Carbon-13 Nuclear Magnetic Resonance Studies of C_{27} Sterol Precursors of Cholesterol[1]

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Received September 12, 1978

The natural-abundance ^{13}C nuclear magnetic resonance spectra of a number of C_{27} sterols at 25.2 MHz have been studied. Peak assignments for individual carbons of 5α-cholest-8-en-3β-ol, 5α-cholest-8(14)-en-3β-ol, 5α-cholest-7-en-3β-ol, cholest-4-en-3β-ol, 5α-cholesta-8,14-dien-3β-ol, and 5α-cholesta-7,14-dien-3β-ol have been made and were based upon single-frequency off-resonance decoupled spectra, considerations of the results of lanthanide-induced shift experiments, the use of empirical rules for chemical shifts, comparisons with the spectra of structurally related compounds, and, in one case, the use of a selectively deuterated analogue. The diagnostic use of $^3J_{^{13}C-^1H}$ long-range coupling to distinguish the angular quaternary carbons (C-10 and C-13) of sterols has also been explored.

The enzymatic conversion of lanosterol to cholesterol involves three general events: removal of the three "extra" methyl groups at carbon atoms 4 and 14, reduction of the Δ^{24} double bond, and "shift" of the Δ^8 nuclear double bond of lanosterol to the Δ^5 position in cholesterol. The overall enzymatic conversion of lanosterol to cholesterol involves a very large number of potential intermediates.[2,3] These potential intermediates include sterols with nuclear double bonds in various positions including the following: Δ^8, $\Delta^{8(14)}$, Δ^7, Δ^5, $\Delta^{8,14}$, $\Delta^{7,14}$, and $\Delta^{5,7}$. The isolation and identification of sterols differing only in the location of the nuclear double bonds is a less than trivial matter. The purpose of the present communication is to describe the results of carbon-13 nuclear magnetic resonance spectral studies of C_{27} sterols possessing the nuclear bonds in various positions in the sterol nucleus.

General Aspects of Spectral Analyses. The peak assignments for each sterol reported herein were based initially upon single-frequency off-resonance, decoupled (SFORD) spectra, subsequent considerations of characteristic ^{13}C chemical shift regions, analyses of the results of lanthanide induced shift (LIS) experiments, the use of empirical rules for chemical shifts, and comparisons with the spectra of structurally related compounds. In the case of 5α-cholest-8(14)-en-3β-ol, the use of a selectively deuterated analogue was employed to facilitate the peak assignments.[5]

The chemical shift data for the monounsaturated and diunsaturated sterols and their acetate derivatives (Figure 1) are presented in Table I along with data of others for 5α-cholestan-3β-ol (**2**). A convenient starting point in the assignments of the various peaks was the identification of the chemical shifts due to the carbon atoms of the alkyl side chain. The chemical shifts of these carbon atoms were, with the exception of C-20, not expected to vary significantly over all of the sterol derivatives studied in the present investigation. This invariance of spectral positions and chemical shift data from the earlier work of Reich et al.[6] and Eggert et al.[7] on **1a** and **2** permitted the rapid identification and assignments of these carbon atoms. The peak assignments for the carbon atom (C-3 in all cases) bearing a hydroxyl function were very easily made due to their characteristic chemical shift values and well isolated peaks. The peak assignments for the methylene carbon atoms of ring A (C-1, C-2, and C-4) were confirmed by the results of studies of shifts upon acetylation[6] of the 3β-hydroxyl function and LIS values for these carbon resonances. In general the carbon resonance peaks for C-10 and C-13 were very easily established since these quaternary carbons give characteristically sharp peaks in the noise spectra.[7]

Cholest-4-en-3β-ol (3a). Upon change of the nuclear double bond location from Δ^5 to Δ^4, the shieldings of only C-1 to C-10 and C-19 were altered significantly from those values for the corresponding carbons of cholesterol.[6,7] The peak assignments of these carbon atoms in **3a** were suggested by the results of the SFORD spectral analysis and confirmed by observation of acetylation shifts characteristic for allylic alcohols.[8,9]

5α-Cholest-7-en-3β-ol (4a). The chemical shift values for all ring carbons, C-1 through C-17, and the two angular methyl groups (C-18 and C-19) of **4a** were within ±0.2 ppm of the reported values for ergosta-7,22-dien-3β-ol.[10] The results of the peak assignments for **4a** agreed with those for ergosta-7,22-dien-3β-ol published by Abraham et al.[10] The peak assignments for the angular methyl groups (C-18 and C-19) in **4a** were confirmed by the results of LIS experiments.

5α-Cholest-8-en-3β-ol (5a). The peak assignments for this compound were based primarily on the results of comparisons of the spectra of this compound with those of **2** and 5α-cholesta-8,14-dien-3β-ol (**6a**). Four types of quaternary carbon atoms exist in **5a.** Two of these are olefinic carbons (C-8 and C-9) and showed characteristic chemical shift values.[4] Peak assignments for these olefinic carbon atoms were, however, not self-evident since the SFORD spectrum did not distinguish between the two carbon atoms (i.e., C-8 and C-9 both gave singlet peaks in the SFORD spectrum). Assignments were, however, permitted by consideration of the expected downfield shift due to β-carbon substitutions.[4] Since C-9 has four β-carbon substituents and C-8 has only three, C-9 would be expected to be more deshielded than C-8.[10] The reported chemical shift values for the corresponding carbons (C-8 and C-9) of lanostenol (4α,4β,14α-trimethyl-5α-cholest-8-en-3β-ol; (**7**)) reported by Knight[11] strongly support our assignments for C-8 and C-9 since, in the case of **7**, both C-8 and C-9 have the same number (four) of β-carbon substituents and showed the identical chemical shift value (134.4 ppm) for both carbons. This value is very close to the value (134.8 ppm) for C-9 in **5a** in our assignments. Peak assignments for the angular methyl carbons (C-10 and C-13) were derived from comparison with the chemical shift values for these carbon atoms of **5a** with those of the $\Delta^{8,14}$-sterol (**6a**). In view of the similarity of the chemical structure of rings A and B in these two sterols, the chemical shifts for the carbons of rings A and B were expected to be very similar. Based on this assumption, the higher field quaternary peak at 35.6 ppm in the spectrum of the Δ^8-sterol was assigned to C-10 and the lower field quaternary peak at 42.0 ppm was assigned to C-13. The shift deviation ($\Delta\delta$) for C-10 between the Δ^8- and $\Delta^{8,14}$-sterols was only 0.7 ppm while the corresponding shift deviation ($\Delta\delta$) for C-13 was 2.8 ppm, findings compatible with these assignments. The strongest evidence in support of the latter assignments was derived from the results of a LIS experiment. The LIS value for C-10 was 1.0 ppm (relative LIS = 0.12) while the LIS value for C-13 was 0.2 ppm (relative LIS = 0.025). Among the methine carbons of **5a** the peak assignments for C-14 and C-17

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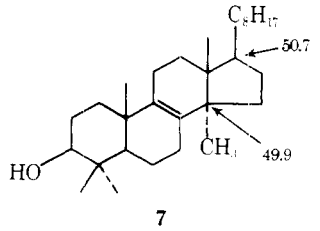
Table I. ^{13}C Chemical Shifts of C_{27} Sterols[a]

carbon atom	Δ^4		Δ^5		Δ^7		Δ^8		$\Delta^{8(14)}$		$\Delta^{7,14}$		$\Delta^{8,14}$	$\Delta^{8(14)}$-15-one	Δ^0	Δ^0
	3a[b]	3b[a]	1a[d]	1b[e]	4a[f]	4b[g]	5a[h]	5b[i]	8a[j]	8b[k]	11a[l]	11b[m]	6a[n]	9[o]	2	2[p]
1	35.3	34.9	37.2	36.9	37.1	36.8	35.1	34.8	36.5	36.2	36.7	36.5	35.2	35.6	37.0	37.0
2	29.4	25.0	31.5	27.7	31.3	27.4	31.5	27.5	31.5	27.5	31.4	27.5	31.3	30.8	31.5	31.5
3	67.8	70.7	71.4	73.8	70.7	73.5	70.9	73.3	71.0	73.4	70.6	73.2	70.7	70.4	71.2	71.4
4	123.2	118.8	42.2	38.1	37.8	33.8	38.2	34.1	38.2	34.0	37.8	33.7	37.9	37.5	38.2	38.2
5	147.3	149.2	140.5	139.4	40.2	40.0	40.7	40.5	44.2	44.0	39.6	39.4	40.8	43.9	44.8	44.9
6	32.2	32.2	121.3	122.4	29.6	39.5	25.4	25.3	28.9	28.7	30.2	30.1	25.1	29.0*	28.7	28.8
7	33.0	32.9	31.8	31.8	117.2	117.1	27.1	27.0	29.6	29.4	119.9*	119.7*	26.4	27.4*	32.1	32.1
8	35.9	35.8	31.8	31.8	139.3	139.2	128.0	128.1	126.1	125.8	134.2	134.2	122.7	150.5	35.4*	35.5
9	54.4	54.1	50.0	50.0	49.4	49.2	134.8	134.4	49.2	49.1	49.6	49.5	140.4	50.7	54.3	54.4
10	37.2	37.2	36.4	36.5	34.1	34.2	35.6	35.6	36.7	36.6	33.8	33.8	36.3	38.5	35.5*	35.5
11	21.0	21.3	21.0	21.0	21.5	21.4	22.7	22.7	19.9	19.8	20.9	20.9	21.7	19.4	21.3	21.3
12	39.8	39.7	39.7	39.7	39.5	39.5	36.9	36.8	37.2	37.1	40.1	40.0	36.8	36.8	40.0	40.1
13	42.4	42.4	42.2	42.2	43.2	43.2	42.0	42.0	42.6	42.6	46.3	46.3	44.8	42.3	42.5	42.6
14	56.1	56.0*	56.6	56.6	54.9	54.9	51.8	51.7	142.4	142.5	151.7	151.6	150.7	139.8	56.4	56.5
15	24.2	24.1	24.2	24.2	22.9	22.9	23.9	23.9	25.7	25.7	119.2*	119.4*	117.1	207.7	24.2	24.3
16	28.2	28.1	28.2	28.2	27.9	27.9	28.7	28.7	27.0	26.9	35.1	35.1	35.7	42.2	28.2	28.3
17	56.1	56.1*	56.1	56.1	56.1	56.1	54.8	54.8	56.8	56.8	58.6	58.6	57.0	50.7	56.2	56.3
18	12.0	11.9	11.8	11.8	11.8	11.8	11.2	11.2	18.2	18.1	16.4	16.5	15.6	18.6	12.1	12.1
19	18.9	18.8	19.2	19.2	12.9	12.9	17.8	17.6	12.8	12.6	12.3	12.2	18.2	12.7	12.3	12.3
20	35.7	35.7	35.7	35.7	36.1	36.1	36.2	36.2	34.4	34.3	34.0	34.1	33.8	34.3	35.7	35.8
21	18.6	18.6	18.7	18.7	18.8	18.8	18.7	18.7	19.0	19.0	18.9	18.9	18.7	19.0	18.7	18.7
22	36.1	36.1	36.1	36.1	36.1	36.1	36.1	36.1	35.9	35.9	36.0	36.0	35.9	36.3	36.1	36.2
23	23.8	23.8	23.8	23.8	23.9	23.9	23.7	23.7	23.7	23.7	23.7	23.7	23.6	23.3	23.8	23.9
24	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39.4	39.5	39.4	39.4	39.4	39.3	39.1	39.5	39.5
25	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.9	27.8	27.7	28.0	28.0
26	22.5	22.5	22.5	22.5	22.5	22.5	22.4	22.5	22.5	22.4	22.5	22.5	22.4	22.3	22.5	22.6
27	22.8	22.8	22.7	22.8	22.7	22.8	22.7	22.7	22.7	22.7	22.7	22.7	22.6	22.5	22.8	22.8
$COCH_3$		170.5		170.1		170.2		170.1		170.1		170.1				
$COCH_3$		20.9		21.3		21.3		21.3		21.2		21.3				

[a] In ppm downfield from Me_4Si; $\delta(Me_4Si) = \delta(CDCl_3) + 76.9$ ppm. Assignment of chemical shifts for close-lying peaks marked with an asterisk in any vertical column may be reversed although those given are preferred. [b] Registry no. 517-10-2. [c] Registry no. 4087-12-1. [d] Registry no. 57-88-5. [e] Registry no. 604-35-3. [f] Registry no. 80-99-9. [g] Registry no. 2465-00-1. [h] Registry no. 566-97-2. [i] Registry no. 5258-86-6. [j] Registry no. 566-99-4. [k] Registry no. 6562-21-6. [l] Registry no. 34227-11-7. [m] Registry no. 6562-05-6. [n] Registry no. 19431-20-0. [o] Registry no. 50673-97-7. [p] Registry no.89-97-7. [p] Data from ref 7.

were not immediately self-evident. Unfortunately, the results of a LIS experiment were not helpful in these particular assignments. The LIS values for C-14 and C-17 were the same (0.2 ppm; relative LIS = 0.025). C-14 is an allylic (α) carbon to the Δ^8-double bond whereas C-17 is a γ carbon to the double bond. In general, the γ effects of double bonds are small relative to α (allylic) or β (homoallylic) shift effects, especially on an exocyclic carbon.[12,13] On the basis of such considerations and comparisons of the chemical shifts for the corresponding carbons of **2** the peak at 54.8 ppm was assigned to C-17 and the peak at 51.8 ppm was assigned to C-14. In view of the 14α-methyl substituent effects on the chemical shift values for C-14 and C-17 of **7**, our assignments for C-14 and C-17 of **5a** appear reasonable. Upon removal of the 14α-methyl group of lanostenol (**7**), the C-17 carbon can be expected to be deshielded due to the absence of a γ-steric effect.[14,15] Upon removal of the 14α-methyl group of **7**, the C-14 carbon atom could be expected to change by ±1–2 ppm due to absence of an α effect.[14] Our data for C-17 of **5a** (54.8 ppm in **5a** vs. 50.7 ppm for C-17 in **7**[11]) are consistent with these considerations. The chemical shifts for C-15 and C-16 of **5a**



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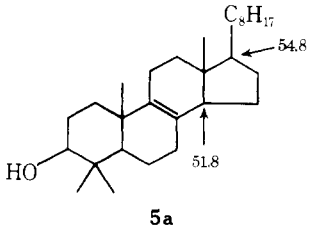


5a

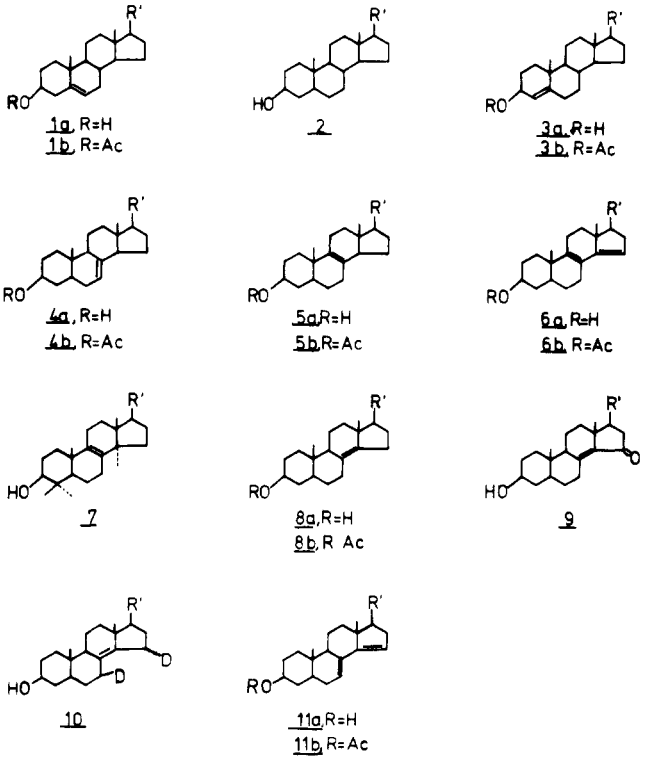
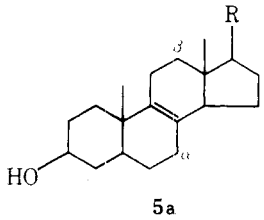


Figure 1. Sterols and steryl acetates considered in ^{13}C nuclear magnetic resonance peak assignment studies (R' = C_8H_{17}).

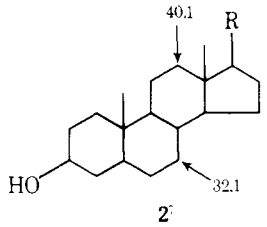
Chart I

case	δ, ppm	assignment	allylic shift, ppm	homoallylic shift, ppm	LIS, ppm
A	27.1	C-7	−5.1		0.3
	36.9	C-12		−3.2	0.2
B	27.1	C-12		−13.0	0.3
	36.9	C-7	4.8		0.2

were expected to be similar to those in **2** since induced shift effects of the Δ^8 double bond on these ring D carbons should be negligible. Based on these considerations the peaks at 23.9 ppm ($\Delta\delta$ = −0.3 ppm; **5a** vs. **2**) and 28.7 ppm ($\Delta\delta$ = 0.5 ppm; **5a** vs. **2**) were assigned to C-15 and C-16, respectively. The peaks at 22.7 and 25.4 ppm were assigned to C-11 and C-6, respectively, on the basis of the results of a LIS experiment and considerations of chemical shift correlations between **5a** and **6a**. The LIS value for C-6 was 0.5 ppm (relative LIS = 0.06) while the LIS value for C-11 was 0.4 ppm (relative LIS = 0.05). Finally, two methylene peaks, those at 27.1 and 36.9 ppm, remained to be assigned. It was not clear from the results of a LIS experiment which of these peaks was due to C-7 methylene carbon and which was due to that at C-12. An assignment could, however, be made by consideration of the magnitudes of the allylic[4,15] and homoallylic[12] shifts for C-12 (Chart I). The assignment pair listed in case A was preferred in view of the fact that the magnitude of the homoallylic shift in case B is too large to be accounted for by the β effect expected upon introduction of the Δ^8 double bond. Five types of methyl carbons exist in **5a**. The peaks at 18.7, 22.4, and 22.7 ppm can be assigned to C-21, C-26, and C-27 on the basis of comparisons with the chemical shift values for the corresponding carbons of **2**. C-19, which is located in a homoallylic position (β) to the Δ^8 double bond, should be deshielded by ~5 ppm.[13] Based on this reasoning, the peaks at 11.2 and 17.8 ppm were assigned to C-18 and C-19, respectively. The results of LIS experiments were compatible with these assignments. The LIS value for C-18 was 0.2 ppm while that for C-19 was 0.7 ppm.



5a



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5α-Cholest-8(14)-en-3β-ol (8a). Four types of quaternary carbons exist in **8a**. Two of these are olefinic carbons (C-8 and C-14) while two are aliphatic angular carbons (C-10 and C-13). The olefinic carbon peaks could easily be distinguished from the angular carbon peaks by their characteristically high chemical shift values. Differentiation between C-8 and C-14 was based upon considerations of the differences between the number of β-carbon substituents for the two carbons in question.[4,10] C-14, which is affected by four β-carbon substituents (C-12, C-16, C-17, and C-18), should resonate at a lower magnetic field than C-8 which has only three β-carbon substituents (C-7, C-10, and C-11). The peaks at 126.1 and 142.4 ppm were therefore assigned to C-8 and C-14, respectively. In many steroids, the angular quaternary carbon C-10 has been found to be more shielded than the other angular methyl carbon, C-13.[6] The peaks at 36.7 and 42.6 ppm were therefore assigned to C-10 and C-13, respectively. These assignments were strongly supported by the results of LIS experiments. The LIS value for C-10 was 1.0 ppm (relative LIS = 0.07) while the LIS value for C-13 was 0.4 ppm (relative LIS = 0.03). Six types of methine carbons (C-3, C-5, C-9, C-17, C-20, and C-25) exist in **8a**. The assignments for C-3, C-20, and C-25 were based upon comparisons with the chemical shift values for the corresponding carbons of **2**.[6,7] The assignments for the three other tertiary (methine) carbon atoms (C-5, C-9, and C-17) were made as follows. The peak at 56.8 ppm was assigned to C-17 on the basis of the fact that this peak was absent in the spectrum of 5α-cholest-8(14)-en-3β-ol-15-one (**9**). Introduction of the 15-keto function into the $\Delta^{8(14)}$-sterol should have a much larger effect on the resonance of C-9 than on C-5. On the basis of these considerations the peak at 49.2 ppm ($\Delta\delta = \delta(\mathbf{9}) - \delta(\mathbf{8a})$ = 1.5 ppm) was assigned to C-9 and the peak at 44.2 ppm ($\Delta\delta = \delta(\mathbf{9}) - \delta(\mathbf{8a})$ = −0.3 ppm) was assigned to C-5. The results of LIS experiments were also compatible with the above assignments for these methine carbons. The LIS values for C-5, C-9, and C-17 were 1.2 ppm (relative LIS = 0.09 ppm), 0.7 ppm (relative LIS = 0.05 ppm), and 0.29 ppm (relative LIS = 0.02 ppm), respectively.

8a contains 12 types of methylene carbons. Peak assignments for the methylene carbon atoms in the side chain (C-22, C-23, and C-24) and those in ring A (C-1, C-2, and C-4) followed those in **2**.[6,7] The remaining methylene peaks at 25.7, 27.0, 28.9, and 29.6 ppm had to be assigned to the remaining methylene carbon atoms (C-6, C-7, C-15, and C-16). Clarification of this matter was facilitated by the results of spectral studies of [7β,15ξ-$^2H_2$]-5α-cholest-8(14)-en-3β-ol[16] (**10**). The peaks at 29.6 and 25.7 ppm in the spectrum of **8a** were absent in the spectrum of **10**, a finding which permitted assignments of these peaks to C-7 and C-15.[5] Differentiation between C-7 and C-15 was made on the basis of the results of LIS experiments. The peak at 29.6 ppm (LIS = 0.44 ppm; relative LIS = 0.032 ppm) was assigned to C-7 while the peak at 25.7 (LIS = 0.29 ppm; relative LIS = 0.021 ppm) was assigned to C-15. The remaining two methylene peaks at 28.9 ppm (LIS = 0.7 ppm; relative LIS = 0.051 ppm) and at 27.0 ppm (LIS = 0.2 ppm; relative LIS = 0.015 ppm) were assigned to C-6 and C-16 on the basis of the results of LIS experiments. Additional evidence in support of the peak assignments for C-6 and C-16 was derived from observation of isotope shifts (~0.1 ppm upfield shift) for the two peaks in question upon deuteration at the neighboring (β) positions at C-7 and C-15 in **10**.

Five types of methyl carbons exist in **8a**. The assignments for the three methyl carbons in the side chain (C-21, C-26, C-27) followed those for **2**.[6,7] The remaining two methyl carbon peaks at 12.8 and 18.2 ppm were assigned to C-19 and C-18, respectively, since the C-18 carbon which is homoallylic (β) to the $\Delta^{8(14)}$ double bond should be deshielded by ~5 ppm. Additional strong support for the assignments for the angular methyl groups was derived from the results of LIS experiments. The LIS value for C-18 was 0.3 ppm (relative LIS = 0.02 ppm) while the LIS value for C-19 was 1.2 ppm (relative LIS = 0.09 ppm).

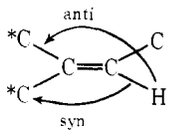
5α-Cholesta-8,14-dien-3β-ol (6a) and 5α-Cholesta-7,14-dien-3β-ol (11a). The spectral analyses for the $\Delta^{8,14}$- and $\Delta^{7,14}$-sterols were carried out analogously to those above with comparisons of the shift values of the dienes with the chemical shifts of the corresponding Δ^8- and Δ^7-sterols.

$^3J^{13}C-^1H$ Long-Range Coupling as a Structurally Diagnostic Effect on Angular Quaternary Carbons (C-10 and C-13). In the SFORD spectra of these sterol monoenes and dienes the peaks due to the angular quaternary carbon atoms (C-10 or C-13) were occasionally split, a situation arising from long-range coupling with the olefinic proton ($^3J_{^{13}C-^1H}$). This splitting was usually observed for the peaks of angular quaternary carbons which were located in an anti position[23] to the olefinic bond as depicted below. The results of this type of analysis with respect to the sterols considered in this study are summarized in Table II. These data indicate that, given a known location of a nuclear double bond, these long-range couplings can be of considerable diagnostic significance for

Table II. Long-Range $^3J_{^{13}C-^1H}$ Coupling in Olefinic Sterols

sterol	double bond position	C-10 ppm	$^3J_{^{13}C-^1H}$[a]	C-13, ppm	$^3J_{^{13}C-^1H}$[a]
3a	Δ^4	37.2	present	42.4	absent
1a	Δ^5	36.4	present	42.2	absent
4a	Δ^7	34.1	absent	43.2	absent
5a	Δ^8	35.6	absent	42.0	absent
8a	$\Delta^{8(14)}$	36.6	absent	42.5	absent
11a	$\Delta^{7,14}$	33.8	absent	46.3	present
6a	$\Delta^{8,14}$	36.3	absent	44.8	present

[a] Residual coupling; $^3J_{^{13}C-^1H} \approx 5$ Hz.



peak assignments of the angular carbons in sterols. Alternatively, if the peak assignments of the angular carbons of a sterol have been established independently, the long-range couplings could be of diagnostic importance with respect to the location of nuclear double bonds in the sterols.

In summary, ^{13}C nuclear magnetic resonance peak assignments for a number of C_{27} sterol precursors of cholesterol have been completed. In addition, the results of studies of long-range coupling data ($^3J_{^{13}C-^1H}$) for quaternary angular carbons of sterols indicate that such data provide important diagnostic information with respect to the determination of the location of nuclear double bonds.

Experimental Section

The ^{13}C nuclear magnetic resonance spectra were recorded on a Varian XL-100-15 spectrometer operating at 25.2 MHz in the Fourier transform mode using 0.2–0.7 M solutions of the sterols in $CDCl_3$. Data were accumulated with a maximum of 0.61 Hz per data point. A 5-mm sample tube was utilized and solvent-signal $CDCl_3$ was used as an internal standard. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (Me_4Si) and are estimated to be accurate to ±0.05 ppm (δ (Me_4Si) = δ ($CDCl_3$) + 76.9 ppm). The probe temperature was ~30 °C. Variation in sample concentration was found to have a negligible influence (<0.2 ppm). LIS experiments were performed using commercially available Eu(fod)$_3$. The ^{13}C NMR spectra (in $CDCl_3$) were first recorded in the proton noise-decoupling mode in order to measure the exact chemical shifts of all of the ^{13}C nuclei present. The degree of substitution of each carbon atom was determined by a second series of spectra in the single frequency off-resonance decoupling (SFORD) mode. Subsequently, an appropriate amount of Eu(fod)$_3$ was added to the $CDCl_3$ solution and the spectral data in the two modes were redetermined. The molar ratio of the shift reagent to the sterol was between 0.2 and 0.3. Relative LIS values are those relative to an assigned value of unity for C_3.

Cholesterol was purified via the dibromide.[18] Compounds **2**,[19] **4a**,[20] **5a**,[19] **6a**,[21] **8a**,[22] **9**,[23] and **11a**[24] were prepared as described elsewhere. Compound **3a** (mp 132–134 °C (lit.[25] mp 132 °C); single component on TLC) was prepared from cholest-4-en-3-one by reduction with lithium aluminum hydride followed by silica gel column chromatography and recrystallization from methanol–water. Acetate derivatives of the sterols were prepared using acetic anhydride and pyridine.

Registry No.—**10**, 69140-05-2; cholest-4-en-3-one, 601-57-0.

References and Notes

(1) These studies have been supported in part from a grant (HL-15376) from the National Institutes of Health and from a grant (C-583) from the Robert A. Welch Foundation. A preliminary account of a portion of this work has been presented (M. Tsuda, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 817 (1977)).
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