The Full Assignment of the Carbon-13 Nuclear Magnetic Resonance Spectrum of 5α-Cholestan-3β-ol with the Aid of the Lanthanide Shift Reagent Yb(dpm)₃

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Shifts induced by Yb(dpm)₃ and La(dpm)₃ in the ^{13}C and [1]H n.m.r. spectra of 5α-cholestan-3β-ol are used in conjunction with off-resonance proton decoupling to assign the 27 carbon resonances. Simple shielding considerations are required to resolve ambiguities in only two pairs of resonances. Three computer-based approaches to metal ion positioning are examined, best results being obtained when the metal ion is allowed to populate, equally, sites symmetrically disposed with respect to the O–C(3)–H(3) plane. The steroid does not appear to rotate freely about the O–C(3) bond in the shift reagent–steroid complex. Spin–lattice relaxation times are presented in support of the assignments.

THE increasing availability of pulse and Fourier transform n.m.r. spectrometers has made the recording of [13]C n.m.r. spectra a routine matter, and the problem of the assignment of such spectra is becoming more acute. In the past, assignments have been based largely upon chemical shift screening data derived from exhaustive studies on analogues and simple systems; where appropriate, off-resonance decoupling has been used to

resolve ambiguities.[1] Such an approach has the disadvantages (i) of involving many man-hours and (ii) in some cases of leading to controvertible assignments. However, lanthanide shift reagents can be conveniently used in the assignment of ^{13}C spectra.[2]

The recent publication[3,4] of two papers demonstrating the utility of lanthanide shift reagents in assigning the [13]C n.m.r. spectra of steroids prompts us to

[1] J. B. Stothers, ' Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York, 1972.

[2] J. Briggs, F. A. Hart, G. P. Moss, and E. W. Randall, *Chem. Comm.*, 1971, 364.

[3] J. W. ApSimon, H. Beierbeck, and J. K. Saunders, *Canad. J. Chem.*, 1973, **51**, 3874.

[4] W. B. Smith, D. L. Deavenport, J. A. Swanzy, and G. A. Pate, *J. Magnetic Resonance*, 1973, **12**, 15.

present our data for the assignment of the [13]C n.m.r. spectrum of 5α-cholestan-3β-ol (1), since our approach differs in some important aspects from the approaches

(I)
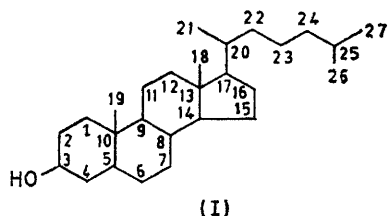
already published. Thus ApSimon et al.,[3] using Eu[III]- and Pr[III]-induced shifts for some keto- and hydroxy-androstanes, note that best fits between observed and calculated induced shifts are obtained by ignoring shifts at carbons close to the site of complexation: further, an unusually short Pr–O distance (1·8 Å) is found to yield the best fit, unique metal ion positions (only) being assumed. The authors ascribe the anomalous shifts at carbons close to the complexation site to 'contact' effects, but we would point out that superimposed upon such effects are diamagnetic 'complex formation' shifts [5] which are by no means negligible, especially (as we have previously demonstrated [6]) for ketones. On the other hand, Smith et al.[4] use Yb[III]-induced shifts (which are known to be largely pseudo-contact [6,7]) to assign the [13]C n.m.r. spectra of all but the side-chain carbon atom resonances of i-cholesterol and epi-i-cholesterol: however, once again, only unique metal ion positions are assumed, and no consideration is given to the 'complex formation' shift.

Our approach, which uses Yb(dpm)₃-induced pseudo-contact shifts, is not restricted to this 'unique' assumption (which we have previously demonstrated to be unsound in the case of ketones [8]) and leads to improved fits between experimental and calculated lanthanide-induced shifts: we further show how the 'unique' assumption can lead to incorrect carbon resonance assignments. A correction is applied for the 'complex formation' shift, and we finally show that our assignments are supported by spin–lattice relaxation time measurements.

Commercial 5α-cholestan-3β-ol (pure by t.l.c. on silica with ether–petroleum) was used without further purification. Spectra were recorded on solutions of known concentration (ca. 0·8M) in deuteriochloroform (dried over preheated B.D.H. molecule sieves, type 3A) using Bruker WH90 (operating at 22·6 MHz) and Varian XL 100 (operating at 25·2 MHz) pulse Fourier transform spectrometers (^{13}C data), and a Perkin-Elmer R32B (operating at 90 MHz) spectrometer (^{1}H data). [13]C Spectra were determined (i) with full proton 'noise' decoupling, (ii) with off-resonance broad-band proton decoupling, and (iii) with off-resonance narrow-band proton decoupling at assorted decoupling powers and

frequencies. Lanthanide-induced shifts were determined by sequential addition of shift reagent to substrate solution and linear least-squares extrapolation (correlation coefficients generally >0·99) to 1 : 1 molar ratios. The reagents La(dpm)₃ and Yb(dpm)₃ were synthesised by the literature method [9] and were dried at 100° over phosphoric oxide in vacuo. Spin–lattice relaxation times (T_1) were measured with a 180-τ-90° pulse sequence (inversion-recovery [10]) and proton noise decoupling, on a ca. 1M solution in deuteriochloroform at 35°. The delay between successive 180° pulses was 30 s.

All 27 carbon atom resonances were resolved and are shown diagrammatically in Figure 1 before and after the addition of 0·27M-Yb(dpm)₃; the relative heights of the lines in Figure 1 correspond to the observed relative signal intensities; shifts are in Hz (measured at 25·2 MHz) relative to the centre of internal $CDCl_3$. The resonances are numbered consecutively from low to high field.

Preliminary studies with the diamagnetic lanthanide complex La(dpm)₃ indicated that significant 'complex formation' shifts occur only for carbon resonances numbered 1 (2·0 p.p.m. mol mol⁻¹, deshielding) and 9 (0·9 p.p.m. mol mol⁻¹, shielding). These shifts are taken into account in the Table which lists the observed shifts in p.p.m. mol mol⁻¹ and shift percentages relative to resonance 1, induced by Yb(dpm)₃ for all carbon resonances, and for H(3), (18), and (19). Off-resonance broad-band decoupling immediately established signals 6 and 14 as corresponding to quaternary carbon atoms since these are the only signals which remained sharp under the decoupling conditions. Using the induced-shift data it was then immediately apparent that resonance 14 corresponds to C(10) and resonance 6 to C(13). The shift data further allowed the assignments of resonances 1, 9, and 16 to C(3), (4), and (2) respectively, the distinction between the latter pair being straightforward because of the large chemical shift difference between the two resonances (Figure 1) arising from the well documented β-substituent deshielding effect expected for C(4). Signals 5 and 10 were assigned to C(5) and (1), respectively, on the basis of the shift data and off-resonance narrow-band decoupling under which conditions signal 5 appears as a doublet (signifying one bound hydrogen atom) and signal 10 as a triplet (signifying two bound hydrogen atoms). A similar combination of shift and narrow-band decoupling data established resonance 26 as corresponding to C(19).

These (definitely) assigned shifts were then used along with the lanthanide-induced shifts for H(3), (18), and (19) to establish, using a computer, optimum positions for the ytterbium ion which would generate induced shifts closest to the observed shifts at the assigned

[5] B. Bleaney, C. M. Dobson, B. A. Levine, R. B. Martin, R. J. P. Williams, and A. V. Xavier, J.C.S. Chem. Comm., 1972, 791; K. Tori and Y. Yoshimura, Tetrahedron Letters, 1973, 3127.

[6] D. J. Chadwick and D. H. Williams, J.C.S. Chem. Comm., 1974, 128.

[7] O. A. Gansow, P. A. Loeffler, R. E. Davis, M. R. Willcott, and R. E. Lenkinski, J. Amer. Chem. Soc., 1973, 95, 3389.

[8] D. J. Chadwick, Tetrahedron Letters, 1974, 1375.

[9] K. J. Eisentraut and R. E. Sievers, Inorg. Synth., 1968, 11, 94.

[10] R. Freeman and H. D. W. Hill, J. Chem. Phys., 1971, 54, 3367.

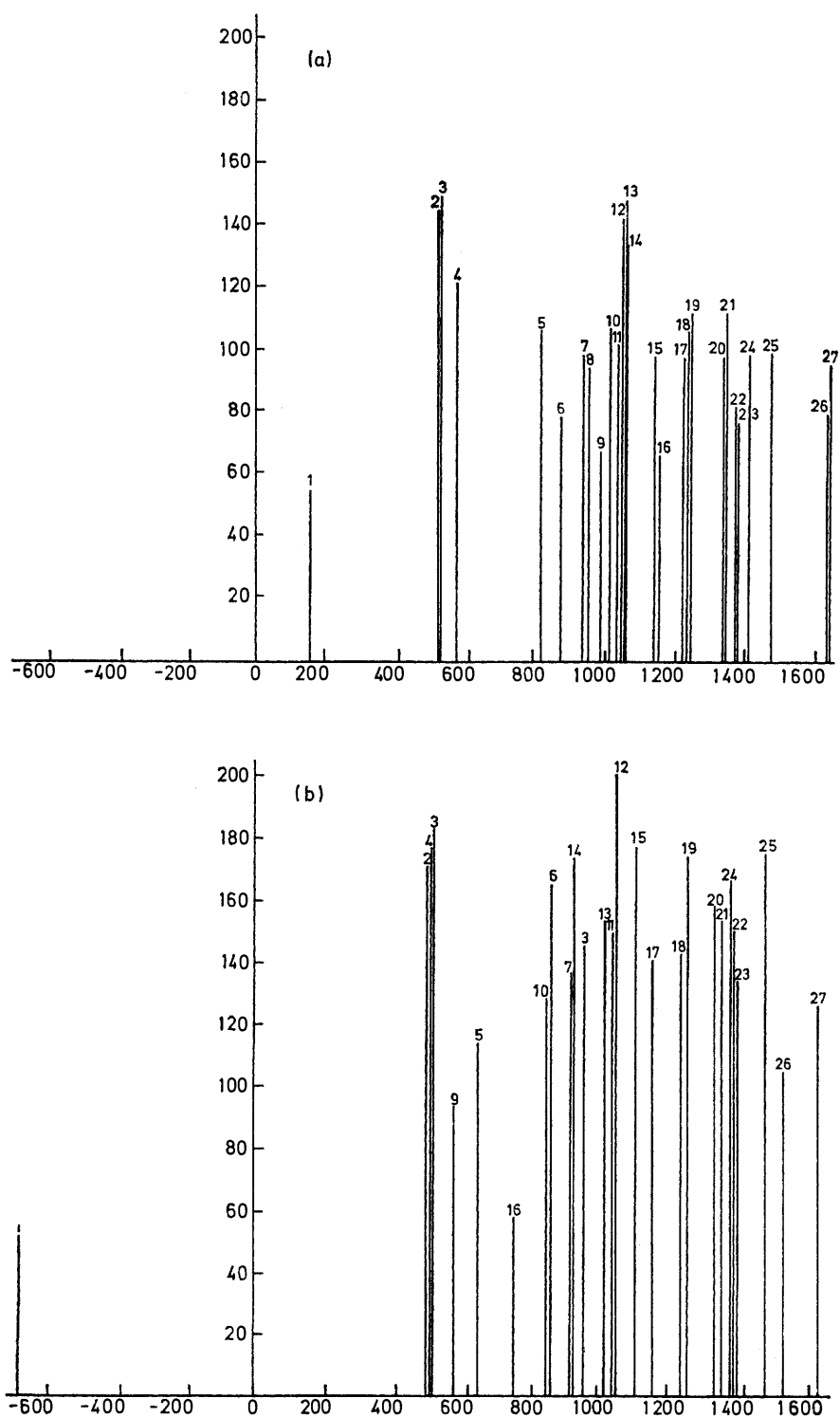FIGURE 1 ^{13}C N.m.r. spectrum of 5α-cholestan-3β-ol (a) before and (b) after addition of Yb(dpm)$_3$ (0·27M)

nuclei, assuming only pseudo-contact contributions and 'effective axial symmetry' in the shift reagent–substrate complex. By 'effective axial symmetry,' we imply not a static symmetry axis, but rather an 'effective' symmetry axis, which results after dynamical averaging about the lanthanide–ligand bond,[11] and allows the use of the simple one-term dipole–dipole formula. In the absence of a published crystal structure for the steroid,

it is highly unlikely that the steroidal side-chain is immobile in solution, its X-ray co-ordinates were nevertheless included in the analysis in order to establish whether or not, despite the possibility of conformational heterogeneity, reasonable spectral assignments could be made. The program [13] scans the positions of the metal ion over spheres (centre 0,0,0) specified by two angles and a variable radius (Figure 2). Previous

Resonance number:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Yb(dpm)$_3$-induced shifts (p.p.m. mol mol^{-1})	110·8	4·1	2·2	9·4	23·9	3·4	4·0	0·8	57·1	24·1	1·2	1·7	6·6	19·4	6·42
Shift ratios	100·0	3·7	2·0	8·5	21·6	3·1	3·6	0·7	51·5	21·8	1·0	1·5	6·0	17·5	5·80
'Obvious' assignments including off-resonance broad band data	C(3)				C(5)	C(13)			C(4)	C(1)				C(10)	
Predicted shift ratios	100·0				22·5	3·5			51·1	22·2				18·0	
Refined predictions	100·0				21·8	2·9			52·5	21·5				17·2	
Additional assignments from Yb(dpm)$_3$ shifts		C(14)	C(17)	C(9)			C(12)	C(23) or C(24)			C(22)	C(20) or C(21)	C(8) or C(7)		C(8) or C(7)
Predicted shift ratios		4·3	2·4	9·7			4·1				1·3				
Refined predictions		3·7	1·9	8·9			3·4				1·0				
Include narrow-band decoupling data								C(23) or C(24)				C(20)	C(8)		C(7)
Predicted shift ratios												1·7	6·9		6·9
Refined predictions												1·3	6·0		5·9
Include shielding								C(24)							
Predicted shift ratios								0·8							
Refined predictions								0·6							
T_1 (s)	0·55	0·55	0·55	0·55	0·55	4·20	0·25	1·05	0·25	0·25	0·4	0·8	0·55	4·2	0·25

Resonance number:	16	17	18	19	20	21	22	23	24	25	26	27	H(3)	H(18)	(H19)
Yb(dpm)$_3$-induced shifts (p.p.m. mol mol^{-1})	56·5	11·1	1·9	0·6	2·7	0·7	0·5	0·5	6·37	1·6	16·2	3·3	75·6	3·4	16·0
Shift ratios	51·0	10·0	1·7	0·5	2·5	0·7	0·4	0·4	5·75	1·5	14·6	3·0	68·3	3·1	14·4
'Obvious' assignments including off-resonance broad band data	C(2)										C(19)		H(3)	H(18)	H(19)
Predicted shift ratios	50·4										15·0		67·9	3·1	13·8
Refined predictions	52·0										13·6		68·8	2·5	12·8
Additional assignments from Yb(dpm)$_3$ shifts		C(6)	C(16)	C(25) or C(26) or C(27)	C(15)	C(23) or C(24)	C(25) or C(26) or C(27)	C(25) or C(26) or C(27)	C(11)	C(20) or C(21)		C(18)			
Predicted shift ratios		11·6	2·2		3·0				6·7			3·2			
Refined predictions		9·9	1·7		2·3				5·8			2·5			
Include narrow-band decoupling data				C(25)		C(23) or C(24)	C(26) or C(27)	C(26) or C(27)		C(21)					
Predicted shift ratios					0·6					1·7					
Refined predictions					0·4					1·3					
Include shielding						C(23)	C(26) or C(27)	C(26) or C(27)							
Predicted shift ratios						1·0									
Refined predictions						0·7	0·4	0·3							
T_1 (s)	0·25	0·25	0·25	2·1	0·25	0·55	2·1	2·1	0·25	1·05	2·1	2·1			

the fractional co-ordinates for 2α,3β-dichloro-5α-cholestane [12] were used since the steroid framework is substantially free from distortion in this system. The co-ordinates were orthonormalised, and co-ordinates generated for a 3β-oxygen atom allowing usual bond lengths and angles. The axes were translated and rotated to place the oxygen at (0,0,0), the C(3)–O bond along the x-axis, and the steroid ring A symmetrically disposed about the x–z plane (Figure 2). Although

studies [14] have indicated that the effective magnetic axis of the lanthanide–substrate complex is coincident with the lanthanide ion–oxygen radius vector: in this study, therefore, the orientation of the principal symmetry axis was not varied independently of the metal ion scan, in order to economise on computing time.

The metal ion was constrained to populate, equally,

[11] J. M. Briggs, G. P. Moss, E. W. Randall, and K. D. Sales, J.C.S. Chem. Comm., 1972, 1180.

[12] H. J. Geise and C. Romers, Acta Cryst., 1966, 20, 257.

[13] A modification of program 'METALSEARCH,' L. O. Ford, Laboratory of Molecular Biophysics, University of Oxford, run on an I.C.L. 1906A computer.

[14] G. E. Hawkes, D. Leibfritz, D. W. Roberts, and J. D. Roberts, J. Amer. Chem. Soc., 1973, 95, 1659; D. J. Chadwick and D. H. Williams, J.C.S. Perkin II, 1974, 1202.

sites disposed symmetrically about the x–z plane [$i.e.$, the O–$C(3)$–$H(3)$ plane] (we consider this assumption to be well founded because of the essentially symmetrical nature of ring A), and the parameters were varied in the ranges R 2·5—3·2 Å in 0·1 Å steps, ϕ 10—170° in 10° steps, and θ 90—270° in 10° steps, goodness of fit
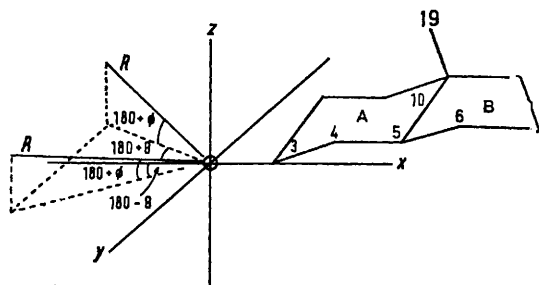

FIGURE 2 Co-ordinate system for computations

between calculated and experimental shifts for the already assigned nuclei being estimated by an agreement factor.[15] The agreement factor (R) used in the present work is given by equation (1). The calculated

$$R = \left\{ \frac{\Sigma([\text{shift}_{obs} - \text{shift}_{calc}]/\text{tolerance})^2}{\text{No. of observations}} \right\}^{\frac{1}{2}} \quad (1)$$

shifts for the methyl hydrogen atoms [H(18) and (19)] were estimated in each case by generating 12 hydrogen atom positions in a circle and averaging the resulting shifts (though it emerges during the analysis that the numerical difference between shifts so estimated and shifts determined merely using the X-ray crystal co-ordinates is well within the tolerances of the experimental data). The best metal ion positions had R at 3·0 Å, ϕ at 110°, and θ at 140 or 220° (agreement factor 0·111), giving a Yb–O–C(3) angle of 136° and a dihedral angle of $\pm 60\cdot5°$ with respect to the C(3)–H(3) bond. Goodness of fit between observed and calculated Yb^{III}-induced shifts was found to be strongly angle dependent but only weakly dependent on distance: thus, the next best solutions had the same values for the angular variables but with R at 2·9 Å. The shifts predicted for the best metal ion positions are in the Table which shows the assignments which follow directly from a comparison of the observed and predicted induced shift magnitudes. The only ambiguity remaining after inclusion of the off-resonance narrow-band decoupling data and simple shielding considerations is between C(26) and (27).

Thus we assign the ^{13}C n.m.r. spectrum of the ring system and side chain of 5α-cholestan-3β-ol by a combination of lanthanide-induced shift and off-resonance decoupling data, with recourse to simple shielding considerations for only two pairs of signals [those due to

C(2) and (4) and (23) and (24)]. Our assignment is in agreement with the published assignment based on shielding and analogue studies [16] except that the published assignments for C(12) and (16) should be reversed, as should those for C(18) and (19). The published C(18) and (19) assignments demand that the resonances ' cross ' during a shift experiment with a down-field shift reagent: we did not observe this. However, when a shift experiment is carried out with Pr(dpm)$_3$, then these two signals can be seen to cross, confirming that the literature assignment is definitely incorrect. Our spectrum is an improvement on the published spectrum in that we are able to resolve the resonance due to C(14) from that due to (17). With these assignments, the metal ion search was then repeated with optimisation on all 27 carbon atoms and on the three sets of hydrogen atoms. An improved solution with R at 3·2 Å, ϕ at 100°, and θ at 130 or 230° (agreement factor 0·098) was generated giving a Yb–O–C(3) angle of 129·3° and a dihedral angle of $\pm 77\cdot0°$. The ' refined ' shifts are listed in the Table.

The metal ion search was then repeated but allowing only $unique$ ion positions (this being identical to the literature procedure of ref. 4). The solutions so obtained gave poorer fits of calculated to experimental induced shifts, and the best metal ion position (R at 2·8 Å, ϕ at 110°, θ at 150°, agreement factor 0·225) predicted shifts which led to incorrect assignments for resonances 2, 4, 7, 8, 13, 17, 21, and 24. The search program was then further modified to allow only unique ion positions and, in addition, to calculated induced shifts as averages over 20 conformations of the steroid with respect to the Yb(dpm)$_3$ obtained by rotation of the steroid about the O–C(3) bond. The solutions so obtained once again gave poorer fits of calculated to experimental induced shifts. Further, no improvement was obtained by doubling the number of allowed steroid conformations. The best ion positions in these latter analyses had R at 2·8 Å, ϕ at 70 or 110°, and θ at 160 or 200° (agreement factor 0·278).

Thus we conclude that the shift data can best be accommodated by making the not unreasonable assumption of equal metal ion populations with respect to the plane bisecting the steroid ring A through C(3) and (10), and that such an approach permits a simple and straightforward means for the assignment of the ^{13}C spectrum. We consider that metal ion positions based on the ' unique position ' assumption are fictional, and may lead to incorrect shift predictions and incorrect spectral assignments. Further, our analysis does not point to ' free ' rotation of the steroid about the O–C(3) bond in the Yb(dpm)$_3$–steroid complex. In a more general case in which the essential symmetry about the site of complexation is no longer present ($e.g.$, a 3-substituted lanostane) we suggest that the computer program of Wing et $al.$[17] be used, since it permits $variable$ populations of sites as part of the optimisation process.

[15] M. R. Willcott, R. E. Lenkinski, and R. E. Davis, $J.$ $Amer.$ $Chem.$ $Soc.$, 1972, **94**, 1742.

[16] H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, $J.$ $Amer.$ $Chem.$ $Soc.$, 1969, **91**, 7446.

[17] R. M. Wing, J. J. Uebel, and K. K. Andersen, $J.$ $Amer.$ $Chem.$ $Soc.$, 1973, **95**, 6046.

Finally, we include spin–lattice relaxation times for the carbon resonances (Table and Figure 3). The absolute values are judged to have an error of $\pm 20\%$ although the relative values between resonances are

the side chain and methyl carbon atoms, the effective correlation time is decreased by internal motion leading to increased T_1 values, and that this effect increases towards the free end of the side chain: these observ-
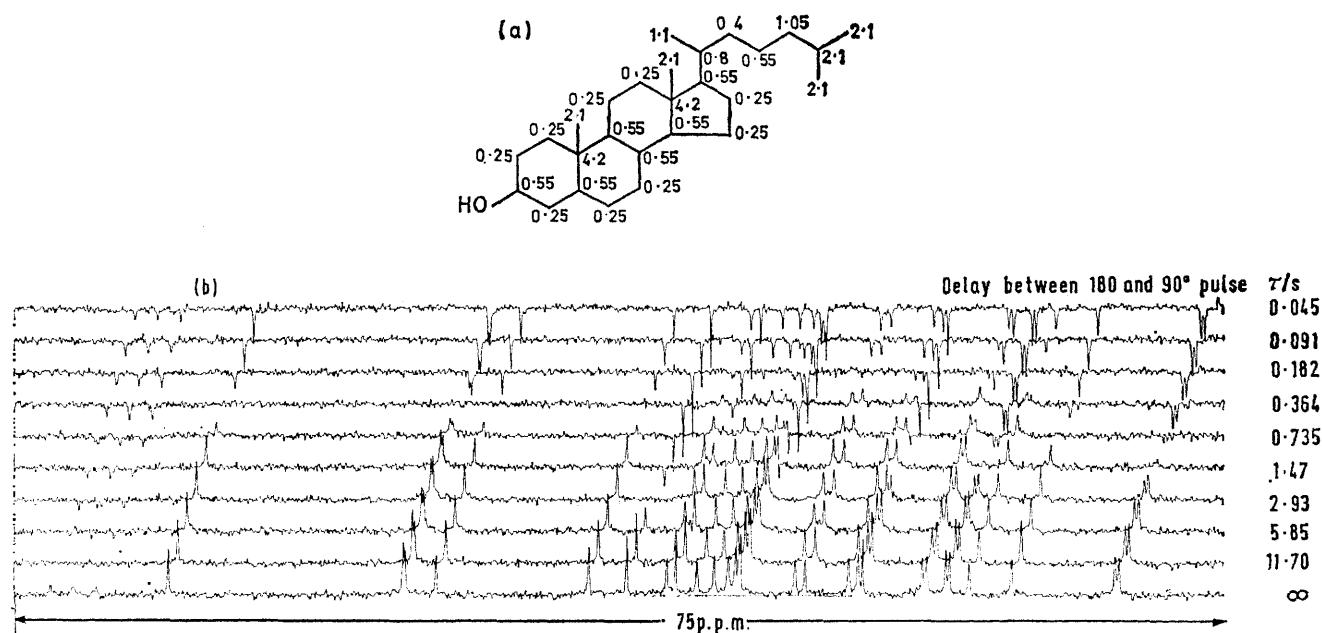

FIGURE 3 (a) Relaxation times T_1/s; (b) spectra obtained with $180°-\tau-90°$ pulse sequence

probably better than this. It is immediately apparent (i) that the T_1 values are entirely compatible with our assignment of resonances; (ii) that for ring carbon atoms, dipolar relaxation by protons is the dominant relaxation mechanism; (iii) that the rotation of the ring system is essentially isotropic; and (iv) that for

ations are in accordance with generally accepted views.[18-20]

[18] A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, 1971, **93**, 2777.

[19] A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, 1971, **55**, 189.

[20] F. W. Wehrli, *J.C.S. Chem. Comm.*, 1973, 379.