was more intense in partially relaxed spectra due to closer proximity to ^1H nuclei, was less shifted (1.2% less) by $Cr(acac)_3$ because these carbons are further from the OH site of $Cr(acac)_3$ complexation, and was less shifted by the lanthanide shift reagents $Eu(fod)_3$ (2.6% less) and $Yb(dpm)_3$ (52% less) than are C-1 and -6. Although the Eu and Yb compounds can complex at three sites in kelevan, at carbonyls C-2′ and C-6′ and at the hydroxyl-substituted carbon C-2, the preferred conformation, due to intramolecular hydrogen bonding between the OH and C=O (C-2′), places the site of shift reagent binding much further from C-3a and -5b than from C-1 and -6. Shift reagent bound at C-6′ has little effect on the relative chemical shifts of C-3a, -5b, -1, and -6. Preferential binding of the shift reagents at the C-2′ carbonyl and the hydroxyl group is indicated by the greater effects of the shift reagents on the chemical shifts of C-2 and C-2′ than on the chemical shift of C-6′.

The ^{13}C spectrum of monohydrokelevan **7** was partially assigned using the same techniques and considerations as for kelevan (**3**). The conversion by CrO_3 oxidation of **7** to monohydrokepone diol **8** showed that monohydrokelevan had either of the two structures **16** or **17**, which differ in the syn (**16**) or the anti (**17**) orientation of the ring hydrogen to the hydroxyl group. No doubling of the peaks in the presence of either $Eu(fod)_3$ or $Yb(dpm)_3$ indicated that only one of these isomers was present in the major product. The Yb shift reagent affected the chemical shift of the CH carbon somewhat less (8%) than it affected the chemical shifts of the carbons diagonally opposite it (C-1,6 in **17** or C-3a,5b in **16**).

To distinguish between these two possible structures, we estimated the effects of replacing a chlorine by a hydrogen at either the 5b or the 6 position on the δ_C values of **3** by calculating the additive substituent effects[15] [$\delta_C(8) - \delta_C(9)$, ppm] of this replacement in Kepone from the ^{13}C chemical shifts of Kepone diol **9** and monohydrokepone diol **8**. The predicted δ_C values for both syn and anti forms of **7** are given with the observed chemical shifts in Table II. Five chemical shifts should differ for the two forms; of these, the average deviation between predicted and observed shifts is 0.19 ppm for **17** but 1.40 ppm for **16**. These data thus show clearly that the anti form **17** is the major product of the photoreaction.

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Registry No.—**1**, 143-50-0; **2**, 2385-85-5; **3**, 4234-79-1; **8**, 52171-96-7; **9**, 4715-22-4; **10**, 69069-44-9; **14**, 1034-41-9; **17**, 69069-45-0.

References and Notes

(1) E. G. Alley, B. R. Layton, and J. P. Minyard, Jr., *J. Agric. Food Chem.*, **22**, 442 (1974).
(2) E. G. Alley and B. R. Layton, in "Mass Spectrometry and NMR Spectroscopy in Pesticide Chemistry", Plenum Press, New York, 1974, pp 81–90.
(3) E. G. Alley, D. A. Dollar, B. R. Layton, and J. P. Minyard, Jr., *J. Agric. Food Chem.*, **21**, 138 (1973).
(4) K. Knoevenagel and R. Himmelreich, *Z. Pflanzenkr. Pflanzenschutz*, **80**, 155 (1973).
(5) S. Begum, A. Gäb, H. Parlar, and F. Korte, *Chemosphere*, **2**, 235 (1973).
(6) H. Parlar, W. Klein, and F. Korte, *Chemosphere*, **1**, 129 (1972).
(7) D. A. Carlson, K. D. Konyha, W. B. Wheeler, G. P. Marshall, and R. G. Zaylskie, *Science*, **194**, 939 (1976).
(8) E. G. Alley, B. R. Layton, and J. P. Minyard, Jr., *J. Org. Chem.*, **41**, 462 (1976).
(9) N. K. Wilson and R. D. Zehr, presented at the 29th Southeastern Regional Meeting of the American Chemical Society, Tampa, Fla., Nov 9–11, 1977, Abstract 209.
(10) R. D. Zehr and N. K. Wilson, *J. Chromatogr.*, submitted for publication.
(11) A. Westlake, W. E. Westlake, and F. A. Gunther, *J. Agric. Food Chem.*, **18**, 159 (1970).
(12) N. K. Wilson and G. W. Sovocool, *Org. Magn. Reson.*, **9**, 536 (1977).
(13) R. L. Harless, D. E. Harris, G. W. Sovocool, R. D. Zehr, N. K. Wilson, and E. O. Oswald, *Biomed. Mass Spectrom.*, **5**, 232 (1978).
(14) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.
(15) N. K. Wilson and R. D. Zehr, *J. Org. Chem.*, **43**, 1768 (1978).
(16) Bayer names for some compounds in this paper are as follows: **1**, 1,2,3,4,6,7,8,9,10,10-decachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decan-5-one; **9**, 5,5-dihydroxy-1,2,3,4,6,7,8,9,10,10-decachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane; **4**, 1,3,4,6,7,8,9,10,10-nonachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decan-5-one; **8**, 5,5-dihydroxy-1,3,4,6,7,8,9,10,10-nonachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane; **5**, 1,3,4,6,7,9,10,10-octachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decan-5-one; **10**, 5,5-dihydroxy-1,3,4,6,7,9,10,10-octachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane.

Carbon-13 Nuclear Magnetic Resonance Studies of Allylic Hydroxysterols. Assignment of Structure to 5α-Cholest-8(14)-ene-3β,7α,15α-triol, an Inhibitor of Sterol Synthesis[1]

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5α-Cholest-8(14)-ene-3β,7ξ,15ξ-triol, a potent inhibitor of sterol biosynthesis in animal cells in culture, has been shown to be formed in 53% yield upon treatment of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene with refluxing aqueous ethanolic KOH [G. J. Schroepfer, Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch, *J. Biol. Chem.*, **252**, 8975 (1977)]. Detailed analyses of the ^{13}C nuclear magnetic resonance spectra of this compound and of other steroidal allylic alcohols and their derivatives have permitted the establishment of configurations of the 7 and 15 hydroxyl functions as α. The resonances of the individual carbon atoms have been determined for six allylic hydroxysterols as well as a number of carbamate and acetate derivatives. Treatment of 5α-cholest-8(14)-ene-3β,7α,15α-triol with acid gave 15-oxo-5α-cholest-8(14)-en-3β-ol in 87% yield. Also described herein are syntheses of 3β-benzoyloxy-8α,14α-epoxy-5α-cholestan-7α-ol, 3β-benzoyloxy-8α,9α-epoxy-5α-cholestan-7α-ol, 7-oxo-5α-cholest-8-en-3β-ol, 5α-cholest-8(14)-ene 3β,15α-diacetate, 5α-cholest-8(14)-ene 3β,15β-diacetate, 5α-cholest-8(14)-ene 3β,7α,15α-triacetate, and 7α,15α-diacetoxy-5α-cholest-8(14)-en-3-one.

Over 20 years ago Barton et al.[2,3] reported that treatment of 3β-acetoxyergosta-7,14,22-triene with perphthalic acid in ether gave, upon washing of the ether solution with dilute aqueous sodium hydroxide, the sodium salt of the half phthalate ester of 3β-acetoxyergosta-8(14),22-dien-7ξ,15ξ-diol. The product was not characterized as such but, upon

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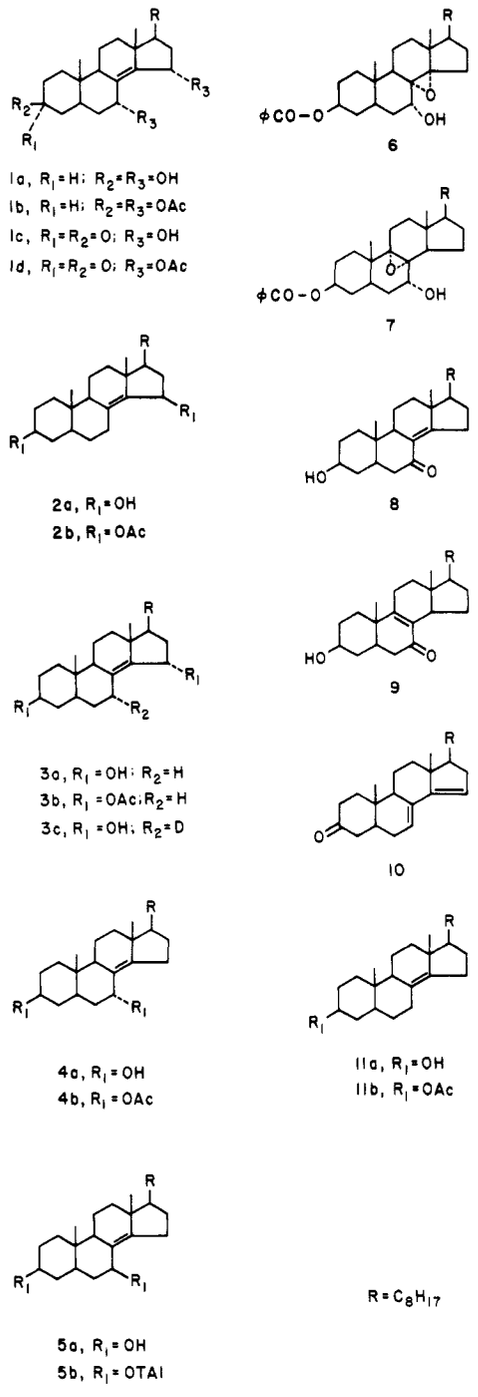
treatment with dilute acid, gave the corresponding half ester in the acid form which was characterized by elemental analysis, optical rotation, and ultraviolet spectral analysis. Heating of the sodium salt of the half ester with hydrochloric acid in methanol gave 3β-acetoxyergosta-8(14),22-dien-15-one. Subsequently, Woodward et al.[4,5] reported that treatment of a mixture enriched with 3β-acetoxy-4,4-dimethyl-5α-cholesta-7,14-diene with monoperphthalic acid gave a product which, on saponification, gave 4,4-dimethyl-5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol. The latter compound gave 4,4-dimethyl-5α-cholest-8(14)-en-3β-ol-15-one on treatment with hydrochloric acid in methanol. More recently, Muccino and Djerassi[6] reported that treatment of 5α-cholesta-7,14-diene with *m*-chloroperbenzoic acid in ether followed by saponification of the crude product with ethanolic KOH gave a product which was formulated to be 5α-cholest-8(14)-ene-7ξ,15ξ-diol. The latter compound, which was not characterized as such, gave the known 5α-cholest-8(14)-en-15-one upon treatment with hydrochloric acid in ethanol. Akhtar et al.[7,8] have also recently reported the preparation of 4,4-dimethyl-5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol by treatment of the corresponding 7,14-diene with perphthalic acid.

We have recently found that a number of 15-oxygenated sterols are very potent inhibitors of sterol synthesis in animal cells in culture.[9-13] In the course of this research we pursued the chemical synthesis of a 3,7,15-trihydroxysterol for biological testing. A key precursor for the preparation of the desired compound was 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene which was obtained in 96% yield by treatment of pure 3β-benzoyloxy-5α-cholesta-7,14-diene with *m*-chloroperbenzoic acid.[14] Unambiguous establishment of structure was based upon the results of X-ray crystallographic analysis of 3β-*p*-bromobenzoyloxy-14α,15α-epoxy-5α-cholest-7-ene.[15] Treatment of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene with refluxing ethanolic KOH gave, in 53% yield, 5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol which was characterized by infrared, NMR, and mass spectroscopy and by conversion to 5α-cholest-8(14)-ene-3,7,15-trione. The $\Delta^{8(14)}$-3β,7ξ,15ξ-triol was found to be a potent inhibitor of sterol synthesis.[12] Apart from the high activity of this compound in the inhibition of sterol biosynthesis, the triol is of considerable interest since we have now found that 5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol, upon treatment with hydrochloric acid in ethanol, gives 5α-cholest-8(14)-en-3β-ol-15-one in high (87%) yield. The latter compound and several of its derivatives have been found to have significant hypocholesterolemic activity in rats and mice.[16-19] In view of the potency of the triol in the inhibition of sterol biosynthesis, its utility as an intermediate in the chemical synthesis of 5α-cholest-8(14)-en-3β-ol-15-one, and the unresolved status of the absolute stereochemistry of this and related $\Delta^{8(4)}$-3β,7,15-triols, we have pursued determination of the establishment of the structure of the concerned triol. For the solution of this problem we have primarily used ^{13}C NMR spectroscopy. Presented herein are ^{13}C NMR data on $\Delta^{8(14)}$-sterol diols and triols and analyses with respect to allylic alcohol substituent effects and their acetylation and trichloroacetyl isocyanate (TAI) shifts. The configurations of the C-7 and C-15 hydroxyl functions of 5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol have been determined by evaluation of the hydroxyl substituent effects obtained during the course of this investigation.



Results and Discussion

The determination of the structure of 5α-cholest-8(14)-ene-3β,7ξ,15ξ-triol by ^{13}C NMR required the syntheses (reported herein) of $\Delta^{8(14)}$-allylic hydroxysterols containing each possible (α and β) hydroxyl substituent at carbon atoms 7 and 15. Eggert et al.[20] have reported the results of studies of the effects of hydroxyl functions at C-7 and at C-15 on the ^{13}C NMR spectra of androstane and cholestane. The observed substituent effects were greatly influenced by the position and configuration of the hydroxyl function. The ^{13}C NMR chemical shifts have also been reported for 3-cyclohexenol, a cyclic allylic alcohol.[21] The assignments reported herein of resonance peaks of carbon atoms in close proximity to the hydroxyl substituent were based, to a large extent, on the data for hydroxyl substituent effects reported by Eggert et al.[20] and, in addition, on shifts induced upon acetylation and upon trichloroacetyl isocyanate formation.[22] Additional evidence in support of the assignments was obtained not only by single-frequency off-resonance decoupling (SFORD) spectra but also from protic solvent induced shifts and spectral comparisons with a selectively deuterated compound.

It is well established that acetylation of hydroxyl functions in cyclic alcohols causes a downfield shift of the α-carbon resonances (~3 ppm), an upfield shift of the β-carbon resonances (~4 ppm), and a smaller upfield shift of the γ-carbon

Table I. ^{13}C Chemical Shifts of $\Delta^{8(14)}$-Sterols

	11a[c]	4a[d]	5a[e]	3a[f]	2a[g]	1a[b,h]	1c[i]
C-1	36.5	36.2	36.1	36.3	36.4	36.2	37.9
C-2	31.5	31.3	31.4	31.3	31.3	31.3	37.4
C-3	71.0	70.8	70.9	70.9	70.9	70.8	211.3
C-4	38.2	37.7	37.6	37.9	37.9*	37.6	43.9
C-5	44.2	37.1	42.0	44.2	44.2	37.6	39.7
C-6	28.9	35.5	40.1	28.8	28.9**	35.1	35.2
C-7	29.6	66.5	73.6	30.9	29.2**	66.7	66.1
C-8	126.1	128.1	127.9	134.1	133.2	136.2	135.0
C-9	49.2	44.0	48.4	49.4	49.2	45.3	44.5
C-10	36.7	36.6	36.5	37.1	37.0	36.4	36.4
C-11	19.9	19.3	20.1	19.9	19.8	19.6	19.5
C-12	37.2	36.7	36.8	37.5	37.3	37.2	37.0
C-13	42.6	42.8	44.3	43.1	42.0	43.2	43.1
C-14	142.4	147.9	144.2	147.2	145.2	151.2	151.9
C-15	25.7	25.0	27.8*	70.0	69.8	70.2	69.9
C-16	27.0	26.8	27.4*	38.5	38.0*	39.1	38.9
C-17	56.8	56.3	55.6	53.5	54.6	53.1	53.1
C-18	18.2	17.9	18.7	19.0*	19.0***	19.0*	18.9*
C-19	12.8	11.9	12.9	12.9	12.7	12.1	11.2
C-20	34.4	34.3	33.9	33.6	34.3	33.6	33.5
C-21	19.0	19.0	19.4	19.2*	19.2***	19.3*	19.3*
C-22	35.9	35.8	35.9	36.0	35.7	36.0	35.9
C-23	23.7	23.6	23.8	23.8	23.6	23.8	23.8
C-24	39.5	39.4	39.5	39.4	39.3	39.4	39.3
C-25	27.9	27.9	28.0	27.9	27.9	28.0	27.8
C-26	22.5	22.5	22.5	22.5	22.5	22.5	22.4
C-27	22.7	22.7	22.8	22.7	22.7	22.8	22.7

[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 here are preferred. [b] 0.03 M (saturated) solution. [c] Registry no. 566-99-4. [d] Registry no. 69140-06-3. [e] Registry no. 65164-27-4. [f] Registry no. 26758-45-2. [g] Registry no. 26660-51-5. [h] Registry no. 69177-17-9. [i] Registry no. 69140-07-4.

resonances (~0.3 ppm).[23] In contrast, acetylation of the hydroxyl function of allylic alcohols effects a downfield shift of the α-carbon (sp^3) resonance (~3 ppm), a large upfield shift of the β-carbon (sp^2) resonance (~5 ppm), and a large downfield shift of the γ-carbon (sp^2) resonance (~3 ppm).[24] These characteristic acetylation-induced shifts have provided strong evidence in support of the peak assignments of the quaternary olefinic carbons (C-8 and C-14) of the various $\Delta^{8(14)}$-hydroxysterols (Tables III and IV). TAI-induced shifts[22] were also studied to confirm the peak assignments of carbon atoms in close proximity to the hydroxyl substituents and, in some cases, as a substitute for analyses of an acetate derivative (Table IV). The resonances of carbon atoms five or more bonds removed from the hydroxyl group were, in general, only slightly shifted from the parent sterol, 5α-cholest-8(14)-en-3β-ol (**11a**), and the peak assignments were usually carried over. The assignments of the individual chemical shift values to specific carbon atoms are presented in Table I and were based upon our assignments of the individual carbon atoms in the spectrum of the parent sterol (**11a**).[25,26] In **4a** and **5a** the assignments for C-1 and C-12 were supported by shift comparisons with the corresponding carbon shifts of **1a, 1c,** and **11a** and consideration of the magnitudes of the ϵ-shift effect (less than 1 ppm) of the 7-hydroxyl group. Assignments for C-4 (37.7 ppm) and C-6 (35.5 ppm) of **4a** were derived from the small shift values for the corresponding carbon atoms of **5a** in which C-4 was not expected to change by more than 1.0 ppm as a result of configurational differences of the hydroxyl group at C-7 (δ effect). Assignments of C-15 (25.0 ppm) and C-16 (26.8 ppm) of **4a** were derived from a comparison of shift values from the corresponding carbon atoms of the parent sterol (**11a**). The ϵ-shift effect of the 7α-hydroxyl group on C-16 in **4a** should be less than 1.0 ppm.[20] Although the exact assignments of C-15 and C-16 (27.4 and 27.8 ppm) of **5a** could not be resolved due to the small shift difference, the remarkable downfield shift effect (δ effect) on the C-15 resonance (2.1 or 1.7 ppm) is significantly greater than the value observed for the corresponding carbon (C-15) of **11a** and **4a.** This finding was interpreted in terms of a δ_1 effect from the 7β-hydroxyl group.[20] The assignments for C-18 (18.7 ppm) and C-21 (19.4 ppm) of **5a** are in satisfactory agreement with the expected ϵ-shift effect of the 7β-hydroxyl group on C-18 and were confirmed by a lanthanide induced shift (LIS) experiment. In the presence of $Eu(fod)_3$ (0.07 M in $CDCl_3$; **5a,** 0.31 M) the induced downfield shifts ($\Delta\delta_{LIS}$) for C-18 and C-21 were observed at 1.09 and 0.39 ppm, respectively.

Assignments for C-4 (37.9 ppm) and C-16 (38.5 ppm) of **3a** were based on the expectation of identical chemical shift values for the carbon atoms of ring A in the two 15-hydroxy epimers (**2a** and **3a**). While ambiguity exists for the assignments of C-4 and C-16 in **2a** (Table I), the assignments for C-4 and C-16 in **3a** can be made with some assurance. Irrespective of the precise assignments for C-4 and C-16 in **3a,** the chemical shift deviations ($\Delta\delta$) for C-1 through C-5 between **2a** and **3a** are within 0.1 ppm. However, if the assignment for C-4 and C-16 in **3a** were reversed, the shift deviation ($\Delta\delta$) for C-4 would be 0.5 or 0.6 ppm. Such a large $\Delta\delta$ for C-4 between the two epimeric (at C-15) sterols (**2a** and **3a**) would not be anticipated. The assignment of C-7 in **3a** was derived directly from a comparison of the spectrum of the 7α-deuterated derivative (**3c**). The peak at 30.8 ppm in the spectrum of **3a** was absent in the spectrum of **3c.** The assignment of C-6 in **3a** was confirmed by observation of an upfield deuterium isotope shift (0.1 ppm) at 28.8 ppm. Definitive assignments for C-6 and C-7 in **2a** and its acetate and carbamate derivatives could not be achieved due to the small shift deviations observed.

A summary of the allylic substituent effects is presented in Table II. The introduction of a hydroxyl substituent at the C-7 or C-15 allylic positions produced downfield shifts (1.8–8.0 ppm) in the β and γ carbons relative to the values observed

Table II. Allylic Hydroxyl Substituent Effects (in ppm)

substituent effects	carbon atom	7-hydroxyl substituent effects (allyl)[a] 7-α	7-β	carbon atom	15-hydroxyl substituent effects (allyl)[b] 15-α	15-β
α	C-7	36.9	44.0	C-15	44.3	44.1
β	C-6	6.6	11.2	C-14	4.8	2.8 sp^2
	C-8	2.1	1.8 sp^2	C-16	11.5	11.0
γ	C-5	−7.1	−2.2	C-8	8.0	6.0 sp^2
	C-9	−5.1	−0.6	C-13	0.5	−0.6
	C-14	5.5	1.75 sp^2	C-17	−3.3	−2.2
δ	C-4	−0.5	−0.6	C-7	1.3	−0.4 or −0.7
	C-10	−0.1	−0.2	C-9	0.4	0.0
	C-11	−0.6	0.2	C-12	0.3	0.1
	C-13	0.2	1.7	C-18	0.8–1.0	0.8–1.0
	C-15	−0.7	2.1 or 1.7	C-20	−0.8	−0.1

[a] $\Delta\delta = \delta$(**4a** or **5a**) − δ(**11a**). [b] $\Delta\delta = \delta$(**2a** or **3a**) − δ(**11a**).

in the parent sterol (**11a**). The magnitude of the downfield shift was, in general, larger for the γ-olefinic carbon than for the β-olefinic carbon (with the notable exception of **5a**). These downfield shifts for allylic hydroxyl substituents on the olefinic carbons of the $\Delta^{8(14)}$-sterols are opposite to the corresponding substituent effects for olefinic carbon atoms of acyclic allylic alcohols[27,28] in which the downfield shift effect on the β-olefinic carbon atom is larger than that on the γ-carbon atom. While the precise reason for this difference is not clear, it most probably results from the differences, in the two systems, in the degree of rotational freedom of the carbinol group which controls the through space and through bond effects on resonances of the olefinic carbon atoms.[29,30]

As mentioned above, the olefinic carbon chemical shifts of allylic alcohols are quite sensitive to configurational and conformational changes. Acetylation shift effects on olefinic carbon atoms of allylic alcohols have been found to be very useful in distinguishing these olefinic carbon resonances. The allylic hydroxyl substituent effects on the olefinic carbon resonances of C-8 and C-14 of **1a** and the shift effects observed upon acetylation are noteworthy. The C-8 olefinic resonance is affected by the β effect of the C-7 allylic hydroxyl and by the γ effect of the C-15 allylic hydroxyl while the C-14 olefinic resonance is affected by the β effect of the C-15 allylic hydroxyl and the γ effect of the C-7 allylic hydroxyl. The predicted olefinic carbon chemical shifts for C-8 and C-14 of **1a**, assuming additivity of the allylic hydroxyl substituent effects observed for the olefinic carbons of compounds **2a–5a**, are 136.2 and 152.7 ppm, respectively. The observed values for C-8 and C-14 of **1a** were 136.2 and 151.2 ppm, respectively. A similar calculation of the expected carbon chemical shifts for C-8 and C-14 of the triacetate **1b** gave 131.8 and 149.1 ppm, respectively. The observed chemical shift values for C-8 and C-14 of **1b** were 131.8 and 147.3 ppm, respectively.

The carbon signals at 35.1 and 37.6 ppm in the spectrum of the triol **1a** were initially attributed to C-4 or C-6. Both resonances showed strong upfield shifts (2.2 and 1.5 ppm, respectively) upon acetylation of **1a** to give **1b**. Upon oxidation of the 3β-hydroxyl function of **1a**, the signal due to C-4 would be expected to be shifted downfield significantly (~6 ppm).[20,31] The signal at 43.9 ppm in the spectrum of **1c** was therefore assigned to C-4 and the peak at 35.1 ppm in the spectra of **1a** and **1c** was accordingly assigned to C-6.

The shift assignments for C-16 of **1a** and **1c** were based upon comparisons of the carbon shifts for the carbon atoms of rings C and D in the two compounds and were confirmed by acetylation and protic solvent (methanol) induced shift effects. Peak assignments for the carbon atoms in ring A of **1c** were based upon the empirical shift rules developed for 5α-cholestan-3β-ol and 5α-cholestan-3-one.[20,31]

A carbon atom bearing a hydroxyl function and carbon atoms immediately adjacent to such a center can be detected by observation of changes in chemical shifts induced by protic solvents.[24,32] Investigations of protic solvent induced shifts have been made in the cases of compounds **1a–5a** using $CDCl_3$ and $CDCl_3$ containing 20% (v/v) CD_3OD. These methanol-induced shifts are presented in Table V. The assignments of C-6 (35.5 ppm) and C-12 (36.7 ppm) in compound **4a** were based upon the presence of a methanol-induced shift (0.2 ppm) in the case of C-6 and the absence of such an effect on C-12. Similarly, the assignments of C-4 (37.6 ppm) and C-12 (36.8 ppm) in **5a** could be made on the basis of the presence of a methanol-induced shift on C-4 (−0.4 ppm) and the absence of the same on C-12. In addition, the assignments of C-12 (37.2 ppm) and C-16 (39.1 ppm) of **1a** were confirmed by the presence of a PSIS (−0.5 ppm) in the case of C-16 and the absence of the same in the case of C-12.

The configurations of the C-7 and C-15 hydroxyl groups in **1a** can be determined through the use of the shift data presented in Table II. Chemical shifts of the carbon atoms of ring B (except the olefinic carbon C-8) of compound **1a** should be affected by the C-7 hydroxyl function and its configuration. The chemical shift values for C-5 (γ), C-6 (β), C-7 (α), and C-9 (γ) of **1a** were compared with the values observed for the corresponding carbon atoms of the $\Delta^{8(14)}$-3β,7α-diol (**4a**) and the $\Delta^{8(14)}$-3β,7β-diol (**5a**). The small $\Delta\delta$ values of **1a** and **4a** relative to the $\Delta\delta$ values of **1a** and **5a** lead to the conclusion that the hydroxyl function at C-7 in **1a** has the α configuration (Table VI). The chemical shifts of carbon atoms of ring D (except the olefinic carbon C-14) of compound **1a** should, for the most part, be governed by the 15-hydroxyl function and its stereochemical orientation and should be independent of the hydroxyl function at C-7. The chemical shift values for C-13 (γ), C-15 (α), C-16 (β), C-17 (γ), and C-20 (δ) of compound **1a** were compared with the values observed for the corresponding carbon atoms of the $\Delta^{8(14)}$-3β,15α-diol (**3a**) and the $\Delta^{8(14)}$-3β,15β-diol (**2a**) (Table VI). The magnitudes of the shift differences for C-15 (α) and C-16 (β) in the two epimeric $\Delta^{8(14)}$-15-hydroxysterols (**2a** and **3a**) are rather small relative to the corresponding observed shift differences induced by the C-7 hydroxyl function in the two epimeric $\Delta^{8(14)}$-7-hydroxysterols on C-7 (α) and C-6 (β) of **4a** and **5a**.[20] However, the smaller $\Delta\delta$ values of **1a** and **3a** relative to the $\Delta\delta$ values of **1a** and **2a** indicate that the hydroxyl function at C-15 of **1a** has the α configuration.

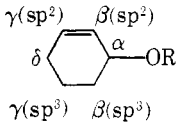
The combination of the results presented previously[12] and those presented herein establish that treatment of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene with base gives 5α-cholest-8(14)-ene-3β,7α,15α-triol. The overall reaction can be envisioned as proceeding via an S_N2' ring opening of the 14α,15α-epoxide function, a process involving the entering nucleophile being syn related such that the hydroxyl ion is

Table III. ^{13}C Chemical Shifts of $\Delta^{8(14)}$-Sterol Acetates[a]

	11b[b]	4b[c]	3b[d]	2b[e]	1b[f]	1d[f]
C-1	36.2	35.9*	36.0	36.0*	35.5	37.8*
C-2	27.5	27.4	27.3	27.3	27.0	37.4*
C-3	73.4	73.2	73.2	73.2	72.7	210.5
C-4	34.0	33.4**	33.8	33.7	32.9*	43.6
C-5	44.0	37.7	43.8	43.9	37.4	39.9
C-6	28.7	33.7**	28.5	28.8**	33.2*	33.6
C-7	29.4	69.7	30.2	29.0**	69.6	69.4
C-8	125.8	124.0	135.1	134.7	131.8	131.2
C-9	49.1	44.7	49.2	49.1	44.7	44.5
C-10	36.6	36.5	36.8	37.1	36.8	37.1
C-11	19.8	19.3	19.8	19.6	19.1	19.4
C-12	37.1	36.3	37.1	37.1	36.4	36.6
C-13	42.6	43.0	42.9	42.2	43.3	43.5
C-14	142.5	150.4	141.6	140.4	147.3	148.2
C-15	25.7	25.2	73.0	72.5	72.3	72.4
C-16	26.9	26.7	36.4	36.1*	36.1	36.2
C-17	56.8	56.2	53.7	54.5	52.7	52.9
C-18	18.1	17.8	19.4*	18.2	18.6**	18.9
C-19	12.6	11.9	12.7	12.4	11.9	11.3
C-20	34.3	34.3	33.5	34.4	33.2	33.4
C-21	19.0	19.0	18.9*	18.8	18.7**	18.9
C-22	35.9	35.8*	35.8	35.6	35.5	35.7
C-23	23.7	23.8	23.6	23.5	23.4	23.5
C-24	39.4	39.4	39.2	39.3	39.0	39.2
C-25	27.9	27.9	27.8	27.8	27.6	27.7
C-26	22.4	22.5	22.4	22.4	22.2	22.4
C-27	22.7	22.8	22.6	22.6	22.5	22.6
$COCH_3$	170.1	170.1	170.2	170.1	169.3	169.5
		170.3	170.5	170.5	169.9	170.5
					170.3	
$COCH_3$		21.3	21.0	21.2	21.0	21.3
	21.2	21.6	21.3	21.2	21.1	21.5
					21.5	

[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 here are preferred. [b] Registry no. 6562-21-6. [c] Registry no. 34271-12-0. [d] Registry no. 69140-08-5. [e] Registry no. 69140-09-6. [f] Registry no. 69140-10-9. [g] Registry no. 69140-11-0.

Table IV. Acetylation and TAI Shifts for Allyl Alcohol Moiety of $\Delta^{8(14)}$-Sterols



R = H or Ac

	acetylation shifts, ($\Delta\delta$) ppm[a]			TAI shifts, ($\Delta\delta$) ppm[b]
	3β,7α [4b]	3β,15α [3b]	3β,15β [2b]	3β,7β [5b]
α(sp³)	3.2 (C-7)	3.0 (C-15)	2.7 (C-15)	6.6 (C-15)
β(sp²)	−4.1 (C-8)	−5.6 (C-14)	−4.8 (C-14)	−6.0 (C-14)
β(sp³)	−2.1 (C-6) (−1.8)	−2.1 (C-16)	−1.9 (C-16)	−2.2 (C-16)
γ(sp²)	2.5 (C-14)	1.0 (C-8)	1.5 (C-8)	3.3 (C-8)
γ(sp³)	0.7 (C-9)	−0.2 (C-13)	−0.1 (C-17)	−0.3 (C-17)
	0.6 (C-5)	0.2 (C-17)	0.2 (C-13)	0.2 (C-13)
δ(sp³)	−0.1 (C-10)	−0.2 (C-10)	−0.1 (C-10)	−0.1 (C-17)
	0.0 (C-11)	0.4 (C-12)	−0.2 (C-12)	−0.2 (C-9)
	0.2 (C-13)	−0.7 (C-7)	−0.8 (C-18)	−0.7 (C-18)
		−0.1 (C-20)		
	0.2 (C-15)	−0.1–0.4 (C-18)	0.1 (C-20)	−0.3 (C-12)

[a] $\Delta\delta = \delta$(acetate) − δ(free sterol). [b] $\Delta\delta = \delta$(TAI derivative) − δ(free sterol).

introduced vicinal and cis to the departing oxygen function at C-14.[33-37] In the case under investigation such a process would involve introduction of the hydroxyl function at C-7 in the α configuration. The hydroxyl group at C-15 (resulting from the epoxide ring opening) would then retain its original α configuration. This proposed mechanism and stereochemical course finds direct analogy in the previously studied reductive rearrangement of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene with lithium aluminum hydride or lithium triethylborohydride.[38,39] Other α,β-unsaturated epoxides,[40] vinyl alcohols,[41] and ethers[41] have also been shown to undergo a similar S_N2' rearrangement upon hydride reduction.

Table V. Protic Solvent Induced Shift Effects ($\Delta\delta$, ppm)[a] on Dihydroxy and Trihydroxy $\Delta^{8(14)}$-Sterols

carbon atom	3α,7α [4a]	3β,7β [5a]	3β,15α [3a]	3β,15β [2a]	3β,7α,15α [1a]
C-1					
C-2	−0.4	−0.45	−0.3	−0.4	−0.4
C-3	−0.3 } 3β	−0.5 } 3β	−0.3 } 3β	−0.3 } 3β	−0.4 } 3β
C-4	−0.4	−0.4	−0.3	−0.4	−0.2
C-5				or 0.0	
C-6	0.2	−0.4			−0.1
C-7	−0.2 } 7α	−0.4 } 7β			−0.1 } 7α
C-8	−0.3	−0.2	+0.4	+0.2	−0.7
C-9				+0.2	
C-10					
C-11					
C-12				+0.2	
C-13				+0.2	
C-14			−0.7	−0.8	+0.5
C-15		−0.2	−0.2 } 15α	−0.3 } 15β	−0.5 } 15α
C-16			−0.1	−0.0	−0.4
C-17		0.2		or −0.4	
C-18					
C-19		−0.2		−0.2	
	0.37 M in C	0.18 M in C	0.3 M in C	0.34 M in C	0.03 M in C
	0.3 M in C–M	0.46 M in C–M	0.33 M in C–M	0.39 M in C–M	0.31 M in C–M

[a] $\Delta\delta$(ppm) = δ(C–M) − δ(C). $\Delta\delta$ for any other positions were within ±0.1 ppm. C = $CDCl_3$ and C–M = $CDCl_3$–CD_3OD (4:1).

Table VI. Chemical Shift Deviation Values for Selected Carbon Atoms of $\Delta^{8(14)}$-Allylic Hydroxysterols, $\Delta\delta$(1a–4a, 1a–5a, 1a–3a, and 1a–2a) (ppm)

carbon atom		Δδ(1a–4a)	Δδ(1a–5a)	carbon atom		Δδ(1a–3a)	Δδ(1a–2a)
C-5	γ	0.5	−4.4	C-13	γ	0.1	1.2
C-6	β	−0.4	−5.0	C-15	α	0.2	0.4
C-7	α	0.2	−6.9	C-16	β	0.6	1.2
C-9	γ	1.3	−3.1	C-17	γ	−0.4	−1.5
C-13	δ	0.4	−1.1	C-20	δ	0.0	−0.7

Experimental Section

Procedures and conditions for the recording of melting points,[12] infrared spectra,[12] ultraviolet spectra (ethanol solvent),[14] optical rotations (chloroform solvent),[13] low resolution mass spectral and combined gas–liquid chromatography–mass spectral analyses,[42] preparation of trimethylsilyl ether derivatives of the sterols,[13] thin-layer chromatographic analyses,[43] and gas–liquid chromatographic analyses[14] have been described previously. High resolution mass spectral measurements were made on a Varian CH-5 spectrometer (courtesy of Professor C. C. Sweeley). Medium pressure liquid chromatography (100 psi) was carried out on columns (118 cm × 1.5 cm) of silica gel (0.032–0.063 mm; ICN Pharmaceuticals, Cleveland, Ohio) at a flow rate of 4 mL/min using the appropriate solvent. Proton magnetic resonance spectra (^1H NMR) were recorded in $CDCl_3$ solution on a Perkin-Elmer HR-12 spectrometer using tetramethylsilane (Me_4Si) as an internal standard. Peaks are reported as ppm (δ) downfield from the Me_4Si standard. Proton chemical shifts for the C-18 and C-19 angular methyl resonances were calculated by the method of Zurcher.[44] 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 either $CDCl_3$ or $CDCl_3$–CD_3OD (4:1; v/v) solutions (0.2–0.7 M unless otherwise stated). 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 Me_4Si and are estimated to be accurate to ±0.05 ppm (δ(Me_4Si) = $\delta(CDCl_3)$ + 76.9 ppm). The probe temperature was ~30 °C. In the case of the mixed solvent, $CDCl_3$–CD_3OD, the $CDCl_3$ resonance shifted 0.3 ppm downfield.[32] The chemical shift values in the mixed solvent have been corrected for this shift in the Me_4Si reference as follows: $\delta(Me_4Si) = \delta(CDCl_3\text{–}CD_3OD) + 77.2$ ppm. Variation in sample concentration was found to have a negligible influence (<0.2 ppm). Trichloroacetyl isocyanate was purchased from Eastman Kodak and utilized to prepare the corresponding carbamate derivatives[22] of the sterols. After recording the ^{13}C NMR spectrum of a given sterol, the sample tube was cooled in an ice-bath and trichloroacetyl isocyanate was added dropwise until no further effervescence was observed. The ^{13}C NMR spectrum of the resulting carbamate derivative was then recorded. 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.

5α-Cholest-7-en-3β-ol, mp 122–123 °C [lit. mp 121.5–122.0 °C,[45] 122 °C,[46] 124–125 °C[47]], single component on TLC and GLC, was prepared by catalytic reduction of 7-dehydrocholesterol.[45] 3β-Benzoyloxy-5α-cholest-7-ene, mp 156–157 °C, clearing at 175 °C [lit. mp 157 °C, clearing at 176 °C[46]], was prepared from the free sterol by treatment with benzoyl chloride in pyridine. 5α-Cholest-8(14)-ene-3β,7α,15α-triol (**1a**),[12] 5α-cholest-8(14)-ene-3β,15β-diol (**2a**),[48–51] 5α-cholest-8(14)-en-3β-ol (**11a**),[47] and its 3β-acetoxy derivative (**11b**)[47] were prepared as described previously. 5α-Cholest-8(14)-ene-3β,15α-diol (**3a**) and [7α-$^2H_1$]-5α-cholest-8(14)-ene-3β,15α-diol (**3c**) were prepared from 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene as described previously.[38,39]

3β-Benzoyloxy-8α,14α-epoxy-5α-cholestan-7α-ol (6) and 3β-Benzoyloxy-8α,9α-epoxy-5α-cholestan-7α-ol (7). To 3β-benzoyloxy-5α-cholest-7-ene (5.00 g; 10.2 mmol) in ethanol-free $CHCl_3$ (75 mL) was added *m*-chloroperbenzoic acid (4.5 g). The reaction mixture was allowed to stand at 4 °C for 9 days with occasional stirring. The filtered reaction mixture was washed with 5% $NaHCO_3$ and dried over $MgSO_4$. Analysis by TLC indicated two major components and trace amounts of starting material. The mixture was subjected to silica gel (95 g; 60–200 mesh) column (100 cm × 2 cm) chromatography using 500-mL portions of benzene and 2.5, 5, 7.5, and 10% ether in benzene as the eluting solvents. Fractions 20 mL in volume (flow rate, ~5 mL/min) were collected. The contents of fractions 39 through 51 were pooled and recrystallized four times from acetone–water to give **6** (1.60 g; 30% yield) melting at 131.0–132.5 °C: IR ν_{max} 3500, 1590, 1610, 1280, 1120, 940, and 715 cm^{-1}; ^1H NMR δ 0.95

(s, 6 H, C-18–CH_3 and C-19–CH_3), 3.67 (m, 1 H, C-7–H), 5.15 (m, 1 H, C-3–H), 8.10 (m, 5 H, aromatic); MS 522 (M, 3), 507 (1), 504, (100), 489 (22), 486 (10), 471 (8), 409 (3), 400 (3), 391 (96), 382 (12), 373 (28), 367 (10), 349 (19), 338 (32), 269 (15), and 251 (18); high resolution MS, 522.3694 (calcd for $C_{34}H_{50}O_4$: 522.3709); single component on TLC in three solvent systems. The contents of fractions 53 through 61 were pooled and recrystallized from acetone–water to give **7** (0.60 g; 11% yield) melting at 176–177 °C: IR ν_{max} 3500, 1728, 1610, 1590, 1285, 1118, 910, and 712 cm^{-1}; ^1H NMR δ 0.69 (s, 3 H, C-18–CH_3), 1.05 (s, 3 H, C-19–CH_3), 4.10 (m, 1 H, C-7–H), 5.12 (m, 1 H, C-3–H), and 8.10 (m, 5 H, aromatic); MS 522 (M, 1), 507 (1), 504 (29), 489 (16), 488 (30), 486 (42), 471 (11), 409 (1), 400 (8), 391 (16), 382 (1), 373 (100), 367 (13), 364 (26), 349 (48), 306 (80), 293 (15), and 251 (52); high resolution MS 522.3688 (calcd for $C_{34}H_{50}O_4$: 522.3709); single component on TLC in three solvent systems.

7-Oxo-5α-cholest-8(14)-en-3β-ol (8) and 7-Oxo-5α-cholest-8-en-3β-ol (9). To epoxide **6** (1.00 g; 1.9 mmol) in ethanol (175 mL) was added, with stirring, water (10 mL) and H_2SO_4 (30 mL). After heating under reflux for 18 h, the mixture was cooled to room temperature and its volume was reduced to one-fourth of its initial value. The mixture was poured into water and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness to yield a light yellow residue (0.46 g) which was subjected to MPLC (medium pressure liquid chromatography) using $CHCl_3$ as the eluting solvent. The contents of fractions 68 through 135 were pooled and recrystallized from methanol–water to yield **8** (0.32 g; 42% yield) melting at 129–131 °C [lit.[52] mp 129–130 °C]: IR ν_{max} 3400, 1670, 1590, 1260, 1045, 948, and 864 cm^{-1}; ^1H NMR δ 0.82 (s, 3 H, C-18–CH_3; calcd 0.83), 0.93 (s, 3 H, C-19–CH_3; calcd 0.97), 3.65 (m, 1 H, C-3–H); MS 400 (M, 100), 385 (10), 382 (1), 367 (1), 315 (7), 287 (35), 273 (14), 259 (11), 245 (14), 234 (64), 232 (3); high resolution MS 400.3330 (calcd for $C_{27}H_{44}O_2$: 400.3341); UV λ_{max} 262 (ϵ = 8500) [lit.[52] 261]; single component on TLC using three solvent systems and on GLC. Epoxide **7** (0.40 g; 0.77 mmol) was treated with acid and worked up in an identical fashion to the case of **6**. The light yellow residue (205 mg) thus obtained was subjected to MPLC using $CHCl_3$ as the eluting solvent. The contents of fractions 92 through 116 were pooled and, after evaporation of the solvent, recrystallized from methanol–water to give **9** (170 mg; 55% yield) which melted at 122–123 °C: IR ν_{max} 3350, 1660, 1590, 1378, 1268, 1050, and 933 cm^{-1}; ^1H NMR δ 0.56 (s, 3 H, C-18–CH_3; calcd 0.57), 1.18 (s, 3 H, C-19–CH_3; calcd 1.21), and 3.70 (m, 1 H, C-3–H); MS 400 (M, 100), 385 (16), 382 (3), 367 (2), 315 (6), 287 (31), 274 (28), 260 (14), 245 (30), 234 (24), 232 (46), and 214 (12); high resolution MS 400.3342 (calcd for $C_{27}H_{44}O_2$: 400.3341); UV λ_{max} 253 (ϵ = 10 000); single component on TLC in three solvent systems and on GLC.

5α-Cholest-8(14)-ene-3β,7β-diol (5a) and 5α-Cholest-8(14)-ene-3β,7α-diol (4a). To ketone **8** (500 mg; 1.25 mmol) in ether (50 mL) was added $LiAlH_4$ (1.00 g; 26.4 mmol). The resulting mixture was stirred at 25 °C for 4 h and, after cooling to 0 °C, ice was cautiously added to decompose the unreacted hydride. The mixture was poured into a saturated solution of NH_4Cl and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness to yield a white residue (470 mg) which was subjected to MPLC using 40% ethyl acetate in benzene as the eluting solvent. The contents of fractions 37 through 67 were pooled and recrystallized from acetone–water to give **5a** (344 mg; 69% yield) melting at 168.0–169.5 °C [lit.[53] mp 163–164 °C]: IR ν_{max} 3390, 1040, and 950 cm^{-1}; ^1H NMR δ 0.74 (s, 3 H, C-19–CH_3; calcd 0.72), 0.86 (s, 3 H, C-18–CH_3; calcd 0.86), 3.76 (m, 1 H, C-3–H), and 4.28 (m, 1 H, C-7–H); MS 402 (M, 6), 387 (1), 384 (72), 369 (29), 366 (4), 351 (10), 289 (2), 271 (100), 257 (24), 253 (25), and 200 (16); high resolution MS 402.3498 (calcd for $C_{27}H_{46}O_2$: 402.3503); $[\alpha]_D$ + 46.0° (*c*, 0.44) [lit.[39] +41.0°]; single component on TLC on silica gel G in three solvent systems and on silica gel G–silver nitrate. The contents of fractions 75 through 98 from the MPLC were combined and recrystallized from acetone–water to give **4a** (44 mg; 9% yield) melting at 156–158 °C [lit.[54] mp 157–158 °C]: IR ν_{max} 3380, 1167, 1050, 1024, and 949 cm^{-1}; ^1H NMR δ 0.68 (s, 3 H, C-19–CH_3; calcd 0.68), 0.85 (s, 3 H, C-18–CH_3; calcd 0.83), 3.71 (m, 1 H, C-3–H), 4.66 (m, 1 H, C-7–H); MS 402 (M, 10), 387 (2), 384 (76), 369 (29), 366 (3), 351 (10), 289 (2), 271 (100), 257 (30), and 253 (25); $[\alpha]_D$ −26.9° (*c*, 0.44) [lit.[38] −21°]; single component on TLC as in the case of **5a**.

3-Oxo-5α-cholest-8(14)-ene-7α,15α-diol (1c). To 5α-Cholesta-7,14-dien-3β-ol (10.0 g; 26.0 mmol; single component on TLC and GLC but containing, as judged by ^1H NMR, ~25% of the $\Delta^{8,14}$-isomer), prepared by saponification of the corresponding 3β-benzoate ester,[14] in dry CH_2Cl_2 (100 mL) was added a suspension of pyridinium chlorochromate (12.0 g) in dry CH_2Cl_2 (100 mL). After stirring under nitrogen at room temperature for 30 min, the reaction mixture was poured into a saturated NaCl solution and thoroughly extracted with $CHCl_3$. The combined extracts were evaporated to dryness and the resulting residue was subjected to silica gel (60/200 mesh) column (100 cm × 2 cm) chromatography using benzene as the eluting solvent. Fractions 20 mL in volume were collected. The contents of fractions 26 through 85 were pooled and recrystallized from acetone–water to give crude **10**, 5α-cholesta-7,14-dien-3-one (6.10 g; 61% yield), melting at 107–110 °C. Pure 5α-cholesta-7,14-dien-3-one, free of its $\Delta^{8,14}$-isomer, melts at 129.0–129.5 °C.[55] The product showed a single component on TLC on silica gel plates in three solvent systems and on GLC. The ^1H NMR spectra indicated (by analysis of the resonances due to the C-7–H and the C-15–H) the presence of ~27% of the $\Delta^{8,14}$-isomer. To crude **10** (5.00 g; 13.1 mmol) from above, in 25% ether in hexane (100 mL) at 0 °C, was added *m*-chloroperbenzoic acid (4.40 g) in 25% ether in hexane (200 mL) with stirring. After standing at 0 °C for 1 h, the solution was maintained at −12 °C for 12 h. The mixture was poured into an NaCl solution (2%) and extracted with ether (300 mL). The ether extract was washed successively with water, cold 1 N NaOH, and water, dried over $MgSO_4$, and evaporated to dryness. The resulting residue was heated under reflux with a mixture of ethanol (270 mL), water (27 mL), and KOH (3.0 g) for 45 min under nitrogen. After reduction of the volume to ~½ its initial value, the mixture was poured into ice–water and the pH of the mixture was adjusted to neutrality by the slow addition of cold aqueous 5% HCl. Ether was added and the separated ether phase was washed thoroughly with water, dried over $MgSO_4$, and evaporated to dryness. The resulting residue was subjected to MPLC using 10% ethyl acetate in $CHCl_3$ as the eluting solvent. The contents of fractions 45 through 100 were pooled and recrystallized from hexane to give **1c** (1.40 g; 26% yield) melting at 121.5–122.5 °C: IR ν_{max} 3320, 1731, 1037, 962, and 875 cm^{-1}; ^1H NMR δ 0.90 (s, 6 H, C-18–CH_3 and C-19–CH_3; calcd 0.90), 4.62 and 4.91 (m, 1 H each, C-7–H and C-15–H); MS 398 (M − H_2O, 100), 383 (75), 380 (74), 365 (26), 295 (16), 285 (70), 267 (98), 257 (12), 253 (18), 231 (23), 215 (22), and 211 (22); high resolution MS on ion at *m/e* 398, 398.3185 (calcd for $C_{27}H_{42}O_2$: 398.3186); high resolution MS on ion at *m/e* 560 (M) of the $(Me_4Si)_2$ derivative, 560.4081 (calcd for $C_{33}H_{60}O_3Si_2$: 560.4085); single component on TLC in three solvent systems and on GLC of the free sterol and its $(Me_4Si)_2$ derivative.

5α-Cholest-8(14)-ene-3β,7α,15α-triol (1a) from 3-Oxo-5α-cholest-8(14)-ene-7α,15α-diol (1c). To ketone **1c** (100 mg; 0.24 mmol) in ether (20 mL) was added $LiAlH_4$ (300 mg). After stirring for 3 h at room temperature, the mixture was cooled to 0 °C and ice was cautiously added to decompose the unreacted hydride. The mixture was poured into a saturated solution of NH_4Cl and thoroughly extracted with ether containing CH_2Cl_2 (10%). The combined extracts were dried over $MgSO_4$ and evaporated to dryness. The resulting residue was recrystallized from acetone–water to yield a white crystalline solid (91 mg). Analyses by TLC showed a single component. However, GLC analysis of the $(Me_4Si)_3$ derivative indicated the presence of a less polar impurity (~10%). The major component had the same retention times as the $(Me_4Si)_3$ derivative of authentic **1a**. Analysis by GLC–MS of the Me_4Si derivatives of the major and minor components indicated that they were isomeric and suggested that the impurity represented 5α-cholest-8(14)-ene-3α,7α,15α-triol. The crude triol (75 mg) was dissolved in hot ethyl acetate (2 mL) and, after cooling to room temperature, hexane (18 mL) was added. The resulting mixture was cooled at −12 °C for 8 h and the resulting crystals were collected and washed with cold hexane. After repeating this procedure three times, pure **1a** (36 mg; 99% purity as indicated by GLC of the $(Me_4Si)_3$ derivative) was obtained which melted at 212.5–214.0 °C [lit.[12] mp 213–214 °C]: IR ν_{max} 3360 and 1038 cm^{-1}; NMR δ 0.80 (s, 3 H, C-19–CH_3; calcd 0.79), 0.90 (s, 3 H, C-18–CH_3; calcd 0.87), 3.62 (m, 1 H, C-3–H), 4.60 (m, 1 H, C-7–H), 4.95 (m, 1 H, C-15–H); MS 400 (M − H_2O, 60), 385 (48), 382 (98), 367 (39), 364 (9), 349 (100), 297 (7), 287 (35), 272 (5), 269 (52), 257 (6), 251 (19), 233 (14), 215 (11), 213 (15), 209 (17); $[\alpha]_D$ −43.0° (*c*, 0.2) [lit.[12] −40.7°].

General Preparation of Acetate Derivatives of Allylic Di- and Trihydroxysterols. The sterol (400 mg) was dissolved in a 1:1 mixture (20 mL) of acetic anhydride and pyridine and, after standing overnight at room temperature, the mixture was poured into ice–water and extracted with 60 mL of ether containing methylene chloride (10%). The separated ether layer was successively washed with water, cold 5% HCl, water, 5% Na_2CO_3, and water, dried over $MgSO_4$ sulfate, and evaporated to dryness. The resulting crude acetate was subjected to silica gel (60/200 mesh) column (35 cm × 1.5 cm) chromatography. Using the appropriate solvent as the eluting solvent, fractions 20 mL in volume were collected.

5α-Cholest-8(14)-ene 3β,15α-Diacetate (3b). After column chromatography (solvent, 1% ether in benzene), the contents of

fractions 10 through 21 were pooled and recrystallized from acetone–water to give **3b** (376 mg; 78% yield) melting at 175.5–177.5 °C: IR ν_{max} 1740, 1671, 1251, 1034, and 950 cm^{-1}; ^1H NMR δ 0.73 (s, 3 H, C-19–CH_3; calcd 0.73), 0.90 (s, 3 H, C-18–CH_3; calcd 0.89), 2.03 (s, 6 H, diacetate), 4.88 (m, 1 H, C-3–H), 5.92 (m, 1 H, C-15–H); MS 426 (M − CH_3COOH, 100), 411 (20), 366 (10), 351 (41), 313 (70), 299 (25), 253 (25), 238 (10); high resolution MS on ion at *m/e* 426, 426.3496 (calcd for $C_{29}H_{46}O_2$: 426.3498); single component on TLC in three solvent systems.

5α-Cholest-8(14)-ene 3β,15β-Diacetate (2b). After column chromatography (solvent, 1% ether in benzene), the contents of fractions 9 through 20 were pooled and recrystallized from acetone–water to yield **2b** (410 mg; 85% yield) which melted at 124–125 °C: IR ν_{max} 1740, 1688, 1250, 1030, and 961 cm^{-1}; ^1H NMR δ 0.76 (s, 3 H, C-19–CH_3; calcd 0.75), 1.03 (s, 3 H, C-18–CH_3; calcd, 1.05), 2.08 (s, 6 H, diacetate), 4.91 (m, 1 H, C-3–H), 5.80 (m, 1 H, C-15–H); MS 426 (M − CH_3COOH, 100), 411 (9), 366 (6), 351 (10), 313 (46), 299 (20), 253 (15); high resolution MS on ion at *m/e* 426, 426, 3491 (calcd for $C_{31}H_{50}O_4$: 426.3498); single component on TLC in three solvent systems.

5α-Cholest-8(14)-ene 3β,7α,15α-Triacetate (1b). After column chromatography (solvent, 5% ether in benzene), the contents of fractions 10 through 18 were pooled and, upon evaporation of the solvent, gave **1b** (405 mg; 75% yield) as a colorless glass which resisted all attempts at crystallization: IR ν_{max} 1745, 1256, 1034, and 951 cm^{-1}; ^1H NMR δ 0.72 (s, 3 H, C-19–CH_3; calcd 0.72), 0.89 (s, 3 H, C-18–CH_3; calcd, 0.89), 1.94, 2.05, and 2.08 (s, 3 H each, methyls of acetoxy functions), 4.92 (m, 1 H, C-3–H), 5.51 (m, 1 H, C-7–H), and 5.88 (m, 1 H, C-15–H); MS 484 (M − CH_3COOH, 29), 469 (3), 442 (56), 424 (10), 409 (14), 364 (74), 349 (84), 339 (26), 283 (83), 251 (38); high resolution MS on ion at *m/e* 484, 484.3547 (calcd for $C_{33}H_{52}O_6$: 484.3552); single component on TLC in two solvent systems.

7α,15α-Diacetoxy-5α-cholest-8(14)-en-3-one (1d). The residue obtained after evaporation of the solvent from the ether extraction of the reaction mixture was recrystallized from acetone–water to give **1d** (395 mg; 82%) melting at 169.5–171.0 °C: IR ν_{max} 1738, 1265, 1220, 1030, and 946 cm^{-1}; ^1H NMR δ 0.91 (s, 3 H, C-19–CH_3; calcd 0.91), 0.93 (s, 3 H, C-18–CH_3; calcd 0.93), 1.97 and 2.08 (s, 3 H each, methyls of acetoxy functions), 5.56 (m, 1 H, C-7–H), and 5.91 (m, 1 H, C-15–H); MS 440 (M − CH_3COOH, 13), 398 (35), 380 (20), 267 (99), 255 (19), and 253 (17); high resolution MS on ion at *m/e* 440, 440.3306 (calcd for $C_{31}H_{48}O_5$: 440.3290); single component on TLC in two solvent systems.

15-Oxo-5α-cholest-8(14)-en-3β-ol from 5α-Cholest-8(14)-ene-3β,7α,15α-triol (1a). Triol **1a** (1.00 g; 2.40 mmol) was heated under reflux for 4 h with a mixture consisting of 200 mL of 95% ethanol–water and 10 mL of concentrated HCl. After reduction of the volume to ~1/3 of its initial value the mixture was diluted with water and thoroughly extracted with ether containing CH_2Cl_2 (5%). The combined extracts were washed with 10% aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated to dryness to give a pale yellow residue (0.91 g) which was subjected to MPLC using 10% ether in benzene as the eluting solvent. The contents of fractions 35 through 50 were pooled and, after evaporation of the solvent, recrystallized from methanol–acetone–water to give 15-oxo-5α-cholest-8(14)-en-3β-ol (0.83 g; 87% yield) melting at 147.5–149.0 °C [lit.[14,16] mp 147.5–149.0 °C]: IR ν_{max} 3350, 1704, and 1620 cm^{-1}; UV λ_{max} 258 (ϵ = 13 600); ^1H NMR δ 0.72 (s, 3 H, C-19–CH_3; calcd 0.70), 0.98 (s, 3 H, C-18–CH_3), 3.66 (m, 1 H, C-3–H), 4.18 (m, 1 H, C-7β–H); MS 400 (M, 100), 385 (25), 382 (15), 367 (35), 287 (24), 269 (53), and 251 (13). The compound showed a single component on TLC and on GLC.

Registry No.—**1c** (Me_4Si)$_2$ derivative, 69140-12-1; **6**, 69140-13-2; **7**, 69140-14-3; **8**, 566-29-0; **9**, 69140-15-4; **10**, 69140-16-5; 5α-cholest-7-en-3β-ol, 80-99-9; 7-dehydrocholesterol, 434-16-2; 3β-benzoyloxy-5α-cholest-7-ene, 4356-22-3; 5α-cholesta-7,14-dien-3β-ol, 34227-11-7; 3β-benzoyloxy-5α-cholesta-7,14-diene, 34227-12-8; 5α-cholest-8(14)-ene-3α,7α,15α-triol, 69177-18-0; 15-oxo-5α-cholest-8(14)-en-3β-ol, 50673-97-7.

References and Notes

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(2) C. S. Barnes, D. H. R. Barton, and G. F. Laws, *Chem. Ind. (London)*, 616 (1953).
(3) D. H. R. Barton and G. F. Laws, *J. Chem. Soc.*, 52 (1954).
(4) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954).
(5) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1131 (1957).
(6) R. R. Muccino and C. Djerassi, *J. Am. Chem. Soc.*, **96**, 556 (1974).
(7) M. Akhtar, C. W. Freeman, D. C. Wilton, R. B. Boar, and D. B. Copsey, *Bioorg. Chem.*, **6**, 473 (1977).
(8) M. Akhtar, K. Alexander, R. B. Boar, J. F. McGuie, and D. H. R. Barton, *Biochem. J.*, **169**, 449 (1978).
(9) G. J. Schroepfer, Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **35**, 1697 (1976).
(10) G. J. Schroepfer, Jr., E. J. Parish, and A. A. Kandutsch, *J. Am. Chem. Soc.*, **99**, 5494 (1977).
(11) G. J. Schroepfer, Jr., D. L. Raulston, and A. A. Kandutsch, *Biochem. Biophys. Res. Commun.*, **79**, 406 (1977).
(12) G. J. Schroepfer, Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch, *J. Biol. Chem.*, **252**, 8975 (1977).
(13) G. J. Schroepfer, Jr., R. A. Pascal, Jr., and A. A. Kandutsch, *Biochem. Pharmacol.*, **28**, 249 (1979).
(14) E. J. Parish, T. E. Spike, and G. J. Schroepfer, Jr., *Chem. Phys. Lipids*, **18**, 233 (1977).
(15) B. N. Conner, E. J. Parish, G. J. Schroepfer, Jr., and F. A. Quiocho, *Chem. Phys. Lipids*, **18**, 240 (1977).
(16) D. L. Raulston, C. O. Mishaw, E. J. Parish, and G. J. Schroepfer, Jr., *Biochem. Biophys. Res. Commun.*, **71**, 984 (1976).
(17) G. J. Schroepfer, Jr., D. Monger, A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, and A. A. Kandutsch, *Biochem. Biophys. Res. Commun.*, **78**, 1227 (1977).
(18) A. Kisic, A. S. Taylor, J. S. Chamberlain, E. J. Parish, and G. J. Schroepfer, Jr., *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **37**, 1663 (1978).
(19) A. Kisic, D. Monger, E. J. Parish, S. Satterfield, D. L. Raulston, and G. J. Schroepfer, Jr., *Artery*, **3**, 421 (1977).
(20) H. Eggert, C. L. VanAntwerp, N. S. Bhacca, and C. Djerassi, *J. Org. Chem.*, **41**, 71 (1976).
(21) J. Polonsky, Z. Baskevitch, H. E. Gottlieb, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, **40**, 2499 (1975).
(22) A. K. Bose and P. R. Srinivasan, *Tetrahedron*, **31**, 3025 (1975).
(23) H. J. Rich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Am. Chem. Soc.*, **91**, 7445 (1969).
(24) E. Wenkert, M. J. Gasic, E. W. Hagaman, and L. D. Kwart, *Org. Magn. Reson.* **7**, 51 (1975).
(25) M. Tsuda, *Fed Proc., Fed. Am. Soc. Exp. Biol.*, **36**, 817 (1977).
(26) M. Tsuda and G. J. Schroepfer, Jr., *J. Org. Chem.*, following paper in this issue.
(27) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, pp 84–88.
(28) J. W. deHaan, *Org. Magn. Reson.*, **5**, 147 (1973).
(29) P.v. R. Schleyer, D. S. Trifan, and R. Bucskai, *J. Am. Chem. Soc.*, **80**, 6691 (1958).
(30) M. Ohoki and H. Iwamura, *Bull. Chem. Soc. Jpn.*, **32**, 307 (1959).
(31) H. Eggert and C. Djerassi, *J. Org. Chem.*, **38**, 3788 (1973).
(32) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972.
(33) G. Stork and W. N. White, *J. Am. Chem. Soc.*, **78**, 4609 (1956).
(34) A. Fry, *Pure Appl. Chem.*, **8**, 409 (1964).
(35) C. W. Jeffold, S. N. Mahajan, and J. Gunsher, *Tetrahedron*, **24**, 2921 (1968).
(36) C. W. Jeffold, A. Sweeney, D. T. Hill, and F. Delay, *Helv. Chim. Acta*, **55**, 2214 (1971).
(37) C. W. Jeffold, A. Sweeney, and F. Delay, *Helv. Chim. Acta*, **55**, 2214 (1972).
(38) E. J. Parish and G. J. Schroepfer, Jr., *Tetrahedron Lett.*, 3775 (1976).
(39) E. J. Parish and G. J. Schroepfer, Jr., *Chem. Phys. Lipids*, **18**, 258 (1977).
(40) R. S. Lenox and J. A. Katzenellenbogen, *J. Am. Chem. Soc.*, **95**, 957 (1973).
(41) B. Fraser-Reid, S. Y.-K. Tam, and B. Radatus, *Can. J. Chem.*, **53**, 2005 (1975).
(42) F. F. Knapp, Jr., M. S. Wilson, and G. J. Schroepfer, Jr., *Chem. Phys. Lipids*, **16**, 31 (1976).
(43) F. F. Knapp, Jr., and G. J. Schroepfer, Jr., *Steroids*, **26**, 339 (1975).
(44) R. F. Zurcher, *Helv. Chim. Acta*, **46**, 2054 (1963).
(45) G. J. Schroepfer, Jr., and I. D. Frantz, Jr., *J. Biol. Chem.*, **236**, 3137 (1961).
(46) P. Bladon in "Cholesterol: Chemistry, Biochemistry and Pathology", R. P. Cook, Ed., Academic Press, New York, 1958, pp 89–92.
(47) W.-H. Lee, R. Kammereck, B. N. Lutsky, J. A. McCloskey, and G. J. Schroepfer, Jr., *J. Biol. Chem.*, **244**, 2033 (1969).
(48) S. Huntoon and G. J. Schroepfer, Jr., *Biochem. Biophys. Res. Commun.*, **40**, 476 (1970).
(49) G. J. Schroepfer, Jr., B. N. Lutsky, J. A. Martin, S. Huntoon, B. Fourcans, W. H. Lee, and J. Vermilion, *Proc. R. Soc. London, Ser. B*, **180**, 125 (1972).
(50) S. Huntoon, B. Fourcans, B. N. Lutsky, E. J. Parish, H. Emery, F. F. Knapp, Jr., and G. J. Schroepfer, Jr., *J. Biol. Chem.*, **253**, 775 (1978).
(51) G. N. Phillips, Jr., F. A. Quiocho, R. L. Sass, P. Werness, H. Emery, F. F. Knapp, Jr., and G. J. Schroepfer, Jr., *Bioorg. Chem.*, **5**, 1 (1976).
(52) L. F. Fieser, K. Nakanishi, and W.-Y. Huang, *J. Am. Chem. Soc.*, **75**, 4719 (1953).
(53) L. G. Partridge, I. Midgley, and C. Djerassi, *J. Am. Chem. Soc.*, **99**, 7686 (1977).
(54) L. F. Fieser and G. Ourisson, *J. Am. Chem. Soc.*, **75**, 4404 (1953).
(55) B. N. Lutsky, J. A. Martin, and G. J. Schroepfer, Jr., *J. Biol. Chem.*, **246**, 6737 (1971).