Tetrahedron Vol 31 pp 437 to 440 Pergamon Press 1975 Printed in Great Britain

ESR OF CATIONIC ISOALLOXAZINE FREE RADICALS

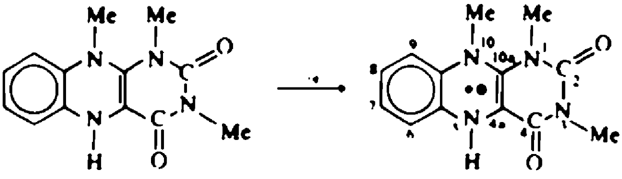
J. Westerling,* H. I. X. Mager and W. Berends
Biochemical and Biophysical Laboratory of the University of Technology, 67 Julianalaan, Delft, The Netherlands

(Received in UK 18 July 1974. Accepted for publication 3 October 1974)

Abstract—The ESR spectra in solution of a number of cationic isoalloxazine free radicals were recorded and interpreted in terms of hyperfine coupling constants. Two previously published interpretations are shown to be incorrect.

INTRODUCTION

The hydroxylation of aromatic compounds by model systems consisting of 1,3,10-trimethyl-5,10-dihydroalloxazine 1 and oxygen or hydrogenperoxide has been studied extensively.[1,2] The cationic radical 2 formed by one-electron oxydation of 1 is an essential intermediate in the hydroxylation reactions. The object of this study is to obtain more information concerning the electronic structure and reactivity of 2.



1

2

Two attempts to interpret the ESR spectra of cationic isoalloxazine radicals have been published.[3,4] Ehrenberg[5] showed that the cationic radicals of lumiflavine and lumiflavine-1,3-$^{15}N_2$ have identical ESR spectra. This means that Tollin's interpretation,[3] involving a high coupling constant for N1, cannot be correct.

Mueller *et al.*[4] assigned the following coupling constants to a number of 3-alkylated cationic isoalloxazine radicals, including 2: $A_{N5} = 8{\cdot}5$, $A_{H5} = 11{\cdot}5$, $A_{H8} = 3{\cdot}4$, $A_{N10} = 4{\cdot}3$, $A_{H10} = 4{\cdot}7$ (gauss).

We tested these values with a computer program for the simulation of ESR spectra similar to the one of Stone and Maki.[6] The simulated spectra obtained bear no resemblance to the experimental spectra. Simulation of the spectrum of 2 using Gaussian lineshapes and linewidths of 1·0 and 1·5 gauss respectively results in spectra consisting of 14 lines, whereas the experimental spectrum has 22 lines. Simulation of the spectrum of the 3-alkylated lumiflavine cation radical[11] was equally unsuccessful. We therefore regard this interpretation as erroneous.

A number of methods for the interpretation of unresolved ESR spectra in solution have been published:

1. Spectrum simulation.[6]
2. Least-squares fitting procedures.[7,8]
3. Comparing total widths of spectra of radicals with different isotopic substitution.
4. Spectrum reduction by the method of Newton *et al.*[9]
5. Fourier analysis.[10]

Only the first and third of these have been applied to alloxazines and isoalloxazines.

In our experience comparison of total widths (method 3) is highly inaccurate when the spectra have unresolved outer lines as in the case of alloxazines and isoalloxazines.

Apparently the spectra of the cationic isoalloxazine radicals cannot be interpreted by these two methods alone. We found that the problem can be solved by using a combination of methods 1, 2 and 4.

RESULTS AND DISCUSSION

The cationic radicals of the following compounds were prepared in 6N HCl solution by half-reduction with sodium dithionite: 1,3,10-trimethylalloxaziniumperchlorate (2)*, 1,3-dimethyl-10-methyl-d_3-alloxaziniumperchlorate (3), 1,3,10-trimethyl-d_9-alloxaziniumperchlorate (4), 10-methyl-isoalloxazine (5) and lumiflavine (6).

Each radical was also prepared in 6N DCl, thus leading to the analogs deuterated in position 5 (2-5-d, 3-5-d and 4-5-d) or positions 1, 3 and 5 (5-1,3,5-d_3 and 6-1,3,5-d_3).

The spectra of 3 and 4 and also those of 3-5-d and 4-5-d are identical as expected in case of a low spin density on positions 1 and 3.

The spectra of 2, 5 and 6 were analysed by the method of Newton *et al.*[9] In this way coupling constants with an accuracy of about 0·2 gauss were obtained for each radical. The coupling constants were refined by means of a least-squares fitting procedure similar to the one of Plato.[7]

The coupling constants of the deuterated radicals 3, 4, 2-5-d, 3-5-d, 4-5-d, 5-1,3,5-d_3 and 6-1,3,5-d_3 were calculated from those of the corresponding nondeuterated radicals using the relationship:

$$A_D = 0{\cdot}154 * A_H$$

---

* Numbers between parenthesis refer to the radicals obtained

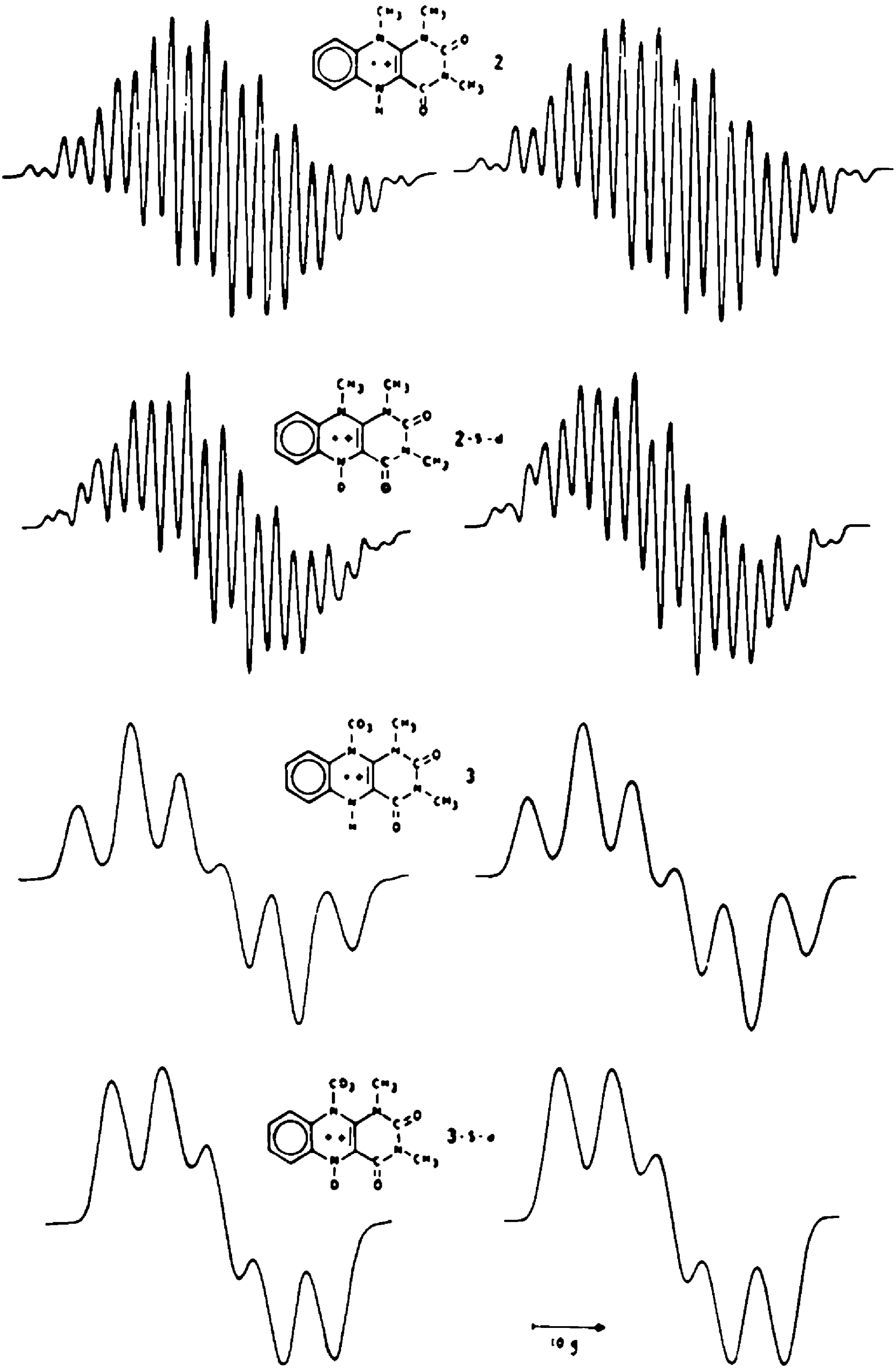


Fig 1(a)

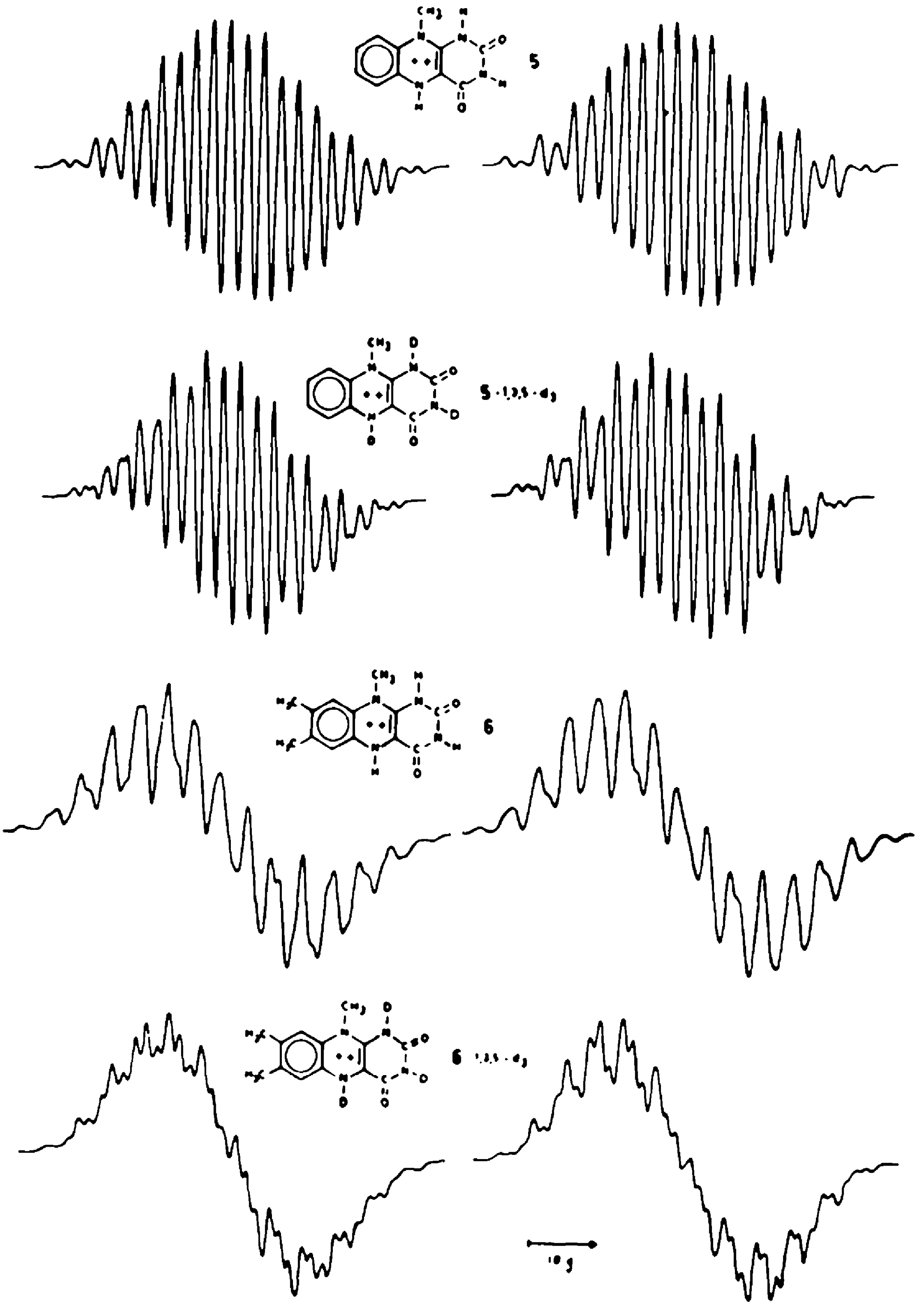


Fig 1(b)

Fig 1 ESR spectra of cationic isoalloxazine radicals. (a) Experimental spectra (b) Simulated spectra

Table 1

Radical	N_5	N_{10}	H_5	H_{10}	H_{Ar}	$H_{Ar'}$	D_5	D_{10}
	Coupling constants in gauss							
2	7·15	5·20	7·55	4·61	2·66	1·60	—	—
2-5-d	7·15	5·20	—	4·61	2·66	1·60	1·16	—
3 and **4**	7·15	5·20	7·55	—	2·66	1·60	—	0·71
3-5-d and **4**-5-d	7·15	5·20	—	—	2·66	1·60	1·16	0·71
5	7·39	4·85	7·75	4·92	2·91	1·67	—	—
5-1,3,5-d_3	7·39	4·85	—	4·92	2·91	1·67	1·19	—
6	7·27	4·66	7·68	4·90	3·40	1·62	—	—
6-1,3,5-d_3	7·27	4·66	—	4·90	3·40	1·62	1·18	—

Finally all spectra were simulated using the refined coupling constants (Table 1).

Good agreement between simulated and experimental spectra is observed (Fig. 1). The minor deviations are largely due to the fact that simulated spectra are symmetrical whereas the experimental spectra are slightly unsymmetrical.

When agreement between simulated and experimental spectra is observed the possibility of a spurious fit still remains.

The chance of finding a spurious fit is minimised by fitting the spectra of radicals with different isotopic substitution. The correctness of the coupling constants of those nuclei that were replaced by their isotopes is proved unequivocally by this method.

Therefore the coupling constants of H_5 and H_{10} in radical **2** and H_5 in the radicals **5** and **6** are established beyond doubt by our results.

This study also illustrates the usefulness of the spectrum-reduction method,[9] which does not seem to have received much attention in the literature.

EXPERIMENTAL

1,3,10-Trimethylalloxaziniumperchlorate was prepared as described previously.[1]

1,3-Dimethyl-10-methyl-d_3-alloxaziniumperchlorate and 1,3,10-trimethyl-d_9-alloxaziniumperchlorate were prepared by the same method using dimethylsulfate-d_6 instead of dimethylsulfate at the proper stages of the synthesis.

10-Methyl-isoalloxazine and lumiflavine were prepared by Ir. R. Addink of this laboratory.

The first-derivative ESR spectra were recorded with a Varian X-band spectrometer model E4. A standard flat aqueous sample cell made of quartz was used. The spectra were recorded on strip-chart and converted into the digital form manually.

Gaussian lineshapes were employed in the least-squares fitting procedure and in the spectrum simulations.

The faultless operation of the computer program for the simulation of spectra was checked by duplicating spectrum simulations of pteridine radicals[11] and neutral isoalloxazine radicals.[12]

The solns of the radicals were prepared as follows.

A soln of 0·02 mmole of the isoalloxazine in 1 ml of 6N HCl or 6N DCl was deaerated by bubbling through argon. Solid sodium dithionite (0·01 mmole) was added and the soln was transferred anaerobically to the sample cell.

Calibration of the field scale of the ESR spectrometer was carried out using a solution of Fremy's salt in water saturated with sodium carbonate.

Acknowledgements—We are grateful to Professor Th. J. de Boer, Dr. P. van der Put and Dr. A. H. Huizer for valuable advice.

REFERENCES

[1] H. I. X. Mager and W. Berends, *Rec. Trav. Chim.* **91**, 611 (1972).
[2] H. I. X. Mager and W. Berends, *Tetrahedron* **30**, 917 (1974).
[3] A. V. Guzzo and G. Tollin, *Arch. Biochem. Biophys.* **103**, 231 (1963).
[4] F. Mueller, P. Hemmerich and A. Ehrenberg, *Flavins and Flavoproteins* (Edited by H. Kamin) pp. 107–122. University Park Press, Butterworths, Baltimore and London (1971).
[5] A. Ehrenberg and L. E. Göran Eriksson, *Arch. Biochem. Biophys.* **105**, 453 (1964).
[6] F. W. Stone and A. H. Maki, *J. Chem. Phys.* **38**, 1999 (1963).
[7] M. Plato, *Z. Naturforschung* **22A**, 119 (1967).
[8] A. Bauder and R. J. Myers, *J. Mol. Spectrosc.* **27**, 110 (1968).
[9] R. Newton, K. F. Schulz and R. M. Elofson, *Can. J. Chem.* **44**, 752 (1966).
[10] R. H. Silsbee, *J. Chem. Phys.* **45**, 1710 (1966).
[11] A. Ehrenberg, P. Hemmerich, F. Mueller and W. Pfleiderer, *Eur. J. Biochem.* **16**, 584 (1970).
[12] F. Mueller, P. Hemmerich, A. Ehrenberg, G. Palmer and V. Massey, *Ibid.* **14**, 185 (1970).
[13] H. Walker and A. Ehrenberg, *FEBS Letters* **3**, 315 (1969).