The σ-inductive effects of C=C and C≡C bonds: predictability of NMR shifts at sp^2 carbon in non-conjugated polyenoic acids, esters and glycerides

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The ^{13}C NMR shift separations in a wide range of non-conjugated polyenoic acids can be reliably predicted from the σ-inductive theory developed previously for monoenes, with only one further adjustable parameter, the dipolar effect of one double bond upon another. Further improvements can be made by allowing the enhanced transmission of charge by an intervening double bond. Shifts in poly-ynes are also predicted, though in this case both the dipolar effect and the transmission gain approximately double. The theory also semi-quantitatively explains the small differences in carbonyl shift, used in the quantitative analysis of glycerides.

Many ^{13}C NMR shifts have been measured over the past 20 years to assist in the analysis of long-chain fatty acids and esters.[1–6] The object of this work has generally been to develop empirical equations for the prediction of shifts in novel lipids, or their derivatives, and thus to extend the range of analytical options. In most of these papers, parameterised equations have been proposed both for individual shifts, δ, at unsaturated carbon, and also for the shift differences, $\Delta\delta$. The latter data are often more reliable because they are insensitive to small errors in referencing. These empirical correlations have been only moderately successful. They have sometimes permitted good assignments to be made for novel lipids,[4] but even in very recent analyses, as the authors[7] freely admit, they have necessitated the use of parameters that vary in an apparently arbitrary way with the nature of the multiple bond (the 'reporter group') and of the relatively remote substituent (the 'generating dipole').

Another weakness of these correlations has been that their theoretical basis has been unclear. They have usually been based on Bachelor's electric field theory,[5,6] in which the 'generating dipole' is understood to be that of the entire remote substituent (*e.g.* an ester group) acting through space so as to polarise the π-electrons of the multiple bond. The resulting local separation of charge is then linearly monitored by the ^{13}C shifts of the carbons at either end of each multiple bond. Whilst this monitoring process is a reasonable approximation in the absence of full *ab initio* calculations of shift, there are nevertheless major problems with the emphasis on electric fields acting through space. In some cases, any reasonable assessment of average chain conformation leads to an incorrect prediction of the sign of the induced polarisation. Also, on this theory, *cis* C=C bonds elsewhere in the chain should have little or no effect upon other multiple bonds, and *trans* C=C bonds and also C≡C bonds can induce no dipole field whatever, through space, because they have no net electric dipole. Only a quadrupole field is possible, and this should be much weaker, and should also decay steeply with distance. Yet several earlier studies[1,2,4] have noted that such bonds in fact generate substantial shifts, with triple bonds being particularly effective in this regard. Indeed, small shifts can even be observed at the ester carbonyls in triglycerides, arising from the presence and positioning of double bonds as many as 11 carbons away. Wollenberg[8] and Bonnet[9] have developed these into an elegant analytical method for the non-destructive analysis of natural mixed-chain triglycerides.

In two recent publications,[10,11] we have argued that the model with dipolar electric fields acting through space should be replaced by one with a σ-inductive electric polarisation acting through bonds, and we have presented both experimental data and theoretical arguments to show that such polarisation need not attenuate as sharply as generally assumed with each added intervening chain methylene group. The C(n + 1) − Cn shift differences $\Delta\delta$ for a variety of 'reporter groups' and 'generating dipoles' all fit closely to the general formula (1),

$$\Delta\delta = \text{Intercept } (I) \times \text{Attenuation factor}^{-n} \qquad (1)$$

where n is the chemical position of the reporter group and the generating dipole is at C1, using the standard numbering system for unsaturated fatty acids and alcohols. In the previous work,[11] we showed that, for a variety of reporter groups and generating dipoles, the attenuation factors were the same, within the error limits. We have thus undertaken a global re-analysis of an extended data set, for 85 compounds, assuming a common attenuation factor, for which the least squares analysis gave 1.78 ± 0.03. From the same analysis, the intercepts I for *cis*-esters and *cis*-acids were 45.1 and 52.0, respectively.

In our previous paper we also presented very similar formulae for the individual shifts of the reporter group carbons (see below). Both theory and experiment show that Cn and C(n + 1) are not quite symmetrically spaced about the high-n asymptote, although the asymmetry is not significant for $n > 12$. The Cn shift is somewhat further from the asymptote. This again has a natural explanation in the σ-inductive theory, but not in the through-space theory.

This σ-inductive model resolves several of the problems raised by the through-space model. Because the electric polarisation is generated largely by the polar bond nearest to the reporter group, rather than by the overall dipole of the substituent, it can readily be generated by the nearer sp^3–sp^m (m = 1,2) bond of a double or triple bond. The well known increase of electronegativity in the series C(sp^3) < C(sp^2) < C(sp) has been estimated quantitatively[12] and leads to the estimate that a C(sp^2)–C(sp^3) bond has about 30% of the dipole of a C(sp^3)–OH bond. Also, because the inductive model contains no geometrical terms, it is well placed to offer a quantitative explanation of the additivity of induced shifts, noted experimentally by several earlier workers.[1,2] In contrast,

Table 1 C=C shift differences in *cis* polyenoic acids and esters; calculated values are in parentheses

18:2(*n*,*m*)[a,b]	(*n* + 1)−*n*[c]	(*m* + 1)−*m*
n = 5, *m* = 12	3.00 (2.70)[e]	0.21 (0.28)[f]
6,12	1.28 (1.25)	0.37 (0.45)
7,12	0.21 (0.24)	0.76 (0.75)
8,12	−0.64 (−0.69)	1.22 (1.28)
9,12	−1.77 (−1.86)	2.12 (2.22)
6,9	−0.55 (−0.52)	2.52 (2.52)
6,10	0.41 (0.43)	1.43 (1.42)
6,11	0.94 (0.96)	0.79 (0.80)
7,13	0.54 (0.54)	0.37 (0.42)
11,14	−2.06 (−2.07)	2.06 (1.84)[h]

18:2(*n*,*m*)[a,d]	(*n* + 1)−*n*[b]	(*m* + 1)−*m*
n = 2,*m* = 6	[g]	3.0 (3.03)[f]
3,7	11.7 (11.84)[h]	2.2 (2.23)
4,8	3.1 (3.28)[e]	1.7 (1.78)
5,9	1.65 (1.31)	1.6 (1.53)
6,10	0.2 (0.21)	1.4 (1.39)
7,11	−0.45 (−0.41)	1.35 (1.31)
8,12	−0.75 (−0.71)	1.25 (1.27)
9,13	−0.95 (−0.96)	1.15 (1.24)
10,14	−1.05 (−1.07)	0.75 (0.84)[h]

A:3(*n*,*m*,*o*)[b]	(*n* + 1)−*n*	(*m* + 1)−*m*	(*o* + 1)−*o*
18:*n* = 6, *m* = 9,*o* = 12	−1.22 (−1.22)[i]	0.32 (0.32)	2.68 (2.71)
18:9,12,15	−2.23 (−2.35)	0.00 (0.07)	4.60 (4.90)[h]
19:7,10,13	−1.90 (−1.84)	0.20 (0.18)	2.69 (2.68)
20:8,11,14	−2.20 (−2.10)	0.12 (0.10)	2.72 (2.66)
20:11,14,17	−2.41 (−2.56)	0.00 (0.02)	4.55 (4.88)[h]

A:4(*n*,*m*,*o*,*p*)[b,j]	(*n* + 1)−*n*	(*m* + 1)−*m*	(*o* + 1)−*o*	(*p* + 1)−*p*
20:*n* = 5, *m* = 8, *o* = 11, *p* = 14	0.00 (−0.23)	0.00 (0.08)	0.68 (0.64)	2.88 (2.78)
22:7,10,13,16	−1.99 (−1.83)	−0.30 (−0.28)	0.58 (0.54)	2.86 (2.76)

A:5(*n*,*m*,*o*,*p*,*q*)[j]	(*n* + 1)−*n*	(*m* + 1)−*m*	(*o* + 1)−*o*	(*p* + 1)−*p*	(*q* + 1)−*q*
20:*n* = 5,*m* = 8,*o* = 11,*p* = 14,*r* = 17	−0.29 (+0.13)	0.01 (0.06)	0.18 (0.15)	0.69 (0.63)	5.02 (5.02)[h]
22:7,10,13,16,19	−1.82 (−1.86)	−0.34 (−0.39)	0.00 (0.05)	0.68 (0.61)	4.98 (5.02)[h]

A:6(*n*,*m*,*o*,*p*,*q*,*r*)[j]	(*n* + 1)−*n*	(*m* + 1)−*m*	(*o* + 1)−*o*	(*p* + 1)−*p*	(*q* + 1)−*q*	(*r* + 1)−*r*
22:4,7,10,13,16,19	2.06 (2.40)	0.34 (0.55)	0.03 (0.12)	0.00 (0.20)	0.68 (0.64)	4.95 (5.03)[h]

[a] Using the standard nomenclature for fatty acids: *n*, *m etc.*, are the double bond positions. [b] Data from ref. 1. [c] *i.e.* $\delta_{C(n+1)} - \delta_{Cn}$. [d] Data from ref. 2. [e] Diene calculation formula is $52.0 \times 1.78^{-n} - 6.82 \times 1.78^{-(m-n-1)}$ for the free acids; for esters the initial multiplier becomes 45.1. [f] Diene calculation formula is (52.0 or 45.1) $T \times 1.78^{-m} \times 6.82 \times 1.78^{-(m-n-1)}$, with $T = 1.28$. [g] Not used, because of end effects (see text). [h] Including end-effect correction based on palmitate shifts. [i] The polyene calculations follow the same patterns as for the dienes. [j] Data from ref. 4.

any additivity theory based on fields acting through space must include an assessment of the average relative angle of the separate generating dipoles.

Results

Shifts generated at and by C=C bonds

Our aim is to predict Δδ at C=C bonds, generated by the combined effects of remote ester or acid groupings and of remote, differently placed C=C bonds. By remote, we mean having sufficient chemical separation that extra γ-gauche interactions, or else their absence, can be discounted. For the same reason, we do not include Δδ data derived from shifts at carbons two or fewer atoms from either of the chain ends. The presence of such interactions is in any case apparent because the average shift of the C=C carbons alters, compared with the carbons of a more centrally placed multiple bond. We do include atoms three or four away from the chain ends, even though these are clearly influenced by γ-gauche interactions, but in such cases we subtract a correction equal to the corresponding shift difference in palmitic acid, a saturated chain. The use of these corrections is noted against the appropriate entries in the Tables. The 'remoteness' criterion also eliminates any consideration of conjugated double bonds and it discourages unmodified applications to triple bonds separated by only one intervening methylene group, because in this case the linearity of the triple bonds must act to reduce steric interactions of other methylene groups with the inner carbons of the triple bonds.

The data selected for fitting to our model is largely that quoted in the recent attempt to use alternative models.[7] This initial data set is confined to *cis* double bonds. In its simplest form, our σ-inductive theory requires only one new parameter in order for it to predict all the Δδ values, for all polyene esters or acids not ruled out by the above caveat. This parameter is the (hypothetical) intercept, *I* (C=C), in eqn. (1) above, when the double bond is the generating dipole. The other parameters in this equation, namely the effect of the ester/acid generating dipoles, and the attenuation factor, are already fixed from the monoene data, as above. We have indeed carried out this fitting process for *I* (C=C), and obtain results very similar to those displayed above. However, one can improve the fit further, for shifts generated through other, intervening multiple bonds. This should not be unexpected. Our theory assumes that an intervening methylene pair attenuates the effect of a more remote dipole by (1.78),[2] whereas one intervening multiple bond may attenuate the same remote interaction by a somewhat different factor, in addition to being a generating dipole in its own right. We have therefore introduced one further fitted parameter to our analysis, namely the gain in transmission, *T*, arising from the multiple bond. *T* is defined as $(1.78)^2$/(the corresponding attenuation factor for the multiple bond).

The Tables show the least-squares fits obtained by varying these two parameters, *I* (C=C) and *T*. Most of the calculated values fall within the estimated error range of the data, and they also explain why the sign of Δδ varies with the placing of the double bonds. The fitted value of *I* (C=C) is 6.82 ppm, which may be compared with 20.9 ppm for the OH group. This is close to the one-third estimate given above. The *T* parameter, only relevant to polyunsaturated chains, affects the calculated shifts by up to 0.3 ppm. Its fitted value is 1.28, which indicates that *cis* double bonds are significantly more effective than dimethylene groups in the transmission of inductive effects. *T* is especially valuable in explaining data from highly unsaturated chains.[4]

We have also carried out a similar fitting procedure for a much less extensive set of data[2] involving *trans* double bonds. We obtain very similar but slightly larger *I* and *T* factors.

Shifts generated at and by C≡C bonds

Bus *et al.* have published a fairly extensive set of data for polyyne esters,[2] which enables us to extend our analysis to the triple bond (C≡C). Table 2 shows the fits obtained, using our earlier monoyne data to give an *I* value of 41.20 ppm for the effect of the ester grouping and the usual 1.78 attenuation factor. The resulting fitted parameters for the corresponding effects of the triple bond are *I* (C≡C) = 12.8 and *T* = 1.79. Thus *I* (C≡C) is close to twice *I* (C=C) and *T* (C≡C) − 1 is a little more than twice *T* (C=C) − 1. These increases seem intuitively reasonable, pending a fuller quantum mechanical analysis. The sp^3–sp bond dipole is approximately twice that of the sp^3–sp^2 dipole and also one might expect $T - 1$ to increase by more than double when one π-bond is replaced by two shorter ones.

Table 2 C≡C shift differences in polyynoic esters; calculated values are in parentheses

$18{:}2(n,m)$[a]	$(n+1)-n$[b]	$(m+1)-m$
$n = 2, m = 6$	*c*	5.05 (4.59)[d]
3,7	10.15 (10.09)[e,f]	2.95 (3.57)
4,8	0.75 (1.83)[e]	2.70 (3.00)
5,9	0.15 (0.04)	2.60 (2.68)
6,10	−1.05 (−0.97)	2.50 (2.50)
7,11	−1.65 (−1.54)	2.45 (2.40)
8,12	−1.95 (−1.86)	2.45 (2.34)
9,13	−2.15 (−2.04)	2.30 (2.31)
10,14	−2.20 (−2.20)	2.00 (2.20)[f]
11,15	−2.30 (−2.20)	4.35 (4.52)[f]
12,16	−2.50 (−2.23)	*c*

[a] Data from ref. 2. [b] *i.e.* $\delta_{C(n+1)} - \delta_{Cn}$. [c] Not used, because of end effects (see text). [d] General calculation formula is $41.2T \times 1.78^{-m} + 12.80 \times 1.78^{-(m-n-1)}$, with $T = 1.78$. [e] General calculation formula is $41.2 \times 1.78^{-n} - 12.80 \times 1.78^{-(m-n-1)}$. [f] Including end-effect correction based on palmitate shifts.

Shifts at C=O

Carbonyl shifts are noted[6] for their sensitivity to changes in solvent and temperature. This means that any predictions of absolute shift for C=O carbons will inevitably be less reliable than for CC shift differences in multiple bonds. Nevertheless, it is of interest to explore whether the principles developed above can also be applied at least to the semi-quantitative explanation of the experimentally observed trends for C=O shifts.

Shifts arising from remote double bonds. The equation giving the shift of C*n* caused by an ester group at C1 (slightly modified from ref. 11) is given in eqn. (2). Because σ-inductive effects arise from any dipolar bond in the chain and transmit in either direction, a similar formula should hold for the effect of a multiple bond dipole on the ester carbonyl shift. The initial constant will be the C=O shift for a long, saturated chain such as stearyl, bound in the same chemical way. We have measured this ‘infinite-*n*’ shift in a range of triglyceride esters and indeed find that it varies by less than 0.01 ppm between different compounds. The shift for the outer, α-chains is 173.277 ppm and for β-chains is 172.863 ppm. Indeed, in all matched cases, α-CO always appears at 0.414 ppm to high frequency of β-CO, because of their different γ-gauche interactions. Our measure-

$$Cn\ \text{shift} = 129.835 - 27.60 \times 1.78^{-n} \qquad (2)$$

Table 3 Carbonyl shifts in glyceryl mono-, di- and tri-esters

Compound and chain	Carbonyl shift	Shift difference from corresponding S chain[c]	Shift difference for α − (corresponding) β	Shift difference from corresponding triglyceride
α chains				
S S **S**[a]	173.277			
S S O	173.286			
S O S	173.281			
S O O	173.278			
S S oh	173.794			0.517 (0.446)[b]
S oh **S**	173.918			0.641 (0.794)
S oh oh	174.385			1.108 (1.240)
O S S	173.261	−0.018[d]		
O S **O**	173.255	−0.031[d]		
O O S	173.253	−0.028[d]		
O O **O**	173.244	−0.034[d]		
O O oh	173.767			0.523 (0.446)
O oh **O**	173.888			0.644 (0.794)
O oh oh	174.362	−0.023[d]		1.118 (1.240)
13-Erucyl[e]		−0.008 (−0.003)		
11-Eicosenoyl	173.268	−0.010 (−0.009)		
11-Eicosenoyl[e]		−0.013 (−0.009)		
9,12-Linoleyl	173.237	−0.041 (−0.033)		
β chains				
S **S** S	172.863		0.414	
S **S** O	172.871		0.415	
O **S** O	172.866		0.412	
S **S** oh	173.435			0.572 (0.794)
S **O** S	172.845	−0.018[d]	0.416	
S **O** O	172.841	−0.030[d]	0.414	
O **O** O	172.832	−0.034[d]	0.412	
O **O** oh	173.401			0.569 (0.794)
9,12-Linoleyl	172.822	−0.041 (−0.033)		
9,12,15-Linolenyl[e]		−0.043 (−0.035)	0.415	

[a] S = stearyl, O = oleyl, oh = no acid chain. Relevant chain in bold type. [b] Differences in parentheses are calculated, as in Table 1, but with a scaling factor of 0.7 derived from the average S–O difference above. [c] Differences in parentheses calculated, as described in text, using data from ref. 11. [d] Average triglyceride S–O shift difference = −0.027. This average is used for calculating S–X differences. [e] Experimental shift differences from ref. 8.

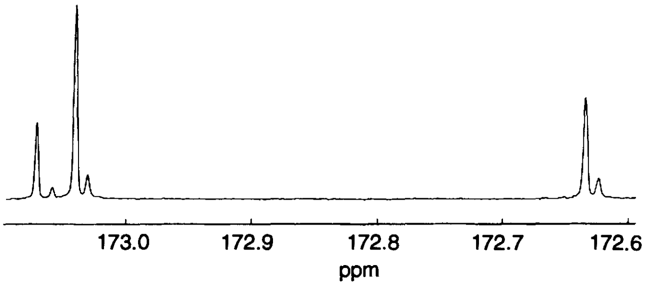

Fig. 1 Ester CO region of the ^{13}C NMR spectrum of a commercial olive oil. Assignments (*l* to *r*) are: saturates (α chains), 11-eicosenoyl (α), oleyl (α), linoleyl (α), oleyl (β chain), linoleyl (β).

ments confirm and extend similar measurements by other authors.[8,9,13] The corresponding shift for monomethyl esters is 174.316 ppm: in this case chain-methyl γ-gauche interactions are negligible. However, all these shifts are also sensitive to the presence of double bonds in the same chain, as is shown in Table 3. Fig. 1 shows the CO region of a typical spectrum for a naturally heterogeneous triglyceride, to illustrate their analytical usefulness. The quantitative analysis of such spectra has been discussed by Wollenberg.[8] The CO shift is also still more sensitive to the absence of a neighbouring chain, in di- and mono-glycerides and these shift changes are discussed later.

The relevant equation for the CO shift will also have a different multiplicative, intercept constant I (C=O). This constant may be considered to arise from the effect of the generating dipole, multiplied by the response of the reporter group. I (C=O) remains to be determined, because although an I (C=C) factor of 6.82 ppm, was found above for C=C double bonds acting on each other, the reporter group has now changed to just one atom in C=O. In contrast, the attenuation factor of 1.78 should remain fixed, because it arises simply from the intervening chain methylenes. The positional integer n must be replaced by $(n - 1)$, because the double bond at Cn is considered to behave simply as a substituent at the C$(n - 1)$ carbon, for present purposes.

Table 3 lists our experimental shift differences between the shifts of ester carbonyls terminating saturated chains and those terminating otherwise identical unsaturated chains. It also includes shift differences calculated from a modified eqn. (2). This equation predicts the correct sign and sequence of carbonyl shifts, and gives I (C=O) = 2.72, suggesting that the C=O bond has only *ca.* 65% of the sensitivity of the C=C bond to the influence of a C=C dipole, as judged from the shifts of the C nearest to the generating dipole.

Shifts arising from the absence of neighbouring chains. Table 3 also offers the more speculative possibility of explaining the carbonyl shift differences between triglycerides and di- or mono-glycerides. These differences, relative to the appropriate chain of glyceryl tristearate, are listed in Table 3 and compared with the shift differences calculated on the admittedly naive theory that all the atoms of the glyceryl moiety, including O, have the same 1.78 transmission factor as methylene groups. There are then no further factors to fit. The generating dipoles for the groups -OH and -OCOR have already been determined in *e.g.*, jojoba lipids,[10,11] and the reporter group is as above. It merely receives incremental charge from a different part of the molecule. Thus, the equation used for calculation is simply the above, modified eqn. (2) with the generating dipole being the difference of the -OCOR and the -OH generating dipoles, multiplied by the 0.7 factor discussed above.

The calculated data in Table 3 show a semi-quantitative relation to experiment. One would expect some discrepancies, particularly when a neighbouring chain has been removed, because one also anticipates some changes in the average γ-gauche effect. From our analysis, this factor alone seems to account for a drop in shift of *ca.* 0.2 ppm upon removal of a neighbouring chain and is thus smaller than the through-bond effect.

Experimental

Carbon-13 NMR spectra were measured on Bruker AC250 and ACP400 spectrometers, with lipid solutions 10% by volume in deuteriochloroform. 128 K data points were used, to maximise digital resolution. Referencing was to internal Me$_4$Si, except that the lipid methyl shift was used to make very small referencing corrections (< 0.02 ppm). A narrowed sweep width was used for the Fig. 1. Iterative fitting was carried out using Microsoft Excel 4 with Solver.

Triglycerides were purchased from Sigma.

Conclusions

All the multiple bond shifts studied so far seem to be quantitatively predictable on the σ-inductive theory, using very few parameters. This probably means that the shifts of as yet unstudied lipids, such as the initial degradation products of natural oils, can also be predicted. Indeed, the theory already explains the remarkable additivity of the shifts arising from the combination of normal ester shifts and of hydroxylation shifts, noted experimentally by Knothe[7] and explicitly by Pfeffer.[14] It could therefore make a useful contribution to lipid analysis, as well as establishing the inductive influence of multiple carbon–carbon bonds.

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