Inhibitors of sterol biosynthesis. Carbon-13 nuclear magnetic resonance studies of 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one and related compounds

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Abstract The natural abundance ^{13}C nuclear magnetic resonance spectra of a number of 9α-fluoro and 9α-hydroxy-$\Delta^{8(14)}$-15-ketosterols and their derivatives have been studied. Peak assignments for individual carbons of 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one, 9α-hydroxy-5α-cholest-8(14)-en-3β-ol-15-one, 5α-cholest-8(14)-ene-3,15-dione, 9α-fluoro-5α-cholest-8(14)-ene-3,15-dione, 9α-hydroxy-5α-cholest-8(14)-ene-3,15-dione, 3β-benzoyloxy-5α-cholest-8(14)-en-3β-ol-15-one, 3β-benzoyloxy-9α-fluoro-5α-cholest-8(14)-en-15-one, 3β-benzoyloxy-5α-cholest-8(14)-en-9α-ol-15-one, 3β-acetoxy-9α-fluoro-5α-cholest-8(14)-en-15-one, and 3β-acetoxy-5α-cholest-8(14)-en-9α-ol-15-one have been made. Also presented herein are: *1*) considerations of the substituent effects of the 9α-hydroxy and 9α-fluoro groups on carbon shieldings, *2*) demonstration that the state of oxidation at C-3 in the various $\Delta^{8(14)}$-15-ketosteroids affects the olefinic carbon shieldings due to an apparent long range through space effect of the electric field on the olefinic carbon shieldings, *3*) the results of analyses of ^{13}C-^{19}F spin-spin couplings, and *4*) the results of considerations of ^{13}C nuclear magnetic resonance studies of the concerned compounds with respect to the conformation of ring B in the various 9α-substituted sterols.—**Tsuda, M., and G. J. Schroepfer, Jr.** Inhibitors of sterol biosynthesis. Carbon-13 nuclear magnetic resonance studies of 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one and related compounds. *J. Lipid Res.* 1981. **22:** 1188–1197.

Supplementary key words 15-oxygenated sterols · 9α-hydroxysterols · 9α-fluorosterols · ^{13}C-^{19}F spin-spin coupling · electric field effect on olefinic carbon shieldings.

5α-Cholest-8(14)-en-3β-ol-15-one (I) (**Fig. 1**) is a potent inhibitor of sterol biosynthesis in animal cells in culture (1, 2). Moreover, this compound and a number of its derivatives have been shown to have significant hypocholesterolemic activity upon administration to intact animals (3–6). We have recently described the chemical syntheses of a number of 9α-fluoro and 9α-hydroxy derivatives of I (7). The effects of some of these compounds on sterol biosynthesis in cells in culture and on serum cholesterol levels of intact animals have been investigated. 9α-Fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II), 9α-fluoro-5α-cholest-8(14)-ene-3,15-dione (V), and 9α-hydroxy-5α-cholest-8(14)-en-3β-ol-15-one (III) have been found to be potent inhibitors of sterol synthesis in L cells (8). Moreover, II has been shown to have significant hypocholesterolemic activity upon oral administration to normal animals (9).

The availability of these 9α-fluoro- and 9α-hydroxysterols provided a unique opportunity to extend our previous ^{13}C nuclear magnetic resonance (NMR) studies of sterols (10–14) to explore the effects of these 9α-substituents. Presented herein are the results of detailed ^{13}C NMR studies on II and III and a number of their derivatives, including peak assignments and the exploration of the substituent effects of 9α-fluoro and 9α-hydroxy groups on carbon shieldings.

EXPERIMENTAL

The preparation of 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II) (7,8), 9α-hydroxy-5α-cholest-8(14)-en-3β-ol-15-one (III) (7,8), 5α-cholest-8(14)-ene-3,15-dione (IV) (15), 9α-fluoro-5α-cholest-8(14)-ene-3,15-dione (V) (7,8), 9α-hydroxy-5α-cholest-8(14)-ene-3,15-dione (VI) (7), 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (VII) (16–18), 3β-benzoyloxy-9α-fluoro-5α-cholest-8(14)-en-15-one (VIII) (7), 3β-benzoyloxy-5α-cholest-8(14)-en-9α-ol-15-one (IX) (7), 3β-acetoxy-9α-fluoro-5α-cholest-8(14)-en-15-one (X) (7), and 3β-acetoxy-5α-cholest-8(14)-en-9α-ol-15-one (XI) (7,8) have been described previously.

The ^{13}C NMR spectra were recorded on a Varian

Abbreviations: NMR, nuclear magnetic resonance; TMS, tetramethylsilane; LIS, lanthanide-induced shift.

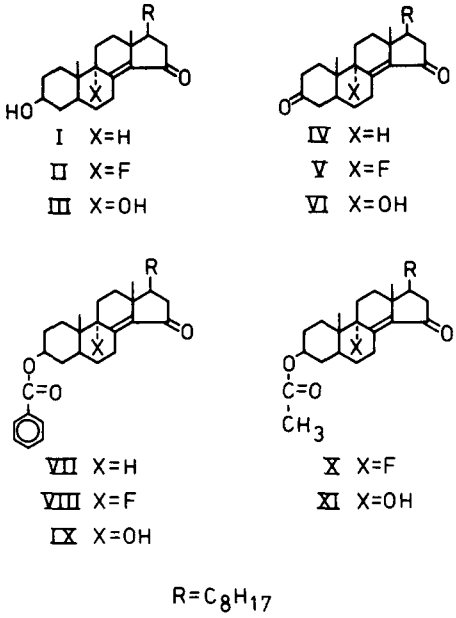
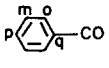


Fig. 1. 9α-Fluoro and 9α-hydroxy derivatives of $\Delta^{8(14)}$-15-ketosterols.

XL-100-15 spectrometer operating at 25.2 MHz in the Fourier transform mode using $CDCl_3$ solutions (0.12 M to 0.7 M) of the sterols. All samples were analyzed in tubes (5 mm o.d.) with a probe temperature of ~30°C. The numbers of spectral accumulations were 3K–6K for noise spectra and 8K–18K for SFORD (single frequency, off-resonance decoupled) spectra. Sweep widths of 5000 Hz with 4K data points were used, corresponding to 1.25 Hz per data point. The time interval between pulses was 1.6 sec and the flip angle was ~45°. Solvent-signal $CDCl_3$ was used as an internal standard. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) and are estimated to be accurate to ±0.05 ppm ($\delta(TMS) = \delta(CDCl_3) + 76.9$ ppm). Lanthanide-induced shift (LIS) experiments were performed using commercially available $Eu(fod)_3$. The ^{13}C NMR spectra (in $CDCl_3$) were first recorded in the proton-decoupling mode in order to measure the exact chemical shifts of all ^{13}C nuclei present. The degree of substitution of each carbon atom was determined

TABLE 1. ^{13}C Chemical shifts of 9α-protio, 9α-hydroxy, and 9α-fluoro-$\Delta^{8(14)}$-15-ketosterols

Carbon Atom	9α-Protio Compounds			9α-Hydroxy Compounds				9α-Fluoro Compounds			
	I	VII	IV	III	XI	IX	VI	II	X	VIII	V
1	36.3	36.1	37.7*[a]	29.6	29.4	29.4	31.2	29.7	29.5	29.5	31.1
2	30.8	27.2	37.5*	30.9	27.0	27.1	37.3*	30.7	26.8	26.9	37.1
3	70.4	73.5	210.1	70.4	72.9	73.6	211.0	70.1	72.6	73.0	209.6
4	37.5	33.6	43.9	37.9	33.7*	33.8*	44.1	37.5	33.4	33.5*	43.7
5	43.9	43.8	45.7	35.2	35.0	35.0	37.5*	35.2	35.4	35.5	37.7
6	29.0	28.9	29.1	28.4	28.3	28.3	28.7	28.1	27.9	27.8	28.2
7	27.4	27.3	26.9	22.7	22.7	22.6	22.3	23.0	23.0	23.0	22.6
8	150.5	149.7	148.3	148.1	147.9	148.3	147.3	143.5	143.3	143.1	141.9
9	50.7	50.5*	50.0	74.3	74.0	73.9	74.0	95.7	95.6	95.4	95.3
10	38.5	38.5	38.5	41.3	41.3	41.3	41.3	41.3	41.3	41.3	41.2
11	19.4	19.4	19.5	27.9	27.9	27.8	27.8	25.4	25.4	25.4	25.5
12	36.8	36.8	36.7	33.6	33.5*	33.4*	33.4	33.3	33.4	33.3*	33.2
13	42.3	42.3	42.3	43.1	43.1	43.1	43.1	42.8	42.9	42.8	42.8
14	139.3	140.1	140.5	141.2	141.2	141.1	141.6	143.3	143.5	143.2	143.8
15	207.8	207.4	207.1	207.9	207.8	208.2	208.2	208.2	207.9	207.8	207.7
16	42.2	42.2	42.1	42.5	42.4	42.4	42.4	42.3	42.2	42.2	42.1
17	50.7	50.6*	50.6	50.4	50.4	50.3	50.3	50.2	50.1	50.0	50.0
18	18.7	18.6	18.6	17.3	17.3	17.2	17.2	16.7	16.7	16.7	16.6
19	12.7	12.7	11.9	15.6	15.5	15.4	14.6	14.9	14.9	15.0	14.1
20	34.3	34.3	34.3	34.4	34.4	34.3	34.3	34.3	34.4	34.3	34.3
21	19.0	19.1	19.0	19.2	19.2	19.1	19.1	19.1	19.1	19.1	19.0
22	35.6	35.6	35.6	35.7	35.7	35.6	35.6	35.6	35.6	35.5	35.5
23	23.3	23.4	23.3	23.5	23.5	23.4	23.4	23.4	23.4	23.4	23.3
24	39.1	39.2	39.1	39.3	39.3	39.2	39.2	39.2	39.3	39.2	39.2
25	27.7	27.7	27.7	27.9	27.9	27.8	27.8	27.9	27.9	27.8	27.8
26	22.3	22.4	22.3	22.5	22.5	22.4	22.4	22.5	22.4	22.4	22.4
27	22.5	22.6	22.5	22.7	22.7	22.6	22.6	22.7	22.6	22.6	22.6
	C=O 165.5 p 132.4 q 130.4 o 129.2 m 127.9			C=O 170.2 CH_3 21.3		C=O 165.8 p 132.5 q 130.5 o 129.2 m 128.0		C=O 170.4 CH_3 21.3		C=O 165.5 p 132.4 q 130.5 o 129.2 m 128.0	

[a] Assignment of chemical shifts for close-lying peaks marked with an asterisk in any vertical column may be reversed, although those given here are preferred.

by a second series of spectral measurements in the 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 ratios of shift reagent to the sterols were from 0.08 to 0.29.

RESULTS AND DISCUSSION

Peak assignments

The peak assignments for each of the sterols considered herein were based upon the results of an initial analysis of its SFORD spectrum followed by considerations of characteristic ^{13}C chemical shift regions, analyses of the results of LIS experiments, the use of empirical shift rules (10–14, 19), and comparisons with the spectra of structurally related compounds (10, 11, 20).

The chemical shift data for compounds II–XI, along with our previously published (10) assignments for compound I, are presented in **Table 1.**[1] The shieldings for carbon atoms that are sufficiently distant from the substituent at the 9α-position were assigned on the basis of direct comparisons with the corresponding parent compounds (bearing no hetero atom at the 9α-position; I, IV, and VII) and further confirmed by analyses of the results of LIS experiments (**Table 2**). In our previously published analysis of the ^{13}C NMR spectrum of compound I, we did not

[1] The assignments for C-1 and C-22 in I have been reversed relative to those presented previously (10). In our previous study, knowledge of the effect of the introduction of a 15-ketone function on the C-22 shielding was clearly limited. The systematic inspection of this effect in the present study led us to revision of the previous assignments between C-1 and C-22 in I. In the $\Delta^{8(14)}$-15-keto system, the peak at 36.3 ppm (C-1 in I, new assignment) was markedly perturbed by the introduction of a 9α-substituent or by oxidation of the 3-hydroxy function, while the peak at 35.6 ppm (C-22 in I, new assignment) always appeared with a constant shielding value in the $\Delta^{8(14)}$-15-keto system and was not perturbed by substitution at the 9α-position or by oxidation of the 3-hydroxy function.

TABLE 2. Relative LIS values for $\Delta^{8(14)}$-15-ketosterols and their derivatives[a]

	Compounds						
Carbon Atom	I (0.71 M) (3β-Hydroxy) R = 0.2[b]	VII (0.5 M) (3β-Benzoate) R = 0.15	III (0.17 M) (3β-Hydroxy) R = 0.08	XI (0.12 M) (3β-Acetate) R = 0.13	IX (0.5 M) (3β-Benzoate) R = 0.19	II (0.35 M) (3β-Hydroxy) R = 0.29	VIII (0.44 M) (3β-Benzoate) R = 0.16
1	0.202	0.060	0.281	0.092	0.062	0.256	0.047
2	0.366	0.073	0.603	0.119	0.069	0.511	0.060
3	1.62	0.112	2.46	0.238	0.106	2.20	0.114
4	0.462	0.067	0.636	0.119	0.057	0.631	0.054
5	0.234	0.067	0.322	0.106	0.075	0.310	0.053
6	0.215	0.112	0.281	0.130	0.112	0.228	0.094
7	0.288	0.231	0.322	0.238	0.230	0.272	0.180
8	0.683	0.570	0.645	0.542	0.511	0.580	0.416
9	0.142	0.090	0.198	0.119	0.093	0.144	0.054
10	0.312	0.127	0.405	0.133	0.107	0.354	0.080
11	0.148	0.089	0.116	0.079	0.094	0.174	0.078
12	0.091	0.096	0.124	0.106	0.106	0.136	0.080
13	0.196	0.177	0.116	0.182	0.174	0.217	0.158
14	−0.223	−0.275	−0.248	−0.214	−0.200	−0.222	−0.162
15	1.00	1.00	1.00	1.00	1.00	1.000	1.00
16	0.605	0.563	0.521	0.504	0.511	0.529	0.450
17	0.247	0.237	0.240	0.209	0.212	0.248	0.188
18	0.089	0.104	0.157	0.106	0.094	0.126	0.080
19	0.207	0.081	0.281	0.092	0.075	0.264	0.065
20	0.124	0.096	0.116	0.092	0.088	0.112	0.073
21	0.054	0.052	0.041	0.041	0.050	0.064	0.033
22	0.065	0.044	0.033	0.024	0.037	0.063	0.040
23	0.054	0.037	0.041	0.014	0.031	0.056	0.021
24	0.013	−0.008	−0.008	−0.014	−0.013	0.008	−0.014
25		0.008	0.000	0.000	0.000	0.007	−0.014
26	−0.019	−0.006	−0.041	0.000	−0.012	−0.008	−0.012
27		−0.008	−0.041	−0.014	−0.006	−0.017	−0.014
CO		−0.052		0.157			−0.080
CH_3				0.320			

[a] Relative to an assigned value of unity for C-15. The absolute LIS values (in ppm) for C-15 in I, VII, III, XI, IX, II, and VIII were 3.72, 6.54, 1.21, 3.69, 7.77, 6.05, and 7.22, respectively.
[b] R = molar ratio of shift reagent to sterol.

make a definitive assignment of the specific chemical shifts for C-6 and C-7. The completion of detailed analyses of the results of LIS experiments on compounds I and VII now permits exact assignments for C-6 and C-7 since the relative LIS values (relative to the LIS of C-15 in these 15-ketosterols) for C-7 were always larger than those for C-6. This finding was particularly striking in the case of the various 3β-acylated derivatives. However, ambiguity exists with respect to the differentiation between the chemical shift assignments for C-4 and C-12 in the 3β-acylated derivatives of the 9α-substituted compounds due to their small shift differences (less than 0.4 ppm).

In the 9α-fluoro compounds (II, V, VIII, and X), the assignments for C-10, C-11, C-18, and C-19 were readily made due to their large and/or clear coupling with ^{19}F at the 9α-position (19, 21–25) (**Table 3; Fig. 2**). Assignments for the olefinic carbons (C-8 and C-14) of the 9α-fluorinated sterols (II, V, VIII, and X) were based upon considerations of the magnitude of their ^{19}F coupling (J_{CCF} for C-8 and J_{CCCF} for C-14). The relative peak positions of the olefinic carbons in 9α-fluoro-5α-cholest-8(14)-en-3β-ol-15-one (II) and in its 3β-acetate (X) and 3β-benzoate (VIII) esters appear to be reversed.

Effects of 9α-substitution on chemical shifts

The substituent effects of 9α-hydroxy and 9α-fluoro groups on the carbon shieldings of 5α-cholest-8(14)-en-3β-ol-15-one (I) and 5α-cholest-8(14)-en-3,15-dione (IV) are summarized in **Table 4.** In general, downfield shifts due to α shift effects of tertiary hydroxyl substitution are smaller in magnitude than those due to a substitution with a secondary hydroxyl group (26). In the present study the observed α-shift values for the 9α-hydroxy and 9α-fluoro substituent are comparable to reported values for tertiary substituents (23, 26).

In a previous study (11), we showed that the introduction of a hydroxyl substituent into an allylic position (C-7 or C-15) of 5α-cholest-8(14)-en-3β-ol causes a deshielding (1.8–8.0 ppm) of the olefinic carbons (C-8 and C-14). However, as shown in **Fig. 3,** introduction of a hydroxyl function into an allylic position (9α) in the α,β-unsaturated $\Delta^{8(14)}$-15-ketosterol system causes shifts of the olefinic carbon peaks in different directions (i.e., an upfield shift of the β-olefinic carbon (C-8) and a downfield shift of the γ-olefinic carbon (C-14)). These substituent effects on the olefinic carbon shieldings were much more pronounced in the case of 9α-fluoro allylic substitution into compound I. Consequently, the olefinic carbon peaks in the spectra of the 9α-fluorinated compounds (II, VIII, X) occurred very close to each other ($\Delta\delta = C_8 - C_{14} = 0.2$ ppm; see Fig. 2). Such allylic substituent effects on olefinic carbon shieldings in α,β-unsaturated ketone systems can be rationalized as due to a partial suppression by the electron withdrawing effect of the 9α-substituent (OH; F) on an originally strong charge polarization induced by the 15-carbonyl group of the $\Delta^{8(14)}$-15-ketosterol system (19, 27) as shown in **Fig. 4.**

The C-19 shieldings were notably deshielded upon introduction of a fluoro or hydroxyl substituent at the 9α-position of I. This downfield shift can be explained in terms of an anti-perpendicular γ substituent effect through quaternary carbons (26, 28).

A finding of special interest in the analyses of the spectra of the various 9α-hydroxy- and 9α-fluoro-$\Delta^{8(14)}$-15-ketosterols was the magnitude (1.3 to 2.0 ppm) of the upfield shift of the C-18 shieldings (ϵ shift effect) in view of the relative remoteness of this carbon from the 9α-substituent in these compounds. The precise reason for this remarkable long range shift effect is not clear, but it may be related to the observation of the unusually long range spin-spin coupling (J_{CCCCCF}) between ^{19}F at the 9α-position and ^{13}C-18 through the π-bond.

TABLE 3. ^{19}F–^{13}C Coupling constants (Hz) in 9α-fluoro-$\Delta^{8(14)}$-15-ketosterols and their derivatives

Carbon Atom	Compounds II (3β-Hydroxy)	X (3β-Acetate)	VIII (3β-Benzoate)	V (3-Ketone)	
C-9	172.1	173.2	173.3	174.0	J_{CF}
C-10	20.2	20.6	19.5	20.8	J_{CCF}
C-11	25.4	25.0	25.6	25.0	J_{CCF}
C-8	18.8	19.7	18.3	18.9	J_{CCF}
C-5	NE[a]	4.2	NE[a]	4.3	J_{CCCF}
C-14	9.1	9.2	8.5	9.2	J_{CCCF}
C-19	3.1	4.3	~4.3	~3.8	J_{CCCF}
C-13	1.72	broadening	split but broad	broadening	J_{CCCCF}
C-18	4.7	4.3	4.5	~5.5	J_{CCCCCF}

[a] The exact coupling constant could not be evaluated due to overlapping with the C-22 peak.

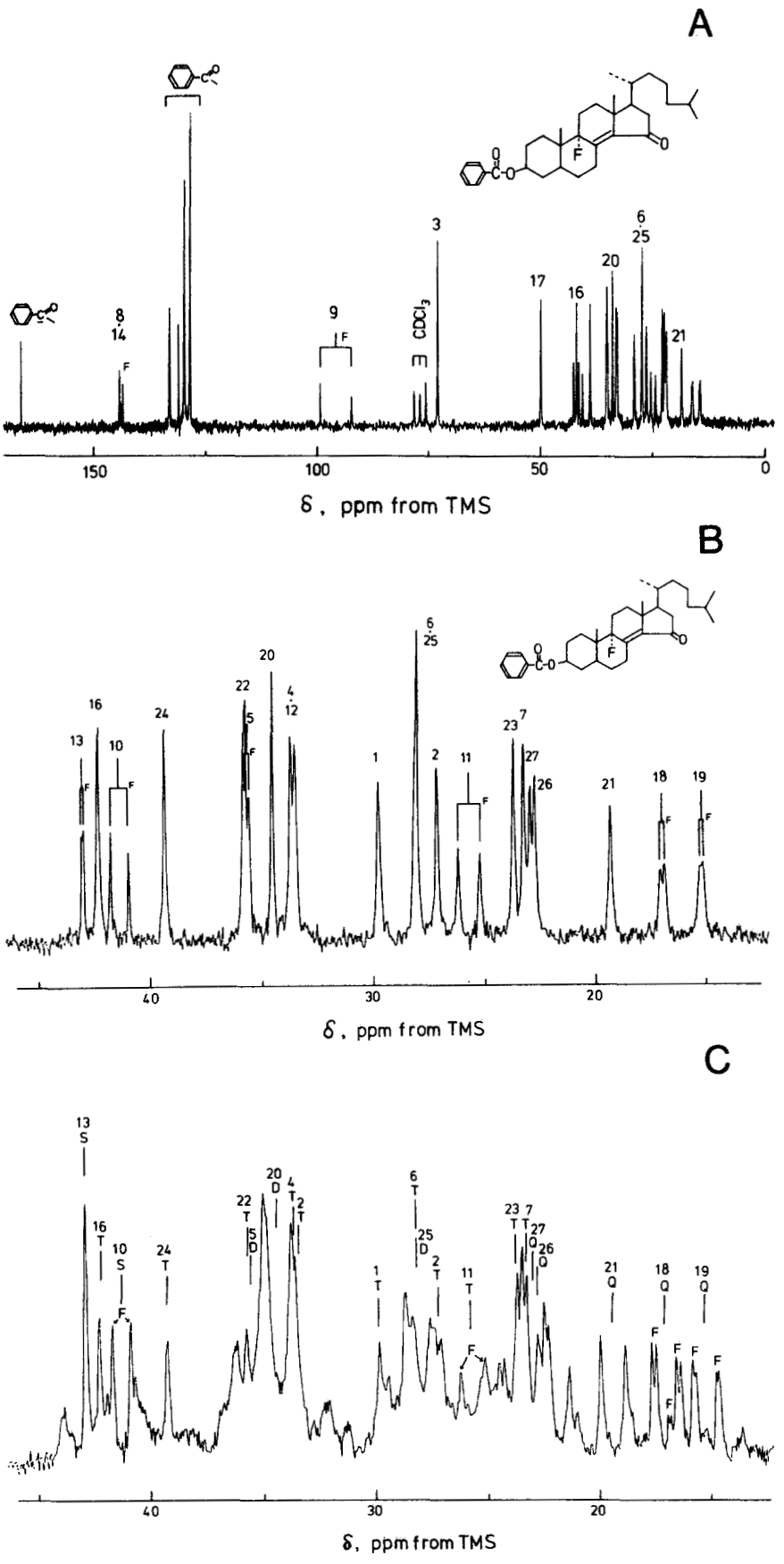


TABLE 4. 9α-Substituent effects (in ppm)[a] on the shieldings of the 3β-hydroxy and 3-keto derivatives of $\Delta^{8(14)}$-15-ketosterols

Substituent Effects	Carbon Atom	5α-Cholest-8(14)-en-3β-ol-15-one		5α-Cholest-8(14)-ene-3,15-dione	
		9α-Hydroxy	9α-Fluoro	9α-Hydroxy	9α-Fluoro
α	C-9	23.6	45.0	24.0	45.3
β	C-8[b]	−2.4	−7.0	−1.0	−6.4
	C-10	2.8	2.8	2.8	2.7
	C-11	8.5	6.0	8.3	6.0
γ	C-1	−6.7	−6.6	−6.5	−6.6
	C-5	−8.7	−8.7	−8.2	−8.0
	C-7	−4.7	−4.4	−4.6	−4.3
	C-12	−3.2	−3.5	−3.3	−3.5
	C-14[b]	1.9	4.0	1.1	3.3
	C-19	2.9	2.2	2.7	2.2
δ	C-2	0.1	−0.1	−0.2	−0.4
	C-4	0.4	0.0	0.2	−0.2
	C-6	−0.6	−0.9	−0.4	−0.9
	C-13	−0.2	0.5	0.8	0.5
	C-15	0.2	0.5	1.1	0.6
ϵ	C-18	−1.3	−1.9	−1.4	−2.0
	C-17	−0.3	−0.5	−0.3	−0.6
	C-16	0.3	0.1	0.3	0.0

[a] A minus value indicates an upfield shift.
[b] sp^2 carbon.

Effects of oxidation state at C-3 on the olefinic carbon shieldings

Comparisons of the chemical shift data for the various 3β-hydroxysterols versus their corresponding 3-ketosterols indicated that the olefinic carbon shieldings of the $\Delta^{8(14)}$-15-ketosterols were affected in a consistent fashion by the state of oxidation at C-3 as shown in Fig. 3. Conversion of the 3β-hydroxysterols to the corresponding 3-ketosterols resulted in an upfield shift for C-8 by 0.8–2.2 ppm, while C-14 was deshielded slightly (0.4–1.2 ppm). These induced shifts upon 3-ketone formation can be explained in terms of a through space effect as shown in Fig. 4. Batchelor et al. (29, 30) have provided evidence in support of the existence of a significant electric field dependent contribution to the ^{13}C chemical shifts, an effect which has been used to explain the nonequivalence of the olefinic carbon shieldings in long-chain fatty acids. More recently, Schneider, Gschwendtner, and Buchheit (31) have observed such a long-range effect on the shieldings of several estr-4-enes possessing various polar functions (ketone, hydroxyl, fluoro, chloro) at carbon atom 17. In the present study the olefinic carbon shieldings (C-8 and C-14) in the spectra of 9α-fluorinated compound II and its 3β-acylated derivatives (VIII and X) were very close to each other ($\Delta\delta = \delta_8 - \delta_{14} = 0.2$ ppm) and showed fluorine coupling ($J_{CCF} \approx 20$ Hz for C-8 and $J_{CCCF} \approx 10$ Hz for C-14). Consequently, the olefinic carbon region of the spectra of II, VIII, and X showed complex splitting patterns (**Fig. 5**). However, in the case of the corresponding 3-keto derivatives (V), the olefinic carbon shieldings were clearly separated ($\Delta\delta = 1.9$ ppm) while the fluorine coupling was retained (18.9 Hz for C-8 and 9.2 Hz for C-14). Thus, such a through space shift effect of the electric field on olefinic carbon shieldings can be of practical utility in spectral analyses.

Spin-spin couplings between ^{13}C and ^{19}F

An analysis of the spin-spin couplings between ^{13}C and ^{19}F (J_{CF}) (Table 3) indicated that C-9 had the largest J value, followed by the β-carbons (C-8, C-10, and C-11). The γ-carbons (C-5, C-14, and C-19) also showed clear couplings with ^{19}F but the other γ-

Fig. 2. Proton noise-decoupled C-13 NMR spectrum of 3β-benzoyloxy-9α-fluoro-5α-cholest-8(14)-en-15-one (VIII). A, full-scale spectrum. The 15-carbonyl carbon peak (δ = 207.8 ppm) appeared as a reversed signal (not shown). B, an expanded spectrum of the chemical shift range between 10 and 45 ppm. C, a SFORD spectrum of the chemical shift range between 10 and 45 ppm (i.e., the SFORD spectrum corresponding to B). In this figure, F indicates the splitting due to the spin-spin coupling between ^{13}C and ^{19}F. S, singlet; D, doublet; T, triplet; and Q, quartet.

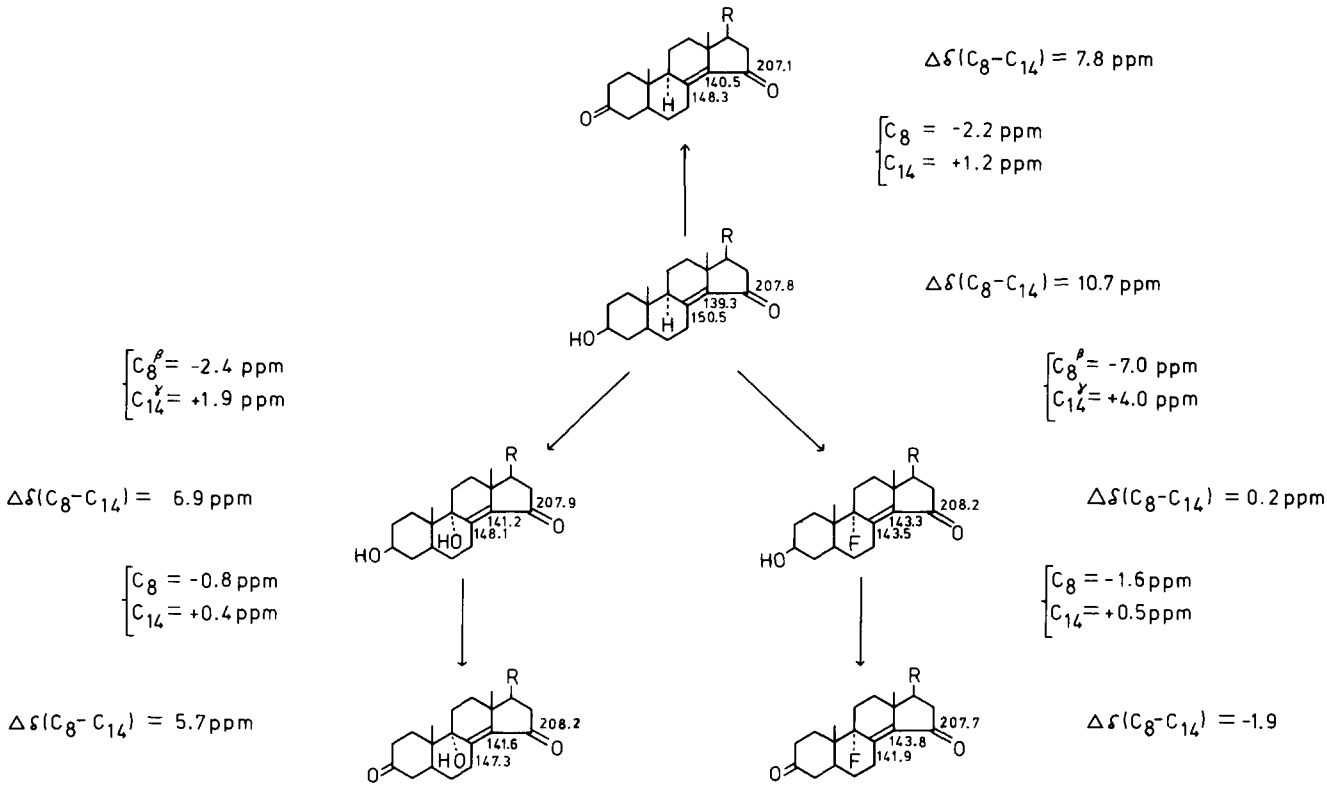


Fig. 3. Effects of allylic hydroxyl substituent at C-9 and oxidation state at C-3 on olefinic carbon shieldings in 9α-fluoro and 9α-hydroxy $\Delta^{8(14)}$-15-ketosterols.

carbons (C-1, C-7, and C-12) did not show either any measurable coupling or a detectable line broadening due to the small coupling of less than 1 Hz. C-13 (a δ carbon) showed a small J value and the splitting due to this coupling was only observed in spectra of high resolution. This peak was accompanied by notable line broadening instead of showing a characteristic sharp line usually observed for quaternary carbon shieldings in noise-decoupled spectra (10, 21, 26). It should be noted that the peak for C-18 also showed clear coupling ($J_{CCCCCF} \approx 4.5$ Hz). This represents unusually high long range coupling between ^{13}C and ^{19}F in such an aliphatic system (even though a through π-bond effect is involved in these molecules). This finding suggests that the 9α-fluorine nucleus interacts with the C-18 nucleus which is remote by a total of five bonds. The observed strong ε shift effect of the 9α-fluoro substituents (as well as the 9α-hydroxy substituent) on the C-18 shieldings also supports this long range (spin-spin) interaction between the 9α-substituent and the C-18 carbon. The fluorine coupling with C-5 was obscured by the overlapping signal of C-22 in compounds II and VIII. Due to the small shift deviations ($\Delta\delta \approx 0.2$ ppm) between the olefinic carbon shieldings (C-8 and C-14) in II, VIII, and X, evaluation of the coupling constants between ^{19}F and these olefinic carbons was not simple and, to obtain the values of J_{CF} listed in Table 3, required confirmation after peak separation through LIS experiments (see Fig. 5).[2]

LIS experiments

LIS experiments (Table 2) were required for exact assignments for C-6 and C-7 for which definitive assignments were not made in our previous publication (10) on I. In order to enhance the contribution of the lanthanide-induced shifts due to the coordination of the europium to the 15-keto function, corresponding 3-acylated derivatives of I were used for the LIS studies. For this purpose, the benzoate ester was more suitable than the acetate ester to block the coordination of the shift reagent to the 3-oxy moiety. The results of the LIS experiments in this study are summarized in Table 2. In all cases, even those of the free sterols, the relative LIS values (LIS for 15-keto ^{13}C = 1.00) for C-7 were larger than those for C-6, a finding which permitted the definitive differentiation of the shieldings due to these carbon atoms. In

[2] J_{CCF}(C-8) and J_{CCCF}(C-14) were almost constant (within ± 1 Hz) in the range of the ratios of Eu(fod)$_3$ to sterol studied (from 0.08 to 0.29).

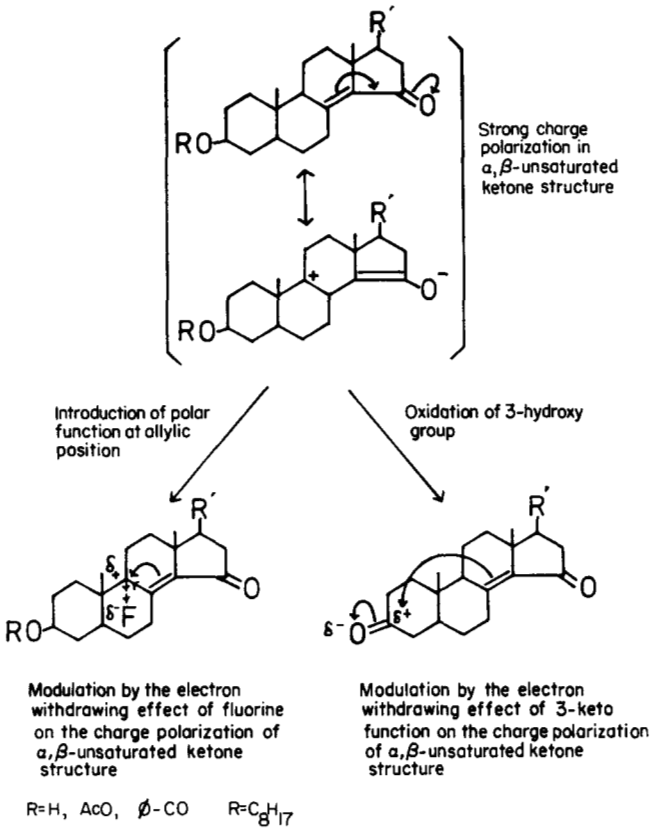


Fig. 4. Effects of allylic substitution with fluorine on charge polarization of $\Delta^{8(14)}$-15-ketone system and effects of 3-ketone function on charge polarization of $\Delta^{8(14)}$-15-ketone system.

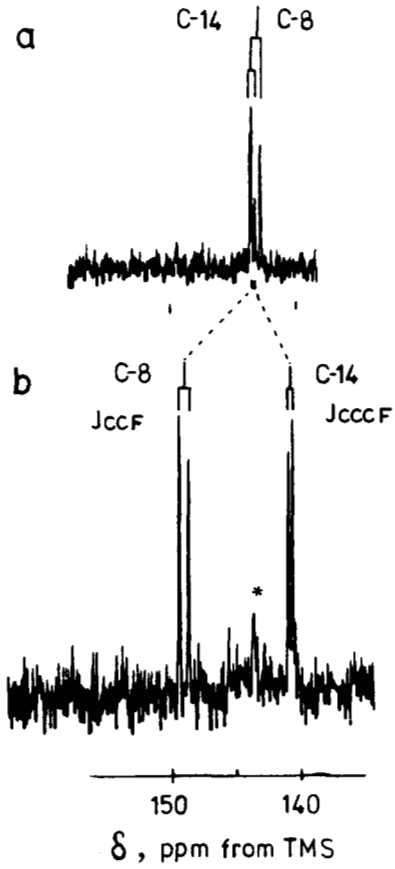


Fig. 5. Olefinic carbon region of the proton noise-decoupled spectrum of 3β-benzoyloxy-9α-fluoro-5α-cholest-8(14)-en-15-one (VIII) (part a, above) and lanthanide-induced shift (LIS) effects on the olefinic carbons of VIII (part b, below; $[Eu(fod)_3]/[VIII] = 0.16$; * = peaks originating from the shift reagent).

the $\Delta^{18(14)}$-15-keteosterols studied in this work, the shifts of the olefinic carbon shieldings induced by the $Eu(fod)_3$ were notable; i.e., a shielding effect on C-14 and a deshielding effect on C-8. The magnitudes of the LIS values for C-8 were 2–3 times larger than those for C-14 (see Fig. 5). These findings are similar to reported protonation-induced shifts in other α,β-unsaturated ketones (32). This similarity in findings suggests that the observed lanthanide-induced shifts for the olefinic carbons include, in addition to the dominant paramagnetic shift induced by the europium atom, a significant contribution of a complex formation shift due to a Lewis acid-base interaction between the lanthanide and the 15-ketone function. As might be expected from consideration of its steric hindrance, the coordination of the 9α-hydroxyl group with the lanthanide was negligibly small.

^{13}C NMR and conformational analysis of ring B

Ring B of 5α-cholest-8(14)-en-3β-ol-15-one (I) and of its 9α-substituted derivatives, can exist in two main possible conformations, a chair form and a boat form as shown in **Fig. 6.** Comparison of these two conformers using Dreiding models indicated that, upon

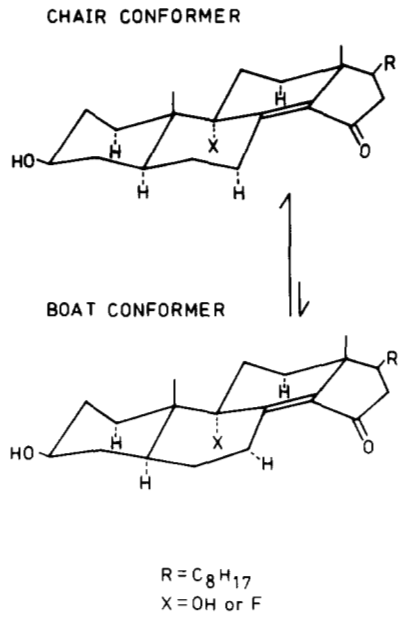


Fig. 6. Chair and boat conformers of 9α-substituted (OH or F) 5α-cholest-8(14)-en-3β-ol-15-one.

introduction of a 9α-substituent (hydroxyl or fluorine) into I, the production of four 1,3-diaxial interactions of the 9α-substituent with the 1α-, 5α-, 7α-, and 12α-hydrogens can occur when ring B has the chair conformation. In the case of the ring B boat conformer, the "flagpole" interaction (33) between the C-19 methyl and the 7β-hydrogen and between the 9α-substituent and the 7α-hydrogen and several eclipsed interactions occur instead of loosing the two repulsive 1,3-diaxial interactions (between the 9α-substituent and the 7α-hydrogen and between the C-19 methyl and the 6β-hydrogen) which occur in the chair conformer. However, these comparisons of the molecular models of these two conformers do not permit a definitive estimation of energy difference between these two conformers. Additional useful information regarding the conformation of ring B can be derived from consideration of the results of the ^{13}C NMR analyses. The upfield shift effect on the gauche γ carbon can be considered to be caused by steric repulsions between a hydrogen on the γ-carbon and a hydrogen or a lone pair of electrons on a substituent. Polarization of the carbon-hydrogen bond, with increasing electron density on the carbon atom, causes a shielding of the ^{13}C nucleus (34, 35). In the compounds considered in the present study, such γ steric effects caused by the 9α-substituents were clearly observed for carbon atoms 1, 5, 7, and 12, as shown in Table 4. If ring B of these compounds existed in a boat form, such a clear γ steric shift would not be expected for the C-7 shielding because of the decreased 1,3-diaxial interaction between the 9α-substituent and the 7α-hydrogen in the boat conformation. Thus, the observed patterns of the γ steric shifts induced by the 9α-substituent in compounds II, III, V, and VI strongly suggest that the conformation of ring B in these compounds is the chair form. Moreover, it appears that, upon inspection of Dreiding models of the ring B chair conformer of the various 9α-substituted compounds, the magnitudes of the steric effects of the γ carbons C-1, C-5, C-7, and C-12 reflect the distance between the 9α-substituent and the proton on the γ carbon in question. It should be noted that the magnitudes of the γ steric shifts observed in the cases of the 9α-hydroxy-substituted compounds were very similar (within ±0.2 ppm) to those observed in the cases of the 9α-fluorine-substituted compounds. This finding suggests that the γ steric shifts in the cases of the 9α-hydroxy-$\Delta^{8(14)}$-15-ketosterols are probably due to interactions between the lone pair of electrons on oxygen and the protons on the γ carbon atoms rather than interactions between the hydroxyl hydrogen and the protons on the γ carbon atoms.

This research was supported in part by grants (HL-15376 and HL-22532) from the National Institutes of Health and by a grant (C-583) from the Robert A. Welch Foundation.

Manuscript received 8 July 1980 and in revised form 15 June 1981.

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