sons for the above example.

In view of the advantages of nonparametric statistics illustrated in this paper, we recommend this procedure for consideration.

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Carbon-13 Chemical Shift Anisotropy Relaxation in Organic Compounds

Sir:

In the last few years numerous studies of ^{13}C spin-lattice relaxation times (T_1's) have shown that these investigations can yield valuable information about molecular dynamics in liquid systems.[1,2] The various applications for these measurements require that all contributing relaxation mechanisms must be identified. For carbon-13 nuclei, relaxation occurs by four processes:

$$R_1^{\mathrm{obsd}} = R_1^{\mathrm{DD}} + R_1^{\mathrm{SR}} + R_1^{\mathrm{SC}} + R_1^{\mathrm{CSA}} \qquad (1)$$

In eq 1, R_1^{obsd} is the observed relaxation rate ($\equiv 1/T_1$). The R_1 terms refer to *d*ipole-*d*ipole, *s*pin-*r*otation, *s*cal*a*r, and *c*hemical *s*hift *a*nisotropy contributions. For essentially all carbons in large molecules and for protonated carbons in small molecules the dipole-dipole term generally has been shown to be predominant.[2a-c]

It has often been assumed that relaxation due to chemical shift anisotropy and modulated scalar coupling affords a negligible contribution to R_1^{obsd} and that R_1^{SR} is the only process that can compete with efficient dipole-dipole relaxation.[1,2b] In this communication we present results for representative organic compounds showing that the CSA mechanism has been underestimated as a contributing term for unsaturated carbons not having directly attached hydrogens.

Relaxation through the anisotropic chemical shift can be separated unambiguously because R_1^{CSA} is proportional to the square of the static magnetic field. Within the extreme narrowing limit this could be written as[1b]

$$R_1^{\mathrm{CSA}} \equiv 1/T_1^{\mathrm{CSA}} = (\gamma_C^2 H_0^2/5)(\sigma_{12}^2 + \sigma_{23}^2 + \sigma_{31}^2)\tau_{\mathrm{eff}} \qquad (2)$$

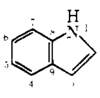
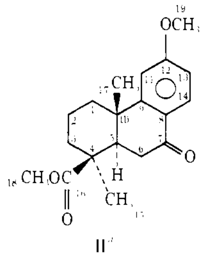
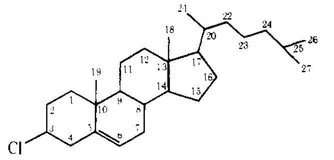
where the σ_{ij} terms represent the anisotropic magnitudes, $\sigma_i\sigma_j/3$, of the three principal terms of the diagonalized shielding tensor σ. An approximation that is often made is that σ is axially symmetric. Then eq 2 reduces to

$$1/T_1^{\mathrm{CSA}} = (2/15)\gamma_C^2 \Delta\sigma^2 H_0^2 \tau_{\mathrm{eff}} \qquad (3)$$

where $\Delta\sigma$ is the difference of the chemical shielding parallel and perpendicular to the axis of the shift tensor ($\sigma_\parallel - \sigma_\perp$).

Although it has been pointed out theoretically that anisotropic chemical shielding could provide significant relaxa-

Table I. ^{13}C Spin Lattice Relaxation Times (T_1's) and Nuclear Overhauser Effects (NOE's) Measured at 38° at High and Low Fields

		67.9 MHz^{b}		22.6 MHz^{c}	
	Carbons	T_1 (sec)	NOE (η)	T_1 (sec)	NOE (η)
 I^{a}	2	5.8	2.0	5.5	2.0
	3	5.8	1.9	6.4	2.0
	4	5.8	2.0	5.6	2.0
	5	5.3	1.8	5.4	2.0
	6	5.8	1.9	6.4	2.0
	7	5.8	1.9	5.4	2.0
	8	49	1.1	83	1.6
	9	47	1.0	85	1.5
 II^{d}	1, 2, 6^{e}	1.0 ± 0.05	1.9 ± 0.1	1.1 ± 0.2	1.9
	3	0.83	2.0	. . .^{e}	. .^{f}
	11, 14, 5^{e}	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	2.0
	13	1.34	2.0	1.5	2.0
	4	16.2	2.0	16.0	1.9
	10	13.8	2.0	15.0	1.9
	7	18.0	0.9	34.5	1.8
	8	25.0	0.8	48.0	1.7
	9	18.0	0.9	29.0	1.9
	12	22.0	1.1	33.0	1.8
	16	27.0	1.0	48.0	1.8
 III^{g}	1, 2, 4, 7, 15, 16, 11, 12^{e}	0.37 ± 0.03	1.9 ± 0.15	0.39 ± 0.06	1.9 ± 0.2
	3, 6, 8, 9, 17^{e}	0.70 ± 0.10	1.9 ± 0.15	0.70 ± 0.16	1.9 ± 0.2
	10, 13^{e}	4.2 ± 0.10	1.9 ± 0.15	4.5 ± 0.20	1.9 ± 0.2
	5	3.2	0.8	5.6	1.6

[a] Indole (4 *M*) in acetone-d_6; degassed by three freeze–pump–thaw cycles. [b] T_1's and NOE's have internal estimated errors less than 10%. Several separate runs for each sample produced deviations less than 5–10%. The T_1 measurements were performed using the fast inversion–recovery sequence (see ref. 10)(FIRFT) and/or the unmodified IRFT sequence. [c] T_1's are accurate to 5–15%, and the accuracy of the NOE's is 5% (compd I), 15% (compd II), and 10% (compd III). [d] Me–OMe–Podocarpate (0.8 *M*) in acetone-d_6; degassed by three freeze–pump–thaw cycles. [e] The stated values represent the range observed for all carbons in the group. [f] C-3 not well resolved at 22.6 MHz. [g] Cholesteryl chloride (1 *M*) in benzene-d_6, undegassed.

Table II. Contributions of Chemical Shift Anisotropy to the Relaxation of sp^2 Carbons at 67.9 MHz

	Carbon	T_1CSA[a] (sec)	$\Delta\sigma$[b] (exptl) (ppm)
I. Indole[c]	C-8	109	220
	C-9	94	230
II. Me-OMe-Podocarpate[d]	C-7	33	220
	C-8	42	200
	C-9	33	220
	C-12	49	180
	C-16	54	. . .[e]
III. Cholesteryl chloride[f]	C-5	5.3	340

[a] For the calculation of τ_{eff} the following values were used: $\gamma_C = 6720$, $\hbar = 1.05 \times 10^{-27}$, $r_{CH} = 1.09 \times 10^{-8}$ cm, $\gamma_H = 26{,}700$. [b] Estimated accuracy ±10%; maximum error ±20% (est) due to the relative low accuracy of the NOE's. [c] $\tau_{eff} \approx 8.1 \times 10^{-12}$ sec/rad using $NT_1 = 5.8$. [d] $\tau_{eff} \approx 2.6 \times 10^{-11}$ sec/rad using $NT_1 = 1.8$. [e] Correlation time unknown; see text. [f] $\tau_{eff} \approx 6.6 \times 10^{-11}$ sec/rad using $NT_1 = 0.72$.

tion in liquids,[3] only a very few studies *at high magnetic field and low temperatures* have been performed where important or dominant ^{13}C CSA relaxation has been established (at higher temperatures SR relaxation replaced the CSA contribution).[4] A variable field study of the acetylenic carbons in diphenyldiacetylene (DPDA) is the only reported example at room temperature where the CSA mechanism has been found to be important.[2b,5]

Table I summarizes the results of the present study. Accurate T_1 and NOE data for the three compounds shown in Table I clearly show that CSA relaxation contributes strongly to the relaxation *of all nonprotonated sp^2 carbons* at 67.9 MHz, with minor contributions also indicated at 22.6 MHz. This can be seen from the field dependence of the T_1's and NOE's[6] given in Table I. In the case of nonprotonated sp^3 carbons there is no observed field dependence whereas for nonprotonated aromatic and olefinic sp^2 carbons, the T_1 values at 67.9 MHz are ca. half those observed at 22.63 MHz, and the NOE's simultaneously drop from 1.5-1.9 to 0.8-1.1(η). This results from a field independent R_1^{DD} term[6] being augmented at high field by a comparable R_1^{CSA} term. Because of the large threefold magnetic field ratio it is possible to accurately calculate R_1^{CSA} contributions of 2-10% at 22.6 MHz. These results indicate that quantitative ^{13}C studies performed at moderate or high magnetic fields may be less accurate than expected due to variable NOE for nonprotonated carbons that can have anisotropic chemical shift tensors.

Evaluation of the anisotropy of the shift tensor, $\Delta\sigma$, from eq 3 by measurements of ^{13}C T_1's and NOE's is limited because of several reasons. Most importantly, accuracy is limited due to the uncertainty in the experimental T_1 and NOE values. Secondly, if the molecular motion is anisotropic the uncertain orientation of the principal axis system for the carbon chemical shift tensor places in doubt the validity of the calculated τ_{eff} for relaxation through this mechanism.

Although the protonated carbon T_1 data for I and II indicate limited motional anisotropy, all three compounds in Table I can be considered to undergo largely rigid, isotropic molecular reorientation. Thus it is possible to calculate approximate chemical shift tensor anisotropies for the sp^2 carbons in I-III; these are given in Table II. In Table II no $\Delta\sigma$ value is given for the carbonyl carbon C-16 of II. The ester group can undergo group segmental motion and thus τ_{eff} for C-16 is unknown. The very large $\Delta\sigma$ for C-5 in cholesteryl chloride (340 ppm) is somewhat surprising. We have no explanation at this time for this fact.

The earlier study[5] of DPDA yielded an anisotropy of ca. 270 ppm for the inner acetylenic carbons. For toluene, a thoroughly studied molecule both in liquid and solid state, an upper limit for $\Delta\sigma$ of 295 ppm was reported[4b] using the effective correlation time obtained from the dipolar relaxation rate. Another investigation[7] reported the anisotropic shielding tensors for the unsaturated carbons in acetonitrile and acetone as 460 and 390 ppm, respectively. However, quite recently a double resonance study of acetonitrile determined $\Delta\sigma$ to be 307 ± 4 ppm.[8]

Where it is possible experimentally the method of proton-enhanced nuclear induction spectroscopy for ^{13}C observation of solids is superior in accuracy and furthermore the cross polarization experiment gives directly the principal elements (σ_{ij}) of the chemical shift tensor.[9] In these studies benzene has been found to have an anisotropy of 180 ± 2 ppm.[9b]

In spite of the shortcomings of the liquid phase high resolution technique for determining $\Delta\sigma$, it is the only method useful for examination of molecules of even moderate complexity.

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On the Role of Cycloartenol in the Formation of Phytosterols. Biosynthesis of [19-^{2}H]Sitosterol in Deuterium Oxide Germinated Peas

Sir

The hypothesis that cycloartenol rather than lanosterol is the key precursor of phytosterols in higher plants rests on two sets of evidence. Firstly it was noted that cycloartenol was present in many plants, while lanosterol was found only rarely (latex of **Euphorbia** family) and then in minor amounts.[1] Also, [1-^{14}C]acetate[2] and [2-^{14}C]mevalonic acid[3] (MVA) were efficiently incorporated into cycloarten-