoalkaloids α-solamargine (**8**) and α-solasonine (**9**) have the same side chains as α-chaconine (**6**) and α-solanine (**7**), respectively. Likewise, the tomatidenol glycosides β-solamarine (**10**) and α-solamarine (**11**) are glycosides of chacotriose and solatriose, respectively. In more than 100 different varieties of the bittersweet nightshade *Solanum dulcamara* α-solasonine and α-solamargine occur as the two major glycoalkaloids. Small amounts of solasodine glycosides were also found in hybrids of *S. vernei* and *S. tuberosum*[39]. Kennebec *S. tuberosum* and clones of the *S. demissum* crosses contain small amounts of solamarines glycosides[40]. The demissidine glycoalkaloids, demissine (**12**) and commersonine (**13**), both have a four-sugar side chain. Demissinine (**12**), first isolated in 1947 from *S. demissum* by Kuhn and Löw[41], has the same lycotetraose side chain as α-tomatine (**14**), the major tomatidine glycoalkaloid found in tomatoes[37]. Commersonine (**13**) was first isolated and characterized from accessions of *S. chacoense* and *S. commersonii* Dun. by Osman *et al.*[42] in 1957. The tetraose side chain commertetraose is not found in any other major glycoalkaloid.

Leptines, a group of closely related glycoalkaloids, are present in special accessions of *S. chacoense* Bitt. and possess the solanidane structure[43,44] (Figure 1.4). The additional β-OH-group at C23 is axially positioned. Leptines are soluble at high pH and are concentrated in the leaves and stem of *S. chacoense*, but not in the tubers of the plant. Therefore, these compounds have not been studied very well[45].

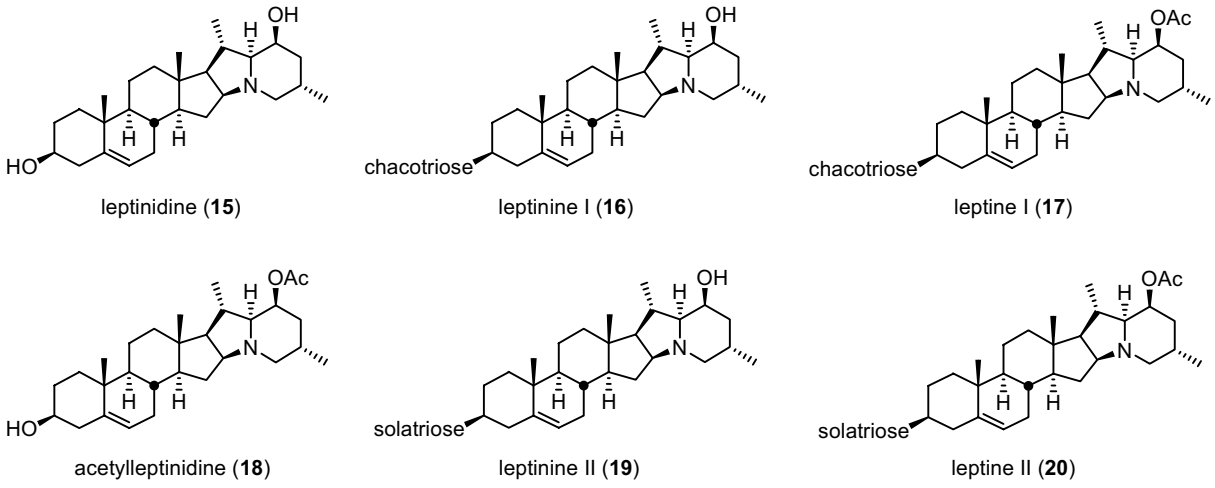
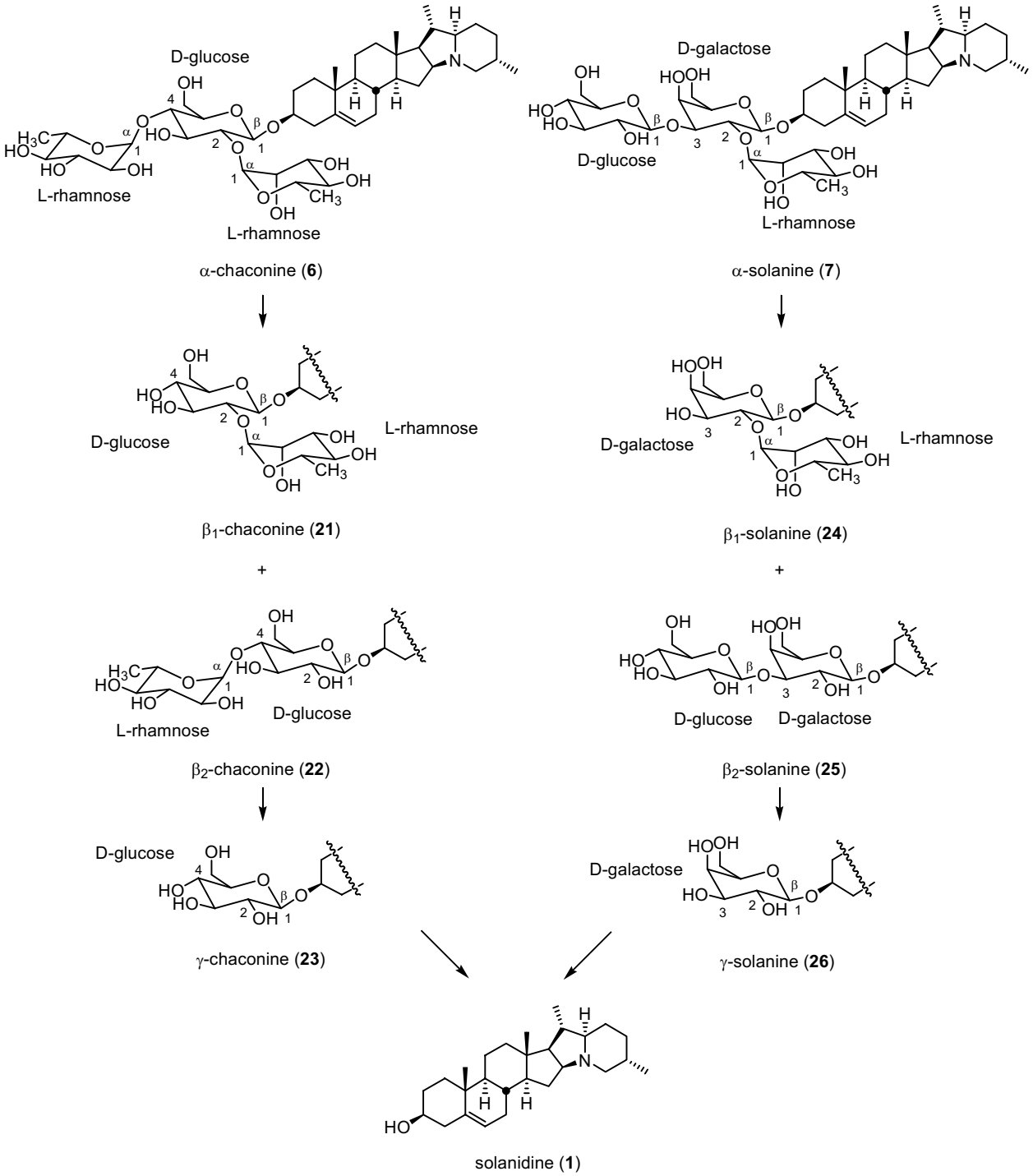


Figure 1.4

Although regularly new alkaloids are isolated from the *Solanum* family one has to keep in mind that of all the glycoalkaloids in commercial potatoes at least 95% are α-chaconine (**6**) and α-solanine (**7**).

1.2.2 Hydrolysis

Naturally occurring glycoalkaloids are called α-compounds. Stepwise cleavage of the glycoside side chain leads to β-, γ- and δ-compounds in case of tetrasaccharides and to β- and γ-compounds in case of trisaccharide sidechains as exemplified for α-chaconine (**6**) and α-solanine (**7**) in Scheme 1.1. Except for β_1-solanine (**24**), all intermediates have been isolated from Nature.



Scheme 1.1

There are indications that potato sprouts contain enzymes (rhamnosidase, glucosidase, and galactosidase) required for the hydrolytic cleavage of rhamnose, glucose, and galactose residues, respectively, from the potato glycoalkaloid[46-49]. Although these enzymes work under laboratory conditions, they seem to have very little activity in the intact mature potato tuber. Generally, only traces of β- and γ-compounds have been found in tubers. Additional study is necessary to gain a better insight under which conditions these enzymes become active.

Contrary to enzymatic hydrolysis, the chemical hydrolysis is studied in more detail[50-52]. Factors of influence on the chemical hydrolysis are time, temperature, and concentration of the acid used. In general, hydrolysis rates increase with the temperature and the concentration of acid (HCl[53], H_2SO_4[54]) and decrease with the amount of water in organic solvent-water solutions[55]. Under strongly acidic conditions and at high temperatures solanidine (**1**) obtained from hydrolysis of α-chaconine (**6**) and α-solanine (**7**) will react further to solasodiene (solanthrene). Further study has revealed that the nature of the alcohol used as solvent, strongly influences the rate and the specificity of the hydrolysis, permitting optimal formation of specific hydrolysis products (β- and γ-compounds)[56,57].

1.2.3 Analysis

Over the years several methods for analysis of glycoalkaloids have been developed and still new methods appear. Each method, new or old, consists of an extraction, purification, and (quantitative) determination step[58,59]. Some analytical methods may include an extra step, such as derivatization or hydrolysis. Several methods combine the extraction and modification steps. Extraction with H_2SO_4, for example, allows simultaneous extraction and hydrolysis[60,61].

Noteworthy, the amounts found by colorimetric and titrimetric methods have been consistently higher than those obtained by GC or HPLC. The reason for this difference is not yet understood. A nice overview and discussion of the methods used for analysis of glycoalkaloids is given by Friedman and McDonald[8].

1.3 Biosynthesis of solanidine

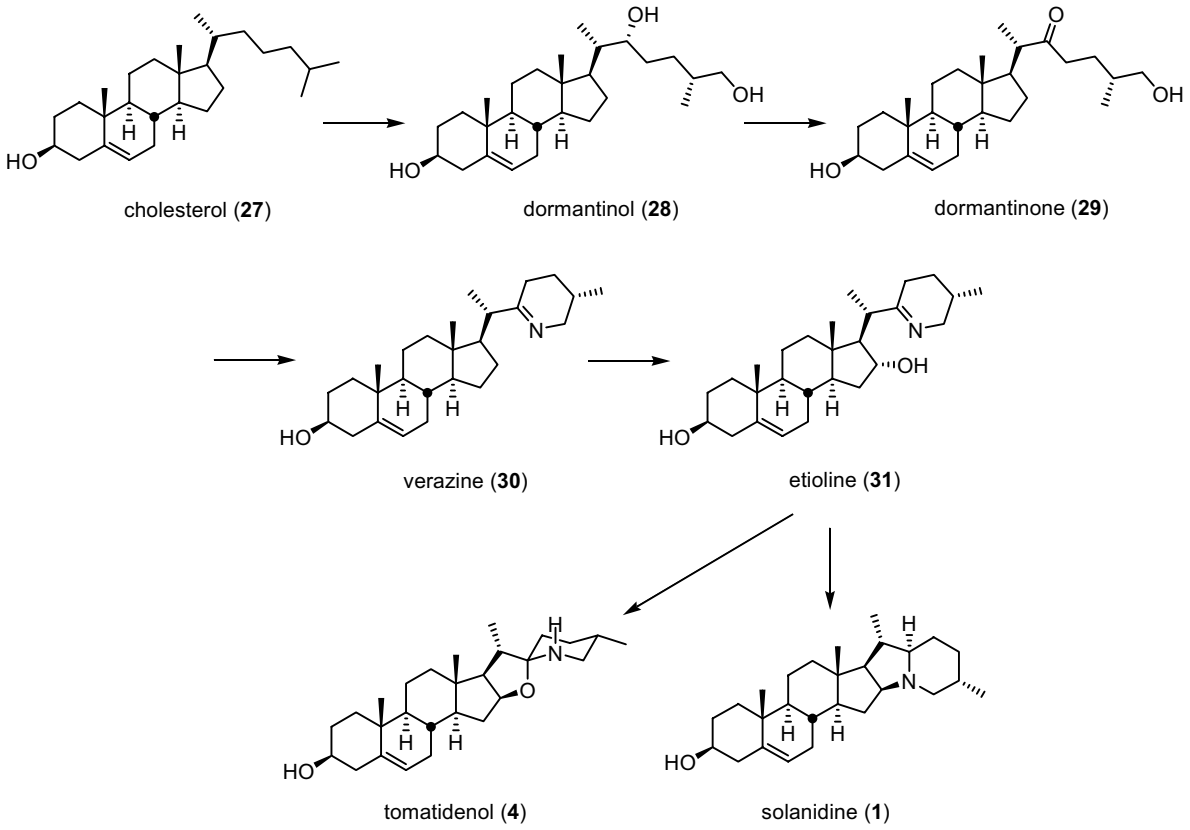
Kaneko *et al.*[62,63], Heftman[64], Petersen *et al.*[65], and Bergenstråhle[66] have proposed a biosynthetic pathway for solanidine (**1**). The starting point of the biosynthesis of glycoalkaloids is cholesterol, which is responsible for the biosynthesis of steroids in general. The exact pathway has not fully been proven.

Kaneko *et al.*[62,63] have proposed the pathway shown in Scheme 1.2. The N-atom originates from amino acids such as alanine, glycine, or arginine. The latter amino acid is probably the principal source of incorporated N[67]. Once etioline (**31**) is formed, the E-ring can close in various ways to give, for instance, solanidine (**1**) or tomatidenol (**4**). It should be noted here that the OH group at C16 has to be inverted first prior to ring closure of **31** to **4**.

Petersen[68] postulated that the aglycones with a $\Delta^{5,6}$ double bond (solanidine (**1**), solasodine (**3**), and tomatidenol (**4**)) and the saturated aglycones (demissidine (**2**), soladulcidine, and tomatidine (**5**)) are formed by identical pathways. The presence or absence of the $\Delta^{5,6}$ double bond depends on whether the biosynthesis starts from cholesterol or cholestanol. Another possibility is that aglycones without a double bond are derived from cholesterol but that its $\Delta^{5,6}$ double bond is saturated at some point.

The last step in the biosynthesis of glycoalkaloids is glycosylation of the aglycones[69,70]. There are strong indications that UDP-glucose, UDP-galactose, UDP-rhamnose, and transglycosylases, required for the transfer of the three sugars from the nucleotide to the acceptor

molecule, are involved[66,71-87].



Scheme 1.2

1.4 Distribution of glycoalkaloids in the plant

Glycoalkaloids are formed in all parts of the plant[88-92]. The biosynthesis of glycoalkaloids occurs in places with the highest metabolic activity such as young leaves, fruit, flowers, and sprouts. The glycoalkaloid biosynthesis reaches its maximum during the flowering period. Ultimately, the highest amounts of glycoalkaloids are found in the tubers and roots. Generally, glycoalkaloids are not transported between different parts of the plant. Each part is responsible for its own synthesis and degradation of glycoalkaloids. The majority of glycoalkaloids in commercial potatoes are found in the first 1.5–2.0 mm of the peel but this can vary from cultivar to cultivar[90,93-97]. The normal ratio in which the two major glycoalkaloids α-chaconine and α-solanine are formed is 40:60, respectively, but this ratio can range from 25:75 to 60:40[42,91,98-102]. Studies show that the glycoalkaloid content of a specific cultivar is genetically controlled and that production of high levels of glycoalkaloids can be inherited[103,104]. Caution has to be taken because desired effects (resistance against pathogens, insects, and physiological stress) of crossing two species can lead to accumulation of alien glycoalkaloids under conditions different from the research conditions[39,105].

Although the nature and relative concentrations of glycoalkaloids are genetically controlled, the total concentrations are certainly influenced by other factors in the pre- and post-harvest period. During the pre-harvest period environmental factors have strong influence on the biosynthesis of glycoalkaloids, especially climatic and seasonal variations. To a lesser extent agricultural practice and soil composition play a role in the biosynthesis. Seasonal factors such as low or high temperature, excessive or too little rains, and lack of or too much sunshine raise the levels of glycoalkaloids[106,107]. It seems that an increased glycoalkaloid production is the plant's reaction to

stress.

Long after harvest, glycoalkaloids levels can still rise in potato tubers since the biosynthesis goes on for some time. Factors of influence are light, storage conditions, and mechanical injury. Some other factors are also of influence but to a much lesser degree. Exposure of the potato to light has a dramatic effect on the amount of glycoalkaloids[108-116]. Reports of an increase in the glycoalkaloid content of more than 300% are not unusual. In many cases an increase in glycoalkaloids is accompanied by greening of the potato. Both effects are probably dependent on the wavelength of light used in the experiment[117]. It should be mentioned that the intensity of greening is variety dependent[118-126]. Another important factor that increases the glycoalkaloid content after harvesting is temperature[110,113,115,127], but reports on this issue are conflicting. Some reports state that higher temperatures increase the glycoalkaloid content[110,123,128,129], while other reports claim that lower temperature raises the amount of glycoalkaloids[130-132]. Temperature proves also to be a stress-inducing factor and this is probably the reason that potatoes start to produce more glycoalkaloids. Related to light and temperature is the storage period of the potato. Generally, the level of glycoalkaloids increases when potato tubers are stored longer under the same conditions, although there are indications that levels reach a maximum and then begin to decline[133-136]. Mechanical injury, just like light, can cause a dramatic increase in glycoalkaloids content[110,137,138]. Cutting the potato produces the highest levels of glycoalkaloids. Susceptibility for mechanical injury varies with the cultivars[139-142]. In cultivars with a high original content of glycoalkaloids these levels increase more than in cultivars with a low original content.

1.5 Physiological aspects of glycoalkaloids

1.5.1 Human studies

Since the first introduction of the potato in Europe people have been aware that ingestion of blighted or sprouted potatoes and other parts of the potato, especially the leaves and berries, can cause illness and even death. Several reviews have been written about the toxic effects of *"solanine"* poisoning[8,24,59,143-149]. The symptoms of poisoning include nausea, vomiting, diarrhea, stomach and abdominal cramps, headache, fever, rapid and weak pulse, rapid breathing, hallucinations, delirium, and coma. There are several cases known in which people died after eating potatoes[26-29,31-33,150]. There is one case known in which after a long school break, 78 schoolboys between the age of 11 and 13 became ill after eating lunch[28]. Seventeen of the boys had to be treated in the hospital, three of them were even in critical condition. Common symptoms were nausea, vomiting, and diarrhea. Most had fever and were confused or drowsy. The severe ill had weak and rapid pulses, difficulty in breathing, high fever, and low blood pressure. The critically ill were unconscious. The symptoms declined after 1 to 2 weeks and the boys were discharged. It was noted that even after 6 days, plasma cholinesterase levels were extremely low. The cause of this illness was traced to potatoes that had *"gone bad"* over the long holiday. Remaining potatoes were found to contain around 330 mg/kg of glycoalkaloids.

It has been estimated that oral doses in the order of 1-5 mg/kg bodyweight of potato glycoalkaloids can be overtly toxic and 3-6 mg/kg bodyweight (BW) has been claimed to cause death[147,151-153].

1.5.2 Animal studies

Early studies with animals on the absorption of glycoalkaloids showed that these compounds enter slowly into the bloodstream after oral admission[154]. Although there are only a few studies

conducted on oral intake of pure compounds, several generalities can be noticed[144,155-159]. For most animals, the intraperitoneal (IP) LD_{50}s of the various glycoalkaloids are around 30-60 mg/kg BW. Intravenous (IV) LD_{50}s are around 10-20 mg/kg BW established from limited studies[154,160]. The oral LD_{50}s are around 500-1500 mg/kg BW. These values are considerably higher than the estimated oral lethal dose of 3-6 mg/kg BW for humans. Studies with hamsters have shown that these animals have a sensitivity similar to humans for glycoalkaloids.

Although IV and IP studies are generally easier and require less material, they may not be indicative of what is actually occurring after ingestion. Surprisingly, there have been only a few studies about the effects of oral intake of glycoalkaloids. This is probably partly due to the large amount of material needed to evoke a reaction and the difficulties in determining low levels of glycoalkaloids in tissues and body fluids. Oral studies with hamsters, highly sensitive to glycoalkaloids, are needed to elucidate the many questions concerning glycoalkaloid toxicity in the human diet. The toxic effects of glycoalkaloids are cell membrane disruption, acetylcholinesterase inhibition, liver damage, heart damage, teratogenicity and embryotoxicity.

1.5.3 Relative toxicities

Comparing glycoalkaloid toxicities is a bit problematic because there are at least two and may be more toxic effects that have generally been combined into one overall effect. In overall toxicity, α-chaconine (**6**) is the most toxic of the potato alkaloids. It exhibits the strongest cell membrane disruption, causes inhibition of acetylcholinesterase, organ damage and teratogenicity in embryos. α-Solanine (**7**) is somewhat less toxic, although it has the same potency in cell membrane disruption as α-chaconine (**6**) but has little or none lytic properties by itself. It is also less teratogenic to embryos than α-chaconine. All this indicates that it also would be less damaging to organs but more data are needed. The intermediate hydrolysis products of α-chaconine (**6**) and α-solanine (**7**) seem to lose toxicity as they lose sugar groups. The aglycone solanidine (**1**) seems to be the least toxic in all effects[161-164]. The solanidanes seem to be more toxic than their corresponding spirosolanes: α-solasonine (**9**), α-solamargine (**8**) and solamarine (**11** and **12**). There is an apparent relationship between chemical structure and biological properties of the glycoalkaloids[163-169]. This might be used to predict potencies of new glycoalkaloids.

As mentioned earlier, the combination of α-chaconine (**6**) and α-solanine (**7**) or α-solanine (**7**) and α-solamargine (**8**) gives a synergistic effect on toxicities[170-175]. Synergism could be the reason that both pairs of glycoalkaloids are synthesized in the plant. The two synergistic pairs are both a combination of chacotriose and solatriose glycosides. This concept applies to many *Solanum* species which seem to produce glycoalkaloids in matched pairs of chacotriose and solatriose (α-solasonine (**9**) and α-solamargine (**8**), leptine I (**17**) and leptine II (**20**), α-solamarine (**10**) and β-solamarine (**11**)). This synergistic effect seems only to apply for their cell membrane disrupting properties and not for acetylcholinesterase inhibition[176].

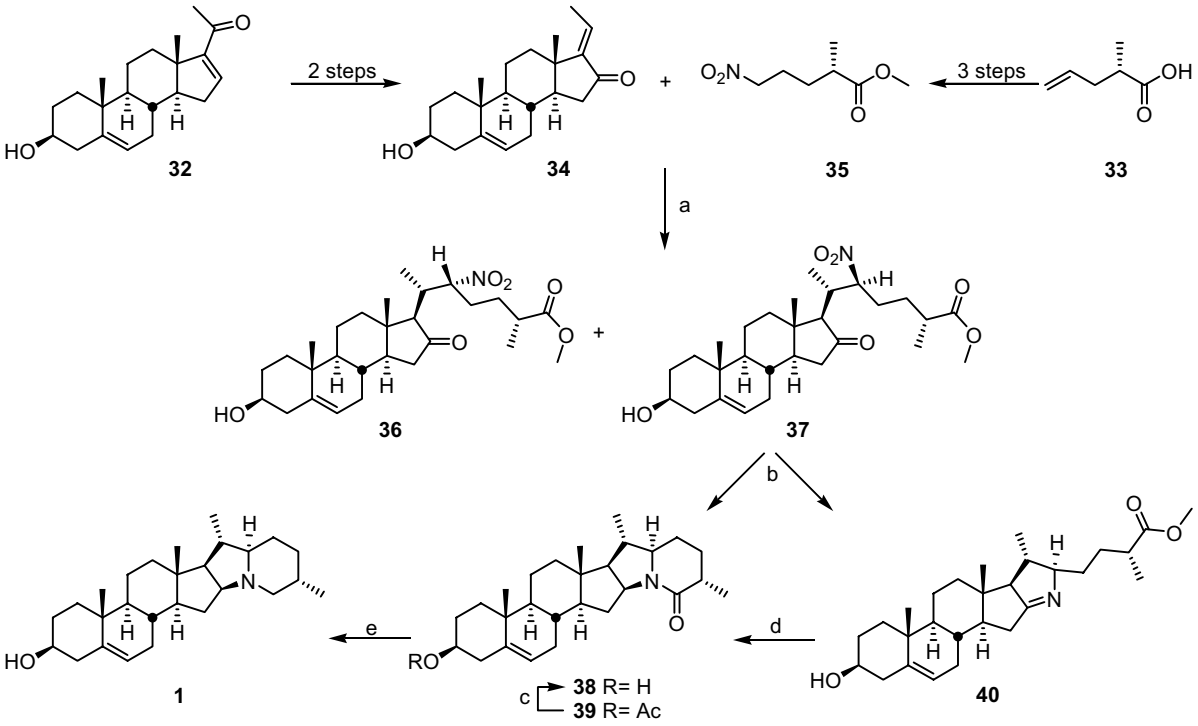
1.5.4 Safety guidelines for potatoes

From the previous part, it may be concluded that the minimum toxic level of glycoalkaloids for humans is around 1-2 mg/kg BW. Lethal doses may be as low as 5-6 mg/kg BW. The common current guideline for potatoes establishes an upper level of 200 mg/kg fresh weight (FW) of glycoalkaloids in the Netherlands. Several researchers have expressed their concern that these levels are too high and need to be lowered[24,147,177-179]. Main objective is that the 200 mg/kg FW level only relates to acute and/or subacute effects and not to possible chronic effects. More study is needed to determine the safety guidelines for the levels of glycoalkaloids in potatoes.

1.6 Synthesis of solanidine

Most of the synthetic research is focussed on the chemical or microbiological transformation of steroidal alkaloids toward pharmacologically more potent compounds. Since more than 95% of the potato steroidal alkaloids have solanidine (**1**) as aglycone, and also this thesis deals with solanidine, only the synthesis of **1** and some solanidine-like compounds will be discussed here.

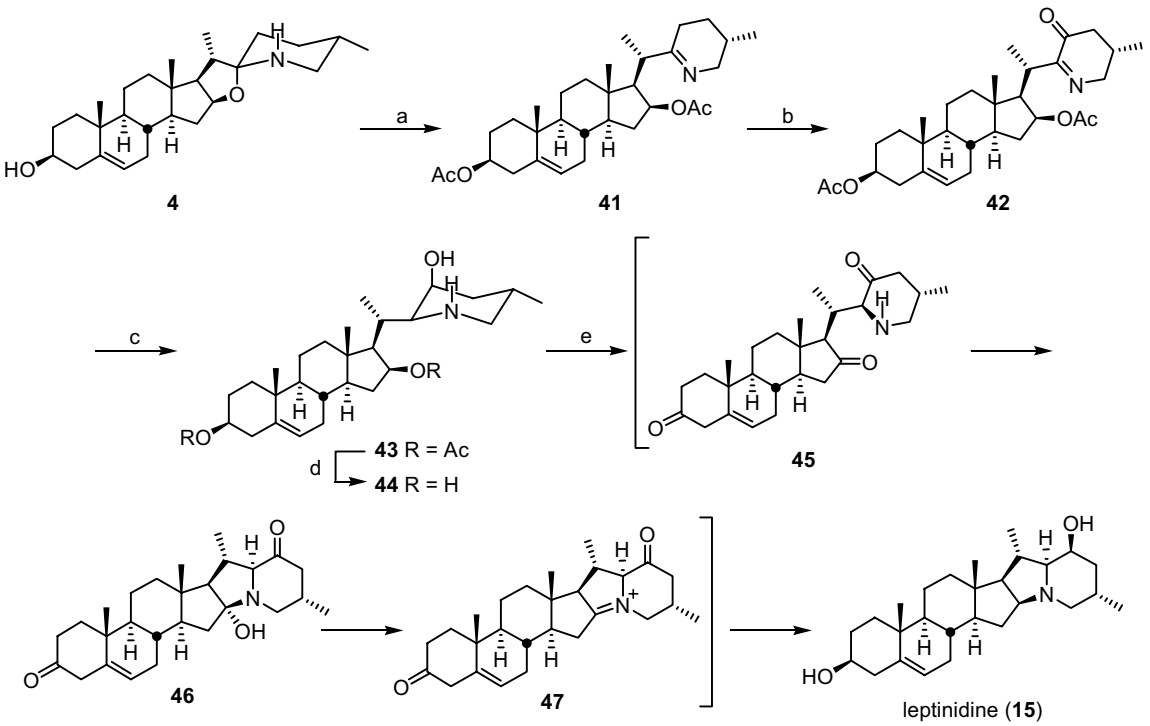
Kessar *et al.*[180,181] have published a total synthesis of solanidine (**1**) that starts from dehydropregnenolone (**32**) and *S*-2-allylpropionic acid (**33**) (Scheme 1.3). After transformation of **32** to the α,β-unsaturated ketone **34** and the conversion of **33** to nitro compound **35**, Michael addition of **35** to **34** yielded a separable 1:1.3 mixture of the epimers **36** and **37**, respectively. Reduction of the nitro group in **37** with Zn in HOAc gave, after column chromatography, a neutral and a basic fraction. The neutral fraction contained a mixture of amide **38** and its acetate **39**, while the basic fraction only contained the imine ester **40**. Hydrolysis of the mixture of **38** and **39** gave pure **38**. After reduction of the imine moiety in **40** with sodium borohydride ($NaBH_4$) and cyclization, another portion of **38** was obtained. Finally, treatment of **38** with lithium aluminum hydride (LAH) gave solanidine (**1**)[181]. When **36** was treated in the same way as **37**, the C22 epimer of solanidine (**1**) was obtained.



a) *t*-BuOK; b) Zn, AcOH; c) HCl, MeOH; d) $NaBH_4$; e) LAH, dioxane

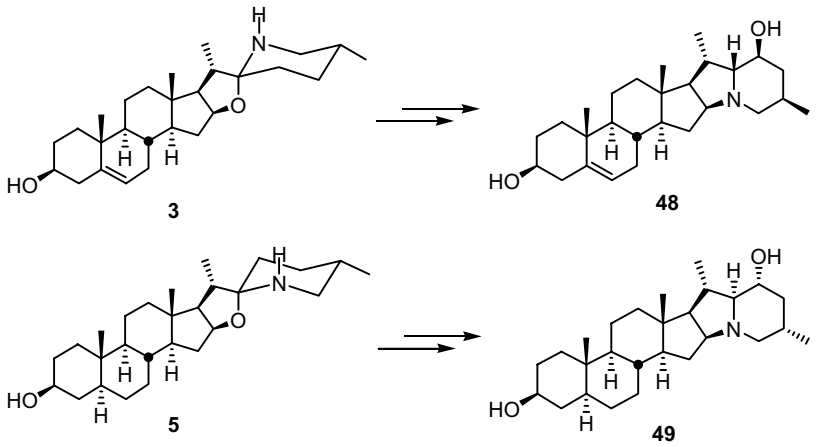
Scheme 1.3

Ripperger and Schreiber[182] have described the partial synthesis of the solanidine derivative leptinidine (**15**) from natural tomatidenol (**4**) (Scheme 1.4). Upon treatment of **4** with Ac_2O and $ZnCl_2$ in HOAc, the resulting imine **41** was oxidized with SeO_2 to give the corresponding 23-keto compound **42**. Reduction of **42** with $NaBH_4$ in MeOH resulted in a mixture of three stereoisomers. After thin-layer chromatography, the main isomer **43** could be obtained in 16% yield. Saponification of **43** afforded triol **44** which was then converted to leptinidine (**15**) via an oxidation-reduction sequence with most likely **45**, **46**, and **47** as intermediates.



a) Ac_2O, $ZnCl_2$; b) SeO_2; c) $NaBH_4$, MeOH; d) KOH; e) CrO_3, NaOAc, AcOH; then $NaBH_4$

Scheme 1.4



Scheme 1.5

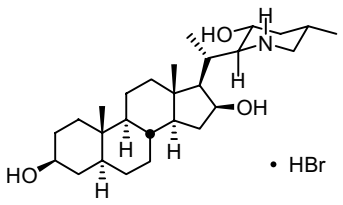


Figure 1.5

Ripperger and Schreiber[182] also used the reaction sequence depicted in Scheme 1.4 to synthesize some other leptinidine derivatives. For instance, compound **48**, the C22,C25 stereoisomer of leptinidine, was prepared from solasodine (**3**), whereas the saturated leptinidine derivative **49** was obtained from tomatidine (**5**) (Scheme 1.5). The absolute configuration of **49** at C22 and C23 was concluded from X-ray analysis of the hydrobromide of (22*S*,23*R*,25*S*)-22,26-

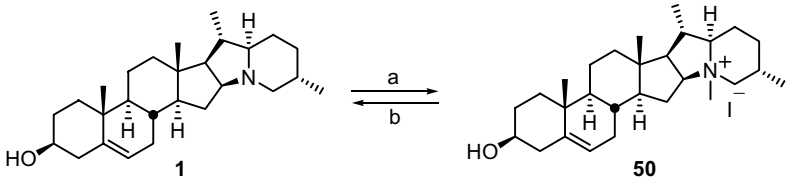
epimino-5α-chlostane-3β,16β,23-triol[183], an intermediate in the synthesis of **49** (Figure 1.5).

1.7 Transformations of solanidine

Soon after the isolation and structure elucidation of solanidine (**1**), its potential as starting material for steroid synthesis was recognized. Over the years several degradation reactions have been published in literature and a summary is given below.

1.7.1 Hofmann degradation

The first attempt to degrade solanidine (**1**) was published by Schöpf and Hermann in 1933[184]. After methylation of **1**, the corresponding methiodide **50** was subjected to a Hofmann degradation but the sole product isolated was recovered **1** and no disruption of the ring system was observed (Scheme 1.6).



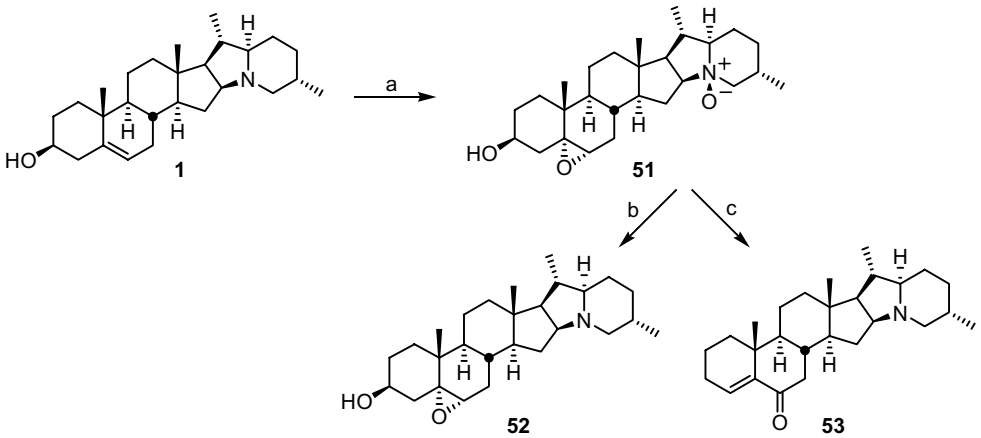
a) MeI, toluene; b) base, Δ

Scheme 1.6

1.7.2 N-oxide reactions

In 1950 Briggs *et al.*[185] reported that treatment of demissidine *N*-oxide with $Na_2Cr_2O_7$, in an effort to oxidize the C-atom next to the nitrogen, only led to the recovery of the original starting material demissidine (**2**). A similar result was obtained when demissidine *N*-oxide was treated with SO_2.

In 1988 Gasi and Miljkovic[186] described that heating of the solanidine derivative **51** resulted in the formation of compound **52** in 32% yield with regeneration of the indolizidine system (Scheme 1.7). More vigorous reaction conditions resulted in a 30% yield of 4-solaniden-6-one (**53**).



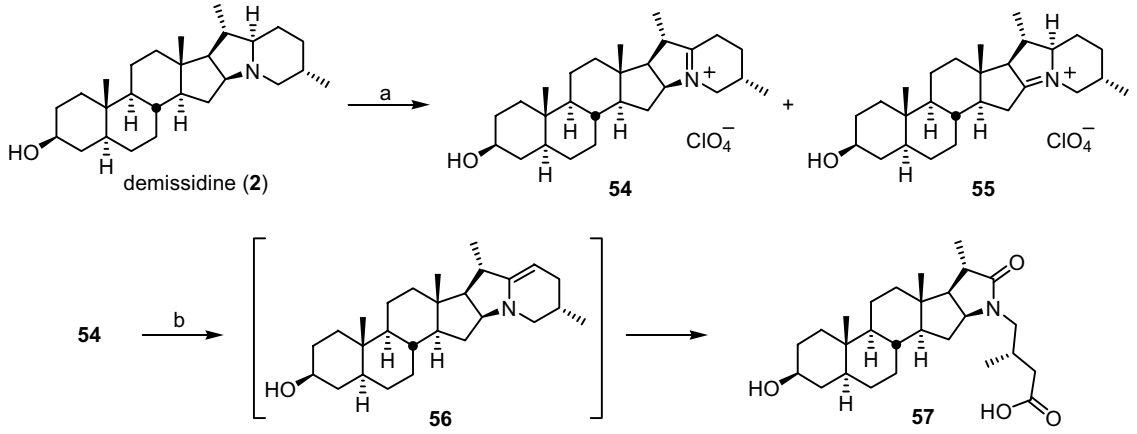
a) monoperphtalic acid, Et_2O, $CHCl_3$, 4ºC; b) 260-270ºC, 1 min. or DMSO, Δ, 30 min.;
c) 270-280ºC, 10 min or DMSO, Δ, 1 h.

Scheme 1.7

1.7.3 Oxidation

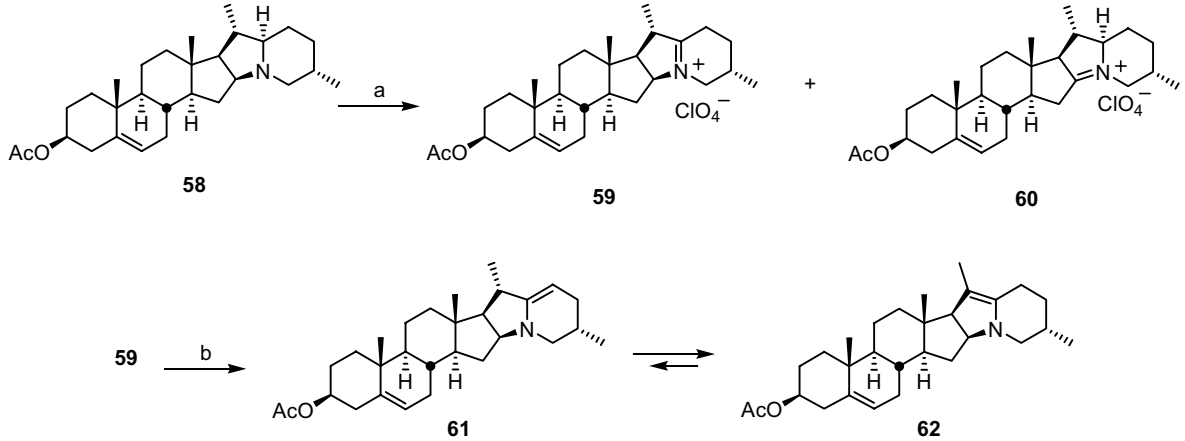
In 1959 Tanabe and Bolger patented the oxidation of some natural solanidanes (isorubijervine and derivatives) with $Hg(OAc)_2$[187]. In 1966 Schreiber and Horstmann described the oxidation of demissidine with $Hg(OAc)_2$ resulting in the isolation of the iminium salts **54** (46%) and **55** (23%) (Scheme 1.8)[188]. This oxidation was also performed with NBS[188,189] and gave, according to the authors, the same mixture of iminium salts **54** and **55** in 85% yield. Oxidation of **54** with H_2O_2 in basic medium gave in 49% yield the F-ring opened product **57**. According to the authors, the formation of **57** must have been preceded by formation of enamine **56**.

In 1994 Gunic and coworkers[190] reported the electrochemical oxidation of $\Delta^{4,5}$-solaniden-3-one, 3β-acetoxy-solanidine (**58**), and 3β-acetoxy-5α-chlorosolanidine in CH_3CN/CH_2Cl_2 1/1 with pyridine as base. As exemplified for **58** in Scheme 1.9, the corresponding iminium salts **59** and **60** were obtained in a 1/1 ratio in good yield. Performing this electrochemical reaction in CH_2Cl_2 with pyridine gives **60** in 95% yield, while the same reaction in acetone gives iminium salt **59** in 95% yield.



a) $Hg(OAC)_2$, HOAc (50%), EDTA; b) H_2O_2, NaOH, MeOH

Scheme 1.8

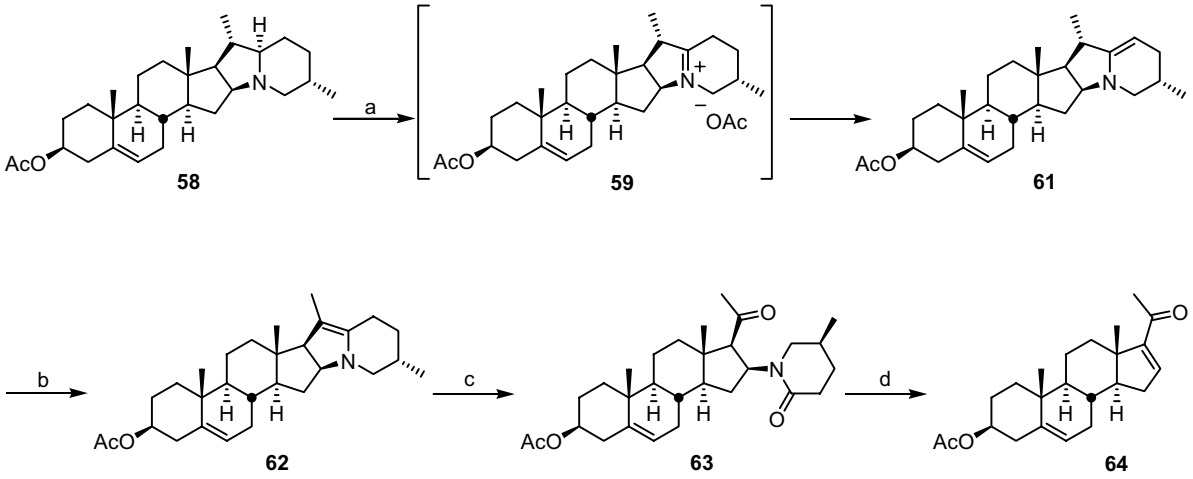


a) CH_3CN/CH_2Cl_2 1/1, pyridine; b) NaOH (30%), acetone, 40ºC

Scheme 1.9

Iminium ion **59** can be isomerized to the thermodynamically more stable enamine **62**. This isomerization is believed to proceed via enamine **61**, which is the kinetic product.

In 1997 Gasi *et al.*[191] reported a short procedure for the degradation of solanidine (**1**) to DPA (**64**) (Scheme 1.10). Instead of applying the electrochemical oxidation, $Hg(OAc)_2$ in acetone was used as oxidizing agent. The advantage of this reagent and solvent system was the ease of use and the selective formation of iminium salt **59**, which spontaneously isomerized to enamine **61** (94%). This enamine was then subjected to another isomerization, which yielded the thermodynamically more stable enamine **62**. $NaIO_4$-oxidation opened up the cyclic enamine **62** and gave lactam **63**. Elimination of the lactam part with Al_2O_3 in benzene afforded in 34% dehydropregnenolon acetate (DPA) (**64**). Using K_2CO_3 in benzene followed by reacetylation produced **64** in a lower yield (11%).

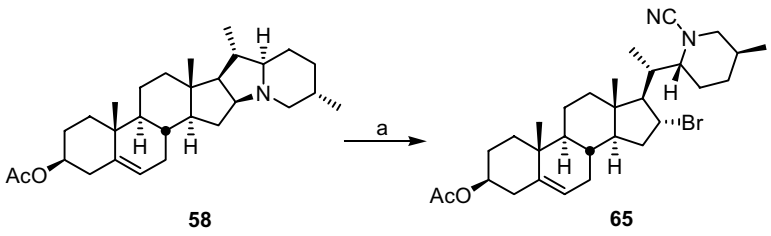


a) $Hg(OAc)_2$, acetone; b) HOAc, Et_2O; c) $NaIO_4$, NaI, *t*-BuOH, H_2O, $NaHCO_3$;
d) Al_2O_3, benzene or i. K_2CO_3, benzene; ii. Ac_2O, pyridine

Scheme 1.10

1.7.4 Von Braun reaction followed by conversion to tomatidenol

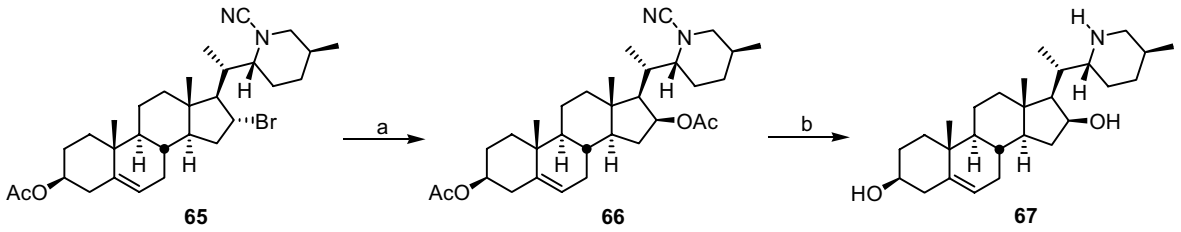
In 1968 Beisler and Sato reported the successful opening of the E ring of solanidine (**1**) via the Von Braun reaction[192,193] (Scheme 1.11). Only in case of acetylated solanidine (**58**) the Von Braun reaction gave the E ring-opened product **65** in 78% yield.



a) BrCN, $CHCl_3$, Δ

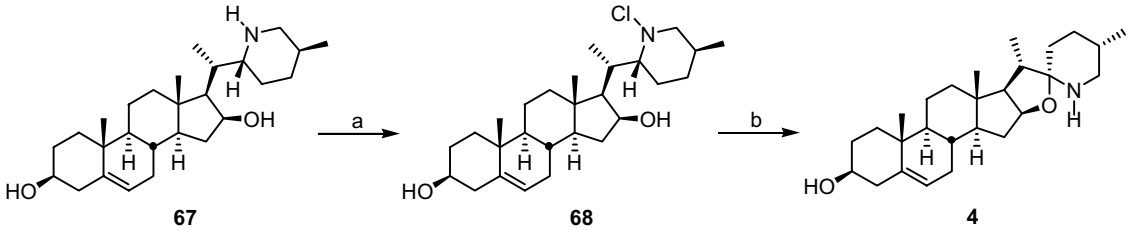
Scheme 1.11

Treatment of the α-bromine **65** with KOAc gave in good yield the β-diacetate **66**, which could be reduced with Red-Al in benzene to **67**[194] (Scheme 1.12). These types of compounds can be ringclosed to spirosolane compounds as shown by Schreiber[195] (Scheme 1.13).



a) KOAc, DMF, 90ºC; b) Red-Al, benzene

Scheme 1.12

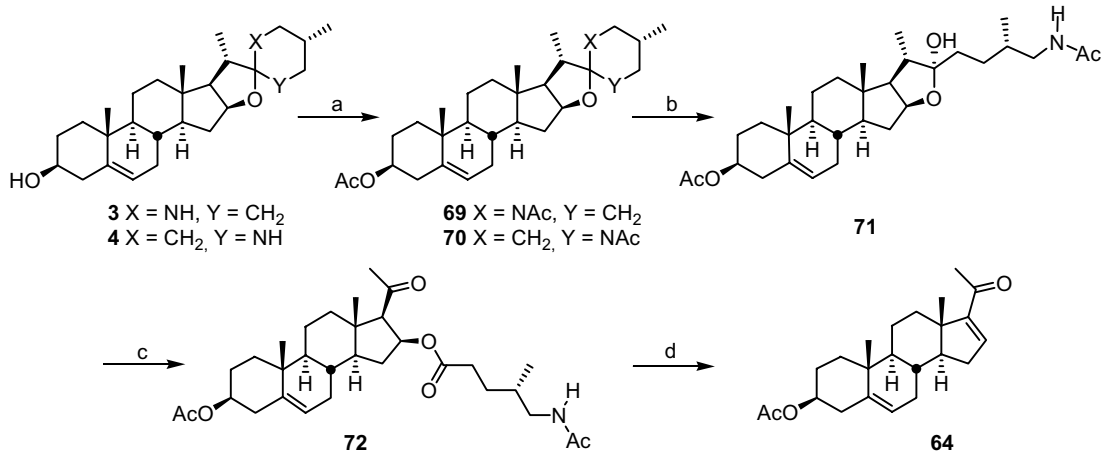


a) NCS, $CHCl_3$; b) NaOMe, MeOH

Scheme 1.13

1.8 Conversions of glycoalkaloids to DPA

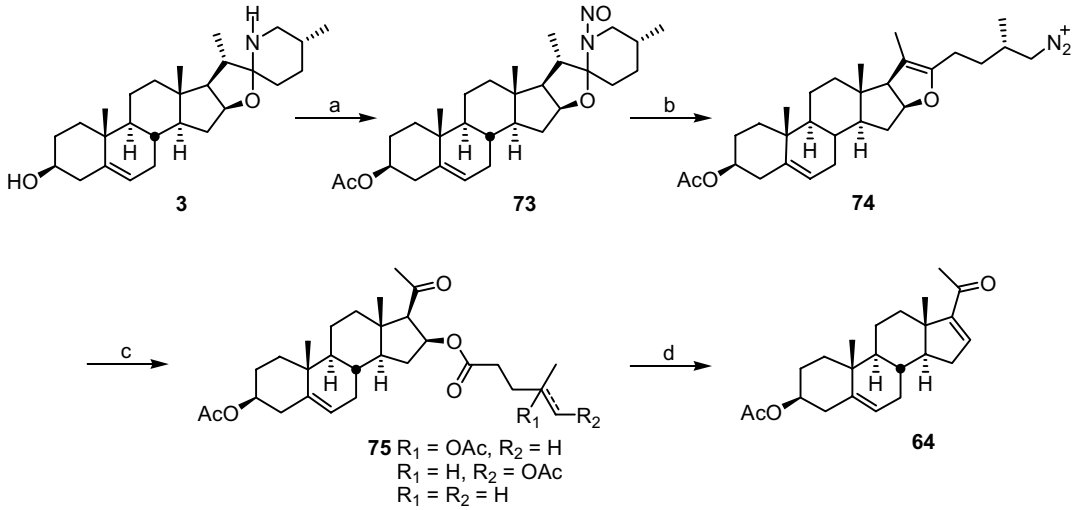
Sato *et al.*[196] reported the degradation of solasodine (**3**) and tomatidine (**5**) to DPA (**64**) and its 5α-H derivative, respectively. Since then several modifications have been applied to increase the yield of DPA[197-202] (Scheme 1.14). Especially the degradation of solasodine (**3**) has been extensively studied in this respect. Acetylation of solasodine (**3**) with 3.8 mole equivalents of Ac_2O in pyridine, followed by an eliminative ringopening of diacetate **69** using boiling HOAc gave the enol ether **71**. Oxidation of **71** with 2 mole equivalents of CrO_3 in HOAc (80%) under cooling and finally, cleavage of the 16β-side chain moiety of **72** by treatment with boiling HOAc gave DPA (**64**) in a continuous operation without isolation of the intermediates in an overall yield of 65-68%. In a similar way tomatidenol (**4**) can be converted via compound **70**, **71**, and **72** to DPA (**64**) in an overall yield of 60-75%[203] (Scheme 1.14).



a) Ac_2O, pyridine, Δ, 1 hour; b) HOAc, Δ, 15 min.; c) CrO_3, HOAc; d) HOAc, Δ

Scheme 1.14

An alternative degradation route of solasodine (**3**) to DPA (**64**) has been reported by Bakker and Vrijhof[204] (Scheme 1.15). Solasodine (**3**) was first nitrosylated with $NaNO_2$ in aqueous HOAc and then acetylated with Ac_2O in pyridine to obtain *N*-nitrososolasodine (**73**). Treatment of **73** with TsOH in MeOH at 65ºC yielded a mixture of intermediates originating from **74**. Oxidation of this mixture of intermediates with CrO_3 in HOAc (80%) gave **75** as a mixture of three compounds. Elimination of the 16β-side chain moiety in **75** with HOAc yields DPA (**64**). Starting from solasodine (**3**) the overall yield of DPA (**64**), without isolation of intermediates, was 60%.



a) $NaNO_2$, HOAc, MeOH, 0ºC; Ac_2O, pyridine; b) TsOH, MeOH, 65ºC;
c) CrO_3, HOAc; d) HOAc, Δ

Scheme 1.15

1.9 Scope and outline of this thesis

In the potato-starch industry, specially cultivated potatoes are used for the production of starch. In earlier days the starch industry only used the starch and fibers of the potatoes. The potato juice containing the proteins and glycoalkaloids was treated as waste. During the seventies the starch factories were equipped with a protein factory and an installation to isolate compounds with a high nutritious value from this potato juice. These compounds, proteins and protamylasse, are used in cattle fodder. The protein fraction also contains the glycoalkaloids and these compounds have to be removed for use of the proteins in high quality cattle fodder or consumer products. A relative simple process has been developed to isolate these proteins. The remaining fraction contains residual proteins with a high amount of glycoalkaloids (200-2000 ppm) and is still regarded as a waste product.

As mentioned before, potato glycoalkaloids consist of more than 95% of α-chaconine (**6**) and α-solanine (**7**). Since both compounds have the steroid-like solanidine (**1**) as aglycone, a large potential of starting material is available for conversion to steroid hormones.

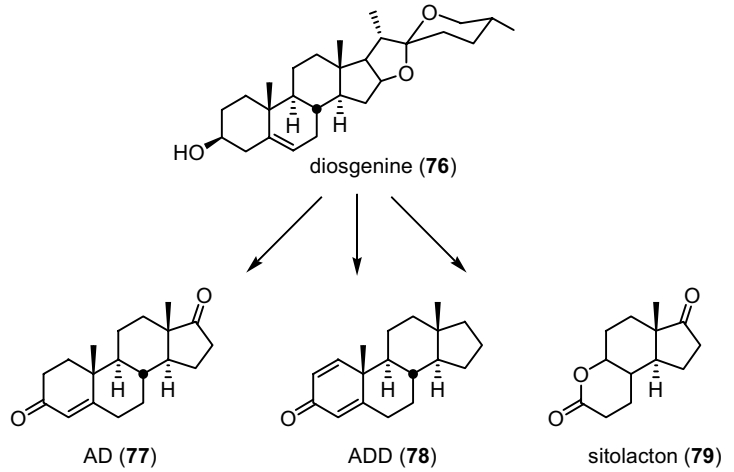
During the last 30 years diosgenine (**76**) is used as the main starting material in the industrial synthesis of progestagens, androgens, estrogens, norsteroids, and a diuretic spironolactone. Until 1975 diosgenine (**76**) was isolated from tubers of *dioscorea* species grown in Mexico. All major steroid companies had their own production facilities in Mexico where diosgenine (**76**) and intermediates were isolated. After the Mexican government nationalized the collection of tubers, prices raised with a factor 3 to 4 and the steroid manufacturers switched to already developed alternatives. Most applied alternative was the microbiological degradation of sitosterol to

androstane derivatives. Sitosterol is readily available from soy-oil but a major disadvantage is that not all classes of steroids can be synthesized from sitosterol (Table 1.1, Scheme 1.16). Protagens cannot be synthesized from intermediates derived from sitosterol and for the other classes mentioned above, different degradation processes must be applied.

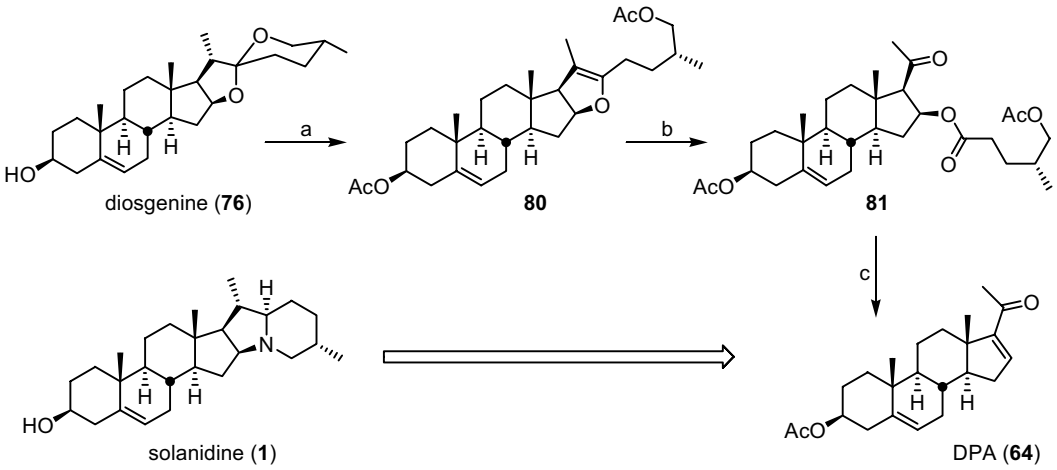
Soon China took over Mexico's role as major supplier of diosgenine (**76**), which is isolated from a cultivar, called *Costus speciosus*. The internal booming economy of China puts a lot of pressure on the production of diosgenine (**76**) and a big and varying part of its production is used for the internal market, so again new alternatives were developed or old alternatives re-examined to become independent from these uncertain external factors. These alternatives should have all in common that they can be implemented in the existing production facilities for economic and pharmaceutical reasons.

Table 1.1

Class of steroids	**Starting material**		
protagens	diosgenine (**76**)	-	-
androgens	diosgenine (**76**)	sitosterol	via AD (**77**)
estrogens	diosgenine (**76**)	sitosterol	via ADD (**78**)
norsteroids	diosgenine (**76**)	sitosterol	via sitolactone (**79**)



Scheme 1.16

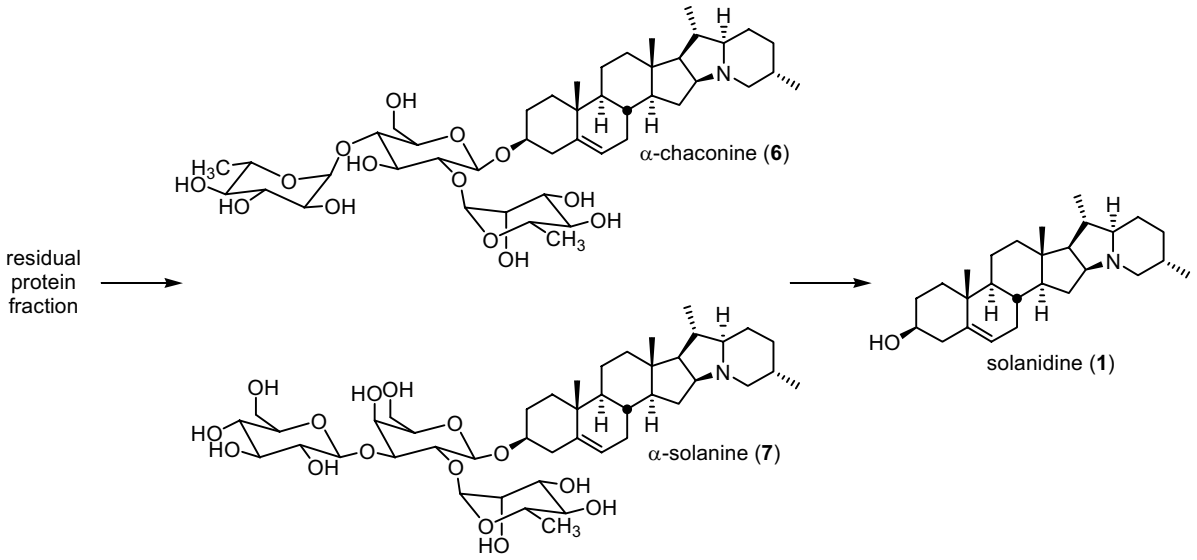


a) Ac_2O, HOAc, Δ; b) CrO_3, HOAc; c) HOAc, Δ

Scheme 1.17

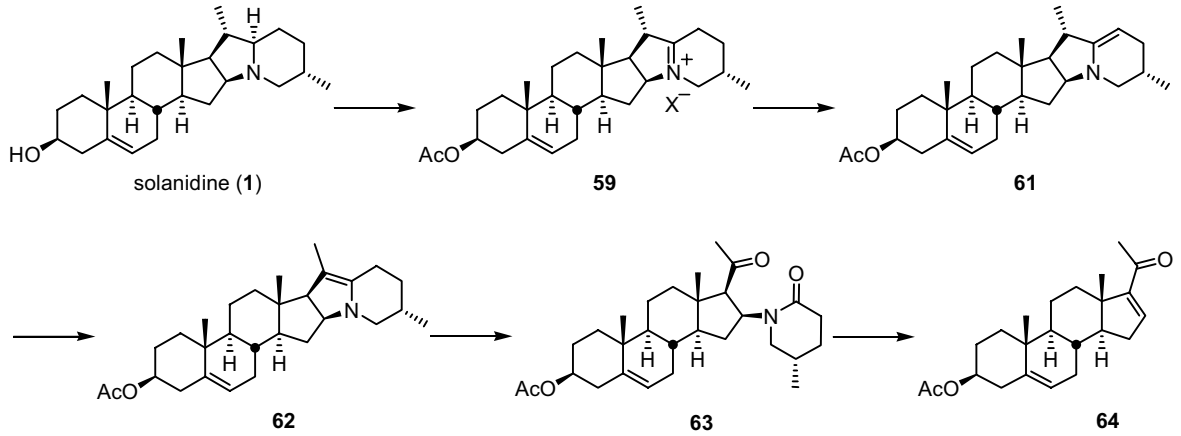
Solanidine (**1**) could be such an alternative on condition that it can be converted in an industrially attractive way to DPA (**64**), which is a key intermediate in the industrial syntheses of progesterone and cortisone derivatives (Scheme 1.17). This thesis focuses on the development of an industrial applicable degradation procedure of α-chaconine (**6**) and α-solanine (**7**) to DPA (**64**).

After the foregoing in which a general introduction and a brief overview on the synthesis and reactions of solanidane compounds has been presented, Chapter 2 gives a detailed description of the isolation of the glycoalkaloids **6** and **7** from the residual spray-dried potato protein fraction and the hydrolysis of these glycoalkaloids to solanidine (**1**) (Scheme 1.18).



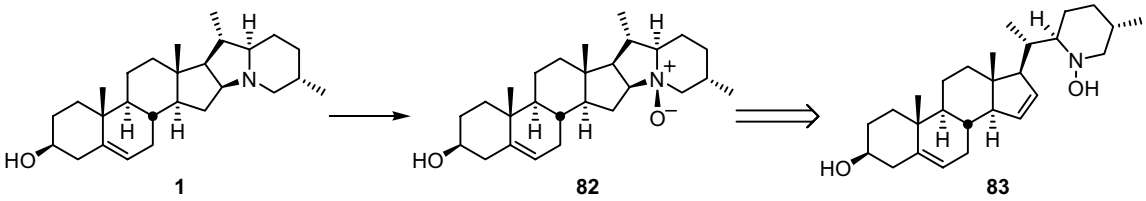
Scheme 1.18

In Chapter 3, the direct oxidation of solanidine (**1**) to **59**, the isomerization of **59** to **61** and **62**, the oxidation of **62** to **63**, and finally the elimination of the amide moiety to **64** are studied (Scheme 1.19).



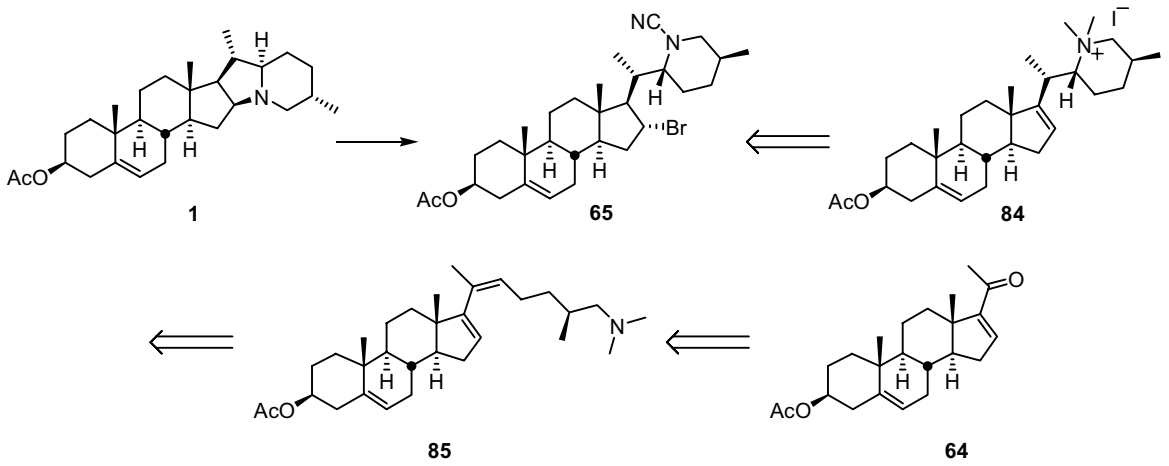
Scheme 1.19

Besides alternatives for the oxidation procedure of **1** to **59**, the synthesis of solanidine *N*-oxide **82** and its possible degradation to **83** via the Cope and Polonovski reaction are discussed (Scheme 1.20).



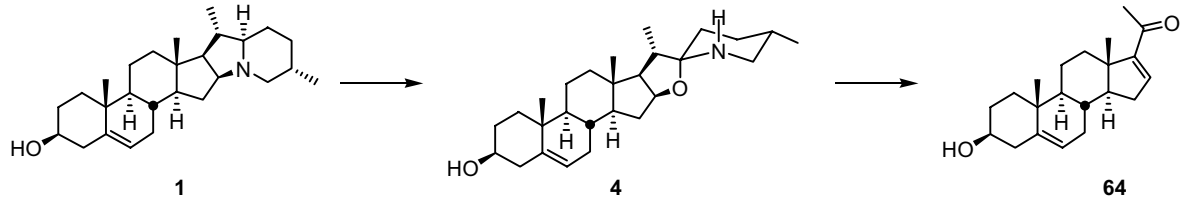
Scheme 1.20

In Chapter 4, the Von Braun reaction on solanidine (**1**) is described and possible alternatives for this reaction are investigated. Further degradation of **65** to **85** via the Hofmann degradation procedure is the second topic described in this chapter (Scheme 1.21).



Scheme 1.21

In Chapter 5, the transformation of solanidine (**1**) to DPA (**64**) via tomatidenol (**4**) is reported (Scheme 1.22).



Scheme 1.22

In Chapter 6, some concluding remarks on the reactivity and chemistry of solanidine (**1**) are given.

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Chapter 2

Isolation and hydrolysis of potato glycoalkaloids

2.1 Introduction

2.1.1 Production of starch

Nowadays the production of starch has become a complicated process due to environmental regulations and the desire to use more and more elements of the potato as starting material for other applications than starch. The complete starch production procedure, including all process recycle steps, as applied in AVEBE's Oostermoer plant is depicted in Figure 2.1[1].

During the starch refining process the proteins together with the glycoalkaloids are separated from the starch. The glycoalkaloid containing protein fraction is then subjected to a number of refining steps in which the proteins are separated from the glycoalkaloids. These proteins are used as nutritional additives in cattle fodder. After the proteins have been isolated the glycoalkaloids together with free amino acids, peptides and minerals end up in the so-called protamylasse fraction, which is formed after evaporation of the water. The amount of glycoalkaloids present in the protein fraction (35.000 ton) varies from 200-2000 ppm dependent on the potatoes processed per campaign.

2.1.2 Glycoalkaloids

In general the isolation and determination of potato glycoalkaloids can be divided in three steps: extraction, separation, and analysis[2]. The first part depends on the nature of the material from which the glycoalkaloids have to be extracted. In the second step all compounds that interfere with the chosen method have to be eliminated. The analysis may include modification of the

glycoalkaloids depending on the analysis techniques used. Several isolation and analytical methods for glycoalkaloids combine the extraction and modification steps[3].

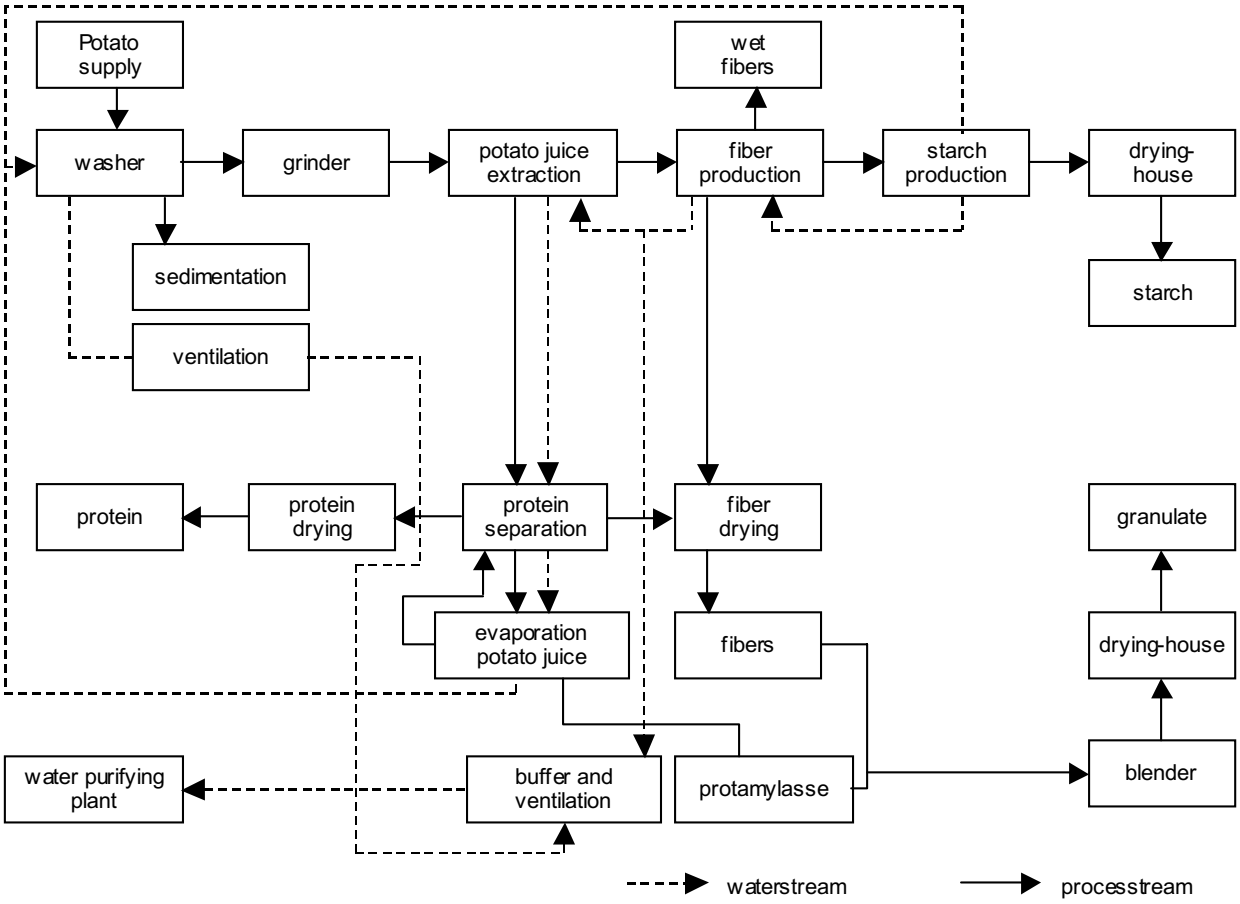
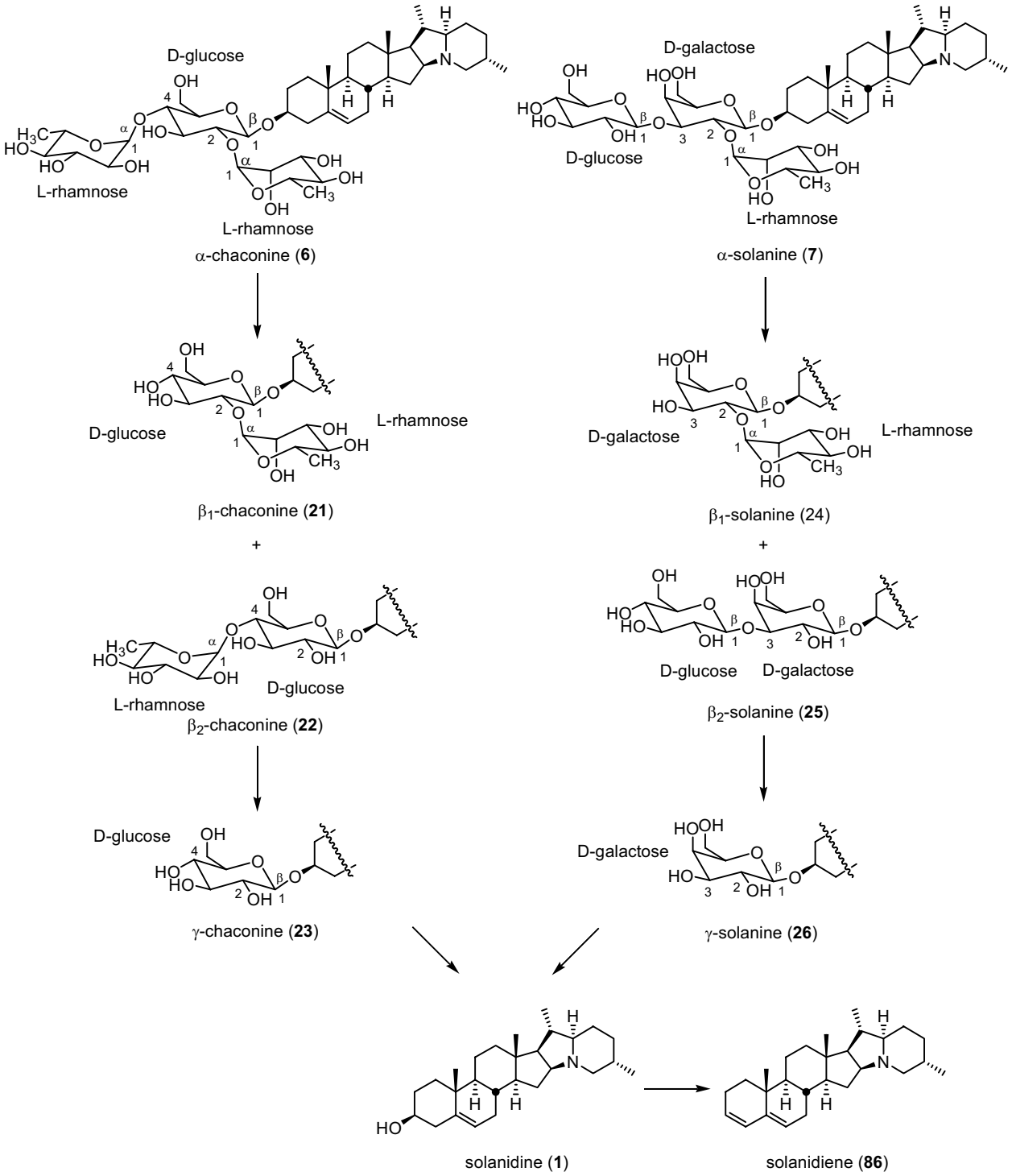


Figure 2.1

Mass extraction methods are based on the specific physical properties of the potato glycoalkaloids[4]. Except the leptines, all potato glycoalkaloids are sparingly soluble in water at pH 7 or higher. For this reason, many extraction methods make use of non-aqueous or acidic solvents. Extractions are usually performed at room temperature and any combination of heat and acid has to be avoided to minimize hydrolysis. More than 20 extraction solvents have been studied and in most studies recovery experiments have been performed[5]. Notice has to be taken that recovery experiments can only ensure that there is little or no loss of glycoalkaloids in clean up and analysis. Although extraction procedures for analysis of glycoalkaloids are described extensively, only a few articles about the mass extractions of potato glycoalkaloids have been published[4,6-8].

In general, methodologies for the isolation and analysis of potato glycoalkaloids and related compounds include preparative thin layer chromatography (TLC)[9,10], silica gel and Sephadex LH-20 column chromatography[11-13], high performance liquid chromatography (HPLC)[14] and reversed-phase HPLC[15], droplet counter current chromatography (DCCC)[11,16,17], medium pressure liquid chromatography (MPLC)[18], colorimetry[19-22], gas chromatography (GC)[23-26], immunoassays (ELISA)[23,27-29], and capillary electrophoresis (CE)[30-34]. The coupling of a mass and/or NMR spectrometer to GC, HPLC, and CE is an important method for the rapid qualitative and quantitative analysis of compounds in plant extracts. At present, the most commonly used method for quantitative and qualitative analysis of glycoalkaloids is reversed-phase HPLC. Since the first pioneering studies[35-37] HPLC has been continuously improved with respect to sample preparation and clean up, column selection and peak detection.

Chemical hydrolysis[38-40] of the potato glycoalkaloids has been studied in more detail than enzymatic hydrolysis[41-46]. The factors that are of influence on the chemical hydrolysis are time, temperature, and acid concentration. In general, hydrolysis rates increase with acid concentration[47,48] and temperature, and decrease with the amount of water in organic solvent-water solutions[5]. Further studies have revealed that the nature of the alcohol used as solvent strongly influences the rate and the specificity of the hydrolysis, permitting optimal formation of the β- and γ-compounds (**21**-**26**) as specific hydrolysis products[49,50] (Scheme 2.1).



Scheme 2.1

Under strongly acidic conditions and high temperatures, solanidine (**1**) will react further to

solanidiene (**86**) (solanthrene) after the hydrolysis of α-chaconine (**6**) and α-solanine (**7**)[5].

2.2 Results and discussion

The first step in the isolation and hydrolysis of potato glycoalkaloids was the analysis of the spray-dried protamylasse. The method of choice for the analysis of protamylasse is well described and easy to perform with the reversed-phase HPLC method of Houben and Brunt[51]. First, a solid-phase extraction step was performed to obtain higher concentrations of the glycoalkaloids. As mentioned by Houben and Brunt[51], peak tailing occurs due to interactions of the glycoalkaloids with the residual silanol groups of the column. Buffer solutions are therefore necessary to keep the glycoalkaloids in solution. As a result, the lifetime of the column decreases and the seals of the HPLC instrument rapidly deteriorate. Due to the reduced column lifetime, the potato samples and standard solutions should be measured at the same day for a reliable quantitative analysis.

For the spray-dried protamylasse a potato glycoalkaloid content of 6% was determined. Figure 2.2 shows a HPLC sample of the spray-dried protamylasse.

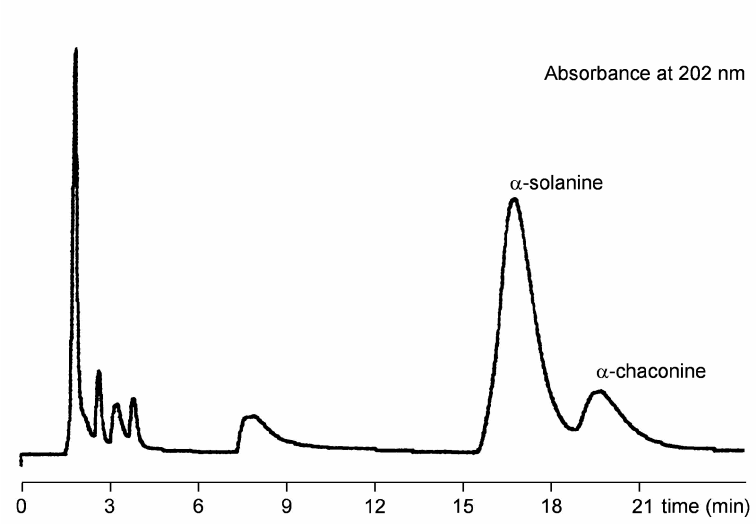


Figure 2.2: Illustration of an protamylasse HPLC sample determined with the method of Houben and Brunt.

To obtain analytical data for the glycoalkaloids, a sample of the spray-dried protamylasse was isolated following the method of Friedman *et al.*[5]. This method should allow the separate isolation of α-chaconine (**6**) and α-solanine (**7**) but in our hands extraction of the pellet and recrystallization from EtOH gave always a mixture of both compounds. This mixture of **6** and **7** was analyzed using nanoelectronspray-ion trap mass spectroscopy[52]. The two dominant peaks in Figure 2.3a correspond with the MH^+ ions of α-chaconine (**6**) (*m/z* 853.2) and α-solanine (**7**) (*m/z* 868.7). Figures 2.3b and 2.3c show the more detailed MH^+ ion and isotope peaks of α-solanine (**7**) and α-chaconine (**6**), respectively.

MS of *m/z* 868.5 (Figure 2.3d) gives information about the structure of α-solanine (**7**). Peak *m/z* 706.5: loss of 162 amu, a terminal hexose. Peak *m/z* 722.5: loss of 146 amu, a terminal deoxyhexose. Peak *m/z* 398.5: loss of 470 amu, the complete sugar unit. The last and connecting sugar is also a hexose: 470-146-162 = 162. Peak 560.4: loss of 308 amu = 146 + 162. From these results the structure of α-solanine (**7**) can be established (Figure 2.4).

MS of *m/z* 852.5 (Figure 2.3e) gives information about the structure of α-chaconine (**6**). Peak *m/z* 706.5: loss of 146 amu, a terminal deoxyhexose. Peak 560.7: loss of 292 amu, two deoxyhexoses (146 + 146 = 292). Peak *m/z* 398, loss of 454 amu, complete sugar unit consisting of

two deoxyhexoses and one hexose (2×146 + 162 = 454). The hexose is the connecting sugar to the aglycone. The two deoxyhexoses are connected to the hexose but the branching cannot be derived from these spectra (Figure 2.4). These mass spectra match the data of α-solanine (**7**) and α-chaconine (**6**) described in literature[5,53].

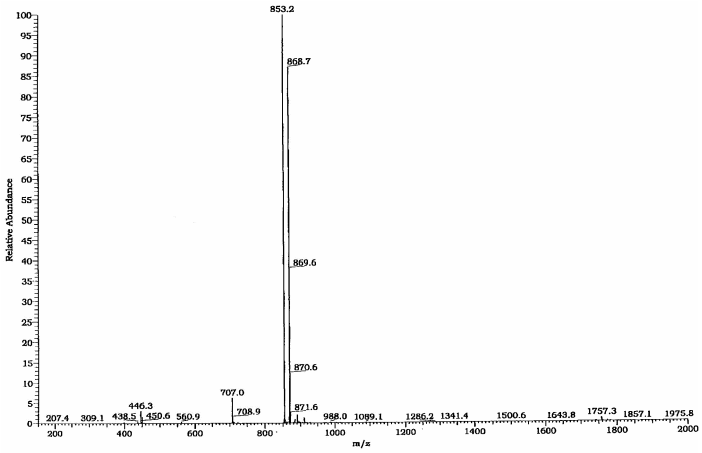


Figure 2.3a: full spectrum of mixture of glycoalkaloids

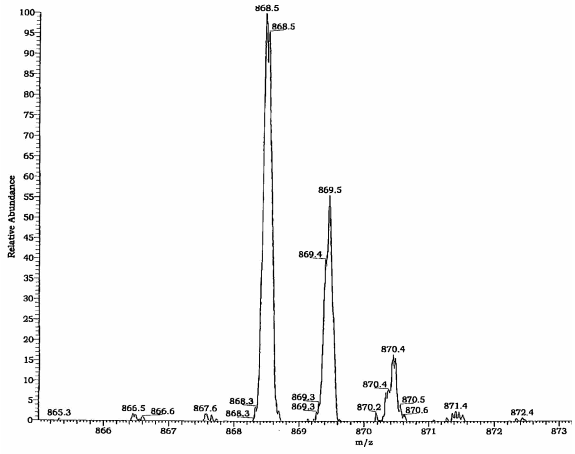


Figure 2.3b: detailed MH^+ α-solanine

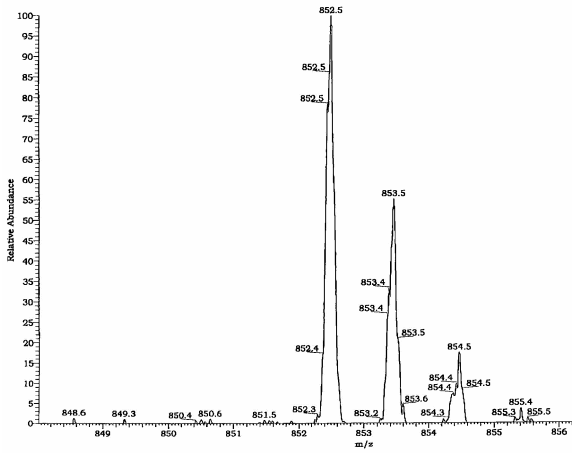


Figure 2.3c: detailed MH^+ α-chaconine

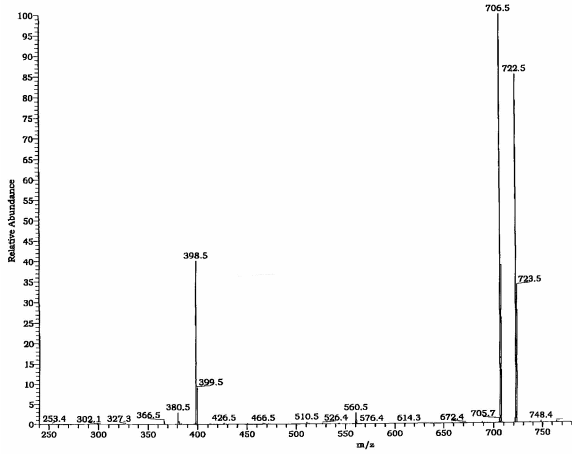


Figure 2.3d: full spectrum of α-solanine

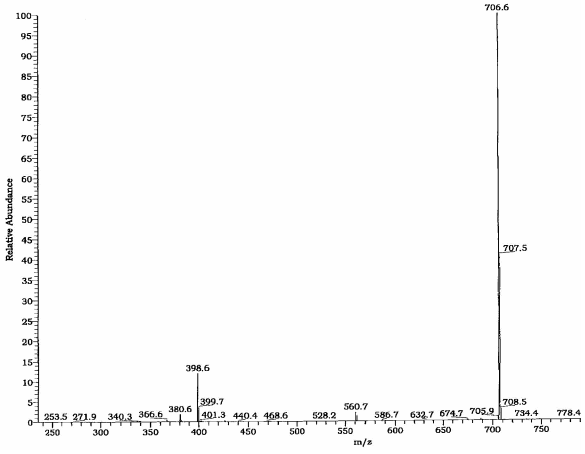


Figure 2.3e: full spectrum of α-chaconine

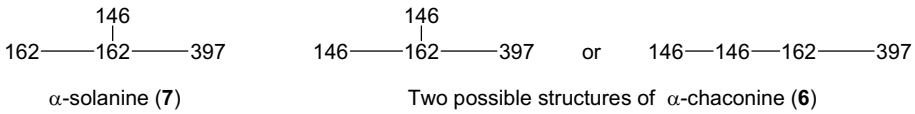


Figure 2.4: structures of glycoalkaloids represented by the mass of the sugar and aglycone units

After analysis of the spray-dried protamylasse, the next steps were the isolation and hydrolysis of glycoalkaloids on large scale. It is known that direct hydrolysis of the spray-dried protamylasse leads to an unidentifiable mixture of products[49,54]. Since the protamylasse consists for only 6% of glycoalkaloids, other compounds present in the protamylasse can react as well with acid thereby forming all kind of products. Therefore, the glycoalkaloids have to be isolated prior to hydrolysis. All known mass extraction methods begin with the extraction of the glycoalkaloids from the fresh material (blossom or tubers) using a mixture of solvents (water, acetonitrile and tetrahydrofuran) with some acid (acetic acid) or followed by an acidic workup step[4,6,7,55]. In the next step precipitation with NH_4OH gives the crude glycoalkaloid fraction. Purification of the crude glycoalkaloids by column chromatography or re-extraction with methanol, precipitation with NH_4OH, and recrystallization from ethanol gives a clean glycoalkaloid fraction.

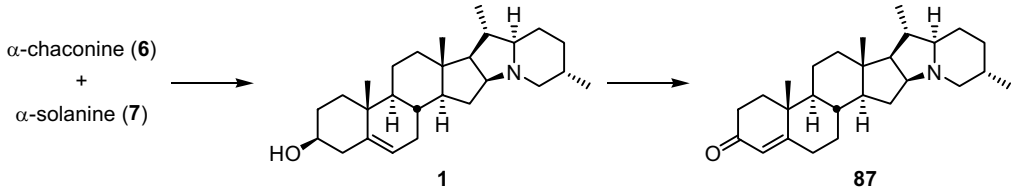
The modified method of Friedman *et al.*[5] for mass analysis was used by us for the extraction of the potato glycoalkaloids from the protamylasse. A crude mixture of α-chaconine (**6**) and α-solanine (**7**) is obtained by using aqueous ethanol in the extraction step. Recrystallization from ethanol gives a clean mixture of both glycoalkaloids. In this way 221.1 g of spray-dried protamylasse yields 10.8 g of glycoalkaloids (85% yield based on 5.7% glycoalkaloids content in spray-dried protamylasse as determined by HPLC).

Chemical hydrolysis has to be performed with acid since alkaline hydrolysis does not hydrolyze α-solanine (**7**)[56]. Van Gelder[54] developed a two-phase system consisting of an immiscible organic liquid phase and an aqueous acid phase in which the substrate was hydrolyzed. The apolar organic liquid served as a protective phase for the apolar aglycones. Recoveries of two-phase hydrolysis were almost 100% regardless of type and purity (presence or absence of tuber extracts) of the glycoalkaloid. Since tetrachloromethane used by van Gelder[54] as organic phase is not acceptable in industry, conventional hydrolysis was employed. Ethanol was used instead of methanol in the conventional hydrolysis (2.5 hours, 70ºC). Solanidine (**9**) was isolated from the acidic ethanol in an almost quantitative yield after precipitation with NH_4OH.

Because both the isolation and hydrolysis of glycoalkaloids can now be performed successfully on a large scale, attempts were made to combine both procedures. In the procedure of Friedman *et al.*[5] HOAc (1%) is added to dissolve the glycoalkaloids but because the protamylasse has a pH of 5-6 it can be dissolved in water without the addition of acid. After the aqueous solution was made basic with NH_4OH and stored overnight at 4ºC, the slurry was centrifuged and the supernatant discarded. The pellet was subjected to hydrolysis but again a complex product mixture was formed. Extraction proved to be necessary prior to hydrolysis and ethanol appeared to be a good alternative for the mixture of $CHCl_3$, MeOH, and HOAc.

Thus the pellets were transferred to a Soxhlett apparatus and extracted with ethanol for 24 hours. After concentration of the extract, a crude glycoalkaloid fraction was obtained. This fraction was hydrolyzed in acidic ethanol for 2.5 hours, precipitated with NH_4OH, filtered, and recrystalized from ethanol to give pure solanidine (**1**). The mother liquor was concentrated *in vacuo* and the remaining solid was recrystallized again. The isolation of solanidine (**1**) is accompanied by the formation of solanidiene (**86**) in 9% yield. Lowering the amount of acid used in the hydrolysis step from 2M to 1M HCl in ethanol reduced the formation of solanidiene (**86**).

The protamylasse delivered by AVEBE in several batches was stored at room temperature. The acidic (pH 5~6) spray-dried protamylasse contained microorganisms[57] and over the years the glycoalkaloid content decreased due to conversion of the glycoalkaloids to solanid-4-en-3-one (**87**) by these microorganisms (Scheme 2.2). This solanid-4-en-3-one (**87**) is lost during the isolation and hydrolysis procedure of solanidine (**1**) because the method is optimized for the isolation of glycoalkaloids and not for isolation of the alkaloids.



Scheme 2.2

2.3 Conclusion

The content of glycoalkaloids in the first batch received from AVEBE, determined with HPLC, amounted to 6%. Solanidine (**1**) was isolated in a fairly simple and effective way with a yield of 95% based on the amount of glycoalkaloids present in the spray-dried potato protein fraction as determined by HPLC. After hydrolytic workup, crystallization from ethanol turned out to be an efficient way to purify the crude solanidine (**1**). Formation of solanidiene (**86**) can be prevented by the use of 1M HCl in ethanol in the hydrolysis reaction.

2.4 Experimental Section

2.4.1 General comments and Materials

All reagents were purchased from Aldrich or Acros and used without further purification, unless stated otherwise. Dry reactions were performed under a steady stream of dry nitrogen or argon with glassware dried at 140ºC. All ^{1}H and ^{13}C NMR spectra were measured with a Bruker AC-E 200 spectrometer. 400 MHz ^{1}H and 100 MHz ^{13}C NMR were measured with a DPX 400 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (δ 0.0). MS and HRMS data were obtained with a Finnigan Mat 95 spectrometer. FT-IR spectra were measured with a BIO-RAD FTS-7 infra-red spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter with the concentrations denoted in units of g/100 ml. Analytical data were obtained using a Carlo Erba Elemental Analyzer 7206. Melting points are uncorrected. Solvents were freshly distilled by common practice. Product solutions were dried over Na_2SO_4 prior to evaporation of the solvent under reduced pressure by using a rotary evaporator. For flash chromatography, Merck Kieselgel silica 60 (230-400 Mesh) or Baker Alumina was used. Reactions were monitored with TLC using Merck silica gel 60F254 plastic sheets. Compounds were visualized on TLC by UV detection and by spraying with acid and subsequent heating.

2.4.2 High-Performance Liquid Chromatography (HPLC) system

Acetonitrile and methanol were of HPLC spectroquality grade and obtained from LAB-SCAN Analytical Sciences. Ultra pure water was obtained from a combined Seradest LFM 20 Serapur Pro 90 C apparatus. All HPLC solvents were degassed by vacuum filtration over a 45 μm membrane filter (Type RC, Schleicher and Schuell). Heptane sulfonic acid sodium salt and diammoniumhydrogenphosphate were obtained from Acros.

The solvents used for extraction were of analytical grade. The glycoalkaloid standards α-solanine (95% pure) and α-chaconine (95% pure) were obtained from Sigma Chemical Co. The spray-dried protamylasse was received from AVEBE and stored at room temperature.

HPLC analyses were carried out on a Varian 2010 HPLC pump fitted with an Spherisorb S5-RP8 (200 × 4.6 mm I.D.) column and a Spheri-5 RP-8S (30 × 4.6 mm I.D.) (5μm) as precolumn.

The pump was fitted with a 10 μm injection loop and an Applied Biosystems 4000 solvent delivery system. A Varian 2050 variable λ detector and a HP 3395 integrator were used. The flow rate was 1 ml/min and detection took place with UV at 200 nm. The eluent for glycoside detection was acetonitrile and an aqueous 0.02% diammoniumhydrogenphosphate solution.

Solid Phase Extraction was performed with a disposable Sep-Pak C-18 column (Waters).

2.4.3 Quantitative analysis of spray-dried potato protein

Extraction fluid I

Water (2500 ml) and glacial acetic acid (30 ml were added to a 3 L measuring flask containing the sodium salt of heptanesulfonic acid (12.00 g).

Extraction fluid II

Acetonitrile (600 ml) was added to an aqueous solution of diammoniumhydrogenphosphate (0.02%, 400 ml).

Wash fluid

Acetonitrile (200 ml) was added to water (800 ml).

Solid phase extraction and HPLC

To concentrate the glycoalkaloids from the spray-dried protamylasse the method of Houben and Brunt[51] was adapted. Spray-dried protamylasse (0.2435 g, m_m) was dissolved in extraction fluid I (10 ml) and centrifuged (2000 rpm) for 5 minutes. The supernatant was transferred with a disposable polyethylene pipette into a 100 ml flask (m_0= 55.963 g). The pellet was reextracted with extraction fluid I, centrifuged (2000 rpm), and the supernatant was added to the 100 ml flask. This procedure was repeated once more and the supernatant was added to the 100 ml flask (m_1= 85.634 g). The combined extracts were stirred for 30 minutes. The supernatant fraction (20 ml) was put on a solid phase extraction column (Sep-Pak C-18 column), conditioned with methanol (5 ml) and extraction fluid I (5 ml) before use. After the supernatant has eluted from the column, the column was washed with wash fluid (5 ml) and dried by removing the wash fluid with a syringe. The glycoalkaloids were eluted from the column by extraction fluid II. In a calibrated tube the eluent is collected (4.0 ml) and used for analysis.

Determination of glycoalkaloids in protamylasse

$$w_{TGA} = \frac{w_1 \times 4 \times (m_1 - m_0)}{m_m \times 20} [\mu g / g]$$

w_{TGA} = amount Total GlycoAlkaloids (TGA) in sample (μg/g)

w_1 = amount TGA in sample (μg /ml)

m_0 = weight of flask (g)

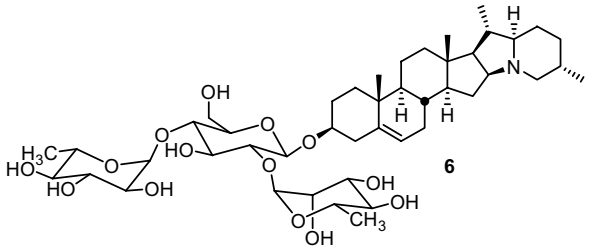
m_1 = weight of flask + supernatant (g)

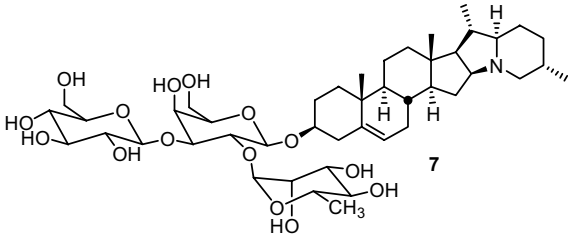
m_m = weight of spray-dried protamylasse sample (g)

2.4.4 Isolation procedures

Isolation of α-chaconine (6) and α-solanine (7) according to Friedman *et al.*[5].

Spray-dried protamylasse (39.4 g) was dissolved in diluted acetic acid (120 ml, 1%) and stirred for 10 minutes at room temperature. The solution was made basic with NH_4OH to pH 9~10, heated at 70ºC for 30 minutes, and stored overnight at 4ºC. The mixture was then centrifuged (3161 rpm) for



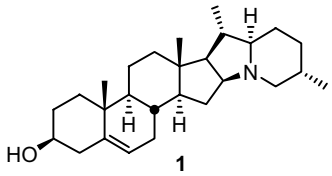


50 minutes and the supernatant was discarded. The solid pellet was washed with cold aqueous NH_4OH (2%) and recentrifuged (3161 rpm) for 30 minutes. The pellet was taken up in a mixture of EtOAc/EtOH/NH_4OH (5%)(250 ml, 80/16/4) and filtered. The remaining filter cake was extracted twice with EtOAc/EtOH/NH_4OH (5%)(250 ml, 80/16/4). The combined filtrates were evaporated under reduced pressure to afford a yellow solid (3.0 g). Crystallization from ethanol (80%) gave 2.16 g of a mixture of **6** and **7**.

MS *m/z* (r.i.) 870.4 (MH^++2, α-solanine), 869.5 (MH^++1, α-solanine), 868.5 (MH^+, α-solanine), 854.5 (MH^++2, α-chaconine), 853.5 (MH^++1, α-chaconine), 852.5 (MH^+, α-chaconine), 723.5 (MH^++1, β_2-solanine), 722.5 (MH^+, β_2-solanine), 707.5 (MH^++1, β-chaconine), 706.5 (MH^+, β-chaconine), 561.5 (MH^+, γ-chaconine), 560.5 (MH^+, γ-chaconine), 399.6 (MH^++1, solanidine), 398.5 (MH^+, solanidine)

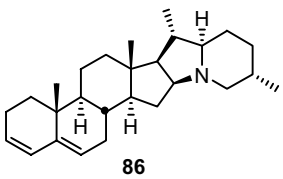
Isolation of solanidine (1) and solanida-3,5-diene (86)

The spray-dried potato protein (225 g) was dissolved in water (700 ml), made basic with NH_4OH (35%) to pH 10-12, and stored overnight at 4ºC. The solution was then centrifuged (6000 rpm) for 30 minutes and the supernatant was discarded. The crude glycoalkaloid mixture was transferred to a Soxhlett apparatus and extracted with ethanol for 24 hours. After evaporation, the resulting brown solid was dissolved in ethanol (350 ml) and hydrolyzed with aqueous HCl (37%, 40 ml) at reflux temperature for 3 hours. The solution was cooled to room temperature and made basic with NH_4OH (35%). The solvent was evaporated to yield a brown solid, which was taken up in H_2O (250 ml) and $CHCl_3$ (250 ml). The water layer was extracted three times with $CHCl_3$ (200 ml) and the combined organic extracts were dried and flash chromatographed (Al_2O_3, $CHCl_3$/MeOH 9/1) to give a crude mixture of **1** and **86**. Crystallization from the eluent gave **1** (2.47 g). After concentration of the mother liquor, crystallization of the remaining residue from EtOH (96%) provided solanid-3,5-diene **86** (0.51 g).



1

1: M.p. 213-215ºC (EtOH 80%) (lit. 215-217ºC[5]); ^{1}H NMR ($CDCl_3$+ CD_3OD) δ 0.72 (d, 3H, *J*= 6.3 Hz, *H*21), 0.73 (s, 3H, *H*18), 0.80 (d, 3H, *J*= 5.7 Hz, *H*27), 0.89 (s, 3H, *H*19), 2.12 (d, 2H, *J*= 5.5 Hz, *H*), 2.53 (m, 1H, *H*), 2.78 (dd, 1H, *J*= 10.2, 2.4 Hz, *H*26β), 3.35 (m, 1H, *H*3), 5.21 (d, 1H, *J*= 4.8 Hz, *H*6); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 16.32 (q, C18), 17.55 (q, C21), 19.20 (q, C19), 19.33 (q, C27), 20.79 (t, C11), 28.44 (t, C23), 30.40 (d, C25), 30.60 (t, C15), 31.07 (t, C24), 31.30 (d, C8), 31.94 (t, C2), 32.95 (t, C7), 36.50 (s, C10), 36.61 (d, C20), 37.13 (t, C1), 39.96 (t+s, C12+C13), 41.78 (t, C4), 50.05 (d, C9), 57.47 (d, C14), 60.63 (t, C26), 62.48 (d, C17), 69.41 (d, C16), 72.12 (d, C3), 74.69 (d, C22), 121.24 (d, C6), 140.79 (s, C5); MS (FAB) *m/z* (r.i.) 400 (10), 399 (26), 398 (100, M^++1), 397 (9), 382 (6), 380 (7), 98 (10). NMR and mass spectral data are in accordance with literature data[58].



86

86: M.p. 172ºC (EtOH) (lit. 172ºC[59]); IR, λ_{max} (neat) 1451, 1370 cm^{-1}; ^{1}H NMR δ 0.83 (d, *J*= 6.4 Hz, 3H), 0.86 (s, 3H), 0.92 (d, *J*= 6.4 Hz, 3H), 0.95 (s, 3H), 2.63 (m, 1H), 2.85 (d, *J*= 8.7 Hz, 1H), 5.30 (m, 1H), 5.55 (m, 1H), 5.91 (d, *J*= 9.7 Hz, 1H); ^{13}C NMR δ 17.0 (q), 18.2 (q), 18.8 (q), 19.5 (q), 20.8 (t), 23.0 (t), 29.2 (t), 31.0 (d), 31.5 (d), 31.9 (t), 33.3 (t), 33.8 (t), 35.3 (s), 36.6 (d), 40.0 (t), 40.4 (s), 48.4 (d), 57.8 (d), 60.2 (t), 62.9 (d), 69.1 (d), 74.7 (d), 123.0 (d), 125.0 (d), 128.9 (d), 141.4 (s); MS (EI) *m/z* (r.i.) 380 (20), 379 (78), 378 (26), 364 (15), 204 (20),

152 (4), 151 (14), 150 (100), 136 (6), 98 (4). NMR spectral data were compared with literature data of solasodiene and cholesta-3,5-diene[60].

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Chapter 3

The mercury acetate and the Cope and Polonovski reaction

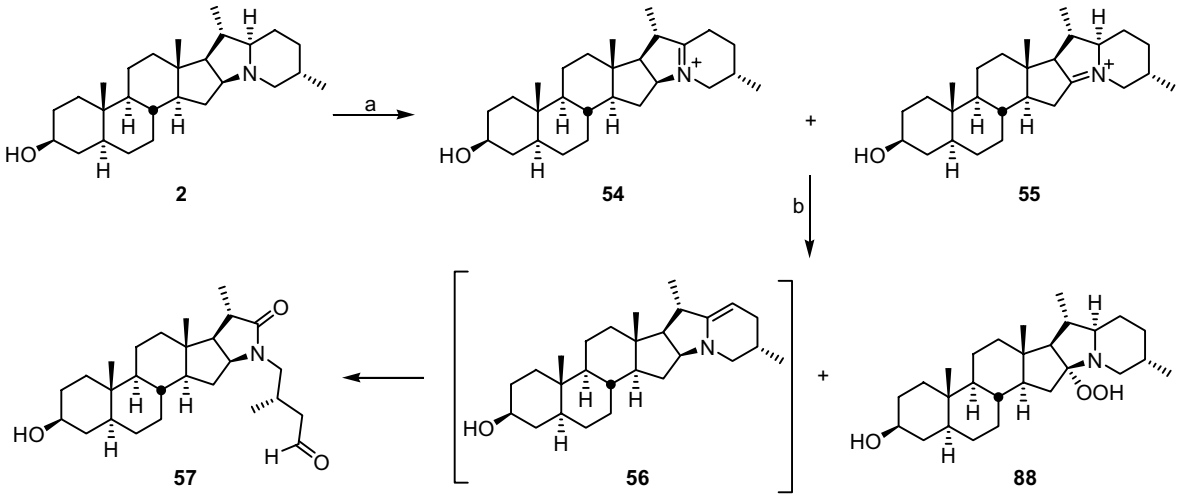
3.1 Introduction

3.1.1 The mercury acetate oxidation route

$Hg(OAc)_2$ has been widely applied in alkaloid chemistry since its introduction by Gadamer in 1919[1]. Leonard *et al.*[2-9] investigated extensively the oxidation of alkaloids by $Hg(OAc)_2$. Riker Research Laboratories patented some reactions of $Hg(OAc)_2$ with rubijervine and isorubijervine leading to the formation of the corresponding $\Delta^{22(N)}$-iminium (major product) and $\Delta^{16(N)}$-iminium salts (minor product) [10]. In 1966, Schreiber and Horstmann[11] reported the $Hg(OAc)_2$ and NBS oxidation of demissidine (**2**) to the corresponding $\Delta^{22(N)}$-iminium (**54**) and $\Delta^{16(N)}$-iminium salts (**55**) (Scheme 3.1). Oxidation of **2** with $Hg(OAc)_2$ and EDTA gave a mixture of **54** and **55** in 46% and 23% yield, respectively. The same oxidation without EDTA gave a mixture of **54** and **55** in 32% and 7% yield, respectively. Oxidation of the mixture of **54** and **55** with H_2O_2 in alkaline media gave a mixture of **57** and **88** in 49% and 27% yield, respectively. Pure **54** and **55** showed a selective formation of **57** and **88**, respectively. According to Schreiber and Horstmann, the formation of **57** can only be explained by the formation and subsequent oxidation of the $\Delta^{22,23}$-enamine **56** (Scheme 3.1).

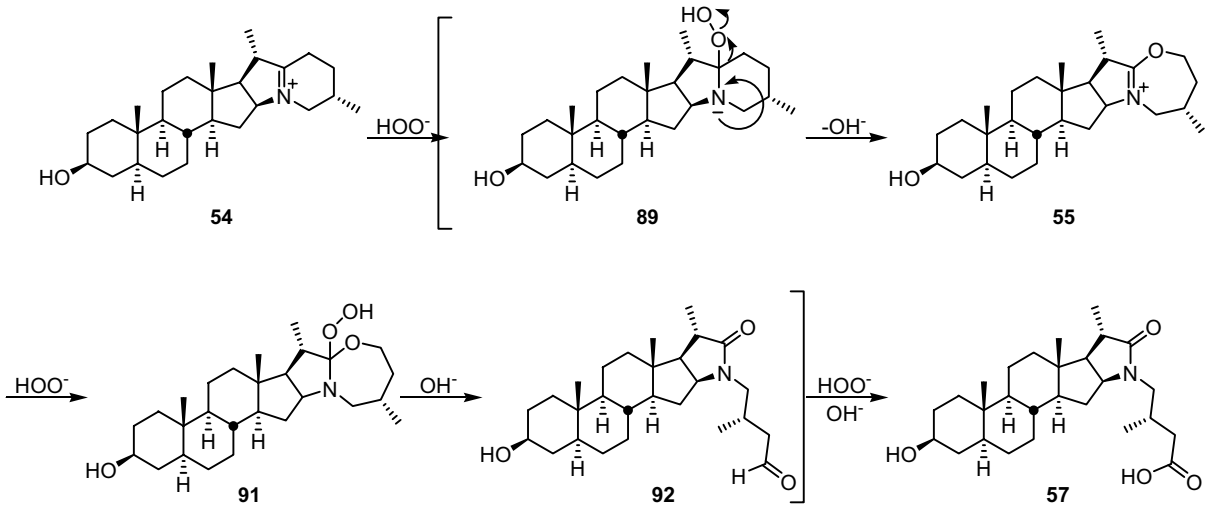
In our opinion, an alternative mechanism which does not involve the intermediacy and oxidation of the enamine **56**, is perhaps more likely. This mechanism depicted in Scheme 3.2 is supported by the formation of **88** as a byproduct in the oxidation step. Iminium ion **54** is attacked by the hydroperoxide anion yielding **89** which in turn rearranges to iminium ion **90**. The hydroperoxide anion then attacks **90** yielding the unstable hemiacetal **91** which immediately undergoes ring

opening under the influence of KOH to compound **92**. Addition of the hydroperoxide anion to the aldehyde function of **91** and subsequent abstraction of the aldehydic hydrogen by KOH finally gives **57**.



a) $Hg(OAC)_2$, HOAc (50%), EDTA; b) H_2O_2, MeOH, NaOH

Scheme 3.1



Scheme 3.2

According to Schreiber and Horstmann[11], NBS oxidation of **2** gave the same results as the $Hg(OAc)_2$ oxidation. The same mixture of iminium salts (**54** and **55**) was obtained and subjected to alkaline H_2O_2 oxidation yielding **57** and **88**.

In principle the $\Delta^{16(N)}$-iminium, $\Delta^{22(N)}$-iminium, and $\Delta^{26(N)}$-iminium salt (see partial structure **93**, Figure 3.1) can be formed during the reaction of **2** or its derivatives with $Hg(OAc)_2$. In practice only the $\Delta^{16(N)}$-iminium and the $\Delta^{22(N)}$-iminium salts are obtained.

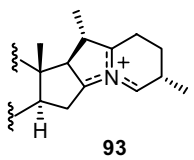
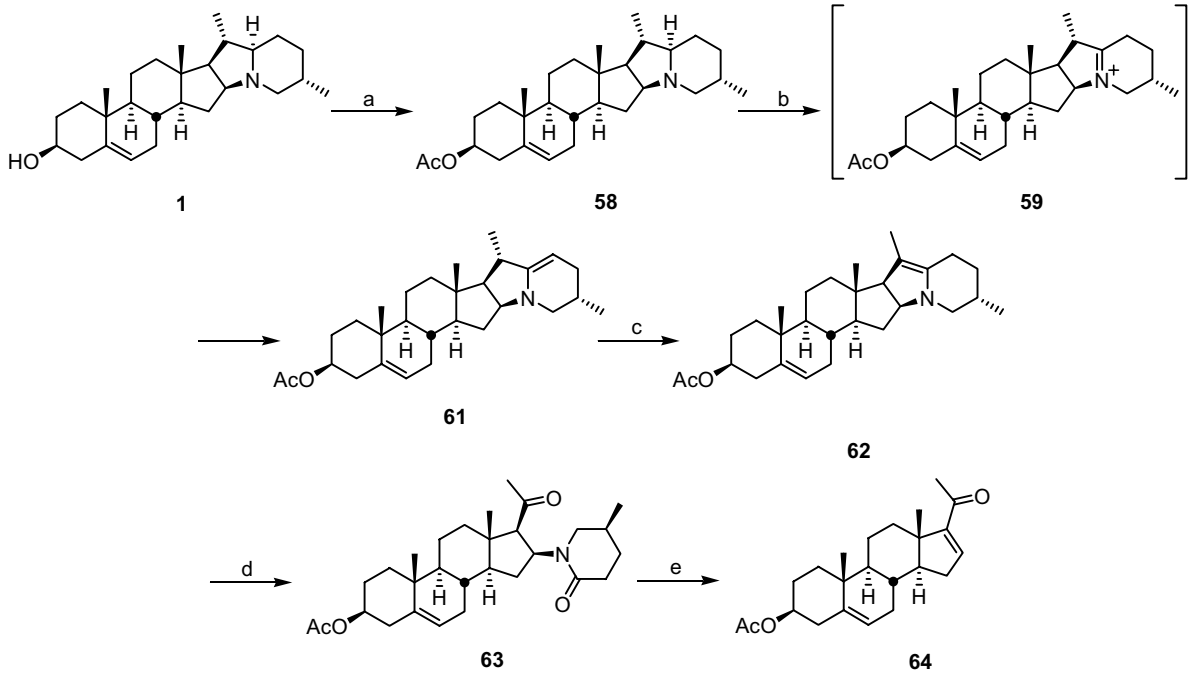


Figure 3.1

Electrochemical[12,13] oxidations of solanidane compounds have shown that the $\Delta^{22(N)}$-iminium salt is exclusively formed in acetone, while the $\Delta^{16(N)}$-iminium salt is the sole product in CH_2Cl_2. In both cases pyridine is added as base. The abstraction of the proton determines the regioselectivity of the reaction. According to Gunic *et al.*[13], the regioselectivity in CH_2Cl_2 is determined by the steric hindrance of C22-H. They concluded that formation of the $\Delta^{16(N)}$-iminium ion is under kinetic control, while formation of the $\Delta^{22(N)}$-iminium ion is under thermodynamic control. The single base available in CH_2Cl_2 is pyridine, which faces considerably more steric obstruction approaching C22-H than C16-H. In acetone, the solvent itself can act as a weak and less voluminous base and it preferentially takes the hydrogen of C22, which is bonded more weakly. Calculations gave a considerable difference in bond energy for C22-H and C16-H: 283.8 kcal/mol and 286.8 kcal/mol, respectively. In our opinion the explanation for this selectivity is due to the different solvatation of pyridine in CH_2Cl_2 and acetone. Pyridine in acetone is a weaker base than in CH_2Cl_2 and only C22-H will be abstracted when acetone is used as solvent. Chemical[14-16] oxidation of solanidine with $Hg(OAc)_2$ in acetone or CH_2Cl_2 showed the same preference in formation of the $\Delta^{16(N)}$-iminium and $\Delta^{22(N)}$-iminium salts as the electrochemical oxidation[14].

In 1997 Gaši *et al.*[17] reported the conversion of solanidine (**1**) to DPA (**64**) via iminium ion **59** and the enamine intermediates **61** and **62** (Scheme 3.3). Oxidation of solanidine acetate (**58**) accompanied by isomerization of the double bond gives enamine **61**. Further isomerization of enamine **61** under acidic conditions leads to enamine **62** which in turn is oxidized with $NaIO_4$, NaI, and $NaHCO_3$ in a mixture of water and *t*-BuOH to ketolactam **63**. Finally, elimination of the lactam moiety present in **63** gives DPA (**64**) in an overall yield of 28%.



a) Ac_2O, pyridine; b) $Hg(OAc)_2$, acetone; c) HOAc (0.1%), Et_2O;
d) $NaIO_4$, NaI, *t*-BuOH, H_2O, $NaHCO_3$; e) Al_2O_3, benzene

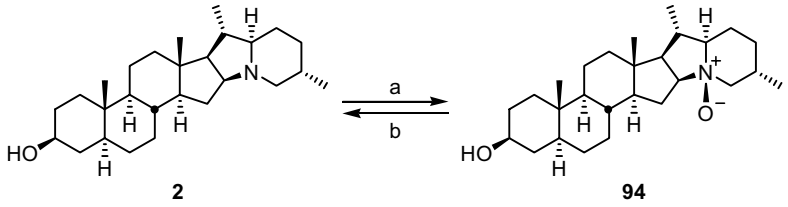
Scheme 3.3

Although this route gives fast access to DPA (**64**), the last two steps have to be improved considerably for industrial application. Furthermore, the use of $Hg(OAc)_2$ in industry is not accepted. In paragraph 3.2 attempts to improve the yield of DPA (**64**) via the $Hg(OAc)_2$ oxidation route and investigations for alternatives for $Hg(OAc)_2$ are described.

3.1.2 The Cope and Polonovski reaction

Though not as varied as their aromatic analogues[18], the chemistry of aliphatic tertiary amine *N*-oxides allows a number of preparative useful transformations among which the Cope reaction[19] and the Polonovski reaction[20]. The Cope and Polonovski reactions both have the ability to open the five or six membered ring giving compounds useful for further transformation into DPA (**64**).

Already in 1950 Briggs *et al.*[21] described the synthesis of demissidine *N*-oxide **94** with perbenzoic acid in chloroform (Scheme 3.4).



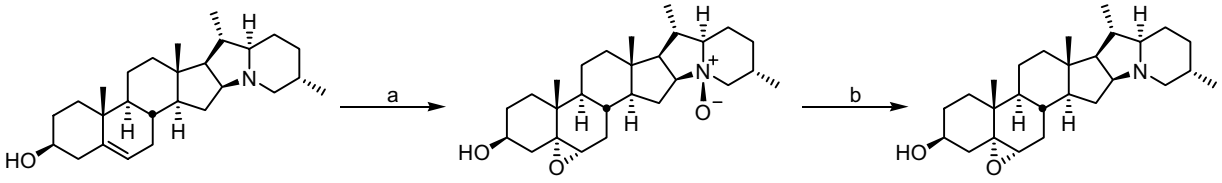
2 **94**

a) $C_6H_5CO_2OH$, $CHCl_3$; b) K_2CrO_4, 50% dioxane

Scheme 3.4

Attempts to rearrange demissidine *N*-oxide **94** by heating in 50% aqueous dioxane and subsequent treatment with potassium chromate only gave recovered demissidine (**2**). Heating of the *N*-oxide **94** at 0.01 mm also failed to give any useful products.

In 1991 Gaši *et al.*[22] described the reaction behavior of 3β-hydroxy-5α,6α-epoxy-solanidane *N*-oxide **51** upon heating (Scheme 3.5). Oxidation of **1** with monoperphthalic acid in CH_2Cl_2 and Et_2O at 4ºC during 24 hours afforded **51** in 74% yield. Applying the reactions conditions for Cope reaction (DMSO, Δ, 30 min) on **51** only gave the deoxygenated compound **52** in 67% yield.



1 **51** **52**

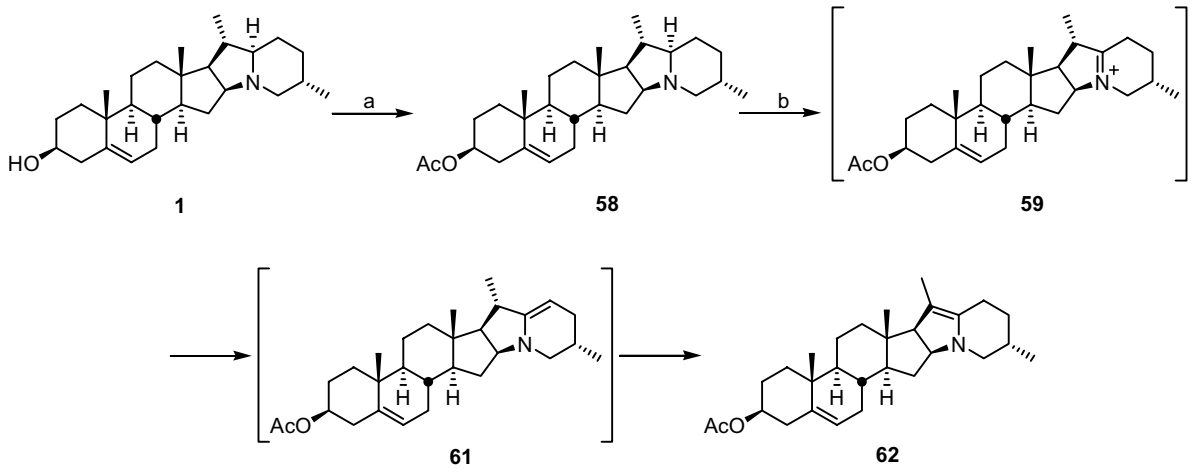
a) monoperphthalic acid, CH_2Cl_2, Et_2O, 4ºC, 24 hours; b) DMSO, Δ, 30 min.

Scheme 3.5

Further study is necessary in order to examine the *N*-oxides to their full potential. Attention will be focused especially on the Cope and Polonovski reactions (Paragraphs 3.4 and 3.5).

3.2 The mercury acetate oxidation route

To investigate the degradation of the E- and F-ring of solanidine (**1**) without unwanted side-reactions of other functional groups in the molecule, protection of these functional groups is necessary. Therefore solanidine (**1**) was converted to acetate **58** prior to oxidation with $Hg(OAc)_2$ or NBS. Because the $Hg(OAc)_2$ used in the oxidation reaction contained some acetic acid, enamine **62** was directly formed in 90% yield which made the separate isomerization step superfluous (Scheme 3.6).

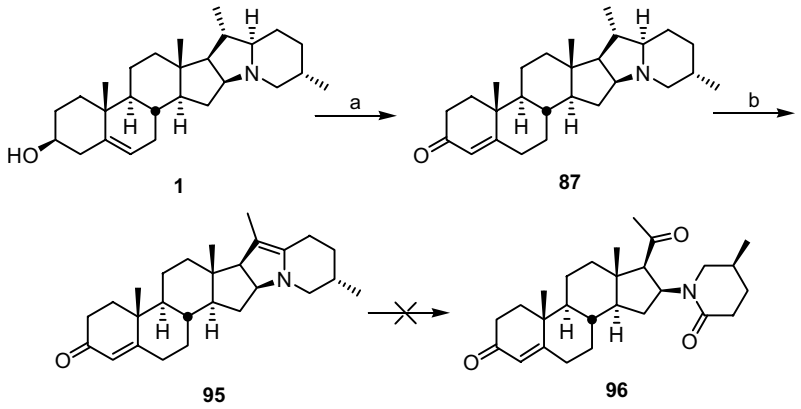


a) Ac_2O, pyridine; b) $Hg(OAc)_2$, acetone

Scheme 3.6

In contrast to the results with demissidine (**2**)[11], oxidation of **58** with NBS, an industrially tolerated reagent, did not yield iminium ion **59**; only the starting material was recovered.

Oxidation of enamine **62** according to the procedure described by Gaši *et al.*[17] was in our hands also unsuccessful and the starting material was recovered almost quantitatively. Many other oxidation reagents were tried (ozone[23], O_2/CuCl[24], MnO_2[25,26], $KMnO_4$[27], $KMnO_4/Al_2O_3$[28], CrO_3/HOAc[29-31], CrO_3/pyridine[32], H_2O_2[11], MMPP[33]), but in all cases no oxidation product could be obtained. To rule out a possible involvement of the $\Delta^{5,6}$ double bond during the oxidation[34], **1** was transformed into enone **87** in which the double bond is less electron rich (Scheme 3.7).



a) Al(*i*-PrO)$_3$, toluene, cyclohexanone; b) $Hg(OAc)_2$, acetone

Scheme 3.7

For this purpose **1** was treated with Al(*i*-PrO)$_3$ in toluene in the presence of cyclohexanone to give solanidan-4-en-3-one **87** in good yield. Oxidation of **87** with $Hg(OAc)_2$ again showed the exclusive formation of the $\Delta^{20,22}$-enamine **95** which was subjected to a range of oxidation reactions[11,17,23,25-31,33,35] in the hope to obtain **96**. However, all attempts failed including the oxidation reaction described by Gaši *et al.*[17].

According to Gaši and co-workers[17] the modest yield of the oxidation of enamine **62** was due to the instability of enamine **62**. However, Mopac[36] PM3 calculations[37,38] showed that enamine **62** is more stable than enamine **61** (Figure 3.2). The energy difference is large enough to make isomerization during the oxidation reaction unlikely and the difficulties in the oxidation reaction

can not only be imputed to the instability of enamine **62**.

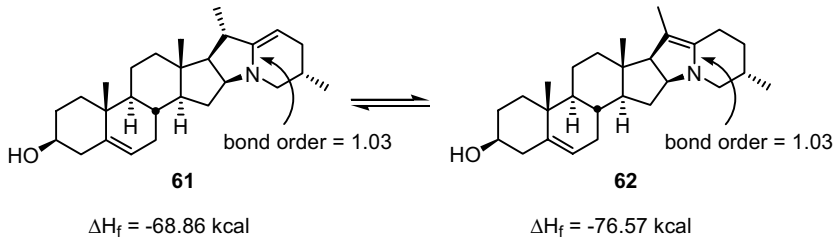


Figure 3.2

These calculations also show that enamine **62** is not really an enamine but more an isolated double bond and a separate amine group. The bond order gives an indication whether the bond has a single, double, or triple bond character. In this case the bond order is 1.03, which indicates that there is a single bond between C22 and N. The lone pair of the N-atom does not stabilize the double bond, as it would normally do in enamines. The $\Delta^{20,22}$ double bond forces the five membered E-ring to be completely flat and as a consequence the D-ring is bent away in such a way that C18 and the six membered ring now shield its top and bottom side, respectively (Figure 3.3). C21 lies in the plane of the $\Delta^{20,22}$ double bond making it even more difficult to approach it by additional steric hindrance. Taken all these factors into account, this may be an explanation for the low reactivity of the $\Delta^{20,22}$ double bond.

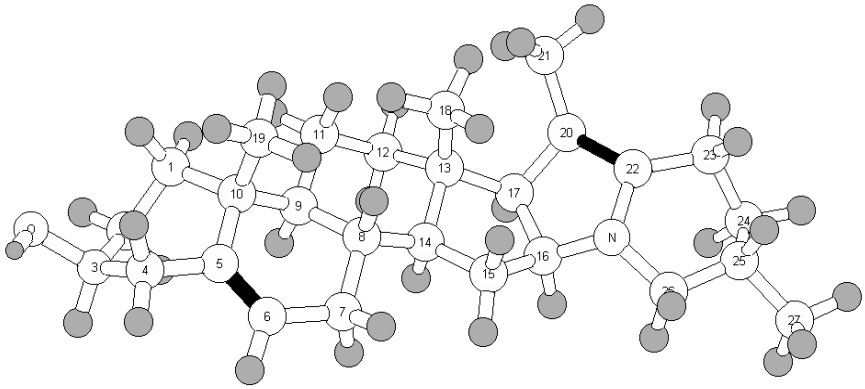
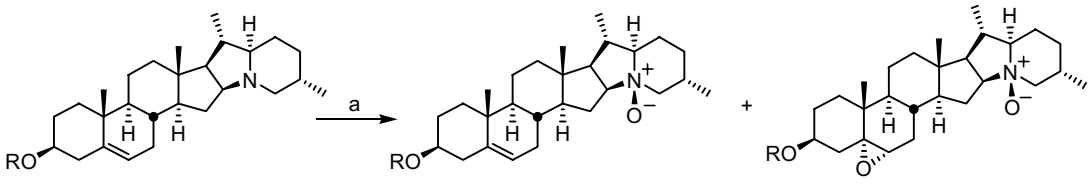


*Figure 3.3: 3D structure of compound **62***

3.3 The synthesis of solanidine *N*-oxide

In 1994 Le thi Quyen *et al.*[39] described the synthesis of several solanidane *N*-oxides, including solanidine *N*-oxide **96**, using *m*CPBA in CH_2Cl_2. In our hands, the formation of solanidine *N*-oxide **96** was always coupled with epoxidation of the $\Delta^{5,6}$ double bond to epoxy *N*-oxide **51** (Scheme 3.8). On larger scale this proved to be more abundant than on a small scale.

Most attempts to prevent epoxidation of the $\Delta^{5,6}$ double bond were unsuccessful. Dimethyldioxirane (DMD)[40,41], H_2O_2[42,43], and H_2O_2/$VO(acac)_2$[44] were not strong enough to oxidize the N-atom of solanidine (**1**). Oxidation of **1** with *m*CPBA[39] or MMPP[33] under different conditions gave mixtures of *N*-oxide **96** and epoxy *N*-oxide **51**. Besides, the separation of these mixtures was difficult and repeated column chromatography was necessary. Treatment of **58** with urea·H_2O_2[45] at 40ºC gave *N*-oxide **97** in 45% yield together with recovery of the starting material. Attempts to improve this yield were unsuccessful.



1 R= H
58 R= Ac

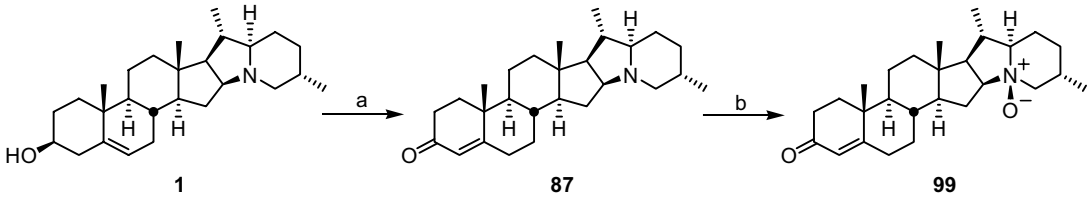
96 R= H
97 R= Ac

51 R= H
98 R= Ac

a_1) *m*CPBA, CH_2Cl_2, RT, 2 hours; a_2) *m*CPBA, CH_2Cl_2, RT, 18 hours;
a_3) MMPP, CH_2Cl_2/MeOH, RT, 2 hours; a_4) urea·H_2O_2, CH_2Cl_2/MeOH, 40ºC

Scheme 3.8

By conversion of solanidine (**1**) to solanidi-4-en-3-one **87**[34] and treatment of **87** with MMPP the epoxidation problem could be circumvented and solanidi-4-en-3-one *N*-oxide **99** was now isolated in 83% yield (Scheme 3.9).



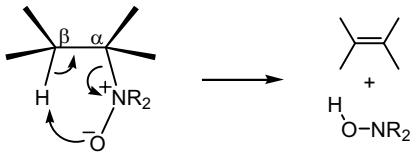
1 **87** **99**

a) Al(*i*-OPr)$_3$, cyclohexanone, toluene, Δ; b) MMPP, CH_2Cl_2

Scheme 3.9

3.4 The Cope reaction

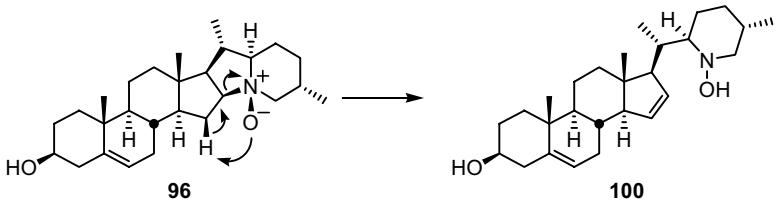
The mechanism of the Cope reaction is considered to be an E_i or E_1 syn elimination process with a five membered, planar transition state[46-49] (Scheme 3.10). The Cope reaction does not open six-membered rings containing a nitrogen atom.



Scheme 3.10

The only segment in *N*-oxide **96** that comes close to an almost syn periplanar configuration is H15-C15-C16-N^+-O^-, and should lead, in the case of a successful Cope reaction, to the formation of hydroxyl amine **100**[22,46] (Scheme 3.11). If the Cope reaction fails, deoxygenation will become the main reaction and solanidine (**1**) is recovered[19,50,51].

Solventless heating of **96** at 100-120ºC[48,51] or 180-200ºC[51] gave deoxygenation as the main reaction. All attempts to perform the Cope reaction with **96** in DMSO[47-49,51-54], THF[47-49,51-53], or DMF[47-49,51-54] showed deoxygenation as the sole reaction process[19]. The explanation for this failure may be that the required syn periplanar configuration cannot be reached due to the rigid indolizidine system. In order to establish the exact stereochemistry of solanidine *N*-oxide **96** an X-ray crystal structure was determined (Figure 3.4).



Scheme 3.11

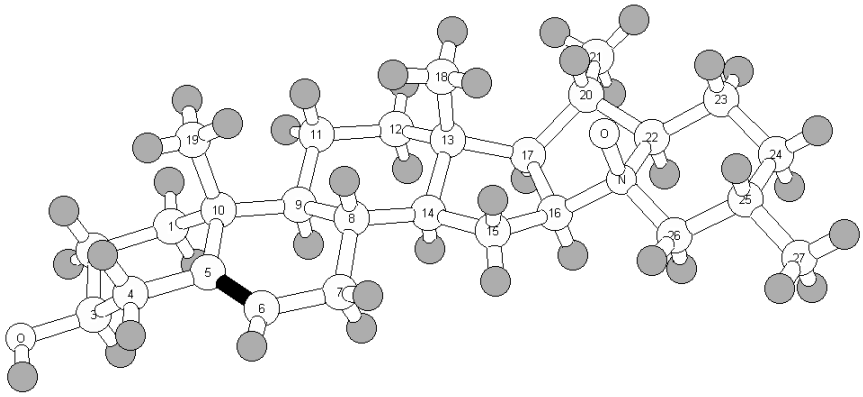


*Figure 3.4: 3D structure of compound **96***

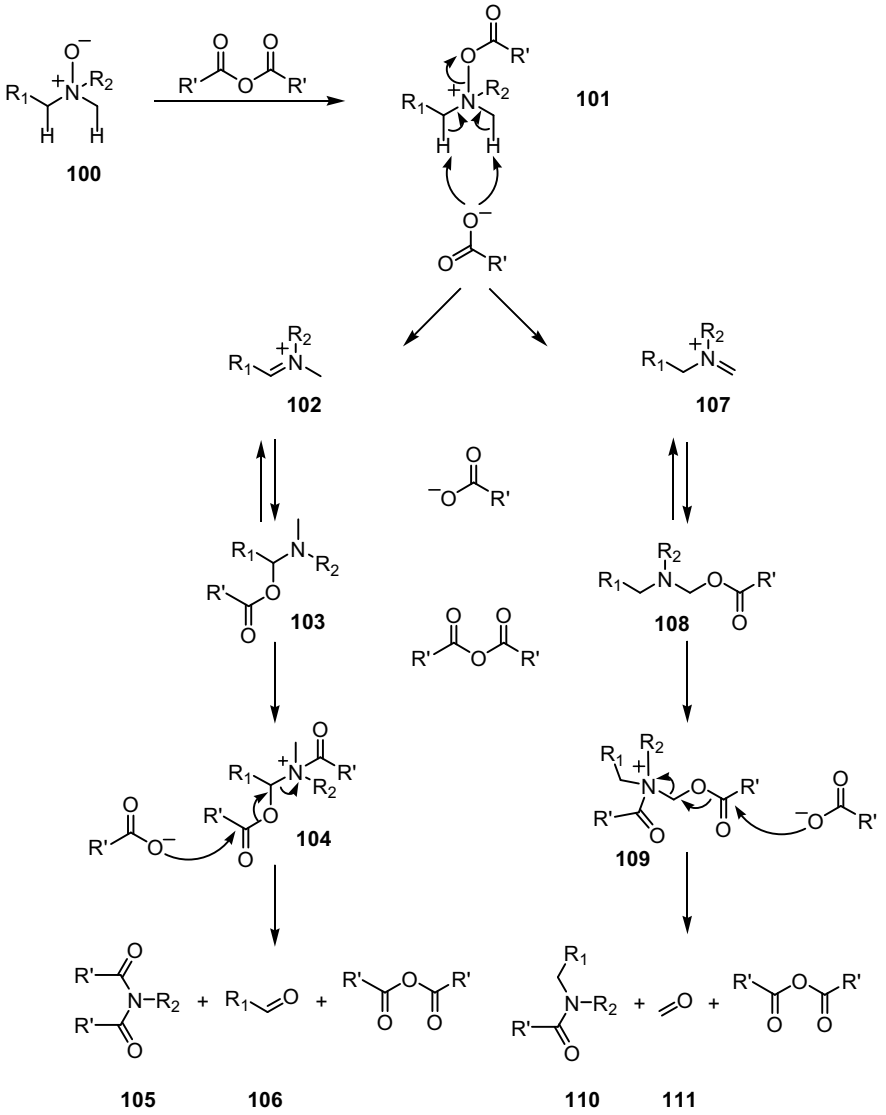
Analyses of the bond angles in the X-ray structure indeed showed that the syn periplanar conditions required for a successful Cope reaction are not met. The H15-C15-C16-N bond angle deviates from the syn periplanar plane 28.5º and the O-N-C16-C15 bond angle 35.4º.

3.5 The Polonovski reaction

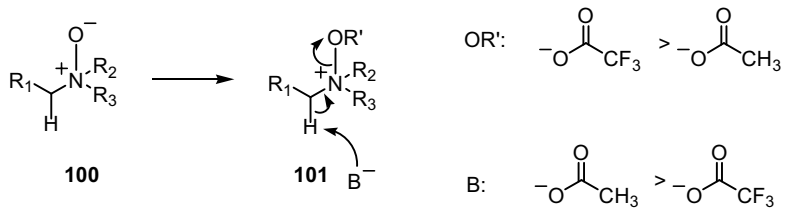
The Polonovski reaction is a complicated reaction because the product formation is not only dependent on the reaction conditions but also on the availability of protons at the α- and β-positions[55]. The first step in the Polonovski reaction of and amine *N*-oxide **100** with an acid anhydride, acid chloride, or chloroformate ester is the formation of an O-acyliminium salt **101**[56] (Scheme 3.11).

The counterion of the acylating reagent deprotonates the α-position (anti elimination) leading to the formation of iminium ions **102** and **107**. That even weak bases as Cl^- or CF_3COO^- are capable of promoting this elimination is probably due for the greater part to the fact that the C-H bond in question is adjacent to a positively charged nitrogen atom through which its acidity increases considerably. Although the regiochemistry of the elimination reaction is governed to a considerable extent by steric hindrance and conformational effects, electronic effects alter the acidities of the α-H atoms as well. A different leaving group also influences the product distribution. Depending on the particular combination of these factors the transition state for the elimination step may alter from E_2-like to one possessing a more or less E_1 or E_{1cb} character[57,58]. As a rule, it is found that the thermodynamically more stable iminium ion is produced when $(CF_3CO)_2O$ is employed and with Ac_2O the product obtained generally depends on the acidity of the α-protons.[55] The elimination step is best described in terms of Variable E_2-Transition State theory[59-61] (Scheme 3.12). In the reaction of a *N*-oxide with $(CF_3CO)_2O$, departure of the leaving group will be advanced with respect to cleavage of the Cα-H bond (E_1-like) with the stability of the resulting iminium ion as the controlling factor. In the reaction of an *N*-oxide with Ac_2O, a highly developed bond may form between the proton being abstracted and the base (E_{1cb}-like). In this

transition state model the acidity of the α-protons is the main factor that directs the regiochemistry. In conformatially rigid polycyclic systems the regioselectivity is not only determined by the use Ac_2O or $(CF_3CO)_2O$. The relative stereochemistry between the N-O and the neighboring Cα-H becomes an important factor in determining the stereochemistry in the elimination reaction. Elimination of a Cα-H is favored in those cases where the Cα-H and N-O bonds have an antiperiplanar relationship.



Scheme 3.11

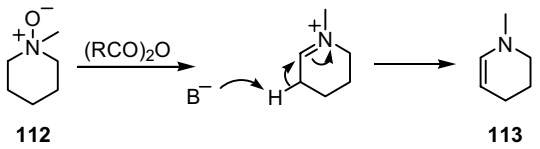


Scheme 3.12

The iminium ions **102** and **107** are in equilibrium with their addition products **103** and **108**, respectively. With $(CF_3CO)_2O$ the equilibrium is entirely displaced toward the iminium ions **102** and **107**. When Ac_2O is used the equilibrium is displaced toward the addition products **103** and **108**,

which can react further with Ac_2O to **104** and **109**. The reaction continues by acetate ion-promoted fragmentation giving the amide **105** or **110** and the aldehyde **106** or **111**, respectively.

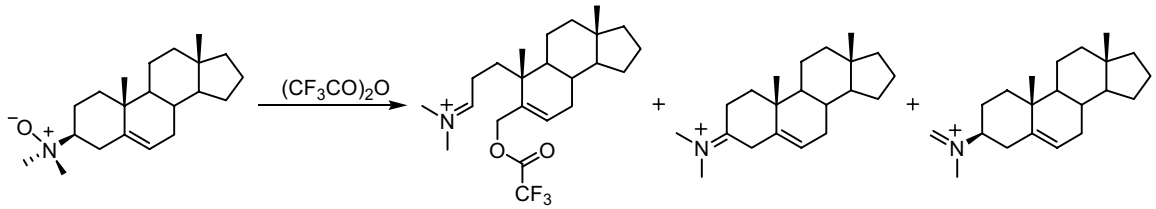
Enamines can also be obtained from amine *N*-oxides, especially with piperidine *N*-oxides **112** yielding labile iminium ions which readily tautomerize to **113**. Addition of base in the Polonovski reaction also favors formation of enamines **113**[55,62-64] (Scheme 3.13).



112 **113**

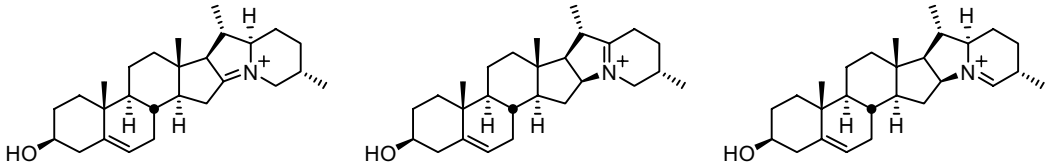
Scheme 3.13

Carbon-carbon fragmentation reactions only occur when two conditions are met. The Cα-C bond to be broken must be (*i*) next to an adjacent electron donating center (double bond, aromatic ring, or hetero atom) and (*ii*) oriented antiperiplanarly to the N-O bond[55,65,66] (Scheme 3.14).



Scheme 3.14

In principle, the Polonovski reaction of solanidine *N*-oxide **96** under thermodynamic conditions can lead to three intermediate iminium ions (Figure 3.5). These iminium ions are similar to those formed in the $Hg(OAc)_2$ oxidation as described in the beginning of this chapter. PM3 calculations clearly indicate that iminium ion **115** has the lowest formation energy and will therefore contribute considerably to the formation of the product. The products expected to be formed upon treatment of **97** with Ac_2O or $(CF_3CO)_2O$ are shown in Scheme 3.15.



ΔH_f = 69.62 kcal | ΔH_f = 62.86 kcal | ΔH_f = 66.91 kcal

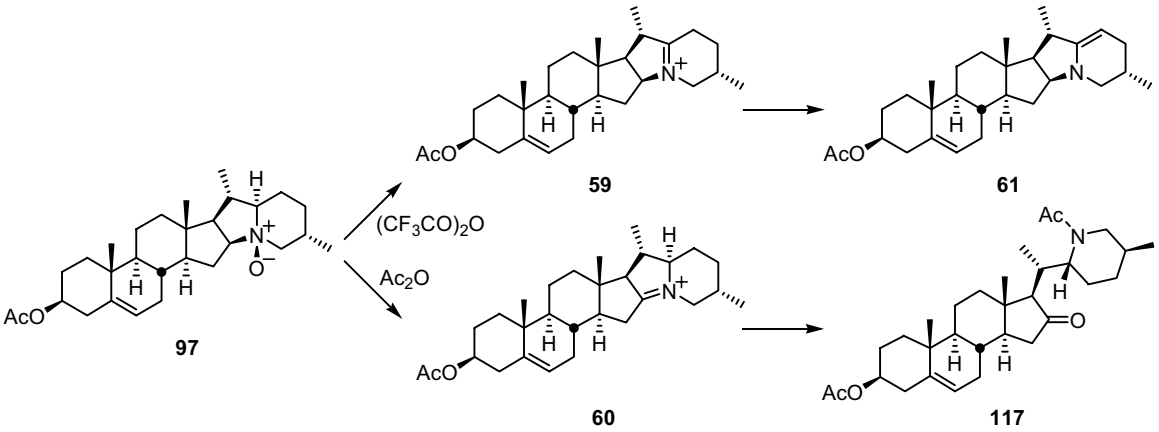
114 **115** **116**

Figure 3.5

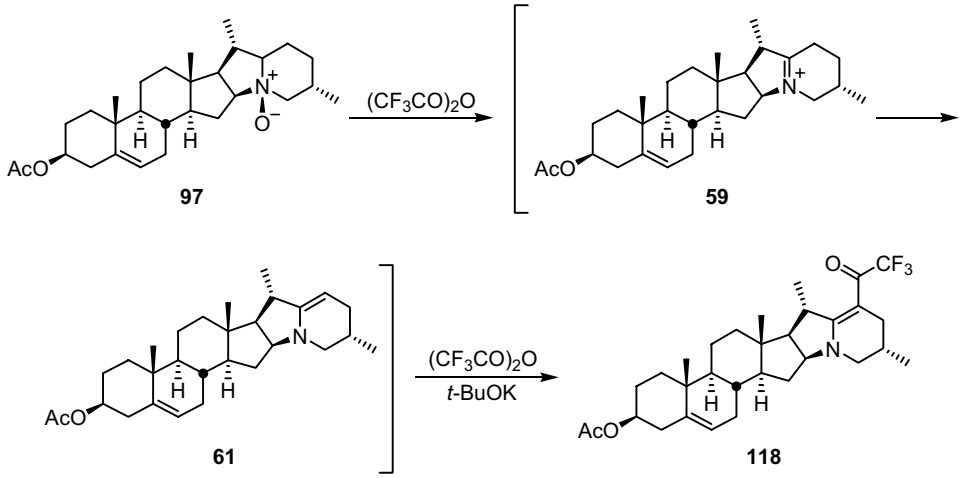
Formation of iminium ions **60** is followed by formation of the corresponding keto compound **117**. Due to the weak nucleophilicity of the trifluoroacetate ion isomerization of **59** to enamine **61** is expected to be the main reaction.

In practice, reaction of **96** with Ac_2O[52,55,67-69], $(CF_3CO)_2O$[52,55,62,70-72], or $(CCl_3CO)_2O$[55] did not give the desired Polonovski products. Only the corresponding C3-OH acetylated compounds and starting material **96** were isolated. The reaction of **97** with Ac_2O[52,55,67-69], $(HCO)O(COCH_3)$[55,73], or $CH_3C(O)Cl$[62,72] did not proceed as well and the starting material **96** was recovered. Treatment of the *N*-oxide/epoxy *N*-oxide **97**/**98** mixture with $(CF_3CO)O(COCH_3)$[55,62], $ClC(O)OEt/K_2CO_3$[58,74-79],

$Cl_3C(O)Cl/K_2CO_3$[62,72], and $(COCl)_2/K_2CO_3$ was unsuccessful. Solanidi-4-en-3-one *N*-oxide (**99**) was treated with $Cl_3C(O)Cl$[62,72], $(CH_3CO)O(OCH)$[55,73], or AcCl[62,72] but also in these cases only starting material was recovered. Only when **97** was treated with $(CF_3CO)_2O/t$-BuOK[52,55,70,71] product formation (41%) was noticed. NMR and mass spectral analysis showed that the isolated product was acylated enamine **118**. The formation of **118** must proceed via iminium ion **59** and enamine **61**. Further reaction of **61** with $(CF_3CO)_2O$ under the influence of *t*-BuOK then results in the formation of **118** (Scheme 3.16). Similar reactions are known in literature[80-88]. The most characteristic signals in the C^{13} NMR spectrum of **118** are found at δ 172.32 (C22) and 90.93 (C23).



Scheme 3.15



Scheme 3.16

In principle the formation of **118** from **97** offers an alternative for the electrochemical- or $Hg(OAc)_2$ oxidation. If the reaction could be stopped at the enamine stage by using 1 equivalent of $(CF_3CO)_2O$, isomerization of **61** to enamine **62** should provide an industrial applicable alternative for the electrochemical- or $Hg(OAc)_2$ oxidation. However, due to the failure of the oxidation of enamine **62** further research was cancelled.

Besides the "classical" Polonovski reaction with Ac_2O, $(CF_3CO)_2O$, and other comparable reagents, several alternative methods have been tried. However, attempts with $FeCl_2$ (aq.) [89,90,91], CF_3CO_2H[92], *t*-BuOK/*t*-BuOH[58,74,93], MsCl and base[94], and Cu(I)Cl[95] all failed to give any iminium salt. Treatment of **97** with $K_2Cr_2O_7 \cdot H_2O$[96] in the hope to obtain the corresponding amide was unsuccessful too. Finally 3-trifuoroacetyl solanidine *N*-oxide, obtained from the reaction of **96** with $(CF_3CO)_2O$, was reacted with *t*-butyldimethylsilyl trifluoromethanesulfonate in pyridine and

THF[97,98], but also this reaction failed.

3.6 Conclusion

The mercury oxidation with $Hg(OAc)_2$ containing traces of HOAc gives in one step the $\Delta^{20,22}$ enamine **62** or **95** starting from either acetylated solanidine (**58**) or solanid-4-en-3-one (**87**), respectively. In our hands oxidation of the enamines **62** and **95** proved to be unsuccessful under the reaction conditions used.

Oxidation of both solanidine (**1**) and 3-acetoxysolanidine (**58**) with either *m*CPBA or MMPP gave mixtures of the *N*-oxide and epoxy *N*-oxide (**96** and **51** starting from **1**, and **97** and **98** starting from **58**) in high yield. The best result was obtained by oxidation of **58** with urea·H_2O_2, which gave the desired *N*-oxide **97** in 45% yield. The conversion of solanidine (**1**) to solanid-4-en-3-one (**87**) and subsequent epoxidation of **87** afforded *N*-oxide **90** in 89% yield.

Subjecting the *N*-oxides **96**, **97**, and **90** to the Cope and Polonovski reactions under various conditions did not give any of the desired products. Only reaction of **97** with $(CF_3CO)_2O$ yielding compound **118** suggests that this reaction could provide an alternative for the electrochemical- or $Hg(OAc)_2$ oxidation.

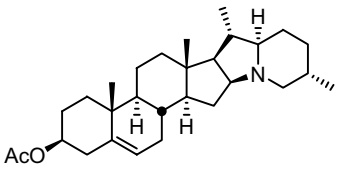
3.7 Experimental Section

3.7.1 General comments and materials[99]

All ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ unless stated otherwise.
Solanidine (**1**) was obtained as described in chapter 2.

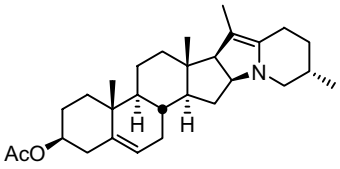
3.7.2 Procedures and spectral data

3β-Acetoxysolanidine (58)



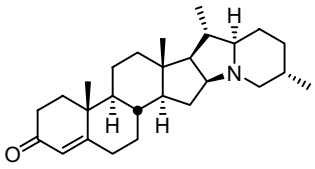
A solution of **1** (10.06 g, 25.30 mmol) in a mixture of pyridine (400 ml) and acetic anhydride (400 ml) was stirred overnight at room temperature and then poured into ice-water. Upon treatment with NH_4OH (pH = 9), a white solid precipitated. The crude product was filtered, washed with water, and dried on air. Recrystalization from $CHCl_3$/MeOH 9/1 gave **58** as a white solid (10.90 g, 98%). M.p. 208-210ºC (lit. 208ºC[11]); ^1H NMR δ 0.76 (d, 3H, *J*= 6.4 Hz), 0.77 (s, 3H), 0.85 (d, 3H, *J*= 6.2 Hz), 0.96 (s, 3H), 1.96 (s, 3H), 2.25 (m, 2H), 2.57 (m, 1H), 2.78 (dd, 1H, *J*= 2.8 and 10.0 Hz), 4.53 (m, 1H), 5.30 (br d, 1H, *J*= 4.4 Hz); ^{13}C NMR δ 16.90 (q), 18.30 (q), 19.35 (q), 19.55 (q), 20.89 (t), 21.47 (q), 27.77 (t), 29.32 (t), 31.10 (d), 31.35 (t), 31.61 (d), 32.07 (t), 33.38 (t), 36.64 (s), 36.72 (d), 37.02 (t), 38.13 (t), 39.90 (t), 40.28 (s), 50.11 (d), 57.54 (d), 60.26 (t), 63.00 (d), 69.04 (d), 73.96 (d), 74.66 (d), 122.60 (d), 139.67 (s), 170.57 (s); MS *m/z* (r.i.) 439 (35), 438 (14), 424 (12), 204 (25), 151 (14), 150 (100); HRMS calculated for $C_{29}H_{45}NO_2$ (M^+) 439.3450, found 439.3443; Anal calculated for $C_{29}H_{45}NO_2$: C 79.22, H 10.32, N 3.19. Found: C 79.10, H 10.37, N 2.97. The NMR spectral data for **58** were identical to those reported in literature[100].

3β-Acetoxy-5,20(22)-solanidiene (62)



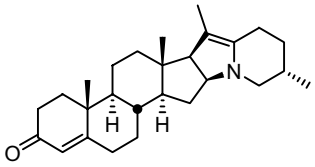
To a suspension of **58** (1.03 g, 2.35 mmol) in acetone (40 ml) was added $Hg(OAc)_2$ (3.98 g, 12.48 mmol). After 3 hours of stirring at room temperature, the suspension was filtered and the filtrate was concentrated to 5 ml. After addition of water (70 ml) and heating to 50ºC, an aqueous solution of NaOH (1.5 ml, 30%) was added. The precipitated white crystals were filtered, washed with water, and dried on air to yield **62** (1.00 g, 97%) as a white solid. M.p. 146-150ºC (lit. 147-151ºC[13,17]); ^{1}H NMR (C_6D_6) δ 0.80 (d, 3H, *J*= 6.4 Hz), 0.86 (s, 3H), 0.90 (s, 3H), 1.56 (s, 3H), 1.73 (s, 3H), 2.99 (dt, 1H, *J*= 1.7 and 9.5 Hz), 3.28 (m, 1H), 4.85 (septet, 1H, *J*= 5.4 Hz), 5.35 (d, 1H, *J*= 5.2 Hz); ^{13}C NMR (C_6D_6) δ 12.52 (q), 13.64 (q), 19.12 (q), 19.37 (q), 20.78 (q), 21.11 (t), 22.58 (t), 27.97 (t), 30.81 (d), 31.67 (d), 32.30 (t), 32.36 (t), 34.00 (t), 36.63 (s), 37.02 (t), 38.43 (t), 39.65 (t), 43.86 (s), 50.16 (d), 55.64 (d), 61.22 (d), 64.26 (t), 70.32 (d), 73.67 (d), 105.05 (s), 122.57 (d), 139.59 (s), 141.27 (s), 169.36 (s). The ^{1}H NMR spectral data for **62** were identical to those reported in literature[13].

Solanid-4-en-3-one (87)



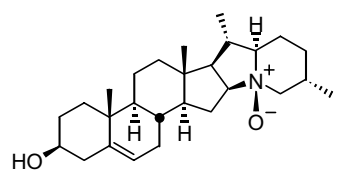
A solution of **1** (0.51 g, 1.27 mmol) in toluene (150 ml) and cyclohexanone (25 ml) was stirred. Then $Al(i\text{-}OPr)_3$ (0.54 g, 2.67 mmol) was added and the mixture was refluxed overnight. After cooling to room temperature, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted three times with EtOAc. The combined organic layers were washed with brine, dried, and evaporated to give a brown oil (0.54 g). The brown oil was flash chromatographed ($CHCl_3$/MeOH 9/1) to give **87** (0.36 g, 72%) as a white solid. M.p. 218ºC (lit. 218ºC[101]); ^{1}H NMR δ 0.74 (s, 3H), 0.79 (d, 3H, *J*= 6.4 Hz), 0.85 (d, 3H, *J*= 5.7 Hz), 1.14 (s, 3H), 5.67 (d, 1H, *J*= 1.5 Hz); ^{13}C NMR δ 16.92 (q), 17.41 (q), 18.26 (q), 19.54 (q), 20.84 (t), 29.25 (t), 31.04 (d), 31.16 (t), 32.14 (t), 32.93 (t), 33.30 (t), 33.97 (t), 35.41 (d), 35.70 (t), 36.62 (d), 38.66 (s), 39.77 (t), 40.37 (s), 53.90 (d), 56.71 (d), 60.14 (t), 62.87 (d), 68.86 (d), 75.55 (d), 123.76 (d), 171.51 (s), 199.56 (s); MS *m/z* (r.i.) 395 (50), 380 (9), 204 (25), 150 (100); HRMS calculated for $C_{27}H_{41}NO$ (M^+) 395.3188, found 395.3181.

Solanida-4,20(22)-dien-3-one (95)



Solanid-4-en-3-one **87** was treated with $Hg(OAc)_2$ as described for the synthesis of 3β-acetoxy-5,20(22)-solanidiene **62** to yield **95** (85%) as a white solid. M.p. 156-158ºC (lit. 158-159ºC[13,14]); ^{1}H NMR (C_6D_6) δ 0.77 (s, 3H), 0.83 (d, 3H, *J*= 7.2 Hz), 0.85 (s, 3H), 1.75 (s, 3H), 2.36 (q, 1H, *J*= 2.3 Hz), 2.43 (q, 1H, *J*= 2.2 Hz), 2.52 (1H, *J*= 10.7 Hz), 3.03 (dt, 1H, *J*= 2.2 and 8.3 Hz), 5.76 (s, 1H); ^{13}C NMR (C_6D_6) δ 12.47 (q), 13.73 (q), 16.85 (q), 19.36 (q), 20.92 (t), 22.57 (t), 30.82 (d), 32.27 (t), 32.32 (t), 32.46 (t), 33.82 (t), 34.09 (t), 35.24 (d), 35.66 (t), 38.20 (s), 39.47 (t), 43.89 (s), 53.70 (d), 54.75 (d), 61.28 (t), 64.17 (d), 70.21 (d), 104.97 (s), 124.14 (d), 141.44 (s), 168.72 (s), 197.07 (s). MS *m/z* (r.i.) 393 (53), 378 (6), 204 (10), 62 (100), 150 (47); HRMS calculated for $C_{27}H_{39}NO$ (M^+) 393.3032, found 393.3029. The ^{1}H NMR and mass spectral data for **95** were identical to those reported in literature[13].

Solanidine *N*-oxide (96)[39]

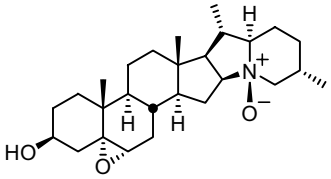


<u>*Method A:*</u> To a solution of **1** (52.0 mg, 0.13 mmol) in CH_2Cl_2 (5 ml) was added 70-75% *m*CPBA (61.4 mg, 0.27 mmol). After 2 hours in the dark, the reaction mixture was concentrated, washed with a dilute solution of $NaHCO_3$, dried, and evaporated *in vacuo*. The remaining

residue was purified by flash chromatography ($CHCl_3$/MeOH 9/1) yielding **96** (48.7 mg, 90%) as a white solid. M.p. 247-250ºC (248-253ºC[39]); ^{1}H NMR (400 MHz) δ 0.85 (d, 3H, *J*= 6.0 Hz), 0.92 (d, 3H, *J*= 6.8 Hz), 0.97 (s, 3H), 1.00 (s, 3H), 2.65 (td, 1H, *J*= 10.4 Hz, 2.4 Hz), 3.26 (d, 1H, *J*= 8.4 Hz), 3.41 (m, 1H), 3.65 (m, 1H), 5.26 (d, 1H, *J*= 5.2 Hz); ^{13}C NMR δ 14.85 (q), 16.75 (q), 18.64 (q), 19.69 (q), 21.35 (t), 22.11 (t), 23.94 (t), 26.63 (d), 31.48 (d), 31.70 (t), 31.97 (t), 32.41 (t), 34.09 (d), 37.02 (t), 37.65 (s), 40.18 (s), 40.67 (t), 42.48 (t), 50.42 (d), 57.49 (d), 61.91 (d), 71.56 (d+t), 82.01 (d), 84.03 (d), 121.31 (d), 141.49 (s). MS *m/z* (r.i.) 413 (61), 397 (33), 396 (48), 395 (27), 344 (49), 204 (16), 150 (86), 114 (66), 113 (100), 69 (20); HRMS calculated for $C_{27}H_{43}NO_2$ (M^+) 413.3285, found 413.3294. The NMR and mass spectral data for **96** were identical to those reported in literature[39].

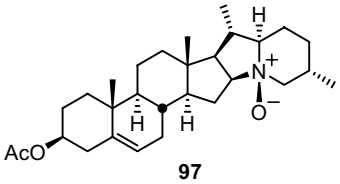
Method B: To a solution of **1** (50.2 mg, 0.13 mmol) in CH_2Cl_2 (4 ml) and MeOH (2 ml) was added 35% ureum·H_2O (188.2 mg, 0.70 mmol). After 10 days, the reaction mixture was evaporated in vacuo, dissolved in $CHCl_3$, washed with a saturated aqueous solution of Na_2SO_3, and extracted with $CHCl_3$ (30 ml). The combined organic layers were dried and evaporated *in vacuo*, and the remaining residue was purified by flash chromatography ($CHCl_3$/MeOH 9/1) yielding **96** (23.7 mg, 45%) as a white solid.

5,6α-Epoxy-3β-hydroxysolanidine *N*-oxide (51)

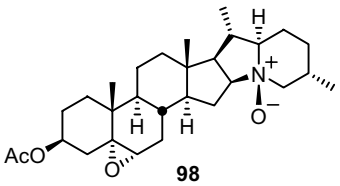


To a solution of **1** (513.6 mg, 1.29 mmol) in CH_2Cl_2/MeOH 10/1 (22 ml) was added 70-75% *m*CPBA (602.0 mg, 2.44 mmol). After 18 hours in the dark, the reaction mixture was concentrated, washed with a dilute solution of $NaHCO_3$, dried, and evaporated *in vacuo*. The remaining residue was purified by flash chromatography ($CHCl_3$/MeOH 9/1) yielding **51** (530.9 mg, 96%) as a white solid. M.p. 287-289ºC (m.p. 287-289ºC[39]); ^{1}H NMR δ 0.83 (d, 3H, *J*= 6.0 Hz), 0.88 (d, 3H, *J*= 6.7 Hz), 0.92 (s, 3H), 1.01 (s, 3H), 2.51 (dt, 1H, *J*= 9.2 and 2.3 Hz), 2.84 (d, 1H, *J*= 4.3 Hz), 3.14 (bs, 1H), 3.25 (d, 1H, *J*= 7.1 Hz), 3.62 (m, 1H), 3.77 (m, 1H); ^{13}C NMR δ 14.50 (q), 15.88 (q), 16.34 (q), 8.27 (q), 20.61 (t), 21.74 (t), 23.39 (t), 26.26 (d), 28.98 (t), 29.06 (d), 30.80 (t), 31.57 (t), 32.36 (t), 33.69 (d), 34.98 (s), 39.64 (t), 39.85 (t), 42.44 (d), 57.19 (d), 59.16 (d), 61.26 (d), 66.02 (s), 68.09 (d), 71.17 (t), 77.29 (s), 81.43 (d), 83.64 (d); MS *m/z* (r.i.) 429 (45, M^+), 412 (33), 360 (43), 150 (62), 114 (65), 113 (100), 69 (44), 55 (25), 43 (36); HRMS calculated for $C_{27}H_{43}NO_3$ (M^+) 429.3243, found 429.3225. The NMR spectral data for **51** were identical to those reported in literature[22].

3β-Acetoxysolanidine *N*-oxide (97) and 3β-Acetate-5,6α-epoxy-solanidine *N*-oxide (98)



97



98

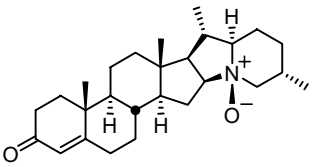
To a solution of **58** (4.89 g, 11.14 mmol) in CH_2Cl_2/MeOH 2/1 (750 ml) was added a suspension of 70-75% *m*CPBA (5.45 g, 22.11 mmol) in CH_2Cl_2 (200 ml). After 18 hours in the dark, the reaction mixture was concentrated, washed with a dilute solution of $NaHCO_3$, dried, and evaporated *in vacuo*. Repeated flash chromatography ($CHCl_3$/MeOH 9/1) of the amorphous residue yielded **97** (1.82 g, 36%) and **98** (1.82 g, 36%), both as white solids.

97: ^{1}H NMR δ 0.65 (d, 3H, *J*= 6.2 Hz), 0.73 (d, 3H, *J*= 6.6 Hz), 0.78 (s, 3H), 0.80 (s, 3H), 1.78 (s, 3H), 3.14 (d, 1H, *J*= 9.6 Hz), 3.47 (1H), 4.32 (m, 1H), 5.12 (br d, 1H, *J*= 3.8 Hz); ^{13}C NMR δ 14.56 (q), 16.38 (q), 18.38 (q), 19.27 (q), 20.96 (t), 21.43 (q), 21.93 (t), 23.69 (t), 26.29 (d), 27.68 (t), 31.04 (d), 31.66 (t), 31.91 (t), 32.03 (t), 33.82 (d), 36.69 (s), 36.93 (t), 38.03 (t), 39.84 (s), 40.28 (t), 49.94 (d), 57.11 (d), 61.51 (d), 71.25 (t), 73.83 (d), 81.45 (d), 83.56 (d), 122.21 (d), 139.70 (s), 170.57 (s).

98: ^{1}H NMR δ 0.80 (d, 3H, *J*= 6.5 Hz), 0.86 (d, 3H, *J*= 6.6 Hz), 0.90 (s, 3H), 0.98 (s, 3H), 1.92 (s,

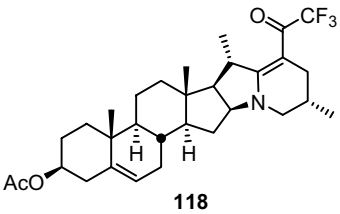
3H), 3.68 (m, 1H), 4.92 (m, 1H); ^{13}C NMR δ 14.46 (q), 15.95 (q), 17.81 (q), 20.73 (2q), 21.14 (d), 21.48 (q), 23.44 (t), 26.11 (d), 26.31 (t), 29.32 (t), 31.08 (d), 32.04 (t), 33.57 (d), 35.36 (t), 37.26 (t), 38.91 (s), 40.02 (t+s), 44.67 (t), 49.99 (d), 55.38 (d), 61.06 (d), 63.45 (d), 70.43 (t), 71.59 (t), 75.23 (s), 81.44 (d), 83.53 (d), 171.76 (s).

Solanidan-4-en-3-one *N*-oxide (99)



To a solution of **89** (130.1 mg, 0.33 mmol) in CH_2Cl_2 (5 ml) was added a solution of MMPP (223.2 mg, 0.45 mmol) in CH_2Cl_2/H_2O 1/1 (11 ml). After 12 hours in the dark, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted twice with CH_2Cl_2. The combined organic layers were dried and the solvent evaporated *in vacuo*. The remaining residue was flash chromatographed (EtOAc/MeOH 9/1) yielding **98** (69.0 mg, 53%) as a white solid. M.p. 197-200ºC; ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.86 (d, 1H, *J*= 6.1 Hz) 0.96 (d, 3H, *J*= 6.6 Hz), 1.13 (s, 3H), 1.19 (s, 3H), 2.74 (dt, 1H, *J*= 10.3 and 2.3 Hz), 3.35 (d, 1H, *J*= 6.9 Hz), 3.73 (m, 1H), 5.71 (d, 1H, *J*= 0.9 Hz); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 14.65 (q), 16.29 (q), 17.29 (q), 18.32 (q), 20.90 (t), 21.92 (t), 23.61 (t), 26.30 (d), 31.56 (t), 23.12 (t), 32.68 (t), 33.82 (d), 33.89 (t), 34.74 (d), 35.60 (t), 38.61 (s), 39.91 (s), 40.08 (t), 53.70 (d), 56.24 (d), 61.32 (d), 71.21 (t), 81.25 (d), 83.49 (d), 123.76 (d), 171.11 (s), 199.52 (s).

(3β)-Acetoxy-23-trifluoroacetylsolanid-5,22-diene (118)



118

To a solution of **97** (100.3 mg, 0.22 mmol) in THF (2 ml) was added $(CF_3CO)_2O$ (35 μl, 0.24 mmol) under argon atmosphere. The solution colored immediately yellow and then slowly to orange. After 30 minutes, *t*-BuOK (60.6 mg, 0.51 mmol) was added and the solution was stirred for 16 hours. The reaction mixture was diluted with H_2O, and extracted three times with $CHCl_3$ (50 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$, dried, and evaporated *in vacuo*. Flash chromatography of the remaining residue (EtOAc/PE 9/1) yielded **118** (45.9 mg, 41%) as an orange oil. ^{1}H NMR (main peaks) δ 0.60 (s, 3H), 1.03 (s, 3H), 1.03 (d, 3H, *J*= 6.4 Hz), 1.19 (d, 3H, *J*= 6.9 Hz), 2.05 (s, 3H), 2.29 (br d, 1H, *J*= 15.0 Hz), 2.78 (dd, 1H, *J*= 8.2 and 12.9 Hz), 3.31 (dd, 1H, *J*= 4.3 and 12.9 Hz), 3.90 (q, 1H, *J*= 6.9 Hz), 4.21 (ddd, 1H, *J*= 5.6, 7.9, and 7.9 Hz), 4.61 (m, 1H), 5.38 (br d, 1H, *J*= 5.2 Hz); ^{13}C NMR δ 13.81 (q), 18.67 (q), 19.73 (q), 20.16 (q), 20.74 (t), 21.82 (q), 26.59 (d), 28.10 (t), 29.05 (t), 31.41 (t), 31.76 (d), 32.32 (t), 37.07 (s), 37.35 (t), 38.47 (2t), 39.61 (d), 42.13 (s), 49.81 (t), 50.43 (d), 55.38 (d), 60.07 (d), 67.36 (d), 74.17 (d), 90.93 (s), 116.21 (q, *J*= 285 Hz), 122.23 (d), 140.42 (s), 170.98 (s), 172.32 (s). The chemical shift of the quartet signal for the C=O group of the trifluoroacetyl moiety could not be determined because its intensity was too low. MS *m/z* (r.i.) 534 (M^++1, 35), 533 (M^+, 100), 465 (28), 464 (M^+-HCF_3, 89), 404 (17), 258 (22), 195 (9), 69 (11), 43 (9); HRMS calculated for $C_{31}H_{42}F_3NO_3$ (M^+) 533,3117, found 533.3120.

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Chapter 4

The Von Braun reaction and the Hofmann degradation in solanidine chemistry

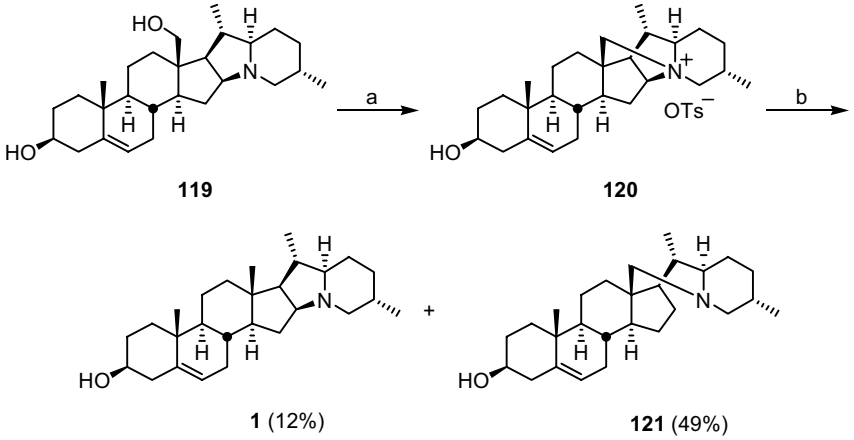
4.1 Introduction

Due to the negative results described in Chapter 3 only a few options, based on known chemistry, are left for the conversion of solanidine (**1**) to DPA (**64**). These options are the Hofmann degradation[1-5] and the Von Braun reaction[6-10]. Both reactions have been widely applied on alkaloids[11-17], including solanidine (**1**)[18-23], since the beginning of the 20th century.

The Hofmann degradation on the methylated iminium salt of solanidine did not give any ring-opened product. Instead solanidine (**1**) was formed back as a result of demethylation[20,23]. The only successful example of this approach is the Hofmann-like degradation of iminium salt **120**, easily obtained in 60% yield from isorubijervine (**119**) upon treatment with tosylchloride in pyridine. Addition of an alkali metal to **120** then gives solanidine (**1**) and **121**, the latter resulting from C16-N bond cleavage[24,25] (Scheme 4.1).

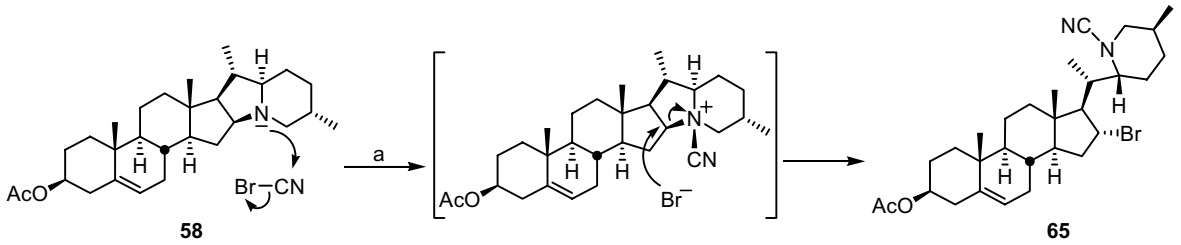
Up to now the Von Braun reaction is the only known reaction able to open the E-ring of 3-acetoxysolanidine (**58**). Attempts to open the E-ring system in solanidine (**1**) itself with BrCN failed. In the Von Braun reaction the lone pair of the nitrogen in **58** attacks bromocyanide, thereby liberating bromide, which in turn attacks from the rearside predominantly at the C16-position to give compound **65** in 77% yield[20,26,27] (Scheme 4.2). In principle the bromide can approach the intermediate from the rearside at C16, C22, or C26. However, the preferential formation of **65** shows that the bromide mainly attacks at C16 resulting in opening of the E-ring[23]. Attack at C22 is difficult due to the steric hindrance by C21, but there is little steric difference in the approach to C16 or C26. Probably the preference for reaction at C16 is due to the relieve of ring strain that goes together with opening of the five-membered E-ring. Until now the opening of the six-membered

ring F has not been mentioned in the literature (vide infra).



a) TsCl, pyridine, 0ºC, 3 days; b) Na, EtOH, Δ

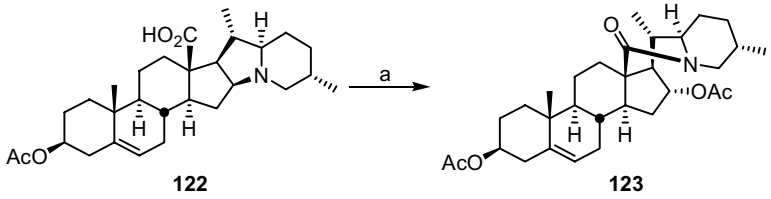
Scheme 4.1



a) CNBr, $CHCl_3$, Δ

Scheme 4.2

The bromocyanide reagent used in the Von Braun reaction is very toxic and therefore not preferred for industrial applications. Alternatives for BrCN have been investigated and the results will be discussed in this chapter. A possible alternative may be found in the application of acylating reagents, based on the successful opening of the E-ring in 3β-acetoxy-5-solanidene-18-oic acid **122** yielding compound **123** in 80% [28] (Scheme 4.3).



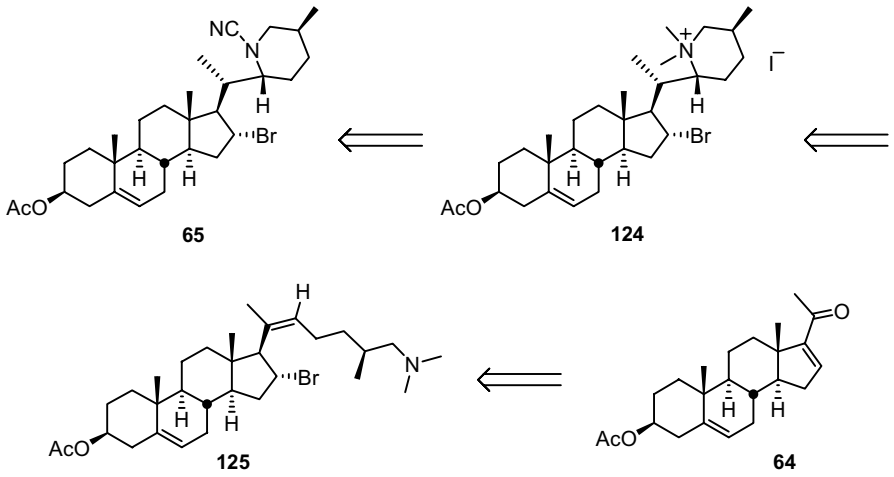
a) Ac_2O, Δ

Scheme 4.3

After the Von Braun reaction, the elimination of the nitrogen containing six-membered ring has to be investigated. A possible route consists of the introduction of a $\Delta^{20,22}$ double bond in compound **65**, followed by oxidation and elimination of the substituent at C16, which should give fast access to a precursor for DPA (**64**) (Scheme 4.4).

The introduction of the double bond in **65** may be established via the Hofmann degradation reaction of **124** to **125** because Zaitsev's rule predicts that formation of the $\Delta^{20,22}$ double bond will

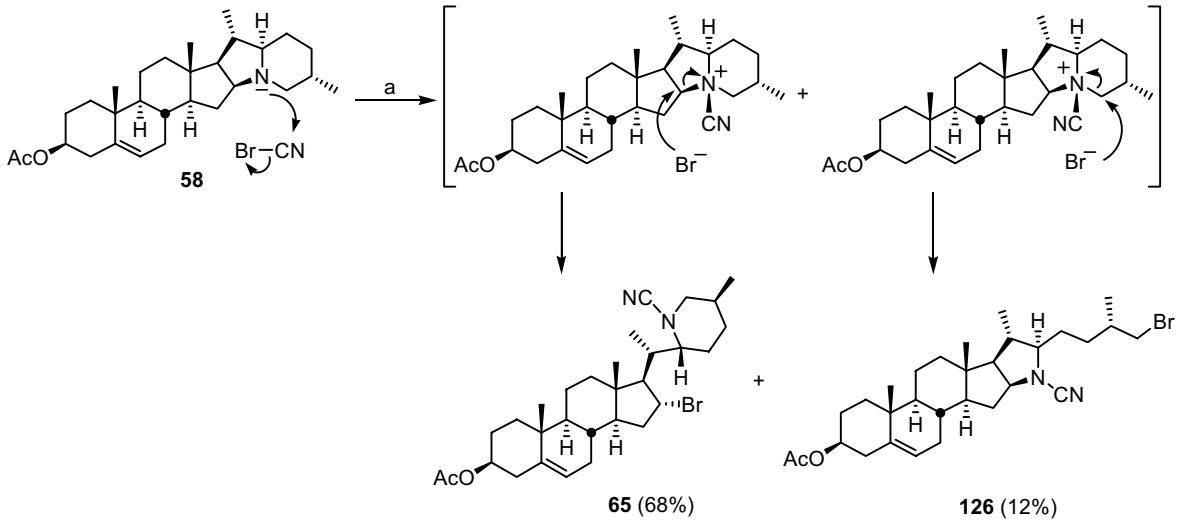
be favored[29-32]. The nitrogen-containing ring will be opened and the nitrogen will end up as a tertiary amine, thus preventing further reactions of the nitrogen. Cleavage of the $\Delta^{20,22}$ double bond in **125** and elimination of bromide would then give DPA (**64**).



Scheme 4.4

4.2 The Von Braun reaction

The Von Braun reaction was performed on 3-acetoxysolanidine (**58**) according to the method of Beisler and Sato[20] and gave **65** in 68% yield. Next to **65**, the F-ring opened side-product **126** was isolated, which must be the result of bromide attack at C26 (Scheme 4.5). Although not described in the literature, it is most likely that this product is always formed in the Von Braun reaction but it has never been isolated before. A clear 5:1 preference was found in the formation of **65** over the formation of **126**.



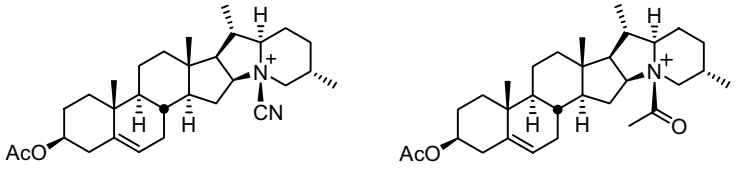
a) BrCN, $CHCl_3$, Δ

Scheme 4.5

Because the use of BrCN in industry requires special safety measures, less toxic alternatives have been investigated. Possible alternatives for BrCN may be acetyl chloride[33,34], ethyl chloroformate[13-15,35-38], trichloroethyl chloroformate[39], benzoylchloride[40], and benzyl chloride[41].

The successful reaction depicted in Scheme 4.3 encouraged the investigation of these acylating reagents. Although these reagents give good results with common tertiary amines, no reaction was observed with 3-acetoxysolanidine (**58**). Other attempts with Ac_2O and $(CF_3CO)_2O$[42], and the triazines trichlorotriazine and chlorodimethoyxytriazine[43-45] were also unsuccessful. In a final attempt **58** was treated with TMSCl, NaI, and Ac_2O[46]. This combination is known to be more reactive toward tertiary amines than ethyl chloroformate but again no reaction was observed.

The fact that ringopening can only be achieved with BrCN can be explained by the unique electronic and steric properties of this reagent. BrCN is not sterically hindered and its carbon atom is electrophilic enough to be attacked by **58**, thereby releasing the bromide ion, which is a good leaving group. The nitrile, as a non-voluminous group, points straight up and the bromide, which is a good nucleophile as well, now can only attack from the bottom side breaking the C16-N or the C26-N bond (Figure 4.2). The most striking difference between a N-atom bearing a nitrile group and an acylated N-atom is the formal charge of the nitrogen atom as shown by MOPAC PM3 calculations. The N-atom of the intermediate *N*-nitrilium ion possesses a formal charge of +0.75 while the N-atom of the corresponding *N*-acylium ion has a charge of only +0.38. This makes the neighboring C-atoms in case of the *N*-nitrilium ion more susceptible to nucleophilic attack than the neighboring C-atoms in the *N*-acylium ion.



N-nitrilium ion — N-atom δ +0.75

N-acylium ion — N-atom δ +0.38

Figure 4.2

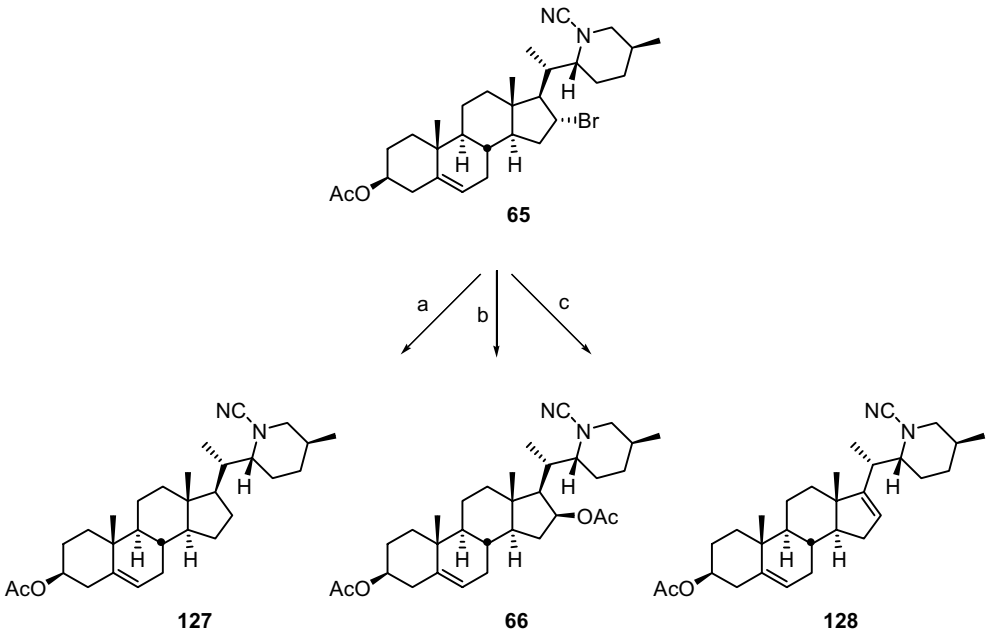
Despite all attempts to replace it, BrCN remains the only reagent able to open the indolizidine ring system of 3-acetoxysolanidine (**58**). Although the objectives against large-scale industrial application of BrCN remain, the reaction itself gives a good yield of **65**, which has good synthetic possibilities for further transformation to DPA (**64**).

4.3 The Hofmann degradation

One of the possibilities for further transformation of the ringopened product **65** is the Hofmann degradation. For this reaction **65** has to be converted to the quaternary ammonium salt **124** followed by treatment with base to obtain **125** (Scheme 4.4).

To avoid disturbing sidereactions in the Hofmann degradation, the bromide was replaced by a hydrogen atom[47] (**127**) or an acetate group[48] (**66**), or by introduction of a double bond through HBr elimination[48] (**128**) (Scheme 4.6).

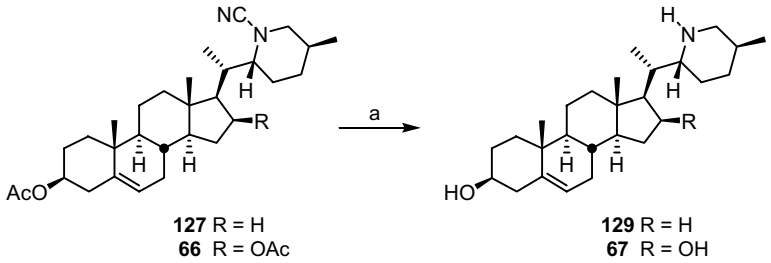
Treatment of **65** with Bu_3SnH and AIBN in benzene[49] at reflux temperature gave **127** in 94% yield. Treatment of **65** with KOAc in DMF at 90ºC gave the acetate **66** in 83% yield[48]. Introduction of the $\Delta^{16,17}$ double bond was achieved by treatment of **65** with *s*-collidine to afford **128** in 98% yield[48].



a) Bu_3SnH, AIBN, benzene, Δ; b) KOAc, DMF, 90ºC; c) *s*-collidine, Δ

Scheme 4.6

In order to develop the shortest possible route from 3-acetoxysolanidine (**58**) to DPA (**64**) attempts were made to methylate **128** with MeI in acetone[50], but these attempts were unsuccessful. The electron-withdrawing nitrile makes the nitrogen atom too little nucleophilic and this implies that the nitrile has to be removed prior to methylation (Scheme 4.7). Reduction of **127** and **66** with Red-Al gave the secondary amine **129** and **67** in 55% in 93% yield, respectively (Scheme 4.7a). A similar treatment of alkene **128** produced **130** in 94% yield (Scheme 4.7b)[51]. The less expensive $LiAlH_4$ reagent[52] also reduces nitrile **128** to **130** but only in 64% yield, which is much lower than the result of the Red-Al reduction[51].

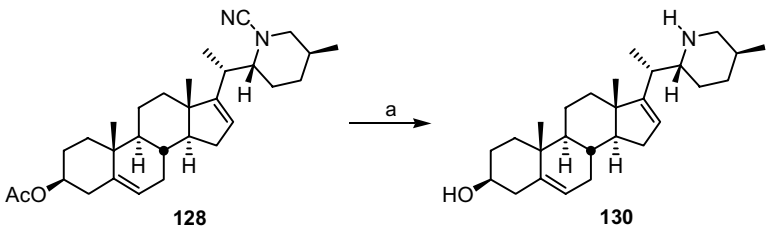


127 R = H
66 R = OAc

129 R = H
67 R = OH

a) Red-Al, toluene, Δ

Scheme 4.7a



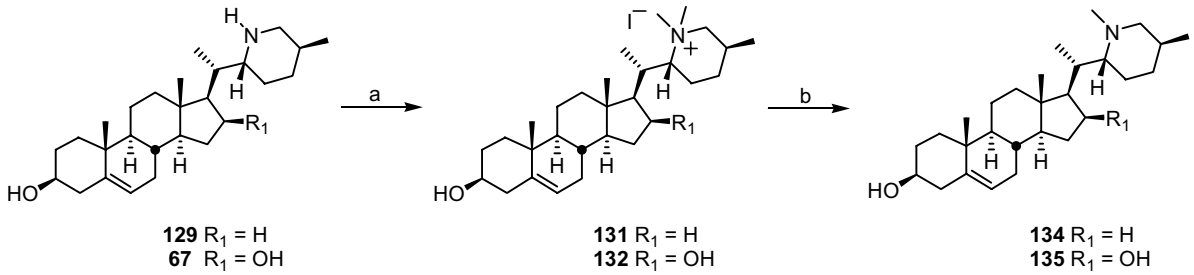
128

130

a) Red-Al, toluene, Δ or $LiAlH_4$, THF, Δ

Scheme 4.7b

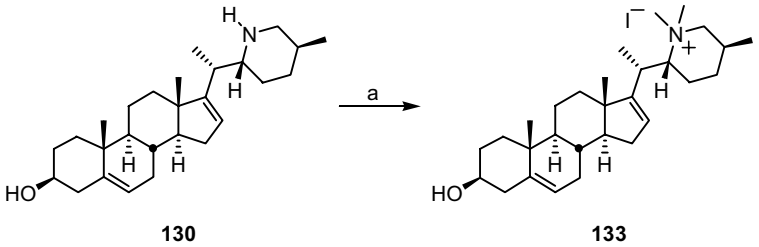
Methylation of **129**, **67**, and **130** with MeI and Na_2CO_3 in water proceeded smoothly and gave the corresponding ammonium salts **131**, **132**, and **133** in quantitative yield (Scheme 4.8a and b). In order to introduce the $\Delta^{20,22}$ double bond, the salts **131**, **132**, and **133** were subjected to treatment with base. When **131** and **132** were treated with KOH in MeOH[53] the demethylated products **134** and **135** could be isolated in 76% and 32% yield, respectively (Scheme 4.8a). Treatment of **132** with LDA led to products which decomposed during isolation.



129 R_1 = H, **67** R_1 = OH; **131** R_1 = H, **132** R_1 = OH; **134** R_1 = H, **135** R_1 = OH

a) MeI, Na_2CO_3, H_2O; b) KOH, MeOH, Δ

Scheme 4.8a



130 **133**

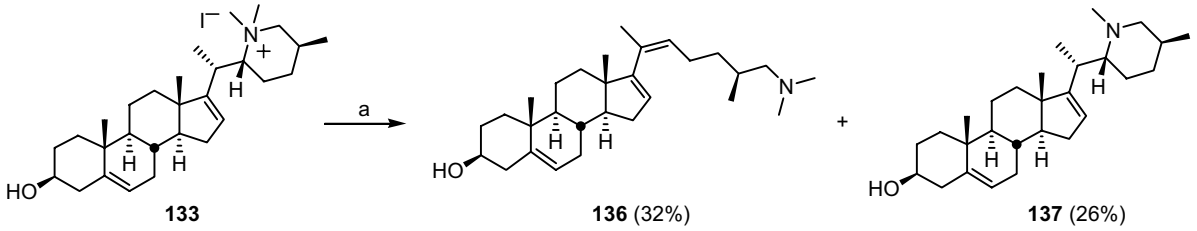
a) MeI, Na_2CO_3, H_2O

Scheme 4.8b

Treatment of **133** with KOH[53], *t*-BuOK[54,55], NaOH[50,56], NaOMe[57], or Et_3N[58] gave products which also immediately decomposed during the isolation process. Only when **133** was treated with LDA the expected product **136** and the *N*-monomethylated product **137** could be isolated in 32% and 26% yield, respectively (Scheme 4.9). The formation of demethylated products can be explained by difficulties in the proton abstraction which is necessary for ringopening. This can be caused by the size of the base, the strength of the base, or a combination of both, and by the unfavorable stereochemistry of the six-membered ring[59,60]. In these cases the competitive nucleophilic substitution resulting in demethylation is strongly favored over the Hofmann degradation. LDA is a poor nucleophilic strong base and small enough to abstract the proton from C20 but only in the case of **133** compound **136** was formed. When the $\Delta^{16,17}$ double bond is absent, as in **131** and **132**, the demethylated products **134** and **135** are formed as the sole products. An important consequence of the presence of the $\Delta^{16,17}$ double bond is the fact that H20 now has become an allylic proton which is more prone to abstraction.

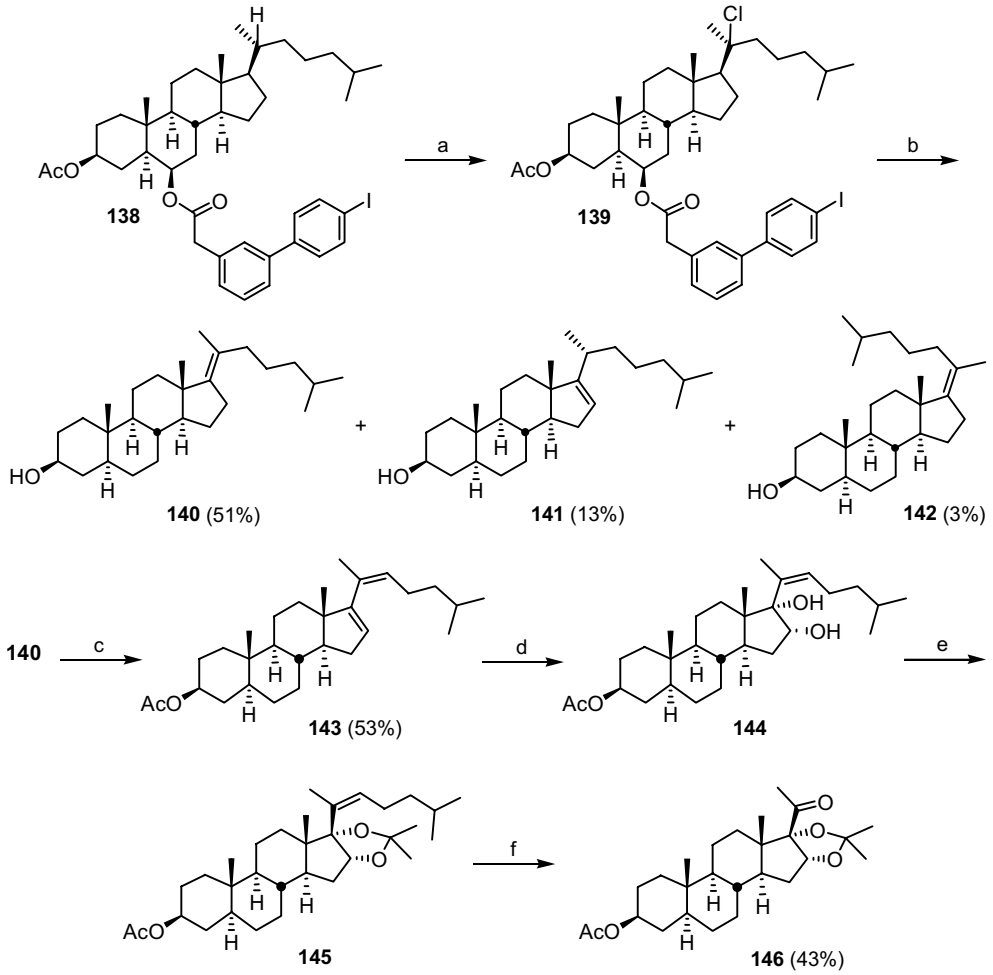
The next step would be the selective oxidation of the $\Delta^{20,22}$ double bond in **136**. However, because of the moderate results obtained by Maitra and Breslow[61] in the degradation of the cholesterol side chain to a 17-acetyl group (**138→146**) (Scheme 4.10), this approach was not investigated further. The $\Delta^{16,17}$ double bond in **143** is more reactive than the $\Delta^{20,22}$ double bond and this means that, prior to oxidation of the $\Delta^{20,22}$ double bond, the $\Delta^{16,17}$ double bond has to be converted to a protected diol. In this way the DPA derivative **146** could be obtained in 43% yield starting from **143**. In the case of compound **136** following the degradation method as depicted in

Scheme 4.9, the $\Delta^{5,6}$ double has to be protected prior to the oxidation reaction and afterwards deprotected again. The large number of steps and the low overall yield make the route from solanidine (**1**) to the DPA derivative **146** involving the Von Braun reaction and the Hofmann degradation unacceptable for industrial application. Especially the Hofmann degradation contributes severely to this low overall yield. In principle the demethylated product **137** can be methylated again to **133**, which can be reused in the Hofmann degradation, but this would not increase the overall yield sufficiently. Besides the low overall yield, the use of several hazardous reagents (BrCN, Red-Al, OsO_4, $RuCl_3$, and $NaIO_4$) is another reason to cancel this approach and to look for better alternatives.



a) LDA, THF, -78ºC

Scheme 4.9



a) $PhICl_2$, *t*-BuOH, CH_2Cl_2, $NaHCO_3$, hν, 60 min; b) DBU, 90ºC; c) NBS, $C_6H_5CO_2OH$, CCl_4; d) OsO_4; e) H_2SO_4, acetone; f) $RuCl_3/NaIO_4$

Scheme 4.10

4.4 Conclusions

The Von Braun reaction is the only reaction capable of opening the E-ring of solanidine (**1**). Less toxic reagents failed to achieve ring opening. Next to the desired E-ring opened compound **66** also the F-ring opened compound **126** was isolated.

Substitution of the bromide in **66** with a H atom or an acetate group, or elimination of the bromide to a $\Delta^{16,17}$ double bond gives in high yield the corresponding products **127**, **66**, and **128**, respectively. Removal of the nitrile substituent proves necessary to obtain the corresponding dimethylated ammonium salts. Removal of the nitrile with Red-Al followed by dimethylation gives the highest yield of the desired dimethyl ammonium salt.

The Hofmann degradation proceeds only with compound **133** in which the $\Delta^{16,17}$ double bond is present. In combination with LDA this degradation of **133** gives the ring-opened product **136** in 32% and the monomethylated product **137** in 26% yield.

The entire degradation procedure of solanidine (**1**) to DPA derivative (**146**) via the Von Braun reaction and Hofmann degradation would involve many steps with a poor overall yield and is therefore not acceptable for industrial application.

4.5 Experimental section

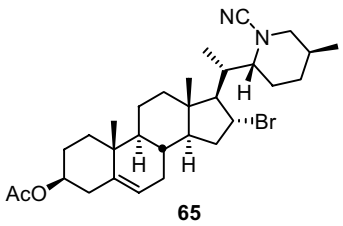
4.5.1 General comments and materials[62]

All ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless stated otherwise. Solanidine (**1**) and 3-acetoxysolanidine (**58**) were prepared as described previously in Chapter 3.

4.5.2 Procedures and Spectral data

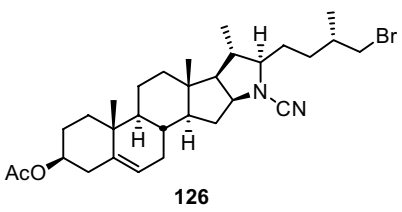
(3β,16α,20*S*)-16-Bromo-20-[(2*R*,5*S*)-1-cyano-5-methylpiperidinyl]pregn-5-en-3-yl Acetate (65)[23] **and (2*S*,4a*R*,4b*S*,6a*S*,6b*R*,7*S*,9a*S*,10a*S*,10b*S*)-8-[(3*R*)-4-Bromo-3-methylbutyl]-9-cyano-4a,6a,7-trimethyl-1,2,3,4,4a,4b,5,6,6a,6b,7,8,9,9a,10,10a,10b,11-octadecahydronaphtho-[2',1':4,5]indeno[2,1-*b*]pyrrol-2-yl Acetate (126)**

A solution of **58** (0.9 g, 2.05 mmol) in $CHCl_3$ (20 ml) was treated with a 3M solution of BrCN (5 ml, 15.00 mmol) in CH_2Cl_2. After heating at reflux temperature for 24 hours under nitrogen atmosphere, the solvent was removed *in vacuo* and the remaining gum crystallized from PE/EA 1/1 to give **65** (0.5 g, 45%). The mother liquor was purified by column chromatography (PE/EA 5/1) to give additional **65** (0.255 g, 23%) and the F-ring opened product **126** (0.135 g, 12%).



65

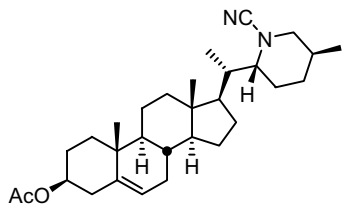
65: M.p. 250-260ºC (EtOH) (lit. 245-260ºC[23]); IR[23] λ_{max} (neat) 1717, 2199, 2841, 2911, 2932 cm^{-1}; ^{1}H NMR δ 0.72 (s, 3H), 0.85 (d, 3H, *J*= 6.5 Hz), 0.96 (d, 3H, *J*= 7.7 Hz), 0.98 (s, 3H), 2.00 (s, 3H), 2.29 (d, 2H, *J*= 7.5 Hz), 2,72 (t, 1H, *J*= 11.0 Hz), 3.17 (d, 1H, *J*= 11.0 Hz), 3.38 (dd, 1H, *J*= 3.3 and 10.9 Hz), 4.04 (t, 1H, *J*= 6.2 Hz), 4.57 (m, 1H), 5.34 (d, 1H, *J*= 4.1 Hz); ^{13}C NMR δ 12.50 (q), 13.98 (q), 18.63 (q), 19.23 (q), 20.72 (t), 21.40 (q), 23.76 (t), 27.63 (t), 30.54 (2d), 31.58 (t), 31.68 (t), 36.46 (s), 36.82 (t), 37.08 (d), 37.97 (t), 39.51 (t), 39.67 (t), 45.16 (s), 49.47 (d), 53.15 (d), 54.16 (d), 58.25 (t), 60.08 (d), 63.77 (d), 73.72 (d), 117.08 (s), 121.97 (d), 139.64 (s), 170.48 (s)); MS *m/z* (r.i.) 425 (8), 410 (7), 124 (14), 112 (100), 98 (31), 58 (71); HRMS calculated for $C_{28}H_{41}N_2Br$ ([M-HOAc]$^{+}$) 484.2453, found 484.2454. The ^{1}H NMR data were in accordance with the literature data[20].



126

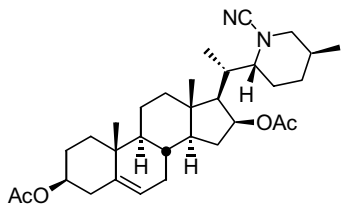
126: ^{1}H NMR δ 0.77 (s, 3H), 0.98 (2s, 6H), 0.99 (d, 3H, *J*= 6.4 Hz), 1.97 (s, 3H), 3.32 (d, 2H, *J*= 5.4 Hz), 3.87 (m, 1H), 4.53 (m, 1H), 5.31 (d, 1H, *J*= 4.3 Hz); ^{13}C NMR d 16.09 (q), 18.54 (q), 19.13 (q), 20.11 (q), 20.32 (t), 21.28 (q), 27.53 (t), 28.18 (t), 29.63 (t), 31.10 (t), 31.26 (d), 31.64 (t), 35.00 (d), 35.42 (d), 36.47 (s), 36.78 (t), 37.87 (t), 38.83 (t), 40.71 (t), 41.28 (s), 49.63 (d), 56.95 (d), 61.18 (d), 65.21 (d), 72.12 (d), 73.56 (d), 116.19 (s), 121.93 (d), 139.52 (s), 170.30 (s)); MS *m/z* (r.i.) 484 (18), 439 (5), 405 (5), 150 (21), 123 (100); HRMS calculated for $C_{28}H_{41}N_2Br$ ([M-HOAc]$^{+}$) 484.2453, found 484.2441.

(3β,20*S*)-20-[(2*R*,5*S*)-1-Cyano-5-methylpiperidinyl]pregn-5-en-3-yl Acetate (127)



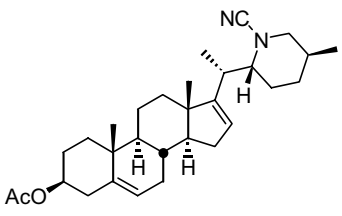
A solution of **65** (0.20 g, 0.37 mmol), Bu_3SnH (0.15 ml, 0.56 mmol), and AIBN (cat. amount) in benzene (5 ml) was heated at reflux temperature for 7.5 hours under a nitrogen atmosphere[49]. After cooling and concentration of the mixture *in vacuo*, ether (25 ml) and saturated aqueous KF (25 ml) were added and the mixture was stirred overnight at room temperature. The layers were separated and the aqueous phase was extracted three times with chloroform (25 ml). The combined organic phases were washed with brine, dried, and concentrated *in vacuo*. Purification of the residue by flash chromatography (PE/EA 9/1) gave **127** (0.11 g, 64%) as a white solid. ^{1}H NMR δ 0.66 (s, 3H), 0.80 (d, 3H, *J*= 6.6 Hz), 0.92 (d, 3H, *J*= 6.7 Hz), 0.95 (s, 3H), 1.97 (s, 3H), 2.25 (d, 2H, *J*= 7.9 Hz), 2.62 (t, 1H, *J*= 11.6 Hz), 2.76 (d, 1H, *J*= 8.9 Hz), 3.32 (dd, 1H, *J*= 2.4 and 11.7 Hz), 4.50 (m, 1H), 5.30 (d, 1H, *J*= 4.3 Hz); ^{13}C NMR δ 11.81 (q), 13.65 (q), 18.72 (q), 19.32 (q), 21.00 (t), 21.47 (q), 23.33 (t), 24.23 (2t), 27.56 (t), 27.74 (t), 30.64 (d), 31.83 (d), 32.34 (t), 36.56 (s), 36.98 (t), 37.34 (d), 38.09 (t), 39.69 (t), 42.69 (s), 49.98(d), 52.68 (d), 56.44 (d), 58.17 (t), 60.40 (d), 73.92 (d), 117.29 (s), 122.42 (d), 139,73 (s), 170.59 (s).

(3β,16β,20*S*)-16-(acetyloxy)-20-[(2*R*,5*S*)-1-Cyano-5-methylpiperidinyl]pregn-5-en-3-yl Acetate (66)



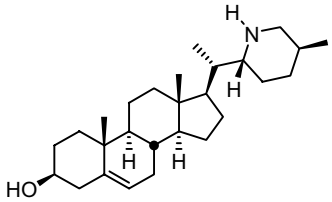
To a solution of **65** (0.4 g, 0.73 mmol) in DMF (40 ml) was added an aqueous KOAc solution (40%, 1.33 ml). The reaction mixture was heated at 90°C for 2 hours and then poured into water. The water layer was extracted three times with ether (15 ml). The combined organic layers were washed with water, dried and evaporated *in vacuo* yielding a white solid. Flash chromatography (PE/EA 5/1) yielded **66** (0.32 g, 83%) as a white solid. M.p. 163-167ºC (160-167ºC[51]); ^{1}H NMR δ 0.79 (d, 3H, *J*= 6.5 Hz), 0.86 (s, 3H), 0.95 (d, 3H, *J*= 5.0 Hz), 1.98 (s, 3H), 2.06 (s, 3H), 2.25 (d, 2H, *J*= 7.6 Hz), 2.55 (t, *J*= 11.5 Hz), 3.30 (dd, 1H, *J*= 2.4 and 11.7 Hz), 4.57 (m, 1H), 5.08 (m, 1H), 5,29 (d, 1H, *J*= 4.1 Hz); ^{13}C NMR δ 12.42 (q), 13.05 (q), 18.61 (q), 19.29 (q), 20.64 (t), 21.47 (2q), 23.84 (t), 27.69 (t), 30.61 (d), 31.32 (d), 31.58 (t), 31.90 (d), 32.16 (t), 34.76 (t), 36.53 (s), 36.90 (t), 38.04 (t), 39.47 (t), 42.72 (s), 49.86 (d), 54.63 (d), 55.63 (d), 58.35 (t), 59.64 (d), 73.80 (d), 73.88 (d), 116.97 (s), 122.10 (d), 139.78 (s), 170.56 (s), 171.04 (s). MS *m/z* (r.i.) 524 (1), 509 (7), 464 (26), 449 (19), 151 (100), 123 (100); HRMS calculated for $C_{32}H_{48}N_2O_4$ ([M]$^{+}$) 524.3614, found 524.3614.

(3β,20*S*)-20-[(2*R*,5*S*)-1-Cyano-5-methylpiperidinyl]pregna-5,16-dien-3-yl Acetate (128)



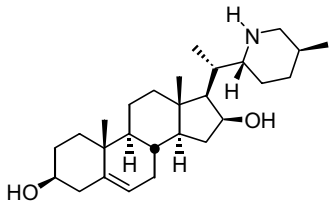
Compound **65** (0.1 g, 0.18 mmol) was dissolved in *s*-collidine (2 ml) and heated at reflux temperature for 16 hours. After cooling, the reaction mixture was diluted with EA, washed with aqueous HCl (10%) and water, and dried. Evaporation of the solvent *in vacuo* yielded **128** (0.083 g, 98%) as a white crystalline product. M.p.170-175ºC (173ºC[63]); ^{1}H NMR δ 0.81 (d, 1H, *J*= 5.8 Hz), 0.82 (s, 3H), 0.99 (s, 3H), 0.99 (d, 3H, *J*= 6.8 Hz), 1.97 (s, 3H), 2.26 (d, 2H, *J*= 7.4 Hz), 2.55 (t, 1H, *J*= 11.9 Hz), 2.77 (m, 1H), 3.30 (m, 1H), 4.55 (m, 1H), 5.32 (d, 1H, *J*= 4.3 Hz), 5.40 (m, 1H); ^{13}C NMR δ 15.67 (q), 17.03 (q), 18.43 (q), 19.17 (q), 20.60 (t), 21.41 (q), 25.30 (t), 27.68 (t), 29.91 (d), 30.33 (d), 31.09 (t), 31.43 (t), 32.59 (t), 34.92 (t), 35.24 (d), 36.73 (s), 36.85 (t), 38.07 (t), 46.83 (s), 50.37 (d), 57.77 (d), 58.47 (t), 61.14 (d), 73.84 (d), 117.08 (s), 122.37 (d), 124.90 (d), 139.88 (s), 156.02 (s), 170.48 (s).

(3β,20*S*)-20-[(2*R*,5*S*)-5-Methylpiperidinyl]pregn-5-en-3-ol (129)



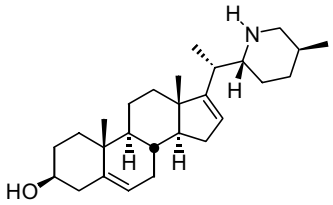
A solution of **127** (0.1 g, 0.214 mmol) in dry toluene (10 ml) was added dropwise to a boiling solution of 3.5 M Red-Al (1 ml, 3.5 mmol) in toluene (10 ml). After heating at reflux for 2 hours, the reaction mixture was cooled and the excess of Red-Al was decomposed with 2M aqueous NaOH. The aqueous phase was extracted three times with EA (15 ml). The combined organic phases were washed with water, dried, and evaporated *in vacuo* to give **129** (0.047 g, 55%) as a white crystalline product. M.p. 216-220ºC (219-220ºC[64]); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.57 (s, 3H), 0.69 (d, 3H, *J*= 6.6 Hz), 0.75 (d, 3H, *J*= 6.7 Hz), 0.87 (s, 3H), 2.34 (d, 1H, *J*= 11.0 Hz), 2.83 (m, 1H), 3.30 (m, 1H), 5.19 (d, 1H, *J*= 4.3 Hz); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 11.32 (q), 12.92 (q), 18.99 (2q), 20.76 (t), 23.89 (t), 24.59 (t), 27.40 (t), 30.85 (t), 31.54 (t), 31.62 (d), 32.00 (d), 33.24 (t), 36.18 (s), 36.96 (t), 39.57 (t), 40.09 (d), 41.55 (t), 42.08 (s), 49.82 (d), 52.68 (d), 54.11 (t), 56.32 (d), 58.64 (d), 70.93 (d), 121.09 (d), 140.64 (s). The NMR data were in accordance with the literature data[64-67].

(3β,16β,20*S*)-20-[(2*R*,5*S*)-5-Methylpiperidinyl]pregn-5-ene-3,16-diol (67)



Compound **67** was prepared from **66** (0.3 g, 0.572 mmol) and 3.5 M Red-Al (3 ml, 10.5 mmol) according to the procedure used in the preparation of **129**. Compound **67** (0.22 g, 93%) was obtained as a white solid. M.p. 214-222ºC (216-220ºC[68]); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.61(d, 3H, *J*= 6.5 Hz), 0.68 (s, 3H), 0.74 (d, 3H, *J*= 7.0 Hz), 0.79 (s, 3H), 1.99 (m, 2H), 2.36 (dd, 1H, *J*= 9.9 Hz, *J*= 3.0 Hz), 2.73 (m, 1H), 3.23 (m, 1H), 4.09 (sextet, 1H, *J*= 3.6 Hz), 5.11 (d, 1H, *J*= 4.5 Hz); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 12.07 (q), 13.46 (q), 18.58 (q), 18.67 (q), 20.18 (t), 26.94 (t), 30.53 (t), 31.01 (t), 31.21 (d), 31.69 (d), 33.32 (t), 34.70 (d), 35.37 (t), 35.97 (s), 36.67 (t), 39.56 (t), 41.24 (t), 41.71 (s), 49.59 (d), 53.73 (t), 54.14 (d), 59.11 (d), 60.08 (d), 70.66 (d), 70.96 (d), 120.71 (d), 140.46 (s). MS *m/z* (r.i.) 414 (1), 140 (5), 126 (3), 98 (100); HRMS calculated for $C_{27}H_{44}NO_2$ ([M-H]$^+$) 414.3360, found 414.3372. The NMR and mass data were in accordance with the literature data[68].

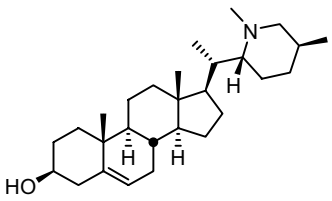
(3β,20*S*)-20-[(2*R*,5*S*)-5-Methylpiperidinyl]pregna-5,16-dien-3-ol (130)



Method A: Compound **130** was prepared from **128** (0.03 g, 0.065 mmol) and 3.5 M Red-Al (3 ml, 10.5 mmol) according to the procedure used in the preparation of **129**. Compound **130** (0.025 g, 94%) was obtained as a white solid. M.p. 180-184ºC (lit. 180ºC[51]) ; ^{1}H NMR δ 0.76 (d, 3H, *J*= 6.3 Hz), 0.77 (s, 3H), 0.91 (d, 3H, *J*= 6.9 Hz), 0.97 (s, 3H), 2.90 (dd, 1H, *J*= 2.2 and 9.8 Hz), 3.45 (m, 1H), 5.34 (m, 2H); ^{13}C NMR δ 16.27 (q), 18.40 (q), 19.36 (q), 19.54 (q), 20.81 (t), 29.37 (t), 30.61 (d), 31.19 (t), 31.26 (d), 31.59 (2t), 33.93 (t), 34.87 (t), 36.75 (s), 37.21 (t), 38.77 (d), 42.27 (t), 47.03 (s), 50.72 (d), 54.90 (t), 57.61 (d), 59.83 (d), 71.45 (d), 121.41 (d), 121.86 (d), 141.15 (s), 158.83 (s).

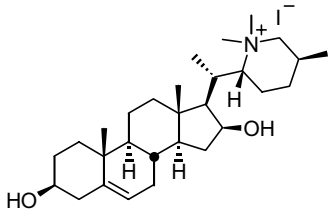
Method B: To a solution of **128** (0.1 g, 0.214 mmol) in dry THF (10 ml) was added $LiAlH_4$ (0.039 g, 1.02 mmol). After refluxing for 1.5 hours, the reaction mixture was cooled and the excess of $LiAlH_4$ was decomposed with 4M aqueous NaOH. The aqueous phase was extracted three times with CH_2Cl_2 (15 ml). The combined organic phases were washed with water, dried, and evaporated *in vacuo* to give **130** (0.048 g, 64%) as a white crystalline product. M.p. 182-184ºC (lit. 180ºC[51]). The NMR data are identical with those obtained from the reduction with Red-Al.

(3β,20*S*)-20-[(2*R*,5*S*)-1,5-Dimethylpiperidinyl]pregn-5-en-3-ol (134)



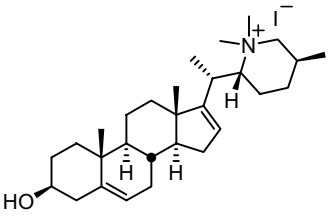
A suspension of **131** (0.07 g, 0.175 mmol), Na_2CO_3 (0.035 g), and MeI (0.07 ml, 1.12 mmol) in water was heated at reflux temperature for 7 hours. After cooling, the white precipitate was filtered and washed with water. The crude material was suspended in methanolic KOH (3.5 M, 5 ml) and heated under reflux for 3 hours. After cooling, the alkaline mixture was concentrated and water was added. The mixture was extracted three times with ether (5 ml) and dried. Evaporation of the solvent *in vacuo* yielded an amorphous material (0.065 g) which was flash chromatographed ($CHCl_3$/MeOH 9/1) yielding **134** (0.055 g, 76%) as a white solid. M.p. 296-301ºC; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.51 (s, 3H), 0.73 (d, 3H, *J*= 6.6 Hz), 0.77 (s, 3H), 0.88 (d, 3H, *J*= 6.7 Hz), 2.03 (m, 2H), 2.34 (t, 1H, *J*= 12.0 Hz), 2.51 (s, 3H), 3.20 (m, 1H), 5.11 (d, 1H, *J*= 4.3 Hz); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 11.44 (q), 12.25 (q), 17.94 (q), 18.98 (q), 20.72 (t), 22.38 (t), 23.79 (t), 27.18 (t), 29.22 (d), 30.79 (t), 31.04 (t), 31.45 (t), 31.61 (d), 34.21 (d), 36.17 (s), 36.95 (t), 39.45 (t), 40.35 (q), 41.47 (t), 42.52 (s), 49.69 (d), 52.15 (d), 56.08 (d), 63.46 (t), 69.24 (d), 70.84 (d), 120.87 (d), 140.70 (s); MS *m/z* (r.i.) 412 (2), 112 (100); HRMS calculated for $C_{28}H_{46}NO$ ($[M-H]^+$) 412.3579, found 412.3579.

(3β,16β,20*S*)-20-[(2*R*,5*S*)-1,1,5-Trimethylpiperidiniumyl]pregn-5-ene-3,16-diol Iodide (132)



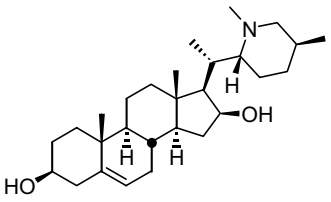
A suspension of **67** (0.04 g, 0.096 mmol), Na_2CO_3 (0.02 g), and MeI (0.04 ml, 0.64 mmol) in water was heated at reflux temperature for 7 hours. After cooling, the white precipitate was filtered and washed with water. Drying on air gave **132** (0.04 g, 99%) as a white solid. ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.52 (s, 3H), 0.52 (d, 3H, *J*= 6.7 Hz), 0.58 (s, 3H), 0.66 (d, 1H, *J*= 6.8 Hz), 2.64 (s, 3H), 2.82 (s, 3H), 3.83 (m, 1H), 4.89 (d, 1H, *J*= 4.6 Hz); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 11.83 (q), 14.26 (q), 16.91 (q), 18.25 (q), 20.02 (t), 20.39 (t), 26.06 (d), 28.27 (d), 30.26 (d), 30.43 (s), 30.89 (2t), 35.70 (s), 36.40 (2t), 39.41 (t), 40.97 (2t), 42.05 (q), 44.30 (d), 46.21 (q), 49.27 (d), 53.56 (q), 53.84 (d), 57.98 (d), 68.93 (d), 70.36 (t), 72.80 (t), 73.63 (d), 120.27 (d), 140.25 (s).

(3β,20*S*)-20-[(2*R*,5*S*)-1,1,5-Trimethylpiperidiniumyl]pregna-5,16-dien-3-ol Iodide (133)



A suspension of **130** (0.048 g, 0.13 mmol), Na_2CO_3 (0.025 g), and MeI (0.05 ml, 0.8 mmol) in water (4 ml) was heated under reflux for 7 hours. After cooling, the white precipitate was filtered and washed with water. Drying on air gave **133** (0.065 g, 97%) as a rather unstable compound through which further purification failed. ^{1}H NMR (DMSO) δ 0.83 (s, 6H), 0.83 (d, 3H, *J*= 6.8 Hz), 0.97 (s, 3H), 0.99 (d, 3H, *J*= 7.0 Hz), 2.99 (s, 3H), 3.14 (s, 3H), 4.62 (d, 1H, *J*= 4.4 Hz), 5.27 (d, 1H), 5.50 (m, 1H).

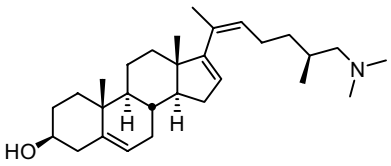
(3β,16β,20*S*)-20-[(2*R*,5*S*)-1,5-Dimethylpiperidinyl]pregn-5-ene-3,16-diol (135)



A suspension of **132** in 3.5 M methanolic KOH (3 ml, 10.5 mmol) was heated at reflux temperature for 3 hours. After cooling, the reaction mixture was filtered and washed with water. Flash chromatography ($CHCl_3$/MeOH 1/1) gave **135** (0.013 g, 32%) as a white solid. M.p. 199-202ºC (196.5-198.5ºC[66]); ^{1}H NMR δ 0.76 (s, 3H), 0.85 (d, 3H, *J*= 7.0 Hz), 0.92 (d, 6H, *J*= 6.6 Hz), 0.96 (s, 3H), 2.32 (s, 3H), 3.46 (m, 1H), 3.94 (m, 1H), 4.43 (q, 1H, *J*= 7.2 Hz), 5.29 (d, 1H, *J*= 4.9 Hz); ^{13}C NMR δ 14.75 (q), 16.27 (q), 18.34 (q), 19.44 (q), 20.74 (t), 27.37 (t), 30.41 (d), 30.98 (d), 31.61 (t), 32.04 (s), 32.11 (t), 33.46 (t), 35.45 (d), 36.67 (t), 37.21 (t), 39.67 (t), 40.92 (s), 42.27 (t), 45.11 (q), 50.14 (d), 55.55 (d), 64.31 (d), 66.08 (t), 71.68 (d), 81.63 (d), 84.11 (d), 121.40 (d), 140.82 (s). The NMR spectral data were in accordance with the literature data[66,69].

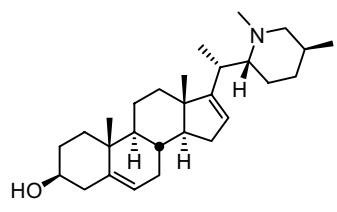
(3β,25*S*)-26-(Dimethylamino)cholesta-5,16,20(22)-trien-3-ol (136) and (3β,20*R*)-20-[(2*R*,5*S*)-1,5-Dimethylpiperidinyl]pregna-5,16-dien-3-ol (137)

To a solution of isopropylamine (0.85 ml, 10 mmol) in THF (2.9 ml) was added 1.6 M *n*-BuLi (6.25 ml, 10 mmol) in hexane at -20ºC. The reaction mixture was cooled to -60ºC and stirring was continued for 30 minutes. To a suspension of **133** (0.29 g, 0.49 mmol) in THF (3 ml) was added freshly prepared 1 M LDA (1.6 ml, 1.60 mmol) at -78ºC. The reaction mixture was allowed to warm to room temperature, stirred for 4 hours, and then quenched with an aqueous saturated NH_4Cl solution. The reaction mixture was extracted three times with CH_2Cl_2 (15 ml). The combined organic layers were washed with saturated brine, dried, and evaporated *in vacuo*. The residue was purified by flash chromatography ($CHCl_3$/MeOH 9/1) to afford **136** (0.070 g, 32%) and **137** (0.055 g, 26%), both as white solids.



136

136: M.p. 164-165ºC (MeOH); IR λ_{max} (neat): 3224, 1457, 1375, 1063 cm^{-1}.^{1}H NMR δ 0.90 (d, 3H, *J*= 6.5 Hz), 0.94 (s, 3H), 1.00 (s, 3H), 1.62 (s, 3H), 2.19 (s, 6H), 3.50 (m, 1H), 5.34-5.59 (m, 3H); ^{13}C NMR ($CDCl_3$) 15.3 (q), 16.2 (q), 18.1 (q), 19.3 (q), 21.0 (t), 25.8 (t), 30.3 (d), 30.6 (d), 30.9 (t), 31.5 (t), 31.6 (t), 35.0 (t), 36.1 (t), 36.6 (s), 37.1 (t), 42.3 (t), 45.8 (2q), 46.7 (s), 50.3 (d), 57.6 (d), 67.0 (t), 71.7 (d), 121.5 (d), 124.8 (d), 126.6 (d), 130.5 (s), 141.0 (s), 156.4 (s); MS *m/z* (r.i.) 410 (2), 397 (4), 382 (1), 204 (2), 150 (8), 112 (100); HRMS calculated for $C_{29}H_{46}NO$ ([M-H]$^+$) 425.3658, found 425.3658.



137

137: M.p. 148-151ºC; IR λ_{max} (neat): 3382, 1455, 1376 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.73 (s, 3H), 0.81 (d, 3H, *J*= 6.4 Hz), 0.90 (s, 3H), 1.04 (d, 3H, *J*= 6.9 Hz), 2.59 (s, 3H), 3.25 (m, 1H), 5.29 (m, 1H), 5.45 (m, 1H); ^{13}C NMR ($CDCl_3$) 12.7 (q), 17.0 (q), 19.0 (q), 19.3 (q), 20.5 (t), 23.6 (t), 30.2 (d), 31.0 (t), 31.4 (t), 31.5 (t), 32.2 (t), 32 .5 (d), 34.5 (t), 36.6 (t), 37.1 (s), 41.8 (q), 42.2 (t), 46.3 (s),

50.4 (d), 58.6 (d), 65.3 (t), 66.9 (d), 71.5 (d), 121.3 (d), 126.5 (d), 141.0 (s), 155.9 (s); MS *m/z* (r.i.) 425 (8), 410 (7), 124 (14), 112 (100), 98 (31), 58 (71); HRMS calculated for $C_{28}H_{44}NO$ ($[M-H]^+$) 410.3421, found 410.3423.

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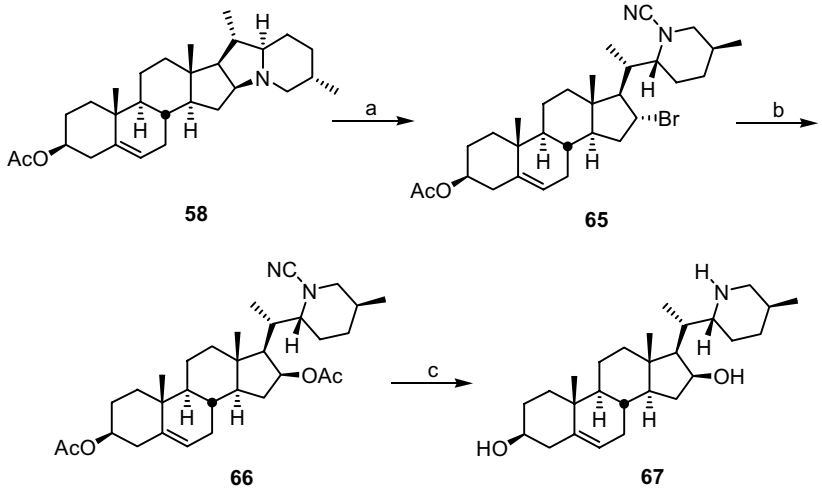
Chapter 5

Synthesis of DPA via the tomatidenol route

5.1 Introduction

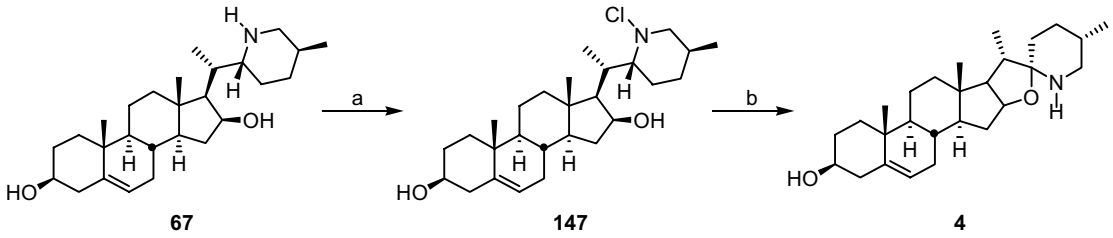
In the quest for an industrially applicable degradation route from solanidine (**1**) to DPA (**64**), an alternative for the Von Braun reaction to **65** has not been found. Further breakdown via the Hofmann degradation gives the desired triene **136** in only 32% yield and, what's more, further breakdown to DPA (**64**) is laborious because of extra protection and deprotection steps. Industrial application of this route is therefore not very likely. A better industrially applicable alternative may be the conversion of solanidine (**1**) to spirosolanes. In 1971 Schramm and Riedl[1] already mentioned that the degradation of solanidine (**1**) to DPA (**64**) can be accomplished via the tomatidine series but up to now the complete procedure has not been published in a single article. A smart integration of known reactions of solanidine (**1**) and tomatidenol (**4**) depicted in Schemes 5.1, 5.2, and 5.3 or 5.4 into one procedure may lead to an industrially applicable route to DPA (**64**).
3-Acetoxysolanidine (**58**) can be converted to compound **67** as already described in Chapter 4 (Scheme 5.1). Chlorination of **67** with NCS gives **147** and subsequent treatment of **147** with NaOMe then gives tomatidenol (**4**) in 95%[2-9] (Scheme 5.2).

Several degradation routes to DPA (**64**) starting from spirosolanes, such as *N*,*O*-diacetyl-tomatidinol[10-14] (**148**) (Scheme 5.3), *N*,*O*-diacetylsolasodine[15-22], or *N*-nitrosyl-3-acetyl-solasodine[10,23] (**151**) (Scheme 5.4), have been developed.



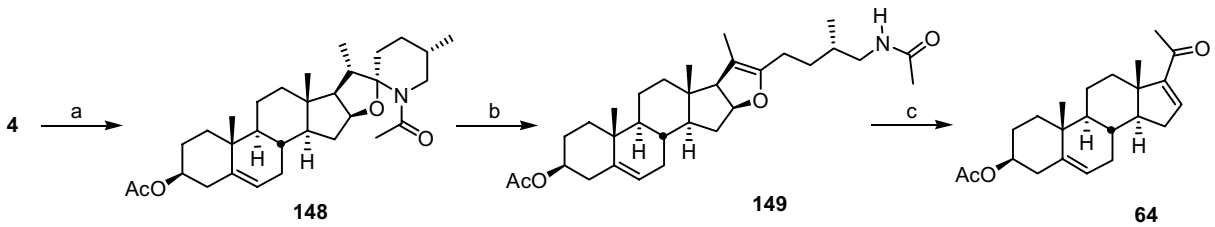
a) BrCN, $CHCl_3$, Δ; b) KOAc, DMF; c) Red-Al, toluene, Δ

Scheme 5.1



a) NCS, CH_2Cl_2; b) NaOMe, MeOH

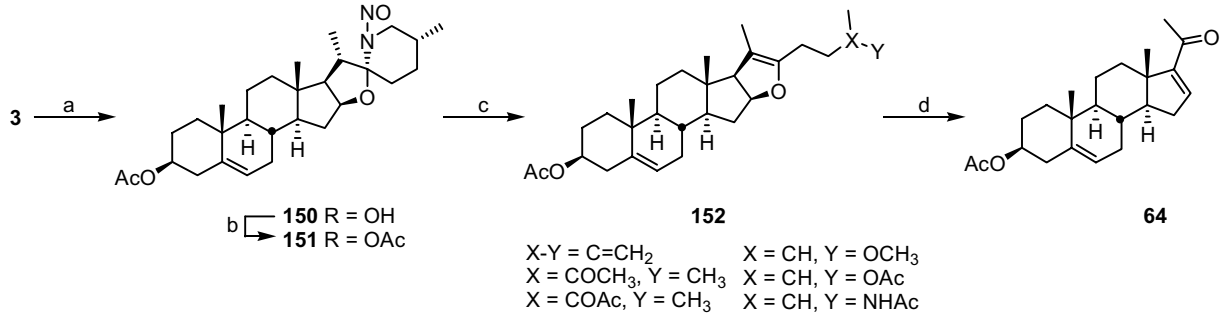
Scheme 5.2



a) Ac_2O, pyridine; b) HOAc, Δ; c) CrO_3, HOAc, Δ

Scheme 5.3

The routes depicted in Schemes 5.1, 5.2, and 5.3 were integrated into one procedure and gave similar results as those reported in the literature. Treatment of 3-acetoxysolanidine (**58**) with BrCN gave **65** in 68% yield[24]. Substitution of the bromide at C16 with KOAc yielded acetate **66** in 83%[25]. Subsequent reduction of **66** with Red-Al then gave **67** in 93% yield[26]. Chlorination of **67** with NCS afforded **147** in 95% yield and elimination of HCl from **147** with NaOMe resulted in ringclosure to give tomatidenol (**4**) in 95% yield[2-7]. Further degradation of **4** to **64** was achieved by employing the method used by Schreiber and Rönsch[14] (Scheme 5.3). Acetylation of tomatidenol (**4**) with Ac_2O in pyridine gave **148** in 98% yield. Treatment of **148** with HOAc at reflux temperature gave compound **149** in 85% yield. Subsequent oxidation of **149** with CrO_3 in HOAc and elimination of the resulting C16-ester gave DPA (**64**) in 76%. The overall yield starting from 3-acetoxysolanidine (**58**) via tomatidenol (**4**) to DPA (**64**) over 9 steps was 30%.



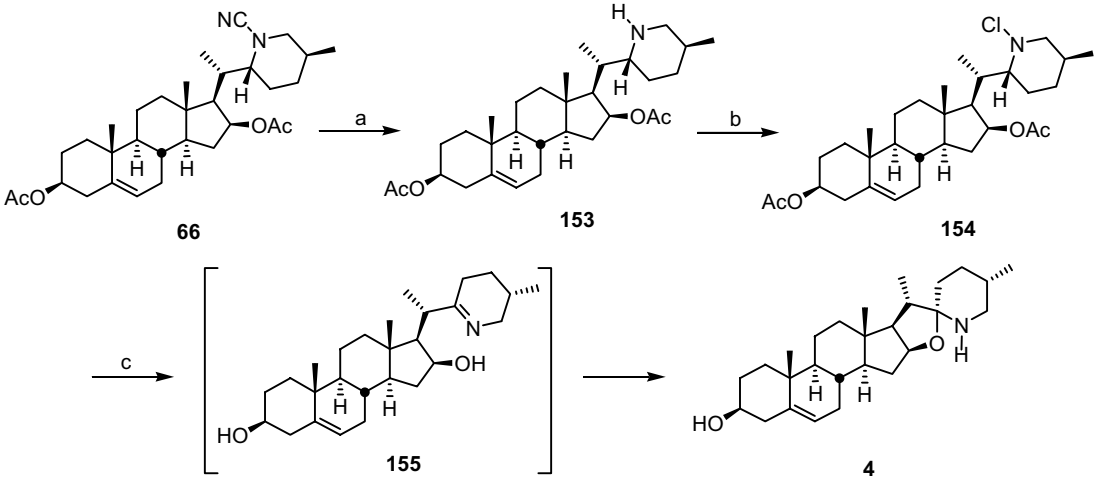
a) $NaNO_2$, HOAc, EtOH; b) Ac_2O, pyridine; c) *p*-TsOH, HOAc, MeOH; then HOAc, Δ; d) CrO_3, HOAc, Δ

Scheme 5.4

5.2 Shortcuts to tomatidenol (4) starting from 3-acetoxysolanidine (58)

The routes depicted in Schemes 5.1, 5.2, and 5.3 form the starting point for the research described in this chapter. Some of the reactions in these schemes are not acceptable or favored in industry (like the use of the expensive Red-Al reagent and the chlorination/dehydrochlorination process) and therefore alternatives will be investigated. More important, development of shortcuts in the routes depicted in Schemes 5.1 and 5.2, which can compensate for these problems will be explored as well.

A first improvement was found in the replacement of Red-Al by activated Zn in HOAc[27] in the reduction of **66**. Best results were obtained by using freshly prepared activated Zn[28] and **153** was obtained in a yield of 90% (Scheme 5.5). A further characteristic of the use of Zn/HOAc instead of Red-Al is that acetate groups are not reduced. $LiAlH_4$[29] also reduces nitrile **66** to **67** but the yield (64%) is lower than in case Red-Al or Zn/HOAc[26] are used. Chlorination of **153** with NCS leads to chloride **154** in 95% yield and subsequent treatment of **154** with NaOMe in MeOH gives tomatidenol (**4**) in 95% yield[2-9].

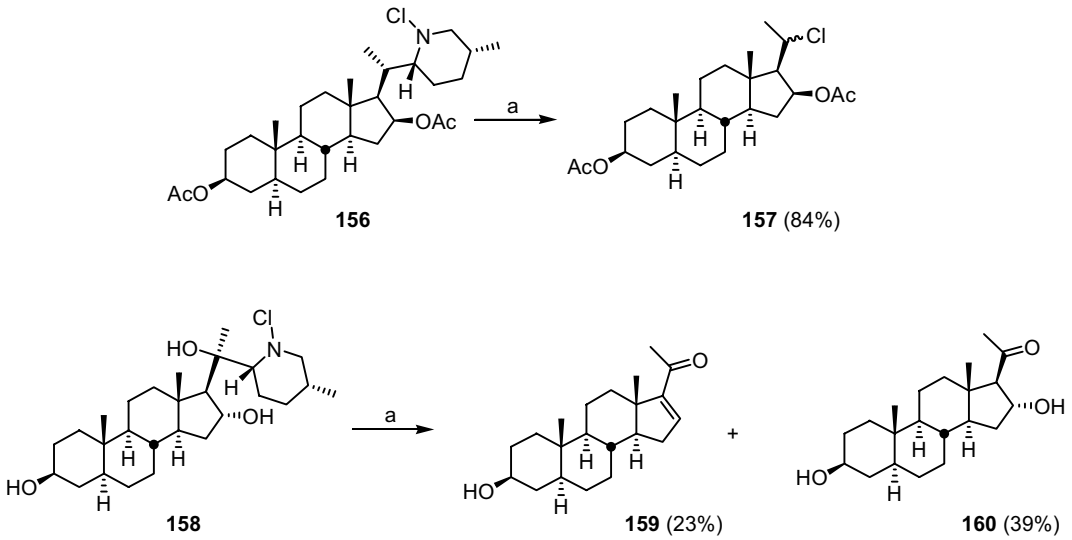


a) Zn, HOAc, H_2O; b) NCS, CH_2Cl_2; c) NaOMe, MeOH

Scheme 5.5

A possible alternative for a shorter route from **154** to DPA (**64**) is degradation via irradiation of halogenated pregnane derivates as demonstrated for **156**[30] and **158**[31] (Scheme 5.6). Although these irradiation routes give fast access to the pregnane derivatives **157**, **159**, and **160**, it will be difficult to apply irradiation on a large scale in industry. Attempts to perform the UV-irradiation

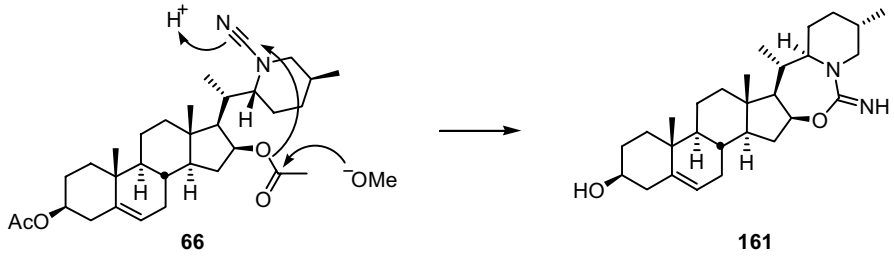
reaction with **154** gave decomposition of the compound even before the reaction was started[30]. The only difference is the $\Delta^{5,6}$ bond, which probably reacts with the acid leading to a reactive intermediate which degrades further. Therefore this route has not been investigated



a) CF_3CO_2H, hν, argon

Scheme 5.6

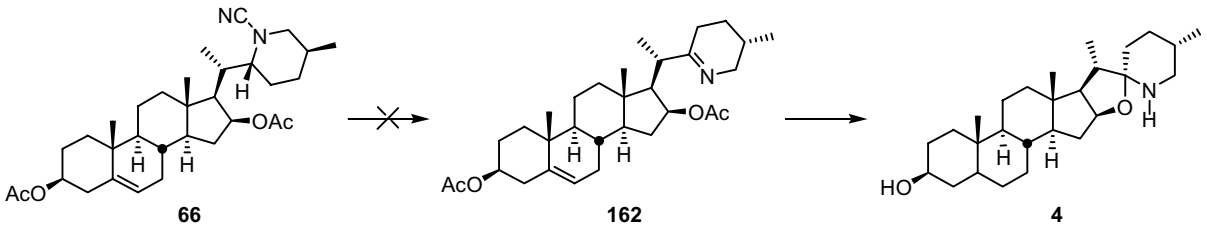
In the search for a shorter route toward DPA (**64**) from solanidine (**1**) the introduction of a $\Delta^{22(N)}$ double bond, as in **155**, is essential for the formation of tomatidenol (**4**). The chlorination/ dehydrochlorination process is an economically unfavorable two-step process and direct introduction of the $\Delta^{22,N}$ double bond would be more efficient. The shortest route from nitrile **66** to tomatidenol (**4**) would be a direct conversion. So, instead of the HCl elimination, elimination of HCN, which is also a fairly good leaving group, was attempted. Treatment of **66** with NaOMe did not afford **4**. Instead a single product was isolated and identified as compound **161**. A mechanistic explanation for the formation of **161** is depicted in Scheme 5.7. The alcoholate resulting after saponification of the C16-acetate attacks the cyanide group thereby forming an imidocarbamate[32].



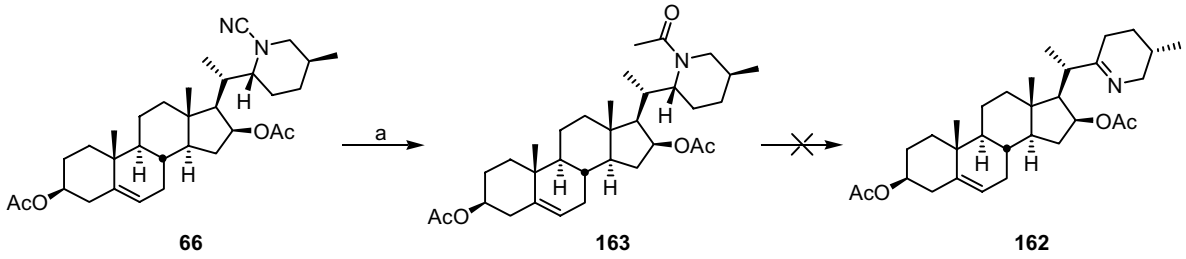
Scheme 5.7

Another shortcut would be the conversion of **66** to imine **162**, which then can be converted to **4** (Scheme 5.8). However, attempts to introduce the $\Delta^{22(N)}$ double bond with CAN[33] or MnO_2[34,35] were unsuccessful.

Another option would be conversion of nitrile **66** to amide **163**, followed by oxidation to **162** (Scheme 5.9). The amide **163** could be obtained in 95% yield by treatment of nitrile **66** with Zn in anhydrous HOAc[1,36].



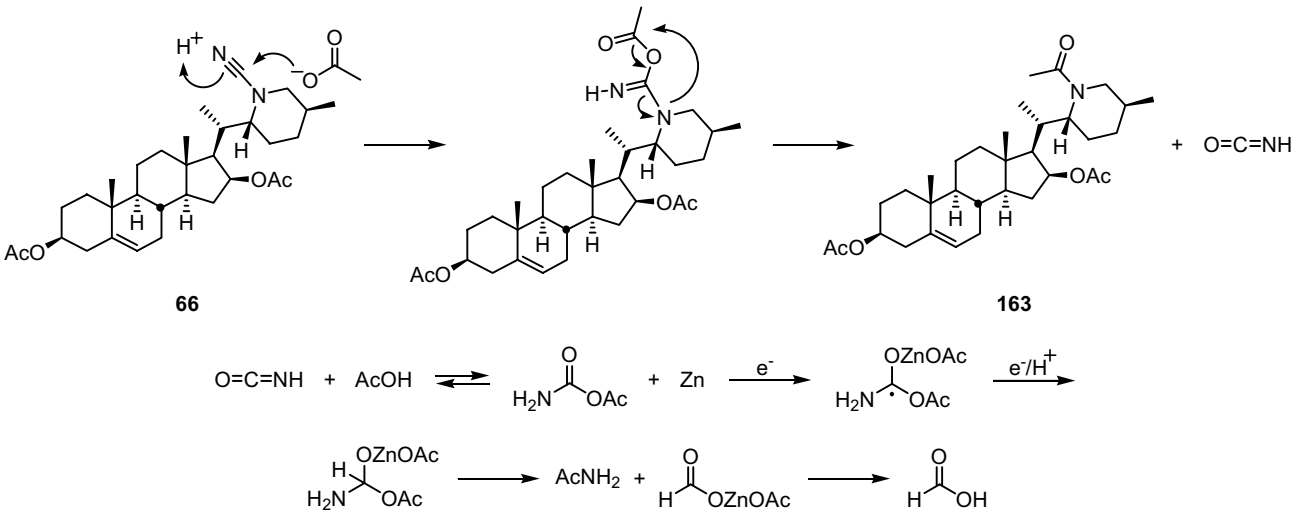
Scheme 5.8



a) Zn, HOAc

Scheme 5.9

The mechanism proposed for this reaction is depicted in Scheme 5.10 and is shown to be an ionic mechanism[36]. It involves a preliminary HOAc addition to the cyano group, followed by an intramolecular transacetylation releasing isocyanic acid. The actual product is not isocyanic acid but formic acid, which is formed by reduction of isocyanic acid with Zn. The introduction of the double bond in **163** leading to **162** was attempted with CAN[33], MnO_2[34,35], and HCl(aq.)[37] but unfortunately without success.



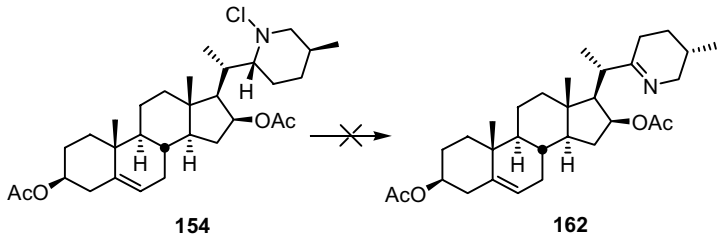
Scheme 5.10

As the preceding results indicated, imine **162** can only be formed by chlorination and subsequent dehydrochlorination with NaOMe but under these circumstances **162** reacts immediately further to tomatidenol (**4**). Therefore other ways to synthesize **162** have been investigated.

5.3 Studies to other useful shortcuts

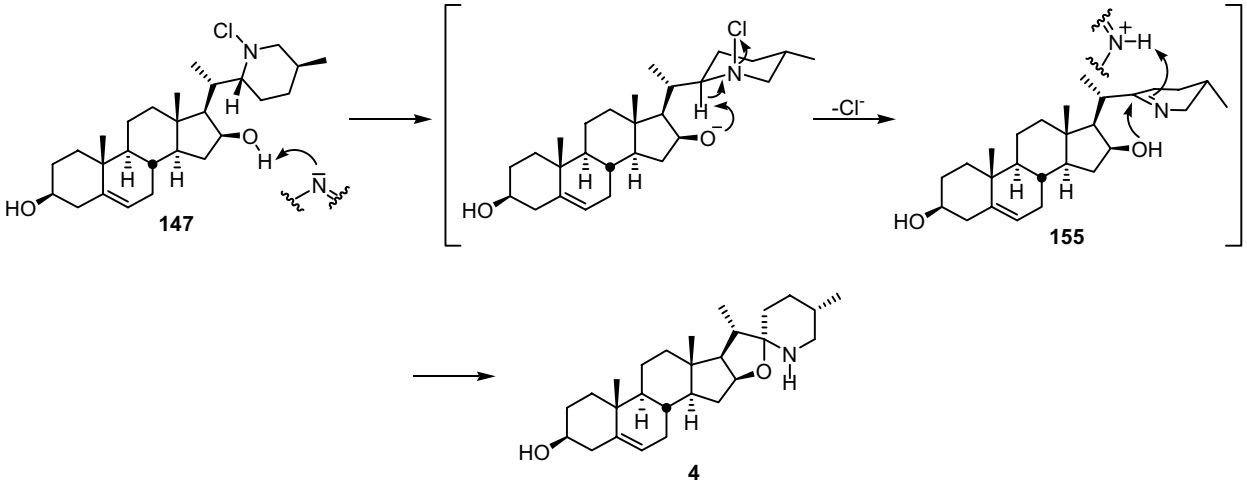
To continue the research for shorter ways to DPA (**64**), it seemed worthwhile to investigate

the usefulness of **162** as an intermediate. To achieve the formation of the $\Delta^{22(N)}$ bond and to prevent further reaction to tomatidenol (**4**), it will be necessary to maintain the acetate group at C16. Therefore **154** was treated with several bases (NaOAc/EtOH[38], K_2CO_3/DMF[39], LiBr/Li_2CO_3/DMF[39], NaOMe/toluene, *t*-BuOK[40], Et_3N[41], Et_2NLi[42], KHMDS[42,43], NaH/DMSO[44]) but all attempts were unsuccessful (Scheme 5.11).



Scheme 5.11

Treatment of **154** with other reagents able to eliminate HCl (Zn(Cu)/toluene[45] and $BF_3{\cdot}Et_2O$[46]) only gave recovery of the starting material. It thus proved to be impossible to eliminate HCl from **154**, when an acetate group at C16 is present[9]. On the other hand, elimination of HCl proved to be relatively easy if a free hydroxyl group is present at C16 as the conversion of **147** to tomatidenol (**4**) in 73% yield with DBU[47] clearly demonstrated. The formation of tomatidenol (**4**) from **147** or **154** in the presence of NaOMe in MeOH supported this view[2,3,6,7,31] (see Scheme 5.1 and 5.4). A possible mechanism explaining these results is depicted in Scheme 5.12. DBU deprotonates the C16-hydroxyl group and the resulting alkoxy anion then intramolecularly eliminates HCl to give imine **155** which reacts further to tomatidenol (**4**).

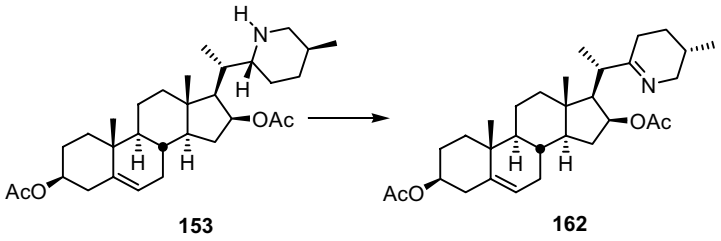


Scheme 5.12

Since the attempts to introduce the $\Delta^{22(N)}$ double bond in **154** by elimination were unsuccessful, another approach has been investigated. Oxidation of secondary ring amines is a common procedure and can be performed with various reagents. Thus, it might be possible to oxidize **153** directly to imine **162** (Scheme 5.13).

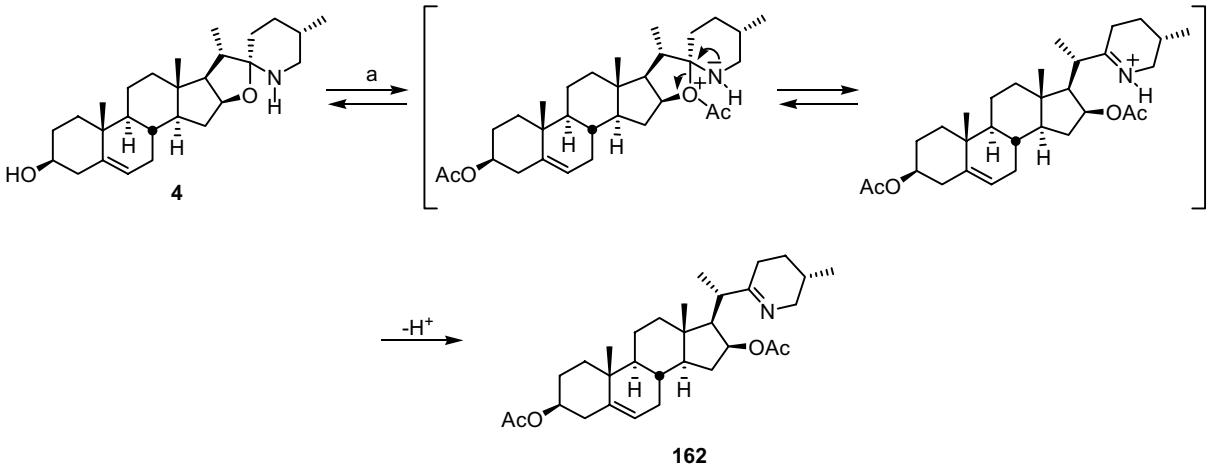
Unfortunately, oxidation of **153** with CrO_3/pyridine[48], $KMnO_4$[49], CAN[33], or $KMnO_4/Al_2O_3$[33] was in all cases unsuccessful and only the starting material **153** was recovered. Adam and Huong[50] described several successful spirosolane formations (tomatidine (**5**), solasodine (**3**), soladulcidine, and solasodenone) through oxidation of the secondary amine with MnO_2[51] followed by stereospecific cyclization[7,52]. However, several attempts with differently activated MnO_2[50,53] failed

to give any of the desired imine **162**. Only with MnO_2 freshly prepared according to the method of Attenburrow *et al.*[51] some imine **162** could be detected in the reaction mixture but the major product was still the starting material **153**.



Scheme 5.13

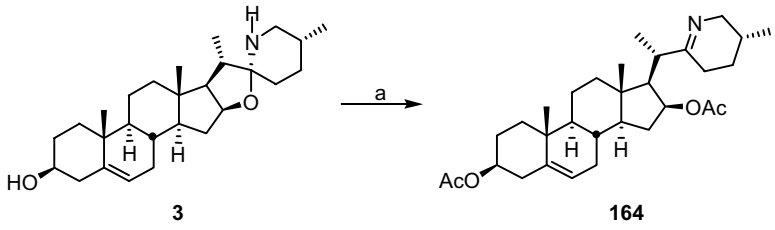
Although the diacetylated imine **162** could not be obtained from nitrile **66**, chloride **154**, or amine **153**, it can become available from tomatidenol (**4**) by treatment with $ZnCl_2$ and Ac_2O in HOAc[54] (Scheme 5.14).



a) $ZnCl_2$, HOAc, Ac_2O

Scheme 5.14

The availability of **162** gives the possibility to investigate two alternative degradation routes toward DPA (**64**). However, the limited amount of solanidine (**1**), obtained from the spray-dried protein mixture, and the fact that tomatidenol (**4**) is not commercially available gave severe problems for further research.



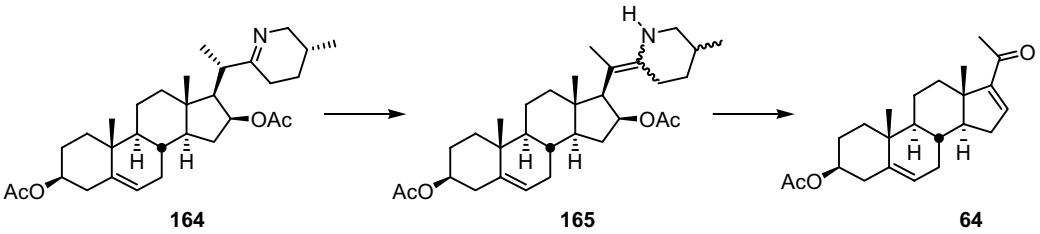
a) $ZnCl_2$, HOAc, Ac_2O

Scheme 5.15

On the other hand, solasodine (**3**) is commercially available and can be considered as an

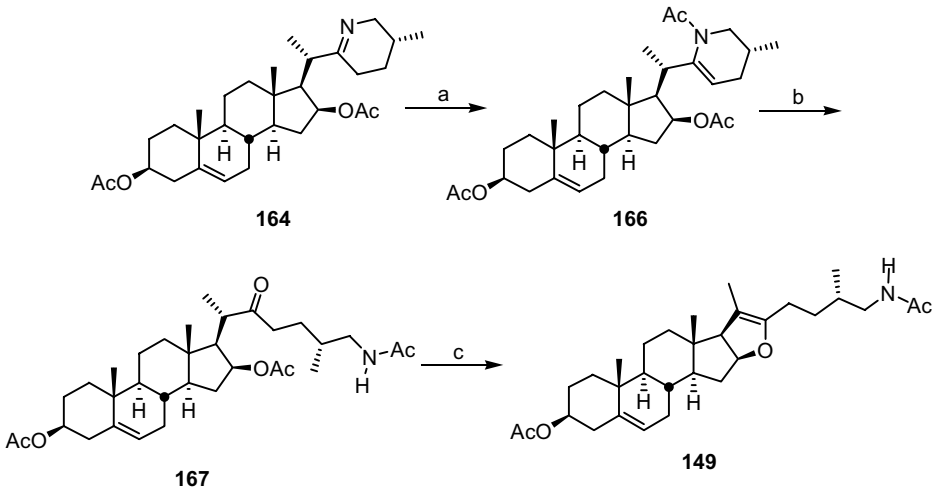
acceptable alternative due to its structural resemblance with **4**. Therefore solasodine (**3**) was used as a model compound to investigate two alternative degradation routes towards DPA (**64**). Any successful results could then be repeated with tomatidenol (**4**) itself. Treatment of solasodine (**3**), with $ZnCl_2$ in Ac_2O and HOAc in the same manner as previously described for tomatidenol (**4**) gave imine **164** in 96% yield[20] (Scheme 5.15).

The first route involves isomerization of the $\Delta^{22(N)}$ double bond to the $\Delta^{20,22}$ position (**164→165**) and oxidation of the $\Delta^{20,22}$ bond[55-59] followed by elimination of the C16 acetate (**165→64**) (Scheme 5.16).



Scheme 5.16

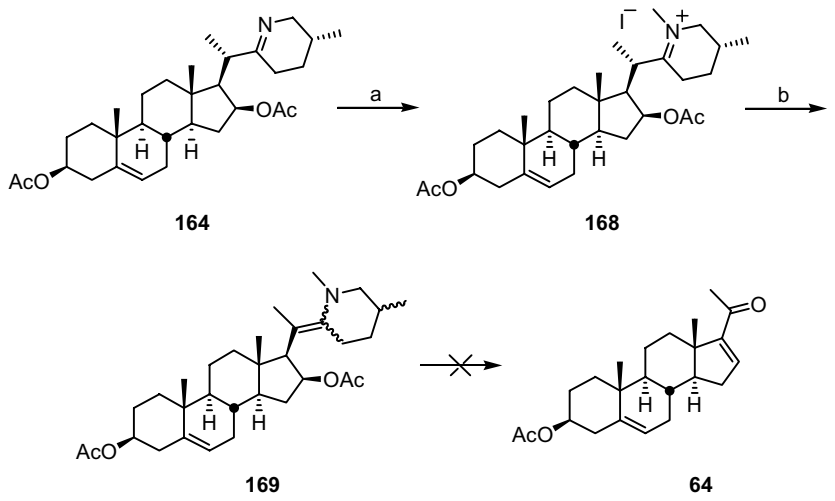
In the second route imine **164** has to be converted to compound **166** with an endocyclic $\Delta^{22,23}$ double bond[16,60]. According to the literature[16,60], hydrolysis of this $\Delta^{22,23}$ enamide proceeds well and gives ringopened product **167** with a keto functionality at C22 (Scheme 5.17). Subsequent deprotection of the C16 acetate results in ringclosure and elimination of the C22-hydroxyl function then gives **149**. This compound would also have been obtained starting from tomatidenol (**4**).



a) Ac_2O, pyridine, 4 days; b) HCl, HOAc; c) K_2CO_3, MeOH, Δ then HOAc

Scheme 5.17

In the first route isomerization of the endocyclic $\Delta^{22(N)}$ double bond to the exocyclic $\Delta^{20,22}$ position could only be achieved after methylation of **164** to **168**. Treatment of **168** with aqueous $NaHCO_3$ in acetone[20] than gave **169** in 80% overall yield (Scheme 5.18). Treatment of **164** with NaOH in EtOH under reflux conditions gave the thermodynamically more stable endocyclic $\Delta^{22,23}$ compound[20]. Oxidation of **169** was attempted with CrO_3/HOAc[18], $KMnO_4/Al_2O_3$[61], ozone[62], and O_2/CuCl[63] but all attempts were unsuccessful. Hydrolysis of **169** with HCl or HBr in HOAc was tried but failed as well.



a) MeI, benzene, acetone; b) $NaHCO_3$, H_2O, acetone

Scheme 5.18

The reasons for these failures are probably the same as described in Chapter 3 for the failure of the oxidation of 3-acetoxysolanidi-5,20-ene (**62**), namely the lack of reactivity of the $\Delta^{20,22}$ double bond together with steric hindrance. Mopac calculations on **169** indicate that it becomes difficult for other reagents to approach the $\Delta^{20,22}$ double bond due to C18, the F-ring itself, and the *N*-methyl, as exemplified by the optimized 3D-structure for one of the isomers of **169** (Figure 5.1). Similar negative results were found by Sato and Ikekawa in their attempt to oxidize **169** with an acetyl instead of a methyl group attached to the nitrogen[16].

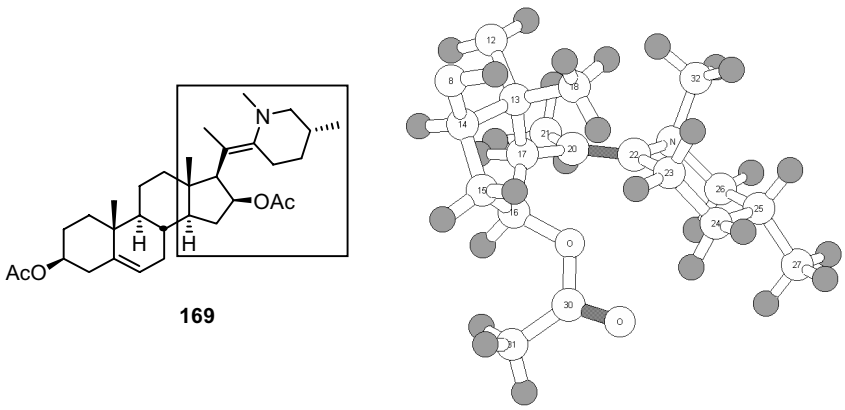
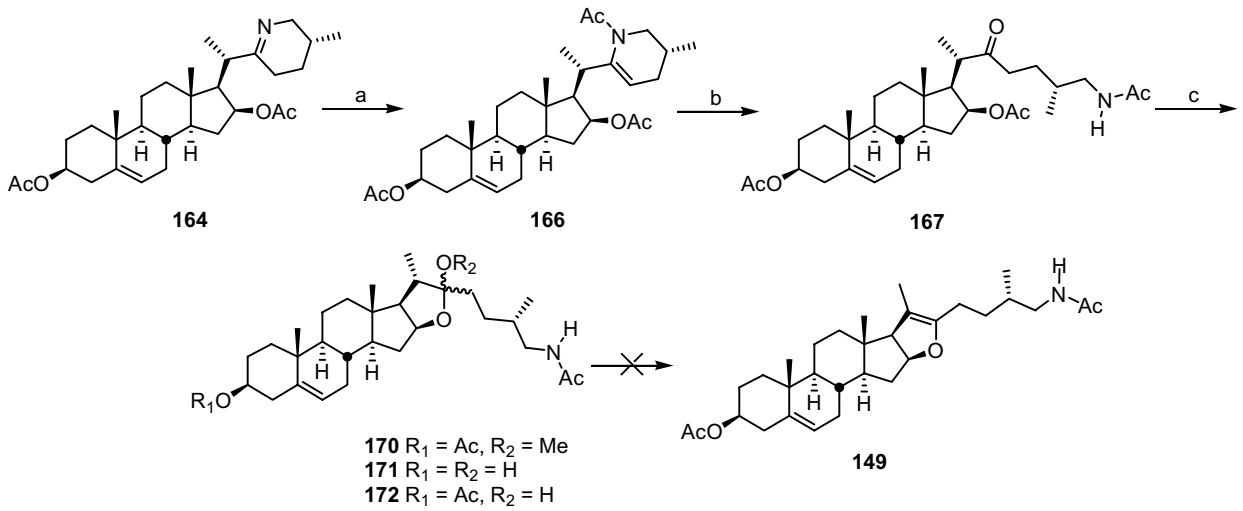


Figure 5.1

The second route for degradation of compound **164** is depicted in Scheme 5.19. Treatment of **164** with Ac_2O and pyridine acetylates the nitrogen and simultaneously shifts the $\Delta^{22(N)}$ double bond to the $\Delta^{22,23}$ position yielding the thermodynamic compound **166** quantitatively[16,60]. Mild hydrolysis of **166** with aqueous HCl and HOAc gives **167** in quantitative yield[16,60]. Deprotection of the C16 acetate with methanolic K_2CO_3 followed by addition of HOAc gave, instead of the expected **149**, a 82% yield of **170** [16]. A renewed attempt to eliminate MeOH from **170** with HOAc at reflux temperature also failed.

Treatment of **167** with K_2CO_3 in a mixture of water and EtOH overnight gave hemiacetal **171** in 78% yield. The formation of **171** in about the same yield was also achieved upon treatment of **167** with NaOH in dioxane. In this case the elimination of water from **171** with HOAc at reflux temperature[16] failed. These results are confirmed by Cambie *et al.*[64], who obtained **172** in 21% yield

as a byproduct next to a 72% yield of the main product **149**, after treatment of *N,O*-diacetylsolasodine with perchloric acid. Also the attempts of Cambie *et al.*[64] to dehydrate the C22 hydroxy group of **172** were unsuccessful. Apparently this dehydration isn't as facile as Sato and Ikekawa[16] suggested.



a) Ac_2O, pyridine, 4 days; b) HCl, HOAc;
c_1) K_2CO_3, MeOH; c_2) K_2CO_3, EtOH; c_3) NaOH, dioxane

Scheme 5.19

5.4 Conclusion

DPA (**64**) was synthesized from solanidine (**1**) in an overall yield of 30% following the routes depicted in Schemes 5.1, 5.2, and 5.3. An industrially applicable alternative for the Red-Al reduction has been found by using Zn/HOAc. The numerous attempts to shorten the degradation route as depicted in Schemes 5.1, 5.2, and 5.3 all failed.

The chlorination/dehydrochlorination step is necessary to synthesize tomatidenol (**4**). Attempts to eliminate the chloride without affecting the acetates were unsuccessful. The hydroxyl group at C16 is necessary to eliminate the chloride intramolecularly thereby forming imine **162**, which immediately reacts further to tomatidenol (**4**). This reaction can also be performed with DBU instead of NaOMe with the same yield.

Several unsuccessful attempts were undertaken to synthesize imine **162** from nitrile **66** and amine **153**. Treatment of nitrile **66** with NaOMe resulted in the formation of **161**. Due to the lack of tomatidenol (**4**) further research on the properties of imine **162** as a possibly useful intermediate in the synthesis of DPA (**64**) was done with imine **164** easily obtained from the commercially available solasodine (**3**). Isomerization of the $\Delta^{22(N)}$ double bond in methylated derivative **168** to the exocyclic $\Delta^{20,22}$ position gave compound **169**, but oxidation of **169** with several reagents failed. Isomerization of the $\Delta^{22(N)}$ double bond in the acylated derivative **164** to the endocyclic $\Delta^{22,23}$ position in **166** followed by oxidation of **166** to **167** and recyclization to the hemiacetals **170** and **171** proceeded smoothly. Elimination of the C22 hydroxyl in **171** was unsuccessful and not as easy as suggested in the literature. Despite all our efforts, shortening of the route from solanidine (**1**) via tomatidenol (**4**) to DPA (**64**) could not be accomplished.

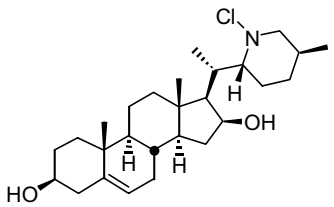
5.5 Experimental Section

5.1.1 General comments and materials[65]

All ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ unless stated otherwise. Solanidine (**1**), 3*β*-acetoxysolanidine (**58**), (3β,16α,20*S*)-16-bromo-20-[(2*R*,5*S*)-1-cyano-5-methylpiperidinyl]-pregn-5-en-3-yl acetate (**65**), and (22*S*,25*R*)-22,26-cyanoepiminocholest-5-ene-3*β*,16*β*-diol diacetate (**66**) were obtained as described in previous chapters. Solasodine was purchased from ResearchPlus Inc.

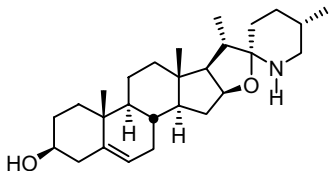
5.1.2 Procedures and spectral data

(3β,16β,20*S*)-20-[(2*R*,5*S*)-1-Chloro-5-methylpiperidinyl]pregn-5-ene-3,16-diol (147)



To a solution of **67** (606.6 mg, 1.47 mmol) in $CHCl_3$ (25 ml) was added dropwise a solution of NCS (204.0 mg, 0.16 mmol) in $CHCl_3$ (10 ml) at -10ºC over a period of 30 minutes. After 2 hours at room temperature, the clear solution was washed with water, dried, and evaporated *in vacuo* to obtain a white amorphous solid. Recrystallization from acetone/PE gave **147** (630.6 mg, 96%) as a white solid. ^{1}H NMR δ 0.85 (d, 3H, *J*= 6.4 Hz), 0.92 (s, 3H), 0.98 (s, 3H), 1.04 (d, 3H, *J*= 7.2 Hz), 3.49 (m, 2H), 4.33 (q, 1H, *J*= 6.1 Hz), 4.86 (br s, 1H, OH), 5.31 (d, 1H, *J*= 4.8 Hz); ^{13}C NMR δ 12.95 (q), 19.07 (q), 19.34 (q), 20.00 (q), 20.84 (t), 27.84 (t), 31.44 (d), 31.50 (t), 31.76 (t), 32.31 (t), 33.73 (d), 35.30 (t), 36.42 (s), 37.15 (t), 40.40 (t), 42.18 (t), 42.77 (s), 49.99 (d), 53.40 (d), 54.36 (d), 59.00 (d), 71.56 (d), 71.77 (d), 72.12 (t), 78.40 (d), 121.38 (d), 140.82 (s); MS *m/z* (r.i.) 413 (37, $[M\text{-}HCl]^+$), 385 (27), 271 (6), 139 (13), 138 (84), 114 (100), 113 (69), 98 (62), 61 (9), 43 (9); HRMS calculated for $C_{27}H_{43}NO_2$ 413.3294, found 413.3293. The NMR and mass data are in accordance with literature data[66].

(3β,22α,25α)-Spirosol-5-en-3-ol (Tomatidenol) (4)

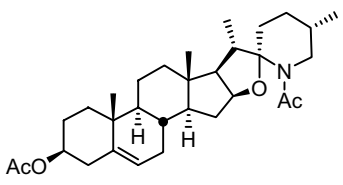


<u>*Method A:*</u> To a solution of Na (240.5 mg) in MeOH (20 ml) was added a solution of **147** (223.3 mg, 0.50 mmol) in MeOH (5 ml). After refluxing for 1 hour, the reaction mixture was evaporated *in vacuo* and H_2O (40 ml) was added. The mixture was extracted three times with $CHCl_3$ (100 ml), dried, evaporated *in vacuo*, and crystallized from acetone to yield **4** (174.1 mg, 85%) as a white solid. M.p. 236-238ºC (234-238ºC[14]); ^{1}H NMR δ 0.79 (s, 3H), 0.80 (d, 3H, *J*= 6.8 Hz), 0.92 (d, 3H, *J*= 6.5 Hz), 0.97 (s, 3H), 3.42 (m, 1H), 4.09 (q, 1H, *J*= 7.1 Hz), 5.28 (d, 1H, *J*= 4.9 Hz); ^{13}C NMR δ 15.84 (q, C21), 16.79 (q, C18), 19.40 (q, C19), 19.47 (q, C27), 20.92 (t, C11), 26.63 (t, C23), 28.43 (t, C24), 30.85 (d, C25), 31.42 (t, C2), 31.45 (d, C8), 32.13 (t, C7), 32.67 (t, C15), 36.70 (s, C10), 37.28 (t, C1), 39.93 (t, C12), 40.65 (s, C13), 42.15 (d, C4), 42.94 (d, C20), 50.02 (t, C26), 50.13 (d, C9), 56.06 (d, C14), 61.97 (d, C17), 71.44 (d, C3), 78.71 (d, C16), 99.00 (s, C22), 121.30 (d, C6), 140.99 (s, C5). MS *m/z* (r.i.) 413 (66, M^+), 386 (20), 385 (44), 384 (24), 187 (11), 155 (13), 138 (91), 114 (100), 113 (53); HRMS calculated for $C_{27}H_{43}NO_2$ 413.3294, found 413.3298. The NMR and mass data are in accordance with literature data[67].

<u>*Method B:*</u> To a solution of **147** (150.2 mg, 0.33 mmol) in dry CH_2Cl_2 (3 ml) and dry Et_2O (2 ml) was added DBU (21 µl, 0.14 mmol) at -10ºC. After 2 hours, the reaction mixture was allowed to warm to room temperature, evaporated *in vacuo*, and crystallized from acetone to yield **4** (100.8 mg, 73%) as a white solid. M.p. 235-238ºC (234-238ºC[14]). The NMR and mass data are identical to

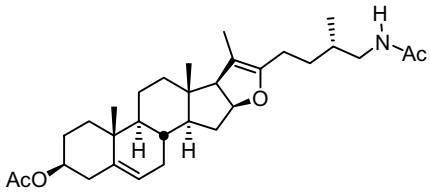
those obtained with method A.

(3β,22α,25α)-28-Acetylspirosol-5-en-3-yl Acetate (148)



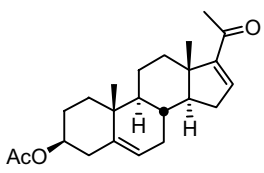
Tomatidenol (**4**) (174.1 mg, 0.42 mmol) was dissolved in a mixture of Ac_2O (2 ml) and pyridine (10 ml). After 24 hours, the reaction mixture was poured into ice-water. The suspension was filtered, and the filter cake was washed with water and recrystallized from acetone/H_2O to yield **148** (205.5 mg, 98%) as a white solid. M.p. 161-163ºC (163-165ºC[14]); ^{1}H NMR δ 0.81 (s, 3H), 0.84 (d, 3H, *J*= 5.4 Hz), 1.00 (s, 3H), 1.19 (d, 3H, *J*= 6.9 Hz), 2.01 (s, 3H), 2.08 (s, 3H), 4.17 (q, 1H, *J*= 6.6 Hz), 4.57 (m, 1H), 5.35 (d, 1H, *J*= 4.4 Hz); ^{13}C NMR δ 16.00 (q), 17.78 (q), 19.02 (q), 19.34 (q), 20.70 (q), 21.46 (t), 24.75 (q), 27.73 (t), 28.13 (t), 28.36 (t), 31.50 (d), 31.73 (d), 32.03 (t), 32.75 (t), 36.71 (s), 36.96 (t), 38.09 (t), 38.52 (d), 39.43 (t), 41.13 (s), 50.00 (d), 52.12 (t,), 56.40 (d), 64.47 (d), 73.89 (d), 78.84 (d), 101.03 (s), 122.29 (d), 139.81 (s), 170.41 (s), 170.59 (s). MS *m/z* (r.i.) 497 (72, M), 482 (100), 454 (15), 428 (37), 155 (31), 114 (15), 43 (9); HRMS calculated for $C_{31}H_{47}NO_4$ 497.3505, found 497.3508. The NMR and mass data are compared with literature data of *N*,*O*-diacetyltomatidine[66].

(3β,25*S*)-26-(Acetylamino)furosta-5,20(22)-dien-3-yl Acetate (149)



Compound **148** (105.8 mg, 0.16 mmol) was dissolved in HOAc (2.5 ml). After heating at reflux for 15 minutes, the reaction mixture was poured into water (20 ml). The suspension was evaporated *in vacuo* and dissolved in $CHCl_3$. The organic layer was washed with water, dried, and evaporated *in vacuo* to yield an off-white solid. Recrystallization from acetone/PE gave **149** (89.5 mg, 85%) as a white solid. M.p. 133-136ºC (133-137ºC[68]); ^{1}H NMR δ 0.86 (d, 3H, *J*= 6.6 Hz), 0.98 (s, 3H), 1.21 (s, 3H), 1.53 (s, 3H), 1.93 (s, 3H), 1.98 (s, 3H), 3.07 (t, 2H, *J*= 6.1 Hz), 4.55 (m, 1H), 4.69 (m, 1H), 5.33 (d, 1H, *J*= 4.3 Hz), 5.79 (m, 1H); ^{13}C NMR δ 11.63 (q), 13.95 (q), 17.52 (q), 19.31 (q), 20.91 (t), 21.43 (q), 23.08 (t), 23.35 (q), 27.70 (t), 31.16 (d), 31.59 (t), 32.12 (t), 32.77 (d), 34.09 (t), 36.66 (s), 36.95 (t), 38.05 (t), 39.40 (t), 43.20 (s), 45.28 (t), 49.91 (d), 54.90 (d), 64.10 (d), 73.84 (d), 84.27 (d), 103.94 (s), 122.27 (d), 139.67 (s), 151.34 (s), 170.15 (s), 170.56 (s). MS *m/z* (r.i.) 497 (100, M^+), 437 (29), 281 (27), 253 (31), 252 (29), 240 (22), 215 (32), 214 (46), 114 (36), 43 (47); HRMS calculated for $C_{31}H_{47}NO_4$ 497.3505, found 497.3511. The NMR and mass data are in accordance with literature data[64].

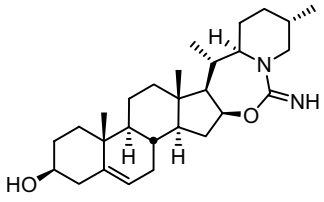
(3β)-3-Acetoxypregna-5,16-dien-20-one (Dehydropregnenolone Acetate, DPA) (64)



To a solution of **149** (89.5 mg, 1.94 mmol) in HOAc (5 ml) was added dropwise a solution of CrO_3 (44.8 mg, 4.54 mmol) in H_2O (0.2 ml) and HOAc (0.6 ml) in 15 minutes at 0ºC. After 2 hours, the reaction mixture was neutralized with aqueous Na_2CO_3 and saturated with NaCl. The mixture was extracted three times with Et_2O (15 ml), dried, and evaporated *in vacuo* to yield a yellow oil. This oil was dissolved in HOAc (2 ml) and refluxed for 1 hour, after which the acid was removed *in vacuo*. The residue was crystallized from MeOH to yield **64** (35.4 mg, 76%) as a white solid. M.p. 172-175ºC (172-174ºC[23]); ^{1}H NMR δ 0.88 (s, 3H, H18), 1.02 (d, 3H, H19), 1.99 (s, 3H, OC(O)*CH*$_3$), 2.23 (s, 3H, H21), 4.56 (m, 1H, H3), 5.35 (d, 1H, H6, *J*= 4.8 Hz), 6.68 (m, 1H, H16); ^{13}C NMR δ 15.70 (q, C18), 19.22 (q, C19), 20.60 (t, C11), 21.44 (q, OC(O)*CH*$_3$), 27.15 (q, C21), 27.69 (t, C7), 30.12 (d, C8), 31.51 (t, C2), 32.24 (t, C15), 34.56 (t, C12), 36.75 (s, C10), 36.84 (t, C1), 38.11 (t, C4), 46.04 (s, C13), 50.34 (d, C9), 56.31 (d, C14), 73.86 (d, C3), 121.99 (d, C6), 140.22 (s, C5), 144.55 (d, C16), 155.28 (s, C17), 170.60 (s, O*C(O)*CH$_3$), 196.91 (s, C20); MS

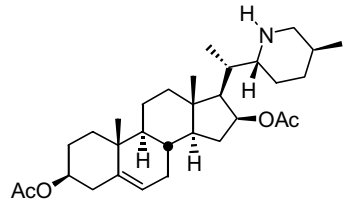
m/z (r.i.) 356 (0.1, M^+), 312 (3, $[M-CH_3C(O)H]^+$), 296 (100, $[M-HOAc]^+$), 281 (11), 145 (6), 43 (14); HRMS calculated for $C_{23}H_{32}O_3$ 356.2351, found 356.2342; calculated for $C_{21}H_{28}O_2$ 312.2089, found 312.2087; calculated for $C_{21}H_{28}O$ 296.2140, found 296.2140. NMR data are in accordance with literature data[69].

(3*S*,6a*S*,6b*S*,7a*S*,12*S*,14a*R*,15a*R*,15b*S*,17a*S*,17b*R*)-9-Imino-12,15,15b,17b-tetramethyl-2,3,4,6,6a,6b,7,7a,11,12,13,14,14a,15,15a,15b,16,17,17a,17b-icosahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*f*]pyrido[1,2-*c*][1,3]oxazepin-3-ol (161)



Treatment of compound **66** (121.3 mg, 0.23 mmol) in MeOH with NaOMe (2.2M) according to method a for the synthesis of solasodine (**3**) gave compound **161** (101.3 g, 81%) as a brown solid. ^{1}H NMR ($CDCl_3$) δ 0.77 (d, 3H, *J*= 6.1 Hz), 0.78 (s, 3H), 0.95 (s, 3H), 0.95 (d, 3H, *J*= 6.5 Hz), 4.63 (q, 1H, *J*= 7.6 Hz), 5.27 (d, 1H, *J*= 4.9 Hz); ^{13}C NMR δ 13.02 (q), 17.87 (q), 18.92 (q), 19.35 (q), 20.82 (t), 27.62 (d), 30.15 (t), 30.96 (d), 31.52 (t), 31.89 (t), 33.21 (t), 34.44 (t), 34.81 (d), 36.45 (s), 37.12 (t), 40.22 (t), 42.18 (t), 42.60 (s), 49.83 (d), 53.01 (d), 57.55 (t), 57.86 (d), 65.92 (d), 71.58 (d), 80.56 (d), 121.19 (d), 140.83 (s), 163.53 (s); MS *m/z* (r.i.) 440 (15, M), 422 (7), 397 (27), 393 (15), 322 (34), 204 (11), 150 (47), 123 (34), 98 (100); HRMS calculated for $C_{28}H_{44}N_2O_2$ 440.3403, found 440.3400.

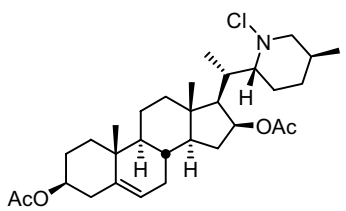
(3β,16β)-16-(Acetyloxy)-20-[(2*R*,5*S*)-5-methylpiperidinyl]pregn-5-en-3-yl Acetate (153)



Zn powder was activated as follows[28]: To a vigorously stirred suspension of Zn (2 g, 16 mmol) in HOAc (10 ml) was added a solution of HCl (2M, 10 ml), followed by an aqueous solution of $CuSO_4$ (5%, 0.4 ml). After about 1 minute, the reaction mixture was decanted and the Zn washed several times with HOAc.

To a suspension of freshly activated Zn (210.7 mg) in HOAc (1.3 ml) and H_2O (3.0 ml) was added **66** (107.6 mg, 0.21 mmol) and the reaction mixture was heated at reflux temperature. After 2 hours, the reaction mixture was filtered through Hyflow and evaporated *in vacuo*. The residue was dissolved in $CHCl_3$, washed with aqueous NaOH (0.05 M) and brine, dried, and evaporated *in vacuo* to obtain a white amorphous solid. Purification by column chromatography with $CHCl_3$/MeOH 9/1 followed by crystallization from EtOAc gave **153** (92.0 mg, 90%) as a white solid. M.p. 170-173ºC (172-173ºC[66]); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.77 (d, 3H, *J*= 6.5 Hz), 0.83 (s, 3H), 0.91 (d, 3H, *J*= 5.6 Hz), 0.93 (s,3H), 1.93 (s, 3H), 2.02 (s, 3H), 3.27 (dd, 1H, *J*= 2.7 and 11.7 Hz), 3.50 (s,1H, NH), 4.48 (m, 1H), 5.04 (td, 1H, *J*= 3.8 and 7.6 Hz), 5.25 (d, 1H, *J*= 4.4 Hz); ^{13}C NMR δ 12.23 (q), 12.89 (q), 18.42 (q), 19.14 (q), 20.53 (t), 21.25 (2q), 23.73 (t), 27.56 (t), 30.50 (d), 31.23 (d), 31.46 (t), 31.83 (d), 32.00 (t), 34.63 (t), 36.43 (s), 36.77 (t), 37.89 (t), 39.34 (t), 42.62 (s), 49.74 (d), 54.50 (d), 10.46 (d), 58.14 (t), 59.57 (d), 73.99 (d), 74.06 (d), 122.04 (d), 139.63 (s), 171.02 (s), 171.26 (s). The NMR data are in accordance with literature data[66].

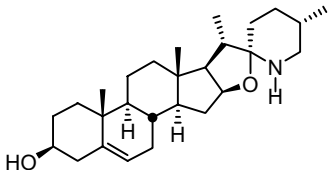
(3β,16β)-16-(Acetyloxy)-20-[(2*R*,5*S*)-1-chloro-5-methylpiperidinyl]pregn-5-en-3-yl Acetate (154)



To a solution of **153** (234.6mg, 0.47 mmol) in CH_2Cl_2 (20 ml) was added dropwise a solution of NCS (77.6 mg, 0.58 mmol) in CH_2Cl_2 (15 ml) at -10ºC in 30 minutes. After 30 minutes at room temperature, the clear solution was washed with water, dried, and evaporated *in vacuo* to afford a white amorphous solid. Recrystallization from $CHCl_3$/hexane gave **154** as white crystals (236.9 mg, 95%). ^{1}H NMR δ

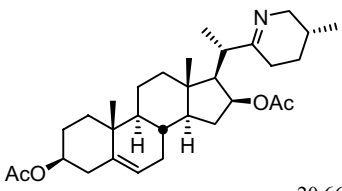
0.81 (d, 3H, *J*= 6.7 Hz), 0.84 (s, 3H), 1.00 (s, 3H), 1.10 (d, 3H, *J*= 6.9 Hz), 1.99 (s, 3H), 2.01 (s, 3H), 3.41 (d, 1H, *J*= 10.0 Hz), 4.57 (m, 1H), 5.30 (m, 1H), 5.33 (d, 1H, *J*= 4.8 Hz); ^{13}C NMR δ 12.43 (q), 18.91 (q), 19.30 (2q), 20.70 (q), 21.39 (t), 21.45 (q), 27.72 (t), 29.71 (t), 31.36 (d), 31.59 (t), 32.26 (t), 33.70 (d), 35.13 (t), 36.54 (s), 36.70 (d), 36.89 (t), 38.06 (t), 39.50 (t), 42.66 (s), 49.91 (d), 54.62 (d), 57.66 (d), 71.48 (t), 72.89 (d), 73.86 (d), 76.01 (d), 122.32 (d), 139.70 (s), 170.50 (s), 170.54 (s). The NMR data are in accordance with literature data[66].

(3β,22α,25α)-Spirosol-5-en-3-ol (Tomatidenol) (4)



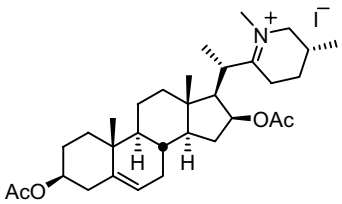
To a solution of Na (120.1 mg) in MeOH (60 ml) was added a solution of **152** (34.5 mg, 0.65 μmol) in MeOH (5 ml). After reflux for 1 hour, the reaction mixture was concentrated *in vacuo*, and the remaining residue was taken up in H_2O (10 ml). The mixture was extracted three times with $CHCl_3$ (25 ml), dried, and evaporated *in vacuo* to yield **4** (27.5 mg, 85%) as a white solid. M.p. 234-238ºC (134-138ºC[14]). The NMR and mass data are identical with those obtained in method A as previously described for the synthesis of tomatidenol (**4**).

(3β,16β)-16-(Acetyloxy)-20-[(5*R*)-5-methyl-3,4,5,6-tetrahydro-2-pyridinyl]pregn-5-en-3-yl Acetate (164)



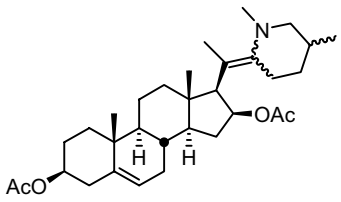
Solasodine (**3**) (8.81 g, 7.47 mmol) was dissolved in a mixture of Ac_2O (210 ml), HOAc (90 ml), and $ZnCl_2$ (30.12 g). After 16 hours, the mixture was poured into icewater and basified (pH 10) with NH_3. The resulting precipitate was filtered, washed with water, and recrystallized from MeOH to yield **164** (10.17 g, 96%) as pale yellow crystals. M.p. 189-193ºC (189-193ºC[20,66]); ^{1}H NMR (C_6D_6+CD_3OD) δ 0.84 (d, 3H, *J*= 6.4 Hz), 0.86 (s, 3H), 0.99 (s, 3H), 1.07 (d, 3H, *J*= 7.0 Hz), 1.95 (s, 3H), 2.00 (s, 3H), 3.56 (d, 1H, *J*= 16.7 Hz) 4.56 (m, 1H), 5.16 (m, 1H), 5.33 (d, 1H, *J*= 4.3 Hz); ^{13}C NMR δ 12.95 (q), 18.85 (q), 19.12 (q), 19.29 (q), 20.72 (t), 21.28 (q), 21.44 (q), 27.27 (d), 27.70 (t), 27.90 (t), 28.32 (t), 31.31 (d), 31.65 (t), 34.61 (t), 36.10 (s), 36.85 (t), 38.04 (t), 39.51 (t), 40.77 (d), 42.00 (s), 49.82 (d), 54.17 (d), 56.31 (d), 56.63 (t), 73.84 (d), 75.13 (d), 122.30 (d), 139.67 (s), 170.28 (s), 170.50 (s), 173.80 (s); MS *m/z* (r.i.) 497 (49, M), 454 (68), 438 (100), 422 (39), 125 (59), 111 (19); HRMS calculated for $C_{31}H_{47}NO_4$ 497.3505, found 497.3503. The NMR and mass spectral data are in accordance with literature data[66].

(3β,16β)-3,16-Bis(acetyloxy)-20-[(5*R*)-1,5-dimethyl-3,4,5,6-tetrahydro-2-pyridiniumyl]pregn-5-ene Iodide (168)



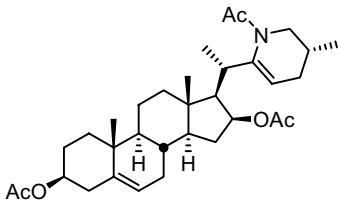
To a solution of **164** (1.07 g, 2.15 mmol) in benzene (50 ml) and acetone (100 ml) was added MeI (10 ml, mmol). After 3 hours reflux, the mixture was concentrated *in vacuo* and the remaining residue was dissolved in acetone. Precipitation with *n*-hexane and air-drying gave **168** (1.12 g, 82%) as a white solid. M.p. 266-268ºC (268-270ºC[20]); ^{1}H NMR δ 0.94 (s, 3H), 1.00 (s, 3H), 1.08 (d, 3H, *J*= 4.5 Hz), 1.59 (d, 3H, *J*= 6.8 Hz), 2.00 (s, 3H), 2.06 (s, 3H), 3.32 (q, 1H, *J*= 5.4 Hz), 3.78 (s, 3H), 4.33 (m, 1H), 4.56 (m, 1H), 5.13 (m, 1H), 5.32 (d, 1H, *J*= 4.4 Hz); ^{13}C NMR δ 13.30 (q), 17.88 (q), 18.01 (q), 19.29 (q), 20.70 (t), 21.45 (q), 21.87 (q), 24.47 (t), 27.10 (d), 27.64 (t), 29.05 (t), 31.20 (d), 31.51 (t), 34.96 (t), 36.51 (s), 36.83 (t), 37.07 (d), 37.99 (t), 39.66 (t), 43.47 (s), 45.52 (q), 49.10 (d), 53.61 (d), 54.89 (d), 62.87 (t), 73.68 (d), 74.54 (d), 121.76 (d), 139.84 (s), 169.85 (s), 170.57 (s), 192.26 (s).

(3β,16β)-16-(Acetyloxy)-20-[(5*R*)-1,5-dimethylpiperidinylidene]pregn-5-en-3-yl Acetate (169)



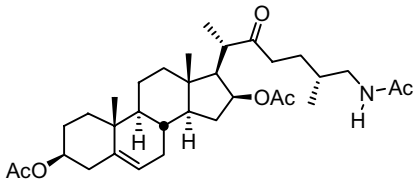
To a solution of **168** (695.3 mg, 1.09 mmol) in acetone (150 ml) was added an aqueous solution of $NaHCO_3$ (100 ml, 1 M). The resulting white suspension was filtered, washed with water, and air-dried yielding **169** (536.8 mg, 97%) as a white solid. M.p. 194-196ºC (194-197ºC[20]); ^{1}H NMR (C_6D_6) δ 0.93 (s, 3H), 0.95 (s, 3H), 1.21 (d, 3H, *J*= 6.8 Hz), 1.80 (s, 3H), 1.84 (s, 3H), 1.93 (br s), 2.53 (s, 3H), 4.54 (d, 1H, *J*= 2.8 Hz), 4.88 (quintet, 1H, *J*= 5.3 Hz), 5.35 (d, 1H, *J*= 4.4 Hz), 5.53 (q, 1H, *J*= 6.2 Hz); ^{13}C NMR (C_6D_6) δ 12.62 (q), 19.11 (q), 19.33 (q), 20.80 (t), 20.80 (2q), 21.60 (q), 26.51 (d), 27.96 (2t), 31.34 (d), 31.68 (t), 34.52 (t), 36.48 (s), 36.90 (t), 38.37 (2t), 39.21 (q), 39.76 (t), 42.09 (s), 49.88 (d), 54.22 (d), 58.96(d), 59.99 (t), 73.58 (d), 74.22 (d), 95.80 (s), 122.49 (d), 139.48 (s), 150.06 (s), 168.96 (s), 169.35 (s); MS *m/z* (r.i.) 512 (33), 511 (67), 468 (40), 452 (100), 127 (54), 126 (83), 98 (70); HRMS calculated for $C_{32}H_{49}NO_4$ 511.3662, found 511.3664. The NMR and mass spectra were compared to literature data of comparable compounds[60].

(3β,16β)-20-[(5*R*)-1-Acetyl-5-methyl-1,4,5,6-tetrahydro-2-pyridinyl]-16-(acetyloxy)pregn-5-en-3-yl Acetate (166)



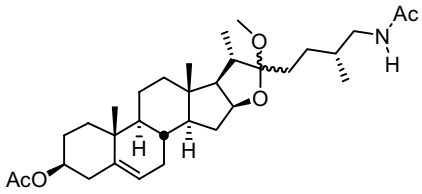
To a mixture of pyridine (10 ml) and Ac_2O (10 ml) was added **164** (1.01 g, 2.03 mmol) after which a white suspension was formed. After 4 days, the now clear solution was poured into icewater. The resulting precipitate was filtered, washed with water, dried, and recrystallized from Et_2O/hexane to yield **166** (1.09 g, 99%) as a white solid. M.p. 168-172ºC (166-169ºC[16]); ^{1}H NMR δ 0.84 (s, 3H), 0.88 (d, 3H, *J*= 6.8 Hz), 0.92 (s, 3H), 1.23 (d, 3H, *J*= 6.8 Hz), 1.94 (s, 3H), 1.99 (s, 3H), 2.12 (s, 3H), 3.14 (dd, 1H, *J*= 2.9 and 12.4 Hz), 4.52 (m, 1H), 5.04 (m, 1H), 5.15 (t, 1H, *J*= 3.7 Hz), 5.31 (d, 1H, *J*= 4.4 Hz); ^{13}C NMR δ 12.49 (q), 18.19 (q), 19.23 (2q), 20.66 (q), 21.19 (t), 21.41 (q), 23.66 (q), 27.64 (t), 28.79 (d), 30.77 (t), 31.31 (d), 31.51 (t), 33.30 (d), 34.96 (t), 36.47 (s), 36.83 (t), 37.98 (2t), 39.68 (t), 42.60 (s), 49.86 (d), 54.65 (d), 62.00 (d), 73.76 (d), 75.670 (d), 112.30 (d), 122.15 (d), 139.66 (s), 148.00 (s), 169.79 (s), 170.46 (s), 170.74 (s). The NMR and mass spectra were compared to literature data of comparable compounds[60].

(3β,16β,25*R*)-26-(Acetylamino)-16-(acetyloxy)-22-oxocholest-5-en-3-yl Acetate (167)



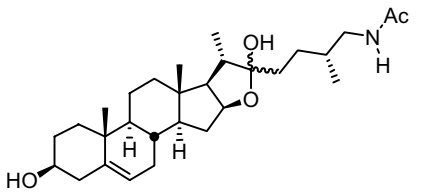
Compound **166** (1.09 g, 2.02 mmol) was added to a mixture of aqueous HCl (5 ml, 5M) and HOAc (20 ml). After 1.5 hours, the reaction mixture was diluted with water (200 ml) and neutralized with solid $NaHCO_3$. The mixture was extracted three times with $CHCl_3$ (150 ml), dried, and evaporated *in vacuo* to obtain a yellow oil. Crystallization from acetone/hexane gave **167** (1.12 g, 99%) as white crystals. M.p. 176-179ºC (175-178ºC[16]); ^{1}H NMR δ 0.84 (s, 3H), 0.86 (d, 3H, *J*= 7.5 Hz), 1.00 (s, 3H), 1.11 (d, 3H, *J*= 7.1 Hz), 1.93 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 3.06 (t, 1H, *J*= 5.9 Hz), 4.57 (m, 1H), 4.95 (m, 1H), 5.33 (d, 1H, *J*= 4.4 Hz), 5.96 (m, 1H, N*H*); ^{13}C NMR δ 13.18 (q), 16.76 (q), 17.73 (q), 19.24 (q), 20.65 (t), 21.12 (q), 21.39 (q), 23.30 (q), 27.03 (t), 27.64 (t), 31.19 (d), 31.54 (t), 32.80 (d), 34.73 (t), 36.49 (s), 36.89 (t), 37.97 (t), 38.04 (t), 39.32 (t), 41.84 (s), 43.52 (d), 44.94 (t), 49.69 (d), 53.87 (d), 60.02 (d), 73.78 (d), 75.58 (d), 122.24 (d), 139.58 (s), 169.88 (s), 170.36 (s), 170.51 (s), 213.45 (s); MS *m/z* (r.i.) 107 (11), 497 (7), 437 (6), 422 (14), 252 (84), 114 (100), 43 (10); HRMS calculated for $C_{33}H_{51}NO_6$ 107.3716, found 107.3720. The NMR and mass spectra were compared to literature data of comparable compounds[60].

(25*R*)-26-(Acetylamino)-22-methoxyfurost-5-en-3-yl Acetate (170)



To a solution of **167** (249.5 mg, 0.44 mmol) in MeOH (10 ml) was added an aqueous solution of K_2CO_3 (2 ml, 0.3 M). The reaction mixture was refluxed for 3 hours, cooled to room temperature, and then mixed with aqueous HOAc (1.5 ml, 20%). After 1 hour, the reaction mixture was neutralized by addition of aqueous $NaHCO_3$, extracted three times with $CHCl_3$ (25 ml), dried, and evaporated *in vacuo*. The remaining residue was crystallized from aqueous methanol to yield **170** (190.9 mg, 82%) as a white solid. M.p. 138-140ºC (138-140C°[64]); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.71 (s, 3H), 0.81 (d, 3H, *J*= 6.4 Hz), 0.92 (d, 3H, *J*= 5.0 Hz), 0.94 (s, 3H), 1.87 (s, 3H), 3.30 (s, 3H), 3.33 (m, 1H), 4.50 (q, 1H, *J*= 7.2 Hz), 5.23 (d, 1H, *J*= 3.8 Hz), 7.23 (m, 1H, N*H*); ^{13}C NMR δ 15.17 (q), 16.07 (q), 17.36 (q), 17.73 (q), 19.16 (q), 20.63 (t), 22.38 (q), 27.58 (t), 30.97 (d), 31.23 (t), 31.63 (t), 31.85 (t), 33.14 (d), 35.36 (t), 36.46 (s), 37.07 (t), 39.49 (t), 39.69 (d), 40.44 (s), 41.69 (t), 44.74 (t), 49.51 (q), 49.90 (d), 56.21 (d), 62.25 (d), 71.00 (d), 81.00 (d), 110.41 (s), 120.96 (d), 140.82 (s), 171.55 (s), 171.64 (s) . The NMR spectra were compared to literature data of comparable compounds[64].

***N*-[(3β,25*R*)-3,22-dihydroxyfurost-5-en-26-yl]acetamide (171)**



<u>*Method A:*</u> To a solution of **167** (128.5 mg, 0.23 mmol) in EtOH (10 ml) was added an aqueous solution of K_2CO_3 (1 ml, 1.5 M). After heating for 3 hours at reflux temperature, the reaction mixture was poured onto ice, allowed to come to room temperature overnight, extracted three times with $CHCl_3$ (50 ml), dried, and evaporated *in vacuo* to yield **171** as a colorless glass. Crystallization from acetone/hexane gave **171** (100.0 mg, 78%) as a white solid. M.p. 120-122ºC (119-122ºC[16]); ^{1}H NMR δ 0.73 (s, 3H), 0.84 (d, 3H, *J*= 6.6 Hz), 0.95 (s, 3H), 0.96 (d, 3H, *J*= 6.7 Hz), 1.91 (s, 3H), 3.44 (m, 1H), 4.53 (q, 1H, *J*= 7.2 Hz), 5.27 (d, 1H, *J*= 4.4 Hz), 5.87 (t, 1H, *J*= 5.9 Hz, N*H*); ^{13}C NMR δ 15.54 (q), 16.30 (q), 17.72 (q), 19.42 (q), 20.81 (t), 23.35 (q), 27.66 (t), 31.42 (d), 31.55 (t), 31.87 (t), 32.03 (t), 33.38 (d), 35.68 (t), 36.63 (s), 37.23 (t), 39.66 (t), 40.13 (d), 40.60 (s), 42.23 (t), 44.82 (t), 50.03 (d), 56.42 (d), 62.57 (d), 71.60 (d), 81.29 (d), 110.43 (s), 121.27 (d), 140.88 (s), 170.54 (s). The NMR spectra were compared to literature data of comparable compounds[64].

<u>*Method B:*</u> To a solution of **167** (249.5 mg, 0.44 mmol) in dioxane (10 ml) was added an aqueous solution of K_2CO_3 (6 ml, 0.3 M). After heating for 3 hours at reflux temperature, an aqueous solution of NaOH (1.5 ml, 2 M) was added and reflux was continued for 2 hours. After 16 hours at room temperature, the reaction mixture was concentrated *in vacuo*, dissolved in $CHCl_3$/H_2O 1/1 (50 ml), extracted three times with $CHCl_3$ (25 ml), dried, and evaporated *in vacuo* to yield a yellow foam. Recrystallization from acetone/hexane gave **171** (190.4 mg, 76%) as a white solid. The NMR data are identical to those obtained with method A.

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Chapter 6

Discusion

6.1 Discussion

The aim of the project was to develop an industrial method to convert the potato glycoalkaloids α-solanine (**6**) and α-chaconine (**7**) to DPA (**64**). Both glycoalkaloids contain solanidine (**1**) as aglycon and can be made available on ton scale. DPA is a key intermediate for the industrial synthesis of progesterone and cortisone derivatives.

The concept of the conversion of solanidine (**1**) to a nitrogen-free steroid is not new and was already recognized around 1930. Attempts were made to open the E- or F-ring of solanidine (**1**) via the Von Braun reaction[1-4], the Hofmann degradation[5,6], and the *N*-oxide[7] (demissidine *N*-oxide). Since that time a lot of research has been carried out on this subject but so far no satisfactory industrial method has been developed. The reason for this failure is mainly due to the poor availability of the starting material, the lack of reactivity of this rigid molecule, and the toxicity and hazardous nature of the reagents used.

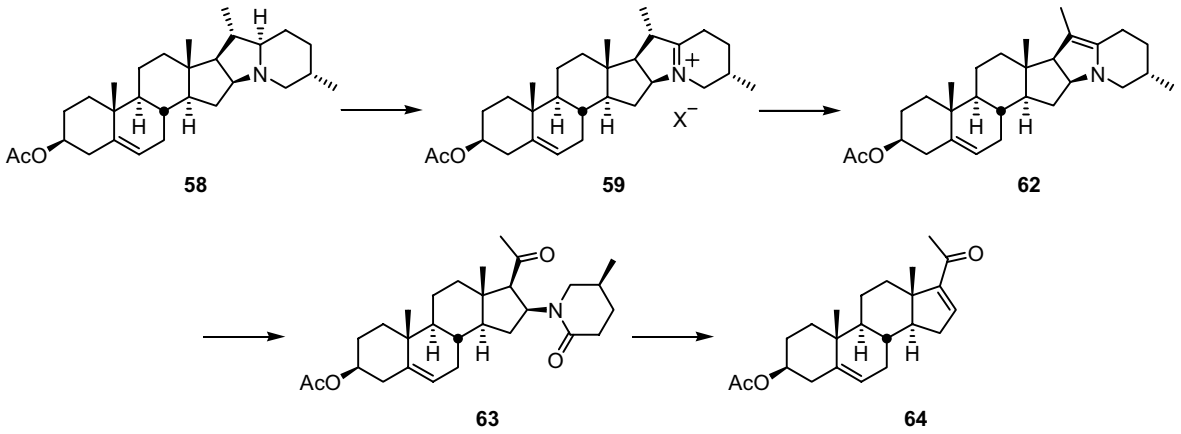
In order to implement a newly developed method into an existing industrial route there are a number of other conditions that have to be taken into account. Economic reasons will be the determining factor for implementation of the developed synthetic route as a replacement for the existing routes to DPA (**64**). As long as the price of diosgenine (**76**), the present starting material for the synthesis of DPA (**64**), is reasonably low, alternatives will not be implemented. At the moment almost all diosgenine (**76**) is isolated from a plant cultivated in China. In the future prices may rise due to the increasing demand of the booming Chinese internal market and, as a consequence, the surplus of diosgenine will decrease through which export prices will increase. This will improve the

position and possibilities of alternative starting materials.

Another important factor is the availability of the starting material, e.g. the potato glycoalkaloids **6** and **7**. In Chapter 2 a short description is given how these glycoalkaloids can be concentrated in the protein fraction, isolated by a fairly simple method, and then hydrolyzed to obtain solanidine (**1**). This method is suitable for laboratory scale production but for industrial scale production there are some drawbacks. The amount of water is probably the biggest problem because the glycoalkaloids are present in a very diluted solution. A solution may be the use of a membrane to separate the glycoalkaloids from water. Another option is the direct conversion of the glycoalkaloids to solanid-4-en-3-one (**87**) by microorganisms[8]. This is briefly mentioned in Chapter 2 but it could be of advantage for the isolation process because solanid-4-en-3-one (**87**) has a much lower solubility in water and will precipitate easier than the glycoalkaloids. Direct isolation of solanid-4-en-3-one (**87**) from the protein fraction would also make the protection of the hydroxyl group at C3 in solanidine (**1**) superfluous, but whether this is a real advantage depends on the ease by which further degradation of **87** will proceed.

The obvious handle to open the E- or F-ring is the tertiary nitrogen atom and most methods to degrade a tertiary amine have been applied on solanidine without much success. Only the Von Braun reaction[6,9,10]on 3-acetoxysolanidine (**58**) and the $Hg(OAc)_2$ and H_2O_2[11] oxidation have been used successfully until now.

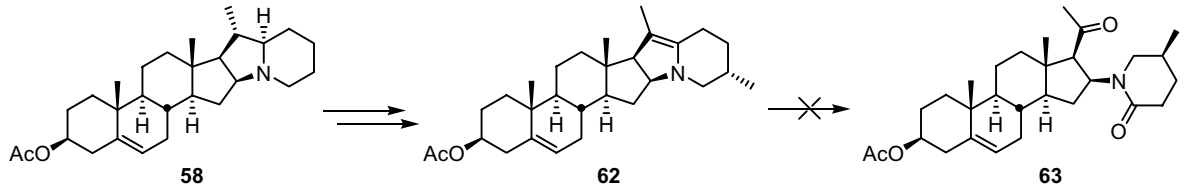
Our original research proposal for the conversion of solanidine (**1**) to DPA (**64**) started with the oxidation of **58** to **59** followed by isomerization (**59→62**), oxidation (**62→63**), and finally elimination (**63→64**) (Scheme 6.1).



Scheme 6.1

In literature three oxidation methods of **58** to **59** are described: electrochemical oxidation[12,13], $Hg(OAc)_2$ oxidation[9,14,15], and oxidation with NBS[11]. The electrochemical oxidation method works equally well as the $Hg(OAc)_2$ oxidation but was not regarded as a subject for further research. Although it can be performed well at laboratory scale, large scale implication may be difficult when experience with electrochemistry on industrial scale is lacking. When the research described in this thesis started (1997), an article by Gasi *et al.*[16] appeared describing the conversion of solanidine (**58**) to DPA (**64**) following the same strategy as depicted in Scheme 6.1. This had a major impact on our research because an important route in our proposal had already been studied and published. However, the overall yield of this route is poor, as a result of difficulties encountered in the last step. Another important fact is that $Hg(OAc)_2$, used for the oxidation of the tertiary amine, is not allowed for industrial use. Despite these facts, attempts were tried to improve the yields of the oxidation and elimination steps, and to obtain intermediates which could be useful for the identification of intermediates obtained by other methods. The first steps in this route proceeded

very well but we were unable to oxidize compound **62** to **63** (Scheme 6.2). Different reagents were tried but in our hands the $\Delta^{20,22}$ double bond in compound **62** resisted oxidation.



Scheme 6.2

Calculations showed that enamine **62** is not a real enamine and that its $\Delta^{20,22}$ double bond behaves as an isolated double bond. Together with the steric hindrance provided by C18 and the six-membered ring, this behavior makes the double bond difficult to oxidize. These findings were later on supported by the failure of oxidation of this $\Delta^{20,22}$ double bond in other, E-ring opened compounds (Chapter 4 and 5). Examples of the oxidation of the $\Delta^{20,22}$ double bond in similar compounds are rare and only known for the cholesterol-type compounds **145**[17], **149**[18], and **173**[19,20] under strongly oxidizing conditions (Figure 6.1).

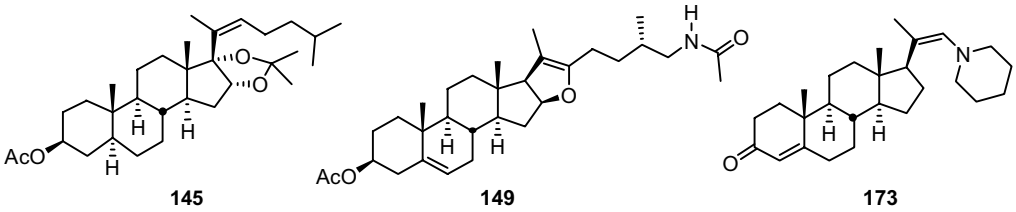
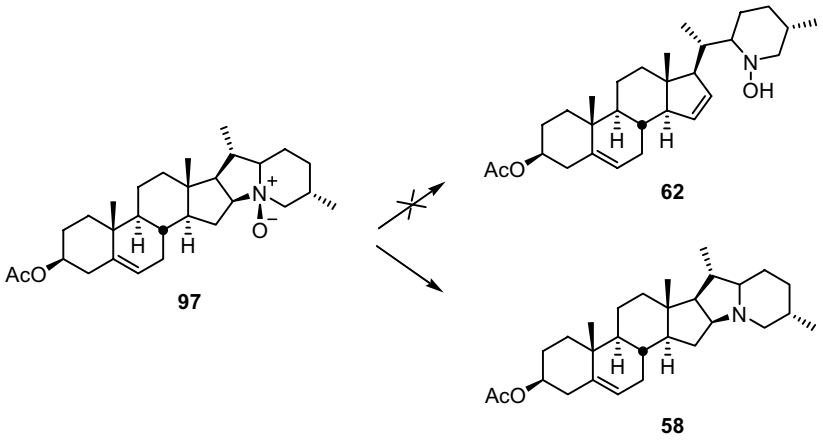


Figure 6.1

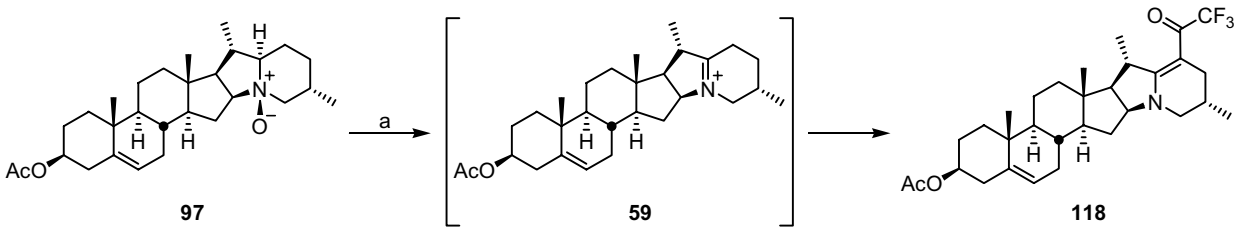
Due to these developments new ways for the ringopening had to be found, and the Cope and Polonovski reactions were studied first. In both reactions the corresponding solanidine *N*-oxide **59** is the starting material. The Cope reaction proceeds via a β-syn elimination while the Polonovski reaction proceeds via an α-anti elimination process. Treatment of **1** or **58** with *m*CPBA[21] or MMPP[22] gave mixtures of the corresponding *N*-oxide **96** or **97** and its epoxidized *N*-oxide **51** or **98**, respectively. Treatment of **58** with H_2O_2·urea[23] gave the desired *N*-oxide **97** in only 45% yield. Although this yield is not high, the *N*-oxide is the sole product formed and the starting material can be recovered. To avoid these epoxidation problems solanidine (**1**) was converted to solanidi-4-en-3-one (**87**)[24] and then oxidized with MMPP to the corresponding *N*-oxide **99**. This at least gave the opportunity to investigate the reactivity of the E,F-ring system in the Cope and Polonovski reactions.

Subjecting the *N*-oxides **96**, **97**, and **99** to the Cope reaction led to degradation of the starting material. In most cases deoxygenation was the main reaction with solanidine (**1**), 3-acetoxysolanidine (**58**), or solanidi-4-en-3-one (**87**) as isolated products. In Scheme 6.3 this process is given for **97**. The inflexibility of the rigid E,F-ring system is prohibitive for this reaction because the system can not adopt the required conformation for the Cope reaction.



Scheme 6.3

Subjecting the *N*-oxides to the Polonovski reaction using Ac_2O gave in most cases the competitive deoxygenation product as well. The new compound **118** was obtained when *N*-oxide **97** was treated with $(CF_3CO)_2O$ and *t*-BuOK (Scheme 6.4).



a) $(CF_3CO)_2O$, *t*-BuOK

Scheme 6.4

The unsatisfactory results from the $Hg(OAc)_2$ route and Cope and Polonovski reactions forced us to return to the "older" literature for the successful opening of the E-ring. The idea was to start with the Von Braun reaction using BrCN and then search for alternatives for this reagent. The Von Braun reaction gave the known ringopened product **65** together with a small amount (12%) of **126**. After formation of the initial ammonium ion, there is little difference in steric hindrance between the C-atoms around the N-atom and the bromide can attack from the less hindered rear-side at C16 as well as at C26. Relief of ring strain then drives the reaction to completion and opening the five-membered ring is preferred over ringopening of the six-membered ring.

At first sight, it was rather strange that no alternative for BrCN could be found, but calculations of the formal charge on the N-atom of the initial ammonium ions revealed a possible reason. This formal charge has to be large enough to weaken the C-N bond to such a degree that it can be broken through attack of a nucleophile. Thus the formal charges on the N-atom of some ammonium ions were calculated (Figure 6.2), and apparently this formal charge has to be at least +0.70 before a reaction can take place as can be seen for the *N*-trifluoroacetoxy ammonium ion. In this particular case, using $(CF_3CO)_2O$ in the presence of *t*-BuOK, the C-N bond is not broken due to the weak nucleophilic character of the $CF_3CO_2^-$ anion and as a result elimination takes place to iminium ion **59** (Scheme 6.4). In our investigations no alternatives for the Von Braun reaction could be found and until now BrCN is the only option to open the E,F-ring system.

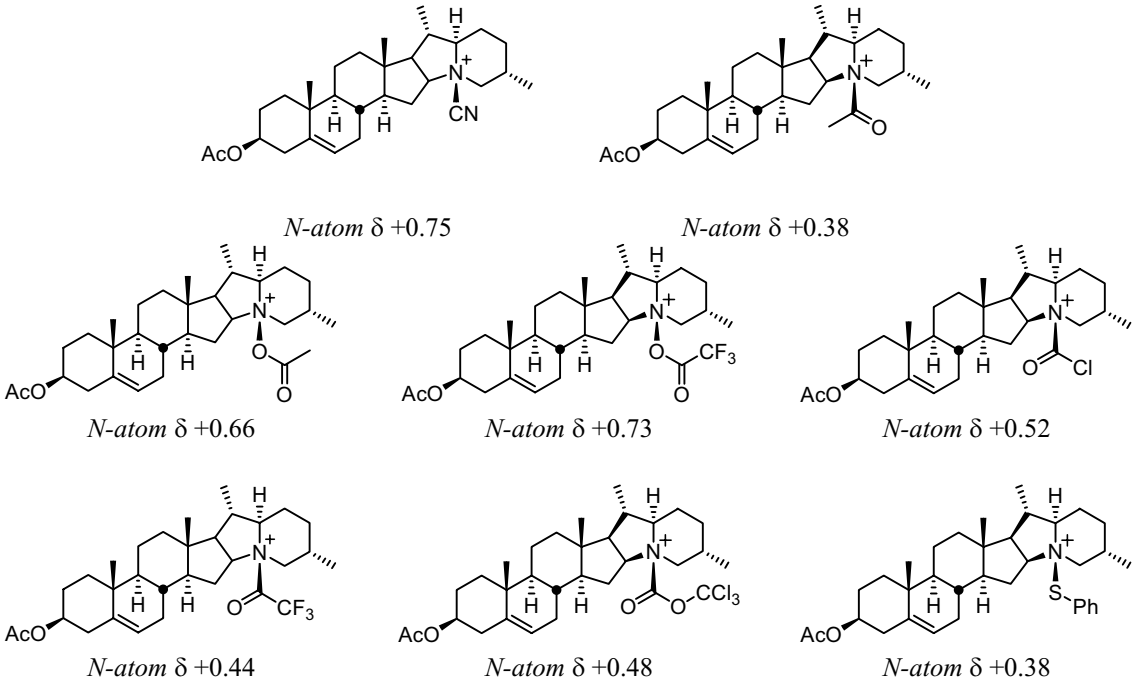
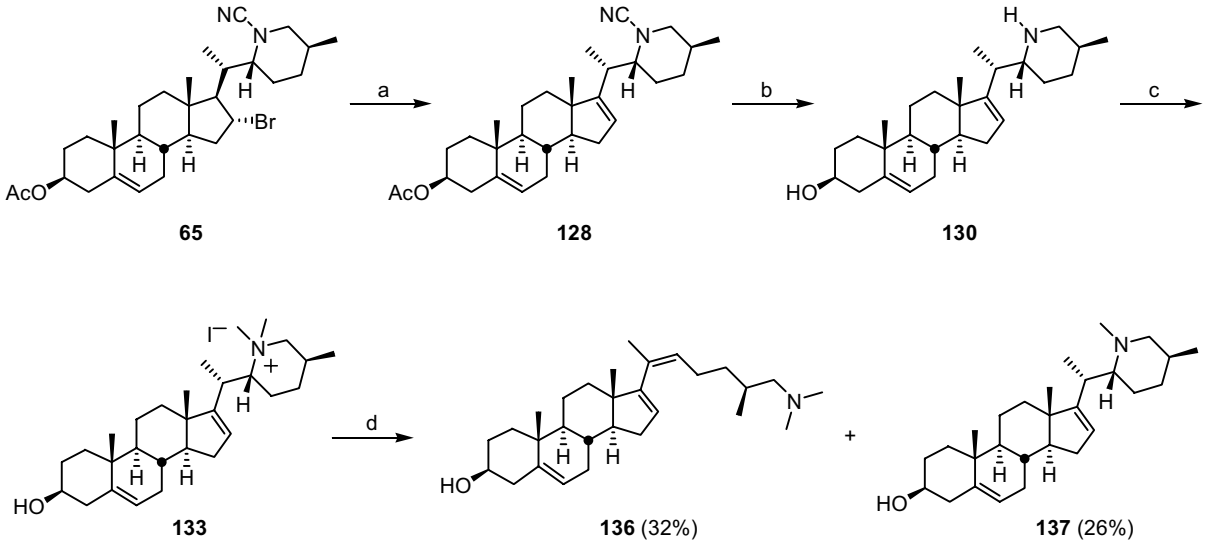


Figure 6.2

Further degradation of **65** should lead to a carbonyl at C20 and one way to do this, is the oxidation of the $\Delta^{20,22}$ double bond, although some difficulties can be expected (see Scheme 6.2). The $\Delta^{20,22}$ double bond can be introduced *via* Hofmann degradation, but this reaction has been only moderately successful in the formation of triene **136** (Scheme 6.5). This triene could be obtained in 32% yield and further protection and deprotection steps would be necessary for its conversion to DPA (**64**). Ultimately an industrially unacceptable long route would be the result, but **136** remains an interesting compound for further research because it contains 5 functionalities with different reactivities.

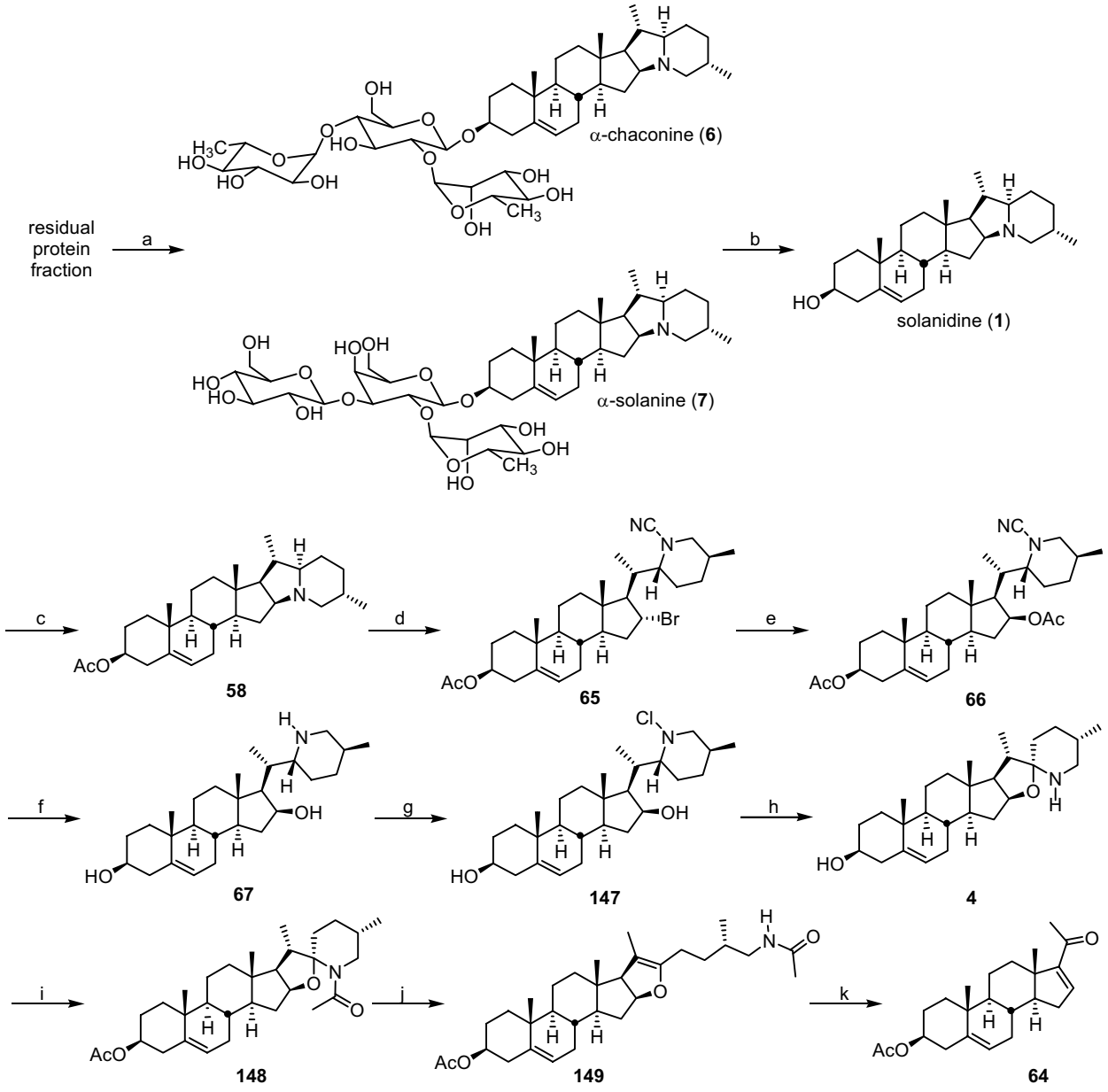


a) *s*-collidine, Δ; b) Red-Al, toluene, Δ; c) MeI, Na_2CO_3, H_2O; d) LDA, THF, -78ºC

Scheme 6.5

Due to the moderate results of the Hofmann degradation, attempts toward a better route to

DPA (**64**) were investigated with tomatidenol (**4**) as an intermediate. The synthesis of tomatidenol (**4**) from solanidine (**1**) was already known but the complete synthesis from solanidine (**1**) to DPA (**64**) in one sequence has never been described in literature. For actual application in industry this route is relatively long (Scheme 6.6). It takes 2 steps, isolation and hydrolysis, to obtain solanidine (**1**) from the residual protein fraction and 9 further steps for the conversion of **1** to DPA (**64**). These steps consist of acetylation of solanidine (**1**) to **58**, treatment with BrCN to **65**, substitution of the bromide by acetate (**66**), reduction of the nitril (**67**), chlorination of the free amine (**147**), ringclosure with NaOMe to tomatidenol (**4**), acetylation of **4** to **148**, HOAc treatment to open the F-ring (**149**), and finally oxidation and elimination of the resulting C16-ester to DPA (**64**).



a) NH_3OH; b) HCl, EtOH, Δ; c) Ac_2O, pyridine; d) BrCN, $CHCl_3$, Δ; e) KOAc, DMF, Δ; f) Red-Al, toluene, Δ; g) NCS, CH_2Cl_2, 0°C; h) NaOMe, MeOH, Δ; i) Ac_2O, pyridine; j) HOAc: k) CrO_3 in HOAc followed by Δ

Scheme 6.6

Integration of separate reaction steps to a one pot procedure would be of great benefit to implementation of this synthesis in industry, and the possibilities for shortcuts in this route have been investigated. The bottle neck in the degradation route depicted in Scheme 6.6 will be the use

of toxic BrCN through which special handling conditions are required. Furthermore, Red-Al is an expensive reagent to reduce nitrile **66**, although Red-Al can be replaced by the cheaper Zn, HOAc. Another difference between these two reagents is that Red-Al also reduces the acetates to hydroxyl groups, and Zn, HOAc leaves the acetates untouched.

The chlorination and dehydrochlorination steps are the only steps that possibly could be combined. Ringclosure to tomatidenol (**4**) by addition of DBU to the reaction mixture is only effective when a free hydroxyl group at C16 is present. This requires the use of Red-Al as a reducing agent to provide for this hydroxyl group at C16, or an extra saponification step with NaOMe after the Zn, HOAc reduction. The advantage using the cheaper Zn, HOAc will disappear through the necessity of this extra reaction step.

For the conversion of tomatidenol (**4**) to DPA (**64**) exactly the same method is used as for the degradation of diosgenine (**76**) to DPA (**64**). At this stage tomatidenol (**4**) then can be inserted in the diosgenine (**76**) degradation process to DPA (**64**). The extra steps remaining are those for the conversion of solanidine (**1**) to tomatidenol (**4**).

The attempted shortcuts all have been focussed on the introduction of the $\Delta^{22,N}$ double bond in the nitrile **66**, the amine **67**, and the diacetate of chloride **147**, *via* elimination or oxidation, because the resulting imine could be an important intermediate for further degradation to tomatidenol (**4**) or DPA (**64**). Treatment of **66** with NaOMe would lead to the most direct synthesis of tomatidenol (**4**), but instead of elimination of the nitrile group the C16-alkoxy ion attacked the nitrile group leading to the formation of compound **161**. Attempts to oxidize compounds **67** and **163** to the corresponding imines using several mild oxidizing reagents unfortunately all failed, probably due to steric hindrance and/or lack of oxidizing power. Stronger oxidation reagents are not useful because they will also affect the $\Delta^{5,6}$ double bond. Therefore further research was concentrated on the elimination of HCl from the diacetate of **147**. Although many attempts with different elimination methods were investigated, all failed to afford the corresponding imine.

At the same time the reactivity of the imine as a useful intermediate in the synthesis of DPA (**64**) was investigated. The aim of this research was the synthesis of new intermediates which could be synthesized from one of the compounds in the conversion of 3-acetoxysolanidine (**58**) to tomatidenol (**4**). Isomerization of the $\Delta^{22,N}$ double bond to the $\Delta^{20,22}$ position followed by oxidation failed due to steric hindrance and the lack of reactivity of the double bond. This result is comparable with the failure to oxidize the $\Delta^{20,22}$ double bond in the $Hg(OAc)_2$ route. Isomerization of the $\Delta^{22,N}$ double bond to the $\Delta^{22,23}$ position followed by hydrolysis and ringclosure gave hemiacetal **170** or **171**, but all attempts to achieve elimination of the substituent at C22 failed, which shows that also this imine probably has few perspectives as intermediate.

Although the insight in the reactivity of the E,F-ring system of solanidine has increased, this has not yet led to a better synthesis of DPA starting from solanidine. The degradation of 3-acetoxysolanidine (**58**) to DPA (**64**) as depicted in Scheme 6.6 is at present the only available method available.

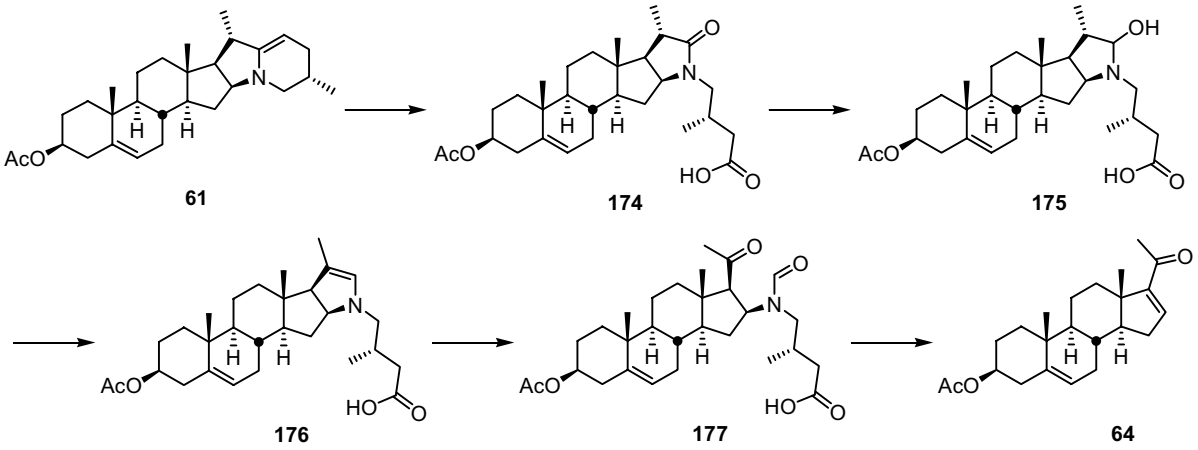
6.2 Outlook

With our current knowledge, the Von Braun reaction is the only reaction able to open the E-ring of 3-acetoxysolanidine (**58**). To find alternatives for the BrCN reagent, calculations can provide information whether the substituent on the nitrogen is capable to generate a formal charge large enough to break the C-N bond. A combination of the trifluoracetoxy group at the N-atom with a good nucleophile like the bromide ion may be worth a try out.

A reaction of *N*-oxide **97** (see Scheme 6.4) without excess of $(CF_3CO)_2O$ could give

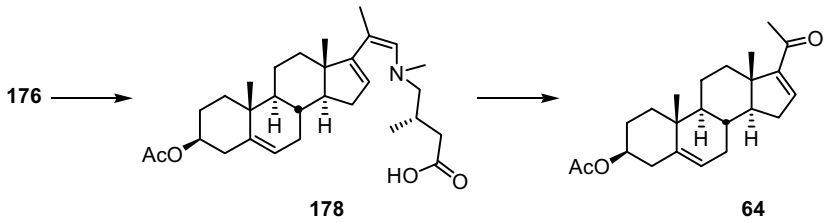
enamine **61** and oxidation of the enamine in this position might be easier than the oxidation of its $\Delta^{21,22}$ isomer. Perhaps the trifluoroacetyl compound **118** will open up also new possibilities. Both oxidations would lead to the formation of a lactam like **174** (Scheme 6.7). Enamine **61** also can be isolated from the oxidation of 3-acetoxysolanidine (**58**) with $Hg(OAc)_2$, which will enable an easy access to this compound to investigate its viability as an intermediate.

Schreiber and Horstmann[11] claimed to have synthesized an intermediate similar to compound **174** by treatment of demissidine (**2**) with NBS, followed by alkaline H_2O_2 oxidation (Chapter 3). Reduction of the keto functionality[25-28] to the corresponding alcohol **175** followed by elimination of water[29-31] then would give enamine **176** (Scheme 6.7).

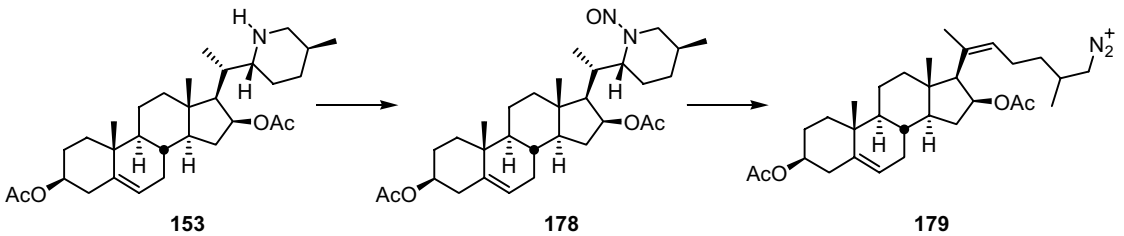


Scheme 6.7

The first possibility to degrade **176** to DPA would be oxidation of enamine **176** to **177** followed by Hofmann degradation to DPA (**64**). The second possibility could be Hofmann degradation on **176** to **178** followed by oxidation of **178** to DPA (**64**) (Scheme 6.8).



Scheme 6.8



Scheme 6.9

Finally it may be useful to try out the Bakker-Vrijhof[18] route with compound **153** as starting material (Scheme 6.9). In compound **153** no neighboring O-atom is present to stabilize possible cationic intermediates, but anchimeric assistance from the C16-acetate may compensate for this.

6.3 References

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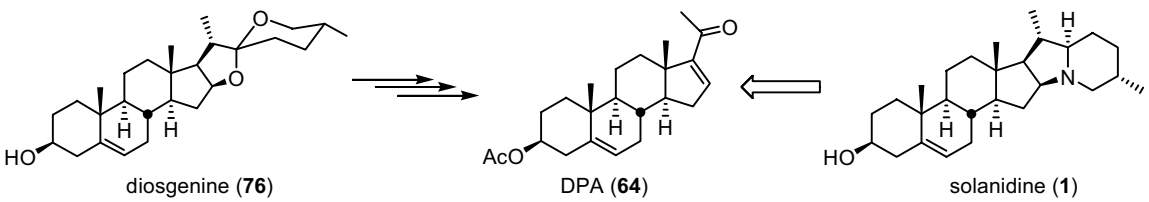
Summarry

Since the first structure elucidation of solanidine (**1**) about 70 years ago, there has been an interest to convert this aglycon to an intermediate suitable for the synthesis of steroids. A renewed interest in this conversion is stimulated by the rising prices of the present starting material diosgenine (**76**). Diosgenine is found in *Costus speciosus* grown in China and the availability and monopoly position of China for diosgenine (**76**) is of growing concern.

The use of an alternative starting material for diosgenine (**76**) has been taken under consideration by the possibility to isolate large amounts (ton scale) of potato glycoalkaloids from a waste stream of the potato starch production. In the potato process industry the potato glycoalkaloids α-chaconine (**6**) and α-solanine (**7**) are first concentrated in the protein fraction. The latter can be upgraded by removal of the glycoalkaloids which ends up in a waste fraction. A procedure is available to isolate these glycoalkaloids from this waste and in principle they can be made available as alternative starting material for the production of dehydropregnenolon acetate (DPA) (**64**) (Scheme 1).

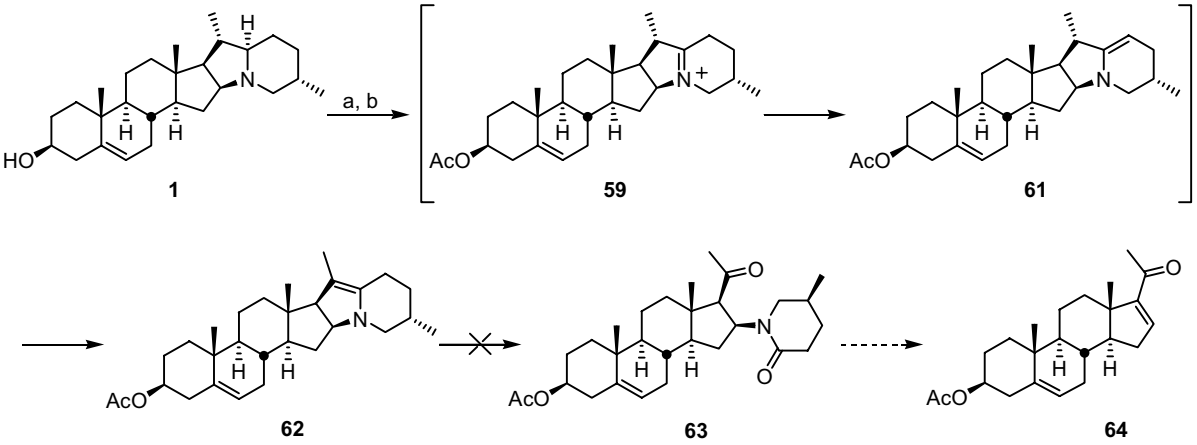


Scheme 1

To obtain the starting material for our research, an improved simple and effective method

has been developed for the isolation of the potato glycoalkaloids, α-chaconine (**6**) and α-solanine (**7**), from the residual protein waste fraction which was obtained from AVEBE. The hydrolysis of these glycoalkaloids to solanidine (**1**) has been accomplished by mild acid treatment (Chapter 2).

The conversion of solanidine (**1**) to DPA (**64**) was first tried by a recently published method using $Hg(OAc)_2$ as oxidation reagent. Although the first steps could be improved by combining the $Hg(OAc)_2$ oxidation (**1**→**61**) with the isomerization step (**61**→**62**) in a one pot synthesis (**1**→**62**), the crucial oxidation of the enamine (**62**→**63**) could not be accomplished (Scheme 2). Calculations showed that what was presumed to be the enamine **62** in fact behaves as an amine with an isolated double bond.

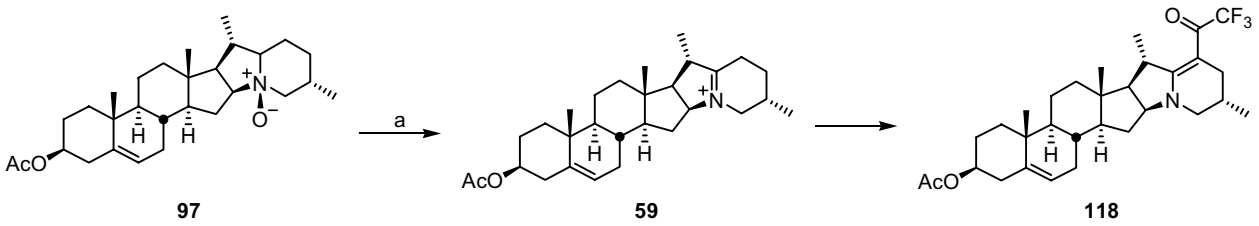


a) Ac_2O, pyridine; b) $Hg(OAc)_2$, acetone
overall yield 90%

Scheme 2

In the second attempt to convert solanidine (**1**) to DPA (**64**) the Cope and Polonovski reactions were studied and to do so it was necessary to synthesize solanidine *N*-oxide first. To avoid epoxidation problems solanidine (**1**) was converted to solanidi-4-en-3-one (**87**), which could be oxidized with MMPP to the corresponding solanidi-4-en-3-one *N*-oxide (**99**) in good yield. It turned out that the Cope reaction can not give the desired results because a 5-membered planar transition state necessary for the Cope reaction is not possible in these systems. In such situation deoxygenation, being a competitive process, takes over and solanidi-4-en-3-one was recovered in all attempts. The Polonovski reaction can be carried out with Ac_2O or $(CF_3CO)_2O$ under rather extreme conditions. With Ac_2O no reaction was observed for solanidine *N*-oxide (**96**) or 3-acetoxysolanidine *N*-oxide (**97**). Treatment of 3-acetoxysolanidine *N*-oxide (**97**) with $(CF_3CO)_2O$ yielded compound **118** as the result of isomerization and trifluoroacylation after initial formation of iminium ion (**59**) (Scheme 3). No further attempts were undertaken to convert compound **118** into DPA (**64**).

The third option was to open the E,F-ring system using the Von Braun reaction. Besides the desired major E-ring opened compound **65** also the minor F-ring opened compound **126** was isolated. Alternatives for the hazardous Von Braun reagent BrCN were investigated but without success. Calculations confirmed the unique properties of BrCN and led to the conclusion that this is one of the few reagents capable of opening ring E of solanidine. The next step was the conversion of **65** to DPA (**64**) via the Hofmann degradation. The C16 bromide of **65** was substituted with a hydrogen atom or an acetate group, or eliminated to give a $\Delta^{16,17}$ double bond. Subsequent methylation then provided the corresponding ammonium ions.

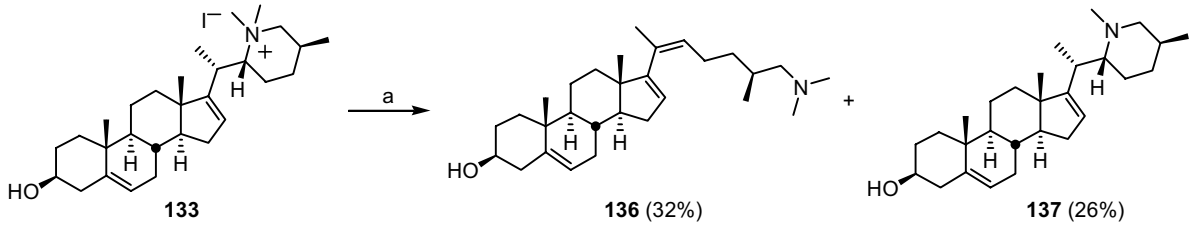


97 **59** **118**

a) $(CF_3CO)_2O$, *t*-BuOK, *t*-BuOH, 41%

Scheme 3

Only Hofmann degradation of ammonium salt **133** containing the $\Delta^{16,17}$ double bond with LDA yielded triene **136** and the monomethylated diene **137** which in principle can be remethylated and subjected again to Hofmann degradation (Scheme 4). In the other cases no reaction was observed, or the monomethylated compounds were isolated. Although Hofmann degradation gives the desired triene **136** further degradation to DPA (**64**) requires several protection and deprotection steps which are not very attractive for industrial application.



133 **136** (32%) **137** (26%)

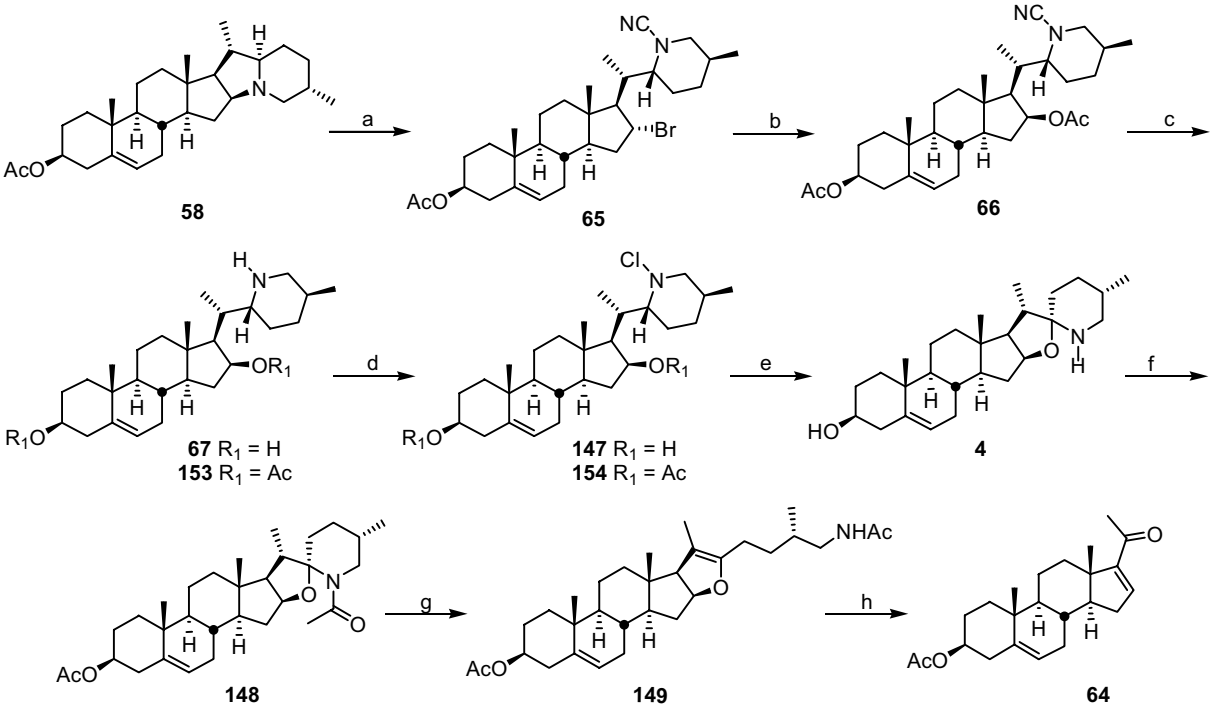
a) LDA, THF, -78ºC

Scheme 4

As a last option, the known conversion of solanidine (**58**) to spirosolane compounds followed by conversion to DPA (**64**) has been investigated. Although all individual steps of this route are known, it has never been published as one route. Therefore compound **65** was converted to tomatidenol (**4**) by substitution of the bromide (**65→66**), reduction of the nitril (**66→67** or **66→153**), chlorination of the secondary amine (**67→147** or **153→ 154**), and dehydrochlorination with base (**147→4** or **153→4**) (Scheme 5). Tomatidenol (**4**) was then converted to DPA (**64**) in an overall yield of 30% through acetylation of the secondary amine and free hydroxyl group (**4→148**), ringopening and elimination upon treatment with HOAc (**148→149**), and finally oxidation and elimination (**149→64**).

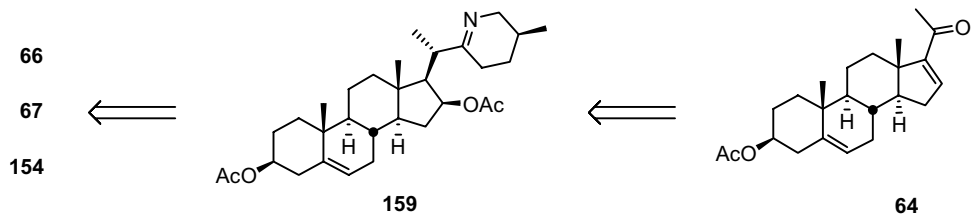
To compete with existing industrial processes this route should be shortened and expensive reagents should be avoided. Shortcuts were attempted to convert compounds from the first part of the route (3-acetoxysolanidine (**58**) to tomatidenol (**4**)) to compounds from the second part of the route (tomatidenol (**4**) to DPA (**64**)).

Replacement of the expensive Red-Al reagents with Zn in HOAc proved to be a good alternative for an eventual industrial application. A significant improvement could be the conversion of nitrile **66**, amine **67**, or chloride **154** to an intermediate with a $\Delta^{22(N)}$ double bond. This double bond might be isomerized and oxidized to a C20 carbonyl group (Scheme 6).



a) BrCN, $CHCl_3$, Δ; b) KOAc, DMF; c) Red-Al, toluene, Δ or Zn, HOAc; d) NCS, CH_2Cl_2; e) NaOMe, MeOH; f) Ac_2O, pyridine; g) HOAc, Δ; h) CrO_3, HOAC, Δ
Overall yield 30%

Scheme 5



Scheme 6

Many reactions to introduce the $\Delta^{22(N)}$ double were attempted, but all failed. The results showed that the C16 hydroxyl group assists in the formation of the $\Delta^{22(N)}$ double bond but also that the presence of a free hydroxyl group at C16 results in ringclosure to tomatidenol (**4**). Further, a good leaving group must also be present at the nitrogen atom to make this reaction possible.

Imine **164** was obtained from solasodine (**3**) and its properties as an intermediate in the synthesis of DPA (**64**) were investigated. These efforts were not successful and our expectations about imine **164** as a good intermediate were not redeemed.

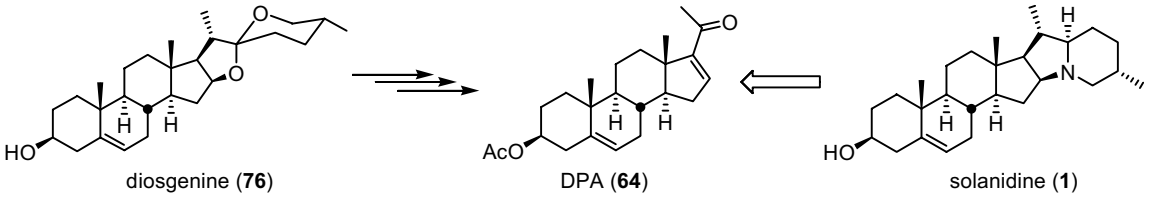
Despite all our efforts, further shortening of the route from 3-acetoxysolanidine (**58**) via tomatidenol (**4**) to DPA (**64**) could not be accomplished.

Samenvatting

De structuur van solanidine (**1**) is zo'n 70 jaar geleden opgehelderd en sindsdien bestaat er belangstelling voor de omzetting van dit aglycon in een intermediair dat geschikt is voor de (industriële) synthese van steroïd hormonen. De hernieuwde belangstelling voor deze omzetting wordt gestimuleerd door de stijgende prijzen van de huidige uitgangsstof voor steroïd hormonen, diosgenine (**76**). Diosgenine wordt geïsoleerd uit *Costus speciosus* dat wordt gecultiveerd in China. Diosgenine (**76**) kan worden omgezet in dehydropregnenolon acetaat (DPA) (**64**) dat een belangrijk intermediair is voor de industriële synthese van progesteron en cortison derivaten. De beschikbaarheid en monopolie positie van China voor diosgenine (**76**) baart echter zorgen.

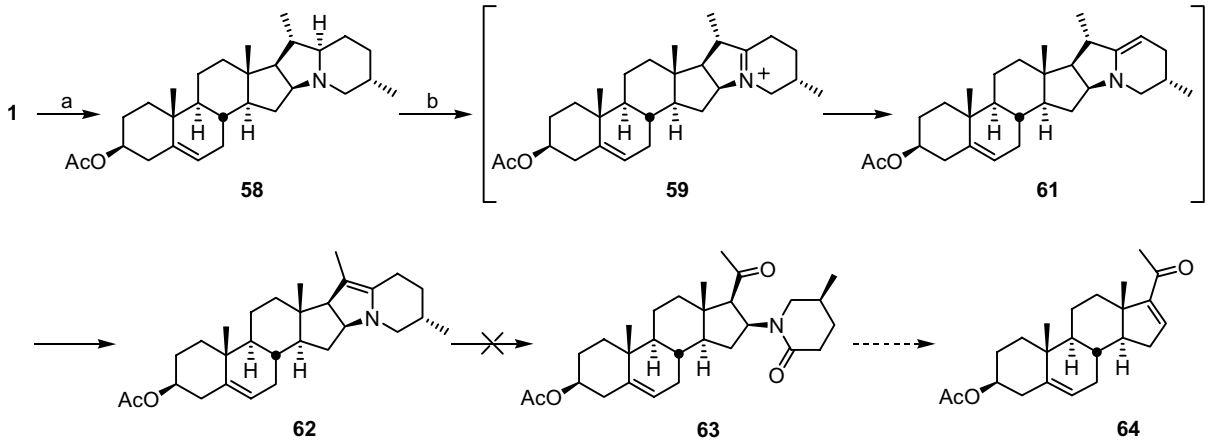
Nieuw onderzoek naar solanidine (**1**) als alternatief voor diosgenine (**76**) wordt eveneens gestimuleerd door de mogelijkheid om grote hoeveelheden (ton schaal) aardappel glycolalkaloïden te isoleren uit een afvalstroom van het aardappelzetmeel proces. In de aardappelzetmeel industrie komen de glycolalkaloïden α-chaconine (**6**) en α-solanine (**7**) in eerste instantie terecht in de eiwitfractie. Deze eiwitfractie wordt gezuiverd en opgewaardeerd door de glycolalkaloïden hieruit te verwijderen. Hierdoor ontstaat een afvalstroom met een hoog gehalte aan glycoalkaloïden. Het moet mogelijk zijn deze glycoalkaloïden uit deze afvalstroom te isoleren waardoor een alternatieve grondstof voor de synthese van DPA (**64**) beschikbaar komt (Schema 1).

Om de grondstof voor ons onderzoek te verkrijgen is een simpele en effectieve methode ontwikkeld voor de isolatie op laboratorium schaal van de glycolalkaloïden α-chaconine (**6**) en α-solanine (**7**) uit afvalproducten verkregen van AVEBE. Door deze glycoalkaloïden mild te behandelen met zuur kan solanidine worden vrijgemaakt en geïsoleerd (Hoofdstuk 2).



Schema 1

Een eerste poging tot de synthese van DPA (**64**) uit solanidine (**1**) is uitgevoerd volgens een recent gepubliceerde methode waarin $Hg(OAc)_2$ als oxidatie reagens wordt gebruikt. Hoewel de eerste stappen in de synthese verbeterd zijn door de $Hg(OAc)_2$ oxidatie (**58→61**) en de isomerisatie stap (**61→62**) te combineren tot een "één-pots" synthese (**58→62**), is het niet gelukt de cruciale oxidatie van de $\Delta^{20,22}$ dubbele binding in het enamine (**62→63**) te realiseren (Schema 2). Berekeningen geven aan dat enamine **62** in feite een amine is aan een geïsoleerde dubbele band.



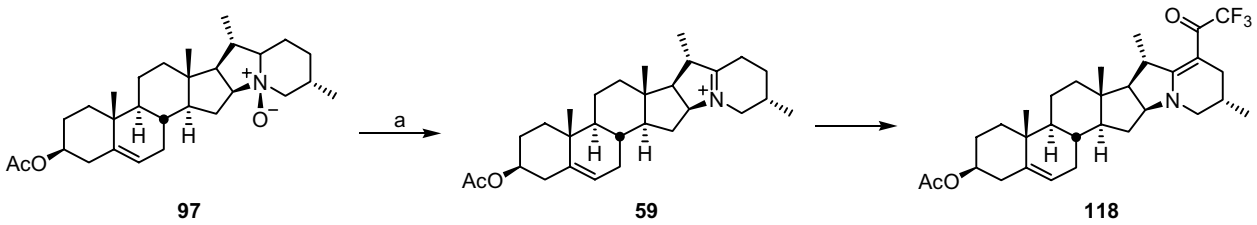
a) Ac_2O, pyridine; b) $Hg(OAc)_2$, aceton
totaal opbrengst 90%

Schema 2

In de tweede poging solanidine (**1**) om te zetten in DPA (**64**) zijn de Cope en Polonovski reacties van solanidine *N*-oxide bestudeerd. Om epoxidatie van de $\Delta^{5,6}$ dubbele binding te voorkomen werd solanidine (**1**) omgezet in solanidi-4-en-3-on (**87**), dat vervolgens werd geoxideerd met MMPP tot solanidi-4-en-3-on *N*-oxide (**99**) in een goede opbrengst. Het blijkt dat de Cope reactie niet het gewenste resultaat geeft omdat de juiste conformatie, nodig voor de Cope reactie, niet bereikt kan worden. In deze situatie treedt deoxygenatie als competitief proces op en wordt solanidi-4-en-3-on teruggevormd. De Polonovski reactie kan worden uitgevoerd door **97** te behandelen met Ac_2O of $(CF_3CO)_2O$. In het geval van Ac_2O is geen enkele reactie met solanidine *N*-oxide (**96**) of 3-acetoxysolanidine *N*-oxide (**97**) waargenomen. Behandeling van 3-acetoxysolanidine *N*-oxide (**97**) met $(CF_3CO)_2O$ geeft verbinding **118** die gevormd wordt door isomerisatie van iminium ion (**59**) gevolgd door trifluoroacylering (Schema 3). Er zijn geen verdere pogingen ondernomen om deze verbinding om te zetten in DPA (**64**).

In de derde poging is de Von Braun reactie gebruikt om het E,F-ringsysteem van solanidine te openen. Behalve het gewenste E-ring geopende hoofdproduct **65** is ook het F-ring geopende bijproduct **126** geïsoleerd. Alternatieven voor het gevaarlijke Von Braun reagens BrCN zijn onderzocht maar zonder succes. Berekeningen geven aan dat BrCN één van de weinige reagentia is dat het E,F-ringsysteem van solanidine kan openen. De volgende stap is de omzetting van **65** in DPA (**64**) via de Hofmann degradatie. Het broom atoom in **65** is daartoe vervangen door een

waterstof atoom, een acetaat groep of geëlimineerd tot een $\Delta^{16,17}$ dubbele band. De verkregen verbindingen zijn vervolgens gemethyleerd tot de overeenkomstige ammoniumionen.

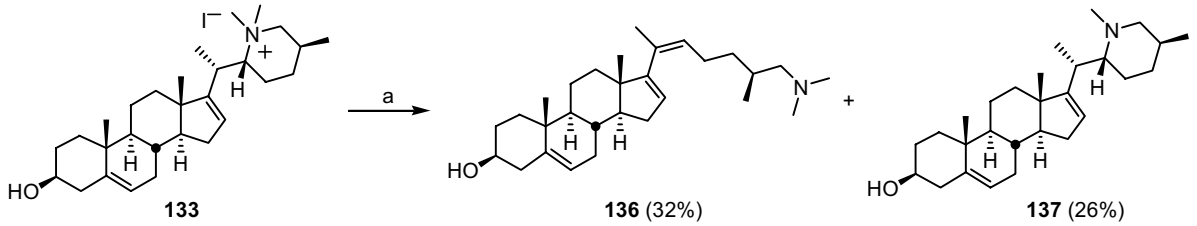


97 **59** **118**

a) $(CF_3CO)_2O$, *t*-BuOK, *t*-BuOH, 41%

Schema 3

Slechts de Hofmann degradatie uitgevoerd met LDA en het ammonium zout **133** met de $\Delta^{16,17}$ dubbele band geeft eliminatie tot trieen **136**, echter wel tesamen met het monogemethyleerde dieen **137**. Verbinding **137** kan in principe opnieuw gemethyleerd worden en Hofmann degradatie ondergaan (Schema 4). In de andere gevallen is geen reactie waargenomen of is de monogemethyleerde verbinding **137** geïsoleerd. Alhoewel Hofmann degradatie leidt tot de vorming van **136**, vraagt verdere degradatie tot DPA (**64**) om meerdere beschermings- en ontschermingsstappen die niet erg aantrekkelijk zijn voor industriële toepassing.



133 **136** (32%) **137** (26%)

a) LDA, THF, -78ºC

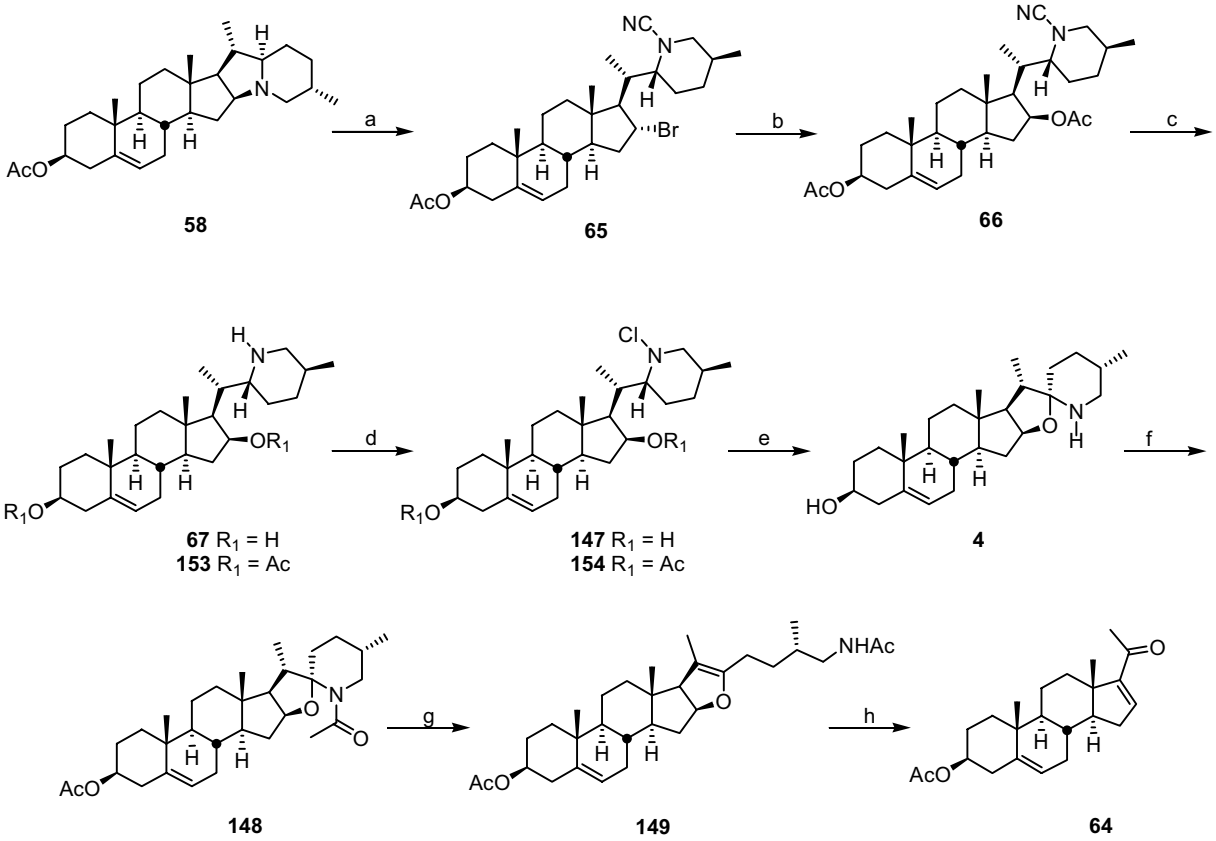
Schema 4

Als laatste mogelijkheid is de omzetting van 3-acetoxysolanidine (**58**) tot spirosolaan verbindingen en vervolgens tot DPA (**64**) onderzocht. Hoewel alle afzonderlijke stappen in deze route reeds bekend zijn, is de route nooit in zijn geheel gepubliceerd. Verbinding **65**, verkregen na behandeling van **58** met BrCN, is daartoe omgezet in tomatidenol (**4**) via substitutie van het bromide (**65→66**), reductie van het nitril (**66→67** of **66→153**), chlorering van het secundaire amine (**67→147** of **153→ 154**) en dehydrochlorering met base (**147→4** of **153→4**) (Schema 5). Tomatidenol (**4**) is omgezet in DPA (**64**) door gelijktijdige acylering van zowel de amine als de hydroxyl groep (**4→148**), ringopening en de daarmee gepaardgaande vorming van de $\Delta^{20,22}$ dubbele band door behandeling met HOAc (**148→149**), gevolgd door oxidatie van deze dubbele band en eliminatie van de gevormde ester (**149→4**). De hele synthese route is gerealiseerd in een totale opbrengst (**58→64**) van 30%.

Om te kunnen concurreren met reeds bestaande industriële processen, moet deze route worden ingekort en het gebruik van dure en gevaarlijke reagentia moet worden vermeden. Pogingen hiertoe door verbindingen uit het eerste deel van de route (3-acetoxysolanidine (**58**) tot tomatidenol (**4**)) om te zetten in verbindingen van het tweede deel van de route (tomatidenol (**4**) tot DPA (**64**)) zijn uitvoerig bestudeerd maar hebben weinig opgeleverd.

Vervanging van het dure Red-Al reagens door Zn in HOAc is een goed alternatief voor industriële toepassing. Een significante verbetering zou de omzetting van nitril **66**, amine **67** of

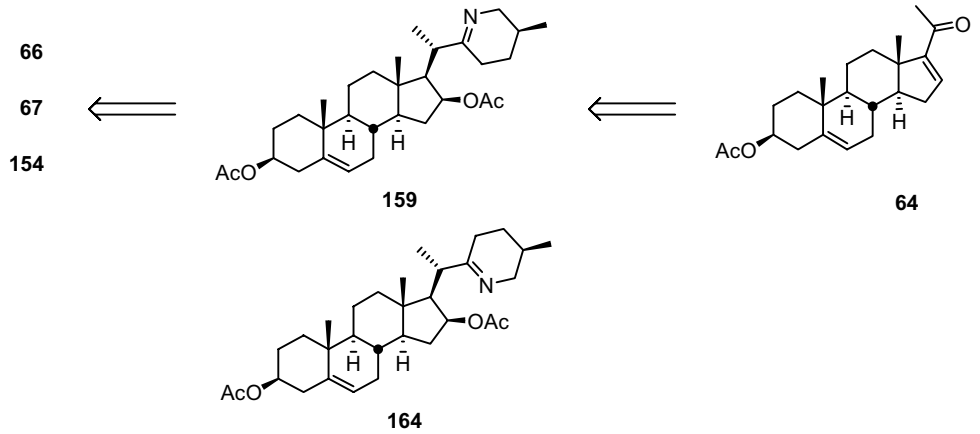
chloride **154** tot intermediair **159** met een $\Delta^{22(N)}$ dubbele band zijn. Dit dieen kan in principe weer geïsomeriseerd en geoxideerd worden tot een C20 carbonylgroep (Schema 6). De pogingen om de $\Delta^{22(N)}$ dubbele band te introduceren zijn echter alle zonder succes gebleven. De resultaten tonen ook aan dat de C16 hydroxyl groep assisteert in de vorming van de $\Delta^{22(N)}$ dubbele band die daarna echter direct verder reageert tot tomatidenol (**4**). Een ander vereiste is de aanwezigheid van een goede vertrekkende groep op het stikstofatoom.



a) BrCN, $CHCl_3$, Δ; b) KOAc, DMF; c) Red-Al, tolueen, Δ of Zn, HOAc; d) NCS, CH_2Cl_2; e) NaOMe, MeOH; f) Ac_2O, pyridine; g) HOAc, Δ; h) CrO_3, HOAc, Δ

Totaal opbrengst 30%

Schema 5



Schema 6

Het imine **164**, het C25 epimeer van imine **159**, kon uitgaande van solasodine (**3**) wel

gesynthetiseerd worden. Uit onderzoek naar de reactiviteit en eigenschappen van **164** moet echter geconcludeerd worden dat de bruikbaarheid van deze imines als intermediair in de synthese van DPA (**64**) nihil is.

Ondanks alle inspanningen, is een significante inkorting van de route uitgaande van 3-acetoxysolanidine (**58**) via tomatidenol (**4**) tot DPA (**64**) niet gerealiseerd.

Curriculum Vitea

De schrijver van dit proefschrift is op 6 augustus 1972 geboren te Heerlen. Na het behalen van het MAVO diploma aan MAVO Scharn te Maastricht en het HAVO diploma aan het St. Maartenscollege te Maastricht werd in 1990 begonnen met het Hoger Laboratorium Onderwijs (H.L.O.) aan de Hogeschool Heerlen, destijds gevestigd te Sittard. Hij koos voor de chemische studierichting en specialiseerde zich verder in de preperatieve organische chemie. Zijn stage en afstudeerperiode werden doorgebracht aan de Katholieke Universiteit Nijmegen onder begeleiding van dr. J. Tijhuis, L. Thijs en prof. dr. B. Zwanenburg. In 1994 werd het getuigschrift ontvangen en werd de studie vervolgd met het doorstroomprogramma Scheikunde aan de Katholieke Universiteit Nijmegen. Na een uitgebreid hoofdvak Organische Chemie onder begeleiding van dr. P. Hermsen, L. Thijs en prof. dr. B. Zwanenburg werd in 1997 het doctoraalexamen afgelegd. Van 1997 tot 2001 was hij werkzaam als onderzoeker in opleiding (OIO) bij de leerstoelgroep Organische Chemie van Wageningen Universiteit. Het tijdens deze periode uitgevoerde onderzoek, onder begeleiding van dr. J.B.P.A. Wijnberg en prof. dr. Ae. de Groot, staat beschreven in dit proefschrift. Van mei 2002 tot mei 2003 was hij werkzaam als post-doc bij de leerstoelgroep Organische Chemie van Wageningen Universiteit.

Dankwoord

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Natuurlijk wil ik ook al mijn (ex-)collega's van het Laboratorium voor Organische Chemie die ik in het bovenstaande niet persoonlijk genoemd heb, bedanken voor de prettige werksfeer, de borrels, de fietstochten, de sportdagen, de AIO-reizen naar Zweden en Amerika en alle andere zaken die mijn promotietijd hebben veraangenaamd.

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