chains of sonicated bilayers, on the other hand, have much greater freedom, and the motional state of these chains very nearly resembles a liquid state.

This paper also demonstrates that the stochastic line width theory of Anderson[21] offers the most straightforward way of calculating nmr line widths from spin species undergoing restricted motions and can be readily extended to treat restricted local motions combined with overall Brownian tumbling. Even relatively complex spectra, such as those of methyl groups, may be conveniently simulated for all types of restricted motions. Biological investigations using nmr, for example, studies on tissues, nerves, membranes, or very large molecules frequently demand an understanding of the effects of restricted motion on nmr line widths, and calculations of the type performed here may be necessary.

Acknowledgment. The authors would like to acknowledge many helpful discussions with Mr. G. W. Feigenson and would like to thank Dr. R. W. Vaughan for the use of his multipulse spectrometer.

Assignments in the Natural-Abundance Carbon-13 Nuclear Magnetic Resonance Spectrum of Chlorophyll a and a Study of Segmental Motion in Neat Phytol

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Contribution No. 2306 from the Department of Chemistry, Indiana University, Bloomington, Indiana 47401. *Received May 22, 1973*

Abstract: We have recorded the proton-decoupled natural-abundance ^{13}C spectra (at 15.18 MHz) of phytol, phytyl acetate, and chlorophyll a. The 20 carbons of phytol yield 19 peaks. We have assigned 16 out of the 18 single-carbon resonances of phytol to specific carbons with the use of the chemical shift parameters of Grant and Paul, comparisons with the spectrum of pristane and of a phytol–pristane mixture, and ^{13}C spin–lattice relaxation times (T_1) of individual carbons of neat phytol. Even some resonances separated by less than 0.1 ppm arising from structurally very similar carbons near the center of the phytol molecule were specifically assigned. The ^{13}C resonances of phytyl acetate were assigned with the use of single-frequency off-resonance proton decoupling and a comparison with the spectrum of phytol. Phytyl acetate was then used as a model for identifying and assigning all the phytyl carbon resonances in the ^{13}C spectrum of chlorophyll a dissolved in a chloroform–methanol mixture. The T_1 values of neat phytol (at 52°) yielded information about the segmental motions in the branched phytyl chain. The observed behavior is compared with that of the unbranched 1-decanol molecule. While the effective rotational correlation time of the carbons of neat 1-decanol increases monotonically toward the hydroxyl end of the molecule, in the case of phytol there are localized deviations from monotonic behavior, as a result of branching and the presence of an olefinic bond.

Proton nuclear magnetic resonance has been used for investigating the properties of chlorophylls in solution.[1,2] However, even at 220 MHz (51.7 kG), proton nmr spectra of chlorophylls contain very few resolved single-hydrogen resonances, as a result of the small range of proton chemical shifts and the splittings arising from homonuclear scalar coupling. An investigation of the proton-decoupled ^{13}C nmr spectrum of vitamin B_{12} and other corrinoids[3] indicated that ^{13}C nmr is an excellent alternative for studying organic molecules of high complexity. For example, in the proton-decoupled natural-abundance ^{13}C nmr spectrum of cyanocobalamin, more than 40 of the 63 carbons are resolved into single-carbon resonances, even at the relatively low magnetic field of 14.1 kG.[3] Katz and coworkers have reported the ^{13}C nmr spectra of 15% ^{13}C-enriched chlorophyll a and methyl pheophorbide a, recorded at 55 MHz (51.7 kG).[2,4,5] Strouse, Kollman, and Matwiyoff[6] have reported ^{13}C spectra of 90% ^{13}C-enriched chlorophyll a and chlorophyll b at 25.2 MHz (23.5 kG). In the latter work, the use of samples highly enriched in ^{13}C permitted the observation of splittings arising from ^{13}C–^{13}C scalar coupling, which were helpful in assigning the resonances to specific carbons. However, these splittings greatly complicated the spectra and reduced the number of identifiable peaks.

When the percentage of the ^{13}C isotope is small, each carbon yields a single peak (though not necessarily resolved from others) in a proton-decoupled ^{13}C nmr spectrum. There are 55 carbons, all of them nonequivalent, in the chlorophyll a molecule (Figure 1). It seems that when dealing with a system of this complexity, samples of the naturally occurring isotopic composition (1.1% ^{13}C) are most attractive for ^{13}C nmr studies, in terms of spectral simplicity and ease of sample preparation, provided that there is sufficient spectrometer sensitivity for detecting the ^{13}C resonances.

Many resonances in the proton-decoupled ^{13}C nmr spectrum of chlorophyll a have already been assigned to specific carbons.[2,6] However, only carbons 1, 2,

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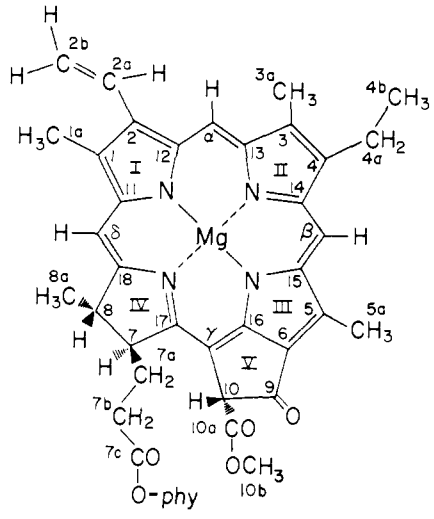

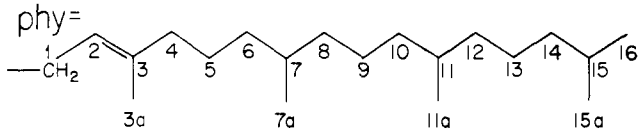

Figure 1. Structure of chlorophyll a.

and 3 of the phytyl chain have been assigned.[2] The specific assignment of most phytyl carbon resonances appears difficult at first glance because of their structural similarity (Figure 2A).

We have recorded the proton-decoupled natural-abundance ^{13}C nmr spectra of phytol (Figure 2A), phytyl acetate, and chlorophyll a at 15.18 MHz (14.2 kG). Even at this low resonace frequency, most carbons of these compounds yield resolved single-carbon resonances. We have specifically assigned nearly every resolved single-carbon resonance of phytol with the use of known chemical shifts of hydrocarbons,[7] partially relaxed Fourier transform (PRFT) nmr spectra,[8,9] and comparisons with the spectra of pristane (Figure 2B) and of a phytol–pristane mixture. We assigned the resonances of phytyl acetate with the use of single-frequency off-resonance proton decoupling[10] and comparisons with the phytol assignments. Finally, we assigned the phytyl resonances of chlorophyll a by means of a comparison with the spectrum of phytyl acetate.

Experimental Section

Chlorophyll a was prepared using the method of Jacobs, Vatter, and Holt[11] with the following modifications: large quantities of sodium chloride were used to prevent emulsion formation in the extraction stages; the methanol washes were reextracted three times using petroleum ether; the ether solutions were dried over anhydrous Na_2SO_4 prior to the evaporation of solvent; and kale and lettuce were used in addition to spinach. Phytol (approximately 97%) was obtained from Sigma Chemical Company, St. Louis, and

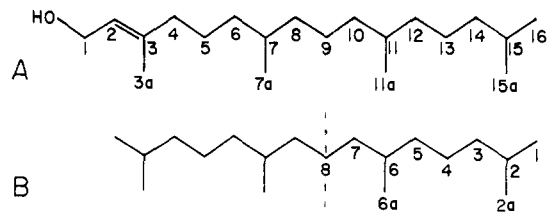

Figure 2. (A) Structure of phytol. (B) Structure of pristane.

was used as received for chemical shift measurements. For PRFT measurements, the phytol was purified by fractional distillation. Phytyl acetate was prepared by adding a solution of acetyl chloride in chloroform to a solution of phytol in pyridine at 0°. It was purified by chromatography on a column of alumina. 2,6,10,14-Tetramethylpentadecane (pristane) was purchased from Aldrich Chemical Co., Milwaukee, Wis.

The chlorophyll sample consisted of approximately 1.45 g of chlorophyll a in 7.9 ml of chloroform (without stabilizers) and 0.6 ml of methanol. The methanol was added to suppress aggregation.[8] The solvents were degassed before use. All chlorophyll spectra were obtained in 20-mm (o.d.) sample tubes.[12] Chemical shifts of chlorophyll were measured digitally with respect to the ^{13}C resonance of chloroform, which was taken at 77.3 ppm downfield from tetramethylsilane (TMS). Chemical shifts of phytol, phytyl acetate, and pristane were measured digitally with respect to an internal TMS standard, using chloroform solutions in 20-mm sample tubes. The PRFT spectra of neat phytol were obtained in 13-mm (o.d.) sample tubes.

The Fourier transform nmr equipment has been described elsewhere,[9,12,13] except that most ^{13}C nmr spectra were recorded before the incorporation of some of the instrumental improvements described in ref 13. Thus, the signal-to-noise ratios are not representative of the current performance of our Fourier transform nmr system.

Results and Discussion

Proton-decoupled natural-abundance ^{13}C nmr spectra of phytyl acetate and chlorophyll a are shown in Figure 3. The chlorophyll peaks (Figure 3B) are numbered consecutively, starting downfield. The phytyl acetate resonances and their assignments (see below) are shown in Figure 3A. We use the phytyl carbon designations of Figure 2A.

In Figure 4A we show the upfield region of the spectrum of phytol. Chemical shifts are given in Table I. It should be noted that even at our low resonance frequency of 15.18 MHz, only carbons 7a and 11a do not yield individual resonances. We show below that all but two of the 18 resolved single-carbon resonances can be assigned to specific carbon atoms. Even the resonances of C-8 and C-10, which are separated by less than 0.1 ppm, can be assigned on a one-to-one basis.

Phytol Assignments. The resonance at 19.80 ppm (Figure 4A) was identified as the only two-carbon peak on the basis of its intensity. Specific assignments were made with the use of reported chemical shift parameters of model compounds,[7,14,15] comparisons with the ^{13}C spectrum of pristane, and ^{13}C spin–lattice relaxation times of individual carbons.

Chemical shift variations of alkane carbons have been condensed by Grant and Paul into a set of empirical parameters.[7] These parameters yield calcu-

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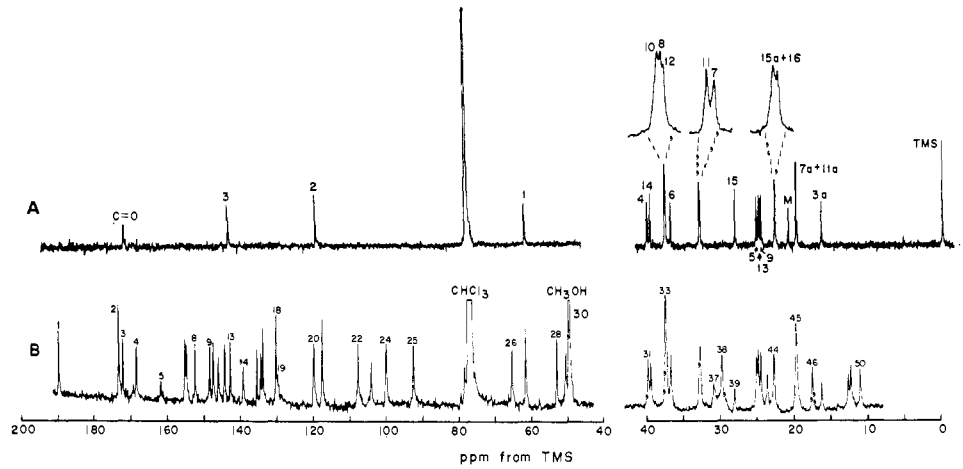

Figure 3. Proton-decoupled natural-abundance ^{13}C Fourier transform nmr spectra at 15.18 MHz. (A) Spectrum of 0.56 *M* phytyl acetate in chloroform (with TMS), recorded at 60°, after 16 accumulations with a recycle time of 90 sec and 16,384 points in the time domain. Spectral widths of 3048.78 Hz (0.372-Hz digital resolution) and 1000 Hz (0.122-Hz digital resolution) were used for the downfield region and upfield region, respectively. Phytyl peak assignments are given using the carbon designations of Figures 1 and 2A. Peak M is the acetate methyl carbon resonance. The inserts shown above some peaks were printed with an eightfold expansion of the horizontal scale. (B) Spectrum of 1.45 g of chlorophyll a in 7.9 ml of chloroform and 0.6 ml of methanol, recorded at 35°, after 1024 accumulations with 4096 points in the time domain, using spectral widths of 250 (downfield) and 52.5 ppm (upfield) and recycle times of 8.44 (downfield) and 2.32 sec (upfield).

Table I. ^{13}C Nmr Parameters of Phytol and Pristane

Assignment		Chemical shift[a]				
Phytol[b]	Pristane[c]	Phytol[d]	Mixture[e]	Pristane[f]	Estimated[g]	NT_1[h]
1		59.39	59.44		59.0[i]	0.80
2		123.4[j]	*k*		124.8[i]	0.56
3		139.9[j]	*k*		137.6[i]	4.8[l]
4		39.95	39.96		40.0[i]	0.62
5		25.28	25.28		25.1[m]	0.64
6		36.80	36.80		37.3[n]	0.58
7		32.80	32.81		32.0[o]	0.45
8		37.49	37.50		37.3[n]	0.60[p]
9	8	24.56	24.56	24.58	25.1	0.62
10	7	37.55	37.57	37.60	37.3	0.60[p]
11	6	32.89	32.90	32.92	32.0	0.53
12	5	37.42	37.43	37.44	37.2	0.79
13	4	24.85	24.86	24.89	24.7	1.2
14	3	39.50	39.50	39.53	39.4	1.7
15	2	28.04	28.05	28.07	28.3	1.8
3a		16.17	16.18		16.3[i]	7.1
7a,11a	6a	19.80	19.80	19.82	19.9	2.8
15a, 16	2a, 1	22.64, 22.72	22.64, 22.72	22.66, 22.74	22.0	6.0

[a] In ppm downfield from internal TMS. Unless otherwise stated, experimental values were obtained using a spectral width of 1000 Hz and 16,384 addresses in the time domain, resulting in a digital resolution of 0.122 Hz (0.008 ppm). [b] Carbon designations are those of Figure 2A. [c] Carbon designations are those of Figure 2B. [d] 0.55 *M* phytol in chloroform at 48°. [e] 0.32 *M* phytol and 0.12 *M* pristane in chloroform at 48°. [f] 0.75 *M* pristane in chloroform at 50°. [g] Estimated chemical shifts of phytol. Unless otherwise stated, calculated by the method of Grant and Paul.[7] The constant term was taken as −2.5 ppm.[14] [h] NT_1 (in sec) of neat phytol at 52°. In the case of the nonprotonated carbon 3, the T_1 value is given. Estimated accuracy is ±10% for well-resolved resonances and somewhat less for partly resolved ones. [i] Chemical shift of a structurally similar carbon of geraniol.[14,15] [j] Obtained with a spectral width of 3048.78 Hz and 8192 points in the time domain, resulting in a digital resolution of 0.744 Hz (0.049 ppm). [k] Not measured. [l] T_1 (in sec) of a nonprotonated carbon. [m] Based on the structural similarity of this carbon and C-9. [n] Based on the structural similarity of this carbon and C-10. [o] Based on the structural similarity of this carbon and C-11. [p] C-8 and C-10 do not yield resolved resonances in the spectrum of neat phytol. The given T_1 value was calculated from the PRFT behavior of the two-carbon peak.

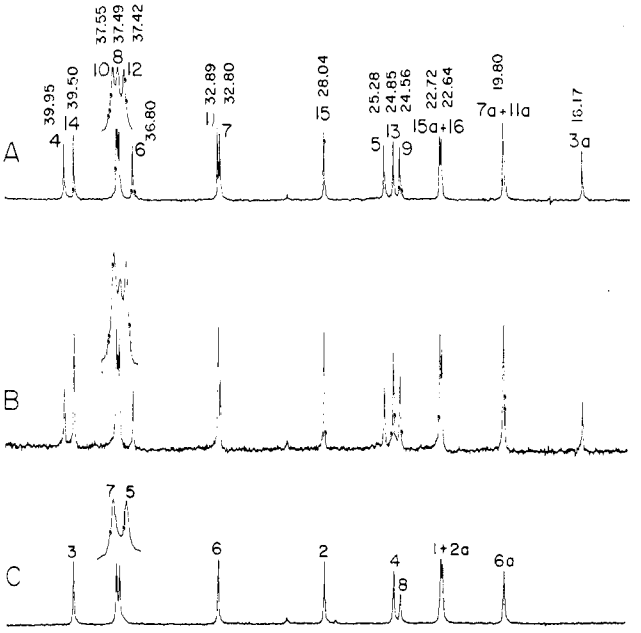

Figure 4. Upfield region (about 14 ppm to about 43 ppm downfield from TMS) in the proton-decoupled natural-abundance ^{13}C Fourier transform nmr spectra of phytol and pristane in chloroform at 48°, recorded at 15.18 MHz using a 1000-Hz spectral width and 16,384 points in the time domain (0.122-Hz digital resolution). The inserts shown above some resonances were printed with a fourfold expansion of the horizontal scale. (A) 0.55 *M* phytol, after 32 accumulations, with a recycle time of 31.6 sec. Large numbers are assignments, using the carbon designations of Figure 2A. Small numbers are chemical shifts, in ppm, with respect to internal TMS. (B) A solution 0.32 *M* in phytol and 0.12 *M* in pristane, recorded in the same way as spectrum A above. (C) 0.78 *M* pristane, after 16 accumulations, with a recycle time of 60 sec. Assignments are indicated using the carbon designations of Figure 2B. Spectra A and B do not show the resonances of C-1, C-2, and C-3 of phytol, at 59.39, 123.4, and 139.9 ppm, respectively.

lated chemical shifts within about ±1 ppm of the experimental values. The parameters of Grant and Paul can be used to estimate the ^{13}C chemical shifts of those phytol carbons that are far removed from the hydroxyl group and from the olefinic carbons. The calculated values for carbons 9–16, 11a, and 15a are given in Table I. The estimated chemical shifts of carbons 5, 6, 7, 8,

and 7a (Table I) are based on their structural similarity with carbons 9, 10, 11, 10, and 11a, respectively. The estimated values for carbons 1–4 and 3a are the experimental values of structurally similar carbons of geraniol.[14,15]

The ^{13}C spin–lattice relaxation of protonated carbons in a large molecule such as phytol is overwhelmingly dominated by ^{13}C–^{1}H dipole–dipole interactions with the directly bonded hydrogens.[9] T_1 is given by

$$1/(NT_1) = \hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\tau_{eff} \quad (1)$$

where N is the number of directly attached hydrogens, γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^{1}H, and τ_{eff} is the effective correlation time for rotational reorientation of the C–H groups. Equation 1 applies in the "extreme narrowing" limit (that is, when $1/\tau_{eff}$ is much greater than the ^{1}H-resonance frequency), a condition that is satisfied here.[9] As a result of the invariance of C–H bond lengths,[16] NT_1 gives a direct measure of $1/\tau_{eff}$, the effective rate of segmental motion. Doddrell and Allerhand[17] have used ^{13}C T_1 measurements to monitor the decrease in segmental mobility, caused by hydrogen bonding, when going from the methyl end to the hydroxyl end of neat liquid 1-decanol. It was pointed out that NT_1 values of individual protonated carbons could be used to make specific assignments of ^{13}C resonances of hydrocarbon chains with restricted mobility at one end.[17] In *neat* liquid phytol, as in neat 1-decanol, intermolecular hydrogen bonding should restrict rotational motions at the hydroxyl end of the molecule. NT_1 of protonated carbons of neat phytol should get progressively longer when going away from C-1. We have measured the ^{13}C T_1 values of all resolved resonances in the ^{13}C spectrum of neat phytol by means of PRFT spectra.[8] Details of the procedure have been reported.[9] The resulting NT_1 values are given in Table I.

The 20 carbons of phytol yield 19 resolved resonances. A comparison of the structures of phytol (Figure 2A) and pristane (Figure 2B) strongly suggests that carbons 9–16, 11a, and 15a of phytol should have nearly identical chemical shifts with those of similar carbons of pristane. A comparison of the ^{13}C chemical shifts of phytol and pristane, under the same conditions of bulk magnetic susceptibility, can be used to identify phytol resonances that are not present in the spectrum of pristane. These resonances can then be assigned to carbons that are to the left of C-9 in Figure 2A. In Figure 4C we show the ^{13}C spectrum of pristane in chloroform. Chemical shifts measured with respect to internal TMS (Table I) clearly indicate that the phytol resonances that will be assigned to carbons 1–8 and 3a (see below) are absent from the pristane spectrum and that all other phytol resonances are present in the spectrum of pristane. A verification of this result can be seen in Figure 4B, which shows the saturated-carbon region of the spectrum of a solution of phytol and pristane in chloroform. The stronger peaks are the sums of phytol and pristane resonances. The weaker peaks are just phytol resonances. The intermediate amplitude of the peak arising from C-9 of phytol plus C-8 of pristane is due to the fact that C-8 of pristane has half the intensity of the other pristane carbons.

The estimated chemical shifts based on model compounds[14,15] and on the empirical parameters of Grant and Paul[7] (Table I) yielded the specific assignments of phytol carbons 1–3, 15, and 3a (Table I). Also on the basis of estimated chemical shifts, the two-carbon resonance at 19.80 ppm was assigned to C-7a and C-11a. The peaks at 22.64 and 22.72 ppm were assigned to C-15a and C-16 but not on a one-to-one basis. The estimated chemical shifts were sufficient to assign C-4 and C-14 to the peaks at 39.95 and 39.50 ppm but not on a one-to-one basis. However, the resonance at 39.50 ppm had a T_1 about three times that of the other resonance and it was therefore assigned to C-14. The estimated chemical shifts also indicated that the group of three resonances at 24.56, 24.85, and 25.28 ppm corresponds to carbons 5, 9, and 13. Of these three peaks, only the one at 25.28 ppm is absent from the spectrum of pristane (Figure 4C), and it is thus assigned to C-5. The resonance at 24.85 ppm has a T_1 value that is twice that of the one at 24.56 ppm. Therefore, the former is assigned to C-13 and the latter to C-9.

On the basis of estimated chemical shifts, C-7 and C-11 correspond to the pair of resonances at 32.80 and 32.89 ppm. The peak at 32.80 ppm is absent from the spectrum of pristane (Figure 4C) and is therefore assigned to C-7.

By elimination, the remaining four unassigned resonances, one at 36.80 ppm and a closely spaced group of peaks at 37.42, 37.49, and 37.55 ppm, must be those of carbons 6, 8, 10, and 12, as the estimated chemical shifts indeed predict. Only the resonance at 36.80 ppm and the central peak of the triplet are absent from the spectrum of pristane (Figure 4C). Therefore, these are the resonances of C-6 and C-8. The outer components of the triplet are the resonances of C-10 and C-12. The great structural similarity of C-8 and C-10 requires that these resonances be assigned to two peaks of the closely spaced triplet. Thus, C-6 is assigned to the resonance at 36.80 ppm, and C-8 is assigned to the central component of the triplet. The assignment of the resonance at 37.42 ppm (upfield component of the triplet) to C-12 is based on its relatively long T_1 value (Table I). Even though the differences in the T_1 values of all four resonances are barely outside experimental error, the PRFT patterns clearly indicate that the resonance assigned to C-12 has the longest T_1 value. For example, in the PRFT spectrum with a τ value (interval between a 180° radiofrequency pulse and the following 90° pulse[18]) of 211 msec, the resonance assigned to C-12 is the only one of this group that has a negative intensity. Finally, by elimination, the downfield component of the triplet (at 37.55 ppm) is assigned to C-10.

The phytol assignments yield corresponding assignments in the ^{13}C spectrum of pristane (Table I and Figure 4C). Our pristane assignments are in agreement with those reported without proof by Lindeman and Adams,[19] except for an inversion in the assignments for C-5 and C-7.

Segmental Motion in Phytol. In Figure 5 we show a map of the NT_1 values of the protonated carbons and the T_1 value of the lone nonprotonated carbon of neat

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phytol. In neat 1-decanol, the values of NT_1 decrease monotonically toward the hydroxyl end of the molecule.[17] The situation is more complicated when dealing with phytol. In the first place, T_1 values of nonprotonated carbons are much longer than those of protonated ones which have comparable values of τ_{eff}.[9] For this reason the T_1 of C-3 is much longer than that of its protonated neighbors on the main chain. Secondly, branching along a hydrocarbon chain and the presence of an olefinic bond cause relatively localized changes in the barriers to internal rotation about C–C bonds.[20] As a result, even though the general trend is for $1/\tau_{eff}$ (and NT_1) of the phytol carbons to increase when going from C-1 to C-16, there are localized deviations (Figure 5). For example, C-1 and C-3a have longer NT_1 values than C-2 and C-7a, respectively, as a result of the relatively low barrier to internal rotation in the vicinity of an olefinic carbon.[20] Also, C-7 and C-11 have shorter NT_1 values than C-6 and C-10, respectively, because of increased restrictions to internal rotation accompanying methyl substitution.[20] Obviously, caution is required when using ^{13}C T_1 values for assigning ^{13}C resonances of complex hydrocarbon chains. In our work on phytol, we have used ^{13}C NT_1 values only to distinguish structurally similar carbons, such as C-10 and C-12.

Phytyl Acetate Assignments. Phytol is a good model compound for assigning only those phytyl resonances of chlorophyll a which arise from carbons that are not close to the polar end of the chain. Esterification should significantly affect the chemical shifts of C-1 and the olefinic carbons. Therefore, phytyl acetate is a better model compound for our purpose than phytol. The proton-decoupled ^{13}C spectrum of phytyl acetate in chloroform is shown in Figure 3A. The saturated-carbon resonances were readily assigned by comparing this spectrum with that of phytol. The peak at 170.6 ppm was assigned to the carbonyl carbon, on the basis of its chemical shift.[14] The resonances at 118.5 and 142.4 ppm were assigned to C-2 and C-3 of the phytyl chain, respectively, on the basis of their chemical shifts[14] and their behavior in single-frequency off-resonance proton-decoupling experiments.[10] C-1 (at 61.39 ppm), C-2, and C-3 are the only carbons with significantly different chemical shifts in phytyl acetate and in phytol. C-3a is displaced downfield by about 0.2 ppm when going from phytol to phytyl acetate. All other phytyl acetate resonances are within 0.1 ppm from the corresponding peaks of phytol.

Assignments of ^{13}C Resonances of Chlorophyll a. The proton-decoupled natural-abundance ^{13}C spectrum of chlorophyll a in a chloroform–methanol solvent mixture is shown in Figure 3B, with the peaks numbered consecutively, starting downfield. The chemical shifts are given in Table II. Chlorophyll carbon designations are those of Katz and Janson[2] (Figure 1). A prefix P is used to designate carbons of the phytyl chain and a prefix C for all other carbons.

The 25 unsaturated carbons of chlorophyll a give rise to 25 fully or partly resolved resonances downfield from the chloroform peak. A comparison with the spectrum of phytyl acetate (Figure 3A) indicated that the peak at 143.0 ppm (peak 13 in Figure 3B) is the resonance of P-3. This assignment is in disagreement with the reported one[2] at 131.9 ppm. Off-resonance single-frequency decoupling indicated that peak 20 is a methylene carbon resonance; peaks 18, 21, 22, 24, and 25 are methine resonances, and all other peaks downfield from $CHCl_3$ are nonprotonated carbon resonances. Thus, peak 20 must be assigned to C-2b. Peaks 22, 24, and 25 have already been assigned[2,6] to C-β, C-α, and C-δ, respectively. Peak 18 (at 130.4 ppm) and peak 21 (at 117.7 ppm) must be the resonances of the only remaining unsaturated methine carbons, C-2a and P-2. Carbon 2 of phytyl acetate resonates at 118.5 ppm. Therefore, peak 21 must be assigned to P-2. By elimination, peak 18 is the resonance of C-2a. Peak 1 has been assigned[2] to C-9, the only keto carbonyl, on the basis of known ^{13}C chemical shifts.[14] No additional specific assignments in the unsaturated carbon region

(20) J. P. Lowe, "Progress in Physical Organic Chemistry," Vol. 6, Interscience, New York, N. Y., 1968, pp 1–80.

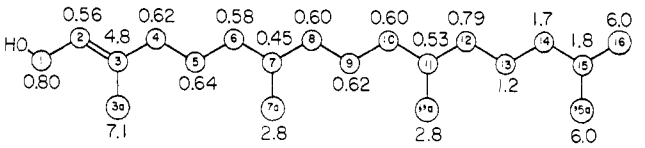

Figure 5. NT_1 map for neat phytol at 52°. The values of NT_1 (large numbers, in sec) are shown for all carbons except for C-3, whose T_1 value is indicated. Small numbers are carbon designations of Figure 2A.

Table II. ^{13}C Chemical Shifts of Chlorophyll a

Peak[a]	Chemical shift[b]	Assignment[c]	Peak[a]	Chemical shift[b]	Assignment[c]
1	189.9	C-9[d,e]	26	65.3	C-10[d,e]
2	173.5	*f*	27	61.5	P-1
3	172.4	*f*	28	53.0	C-10b[d,e]
4	168.7	*g*	29	50.4	C-7[h]
5	161.9	*g*	30	49.7	C-8[h,i]
6	155.5	*g*	31	39.8	P-4
7	155.1	*g*	32	39.4	P-14
8	152.7	*g*	33	37.4	P-10, P-8, P-12
9	148.6	*g*	34	36.6	P-6
10	147.6	*g*	35	32.8	P-11
11	146.2	*g*	36	32.7	P-7
12	144.5	*g*	37	30.9	C-7a
13	143.0	P-3	38	29.8	C-7b
14	139.3	*g*	39	28.0	P-15
15	135.7	*g*	40	25.0	P-5
16	134.6	*g*	41	24.8	P-13
17	134.0	*g*	42	24.4	P-9
18	130.4	C-2a	43	23.6	C-8a[d]
19	129.8	*g*	44	22.7	P-15a, P-16
20	119.9	C-2b	45	19.7	C-4a, P-7a, P-11a
21	117.7	P-2	46	17.5	C-4b[e]
22	107.8	C-β[d,e]	47	16.2	P-3a
23	104.2	C-γ[d,e]	48	12.6	C-1a, C-5a
24	100.1	C-α[d,e]	49	12.3	
25	92.6	C-δ[d,e]	50	11.0	C-3a[d,e]

[a] Peak numbers are those of Figure 3B. [b] In ppm downfield from TMS, obtained from a spectrum of 1.45 g of chlorophyll a in 7.9 ml of chloroform and 0.6 ml of methanol, at 35°, using a spectral width of 250 ppm and 4096 points in the time domain (digital resolution of 0.122 ppm). Estimated accuracy is ±0.2 ppm. [c] Carbon designations are those of Figure 1. A prefix C designates a nonphytyl carbon, and prefix P refers to a carbon of the phytyl chain. [d] Assignment taken from ref 2. [e] Assignment taken from ref 6. [f] Probably C-7c or C-10a. [g] Nonprotonated unsaturated carbon of the ring system. [h] Specific assignments of C-7 and C-8 are based on reported assignments for chlorophyll a in dioxane.[2] Because of possible solvent effects (see text), our assignments may be reversed. [i] Coincident with strong methanol resonance (see text).

can be extracted from the available information. A comparison of our ^{13}C chemical shifts of chlorophyll a with those of Katz and Janson[2] indicates that there are appreciable solvent effects on the unsaturated carbon chemical shifts. For example, the resonance of C-2b (peak 20 in Figure 3B) is downfield from that of P-2 (peak 21). In dioxane, the reverse has been reported.[2]

The 30 saturated carbons of chlorophyll a give rise to the 25 resonances upfield from chloroform (peaks 26–50 in Figure 3B). Peak 26 has been assigned to C-10.[2,6] Assignment of the rest of the saturated-carbon region is greatly facilitated by a comparison with the spectrum of phytyl acetate (Figure 3A). Peak 27 must be assigned to P-1. Peaks 31–36, 39–42, 44, 45, and 47 contain phytyl carbon resonances whose assignments (Table II) follow directly from the phytol assignments (Table I). Peak 45 is a three-carbon resonance, but only two phytyl carbons contribute to it. The 12 saturated carbons of chlorophyll a other than those of the phytyl group give rise (Figure 3B) to peaks 26, 28, 29, 37, 38, 43, 46, 48, 49, and 50 and one-carbon contributions to peaks 45 and 30. Peak 30 contains a chlorophyll resonance coincident with a strong methanol peak. The presence of a chlorophyll resonance at this position was inferred from spectra of chlorophyll a in dioxane[2] and in pure chloroform.[21] We have listed above a total of 12 peaks for 12 carbons. Variations in peak heights arise from differences in line widths and from partial saturation of some resonances that have long T_1 values (because of the relatively short recycle time used for recording the upfield region of the spectrum). Peaks 26, 28, 29, 30, 43, 46, and 50 can be assigned[2,6] to C-10, C-10b, C-7, C-8, C-8a, C-4b, and C-3a, respectively. However, the specific assignments of peaks 29 and 30 are tentative and could turn out to be inverted. Peaks 37 and 38 arise from carbons 7a and 7b.[2] Katz and Janson[2] specifically assigned peak 37 to C-7a but only tentatively. We confirmed this assignment by means of T_1 measurements. Resonance 37 has a much shorter relaxation time (about 0.18 sec) than resonance 38 (about 0.34 sec). C-7a should have a relatively short T_1 value because it is directly anchored to a bulky ring system.[9] C-7b, with its additional degree of internal rotation, should have a longer T_1 than C-7a.[9] The nonphytyl component of peak 45 can be assigned to C-4a, on the basis of the ^{13}C spectrum of methyl pheophorbide a.[2,5] Peaks 48 and 49 have been assigned to C-1a and C-5a but not on a one-to-one basis.[2]

(21) R. A. Goodman and A. Allerhand, unpublished results.

Acknowledgment. This research was supported by the National Science Foundation (Grant GP-17966), by the donors of the Petroleum Research Fund, administered by the American Chemical Society, by the National Institutes of Health (Grant NS-10977), and by Eli Lilly and Co. One of us (E. O.) thanks the European Molecular Biology Organization and the Gilbert Foyle Trust of Great Britian for partial support. We thank Dr. D. Brune for his helpful suggestions about preparation of chlorophyll. We thank Dr. J. J. Katz for making available data in advance of publication.

Attractive Nonbonded Interactions in 1-Substituted Propenes. Consequences for Geometric and Conformational Isomerism

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Abstract: Heteroatoms at the 1 position of propene can interact with the methylene group through space and through the olefinic bond. This interaction can be attractive in nature and responsible for both the lower energy of the cis isomer relative to the trans isomer and also the lower rotational barrier of the cis isomer relative to the rotational barrier of the trans isomer. The proposed model is tested by SCF–MO semiempirical calculations and the qualitative generalizations are found to be in good accord with the observed experimental trends.

Organic chemistry provides a number of instances where one cannot invoke the concept of steric or nonbonded repulsion to predict the relative stabilities of geometrical isomers of 1-substituted propenes. Various cases illustrating the general preference for the cis over the trans isomer of substituted propenes are listed in Table I. Furthermore, rotational isomerism in 1-substituted propenes is characterized by certain intriguing regularities. Specifically, it is found that in such molecules the rotational barrier of the cis isomer is significantly lower than the rotational barrier of the trans isomer. Experimental data pertaining to this interesting effect have been collected in an excellent

Table I. Equilibrium Composition of Cis and Trans Isomers of 1-Substituted Propenes

Compd	% cis at equil	Ref
$CH_3CH{=}CHOMe$	71	*a*
$CH_3CH{=}CHOEt$	81	*a*
$CH_3CH{=}CHOPh$	65	*b*
$CH_3CH{=}CHCl$	76	*c*
$CH_3CH{=}CHBr$	68	*d*

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