Communications to the Editor

^{14}N and ^{1}H ENDOR and TRIPLE Resonance Experiments of Flavin and Thiaflavin Radical Cations in Liquid Solution

M. Bock, W. Lubitz, and H. Kurreck*

Institut für Organische Chemie der Freien Universität Berlin 1000 Berlin 33, West Germany

H. Fenner and R. Grauert

Institut für Pharmazie der Freien Universität Berlin 1000 Berlin 33, West Germany

Received March 2, 1981

Since Michaelis and co-workers[1,2] demonstrated that paramagnetic species are involved in flavoenzyme catalysis, flavin radicals have been the subject of intense spectroscopic studies.[3-8] ESR experiments yielded valuable information regarding the structure and properties of these radicals. However, these studies were limited by the low resolution of standard ESR techniques, and even the solid-state ENDOR experiments essentially yielded only hyperfine data for the methyl groups.[9-12] It was therefore a challenge to perform *high-resolution* ENDOR (electron nuclear double resonance) spectroscopy with its significantly increased spectral resolution on flavin radicals in *liquid solution*. The aim of these experiments was to evaluate the isotropic hyperfine coupling constants of all magnetic nuclei contributing to the ESR pattern. This approach should, in principle, give a more detailed insight into the electronic structures and radical geometries.

In this paper we report first successful ENDOR-in-solution experiments on flavin cation radicals **1** and **2** from riboflavin and lumiflavin, respectively. The data of the model compound lumiflavin radical cation **2** are compared with its sulfur analogue, 5-thia-5-deaza-3-methyllumiflavin radical cation **3**.[13] Substitution of N-5 by sulfur yields flavin derivatives which serve as models for studying $1e^-$-transfer reactions in flavin catalysis.[14]

The diamagnetic precursors of **2** and **3** were synthesized according to the procedure described elsewhere;[15,16] riboflavin is available from commercial sources (Merck, Darmstadt). Radical cations were studied in toluene/trifluoroacetic acid (ca. 5% by volume). Compound **3** was generated by dibenzoyl peroxide oxidation.[17] Compounds **1** and **2** were obtained from the corresponding oxidized flavins by reduction with sodium dithionite. Sample preparation was performed under high vacuum conditions. ESR, ENDOR, and TRIPLE spectra were recorded on a Bruker ER 220D ESR spectrometer equipped with a Bruker ER 200 ENB ENDOR cavity and home-built NMR facilities described elsewhere.[18]

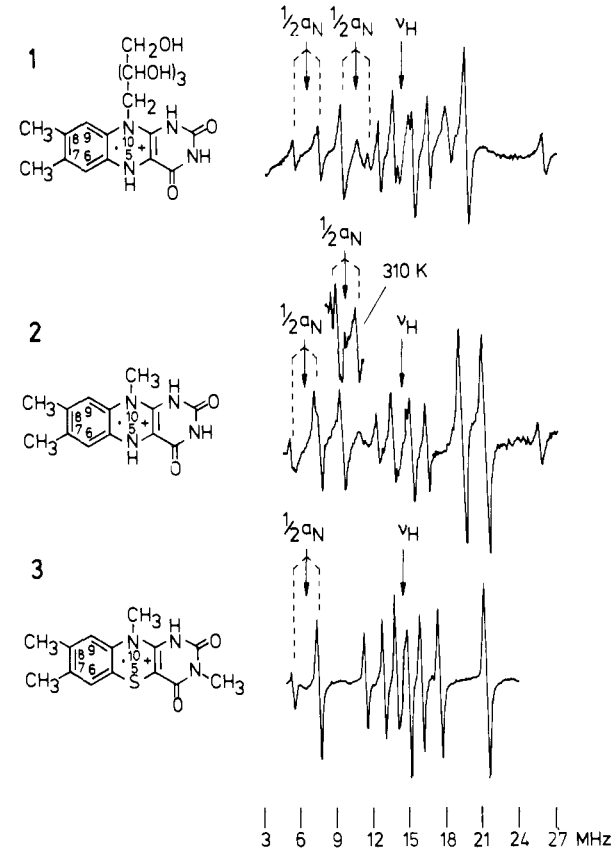

Figure 1. ENDOR spectra of the radical cations **1–3** in toluene/CF_3COOH at 265 ± 3 K (insert in **2**, 310 K). Radical concentration was roughly 10^{-3}–10^{-4} M; radio frequency power 100 W (14 MHz); microwave power 200 (**1**, **2**) and 60 mW (**3**). FM of the NMR field (10 kHz): amplitude 70 kHz; 100 scans; scan time 30 s; time constant 40 ms. The low frequency ENDOR line of the largest ^{1}H hyperfine coupling of **1** and **2** could only be detected under modified experimental conditions. The smallest coupling of **3** could be extracted from a general TRIPLE experiment.[22]

According to the ENDOR resonance condition $\nu_{ENDOR} = |\nu_{free\ nucleus} \pm a_n/2|$ ENDOR line pairs are centered either around the free nuclear frequency or around half the hyperfine coupling constant a_n. Usually $a_H/2 < \nu_H$ (ν_H = 14.7 MHz in our experiments) and the former condition holds for protons, whereas for nitrogen $a_N/2 > \nu_N$ is valid, and the nitrogen lines are centered around $a_N/2$, spaced by $2\nu_N$.[19]

(1) Michaelis, L.; Schubert, M. P.; Smythe, C. V. *J. Biol. Chem.* **1936**, *116*, 587.
(2) Michaelis, L.; Schwarzenbach, G. *J. Biol. Chem.* **1938**, *123*, 527.
(3) Beinert, H. *J. Am. Chem. Soc.* **1956**, *78*, 5323.
(4) Müller, F.; Brüstlein, M.; Hemmerich, P.; Massey, V.; Walker, W. H. *Eur. J. Biochem.* **1972**, *25*, 573.
(5) Eriksson, L. E. G.; Ehrenberg, A. *Acta Chem. Scand.* **1964**, *18*, 1437.
(6) Ehrenberg, A.; Müller, F.; Hemmerich, P. *Eur. J. Biochem.* **1967**, *2*, 286.
(7) Walker, W. H.; Ehrenberg, A. *FEBS Lett.* **1969**, *3*, 315.
(8) Müller, F.; Hemmerich, P.; Ehrenberg, A.; Palmer, G.; Massey, V. *Eur. J. Biochem.* **1970**, *14*, 185.
(9) Ehrenberg, A.; Eriksson, L. E. G.; Hyde, J. S. *Biochim. Biophys. Acta* **1968**, *167*, 482.
(10) Eriksson, L. E. G.; Hyde, J. S.; Ehrenberg, A. *Biochim. Biophys. Acta* **1969**, *192*, 211.
(11) Eriksson, L. E. G.; Walker, W. H. *Acta Chem. Scand.* **1970**, *24*, 3779.
(12) Walker, W. H.; Salach, J.; Gutman, M.; Singer, T. P.; Hyde, J. S.; Ehrenberg, A. *FEBS Lett.* **1969**, *5*, 237. Eriksson, L. E. G.; Ehrenberg, A.; Hyde, J. S. *Eur. J. Biochem.* **1970**, *17*, 539. Salach, J.; Walker, W. H.; Singer, T. P.; Ehrenberg, A.; Hemmerich, P.; Ghisla, S.; Hartmann, U. *Ibid.* **1972**, *26*, 267. Eriksson, L. E. G.; Ehrenberg, A. *Biochim. Biophys. Acta* **1973**, *293*, 57. Fritz, J.; Müller, F.; Mayhew, S. G. *Helv. Chim. Acta* **1973**, *56*, 2250.
(13) Fenner, H.; Grauert, R.; Hemmerich, P. *Liebigs Ann. Chem.* **1978**, 193.
(14) Fenner, H.; Grauert, R.; Hemmerich, P.; Michel, H.; Massey, V. *Eur. J. Biochem.* **1979**, *95*, 183.
(15) Fenner, H.; Tessendorf, L.; Grauert, R. *Arch. Pharm. (Weinheim, Ger.)* in press.
(16) Tishler, M.; Pfister, K.; Bobson, R. D.; Ladenburg, K.; Fleming, A. J. *J. Am. Chem. Soc.* **1947**, *69*, 1487. Hemmerich, P.; Fallab, S.; Erlenmeyer, H. *Helv. Chim. Acta* **1956**, *39*, 1242.
(17) Grauert, R. Thesis, Freie Universität Berlin, West Germany, 1976.
(18) Fey, H. J.; Kurreck, H.; Lubitz, W. *Tetrahedron* **1979**, *35*, 905.
(19) The optimum ENDOR conditions for the detection of different nuclei in a radical are now well understood: Kirste, B.; Kurreck, H.; Lubitz, W.; Zimmermann, H. *J. Am. Chem. Soc.* **1980**, *102*, 817. Plato, M.; Lubitz, W.; Möbius, K. *J. Phys. Chem.* **1981**, *85*, 1202.

0002-7863/81/1503-5567$01.25/0

Table I. Isotropic Hyperfine Coupling Constants (±0.01 MHz) from ENDOR Spectra of Flavin Radical Cations in Toluene/CF_3COOH at 265 K[a]

	position							
radical	H-6α	CH_3-7β	CH_3-8β	H-9α	H-5α	CH_2/CH_3-10β	N-5	N-10
1	−4.07	(−)0.80	+10.35	+1.60	−23.30	+7.33	(+)21.16	(+)13.00
2	−4.03	(−)0.86	+9.95 (9.53)	+1.53	−22.70 (32.24)	+13.83 (13.17)	+20.73 (23.83)	+13.19 (12.05)
3	+1.06	+3.18	+6.30	(+)0.32	-	+14.06	-	+13.08

[a] For numbering scheme refer to Figure 1. The signs of the smallest couplings are uncertain due to lack of resolution. Simulations of the ESR spectra using the ENDOR data gave satisfying agreements. Hyperfine data for **2** in brackets are taken from ref 21. For assignments see text and ref 5, 6, 14.

In Figure 1 the resolution achieved in our ENDOR experiments is demonstrated for flavin radical cations **1–3**. From the ENDOR spectrum of **3** six hyperfine couplings can be evaluated arising from five different sets of protons and from one ^{14}N nucleus, respectively. For **1** and **2** six proton and two nitrogen hyperfine coupling constants could be detected. An improved ENDOR response of the signals belonging to the larger ^{14}N hyperfine coupling occurred at higher temperatures (see, e.g., insert in Figure 1).[19] The relative signs of the hyperfine coupling constants were measured by electron nuclear nuclear TRIPLE resonance.[20] All isotropic couplings are collected in Table I. Only for **2** some hyperfine coupling constants are already known from ESR simulations.[21]

Since no further ^{14}N ENDOR lines could be detected, spin populations within the pyrimidine moiety of the flavin skeleton seem to be negligibly small. We therefore feel that our ENDOR results present the complete set of isotropic proton and nitrogen hyperfine coupling constants larger than 0.3 and 1 MHz, respectively.

One of the drawbacks of the ENDOR method is the fact that ENDOR line intensities normally do not reflect the number of nuclei belonging to a particular hyperfine coupling. Hence, unambiguous assignments of the couplings to specific molecular positions often call for isotopic labeling of the compound under study. We are currently synthesizing some partially deuterated derivatives of the flavin system. In the present studies some of the assignments could be established from the following facts: For **3** the discrimination between the methyl proton couplings of positions 7 and 8 is based on a comparison with similar radicals bearing only one methyl group in position 7 or 8 respectively.[22] The largest (negative) proton coupling of **1** and **2** has to be assigned to the proton attached to the nitrogen in the 5 position, because the respective ^1H ENDOR lines do not show up when using deuterated trifluoroacetic acid for the sample preparation. Comparison of **3** with **1** or **2** shows that the smaller nitrogen coupling can be ascribed to position 10. Thus in **1** and **2** the larger nitrogen coupling can be assigned to position 5. Since even the nitrogen in position 10 has relatively large spin population, the largest methyl proton coupling of 14 MHz can be assigned to the methyl group at this nitrogen. This assignment is supported by comparison of **2** with **1** because substitution of the methyl by the ribityl group results in a decrease of the respective hyperfine coupling of almost 50%. It should be mentioned that all the other couplings, including those of the nitrogen at position 10, remain essentially unaffected by this substitution. On the other hand, substitution of N by S in position 5 causes a considerable redistribution of the spin density within the benzene fragment (Table I).

As has recently been demonstrated, it is possible to study protein bound organic π radicals under physiological conditions by ENDOR spectroscopy in aqueous solution.[23] We therefore feel encouraged to extend our ENDOR experiments to the investigation of naturally occurring flavoenzymes in order to get a better understanding of structures, bondings, and functions of flavins in biological systems.

Acknowledgment. We thank F. Lendzian (Institut für Molekülphysik, Freie Universität Berlin) for his help concerning the ENDOR measurements. This work was supported by the Deutsche Forschungsgemeinschaft (Normalverfahren) and the Fonds der Chemischen Industrie which is gratefully acknowledged.

(20) Möbius, K.; Biehl, R. In "Multiple Electron Resonance Spectroscopy"; Dorio, M. M.; Freed, J. H., Eds.; Plenum Press: New York, 1979; p 475.
(21) Müller, F.; Hemmerich, P.; Ehrenberg, A. In "Flavins and Flavoproteins"; Kamin, M., Ed; University Park Press: Baltimore, 1971.
(22) Bock, M., unpublished results.
(23) Lendzian, F.; Lubitz, W.; Scheer, H.; Bubenzer, C.; Möbius, K. *J. Am. Chem. Soc.*, in press.

Stereoselective Carbonyl Olefination via Organosilicon Compounds

Yoshihiko Yamakado, Masaharu Ishiguro, Nobuo Ikeda, and Hisashi Yamamoto*

Department of Applied Chemistry, Nagoya University Chikusa, Nagoya 464, Japan

Received April 14, 1981

Of the many reactions available for carbonyl olefination, the silicon method, which is known as Peterson olefination,[1] has been shown in several cases to be superior to the conventional Wittig reaction due to the higher reactivity of α-silyl carbanions.[2] The significant limitation on the broad utility of these reagents often arises from the lack of stereoselectivity of the reactions.[1] In order to circumvent this problem, several indirect approaches were reported.[3] We report here an efficient silicon-mediated alkene synthesis which directly produces (*Z*)-alkenyl derivatives almost exclusively.

We have found that 1,3-bis(trimethylsilyl)propyne (**1**)[4] may be rapidly metalated at −78 °C in dry tetrahydrofuran (THF) with *tert*-butyllithium in essentially quantitative yield.[5] Our observation that the produced anion reacts with cyclohexanone to furnish the enyne **2** in 83% yield, accompanied by a very small amount (<3%) of the corresponding cumulene derivative,[6] es-

(1) For an excellent review, see: (a) Chan, T.-H. *Acc. Chem. Res.* **1977**, *10*, 442. (b) See also: Jarvie, A. W. P. *Organomet. Chem. Rev., Sect. A* **1970**, *6*, 153.
(2) Boeckman, R. K.; Silver, S. M. *Tetrahedron Lett.* **1973**, 3497. Shimoji, K.; Taguchi, H.; Oshima, K.; Yamamoto, H.; Nozaki, H. *J. Am. Chem. Soc.* **1974**, *96*, 1620. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2529.
(3) (a) Hudrlik, P. F.; Peterson, D. *Tetrahedron Lett.* **1974**, 1133. *J. Am. Chem. Soc.* **1975**, *97*, 1464. (b) Chan, T.-H.; Mychajlowskij, W.; Ong, B. S.; Harpp, D. N. *J. Organomet. Chem.* **1976**, *107*, C1.
(4) Prepared from 1-(trimethylsilyl)propyne (Corey, E. J.; Kirst, H. A. *Tetrahedron Lett.* **1968**, 5041) by the following procedure: Treatment of 1-(trimethylsilyl)propyne in ether at −5 °C with tetramethylethylenediamine and an equivalent amount of *n*-butyllithium under argon led to complete metalation. After 30 min, chlorotrimethylsilane (1 equiv) was added, and the mixture was stirred at room temperature for 12 h. After extractive workup followed by distillation [bp 75–76 °C (34 mmHg)], 1,3-bis(trimethylsilyl)-propyne was obtained as a colorless liquid in 70–75% yield; ^1H NMR ($CDCl_3$) δ 0.17 and 0.24 (2s, 9 H each), 1.61 (s, 2H). For another preparation of the compound, see: Jaffe, F. *J. Organomet. Chem.* **1970**, *23*, 53.
(5) Metalation may be also carried out with *n*-butyllithium and tetramethylethylenediamine (1:1) at −78 °C for 1 h.
(6) Cumulene derivatives, which have the higher R_f values on TLC assay, are the major byproducts from this reaction and may be readily removed by simple column chromatography. The preparation of cumulene under different conditions will be published in due course.

0002-7863/81/1503-5568$01.25/0