

Carbon-13 Fourier Transform Nuclear Magnetic Resonance. VIII. Role of Steric and Electric Field Effects in Fatty Acid Spectra

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From the carbon-13 spectra of over 40 fatty acids and their derivatives, a set of substituent shift parameters have been derived which *uniquely* define saturated, unsaturated, polyunsaturated, or cyclopropane fatty acids. Together with model compounds and selectively enriched compounds, lanthanide shift reagents were used to confirm carbon-13 shift assignments. Carbon-13 substituent shifts are interpreted in terms of steric and linear electric field effects and a rationale is given for linear electric field effects. Qualitative agreement between calculated electric field shifts for saturated carbons and the observed shifts has been obtained. Since both steric and linear electric field effects show both angle and distance dependence, they should be considered powerful tools for conformational as well as structural analysis.

Carbon-13 chemical shifts are reported for over 40 fatty acids, their methyl esters, triglycerides, and related compounds. Interest in these molecules derives from several sources, among which are their roles as an energy store for animal life and as a major class of tissue components. In their free acid form they are intermediates in fat transport;² in combined form they represent a very high proportion of fat deposits,² lipoprotein,^{2,3} and biological membrane material.⁴

In this study we have been able to derive a set of substituent shift parameters which describes the shifts of all the carbons in fatty acid chains such that the complete structures of unknown fatty acids may now be determined *solely* from their ¹³C spectra. Application of these substituent shifts will prove useful in the analysis of fatty acid components in biological tissues.

The present study has delineated the significant roles of steric and electric field effects in producing the observed shift differences between alkane and fatty acid spectra. The shifts of the unsaturated carbons, in particular, are strongly dependent on electric field effects and hence on conformation and solvent properties.⁵ The linear electric field effects can be estimated in a simple way, giving good qualitative agreement with our observed values. We have also, in specific instances, been able to ascribe individual shifts induced by substituent changes to a conformational mechanism. Such information will help to further the application of ¹³C shift measurements to conformational analysis⁶ as well as the structural and analytical problems considered here.

Experimental Section

The natural abundance fatty acids, methyl esters, triglycerides, and alcohols used in the present research were obtained from Nu-Chek Prep, Analabs, the Hormel Institute, and Farhan Research Labs. Alkanes were obtained from Eastman Kodak and alkenes from Nu-Chek Prep and the Chemical Samples Co. All were used without further purification. Methyl *cis*-6,7-methyleneoctadecanoate and *cis*-4,5-methylenedecene (*cis*-1-propyl-2-pentylcyclopropane) were synthesized from the corresponding olefins by the method of Rawson, *et al.*⁷ ¹³C-Enriched methyl esters were prepared biosynthetically as described elsewhere.⁸

In general, 500-mg samples were made up in 1.5 ml of a 10% solution of hexamethyldisilane (HMDS, as internal standard), in chloroform and were run in 10-mm tubes. Hexafluorobenzene in a coaxial 5-mm tube provided a heteronuclear field/frequency lock. In the shift reagent studies and some early measurements (see footnotes to Table I) hexafluorobenzene was added internally. For shift studies the internal lock was necessary to avoid severe side-band problems.

Spectra were run on a 22.6-MHz computer-controlled Fourier transform system which has been described previously.⁹ Except where otherwise noted an 8K data set was used. Under these conditions, computer-measured chemical shifts are correct to ± 0.05

ppm and narrow peaks as close as 0.10 ppm can be resolved. (When a 4K data set was used the shifts are quoted to ± 0.1 ppm). In general it was found that differential solvent effects could be minimized by measuring the shifts relative to the ω -1¹⁰ carbon of the chain, since methyl carbons, including those of TMS and HMDS, are more solvent dependent than methylenes. The shift on an HMDS scale is then given by the relation $\delta_C = \delta_{C\omega-1} + 25.20$ ppm (downfield shifts positive; upfield shifts negative). We have found that 25.20 ± 0.05 ppm is the chemical shift of the ω -1 carbon of a saturated straight-chain fatty acid under our standard solvent conditions. When the solvent conditions were the same but one of the α , β , γ , or δ substituents was not a methylene, the shifts were measured directly with respect to HMDS. If the solvent conditions were also different, then another carbon was chosen for referencing and compared with the corresponding carbon of a fatty acid in which the reference carbon had the same α , β , γ , and δ substituents. Results can be converted to a TMS scale, $\delta_{TMS} = \delta_{HMDS} - 2.34$ ppm.

Results

The chemical shifts of representative compounds we have studied are shown in Table I.¹¹ In almost all cases unambiguous assignments have been made. We have set a confidence limit for our ¹³C assignments of 0.20 ppm; hence, where peaks are resolved but their separation is ≤ 0.15 ppm, the shift values have been placed in parentheses.

The well-resolved resonances in fatty acyl chains, C₁ (carbonyl), ester methyl, C₂, C₃, ω (terminal methyl), ω -1, and ω -2 have been assigned previously.^{12,13} The other fatty acid chain carbons were assigned consistent with the substituent shifts presented in Tables II-IV. In establishing these substituent shifts, long-chain alkanes and alkenes (Table V), short-chain saturated fatty acids, and methyl esters ¹³C enriched at odd carbon positions were studied as model compounds. In addition, linear electric field theory, lanthanide induced shifts, and data on analogous compounds by other workers were used.¹⁴⁻¹⁷

In triglycerides the chain resonances were essentially those found in isolated chains except that two peaks each were observed for C₁ and C₂ (Table I). These clearly represent the corresponding carbons on chains 1 and 3 (intensity 2) and on chain 2 (intensity 1).

The two resonances of the cyclopropane ring carbons (Table I) were assigned on the basis of their large upfield shifts and 2:1 intensity ratio. The carbons α to a trans ring were assigned by their lack of a γ upfield shift. The substituent shifts at positions near a *cis* ring were derived by comparison of pairs of resonances in the spectrum of *cis*-4,5-methylenedecene.

Lanthanide Induced Shifts. Figure 1 shows the lanthanide induced shifts (LIS) of the carbons near the carboxyl end of methyl petroselinate plotted against their

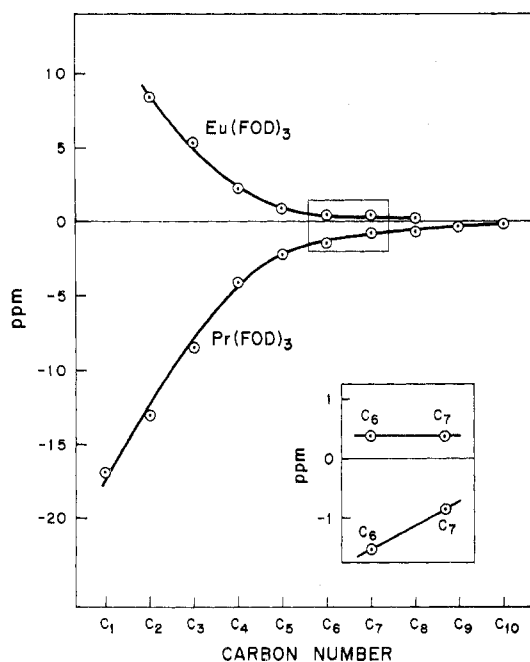


Figure 1. Carbon-13 LIS of methyl petroselinate, 0.5-mmol concentrations of ester and lanthanide. Note: for consistency we have defined a downfield LIS positive; upfield LIS negative.

position in the chain for 0.5 mmol shift reagent and ester. All of the saturated carbons fit a smooth curve with LIS decreasing monotonically with carbon position. From the magnitude of the LIS values we were clearly able to assign the C_5 and C_8 allylic positions. Using $\text{Eu}(\text{fod})_3$, the LIS values for the unsaturated carbons C_6 and C_7 were found to be identical (0.4 ppm) (Figure 1). This behavior could be explained by a slight increase in the electric field from $-\text{COOMe}$ in the complex cancelling the slightly greater LIS of C_6 . When the upfield shift reagent, $\text{Pr}(\text{fod})_3$, was used (Figure 1), C_6 showed 0.6 ppm greater LIS than C_7 . Since the LIS and electric field effect for C_6 would in this case be in the same direction, the assignments of the unsaturated carbons of methyl petroselinate are confirmed.

Although lanthanide shift reagents are valuable tools for ^{13}C chemical shift assignments, failure of the Eu agent above stresses the fact that, in cases where electric fields or related effects are large, they must be used with caution.

Olefin Shifts. Unsaturated carbons were assigned by electric field theory (see Discussion) and by the substituent shifts shown in Tables III and IV. The unsaturated carbon chemical shifts of monoenoic long-chain fatty acids can be calculated as follows.

$$\delta_{\text{C}}(\text{cis}) = 132.40 \pm \Delta\delta/2 \quad (1)$$

$$\delta_{\text{C}}(\text{trans}) = 132.85 \pm \Delta\delta/2 \quad (2)$$

The shift for the carbon nearest the head group has the smaller value. The values of $\Delta\delta$ are given in Table IV. Slightly smaller values are observed for the corresponding methyl esters. Both the mean shift and the nonequivalence, $\Delta\delta$, are solvent dependent.

Carbon-13 substituent shifts due to other double bonds appear in Table IIIB. Most of these values were derived by comparison of the spectra of mono-, di-, tri-, and tetraenes (Table I) and refer to the normal configuration of double bonds found in fatty acids, *i.e.*, cis bonds separated by single methylenes.

The substituent shifts of double bonds on saturated carbon resonances (Table IIE) were derived from the spectra of alkenes,^{15,16} including those in Table I. Accordingly, we have assigned the resonance at 29.80 ppm in methyl

palmitoleate to C_8 and C_{11} . These allylic carbons are shifted upfield 2.45 ppm from the corresponding carbons in an alkane by a steric interaction due to the cis configuration of the double bond. Where carbons α to a double bond were found to be nonequivalent they were assigned by electric field theory. These assignments were verified by our lanthanide shift studies. Carbons allylic to two cis double bonds were assigned by virtue of an extra upfield steric shift of 1.6 ppm.

The results of this study have been useful in reassigning published ^{13}C spectra of several fatty acids and alkenes,^{16,18,19} and in the assignments of methyl 9,11-linoleate as *trans,trans*- and methyl tetradecenoate as *cis*-7 (Table I).

Discussion

Carbon-13 chemical shifts are, to a very large degree, additive, especially in unbranched chains; so any fatty acid spectrum can be considered as a modification of the spectrum of a long chain alkane due to, first, the polar head group and second, the addition of various substituents (olefin, hydroxy, cyclopropane) along the chain. Therefore, the chemical shifts of any fatty acid or methyl ester, under our specific solvent conditions, can be predicted by taking the shifts of the corresponding alkane or olefin (Table V) and adding the substituent shifts in Tables II-IV. The spectra of fatty acids and their methyl esters differ mainly in the C_1 and C_3 resonances. The spectra of corresponding methyl esters (or fatty acids) can be obtained using the substituent shifts for $-\text{COOH}$ or for $-\text{COOMe}$ shown in Table IIB. These shifts are listed relative to the corresponding alkane; *i.e.*, the C_1 substituents are the two oxygens and not a carboxyl. Substituent shifts for branched chains are not available for our solvent system but have been presented by Lindeman and Adams¹⁶ for a different system.

Several authors have listed sets of ^{13}C shift parameters obtained by a regression analysis of their total data on a group of closely related compounds.^{14,15,17,20} Such an analysis, to be meaningful, requires a number of compounds large compared to the number of parameters to be determined. Even so, small but meaningful shift differences often become obscured by the averaging process.

We have chosen to discuss our shift data on the basis of the underlying principles involving both steric and electric field effects. Logical trends in ^{13}C shifts can then replace the averaging process for our large data set.

Under our specific solvent conditions the parameter set values are within ± 0.1 ppm of the observed values for all carbons except $-\text{COOH}$.

Steric Effects. Saturates. One of the most significant features of the ^{13}C chemical shift is the so-called γ effect observed in alkanes.²¹⁻²³ An upfield shift is produced by steric compression of hydrogens when 1,4 carbons (γ carbons) are in *gauche* juxtaposition. Grant and Cheney²¹ proposed a semiempirical formula to describe the magnitudes of the γ shift which has both angle and distance dependence. For *trans* rotamers the γ shift is negligible but for a *gauche* rotamer Grant's formula²¹ predicts a shift of 4.8 ppm. We have applied Grant's formula²¹ to a series of applicable situations described in the current literature and our own work²⁴ and have found, in most instances, that the predicted values are lower than those observed. For instance, the observed *gauche* γ shift for a methyl-methylene interaction in methylcyclohexanes is 6 ppm.²³ In view of this observation, we have based all of our calculations on the value of 6 ppm for 100% *gauche* γ interaction.

From the statistical calculations of Flory²⁵ the rotamer population about the $\omega-1$, $\omega-2$ C-C bond of an alkyl chain

Table I. Representative ^{13}C Chemical Shifts of Lipids¹¹

Registry no.	Compd	C-1 ^a	C-2	C-3	C-4	C-5	C-6	C-7	C-8
57-11-4	Octadecanoic acid (stearic)	182.70	36.65	27.25	31.60	31.80	32.00	32.20	32.20
593-39-5	<i>cis</i> -6-Octadecenoic acid (petroselinic)	182.40	36.55	26.95	31.75	29.35	131.50 ^f	133.15	29.85
112-80-1	<i>cis</i> -9-Octadecenoic acid (oleic)	182.40	36.60	27.25	31.60	31.60	31.60	32.20	29.75
112-79-8	<i>trans</i> -9-Octadecenoic acid (elaidic)	182.55	36.65	27.25	31.60	(31.60)	31.45	32.10	35.05
13038-47-6	Methyl <i>trans,trans</i> -9,11-octadecadienoate	176.15	36.55	27.60	<i>e</i>	<i>e</i>	<i>e</i>	<i>e</i>	35.25
60-33-3	<i>cis,cis</i> -9,12-Octadecadienoic acid (linoleic)	182.55	36.65	27.25	31.60	31.60	31.60	32.15	29.75
4675-61-0	<i>cis</i> -9,10-Methyleneoctadecenoic acid (lactobacillic) ^{b,c}	183.4	36.6	27.2	31.6	(31.9)	(32.0)	32.6	31.3
29203-99-4	<i>trans</i> -9,10-Methyleneoctadecenoic acid ^{b,c}		36.6	27.2	31.6	31.8	31.8	32.1	36.9
3296-43-3	Tri- <i>cis</i> -6-octadecenyl glycerol (tripetroselinin) ^d	175.60 175.20	36.55 36.40	27.05	(31.95)	29.30	131.40	133.00	29.80

^a In ppm downfield from HMDS ($\delta_{\text{TMS}} = \delta_{\text{HMDS}} - 2.34$); concentration 500 mg/1.5 ml CHCl_3 (containing 10% HMDS).

Table II. Substituent Shifts on Saturated Carbons^a

A. Due to Terminal Methyl

Substituent position	Shift, ppm
α	+8.75
β	+9.25
γ	-2.60
δ	+0.35

B. Due to Terminal Carboxyl^b

Carbon position	Shift, ppm	
	Fatty acids	Methyl esters
C ₁ (-COOR)	+166.2	+159.9
C ₂	+11.35	+11.35
C ₃	-7.15	-6.90
C ₄	-0.15	-0.10
C ₅	-0.35	-0.30
C ₆	-0.20	-0.20
C ₇	≤ -0.05	≤ -0.05

C. Due to Terminal Carboxyl^b (on Allylic Carbons Only)

Allylic position	Shift, ppm	Allylic position	Shift, ppm	Nonequivalence of allylic carbons
C ₅ ^d	-0.40	C ₈	+0.10	0.50
C ₆ ^e	-0.05	C ₁₁	+0.05	0.1

D. Due to Cyclopropane Rings^f

Substituent position	Shift, ppm	
	Cis	Trans
α	-1.05	+4.7
β	+0.55	+0.05
γ	0.0	0.0

E. Due to Unsaturated Bonds^f

Substituent position	Shift, ppm	
	Cis	Trans
α (1st)	-2.45	+2.85
α (2nd)	-1.60	
β	+0.10	0.0
γ	-0.40	-0.5
δ	-0.10	

^a CHCl_3 solution; ~ 250 mg/ml. Positive shifts downfield from HMDS. ^b Relative to the alkane containing the same number of carbon atoms. ^c *cis*-5-Tetradecenoic acid. ^d Petroselinic acid. ^e Palmitoleic acid. ^f Relative to an unsubstituted alkyl chain.

is 46% gauche. Using this percentage the shift difference between the ω -2 and ω -3 carbons of long-chain fatty acids is predicted to be 2.8 ppm, in close agreement with the substituent shift of 2.6 ppm (Table IIA).

Table III. Substituent Shifts on Unsaturated Carbons^a

A. Due to Terminal Methyl

Substituent position	Shift, ppm	Substituent position	Shift, ppm
β	+8.00	β'	-1.45
γ	-1.85	γ'	+0.85
δ	+0.3	δ'	-0.2

B. Due to Cis Double Bonds

Substituent position	Shift, ppm	Substituent position	Shift, ppm
α (conj) <i>cis</i> ^b	-5.9	α' <i>cis</i>	+1.7
<i>trans</i> ^c	+0.35	<i>trans</i>	+1.6
β^d	-1.83	β'	+0.30
γ^b	-0.5	γ'	+0.5
e^d	-0.28	e'	+0.23

^a CHCl_3 solution; 250 mg/ml. ^b Reference 15. ^c Trans-*trans* relative to *trans*. ^d 60 mg/ml.

Table IV. Nonequivalences of Unsaturated Carbons due to COOH^a

Double bond position	$\Delta\delta$, ppm	Double bond position	$\Delta\delta$, ppm
C ₅	3.20	C ₉	0.25
C ₆	1.65	C ₁₀	0.1 ^b
C ₇	0.8 ^b	C ₁₁	≤ 0.05
C ₈	0.5 ^b		

^a CHCl_3 solution, ~ 250 mg/ml. ^b Estimated by interpolation.

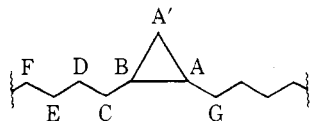
The δ shift in alkyl chains (e.g., the shift difference between the ω -3 and ω -4 carbon resonances, Table IIA) has not previously been explained but can be interpreted in terms of steric interactions. In general, the conformational energies for adjacent C-C bonds are independent. However, for g^+g^- and g^-g^+ combinations there is considerable atomic overlap and these conformations are almost totally forbidden. Thus, g^+g^- interactions perturb the gauche-*trans* populations of intervening bonds in such a way that there is a conformational population difference between the ω -1, ω -2 and ω -2, ω -3 C-C bonds in an alkyl chain, the latter being 6.6% more *trans*. Using this value, a δ shift difference of 0.38 ppm between the ω -4 carbons of saturated fatty acids is predicted, in close agreement with the observed shift of 0.35 ppm. Such δ shifts ensue when the substituent in the δ position is large enough to effectively exclude g^+g^- conformers. Thus, in addition to methyl or methylene, the isosteric -OH and -COOH groups should produce similar δ steric shift contributions.

Steric Effects. Cyclopropanes. Similar conformation-

C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	Other
32.20	32.20	32.20	32.20	32.20	32.20	31.90	34.55	25.20	16.55	
32.20	31.90	32.20	32.20	32.20	32.20	31.90	34.55	25.20	16.50	
<i>132.15</i>	<i>132.45</i>	29.75	32.20	31.85	32.10	31.85	34.50	25.20	16.45	
<i>132.70</i>	<i>133.05</i>	35.05	32.10	31.60	32.20	(31.80)	34.45	25.20	16.50	
<i>134.50</i>	<i>133.20</i>	<i>133.20</i>	<i>134.60</i>	35.25	<i>e</i>	<i>e</i>	34.55	25.20	16.55	53.65 (OMe)
<i>132.45</i>	<i>130.70</i>	28.25	<i>130.50</i>	<i>132.65</i>	29.75	31.90	34.10	25.10	16.45	
18.2	18.2	31.3	32.6	32.2	32.2	31.9	34.5	25.2	16.7	13.4 (exo methylene)
21.3	21.3	36.9	32.1	32.1	32.1	31.8	34.4	25.2	16.5	14.3 (exo methylene)
31.20	(31.75)	31.20	31.20	31.20	31.20	31.95	34.50	25.20	16.30	64.65 (glycerol C-1)

^b 4K data set. ^c Neat solution. ^d 60 mg/ml. ^e Other resonances at 34.10 and 32.10 ppm. ^f Unsaturated carbons are italicized.

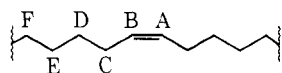
ally induced δ shifts (Table IID) are also apparent in the spectra of cyclopropane fatty acids (Table I). Carbon D, which is β to the cyclopropane ring, experiences a δ shift from the α carbon, G, on the opposite side of the ring. A shift difference between cis and trans isomers of 0.7 ppm is predicted, in good agreement with the observed shift difference of 0.55 ppm. On the other hand, carbon E, which is γ to the ring, experiences no δ shift from ring methylene A', presumably since the internal angle of the cyclopropane ring prevents exclusion of g^+g^- conformers by overlap of carbon A' and E.



The largest steric shifts in cyclopropane fatty acids are those of the carbons α to the ring which are in γ juxtaposition with one another. In the cis isomer these carbons resonate 5.8 ppm upfield from those in the trans isomer.

A configurational dependence of the shifts of the methine ring carbons in the cyclopropane fatty acids is also observed. Trans methine ring carbons absorb 3.1 ppm downfield from cis methine ring carbons in contrast with the 0.5-ppm difference observed between cis and trans olefinic carbons.

Steric Effects. Olefinic Saturated Carbons. Steric interactions are also important in determining the ^{13}C shifts of carbons α to double bonds. For instance, the stereochemistry of the bond adjacent to a double bond (H eclipsed by the double bond) eliminates severe g^+g^- interactions between groups B and F.



Thus, the steric interaction of F with C increases, leading to a predicted upfield " δ -allylic" shift of 0.35 ppm. This effect is the loss of a δ shift (Table IIA); therefore the α shift of a double bond on an $\omega-1$ carbon should be 0.35 ppm less than that for an $\omega-n$ ($n \geq 3$) carbon. The observed shifts for $\omega-1$ allylic carbons are 2.15 ppm, whereas the α shifts for $\omega-n$ carbons are 2.45 ppm. The data of Dorman, *et al.*,¹⁵ for *cis*-2-, *cis*-3-, and *cis*-4-octenes also confirm this hypothesis.

In a *cis* olefin the allylic carbons have a strong γ steric interaction with each other. For instance, C_8 and C_{11} in oleic acid absorb 5.3 ppm upfield of C_8 and C_{11} in elaidic acid. The γ shift for *cis*- vs. *trans*-2-butene is 5.5 ppm.¹⁴ Using the coordinates measured by Kondo, *et al.*,²⁶ for *cis*-2-butene, and Grant's formula,²¹ a shift of 6.9 ppm is predicted which is slightly larger than observed.

When a second *cis* double bond is substituted α to a methylene the " δ -allylic" effect is lost. Thus, the upfield α shift caused by a second double bond is expected to be 0.70 ppm less than that caused by the first double bond. The observed difference is 0.80 ppm (Table IIE). The similarity between the predicted and observed shift differences suggests that no other significant conformational change is occurring.

The upfield substituent shift of a *cis* double bond upon an α methylene (2.45 ppm) is reduced by 0.3 ppm (Table IIE) upon introduction of a second *cis* double bond conjugated with the first. However, a similar small downfield shift for the allylic carbon is observed upon introduction of a *trans* double bond α (conjugated) to another *trans* double bond. Thus, the downfield shift is likely to be inductive rather than steric in origin.

The values in Table IIE show that double bonds cause upfield shifts at carbon E (γ). That the resonance of carbon E should be upfield of the corresponding resonance in a saturated compound is, at first, surprising. Differences would be expected in the γ steric interactions of methylene-methylene and methylene-olefin carbons. However, because of the small γ shifts experienced by unsaturated carbons, the steric shift of the carbon γ to the double bond might be expected to be less than that observed in alkanes and a downfield shift observed. This is not necessarily the case because Grant's equation²¹ contains θ as well as R dependence and the θ values used in calculating the steric shifts of the unsaturated carbon and the γ carbon due to their mutual interaction will not be equal. It is also possible that the carbon γ to a double bond experiences some shift due to interactions with the π electrons or some shift from the sp^2 - sp^3 dipole electric field.

Steric Effects. Olefinic Unsaturated Carbons. For the substituent shifts on olefinic carbon resonances (Table III) we will use the notation of Dorman, *et al.*¹⁵

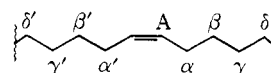


Table V
Carbon-13 Chemical Shifts of Long-Chain Alkanes and Alkenes

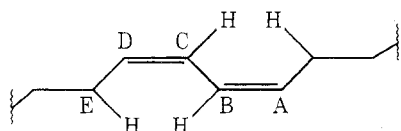
Carbon position	ω^b	$\omega-1$	$\omega-2$	$\omega-3$	$\omega-n$ ($n > 3$)	$\text{C}=(\omega-n)$
Shift, ppm ^a	16.50	25.20	34.45	31.88	32.20	132.40 (<i>cis</i>) 132.85 (<i>trans</i>)

^a Downfield from HMDS. Concentration: 500 mg/1.5 ml CHCl_3 (containing 10% HMDS). ^b Reference 10.

Table IIIA gives the methyl substituent shifts of olefinic carbons based on results of Dorman, *et al.*,¹⁵ but modified by us to include δ and δ' shifts. The total distances of α' , β' , γ' , and δ' substituents from the carbon whose shift is being observed, A, are comparable with those of β , γ , δ , and ϵ substituent shifts for a corresponding carbon in an alkane. The salient features of the values contained in Table IIIA show that the α' , β' , γ' , and δ' substituent shifts are opposite in direction to the α , β , γ , and δ shifts, respectively, and bear no resemblance to the β , γ , δ , and ϵ methyl substituent shifts for a corresponding alkane (Table IIA). This suggests that polarization of the double bond is a very significant factor in determining the primed substituent shifts.

Table IIIB lists the ¹³C substituent shifts for unsaturated carbons due to the presence of a second double bond. When the second double bond is at the α , β , and possibly γ positions, changes in steric shifts will contribute to the total substituent shifts observed. For instance, when both double bonds are *cis*, the β shift involves the loss of an olefinic hydrogen-methylene hydrogen γ steric interaction but the possible gain of a β olefinic hydrogen-olefinic hydrogen interaction.

In *cis,cis* conjugated compounds the hydrogens of the saturated α carbon, E, and an olefinic carbon, B, interact sterically with planar geometry similar to the allylic carbons of a single unsaturated bond.



The difference in α shift (carbon C or B) between the *cis,cis* and *trans,trans* configurations is 6.2 ppm. This shift difference appears to be essentially due to steric considerations, since the α' substituent shifts (D or A) in the *cis,cis* and *trans,trans* compounds are nearly equal, suggesting that inductive, polarization, and electric field contributions are independent of configuration. The 6.2-ppm shift difference is of the same order of magnitude as the corresponding steric shift for allylic carbons (5.3 ppm); so, for similar geometries, the steric shifts of olefinic and saturated carbons are comparable. It seems likely that the generally observed smaller magnitude of olefinic γ shifts, relative to methylene γ shifts, is due to conformational differences. It also appears likely that the steric interaction does not produce significant polarization of the double bond. Thus, in order to explain the observed γ' and δ' shifts of 0.85 and -0.2 ppm, respectively (Table IIIA), one must invoke some other mechanism, such as linear electric field effects arising from changes in the electric field at the double bond upon substitution of a hydrogen by a methyl. Equal and opposite contributions from such a mechanism would also be made to the γ and δ shifts. Thus, steric contributions would account for only part of the observed γ and δ shifts, in contrast to the alkanes for which steric effects appear the only significant contributions to γ and δ effects.

Steric Effects. Triglycerides. The carboxyl carbons on chains 1 and 3 of triglycerides resonate 0.25 ppm downfield of those on chain 2. The carbons at position 2 on chains 1 and 3 resonate 0.15 ppm upfield of those on chain 2.

A simple experiment has shown that steric interactions with the glycerol carbons are a major contributor to the shift differences, since, for a mixture of methyl oleate and propyl oleate in cyclohexane, differences of 0.35 and 0.25 ppm were observed between the pairs of carboxyl and C₂ resonances. The resonance of C₁ is at higher field in the

propyl ester as expected for a γ substituent shift, and C₂ is downfield in the propyl ester as expected for a δ substituent shift.

Electric Field Effects. Unsaturated Carbons. Non-equivalences of the ¹³C chemical shifts of unsaturated carbons in monoenoic fatty acids are caused by electric fields from the head group.⁵ The shifts can be interpreted in terms of changes in electron density at the various carbons as a result of polarization of the bonds by the electric field. Such changes, δ_q , can be estimated as follows

$$\delta_q = \sum \frac{b_{11}E_1}{el} \quad (3)$$

where the sum is over all the bonds of an atom, E_1 is the field resolved parallel to the bond, l the length of bond, b_{11} the empirical longitudinal bond polarizability, and e the electronic charge.^{5,27} The shift is given by

$$\delta_{e1} = (\delta/e)\delta_q \quad (4)$$

where δ/e is the shift/electron for the carbon in question. For isolated double bonds the effect of such polarizations is to cause equal and opposite shifts of the two olefinic resonances, the olefinic carbon nearer the head group being shifted upfield and the other shifted downfield.⁵

Electric field contributions to the spectra of polyunsaturated fatty acids are considerably more complicated. They arise both from the dipolar head group and from polar bonds associated with other double bonds (Table IIIB). The head group electric field effects appear very little different from those observed in monounsaturated chains (Table IV). This should simplify prediction of the spectra of polyunsaturated fatty acids. Double-bond substituent shifts, shown in Table IIIB, contain contributions due to electric field effects and steric shifts. The values for the γ, γ' and ϵ, ϵ' shifts follow the typical pattern for olefinic linear electric field shifts, *i.e.*, equal and opposite shifts decreasing monotonically with increasing dipole-double bond separation. Because the positive end of the dipole is directed toward the double bond, the olefinic carbon closer to the dipole is shifted upfield. Not surprisingly, the α and β shifts do not conform to the same trends, as contributions from other shift mechanisms may dominate at such close distances.

Figure 2 illustrates the origins of the shifts of the various polyolefins and a fatty acid (arachadonic). The dashed lines labeled with Greek letters indicate substituent shifts due to the double bonds. Because of electric field effects the low-field resonances move gradually to lower field with increasing unsaturation and, owing to the very large upfield β shift, most of the resonances are grouped together around 130.6 ppm. The shifts indicated by unlabeled dashed lines for arachadonic acid in chloroform are due to linear electric field effects from the head group. Those shifts indicated for arachadonic acid upon changing solvent from CHCl₃ to acetone are due to complex solvent effects.

Electric Field Effects. Saturated Carbons. The electric field shift dependence of saturated carbons differs considerably from that of unsaturated carbons. For monovalent atoms the sum in eq 3 reduces to a single term.²⁸ The large polarizability of a C=C bond relative to the C-C and C-H bond polarizabilities also allows one to approximate the sum in eq 3 for an unsaturated carbon to a single term. Such an approximation cannot generally be made for a methylene carbon. However, in all cases where perfect tetrahedral, trigonal, etc., bonding exists, the calculation is much simplified by a straightforward theorem which states that the sum of the resolved components of the field at the central nucleus along every bond

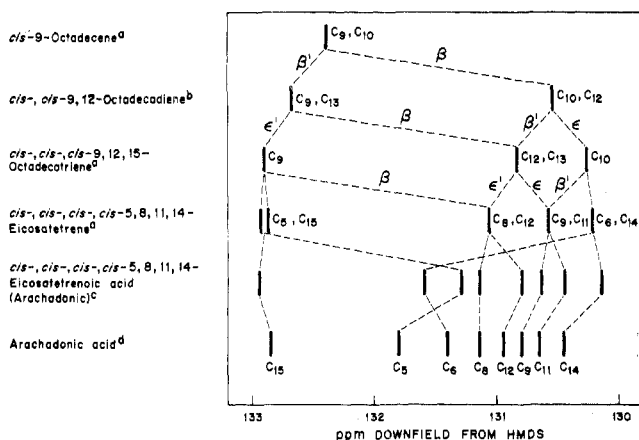


Figure 2. Carbon-13 chemical shifts of olefinic carbons of polyolefins and arachadonic acid showing substituent shifts due to unsaturated bonds and COOH: (a) shift determined using CHCl_3 solution, ~ 60 mg/ml; (b) predicted shifts; (c) CHCl_3 solution, ~ 250 mg/ml; shifts (broken lines) are linear electric field effects due to COOH; (d) acetone solution, ~ 250 mg/ml; shifts are due to solvent effects.

is zero. For methylenes this means that eq 3 can be rewritten as follows

$$\delta_q(C_n) = \left[\frac{b_{11}(\text{C-C})}{el} - \frac{b_{11}(\text{C-H})}{el} \right] [E_{n,n-1} + E_{n,n+1}] \quad (5)$$

$$= a(\text{CH}_2)[E_{n,n-1} + E_{n,n+1}]$$

where $E_{n,n-1}$ and $E_{n,n+1}$ are the fields resolved along the bond from C_n to C_{n-1} and from C_n to C_{n+1} , respectively.

In our description of unsaturated carbon electric field effects the use of optical polarizability data yielded results which agreed with experiment within a factor of 2. Unfortunately, the principal components of bond polarizabilities cannot be calculated from the optical measurements without making at least one assumption. In most studies, the C-H bond has been arbitrarily chosen to be isotropic.²⁹ In the case of unsaturated carbon shifts, the C=C bond polarizability is dominant and therefore the nature of such assumptions is not important. However, in the present case we are concerned with the difference between C-H and C-C bond polarizabilities and the assumption of isotropy leads to difficulties in the interpretation of our data. In fact, it leads to a prediction of very small downfield shifts of the chain resonance signals rather than upfield shifts as observed. We prefer to make the more reasonable assumption that the C-C and C-H bonds have the same axial polarizability ratios. On this basis, b_{11} values of 0.94×10^{-23} and $0.72 \times 10^{-23} \text{ cm}^3$ are calculated for C-H and C-C bonds, respectively, and a value of 5×10^{-8} is obtained for $a(\text{CH}_2)$. The value of $a(\text{CH}_2)$ was also estimated as 3.5×10^{-8} by a CNDO calculation on propane.

Combining eq 4 and 5 we see that the electric field shift is given by eq 6.

$$\delta_{el} = a(\text{CH}_2)[E_{n,n-1} + E_{n,n+1}]\delta/e \quad (6)$$

The shift/electron charge (δ/e) in saturated systems has not been widely investigated. Whitehead, *et al.*,³⁰ obtained a value for δ/e of 74 ppm/electron for substituent shifts at the directly bonded (α) carbon. However, this figure is not necessarily appropriate to the shifts produced by electric fields from a distant source, nor is the commonly used value of 182 ppm/electron obtained for δ/e in aromatic systems.^{31,32} Table VI gives the field effect shifts calculated using $a(\text{CH}_2) = 3.5 \times 10^{-8}$ and both values for δ/e .

The predicted shifts in Table VI agree qualitatively

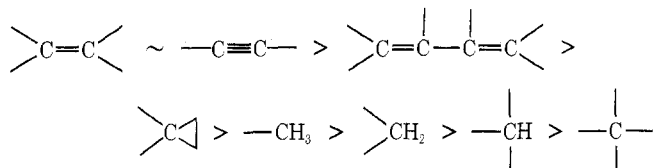
Table VI
Predicted and Observed ^{13}C Linear Electric Field Effect Shifts in Saturated Fatty Acid Chains^a

Position	—Shift predicted, ppm ^b —		Shift observed, ppm ^b
	For $\delta/e = 74$	For $\delta/e = 188$	
C ₄	-0.11	-0.28	-0.45
C ₅	-0.03	-0.09	-0.35
C ₆	-0.02	-0.04	-0.20
C ₇	-0.01	-0.02	≤ -0.05

^a CHCl_3 solution, ~ 250 mg/ml. ^b Negative shifts are upfield.

with the observed results in terms of direction and monotonic decrease with distance from the head group, values calculated using $\delta/e = 182$ ppm being closer to the observed shifts. The direction of the shifts in response to an electric field is consistent with the observation of Hagen and Roberts³³ that methylene resonances shift upfield upon protonation of short-chain carboxylic anions. The upfield shift arises if one assumes a slightly greater polarizability of the C-H bonds than the C-C bonds. Thus, the addition of $-\text{COOH}$ to an alkyl chain, or the protonation of $-\text{COO}^-$, both lead to greater electron densities on the methylene carbons and lower electron densities on the hydrogens.

Magnitudes of the linear electric field shifts of saturated carbons compared with carbons in other linkages are as follows.



The factors determining the size of the shifts are the magnitude of asymmetry of the polarizabilities of adjoining bonds and δ/e .

In general, linear electric field shifts of methylene are very small because of the nearly equal polarizabilities of C-C and C-H bonds. Since a methyl carbon has three C-H bonds adjoining and only one C-C bond, the asymmetry of the polarizabilities is greater. Using the theorem discussed earlier, it follows that the linear electric field shifts of methyl carbons should be 3 times those of methylene carbons and 3 times those of tertiary carbons for the cases where attached atoms are either carbon or hydrogen. The unsaturated carbons have high linear electric field shifts because a highly polarizable unsaturated bond is adjacent to C-C and C-H bonds of much lower polarizability. This results in a large accumulation or depletion of charge on the unsaturated carbon as a result of an electric field. The longitudinal polarizability of a triple bond is greater than that of a double bond (3.5×10^{-24} vs. $2.8 \times 10^{-24} \text{ cm}^3$).²⁹ However, a lower δ/e due to the linear symmetry of the $-\text{C}\equiv\text{C}-$ group³⁴ results in similar electric field shifts for olefinic and acetylenic carbons. For example, the nonequivalences observed for the unsaturated carbons for oleic and stearolic acids are both 0.25 ppm (Table I).

Although molecular polarizabilities can often be enhanced by conjugation of double bonds, the electric field effects observed for the conjugated methyl *trans,trans*-9,11-octadecadienoate are less than those observed in the nonconjugated methyl *cis,cis*-9,12-octadecadienoate (methyl linoleate). The explanation for this lies in the fact that, in conjugated systems, the polarization is increased by further separation of the charge and not by an increase in the magnitude of the charge.

A difference of 0.25 ppm was detected between the shifts of C₆ and C₇ in a methyl ester when they were part of a cyclopropane ring compared with 0.15 ppm for the corresponding difference in a straight saturated chain. This is consistent with the larger polarizability of bonds in the cyclopropane rings (2.4×10^{-24} cm³ vs. 2.1×10^{-24} cm³).³⁵

There are other bond configurations for which large ¹³C linear electric field effects are expected such as C=O, C-S, C=S, C=N, C≡N, and C-X where X is a halide, etc. However, they will not display the large nonequivalence observed for C=C and their analysis will be more complex because their polarity will lead to considerable solvent effects.

Solvent Effects. In some cases solvent effects make large contributions to ¹³C chemical shifts.^{31,36,37} This is particularly so in the case of fatty acids. Thus, as investigators begin to make use of the finer details of ¹³C shifts, as in the present study, the understanding of such solvent shifts will become increasingly important. For this reason we have performed as many measurements as possible under a specific set of solvent conditions. However, some limited data that we have obtained in other solvent systems will be useful in relating our observations to other measurements.

In addition to the large solvent shifts of the carbonyl, considerable solvent dependence is observed for olefinic carbons in fatty acids. These solvent shifts are most obvious in the nonequivalence, $\Delta\delta$, of the carbons. The main factors involved appear to be the solvent dielectric constant modification of the electric field experienced by the double bond. However, the nonequivalences are also modified by solvents which can donate hydrogen bonds and, hence, directly change the headgroup dipole. The largest nonequivalences we have observed were in chloroform (1.40 ppm for methyl petroselinate) and the lowest were in acetone (0.90 ppm for methyl petroselinate). Similar, though smaller, variations in electric field effects are observed for saturated carbons. In cases where no linear electric field effects are expected, *e.g.*, the terminal methyl (ω carbon) of methyl petroselinate, shifts as large as 1.5 ppm are observed upon changing from cyclohexane to CHBr₃ (250 mg/ml) even after corrections for bulk susceptibility have been made. Bergman and Dahm³⁸ have pointed out the dependence of such alkyl carbon shifts on solvent refractive index. Because of the downfield direction of the observed solvent shifts with respect to cyclohexane solution, the dominant contribution is likely to be from second-order field-acting electric field effects (dispersion shifts). Such dispersion shifts have been considered previously by Howard, *et al.*,³⁹ for ¹H nmr and, using equation 11 from their paper, we have obtained a good linear correlation with the observed ¹³C shifts.³⁸

Since second-order electric field effects are strongly distance dependent, carbon dispersion shifts will depend on the number of solvent molecules which can pack around the carbon. Thus, methyls have the largest shifts and hindered, tertiary carbons the smallest. For this reason, reference compounds such as tetramethylsilane (TMS) and hexamethyldisilane (HMDS) are not ideal except when studying methyl resonances. Solvent shifts for methylenes are smaller but not negligible. However, since the solvent shifts for all methylenes in an alkyl chain are very similar, we have used the shift of one of them, the ω -1 carbon, as an internal standard.

Conclusion

We have presented assigned ¹³C spectra of a large number of fatty acids and their derivatives, and discussed their assignments on the basis of the important contribu-

tions of steric effects, linear electric field effects, and solvent effects to the ¹³C chemical shift. We believe that application of these principles will prove of much value in the spectral assignments of numerous natural products, and since steric and electric field shifts both have a geometric and distance factor, such shifts will prove useful in conformational analysis.

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Registry No.—*cis*-5-Tetradecenoic acid, 5684-70-8; palmitoleic acid, 373-49-9; arachidonic acid, 506-32-1.

Supplementary Material Available. Complete ¹³C chemical shift assignments for all fatty acids and derivatives (7 saturated, 15 unsaturated, 5 cyclopropane and 3 hydroxy fatty acids, 5 triglycerides, and 5 alkenes) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for \$3.00 for photocopy or \$2.00 for microfiche, referring to code number JOC-74-1698.

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Base-Catalyzed Racemization of 2,2-Diphenylcyclopropyl nitrile

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The base-catalyzed racemization of 2,2-diphenylcyclopropyl nitrile (1) has been studied in solvents containing various amounts of dimethyl sulfoxide. With sodium methoxide as base and nitrile 1 as substrate, the rate for racemization in 1.5 mol % methanol-98.5 mol % dimethyl sulfoxide is 3.6×10^8 times that observed in methanol. The logarithm of the racemization rate constants depends linearly on the H_- function, which suggests that proton abstraction is not the slowest reaction step in the racemization of the nitrile.

The base-catalyzed racemization and exchange reactions of 2,2-diphenylcyclopropyl nitrile (1) in methanol and *tert*-butyl alcohol was investigated by Walborsky and his coworkers.¹ DeBoer, *et al.*,² studied the base-catalyzed H/D and D/T exchange of cyclopropyl cyanide in methanol with sodium methoxide as base. The work of those authors provides evidence for the existence of an energy barrier to the inversion or delocalization of the cyclopropyl carbanion as a result of "I strain" in the three-membered ring.^{3,4}

The activity of each kind of base should vary with changes in solvent, as should carbanion stability. Perhaps the most dramatic effects of medium on the rate of carbanion generation are found in a comparison of alcohols and dimethyl sulfoxide (DMSO).^{5,6}

In continuation to our interest in the cyclopropyl system, a study of the base-catalyzed racemization of 1 in solvents containing various amounts of DMSO was undertaken.

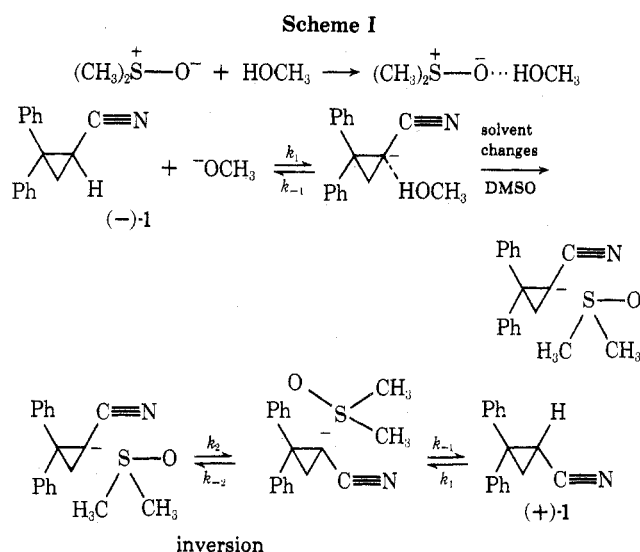
Results and Discussion

The preparation of optically active 2,2-diphenylcyclopropyl nitrile (1) has been described previously.¹ The relative rates of racemization of 1 with sodium methoxide in the presence of different percentages of dimethyl sulfoxide are collected in Table I.

The data in Table I point to the importance of medium on the activity of methoxide anion as base. In passing from methanol to 99.3% DMSO (wt/wt), the rate of racemization by methoxide anion increased by a factor of 3.6×10^8 . This factor is comparable to that reported by Cram⁷ for the enormous increase of the rate of racemization (5×10^7 increase) of the open-chain compound, 2-methyl-3-phenylpropionitrile, with sodium methoxide on increasing the concentration of DMSO in methanol to 98.5% (wt/wt). The big difference in the activity for the methoxide anion in the two solvents is attributed to the presence of solvent-anion hydrogen bonds in methanol ($\text{CH}_3\text{OH} \cdots \text{OCH}_3$) that are absent in DMSO. In the presence of high concentration of DMSO, the alcohol is hydrogen bonded to the latter leaving the methoxide anion relatively poorly solvated and highly reactive.

In methanol, racemization of 1 can occur *via* inversion of the hydrogen-bonded carbanion or through some free carbanion which can become planar by delocalization.

Strong hydrogen bonding between methanol and the three-membered ring carbanion, besides the strain in this ring, is presumably responsible for the slow rate of racemization. At high concentration of DMSO, *i.e.*, low concentration of free CH_3OH , the carbanion, solvated with DMSO, is much more "free" and racemization occurs preferentially (Scheme I). This increase in the rate of racemization of 1 at high concentrations of DMSO emphasizes the importance of solvent-carbanion interactions in determining the configurational stability of carbanions constrained in small ring compounds. Scheme I represents the racemization of 1 in the presence of DMSO.



At base concentrations ranging from 0.02 to 0.09 *M* the reaction is first order in nitrile as shown by the linear plots of $\log k$ *vs.* time. The racemization rate is also found to be first order in sodium methoxide up to 0.08 *M* and deviates from unity above this concentration. The kinetics of the racemization are represented by the equation

$$v_{\text{rac}} = k_0[\text{RH}][\text{CH}_3\text{O}^-]$$

where k_0 is the overall rate constant and $[\text{RH}]$ and $[\text{CH}_3\text{O}^-]$ are the concentrations of RH and CH_3O^-