Tetrahedron Vol. 36, pp 2721 to 2725
Pergamon Press Ltd., 1980 Printed in Great Britain

0040-4020/80/1001 2721 $02.00/0

THE CARBON-13 AND NITROGEN-15 NUCLEAR MAGNETIC RESONANCE SPECTRA OF UROPORPHYRINOGENS I AND III

GERARDO BURTON, PAUL E. FAGERNESS, PETER M. JORDAN and A. I. SCOTT*

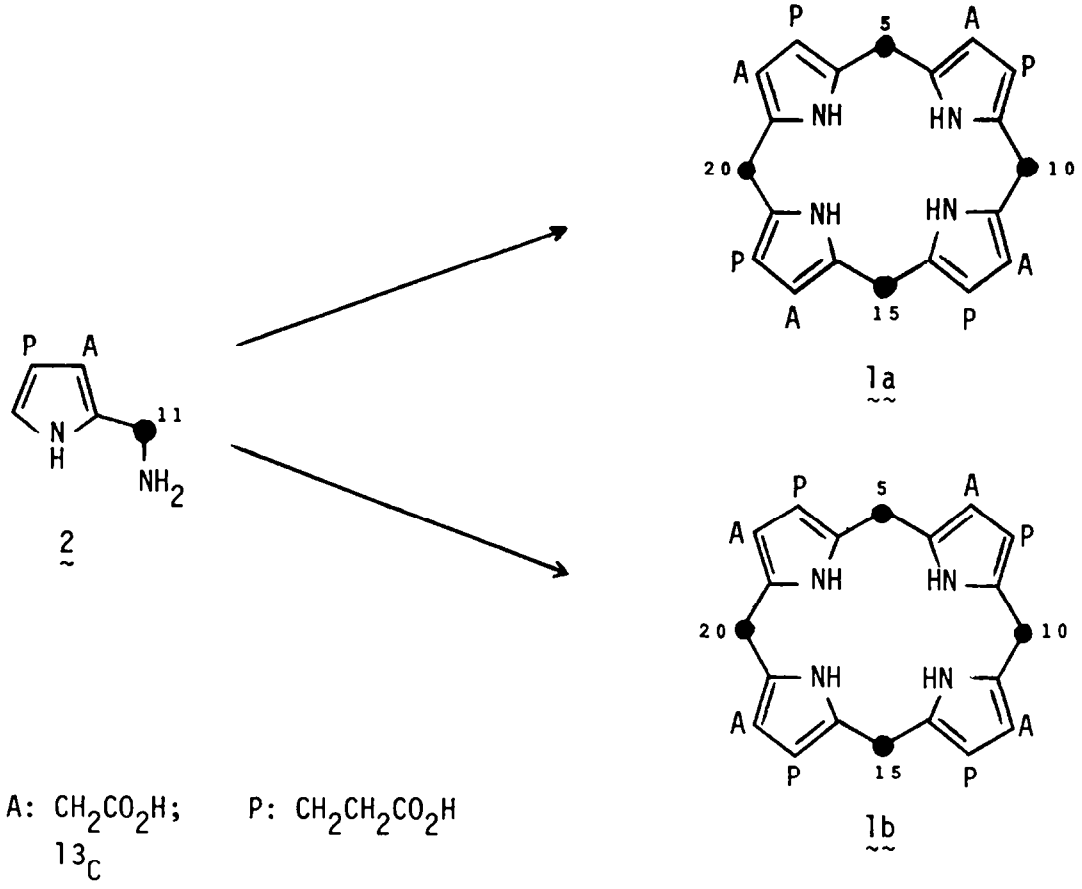
Department of Chemistry, Texas A&M University, College Station, TX 77843, U.S.A

(*Received in U.S.A.* 10 *December* 1979)

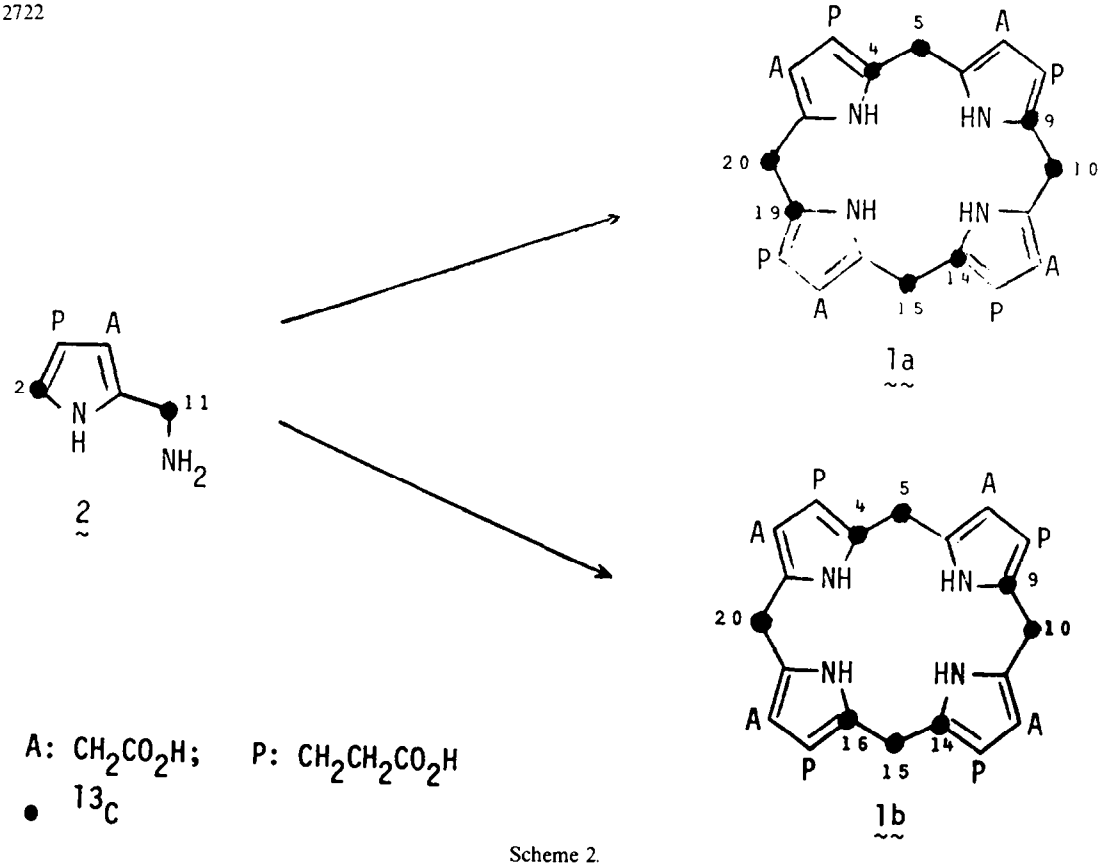
Abstract— Due to their sensitivity to light and air, porphyrinogens are not normally isolated, but are routinely analyzed by oxidation to the corresponding porphyrin. We report herein the ^{13}C- and ^{15}N-NMR spectra of uroporphyrinogens I and III in their "native state", multiply labelled with ^{13}C and ^{15}N, and at natural abundance (^{13}C only).

Uroporphyrinogen (uro'gen) I (**1a**) and III (**1b**), the products of the condensation of the pyrrole porphobilinogen (PBG) (**2**), by the action of the enzymes PBG deaminase and uro'gen III cosynthetase[1] are highly unstable compounds, both being sensitive to air, light and acids.[2] Their instability, in conjunction with the difficulty of obtaining the pure isomers, has limited the available spectroscopic data[3] for these compounds, which are the substrates for the enzymes of copro'gen synthesis and, in the case of uro'gen III, for heme, chlorophyll and vitamin B_{12}.[4]

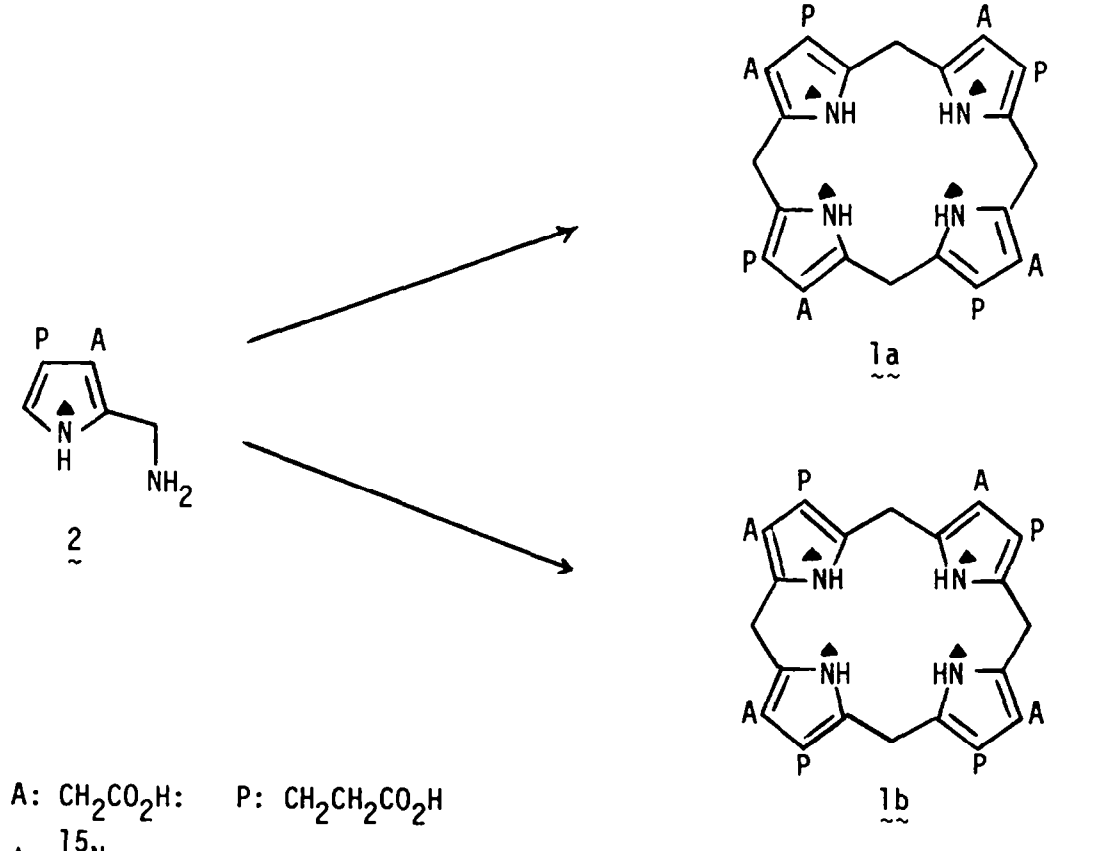
We have recently developed a ^{13}C- and ^{15}N-NMR method for the study of the enzymic reactions yielding the uro'gens I (**1a**) and/or III (**1b**) from PBG (**2**)[5,6] in the NMR tube, which allows us to assign the enriched NMR spectrum of these compounds formed from ^{13}C or ^{15}N labelled PBG. In these studies, 90% enriched 11-^{13}C-PBG, 2,11-$^{13}C_2$-PBG (81% of the molecules containing two ^{13}C atoms) and 99% enriched 1-^{15}N-PBG were used as substrates; the labelling patterns in the final products are shown in Schemes 1–3.



Scheme 1.



Scheme 2.



Scheme 3.

RESULTS AND DISCUSSION

The ^{13}C spectra of [5,10,15,20-$^{13}C_4$]-uro'gens I and III (obtained from 11-^{13}C-PBG) show a single resonance at $\delta = 21.63$ and 21.65 ppm, respectively.† No ^{13}C-^{13}C coupling is observed in the uro'gens derived from single labelled PBG, as is expected from the magnitudes of four bond coupling constants ($^4J_{^{13}C-^{13}C} = 0.8$ Hz).[7]

Two ^{13}C–^{13}C couplings are possible for the uro'gens derived from [2,11-$^{13}C_2$]-PBG, a one bond and a three bond coupling (the latter through the N atom). The three bond coupling is observed in the ^{13}C spectrum of the double labelled PBG and found to be $^3J_{^{13}C-^{13}C} = 1.4$ Hz. This coupling is unresolved in the spectra of the uro'gens, due to the broad lines obtained (half height width 10–12 Hz). The doublets arising from the direct (one bond) coupling in [4,5,9,10,14,15,19,20-$^{13}C_8$]-uro'gen I (meso carbons $\delta = 21.63$ ppm, α-pyrrolic carbons $\delta = 123.93$ ppm) have $^1J_{^{13}C-^{13}C}$ of 50.4 Hz (Fig. 1a); as there is 90% ^{13}C in each position, a line is observed in the center of each doublet corresponding to isolated ^{13}C atoms (9%).

In the case of [4,5,9,10,14,15,16,20-$^{13}C_8$]-uro'gen III(**1b**) (Fig. 1b), a more complex pattern is obtained for the meso carbons. Since this molecule has three ^{13}C atoms directly bonded, ^5C and ^{10}C appear as doublets ($\delta = 21.61$ ppm, $^1J_{^{13}C-^{13}C} = 50.4$ Hz) as in the type I isomer, but ^{15}C is a triplet ($\delta = 21.54$ ppm, $^1J_{^{13}C-^{13}C} = 50.9$ Hz) and ^{20}C a broad singlet ($\delta = 21.65$ ppm). atoms directly bonded, C-5 and C-10 appear as doublets ($\delta = 21.61$ ppm, $^1J_{^{13}C-^{13}C} = 50.4$ Hz) as in the type I isomer, but C-15 is a triplet ($\delta = 21.54$ ppm, $^1J_{^{13}C-^{13}C} = 50.9$ Hz) and C-20 a broad singlet ($\delta = 21.65$ ppm). The differences in chemical shifts between C-15 and C-20 is due probably to an isotope shift induced in the former by the ^{13}C atoms in C-14 and C-16,[8] as no difference is observed in the chemical shifts of the four meso carbons of [5,10,15,20-$^{13}C_4$]-uro'gen III (obtained from 11-^{13}C-PBG).

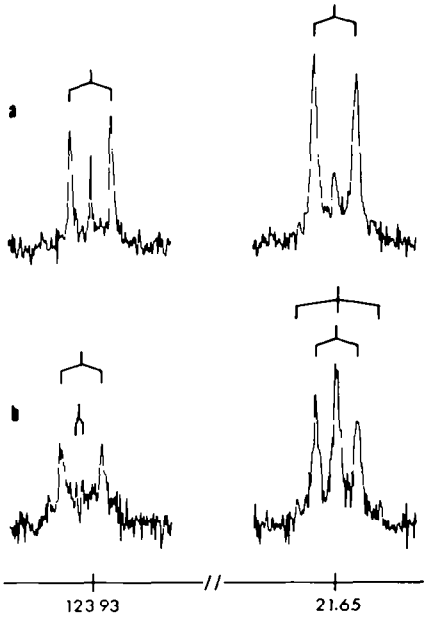


Fig. 1. ^{13}C-NMR spectra at 20.0 MHz of (a) uro'gen I and (b) uro'gen III obtained from 2,11-$^{13}C_2$-PBG. Each is the result of 100,000 pulses with a repetition rate of 0.819 sec. over a spectral width of 5000 Hz.

The pattern for the labelled pyrrolic carbons (4, 9, 14 and 16) is less evident, appearing as a broad doublet at 124.63 ppm ($^1J_{^{13}C-^{13}C} = 50.4$ Hz). A less intense doublet can be observed at 124.75 ppm ($^2J_{^{13}C-^{13}C} = 7.5$ Hz) which is assigned to C-14 and C-10 in those molecules that contain ^{13}C in these positions but not in C-15 (8.1%).

Further examination of the ^{13}C-NMR spectra of non-labelled uro'gen I and III was next carried out at natural abundance (observing ^{13}C at 50.3 MHz). Interpretation of these spectra utilized the ^{13}C ^{13}C coupling information, together with the chemical shift data obtained from the partially enriched ^{13}C spectra, thus allowing assignment of the natural abundance spectra of the uro'gens. In the case of uro'gen I (Fig. 2a), the symmetry of the molecule renders the four pyrrole rings equivalent. Thus only four lines appear in the aromatic region[9] at $\delta = 126.74$ ppm (C 1, 6, 11 and 16), $\delta = 123.51$ ppm (C 4, 9, 14 and 19, similar to the enriched α position discussed above), $\delta = 118.18$ ppm (C 2, 7, 12 and 17) and $\delta = 112.26$ ppm (C 3, 8, 13 and 18). The side chain acetate and propionate moieties may be assigned on the basis of the ^{13}C-NMR spectrum of PBG (**2**) as follows: $\delta = 40.12$ ppm (pyrr-$\underline{C}H_2-CO_2-$), $\delta = 32.91$ ppm (pyrr-$CH_2-\underline{C}H_2-CO_2-$), $\delta = 21.82$ ppm (pyrr-$\underline{C}H_2CH_2CO_2-$), and the meso carbons (C 5, 10, 15 and 20), by analogy with the spectrum of enriched uro'gen I, to δ 21.63 ppm. The carbonyl region exhibits two lines at δ 182.96 and 181.82 ppm corresponding to the acetate and propionate carboxyl groups.

The spectrum of uro'gen III (Fig. 2b) is complicated because of the non-symmetric distribution of the side chains, which makes the carbons of the different pyrrole rings non-equivalent. Although we are unable to assign each resonance in the aromatic region of the spectrum, these can be separated into four groups of complex signals centered at $\delta = 126.03$ ppm (C 1, 6, 11 and 19), $\delta = 124.09$ (C 4, 9, 14 and 16), $\delta = 117.75$ (C 2, 7, 12 and 18) and $\delta = 112.67$ (C 3, 8, 13 and 17). The remaining lines have similar chemical shifts to those of uro'gen I: $\delta = 182.96$ and 181.82 (carboxylates), $\delta = 40.02$ ppm (pyrr-$\underline{C}H_2-CO_2-$), $\delta = 32.92$ ppm (pyrr-$CH_2-\underline{C}H_2-CO_2-$), $\delta = 21.82$ ppm (pyrr-$\underline{C}H_2-CH_2-CO_2-$) and $\delta = 21.66$ ppm (C 5, 10, 15 and 20).

Finally, $^{15}N_4$-uro'gen I and $^{15}N_4$-uro'gen III were synthesized from 1-^{15}N-PBG as above and the ^{15}N-NMR spectra obtained (Figs 3a, b). For the type I isomer a single resonance at δ 154.27 ppm is observed, while uro'gen III shows two lines at $\delta = 153.57$ and 153.43 ppm (Figs 3a, b).

These spectra illustrate *par excellence* the powerful nature of the "in tube" technique for monitoring chemical and enzyme-catalyzed reactions, particularly when potentially labile products are produced, in that an environment is provided for the reaction to proceed under normal conditions, thus eliminating artifacts of isolation, pH change, etc. and where the fluxional changes may be monitored *en train d'aille* without recourse to any additional analytical methodology.

†Small discrepancies were observed in the ^{13}C chemical shifts obtained at 20.0 and 50.3 MHz.

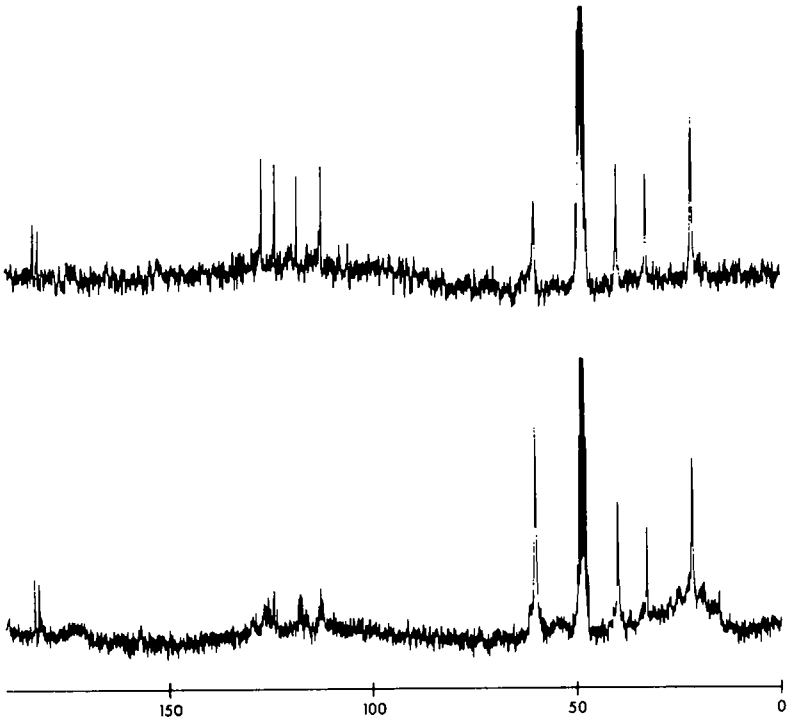


Fig. 2. Natural abundance ^{13}C-NMR spectra at 50.3 MHz of (a) uro'gen I and (b) uro'gen III. Each is the result of 109,000 pulses over a spectral width of 10,000 Hz, using a repetition rate of 0.819 sec.

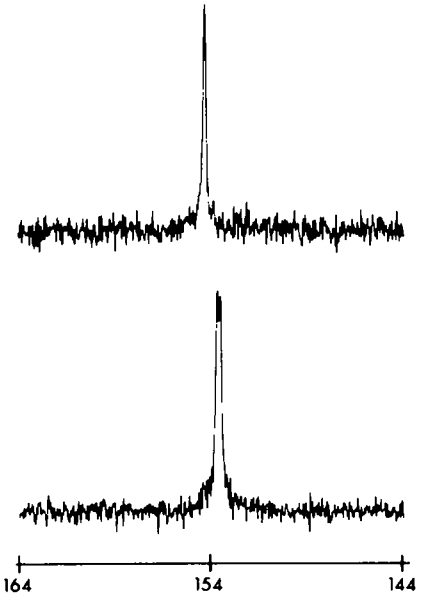


Fig. 3. ^{15}N-NMR spectra at 20.3 MHz of (a) $^{15}N_4$-uro'gen I and (b) $^{15}N_4$-uro'gen III. Each is the result of 60,000 pulses over a spectral width of 6000 Hz using a repetition rate of 1 sec.

EXPERIMENTAL

Carbon-13 NMR spectra were recorded at 0° in H_2O at 20.0 MHz on a Varian FT-80 spectrometer using 10% D_2O for internal lock or at 50.3 MHz on a Varian XL-200 spectrometer with 6% CD_3OD as internal lock. Chemical shifts are given in ppm downfield from $SiMe_4$.

Nitrogen-15 NMR spectra were recorded at 0° in H_2O at 20.3 MHz on a Varian XL-200 spectrometer using 6% CD_3OD as internal lock. Chemical shifts are in ppm. downfield from anhyd NH_3.

[11-^{13}C]-PBG and [1-^{15}N]-PBG were synthesized as previously described.[5,6] [2,11-$^{13}C_2$]-PBG was obtained by incubation of [5-^{13}C]-δ-aminolevulinic acid (δ-ALA)[10] with purified δ-ALA dehydratase.[11]

Enriched uro'gen I was obtained by incubating the appropriately labelled PBG with highly purified PBG deaminase.[12] A typical incubation mixture contained labelled PBG (1 mg/ml), deaminase (8 units/ml) and sodium borohydride (0.15 mg/ml) in 0.067 M phosphate buffer (pH 7.8). After 1 hr at 37° under Argon in the dark, the soln was cooled to 0° and D_2O (10%) or CD_3OD (6%) added. When uro'gen III was desired, an excess of uro'gen III cosynthetase[13] was included in the incubation, which was carried out in the NMR tube, and the spectra recorded immediately.

For the preparation of the non-labelled uro'gens, incubations were performed in a total volume of 5 ml using 0.02 M phosphate buffer (pH 7.8), and concentrated to the appropriate volume by freeze drying upon completion of the reaction.

Acknowledgements We thank the National Institutes of Health (Grant No. AM-20528) for support of this work, the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina for a fellowship (GB), L. M. Pryde and L. K. Hoffman for experimental assistance with enzyme preparation and cell cultures, and the National Science Foundation for the purchase of the FT-80 and XL-200 spectrometers.

REFERENCES

[1] L. Bogorad, *J. Biol. Chem.* **233,** 501, 510 and 516 (1958).
[2] D. Mauzerall, *J. Am. Chem. Soc.* **82,** 2601 and 2605 (1960).
[3] H. Scheer and J. J. Katz, *Porphyrins and Metalloporphyrins* (Edited by K. M. Smith), p. 456. Elsevier, Amsterdam, (1975).
[4] A. R. Battersby and E. McDonald, *Porphyrins and Metalloporphyrins* (Edited by K. M. Smith), p. 96. Elsevier, Amsterdam (1975).
[5] G. Burton, P. E. Fagerness, S. Hosozawa, P. M. Jordan and A. I. Scott, *J. Chem. Soc.* Chem. Commun 202 (1979).
[6] G. Burton, H. Nordlöv, S. Hosozawa, H. Matsumoto, P. M. Jordan, P. E. Fagerness, L. M. Pryde and A. I. Scott, *J. Am. Chem. Soc.* **101,** 3114 (1979).
[7] J. Marshall, D. Müller, S. Conn, R. Seiwell and A. Ihrig, *Acc. Chem. Res.* **1,** 338 (1974).
[8] P. Hansen, O. Poulsen and A. Berg, *Org. Magn. Resonance* 7, 24 (1975).
[9] R. J. Abraham, R. D. Lapper, K. M. Smith and J. F. Unsworth, *J. Chem. Soc.* Perkin II, 1004 (1974).
[10] A. R. Battersby, E. Hunt, E. McDonald and J. Moran, *Ibid.* Perkin I, 2917 (1073).
[11] P. M. Jordan and J. S. Seehra, in preparation.
[12] P. M. Jordan and D. Shemin, *J. Biol. Chem.* **248,** 1019 (1973).
[13] A. I. Scott, H. Nordlöv, P. M. Jordan, M. M. Schneider, S. Hosozawa and L. M. Pryde, in preparation.