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STEROL SYNTHESIS. CHEMICAL SYNTHESIS, STRUCTURE DETERMINATION AND METABOLISM OF 14α-METHYL-5α-CHOLEST-7-EN-3β,15β-DIOL AND 14α-METHYL-5α-CHOLEST-7-EN-3β,15α-DIOL

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14α-Methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol have been prepared by chemical synthesis. Unequivocal establishment of these structures was based upon x-ray crystallographic analysis of 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol and was supported by other spectroscopic data. Spectroscopic data were presented for the following compounds prepared in this study: 3β-benzoyloxy-5α-cholest-8(14)-en-15-one, 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one, 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol, 14α-methyl-5α-cholest-7-en-3β,15β-diol, 14α-methyl-5α-cholest-7-en-3β,15α-diol, 3β,15α-bis-benzoyloxy-14α-methyl-5α-cholest-7-ene, 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol and 3β,15β-bis-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-ene. Studies of the metabolism of [16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15β-diol and [16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15α-diol in liver homogenates of female rats indicated that only the 3β,15β-diol was convertible to cholesterol.

I. Introduction

The enzymatic conversion of lanosterol (4α,4β,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) to cholesterol involves the removal of the three "extra" methyl groups, reduction of the Δ^{24}-double bond, and "shift" of the nuclear double bond from the Δ^8-position in lanosterol to the Δ^5-position in cholesterol. Our recent efforts have been directed towards probing the mechanism involved in the removal of the 14α-methyl group of cholesterol precursors. With this goal in mind we have chosen to pursue studies of the metabolism of 14α-methyl substituted sterols lacking the Δ^{24}-double

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bond and the methyl groups at carbon atom 4. Studies of the metabolism of such substrates allows a marked simplification in the analysis of the products of incubations of these substrates when compared with a comparable analysis of the products derived enzymatically from lanosterol. A possible deficiency of this approach lies in the fact that such compounds may not constitute "natural" substrates for sterol biosynthesis. Until recently, available evidence suggested that the removal of the three "extra" methyl groups of lanosterol proceeded via initial removal of the 14α-methyl group. This suggestion rested on the fact that none of the methyl substituted sterols isolated from animal tissues [1,2] were assigned structures corresponding to 4,14-dimethyl-cholestenols or 14α-methyl-cholestenols. However, the reported isolations of (a) 14α-methyl-5α-cholest-8-en-3β,6α-diol from the cactus *Peniocereus macdougalli* [3], (b) 4α-14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol from grapefruit peel [4], the latex of Euphorbia obtusifolia [5], and from a mutant of *Saccharomyces cerevisiae* [6], (c) 4α,14α-dimethyl-5α-cholest-7-en-3β-ol, (d) 4α-14α-dimethyl-5α-cholest-7-en-3β-ol from feces and meconium of newborn infants [7], (e) 4α,14α-dimethyl-5α-ergosta-8,24-dien-3β-ol from a mutant of *S. cerevesiae* [6], (f) 4α,14α-dimethyl-5α-cholest-8-en-3β-ol from pollen [8], (g) 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol from *Ustilago maydis* grown in the presence of triamol [9] and from a mutant of *S. cerevesiae* [6] and (h) 14α-methyl-5α-ergost-8-en-3β-ol from *Chlorella emersonii* grown in the presence of triparanol [10] indicate that removal of the three "extra" methyl groups can be initiated by removal of either the 4α-methyl function [11–13 and references cited therein] or the 14α-methyl group.

The convertibility of 14α-methyl-5α-cholest-7-en-3β-ol to cholesterol in rat liver homogenate preparations has been demonstrated [14–19]. The results of studies employing mevalonic acid stereospecifically labeled with tritium at carbon atom 2 are compatible with a stereospecific loss of the 15α-hydrogen of lanosterol upon enzymatic formation of 5α-cholest-7-en-3β-ol, 7-dehydrocholesterol, and cholesterol [20–23]. These findings and mechanistic considerations have led our laboratory [2,15–17,24,25] and others [23,26–30] to consider 15-hydroxylated 14α-methyl sterols (or their derivatives) as potential intermediates in the overall enzymatic removal of carbon atom 32 of cholesterol precursors.

The purpose of this paper is to describe the chemical synthesis, structure determination, and metabolism of 14α-methyl-5α-cholest-7-en-3β,15α-diol and 14α-methyl-5α-cholest-7-en-3β,15β-diol. These compounds have recently been found to serve as very potent inhibitors of sterol biosynthesis in L-cells and in primary cultures of mouse liver cells [31].

II. Experimental Procedures and Results

A. General methods

Melting points were recorded in sealed, evacuated capillary tubes using a Thomas

Hoover melting point apparatus. Nuclear magnetic resonance spectra were determined in $CDCl_3$ solution using either a Varian T-60, Perkin-Elmer HR-12, or a Varian HR-220 spectrometer. Tetramethyl silane (TMS) was used as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. Infrared spectra were recorded on a Perkin-Elmer Model 521 spectrophotometer using the KBr pellet technique. Ultraviolet spectra were recorded using ethanol solutions of the sterols. Mass spectral data were obtained on Varian MAT CH-5, MAT SM1B, and MAT 731 mass spectrometers. Data were processed on a Varian 620i computer with STATOS recorder. Samples were introduced via the direct probe method. Radioactivity was measured in a Packard liquid scintillation spectrometer as described previously [32]. Gas-liquid chromatographic analyses were performed on a Barber-Colman Model 5000 instrument using 3% QF-1 on Gas Chrom Q (100–120 mesh) U-shaped columns which were 6 ft in length and with a 5 mm inside diameter. Operating conditions were as follows: column temperature, 220–230°C; injector temperature, 240–270°C; detector temperature 240–270°C. Argon was used as the carrier gas at a flow rate of 60 ml/min. 5α-Cholestane was used as an internal standard. Colorimetric assay of cholesterol was performed according to Abell et al. [33]. Radioactive cholesterol was purified by way of the dibromide by a modification [34] of the method of Fieser [35]. Tris-(2,2,6,6-tetramethylheptane-3,5-dionato)-europium-III (Fu(dpm)$_3$) was obtained from Alfa Inorganics (Danvers, Mass.). The shift reagent was sealed in vacuo and was not opened until just prior to use. For those experiments using the shift reagent, samples were analyzed using the Perkin-Elmer spectrometer. Samples were dissolved in $CDCl_3$ and peaks are reported downfield from the TMS standard. Following the addition of small aliquots of the shift reagent the spectra were recorded several times to ensure complete equilibrium of the substrate and the shift reagent. Shift experiments were generally repeated in duplicate or triplicate when sufficient sample was available. Protons were identified by an examination of the resonance patterns and by analysis of the integration data. In addition, the magnitudes of the linear induced shift data aided in protein assignments.

1. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one

3β-Benzoyloxy-5α-cholest-8(14)-en-15-one was prepared according to the procedure described by Knight et al. [14] and crystallized from ethyl acetate. The compound melted at 157–158°C (literature: 156–158°C [14], 156°C [36], 155–156°C [37]) and showed a single component on plates of neutral alumina, silica gel G, silica gel G-silver nitrate, and silica gel HF_{254} (solvent, benzene). The compound showed a single component with a retention time of 12.45 (relative to 5α-cholestane) upon gas-liquid chromatographic analysis on a 3% QF-1 column. The ultraviolet spectrum showed a λ_{max} at 258 nm due to the α,β-unsaturated ketone with an extinction coefficient of 15,700 (literature: 258 nm, ϵ 15,700 [36]; 258 nm, ϵ 15,650 [37]). The nuclear magnetic resonance spectrum showed no olefinic proton resonances, indicating that the double bond must be tetrasubstituted. The characteristic aromatic resonances at 7.48 and 8.06 ppm are attributable to the protons of the benzoate moiety. The

3α-proton absorption was present at 5.00 ppm as a complex multiplet. The doublet at 4.11 ppm, which integrates for a single proton, is due to a highly deshielded 7β-proton. This deshielding can be attributed to the allylic nature of the 7β-proton plus its close proximity to the 15-ketone function. The coupling constant of 14 cycles/sec displayed by this doublet is characteristic of geminal spin-spin coupling of the chemically identical but magnetically distinguishable protons at carbon atom 7. In the case of saturated steroids, the effect of a 15-ketone function on the C-7-H resonances has been noted previously [38]. In the case of 5α,14α-androstan-15-one (*trans*-C-D ring juncture) deshielding of the C-7β-H by the 15-ketone was observed while deshielding of the C-7α-H by the 15-ketone function was observed in the case of 5α,14β-androstan-15-one (*cis*-C-D ring juncture). The $\Delta^{8(14)}$-15-keto-3β-yl-benzoate showed methyl resonances at 0.78, 0.84, 0.90 and 1.00 ppm. The infrared spectrum was compatible with the assigned structure and did not show an absorption in the region of 1665–1685 cm^{-1} which would be expected in the case of a 6-membered ring ketone with α,β-unsaturation. The latter feature is important in distinguishing a $\Delta^{8(14)}$-15-one from a $\Delta^{8(14)}$-7-one. The mass spectrum showed a prominent molecular ion at *m/e* 504. The precise mass was found to be 504.3607 (calcd. for $C_{34}H_{48}O_3$: 504.3606). Prominent ions in the high mass region of the spectrum were as follows: 504 (61%; M), 489 (5%; M-CH_3), 486 (5%; M-H_2O), 391 (5%; M-side chain), 382 (5%; M-benzoic acid), 373 (8%; M-H_2O-side chain), 367 (92%; M-CH_3-benzoic acid), 364 (10%; M-H_2O-benzoic acid), 360 (3%), 351 (18%), 342 (2%), 335 (3%), 287 (5%), 276 (4%), 272 (6%), 269 (19%; M-side chain-benzoic acid), 253 (20%), and 251 (27%; M-side chain-benzoic acid-H_2O). The base peak was at *m/e* 105. Metastable ions were observed at *m/e* 474.5 (calculated for the transition *m/e* 504–489: 474.5), 468.6 (calculated for the transition *m/e* 504–486: 468.6), 275.5 (calculated for the transition *m/e* 489–367: 275.4), and 346.9 (calculated for the transition *m/e* 382–364: 346.9).

2. 3β-Benzoyloxy-14α-methyl-5α-cholest-7-en-15-one

3β-Benzoyloxy-14α-methyl-5α-cholest-7-en-15-one was prepared by the following modification of the method of Knight et al. [14]. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one (4.50 g) was added to a stirred suspension of freshly sublimed potassium *t*-butoxide (18 g) in freshly distilled *t*-butyl alcohol (200 ml). After 5 min, methyl iodide (50 ml; redistilled) was added and the resulting mixture was stirred at room temperature for 3 h. Water (100 ml) was added and the mixture was extracted twice with ether (200 ml portions). The combined ether solutions were washed successively with water (100 ml) and a saturated solution of sodium chloride, dried over anhydrous sodium sulfate, was evaporated to dryness under reduced pressure. The resulting yellowish residue was dissolved in benzene (4 ml) and applied to a silica gel column (25 × 2.5 cm). Using benzene as the eluting solvent, fractions 10 ml in volume (20 min/fraction) were collected. The contents of fractions 11 through 16 were pooled and, after evaporation of the solvent, recrystallized from ethyl acetate, yielding 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one in the form of long, colorless

crystals (1.10 g) melting at 146–147°C (literature: 146–148°C [14]; 145–147°C [36]). The product showed a single component on thin-layer chromatographic analysis on silica gel HF_{254} plates (solvent, benzene) and on silica gel G plates (solvent, ethyl acetate–benzene, 1 : 3). The compound showed a single component on gas-liquid chromatographic analysis on a 3% QF-1 column (retention time relative to 5α-cholestane: 11.45). The ultraviolet spectrum showed no specific absorption at 258 nm, indicating the absence of the α,β-unsaturated ketone system of the starting material. The infrared spectrum was compatible with the assigned structure. The nuclear magnetic resonance spectrum showed a highly deshielded proton absorption at 6.54 ppm due to the olefinic proton at carbon atom 7. This absorption showed complex spin-spin coupling which could not be resolved on the 220 MHz spectrometer. The chemical shift of this proton is the result of the anisotropy imposed by the spatial proximity of the 15-ketone function. The characteristic aromatic proton absorptions of the benzoate ester occurred at 7.48 and 8.06 ppm. Integration of the methyl proton absorption region accounted for all six methyl groups. The mass spectrum showed a prominent molecular ion at *m/e* 518. High resolution mass spectral analysis indicated a precise mass of 518.3764 (calcd. for $C_{35}H_{50}O_3$: 518.3762). Prominent ions in the high mass region of the spectrum were as follows: 518 (20%; M), 503 (3%; M-CH_3), 500 (22%; M-H_2O), 485 (5%; M-H_2O-CH_3), 475 (3%), 460 (4%), 415 (5%), 405 (11%; M-side chain), 396 (10%; M-benzoic acid), 387 (15%; M-side chain-H_2O), 381 (16%; M-benzoic acid-CH_3), 378 (7%; M-benzoic acid-H_2O), and 363 (26%; M-benzoic acid-H_2O-CH_3).

3. 14α-Methyl-5α-cholest-7 en-3β,15-diols

3β-Benzoyloxy-14α-methyl-5α-cholest-7-en-15-one (100 mg) was dissolved in ether (50 ml) and added to a suspension of lithium aluminum hydride (200 mg) in ether (10 ml). After standing overnight at room temperature, the unreacted reagent was decomposed by the addition of ethyl acetate. Water (50 ml) was added and the resulting mixture was extracted 3 times with ether and the combined ether solutions were dried and evaporated to dryness under reduced pressure. Analysis of the crude reaction mixture by thin-layer chromatography (silica gel H plates; solvent, benzene–ethyl acetate, 2 : 1) showed three components: a trace of the unreacted starting material and two more polar components with R_f values of 0.58 and 0.41 which were designated as diol A and diol B, respectively. The oily residue was applied (in benzene) to an activated silicic acid column (50 × 1 cm) which had been packed as a slurry in benzene. Using a mixture of benzene and ether (9 : 1) as the eluting solvent, fractions 16 ml in volume (15 min/fraction) were collected. Aliquots were taken from each tube for analysis by thin-layer chromatography. Diol A (26 mg), eluted in fractions 9–13, was recrystallized from ethyl acetate yielding white cubes (18 mg) melting at 159–160°C. Diol B (46 mg), eluted in fractions 18–30, was recrystallized from ethyl acetate in the form of long white needles (26 mg) melting at 192–193°C.

This scale of this preparation of the diols could be increased so as to permit detailed studies of the compounds in question.

As indicated below the structure of diol A was established as 14α-methyl-5α-cholest-7-en-3β,15β-diol by x-ray structural analysis of the 3β-*p*-bromobenzoate derivative. The structure of diol B, the 15-hydroxy epimer of diol A, is therefore 14α-methyl-5α-cholest-7-en-3β,15α-diol.

4. 14α-Methyl-5α-cholest-7-en-3β,15β-diol

14α-Methyl-5α-cholest-7-en-3β,15β-diol (diol A) migrated as a single component (R_f 0.58) on thin-layer chromatographic analysis on a silica gel G plate (solvent, benzene–ethyl acetate, 2 : 1). On gas-liquid chromatographic analysis on a column (8 ft. × 4 mm; 220°C) of 3% QF-1 on Gas Chrom Q, the compound had a retention time (relative to 5α-cholestane) of 8.89 and showed a purity in excess of 96%. The mass spectrum showed a prominent molecular ion at *m/e* 416. High resolution mass spectral analysis indicated a precise mass of 416.3688 (calcd. for $C_{28}H_{48}O_2$: 416.3657). Prominent ions in the high mass region of the spectrum were as follows: 416 (40%; M), 401 (23%; M-CH_3), 398 (59%; M-H_2O), 383 (77%; M-H_2O-CH_3), 365 (15%; M-H_2O-H_2O-CH_3), 357 (6%), 355 (7%), 329 (8%), 313 (30%) and 285 (85%; M-side chain-H_2O). The base peak was at *m/e* 232. The infrared spectrum confirmed the absence of absorbancies due to a carbonyl function or an aromatic moiety. The infrared spectrum of 14α-methyl-5α-cholest-7-en-3β,15β-diol differed significantly from that of 14α-methyl-5α-cholest-7-en-3β,15α-ol in the fingerprint region. It is of interest to note that the C-O stretching absorption occurs at 1035 cm^{-1} in the former compound and at 1045 cm^{-1} in the latter compound. The occurrence of the C-O stretching absorption at the lower frequency for 14α-methyl-5α-cholest-7-en-3β,15β-ol (with the pseudoaxial 15-hydroxyl function) corresponds to the frequently observed shift to lower frequency for hydroxyl functions in the axial configuration [39]. The nuclear magnetic resonance spectrum is shown in fig. 1. The 3α-proton shows the expected resonance at 3.60 ppm. Of particular interest is the doublet at ~4.12 ppm due to the 15α-proton. This doublet could be made to collapse to a singlet by irradiating at 7.56 τ with an external radiofrequency oscillator. The integrated area of this doublet corresponds to a single proton. Measurement from Dreiding models of the dihedral angles between the carbinol proton at carbon atom 15 for 14α-methyl-5α-cholest-7-en-3β,15β-diol and the protons at carbon atom 16 show that an angle of approximately 90° is formed by the 16β-proton and the 15α-proton. An angle of approximately 30° exists between the 16α-proton and the 15α-proton. A dihedral angle of 90° should give a coupling constant (J) of approximately 0 while an angle of 30° should result in a J value of approximately 6.2 cycles/sec [40]. This is in close agreement with the observed value of 6.5 cycles/sec for J. This finding is in contrast to that with 14α-methyl-5α-cholest-7-en-3β,15α-diol (see below). The resonance due to the proton at carbon atom 7 in 14α-methyl-5α-cholest-7-en-3β,15β-diol occurs at 5.62 ppm and is shifted considerably more downfield from that of the proton at carbon atom 7 in 14α-methyl-cholest-7-en-3β-ol (C-7-H at 5.26 ppm; Chan et al., submitted for publication). Moreover, the position of the olefinic proton at carbon atom 7 is shifted considerably downfield (5.62 ppm) relative to that (5.49 ppm) in 14α-methyl-5α-cholest-

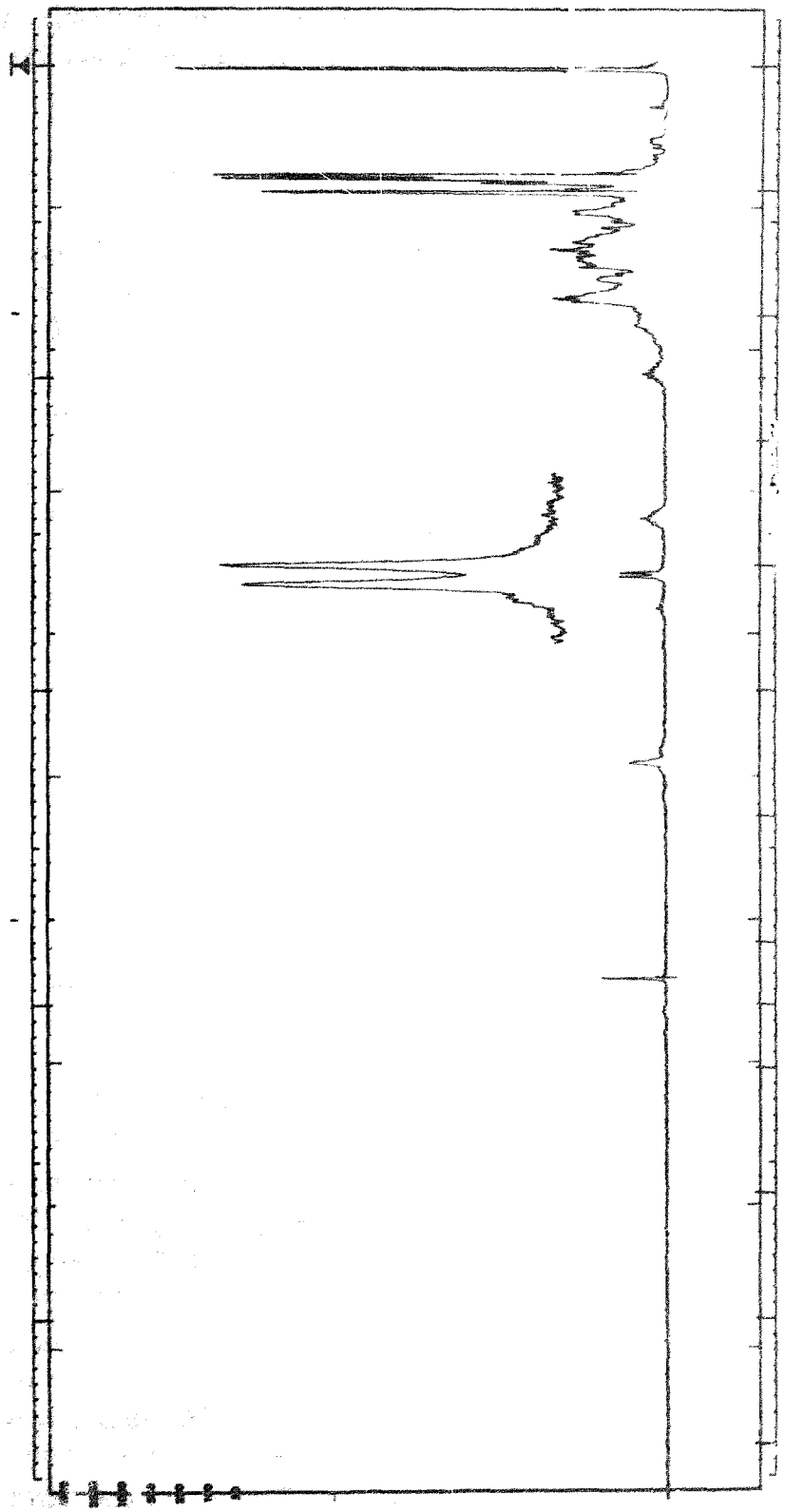

Fig. 1. 220 MHz nuclear magnetic resonance spectrum of 14α-methyl-5α-cholest-7-en-3β,15β-diol. (Resonances are plotted as ppm (δ) downfield from the TMS standard).

7-en-3β,15α-diol (see below), a situation that results from the closer proximity of the 15β-hydroxyl function to Δ^7-double bond than the 15α-hydroxyl function to the Δ^7-double bond. The proton in the 15α-position in 14α-methyl-5α-cholest-7-en-3β,15β-diol resonates at higher field (~4.12 ppm) than the 15β-proton in 14α-methyl-5α-cholest-7-en-3β,15α-diol (~4.33 ppm; see below), a finding compatible with the closer proximity of the 15β-carbinol proton to the π electrons of the Δ^7-double bond than that of the carbinol proton in the 15α-configuration.

5. 3β-p-Bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol and 3β,15β-bis-p-bromobenzoyloxy-14α-methyl-5α-cholest-7-ene

14α-Methyl-5α-cholest-7-en-3β,15β-diol (68 mg) and *p*-bromobenzoyl chloride (391 mg) in pyridine (4 ml; freshly distilled from barium oxide) were stirred at room temperature for 11 h. A saturated solution of sodium bicarbonate (50 ml) was added and the resulting mixture was extracted 3 times with petroleum ether (200 ml portions) and twice with ethyl acetate (200 ml portions). The combined organic extracts were pooled, washed successively with water (50 ml) and a saturated sodium chloride solution (50 ml), and dried over anhydrous sodium sulfate. After evaporation of the solvent under reduced pressure, product was analyzed by thin layer chromatography on silica gel G plates. When a mixture of benzene and ethyl acetate (2 : 1) was used as the developing solvent, the absence of the starting material was established. A single spot was observed at the solvent front. When benzene was used as the developing solvent, 3 components of R_f 0.9, 0.7 and 0.5 were observed. The mixture was subjected to preparative thin-layer chromatography in the same system. The components of R_f 0.9 (22.8 mg), R_f 0.7 (31.0 mg) and R_f 0.5 (48 mg) were eluted from the plates with spectral grade chloroform. The component of R_f 0.9, recovered as an oil, was not studied further.

The material of R_f 0.5, recovered as a white solid, yielded upon crystallization from ethyl acetate–methanol, 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol which melted at 193–195°C (clearing at 219–220°C). The mass spectrum showed two molecular ions at *m/e* 600 and 598. High resolution mass spectral analysis indicated precise mass values of 600.2298 and 598.2971 which correspond to $C_{35}H_{51}O_3{}^{81}Br$ and $C_{35}H_{51}O_3{}^{79}Br$, respectively. Prominent ions in the high mass region of the spectrum were as follows: 600 and 598 (38 and 40%; M), 585 and 583 (15 and 34%; M-CH_3), 582 and 580 (47 and 48%; M-H_2O), 567 and 565 (25 and 40%; in part due to M-H_2O-CH_3), 497 and 495 (15 and 15%; M-side chain), 469 and 467 (40 and 39%), 445 and 443 (15 and 20%), 398 (10%; M-bromobenzoic acid), 389 and 387 (9 and 9%), 383 (30%; M-bromobenzoic acid-CH_3), 380 (22%; M-bromobenzoic acid-H_2O), 365 (43%; M-bromobenzoic acid-H_2O-CH_3), 311 (13%), 295 (10%), 285 (10%; M-bromobenzoic acid-side chain), 267 (37%) and 213 (100%). The infrared spectrum showed major absorption bands compatible with the presence of an ester carbonyl, a hydroxyl function, and an aromatic moiety. The nuclear magnetic resonance spectrum was fully in agreement with the assigned structure. Doublets due to the aromatic moiety were present at ~7.6 and ~7.9 ppm. The resonance due to the

C-7 olefinic proton was noted at 5.58 ppm. The doublet due to the 15α-proton was present at 4.12 ppm as in the case of 14α-methyl-5α-cholest-7-en-3β,15β-diol. This doublet due to the 15α-proton collapsed to a singlet when irradiated at 7.37 τ with an external radiofrequency oscillator. The 3α-proton was shifted to 5.00 ppm due to the influence of the 3β-bromobenzoate function. The crystals of the 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol were found to be suitable for X-ray analysis (see below).

The material of R_f 0.7 from the preparative thin layer chromatography was recovered as an oil and attempts at crystallization were unsuccessful. However, the product was tentatively identified as 3β,15β-bis-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-ene on the basis of infrared, mass spectral, and nuclear magnetic resonance studies. The mass spectrum showed molecular ions at *m/e* 780, 782 and 784 (0.5, 1 and 0.5%, respectively) due to the two bromine isotopes. Prominent ions in the high mass region of the spectrum of diagnostic significance included the following: 580 and 582 (22 and 20%; M-bromobenzoic acid) and 565 and 567 (17 and 15%; M-bromobenzoic acid-CH_3). The nuclear magnetic resonance spectrum confirmed the dibromobenzoate structure by the observed downfield shifts of both the 3α-proton and the 15α-proton. The doublet (5.12 ppm) due to the 15α-proton indicated the 15β-ester function. This doublet collapsed to a singlet upon irradiation at 7.35 τ.

6. Structural analysis of 3β-p-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol

The crystals were found to be suitable for X-ray analysis. The crystal data were as follows: $C_{35}H_{51}O_3Br$, M = 599, monoclinic, a = 6.819(3), b = 9.513(5), c = 25.272(11) Å, β = 94°32(3)′, U = 1639.2 Å^3, μ = 17.5 cm^{-1} (Cu-K$_\alpha$), F (000) = 664, D_m = 1.19 g cm^{-3}, Z = 2, D_c = 1.21 g cm^{-3}, space group $P2_1$. 2101 nonzero reflections were measured on a Picker FACS-1 diffractometer using Cu-K$_\alpha$ radiation [41]. The structure was solved by the heavy atom method and was refined by full-matrix least-squares methods on positional and anisotropic thermal parameters for all but three of the non-hydrogen atoms to an R-factor of 0.091 on all observed data. Positions of 33 hydrogen atoms could be determined from a difference map and were included in the final structure factor calculations but were not refined. The hydrogen atoms in the C(20)-C(27) side chain could not be located nor could the hydroxyl hydrogen. In the crystal there was a rotational disorder around the C(24)-C(25) bond such that the two carbon atoms of the terminal gem-dimethyl group occupy three sites. These atoms were assigned isotropic temperature factors and the occupancy of the three sites was varied to give final values of 0.74 (4), 0.61 (5), and 0.66 (6). A final difference map showed no peak greater than 0.3 electrons/Å^3. Scattering curves for the non-hydrogen atoms were taken from the compilation by Cromer and Mann [42] and the hydrogen curve of Stewart et al. [43] was used. The X-ray data established the structure as 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol. Detailed crystallographic data are available [44]. A stereoscopic display of the molecule is presented in Fig. 2. Figures 3 and 4 present schematic representations of the observed bond lengths and observed bond angles, respectively. Examination of fig. 2 indicates the steric strain

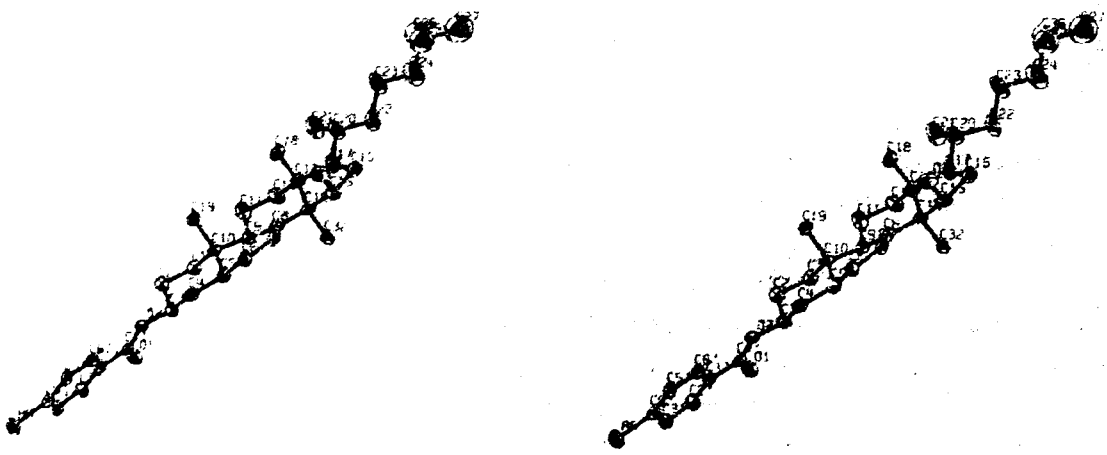

Fig. 2. Stereoscopic display of 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol. To be viewed through a stereoviewer.

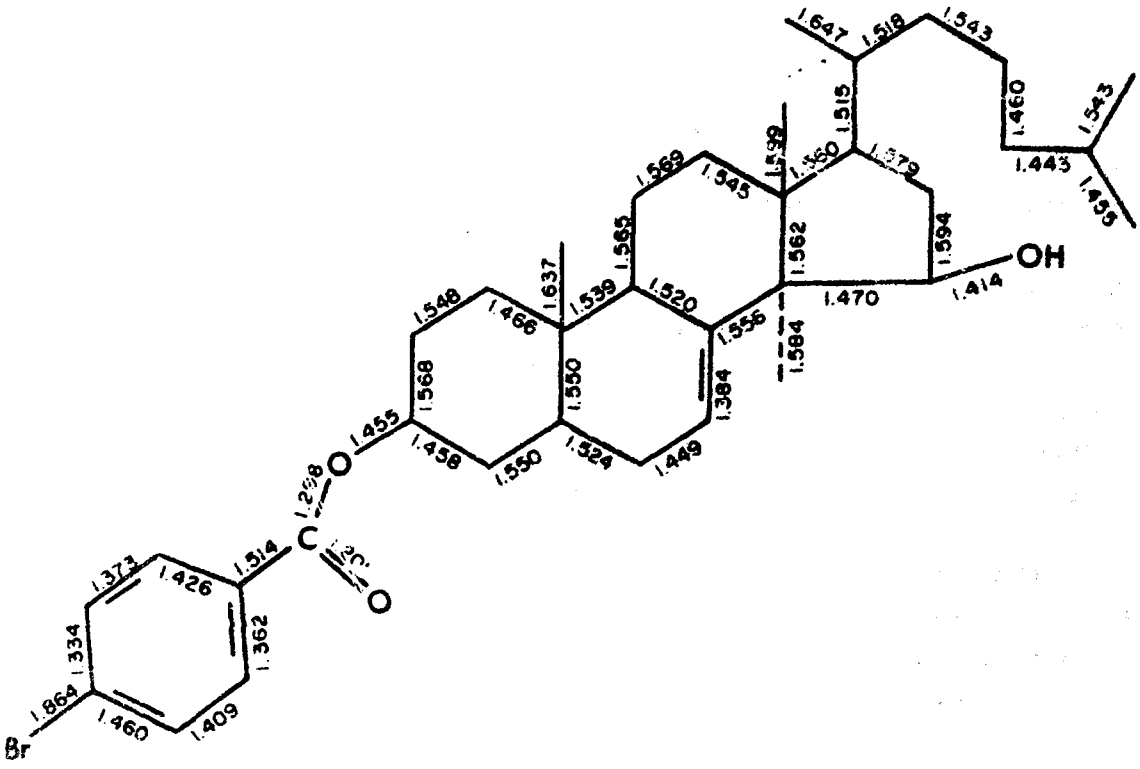

Fig. 3. Schematic representation of observed bond lengths in 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol (esd range from 0.020–0.040 Å).

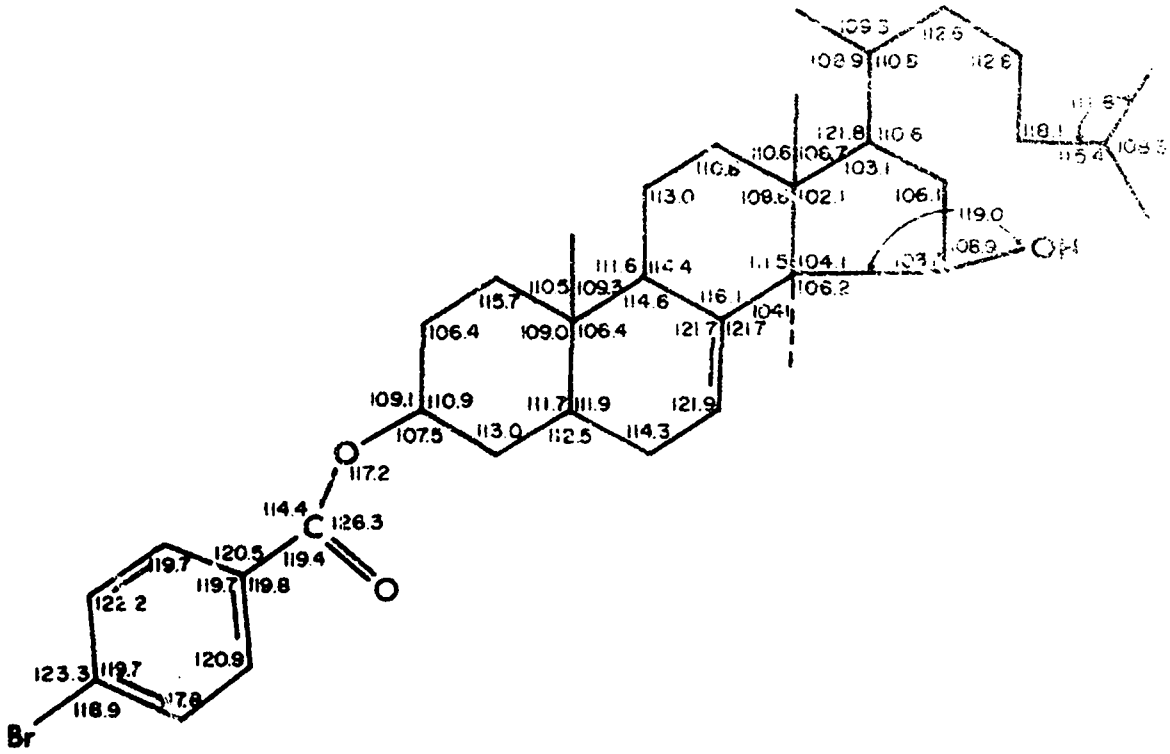

Fig. 4. Schematic representation of observed bond angles in 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol (esd range from 1.0–1.5°).

induced by the strong 1,3 interaction between the 15β-hydroxyl and the angular 18 methyl group. The determined interatomic distance between C(18) and the oxygen attached to C(15) was 2.90(3) Å, a value in agreement with the distance (2.90 Å) measured from Dreiding models. Other points of interest displayed in fig. 2 are the flattened chair conformation of ring B caused by the Δ^7-double bond and the close approach of the oxygen attached to C(15) to the Δ^7-double bond. The effect of this proximity have been observed in the nuclear magnetic resonance studies described herein.

7. 14α-Methyl-5α-cholest-7-en-3β,15α-diol

14α-Methyl-5α-cholest-7-en-3β,15α-diol (diol B) migrated as a single component (R_f 0.41) on thin-layer chromatographic analysis on a silica gel G plate (solvent, benzene-ethyl acetate, 2 : 1). On gas-liquid chromatographic analysis (under the conditions described above for the case of Diol A) the compound had a retention time (relative to 5α-cholestane) of 9.02 and showed a purity in excess of 99%. The mass spectrum showed a prominent molecular ion at *m/e* 416. High resolution mass spectral analysis indicated a precise mass of 416.3667 (calculated for $C_{28}H_{48}O_2$: 416.3657). Prominent ions in the high mass region of the spectrum were as follows: 416 (70% M) 401 (33%; M-CH_3), 398 (7%; M-H_2O), 383 (100%; M-CH_3-H_2O), 365 (23%; M-CH_3-H_2O-H_2O), and 285 (7%; M-side chain-H_2O). The infrared spectrum confirmed the

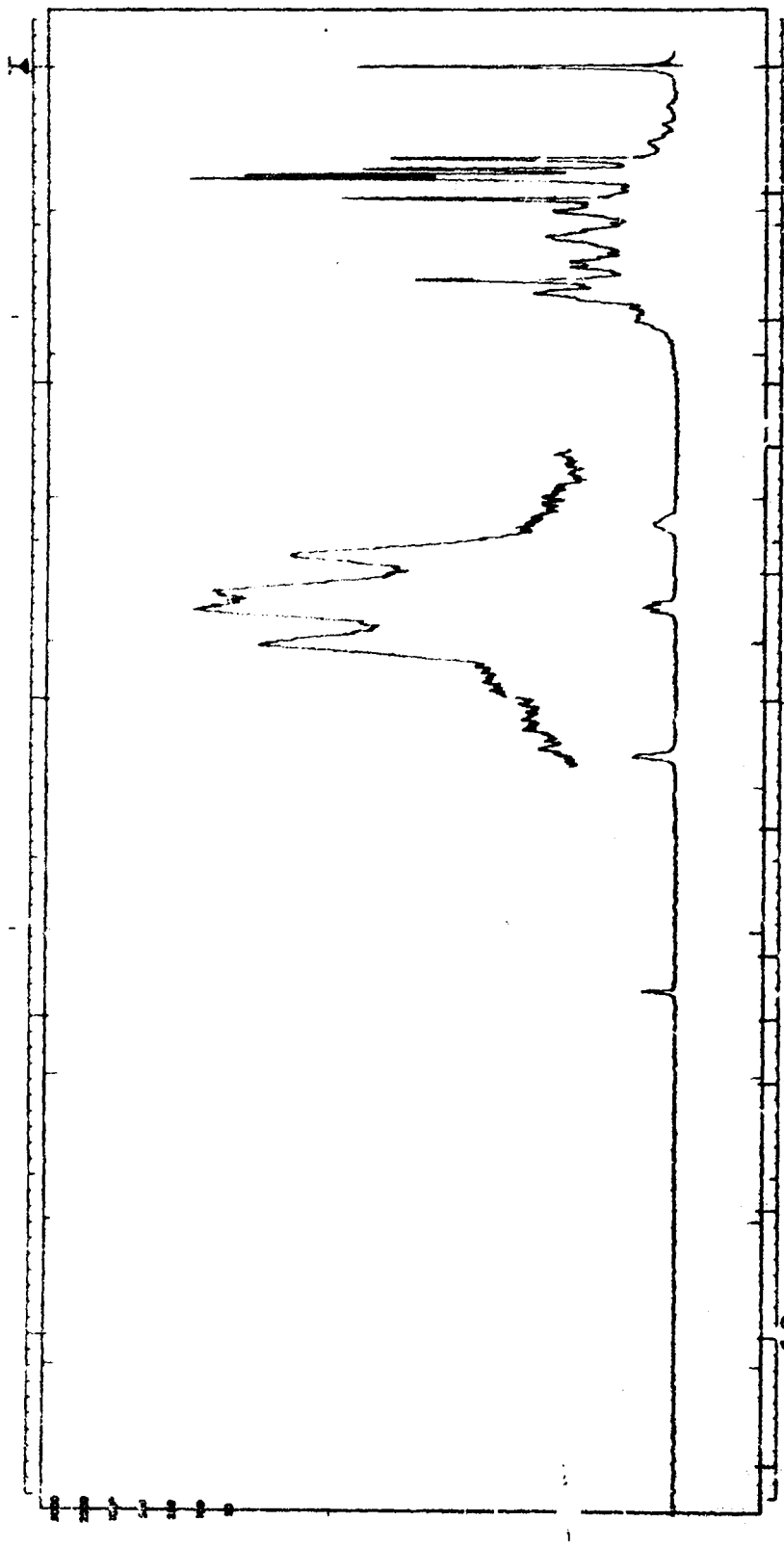

Fig. 5. 200 MHz nuclear magnetic resonance spectrum of 14α-methyl-5α-cholest-7-en-3β,15α-diol. (Resonances are plotted as ppm (δ) downfield from the TMS standard.)

absence of absorbancies due to a carbonyl function and an aromatic moiety. The nuclear magnetic resonance spectrum is shown in fig. 5. The 3α-proton shows the expected resonance at 3.62 ppm. Of particular interest is the doublet of doublets at 4.33 ppm (J = 6.5 and 8.0 cycles/sec). Measurement of the dihedral angles for the 15α-alcohol indicates an angle of approximately 30° between the 15β-proton and the 16β-proton and approximately 150° between the 15β-proton and the 16α-proton. From the Karplus equation [40] a dihedral angle of 150° should result in a coupling constant at about 8.5 cycles/sec which compares favorably with the observed value of 8.0 cps. Irradiation at 8.26 τ with an external radiofrequency oscillator caused the doublet of doublets to collapse to a singlet. The area of the doublet of doublets integrated for a single proton resonance. The resonance due to the proton at carbon atom 7 in 14α-methyl-5α-cholest-7-en-3β,15α-diol occurs at 5.49 ppm, a situation different from that in 14α-methyl-5α-cholest-7-en-3β,15β-diol and in 14α-methyl-5α-cholest-7-en-3β-ol as discussed previously.

14α-Methyl-5α-cholest-7-en-3β,15α-diol, upon treatment with benzoyl chloride in pyridine, yielded a dibenzoate which melted at 165–166°C. Elemental analysis of the dibenzoate showed: $C_{42}H_{56}O_4$ (calcd., C 80.8 H 8.97; found, C 80.4 H 8.94). The mass spectrum of the dibenzoate of diol B showed a molecular ion at *m/e* 624. Other ions in the high mass range of the spectrum compatible with the assigned structure were those at 502 (M-benzoic acid), 487 (M-benzoic acid-CH_3), 381 (M-benzoyl acid-benzoyl residue), and 366 (M-benzoic acid-benzoyl residue-CH_3).

8. [14α-C^2H_3]-3β-Benzoyloxy-14α-methyl-5α-cholest-7-en-15-one, [14α-C^2H_3]-14α-Methyl-5α-cholest-7-en-3β,15β-diol and [14α-C^2H_3]-14α-Methyl-5α-cholest-7-en-3β,15α-diol

3β-Benzoyloxy-5α-cholest-8(14)-en-15-one was methylated with deuterium-labeled methyl iodide in a similar manner to that described earlier for the preparation of the unlabeled compound. Purified [14α-C^2H_3]-3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one melted at 143–144°C. The infrared spectrum showed prominent absorption bands at 2360 (C-D), 1730 (>C=O), 1278 (C-O), 706 and 686 (aromatic) cm^{-1}. The mass spectrum showed a molecular ion at *m/e* 521. The nuclear magnetic resonance (60 MHz) spectrum showed resonances at 0.78 (s, 3H, C-18-CH_3), 0.92 (s, 3H, C-19-CH_3), 5.07 (m, 1 H, C-3-H), 6.55 (m, 1 H, C-7-H), 7.52 and 8.10 (m, 5 H, aromatic).

[14α-C^2H_3]-14α-Methyl-5α-cholest-7-en-3β,15β-diol (m.p., 158.5–159.0°C) and [14α-C^2H_3]-14α-methyl-5α-cholest-7-en-3β,15α-diol (m.p., 191–192°C) were prepared by lithium aluminum hydride reduction of [14α-C^2H_3]-3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one as in the case of the unlabeled material. The infrared spectrum of the 15β-hydroxy compound showed prominent absorption bands at 3400 (O-H), 2241 and 2230 (C-D), and 1050 (C-O) cm^{-1}. The mass spectrum showed a molecular ion at *m/e* 419. The nuclear magnetic resonance spectrum (60 MHz) showed resonances at 0.82 (s, 3H, C-18-CH_3), 0.99 (s, 3 H, C-19-CH_3), 3.62 (m, 1 H, C-3-H), 4.06 (m, 1 H, C-15-H), and 5.58 (m, 1 H, C-7-H). The infrared spectrum of the 15α-hydroxy com-

pound showed prominent absorption bands at 3400 cm^{-1}, 2240 and 2211 (C-D) and 1090 (O-H) cm^{-1}. The mass spectrum showed a prominent molecular ion at *m/e* 419. The nuclear magnetic resonance spectrum (60 MHz) showed resonances at 0.72 (s, 3 H, C-18-CH_3), 0.92 (s, 3 H C-19-CH_3), 3.58 (m, 1 H C-3-H), 4.24 (m, 1 H, C-15-H), and 5.45 (m, 1 H, C-7-H).

9. Lanthanide shift nuclear magnetic resonance studies

Further evidence compatible with the assigned structures for the two epimeric (at carbon atom 15) diols was derived from the results of lanthanide shift nuclear magnetic resonance studies. This approach essentially involves coordination of the lanthanide complex ($Eu(dpm)_3$) to the lone electron pair of a hydroxyl oxygen present in the sterol molecule. The presence of the strong local paramagnetic field generally results in a downfield shift in the resonance of protons in the sterol that are in the vicinity of the coordination site. A stronger local field is experienced by protons closer to the coordination site resulting in a larger downfield shift. The data obtained upon successive additions of the shift reagent can be plotted as a function of the shift reagent to sterol molar ratio. A greater slope of the shift data is observed for protons which are closer to the coordination site. A simple graphical analysis of the shift data can therefore often yield important information concerning the relative proton-coordination site distances. In the present investigation both the C-18 angular methyl group and the C-32 methyl group represent convenient reference points since the relative lanthanide induced shifts of these two methyls should be a function of the stereochemistry of the 15-hydroxyl group. The C-32 methyl resonance was easily identified through studies employing the two epimeric diols labeled specifically in the C-32 methyl group with deuterium.

As indicated in table 1 the estimated C-32 methyl-15-oxygen distance is larger than the C-18 methyl-15-oxygen distance when the 15-hydroxyl has the β-configuration. In the case of the 15α-hydroxy epimer the opposite relations obtain, i.e., the 15-oxygen is further from the C-18 methyl group and closer to the C-32 methyl group. This

Table 1

Estimated interatomic distances (R) from 15-oxygen of 14α-methyl-5α-cholest-7-en-3β,15α-diol and 14α-methyl-5α-cholest-7-en-3β,15β-diol to protons on selected carbon atoms (measured from Dreiding models).

	Distance (A)	
Proton	15α-OH	15β-OH
C-15	2.0	2.0
C-18	4.4	2.9[a]
C-32	2.8	3.7
C-7	3.0	2.0

[a] The actual interatomic distance in the crystal was 2.90 A.

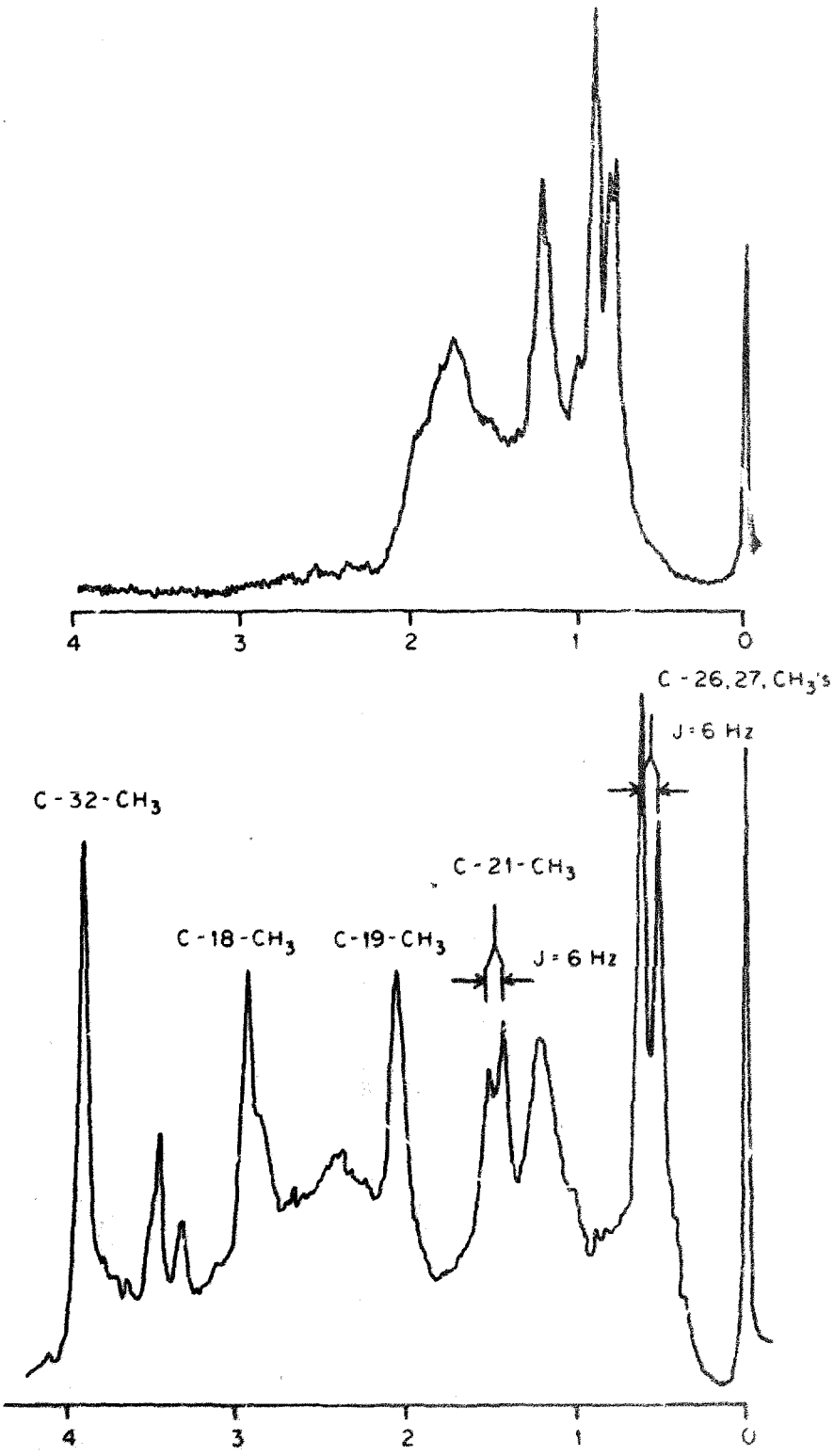

Fig. 6. 60 MHz nuclear magnetic resonance spectra of 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one before (a) and after (b) the addition of shift reagent (molar ratio $Eu(dpm)_3$ to steroid, 1.06). (Resonances are plotted as ppm (δ) downfield from the TMS standard).

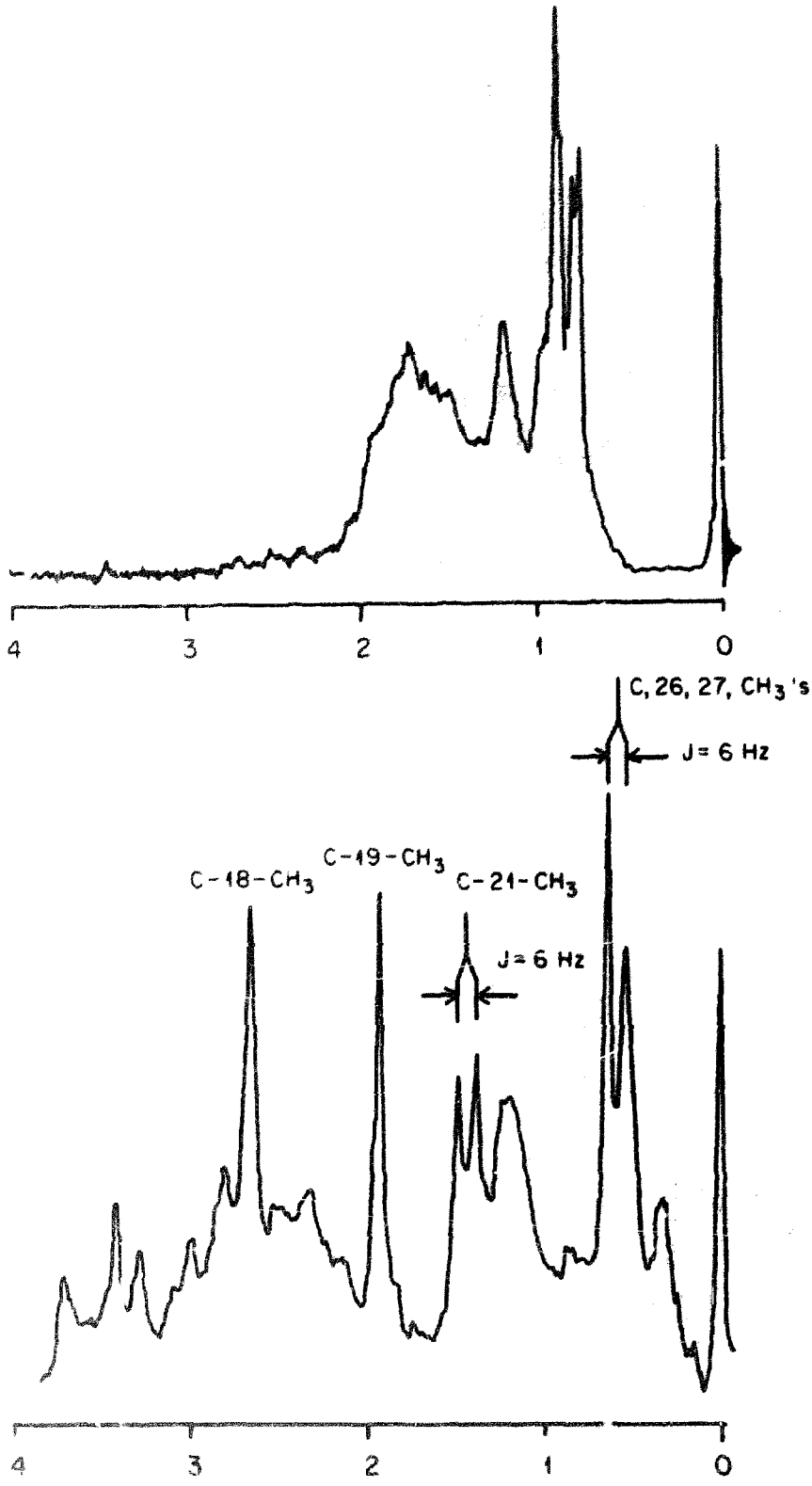

Fig. 7. 60 MHz nuclear magnetic resonance spectra of [14α-C^2H_3]-3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one before (a) and after (b) the addition of shift reagent (molar ratio $Eu(dpm)_3$ to steroid, 1.10). (Resonances are plotted as ppm (δ) downfield from the TMS standard.)

analysis assumes that a similar relative relationship exists for the europium-methyl group distances. Since the position of the europium in space could not be determined the oxygen-methyl distances were used as an approximation. From such an analysis one would expect the C-18 methyl protons to experience a larger shift and the C-32 methyl protons to undergo a smaller shift in that diol in which the 15-hydroxyl group has the β-configuration. A smaller lanthanide induced shift of the C-18 methyl protons and a larger shift of the C-32 methyl protons would be expected for the epimer with the 15α-hydroxy group. Peak assignments were based upon examination of both the resonance pattern and integration data for the unlabeled and the deuterium-labeled sterols.

The 60 MHz spectra of 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one before and after the addition of $Eu(dpm)_3$ are shown in fig. 6. The coupling constants (J) of the secondary C-21 and C-26, C-27 methyl groups could readily be measured in the shifted spectrum. The downfield three proton singlet at 1.48 ppm in the unshifted spectrum is found at 3.86 ppm in the shifted spectrum. That this resonance represents

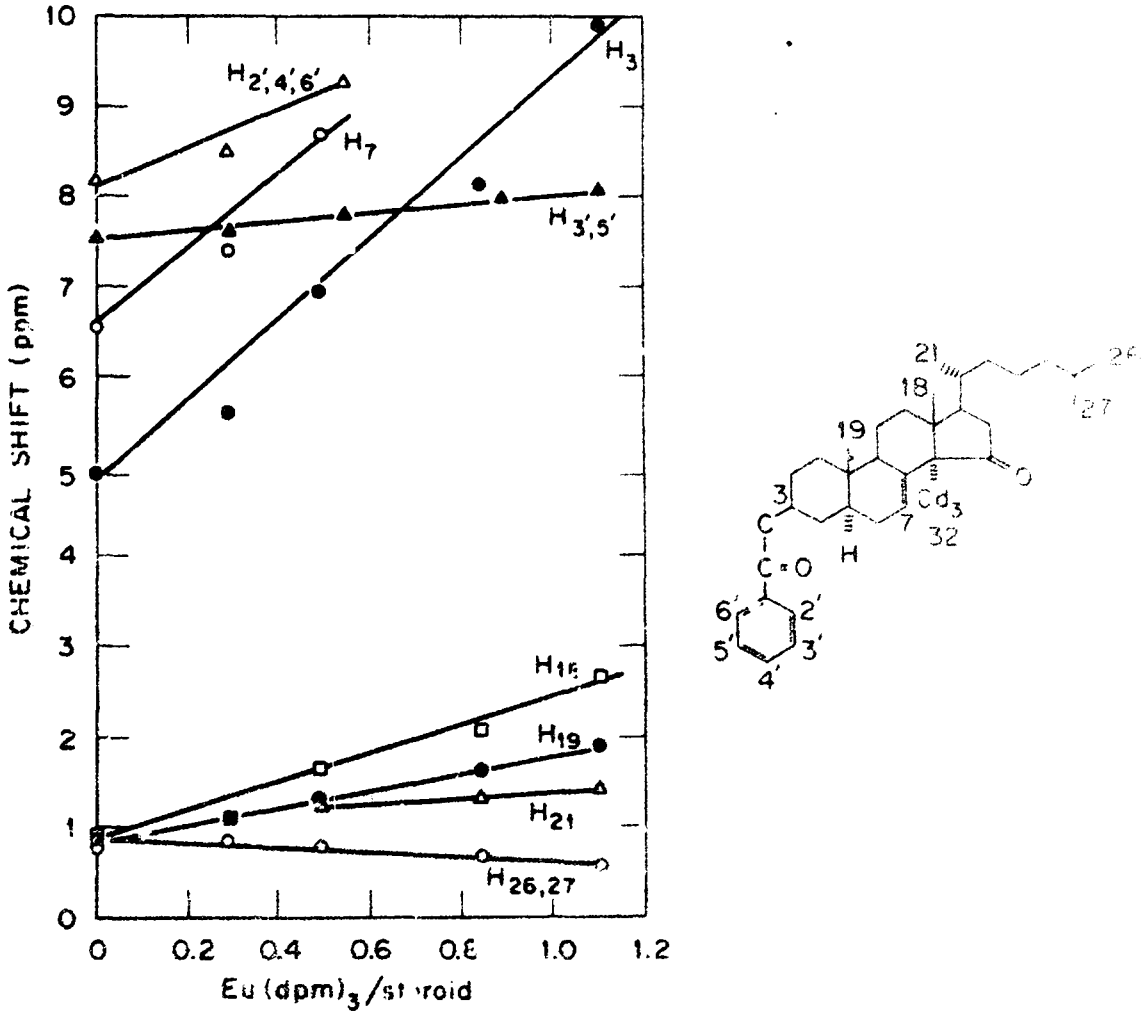

Fig. 8. Plot of chemical shifts of selected protons of [14α-C^2H_3]-3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one as a function of molar ratio of $Eu(dpm)_3$ to steroid.

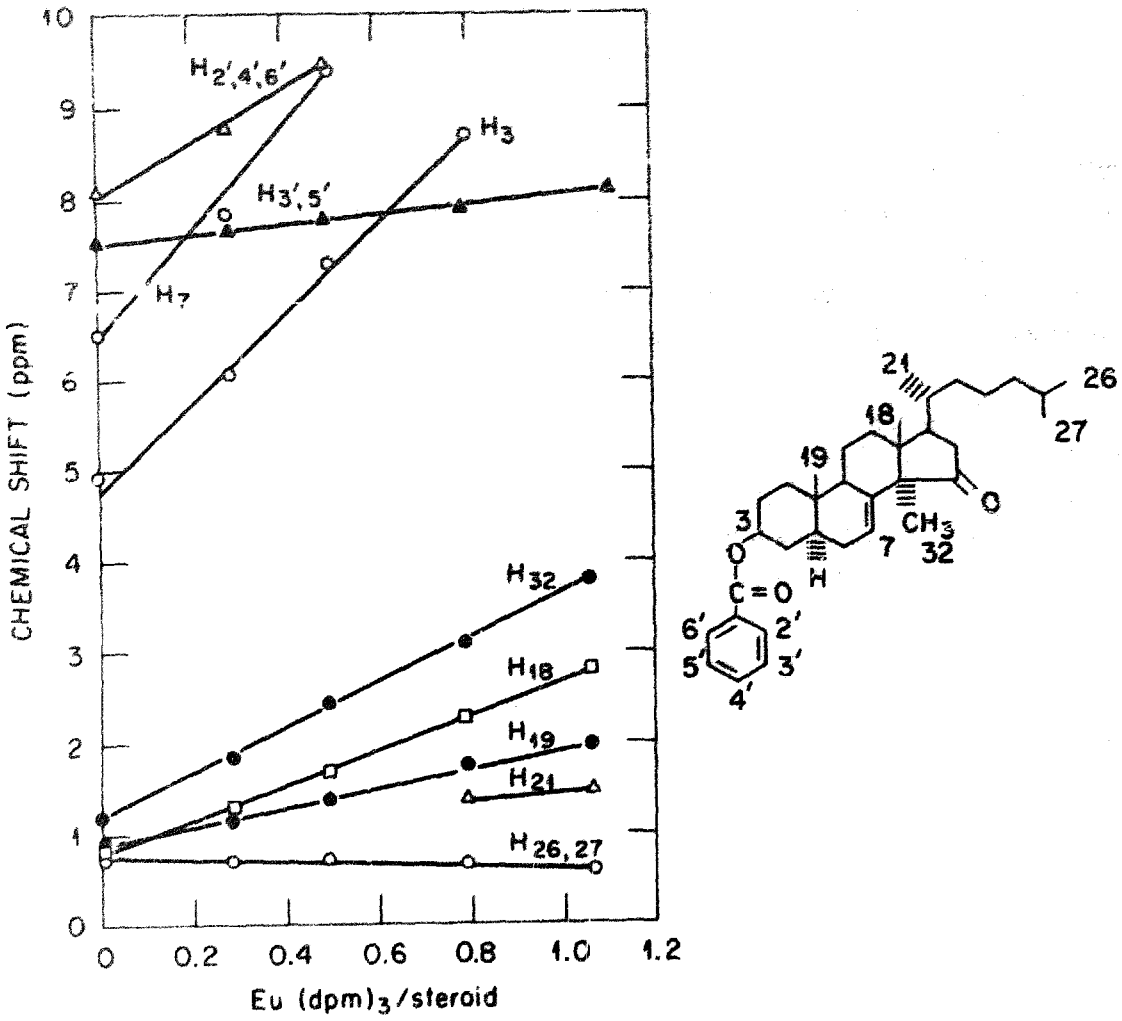

Fig. 9. Plot of chemical shifts of selected protons of 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one as a function of molar ratio of Eu(dpm)$_3$ to steroid.

the C-32 methyl group was indicated by examination of the unshifted and shifted spectra of the deuterium labeled (C-32) analogue (fig. 7). Graphical representations of the shifts of selected protons in the spectra of the labeled and unlabeled steryl ester as a function of the shift reagent/steryl ester molar ratio are presented in figs. 8 and 9, respectively. The consistency observed between the two sets of data demonstrated the reproducibility and reliability of the shift reagent technique in the study of these compounds.

The 60 MHz spectra of 14α-methyl-5α-cholest-7-en-3β,15β-diol and [14α-C^{2}H$_3$]-14α-methyl-5α-cholest-7-en-3β,15β-diol (before and after addition of Eu(dpm)$_3$) were similarly recorded. Plots of the chemical shift data of selected protons in the spectra of unlabeled and deuterium-labeled 14α-methyl-5α-cholest-7-en-3β,15β-diol are shown in fig. 10. From an inspection of these data it is clear that the downfield three proton singlet at 0.99 ppm in the spectrum of the unlabeled diol is the C-32 methyl group.

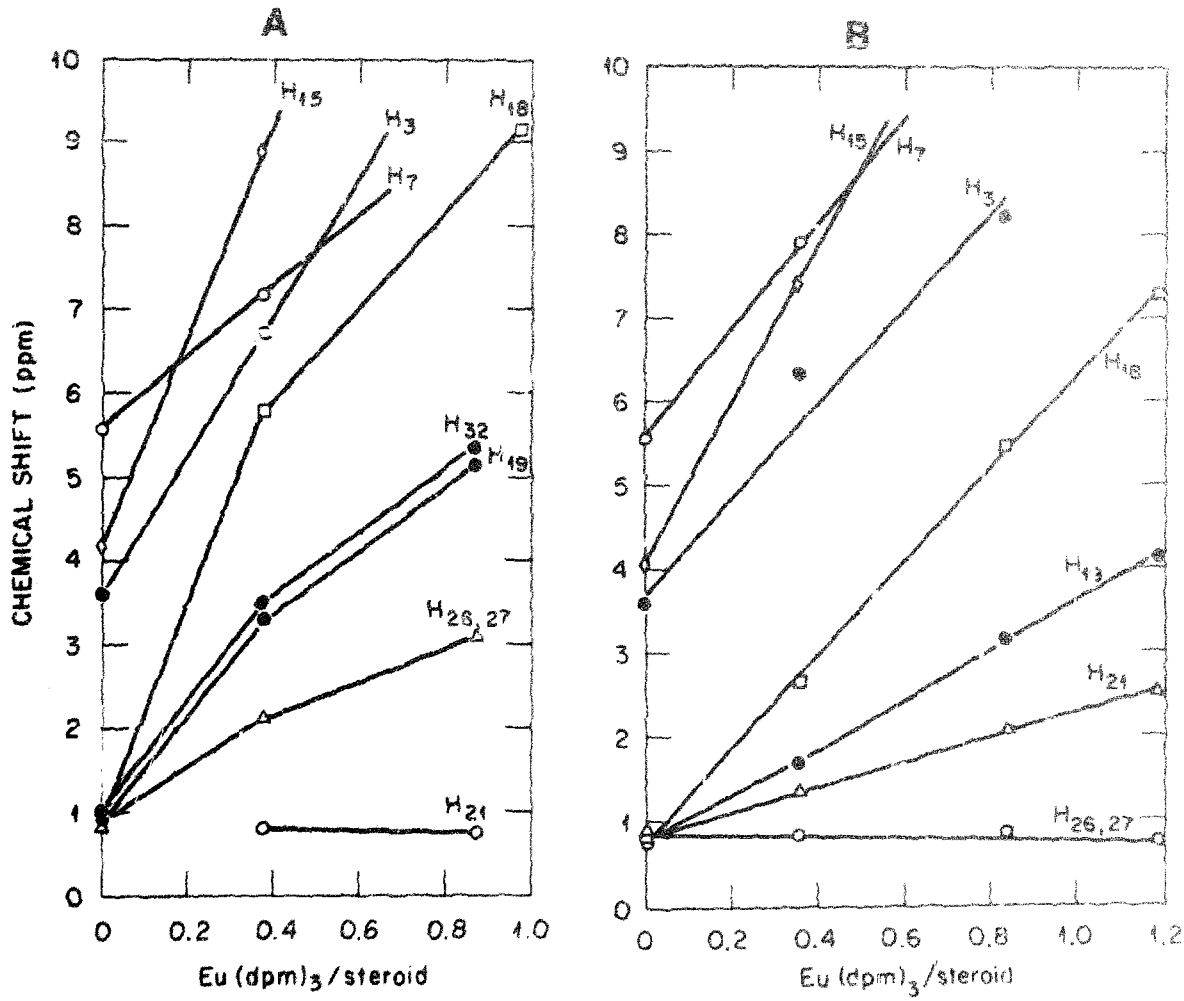

Fig. 10. Plots of chemical shifts of selected protons of 14α-methyl-5α-cholest-7-en-3β,15β-diol (A) and of [14α-C^2H_3]-14α-methyl-5α-cholest-7-en-3β,15β-diol (B) as a function of molar ratio of Eu(dpm)$_3$ to steroid.

The three proton singlet that experienced the greatest induced shift appears to be the C-18 methyl group (Fig. 10).

The 60 MHz spectra of 14α-methyl-5α-cholest-7-en-3β,15α-diol and [14α-C^2H_3]-14α-methyl-5α-cholest-7-en-3β,15α-diol (before and after the addition of Eu(dpm)$_3$) were also recorded and plots of the shift data for the unlabeled and deuterium-labeled 14α-methyl-5α-cholest-7-en-3β,15α-diol are shown in fig. 11. Comparison of the spectra of the unlabeled diol and the labeled diol indicated that the downfield three proton singlet at 1.14 ppm in the spectrum of the unlabeled diol represented the C-32 methyl group.

If the distances between the 15-hydroxyl oxygen and the two methyl groups under consideration are indeed a valid approximation of the *relative* europium-methyl distances, the C-32 methyl group should experience a greater shift in the case of the 15α-hydroxy compound. A comparison of the relative shifts of the C-32 methyl group protons in the two 15-hydroxy epimers as a function of the shift reagent/steroid molar

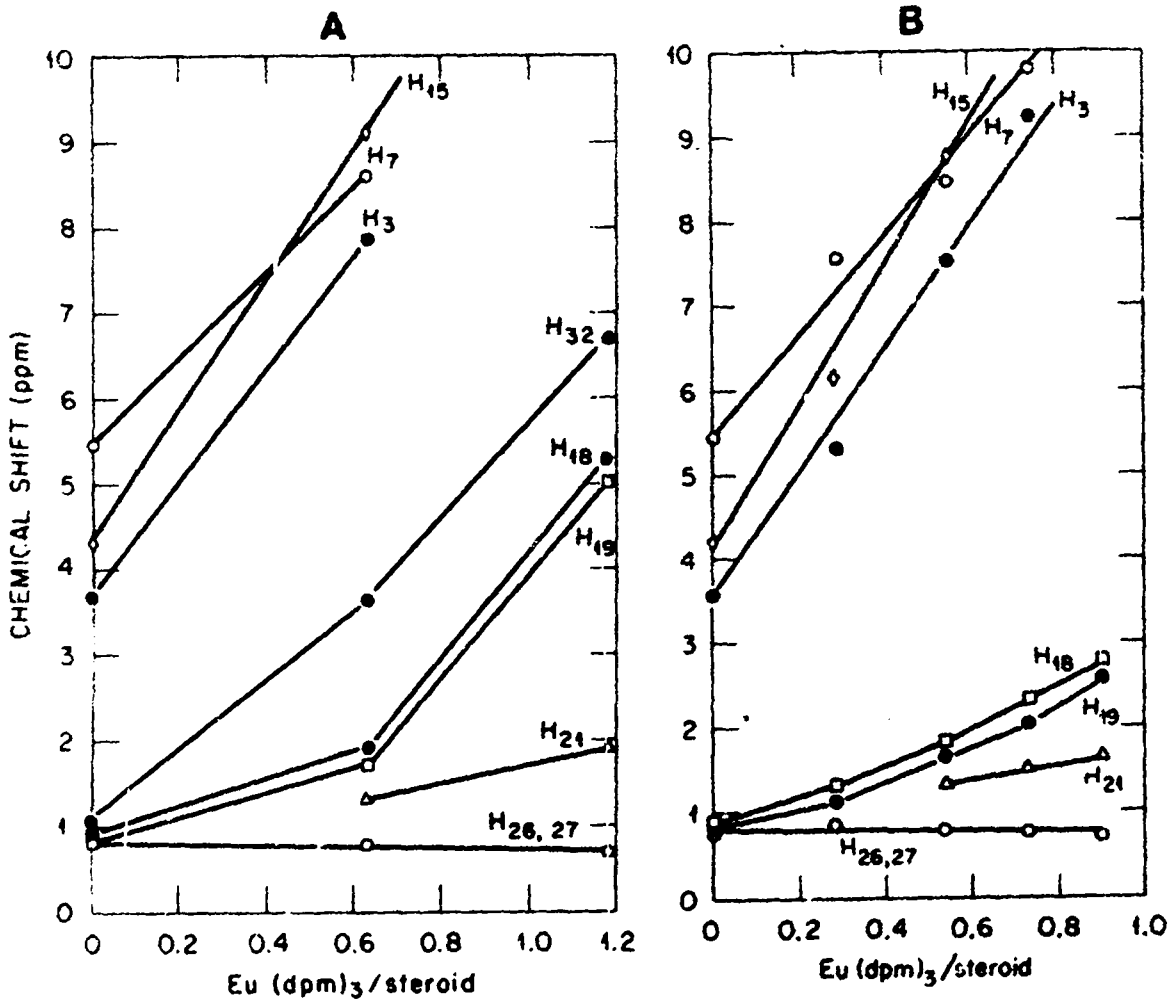

Fig. 11. Plots of chemical shifts of selected protons of 14α-methyl-5α-cholest-7-en-3β,15α-diol (A) and of [14α-C^2H_3]-14α-methyl-5α-cholest-7-en-3β,15α-diol (B) as a function of molar ratio of Eu(dpm)$_3$ to steroid.

ratio is shown in fig. 12. The relative shifts of the C-32 methyl resonance are similar for the two diols, a finding strengthened by the agreement of the shift data obtained on the labeled and unlabeled diols and indicating that the europium-C-32 methyl group distance is similar in the case of the 15α-hydroxy and 15β-hydroxy compounds.

While the experiments noted above indicate that the shifts of the C-32 methyl group resonance do not provide a useful method to gain information regarding the absolute configuration of the 15-hydroxyl function in the compounds under consideration, a similar analysis of the shifts of the C-18 methyl proton resonance proved to be of significant value. The relative shift of the C-18 methyl proton resonances as a function of the shift reagent-steroid molar ratio is shown in fig. 13 for 14α-methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol. The greater slope of the plot of the shift data for the 15β-hydroxy compound is compatible with the closer proximity of the 18-methyl to the europium complexed to the 15β-hydroxyl oxygen. A similar observation was made in the case of the spectral data on [14α-C^2H_3]-

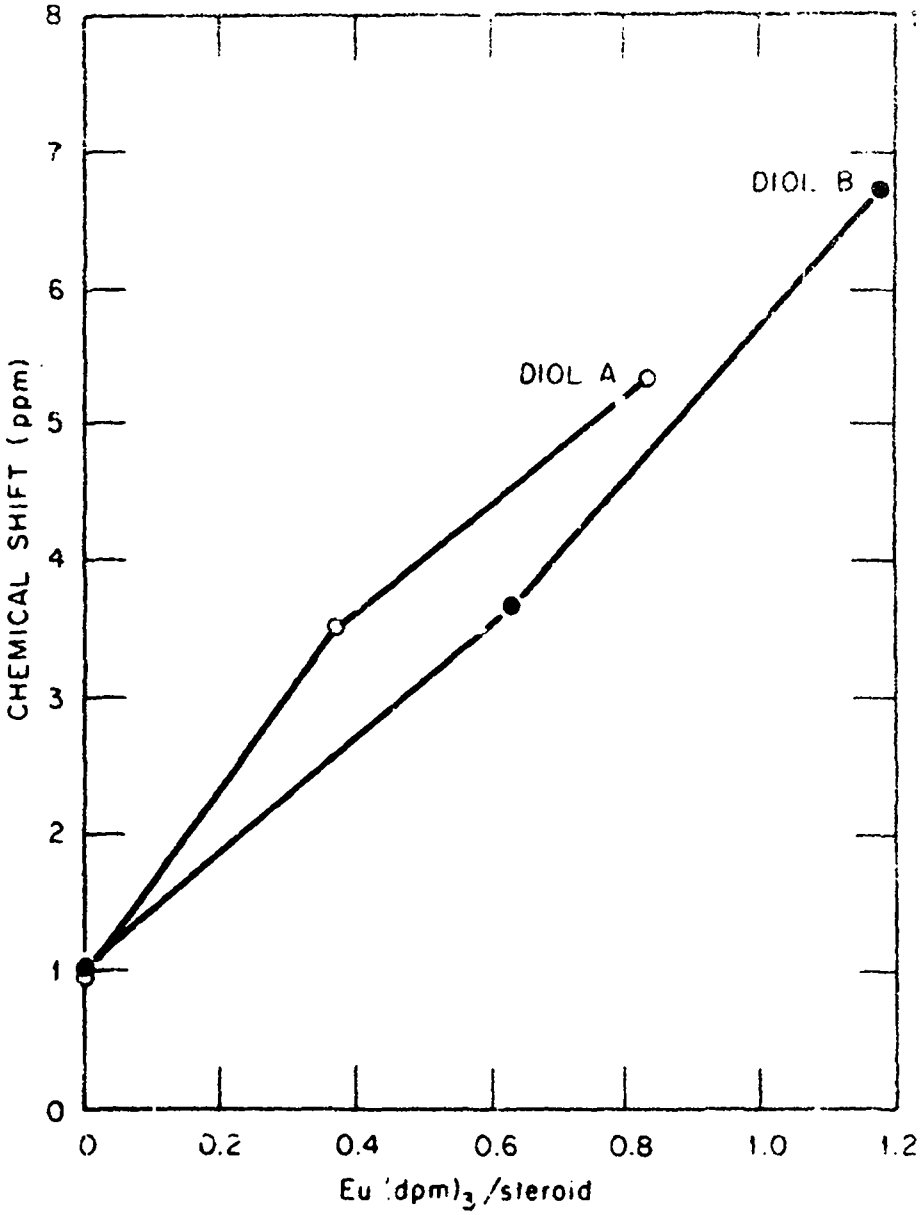

Fig. 12. Plot of chemical shifts of resonances of protons of 14α-methyl methyl group as a function of molar ratio of Eu(dpm)$_3$ to steroid for 14α-methyl-5α-cholest-7-en-3β,15β-diol (diol A) and 14α-methyl-5α-cholest-7-en-3β,15α-diol (diol B).

14α-methyl-5α-cholest-7-en-3β,15β-diol (fig. 14). The excellent agreement between the data illustrated in figs. 13 and 14 indicates the reliability of the shift measurements.

10.[16-^{3}H]-14α-Methyl-5α-cholest-7-en-3β,15β-diol and [16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15α-diol

3β-Benzoyloxy-14α-methyl-5α-cholest-7-en-15-one (50 mg), sodium methoxide (50 mg), tritiated water (10 μl; 1.52 × 10^{10} dpm) and dry dioxane (4 ml) were heated in a sealed vial at 100°C for 6 hr. The reaction mixture was cooled to room temperature, poured into water (50 ml) and extracted 3 times with 30 ml portions of ether. The

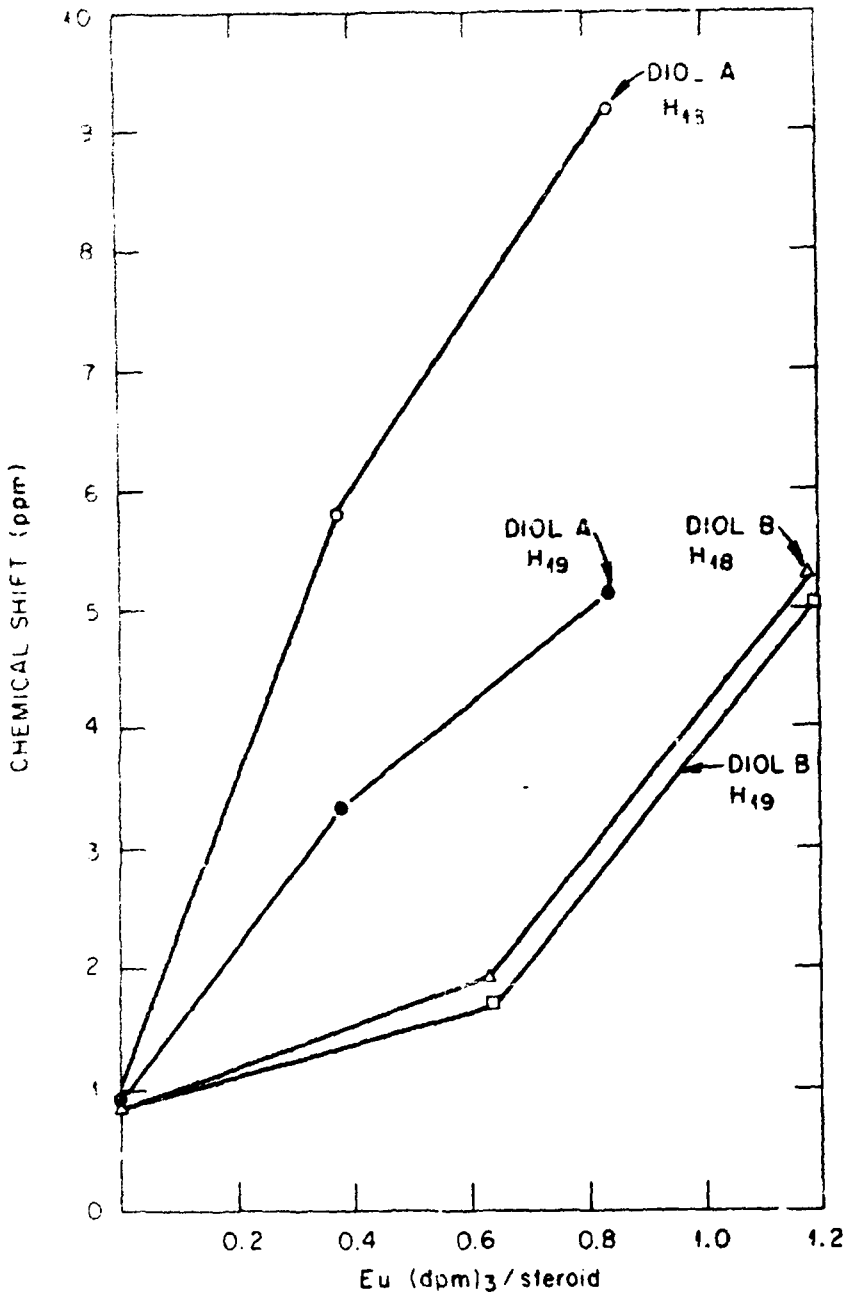

Fig. 13. Plot of chemical shifts of resonances of protons of C-18 and C-19 methyl groups as a function of molar ratio of Eu(dpm)$_3$ to steroid for 14α-methyl-5α-cholest-7-en-3β,15β-diol (diol A) and 14α-methyl-5α-cholest-7-en-3β,15α-diol (diol B).

combined extracts were washed twice with water (50 ml portions) and dried over anhydrous sodium sulfate. The residue (2.5 × 10^8 dpm) obtained upon evaporation of the solvent was dissolved in ether (10 ml) and added to lithium aluminum hydride (200 mg) dissolved in ether (10 ml). The reaction mixture was worked up as in the case of the unlabeled material and subjected to chromatography on an activated silicic acid column (50 × 1 cm). Using a mixture of benzene and ether (9 : 1) as the eluting solvent, fractions 16 ml in volume were collected. [16-^{3}H]-14α-Methyl-cholest-

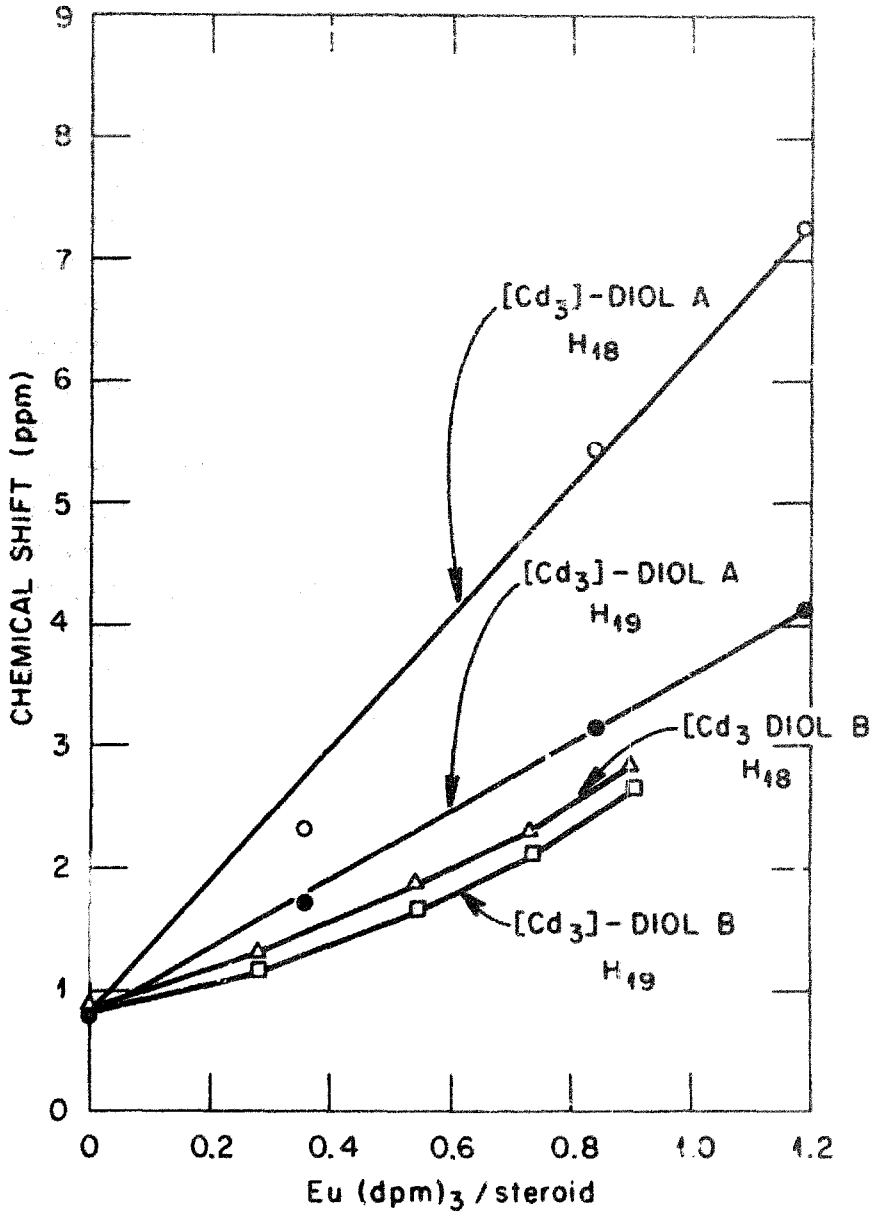

Fig. 14. Plot of chemical shifts of resonances of protons of C-18 and C-19 methyl groups as a function of [14α-C²H₃]-14α-methyl-5α-cholest-7-en-3β,15β-diol ([Cd₃]-diol A) and [14α-C²H₃]-14α-methyl-5α-cholest-7-en-3β,15α-diol ([Cd₃]-diol B).

7-en-3β,15β-diol, eluted in fractions 7–12, was recrystallized from ethyl acetate, yielding 6 mg which melted at 159–160°C. The specific activity was 6.3×10^6 dpm/mg. The radiopurity was judged to be in excess of 97% on the basis of thin-layer radiochromatographic analysis on a silica Gel G plate (solvent, benzene–ethyl acetate, 3 : 1) and of gas-liquid radiochromatographic analysis on an SE-30 column. [16-^{3}H]-14α-Methyl-cholest-7-en-3β,15α-diol, eluted in fractions 15 through 27, was recrystallized from ethyl acetate, yielding 18 mg which melted at 192–193°C. The specific activity was 6.2×10^6 dpm/mg. The radiopurity was judged to be in excess of 98% on the basis of thin-layer and gas-liquid radiochromatographic analyses as in the case of the tritium-labeled 14α-methyl-5α-cholest-7-en-3β,15β-diol.

11. Enzymatic conversion of [16-^{3}H]-14α-Methyl-5α-cholest-7-en-3β,15β-diol to Cholesterol

[16-^{3}H]-14α-Methyl-5α-cholest-7-en-3β,15β-diol (2.58 mg; 4.83 × 10^6 dpm) in propylene glycol (2.1 ml) was incubated with a 10,000 *g* supernatant fraction (500 ml) of a rat liver homogenate [32] fortified with $MgCl_2$ (5.0 mM), nicotinamide (30 mM), adenosine triphosphate (1.0 mM), glucose-6-phosphate (4.9 mM) and nicotinamide adenine dinucleotide phosphate (0.4 mM) for 3 h at 37°C. Extraction of the sterols from the saponified incubation mixture with petroleum ether yielded 2 • 10^6 counts/min which was applied to a silicic acid-Super Cel column (50 × 1 cm). Using benzene as the eluting solvent, fractions 3.8 ml in volume were collected. The resulting chromatogram is shown in fig. 15. After 170 fractions had been collected the eluting solvent was changed to 20% ether in benzene. The recovered unreacted substrate (6.6 • 10^5 counts/min) was recovered in fractions 186–200. The contents of fractions 34–60 were pooled and subjected to chromatography on an alumina-Super Cel-silver nitrate column (50 × 1 cm). Using chloroform-acetone (97 : 3) as the eluting solvent, fractions 3.5 ml in volume were collected. Approximately 20% of the radioactivity was associated chromatographically with cholesterol which was eluted in fractions 24–44. A portion of this material was diluted with unlabeled cholesterol, recrystallized from methanol-ether and purified by way of the dibromide. The specific activites of the [^{3}H]cholesterol before and after this purification were 226 counts/min • mg^{-1} and 225 counts/min • mg^{-1}, respectively.

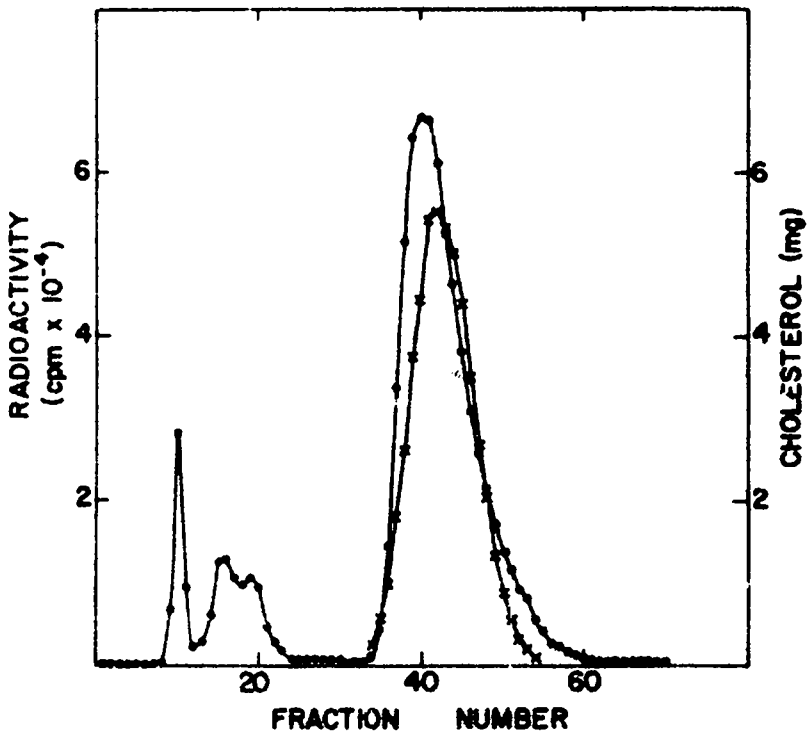

Fig. 15. Silicic acid column chromatographic analysis of labeled unsaponifiable material recovered after incubation of [16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15β-diol with a rat liver homogenate preparation. •——•, radioactivity; ×——×, cholesterol, measured.

[16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15β-diol was incubated with the 10,000 *g* supernatant fraction of the rat liver homogenate under the conditions described above except that the enzyme source was heated at 100°C for 15 min prior to the addition of the substrate. Upon silicic acid column chromatographic analysis of the recovered unsaponifiable material, the only labeled component recovered had the mobility of the incubated substrate.

The results of additional experiments indicated no effect of the inclusion of ATP in the incubation media. In two separate incubations in the presence of ATP, the percentages of the recovered radioactivity in the unsaponifiable fraction which was associated with cholesterol were found to be 19.1 and 20.7. In two parallel incubations in the absence of added ATP, the percentages of the recovered radioactivity in the unsaponifiable fraction which was associated with cholesterol were 20.4 and 23.8.

Five separate incubations of [16-^{3}H]-14α-methyl-5α-cholest-7-en-3β,15α-diol with rat liver homogenate preparations (which were active for the conversion of the 3β,15β-diol to cholesterol) did not lead to the formation of labeled cholesterol.

III. Discussion

14α-Methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol were prepared from 3β-methyl-14α-methyl-5α-cholest-7-en-15-one by reduction with lithium aluminum hydride. Treatment of 14α-methyl-5α-cholest-7-en-3β,15β-diol with *p*-bromobenzoyl chloride in pyridine yielded 3β,15β-bis-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-ene and 3β-*p*-bromobenzoyloxy-14α-methyl-5α-cholest-7-en-15β-ol. The detailed structure of the latter compound was unambiguously established by X-ray analysis. This analysis thereby also established the structure of its 15α-hydroxy epimer and also confirms the stereochemistry and structure of the 3β-benzoyloxy-14α-methyl-5α-cholest-7-en-15-one from which the two epimeric 15-hydroxysterols were prepared.

It is interesting to note that 14α-methyl-5α-cholest-7-en-3β,15β-diol was less polar than 14α-methyl-5α-cholest-7-en-3β,15α-diol on thin layer chromatography on silica gel column chromatography on silicic acid, and on gas-liquid chromatography. In the former compound the 15-hydroxyl function is pseudoaxial while in the latter case the 15-hydroxyl group is pseudoequatorial. In this particular case the chromatographic behavior of the two epimeric diols corresponds to the generality developed for cyclohexane systems in which axially substituted derivatives are usually less polar than the corresponding equatorially substituted epimers [39,45].

Differences in the infrared spectra of the epimeric 15-hydroxysterols were also noted and have been commented upon previously. The nuclear magnetic resonance spectra of the two epimer diols were significantly different and detailed analyses of the spectra indicated the absolute configuration of the hydroxyl function at carbon atom 15 in the two compounds. The results of experiments utilizing the shift reagent, Eu(dpm)$_3$, indicate that the chemical shift of the three proton singlet due to the C-18

methyl group as a function of the shift reagent to steroid molar ratio is markedly greater in the case of the 15β-hydroxy compound than with the corresponding 15α-hydroxy compound. This approach constitutes a potentially very powerful and simple method for the establishment of configuration of the hydroxyl function in 15-hydroxysterols.

The mass spectra of 14α-methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol differ in a number of respects. One major difference was in the ratio of abundancies of the molecular ion (M) to the ion due to loss of water (M-18). In the case of 14α-methyl-5α-cholest-7-en-3β,15β-diol with the pseudoaxial 15-hydroxyl function, this ratio was 0.68. In the case of 14α-methyl-5α-cholest-7-en-3β,15α-diol with the pseudoequatorial 15-hydroxyl function, the same ratio was 10.0. The use of this analysis has been proposed [46] as a method for the determination of the configuration of steroidal alcohols with the axially substituted hydroxysteroid giving the lower ratio.

The results reported herein indicate that 14α-methyl-5α-cholest-7-en-3β,15β-diol, but not 14α-methyl-5α-cholest-7-en-3β,15α-diol, is convertible to cholesterol upon incubation with rat liver homogenates of female rats. Gibbons et al. [30] have recently reported that both 15-hydroxy epimers of 4,4,14α-trimethyl-5α-cholest-8-en-3β-ol are convertible to cholesterol upon incubation with liver homogenate preparations of male rats. In consideration of the results of our study, the reports from three laboratories indicating the stereo-specifc removal of the 15α-hydrogen of lanosterol upon enzymatic formation of 5α-cholest-7-en-3β-ol, 7-dehydrocholesterol, and cholesterol are of considerable importance. Since all hydroxylation reactions at saturated carbon atoms of the sterol nucleus studied to date have been shown to involve introduction of the hydroxyl function with "retention of configuration", the findings that the hydroxyl group at carbon atom 15 in the epimer which is enzymatically convertible to cholesterol has the β-configuration while the hydrogen that is lost from carbon atom 15 in the overall conversion of lanosterol to cholesterol has the α-configuration strongly suggest that 14α-methyl-5α-cholest-7-en-3β,15β-diol may not be a significant intermediate in the biosynthesis of cholesterol.

While we have not investigated in detail the possible enzymatic formation of 14α-methyl-5α-cholest-7-en-3β,15β-diol from 14α-methyl-5α-cholest-7-en-3β-ol, we have noted the formation of polar metabolites (with the expected chromatographic mobility of dihydroxysterols) upon incubation of the latter compound with liver homogenate preparations of female rats [19]. The presence of a steroid 15β-hydroxylase in the liver microsomes of female rats has been reported by Gustafsson and Ingelmann-Sundberg [47,48].

Perhaps the most important features of the present work are the descriptions of the syntheses and unequivocal establishments of structures of 14α-methyl-5α-cholest-7-en-3β,15β-diol and 14α-methyl-5α-cholest-7-en-3β,15α-diol. These compounds have been found to be very potent inhibitors of sterol biosynthesis in L cells and in primary cultures of mouse liver cells [31].

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