Organic Magnetic Resonance, 1976, Vol. 8, pp. 607 to 610.

Pteridine Studies

III†—^{13}C n.m.r. 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*

Laboratory of Organic Chemistry, Agricultural University, Wageningen, The Netherlands

(Received 17 March 1976; accepted (revised) 12 May 1976)

Abstract—^{13}C n.m.r. spectral data of pteridine and nineteen of its derivatives (containing one or more chloro, methylthio, methyl, *t*-butyl or phenyl substituents) are reported. The ^{13}C n.m.r. spectrum of the title compound has been assigned conclusively. ^{13}C n.m.r. substituent effects are shown to be very useful in discerning between 6- and 7-substituted pteridines. Additionally, the ^{13}C n.m.r. spectra of several covalent amination products, i.e. the 3,4-dihydro-4-amino- and the 5,6,7,8-tetrahydro-6,7-diaminopteridine derivatives, formed by dissolving the appropriate pteridine in liquid ammonia, have been recorded. The ^{13}C n.m.r. spectra of the corresponding covalent hydrates are also reported.

CARBON-13 n.m.r. has been reported to be a useful tool in elucidating the structure of naturally occurring pteridines. Recently ^{13}C n.m.r. 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 n.m.r. chemical shifts of several pteridines (e.g. lumazine, leucopterin, xanthopterin) considerably.[5] Assignment of these ^{13}C n.m.r. signals was achieved by the usual techniques and by relating the ^{13}C n.m.r. spectra with previously recorded[6,7] ^{1}H n.m.r. 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 n.m.r.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.

RESULTS AND DISCUSSION

Pteridine (1a)

The four *intense* signals of the ^{13}C n.m.r. 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 and 186 Hz, respectively. The signal at 159·5 ppm having the largest coupling constant [^1J(CH) = 206 Hz] 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 ^1J(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 [^1J(C-2, H) = 206 Hz[11]], 1,3,5-triazine [^1J(CH) = 207 Hz[12]], purine [^1J(C-2, H) = 207 Hz[13]] quinazoline [^1J(C-2, H) = 204 Hz[14]]. Now that the position of the n.m.r. resonance of C-2 is known, the position of the ^{1}H n.m.r. signal of H-2 in the ^{1}H n.m.r. 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 n.m.r. signals at 148·4 and 153·0 ppm, we measured the ^{13}C n.m.r. 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 n.m.r. spectral assignments presented in this paper were found to be in sound agreement with the interpretation of the ^{1}H n.m.r. spectrum of pteridine[17] which was firmly based on a study with deuterium labelled pteridines.

Pteridine derivatives (1b-1t)

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 n.m.r. spectrum of 2-chloropyrimidine[14] (downfield shifts of 2·7 and 2·4 ppm for C-4 and C-2, respectively).

^{13}C n.m.r. spectroscopy—unlike ^{1}H n.m.r. 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

* Author to whom correspondence should be addressed.
† For Part II of our pteridine studies, see Ref. 1.
§ See Ref. 2.

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.

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 ^1H selective decoupling completely clarifies the ^1H n.m.r. spectrum of these 6- (or 7-)phenylpteridines.

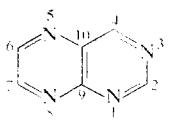
Ammonia adducts

It has been demonstrated by several investigators using both u.v. and ^1H n.m.r. spectroscopy[19,20] that pteridine forms with ammonia a 1:1 σ-adduct (**2a**) and a 2:1 σ-adduct[18] (**3a**). Until now no ^{13}C n.m.r. spectral data on these covalent adducts have been published. To obtain a ^{13}C n.m.r. spectrum of the covalent 3,4-monoadduct (**2a**) (see Table 2) proved to be difficult. During the time between its preparation and the acquisition of the last free induction decay a considerable quantity of precipitate was formed. This results in the spectra being difficult to analyse because of the relatively bad signal to noise ratio. ^{13}C n.m.r. 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.

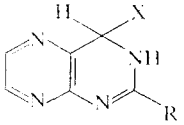
Furthermore, the spectrum exhibits the typical pattern of a pyrimidine derivative in that C-2 now resonates at *lower* field than C-4. Because of the saturation of the pyrazine ring upon diadduct formation, the electron attracting Natoms of the pyrazine ring have adopted the electron releasing character 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 ^1J(C-2, H) and the ^1J(C-4, H) (198 and 176 Hz, respectively) makes it possible to differentiate between the signals from C-2 and C-4.

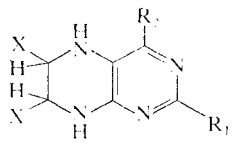
The results of our investigations clearly show that a restrictive condition with respect to diadduct formation in liquid ammonia is that positions 6 and 7 of the pteridine derivative must be unsubstituted.[20] Therefore, of all pteridines listed in Table 1, only a limited number gave the 6,7-diamino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



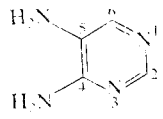
1a



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

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.

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

Hydrates

After studying the ^{13}C n.m.r. 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 n.m.r. 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 n.m.r. 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 n.m.r. 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 1 N 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 1 N 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 n.m.r. spectra of the dihydrate cation (**3h**$^{\oplus}$) and the cation of 4,5-diaminopyrimidine (**4**$^{\oplus}$), both recorded for a 1 N HCl solution, are virtually the same.

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 n.m.r. 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 °C.

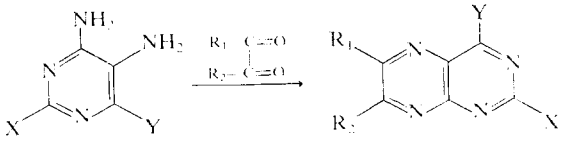
In $CDCl_3$ solution ^{13}C n.m.r. chemical shifts were measured from internal TMS. In NH_3 and H_2O solution ^{13}C n.m.r. chemical shifts were measured from internal trimethylamine and internal dioxane respectively, and they were converted to the TMS scale by adding 47·5 and 67·4 ppm respectively. 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.

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-methylthipoteridine[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. T.l.c. and ^{13}C n.m.r. revealed its formation in a minute amount in addition to the major isomer (**1n**) (ratio 1:10). Dechloro-methoxylation 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%	Found C%	Found H%	Required C%	Required H%
X = Y = H[25]	X = Y = R_1 = H, R_2 = C_6H_5 (**1g**)	158–160	95	69·33	4·06	69·22	3·87
X = Cl, Y = CH_3[26]	X = Cl, Y = CH_3, R_1 = R_2 = H (**1h**)	155–157	80	46·65	2·83	46·55	2·79
X = H, Y = C_6H_5[24]	X = R_1 = H, Y = R_2 = C_6H_5 (**1k**)	154–155	92	76·01	4·31	76·04	4·25
X = Cl, Y = *t*-Bu[28]	X = Cl, Y = *t*-Bu, R_1 = H, R_2 = C_6H_5 (**1n**)	174–176	60	64·39	5·13	64·32	5·06
—	X = OCH_3, Y = *t*-Bu, R_1 = H, R_2 = C_6H_5 (**1p**)	142–144	75	69·50	6·21	69·36	6·16
X = Cl, Y = C_6H_5[20]	X = Cl, R_1 = H, Y = R_2 = C_6H_5 (**1q**)	198–199	72	67·81	3·68	67·82	3·48
X = H, Y = C_6H_5[24]	X = H, Y = R_1 = R_2 = C_6H_5 (**1t**)	174–175	86	79·72	4·65	79·98	4·47

General procedure for measuring the ^{13}C n.m.r. 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 n.m.r. tube.

Acknowledgement—We are indebted to Drs C. A. Landheer, for mass spectrometric data, to Mr H. Jongejan, who carried out the microanalyses and to Mr W. Ch. Melger for chromatographic advice.

REFERENCES

1. See for Pteridine studies Part II: A. Nagel and H. C. van der Plas, *Chem. Pharm. Bull.* **23,** 217 (1975).
2. Part IX of NMR studies on σ-adducts in heterocyclic systems with nucleophiles. Previous paper in this series, D. E. Klinge, H. C. van der Plas and A. van Veldhuizen *Rec. Trav. Chim. Pays-Bas* **95,** 21 (1976).
3. J. A. Lyon, P. D. Ellis and R. B. Dunlap, *Biochemistry*, **12,** 2425 (1973).
4. W. Frick, R. Weber and M. Viscontini, *Helv. Chim. Acta.* **57,** 2658 (1974).
5. G. Müller and W. von Philipsborn, *Helv. Chim. Acta* **56,** 2680 (1973).
6. A. Dieffenbacher and W. von Philipsborn, *Helv. Chim. Acta* **52,** 743 (1969).
7. R. Wagner and W. von Philipsborn, *Helv. Chim. Acta* **53,** 299 (1970).
8. U. Ewers, H. Günther and L. Jaenicke, *Chem. Ber.* **106,** 3951 (1973).
9. U. Ewers, H. Günther and L. Jaenicke, *Angew Chem. Int. Ed. Engl.* **14,** 354 (1975).
10. G. C. Levy and G. L. Nelson, *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*, Wiley-Interscience, New York, 1972, p. 25.
11. K. Tori and T. Nakagawa, *J. Phys. Chem.* **68,** 3163 (1964).
12. S. Braun and G. Freij, *Org. Magn. Reson.* **7,** 194 (1975).
13. J. M. Read Jr and J. H. Goldstein, *J. Am. Chem. Soc.* **87,** 3440 (1965).
14. Measured in this laboratory.
15. Compare assignments made by u.v. spectroscopy: A. Albert, D. J. Brown and H. C. S. Wood, *J. Chem. Soc.* 3832 (1954); A. Albert, D. J. Brown and G. W. H. Cheeseman *J. Chem. Soc.* 1620 (1954).
16. C. J. Turner and G. W. H. Cheeseman, *Org. Magn. Reson.* **6,** 663 (1974).
17. S. Matsuura and T. Goto, *J. Chem. Soc.* 1773 (1963); *J. Chem. Soc.* 623 (1965).
18. A. Albert and K. Ohta, *J. Chem. Soc. C* 2357 (1971).
19. B. E. Evans, *J. Chem. Soc. Perkin Trans* 1 357 (1974).
20. A. Nagel, H. C. van der Plas and A. Van Veldhuizen, *Rec. Trav. Chim. Pays-Bas* **94,** 45 (1975).
21. J. Clark, *J. Chem. Soc. C* 313 (1968).
22. A. Albert and H. Yamamoto, *J. Chem. Soc. C* 2289 (1968).
23. J. Clark, P. N. T. Murdoch and D. L. Roberts, *J. Chem. Soc. C* 1408 (1969).
24. J. Clark and P. N. T. Murdoch, *J. Chem. Soc. C* 1883 (1969).
25. D. J. Brown, *J. Appl. Chem.* **2,** 239 (1952).
26. R. N. Prasad, C. W. Noell and R. K. Robins, *J. Am. Chem. Soc.* **81,** 193 (1959).
27. H. Raudnitz, *J. Chem. Soc.* 763 (1948).
28. G. K. Helkamp and N. S. Kondo, *Biochim. Biophys. Acta* **157,** 242 (1968).