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ESR OF PTERIN AND LUMAZINE RADICALS IN SOLUTION

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Abstract—Cationic tetrahydrolumazine radicals and cationic tetrahydropterin radicals were detected by electron spin resonance when 5-alkyl-5,6,7,8-tetrahydrolumazines and 5-alkyl-5,6,7,8-tetrahydropterins were oxidized with hydrogen peroxide in formic acid. The hyperfine interactions of both types of radicals are essentially the same.

Two consecutive radical species were observed during the oxidation of 3,5,8-trialkyl-5,6,7,8-tetrahydrolumazines in formic acid. They were identified as cationic tetrahydrolumazine radicals and cationic dihydrolumazine radicals.

The ESR spectra of neutral trihydro- and monohydro-lumazine radicals, which have not been obtained before, were recorded during the oxidation of 5-alkyl-5,6,7,8-tetrahydrolumazines in chloroform. Starting from 5-butyl-1,3-dimethyl-5,6,7,8-tetrahydrolumazine three different radicals were observed.

The spectra were interpreted in terms of hyperfine coupling constants and nuclear spins of the atoms involved.

A role for pteridine radicals in biological processes has been suggested by a number of authors.[1-3] The naturally occurring pteridines are either pterins† or lumazines.† Tetrahydro- and dihydro-pterins are found in biological systems in which electron transfer could occur via trihydropterin radicals. The search for these radicals in biological systems will be aided considerably if more insight can be gained in their chemical and physical properties.

Unfortunately the detection by ESR spectroscopy of pteridine radicals in aqueous solutions is considerably hampered by their instability.[4]

In trifluoroacetic acid the cationic forms of pterin and lumazine radicals are sufficiently stable for detection by ESR. Well resolved spectra are observed in the case of cationic dihydropterin- and dihydrolumazine radicals.[1] Cationic tetrahydropterin radicals[1,5-7] have poorly resolved spectra which provide little information about the chemical and electronic structure of the radicals. ESR spectra of cationic tetrahydro-, neutral trihydro- and neutral monohydrolumazine radicals have not been reported.

Because of our interest in oxidation reactions of 5-alkyl-tetrahydrolumazines[8] we decided to undertake an ESR study of the radical intermediates formed during the oxidation of these compounds in acidic and neutral solvents.

RESULTS AND DISCUSSION

Oxidation of 5 - alkyl - tetrahydropteridines in formic acid

Oxidation of 5 - methyl - 6,7 - diphenyl - tetrahydropterin **1a** (Fig. 1) with hydrogen peroxide in TFA or TFA-d leads to a radical with a very poorly resolved ESR spectrum.[1,6,7]

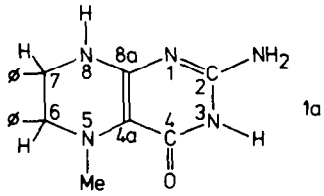


Fig. 1.

The same spectrum is observed in formic acid or formic acid-d_2 (Fig. 2). It consists of seven equally spaced lines with approximate intensity ratios: 1:5:11:14:11:5:1, as expected for one nitrogen and four hydrogens all having equal coupling constants. The coupling constants of the exchangeable protons $N_8(H)$, $C_2(1'\text{-}H)$ and $N_3(H)$ must be much smaller than the observed linewidth as the spectrum is the same in deuterated and nondeuterated solvents.

It has been postulated[1,7] that the spectrum originates from a cationic tetrahydro radical **1b** (Fig. 2).

Three interpretations were published (Table 1).

We investigated three other 5 - methyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydropteridine derivatives: **2a**, **3a** and **4a** (Table 2, Fig. 3).

Upon oxidation with hydrogen peroxide in formic acid or formic acid-d_2 compound **2a** gives the spectrum of the cationic radical **2b** which is the same as the spectrum of **1b**. In contrast with **1a** and **2a**, compound **3a** gives a well resolved spectrum of the cationic tetrahydrolumazine radical **3b** (Fig. 2) when oxidized in formic acid.

Oxidation of **4a** in formic acid gives the cationic tetrahydro radical **4b**. The spectra of **4b** and **3b** are identical. Consequently the spin densities on N_3 and its substituent must be small.

The spectrum of **3b** and **4b** is accurately simulated (Fig. 2) using 6 hydrogen coupling constants and 2 nitrogen coupling constants. Three of the hydrogens have the same coupling constant (11.8 Gauss) (Table 3).

Specific deuteration of **3b** is necessary in order to assign the coupling constants to specific nuclei of the radical molecule.

Oxidation of **3a** in formic acid-d_2 gives the spectrum of the cationic radical **3c** (Fig. 2). This spectrum has one

†Pterin: 2-amino-4(3H)-pteridinone. Lumazine: 2,4(1H, 3H)-pteridinedione. Cationic tetrahydropteridine radicals: the radicals obtained by removal of one electron from 5,6,7,8-tetrahydropteridines. Neutral trihydropteridine radicals: the radicals obtained by removal of one electron and one proton from 5,6,7,8-tetrahydropteridines. Cationic dihydropteridine radicals: the radicals obtained by removal of three electrons and two protons from 5,6,7,8-tetrahydropteridines. Neutral monohydropteridine radicals: the radicals obtained by removal of three electrons and three protons from 5,6,7,8-tetrahydropteridines.

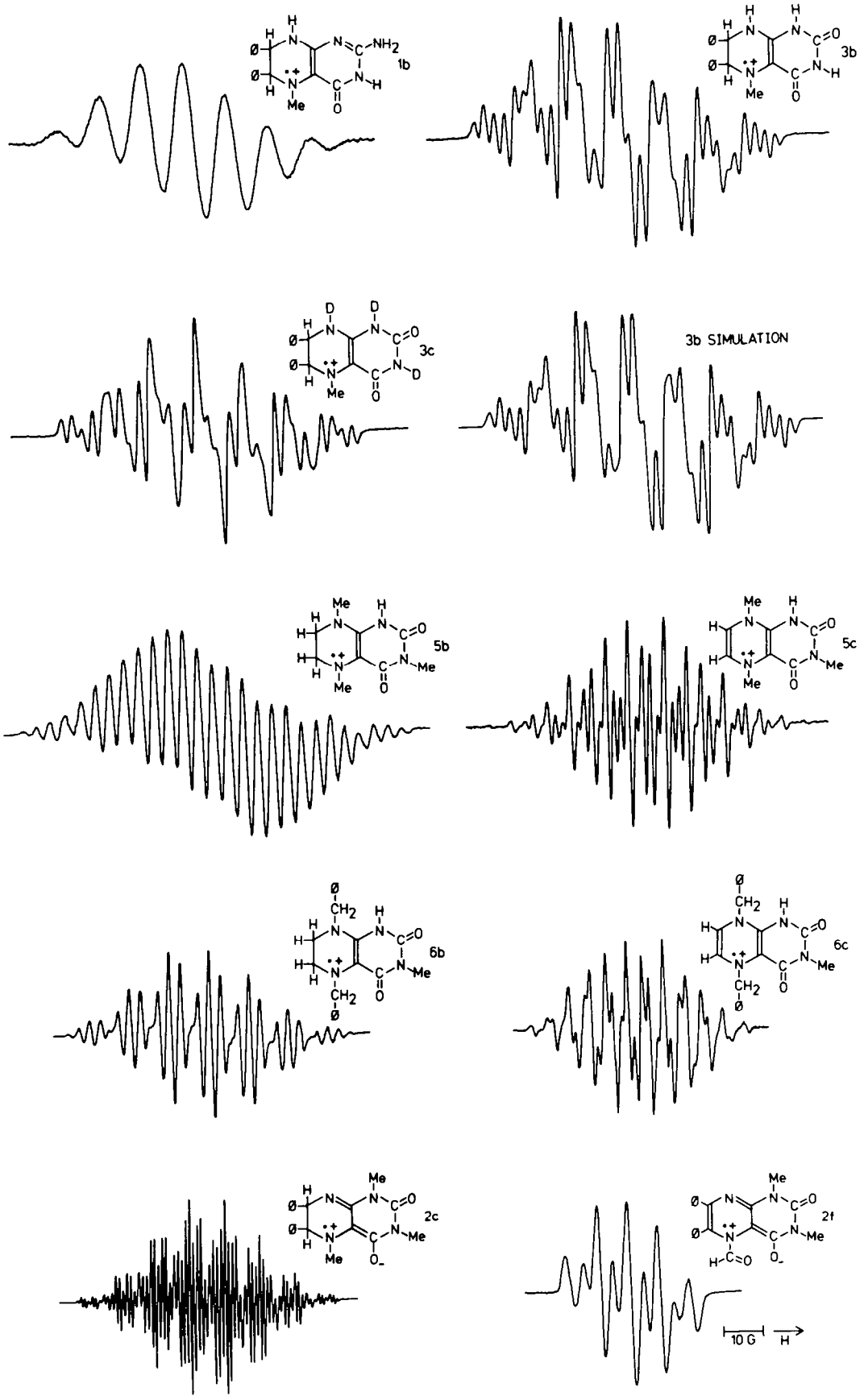


Fig. 2. Experimental ESR spectra of cationic and neutral pteridine radicals and the simulation of the spectrum of 3b.

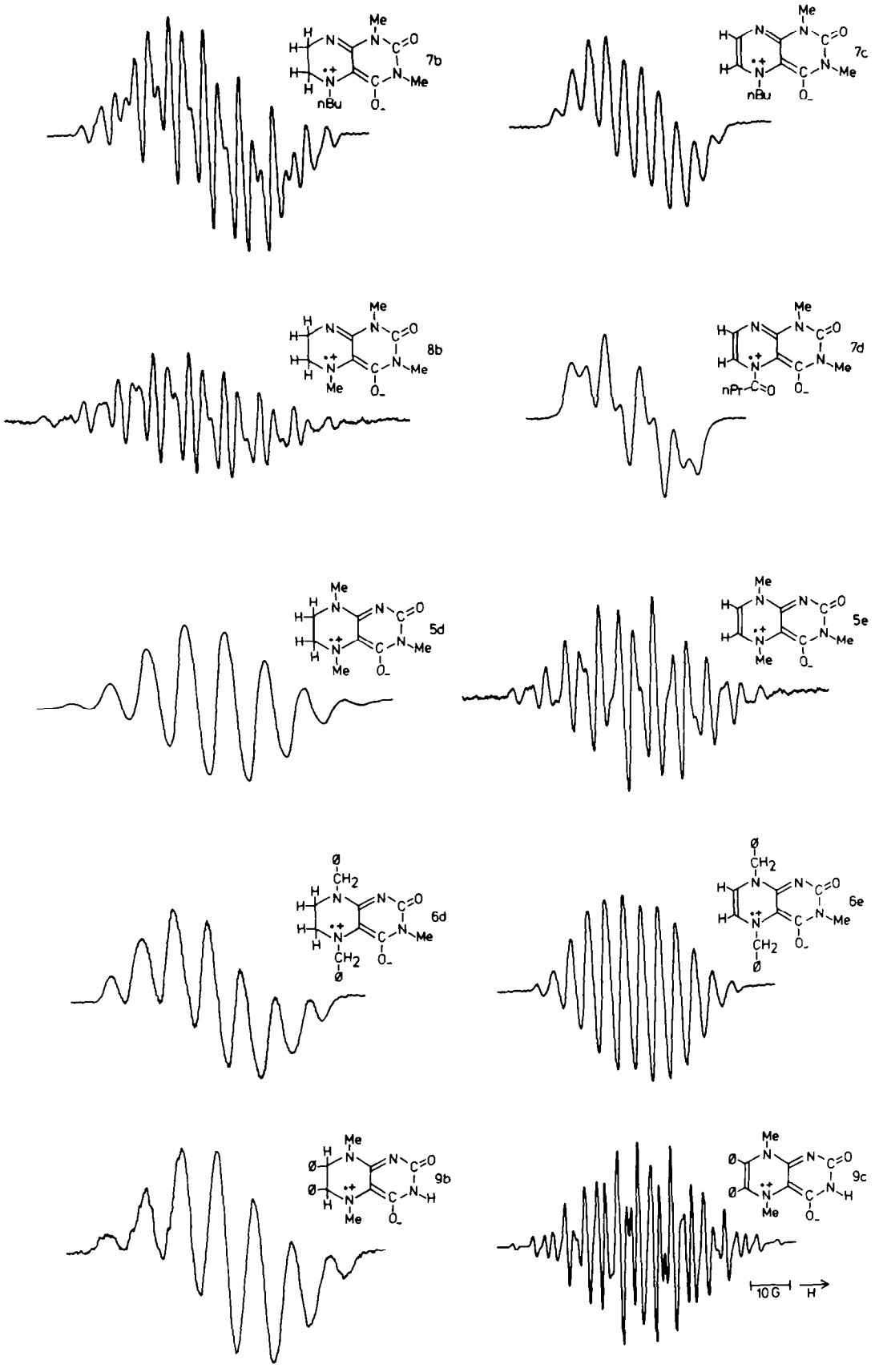


Fig. 2. (*Contd.*). Experimental ESR spectra of cationic and neutral pteridine radicals and the simulation of the spectrum of **3b**.

Table 1. Coupling constants in gauss of radical **1b**

N_5	N_5(1'-H)	C_6(H)	C_7(H)	N_8	N_8(H)	Reference
10	10	10				6
9.5	9.5	9.5				7
9.8	9.8	13.07				1
10.7	11.8	7.6	3.1	2.2	2.4	this work

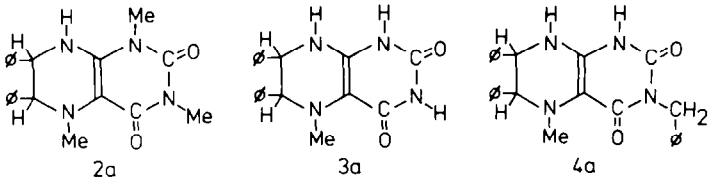
Table 2. Numbering of the 5,6,7,8-tetrahydropteridines and the radicals produced upon oxidation

Tetrahydropteridine	Cationic tetrahydro radical. HCOOH	Cationic tetrahydro radical. DCOOD	Cationic dihydro radical. HCOOH	Neutral trihydro radical.	Neutral monohydro. radical.	Neutral N_5-acyl radical.
5-methyl-6,7-diphenyl-5,6,7,8-tetrahydropterin	1a	1b				
1,3,5-trimethyl-6,7-diphenyl-5,6,7,8-tetrahydrolumazine	2a	2b		2c		2f
1,3-dimethyl-5-methyl-D_3-6,7-diphenyl-6,7-dideutero-5,8-dihydrolumazine	2d			2e		2g
5-methyl-6,7-diphenyl-5,6,7,8-tetrahydrolumazine	3a	3b	3c			
3-benzyl-5-methyl-6,7-diphenyl-5,6,7,8-tetrahydrolumazine	4a	4b	4c			
5-methyl-D_3-6,7-diphenyl-6,7-dideutero-5,8-dihydrolumazine	3d		3e			
3,5,8-trimethyl-5,6,7,8-tetrahydrolumazine	5a	5b	5c	5d	5e	
5,8-dibenzyl-3-methyl-5,6,7,8-tetrahydrolumazine	6a	6b	6c	6d	6e	
5-butyl-1,3-dimethyl-5,6,7,8-tetrahydrolumazine	7a			7b	7c	7d
1,3,5-trimethyl-5,6,7,8-tetrahydrolumazine	8a			8b		8c
5,8-dimethyl-6,7-diphenyl-5,6,7,8-tetrahydrolumazine	9a			9b	9c	

Table 3. Coupling constants in gauss of cationic tetrahydro- and dihydro-lumazine radicals in formic acid and formic acid-d_2. Alternative assignments are placed in parentheses

	N_5	N_8	N_5 (1'-H)	N_5 (1'-D)	N_8(H)	N_8(D)	C_6(H)	C_6(D)	C_7(H)	C_7(D)	Linewidth
3b, 4b	10.7	2.2	11.8		2.4		7.6		3.1		1.7
3c, 4c	10.7	2.2	11.8			0.37	7.6		3.1		1.7
3e	10.7	2.2		1.82		0.37		1.17		0.48	1.7

	N_5	N_8	N_5 (1'-H)	N_8 (1'-H)	C_6(H)	C_7(H)	Linewidth
5b	11.3	3.4	11.3	4.0	9.9	2.7	2.0
5c	9.1	5.6	9.3	5.4		5.8	1.2
6b	10.1	2.9	9.8 (6.8)	2.6 (2.4)	6.8 (9.8)	2.4 (2.6)	1.6
6c	9.1	5.6	5.6	4.1		5.0	1.2



2a 3a 4a

Fig. 3.

line less and is approximately 2 gauss less wide between the outer lines than the one of **3b**. Apparently only one of the three protons that have been exchanged for deuterium is ESR-active (i.e. the coupling constant is resolved).

Oxidation of **4a** in formic acid-d_2 leads to the radical **4c** which is the N_1,N_8-dideuterated analogue of **4b**. As expected the spectra of **4c** and **3c** are identical.

When 5 - methyl - D_3 - 6,7 - diphenyl - 6,7 - dideutero - 5,8 - dihydrolumazine **3d** is oxidized in formic acid-d_2 the radical **3e**, a deuterated analogue of **3b**, is formed. The hydrogens of **3b** that have been replaced by deuterium in **3e** are: N_5(1'-H), C_6(H), C_7(H), N_1(H), N_3(H) and N_8(H). The spectrum of **3e** shows that there are no ESR active protons in this radical. The hyperfine pattern is due to two nitrogens and six deuterons (Table 3). The absence of hydrogen coupling constants in the spectrum of **3e** proves that there is no interaction between the unpaired electron and the hydrogen atoms of the two phenyl groups.

The experimental evidence concerning the spin distribution in radical **3b** can be summarized as follows:

1. There is no measurable spin density on N_3 and N_3(H).
2. Of the other two exchangeable protons N_1(H) and N_8(H) only one is ESR-active.
3. There is no measurable interaction of the unpaired electron with any of the phenyl protons.
4. Two nitrogen atoms and six hydrogen atoms, of which three are equivalent, have resolved coupling constants.

Clearly the three equivalent protons are those of the N_5 ME group.

Experiments with the structurally related alloxazine,[9] isoalloxazine[10–15] and cationic dihydropteridine radicals[1] indicate that the coupling constant of a nitrogen-bound proton is of the same magnitude as the coupling constant of that N-atom. The same approximate equality is found for the protons of a nitrogen-bound Me group. The proton coupling constants of other beta protons such as those of a nitrogen-bound methylene group are somewhat smaller than the nitrogen coupling constant.[1]

Application of these empirical rules to radical **3b** leads to the conclusion that N_5 must have the larger of the two nitrogen coupling constants (10.7 gauss) (Table 3) because the N_5 methyl protons have coupling constants of 11.8 gauss. The larger of the remaining hydrogen coupling constants (7.6 gauss) must be attributed to C_6(H), a beta proton of N_5.

The nitrogen coupling constant of 2.2 gauss and the two hydrogen coupling constants of 2.4 and 3.1 gauss must be assigned to N_8, N_8(H) and C_7(H).

The alternative assumption that N_1 rather than N_8 gives rise to the coupling constant of 2.2 gauss accounts for only one of the two smaller hydrogen coupling constants.

Since the spin densities on ring positions 1, 2 and 3 in the radicals **3b** and **4b** are small, the unpaired electron is almost entirely located in that part of the molecule that is identical in all four radicals **1b**, **2b**, **3b** and **4b**. It is then reasonable to assume that the spectra of **1b** and **2b** arise from essentially the same hyperfine coupling constants as found for **3b** and **4b** (Tables 1 and 3). The difference between the spectra of **1b** and **2b** on the one hand and **3b** and **4b** on the other hand must consequently be a difference in linewidth only.

Indeed this turns out to be the case. When the spectra of **3b** and **4b** are recorded using a modulation amplitude of 8.0 gauss the spectra obtained are identical with those of **1b** and **2b**. Similarly the spectra of **1b** and **2b** can be produced by means of a theoretical spectrum reconstruction using the coupling constants found for **3b** and **4b** and a linewidth of 4.0 gauss.

Oxidation of 5,8 - dialkyl - tetrahydrolumazines in formic acid. Well resolved ESR spectra (Fig. 2) are obtained when 3,5,8 - trimethyl - 5,6,7,8 - tetrahydrolumazine **5a** and 5,8 - dibenzyl - 3 - methyl - 5,6,7,8 - tetrahydrolumazine **6a** are oxidized with hydrogen peroxide in formic acid.

During the first minutes after mixing the reactants the spectra of the cationic tetrahydrolumazine radicals **5b** and **6b** (Fig. 2) are observed.

The hyperfine structure of the spectrum of **5b** arises from two nitrogens, two groups of the two equal hydrogens and two groups of three equal hydrogens. The spectrum of **6b** reveals two ESR active nitrogens and four groups of hydrogens each containing two hydrogens with equal coupling constants (Table 3).

Oxidation of **5a** and **6a** in formic acid-d_2 gives the tetrahydro radicals with a deuterium atom on N_1. The spectra are the same as in formic acid, so the spin densities on N_1 and H_1 are small. Consequently the observed coupling constants of **5b** and **6b** are to be attributed to the nitrogen atoms, the methyl protons and the methylene protons in the pyrazine part of the molecule.

In order to decide which of the pyrazine nitrogens has the higher coupling constant a comparison can be made with 5-alkylated (this work) and 8-alkylated[1] lumazine radicals. In both cases N_5 has a larger coupling constant than N_8.

Apparently the unequal spin distribution in the pyrazine ring is caused by the unsymmetrical structure of the pyrimidine ring and not by the nature of the substituents on N_5 and N_8.

Consequently the larger coupling constants in **5b** and **6b** are to be assigned to N_5, N_5(1'-H) and C_6(H) and the smaller ones to N_8, N_8(1' – H) and C_7(H) (Table 3).

Prolonged oxidation of **5a** and **6a** with hydrogen peroxide in formic acid leads to the disappearance of the spectra of **5b** and **6b** followed by the appearance of the spectra of the cationic dihydrolumazine radicals **5c** and **6c** (Fig. 2). Interpretation of the spectrum of **5c** shows

that two nitrogens and seven hydrogens contribute to the spectrum. Two nitrogens and five hydrogens are ESR-active in the spectrum of **6c** (Table 3).

Cationic 5,8 - dialkyl - dihydropteridine radicals have not been reported before. However a study has been made of cationic dihydropteridine radicals without substituents on N_5 and N_8 and those with one alkyl group on either N_5 or N_8.[1]

The properties of **5c** and **6c** are in complete agreement with those of the previously studied cationic dihydropteridine radicals. The spectra of **5c** and **6c** remain unchanged when the oxidation is carried out in formic acid-d_2, so the spin density on N_1 is small. The spectra of **5c** and **6c** are more persistent that those of the radicals **5b** and **6b**. Large coupling constants are found for N_5, N_8, N_5(1'-H), N_8(1'-H) and C_7(H), while the coupling constant of C_6(H) is small.

A high steady state concentration of the secondary radicals **5c** and **6c** is observed when 1 mmol of tetrahydrolumazine is oxidized with 0.5 mmol of peroxide, whereas 1.5 mmol is theoretically required for their formation.

Oxidation of 5-alkyl-tetrahydrolumazines in chloroform. When 1,3,5 - trimethyl - 6,7 - diphenyl - tetrahydrolumazine **2a** (Fig. 3) is oxidized with t-butyl-hydroperoxide in chloroform under anaerobic conditions the spectrum of the neutral trihydroradical **2c** is obtained (Fig. 2). On adding solid cupric acetate monohydrate to a solution of **2a** the same spectrum is observed. The spectrum does not depend on the nature of the oxidant and incorporation of any part of the oxidant in the lumazine radical is unlikely.

The spectrum is accurately simulated using the coupling constants in Table 4.

Oxidation of 1,3 - dimethyl - 5 - methyl - D_3 - 6,7 - diphenyl - 6,7 - dideutero - 5,8 - dihydrolumazine **2d** with t - butyl - hydroperoxide in chloroform produces the spectrum of the neutral deuterated radical **2e** in which three equivalent hydrogens and two nonequivalent hydrogens with coupling constants of 8.72, 6.80 and 5.94 gauss have been replaced by deuterium atoms. Therefore the three equivalent hydrogens are those of the N_5 methyl group and the two nonequivalent ones are C_6(H) and C_7(H). Again N_5 is the nitrogen atom with the largest hyperfine interaction.

The fact that C_7(H) interacts strongly with the free electron suggests that the remaining nitrogen coupling constant must be assigned to N_8 and not to N_1.

The three proton coupling constants of 0.85 gauss can be explained by the assumption that there is a small amount of direct contact interaction between the protons of the N_1 Me group and the unpaired electron.

The spin densities on N_8 and N_5 can be explained by the two mesomeric structures of Fig. 4. A structure similar to the one on the right hand side of Fig. 4 was proposed for neutral isoalloxazine radicals,[14,16] where the largest spin density is also found on N_5.

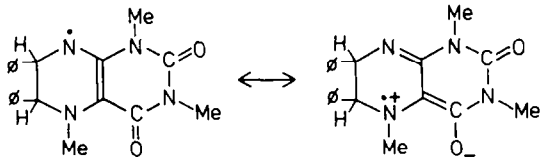


Fig. 4.

Oxidation of 5 - butyl - 1,3 - dimethyl - tetrahydrolumazine **7a** and 1,3,5 - trimethyl - tetrahydrolumazine **8a** in chloroform with t-butyl-hydroperoxide or cupric acetate monohydrate affords the ESR spectra of the corresponding neutral trihydro radicals **7b** and **8b** (Fig. 2). Their hyperfine interactions are of the same magnitude as those of **2c** (Table 4).

As expected **8b** has two ESR active protons more than **2c**. Similarly **7b** has one proton less than **8b** with a coupling constant of about nine gauss. The hyperfine interaction of the Me group on N_1 is not detected in the spectra of **7b** and **8b** because the linewidth is too large.

Prolonged oxidation of **7a** with t-butyl-hydroperoxide results in the disappearance of the spectrum of **7b** followed by the appearance of the spectrum of the neutral monohydro radical **7c** (Fig. 2). Coupling constants are found for N_5, N_5(1'-H), N_8 and one proton which is probably C_7(H) (Table 4).

When **2a** and **8a** are subjected to prolonged oxidation with t-butyl-hydroperoxide in chloroform the radicals **2c** and **8b** are superseded by the radicals **2f** (Fig. 2) and **8c** respectively.

The total width between the outer lines of the spectra of **2f** and **8c** is smaller than expected for 5-alkylated monohydrolumazine radicals. Furthermore the intensity

Table 4. Coupling constants in gauss of neutral trihydro, monohydro and 5-acyl-lumazine radicals in chloroform. Alternative assignments are placed in parentheses

	N_5	N_8	N_5 (1'-H)	N_5 (1'-D)	N_8 (1'-H)	C_6(H)	C_6(D)	C_7(H)	C_7(D)	N_1 (1'-H)	Line-width
2c	8.76	2.78	8.72			6.80		5.94		0.84	0.6
2e	8.76	2.78		1.34			1.05		0.91	0.84	0.6
2f	7.4	4.0	7.7								2.5
2g	7.4	4.0		1.19							2.5
7b	8.6	3.1	8.4 (5.7)			5.7 (8.4)		4.6			2.1
7c	8.3	3.8	5.0					3.4			3.1
7d	8.2	4.5						3.2			3.6
8b	8.8	3.3	9.1			8.3		5.4			2.2
5e	8.4	5.0	8.6		5.2			5.4			1.9
6e	8.5	4.2	5.4		4.2			5.0			2.0
9c	8.4	4.9	8.2		5.4						0.9

of the outer lines as compared with the total intensity of the spectrum proves that apart from two nitrogens only one hydrogen is ESR-active in **2f** (Table 4).

Prolonged oxidation of **2d** (Table 2) produces a second radical **2g** in which the single ESR-active proton of **2f** has been replaced by deuterium (Table 4).

When 1 mmol of **2a**, **7a** or **8a** is oxidized in chloroform with 0.5 mmol of dibenzoylperoxide the same radicals **2f**, **7c** and **8c** are formed as with t-butylhydroperoxide.

Oxidation of 1 mmol of **7a** with 1.5 mmol of dibenzoylperoxide leads to the radical **7d** (Fig. 2).

Two nitrogen coupling constants and one hydrogen coupling constant are required for the simulation of the spectrum of **7d**. The nitrogen coupling constants are of the same magnitude as those of **2f** but the hydrogen coupling constant is much smaller (Table 4).

We propose that the radicals **2f**, **7d** and **8c** have acyl groups on N_5 (Fig. 2) formed by oxidation of the N_5-alkyl groups of the tetrahydrolumazines.

The hydrogen coupling constants in the spectra of **2f** and **7d** arise from $N_5(1'\text{-H})$ and $C_7(H)$ respectively, so a large difference between their values is to be expected.

Radical **2g** has a deuteroformyl group on N_5 because it is formed by oxidation of **2d** which has a trideuteromethyl group on N_5.

The spectrum of **8c** is not suitable for interpretation owing to the large linewidth but it total width is in agreement with the presence of a formyl group on N_5. The total width is about 7 gauss larger than that of the spectrum of **7d** since **8c** has a $N_5(1'\text{-H})$ proton more.

Similarly the spectrum of **8c** is about 3 gauss wider than that of **2f** because it has an extra proton coupling constant from $C_7(H)$.

Oxidation of 5,8 - dialkyl - tetrahydrolumazines in chloroform. Oxidation of **5a**, **6a** and 5,8 - dimethyl - 6,7 - diphenyl - tetrahydrolumazine **9a** in chloroform with t-butyl hydroperoxide proceeds analogously with the oxidation of **5a** and **6a** in formic acid. Two consecutive radical species are observed.

At first the corresponding neutral trihydro radicals **5d**, **6d** and **9b** are present in the reaction mixture. They are followed by the neutral monohydro radicals **5e**, **6e** and **9c** (Fig. 2). The spectra of **5d**, **6d** and **9b** are not suitable for interpretation. In **9c** N_5, N_8 and their methyl groups are ESR active. The hyperfine interactions in the spectra of **5e** and **6e** arise from the same nuclei plus the hydrogen atom on C_7 (Table 4). These findings confirm that **5e**, **6e** and **9c** are monohydrolumazine radicals.

Conversion of neutral trihydro- and monohydro radicals to cationic tetrahydro- and dihydro radicals by protonation. The neutral lumazine radicals can be converted to the corresponding cationic radicals by anaerobic addition of 0.1 ml of TFA to a solution of the neutral radical in 1 ml of chloroform.

Thus **5d**, **5e**, **6d**, **6e** and **2c** gave **5b**, **5c**, **6b**, **6c** and **2b** respectively. Upon addition of TFA radicals **7b**, **8b** and **9b** gave the same spectra as obtained by oxidation of **7a**, **8a** and **9a** with hydrogen peroxide in formic acid.

CONCLUSIONS

Well resolved ESR spectra of cationic 5 - alkyl - tetrahydrolumazine radicals were obtained by oxidation of the corresponding tetrahydrolumazines in formic acid. Artificial line-broadening of the spectrum of the 5 - methyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydrolumazine cation radical gave a spectrum identical with that of the 5-methyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydropterin cation radical. Therefore the hyperfine coupling constants of the pterin radical are essentially the same as those of the lumazine radical. The spectrum of the lumazine radical was interpreted and the coupling constants were assigned to specific nuclei of the radical molecule by means of specific deuteration.

Oxidation of 3,5,8 - trialkyl - tetrahydrolumazines in formic acid led to the successive appearance of two radical species, the first of which was a cationic tetrahydrolumazine radical. The number of ESR-active nuclei of the second radical and the size of the coupling constants indicate that it has the cationic 5,8-dihydrolumazine structure.

Upon oxidation of 5,8 - dialkyl - tetrahydrolumazines and 5 - butyl - 1,3 - dimethyltetrahydrolumazine with t-butyl-hydroperoxide in chloroform two consecutive radicals were observed. They were identified as neutral trihydro- and neutral monohydrolumazine radicals respectively.

Oxidation of 1,3,5 - trimethyl - tetrahydrolumazines gave the neutral trihydro radicals but the neutral monohydro radicals could not be detected. Instead of the monohydro radical a different paramagnetic species having two ESR-active protons less was formed. A structure with a formyl group on N_5 was proposed.

A structure with an acyl group on N_5 was also proposed for the radical generated from 5 - butyl - 1,3 - dimethyl - tetrahydrolumazine by oxidation with a 1.5 fold molar excess of dibenzoylperoxide.

Relatively large quantities of the corresponding 5 - formyllumazine radical are formed upon autoxidation of 1,3,5 - trimethyl - 6,7 - diphenyl - 5,6,7,8 - tetrahydrolumazine **2a** in chloroform. The formation of 1,3 - dimethyl - 6,7 - diphenyl - lumazine in autoxidation reactions of **2a**, which was proposed[8] to arise from oxidation of 1,3 - dimethyl - 6,7 - diphenyl - 7,8 - dihydrolumazine, can also be explained by loss of a formyl radical from the 5-formyllumazine radical. The formyl radical is too unstable for detection in solution at room temperature. It can, however, be detected with the aid of the spin trapping technique. Experiments based on this technique are in progress.

The occurrence of neutral and cationic lumazine radicals as intermediates in the oxidation of 5 - alkyl - tetrahydrolumazines with peroxides has been clearly demonstrated by this study. The chemical structures of the radicals were deduced from the number of ESR-active protons and nitrogen atoms and the size of their coupling constants.

EXPERIMENTAL

ESR-measurements. After 1 ml of HCOOH or $CHCl_3$ was flushed with argon for 15 min, 0.1 mmol of the tetrahydrolumazine and the required amount of peroxide were added anaerobically. In the case of HCOOH solns, 0.05 mmol of a 30% aqueous H_2O_2 soln was used as the oxidant. In $CHCl_3$ all radicals except **7d** were prepared by oxidation with 0.05 mmol of t-BuOOH. The radicals **2f**, **7c** and **8c** could also be obtained by oxidation with 0.05 mmol of BzOOBz. The generation of **7d** required 0.15 mmol of BzOOBz. The resulting soln was transferred to the sample cell and its initial ESR spectrum was measured at room temp. (20–22°) within 10 min after mixing the reactants. For the detection of secondary radical species consecutive eight minute scans were carried out until the spectrum of the second radical reached its maximum intensity.

A standard quartz cell for aqueous samples was used for the measurements in formic acid. Measurements with $CHCl_3$ solns were carried out in a glass tube of 4 mm i.d. and 5 mm o.d. No

ESR spectrum originating from the sample cell was observed under the experimental conditions.

The first derivative ESR spectra were recorded with a Varian model E9 X-band spectrometer.

The spectra were recorded on strip-chart and converted into the digital form manually.

Calibration of the field scan range of the ESR spectrometer was carried out using a soln of Fremy's salt in water saturated with Na_2CO_3.

Interpretation of the ESR spectra. The digital spectra were first analysed by the method of Newton *et al.*[17] This method provides a series of potential hydrogen coupling constants and a series of potential nitrogen coupling constants. These series consist of both physically relevant and unrelevant coupling constants. In order to determine which coupling constants are physically relevant a pattern search was carried out for the best least-squares fit between simulated and experimental spectrum. The data needed for the pattern search were:

(1) The numbers of N and H atoms involved in the experimental spectrum. They were determined by double integration of the first derivative ESR spectrum. The number of hydrogens H and the number of nitrogens N were calculated from the equation: $S/L = 2^H \times 3^N$, in which S is the double integral of the entire spectrum and L is the double integral of one of the two outer lines. The numbers of H and N atoms so obtained must of course be compatible with the chemical structure of the 5,6,7,8 - tetrahydropteridine from which the radical was produced by oxidation.

(2) The values that the coupling constant of any H or N atom may take during the pattern search. The two series of potential coupling constants obtained with the method of Newton *et al.* were used for this purpose.

(3) The linewidth and lineshape to be employed in the spectrum simulations. The linewidth was estimated from the experimental spectrum. Gaussian lineshapes were employed because no satisfactory simulations could be obtained using Lorentzian lineshapes.

During the pattern search all coupling constants were varied independently of each other.

Sets of coupling constants that were not compatible with the total width of the experimental spectrum were rejected.

Spectrum simulations were made with the remaining sets of coupling constants and the least-squares difference between each simulation and the experimental spectrum was computed. Finally the best simulation was represented graphically and compared visually with the experimental spectrum.

The coupling constants and linewidth of the radicals containing deuterium were not determined in the way described here. Instead the hydrogen and nitrogen coupling constants and the linewidth were assumed to be the same as in their nondeuterated analogues. The deuterium coupling constants were calculated using the relationship: $A_D = 0.154 \times A_H$. The theoretical spectrum reconstructions made with these data are in good agreement with the experimental spectra of the deuterated radicals.

The spectra of **8c**, **5d**, **6d** and **9b** show very little hyperfine structure. Interpretation was not attempted because there are too many spurious fits for these spectra. They cannot be interpreted safely unless various selectively deuterated or ^{15}N-substituted analogues are available.

Preparation of 1,3,5 - *trimethyl* - 6,7 - *diphenyl* - 5,6,7,8 - *tetrahydrolumazine* (**2a**). 1,3 - dimethyl - 6,7 - diphenyllumazine[18] (5.0 g, 14.5 mmol) was hydrogenated over Pt(1.2 g PtO_2) in a mixture of 96% EtOH (135 ml), 35% aq. CH_2O (15 ml) and conc. HCl (10 ml) at room temp. and atmospheric pressure for 3 days. The catalyst was filtered off and washed with 96% EtOH (50 ml). The combined filtrates were evaporated to dryness *in vacuo*. The residue was suspended in H_2O (100 ml) and filtered off. Conc. NH_4OH was added to the filtrate to adjust the pH to 7.1. The resulting ppt. was filtered off and washed 6 times with 15 ml of AcOEt. It was purified by continuous extraction with boiling AcOEt (50 ml); colourless crystals separated from the extract, yield 1.87 g (35%).

The analytical data of compound **2a** were published previously.[8]

Preparation of 3,5,8 - *trimethyl* - 5,6,7,8 - *tetrahydrolumazine* (**5a**). A homogenised mixture of 1 - methyl - 4,5 - dichloro - uracil[19] (1.96 g, 10 mmol) and N,N' - dimethyl - ethylenediamine (2.644 g, 30 mmol) was heated rapidly to the boiling point, refluxed for 30 sec and cooled to room temp. The mixture was dissolved in H_2O (50 ml) and neutralised by dropwise addition of conc HCl. The soln was stirred with 0.3 g of activated charcoal for 5 min, filtered and extracted four times with 50 ml of $CHCl_3$. The aqueous layer was discarded. The $CHCl_3$ extracts were dried by stirring with anhyd $MgSO_4$ for 15 min and evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml MeOH and allowed to crystallize at −18°. The crystals were filtered off, washed with peroxide-free ether and dried *in vacuo* over P_2O_5, yield 0.63 g (30%), m.p. 217–219°. $C_9H_{14}N_4O_2$ (210.2) (Calcd: C, 51.42; H, 6.71; N, 26.65. Found: C, 51.71; H, 7.10; N, 26.44%). Mass spectrum *m/e* (%): 210 (M +, 100); 195(53); 181(6); 152(8); 149(7); 139(6); 124(13); 110(19). PMR($CDCl_3$): $\delta = 2.61$ (3, S, N-Me); 3.20 (3, S, N-Me); 3.29 (3, S, N-Me); 2.90–3.50 (4, M, C-H).

Compounds **6a**, **7a** and **8a** were prepared by methods described in the literature.[20]

Compounds **1a**,[21] **3a**,[21] **4a** and **9a** were gifts from Ir. J. A. Jongejan of this laboratory,

The deuterated compounds **2d** and **3d** were prepared in the same way as **2a** and **3a** using D_2, EtOD, CD_2O/D_2O and DCl/D_2O for the reductive methylation.

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