Carbon-13 Nuclear Magnetic Resonance Spectral Assignments in Large Molecules with the Aid of Spin–Lattice Relaxation Data

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Summary ^{13}C Spin–lattice relaxation times are used to assign quaternary carbon n.m.r. signals from codeine and brucine.

HITHERTO the most commonly used method for the assignment of ^{13}C n.m.r. spectral lines has been single-frequency off-resonance proton decoupling[1] combined with empirical chemical shift estimates. Little attention, however, has so far been given to a spectral parameter, which has become readily obtainable with the advent of pulsed high-resolution techniques, the spin–lattice relaxation time T_1.[2,3]

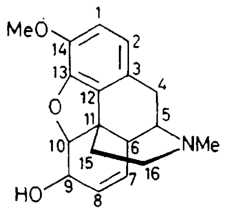
Allerhand and his co-workers[4] have recently shown that in large isotropically reorienting molecules, under the condition of extreme narrowing, the spin–lattice relaxation times of carbon atoms which are part of the rigid backbone of the molecule are inversely proportional to the number of directly attached protons as is predicted for dipolar relaxation.

Interest here is focussed on the non-proton-bearing carbon atoms, because these are particularly difficult to assign by conventional methods. In sucrose and cholesteryl chloride it has been shown[4] that such quaternary carbon atoms are completely relaxed *via* the dipolar mechanism. By assuming that intermolecular contributions are negligible in ^{13}C relaxation of large molecules,[4] the relaxation rate $(1/T_1)$ for such carbon atoms may be described by equation (1), *i.e.* $1/T_1$ is proportional to the sum of the

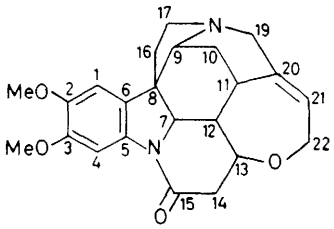
$$1/T_1 = h/2\pi\gamma_C^2\gamma_H^2 \sum_i r_{CH_i}^{-6}\,\tau_C \qquad (1)$$

inverse sixth power of the internuclear distances between the considered carbon atom and proton *i*. Since the nearest protons are generally those bound to α carbon atoms, to a first approximation a proportionality between the relaxation rate and the number of α-protons is to be expected. This, however, will not be precisely true, because bond angles and C–C as well as C–H bond distances can be different.

The application of the technique is demonstrated by the results obtained for codeine (I) and brucine (II) where



(I)



(II)

^{13}C *Chemical shifts and spin-lattice relaxation times*[a] *for compounds* (I)[b] *and* (II)[c]

Carbon	Codeine (I) δ_c[d]	T_1/s	Carbon	Brucine (II) δ_c[d]	T_1/s[e]
3	127·04	1·82	2	149·13/146·14	9·35/10·70
11	42·89	1·53	3	146·14/149·13	10·70/9·35
12	130·91	5·1	5	135·97	7·25
13	146·60	8·9	6	123·46	11·75
14	141·73	5·2	8	51·83	2·62
			15	168·64	4·30
			20	140·29	2·90

[a] The measurements were carried out in degassed deuteriochloroform solutions at 25 °C; [b] 1·6M; [c] 1·2M; [d] relative to internal $SiMe_4$; [e] determined by the progressive saturation technique in the pulsed mode.[3]

unequivocal assignment of quaternary carbon atoms by classical methods failed. Chemical shifts and spin–lattice relaxation times are listed in the Table. The relaxation times for (I) and (II) were determined for solutions in deuteriochloroform by the inversion-recovery[2] and progressive saturation techniques,[3] respectively. Most of the chemical shifts were assigned with the aid of magnitudes and multiplicities of the residual splittings in the off-resonance decoupled spectra. A more detailed account will be provided in a later publication.

The relaxation times for proton-bearing carbon atoms in both structures (I) and (II) were less than 1 s, suggesting that the dipolar mechanism dominates. As previously observed for cholesteryl chloride,[4] methylene carbon atoms are relaxed twice as fast as methine systems, this being indicative of isotropic reorientation. From average values of T_1(CH) of 0·15 and 0·23 s, respectively, reorientational correlation times of 3×10^{-10} and 2×10^{-10} s were computed. For such slow overall motion the dipolar mechanism for the relaxation of quaternary carbons is expected to prevail over competing spin rotational interaction. Since the nuclear Overhauser enhancement (NOE) is intimately connected with dipolar spin-lattice relaxation,[5] knowledge of this effect enables one to separate the observed relaxation rate $[R_1^{\text{tot}}\ (\equiv 1/T_1^{\text{tot}})]$ into a dipolar contribution, R_1^{D}, and a contribution, R_1', arising from other mechanisms according to equation (2) where R_1^{D} can be dissected from

$$R_1^{\text{tot}} = R_1^{\text{D}} + R_1' \tag{2}$$

R_1^{tot} by using the relationship (3),[5] where η is the observed

$$R_1^{\text{D}} = R_1^{\text{tot}}\,(\eta/\eta_0) \tag{3}$$

Overhauser enhancement and η_0 is the theoretical maximum NOE, given by equation (4). Spectra obtained

$$\eta_0 = 0{\cdot}5\,(\gamma_{\text{H}}/\gamma_{\text{C}}) = 1{\cdot}988 \tag{4}$$

with long pulse intervals in order to allow complete return of the magnetization to thermal equilibrium yielded equal integrated intensities for all signals, including those due to quaternary carbon atoms, thus proving purely dipolar relaxation.

This allowed unambiguous assignments to be made for carbon atoms 3, 12, 13, and 14 in (I) and 2(3), 5, 6, and 20 in (II). In compound (I) carbon atoms 12 and 13 are expected to exhibit the largest T_1 values because of absence of α-CH systems. For shielding reasons the resonances at 130·91 and 146·60 p.p.m. are assigned to C-12 and C-13. The lower value found for C-12 may be explained by the proximity of the methylene protons of C-15. Among the four aromatic carbon atoms C-3 should be relaxed fastest because three α-protons contribute to its relaxation. The remaining quaternary carbon signals at 127·04 and 141·73 p.p.m. are therefore assigned to C-3 (T_1 1·82 s) and C-14 (5·2 s). Carbon atom **11**, whose designation is unambiguous for chemical shift reasons, exhibits the shortest T_1 (1·53 s) as anticipated for its four α-protons. The methyl relaxation times were lengthened as a consequence of group rotation. The methoxy-carbon atom, having two degrees of freedom for internal motion, showed a characteristically longer T_1 than the *N*-methyl carbon atom.

Similar reasoning applies to (II), where all except two quaternary carbon atoms were assigned with the combined aid of relaxation and shielding arguments.

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