σ-ADDUCTS
OF PTERIDINES AND 3-DEAZAPTERIDINES, STRUCTURE AND REACTIVITY

Promotor: dr.H.C.van der Plas, hoogleraar in de organische scheikunde

A.NAGEL

σ-ADDUCTS OF PTERIDINES AND 3-DEAZAPTERIDINES, STRUCTURE AND REACTIVITY

proefschrift

ter verkrijging van de graad
van doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr.H.C.van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 23 juni 1978
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

ISN 104682-03

STELLINGEN

1. De in het "Handbook of Chemistry and Physics" voor chinazoline en pteridine vermelde pK_a waarden hebben geen betrekking op de aza-aromaten zelf, doch op de covalente hydraten ervan.

   Handbook of Chemistry and Physics, 57^{th} edition (1977).

2. Het reactiemechanisme dat wordt voorgesteld voor de vorming van 2-aminochinoxaline en benzimidazool bij inwerking van kaliumamide in vloeibare ammoniak op 6-broomchinoxaline, is onjuist.

   W.Czuba en H.Poradowska, Roczniki Chemii, 48, 1233 (1974).
   Dit proefschrift.

3. In hun artikel over de aminolyse van 2-ethylsulfonyl-4-chloor-6-R-pyrimidinen (R=H, alkyl,aryl) hebben Sawayama et al. ten onrechte het S_N(ANRORS) mechanisme buiten beschouwing gelaten.

   T.Sawayama, R.Yamamoto, H.Kinugasa en H.Nishimura,
   Heterocycles, 8, 249 (1977).

4. De structuur van de door Ogilvie en medewerkers gesynthetiseerde ribonucleotiden U_pU en U_pU_pU is onvoldoende bewezen.

   K.K.Ogilvie, N.Theriault en K.L.Sadana,
   J.Am.Chem.Soc., 99, 7741 (1977).

5. De bewering van Komin en Wolfe dat heterocyclische radicalen in een $S_{RN}1$ proces electrofieler zijn ten opzichte van enolaat anionen dan fenylradicalen is niet in overeenstemming met de door hen verkregen resultaten bij de met kalium gestimuleerde reactie van 2-broompyridine met het enolaat ion van aceton in vloeibare ammoniak.

   A.P.Komin en J.F.Wolfe, J.Org.Chem., 42, 2481 (1977).

6. De werkwijze van Bergmann et al. ter optimalisering van de enzymatische oxidatie snelheid van het 8-(3'-N-methylpyridinium)hypoxanthine kation als functie van de pH, kan misleidende resultaten ten gevolge hebben.

   F.Bergmann, L.Levene en H.Gorrin, Biochim.Biofys.Acta, 484, 275 (1977).

7. Het scheppend vermogen van de organisch syntheticus zou kunnen worden bevorderd door in zijn opleiding een artistiek vak op te nemen.

8. Het is te betreuren dat in Nederland drinkwater wordt gebruikt ter verwijdering van afvalstoffen.

9. Cultuur is het produkt van de geestelijke en fysieke activiteit van de mens. Het merendeel der mensheid is helaas gedoemd produkt van zijn cultuur te blijven.

A.Nagel

σ-Adducts of pteridines and 3-deazapteridines, structure and reactivity

Aan mijn moeder,
aan Ykes en Tim

VOORWOORD

Op deze plaats wil ik iedereen die in meer of mindere mate betrokken is geweest bij de voltooiing van dit proefschrift, van harte bedanken. Bijzonder veel dank ben ik verschuldigd aan Prof.Dr.H.C.van der Plas. Zijn kritische instelling en fundamentele aanpak van de talloze problemen die tijdens dit onderzoek aan het licht kwamen, zijn van groot belang geweest voor mijn wetenschappelijke vorming. Zeer veel dank ben ik hem ook verschuldigd voor de vele uren die hij heeft vrijgemaakt voor de kritische beoordeling van het manuscript.
Voorts bedank ik een aantal mensen die nauw betrokken zijn geweest bij het onderzoek, te weten de heren W.Combé en drs.C.A.Landheer, voor het verzamelen en interpreteren van de vele massaspectrometrische gegevens, Hugo Jongejan, die alle microanalyses heeft uitgevoerd, Gerrit Lelyveld en Pim Melger voor de verstrekking van het materiaal voor en het oplossen van problemen op het gebied van de chromatografie. Bep van Veldhuizen heeft vele NMR spectra opgenomen en geinterpreteerd. In het bijzonder dank ik Bart Geurtsen voor de plezierige samenwerking bij de uitvoering van enkele experimenten. Ook Aalt van der Kuilen is nauw betrokken geweest bij het onderzoek, eerst als HBO-B stagiair en, na zijn examen, enige tijd als vaste medewerker.Het illustratieve gedeelte van dit proefschrift komt op naam van Bert van Amersfoort. Mevrouw M.Snell ben ik bijzonder veel dank verschuldigd voor alle typografische zorg die ze aan dit proefschrift heeft besteed.

CONTENTS

The following chapters have been published in the literature:

Chapter 2 Recl.Trav.Chim.(Pays-Bas), 94, 95 (1975)

Chapter 3 Org.Magn.Reson., 8, 607 (1976)

Chapter 4 Chem.Pharm.Bull.(Japan), 23, 2678 (1975)

Chapter 5 Heterocycles, 7, 205 (1977)

Chapter 6 Tetrahedron Lett., 2021 (1978)

Chapters 7 and 8 have been submitted to the
Journal of Heterocyclic Chemistry

1 INTRODUCTION

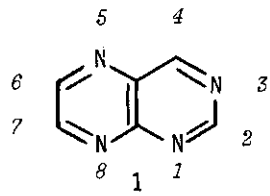
1.1 GENERAL

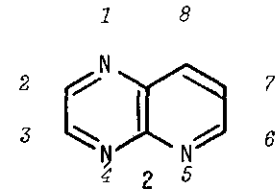
Considerable attention is paid at the Laboratory of Organic Chemistry in Wageningen to the investigation of the behaviour of azaheterocycles towards nucleophilic reagents, in particular potassium amide in liquid ammonia. Pyridines[1], quinolines[2], isoquinolines[3], pyrimidines[4-6], quinazolines[5], pyrazines[7] and triazines[8,9] have been investigated in more or less detail. So far, however, the reactions of derivatives of pteridine and '3-deazapteridine' and their derivatives were not included in these studies.

Generally, pteridine chemistry has been extensively developed, since many pteridines are widely distributed in nature and a fundamental study of these compounds could shed some light on the chemical processes in which these substances are formed and metabolized in nature.

The structural entity of these compounds is the pyrazino[2,3-*d*]pyrimidine ring skeleton (1), trivially named pteridine[10].

The early history[11], the biosynthesis [12-15] and the chemical synthesis[16-24] of the pteridines have been the subject of several reviews.





The ring system has extremely interesting properties, since - as the result of the high C=N/C=C ratio - the pteridine nucleus has a relatively high π-deficient character, which is reflected in a complete inability of pteridine to undergo electrophilic substitution and in the utmost vulnerability towards nucleophilic attack[25-27].

The ring system '3-deazapteridine' (2) - a name that is not accepted officially and, according to the IUPAC-rules[28], will be further referred to as pyrido[2,3-*b*]pyrazine (2) - was next to the pteridines involved in this study. This enabled

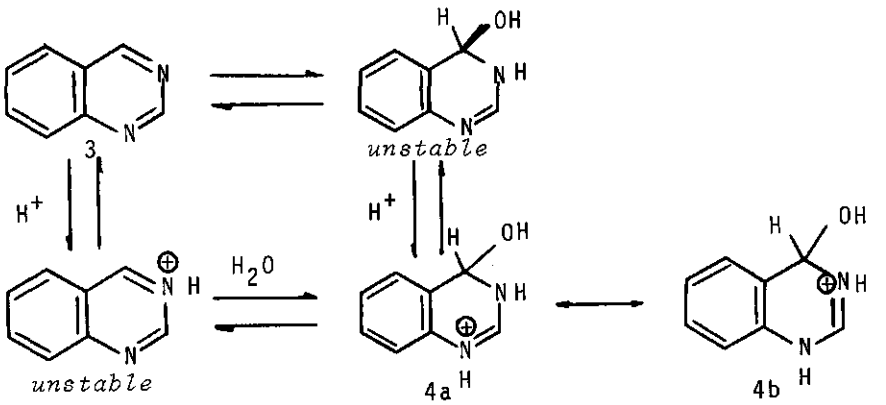
us to evaluate the effect of lowering the C=N/C=C ratio on the reactivity towards nucleophiles.

1.2 NUCLEOPHILIC ADDITIONS TO PTERIDINES

The addition of nucleophilic species to pteridines is a subject of continuing research. These studies parallel the development of new physical methods. Initially the measurement of pK_a values and UV spectra [29] were successfully applied to detect the addition of nucleophiles to pteridines and to obtain detailed information about the structure of the σ-adducts of pteridines and nucleophiles. Later, the attention was focussed on the application of ^1H NMR spectroscopy[30] and recently ^{13}C NMR spectroscopy has been found an extremely useful tool for the study of σ-adducts[31].
The first example of the formation of an adduct between a pteridine and a nucleophile was reported in 1952, when Albert found 6-hydroxypteridine to be covalently hydrated in aqueous solution[32].
In subsequent work pteridine and a number of its derivatives appeared to easily undergo addition reactions with nucleophilic reagents, such as sodium bisulphite, thiophenols, thiobarbituric acid, alcohols, hydrogen cyanide, dimedone and ethyl acetoacetate, hydroxylamine, methylamine and ammonia.
In the following sections a survey will be presented concerning the addition of these oxygen-, sulphur-, carbon- and nitrogen nucleophiles to pteridines. Especially the factors governing the mode and the extent of addition, will be discussed.

1.2.1 COVALENT HYDRATION

Covalent hydration - the addition of water to a C=N bond in an azaheterocyclic system - was found to be a reversible reaction. This phenomenon is probably best illustrated by the well-studied covalent hydration of quinazoline (3)[33].
The UV spectrum of quinazoline surprisingly shows a hypsochromic shift recorded for an acidic aqueous solution. The pK_a of quinazoline is found - by common procedures - to be 3.51, an abnormally high value compared to its 4-alkyl derivatives (for example 4-methylquinazoline: pK_a= 3.06). This apparently anomalous behaviour is explained by the fact that in neutral solution, quinazoline is stable as anhydrous species 3, but the cation present in aqueous acid, is only stable as the covalent hydrate 4 (scheme 1).The 4-methyl group decreases hydration in the quinazoline cation considerably, due to its steric effect.



scheme 1

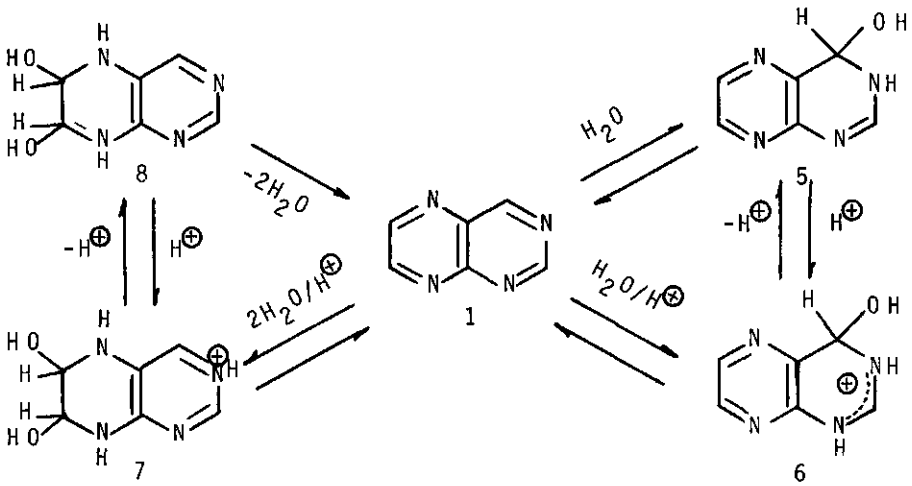
The driving force for the formation of 4 is the strong stabilization of 4 by an amidinium-type resonance (4a ⟷ 4b). By application of a rapid-reaction technique the kinetics of the hydration could be established[33]. The true anhydrous pK_a of quinazoline turned out to be 1.95[34].

With pteridine the covalent hydration is much more complex[29,30,35], since the temperature appears to play an important role in the position of attack of the nucleophile. By application of ^1H NMR spectroscopy[30] the complete hydration pattern, as displayed in scheme 2, could be established. Several steps in this hydration scheme are sufficiently slow to be followed by ^1H NMR spectroscopy. From the spectral data it is evident that pteridine is only partially hydrated in neutral aqueous solution *i.e.* 5, but is immediately hydrated to 6 in aqueous acid. The cation 6 turned out to be an unstable species, as it converts - *via* 1 - to the stable cationic dihydrated species 7.

The stability of 7 is explained by the 4-aminopyridinium-type resonance stabilization, virtually of less importance in the neutral dihydrate 8.

8 is unstable and reconverts irreversibly into pteridine.

The covalent hydration of pteridine is subjected to general acid base catalysis and a cyclic mechanism was reported to be involved in the hydration[36].

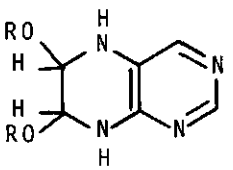


scheme 2

The conversion of 1 into 7 *via* an initial reversible formation of 6 provides us with a good example of a kinetically controlled addition (formation of 6 from 1) *versus* a thermodynamically favoured addition reaction (formation of 7 from 1).
The effect of substituents on the rate and site of hydration has been the subject of several studies in the literature, and was reviewed recently[37].
Partial inhibition of covalent hydration by a CH_3 group attached to the carbon atom undergoing covalent hydration is explained by steric[38] and electronic factors[37,39,40]. Substituent effects on the site and extent of covalent hydration have been reported for a series of 4-substituted pteridines[41].
Recent results described in the literature show that there exists a linear free relationship for the covalent addition of a number of nucleophiles to an aza-aromatic substrate[42]. Thus, in principle, pK_a values of the conjugate acid of the adding nucleophile can be correlated on a logical basis with the equilibrium constant for the addition reaction.
Of particular value for the pteridines is that the considerable interest in the extent to which reaction rates are paralleled by product stabilities in nucleophilic reactions[43] will provide a fundamental approach to the understanding of the factors that bring about the enormous rate accelerations caused by enzymes.

1.2.2 THE ADDITION OF ALCOHOLS

In view of the ease of addition of water it is not surprising that the more nucleophilic alcohols[44-46] - especially primary and secondary alcohols - not only add to pteridine in the presence of acid, but also in a neutral solution. Upon addition, stable covalent 2:1 di-σ-adducts 9, in which the alcohol has been attached to C-6 and C-7, are formed. Since the adducts of type 9 were found to be preceded by those of type 10, it is very likely that the di-adducts are formed in a thermodynamically controlled reaction.



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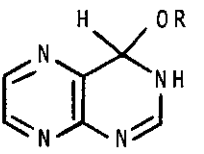
R=CH_3, C_2H_5, $CH(CH_3)_2$



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The stability of the covalent adducts of structure 9 (R=CH_3, C_2H_5) made it possible to determine their spectroscopic properties and melting points. *t*-Butylalcohol was found not to add to pteridine under any of the conditions mentioned.

1.2.3 ADDITION OF SULPHUR NUCLEOPHILES

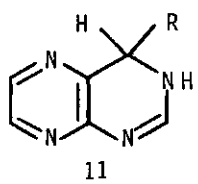
The application of nucleophiles like sodium bisulphite[47], 2-thiobarbituric acid and thiophenols[45,48] - being stronger nucleophilic than the oxygen containing nucleophiles - causes pteridines that are unreactive towards attack by water or alcohols to undergo covalent addition reactions. Thus, pteridin-7-one[45] smoothly adds the named sulphur nucleophiles in a 1:1 ratio to C-6. Similarly pteridin-4-one[48], resisting the addition of water and even alcohols, yields adducts with sulphur nucleophiles in a 2:1 ratio at C-6 and C-7 exclusively.

1.2.4 ADDITION OF CARBON NUCLEOPHILES

The addition of Michael reagents to pteridine[49] and several of its derivatives[45,48,50] leads to a quite complicated and divergent pattern. The reactions that take place strongly depend on the nature of the carbon nucleophile. These reactions can be divided into three categories:

i. Addition across the 3,4-double bond to yield the 4-substituted 3,4-dihydropteridines[11]. This occurs with dimedone, barbituric acid, diethylmalonate

and ethyl benzoylacetate.



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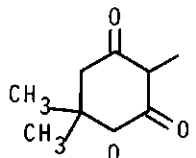
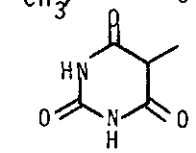
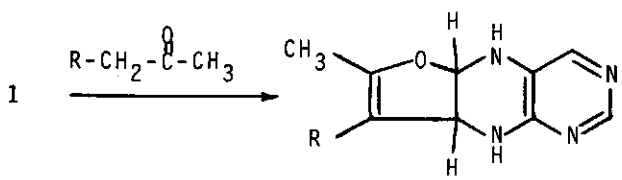




R= $-CH(COC_6H_5)CO_2C_2H_5$

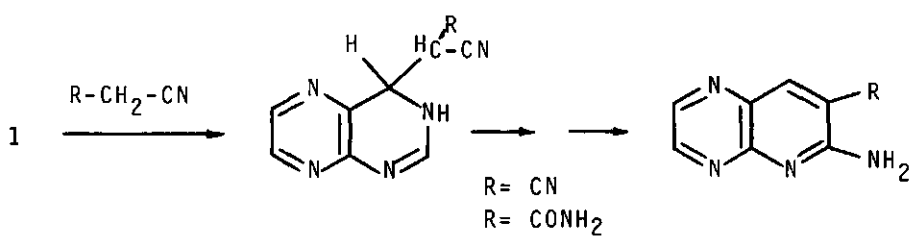
R= $-CH(CO_2C_2H_5)_2$

ii. Addition of one molecule of the Michael reagent to the 5,6- *and* 7,8-bonds of pteridine, to yield 8-substituted 5,5*a*,8*a*,9-tetrahydro-7-methylfuro[2,3-*g*]-pteridines. This reaction course takes place with the dicarbonyl compounds benzyl acetoacetate, ethyl acetoacetate and acetylacetone.



$R=CO_2CH_2C_6H_5$; $CO_2C_2H_5$; $COCH_3$.

iii. Addition of the reagent to the 3,4-bond of 1, followed by scission of the 3,4-dihydro bond formed, can yield an open-chain intermediate that recyclizes into a pyrido[2,3-*b*]pyrazine derivative. This ring transformation was found to occur with malonitrile and cyanoacetamide.



R= CN

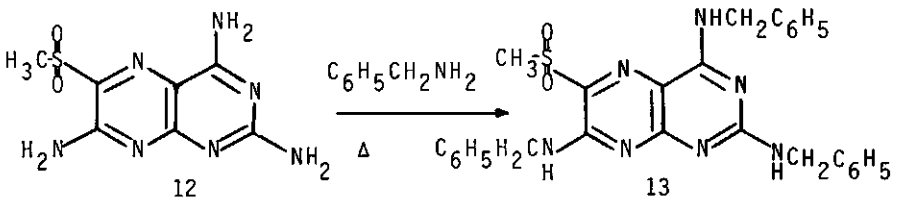
R= $CONH_2$

1.2.5 ADDITION OF NITROGEN NUCLEOPHILES

Only a few addition reactions of amines and pteridines have been reported[39,50-54]. With pteridine itself 2:1 σ-adducts with ammonia and methylamine[52] were found. 4-Methylpteridine was found to form a 2:1 σ-adduct - on C-6 and C-7 - with benzylamine[50]. The 1:1 σ-adduct of ammonia and pteridine[51] - exclusively to the 3,4-bond - is formed amongst other products, in a dilute buffered solution, but in liquid ammonia at low temperature this 1:1 σ-adduct is the sole detectable species[53]. Attempts to add the strongly nucleophilic amide ion failed due to decomposition of 1. The 6- and 7-chloropteridines were reported to yield 1:1 σ-adducts to the 3,4-bond with ammonia, benzylamine and cyclohexylamine, preferably at low temperature and in apolair solvents[54].

1.3 NUCLEOPHILIC SUBSTITUTION OF PTERIDINES

In general, nucleophilic substitution readily takes place with pteridines[17], as the carbon atoms are activated towards nucleophilic attack by the inductive and mesomeric effect of one or more nitrogen atoms of the pteridine ring skeleton. The 6- and 7-chloropteridines were reported to undergo nucleophilic substitution by ammonia, amines and thiols, especially in polar solvents and at elevated temperatures[54]. So far no systematic study on the reactivity of chloropteridines has been reported in the literature[55]. The extreme reactivity of 2-chloropteridine towards *t*-butylamine has been the subject of a kinetic study[56]. The great ease of nucleophilic displacement can be exemplified with 6-methylsulfonyl-2,4,7-triaminopteridine (12), which can undergo nucleophilic substitution by amines[57], such as benzylamine, at C-2, C-4 and C-7[58], yielding 13.

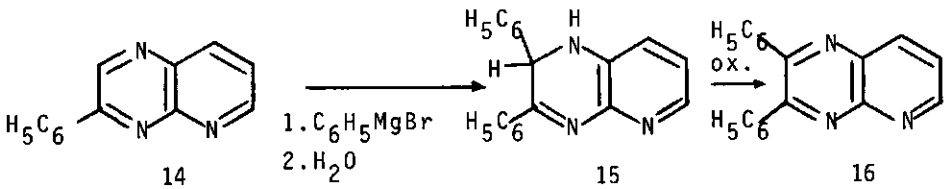


This amine exchange is in agreement with the concept of hard and soft acids and bases: the hard base (benzylamine) replaces the hard leaving groups. On the other hand very soft nucleophiles like thiophenols were found to replace the methylsulphonyl moiety, being a soft leaving group.

1.4 NUCLEOPHILIC REACTIONS WITH PYRIDO[2,3-*b*]PYRAZINES

The reactions of this ring system have been less intensively investigated than the pteridines. Although for approximately fifty years several groups have studied the chemistry of the parent substance (2) and a number of its derivatives in more or less detail[59-79], so far the chemistry of the pyrido[2,3-*b*]pyrazines has not been reviewed.

The cation of 2 is able to add water to C-2 and C-3 to a small extent, yielding a cationic covalent dihydrate[68]. Only a few nucleophilic substitutions of halogeno derivatives with water and ammonia have been reported[73], exemplified by the (autocatalytic) hydrolysis of 6-chloropyrido[2,3-*b*]pyrazine to pyrido [2,3-*b*] pyrazin-6-one by brief boiling in aqueous acid. In recent investigations the action of carbon nucleophiles was studied[60]. Thus Grignard reagents add to 14, to yield the covalent σ-adduct 15, that can be oxidized to 16.



14 15 16

1.5 PURPOSE OF THIS INVESTIGATION

The initial aim of this investigation was to elucidate the factors that influence the formation and reactivity of σ-adducts formed between pteridines and liquid ammonia and between pteridines and potassium amide in liquid ammonia. ^{1}H and ^{13}C NMR spectroscopy were used to study the formation and the reactivity of these σ-adducts. During this study it was found that under influence of potassium amide pteridines and the related pyrido[2,3-*b*]pyrazines easily undergo ring contraction of the pyrazine moiety. The mechanism of these ring contractions was investigated with ^{15}N-, ^{2}H- and ^{13}C-labelled substrates as well as with ^{15}N-labelled potassium amide. The mechanism of the substitution reaction of 2-substituted pteridines was studied with ^{15}N-labelled pteridines and with ^{15}N-labelled reagents.

In Chapter 2 ^{1}H NMR evidence is reported on the covalent addition of liquid ammonia to pteridine and several of its derivatives. Pteridine, in liquid ammonia at room temperature, is transformed into the stable 6,7-diamino-5,6,7,8-tetrahydropteridine in a thermodynamically controlled reaction, which is preceded by

the kinetically controlled formation of 4-amino-3,4-dihydropteridine. The effect of substituents on the covalent amination is interpreted by means of steric and electronic factors.

In Chapter 3 ^{13}C NMR data of pteridine and a number of its derivatives are presented. ^{13}C NMR substituent effects were measured and successfully applied to make an unequivocal assignment of the position of a substituent in 6- and 7-substituted pteridines. Furthermore ^{13}C NMR spectroscopic data on covalent σ-adducts, formed between pteridine and several derivatives with NH_3 and water, are presented and discussed.

In Chapter 4 the ring contraction of pteridines into purines by potassium amide in liquid ammonia is reported and a tentative mechanism is proposed.

In Chapter 5 the mechanism of the ring contraction of pteridines into purines - exemplified by the conversion of 2-methylthio-4,6,7-triphenylpteridine into 6,8-diphenyl-2-methylthiopurine - is investigated in more detail. Firm conclusions could be drawn from ^{15}N- and ^{2}H-labelling experiments on the mechanistic pathway of this ring contraction.

In Chapter 6 evidence is presented that the amino-dechlorination of 2-chloro-4,6,7-triphenylpteridine by potassium amide in liquid ammonia takes place by an S_N(ANRORC) mechanism. Furthermore a novel ring contraction *i.e.* of 6-chloropyrido[2,3-*b*]pyrazine into 1H-imidazo[4,5-*b*]pyridine is described.

In Chapter 7 the synthesis of a number of pyrido[2,3-*b*]pyrazines is described and the reactivity of these compounds towards amide ion is investigated. The mechanism of the ring contraction of 6-chloropyrido[2,3-*b*]pyrazine into 1H-imidazo[4,5-*b*]pyridine is investigated in detail by means of ^{15}N- and ^{13}C-labelled compounds and ^{15}N-labelled potassium amide.

In Chapter 8 ^{1}H and ^{13}C NMR spectral data are presented for pyrido[2,3-*b*]pyrazine and a number of its derivatives, as well as for some covalent σ-adducts, formed from these substances with potassium amide in liquid ammonia or with water.

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2 ^{1}H NMR STUDIES OF σ-ADDUCTS OF PTERIDINE AND SOME OF ITS DERIVATIVES IN LIQUID AMMONIA

A.Nagel, H.C.van der Plas and A.van Veldhuizen

2.1 INTRODUCTION

Recently, ^{1}H NMR studies have shown that the relatively strong nucleophilic amide anion reacts with diazaaromatics such as pyrimidine[1-3], pyrazine[3] and pyridazine[3] and some of their bromo and chloro derivatives[4-7] at low temperature with the formation of very stable anionic σ-adducts. There exists an overwhelming amount of literature[8-10] to prove that the addition of nucleophiles to hetarenes occurs the more easily as the number of nitrogen atoms increase. Tetraazaaromatics, as for example pyrazinopyrazines and pteridines, already undergo covalent addition with weaker nucleophiles such as amines[11], methanol[12] and water[13]. Nowadays it is well established that 8-azapurine, being more electrophilic than the tetraazaaromatics gives addition products with molecules like acrylonitrile, diethyl malonate and acetone[14].
In connection with these results and those found in this laboratory[4-7] on σ-adducts with halogenopyrimidines, pyrimidinium salts and halogenopyrazines, we became interested in the possible addition of amide ions to a fused pyrimidopyrazine *i.e.* pteridine and some of its derivatives. Unfortunately, however, pteridine seems to decompose when dissolved in liquid ammonia, containing two equivalents of potassium amide, at -33^{o}C and even at -60^{o}C. For that reason we turned our attention to a ^{1}H NMR study of pteridine and some of its derivatives, when dissolved in liquid ammonia, free of amide ions.

2.2 RESULTS

2.2.1 PTERIDINE

In comparison with the simple ^{1}H NMR spectrum of a solution of pteridine in $CDCl_3$[15] (see Table), in liquid ammonia as the solvent the spectrum is rather complicated. It consists of twelve peaks, some of which are, when compared with those in $CDCl_3$, considerably shifted to the upfield region, other peaks are

shifted much less. Figure 1a shows the complete ^1H NMR spectrum obtained five minutes after dissolving the pteridine in liquid ammonia at -40°C.

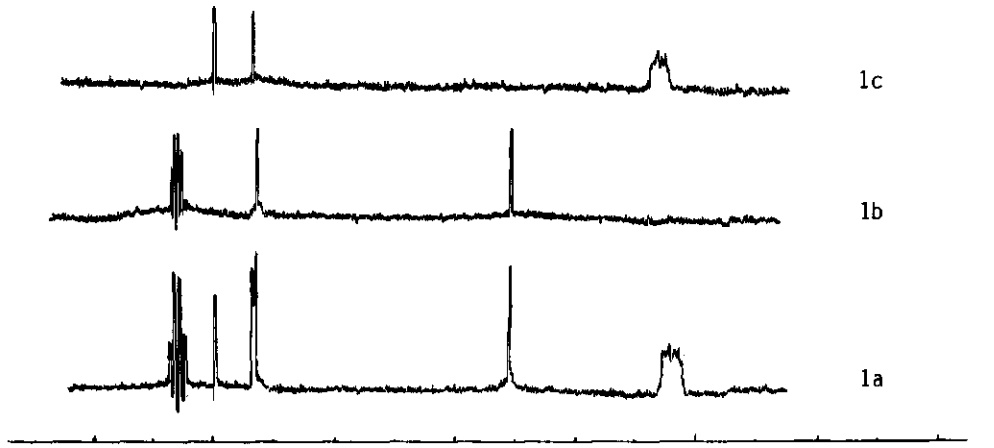
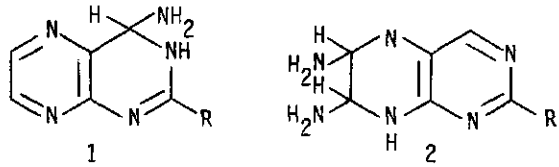


Fig. 1

It can be interpreted as originating from a mixture of two σ-adducts: 4-amino-3,4-dihydropteridine 1 (R = H) and 6,7-diamino-5,6,7,8-tetrahydropteridine 2 (R = H).



Resonance signals of 1 (R = H) appear at δ = 8.43 and δ = 8.33 [AB, 2H (H6, H7)], δ = 7.64 [s, 1H (H2)] and at δ = 5.52 [s, 1H (H4)] . The great upfield shift of ∿ 4 ppm in comparison with that of ∿ 2 ppm, combined with those published in the recent literature for adducts formed from pteridine with water[16] and with methanol[12], strongly supports the conclusion that the incoming amino group has added at C4.

The resonance signals of the 2 : 1 σ-adduct 2 (R = H) are found at δ = 7.96 [s,1H (H2) , at δ = 7.69 s, 1H (H4)] and at δ = 4.20 and 4.14 AB, 2H (H6, H7) . The great upfield shift for H6 and H7 (Δδ ∿ 5 ppm) proves in which positions both amino groups have been added. Since no other resonances than those described above are observed - we did not observe the presence of the N-H protons in the adducts 1 (R = H) and 2 (R = H) probably due to a rapid exchange equilibrium with the solvent molecules - it allowed us to calculate from the integrated

spectrum the ratio in which both species are present. After five minutes of its preparation the solution appears to consist of 40% of the 2 : 1 σ-adduct 2 (R = H) and 60% of the 1 : 1 σ-adduct 1 (R = H).

However, on standing for some time at constant temperature (-40°C) the ratio slowly increases in favour of 2 (R = H), as shown by the gradual increase of the resonances at δ = 7.96, 7.69, 4.20 and 4.14 ppm and a simultaneous decrease of those at δ = 8.43, 8.33, 7.64 and 5.52 ppm. After 3.5 hours at -40°C the ratio of 2 (R = H) to 1 (R = H) was changed into 60/40 respectively.

^{1}H NMR spectra of a solution of pteridine in liquid ammonia at temperatures far below the boiling point of the solvent indicate the presence of a mixture of the adducts 1 (R = H) and 2 (R = H) in which the σ-adduct 1 (R = H) predominates. In fact, at -60°C the species 1 (R = H) is the only one present, as indicated by the simple ^{1}H NMR spectrum as shown in Figure 1b[16]. On the other hand, after allowing the contents of the tube to come to room temperature and measuring the spectrum again at -40°C, only the presence of the 2 : 1 σ-adduct 2 (R = H) is observed (Figure 1c). As was found previously[17] no 1 : 1 σ-adduct, formed on addition of *one* molecule of ammonia to the pyrazine ring of the pteridine, could be detected.

The fast formation of 1 (R = H) clearly indicates that this reaction is kinetically controlled - due to the high degree of polarisation of the 3,4-azomethine linkage - and that the addition of the nucleophile across the 5,6- and 7,8-azomethine bonds is thermodynamically favoured.

The formation of the ammonia di-adduct has been reported in the literature[11], as formed by reaction of pteridine with ethanolic ammonia. However, paper chromatography showed that this σ-adduct consisted of several unidentifiable compounds[11]. In our reaction the di-adduct formed was pure, as indicated unambiguously by its ^{1}H NMR spectrum. It can be easily reconverted into pteridine on sublimation.

In relation with our work on the occurrence of a ring-opening and ring-closure mechanism in nucleophilic displacement reactions with pyrimidines and quinazolines, we asked ourselves whether it would be possible that the adduct 1 (R = H) is in a far to the left lying, but rapid equilibrium with 2,3-disubstituted pyrazines, formed by opening of the 3,4-dihydropyrimidine ring. A somewhat similar suggestion was originally made in the covalent hydration with pteridines[13] but later proved to be erroneous[18,19]. If this equilibrium indeed exists one would expect that in liquid ammonia, containing ^{15}N, a 1 : 1 σ-adduct, containing ^{15}N in the pyrimidine ring would be formed. In order to investigate this,

Table ^1H *NMR spectral data (δ Values)*

Pteridine	H2	H4	H6[f]	H7[f]	solvent
unsubstituted	9.66(s)	9.82(s)	9.13(s)	9.32(d)	$CDCl_3$
4-amino-3,4-dihydro-	7.64(s)	5.52(s)	8.33(d)	8.43(d)	NH_3 liq.
6,7-diamino-5,6,7,8-tetrahydro-	7.96(s)	7.69(s)	4.14(d)	4.20(d)	NH_3 liq.
2-chloro-	-	9.68(s)	9.09(d)	9.28(d)	$CDCl_3$
4-amino-2-chloro-3,4-dihydro	-	5.12(s)	7.75(d)	7.86(d)	NH_3 liq.
6,7-diamino-2-chloro-5,6,7,8-tetrahydro-	-	7.34(s)	∿ 4.1[a]		NH_3 liq.
2-methylthio[b]-	-	9.44(s)	8.87(d)	9.09(d)	$CDCl_3$
6,7-diamino-2-methylthio-5,6,7,8-tetrahydro[b]-	-	2.25(s)	∿ 4.3		NH_3 liq.
2-chloro-4-phenyl[c]-	-	-	9.03(d)	9.17(d)	$CDCl_3$
6,7-diamino-2-chloro-4-phenyl-5,6,7,8-tetrahydro[d]-	-	-	∿ 4.1		NH_3 liq.
6,7-diphenyl-2-methylthio[e]-	-	9.48(s)	-	-	$CDCl_3$

[a] No simple AB pattern is observed. We attribute this to a mixture of conformers.
[b] $-SCH_3$ protons: δ = 2.79(s), shifted to δ = 2.64(s) for the 2:1 σ-adduct.
[c] In $CDCl_3$ the phenyl protons show somewhat different δ values for the α- and β,γ -hydrogen.
[d] The phenyl protons show a slight upfield shift of Δ ∿ 0.21 ppm; the main characteristic being the smaller region in which resonances occur, perhaps due to loss of aromaticity of the pyrazine ring and for out of plane-turning of the phenyl group.
[e] $-SCH_3$ protons: δ = 2.82(s).
[f] H6 and H7 may be interchanged.

pteridine was reacted with ^{15}N-ammonia at low temperature; the solution was warmed subsequently to room temperature and the 2 : 1 σ-adduct, obtained after evaporation of the liquid ammonia, was converted into pteridine in boiling benzene. After purification by sublimation, the pteridine obtained was found *not* to contain an excess of ^{15}N.

2.2.2 PTERIDINE DERIVATIVES

It was observed that the ^{1}H NMR spectrum of a solution of 2-chloropteridine in liquid ammonia at -40°C shows only the resonance signals of the 1 : 1 σ-adduct *i.e.* 4-amino-2-chloro-3,4-dihydropteridine 1 (R = Cl), see Table. In contrast to the observations made with pteridine no indication for the formation of a di-adduct was obtained. It clearly indicates that the addition of the amino group to C4 at this temperature is strongly favoured kinetically, which is in accordance with the rate-enhancement effect of the chlorine atom. The presence of a Meisenheimer adduct, in which the amino group being attached to the same carbon atom as the chlorine atom, was not detected. Again a marked difference was observed between the spectrum of 2-chloropteridine in liquid ammonia at -40°C and that obtained at the same temperature, after the contents of the tube has been allowed to come to room temperature. Under the last mentioned conditions only the resonance signals of the 2 : 1 σ-adduct 2 (R = Cl) were observed.

In sharp contrast, 2-methylthiopteridine does not dissolve in liquid ammonia at -40°C as appears from the ^{1}H NMR spectrum, which shows only a low noise base line. This result seems to lead to the interesting conclusion that only those pteridines are able to dissolve in liquid ammonia, which form σ-adducts (*i.e.* pteridine and 2-chloropteridine). In the case of 2-methylthiopteridine the electron-donating methylthio group disfavours the addition at C4. It was further substantiated that when the solvent is allowed to come to room temperature, 2-methylthiopteridine dissolved with the formation of the 2 : 1 σ-adduct 2 (R = SCH_3).

In contrast to the ease of adduct formation with 2-chloropteridine, 2-chloro-4-phenylpteridine in liquid ammonia at -40°C does not form an adduct. Since the phenyl group is assumed to have no considerable effect on the electron distribution in the pteridine ring, it must lead to the conclusion that steric interference of the solvent molecule approaching position 4, with the phenyl group in that position prohibits the covalent amination at C4 and therefore the solubility of 2-chloro-4-phenylpteridine in liquid ammonia. However, at

room temperature the compound readily dissolves with the formation of the 2 : 1 σ-adduct *i.e.* 2-chloro-6,7-diamino-4-phenyl-5,6,7,8-tetrahydropteridine, as shown by the ^{1}H NMR spectrum (see Table).
In consistency with the steric effect of the phenyl group prohibiting covalent amination on a position, which already carries the phenyl group, we observed that 6,7-diphenyl-2-methylthiopteridine in liquid ammonia at low temperature (-40°C) as well as at room temperature fails to show ^{1}H NMR resonances. It indicates that at both temperatures no adduct is formed and so preventing this pteridine to be appreciably soluble in the solvent.

2.3 EXPERIMENTAL SECTION

Melting points are uncorrected. ^{1}H NMR spectra were recorded with a JEOL JNM C-60H spectrometer, using TMS as an internal standard $\delta = 0$. Mass spectra were recorded on an AEI MS-902 instrument.

2.3.1 PREPARATION OF THE PTERIDINES

Pteridine[20] and 2-methylthiopteridine[21] were prepared according to methods given in the literature. The recorded preparation of 2-chloropteridine[22] was slightly modified in order to improve the yield.

2-Chloro-4,5-diaminopyrimidine

5-Amino-2,4-dichloropyrimidine[23] (3.28 g, 20 mmol) was heated with an excess (20 ml) of aqueous ammonia (25%) at 125°C for 1 h in a sealed tube. The crystals obtained after cooling the contents of the tube in an ice bath were filtered off and the filtrate was evaporated to dryness. The solid residue, together with the crystals, were recrystallized from boiling water, using charcoal. The product thus obtained in almost quantitative yield proved to be pure by TLC (ethyl acetate), in contradiction to the mixture obtained following a procedure given in the literature[24].

2-Chloropteridine

In the only procedure described[22] for the preparation of this compound, 2-chloro-4,5-diaminopyrimidine and polyglyoxal (0.1 molar excess) are used as the starting materials and methanol as the solvent for this condensation. We proved (TLC , ^{1}H NMR) that the low yields reported[22] (less than 20%) had to be attributed to the formation of an appreciable amount of 2-methoxypteridine formed as the sole by-product. This is not remarkable since 2-chloropteridine is rather reactive towards methanol: in boiling methanol it appears

to be quantitatively converted into 2-methoxypteridine in half an hour (TLC, ^{1}H NMR, m.p.).

In order to minimize this methoxy-dechlorination 2-chloropteridine was prepared by reacting 2 equivalents of glyoxal (trimer monohydrate)[25] in a suspension of 2-chloro-4,5-diaminopyrimidine in boiling methanol (10 ml/g of the pyrimidine) for a short time only.

After 5-7 min a clear solution was obtained. The solvent was evaporated to dryness *in vacuo* and the yellow product thus obtained was extracted with several portions of hot ethyl acetate. After evaporation of the solvent from the combined extractions, crude 2-chloropteridine crystallized, and was separated into its two components by quick column chromatography (silica gel activity I, 10 g per g of the crude product, using dry ethyl acetate as the eluens) with no appreciable loss of material. 2-Chloropteridine appears to have the higher mobility. Yield: 65-70%.

6,7-Diphenyl-2-methylthiopteridine

4,5-Diamino-2-methylthiopyrimidine[26] (0.69 g, 4.4 mmol) and benzil (1.02 g, 4.8 mmol) in 21 ml of ethanol were refluxed for 24 hours. After cooling in an ice bath the crystals were collected and washed with cold ethanol (5 ml) and ether (10 ml) in order to remove the excess of benzil. The washed material was dried *in vacuo* at 50°C; yield 1.25 g (86%). The product proved to be pure by TLC (ethyl methyl ketone/cyclohexane 1 : 1); m.p. 208-209°C.

Efforts to sublime 6,7-diphenyl-2-methylthiopteridine at 0.01 nm Hg failed. We observed however, that it readily sublimes in the ultra highvacuum chamber of the mass spectrometer.

Analysis: $C_{19}H_{14}N_4S$(330.40); calcd. C, 69.06; H, 4.27; found C, 69.18, H, 4.52.

5-Amino-2,4-dichloro-6-phenylpyrimidine

2,4-Dichloro-5-nitro-6-phenylpyrimidine[27] (2.7 g, 10 mmol) was reduced by iron (1.5 g) in glacial acetic acid (7.5 ml) according to the prescriptions given in the literature[23]. The product thus obtained was recrystallized from aqueous ethanol to yield slightly yellow needles (1.6 g, 67%); m.p. 133-134°C.

Analysis: $C_{10}H_7Cl_2N_3$(240.09): calcd. C, 50.02; H, 2.94; found C,50.33, H, 3.29.

4,5-Diamino-2-chloro-6-phenylpyrimidine

This compound was prepared in 85% yield from 5-amino-2,4-dichloro-6-phenylpyrimidine using the same procedure as described above (15 ml of 25% aqueous ammonia per g of substance) for the preparation of 2-chloro-4,5-diaminopyrimidine. The crude product was crystallized from water using charcoal. It forms

thin, colourless needles; m.p. 205-206°C.
Analysis: $C_{10}H_9ClN_4$(220.66): calcd. C, 54.43; H, 4.11; found C, 54.74; H, 4.38.

2-Chloro-4-phenylpteridine

A solution of 4,5-diamino-2-chloro-6-phenylpyrimidine (0.26 g, 1.2 mmol) and glyoxal[25](trimer monohydrate)(0.18 g, 2.4 mmol) in 10 ml of ethanol was refluxed for 30 min. After cooling in an ice bath 2-chloro-4-phenylpteridine crystallized in yellow needles (0.18 g). Upon concentration of the filtrate a second crop was obtained (0.06 g) raising the total yield to 0.24 g (=82%). The compound melts sharply at 164-165°C; it proved to be much more stable towards solvolysis than 2-chloropteridine.
Analysis: $C_{12}H_7N_4Cl$(242.67): calcd. C, 59.39; H, 2.91; found C, 59.57; H, 3.17.

2.3.2 GENERAL PROCEDURE FOR MEASURING THE PMR SPECTRA IN LIQUID AMMONIA

100 mg of substance were added with stirring to 1 ml of dry liquid ammonia, in a 5 ml three-necked flask, equipped with a dry ice/acetone condenser. Two min after addition of the solid, the contents of the flask were partially siphoned over into a PMR-tube, which was then sealed by means of a torch. In this way the contents of the tube could be allowed to come to room temperature without evaporation of the ammonia.
The average time required for the greater part of the pteridines under investigation to dissolve in liquid ammonia at room temperature was about 1-5 min.
All spectra were recorded at -40°C, unless otherwise stated.

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3 ^{13}C NMR DATA OF PTERIDINE, SOME OF ITS DERIVATIVES AND THEIR COVALENT σ-ADDUCTS WITH AMMONIA AND WATER

J.P.Geerts, A.Nagel and H.C.van der Plas

3.1 INTRODUCTION

Carbon-13 NMR has been reported to be a useful tool in elucidating the structure of naturally occurring pteridines. Recently ^{13}C NMR spectral data of the biologically important folic acid[3] and the reduced forms, *i.e.* 7,8-dihydro- and 5,6,7,8-tetrahydrofolate[4], were reported.
However, the low solubility in common organic solvents, caused by the substitution of one or more hydrogen atoms of the parent compound, *i.e.* pteridine (1a), by hydroxyl and/or amino groups, necessitates the use of acids or dilute mineral alkali as solvents. In these solvents protonation or anion formation occurs, affecting the ^{13}C NMR chemical shifts of several pteridines (e.g. lumazine, leucopterin, xanthopterin) considerably[5]. Assignments of these ^{13}C NMR signals was achieved by the usual techniques and by relating the ^{13}C NMR spectra with previously recorded[6,7] ^{1}H NMR spectra of these molecules.
However so far no straightforward interpretation of the pteridine ring system has been made[3,8,9]. Our recent interest in the chemistry of pteridines, especially the behaviour of these substrates towards nucleophiles, induced us to investigate in detail the ^{13}C NMR spectrum of pteridine (1a) and some of its derivatives, dissolved in $CDCl_3$, and of several covalent amination products, obtained by dissolving the appropriate pteridine in liquid ammonia[1,2].

3.2 RESULTS AND DISCUSSION

3.2.1 PTERIDINE (1a)

The four *intense* signals of the ^{13}C NMR spectrum of pteridine (1a) dissolved in $CDCl_3$, found at 148.4, 153.0, 159.5 and 164.1 ppm (Table 1) are associated with one bond ^{13}C — ^{1}H coupling constants of 188, 186, 206, 186 Hz, respectively. The signal at 159.5 ppm having the largest coupling constant ^{1}J(CH) = 206 Hz

Table 1. Summary of the ^{13}C chemical shifts[a]

Pteridine		C-2	C-4	C-6	C-7	C-9	C-10
Parent	(1a)	159.5	164.1	148.4	153.0	154.4	135.3
2-Chloro	(1b)	161.3	166.1	148.0	153.3	155.2	133.9
7-Methyl	(1c)	159.2	162.9	149.0	163.2	153.7	133.1
2-Methylthio	(1d)	174.8	163.0	145.6	152.0	154.4	133.0
2-Phenyl	(1e)	164.9	163.6	146.7	152.4	154.4	133.8
4-Phenyl	(1f)	158.5	169.0	146.6	151.6	154.9	133.6
7-Phenyl	(1g)	159.4	162.7	146.2	158.9	153.6	133.4
2-Chloro-4-methyl	(1h)	160.4	177.3	146.6	152.9	154.5	133.4
2-Chloro-4-phenyl	(1i)	160.9	171.4	146.6	152.1	156.2	b
6,7-Dimethyl	(1j)	158.1	161.8	157.4	163.1	153.0	132.9
4,7-Diphenyl	(1k)	158.9	168.1	144.7	157.8	154.5	b
2-Methylthio-4-phenyl	(1l)	174.2	168.2	144.4	151.1	155.4	b
4-*t*-Bu-2-chloro-6-phenyl	(1m)	159.4	184.5	151.2	149.7	154.2	b
4-*t*-Bu-2-chloro-7-phenyl	(1n)	160.4	184.1	142.5	157.8	154.2	b
4-*t*-Bu-2-methoxy-6-phenyl	(1o)	164.0	184.6	148.6	148.4	154.8	b
4-*t*-Bu-2-methoxy-7-phenyl	(1p)	164.6	184.2	139.5	157.3	155.7	b
2-Chloro-4,7-diphenyl	(1q)	161.2	170.2	144.6	158.3	156.0	b
4,6-Diphenyl-2-methylthio	(1r)	173.4	167.2	151.2	149.1	154.2	b
4,7-Diphenyl-2-methylthio	(1s)	174.0	167.2	142.6	157.7	154.9	b
4,6,7-Triphenyl	(1t)	158.5	167.6	155.0	159.8	153.1	b

[a] All samples were measured for $CDCl_3$ solutions.
[b] Could not be detected because of signal overlap by the phenyl group.

is assigned to C-2 since it is known that substitution on carbon by electronegative atoms causes a significant enhancement of the s character of the C—H bond, leading to an increase in the $^{1}J(CH)$ coupling constant[10].
This large value for the $^{13}C—^{1}H$ coupling constant is found in many related compounds, containing the same structure element N—CH—N, *e.g.* pyrimidine $^{1}J(C\text{-}2,H)$ = 206 Hz[11], 1.3.5-triazine [$^{1}J(CH)$ = 207 Hz[12]], purine [$^{1}J(C\text{-}2,H)$ = 207 Hz[13]], quinazoline [$^{1}J(C\text{-}2,H)$ = 204 Hz[14]]. Now that the position of the NMR resonance of C-2 is known, the position of the ^{1}H NMR signal of H-2 in the ^{1}H NMR spectrum of 1a can be established, using the selective heteronuclear decoupling technique. Because of the fact that H-6 and H-7 give rise to a pair of doublets, the remaining singlet must be ascribed to H-4. Irradiation at the H-4 frequency showed that the carbon resonance at 164.1 ppm arises from C-4.
It is of interest that, in contrast to pyrimidine, C-2 resonates at a *higher* field than C-4. In order to assign the ^{13}C NMR signals at 148.4 and 153.0 ppm, we measured the ^{13}C NMR spectrum of 7-methylpteridine (1c), the structure of which has been firmly established[15] (see Table 1). Comparison of the resonances of 1a and 1c and taking into account the literature data on the α- and β-substituent effects (+9.2 and 0.0 ppm, respectively) found in methylpyrazine[16] allowed us to assign the remaining resonances at 148.4 and 153.0 ppm to C-6 and C-7, respectively. The assignments of the signals of C-9 and C-10 were based on those already established for similar systems such as quinoxaline, quinazoline and purine[8].
By using heteronuclear double resonance ^{13}C NMR spectral assignments presented in this paper were found to be in sound agreement with the interpretation of the ^{1}H NMR spectrum of pteridine[17] which was firmly based on a study with deuterium labelled pteridines.

3.2.2 PTERIDINE DERIVATIVES (1b-1f)

Of the recorded monosubstituted compounds (1b-1g), it is noteworthy that in 2-chloropteridine (1b) the chloro atom is found to shift the *meta*-oriented C-4 more downfield (2.0 ppm) than C-2 (1.8 ppm). The same effect was found in the ^{13}C NMR spectrum of 2-chloropyrimidine[14] (downfield shifts of 2.7 and 2.4 ppm for C-4 and C-2, respectively).
^{13}C NMR spectroscopy - unlike ^{1}H NMR spectroscopy - can be successfully applied in establishing the position of the phenyl group in the pteridine ring (C-6 or C-7) obtained when a 4,5-diaminopyrimidine derivative is condensed with

phenylglyoxal in ethanol. This structure assignment is essentially based on the well known fact that the phenyl group shifts the carbon atom to which it has been attached about 5 ppm downfield, and the adjacent carbon atom about 2 ppm upfield. Consequently in a 6-phenyl isomer the signals of C-6 and C-7 must approach each other relative to the corresponding signals in 1a, while in a 7-phenyl isomer they must move apart.
This is clearly demonstrated by comparison of the data of the 2,4-disubstituted 6-phenylpteridines (1m, 1o and 1r), and the corresponding 7-phenylpteridines (1n, 1p and 1s) where there is a striking difference in the region of the absorptions of C-6 and C-7. As a corollary ^{1}H selective decoupling completely clarifies the ^{1}H NMR spectrum of these 6- (or 7-)phenylpteridines.

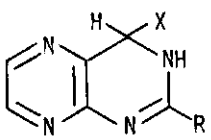
3.3 AMMONIA ADDUCTS

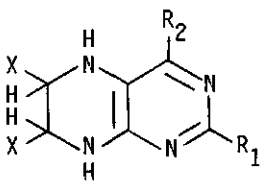
It has been demonstrated by several investigators using both UV and ^{1}H NMR spectroscopy[19,20] that pteridine forms with ammonia a 1 : 1 σ-adduct (2a) and a 2 : 1 σ-adduct (3a)[18]. So far no ^{13}C NMR spectral data on these covalent adducts have been published. To obtain a ^{13}C NMR spectrum of the covalent 3,4-monoadduct (2a) (see Table 2) proved to be difficult. In between the time of its preparation and the acquisition of the last free induction decay a considerable quantity of precipitate was formed, resulting in a difficult interpretation of the spectra because of the relatively bad signal to noise ratio.
^{13}C NMR spectral data of 3a and some of its derivatives have also been obtained (see Table 2). The general picture of the spectrum of this 2 : 1 σ-adduct totally differs from that found for the parent pteridine (1a) as seen by the appearance of strong signals at 60.9 and 62.8 ppm in the sp^3 carbon region resulting from C-6 and C-7.
Furthermore, the spectrum exhibits the typical pattern of a pyrimidine derivative in that C-2 now resonates at *lower* field than C-4. Because of the saturation of the pyrazine ring upon diadduct formation, the electron attracting N-atoms of the pyrazine ring have adopted the electron releasing character of an amino group, as indicated by the upfield shift found for the resonances of the pyrimidine fragment of the molecule. This phenomenon is clearly illustrated by the resemblance found when one compares the spectrum of 3a with that of the structurally closely related 4,5-diaminopyrimidine (4)(see Table 2).
Again the difference in magnitude of the ^{1}J(C-2,H) and the ^{1}J(C-4,H)(198 and 176 Hz, respectively) makes it possible to differentiate between the signals from

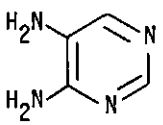
C-2 and C-4.

The results of our investigations clearly show that a restrictive condition with respect to diadduct formation in liquid ammonia is that positions 6 and 7 of the pteridine derivative must be unsubstituted[20]. Therefore, of all pteridines listed in Table 1, only a limited number gave the 6,7-diamino adducts (3a-3g)(see Table 2).

All the assignments based on the ^{13}C NMR spectra are fully consistent with results obtained earlier by ^{1}H NMR spectroscopy[18-20].







2a R=H, X=NH_2
2b R=Cl, X=NH_2
2c R=H, X=OH

3a R_1= R_2= H, X = NH_2
3b R_1= C_6H_5, R_2=H, X = NH_2
3c R_1= H, R_2= C_6H_5, X = NH_2
3d R_1= SCH_3, R_2= H, X = NH_2
3e R_1= OCH_3, R_2= H, X = NH_2
3f R_1= Cl, R_2= CH_3, X = NH_2
3g R_1= Cl, R_2= C_6H_5, X = NH_2
3h R_1= R_2= H, X = OH

4

3.4 HYDRATES

After studying the ^{13}C NMR spectra of covalent adducts of ammonia and pteridine, we became interested in comparing these spectral data with those of the corresponding complexes of pteridine and water[9]. The knowledge acquired from the study on the ammonia adducts 2a and 3a allowed straightforward interpretation of the ^{13}C NMR spectra of the mono- and dihydrate of pteridine 2c and 3h. When 1a is dissolved in water at pH = 6.8 and the ^{13}C NMR spectrum of the solution is recorded without delay, signals of smaller intensity belonging to 4-hydroxy-3,4-dihydropteridine (2c) are found in addition to those of the parent compound (1a). The spectrum of this monohydrate closely resembles that of the 3,4-monoammonia adduct (2a) of pteridine. Only the chemical shift of the sp^3 hybridized C-4 reflects the difference between O- and N-substitution to a considerable extent.

The ^{13}C NMR spectrum of this solution taken after a prolonged period of time (7 h) reveals a number of additional peaks, two of which are found in the sp^3 carbon region, indicating the formation of the dihydrate (3h). A sample consisting almost entirely of the dihydrate (3h) could be prepared by dissolving 1a

in 1N HCl solution [21] and by neutralizing the solution (pH 7), after standing for 60 min. The spectrum of this solution closely resembled that of the diammonia adduct (3a).

The 1N HCl solution of pteridine did not show signals belonging to the parent compound. The three signals at high field indicate that in this solution cations of the mono and dihydrate ($2c^{\oplus}$, $3h^{\oplus}$) have been formed. Interestingly the low field part of the ^{13}C NMR spectra of the dihydrate cation ($3h^{\oplus}$) and the cation of 4,5-diaminopyrimidine ($4^{\oplus}$), both recorded for a 1N HCL solution, are virtually the same.

Table 2 Summary of the ^{13}C chemical shifts of adducts of pteridines

	Solvent	C-2	C-4	C-6[a]	C-7[a]	C-9	C-10
3a	NH_3	148.9	135.8	60.9	62.8	150.5	125.3
3b	NH_3	153.4	136.1	61.2	63.0	150.6	124.1
3c	NH_3	148.7	143.7	61.0	62.3	151.1	121.3
3d	NH_3	157.9	136.4	60.9	62.9	151.1	122.0
3e	NH_3	148.0	136.3	60.7	62.8	152.5	124.4
3f	NH_3	147.3	145.9	60.8	62.4	151.6	120.7
3g	NH_3	148.3	144.7	61.0	62.5	153.2	120.4
3h	H_2O	148.3	135.7	73.5	75.0	150.1	124.7
$3h^{\oplus}$	1 N HCl	144.1	123.8	73.1	75.4	153.8	125.2
2a	NH_3	151.5	61.4	144.2	140.4	b	b
2b	NH_3	158.5	69.6	142.5	135.9	155.2	140.6
2c	H_2O	151.9	73.9	145.8	142.0	b	b

	Solvent	C-2	C-4	C-5	C-6
4	D_2O	149.5	155.3	126.6	139.1
$4^{\oplus}$	1 N HCl	144.0	157.6	127.6	124.8

[a] Signals may be interchanged
[b] Signals did not exceed signal-to-noise level.

3.5 EXPERIMENTAL

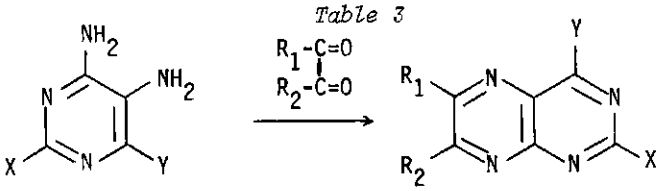
^{13}C spectra were measured on a Varian XL-100-15 spectrometer operating at 25.2 MHz, equipped with a pulse unit and a 620 L-16K on line computer system. In $CDCl_3$ solution the deuterium resonance of the solvent was used as an internal field-frequency lock signal. In the case of liquid ammonia or water as solvent, field-frequency lock was obtained from the ^{19}F NMR signal of a capillary of hexafluorobenzene positioned along the longitudinal axis of the 12 mm (o.d.) sample tubes employed. Spectra were taken at ambient temperature, but when measuring liquid ammonia samples the probe temperature was -50^{o}. In $CDCl_3$ solution ^{13}C NMR chemical shifts were measured from internal TMS. In NH_3 and H_2O solution ^{13}C NMR chemical shifts were measured from internal trimethylamine and internal dioxane respectively, and they were converted to the TMS scale by adding 47.5 and 67.4 ppm respectively. Typical spectral parameters were as follows: spectral width 5120 Hz (1.25 Hz/point) acquisition time 0.8 s, pulse delay 1.2 s, pulse width 10 μs. For most of the samples sufficient signal-to-noise ratio was obtained after accumulating and transforming 2000-4000 free induction decays.

3.5.1 SYNTHESIS OF THE RECORDED PTERIDINES

The following compounds were prepared according to procedures given in the literature, pteridine[22](1a), 2-chloropteridine[20](1b), 7-methylpteridine[15](1c), 2-methylthiopteridine[15](1d), 2-phenylpteridine[23](1e), 4-phenylpteridine[24](1f), 2-chloro-4-phenylpteridine[20](1i), 6,7-dimethylpteridine[15](1j), 2-methylthio-4-phenylpteridine[1](1l), 4,6-diphenyl-2-methylthiopteridine[1](1r) and 4,7-diphenyl-2-methylthiopteridine[1](1s).

The following pteridines (see Table 3) were obtained by condensation of the appropriate 4,5-diaminopyrimidine derivative and glyoxal[27], phenylglyoxal or benzil. With the two former compounds the condensation reaction proceeded smoothly in boiling ethanol. The preparation of 4,6,7-triphenylpteridine (1t) was carried out in boiling 2-ethoxyethanol. 4-*t*-Bu-2-chloro-6-phenylpteridine (1m) was not isolated. TLC and ^{13}C NMR revealed its formation in a minute amount in addition to the major isomer (1n) (ratio 1:10). Dechloromethoxylation of this mixture afforded the isomeric 4-*t*-Bu-2-methoxy-6- and 7-phenylpteridines (1o and 1p), which were measured as a mixture.

Table 3



Starting pyrimidine	*Pteridine derivative*	*m.p. (°C)*	*Yield %*
X = Y = H[25]	X=Y=R_1=H, R_2=C_6H_5 (1g)	158-160	95
X = Cl, Y = CH_3[26]	X=Cl, Y=CH_3, R_1=R_2=H (1h)	155-157	80
X = H, Y = C_6H_5[24]	X=R_1=H, Y=R_2=C_6H_5 (1k)	154-155	92
X = Cl, Y = *t*-Bu[28]	X=Cl, Y=*t*-Bu, R_1=H, R_2=C_6H_5 (1n)	174-176	60
	X=OCH_3, Y=*t*-Bu, R_1=H, R_2=C_6H_5 (1p)	142-144	75
X = Cl, Y = C_6H_5[20]	X=Cl, R_1=H, Y=R_2=C_6H_5 (1q)	198-199	72
X = H, Y = C_6H_5[24]	X=H, Y=R_1=R_2=C_6H_5 (1t)	174-175	86

3.5.2 GENERAL PROCEDURE FOR MEASURING THE ^{13}C NMR SPECTRA IN LIQUID AMMONIA

The procedure followed was reported previously[20]. In this study the formation of the 6,7-diamino-5,6,7,8-tetrahydropteridines was accelerated by preparing a solution of the appropriate pteridine derivative in liquid ammonia at room temperature in a suitable all glass vessel. The cooled solution was siphoned over into a ^{13}C NMR tube.

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4 ON THE CONVERSION OF PTERIDINES INTO PURINES*

A.Nagel and H.C.van der Plas

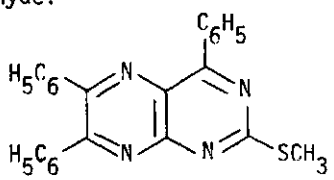
4.1 INTRODUCTION

In a previous paper we reported[1-3] on the covalent amination of pteridine and some of its derivatives by liquid ammonia. By ^{1}H NMR (nuclear magnetic resonance) spectroscopy good evidence was obtained for addition of the nucleophile across the C-4 — N-3 bond or both the C-6 — N-5 and the C-7 — N-8 bonds. The addition across the C-4 — N-3 bond is a kinetically favoured process. In connection with those studies on σ-adduct formation and as a continuation of our investigations on the mechanism of displacement reactions with strong nucleophiles[4] (potassium amide, lithium piperidide) in diazaaromatics, containing a group with considerable leaving character, we became interested in the behaviour of substituted pteridines towards potassium amide in liquid ammonia.
For that purpose we studied initially 2-methylthio-4,6,7-triphenylpteridine (1). This compound features the presence of the relatively large phenyl group on positions which are known to be easily attacked in the parent compound *i.e.* pteridine, by the nucleophile. The bulky phenyl group will retard or prevent addition to the positions 4, 6 and 7, making the amino-dethiomethylation at C-2 a process, which is highly competitive with the addition reaction.

4.2 RESULTS AND DISCUSSION

Treatment of 2-methylthio-4,6,7-triphenylpteridine (1) in liquid ammonia with 4 equivalents of potassium amide at -33^{o} for 3 h results in the formation of 2-amino-4,6,7-triphenylpteridine (2) in moderate yield (25%). The structure of 2 was proved by identity with the product obtained by amination of 2-chloro-4,6,7-triphenylpteridine with ethanolic ammonia. Thin-layer chromatography (TLC) analysis of the reaction product of 1 with potassium amide indicated that besides 2 and unreacted 1 (50%) another species, having a characteristic blue fluorescence, is present. After isolation and purification by column chromatography we were able to identify this compound as 6,8-diphenyl-2-methylthiopurine

(3). The yield of the purine 3 amounted to 8%. The structure assignment of 3 was based on its spectroscopic properties (^{1}H NMR (DMSO-d_6) δ 2.67 ppm 3H, δ 7.6 ppm 10H; accurate mass measurement indicates the molecular formula $C_{18}H_{14}N_4S$, first fragmentation to $C_{17}H_{11}N_4$, (M-SCH_3); UV spectrum λ_{max} 264 nm, $\varepsilon = 7.7.10^3$; λ_{max} 358 nm, $\varepsilon = 4.4.10^3$), and was confirmed by infrared (IR)-identity and undepressed melting point with an authentic specimen obtained by condensation of 4,5-diamino-2-methylthio-6-phenylpyrimidine (6) with benzaldehyde.



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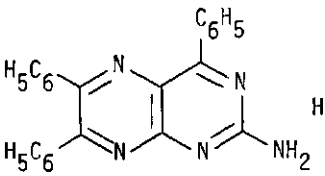

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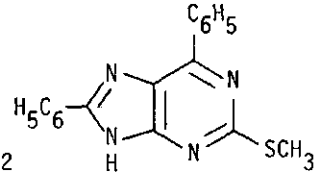
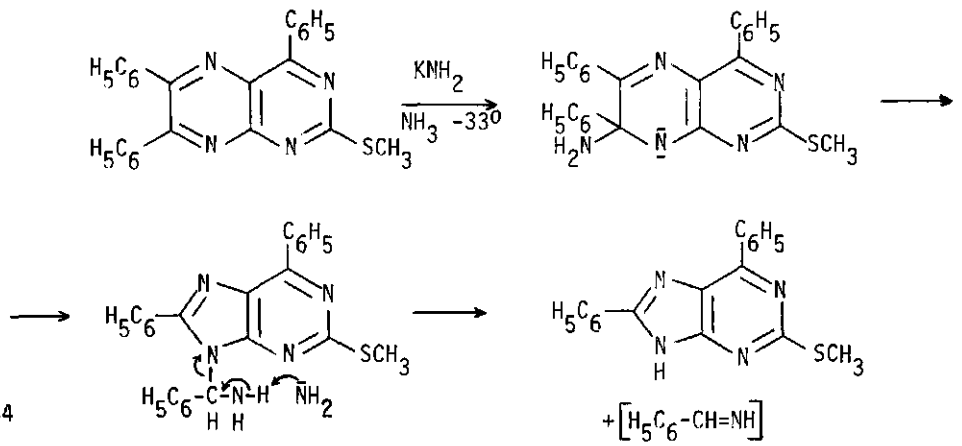


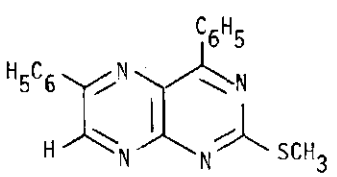
3

This ring contraction of a pteridine into a purine system is unprecedented, in contrast to the ring expansion of purines into pteridines, which has already been described[5]. However, the reported ring contraction bears close relationship to the known conversions of 2,3-diphenylquinoxaline into 2-phenylbenzimidazole[6] and of 2-chloro-3-phenylquinoxaline into benzimidazole[7] by potassium amide in liquid ammonia.

With regards to the mechanism of the conversion of 1 into 3 we assume that it proceeds *via* an initial addition of the amide ion to the C=N bond of the pyrazine ring. Whether this attack will take place at C-6 of C-7 of the pteridine 1 is, as yet, unknown. It is evident that the presence of the phenyl groups on both positions certainly has a considerable blocking effect on the addition. The low yield of 3 probably reflects this hindered attack. The σ-adduct formed is believed to undergo a rearrangement leading to 3 under elimination of benzylideneimine (see in the scheme below).



Although in this scheme the expulsion of C-7 is depictured, it is clear that there is no real evidence for it; the possible expulsion of C-6 cannot be excluded. In order to obtain more information about the possibility of a C-6 and/or C-7 expulsion during the ring contraction we synthesized the isomeric 4,6- and 4,7-diphenyl-2-methylthiopteridines (4 and 5 respectively) and treated both compounds with potassium amide in liquid ammonia at -33°for 1 h. It appears that both isomers are converted into the same purine derivative *i.e.* 3 in a yield (20%) being thus considerably higher than that obtained from 1. Besides the recovery of 60% of unreacted 4 and 5 no indication for the expulsion of the carbon atom, carrying the phenyl group, yielding 2-methylthio-6-phenylpurine was obtained. Thus it is evident that both pteridine derivatives 4 and 5 undergo a reaction in which the carbon atom, carrying the hydrogen atom, is expelled during the ring contraction. Attempts to measure by ^{1}H NMR spectroscopy the adduct of 4 (or 5) with the amide ion by the method described previously[8], failed, probably due to the low solubility of the compounds.



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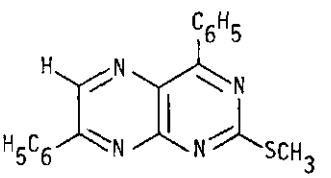

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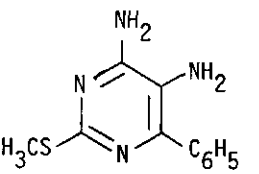

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4.3 SYNTHESIS OF THE STARTING SUBSTANCES 1, 4 and 5

The hitherto undescribed 2-methylthio-4,6,7-triphenylpteridine (1) is conveniently prepared by condensation of 4,5-diamino-2-methylthio-6-phenylpyrimidine (6) with benzil. The required pyrimidine derivative 6 was prepared by methylation of the corresponding mercapto compound. Condensation of 6 with phenylglyoxal yielded a mixture of diphenyl-2-methylthiopteridines. The ^{1}H NMR spectrum of this mixture dissolved in $CDCl_3$, showed, besides the phenyl multiplets, a high field resonance at δ 2.78 ppm and two singlets at δ 9.25 and δ 9.44 ppm. Based on the facts that i H-7 in pteridine shows a lower field absorption resonance[9-11] than H-6 and ii the phenyl group substituted in the pyrazine ring influences the chemical shifts of both adjacent hydrogen atoms in an identical manner (see table), we ascribed the low field adsorption at 9.44 ppm to H-7 of 4 and the absorption at δ 9.25 ppm to H-6 of 5.

Table

	H-6[a]	H-7[a]	
2-Methylthio-4-phenyl-pteridine[b]	8.84	9.03	-
Compound 4[b]	-	9.44	-0.41
Compound 5[b]	9.25	-	-0.41

a) δ-values in ppm
b) Spectrum measured in $CDCl_3$ using TMS as an internal standard (δ = 0.00 ppm)

Based on the ratio of the integrals of the absorptions of both hydrogen atoms at δ 9.25 ppm and δ 9.44 ppm the ratio 4 : 5 is about 1 : 10. After separation of the mixture the ultraviolet (UV) spectra of 4 (λ_{max} 293 nm, $\varepsilon = 30.2 \cdot 10^3$; λ_{max} 397 nm, $\varepsilon = 11.6 \cdot 10^3$) and 5 ($\lambda_{max}$ 280 nm, $\varepsilon = 24.2 \cdot 10^3$; λ_{max} 389 nm, $\varepsilon = 14.2 \cdot 10^3$) were measured and found to be in good agreement with data published in the literature on 6- and 7-substituted pteridines[12,13]

4.4 EXPERIMENTAL

4,5-Diamino-2-methylthio-6-phenylpyrimidine (6)

4,5-Diamino-6-phenyl-2-thiopyrimidine[14] (2.8 g; 12.8 mmol) dissolved in 25 ml 1 N KOH was shaken vigorously with methyliodide (0.88 ml; 14.1 mmol) for 15 min. After refrigeration the filtered crude product was recrystallized from 25 ml of ethanol to yield 2.0 g (67%) of pure 1, m.p. 188-190°. *Anal.* Calcd. for $C_{11}H_{12}N_4S$ (232.30): C, 56.87; H, 5.21. Found: C, 56.46; H, 5.17.

2-Methylthio-4,6,7-triphenylpteridine (1)

To the above described diamine (see section 1) (2.0 g; 8.6 mmol) in 25 ml of ethanol were added 2.2 g of benzil (10.4 mmol). After refluxing for 16 h the contents of the flask were cooled in an ice-bath and filtered. The solid material was purified by chromatography (silica gel, activity I) using chloroform as eluent. After evaporation of the solvent the solid material was recrystallized from a mixture of benzene and methanol (40 ml; 1/1 v/v). The yield amounted to 1.5 g (43%) of pure 2-methylthio-4,6,7-triphenylpteridine, m.p. 232-234°. *Anal.* Calcd. for $C_{25}H_{18}N_4S$ (406.19): C, 73.86; H, 4.46. Found: C, 74.06; H, 4.48.

2-Chloro-4,6,7-triphenylpteridine

2-Chloro-4,5-diamino-6-phenylpyrimidine[2] (440 mg; 2.0 mmol) and benzil (505 mg;

2.4 mmol) were dissolved in warm 2-ethoxyethanol (5 ml) and the solution was kept at 120° for 5 h. To the hot solution were added 5 ml of methanol and 5 ml of water, after which the pteridine crystallized. The crude material was purified by the same procedure as described above for the methylthio derivative (1) (see section 2) m.p. 209-210°, yield 458 mg (58%). *Anal.* Calcd. for $C_{24}H_{15}ClN_4$ (394.85): C, 73.00; H, 3.83. Found: C, 72.82; H, 4.12.

2-Amino-4,6,7-triphenylpteridine

2-Chloro-4,6,7-triphenylpteridine (400 mg, 1.0 mmol) was heated at 100° for ½ h together with 5 ml of ethanolic ammonia (saturated at room temperature) in a sealed tube. Upon cooling the pure product crystallized in quantitative yield, m.p. 271-272°. *Anal.* Calcd. for $C_{24}H_{17}N_5$ (375.42): C, 76.78; H, 4.56. Found: C, 76.38; H, 4.84.

4,6- and 4,7-Diphenyl-2-methylthiopteridine (4, resp. 5)

4,5-Diamino-2-methylthio-6-phenylpyrimidine (464 mg, 2.0 mmol) and phenylglyoxal monohydrate (320 mg, 2.1 mmol) were dissolved in 5 ml of hot 2-ethoxyethanol. The solution was boiled for 5 min. Upon addition of water (5 ml) and methanol (10 ml) a yellow crystalline material deposited (565 mg, 85%). It could be separated into its components by column chromatography (silica gel activity I, eluent chloroform) to yield 424 mg of 4,7-diphenyl-2-methylthiopteridine (5), m.p. 165-166° and 42 mg of 4,6-diphenyl-2-methylthiopteridine (4), m.p. 218-220°. *Anal.* Calcd. for 5: $C_{19}H_{14}N_4S$ (330.40): C, 69.06; H, 4.27. Found: C, 69.01; H, 4.27. Calcd. for 6, $C_{19}H_{14}N_4S$ (330.40): C, 69.06; H, 4.27. Found: C, 69.04; H, 4.31.

6,8-Diphenyl-2-methylthiopurine (3)

To 464 mg (2.0 mmol) of 4,5-diamino-2-methylthio-6-phenylpyrimidine (6) and 80 mg copper (II) acetate in 10 ml of 50% aqueous methanol a solution of 212 mg (2.0 mmol) of benzaldehyde in 2.5 ml of methanol was added. The solution was gently refluxed for 15 min. A precipitate was obtained, which was filtered and freed from copper (II) ions by treatment of an aqueous ethanolic suspension of this precipitate by hydrogen sulphide. After filtration and evaporation to dryness *in vacuo* the remaining solid was extracted with hot chloroform. The filtered chloroform solution slowly deposited the purine derivative upon cooling, m.p. 305° (decomp.), yield 398 mg (61%). *Anal.* Calcd. for $C_{18}H_{14}N_4S$ (318.39): C, 67.90; H, 4.43. Found: C, 67.80; H, 4.51.

2-Methylthio-4-phenylpteridine

4,5-Diamino-2-methylthio-6-phenylpyrimidine (6)(70 mg, 0.3 mmol) and glyoxal trimer monohydrate (75 mg, 1.0 mmol) were dissolved in 9 ml of ethanol. The solution was refluxed for 30 min. The solvent was removed *in vacuo* and the crude product was purified by chromatography on silica gel activity I, using chloroform as the eluent. Yield, 62 mg (81%), m.p. 177-179° (from ethanol). *Anal.* Calcd. for $C_{13}H_{10}N_4S$ (254.31): C, 61.39; H, 3.96. Found: C, 61.50; H, 3.89.

4.5 GENERAL PROCEDURE FOR THE REACTIONS OF THE COMPOUNDS 1, 4 and 5 WITH POTASSIUM AMIDE IN LIQUID AMMONIA

To 10 ml of dry liquid NH_3 (distilled from KNH_2),in a 50 ml three-neck round-bottomed flask, equipped with a dry-ice/acetone condenser, were added a few crystals of $Fe(NO_3)_3 \cdot 9H_2O$ catalyst and 160 mg (4 mmol) of potassium. After stirring for 30 min at -33° the pteridine derivative (1 mmol) was added under the exclusion of moisture. The reaction was terminated after 3 h by the addition of 220 mg (4 mmol) of ammonium chloride. After the ammonia was evaporated, the residue was thoroughly extracted with warm chloroform (5x20 ml). The filtered chloroform extracts were concentrated *in vacuo* and the residual gum (or solid) separated into its components by column or preparative thin layer chromatography. The eluentia used were chloroform or a mixture of chloroform/methanol in the ratio 95 : 5.

4.6 REFERENCES

* Dedicated to the memory of Prof.Eiji Ochiai.

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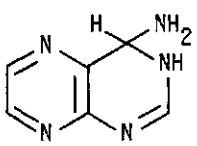
5 ON THE MECHANISM OF THE CONVERSION OF 2-METHYLTHIO-4,6,7-TRIPHENYLPTERIDINE INTO 2-AMINO 4,6,7-TRIPHENYLPTERIDINE AND 6,8-DIPHENYL-2-METHYLTHIOPURINE*

A.Nagel and H.C.van der Plas

5.1 INTRODUCTION

In an earlier investigation we reported on the addition of liquid ammonia to pteridine and some of its derivatives[1-4]. ^{1}H NMR evidence was presented for the formation of two different species *i.e.* the 1 : 1 σ-adduct 4-amino-3,4-dihydropteridine (1) and the thermodynamically favoured 2 : 1 σ-adduct 6,7-diamino-5,6,7,8-tetrahydropteridine (2).



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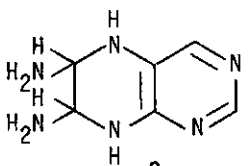
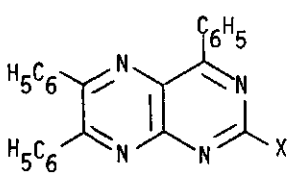
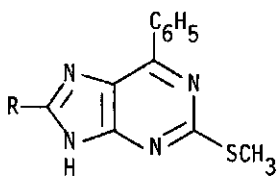


2

Furthermore, we observed that when 2-methylthio-4,6,7-triphenylpteridine (3) is reacted with potassium amide, amino-de(methylthio)lation into 2-amino-4,6,7-triphenylpteridine (4) and ring contraction into 6,8-diphenyl-2-methylthiopurine (5a) takes place[5]. The same purine derivative is also obtained from 4,6-diphenyl- and 4,7-diphenyl-2-methylthiopteridine[5].



3: X=SCH_3
4: X=NH_2

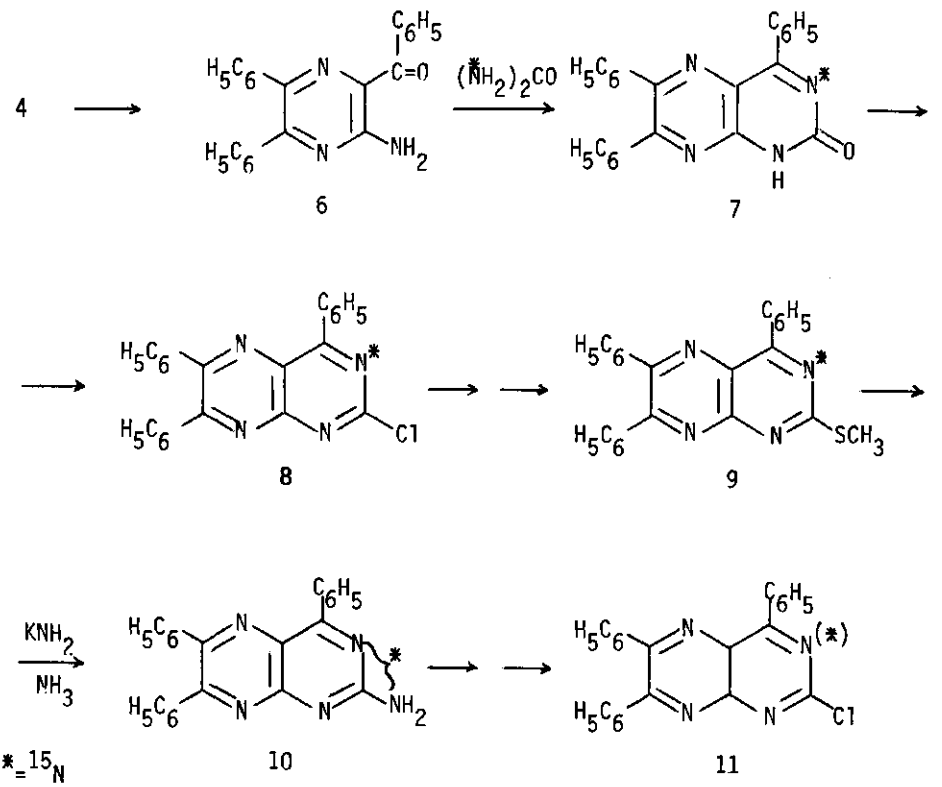


5a: R=C_6H_5
5b: R=C_6D_5

As an amide-catalysed ring contraction of pteridines into purines has never been observed before[6] we became interested in the scope and mechanism of this conversion. In this paper we concentrate us on the intriguing problem whether C-6 and/or C-7 is expelled from the pyrazine ring. There is ample evidence that the nucleophilic displacement in 2-substituted pyrimidines by an amide ion occurs *via* a ring opening - ring closure [S_N(ANRORC)]mechanism[7]. It induced us to study the occurrence of this process in the amino-de(methylthio)lation (3 → 4).

5.2 ON THE AMINO-DE(METHYLTHIO)LATION

In order to study the occurrence of the S_N(ANRORC) mechanism we prepared 2-methylthio-4,6,7-triphenylpteridine (9) which is enriched with ^{15}N in N-3 of the pyrimidine ring. If the amino-de(methylthio)lation occurs without ring opening, all ^{15}N remains in the ring, while in the case of an S_N(ANRORC) mechanism ^{15}N becomes a part of the exocyclic nitrogen atom. The introduction of a ^{15}N label at N-3 in 9 could be achieved as outlined in scheme 1.



Scheme 1

Acid hydrolysis of 4 yielded as main product 2-amino-3-benzoyl-5,6-diphenylpyrazine (6) and only a small amount of 4,6,7-triphenylpteridin-2-one[8]. Formation of 7, being labelled at N-3, was performed by reaction of 6 with 1,3-^{15}N labelled urea.

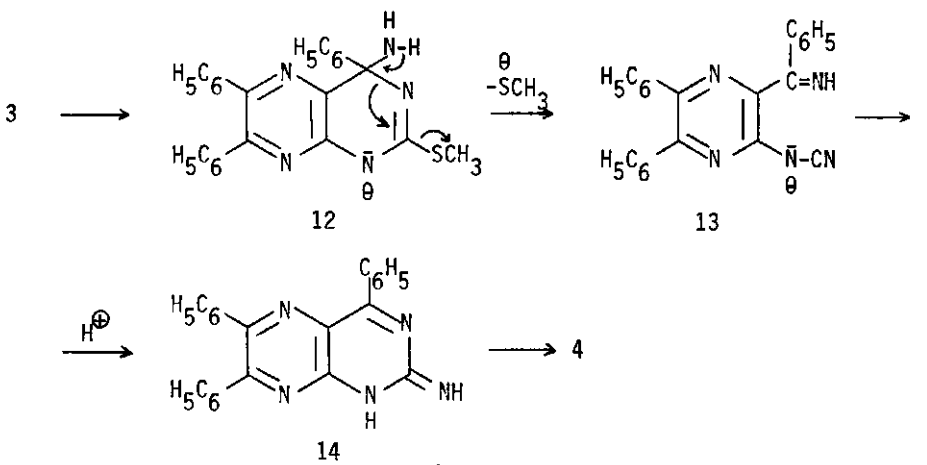
In this reaction no trace of a [^{15}N-1,^{15}N-3] pteridin-2-one was formed as proved by mass spectrometry[9]. By the reaction of 7 with a mixture of $POCl_3$ and PCl_5 8 was formed which then was converted into 9 by treatment with hydrogen sulphide in basic medium and a subsequent methylation of the thio compound formed with methyliodide[10]. This laborious way to prepare 9 led us to develop techniques for small scale operations with KNH_2, containing ^{15}N, in liquid $^{15}NH_3$. So we could study besides the amino-de(methylthio)lation of the ^{15}N labelled 9 with unlabelled KNH_2 (experiment 1) that of unlabelled 3 with $K^{15}NH_2$ (experiment 2). In experiment 1 compound 9 (10% of excess of ^{15}N) was reacted with 4 equivalents of KNH_2 in liquid NH_3 and the 2-amino derivative 10 was isolated by column chromatography. Attempts to establish, by acid hydrolysis into 4,6,7-triphenylpteridin-2-one, whether ^{15}N is present in the exocyclic nitrogen atom in 10 failed due to the formation of 6, leading thus to a complete loss of ^{15}N. Diazotization with sodium nitrite in an aqueous acid was also not successful[11]. We found however that the conversion of 10 into the corresponding pteridin-2-one could nicely be achieved when the diazotization was carried out at room temperature using glacial acetic acid as solvent and adding the sodium nitrite as a solid[12]. The crude pteridin-2-one was converted into 11 by a mixture of $POCl_3$ and PCl_5. Measurement of the ^{15}N excess in 11 by mass spectrometry showed that 11 contained 5.0% of excess of ^{15}N.

This means that 50% of compound 9 reacts in the amino-de(methylthio)lation according to an S_N(ANRORC) mechanism (see table 1)[13]. We assume that the remaining 50% reacts *via* an S_N(AE) pathway[13]. When compound 3 was reacted with 10 equivalents of $K^{15}NH_2$ (6.2% of excess of ^{15}N) in liquid $^{15}NH_3$, it was found from the results of the ^{15}N measurements that 3 under these conditions reacts into 10 according to the S_N(ANRORC) mechanism for 85% (exp.2). Apparently the percentage according to which this ring opening - ring closure mechanism occurs, is strongly dependent on the concentration of KNH_2[14,15].

Table 1

Exp.	Substrate (1 mmol in 25 ml of NH_3)	Reagent	% of excess of ^{15}N in substrate	(10)	(11)	% S_N(ANRORC)
1	9	4 eq.KNH_2	10.0	10.0	5.0	50
2	3	10 eq.KNH_2	0	6.2	5.7	85

From the results obtained it is evident that C-4 in 3 is, in despite of the presence of the phenyl group, vulnerable to a nucleophilic addition of an amide ion. Similar observations have been made with 4,6-diphenyl-2-halogeno-pyrimidines[14,15]. The adduct 12 undergoes the ring opening leading to the open-chain intermediate 13 which recyclizes *via* 14 into 4 (scheme 2).

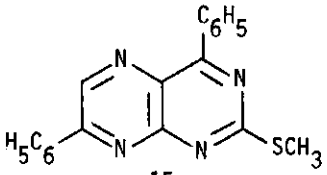


Scheme 2

5.3 ON THE RING CONTRACTION OF 3 INTO 5a

In order to discern whether C-6 and/or C-7 is expelled during the above mentioned ring contraction, we tried to synthesize a compound in which *one* of the phenyl groups either at position 6 or at position 7 is deuterated. The obvious method to synthesize this compound was the phenylation of the relatively easily available 4,7-diphenyl-2-methylthiopteridine (15) or of its structural isomer 4,6-diphenyl-2-methylthiopteridine (16) with deuterated phenyllithium and subsequent oxidation of the intermediary dihydro compound obtained. Phenylation of

pteridines has never been published[16], but this method is successfully used for the preparation of phenyldiazines[17].



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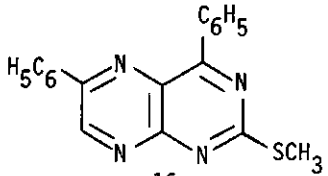

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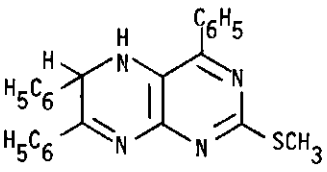

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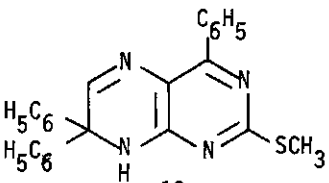
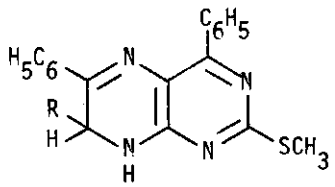


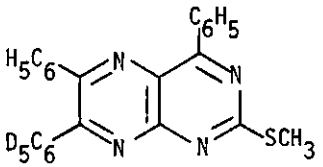
18

From introductory experiments we learned that treatment of 15 with phenyllithium and work-up of the reaction mixture with water gave us a compound with m/e 408; it indicates the formation of a 2-methylthio-triphenyldihydropteridine. Since this compound was found to be very resistant to oxidation with O_2, $KMnO_4$ in acetone and Fe^{3+}, it was evident that this compound cannot have structure 17. Furthermore heating of this compound with hydrochloric acid gave, surprisingly, benzophenone, indicating that the addition of phenyllithium had taken place to a carbon atom already carrying a phenyl group (either C-4 or C-7). This phenomenon is not unprecedented and has been observed in related reactions[18]. A conclusive structure assignment was based on its ^{13}C NMR spectrum and shows that the phenylation product of 15 is 7,8-dihydro-2-methylthio-4,7,7-triphenylpteridine (18) (See Experimental).

Now it has been established that position 7 in the pteridine ring is the preferred position of attack by phenyllithium, it is evident that 16 is a more appropriate compound to serve our purpose. Reaction of 16 with phenyllithium yields indeed 7,8-dihydro-2-methylthio-4,6,7-triphenylpteridine (19a). This compound could not be isolated, since it very easily undergoes oxidation by air. Treatment with $KMnO_4$ in acetone gives 3 in quantitative yield. Analogously, by the action of pentadeuterophenyllithium on 16 and oxidation of 19b we were able to obtain 20.

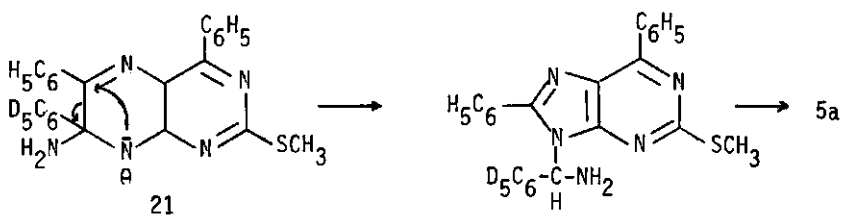


19a: R=C_6H_5
19b: R=C_6D_5



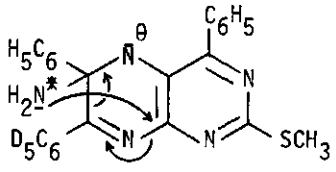
20

After reaction of 20 (99.8% d_5) with KNH_2 in liquid NH_3 the purine was isolated and its deuterium content was established by mass spectrometry. From the data it appeared to consist of a mixture of compound 5a (m/e 318) and 5b (m/e 323) in the ratio 5a/5b = 87/13. From this result the conclusion seems justified that mainly C-7 is expelled confirming our earlier proposal, that the ring contraction starts with initial attack at C-7 *i.e.* 21. Ring opening as indicated gives purine 22 which by a base-catalysed elimination of pentadeuterobenzylideneimine yields 5a. However to exclude the alternative mechanism in which amide anion attacks C-6 in 3 yielding the adduct 23 which then undergoes a ring closure to the purine with a concomitant elimination of pentadeuterobenzonitrile, we reacted 3 with $K^{15}NH_2$ in liquid $^{15}NH_3$.
By mass spectrometry it was shown that the purine formed did not contain any ^{15}N enrichment thus excluding the intermediacy of 23 as reactive species in the ring contraction.



21 22

Both the phenylation reactions as well as the results of the deuterium and ^{15}N labelling experiments fully confirm that C-7 is more vulnerable for a nucleophilic attack than C-6. Attempts to prove the existence of this adduct by ^1H- and ^{13}C NMR measurements failed, probably due to the low solubility of 16 in liquid NH_3.



23

Combining the results discussed in sections a and b it is evident that the pteridine 3 is *multi*reactive towards the amide ion. It undergoes addition at position 2 (yielding 4 according to an S_N(AE) process), at position 4 (yielding 4 *via* an S_N(ANRORC) mechanism), at postion 7 (yielding the purine 5a) and at position 6 (also yielding the purine 5a). The order of reactivity is approximately C-4 > C-2 > C-7 > C-6, based on quantitative product studies and on the distribution of the ^{15}N and the D content in the amino compounds as well as in the purine derivatives.

5.4 EXPERIMENTAL

Melting points are uncorrected. ^1H NMR spectra were recorded with a JEOL JNM C-60H spectrometer. ^{13}C NMR spectra were measured on a Varian XL-100-15 spectrometer operating at 25.2 MHz, equipped with a pulse unit and a 620 L-16K on line computer system.

5.4.1 SYNTHESIS OF THE STARTING MATERIALS

2-Amino-3-benzoyl-5,6-diphenylpyrazine (6)

2-Amino-4,6,7-triphenylpteridine[5] (375 mg, 1.0 mmol) and 5 ml 6N HCl were heated for 10 h at 150° in a sealed tube. After cooling the contents of the tube were extracted with $CHCl_3$. The extracts were dried over $MgSO_4$ and evaporated. The solid obtained was recrystallized from methanol yielding 252 mg (72%) of 6 as tiny yellow needles, m.p. 193°. *Anal.*Calcd. for $C_{23}H_{17}N_3O$ (351.39): C, 78.61; H, 4.88. Found: C, 78.42; H, 5.00.

2-Chloro-4,6,7-triphenyl [^{15}N*-3*] *pteridine (8)*

700 mg of 6 (2.0 mmol) were stirred with 480 mg (8.0 mmol) of $^{15}N,^{15}N$-urea containing 30.3% ^{15}N at 200° for 1 h. Recrystallization of the product from aqueous DMF yielded the pteridin-2-one (7) as yellow needles m.p. 299-300° (490 mg, 65%) (See for the formation of the unlabelled compound from 2-amino-4,6,7-triphenylpteridine, section 5).

Treatment of 7 with $POCl_3$ and PCl_5 for 1 h at 100°, was followed by thorough decomposition of the reagents with water. Extraction of the aqueous layer with $CHCl_3$ yields 8 (m.p. 209-210°) in 35%. It proved to be identical with an authentic specimen[5].

2-methylthio-4,6,7-triphenyl[^{15}N-3]pteridine (9)

400 mg (1.0 mmol) of 8 were suspended in a mixture of 10 ml of ethanol and 10 ml of water containing 100 mg (2.5 eq.) NaOH. The solvent was saturated at 0°C with H_2S. The mixture was heated slowly and finally boiled for 10 min with vigorous stirring. To the filtered red-coloured solution was added 20 ml of glacial acetic acid. After cooling overnight the filtered product was dissolved in 2 ml N KOH and the solution was shaken vigorously with methyliodide (0.2 ml; 3.5 mmol). The resulting suspension was extracted with $CHCl_3$ and the extract purified by column chromatography. Pure 9 was obtained, m.p. 233-234° in a yield of 30% (130 mg)(lit.[5] 232-234°).

5.4.2 PHENYLATION REACTIONS

5.4.2.1 phenylation of 4,6-diphenyl-2-methylthiopteridine (16)

When a solution of 66 mg (0.2 mmol) of 16 in 10 ml of sodium-dried benzene is treated with 0.2 ml of phenyllithium (1.3 N) at room temperature a green solution is obtained. After treatment with water (10 ml), the benzene layer is separated, dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil is dissolved in acetone, and $KMnO_4$ is added until the permanganate colour remains.
The acetone is removed *in vacuo* and the residue is dissolved in $CHCl_3$, filtered and chromatographed on silica gel using $CHCl_3$ as the eluent. A yellow product was obtained as tiny crystals (62 mg, 75%) which proved to be identical with an authentic specimen of 2-methylthio-4,6,7-triphenylpteridine (3)[4].
Following the same procedure and using pentadeuterophenyllithium as the reagent, compound 20 was obtained.

5.4.2.2 phenylation of 4,7-diphenyl-2-methylthiopteridine (15)

The phenylation of this compound was performed in the same way as described in a). After isolation an orange-coloured syrup was obtained,which was characterized by ^{13}C NMR spectroscopy as 18 (C-2 170.3; C-4 158.3; C-6 154.2; C-7 65.1; C-9 152.6; C-10 116.7)[19].

5.4.3 DIAZOTIZATION OF 2-AMINO-4,6,7-TRIPHENYLPTERIDINE (4)

To a solution of 30 mg of 4 in 5 ml of glacial acetic acid, in small portions 200 mg of solid $NaNO_2$ were added in a period of 15 min. The solution was stirred

well. After the addition 5 ml of water were added and the precipitate was collected by suction, washed with water, alcohol and ether to yield the corresponding pteridin-2-one (21 mg, 70%), m.p. 299-300°).

Anal. Calcd. for $C_{24}H_{16}N_4O$ (376.40): C, 76.58; H, 4.28. Found: C, 76.39; H, 4.58.

5.4.4 AMINATION PROCEDURE

The reactions in liquid ammonia with potassium amide were carried out as described before[5]. The all glass apparatus used for the experiments in liquid $^{15}NH_3$ was essentially the same. $^{15}NH_3$ was prepared by treating $^{15}NH_4NO_3$ with a concentrated solution of KOH in H_2O at 100° for 2 h. After the experiment it was reconverted into $^{15}NH_4NO_3$ in an average yield of 85%.

5.5 REFERENCES

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7. For a recent discussion see A.P.Kroon and H.C.van der Plas, Rec.Trav.Chim. Pays-Bas 92, 145 (1973) and references therein.
8. Acid hydrolysis of pteridines has successfully been used for the preparation of certain pyrazines, see: J.Clark and C.Smith, J.Chem.Soc.(C), 1971 (1948).
9. From the ratio of m+2/m, measured in the 2-chloro compound - being obtained from the pteridin-2-one - it was found that 3.5% of excess of ^{15}N is present. It could be calculated that this percentage of enrichment of the m+2 peak must be ascribed to the presence of m+1 molecules derived from $C_{24}H_{15}{}^{15}N^{14}N_3Cl$.
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13. Compare the mechanism proposed by E.C.Taylor Jr. in J.Am.Chem.Soc. 74, 1651, (1952).
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19. The spectrum was interpreted by additivity relationships using the data presented in ref.3.

* Dedicated to Prof. R.B.Woodward, for his 60th birthday.

6 DUAL REACTIVITY OF 2-CHLORO-4,6,7-TRIPHENYLPTERIDINE AND 6-CHLOROPYRIDO[2,3-*b*]PYRAZINE TOWARDS KNH_2 IN LIQUID NH_3

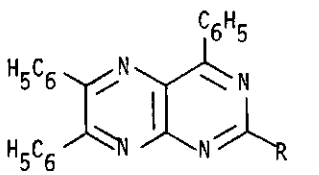
A.Nagel and H.C.van der Plas

6.1 INTRODUCTION

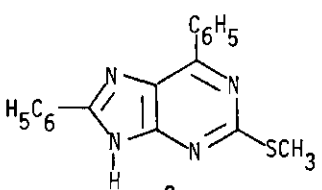
Our first report[1] on the action of strong nucleophiles on pteridines concerned the conversion of 2-methylthio-4,6,7-triphenylpteridine (1a) into 2-amino-4,6,7-triphenylpteridine (1b) and 6,8-diphenyl-2-methylthiopurine (2) (ratio 3/1) by KNH_2 in liquid NH_3 at -33^{o}.
Recently we proved that in these conversions *all* carbon positions *i.e.* C-2, C-4, C-6 and C-7 are attacked: formation of *1b* takes place by initial attack of the nucleophile at *both* C-4 and C-2 and the formation of the purine derivative 2 by an attack at C-7 and, to a less extent, at C-6.

6.2 RESULTS

The purpose of this communication is to report that 2-chloro-4,6,7-triphenylpteridine (1c)[2,3] shows a rather different reactivity towards KNH_2 in liquid NH_3 than 1a. Amino-dechlorination of 1c into 1b takes place (70%) besides dechlorination into 4,6,7-triphenylpteridine[4] (1d). The formation of a purine derivative was not observed.



1a: R=SCH_3 1b: R=NH_2
1c: R=Cl 1d: R=H



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By studying the amination of 2-chloro-4,6,7-[^{15}N-3] pteridine[5] with unlabelled KNH_2 and in a complementary experiment, by reacting 1c with $K^{15}NH_2$ in liquid $^{15}NH_3$ it was proved that the amino-dechlorination proceeds for 100% *via* a ring opening - ring closure sequence [S_N(ANRORC) mechanism]. This result means that the 2-chloro substituent in 1c has directed the attack of the nucleophile *exclusively* to C-4. Moreover the ^{15}N labelling experiments indicate that neither attack on C-2, nor attack on C-6 and/or C-7 in the pyrazine ring, leading to

ring contraction, takes place.

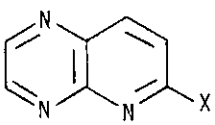
The high reactivity at position 4 in 1c is in good accordance with the general phenomenon that the presence of a chloro substituent on a carbon position adjacent to nitrogen in an azaheterocyclic ring activates the position *meta* to the chloro atom for amide attack[6]. Numerous reactions have been found, showing that attack on that *meta* position is often the introductory step in ring transformation (see, for example, the conversion of 2-chloroquinoline into 2-methylquinazoline[7]).

This result induced us to study the reaction of 6-chloropyrido[2,3-*b*]pyrazine[8] (3a) with KNH_2 in liquid NH_3 in order to obtain 2-methylpteridine. If this ring transformation would be successful, it would provide us with a new synthesis of a pteridine ring system.

It was found, however, that when 3a (166 mg, 1.0 mmol) was reacted with KNH_2 (4 eq.) in liquid NH_3 (50 ml) for 1 h no trace of a pteridine derivative was obtained but two products could be isolated (60% yield), identified as pyrido[2,3-*b*]pyrazine[9] (3b) and 1H-imidazo[4,5-*b*]pyridine[10](4b), ratio 3b/4b=1/3. Since pteridines undergo ring contraction into purines under the applied conditions[1], the reaction mixture was carefully investigated on the presence of purines. No indication for the presence of purines was found.

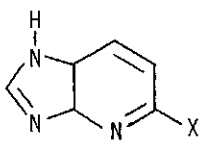
The main process in this reaction *i.e.* the ring contraction of 3a into 4b is essentially different from the conversion of 1a into 2, since in the former reaction the leaving group participates in the ring contraction, while in the latter the leaving group remains.

The transformation of 3a into 4b bears close relationship to the conversion of 6-bromoquinoxaline into benzimidazole as reported in the literature[11].



3a: X=Cl

3b: X=H



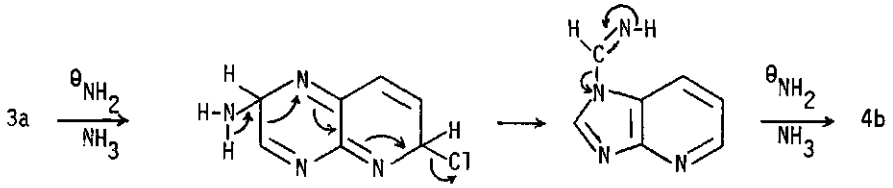
4a: X=Cl

4b: X=H

4c: $X=NH_2$

Concerning the mechanism of the ring contraction the following experimental facts have been established: i) 3b is *not* the precursor of 4b, since 3b is stable under the reaction conditions, ii) an initial ring contraction of 3a

into 5-chloro-1H-imidazo[4,5-*b*]pyridine (4a) (analogous to the conversion of 1a into 2) followed by a base-induced dechlorination is highly unlikely, since treatment of 4a[12] with KNH_2 in liquid NH_3, slowly gives 5-amino-1H-imidazo-[4,5-*b*]pyridine (4c)[12] and does not lead to the dechlorinated product 4b under these reaction conditions. These experiments suggest that the ring contraction starts by a nucleophilic attack of the amide ion at C-2 in 3a, leading to 5, which rearranges into the 1-methyleneiminoimidazo[4,5-*b*]pyridine. Under the basic reaction conditions the N-substituent is easily lost yielding 4b.



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All attempts to prove the existence of the σ-adduct 5 (or its conjugate base) by means of ^{1}H NMR [13] and ^{13}C NMR failed[14]; the high concentration of potassium amide, being necessary for obtaining signals of reasonable intensity, causes a complete decomposition of 3a.

The influence of substituents attached to the pyrazine ring on the ring contraction and the problem which carbon atom is then expelled are now under investigation.

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7 ON THE MECHANISM OF THE CONVERSION OF 6-CHLOROPYRIDO[2,3-*b*]PYRAZINE INTO 1H-IMIDAZO[4,5-*b*] PYRIDINE BY KNH_2 IN LIQUID NH_3

A.Nagel, H.C.van der Plas, G.Geurtsen and A.van der Kuilen

7.1 INTRODUCTION

Extensive investigation on the behaviour of 2-X-4,6,7-triphenylpteridines (X=Cl, H, SCH_3) towards potassium amide in liquid ammonia has shown[1-3] that this ring system can undergo i a ring contraction into a purine derivative in case of X=H, SCH_3 (initiated by attack of amide to the pyrazine ring carbon atoms, followed by expulsion of - mainly - C-7) and ii a nucleophilic substitution at C-2 (X=Cl, SCH_3) which process takes place by initial attack of amide to C-4 [S_N(ANRORC) mechanism]. In order to be more informed about the general scope of the contraction of the pyrazine ring we directed our attention to the behaviour of the pyrido[2,3-*b*]pyrazine derivatives towards potassium amide in liquid ammonia. It was found that, in contrast to pteridine, pyrido[2,3-*b*]pyrazine (2a) is stable in a dilute solution of potassium amide in liquid ammonia; however its 6-chloro derivative (1a, X=Cl) was recently found[1] to undergo two different reactions: dechlorination into 2a and ring contraction into 1H-imidazo[4,5-*b*]pyridine (3a, X=H). Thus the formation of 3a involves ring contraction with a simultaneous dehalogenation.

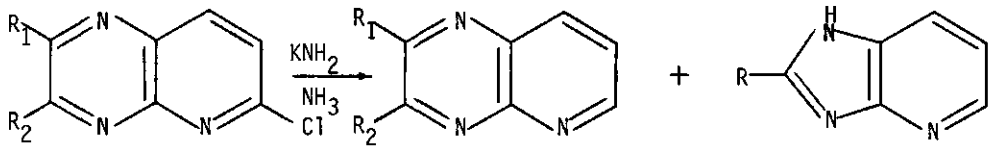
In this paper we wish to present the results of a more detailed study with a number of differently substituted 6-halogenopyrido[2,3-*b*]pyrazines (1) on the mode of formation of both products 2 and 3. Therefore we studied first the influence of alkyl (methyl, t-butyl) or aryl (phenyl, 9,10-phenanthro) groups present in the pyrazine ring on both reactions as well as the influence of different halogeno atoms at position 6 of 1. Furthermore we investigated by means of ^{13}C- and ^{15}N labelling the mechanism[1] of the ring contraction of 1a (X=Cl) into 3a (X=H).

7.2 RESULTS AND DISCUSSION

7.2.1 THE INFLUENCE OF SUBSTITUENTS IN THE PYRAZINE RING OF THE PYRIDO[2,3-*b*]PYRAZINES (1, X=Cl)

The choice of the substituents (alkyl and aryl groups) was mainly determined

by the availability of the diketones or keto-aldehydes. Condensation with 6-chloro-2,3-diaminopyrimidine gives the desired pyrido[2,3-*b*]pyrazine derivatives (1, X=Cl). The preparation of the compounds 1a, 1c and 1g (X=Cl) by this method have already been described.



1 2 3

1	R_1	R_2	2	R_1	R_2	3	R
a	H	H	a	H	H	a	H
b	H	C_6H_5	b	H	C_6H_5	b	C_6H_5
c	C_6H_5	C_6H_5	c	C_6H_5	C_6H_5	c	*t*-C_4H_9
d	H	*t*-C_4H_9	d	H	*t*-C_4H_9		
e	*t*-C_4H_9	H	e	phenanthro (9,10)			
f	CH_3	CH_3					
g	phenanthro (9,10)						

With phenylglyoxal monohydrate only the 3-substituted isomer was formed (1b, X=Cl). A mixture of 3- and 2-substituted isomers (1d and 1e) was obtained when the condensation was performed with *t*-butylglyoxal hemihydrate. By chromatography on silica gel this mixture could easily be separated. Reaction with biacetyl gives in good yield the dimethyl derivative 1f.

These results parallel those obtained in the formation of the pteridine analogues[4] from keto-aldehydes or diketones with 4,5-diaminopyrimidines.

The reactivity of the compounds 1b-1g (X=Cl) towards potassium amide in liquid ammonia was found in several verses to be rather different from that of 1a (X=Cl).

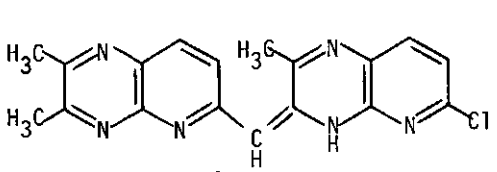
A phenyl substituent attached to C-3 (1b) has almost no influence on the occurrence of dechlorination and ring contraction. The dechlorination product 3-phenylpyrido[2,3-*b*]pyrazine (2b) and the ring contraction product 2-phenyl-1H-imidazo [4,5-*b*]pyridine (3b) were formed in nearly the same ratio (2b/3b=1/3) as obtained from the conversion of 1a into 2a and 3a.

6-Chloro-2,3-diphenylpyrido[2,3-*b*]pyrazine (1c, X=Cl) yielded mainly dechlorina-

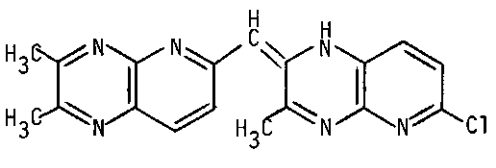
ted material 2c; the ring contraction, giving rise to the formation of 2-phenyl-1H-imidazo[4,5-*b*]pyridine (3b) took place to only a small extent. Also in the reaction of 6-chloro-2,3-[phenanthro(9,10)]-pyrido[2,3-*b*]pyrazine (1g, X=Cl) with potassium amide in liquid ammonia almost quantitatively the dechlorinated material 2e was formed.

A completely anomalous behaviour towards potassium amide was featured with 6-chloro-2,3-dimethylpyrido[2,3-*b*]pyrazine (1f). 1f underwent a fast reaction into a red coloured product to which we tentatively assigned structure A or B, probably resulting from initial deprotonation of the 3-CH_3 group and a subsequent attack of the anion formed, on C-6 of a second molecule 1f[5]. The structure assigned was based on the following evidence:

The ^1H NMR spectrum showed the presence of three methyl groups at δ 2.74, δ 2.69 and δ 2.52 ppm, a singlet at δ 5.81 ppm (=CH) and two pair of doublets at δ 7.88 and 8.73 ppm (J=9Hz) and at δ 7.77 and δ 7.11 ppm (J=8Hz). These doublets refer to AB groups in the pyridine rings.



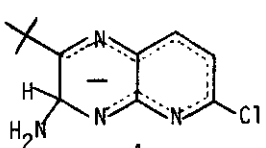
A



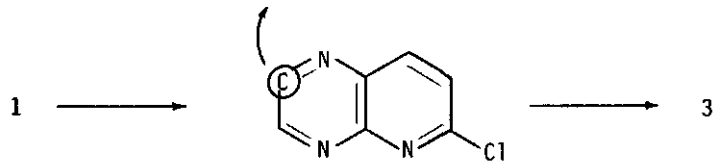
B

A strikingly different reaction pattern is observed with the isomeric 2-*t*-butyl-6-chloropyrido[2,3-*b*]pyrazine (1e, X=Cl) and 3-*t*-butyl-6-chloropyrido[2,3-*b*]-pyrazine (1d, X=Cl). Whereas 1d showed a completely parallel behaviour as 1b - two reaction products *i.e.* 2d and 3c are formed - 1e appeared to be completely *stable* under the reaction conditions. The complete inertness of the chloro atom of 1d towards amide ions - even no dechlorination could be detected - was possibly due to formation of the negatively charged 1 : 1 σ-adduct 4, which species would be deactivated for a nucleophilic attack.

Attempts to detect 4 by ^1H NMR spectroscopy were very successful. A solution of 1e (X=Cl) in liquid ammonia, containing potassium amide, showed a pair of doublets at δ 6.99 (1H) and at δ 5.76 ppm (1H), and a singlet at δ 4.90 ppm (1H). This singlet shows an upfield shift of Δδ 4.1 ppm when compared with a solution of 1e (X=Cl) in $CDCl_3$, indicating the presence of a sp^3 tetrahedral centre in 4.



4



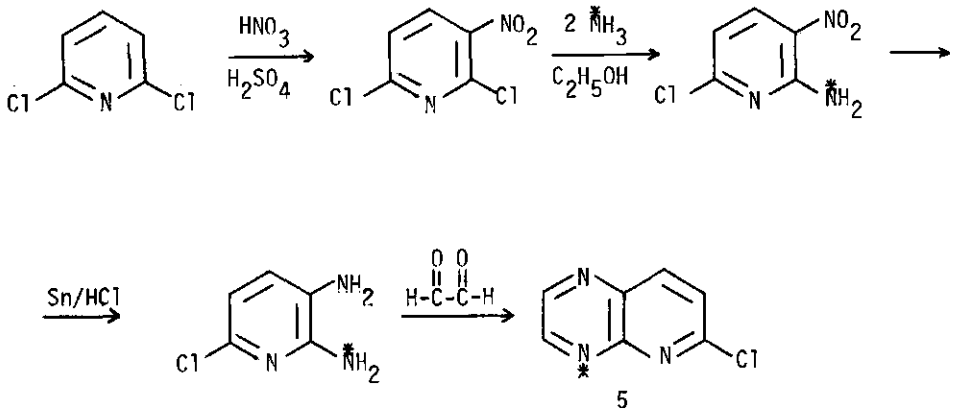
From the results of the above described experiments strong arguments can be taken, that the ring contraction of 1 into 3 only proceeds *via* elimination of C-2, since in cases where this position is free or not heavily blocked the ring contraction is the main process.

7.2.2 THE EFFECT OF THE NATURE OF THE HALOGEN ATOM IN THE PYRIDO[2,3-*b*]PYRAZINES (1, X=F, Br)

In order to study the effect of the nature of the halogen atom on the possible formation of the compounds 2 and 3 we studied the reactivity of the 2,3-diphenyl-6-X-pyrido[2,3-*b*]pyrazines (1c, X=F, X=Br). 1c (X=F) was prepared from 1c (X=Cl) by a halogen-exchange with potassium fluoride in hot DMSO. 1c (X=Br) was synthesized by the action of phosphoryl bromide on 2,3-diphenylpyrido[2,3-*b*]-pyrazin-6-one.
6-Bromo-2,3-diphenylpyrido[2,3-*b*]pyrazine (1c, X=Br) appeared to undergo only reductive debromination; thus it reacts quite analogous as the compound 1c (X=Cl). 2,3-Diphenyl-6-fluoropyrido[2,3-*b*]pyrazine, 1c (X=F), exclusively undergoes amino-defluorination into 6-amino-2,3-diphenylpyrido[2,3-*b*]pyrazine. Neither defluorination into 2c, nor any ring contraction product could be isolated from the reaction mixture. These results indicate that only the 6-chloro substituent is able to induce a ring contraction of the pyrido[2,3-*b*]pyrazine ring system into 1H-imidazo[4,5-*b*]pyridine derivatives.

7.2.3 STUDY OF THE MECHANISM OF THE RING CONTRACTION OF 1a INTO 3a BY ^{15}N- AND ^{13}C LABELLING EXPERIMENTS

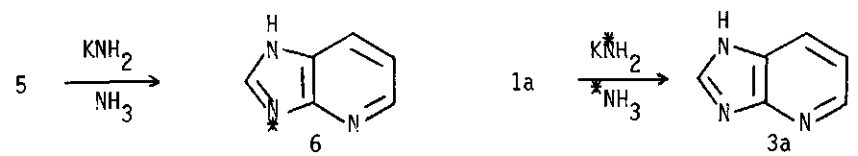
6-Chloro ^{15}N-4 pyrido[2,3-*b*]pyrazine (5) was synthesized as outlined in scheme 1.



*= ^{15}N

Scheme 1

This scheme was in principle based on reactions described earlier[6]. On reaction of 5 with potassium amide, the 1H-imidazo[4,5-*b*]pyridine (6) was found to contain by mass spectrometric determination the *same* excess of ^{15}N label as 5 (table). In a complementary experiment 1a (X=Cl) was reacted with ^{15}N labelled potassium amide. Compound 3a did *not* contain *any* excess of ^{15}N label (scheme 2).



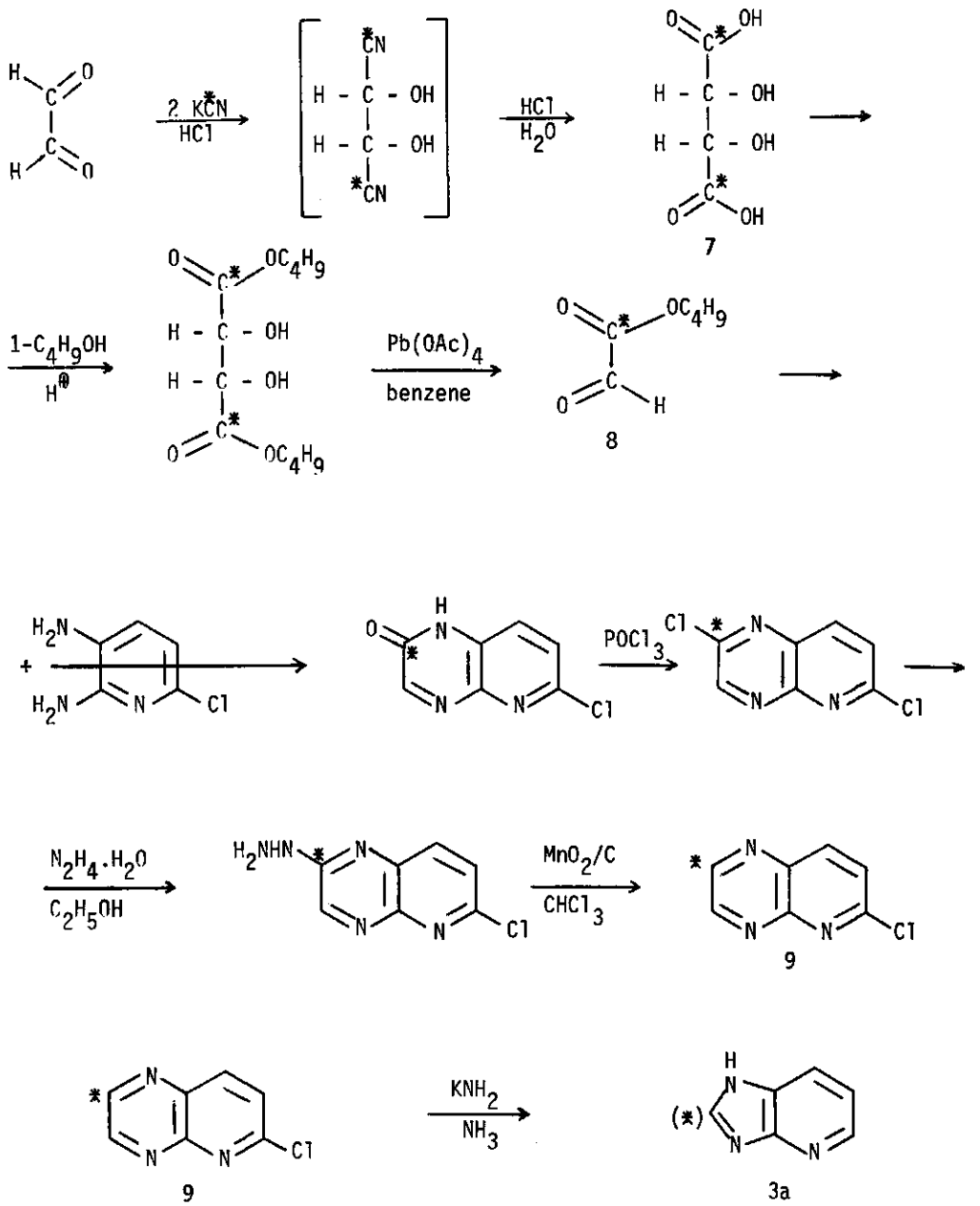
*= ^{15}N

Scheme 2

These experiments indicate that in the ring contraction of 1a (X=Cl) into 3a the nitrogen atom of the amide ion is not incorporated in 3a and that both N-1 and N-4 of 1a are present in 3a.

Although experiments with *substituted* pyrido[2,3-*b*]pyrazines indicate a strong preference for an expulsion of C-2 during the ring contraction, we wanted to prove that also with the *parent* substance 1a (X=Cl) C-2 is exclusively expelled during the ring contraction. Therefore we synthesized 6-chloro[^{13}C-2]pyrido[2,3-*b*]pyrazine (9), as is outlined in scheme 3.

By quantitative mass spectrometric and ^{13}C NMR measurements the enrichment of ^{13}C at C-2 was established (see table).


2 KCN
HCl
HCl
H2O
7
1-C4H9OH
H⊕
Pb(OAc)4
benzene
8
POCl3
N2H4·H2O
C2H5OH
MnO2/C
CHCl3
9
KNH2
NH3
3a

*= ^{13}C

Scheme 3

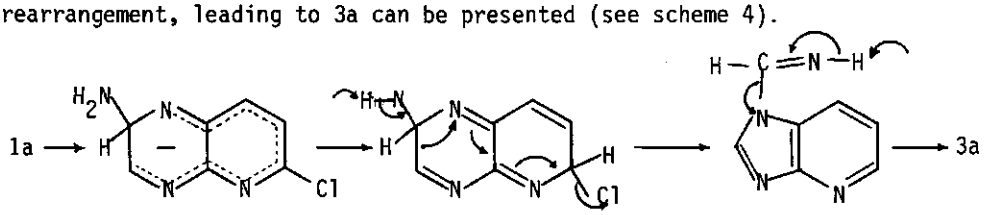
After allowing 9 to react with potassium amide in liquid ammonia we found that in the isolated 1H-imidazo[4,5-*b*]pyridine (3a) *no* excess of ^{13}C label could be detected (see table).

Table

Results of the ^{15}N- and ^{13}C labelling experiments

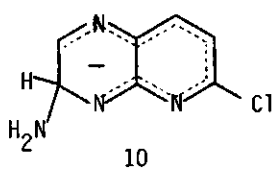
% excess of	comp.; %	comp.; %
^{15}N-4	5; 7.0	6; 7.0
$^{15}NH_2/^{15}NH_3$; 6.7	1a; 0.0	3a; 0.0
^{13}C-2	9; 5.7	3a; 0.2

This means that also in the ring contraction of the *parent* substance 1a (X=Cl) into 3a C-2 is expelled exclusively. The mechanism to explain these results involves an initial addition of amide ion at C-2. From this σ-adduct a further rearrangement, leading to 3a can be presented (see scheme 4).



Scheme 4

We want to point out that although it has been unequivocally proved that the ring contraction involves a reactive σ-adduct at C-2, these experiments do not exclude that in a solution of 1a (X=Cl) in liquid ammonia containing potassium amide, a small concentration of a stable σ-adduct at C-3 (10) is present.



10

7.3 EXPERIMENTAL

7.3.1 AMINATION PROCEDURE

The pyrido[2,3-*b*]pyrazine derivatives (1.0 mmol) were allowed to react with 4 eq. of potassium amide, dissolved in 50 ml of liquid ammonia, at -33°C, for a

period of one hour. GLC analysis (glass column, 180 cm, 2.1 g chromosorb W-HP 100-120 + 10% OV-275, at 250°C) of the reaction products of 1a (X=Cl) showed a reaction time of 1 h to give optimum yields. In general 20-30% of starting material was recovered.

7.3.2 SYNTHESIS OF THE PYRIDO[2,3-*b*]PYRAZINES

The following compounds were synthesized according to procedures described in the literature: 6-chloropyrido[2,3-*b*]pyrazine (1a)[7], pyrido[2,3-*b*]pyrazine (2a)[8], 1H-imidazo[4,5-*b*]pyridine (3a)[9], 2-phenyl-1H-imidazo[4,5-*b*]pyridine (3a)[10], 6-chloro-2,3-diphenylpyrido[2,3-*b*]pyrazine (1c)[11], 2,3-diphenylpyrido[2,3-*b*]-pyrazine (2c)[12], 6-chloro-2,3-[phenanthro (9,10)]pyrido[2,3-*b*]pyrazine (1g)[11], 2,3-[phenanthro(9,10)]pyrido[2,3-*b*]pyrazine (2e)[11], 3-phenylpyrido[2,3-*b*]pyrazine (2b)[13], 6-chloro-2,3-diaminopyridine[6].

6-Chloro-3-phenylpyrido[2,3-b]pyrazine (1b)

0.76 g (5.0 mmol) of 6-chloro-2,3-diaminopyridine and 0.75 g (5.1 mmol) of phenylglyoxal monohydrate were dissolved in 50 ml of boiling ethanol. To the hot solution were added 50 ml of water, whereupon 1b crystallized, m.p. 147-9°C. Analysis: $C_{13}H_8ClN_3$ (241.68): calcd. C, 64.40; H, 3.34; found C, 64.90; H, 3.14.

2,3-Diphenyl-6-fluoropyrido[2,3-b]pyrazine (1c, X=F)

2.0 g of KF (stored at 110°C) were added in one portion to a solution of 0.5 g of 1c (X=Cl) in dry DMSO (5 ml). The mixture was heated for 10 min under vigorous stirring. To the cooled contents of the flask 20 ml of water were added. The organic material was extracted with $CHCl_3$, concentrated and recrystallized from hexane, to give a slightly yellow coloured compound in 50% yield, m.p. 142-3°C. Analysis: $C_{19}H_{21}FN_3$(301.31): calcd. C, 75.73; H, 4.01; found C, 75.53, H, 4.12.

6-Bromo-2,3-diphenylpyrido[2,3-b]pyrazine (1c, X=Br)

2,3-Diphenylpyrido[2,3-*b*]pyrazin-6-one[15] was reacted by the standard procedure with $POBr_3$. The crude material was recrystallized from hexane to yield (70%) slightly yellow needles, m.p. 164-5°C. Analysis: $C_{19}H_{12}BrN_3$(362.23): calcd. C, 63.00; H, 3.34; found C, 63.26; H, 3.11.

2- and 3-t-Butyl-6-chloropyrido[2,3-b]pyrazines (1e, 1d)

Equimolar amounts of *t*-butylglyoxal hemihydrate and 6-chloro-2,3-diaminopyridine were dissolved in boiling ethanol. The solvent was removed *in vacuo* after allowing to reach room temperature. The solid obtained was chromatographied on silica gel, eluent $CHCl_3$. Compound 1d, isolated in 80% yield, was found to have the

higher mobility. The yield of 1e varied from 10-20%. 1d (X=Cl), m.p. 133-4°C (ethanol/water). Analysis: $C_{11}H_{12}ClN_3$(221.69): calcd. C, 59,59; H, 5.46; found C, 59.88; H, 5.20.

6-Chloro-2,3-dimethylpyrido[2,3-b]pyrazine (1f)

The slightly coloured solid obtained after the reaction of 6-chloro-2,3-diaminopyridine with biacetyl in ethanol, was purified by column chromatography (silica gel, $CHCl_3$) and by recrystallization from hexane, m.p. 176-8°C, yield 80%. Analysis: $C_9H_8ClN_3$(193.64): calcd. C, 55.82; H, 4.16; found C, 55.95; H, 4.06.

The dimer A (or B)

1f was allowed to react for 5 min with KNH_2 in liquid NH_3 as described in 7.3.1. The red coloured product was obtained in quantitative yield and recrystallized from ethanol, m.p. 229-30°C. Analysis: $C_{18}H_{15}ClN_6$(350.81): calcd. C, 61.62; H, 4.31; found C, 61.30; H, 4.20.

3-t-Butylpyrido[2,3-b]pyrazine (2d)

Condensation of 2,3-diaminopyridine and *t*-butylglyoxal hemihydrate in ethanol afforded 2d in quantitative yield. The compound was found to be a liquid, b.p. 194°C (dec.) and was analyzed as the picrate, m.p. 141-2°C. Analysis: $C_{17}H_{16}N_6O_7$ (416.35): calcd. C, 49.03; H, 3.87; found C, 49.22, H, 3.60.

2-t-Butylimidazo[4,5-b]pyridine (3c)

The mixture of products, obtained after the reaction of 1d (X=Cl) with KNH_2 in liquid NH_3, was purified by column chromatography on silica gel. When eluted with $CHCl_3$, fractions were obtained containing 2d. 3c was eluted with EtOAc. Recrystallization from acetone gave m.p. 200°C (subl.), 220°C (dec.). Analysis: $C_{10}H_{12}N_3$(174.22): calcd. C, 68.94; H, 6.94; found C, 68.74; H, 6.81.

6-Chloropyrido[2,3-b]pyrazin-2-one

To a solution of 6-chloro-2,3-diaminopyridine (1.52 g, 10 mmol) in 30 ml of ethanol was added freshly distilled 1-butylglyoxalate (1.43 g, 1.1 eq.). The mixture was stirred while standing for 2 days at room temperature. The product was collected by filtration and washed with ethanol and ether to obtain 1.65 g (90%) of yellowish-brown crystals. These could be recrystallized from water using charcoal, to a colourless micro-crystalline material, m.p. 220°C (subl.), 275°C (dec.). Analysis: $C_7H_4ClN_3O$(181.58): calcd. C, 46.30; H, 2.22; found C, 46.19; H, 2.49.

2,6-Dichloropyrido[2,3-b]pyrazine

To 2.6 g (20 mmol) of thoroughly dried 6-chloropyrido[2,3-*b*]pyrazin-2-one were added 160 ml of $POCl_3$. The mixture was refluxed under the exclusion of moisture till all the solid material had disappeared. 120 ml of $POCl_3$ were removed *in vacuo*. To the remaining slightly yellow-green syrup were added 300 g of ice, and the excess of $POCl_3$ was carefully decomposed. The acidic solution was extracted with 3 portions of 50 ml of ether and the combined etherial layers were washed with water and $NaHCO_3$, dried and concentrated *in vacuo*. The remaining slightly coloured solid was purified by column chromatography on silica gel (eluent CH_2Cl_2) and recrystallized from ethanol:water to yield 2.6 g (90%) of colourless needles, m.p. 163°C (dec.). Analysis: $C_9H_3Cl_2N_3$(200.03): calcd. C, 42.03; H, 1.51; found C, 42.22; H, 1.46.

6-Chloro-2-hydrazinopyrido[2,3-b]pyrazine

To 2.0 g (10 mmol) of 2,6-dichloropyrido[2,3-*b*]pyrazine, dissolved in 120 ml of ethanol,were slowly added under vigorous stirring 2.0 g (4 eq.) of hydrazine hydrate at room temperature. After 30 min. the mixture was refluxed for 2 h. The contents of the reaction flask were cooled and the solid removed by filtration. It could not be recrystallized. By reaction with 2,4-dinitrobenzaldehyde in ethanolic solution an intensively yellow coloured 2,4-dinitrophenylhydrazone was obtained, m.p. 170°C (dec.). Analysis: $C_{14}H_8ClN_7O_4$(373.72): calcd. C, 44.99; H, 2.16; found C, 45.02; H, 2.27.

6-Chloropyrido[2,3-b]pyrazine (1a, X=Cl)

To a suspension of the above described hydrazino compound (1.9 g, 10 mmol) in 100 ml of $CHCl_3$ was added freshly prepared MnO_2/C-catalyst (2g) in small portions at room temperature, under vigorous stirring. After the addition had been completed, the solvent was refluxed for 2 h. The suspension was filtered, while still hot, over hyflo supercel. The solid material was extracted with hot $CHCl_3$ (100 ml). The combined filtrates were dried on $MgSO_4$, filtered and the solvent was removed *in vacuo*. Thus pure 1a (X=Cl) could be obtained in a yield of 70%. The product was recrystallized from water and proved to be identical with an authentic specimen by its m.p., mixed m.p. and ^1H NMR spectrum.

7.3.3 SYNTHESIS OF THE ^{15}N- AND ^{13}C LABELLED COMPOUNDS

6-Chloro[^{15}N-4]pyrido[2,3-b]pyrazine

This compound was prepared as described in the literature for the unlabelled compound[6,7]. The required 6-chloro[^{15}N-2]2,3-diaminopyridine was obtained

via the reduction of [^{15}N-2]amino-6-chloro-3-nitropyridine as described in the literature for the unlabelled specimen[6]. The latter could be readily obtained by the amino-dechlorination of 2,6-dichloro-3-nitropyridine with ethanolic ammonia containing $^{15}NH_3$, following the procedure already described for the unlabelled material[6].

Di-1-butyl[di^{13}C-2,3]tartrate (7)

To a solution of a mixture of 4.6 g KCN (71 mmol) and 0.5 g $K^{13}CN$ (7.6 mmol) in water (20 ml) and acetonitrile (5 ml), cooled to -8°C, are added dropwise 7 ml of a 30% solution of glyoxal in water. The temperature is not allowed to rise above -5°C. After the addition has been completed the solution is allowed to reach room temperature and 10 ml of concentrated HCl was added dropwise. To the resulting colourless solution 45 ml of concentrated HCL are added in one portion and the mixture kept at 70°C for 2 h.
The solvents are removed *in vacuo* and the remaining solid is dried at 70°C. To the dry solid 40 ml of 1-butanol and 3 drops of concentrated H_2SO_4 are added. The mixture is refluxed for 1 h and distilled azeotropically to remove the water and the excess of 1-butanol. The cooled residue is diluted with ether and washed with a solution of $NaHCO_3$ in water. The ethereal layer is dried over $MgSO_4$ and concentrated *in vacuo*. The remaining crude di-1-butyl tartrate is distilled *in vacuo*, b.p. 136°C, 0.4 mm Hg. The yield of pure material is 6.0 g (29%).

1-Butyl[^{13}C-2]glyoxalate (8)

6.0 g (23 mmol) of 7 were dissolved in 25 ml of dry benzene in an atmosphere of nitrogen. Under vigorous stirring 11.5 g (1.1 eq.) of $Pb(OAc)_4$ were added in small portions. The temperature was not allowed to rise above 30°C. After the addition had been completed, the stirring was continued for 1 h. The organic material was isolated by filtration and the benzene and acetic acid were removed *in vacuo* in an atmosphere of nitrogen. The product distilled at 55-65°C, 13 mm Hg. Yield 4.8 g (80%).

6-Chloro[^{13}C-2]pyrido[2,3-b]pyrazine (9)

This compound was prepared *via* the route described for the unlabelled material. All ^{13}C labelled compounds were proved to be identical with the unlabelled specimens, by comparing their physical and chemical properties.

7.4 REFERENCES

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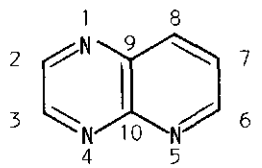
8 NMR DATA OF PYRIDO[2,3-*b*]PYRAZINES AND THEIR σ-ADDUCTS WITH AMIDE ION AND WATER

A.Nagel, H.C.van der Plas, G.Geurtsen and A.van Veldhuizen

8.1 INTRODUCTION

Recently the ^{13}C NMR spectra of pteridines and their covalent σ-adducts with ammonia and water have been analyzed[1,2].
In our study on the course of the ring contraction of pyrido[2,3-*b*]pyrazines (3-deazapteridines) into 1H-imidazo[4,5-*b*]pyridines we suggested as initial step the formation of a σ-adduct between the pyrido[2,3-*b*]pyrazine and amide ion[2]. With the aim to obtain more detailed information about the formation and structure of these σ-adducts we measured the ^{13}C NMR spectra of solutions of pyrido[2,3-*b*]pyrazine and a number of its derivatives in $CDCl_3$ and compared these data with those of solutions of the compounds in liquid ammonia, containing potassium amide.

8.2 RESULTS AND DISCUSSION



1a

The proton coupled ^{13}C NMR spectrum of pyrido[2,3-*b*]pyrazine (1a) - dissolved in $CDCl_3$ - shows five *intense* signals found at 125.6, 138.8, 146.3, 148.0 and 154.5 ppm (table 1), associated with one bond ^{13}C-^{1}H coupling constants (^{1}JC-H) of 168, 169, 185, 185 and 181 Hz respectively. The most downfield signal in the spectrum, at 154.5 ppm, is found to be associated with two long-range ^{13}C-^{1}H coupling constants of 8.6 and 3.5 Hz.
Long-range coupling constants are found for the resonances at 125.6 ppm (9.2 Hz) and 138.8 ppm (6.4 Hz).
The chemical shifts and the one bond coupling constants are in excellent agreement with those established for quinoline (C-2: 150.2 ppm, ^{1}JC-H = 178 Hz,

Table 1

		C-2	C-3	C-9	C-10	C-6	C-7	C-8
pyrido[2,3-*b*]pyrazine	1a	146.3	148.0	138.6	151.6	154.5	125.7	138.8
^{13}C-2-pyrido[2,3-*b*]pyrazine	1a*	146.3*	148.0	138.6	151.6	154.5	125.7	138.8
3-phenyl-	1b	144.3	154.5	135.8	150.8	154.5	124.7	138.1
3-*t*-butyl-	1c	144.8	167.6	135.9	150.4	153.9	124.6	138.2
6-chloro -	1d	146.3	148.6	137.7	150.9	154.7	127.5	141.1
6-chloro[^{13}C-2]-	1d*	146.3*	148.6	137.7	150.9	154.7	127.5	141.1
6-amino[a]-	1e	139.8	146.4	134.6	152.4	161.0	117.7	138.4
pyrido[2,3-*b*]pyrazin-2-one[a]	1f	154.5	155.3	127.9	143.0	145.1	125.7	124.8
2-chloro-	1g	148.3	148.3	137.7	149.9	154.3	126.5	137.7
2-chloro[^{13}C-2]-	1g*	148.3*	148.3	137.7	149.9	154.3	126.5	137.7
2,6-dichloro-	1h	148.7	148.9	136.7	149.2	154.7	128.4	140.0
2.6-dichloro[^{13}C-2]-	1h*	148.7*	148.9	136.7	149.2	154.7	128.4	140.0
6-chloropyrido[2,3-*b*]pyrazin-2-one[a]	1i	154.5	156.3	127.5	142.1	143.7	126.3	128.4
6-chloro-3-phenyl-	1j	144.3	155.2	136.0	150.3	154.8	126.5	140.6
3-*t*-butyl-6-chloro-	1k	144.8	168.7	135.1	149.9	154.3	126.3	140.6
2-*t*-butyl-6-chloro-	1l	165.7	147.1	135.9	149.4	153.4	126.9	140.9
pyrido[2,3-*b*]pyrazin-6-one[a]	1m	139.5	144.6	132.8	146.2	162.4	127.7	140.0
6-chloro-2-hydrazino[a]-	1n	144.3	142.7	136.3	146.0	153.7	126.0	137.2
2,3-diphenyl-	1o	154.7	156.3	136.2	149.9	154.1	125.2	138.0
2,3-diphenyl-6-fluoro-	1p	154.3	156.9	135.3	148+4	163.2	114.7	143.5
2,3-diphenyl-6-chloro-	1q	154.8	156.9	135.2	149.2	154.3	126.9	140.4
2,3-diphenyl-6-bromo-	1r	155.0	157.0	135.5	149.7	145.5	130.4	139.9

Samples were measured for $CDCl_3$ solutions.

[a]*Measured for DMSO(d_6)solution.*

**Increase found for the signal in the ^{13}C NMR spectrum of the ^{13}C-labelled compound*

$^{2}J_{C_2\text{-}H_3}$ = 3.7 Hz, $^{3}J_{C_2\text{-}H_4}$ = 7.9 Hz; C-3: 120.9 ppm, $^{1}J_{C\text{-}H}$ = 165 Hz, $^{2}J_{C_3\text{-}H_2}$ = 9.6 Hz; C-4: 135.7 ppm, $^{1}J_{C\text{-}H}$ = 162 Hz, $^{3}J_{C_4\text{-}H_2}$ = 5.4 Hz).

Based on these data we assigned the signals in the ^{13}C NMR spectrum of 1a at 154.5, 125.6 and 138.8 ppm to C-6, C-7 and C-8 respectively. The two remaining signals at 146.3 ($^{1}J_{C\text{-}H}$ = 185 Hz) and 148.0 ppm ($^{1}J_{C\text{-}H}$ = 185 Hz) are ascribed to C-2 and C-3 respectively. That this assignment is not reversed is substantiated on the increase of the signal at 146.3 ppm, when the ^{13}C NMR spectrum of [^{13}C-2]pyrido[2,3-*b*]pyrazine (1a*) is measured[3]. Two smaller signals at 138.6 ppm and 151.6 ppm were assigned to C-9 and C-10 respectively. These assignments were based on the values established for similar systems such as quinoxaline and quinazoline[4].

From the ^{13}C NMR spectral data presented in table 1 some substituent effects deserve comment. Striking long-range effects are caused by amino and oxo groups. Thus the 6-amino group in 1e causes C-2 to have an upfield shift of 6.5 ppm, while C-3 is almost unaffected. A similar effect is exerted by the 2-oxo group in 1f, that gives rise to an upfield shift of 9.4 ppm for C-6, leaving C-7 unaffected. Apparently the electron-donating capability of the amino or oxo group enhances the electron density in those positions.

When compared with 1a, C-8 in 6-chloropyrido[2,3-*b*]pyrazine (1d) - *meta* oriented to the chloro atom - is shifted more downfield (2.3 ppm) than C-6 (0.2 ppm). This is also observed with C-4 in 2-chloropteridine[1] and is apparently a general phenomenon. It reflects the somewhat enhanced reactivity of the position *meta* oriented to the chloro atom in 2-chloroquinoline[5], 2,6-dichloropyridine[6] and 2-chloropteridine[7] towards nucleophiles, such as the amide ion.

As was reported for pteridine derivatives[1], the α-substituent effect of a *t*-butyl group was found to be approximately - 20 ppm, the β-substituent effect about +2 ppm.

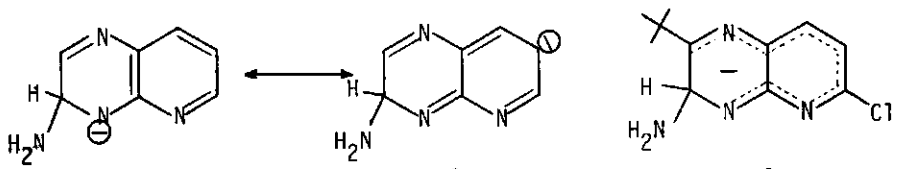
Because of the very slight difference between the chemical shifts (0.1 ppm) in the ^{1}H NMR spectra of pyrido[2,3-*b*]pyrazines it is not possible to assign unequivocally whether a compound is a 2- or 3-substituted derivative. However, it is now certain that ^{13}C NMR substituent effects should provide a more sound base than ^{1}H NMR data, in establishing such structures as 1k and 1l.

8.3 COVALENT σ-ADDUCTS

8.3.1 COVALENT AMINATION

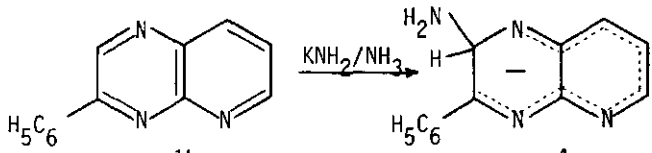
Close resemblance was found for the ^{1}H NMR spectrum of pyrido[2,3-*b*]pyrazine (1a), dissolved in $CDCl_3$ and in liquid ammonia. This indicates that 1a, in

contrast to pteridine[7], is not able to give a σ-adduct with ammonia, not even at elevated temperature.
However, the ^{13}C NMR spectrum of a solution of 1a in liquid ammonia, containing 2 equivalents of potassium amide, completely differs from that of 1a, dissolved in $CDCl_3$(table 2). An enormous upfield shift of 83.7 ppm is observed for C-3, while ^1JC-H decreases to 150 Hz. This is ascribed to rehybridization of C-3, due to formation of the 3-amino-3,4-dihydropyrido[2,3-*b*]pyrazinide ion. Similar magnitudes of upfield shifts have been observed before,on adduct formation of pyrimidines[8] with the amide ion.
Consistent with σ-adduct formation at C-3 is the relatively large upfield shift of C-7, reflecting the enhancement of negative charge in the pyridine nucleus, caused by the contribution of the resonance structure 2a.



2 2a 3

Similar upfield shifts for C-3 and C-7 are found for a solution of 2-*t*-butyl-6-chloropyrido[2,3-*b*]pyrazine (1l) in liquid ammonia, containing 2 equivalents of potassium amide, indicating the formation of the stable σ-adduct 3.
Recently 3-*t*-butyl-6-chloropyrido[2,3-*b*]pyrazine (1k) was found to be converted into 2-*t*-butyl-1H-imidazo[4,5-*b*]pyridine by potassium amide in liquid ammonia. This ring contraction was explained by an initial addition of the amide ion to C-2, followed by a rearrangement with expulsion of C-2. Attempts to obtain spectroscopic evidence for the existence of a covalent σ-adduct between 1k and amide ion failed, due to the fast occurring ring contraction. When measuring the ^1H NMR spectrum of 3-*t*-butylpyrido[2,3-*b*]pyrazine (1c) in the liquid ammonia potassium amide system, the spectrum of this solution was nearly the same as that of 1c, dissolved in $CDCl_3$. The conclusion is justified that 1c does not undergo addition of an amide ion, neither at C-2, nor at C-6.
In contrast, 3-phenylpyrido[2,3-*b*]pyrazine (1b) was found by ^1H NMR spectroscopy to be completely converted into the 2-amino-1,2-dihydro-3-phenylpyrido-[2,3-*b*]pyrazinide ion (4), when dissolved in liquid ammonia, containing two equivalents of potassium amide. This is established by the large upfield shift for H-2 and the smaller upfield shifts for H-6, H-7 and H-8. Moreover the coupling constants for H-6, H-7 and H-8 are found to be unchanged.



1b 4

		H-2	H-6	H-7	H-8	solvent
3-phenylpyrido[2,3-*b*]pyrazine	1b	9.35 (s)	9.08 (q)	7.51 (q)	8.37 (q)	$CDCl_3$
2-amino-1,2-dihydro-3-phenylpyrido[2,3-*b*]pyrazinide ion	4	5.52 (s)	8.05 (q)	6.62 (q)	6.94 (q)	NH_3/KNH_2

This is the first spectroscopic evidence that addition at C-2 of the pyrido[2,3-*b*]pyrazine ring system can take place. It further indicates that the previous suggestion that the ring contraction of 1k into 2-*t*-butyl-1H-imidazo[4,5-*b*]pyridine takes place by an initial addition at C-2, seems reasonable.
Attempts to establish the ^{13}C NMR spectrum of 4 were unsuccessful, due to decomposition of the concentrated solution in the time required for the measurement.

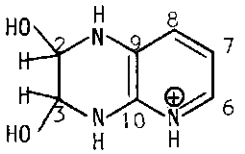
Table 2

		C-2	C-3	C-9	C-10	C-6	C-7	C-8
3-amino-3,4-dihydropyrido[2,3-*b*]pyrazinide ion	2	148.4	64.3	125.6	159.7	149.8	102.7	132.5
3-amino-2-*t*-butyl-6-chloropyrido[2,3-*b*]pyrazinide ion	3	164.2	61.7	124.9	159.5	146.2	99.9	133.2
2,3-dihydroxy-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazine cation	5	73.3	74.5	a	a	125.4	115.9	124.2
2,3-diaminopyridine cation	6			132.7 (C-5)	146.6 (C-2)	125.5	115.0 (C-5)	125.5 (C-4)

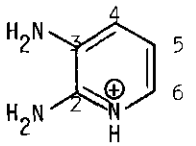
a Signals did not exceed signal-to-noise level

8.3.2 COVALENT HYDRATION

It is proved by ^{1}H NMR spectroscopy that 1a is not hydrated in a neutral aqueous solution[9] and that in dilute aqueous acid 1a exists to a small extent as the cationic 2 : 1 σ-adduct *i.e.* 2,3-dihydroxy-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazine ($5^{\oplus}$).



$5^{\oplus}$



$6^{\oplus}$

We measured ^{13}C NMR spectra of 5 and found that they resemble to a great extent those reported for the pteridine analogue *i.e.* 6,7-dihydroxy-5,6,7,8-tetrahydropteridine cation. Moreover the low field region of the ^{13}C NMR spectra of $5^{\oplus}$ and the cation of 2,3-diaminopyridine ($6^{\oplus}$) are strikingly similar.
In order to obtain ^{13}C NMR data of the neutral peaks of 5, we carefully neutralized the acidic aqueous solution containing $5^{\oplus}$ with ammonia.
However the ^{13}C NMR spectrum of the resulting solution, measured without delay, only showed signals due to 1a, indicating that dehydration of 5 into 1a is completed in the time required for the acquisition of the last free induction decay.

8.4 EXPERIMENTAL

The σ-adduct measurements were performed as described before[8].
All compounds, except 2-chloropyrido[2,3-*b*]pyrazine (1g) were synthesized to reported procedures[10].

2-Chloropyrido[2,3-b]pyrazine (1g)

Pyrido[2,3-*b*]pyrazine-2-one[11](1f) was treated with $POCl_3$ by the usual procedure.[10] 1i was recrystallized from hexane, m.p. 115-116°C. Analysis: $C_7H_4ClN_3$(165.58):calcd. C, 50.77; H, 2.44; found C, 50.96; H, 2.26.

8.5. REFERENCES

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9 GENERAL DISCUSSION

The covalent addition of nucleophiles to pteridines is a well understood phenomenon. In particular the covalent hydration[1] of pteridines and pteridinium ions has been studied intensively, mainly using UV and ^{1}H NMR spectroscopic methods. At the start of this study only little information was available concerning the addition of nitrogen nucleophiles to pteridines[2]. Therefore, initially our attention was paid to the addition of liquid ammonia and to the addition of amide ion in liquid ammonia. The results of our ^{1}H NMR study of the covalent amination of pteridine in liquid ammonia[3] have proved that at -60 ^{o}C instantaneous addition of NH_3 takes place at C-4, yielding the 1 : 1 σ-adduct 4-amino-3,4-dihydropteridine, but that at temperatures up to +25 ^{o}C the 2 : 1 σ-adduct 6,7-diamino-5,6,7,8-tetrahydropteridine is formed. Since the 1 : 1 σ-adduct is present as the sole species for 1 h at -60 ^{o}C, it is considered to be the kinetically favoured σ-adduct. The 2 : 1 σ-diadduct is stable for at least 3 months in liquid NH_3 at room temperature, and is apparently formed in a thermodynamically controlled reaction.

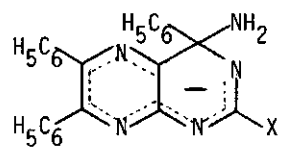
Attempts to isolate the 2 : 1 σ-diadduct - by simply evaporating the NH_3 below 0 ^{o}C - were unsuccessful. Even at -5 ^{o}C it reconverts into pteridine.

We also found that this diaaduct could not be converted into 6,7-diaminopteridine by mild oxydation, nor brought into a reaction with diketones such as glyoxal[4].

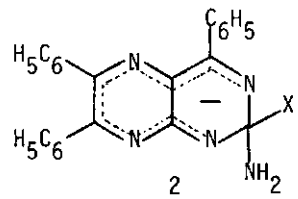
The ^{13}C NMR data[5] presented in this thesis on pteridine and pteridine derivatives and on their covalent σ-adducts with NH_3, are fully consistent with the results obtained by ^{1}H NMR spectroscopy. Both ^{1}H and ^{13}C NMR clearly illustrate the influence of steric and electronic factors on the formation of these covalent σ-adducts. A carbon atom already carrying a substituent R (R≠H) was never found to yield a detectable amount of a covalent amination product. In general, pteridines which form a σ-adduct are found to be appreciably soluble in liquid ammonia.

The behaviour of pteridine derivatives towards the strongly nucleophilic potassium amide was found to be quite different from the behaviour towards liquid ammonia. Pteridine[3], 2-methylthiopteridine[4] and even 6,7-diphenyl-2-methylthiopteridine[4] completely decompose into a number of unidentified products.

If the 4, 6 and 7-positions in the pteridine nucleus are occupied by a blocking group such as the phenyl group, the reaction with potassium amide takes an unexpected course. In the reaction of 2-X-4,6,7-triphenylpteridine with potassium amide, besides aminolysis into the corresponding 2-amino-4,6,7-triphenylpteridine, surprisingly, ring contraction into 6,8-diphenyl-2-X-purine (X=SCH_3) took place[6]. By extensive ^{15}N- and ^{2}H-labelling experiments[7] we could prove that the 2-amino product is partially formed by an initial attack of amide ion at C-4, *via* a ring-opening ring-closure sequence [S_N(ANRORC) mechanism] as well as attack at C-2 [S_N(AE)], and that the purine derivative is formed by attack at C-7 *and* C-6, followed by a rearrangement leading to expulsion of C-7 or C-6, respectively. It was found that the expulsion of C-7 is the more favoured, indicating the higher reactivity of C-7 for nucleophilic attack. This is due to the fact that the σ-adduct at C-7 (4) is more resonance stabilized than the σ-adduct at C-6 (3). In spite of numerous efforts *no* σ-adduct *neither* at C-4 (1) or C-2 (2) *nor* at C-6 (3) or C-7 (4) could be detected by ^{1}H NMR and by ^{13}C NMR spectroscopy. Apparently the σ-adducts 1-4, if formed, quickly decompose into the stable reaction products.



1



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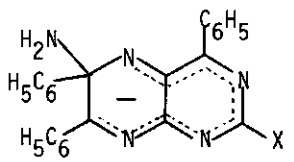

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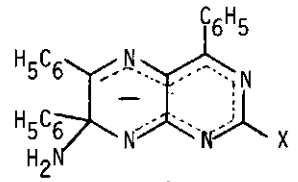
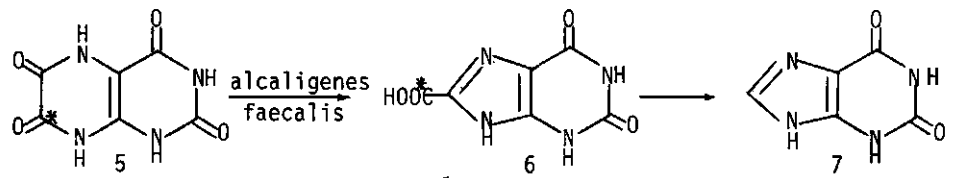


4

An interesting question is to which extent the S_N(ANRORC) mechanism operates in the aminolysis of 2-X-4,6,7-triphenylpteridines, when changing the character of the leaving group X. We found that if X=Cl, the S_N(ANRORC) mechanism is completely preferred to the S_N(AE) mechanism[8].

Very recently we also investigated the aminolysis of 2-fluoro-4,6,7-triphenylpteridine[4] - to our knowledge the first reported pteridine in which the fluoro atom is attached to a carbon atom of the pteridine nucleus - with ^{15}N-labelled potassium amide and found that the 2-amino derivative is formed *via* the

S_N(ANRORC)mechanism to the extent of 40%. This illustrates the extreme reactivity of C-4 of the pteridine nucleus for nucleophilic attack.
The new ring transformation of pteridines into purines reported in this thesis, as effected by the strongly nucleophilic amide ion, bears close relationship to the enzyme-catalyzed ring contraction of tetrahydroxypteridine (5) into xanthine-8-carboxylic acid[9] (6) (scheme 1). It was proved by ^{14}C-7 labelling that in



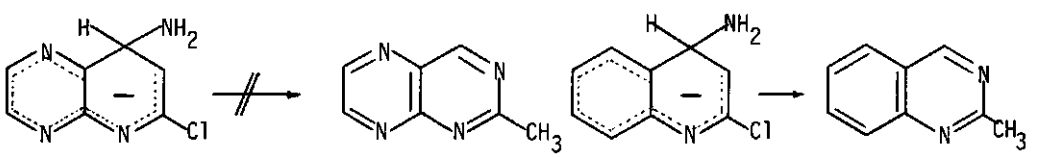
5 6 7

*= ^{14}C

scheme 1

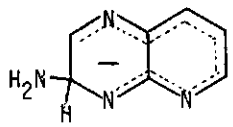
this enzymatic conversion C-7 is expelled exclusively.

In this thesis much attention was paid to the chemistry of 6-chloropyrido[2,3-*b*]-pyrazine, being the 3-deaza analogue of 2-chloropteridine.
Frontier Orbital Density Calculations[10] have indicated that C-8 of the parent substance pyrido[2,3-*b*]pyrazine is the most reactive carbon atom towards nucleophilic attack by the amide ion. Therefore it can be expected that with potassium amide a 1 : 1 anionic σ-adduct of type 8 can be formed.

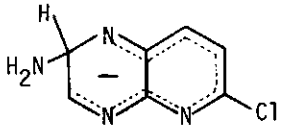


8 10 9 11

This σ-adduct 8 reminds to the σ-adduct 9, being postulated as intermediate in the ring transformation of 2-chloroquinoline into 2-methylquinazoline (11) by potassium amide in liquid ammonia[11]. By analogy we tried to convert 6-chloropyrido[2,3-*b*]pyrazine with potassium amide into 2-methylpteridine (10) (or further reaction products of 10) in order to achieve a new synthesis of pteridines. These attempts were unsuccessful[8]. Besides dehalogenation, ring contraction into 1H-imidazo[4,5-*b*]pyridine took place.
By NMR measurements, reported in chapter 8, we were able to prove that, when dissolved in liquid ammonia containing potassium amide, pyrido[2,3-*b*]pyrazine is converted into the anionic σ-adduct 12. In contrast to the σ-adduct at C-8 we believe that 12 is formed in a thermodynamically favoured process.



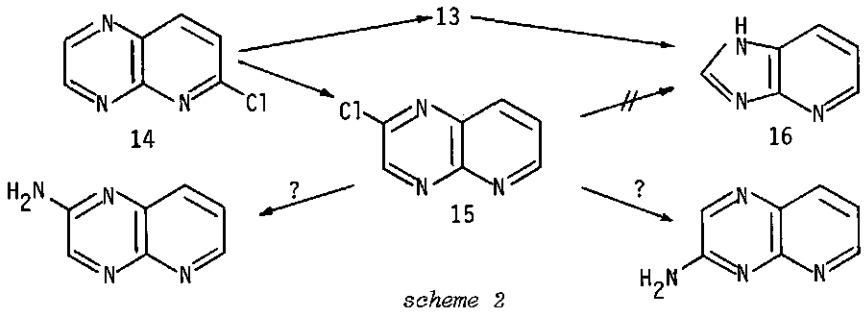
12



13

However the ring contraction of 6-chloropyrido[2,3-*b*]pyrazine (14) into 1H-imidazo[4,5-*b*]pyridine (16) was unequivocally proved by ^{15}N and ^{13}C labelling experiments to proceed *via* the σ-adduct 13. Unfortunately the existence of this σ-adduct (13) could not be established by NMR spectroscopy. Apparently the rate of formation of 13 is slower than its rearrangement.

As a final comment on the ring contraction of 14 into 16 the possibility of halogen migration[12] may be suggested, making this conversion quite analogous to the ring contraction of 2-chloroquinoxaline into benzimidazole[13](scheme 2).



scheme 2

2-Chloropyrido[2,3-*b*]pyrazine (15), however, is found to react quantitatively and almost instantaneously into an unknown aminopyrido[2,3-*b*]pyrazine on treatment with potassium amide in liquid ammonia. Thus 15 can not be the precursor of 16, excluding the possibility of halogen migration.

The aminolysis of 15 rises the interesting question concerning the structure of the aminopyrido[2,3-*b*]pyrazine formed and concerning the mechanism *via* which this amino-dechlorination takes place.

Since ^{1}H NMR spectroscopic data do not allow an unequivocal assignment between a 2-aminopyrido[2,3-*b*]pyrazine - to be formed by an $S_N(AE)$ mechanism - and a 3-aminopyrido[2,3-*b*]pyrazine - to be formed by an $S_N(AE)^{cine}$ substitution - we reacted 2-chloro[^{13}C-2]pyrido[2,3-*b*]pyrazine (17) with potassium amide in liquid ammonia and examined the ^{13}C-labelled aminopyrido[2,3-*b*]pyrazine formed by ^{13}C NMR spectroscopy.

* = ^{13}C 17 18

The increase of the resonance signal for C-2 - carrying the amino substituent, as established by its chemical shift - clearly illustrates that the product formed is 2-amino[^{13}C-2]pyrido[2,3-*b*]pyrazine (18) and thus an $S_N(AE)$ mechanism is operative in the aminolysis of 2-chloropyrido[2,3-*b*]pyrazine.

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SUMMARY

In the introduction of this thesis the reactions of pteridines and pyrido[2,3-*b*]-pyrazines with nucleophiles are reviewed.
In the following chapters the results of an NMR investigation on the formation of σ-adducts between these azaaromatic ring systems and nitrogen nucleophiles, especially KNH_2/NH_3, are described. In order to establish the structures of these - not isolable - σ-adducts, the ^1H and ^{13}C NMR spectra of pteridine, pyrido[2,3-*b*]pyrazine and a number of derivatives of both these heterocyclic systems, containing one or more OCH_3, SCH_3, CH_3, *t*-C_4H_9, OH, NH_2, $NHNH_2$, F, Cl, Br and C_6H_5 substituents, were extensively analyzed.
All resonance signals in the NMR spectra were unequivocally assigned.

By means of ^1H and ^{13}C NMR, pteridines are shown to form in principle two different σ-adducts with NH_3 : at -60°C one molecule of NH_3 adds to C-4, yielding 4-amino-3,4-dihydro-2-R-pteridines (R=H, Cl), or alternatively, at temperatures up to +25°C, the addition of two molecules of NH_3 to C-7 and C-6 takes place, causing the formation of 6,7-diamino-4-R-2-X-5,6,7,8-tetrahydropteridines (R=X=H, R=H, X=Cl, OCH_3, SCH_3, C_6H_5, R=CH_3, X=Cl. R=C_6H_5, X=Cl,H).
This detailed NMR spectral information allowed straightforward interpretation of the ^{13}C NMR spectra of the covalent hydrates 3,4-dihydro-4-hydroxypteridine, 6,7-dihydroxy-5,6,7,8-tetrahydroxypteridine and their cationic species.

Due to the rapid decomposition of pteridine in KNH_2/NH_3, no σ-adduct could ever be detected. In sharp contrast, three σ-adducts between KNH_2 and pyrido[2,3-*b*]pyrazines are described *i.e.* the 3-amino-3,4-dihydropyrido[2,3-*b*]pyrazinide ion, the 3-amino-2-*t*-butyl-3,4-dihydro-6-chloropyrido[2,3-*b*]pyrazinide ion and the 2-amino-1,2-dihydro-3-phenylpyrido[2,3-*b*]pyrazinide ion.

The results are subsequently presented concerning the investigation of the reaction of KNH_2/NH_3 with 2-X-4,6,7-triphenylpteridines (X=SCH_3, Cl, F, H). Two reactions are found to take place : aminolysis at C-2, yielding 2-amino-4,6,7-triphenylpteridine (X=SCH_3, Cl, F) and ring contraction, giving rise to the formation of 2-X-6,8-diphenylpurines (X=SCH_3, H). By studying the aminolysis with both ^{15}N labelled pteridines and with $K^{15}NH_2/^{15}NH_3$ it is proved that the

displacement at C-2 in the case of X=SCH_3, occurs *via* a ring-opening and ring closing sequence [S_N (ANRORC)] mechanism to the extent of 50-85% (depending on [KNH_2]); in the case of X=F this amounts to 40% and in the case of X=Cl to 100%.

It is further proved that in the ring contraction of 2-methylthio-4,6,7-triphenylpteridine 85% of C-7 is expelled and 10% of C-6, both processes being preceded by addition of amide ion to C-7 and C-6 respectively.

The possible elimination of C-7 *and* C-6 is clearly demonstrated by the fact that *both* 4,6- *and* 4,7-diphenyl-2-methylthiopteridines undergo ring contraction to the same product *i.e.* 6,8-diphenyl-2-methylthiopurine. As a consequence in the former isomer only C-7 is eliminated, while in the latter exclusively C-6 is expelled.

In the next chapter the reactions of 6-chloro-2-R_1, 3-R_2-pyrido[2,3-*b*]pyrazines [R_1=H, R_2=C_6H_5, *t*-C_4H_9, R= *t*-C_4H_9, R_2=H, R_1=R_2=H, CH_3, C_6H_5, phenanthro(9,10)] with KNH_2/NH_3 are described.

These compounds undergo ring contraction into 2-R-1H-imidazo[4,5-*b*]pyridines (R=H, C_6H_5, *t*-C_4H_9), besides reductive dechlorination. It is found that ring contraction of 2,3-diphenyl-6-X-pyrido[2,3-*b*]pyrazines takes place exclusively if X=Cl; in the case of X=F only aminolysis is found, and in the case of X=Br reductive debromination occurs exclusively.

The investigation on the mechanism of the ring contraction of 6-chloropyrido-[2,3-*b*]pyrazine into 1H-imidazo[4,5-*b*]pyridine is performed by using both ^{15}N-4 and ^{13}C-2 labelled compounds and $K^{15}NH_2/^{15}NH_3$. The results can be explained by the initial formation of a σ-adduct of amide ion at C-2 - unfortunately not detectable by spectroscopic methods - in which σ-adduct, by an intramolecular rearrangement, the chlorine atom and C-2 are expelled simultaneously.

SAMENVATTING

In de inleiding van dit proefschrift wordt een beknopt literatuuroverzicht gegeven van de inwerking van nucleofielen op pteridine, pyrido[2,3-*b*]pyrazine en een aantal derivaten van deze aza-aromaten.

Daarna wordt het door ons verrichte NMR onderzoek naar de vorming van σ-adducten van deze verbindingen met stikstof nucleofielen, met name NH_3 en KNH_2/NH_3, uitvoerig beschreven. Teneinde de structuur van deze - niet isoleerbare - σ-adducten te kunnen vaststellen, zijn de ^1H en ^{13}C NMR spectra van pteridine, pyrido[2,3-*b*]pyrazine en van een aantal derivaten van deze verbindingen, die een of meer OCH_3, SCH_3, CH_3, *t*-C_4H_9, OH, NH_2, $NHNH_2$, F, Cl, Br en C_6H_5-substituenten bevatten, nauwkeurig geanalyseerd. Op ondubbelzinnige wijze konden alle resonantie signalen in de spectra worden toegekend.

Met ^1H en ^{13}C NMR wordt voor pteridine en een aantal derivaten aangetoond dat in NH_3 twee verschillende σ-adducten gevormd kunnen worden: bij -60°C treedt additie op van één molecuul NH_3 aan C-4, hetgeen leidt tot de 4-amino-3,4-dihydro-2-R-pteridinen (R=H, Cl), terwijl bij temperaturen hoger dan -60°C additie van twee moleculen NH_3 op C-7 en C-6 plaatsvindt, waarbij 6,7-diamino-4-R-2-X-5,6,7,8-tetrahydropteridinen (R=X=H, R=H, X=Cl, OCH_3, SCH_3, C_6H_5, R=CH_3, X=Cl, R=C_6H_5, X=H, Cl) ontstaan.
Op grond van de informatie verkregen bij de interpretatie van de NMR spectra van deze σ-adducten, konden de ^{13}C NMR spectra van de covalente hydraten 3,4-dihydro-4-hydroxypteridine en 6,7-dihydroxy-5,6,7,8-tetrahydropteridine in zowel neutraal als zuur milieu worden verklaard.

Het blijkt dat pteridine ontleedt in aanwezigheid van KNH_2, zodat geen adducten konden worden gemeten. Dit is in tegenstelling tot het pyrido[2,3-*b*]pyrazine ringsysteem, waarvan drie σ-adducten met KNH_2 worden beschreven, te weten het 3-amino-3,4-dihydropyrido[2,3-*b*]pyrazinide ion, het 3-amino-2-*t*-butyl-3,4-dihydro-6-chloorpyrido[2,3-*b*]pyrazinide ion en het 2-amino-1,2-dihydro-3-fenylpyrido[2,3-*b*]pyrazinide ion.

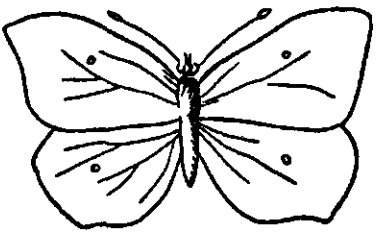
Voorts worden de resultaten vermeld van het onderzoek naar de inwerking van KNH_2/NH_3 op 2-X-4,6,7-trifenylpteridinen (X=SCH_3, Cl, F, H). Er blijken twee verschillende reacties op te treden: een aminolyse op C-2 tot 2-amino-4,6,7-

trifenylpteridine (X=SCH_3, Cl, F) en een ringcontractie, leidend tot de vorming van 2-X-6,8-difenylpurinen (X=SCH_3, H). Door de aminolyse te bestuderen, zowel met ^{15}N-gemerkt substraat als ook met $K^{15}NH_2$ wordt aangetoond dat de substitutie reactie bij X=SCH_3 verloopt voor 50-85% (afhankelijk van [KNH_2]) *via* een ring-opening — ring-sluiting mechanisme [S_N(ANRORS)]; in het geval van X=F is dit percentage 40% en bij X=Cl is dat 100%.
Verder wordt bewezen dat de ringcontractie van het 2-methylthio-4,6,7-trifenylpteridine voor 85% verloopt *via* de additie van het amide ion aan C-7 en voor 15% aan C-6, gevolgd door eliminatie van het C-7(6)-fenyl-fragment.
Dat zowel C-7 als C-6 geëlimineerd kunnen worden, wordt bevestigd door het feit dat de *beide* isomere 4,6- en 4,7-difenyl-2-methylthiopteridinen ringcontractie ondergaan naar hetzelfde product *i.e.* 6,8-difenyl-2-methylthiopurine. Bij deze ringcontractie wordt dus bij het ene isomeer uitsluitend C-7, bij het andere isomeer alleen C-6 geëlimineerd.

Vervolgens wordt het gedrag van de 2-R_1, 3-R_2-6-chloorpyrido[2,3-*b*]pyrazinen [R_1=H, R_2=C_6H_5, *t*-C_4H_9, R_1=R_2=H_2CH_3, C_6H_5, fenanthro(9, 10)]ten opzichte van KNH_2/NH_3 beschreven. Het blijkt dat deze verbindingen naast aanzienlijke dechlorering, ringcontractie ondergaan tot 2-R-1H-imidazo[4,5-*b*]pyridinen (R=H, *t*-C_4H_9, C_6H_5). Het blijkt voorts dat de ringcontractie van 2,3-difenyl-6-X-pyrido[2,3-*b*]pyrazinen alleen optreedt bij X=Cl; in het geval van X=F treedt uitsluitend aminolyse op, terwijl bij X=Br volledige debromering plaatsvindt.
Onderzoek naar het mechanisme van de ringcontractie van 6-chloorpyrido [2,3-*b*]-pyrazine tot 1H-imidazo[4,5-*b*]pyridine is uitgevoerd met behulp van ^{15}N-4 en ^{13}C-2 gemerkte substraten als ook met behulp van $K^{15}NH_2$.
De resultaten laten zich verklaren door de initiële vorming van een - spectroscopisch helaas niet waar te nemen - σ-adduct van amide op C-2, waaruit, na een intramoleculaire omlegging, onder gelijktijdig verlies van het chloor atoom, C-2 wordt geëlimineerd.

CURRICULUM VITAE

In 1965 legde ik het eindexamen gymnasium β af aan het Openbaar Dalton Lyceum te Voorburg, waarna begonnen werd in september van dat jaar met de studie in de scheikunde aan de Rijksuniversiteit te Leiden.
Tot januari 1970, toen het kandidaatsexamen, letter g, werd afgelegd, was ik, ten einde mijn studie te bekostigen, werkzaam als classificeerder, automonteur, fotomodel, documentalist, ober, analist, lopende-band-werker en chauffeur.
De academische studie werd voortgezet onder leiding van de hoogleraren Dr.E.Havinga (organische chemie), drs.P.J.van den Berg (chemische technologie, Delft) en dr.A.J.Staverman (fysische chemie); het doctoraalexamen met als hoofdvak organische chemie werd in oktober 1973 afgelegd.
Van januari 1970 tot oktober 1973 was ik als student-assistent verbonden aan het geïntegreerd propaedeutisch chemisch practicum van de Rijksuniversiteit te Leiden.
Vanaf november 1973 ben ik als wetenschappelijk medewerker werkzaam op het Laboratorium voor Organische Chemie van de Landbouwhogeschool te Wageningen. Daar ben ik als practicumleider mede verantwoordelijk geweest voor het propaedeutisch practicum en als assistent werkzaam geweest op de KA en KB practica. In de resterende tijd werd onder leiding van Prof.Dr.H.C.van der Plas het in dit proefschrift beschreven onderzoek verricht.



To the "golden" brimstone-butterfly

- messenger of spring -

that played a dominant role in the

structural elucidation of pteridines