Vol. 46, No. 1, 1972 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

CARBON-13 NMR SPECTRA OF CARBON-13 ENRICHED CHLOROPHYLLS a AND b*

C. E. Strouse, V. H. Kollman and N. A. Matwiyoff

Los Alamos Scientific Laboratory, University of California,
Los Alamos, New Mexico 87544

Summary - The proton decoupled ^{13}C NMR (CMR) spectra of chlorophylls a and b enriched to 90% ^{13}C have been obtained at 25.2 MHz and, despite the complexity of the spectra, many of the assignments of the ^{13}C resonances have been made.

Received November 3, 1971

INTRODUCTION

With the advent of more stable and sensitive NMR spectrometers in recent years, CMR spectroscopy has been recognized as a valuable tool in the study of biological materials.[1] The large range of ^{13}C chemical shifts and the relatively narrow natural linewidths make CMR generally more useful in the study of complex materials than proton NMR. In addition, CMR gives more direct information about the carbon skeleton of organic molecules and, of course, the relatively smaller number of carbon atoms than the protons in biological materials results in more easily interpreted NMR spectra. In spite of these advantages, the application of CMR to large molecules has been delayed by problems related to the low sensitivity of the technique and the low natural abundance of ^{13}C (1.1%). Fourier transform techniques[2] have recently made studies of this type more practical. Enriched samples, of course, greatly enhance the sensitivity of the CMR experiment but present the potential disadvantage that the large number of accompanying ^{13}C-^{13}C spin-spin interactions could result in exceedingly complex ^{13}C NMR spectra.

The application of CMR to the study of ^{13}C-labeled biological systems is a part

---

* This work performed under the auspices of the U. S. Atomic Energy Commission.

of an active program now underway in this laboratory involving the production and utilization of stable isotopes. In an early phase of this project a strain of green algae was grown on 90% $^{13}CO_2$ [3] from which have been isolated a number of natural products including the chlorophylls a and b. We report here their CMR spectra. Visible, infrared, and proton NMR techniques[4] have already been applied to studies of chlorophyll derivatives. The ^{13}C resonances of these highly labeled natural products are of general interest because they demonstrate that, despite the large number of possible ^{13}C-^{13}C interactions, the ^{13}C chemical shifts are sufficiently large to allow the unique assignment of the resonances in many of the "critical" regions of the spectrum. The spectra are of special interest in that they provide additional insight into the structure of the chlorophylls in solution.

METHODS AND MATERIALS

All spectra recorded in this study were obtained from a Varian XL-100 spectrometer operated in the cw mode at 25.2 MHz and locked to the deuterium resonance (15.4 MHz) of the $CDCl_3$ solvent. Most spectra were proton noise decoupled and signal averaged through an interface to a Data General Supernova Computer. Chlorophyll samples were prepared by the method of Strain and Svec[6] and sealed in 12 mm (o.d.) NMR tubes.

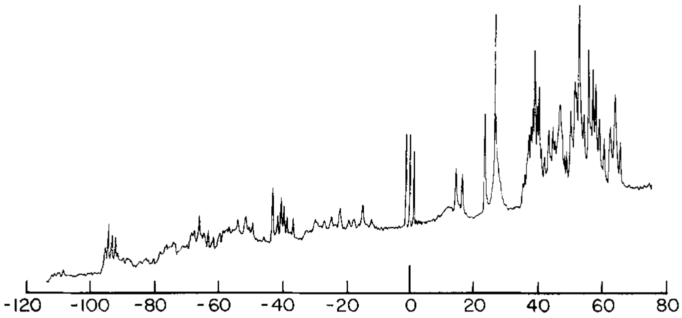

Fig. 1 - Proton noise decoupled ^{13}C NMR spectrum of chlorophyll a. This spectrum is an accumulation of 256 two-minute scans.

RESULTS AND DISCUSSION

Figure 1 shows a proton decoupled CMR spectrum of 90% ^{13}C chlorophyll a (~ 0.06 M) in a $CDCl_3$ solution containing an amount of CH_3OH (~ 8 M) sufficient to insure that the chlorophyll was predominately in the deaggregated form. The sharp triplet at 0 ppm is due to the solvent, $DCCl_3$, ^{13}C resonance and all shifts have been

Table 1

Chemical Shifts	A ppm	B ppm
C_R	66.1	-110.5
C_1, C_5	64.4	64.2, 64.7
C_{10}	13.9	13.9
C_{30}, C_{31}	27.6	28.1
α	-22.3	-32.5
β	-30.0	-25.8
δ	-15.1	-15.8
C_2	-42.6	-42.9
C_9	-111.9	-113.1
C_{11}	-94.9	-95.2
C_{12}	23.8	23.5
C_{22}	-96.0	-96.0
C_{23}	15.6	15.6
C_{24}	-39.8	-39.8
Coupling Constants	Hz	
Methine - C_N	70	70
C_2-C_2	68	69
C_{11}-C_{10}	58	56
C_{22}-C_{21}	55	53
C_{24}-C_{23}	50	50
C_{24}-C_{25}	75	74
C_R-C_P	--	50

measured with respect to it. A summary of the assignments appears in Table 1 and Fig. 2.

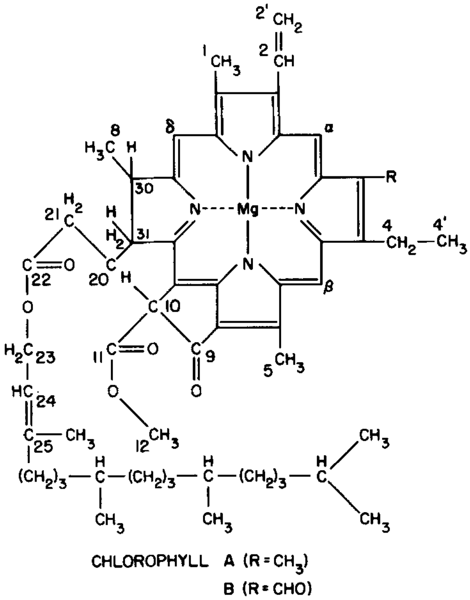

Fig. 2 - Structure and numbering scheme for chlorophyll a.

In making the assignments we have relied on comparisons of the ^{13}C chemical shifts with those of small molecules and on the nature of the multiplets arising from ^{13}C-^{13}C splitting. Two characteristics of the ^{13}C-^{13}C interactions in small molecules we have studied result in deceptively simple multiplet patterns in many regions of the chlorophyll spectra: The coupling constants are small and, for carbon atoms of a given hybridization state, do not exhibit a large variation with the chemical environment of the ^{13}C atom. This could result in deceptively simple multiplet patterns; e.g., the C_i carbon of the unit C_j-C_i-C_j would be a doublet of doublets if the carbon atoms j differed in their gross hybridization states but would resemble a triplet if the C_j atoms exhibited grossly the same hybridization state even though they were not chemically and magnetically equivalent.

The methyl and methylene resonances occur in the region 30 to 70 ppm, the olefin and pyrrole resonances in the 0 to -90 ppm region, and the carbonyl resonances in the region -90 to -120 ppm. The complex high field region consists of the overlapping methyl and methylene resonances, most of which arise from the large number of aliphatic carbons in the phytyl side chain. Studies in progress of methyl pheophorbide and its zinc(II) derivative have provided assignments of the C_4, C_4, C_8, C_{20}, and C_{21} resonances which also occur in this region. Examples of the detailed assignments are provided in the following:

The spectral region 0 to 35 ppm characteristic of alkyl groups attached to electronegative substituents contains a sharp singlet at 23.8 ppm which can readily be assigned to C_{12}, the only carbon atom not directly attached to another carbon. (The sharp singlet at 26 ppm which overlaps the broad resonance at 27.6 ppm is due to the methanol.) Of the potential doublets in the spectrum, that at ±15.6 ppm is assigned to C_{23} because it is absent from the spectra of the pheophorbides. The α, β and δ methine carbon resonances have been assigned to the three resolved 1:2:1 triplets (arising from the interaction with two α-carbons of the pyrrole rings) at -23.3, -30.0, and -15.1 ppm, respectively, from $^{13}CDCl$. These assignments were confirmed by irradiating the ^{13}C resonances while observing the proton NMR spectrum. The fourth methine resonance which should be further split by interaction with the C_{10} carbon is probably also in this region. The proton coupled spectrum suggests that it overlaps the β methine ^{13}C resonance. Changes in the methine region of the ^{13}C spectrum in going from chlorophyll a to b parallel those observed in the proton NMR spectrum. In both cases the δ resonance is shifted very little, the α resonance is shifted upfield, and the β resonance experiences a pronounced downfield shift.

At the low field extreme of the spectrum are the resonances of the carbonyl carbon atoms. The broad resonance at -112 ppm is assigned to C_9 which is a potential triplet or doublet of doublets further split by relatively large long range $^{13}C-C-^{13}C$ interactions. The remaining carbonyl resonances, C_{11} and C_{22}, should be doublets and are assigned to the lines centered at -94.9 and -96.0 ppm, respectively. The distinction between the C_{11} and C_{22} resonances is not completely certain for it is based on the assumption that the C_{22} resonance should be less affected by the addition of methanol to the solution. In the spectrum of chlorophyll b, an additional doublet appears in the spectrum at -110.5 ppm and it is assigned to the aldehyde group at position R.

We defer a detailed discussion of the chemical implications of the ^{13}C NMR spectra of the chlorophylls until the completion of our work with other chlorophyll derivatives and with the chlorophylls presently being prepared at the 20% ^{13}C level. Those studies should allow us to assign all the resonances in the chlorophyll spectra together with an unravelling of most of the $^{13}C-^{13}C$ spin-spin interactions. However, it is of interest to point out at this time that the ^{13}C NMR spectra suggest an important role for the phytyl side chain in the aggregation of chlorophyll a. Extensive proton NMR studies have been conducted in which large spectral changes, especially in the region of ring V, are observed upon addition of methanol to DCCl solutions of chlorophyll a. These studies suggest that intermolecular coordination of Mg(II) occurs via the carbonyl oxygen atoms.[4] Our ^{13}C NMR studies in progress on the aggregation - deaggregation of chlorophyll a show that the phytyl resonances, and particularly the resonance of olefinic carbon C_{24}, are strongly affected by aggregation. A study of the severe broadening and shift of the lines assigned to this olefinic carbon atom in the absence of methanol may provide new information on the structure of aggregated chlorophyll.

References

1. D. Doddrell and A. Allerhand, Proc. Nat. Acad. Sci. (USA) **68**, 1083 (1971) and references therein.

2. R. R. Ernst and W. A. Anderson, Rev. Sci. Inst., 1966, **37**, 93; R. R. Ernst, J. Mag. Res., 1970, **3**, 10.

3. C. T. Gregg, National Symposium on Carbon-13 Proceedings, Los Alamos, NM (USA), June, 1971.

4. J. J. Katz, G. D. Norman, W. A. Svec, and H. H. Strain, J. Am. Chem. Soc., 1968, **90**, 6841; R. C. Dougherty, H. H. Strain, W. A. Svec, R. A. Uphaus, and J. J. Katz, J. Am. Chem. Soc., 1970, **92**, 2826, and references therein.

5. H. H. Strain and W. A. Svec, Chapter 2, "The Chlorophylls," ed. L. R. Vernon and G. R. Seely, Academic Press, New York, 1966.