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274. ^{13}C-NMR. Spectra of Pteridines[1])

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(1. X. 73)

Summary. ^{13}C-NMR. spectra of pterin, xanthopterin, isoxanthopterin, leucopterin, lumazine and of the model compounds isocytosine and desamino-isocytosine have been measured as anions and cations in 1M NaOD, CF_3COOH, H_2SO_4 and FSO_3H solutions. The spectra were analysed by means of heteronuclear double resonance, with the aid of non-decoupled spectra, and by spectral comparison. The results are interpreted in terms of the ionisation state of the pteridines in the four solvents and are compared with those obtained from ^{1}H-NMR. spectroscopy.

1. Introduction. – We have shown previously how ^{1}H-NMR. spectroscopy can be utilized in structural studies of pteridines [3a–e]. In particular, it was possible to demonstrate that specific information can be obtained about the ionisation of the various structural types of pteridines and pyrimidines in a series of solvents of increasing acidity [1] [3a]. A fundamental disadvantage of this spectroscopic technique, however, is that many pteridines possess only a few non-exchangeable hydrogen atoms. Spectroscopic and structural information is therefore limited unless proton exchange is suppressed by low temperature and weakly nucleophilic solvents. Since the pteridine skeleton contains six nonequivalent carbon atoms in very different chemical environments, more detailed structural information may be expected from a study of ^{13}C-NMR. spectra in this series. Here we wish to report first results on a number of key compounds and model substrates.

2. Experimental Results. – Because of the amphoteric character of 2-amino-4-oxo-3,4-dihydropteridines, and their poor solubility in neutral media, and as a consequence of the relative insensitivity of carbon resonance spectroscopy, the choice of suitable solvents requires particular attention. Most compounds exhibit sufficient solubility in 1M aqueous sodium deuteroxide, and this system has the advantage that the D_2O signal can be used as an internal field-frequency lock signal. Trifluoroacetic acid which has been used successfully in ^{1}H-NMR. studies [1] [3a, b] is less

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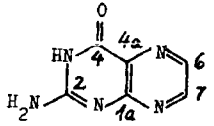
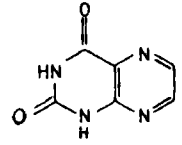
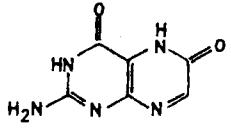
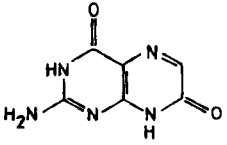
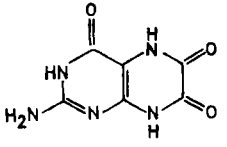
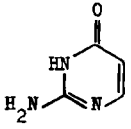
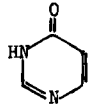
useful in carbon resonance since one of the intense solvent quartets obscures an important spectral region. This solvent, however, is indispensable for a study of monoprotonated pteridines and pyrimidines. Higher protonated species may be conveniently studied in conc. sulfuric acid and fluorosulfonic acid (FSO_3H). Substrate concentrations varied between 0.1 and 0.2M for $NaOD/D_2O$ solutions and from 0.15 to 0.4M for acidic media. All spectra have been measured in the pulsed mode followed by *Fourier*-transformation, and further instrumental details are given in the experimental section. The analysis of the spectra was achieved by a combined investigation of proton-noise-decoupled, CW-off-resonance decoupled and single resonance spectra. In addition, spectral comparison with model pyrimidines and purines [4] was found useful. Chemical shifts are given in Table 1, the one-bond and long-range C,H-coupling constants are listed in Table 2.

3. Discussion. – 3.1. *Anions.* In $NaOD/D_2O$ solution 2-amino-4-oxo-3,4-dihydropteridine (pterin, **1**) exhibits well separated resonances for the six different carbon atoms. The total shift range is rather large (44 ppm), and this is generally true for all anionic species of pteridines (non-hydrogenated pteridines: $\delta_C = 114$–175 ppm). In the spectrum of **1** the two intense signals at 139.7 and 149.5 ppm are assigned to C(6) and C(7) respectively. This follows from their doublet structure and reduced C,H coupling constants under off-resonance decoupling conditions and from the known assignment of H–C(6) and H–C(7) in the 100 MHz ^{1}H-NMR. spectrum [3a]. The two signals at 157.7 and 130.5 ppm appear as narrow doublets in the non-decoupled carbon spectrum. These splittings (10–12 Hz) result from spin coupling with the pyrazine protons. The phenomenon is generally observed in pteridines with hydrogen atoms at C(6) and/or C(7) and is extremely useful for assignment purposes. As shown by double resonance experiments the signals at 157.7 and 130.5 ppm are assigned to C(1a) and C(4a) respectively, and thus the splitting arises from transoid vicinal three-bond C,H coupling. The assignment of the low-frequency signal to C(4a) is in agreement with results obtained in purines [4]. The two remaining signals at highest frequencies (174.1 and 165.4 ppm) belong to the amide carbonyl carbon atom C(4) and the guanidine carbon atom C(2) respectively. Their assignment is usually not straight-forward but can be achieved for the pteridine anions by spectral comparison within the whole series and with reference to the spectra of 2-amino-4-oxo-3,4-dihydropyrimidine (isocytosine, **4**) and 4-oxo-3,4-dihydropyrimidine (**5**), *cf.* Table 1. The spectra of the anions of xanthopterin (**6**), isoxanthopterin (**7**) and leucopterin (**8**) were analysed similarly. The $^1J_{CH}$ coupling constants for C(6) and C(7) in pteridine anions vary between 180 and 188 Hz and, as shown below, increase with increasing protonation of the ring nitrogen atoms. These data are a valuable aid in the assignment of carbon signals in cases of chemical shift ambiguities. In an attempt to interprete the ^{13}C chemical shifts of pteridines dissolved in 1M sodium deuteroxide one has to take into consideration the different ionisation states (see Table 1). It is known from UV. spectroscopic data that above pH 13 pterin (**1**) exists as the *mono*-anion, whereas xanthopterin (**6**) and isoxanthopterin (**7**) form *di*-anions in which the second ionisation occurs in the pyrazine ring. For leucopterin (**8**) threefold deprotonation has been discussed [5].

3.2. *Cations.* In concentrated sulfuric acid the majority of pteridines dissolve to form di-cations. Their solubility is considerably higher than that of the mono-anions

Table 1. *^{13}C-Chemical Shifts [δ, ppm]* [a])

Compound	Solvent	C(2)	C(4)	C(4a)	C(6)	C(7)	C(1a)	ionic species [d])
pterin (**1**)	1 M NaOD	165.4	174.1	130.5	139.7	149.5	157.7	(–)
	CF_3COOH	158.7 ? [b])	152.1	126.5	143.7	151.6	147.6	(+)
	H_2SO_4	153.0	152.2	116.8	136.7	158.8	150.2	(++)
	FSO_3H	151.7	151.2	115.9	136.5	159.0	149.7	(++)
6-methylpterin (**2**)	CF_3COOH	[b])	151.8	125.0	155.7 CH_3: 47.8	152.1	145.5	(+)
7-methylpterin (**3**)	CF_3COOH	[b])	152.0	123.4	144.2	[b]) CH_3: 46.5	147.1	(+)
lumazine (**9**)	CF_3COOH	161.7	151.3	126.5	141.7	150.2	148.7	(+)
	FSO_3H	155.4	151.9	118.2	137.5	157.9	148.8	(++)
xanthopterin (**6**)	1 M NaOD	165.0	174.5	125.9	161.3	147.0	149.0	(– –)
	CF_3COOH	155.8	150.6	112.7	156.9	156.9	137.3	(+)
	H_2SO_4	154.3	151.2	111.7	155.2	152.0	142.1	(++)
	FSO_2H	153.4	151.0	111.1	154.6	151.5	141.9	(++)

isoxanthopterin (**7**)	1 M NaOD	164.9	174.5	118.0	138.5	170.8	159.6	(– –)
	H_2SO_4	153.4	152.0	109.4	133.3	164.9	149.4	(+)
	FSO_3H	152.4	151.7	109.6	131.9	165.9	149.4	(+)
leucopterin (**8**)	1 M NaOD	161.8	172.5	113.8	160.6	168.9	154.0	(– – –)
	H_2SO_4		156.7; 154.2; 152.3; 150.4[c]	99.4			137.1	?
				C(5)	C(6)			
isocytosine (**4**)	1 M NaOD	164.8	177.1	103.7	156.3			(–)
	1 M DCl	152.8	163.1	105.4	142.5			(+)
	H_2SO_4	151.2	169.5	101.9	152.7			(++)
				C(5)	C(6)			
 (**5**)	1 M NaOD	159.1	175.1	113.0	155.3			(–)
	0.1 M DCl	151.6	166.9	115.6	150.2			(+)
	H_2SO_4	152.7	165.0	117.6	147.7			(++)

[a]) ±0.3 ppm.
[b]) the resonances are masked by solvent peaks.
[c]) these four lines have not been assigned.
[d]) (–) = mono-anion, (– –) = di-anion, (+) = mono-cation, (++) = di-cation, etc.

Table 2. *$^{13}C,^{1}H$-Coupling Constants*[a) b)]

Compound	Solvent	J_{CH} [Hz]
pterin (**1**)	1 M NaOD	$^{1}J_{66} = 188$; $^{1}J_{77} = 182$; $^{2}J_{67} = 10$; $^{2}J_{76} = 12$; $^{3}J_{4a6} \sim 5$
	CF_3COOH	$^{1}J_{66} = 196$; $^{1}J_{77} = 189$; $^{2}J_{67} = {}^{2}J_{76} = 10$; $^{3}J_{4a6} = 10$; $^{3}J_{1a7} = 12$
	H_2SO_4	$^{1}J_{66} = 205$; $^{1}J_{77} = 205$; $^{2}J_{67} = 15$; $^{2}J_{76} = 5$; $^{3}J_{4a6} = 5$; $^{3}J_{1a7} = 12$
	FSO_3H	$^{1}J_{66} = 205$; $^{1}J_{77} = 207$; $^{2}J_{67} = 13$; $^{2}J_{76} = 5$; $^{3}J_{1a7} = 12$
6-methylpterin (**2**)	CF_3COOH	$^{1}J_{77} = 190$; $^{3}J_{1a7} = 12$
7-methylpterin (**3**)	CF_3COOH	$^{1}J_{66} = 192$; $^{3}J_{4a6} = 13$
lumazine (**9**)	CF_3COOH	$^{1}J_{66} = 194$; $^{1}J_{77} = 191$; $^{2}J_{67} = {}^{2}J_{76} = 11$; $^{3}J_{4a6} = 8$; $^{3}J_{1a7} = 13$
	FSO_3H	$^{1}J_{66} = 208$; $^{1}J_{77} = 207$; $^{2}J_{67} = 12$; $^{2}J_{76} = 5$; $^{3}J_{4a6} = 5$; $^{3}J_{1a7} = 13$
xanthopterin (**6**)	1 M NaOD	$^{1}J_{77} = 180$; $^{3}J_{1a7} = 10$
	CF_3COOH	$^{1}J_{77} = 206$; $^{2}J_{67} = 10$; $^{3}J_{1a7} = 15$
	H_2SO_4	$^{1}J_{77} = 203$; $^{2}J_{67} = 10$; $^{3}J_{1a7} = 15$
	FSO_3H	$^{1}J_{77} = 207$; $^{2}J_{67} = 10$; $^{3}J_{1a7} = 15$
isoxanthopterin (**7**)	1 M NaOD	$^{1}J_{66} = 183$; $^{3}J_{4a6} = 10$
	H_2SO_4	$^{1}J_{66} = 208$; $^{3}J_{4a6} = 7$
	FSO_3H	$^{1}J_{66} = 208$; $^{3}J_{4a6} = 5$
isocytosine (**4**)	1 M NaOD	$^{1}J_{55} = 168$; $^{1}J_{66} = 170$; $^{2}J_{56} = 7$; $^{3}J_{26} = 10$; $^{3}J_{46} = 7$
	H_2SO_4	$^{1}J_{55} = 190$; $^{1}J_{66} = 197$; $^{3}J_{26} = 10$; $^{3}J_{46} = 8$
4-oxo-3,4-dihydro pyrimidine (**5**)	1 M NaOD	$^{1}J_{22} = 197$; $^{1}J_{55} = 167$; $^{1}J_{66} = 176$; $^{2}J_{56} = 5$; $^{3}J_{26} = 11$ $^{3}J_{42} = {}^{3}J_{46} = 8$; $^{3}J_{62} = 9$
	0.1 M DCl	$^{1}J_{22} = 209$; $^{1}J_{55} = 172$; $^{1}J_{66} = 185$; $^{2}J_{56} = 5$; $^{3}J_{26} = 9$; $^{3}J_{42} = {}^{3}J_{46} = 8$; $^{3}J_{62} = 10$
	H_2SO_4	$^{1}J_{22} = 222$; $^{1}J_{55} = 187$; $^{1}J_{66} = 200$; $^{2}J_{56} = 5$; $^{3}J_{26} = 7$; $^{3}J_{62} \approx {}^{2}J_{65} \approx 5$

a) $J_{66} = J_{C(6)H(6)}$, $J_{67} = J_{C(6)H(7)}$ etc., numbering system see table 1.

b) The long-range C,H-coupling constants were assigned using the following criteria: a) it is justified to assume that only $^{13}C \sim C{-}H$ coupling is observed since N–H proton exchange is fast for N(1)H and N(8)H as shown by proton NMR. at 30° in all solvents used; b) a determination of the long-range C,H-coupling constants in **4** and **5** by means of selective decoupling; c) selective C,H-decoupling experiments in the pteridines whenever assigments were not unequivocal.

in NaOD/D_2O. Furthermore, dipolar relaxation of the quaternary carbons atoms by solvent protons shortens the relaxation times. Consequently, spectra with a much better signal-to-noise ratio are obtained. The same applies for fluorosulfonic acid (FSO_3H) solutions. Both solvents appear to yield the same protonation states as shown by ^{1}H-[3a] [6] and ^{13}C-NMR. spectroscopy (*cf.* Fig.).

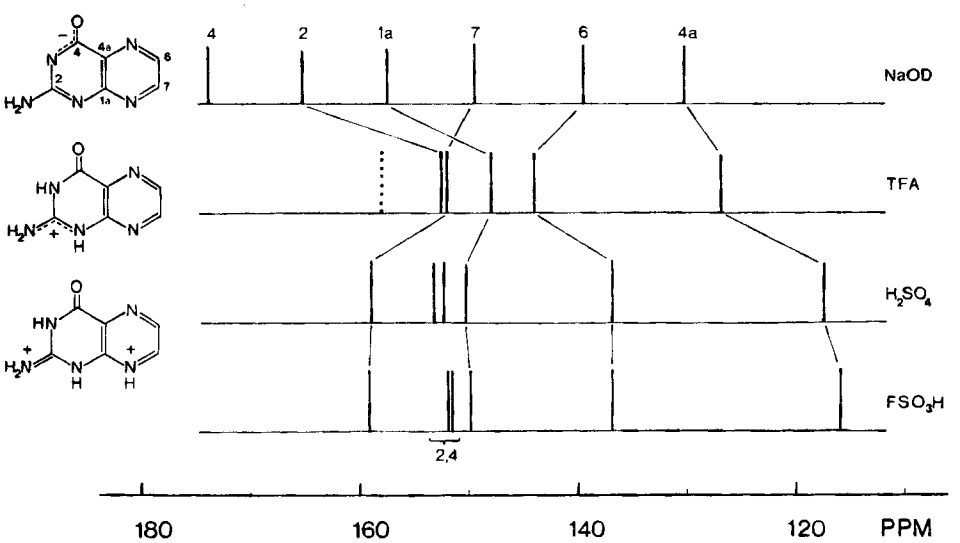


Fig. *Schematic spectra and* ^{13}*C-chemical shift correlation for the mono-anion, mono-cation and di-cation of pterin* (**1**)

In the N(1), N(8)-dication $\mathbf{1}^{++}$ the C(7) resonance appears at higher frequency than C(2) and C(4)[2]. This is also observed in the spectra of xanthopterin (**6**) and lumazine (**9**) which likewise become protonated at N(8). The second protonation which occurs in the pyrazine ring is further reflected in the $^1J_{CH}$ coupling constants of C(7) and C(6) which are now larger than 200 Hz. The conclusions from ^{13}C-NMR. data are thus in agreement with those obtained from ^{1}H-NMR. studies [3a]. In the case of isoxanthopterin (**7**) proton spectra have suggested monoprotonation at the C(7) carbonyl group in FSO_3H solution [6]. This is now confirmed by the low chemical shift of the olefinic C(6) carbon (131.9 ppm). The analogous carbon atom C(7) of xanthopterin (**6**) – which is protonated at N(8) – appears at 151.5 ppm. Protonation of isoxanthopterin (**7**) at the C(7) carbonyl oxygen atom is also reflected in the coupling constant of C(6) ($^1J_{CH}$ = 208 Hz). The assignment of the quaternary carbon atoms in the pteridine cations was carried out by the same methods as described under 3.1.

Twofold protonation in the pyrimidine ring – not observed for pteridines dissolved in H_2SO_4 or FSO_3H – has been demonstrated, by low-temperature ^{1}H-NMR., to occur in the model compound isocytosine (**4**) [1]. Confirmation is now afforded by the very low shielding of C(4) (169.5 ppm) when compared with the corresponding resonances

[2] The resonances of C(7) and C(6) (in H_2SO_4) have been distinguished by means of selective coherent irradiation at the frequencies of H–C(7) (10.28 ppm) and H–C(6) (9.54 ppm).

(151.0–151.9 ppm) in **1**, **6**, **7** and **9**. Thus, in the second protonation stage of pteridines the amide carbonyl C(4)=O cannot compete with any of the basic centres of the pyrazine ring. Even a further increase in the acidity of the medium leads to a third protonation occurring at N(5) of the pyrazine ring as shown by the low-temperature proton spectrum of **1** in FSO_3H-SbF_5-SO_2 solution [7]. This shows that the basicities of the various centres in **1** are as follows: N(1)>N(8)>N(5)>C(4)=O.

Trifluoroacetic acid is known to effect mono-protonation at N(1) of pterin (**1**) and xanthopterin (**6**) [3a, b] [6]. The much less basic isoxanthopterin (**7**) is almost insoluble in CF_3COOH and becomes protonated only in stronger acids such as H_2SO_4 and FSO_3H. Carbon spectra obtained in CF_3COOH are more difficult to interpret since the C(2) and C(4) signals are often masked by the strong $CF_3{}^{13}COOH$ multiplet. Nevertheless, a number of mono-cations have been studied and the assignments are again based on off-resonance decoupled and non-decoupled spectra as well as on chemical shift correlations within the cationic series. The results are listed in Tables 1 and 2. The spectra of the mono-cations of 6-methylpterin (**2**) and 7-methylpterin (**3**) are important for a correct assignment of the C(6) and C(7) signals in pterin (**1**) since the corresponding protons in **1** have nearly the same chemical shift. Furthermore, 6-methylpterin is a suitable model compound for biologically important pteridines such as biopterin and folic acid.

A comparison of the ^{13}C chemical shifts of pteridines in the four different solvents is illustrated in Fig. for pterin (**1**). The best separation of the individual carbon signals is obtained with the mono-anion. The mono-cation is formed in CF_3COOH. Concentrated H_2SO_4 and FSO_3H produce the same di-cation with almost identical chemical shifts in both solvents.

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4. Experimental Part. – The carbon spectra were measured on a *Varian* XL-100-15 spectrometer (25.2 MHz) equipped with a pulse unit and a 620i-8K on-line computer system. The probe temperature was 32° when proton high-power decoupling was used, otherwise 28°. Sample tubes of 12 mm outer diameter required a typical sample volume of 2 ml. For $NaOD/D_2O$ and DCl/D_2O solutions the deuterium resonance of the solvent was used as an internal field-frequency lock signal. A 5 mm tube filled with D_2O and placed inside a 12 mm tube served as an internal lock for CF_3COOH, H_2SO_4 and FSO_3H solutions. Typical parameters for the pulse experiments were as follows: spectral width 5000 (2500) Hz, aquisition time 0.4 (0.8) s, pulse delay 0.2–0.6 s, pulse width 22–25 μs. For the dilute solutions and non-decoupled spectra up to 60000 free induction decays had to be accumulated. Chemical shifts were all determined relative to dioxane as an *internal* standard for D_2O solutions and as an *external* standard for CF_3COOH, H_2SO_4 and FSO_3H. These shifts were then recalculated relative to tetramethylsilane (ext.) using dioxane/TMS relative shifts of 67.8 ppm and 67.5 ppm respectively. The δ-values are within ± 0.3 ppm, the coupling constants within ± 2 Hz.

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9. EUCHEM-Konferenz über Stereochemie

Bürgenstock bei Luzern, 5. Mai–12. Mai 1974

Die Teilnehmerzahl wird begrenzt sein. Interessenten werden gebeten, ihre Anmeldung bis spätestens 15. Januar 1974 an den Präsidenten: Prof. J. M. Lehn, Institut de Chimie, Université de Strasbourg, 1, rue Blaise-Pascal, Strassburg, Frankreich zu richten.

4th International Symposium on Medicinal Chemistry

Noordwijkerhout (The Netherlands) September 9–13, 1974

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Further informations: Merck Sharp & Dohme B.V., Professional and Government Liaison, Waarderweg 39, P. O. Box 581, Haarlem, The Netherlands

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