Pteridine Studies (IV)¹

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-(methylthio)purine²

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The ring contraction of 2-(methylthio)-4,6,7-triphenylpteridine (3) into 2-(methylthio)-6,8-diphenylpurine (5a) by KNH₂ in NH₃ at -33°C has been studied using selectively deuterium labelled pteridines. It was found that the purine obtained from 2-(methylthio)-4,6-diphenyl-7-(pentadeuterophenyl)pteridine - prepared by phenylation of 2-(methylthio)-4,6-diphenylpteridine with pentadeuterophenyllithium - only contained 13% of the deuterium label, indicating that C-7 is mainly expelled during the ring contraction. The mechanism is discussed. Furthermore the amination of 3 was studied using both ¹⁵N-3 labelled compounds as well as K¹⁵NH₂ in ¹⁵NH₃. It was found that the amination of 3 takes place for 50-85% - depending on [KNH₂] - according to a ring opening-ring closure mechanism (SN(ANRORC)) forming 2-amino-4,6,7-triphenylpteridine (4). Thus in 3 the pteridine nucleus is found to be attacked by the amide ion on C-4, C-2, C-6 and C-7 in the approximate order of reactivity: C-4 ≈ C-2 > C-7 > C-6.

Introduction

In an earlier investigation we reported on the addition of liquid ammonia to pteridine and some of its derivatives⁴. ¹H-nmr evidence was presented for the formation of two different species i.e. the 1 : 1 σ-adduct 4-amino-3,4-dihydro-
pteridine (1) and the thermodynamically favoured 2 : 1 σ-adduct 6,7-diamino-5,6,7,8-tetrahydropteridine (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-(methylthio)purine (5a) takes place. The same purine derivative is also obtained from 4,6-diphenyl- and 4,7-diphenyl-2-(methylthio)pteridine. As an amide-catalyzed ring contraction of pteridines into purines has never been observed before we became interested in the scope and mechanism of this conversion. In this paper we concentrate on the intriguing problem whether C-6 and/or C-7 is expelled from the pyrazine ring (see section b). There is ample evidence that the nucleophilic displacement in 2-substituted pyrimidines by an amide ion occurs via a ring opening-ring closure (SN(ANRORC)) mechanism. It induced us to study the occurrence of this process in the amino-de(methylthio)lation (3 + 4) (see section a).

a) 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 pyri-
midine 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.

\[
\begin{align*}
&\text{Scheme I} \\
&\begin{array}{ccc}
&\begin{array}{c}
\text{Acid hydrolysis of 4 yielded as main product 2-amino-3-benzoyl-5,6-diphenylpyrazine (6) and only a small amount of 4,6,7-triphenylpteridin-2-one}^8.
\end{array} \\
&\text{Formation of 7, being labelled at N-3, was performed by reaction of 6 with 1,3-$^{15}$N-labelled urea.}
\end{array} \\
&\text{In this reaction no trace of apN-3, 15N-dpteridin-2-one was formed as proved by mass spectrometry}^9.
\end{align*}
\]

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 methyl-iodide$^{10}$. 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. 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. 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). We assume that the remaining 50% reacts via an $S_N$(AE) pathway. When compound 3 was reacted with 10 equivalents of KNH$_2$ (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$_2$.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Substrate (1 mmole in 25 ml of NH$_3$)</th>
<th>Reagent</th>
<th>% of excess of $^{15}$N in substrate</th>
<th>% $S_N$(ANRORC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>4 eq KNH$_2$</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>10 eq KNH$_2$</td>
<td>0</td>
<td>6.2</td>
</tr>
</tbody>
</table>

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-halogenopyrimidines. The adduct 12 undergoes the ring opening leading to the open chain.

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intermediate 13 which recylizes via 14 into 4 (scheme 2).

\[
\begin{align*}
\begin{array}{c}
\text{Scheme I1} \\
\text{b) On the ring contraction of 3 into 5a}
\end{array}
\end{align*}
\]

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-(methylthio)pteridine (15) or of its structural isomer 4,6-diphenyl-2-(methylthio)pteridine (16) with deuterated phenyllithium and subsequent oxidation of the intermediary dihydro compound obtained. Phenylation of pteridines have never been published\textsuperscript{16}, but this method is successfully used for the preparation of phenyldiazines\textsuperscript{17}. 

\[
\begin{align*}
\begin{array}{c}
\text{Scheme II} \\
\end{array}
\end{align*}
\]
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 penta-deuterophenyllithium on 16 and oxidation of 19b we were able to obtain 20.

![Chemical Structure Diagram]

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

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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-catalyzed elimination of pentadeutobenzylideneimine 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 pentadeutobenzonitrile, 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 $^{15}\text{N}$ enrichment thus excluding the intermediacy of 23 as reactive species in the ring contraction.

Both the phenylation reactions as well as the results of the deuterium and $^{15}\text{N}$-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 $^1\text{H}$- and $^{13}\text{C}$-NMR measurements failed, probably due to the low solubility of 16 in liquid NH$_3$.

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

**Experimental**

Melting points are uncorrected. $^{1}$H-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.

1. 2-Amino-3-benzoyl-5,6-diphenylpyrazine (6)

2-Amino-4,6,7-triphenylpteridine\( ^{5} \) (375 mg, 1.0 mmole) and 5 ml 6N HCl were heated for 10 hours at 150° in a sealed tube. After cooling the contents of the tube were extracted with CHC\(_{3}\). 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°. Analysis calcd. for C\(_{25}\)H\(_{17}\)N\(_{2}\)O (351.39): C: 78.61, H: 4.88; found C: 78.42, H: 5.00.

2. 2-chloro-4,6,7-triphenyl\( ^{15}\)N-3|pteridine \( ^{6} \)

700 mg of 6 (2.0 mmoles) were stirred with 480 mg (8.0 mmoles) of $^{15}$N,$^{15}$N-urea containing 30.3% $^{15}$N at 200°C for 1 hour. Recrystallization of the product from aqueous DMF yielded the pteridin-2-one (7) as yellow needles m.p. 299-300°C (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 hour at 100°C, was followed by thorough decomposition of the reagents with water. Extraction of the aqueous layer with CHC\(_{3}\) yields 8 (m.p. 209-210°C) in 35%. It proved to be identical with an authentic specimen\( ^{5} \).

3. 2-(methylthio)-4,6,7-triphenyl\( ^{15}\)N-3|pteridine \( ^{6} \)

400 mg (1.0 mmole) 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
H₂S. 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 methyl iodide (0.2 ml; 3.5 mmol). The resulting suspension was extracted with CHCl₃ and the extract purified by column chromatography. Pure 9 was obtained, m.p. 233-234°C in a yield of 30% (130 mg). (lit. 5 232-234°C).

4. Phenylation reactions

a) Phenylation of 4,6-diphenyl-2-(methylthio)pteridine (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 MgSO₄ and concentrated in vacuo. The residual oil is dissolved in acetone, and KMnO₄ is added until the permanganate colour remains. The acetone is removed in vacuo and the residue is dissolved in CHCl₃, filtered and chromatographed on silicagel using CHCl₃ 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 pentadeutero phenyllithium as the reagent, compound 20 was obtained.

b) Phenylation of 4,7-diphenyl-2-(methylthio)pteridine (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 ¹³C-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. 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 NaNO₂ were added in a period of 15 minutes. 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°C).

Analysis: calcd. for C₂₄H₁₆N₄O (376.40): C: 76.58, H: 4.28; found: C: 76.39; H: 4.58.

6. Amination procedure

The reactions in liquid ammonia with potassium amide were carried out as described before⁵. The all glass apparatus used for the experiments in liquid ¹⁵NH₃ was essentially the same. ¹⁵NH₃ was prepared by treating ¹⁵NH₄NO₃ with a concentrated solution of KOH in H₂O at 100°C for 2 hours. After the experiment it was reconverted into ¹⁵NH₄NO₃ in an average yield of 85%.

Acknowledgements

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REFERENCES

1. For part III of our Pteridine Studies see ref. 3.


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$_{14}$^{1}Cl.

10. The formation of 2-(methylthio)pyrimidines from 2-chloropyrimidines by a two step sequence instead of direct replacement with sodium methylmercaptide has been reported to be a preferred procedure, see D. J. Brown in *The Pyrimidines*, supplement 1, Wiley Interscience N.Y. 1970, p. 149.

11. Diazotization of aminopteridines has been used with various results, see: R. N. Butler, *Chem. Rev.* 75, 241 (1975).

12. Personal communication of M. Tisler, Department of Chemistry, University of Ljubljana, Yugoslavia.


19. The spectrum was interpreted by additivity relationships using the data presented in ref. 3.

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