

Effects of *bis* Homoallylic and Homoallylic Hydroxyl Substitution on the Olefinic ^{13}C Resonance Shifts in Fatty Acid Methyl Esters¹

Substitution of a hydroxyl group at the *bis* homoallylic position (OH group located three carbons away from the olefinic carbon) in C_{18} unsaturated fatty acid esters (FAE) induces a 0.73 ± 0.05 ppm upfield and a 0.73 ± 0.06 ppm downfield shift on the δ and ϵ olefinic ^{13}C resonances relative to the unsubstituted FAE, respectively. If the hydroxyl group is located on the carboxyl side of the double bond of the *bis* homoallylic hydroxy fatty acid esters (BHAHFA), the olefinic resonances are uniformly shifted apart by $[|1.46 + |\Delta\delta\text{db}_u||]$ where $\Delta\delta\text{db}_u$ represents the absolute value of the double bond resonance separation in the unsubstituted FAE and 1.46 ppm is the sum of the absolute values of the δ and ϵ shift parameters. With hydroxyl substitution on the terminal methyl side of the double bond, the olefinic shift separation is equal to $[|1.46 - |\Delta\delta\text{db}_u||]$. In homoallylic (OH group located two carbons away from the olefinic carbon) substituted FAE the γ and δ induced hydroxyl shifts for the *cis* double bond resonances are +3.08 and -4.63 ppm, respectively while the *trans* double bond parameters are +4.06 and -4.18 ppm, respectively. The double bond resonance separation in homoallylic hydroxy fatty acid esters (HAHFA) can be calculated from the formula $[|7.71 - |\Delta\delta\text{db}_u||]$ for *cis* and $[|8.24 - |\Delta\delta\text{db}_u||]$ for the *trans* case when the OH substitution is on the carboxyl side of the double bond. Conversely, when the OH resides on the terminal methyl side, the double bond shift separations for *cis* and *trans* isomers are $[|7.71 + |\Delta\delta\text{db}_u||]$ and $[|8.24 + |\Delta\delta\text{db}_u||]$, respectively. The derived shift parameters can verify the positions of both the double bond and hydroxyl substitution from the olefinic resonance separation in long-chain fatty acid derivatives, obviating the need for destructive analytical methods.

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Although there are a number of articles concerning the ^{13}C nuclear magnetic resonance (NMR) assignments of fatty acid structures (1,2) including unsaturated fatty acids (3-6) and hydroxy fatty acids (7), no attempts have been made to systematize the ^{13}C NMR assignments of unsaturated hydroxy fatty acids. Spectral assignments for methyl ricinoleate, methyl ricinelaidate, and homoallylic hydroxy fatty acid esters (HAHFA) have been mentioned by Rakoff *et al.* (8) and so have assignments of methyl 12-hydroxy-10-octadecenoate by Frankel *et al.* (9).

Earlier work by Tulloch and Mazurek (7) demonstrated that the position of hydroxylation in saturated fatty acids could be pinpointed by examining the induced shifts in natural abundance ^{13}C spectra of the hydroxylated and adjacent carbon resonances. In these spectra the carbon carrying the hydroxyl group (α carbon) was found to shift 42.2 ppm downfield, the β carbon downfield 7.80 ppm, and γ and δ shifted upfield 4.0 and 0.6 ppm, respectively. Batchelor *et al.* (5) have undertaken an extensive investigation of the electric field effects of the carboxyl group on the ^{13}C double bond resonances and of the effects of other substituents such as cyclopropane and additional double bonds on saturated and unsaturated carbon resonances in long-chain fatty acid derivatives (1). Their findings demonstrated that of the two unsaturated carbons, the carbon nearest the carboxylate head group consistently had the higher field ^{13}C chemical shift. It was also revealed that the separation of the double bond ^{13}C resonances uniquely depends upon the position of the double bond relative to the carboxyl group. These observations were attributed to a linear electric field effect induced by the terminal carboxyl group. In the present work we report the results of hydroxyl substitution on the chemical shift separation of unsaturated carbon resonances in long-chain fatty acid esters together with some strategies for exploiting derived shift parameters for structural identification of these derivatives.

MATERIALS AND METHODS

Materials. Unsaturated and hydroxy-substituted fatty acids and esters such as those of petroselinic, ricinoleic, ricinelaicid, oleic, and elaidic acids were obtained commercially. Most of the ^{13}C NMR data were taken from various literature sources (3,4,7-9). Chemical shift assignments for the unsaturated carbon resonances in *cis* and *trans* HAHFA, methyl 12-OH-9-octadecenoate, were obtained from Rakoff *et al.* (8). Methyl 9-hydroxy-12-octadecenoate (isoricinoleate) was obtained by extracting seeds of *Holarrhena antidysenterica* (NU-46607) and purification as described (10).

NMR spectroscopy. ^{13}C NMR spectra were obtained with a 9.3T JEOL GX-400 NMR spectrometer (Peabody, MA) operating at 100.4 MHz. All spectra were obtained with a 25KHz spectral width, 16K data points, a 15 μsec 90 degree pulse, and a 5 sec repetition rate. Samples were examined in CDCl_3 . All resonances were referenced to CDCl_3 at 77.00 ppm relative to external TMS which is assigned a value of 0.00 ppm. Reported chemical shifts are ± 0.03 ppm.

Mass spectrometry. Mass spectra were obtained on a Hewlett-Packard 5990B GC/MS fitted with an Ultra (methyl silicone) 12-m capillary column (Hewlett-Packard,

TABLE 1

Chemical Shifts^a of the Monounsaturated Fatty Acid Methyl Esters and BHAHFA Type IIa and b Fatty Acid Methyl Esters

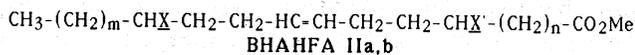
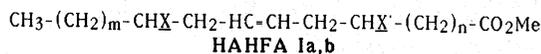
Compound name	Carbon number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Methyl <i>cis</i> -12-octadecenoate	174.35	34.20	25.10	29.30	29.40	29.55	29.65	29.60	29.40	29.85	27.30	130.00	130.00	27.30	29.55	31.65	22.70	14.1
9-OH substitution increment					-0.16	-0.06	-4.06	7.71	42.31	7.61	-3.70	-0.81	0.69					
Methyl 9-OH- <i>cis</i> -12-octadecenoate	174.33	34.11	25.94	29.10	29.24	29.61	25.5	37.36	71.71	37.46	23.60	129.19	130.69	27.23	29.41	31.56	22.60	14.09
Methyl <i>cis</i> -6-octadecenoate	173.80	33.97	24.60	29.21	26.83	128.98	130.28	27.22	29.72	29.33	29.56	29.66	29.66	29.66	29.33	31.92	22.69	14.09
10-OH substitution increment						0.87	-0.63	-3.69	7.82	42.10	7.66	-4.02	0.04	-0.08				
Methyl 10-OH- <i>cis</i> -6-octadecenoate	174.15	33.89	24.49	29.06	26.75	129.85	129.65	23.53	37.54	71.43	37.22	25.64	29.70	29.58	29.24	31.85	22.62	14.04
Methyl <i>cis</i> -5-octadecenoate ^b	174.25	33.55	25.00	26.65	128.40	131.30	27.35	29.75	29.45	29.65	29.75	29.75	29.75	29.65	29.45	32.00	22.75	14.15
9-OH substitution increment					0.65	-0.75	-3.80	8.00	42.06	7.59	-4.05	-0.02	-0.08					
Methyl 9-OH- <i>cis</i> -5-octadecenoate	174.28	33.45	24.89	26.56	130.55	129.09	23.55	37.75	71.51	37.24	25.70	29.73	29.67	29.61	29.35	31.92	22.70	14.12
^a ppm.																		
^b Reference 3.																		

Palo Alto, CA). The column was temperature programmed from 150° to 250°C at 4°C per min.

Methyl 10-hydroxy-6-octadecenoate and methyl 9-hydroxy-5-octadecenoate were synthesized by the following procedure: The synthesis of the δ -6,7-structure began with 5-chloro-1-pentanol that was protected as a tetrahydropyranyl (THP) ether, and was then treated with lithium acetylide in dimethyl sulfoxide (iodide catalyst). The resulting alkyne was cyanoethylated (1:butyllithium, ethylene oxide; 2:methanesulfonyl chloride, triethylamine, 3:sodium cyanide, dimethyl sulfoxide), and the resulting nitrile was then condensed with *n*-octylmagnesium bromide to produce a ketoalkyne of the correct chain length bearing a THP-protected primary alcohol. Deprotection (methanolic HCl) gave a ketoalkynol that was oxidized to the acid (chromic acid, acetone) and esterified (methanol, boron trifluoride). This ketoalkyne ester was reduced at the carbonyl by sodium borohydride and hydrogenated to a *cis*-alkene over palladium on carbon in methanol. The δ -5,6-structure was synthesized in analogous fashion using 4-chloro-1-butanol and 1-bromononane as alternate starting materials. The positions of the hydroxyl group and double bonds were verified by oxidative cleavage and mass spectrometry. Double bond position was determined by the chromic acid micro column technique (11). The scission products (carboxylic acids) were identified by comparison of retention times with standards using gas-liquid chromatography, and also by mass spectrometry. The hydroxyl group was localized by mass spectrometry after hydrogenation of the double bond (12).

RESULTS AND DISCUSSION

Figure 1 gives a schematic representation of the two types of unsaturated hydroxy fatty acids examined in this study. Each of these two structures, *bis* homoallylic (BHAHFA), OH located three carbons away from the olefinic carbon and homoallylic (HAHFA), OH located 2 carbons away from the olefinic carbon fatty acid esters, can contain a hydroxyl substituent on the terminal methyl side [a] or carboxyl side [b] of the double bond. Table 1 lists as an example the observed ¹³C chemical shifts for the newly synthesized BHAHFA II methyl 9-OH-*cis*-5-octadecenoate, methyl 10-OH-*cis*-6-octadecenoate as well as the previously isolated methyl 9-OH-*cis*-12-octadecenoate (isoricinoleate) and the corresponding unsaturated methyl esters from which they are derived (3). The incremental ¹³C shifts due to the substitution of the hydroxyl group



Ia X-OH, X'-H
 Ib X-H, X'-OH
 IIa X-OH, X'-H
 IIb X-H, X'-OH

FIG. 1. Schematic representation of monounsaturated hydroxy fatty acid methyl ester structures.

are given for each structure. Note that the OH induced shifts in the BHAHFA associated with the ^{13}C resonances representing the adjacent saturated carbons are similar to those reported earlier (7). However, the double bond carbon resonances, those δ and ϵ to the OH substituted carbon, have undergone a relatively large shift change as compared to their saturated counterparts, presumably because of the greater polarizability of the unsaturated carbons by the electric field originating at the molecular dipole of the OH group (1,5).

Table 2 lists the observed OH induced shift parameters for the double bond carbon resonances in HAHFA Ia,b and BHAHFA IIa,b. The values represent the average difference in chemical shift between the olefinic carbon resonances in the substituted and non-substituted esters. By combining the shift separation values for the monounsaturated fatty acid methyl esters as given in Table 3 with the shift parameters in Table 2 we can predict the double bond resonance separation in type IIa and b BHAHFA (Fig. 2 and 3). Figure 2 illustrates when the OH group is on the terminal methyl side of the double bond, the double bond resonance separation shows a continuous narrowing as the double bond position moves from carbon 12 to 6. The narrowing in the olefinic shift separations results from the upfield movement (-0.73 ppm) of the lower field shift (δ carbon) and the downfield movement ($+0.73$ ppm) of the higher field olefinic resonance (ϵ carbon). Therefore, in type IIa structures with unsaturation in positions 6–12, the double bond resonance assignments

TABLE 2

Hydroxyl Shift Parameters (ppm) for Substituted Fatty Acid Methyl Esters

Double bond position relative to OH	HAHFA		BHAHFA
	<i>cis</i>	<i>trans</i>	<i>cis</i>
γ	3.08 ^a	4.06 ^a	
δ	-4.63 ^a	-4.18 ^a	-0.73 ± 0.05
ϵ			0.73 ± 0.06

^aBased on shifts for the *cis* and *trans* isomers of methyl 12-hydroxy-9-octadecenoate and methyl 10-hydroxy-*trans*-12-octadecenoate.

TABLE 3

Predicted ^{13}C Double Bond Chemical Shift Separations (ppm) in *cis* and *trans* HAHFA Ia and b Based on Induced Shift Parameters Given in Table 2

Double bond position	$\Delta\delta_{\text{db}_a}$		$\Delta\delta_{\text{db}_s}$				
	<i>cis</i>	<i>trans</i>	<i>trans</i> Ia,b	<i>cis</i> Ia	<i>cis</i> Ib	<i>trans</i> Ia	<i>trans</i> Ib
12	0.00	0.00	1.15	7.71	7.71	8.24	8.24
11	0.10	0.10	1.25	7.61	7.81	8.14	8.34
10	0.15	0.15	1.30	7.56	7.86	8.09	8.39
9	0.30	0.25	1.40	7.41	8.01	8.01	8.49
8	0.45	0.45	1.60	7.26	8.16	7.79	8.69
7	0.75	0.75	1.90	6.96	8.46	7.49	8.99
6	1.40	1.45	2.60	6.31	9.11	6.79	9.69
5	2.90	2.90	4.05	4.81	10.61	5.34	11.14
4	4.40	4.40	5.55	3.31	12.11	3.84	12.64

^aBased on shifts given by Bus *et al.* (4).

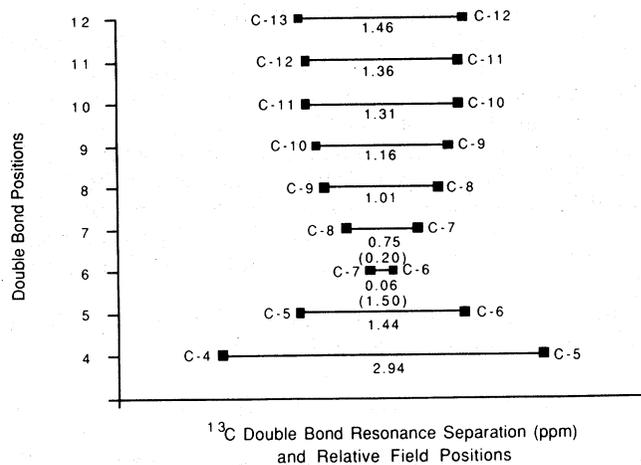
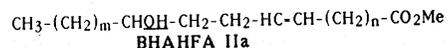


FIG. 2. Predicted and actual ^{13}C double bond resonance separations, chemical shift assignments and relative field positions for BHAHFA when the OH group is on the terminal methyl side of the double bond (type IIa). Value given in parentheses over the line is the actual shift separation value.

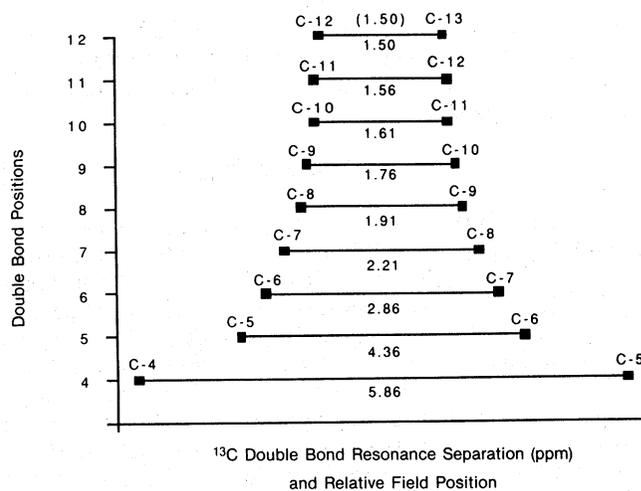
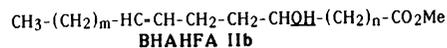


FIG. 3. Predicted and actual ^{13}C double bond resonance separations, chemical shift assignments and relative field positions for BHAHFA when the OH group is on the carboxyl side of the double bond (type IIb). Value given in parentheses over the line is the actual shift separation value.

are opposite to those reported for the unsubstituted mono-unsaturated counterparts (5). That is, in type IIa compounds (double bond positions 6–12 and beyond) the higher field olefinic resonance represents the olefinic carbon farthest from the carboxyl group. At the 6-position the olefinic shift separation reaches a minimum (0.1 ppm) due to the countering dipolar effects of the terminal carboxyl group (5). Beyond the 6-position *e.g.*, double bond

positions 4, 5, etc. the higher and lower field olefinic resonance assignments revert to those previously established for the corresponding fatty esters (3). In essence, these shifts have crossed over each other.

In Figure 3 we observe that the predicted double bond shift assignments for type IIb compounds do not change from the corresponding unsubstituted precursors (4,5), since the upfield resonance (δ) moves upfield (-0.73 ppm) and the downfield resonance (ϵ) moves downfield (0.73) with OH substitution on the carboxyl side of the double bond. Since no shift crossovers can occur in this example, only a smooth increment in double bond resonance separation is observed as the double bond moves from C-12 to C-4.

To calculate the double bond shift separation in BHAHFA we use the following:

$$\text{For type IIa} \quad \Delta\delta_{db_s} = [|\epsilon - \delta| + |\Delta\delta_{db_u}|] \quad [1]$$

Where $\Delta\delta_{db_s}$ is the double bond resonance separation in the BHAHFA, $|\epsilon - \delta|$ is 1.46 ppm for *cis* type II fatty acid esters and $\Delta\delta_{db_u}$ is the observed ^{13}C shift separation in the monounsaturated analogues.

$$\text{For type IIb} \quad \Delta\delta_{db_s} = [|\epsilon - \delta| - |\Delta\delta_{db_u}|] \quad [2]$$

HAHFA double bond resonance separations are given by:

$$\Delta\delta_{db_s} = [|\gamma - \delta| - |\Delta\delta_{db_u}|] \quad [3]$$

For type Ia *cis* or *trans* where $|\gamma - \delta|$ is equal to 7.71 and 8.24 ppm for the *cis* and *trans* isomers, respectively (Table 2). For type Ib the same equation as above applies except the sign is positive:

$$\Delta\delta_{db_s} = [|\gamma - \delta| + |\Delta\delta_{db_u}|] \quad [4]$$

Predicted shift separations for *cis* and *trans* type a or b HAHFA are given in Table 3. In most instances one can pinpoint the position of hydroxyl substitution in long-chain fatty acid derivatives directly from the documented α shift (7). Having established this position, it is easy to ascertain if a double bond is located within 3 or 2 carbons from this site as well as on which side of the double bond it resides. Note that it is not possible to determine if we have type a or b substitution in either BHAHFA or HAHFA derivatives when the double bond is at the 12-position since the chemical shifts are identical for the

olefinic carbons in the spectra of the corresponding unsubstituted fatty acid esters (FAE). Also, we have not accounted for possible effects of H-bonding of the OH group with the carboxyl when OH substitution is found at the C-4 or position closer to the carboxyl group. ^{13}C chemical shift data available from the literature on allylic hydroxy FAE do not appear to show the same predictable shift increments exhibited by derivatives I and II.

The shift parameters described in this work can be useful for the non-destructive identification of structures of type Ia,b and IIa,b FAE. It is particularly valuable when sample size is limited and material cannot be sacrificed for destructive analyses. While alternative NMR methods such as 2D INADEQUATE experiments (13) are often used to elucidate such structures, they require orders of magnitude of more sample and time than is required for this simple analysis.

ACKNOWLEDGMENTS

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