

Line Intensity Ratios in Electron Spin Resonance Spectra. A Remarkable Change Accompanying Saturation¹

G. Brunton,² D. Forrest,³ D. Griller, K. U. Ingold,* and A. H. Reddoch

Contribution from the Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, K1A 0R6, Canada. Received November 30, 1977

Abstract: In certain large organic radicals having a highly localized unpaired electron the intensities, even at moderate levels of microwave power, of the ¹³C satellite lines relative to the main line(s) may be very much greater than would be anticipated from the number of contributing ¹³C nuclei. This phenomenon, which can complicate attempts to identify organic radicals, can be qualitatively accounted for in terms of differential saturation behavior. The species producing the main line(s) has only weak relaxation mechanisms while the nuclear hyperfine interaction in the ¹³C containing radicals provides much stronger relaxation.

The relative intensities of the lines in the ESR spectrum of an organic radical are normally⁴ roughly proportional to the relative number of nuclei which produce each line after correcting for spin multiplicity. For example, the intensities of satellite lines due to a single ¹³C atom ($I = 1/2$, natural abundance 1.1%) are usually 0.55% of the intensities of the corresponding lines due to ¹²C. We have found that in certain radicals this simple rule breaks down even at quite moderate microwave power levels. This phenomenon can complicate attempts to identify organic radicals by the counting of nuclei

responsible for satellite lines. Our observations can be qualitatively understood in terms of the elementary theory of saturation but a quantitative treatment would be very complex and will not be attempted here.

Experimental Section

Radicals were generated photochemically directly in the cavities of a Varian E-4 EPR spectrometer and a modified Varian E-12, using previously described methods.⁶⁻⁹ Microwave power levels were read from the incident power meter.

Results and Discussion

The nature of the saturation effect can be seen in Figure 1 which shows the partially overmodulated ESR spectrum of the hindered aryl radical, **1**. The main line due to this radical is flanked by the two ¹³C_α satellites in natural abundance recorded at 100 times the gain used for the central line. The ¹³C_α/¹²C_α ratio of peak heights increases by a factor of 32 from 0.005 at a power level of 0.6 mW to a value of 0.16 at 100 mW.¹⁰ The central line broadens at high power, but the satu-

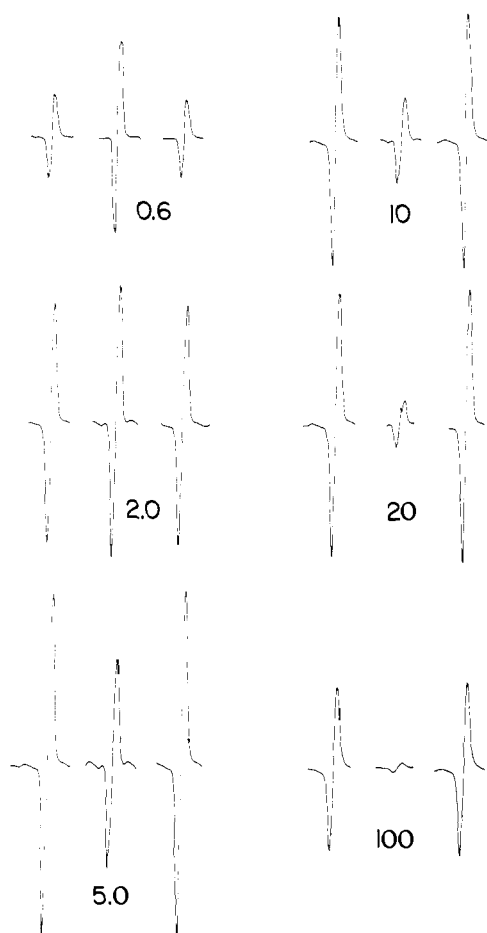


Figure 1. Partially overmodulated (modulation amplitude = 2.5 G) ESR spectrum of **1** in cyclopropane at $-95\text{ }^\circ\text{C}$ showing the main line and the two ¹³C_α satellites ($a^{13}\text{C}_\alpha = 113.8\text{ G}$)⁵ in natural abundance, the latter being recorded with 100 times the gain used for the central line. The numbers refer to the microwave power in mW as read from the incident power meter.

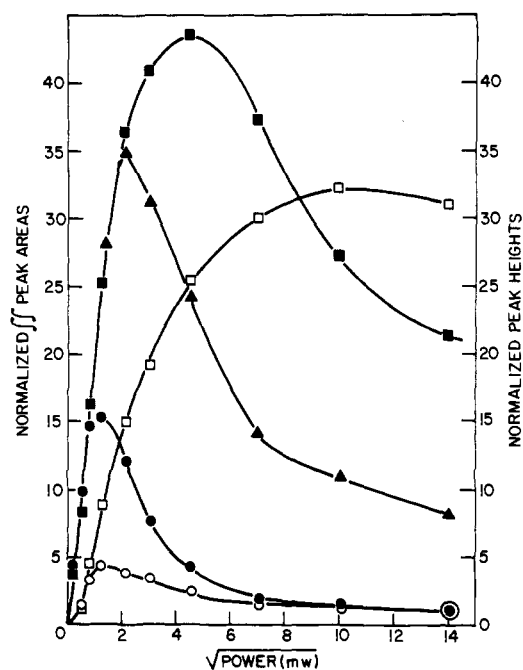


Figure 2. Normalized derivative peak heights and normalized doubly integrated peak areas for **1** in cyclopropane at $-95\text{ }^\circ\text{C}$ plotted against the square root of the microwave power (as read from meter). Modulation amplitude = 2.5 G. Peak heights: ¹²C central line, ●; ¹³C_α high-field satellite, ■; ¹³C_{ortho} high-field satellite, ▲. Doubly integrated peak areas: ¹²C central line, ○; ¹³C_α high field satellite, □.

Table I. Normalized Peak Height Ratios for Some Free Radicals^a

Power, mW	2 ^b		3 ^c			4H ^d and 4D ^d			(CD₃O)₂ĊH ^e		(CH₃)₂ĊH ^f	
	Ht	¹³ C _α	Ht	¹³ C _α	²⁹ Si _β	Ht	¹³ C _α (H)	¹³ C _α (D)	Ht	¹³ C _α	Ht	¹³ C _α
1	0.79	0.9	0.89	1.2	1.1				0.73	1.0		
2	1.0	1.1	1.0	1.4	1.2	0.88	1.0	1.0	0.95	1.0	0.73	1.0
5	0.96	1.8	0.81	2.1	1.4	1.0	1.0	1.1	1.0	1.0	0.93	0.9
10	0.79	2.7	0.52	3.2	1.6	1.0	1.0	1.0	0.83	1.3	1.0	0.9
20	0.54	4.5	0.29	4.8	1.8	0.82	1.3	1.1	0.65	1.3	0.63	1.4
50	0.21	13	0.17	5.7	1.9	0.53	1.2	1.4	0.39	1.3	0.37	0.9
100	0.11	22	0.09	6.9	2.1	0.33	1.6	1.7			0.21	1.3
200	0.07	30	0.06	6.6	2.1							

^a Power is the incident power meter reading in mW. Ht is the relative peak heights of the principal line(s) in the ESR spectrum at the different power levels. ¹³C_α is the ¹³C_α/Ht ratio of the peak heights normalized for the number of nuclei, nuclear spin, and isotopic abundance ratio. Ditto for ²⁹Si_β. ^b In cyclopropane at -95 °C, modulation amplitude = 4 G, a¹³C_α = 122.5 G. ^c In cyclopropane at 25 °C, modulation amplitude = 0.8 G, a¹³C_α = 30.73 G, a²⁹Si_β (2Si) = 20.42 G. ^d In benzene at 25 °C, modulation amplitude = 1.6 G, a¹³C_α = 99 G. ^e In cyclopropane at -110 °C, modulation amplitude = 1.6 G, a¹³C_α = 97.9 G. ^f Using (CH₃)₂¹³CHBr containing 50 atom % ¹³C in cyclopropane at -55 °C, modulation amplitude 0.5 G, a¹³C_α = 41.3 G. Similar data obtained in paraffin oil at 20 °C.

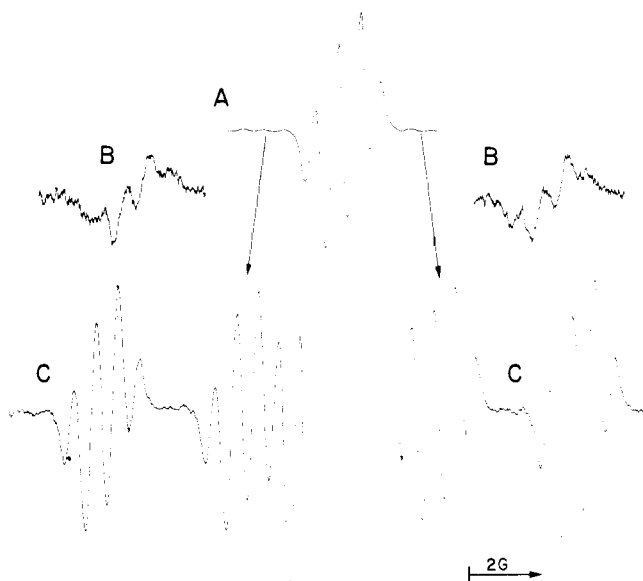
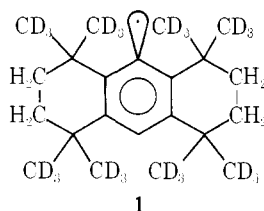


Figure 3. ESR spectrum of **1** in cyclopropane at -95 °C at a modulation amplitude = 0.125 G and with 0.2 mW power.¹⁹ A = main lines, a^H (3 H, 1 para and 2 other) = 0.60 G. B = ¹³C_α satellite lines, gain × 200. C = ¹³C_{ortho} and ¹³C_{meta} satellite lines, gain × 100.



ration effect is still quite evident when the double integrals of ¹³C_α and ¹²C_α lines are examined (see Figure 2).

The behavior of radical **1** is not unique. This can be seen from the normalized peak heights and peak height ratios which are summarized in Table I for the following radicals: 2,4,6-tri(1'-adamantyl)phenyl, **2**;⁷ [(CD₃)₃Si]₂NC[Si(CD₃)₃]₂, **3**;⁸ OCH₂OCH₂OĊH, **4H**;⁹ OCD₂OCD₂OĊD, **4D**; (CD₃O)₂ĊH; and (CH₃)₂ĊH. The intensity-ratio enhancement is most marked with large radicals where the electron spin is highly localized and isolated from nuclear interactions. This suggests that we are dealing with differential saturation where the species that produces the main line(s) in the spectrum has only weak relaxation mechanisms while the other ¹³C (and ²⁹Si) labeled species have much stronger relaxation as a result of the nuclear hyperfine interaction.

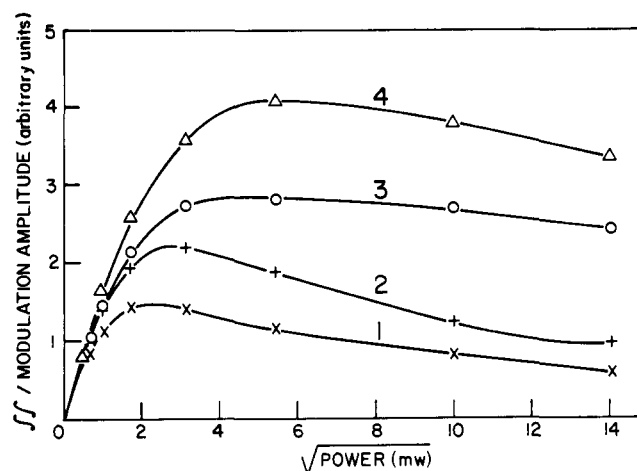


Figure 4. Ratio of (double integrated derivative peak area)/(modulation amplitude) for the main line in the ESR spectrum of **3** plotted against the square root of the microwave power (as read from meter) in cyclopropane at 25 °C. Curves 1 and 2, modulation frequency = 10 kHz; curves 3 and 4, modulation frequency = 100 kHz. Curves 1 and 3, modulation amplitude = 2.5 G, curves 2 and 4, modulation amplitude = 5.0 G. Each point is the average of two or three spectra. Typical standard deviation of mean is 1%.

From the well-known expression^{11,12} for the line shape of a partially saturated, homogeneously broadened line, it is simple to obtain expressions for the integrated intensity and for the height of the derivatives. For two species containing different isotopes the ratios of these expressions become

$$R_I = (1 + \gamma^2 H_1^2 T_1 T_2)^{1/2} / (1 + \gamma^2 H_1^2 T_1' T_2')^{1/2}$$

and

$$R_H = T_2'^2 (1 + \gamma^2 H_1^2 T_1 T_2)^{3/2} / T_2^2 (1 + \gamma^2 H_1^2 T_1' T_2')^{3/2}$$

In the limit of large microwave power these ratios become $R_I = (T_1 T_2 / T_1' T_2')^{1/2}$ and $R_H = (T_1 / T_1')^{3/2} (T_2' / T_2)^{1/2}$. If, as is so often the case, $T_1 = T_2$, both limits become T_1 / T_1' . Thus, the height and intensity ratios can become quite large in those radicals where the relaxation time can be markedly reduced by adding a nuclear hyperfine interaction to an, otherwise isolated, electron. If reasonable values for some of the parameters in the foregoing equations are adopted,¹³ the microwave power at the peak of the saturation curve for the derivative height of the ¹³C_α satellites of **1** can be estimated to be 30 mW. The agreement with experiment is satisfactory and supports the view that interaction with the ¹³C nuclei is credible as the dominant source of relaxation in the ¹³C_α satellites. The relaxation time for the main line in the spectrum must be ap-

precipably longer than for the ^{13}C satellite. This leads to better resolution of the main line(s) than of the ^{13}C satellite lines (see Figure 3). Theory suggests¹³ and our data support the view that differential saturation should be greatest in large radicals with a highly localized electron.¹⁶ The phenomenon should also be more pronounced in more viscous solvents,¹³ and this too was observed.

Although our analysis illustrates the general nature of differential saturation, it is not capable of providing a quantitative account of the measurements. These were, of necessity, made under conditions of partial saturation and to improve signal intensity the lines were generally overmodulated, the modulation frequency being 100 kHz. Moreover, in some cases there was partially resolved hyperfine structure, which means that the lines were not homogeneously broadened. There are no available treatments of line shape which include simultaneously the effect of saturation, modulation amplitude, modulation frequency, and inhomogeneous broadening.¹⁷ The effects of modulation amplitude (2.5 and 5.0 G) and frequency (10 and 100 kHz) on the saturation behavior of the main lines in the spectrum of **3** are illustrated in Figure 4.

Finally, we note that magnetic nuclei in quite remote positions can also produce differential saturation effects of sufficient magnitude to make radical identification difficult, e.g., $^{13}\text{C}_{\text{ortho}}$ and $^{13}\text{C}_{\text{meta}}$ in **1** (Figures 2 and 3) and the $^{29}\text{Si}\beta$ in **3** (see Table I).

References and Notes

(1) Issued as N. R. C. C. No. 16706.

- (2) N. R. C. C. Research Associate, 1974–1976.
- (3) N. R. C. C. Research Associate, 1975–1977.
- (4) That is, when dynamic line broadening effects⁶ are absent.
- (5) See, e.g., J. E. Wertz and J. R. Bolton, "Electron Spin Resonance", McGraw-Hill, New York, N.Y., 1972, Chapter 9.
- (6) G. Brunton, J. A. Gray, D. Griller, L. R. C. Barclay, and K. U. Ingold, *J. Am. Chem. Soc.*, in press.
- (7) G. Brunton, D. Griller, L. R. C. Barclay, and K. U. Ingold, *J. Am. Chem. Soc.*, **98**, 6803 (1976).
- (8) G. Brunton, J. F. Taylor, and K. U. Ingold, *J. Am. Chem. Soc.*, **98**, 4879 (1976).
- (9) G. Brunton, K. U. Ingold, B. P. Roberts, A. L. J. Beckwith, and P. J. Krusic, *J. Am. Chem. Soc.*, **99**, 3177 (1977).
- (10) The $^{13}\text{C}_\alpha/^{12}\text{C}_\alpha$ ratio of peak heights for undeuterated **1** also increased with increasing microwave power, but since this is a less persistent radical⁶ quantitative measurements could not be made.
- (11) A. M. Portis, *Phys. Rev.*, **91**, 1071 (1953).
- (12) E. R. Andrew, "Nuclear Magnetic Resonance", Cambridge University Press, New York, New York, N.Y., 1955, Equations 2.54 and 3.15. See also ref 3, Equation D-3.
- (13) The relaxation time is¹⁴ $1/T_1 = (3/10)\hbar^2 b^2 M_1^2 2\tau_c (1 + \omega_0\tau_c)^{-1}$, the rotational correlation time is¹⁴ $\tau_c = 4\pi\eta a^3/3kT$, and the microwave magnetic field is¹⁵ $H_1^2 = 2 \times 10^{-3} QP$. The anisotropic hyperfine interaction for ^{13}C ($M = 1/2$) is $b \approx 30$ G, the microwave frequency $\omega_0 = 2\pi \times 9 \times 10^9 \text{ s}^{-1}$, viscosity $\eta = 0.3 \text{ cP}$ at a temperature $T = 300 \text{ K}$, the cavity $Q = 2000$, and we take $T_1 = T_2$ and the radius $a = 5 \text{ \AA}$.
- (14) G. E. Pake and T. S. Estle, "The Physical Principles of Electron Paramagnetic Resonance", 2nd ed, W. A. Benjamin, New York, N.Y., 1962.
- (15) C. P. Poole, "Electron Spin Resonance", Interscience, New York, N.Y., 1967.
- (16) That is, in radicals having large values for a and b .¹³
- (17) The problem of the line shape of an inhomogeneously broadened line under saturation has been treated recently,¹⁸ but only for slow passage and small modulation amplitudes.
- (18) M. K. Bowman, H. Hase, and L. Kevan, *J. Magn. Reson.*, **22**, 23 (1976).
- (19) Under similar conditions but with power = 10 mW the ratio, to the ^{12}C lines, of the normalized maximum peak heights are $^{13}\text{C}_{\text{meta}} \sim 6$, $^{13}\text{C}_{\text{ortho}} \sim 6.7$, $^{13}\text{C}_\alpha = 10.6$, and the fine structure is still observed, while with power = 170 mW the ratios are $^{13}\text{C}_{\text{meta}}$, not measurable; $^{13}\text{C}_{\text{ortho}} \sim 4$; $^{13}\text{C}_\alpha \sim 15$, and all lines are now broad singlets.

Studies of the pH Dependence of ^{13}C Shifts and Carbon–Carbon Coupling Constants of [U- ^{13}C]Aspartic and -Glutamic Acids

Robert E. London,* Thomas E. Walker, Victor H. Kollman, and N. A. Matwiyoff

Contribution from the Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545. Received August 5, 1977

Abstract: ^{13}C NMR studies of the chemical shifts and carbon–carbon spin–spin coupling constants of 90% [U- ^{13}C]aspartic and -glutamic acids are reported. Effects of titration of the two carboxyl groups are separated computationally and the results compared with those for asparagine and glutamine, aspartate and glutamate containing peptides, and a series of amino-*n*-butyric acids. The results indicate that the carboxyl carbon shift resulting from titration of the carboxyl group is strongly dependent on its distance (number of bonds) from an amino group. Alternatively, remote methyl groups exhibit a much smaller titration induced shift than carboxyl groups in the corresponding position. Significant remote effects of pH titration on the one-bond carbon–carbon coupling are also observed, particularly for couplings involving the side-chain carboxyl carbons. These results are discussed in terms of polarization of the C–O bonds in response to titration of a remote carboxyl group. Values of $^3J_{\text{CC}}$ in aspartate and glutamate indicate a strong conformational dependence. Rotamer populations predicted on the basis of the observed couplings and theoretical INDO calculations are in good agreement with values based on an analysis of the $^3J_{\text{HH}}$ and $^3J_{\text{CH}}$ couplings. For a given conformation of glutamic acid, it is found that $^3J_{14}$ is considerably smaller than $^3J_{25}$. This result is consistent with observations on a number of other ^{13}C -labeled amino acids.

Introduction

^{13}C -Labeled amino acids have been used as nonperturbing probes of proteins^{1–11} and peptides^{14–22} into which they have been incorporated. Several of these studies have utilized uniformly labeled amino acids, the information content of which is dependent on the degree of isotropic labeling. At low levels ($\leq 20\%$) the labeling results in enhanced intensity, although small ^{13}C satellites due to $^1J_{\text{CC}}$ coupling can frequently be

observed. At intermediate labeling levels the spectra become particularly complex owing to the different isotopic isomers which are present. Thus resonances corresponding to the singly, doubly, triply, etc., labeled amino acids can be observed.²³ At higher enrichments the spectrum is simplified since the fully labeled compound becomes the dominant isotopic isomer.^{24–27} It then becomes possible to obtain values for the long-range carbon–carbon coupling constants which can be used to study molecular conformation. Owing to the complexity of the