

structure **7**. The expected labeling pattern is as shown in Figure 2. In the proton-decoupled spectrum, the C-15 resonance is a triplet ($J = 72$ Hz) due to 1,2 coupling with two adjacent enriched sites. (A lower intensity doublet is also present owing to those molecules having only one adjacent enriched site.) The C-5 and C-10 resonances both occur as doublet of doublets due to 1,2 and 1,4 couplings while C-20 shows only a 1,4 interaction. The four-meso-hydrogen ^1H NMR resonances occurred as doublets ($J = 183$ Hz) at δ 8.54, 7.46, 7.36, and 6.78. As discussed by Bonnett et al.,^{12a} the upfield meso-hydrogen resonance of an isobacteriochlorin may be assigned to that between the reduced (methylated) rings and the downfield resonance to that between the nonreduced rings. Thus the δ 8.54 hydrogen would be coupled to C-15 in structure **7** and to C-20 in structure **6**. The former case was confirmed by a selective heteronuclear decoupling experiment. Additionally, the δ 6.78 meso hydrogen was shown to be coupled to the upfield meso carbon which is therefore C-5.

The structural proposal (**7**) for sirohydrochlorin was examined by two sets of biosynthetic experiments. Specimens of sirohydrochlorin labeled (in $\sim 10\%$ radiochemical yield) by separate incubations⁶ with δ -[4- ^{14}C]aminolevulinic acid and S-[$^3\text{H}_3\text{C}$]adenosylmethionine were purified chromatographically as the octamethyl esters, hydrolyzed, and reincubated as singly and doubly labeled species after sodium amalgam reduction (**7** \rightarrow **9**). The singly labeled species was incorporated by the "corrin synthetase"⁶ preparation into cobyrinic acid **3** (isolated as cobester) in 0.4–1.9% radiochemical yield. Intact incorporation of the reduced version (**9**) is clearly revealed by retention of the $^3\text{H}/^{14}\text{C}$ ratio (3.45) in the isolated cobester (3.41). When *unreduced* **7** was incubated with the corrin synthetase preparation, similar incorporations (0.3–2.8%) were observed. The absolute stereochemistry of **7** is therefore established via bioconversion to corrin.¹³

Other workers^{14,15} have recently isolated a metabolite from *P. shermanii* with similar UV and mass spectral characteristics to those of sirohydrochlorin. The Cambridge group postulated structure **7** for their metabolite after assuming that it was on the B₁₂ pathway and further that ring C was not methylated. This paper¹⁶ shows unequivocally that sirohydrochlorin is indeed identical with a *P. shermanii* metabolite, that it has structure **7**, and that it is an intermediate on the corrin pathway. The possibility that siroheme represents a prebiotic sulfate-reducing agent⁸ and, further, that both sirohydrochlorin and vitamin B₁₂ producing anaerobic organisms predate the evolution of heme-synthesizing aerobes¹⁷ suggest that the reductive methylation of reduced porphyrins may be a phenomenon of considerable antiquity (three billion years).

Acknowledgments. We thank Professor A. Gossauer (Braunschweig) for arranging mass spectral determinations. The 270-MHz spectra (Brüker) were obtained at the Yale High Resolution Facility supported by National Institute of Health Grant CA 11095.

References and Notes

- A. I. Scott, B. Yagen, N. Georgopapadakou, K. S. Ho, S. Killoze, E. Lee, S. L. Lee, G. H. Temme, III, C. A. Townsend, and I. M. Armitage, *J. Am. Chem. Soc.*, **97**, 2548 (1975); see *ibid.*, **98**, 2371 (1976), for a correction of authors. A. I. Scott, *Philos. Trans. R. Soc. London, Ser. B.*, **273**, 303 (1976).
- A. I. Scott, *Acc. Chem. Res.*, in press.
- A. R. Battersby, M. Ihara, E. McDonald, F. Satoh, and D. C. Williams, *J. Chem. Soc., Chem. Commun.*, 436 (1975).
- H. Dauner and G. Müller, *Hoppe-Seyler's Z. Physiol. Chem.*, **356**, 1353 (1975).
- A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, P. J. Whitman, and R. J. Cushley, *J. Am. Chem. Soc.*, **94**, 8267, 8269 (1972); A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, R. J. Cushley, and P. J. Whitman, *ibid.*, **96**, 8069 (1974).
- A. I. Scott, B. Yagen, and E. Lee, *J. Am. Chem. Soc.*, **95**, 5761 (1973).
- L. M. Siegel, M. J. Murphy, and H. Kamin, *J. Biol. Chem.*, **248**, 251 (1973).
- M. J. Murphy, L. M. Siegel, H. Kamin, and D. Rosenthal, *J. Biol. Chem.*, **248**, 2801 (1973); M. J. Murphy and L. M. Siegel, *ibid.*, **248**, 6911 (1973).
- Y. Chang, P. S. Clezy, and D. B. Morell, *Aust. J. Chem.*, **20**, 959 (1967).
- A. Pelter, J. A. Ballantine, V. Ferrito, V. Jaccarini, A. F. Psaila, and P. J. Schembri, *J. Chem. Soc., Chem. Commun.*, 999 (1976).
- H. Scheer and J. J. Katz in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, Chapter 10.
- (a) R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc. C*, 1168 (1967); (b) H. H. Inhoffen, J. W. Buchler, and R. Thomas, *Tetrahedron Lett.*, 1141 (1969).
- A. I. Scott, E. Lee, and C. A. Townsend, *Bioorg. Chem.*, **3**, 229 (1974).
- (a) A. R. Battersby, K. Jones, E. McDonald, J. A. Robinson, and H. R. Morris, *Tetrahedron Lett.*, 2213 (1977); (b) A. R. Battersby, E. McDonald, H. R. Morris, D. C. Williams, V. Ya. Bykhousky, N. I. Zaitseva, and V. N. Bukin, *ibid.*, 2217 (1977).
- R. Deeg, H.-D. Kriemler, K.-H. Bergmann, and G. Müller, *Hoppe-Seyler's Z. Physiol. Chem.*, **358**, 339 (1977).
- Supported by Grant AM20528-01 from the National Institute of Health. This work was presented in part at the International Symposium on Stereochemistry, Kingston, Ontario (June, 1976), and in detail at the 11th Middle Atlantic Regional Meeting of the American Chemical Society, Newark, Delaware, April 1977.
- For a discussion of this and related philosophical aspects of corrin biosynthesis, see, for example, A. Eschenmoser, *Chem. Soc. Rev.*, **5**, 377 (1976); N. Georgopapadakou, Ph.D. Thesis, Yale University, 1975; A. I. Scott, *Acc. Chem. Res.*, in press; and ref 8.

A. I. Scott,* A. J. Irwin

Chemistry Department, Texas A&M University
College Station, Texas 77843

L. M. Siegel

Department of Biochemistry
Duke University Medical Center
and the Veterans Administration Hospital
Durham, North Carolina 27710

J. N. Shoolery

Varian Associates
Palo Alto, California 94303
Received August 29, 1977

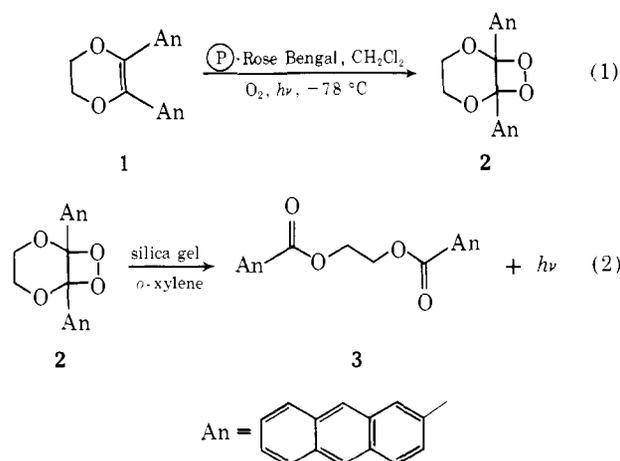
Enhanced Chemiluminescence from the Silica Gel Catalyzed Decomposition of a 1,2-Dioxetane

Sir:

In 1969 Kopecky and Mumford first demonstrated that the thermolysis of 1,2-dioxetanes yields electronically excited carbonyl products.¹ It has subsequently been shown that simple isolable 1,2-dioxetanes afford predominantly triplet excited states upon spontaneous decomposition in solution.² Transition metals,³ electron-rich olefins,⁴ and amines⁴ catalyze the cleavage of 1,2-dioxetanes. However, these processes give ground-state products and therefore result in reduced light emission. We now report the first observation⁵ of *enhanced* chemiluminescence associated with the catalytic decomposition of a stable, crystalline 1,2-dioxetane.

2,3-Di(2-anthryl)-1,4-dioxene (**1**,⁶ 100 mg) was photo-oxygenated for 30 min at -78°C in 100 mL of CH_2Cl_2 using 1 g of polymer-bound Rose Bengal⁷ as sensitizer with two 500-W tungsten-halogen lamps and a UV-cutoff filter. The reaction mixture was filtered and concentrated at 10°C on a rotary evaporator to 50 mL. Addition of 1 mL of pentane and cooling to -25°C gave pure **2** as a pale yellow solid in 51% yield: UV (*o*-xylene) λ_{max} 327 nm ($\log \epsilon$ 3.68), 343 (3.83), 361 (3.93), 380 (3.82);⁸ ^1H NMR (100 MHz, CDCl_3 , 0°C) δ 4.51 (m), 4.99 (m), 6.5–8.6 (m, aromatic). Additional **2** could be obtained by concentrating the reaction solution further;⁹ however, this material also contained cleavage product **3**.

Thermolysis of the dianthryl-substituted 1,2-dioxetane **2** in *o*-xylene results in quantitative formation of the diester **3**¹⁰ and is accompanied by light emission. The rates of decomposition of **2**, the formation of **3**, and the emission of light are first order and identical ($k = 6.56 \times 10^{-3} \text{ s}^{-1}$ at 84.1°C). The



spectrum of the chemiluminescence was acquired by photon counting techniques, and is completely congruent with the fluorescence spectrum of **3** (Figure 1). The chemiluminescence efficiency for the formation of singlet excited **3** was found to be 0.2% at 84.1 °C.

Addition of silica gel to an *o*-xylene solution of **2** leads to an immediate and dramatic increase in light intensity (as much as 10⁴-fold at 25 °C with a 150-fold increase in rate). There is a resultant red shift and loss of structure in the chemiluminescence spectrum, which is similar to the fluorescence spectrum of **3** adsorbed on silica in an *o*-xylene suspension¹¹ (Figure 1). Experiments have shown that the light emission occurs from the surface of the silica and that there was no *o*-xylene-soluble catalytic entity present in the silica. Soxhlet extraction of the silica from spent chemiluminescence mixtures allowed isolation of **3** in 71% yield.¹²

The kinetics of the catalyzed chemiluminescence were examined at 84.1 °C using stirred suspensions of silica gel (Baker, sieved to 100–140 mesh) in *o*-xylene. The results are presented in Figure 2. The integrated light emission per mole of 1,2-dioxetane **2** increases very rapidly with the weight of silica in suspension, until a plateau is reached corresponding to 136-fold enhancement of the observed chemiluminescence compared to the uncatalyzed decomposition of **2** at 84.1 °C. This suggests that above 40 g/L of silica the chemiluminescence is derived entirely from the cleavage of 1,2-dioxetane **2** on the surface of the silica with increased efficiency for the formation of singlet excited **3**. An increased fluorescence quantum yield for **3** on silica could contribute at most a 6-fold enhancement. First-order plots for the decay of the luminescence were linear ($r \geq 0.9990$) for at least 9 half-lives over the entire range of silica concentrations employed. The linear dependence of the rate constant on the weight of silica in the plateau region is in accord with a surface reaction.¹³

Similar behavior was observed using unsieved (60–200 mesh) silica from a different batch, with small differences in rate constant and a maximal light yield corresponding to a 167-fold enhancement. Assuming that the fluorescence quantum yield of **3** bound to silica is substantially independent of temperature,¹⁴ the chemiluminescence efficiency for the production of singlet excited **3** by the catalytic reaction in this case is estimated to be 12% at 84.1 °C.¹⁵

While surface catalysis of peroxide decomposition (e.g., by the vessel walls) is well established, and silica and related catalysts effect the cleavage of peroxides,¹⁶ the mechanism of catalysis appears different in this case. Since glass wool does not increase the rate of decomposition of **2**, and the simple trimethyl-1,2-dioxetane appears unaffected by silica, mere facilitation of O–O bond cleavage seems an inadequate explanation of our results. One possible rationalization rests on

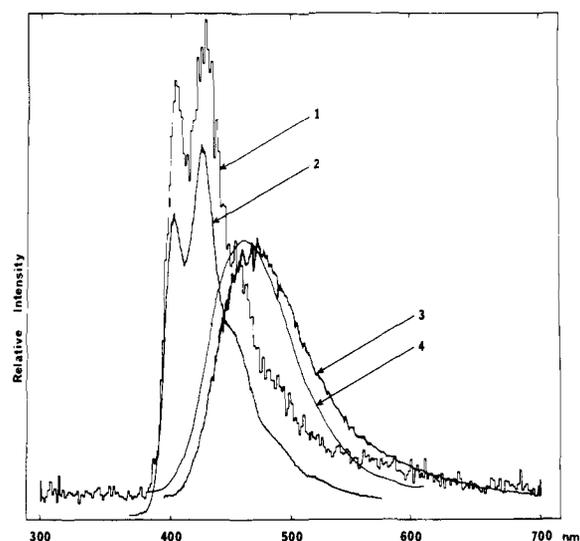


Figure 1. Emission spectra: (1) chemiluminescence from the thermolysis of **2** in *o*-xylene (9.2×10^{-5} M) at 55 °C, acquired with an Ortec 9315 photon counter, a Jarrell-Ash 82-410 scanning monochromator, and an RCA C31034 PM tube operated at -50 °C; (2) fluorescence from **3** in *o*-xylene under the same conditions as those of curve 1; (3) chemiluminescence from the silica gel catalyzed (25 g/L) decomposition of **2** (4.6×10^{-5} M) in *o*-xylene at 25 °C; (4) fluorescence of **3** adsorbed on silica gel in an *o*-xylene suspension at 25 °C.

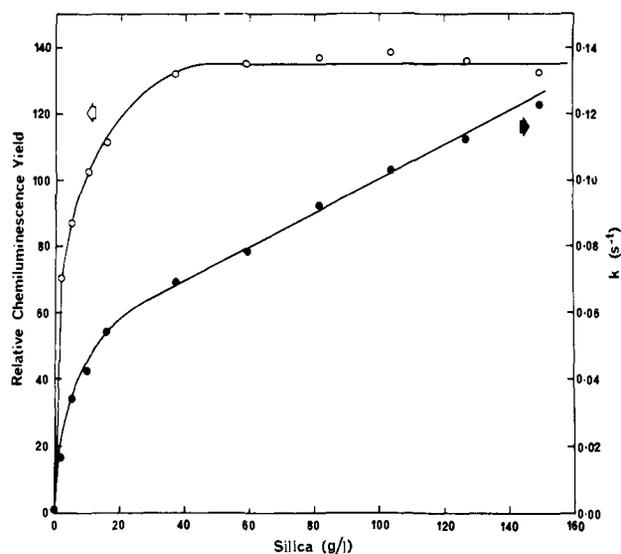


Figure 2. Chemiluminescence from 1,2-dioxetane **2** (6×10^{-6} M) in *o*-xylene with silica gel at 84.1 °C: O, integrated light intensity per mole of **2** relative to that from the uncatalyzed reaction (left-hand scale); ●, rate constant for the decay of chemiluminescence (right-hand scale).

an analogy between the surface crossing leading to chemiexcitation, and the surface crossings involved in the radiationless processes of electronically excited molecules. Methyl 2-anthraate, an excellent model for **3**, exhibits solvent dependent S₁ → T₂ intersystem crossing, resulting from an increasing T₂ energy relative to S₁, with increasing solvent polarity.¹⁷ Similar behavior in polar solvents or in the presence of silica gel is well documented for ketones.^{18,19} It is felt that the changes in excited state energies,²⁰ which are significant for intersystem crossing in **3** or methyl 2-anthraate, are also important earlier along the reaction coordinate for cleavage of **2**, specifically near the transition state or surface crossing point. These environmentally caused changes are reflected in the increased rate and chemiluminescence efficiency for scission of **2**. If this interpretation is correct, the decomposition of **2** should be solvent dependent, without the requirement for a surface-active catalyst. This and other tests of the relationship between the

properties of 1,2-dioxetanes and the photophysics of their cleavage products are presently in hand.

Other catalysts with acidic sites (e.g., alumina but not Norit) are also effective in catalyzing the chemiluminescent cleavage of **2**. The monoanthryl-substituted 1,2-dioxetane described earlier shows similar behavior.⁵ Since the catalyst may be removed by filtration, these heterogeneous systems offer intriguing possibilities for a practical chemiluminescent light source, which may be switched on and off. Studies of the scope and mechanism of the catalytic process are in progress.

Acknowledgment. Support from the U.S. Army Research Office and the National Science Foundation is gratefully acknowledged. We also thank Dr. John Wilinski for assistance in obtaining FT 100-MHz ¹H NMR spectra.

References and Notes

- (1) K. R. Kopecky and C. Mumford, *Can. J. Chem.*, **47**, 709 (1969).
- (2) (a) K. R. Kopecky, J. E. Filby, C. Mumford, P. A. Lockwood, and J.-Y. Ding, *Can. J. Chem.*, **53**, 1104 (1975); (b) E. H. White, J. D. Miano, C. J. Watkins, and E. J. Breaux, *Angew. Chem., Int. Ed. Engl.*, **13**, 229 (1974); (c) F. McCapra, *Prog. Org. Chem.*, **8**, 231 (1973); (d) N. J. Turro, P. Lechtken, N. E. Schore, G. Schuster, H.-C. Steinmetzer, and A. Yekta, *Acc. Chem. Res.*, **7**, 97 (1974); (e) T. R. Darling and C. S. Foote, *J. Am. Chem. Soc.*, **96**, 1625 (1974); (f) W. H. Richardson, F. C. Montgomery, M. B. Yelvington, and H. E. O'Neal, *ibid.*, **96**, 7525 (1974); (g) T. Wilson, *MTP Int. Rev. Sci., Phys. Chem., Ser. 2, Chem. Kinet.*, **9**, 265 (1976).
- (3) (a) P. D. Bartlett, A. L. Baumstark, and M. E. Landis, *J. Am. Chem. Soc.*, **96**, 5557 (1974); (b) P. D. Bartlett, *Chem. Soc. Rev.*, **5**, 149 (1976).
- (4) D. C.-S. Lee and T. Wilson in "Chemiluminescence and Bioluminescence," M. J. Cormier, D. M. Hercules, and J. Lee, Ed., Plenum Press, New York, N.Y., 1973, p 265.
- (5) A. P. Schaap, P. A. Burns, and K. A. Zaklika, *J. Am. Chem. Soc.*, **99**, 1270 (1977).
- (6) **1** was prepared by condensation of 2-anthraldehyde to the benzoin with KCN in aqueous ethanol followed by TsOH-catalyzed reaction of the benzoin with ethylene glycol: yellow needles; mp 285 °C; IR (KBr) 1635 cm⁻¹; ¹H NMR (60 MHz, CDCl₃) δ 4.17 (s, 4 H, (CH₂)₂), 6.80–7.80 (m, 14 H, aromatic), 7.87 (s, 4 H, aromatic); mass spectrum (70 eV) *m/e* (rel intensity) 438 (83), 219 (9), 205 (base peak), 177 (51). Anal. Calcd for C₃₂H₂₂O₂: C, 87.64; H, 5.06. Found: C, 87.90; H, 5.10.
- (7) This sensitizer is available under the tradename SENSITOX from Hydron Laboratories, Inc., New Brunswick, N.J. See A. P. Schaap, A. L. Thayer, E. C. Blossley, and D. C. Neckers, *J. Am. Chem. Soc.*, **97**, 3741 (1975).
- (8) The UV spectrum of **2** is similar to that of 2-methylanthracene.
- (9) The addition of singlet oxygen to **1** to yield the 1,2-dioxetane **2** as the major product contrasts with the photooxygenation of 2-(2-anthryl)-1,4-dioxene which gives both the corresponding 1,2-dioxetane and an endoperoxide.⁹ **2** will be referenced in *Chemical Abstracts* as 1,6-di(2-anthryl)-2,5,7,8-tetraoxabicyclo[4.2.0]octane.
- (10) Yellow solid; mp 253 °C; UV (*o*-xylene) λ_{max} 326 nm (log ε 3.53), 341 (3.75), 359 (3.83), 379 (3.84), 400 (3.85); IR (KBr) 1704 cm⁻¹; ¹H NMR (100 MHz, CDCl₃) δ 4.82 (s, 4 H), 6.6–9.0 (m, 18 H); mass spectrum (70 eV) *m/e* (rel intensity) 470 (1), 249 (2), 222 (2), 205 (2), 178 (7), 177 (7), 176 (4), 155 (3), 92 (10), 91 (30), 83 (base peak). Anal. Calcd for C₃₂H₂₂O₄: C, 81.68; H, 4.71. Found: C, 81.43; H, 4.65.
- (11) Determined by front face excitation. At 25 °C the fluorescence efficiency of **3** increases from 0.23 in *o*-xylene solution to a value of 0.5 in silica gel-*o*-xylene suspension (455 g/L).
- (12) The isolation procedure was hampered by the poor solubility of **3**.
- (13) C. H. Bamford and C. F. H. Tipper, "Chemical Kinetics", Vol. 2, Elsevier, Amsterdam, 1969.
- (14) The value of the fluorescence efficiency of **3** bound to silica at 84.1 °C was taken to be identical with that at 25.0 °C.¹¹ No strong dependence of φ_F on temperature is expected. Any dependence should lead to reduced values of φ_F at higher temperatures so that the chemiluminescence efficiency may have been slightly underestimated. In *o*-xylene solution φ_F of **3** is reduced only ~25% at 84.1 °C compared to φ_F at 25.0 °C.
- (15) Relative to the radioactive light standard of Hastings: J. W. Hastings and G. Weber, *J. Opt. Soc. Am.*, **53**, 1410 (1963).
- (16) (a) J. A. Turner and W. Herz, *J. Org. Chem.*, **42**, 2006 (1977); (b) J. E. Leffler and D. W. Miller, *J. Am. Chem. Soc.*, **99**, 480 (1977); (c) W. Herz, R. C. Ligon, J. A. Turner, and J. F. Blount, *J. Org. Chem.*, **42**, 1885 (1977).
- (17) T. C. Werner, T. Matthews, and B. Soller, *J. Phys. Chem.*, **80**, 533 (1976).
- (18) (a) P. A. Leermakers and H. T. Thomas, *J. Am. Chem. Soc.*, **87**, 1620 (1965); (b) R. Rusakowicz, G. W. Byers, and P. A. Leermakers, *ibid.*, **93**, 3263 (1971).
- (19) A. A. Lamola, *J. Chem. Phys.*, **47**, 4810 (1967).
- (20) **3** shows behavior similar to methyl 2-anthroate, e.g., UV absorption of **3** (*o*-xylene-silica gel) λ_{max} 326, 343, 361, 385, and 403 nm, with a singlet energy of 67.4 kcal/mol (compare with 70.8 kcal/mol in *o*-xylene).
- (21) Alfred P. Sloan Research Fellow, 1974–1978.

K. A. Zaklika, Paul A. Burns, A. Paul Schaap*²¹
 Department of Chemistry, Wayne State University
 Detroit, Michigan 48202
 Received July 15, 1977

Correlation of Chemical Shifts of Protons and Carbon-13

Sir:

We would like to draw attention to the chemical significance of a method for correlating proton and carbon-13 chemical shifts based on spin-spin coupling, information which is traditionally obtained from coherent proton decoupling over a range of different frequencies.¹⁻³ It uses a two-dimensional Fourier transform technique recently introduced by Ernst^{4,5} where one species (the *S* spins) is detected indirectly by observation of a second species (the *I* spins). A rigorous treatment of this experiment has recourse to the density matrix formalism,^{6,7} although considerable insight may be obtained by a simpler approach based on spin population arguments.⁸

The method has already been applied to the problem of correlating proton (*S*) and carbon (*I*) shifts in some simple molecules.^{8,9} The experiment consists of an initial 90° pulse applied to protons, an evolution period *t*₁ to allow free precession of the protons, a second 90° proton pulse which creates nonequilibrium spin populations, and then (simultaneously or suitably delayed) a 90° carbon-13 pulse to monitor the carbon-13 free induction decay. As the second proton pulse acts on magnetization vectors with different orientations in the transverse plane, it can be thought of as behaving in a frequency-selective manner, pumping spin populations differentially. This is how information about the proton spectrum is transmitted via the carbon-13 signal to the receiver, which is tuned to carbon-13 not protons. There is no *net* transfer of magnetization, the "new" carbon-13 resonances having positive or negative intensities with an algebraic sum of zero.^{4,5} Two-dimensional Fourier transformation generates a spectrum in two frequency dimensions containing information about protons (in the *F*₁ dimension) and carbon-13 (in the *F*₂ dimension)—essentially a shift correlation chart containing some additional information about spin coupling.

Spectra were obtained from natural-abundance carbon-13 samples on a Varian CFT-20 spectrometer provided with a program for double Fourier transformation.¹⁰ The carbon-13 free induction signals were detected under conditions of coherent off-resonance proton decoupling, causing incomplete coalescence of the proton splitting of the carbon-13 resonances in the *F*₂ dimension without allowing the antiphase components of these multiplets to come close enough together for mutual cancellation.⁸ No attempt was made to remove the corresponding doublet splitting of the proton spectra in the *F*₁ di-

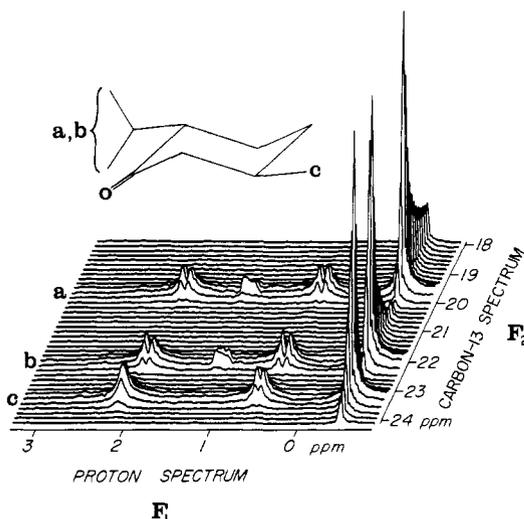


Figure 1. Two-dimensional Fourier transform spectrum (absolute value mode) of the methyl groups in menthone. The assignment of a and b remains ambiguous.