

SYNTHESIS OF ANALOGS OF α -ECDYSONE. A SIMPLIFIED
SYNTHESIS OF $2\beta,3\beta,14\alpha$ -TRIHYDROXY-7-EN-6-ONE-
5 β -STEROIDS

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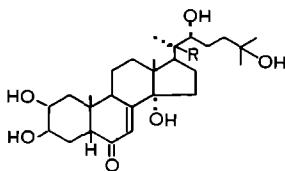
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ABSTRACT

Several compounds that contain the steroid nucleus of α -ecdysone were synthesized, namely; $2\beta,3\beta,14\alpha$ -trihydroxy-5 β -cholest-7-en-6-one, $2\beta,3\beta,14\alpha$ -trihydroxy-27-nor-5 β -cholest-7-en-6-one, $2\beta,3\beta,14\alpha$ -trihydroxy-(24R)-5 β -ergost-7-en-6-one, and $2\beta,3\beta,14\alpha$ -trihydroxy-(24R)-5 β -stigmast-7-en-6-one, and their mass spectral data and that of their 5 α -isomers are presented. Also 11 derivatives of the $2\beta,3\beta,14\alpha$ -trihydroxy-5 β -cholest-7-en-6-one were prepared as were additional compounds with specific structural features that would help to better define the relationship of structure to biological activity in insects.

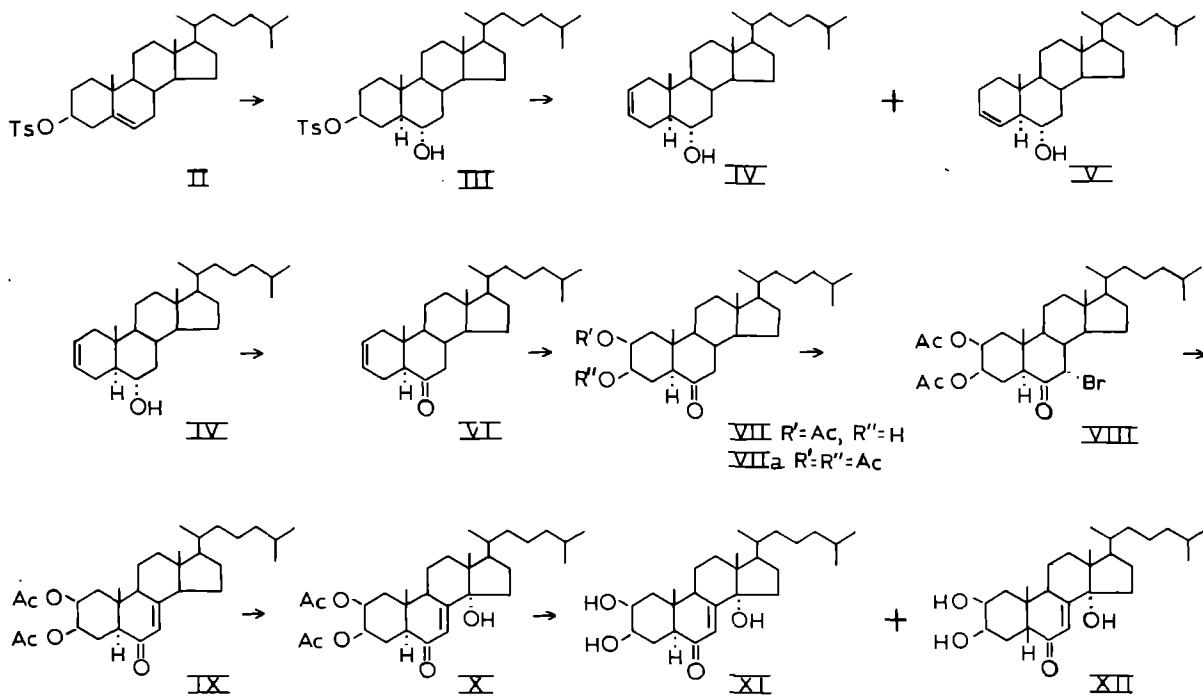
In a previous report (1), we showed that certain synthetic compounds containing structural features similar to the insect molting hormone when ingested were effective inhibitors of larval growth and development in several species of insects though the insects hormones, α -ecdysone (I) and 20-hydroxyecdysone (Ia), were inactive or considerably less active. The most active synthetic analog, $2\beta,3\beta,14\alpha$ -trihydroxy-5 β -cholest-7-en-6-one (XII), also inhibited ovarian maturation and thus is a chemosterilant.

Compound XII has been synthesized by others (2,3); however, the published methods of synthesis for this analog and previous methods



I, R = H
Ia, R = OH

of synthesizing the α -ecdysone nucleus from Δ^5 - 3β -hydroxy sterols or steroids have required a great number of steps. We have shortened the sequence of reactions at the initial stages and have obtained an improved overall yield. This paper reports on the synthesis of several other compounds containing the steroid nucleus of α -ecdysone and presents the mass spectral data of these 5α - and 5β -steroids, their physical properties, and the physical properties of the various intermediates leading to these 5α - and 5β -steroids. In addition,



we describe the preparation of eleven derivatives of compound XII and their physical properties. Three additional 5β -steroids (XIII, XV and XVIII) with specific structural features were synthesized to better define the relationship of structure to biological activity. In a separate paper in this issue of Steroids, we report on the relationship of the structure of these synthetic ecdysone analogs to certain biological activities in insects (4).

The reaction of cholesterol tosylate (II) with diborane in tetrahydrofuran followed by hydrogen peroxide treatment (5) in the presence of sodium bicarbonate solution gave the 6α -hydroxytosylate (III) in quantitative yield. The detosylation of III with equal quantities of lithium carbonate and lithium bromide in dimethylformamide gave IV in 85% yield, V in 10% yield, and about 5% hydrocarbon (6). The mass spectra of both compounds were interesting. Purified IV showed a strong metastable at m/e 351 indicating a loss of H_2O (M-18) from m/e 386 to m/e 368 and a weak metastable at 357 indicating a loss of methyl (M-15). The Δ^3 - 6α -hydroxy compound (V) showed a strong metastable at m/e 357 indicating a loss of methyl (M-15) from m/e 386 to m/e 371 and a weak metastable at m/e 351. That V was indeed Δ^3 - 6α -hydroxy- 5α -cholestene was verified by its oxidation to a 6-ketone that differed from the oxidative product of IV (proof that the differences in IV and V were not caused by position isomerism at C-6), and both ketones were not separable by thin-layer chromatography (TLC) or by gas-liquid chromatography (GLC) on two chromatographic systems. This behavior would not be expected if the compounds differed at the A/B-ring junction, with or without double bond in similar positions. Furthermore, the methyl resonances of IV and V were superimposable. The mixture of IV and V need not be separated

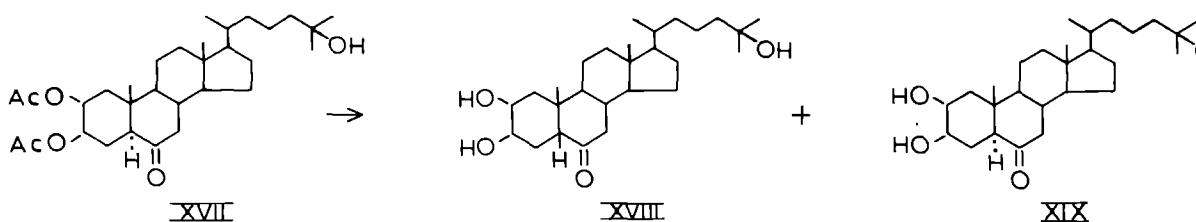
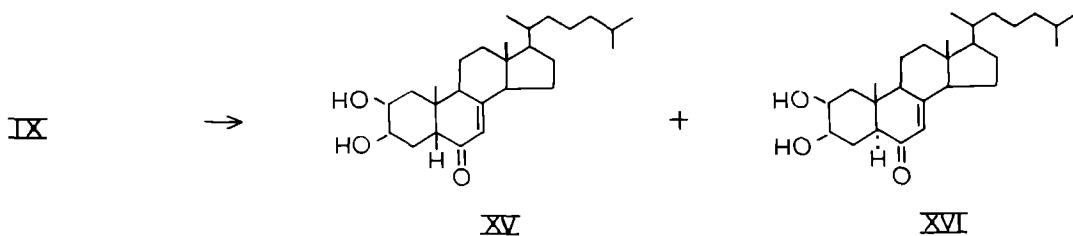
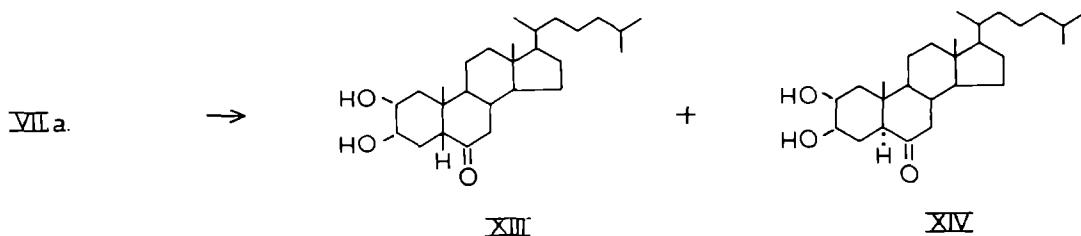
since in the future sequence of reactions, the products resulting from V were removed during purification. The oxidation of IV with chromic acid solution in acetone (7) gave VI in an overall yield of about 70%. Hydroxylation of VI with iodine, silver acetate, and wet acetic acid (8) gave the 2 β -acetoxy-3 β -hydroxy-6-ketone (VII) and subsequent acetylation, and recrystallization gave VIIa in a 49% yield. Bromination of VIIa in a solution of acetic acid and hydrogen bromide yielded VIII, and debromination with lithium carbonate in dimethylformamide gave the 2 β ,3 β -diacetoxy-5 α -cholest-7-en-6-one (IX), λ max. 245 μ in methanol, ϵ 12,000.

Treatment of the diacetoxy Δ^7 -6-keto compound IX with selenium dioxide introduced the 14 α -hydroxy (9) to give compound X in 65% yield or in an overall 13% yield from cholesterol. The saponification and isomerization of X with 2% potassium carbonate in 90% methanol at 50° for 30 min. gave about 70% of the desired 2 β ,3 β ,14 α -trihydroxy-5 β -cholest-7-en-6-one (XII) and its 5 α -isomer (XI) in 20% yield (10). The major product of the remaining mass appeared to be 2 β ,3 β ,14 α -trihydroxy-5 β -cholest-8-en-6-one and its 5 α -isomer. These two Δ^8 -compounds were not very stable and were not easily separated from their respective Δ^7 -component.

Our method of synthesis has produced 10 g of pure XII from 100 g of cholesterol, an overall 9% yield. We also used this method to obtain milligram quantities of tritium-labeled XII from 1 g of tritium-labeled cholesterol. Also, other steroids that contain the α -ecdysone nucleus though differing in carbon number in their side chain, were synthesized by this procedure. The intermediates of these compounds and their physical properties are shown in the experimental section in order of the sequence of reactions leading to the final products

(11). Derivatives of XII and their structural formulas are also presented in the experimental section.

It is noteworthy that the best yield of the 5β -isomers was obtained from the isomerization of 5α -steroids that contained the 14α -hydroxyl group. Even when stronger isomerization conditions were employed, such as in the conversion of VIIa to XIII and XIV, or the



isomerization of XVII to XVIII and XIX, the 5β -steroid was only produced in 60% yield. In the isomerization of IX, which contains a Δ^7 -double bond, to XV and XVI, the yield of the 5β -isomer was even smaller, and it was accompanied by other products, especially the

Δ^8 -isomers. In this case, the production of the Δ^8 -isomer occurred even when the conditions of isomerization were extremely mild. Interestingly, mixtures of the Δ^7 -5 α - and 5 β -isomers that contained the 14 α -hydroxyl group were easier to separate from each other than the corresponding 5 α - and 5 β -isomers without the 14 α -hydroxyl.

We have carried out the synthesis of the 2 β ,3 β ,14 α -trihydroxy-5 β -cholest-7-en-6-one (XII) with a minimum amount of purification. Only the intermediates VI, VIIa, IX and X were purified and this was accomplished by crystallization (12). Column chromatography was necessary only to separate the final products XI and XII. It is possible that the final mixture remaining after the selective crystallization of XI from XII with methanol without further purification would be as active in producing biological effects in insects as the purified substance.

A biological examination of some of the analogs suggested that certain of the compounds planned for this study would be devoid of appreciable biological activity. Nevertheless, to obtain a more complete evaluation of the relationship of structure to biological activity, we carried out the planned syntheses and biological studies. We have since synthesized ecdysone analogs that are considerably more active than any reported here and which are also specific in their activity for certain species of insects.

EXPERIMENTAL

Melting points were taken on Kofler block (13) and are corrected. Rotations were determined at 23° in about 1% solutions in chloroform unless otherwise stated. Infrared spectra were obtained with a Perkin-Elmer Model 221 prism grating spectrophotometer, and ultraviolet spectra were taken in methanol, hexane, or cyclohexane with a Bausch and Lomb spectrophotometer 505. NMR spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer by using deuterated chloroform or pyridine as the solvent and TMS as an internal NMR

standard. The mass spectra were measured by using a LKB model 9000 gas chromatograph mass spectrometer (LKB Produkter AB, Stockholm); the samples were introduced directly into the ionization chamber. The ionization energy was 70 ev. The diborane in tetrahydrofuran was purchased from Ventron Corporation.

Sterols used in the Syntheses. Cholesterol (m.p. 149-150°) was obtained from Fisher Scientific Co. and was used without any further purification. The β -sitosterol (< 99% purity, m.p. 139-140°, α_D -34°) was prepared from stigmasterol by the method of Steele and Mosettig (14). The campesterol (< 95% purity, m.p. 160-161°, α_D -33°) was obtained by fractional crystallization of soybean sterols from which the stigmasterol had been removed. A Wolff-Kishner reduction (Huang-Minlon modification) of 25-keto-27-norcholesterol yielded 27-norcholesterol (< 99% purity, m.p. 138-139°, α_D -38°).

Cholesterol tosylate (II) -- A solution of 100 g of cholesterol, 250 ml of pyridine, and 100 g of p-toluenesulfonyl chloride was allowed to stand overnight at room temperature. Then the mixture was poured into ice and water, and the resultant precipitate was allowed to stand in ice and water for 3 hr with occasional stirring. The precipitate was collected, washed thoroughly with water, air dried, and then dried overnight at 65° under vacuum to give 139 g of cholesterol tosylate, m.p. 130-132°. Recrystallization of a 200-mg sample gave rods m.p. 133-135°, α_D -40°.

3 β ,6 α -Dihydroxy-5 α -cholestane 3 β -tosylate (III) -- To a stirred solution of 138.8 g of cholesterol tosylate in 800 ml of dry tetrahydrofuran at 5°, was added over a 10-min period 259 ml of 1 molar diborane in tetrahydrofuran (5). The mixture was kept at 5° for 30 min. and then allowed to stand for 2.5 hr at room temperature. The mechanically stirred solution was rechilled to 5°, and cold water (about 15 ml) was added dropwise to react with excess diborane; then 225 ml of 5% sodium bicarbonate solution and 90 ml of 30% hydrogen peroxide were added immediately. The reaction mixture was kept at 5° for 15 min, and then removed from the ice bath and allowed to stand for 45 min (the temperature not permitted to rise above 30°). The reaction mixture was poured into cool water and the precipitate was collected, air dried for 2 hr and then dried overnight at 65° in vacuo to give 139 g of crude III, m.p. 129-132°. Recrystallization of a 100-mg sample from hexane containing a trace of methanol gave analytically pure III m.p. 132-134°, α_D +28°. Anal. Calcd. for C₃₄H₅₄O₄S: C, 73.07; H, 9.74. Found: C, 72.90; H, 9.50.

5 α -Cholest-2-en-6 α -ol (IV) and 5 α -Cholest-3-en-6 α -ol (V) -- A mixture of 138.9 g of the hydroxy-tosylate (II), 800 ml of dimethylformamide, and 70 g each of lithium carbonate and lithium bromide was refluxed for 1 hr. The solution was filtered while hot, and the filtrate was cooled and then poured into ice and water. The precipitate was collected, air dried for 2 hr, and then dried overnight under vacuum at 65° to give 97 g of material. Analysis by TLC indicated a major and two minor components. A 1.0-g sample was chromatographed over 60 g of hexane-washed alumina (activity grade II; Woelm) and eluted as follows: 1, 100-ml fraction of hexane; 2-12,

100-ml fractions of hexane-ether (4:1). The fractions were monitored by TLC and infrared analysis. Fraction 2 consisted of hydrocarbon (50 mg) and was discarded, fractions 7-12 were free of the faster moving components and were set aside, and fractions 4-6 were still mixtures (300 mg) and were rechromatographed. The fractions from the first and second chromatography that contained the slower moving component were combined and recrystallized from acetonitrile to give 840 mg of the Δ^2 -compound (IV), m.p. 119-121°, $\alpha_D +83^\circ$, ν_{\max} in CS_2 3615 cm^{-1} (hydroxyl) 3022, 1652, and 662 cm^{-1} (double bond).

Mass spectrum m/e (rel. intensity) 386 (36), 371 (22), 368 (100), 353 (24), 314 (46), 255 (24), 231 (30) strong metastable 351, weak metastable 357. Anal. Calcd. for $\text{C}_{27}\text{H}_{46}\text{O}$: C, 83.87; H, 11.99. Found: C, 83.70; H, 12.00.

The fractions that contained the faster moving component were combined and recrystallized from acetonitrile to give 84 mg of the 5 α -cholest-3-en-6-ol (V), m.p. 124-126°, $\alpha_D +71^\circ$, ν_{\max} in CS_2 3615 cm^{-1} (hydroxyl), 3028, 1645, and 670 cm^{-1} (double bond). Mass spectrum m/e (rel. intensity) 386 (25), 371 (100), 368 (45), 353 (12), 331 (25), 231 (14) strong metastable 357, weak metastable 351. The methyl resonances of IV and V were superimposable.

Anal. Calcd. for $\text{C}_{27}\text{H}_{46}\text{O}$: C, 83.87; H, 11.99. Found: C, 83.66; H, 12.04.

5 α -Cholest-2-en-6-one (VI) -- To a crude mixture of 96.0 g of IV and V in 1500 ml of acetone at 20° was added dropwise an 8 N solution of chromic acid in dilute sulfuric acid until a persistent orange-brown coloration indicated that oxidation was complete (7). The mixture was diluted with water and the crystalline material was collected, washed with water, and recrystallized from dilute acetone to give a total of 73.3 g of VI, m.p. 104-106°. A sample of this material recrystallized once from dilute acetone gave rods, m.p. 107-109°, $\alpha_D +35^\circ$, ν_{\max} in CS_2 1712 cm^{-1} (carbonyl), 3030, 1655, and 665 cm^{-1} (double bond) (Lit. (15), m.p. 109-110°).

2 β ,3 β -Dihydroxy-5 α -cholestan-6-one 2 β -acetate (VII). To 67.2 g of VI dissolved in 2 liters of acetic acid at room temperature was added 58.82 g of silver acetate, and then 44.8 g of iodine in small portions was added over a 2-hr period (8). After all the iodine had been consumed, 6.8 ml of water in 50 ml of acetic acid was added, and the mixture was mechanically stirred for 40 hr at room temperature. At the end of 40 hr, sodium chloride was added to the mixture, and it was stirred for an additional 30 min, and then filtered, and the precipitate was washed with hot benzene, and the filtrate was concentrated to dryness *in vacuo* to give 81 g of semicrystalline residue. Recrystallization of a 200-mg sample from acetonitrile gave rods, m.p. 213-215°, $\alpha_D +15^\circ$ (Lit. (2), m.p. 218-219°).

2 β ,3 β -Dihydroxy-5 α -cholestan-6-one 2 β ,3 β -diacetate (VIIa) -- The crude semicrystalline material (80.5 g) from the preparation of VII acetylated by the pyridine acetic anhydride method overnight at room temperature afforded after crystallization from hexane, 43 g of VIIa, m.p. 188-190°, $\alpha_D +10^\circ$, ν_{\max} in CS_2 1735 cm^{-1} (acetate) 1710 cm^{-1} (ketone). (Lit. (15,3) m.p. 191-192°; m.p. 187-188°, $\alpha_D +5^\circ$).

2 β ,3 β -Dihydroxy-7 α -bromo-5 α -cholestan-6-one 2 β ,3 β -diacetate (VIII) - To 43 g of VIIa in a mixture of 900 ml of acetic acid, 279 ml of ether, and 5 ml of 32% hydrogen bromide in acetic acid at 40°, was added dropwise 12.4 g of bromine in 200 ml of acetic acid over a 75-min period. The temperature of the mixture was gradually being raised during the addition and had reached 55° when all of the bromine had been added. The temperature was brought to 70° and kept there for 2.5 hr. The ether was removed *in vacuo*, and the solution was cooled and poured into ice and water and the precipitate was collected, air dried, and then dried overnight at 65° under vacuum to give 43.5 g of crude VIII. A 1-g sample recrystallized twice from hexane gave 550 mg of rods, m.p. 119-120°, α_D +47° (Lit. (2) m.p. 118-119°).

2 β ,3 β -Dihydroxy-5 α -cholest-7-en-6-one 2 β ,3 β -diacetate (IX) -- A mixture of 42.5 g of crude VIII, 500 ml of dimethylformamide, and 42.5 g of lithium carbonate was refluxed for 1 hr. After the lithium carbonate was removed by filtration, the solution was cooled, diluted with ice water, and the precipitate was collected and dried. Recrystallization from hexane-ether gave 23.5 g of IX as rods, m.p. 212-214°, α_D +39°, λ max in methanol 245 μ , ϵ 12,000, ν max in CS₂ 1745 cm⁻¹ (acetate), 1676 cm⁻¹ (ketone), and 1618 cm⁻¹ (double bond). (Lit. (2) m.p. 213-214°, ϵ 13,000.

2 β ,3 β ,14 α -Trihydroxy-5 α -cholest-7-en-6-one 2 β ,3 β -diacetate (X) -- To 23.4 g of IX in 820 ml of dry dioxane at 80° and under inert atmospheric conditions was added in one portion 23.4 g of selenium dioxide. The reaction mixture was kept at 80-85° for 30 min, and then filtered. The filtrate was cooled, diluted with ice and water, and the crude precipitate was collected and dried. Fractional recrystallization from hexane-acetone gave 15.5 g of X, as needles, m.p. 230-232°, α_D +79°, λ max in methanol 241 μ , ϵ 11,200, ν max in Nujol 3540 (unassociated hydroxyl) and 3430 cm⁻¹ (associated hydroxyl), 1735 (acetate), 1673 (ketone), and 1625 cm⁻¹ (double bond) (Lit. (2,3) m.p. 231-232°, ϵ 11,500; m.p. 224-226°, α_D +75°, ϵ 12,800).

2 β ,3 β ,14 α -Trihydroxy-5 α -cholest-7-en-6-one (XI) -- To 15.5 g of X in 1200 ml methanol at 50° was added 30 g of potassium carbonate in 150 ml of water and 200 ml of methanol. The mixture was kept at 50° for 30 min and the solution was reduced to about half its original volume *in vacuo*. Water was added to complete the precipitation of the compounds. The precipitate was collected by filtration and analyses by TLC on silica gel G plate, developed in the solvent system chloroform-ethanol (10:1) showed two spots. The upper spot was the 5 α -isomer and the lower spot, the major component, showed sign of a partially separate component at the bottom of the spot. The crude mixture was recrystallized from methanol to give 1.0 g of 2 β ,3 β ,14 α -trihydroxy-5 α -cholest-7-en-6-one (XI), m.p. 245-249°, α_D +71° in dioxane, λ max 244 μ in methanol, ϵ 12,950. (Lit. (2,3) m.p. 254-257°, ϵ 11,800; m.p. 249-252° α_D +75°, ϵ 12,800).

The remaining mass (12 g) that contained about 80% of the 5 β -isomer (XII) was purified by column chromatography.

2 β ,3 β ,14 α -Trihydroxy-5 β -cholest-7-en-6-one (XII) -- The remaining mass (12 g) after the separation of XI, was chromatographed in 4 g portions on a chloroform-washed Unisil column (upper part of

column 3.5 X 54 cm and lower portion 2.5 X 95 cm). The 4 g of material was placed on the column in a minimum amount of chloroform and then the column was eluted with chloroform - 95% ethanol (12:1). The first fraction of 500 ml of eluant was collected and discarded; then 100, 10-ml fractions each were collected. The fractions were monitored by TLC; fractions 5-20 usually contained small quantities of material that had no hydroxyl group at the C-14 position, fractions 25-35 contained pure XI, fractions 36-40 contained a mixture of XI and XII, fractions 41-70 contained pure XII, and fractions 71-85 contained XII and increasing amounts of the 5β - Δ^8 -steroid. (The Δ^8 -compound was not always separated by TLC from XII; the fractions containing XII were then monitored by infrared analyses). After three column chromatographs the fraction containing XI were combined and recrystallized from methanol to give an additional 1.5 g of XI. The fractions that showed pure XII were combined and recrystallized from ethyl acetate to give 8.5 g of $2\beta,3\beta,14\alpha$ -trihydroxy- 5β -cholest-7-en-6-one (XII), m.p. 208-210°, $\alpha_D +77^\circ$, λ_{max} 245 μ in methanol, $\epsilon_{12,700}$, ν_{max} in Nujol 1657 cm^{-1} (ketone), 1615 cm^{-1} (double bond) (Lit. (2,3) m.p. 207-209°, $\epsilon_{12,200}$; m.p. 194-198°, $\alpha_D +78^\circ$, $\epsilon_{13,600}$).

A reisomerization of 2.5 g of XI yielded, after separation, an additional 1.5 g of XII. Thus, from 100 g of cholesterol, 10 g of XII was obtained.

Notes on XII

Some of XII could be separated from the Δ^8 -isomer by recrystallization from ethyl acetate, since during the crystallization process the 14α -hydroxyl group of the Δ^8 -compound is eliminated yielding material that did not readily crystallize from this solvent.

Treatment of $2\beta,3\beta$ -dihydroxy- 5β -cholest-7-en-6-one (XV) with selenium dioxide gave XII after recrystallization from ethyl acetate with a m.p. 213-214°. In some instances, we obtained XII via saponification and isomerization of X that melted at 202-205°, and we have had XII that melted at 208-210° and after being refrigerated for 3 months melted at 200-203°. This compound, however, did not differ from the original high melting material by infrared and TLC analyses or the house fly assay.

The infrared spectrum in Nujol of compound XII or other steroids containing the ecdysone nucleus when recrystallized from aqueous acetone or aqueous methanol did not exhibit the typical double bond absorption in the 1615 cm^{-1} region and the ketone absorption had shifted from 1657 cm^{-1} to 1645 and 1625 cm^{-1} region. However, only a recrystallization from ethyl acetate and the material again exhibited ketone absorption at 1657 cm^{-1} and a double bond absorption at 1615 cm^{-1} .

$2\beta,3\beta$ -Dihydroxy- 5α -cholestan-6-one (XIV) and $2\beta,3\beta$ -Dihydroxy- 5β -cholestan-6-one (XIII) --A mixture of 5 g of VIIa, 250 ml of methanol, 2.5 ml of water, and 2.5 g of potassium hydroxide stood overnight in nitrogen atmosphere at room temperature. The solution was diluted with water, and the precipitate was collected, dried, and chromatographed on a Unisil column as used in the purification

of XII; the solvent system was chloroform - 95% ethanol (40:1). The fractions were monitored by TLC. Recrystallization of the faster moving component from acetonitrile gave the $2\beta,3\beta$ -dihydroxy- 5α -cholestan-6-one (XIV) in 40% yield, m.p. 209-211°, $\alpha_D +4^\circ$. (Lit. (15, 16) m.p. 212-213°; m.p. 213-216°, $\alpha_D +5^\circ$, also m.p. 207-209°).

Recrystallization of the combined fractions of the slower moving component gave the 5β -isomer (XIII) in 60% yield, m.p. 180-182°, $\alpha_D -57^\circ$. (Lit. (16) m.p. 179-180°, $\alpha_D -58^\circ$).

$2\beta,3\beta,25$ -Trihydroxy- 5α -cholestan-6-one (XIX) and $2\beta,3\beta,25$ -Trihydroxy- 5β -cholestan-6-one (XVIII) --Saponification and isomerization of XVII as in the preparation of XIII and XIV followed by column chromatography (Unisil, solvent system chloroform - 95% ethanol (40:1)) and recrystallization of the faster moving component from ethyl acetate gave the 5α -triol (XIX) 34% yield, m.p. 202-204°, $\alpha_D +7^\circ$.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.40; H, 10.71

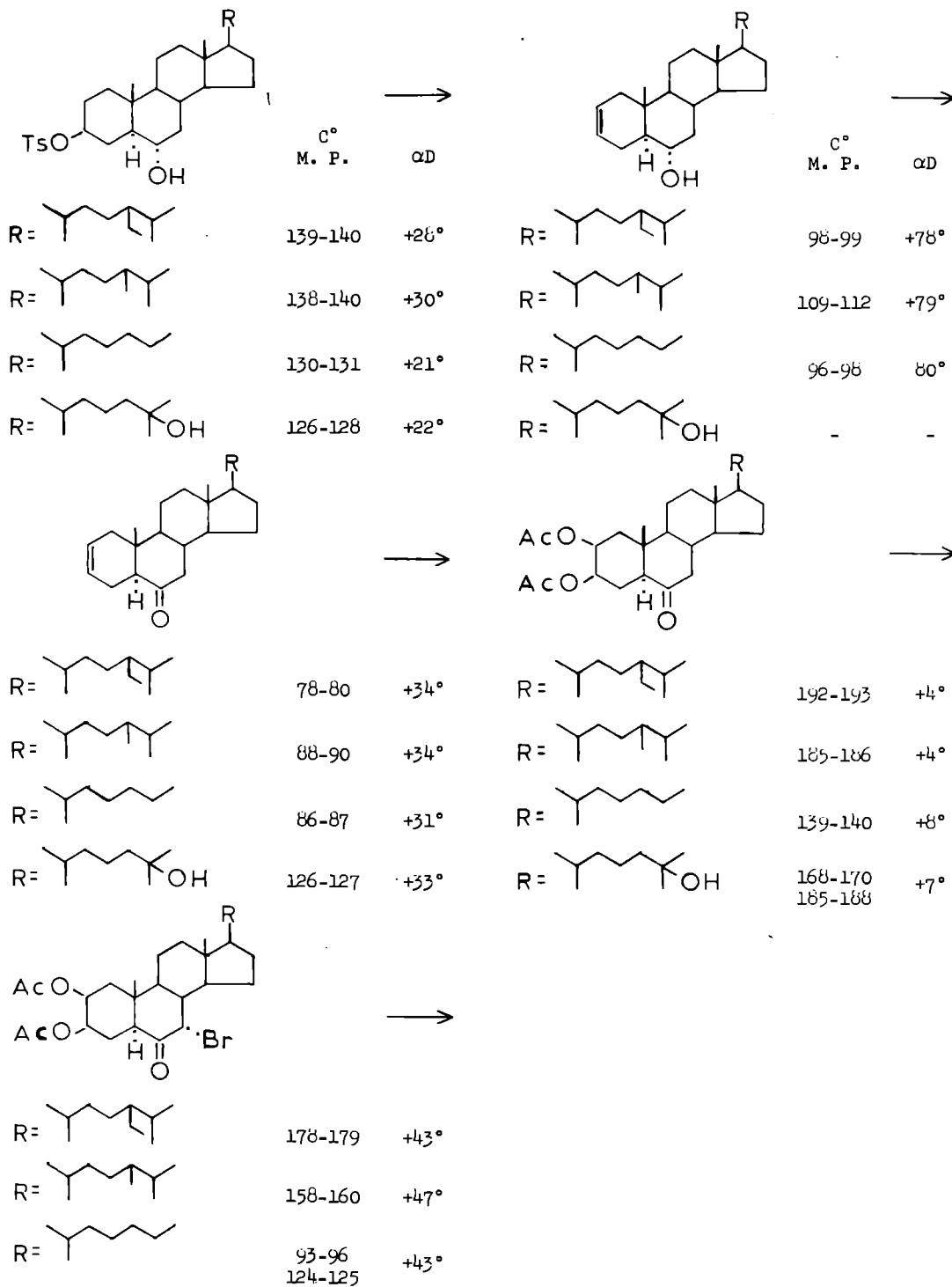
Crystallization of the slower moving component gave the 5β -triol XVIII in 66% yield, m.p. 176-177°, $\alpha_D -53^\circ$.

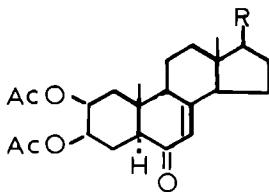
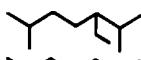
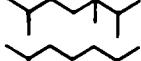
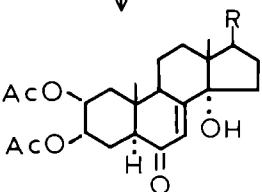
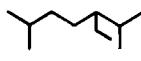
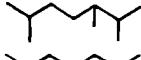
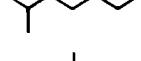
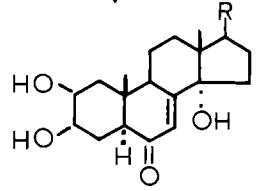
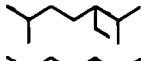
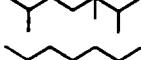
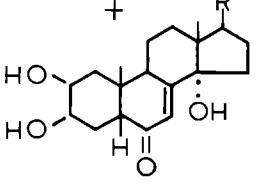
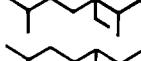
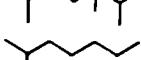
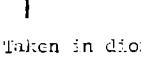
Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.57; H, 10.60.

$2\beta,3\beta$ -Dihydroxy- 5α -cholest-7-en-6-one (XVI) and $2\beta,3\beta$ -Dihydroxy- 5β -cholest-7-en-6-one (XV). A mixture of IX (7.2 g), 1000 ml of methanol, 110 ml of water, and 3.3 g of potassium carbonate was kept at 50° for 30 min. Most of the methanol was removed in vacuo, the solution was diluted with water and the precipitate was collected, dried, and chromatographed over Unisil column as in separation of XII (solvent system chloroform - 95% ethanol (40:1)). Recrystallization of the faster moving component from acetonitrile gave the Δ - 5α -diol (XVI) in 50% yield, m.p. 208-210°, $\alpha_D +5^\circ$, λ_{max} in methanol 244 μ , ϵ 13,500, ν_{max} in Nujol 1657 (carbonyl), and 1615 cm^{-1} (double bond). (Lit. (2) m.p. 208-210°, ϵ 14,000).

7 Recrystallization of the slower moving component gave the Δ^7 - 5β -diol (XV) in 35% yield, m.p. 203-205°, $\alpha_D +34^\circ$, λ_{max} 245 μ in methanol, ϵ 14,000, ν_{max} in Nujol 1657 (carbonyl), and 1615 cm^{-1} (double bond). (Lit. (2), m.p. 201.5-203.5, ϵ 14,600).

The corresponding 5α - and 5β -steroids of 27-norcholesterol, β -sitosterol, and campesterol were synthesized as described for the synthesis of XII. These compounds and their precursors and their physical properties are shown below according to the sequence of reaction.



	C° M. P.	αD	λ Max (mμ) in Methanol	ε _{Max}
R = 	211-212	+39°	245	11,500
R = 	212-214	+40°	244	11,000
R = 	184-185	+30°	244	11,800
↓				
				
R = 	234-236	+65°	242	11,000
R = 	238-241	+80°	242	11,400
R = 	203-205	+72°	242	12,000
↓				
				
R = 	235-238	+63° <u>1/</u>	245	12,000
R = 	247-250	+65° <u>1/</u>	245	11,800
R = 	227-230	-	245	12,600
+				
				
R = 	240-242	+64°	245	11,500
R = 	244-246	+72°	245	12,000
R = 	228-231	+60°	245	13,000

1/ Taken in dioxane

Nuclear Magnetic Resonance and Mass Spectral Data of the Synthetic Compounds that Contain the Steroid Nucleus of α -Ecdysone (probe temperature was 90-100°)

2 β ,3 β ,14 α -Trihydroxy-5 β -cholest-7-en-6-one --m/e (relative intensity): 432 (5), 414 (89), 404 (31), 399 (100), 381 (51), 371 (13), 302 (45), 301 (95), 287 (24), 250 (16), 249 (45), 213 (24). NMR, δ 0.75 (18-CH₃), 1.08 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-5 α -cholest-7-en-6-one --m/e (relative intensity): 432 (2), 414 (23), 396 (51), 381 (28), 365 (20), 302 (26), 301 (68), 284 (43), 283 (100), 269 (24), 213 (17). NMR, δ 0.73 (18-CH₃), 1.40 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-27-nor-5 β -cholest-7-en-6-one --m/e (relative intensity): 418 (19), 400 (98), 390 (100), 385 (16), 372 (17), 367 (35), 357 (27), 339 (8), 302 (9), 301 (27), 283 (8), 250 (39), 249 (98), 231 (28), 223 (28), 213 (20), 210 (20). NMR, δ 0.72 (18-CH₃), 1.06 (19-CH₃).

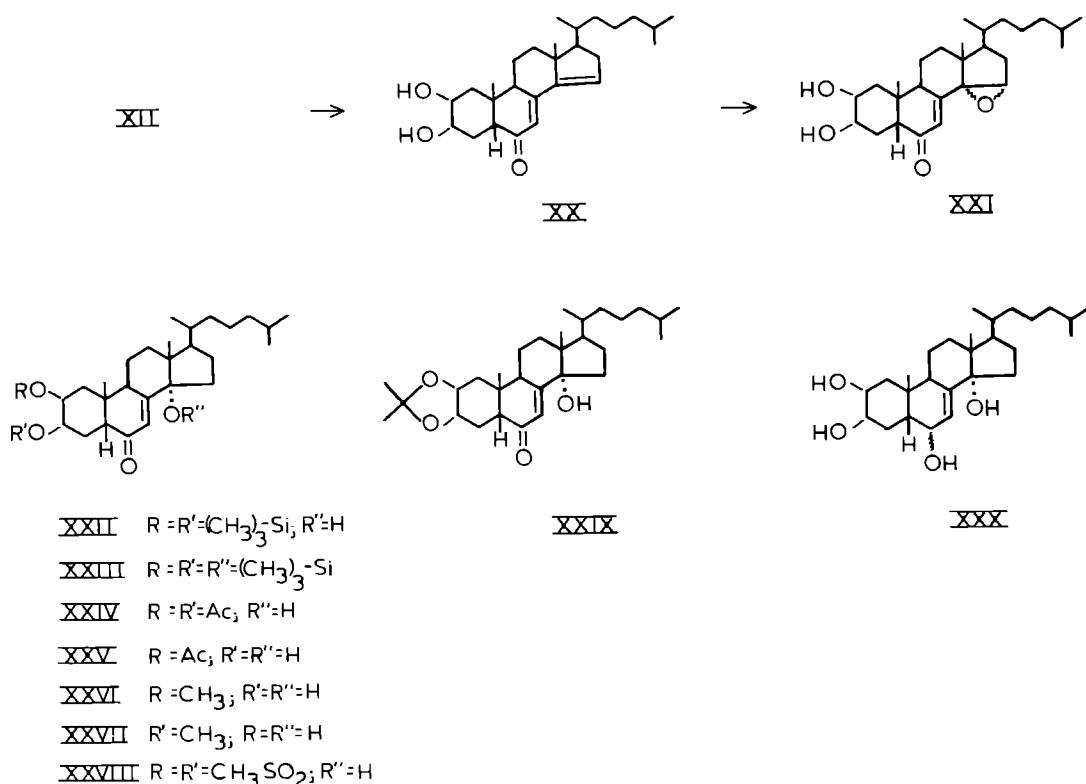
2 β ,3 β ,14 α -Trihydroxy-27-nor-5 α -cholest-7-en-6-one --m/e (relative intensity): 418 (44), 400 (28), 390 (40), 385 (13), 375 (19), 367 (9), 357 (12), 302 (17), 301 (47), 264 (50), 250 (54), 249 (100), 231 (55), 223 (18), 213 (17), 210 (9). NMR, δ 0.73 (18-CH₃), 1.38 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-(24R)-5 β -ergost-7-en-6-one --m/e (relative intensity): 446 (15), 428 (33), 418 (91), 403 (9), 400 (18), 386 (9), 385 (22), 367 (5), 302 (8), 301 (19), 250 (38), 249 (100), 231 (28), 223 (30), 213 (17), 210 (20). NMR, δ 0.75 (18-CH₃), 1.08 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-(24R)-5 α -ergost-7-en-6-one --m/e (relative intensity): 446 (13), 428 (67), 418 (92), 413 (13), 403 (7), 400 (15), 395 (22), 385 (20), 367 (5), 302 (10), 301 (27), 250 (39), 249 (100), 231 (28), 223 (27), 213 (20), 210 (21). NMR, δ 0.73 (18-CH₃), 1.39 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-(24R)-5 β -stigmast-7-en-6-one --m/e (relative intensity): 460 (2), 442 (18), 432 (6), 424 (89), 425 (31), 422 (18), 410 (14), 409 (40), 396 (6), 367 (10), 354 (31), 302 (8), 301 (14), 284 (59), 283 (100), 269 (23), 255 (11), 249 (9), 225 (22), 213 (30). NMR, δ 0.73 (18-CH₃), 1.08 (19-CH₃).

2 β ,3 β ,14 α -Trihydroxy-(24R)-5 α -stigmast-7-en-6-one --m/e (relative intensity): 460 (2), 442 (11), 425 (16), 424 (41), 409 (20), 302 (14), 301 (28), 284 (60), 283 (100), 269 (19), 255 (7), 249 (6), 225 (6), 213 (7). NMR, δ 0.73 (18-CH₃), 1.38 (19-CH₃).

Derivatives of 2 β ,3 β ,14 α -Trihydroxy-5 β -cholest-7-en-6-one (XII)

2 β ,3 β -Dihydroxy-5 β -cholesta-7,14-dien-6-one (XX) --To a solution of 400 mg of XII in 6.3 ml of dry pyridine at 0° was added 1.3 ml of trifluoroacetic anhydride, and the reaction mixture was allowed to stand at room temperature overnight. The solution was poured into cracked ice and water, and the precipitate was collected and dried. The material was chromatographed over 15 g of benzene washed-Unisil. The following 100-ml fractions were collected: 1, benzene; 2-4, ether; and 5, ether-methanol (95:5). On the basis of TLC analyses fractions 3 and 4 combined and recrystallized from ether-hexane gave 250 mg of XX, m.p. 196-197°, α_D -61°, λ max 300 m μ in methanol, ϵ 13,400.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.11; H, 10.15.

14 ξ , 15 ξ -Epoxy-2 β , 3 β -dihydroxy-5 β -cholest-7-en-6-one (XXI) -- To 186 mg of XX in 10 ml of chloroform at 0° was added 163 mg of m-chloroperbenzoic acid, and the mixture was kept at 0° for 30 min and then poured into ice and 2% solution of sodium bicarbonate. The chloroform phase was separated from the aqueous phase, washed with water, dried over sodium sulfate, and concentrated to dryness in vacuo. Recrystallization of the residue from ether-hexane gave 150 mg of XXI as rectangular plates, m.p. 189-191°, α_D -32°, λ max 240 μ in methanol, ϵ 11,000.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.20; H, 9.75.

14 α -Hydroxy-2 β , 3 β -bis(trimethylsiloxy)-5 β -cholest-7-en-6-one (XXII) -- A mixture of 300 mg of XII, 6 ml of pyridine, 1 ml of N,N-bis(trimethylsilyl) acetamide was allowed to stand 18 hr at 65°. Solvent, excess reagent, and reagent by product was removed at reduced pressure at 50°. Recrystallization of the residue from acetonitrile gave 269 mg of XXII, m.p. 187-188°, λ max 238 μ in hexane, ϵ 12,000, α_D +64°, ν max in CS_2 3595, and 3450 cm^{-1} (hydroxyl), 1660 (carbonyl) 1623 cm^{-1} (double bond); second crop, 64 mg, m.p. 186-187°.

Anal. Calcd. for $C_{33}H_{60}O_4Si_2$: C, 68.69; H 10.48. Found: C, 68.23; H, 10.19.

2 β , 3 β , 14 α -tris(trimethylsiloxy)-5 β -cholest-7-en-6-one (XXIII) - A mixture of 200 mg of XII, 5 ml of dimethylformamide, and 1.2 ml of N,N-bis(trimethylsilyl) acetamide was allowed to stand at 80° for 18 hr. (17). Solvent was removed at 70° with a stream of nitrogen. A TLC of the residue showed a major spot that was less polar than the bis(trimethylsilyl) ether derivative (XXII). The compound was chromatographed over hexane washed Unisil and eluted from the column with benzene-hexane (1:1). The fractions monitored by TLC gave 257 mg of noncrystalline XXIII, α_D +65°, λ max in hexane 238 μ , ϵ 11,200, ν max in CS_2 1670 (carbonyl), 1625 cm^{-1} (double bond), mass spectrum, m/e (rel. intensity) (M^+) 648 (62), 633 (18), 630 (9), 620 (43), 558 (43), 530 (14), 453 (10), 441 (17), 348 (21), 235 (27), 147 (100).

2 β , 3 β , 14 α -Trihydroxy-5 β -cholest-7-en-6-one 2 β , 3 β -diacetate (XXIV) -- A mixture of 200 mg of XII, 2 ml of pyridine, and 0.6 ml of acetic anhydride, after standing overnight at 80°, yielded 200 mg of XXIV after recrystallization from dilute methanol, m.p. 171-173°, α_D +76°, λ max in methanol 244 μ , ϵ 12,500.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.30; H, 9.28.

2 β ,3 β ,14 α -Trihydroxy-5 β -cholest-7-en-6-one 2 β -acetate (XXV) - A mixture of 100 mg of XII, 3 ml of pyridine, and 0.5 ml of acetic anhydride stood at 0° for 2 hr. The solution was then diluted with water, and the precipitate was collected and dried. By TLC, the material showed some diacetoxy compound and the mixture was chromatographed over 6 g of benzene-washed Unisil. The diacetoxy compound (XXIV) was eluted with benzene-chloroform (1:1) and the monoacetate with chloroform - 1% ethanol. Recrystallization of the monoacetate from dilute methanol gave 95 mg of XXV, m.p. 214-217°, α_D +71°.

3 β ,14 α -Dihydroxy-2 β -methoxy-5 β -cholest-7-en-6-one (XXVI) -- A mixture of 200 mg of XII, 12 ml of dimethylformamide, 2 g of lithium carbonate, and 1.0 ml of dimethyl sulfate was stirred at room temperature for 72 hr. The solution was filtered, and the filtrate was diluted with cold water and the precipitate was collected, washed with water, and dried. The material was chromatographed over 6 g of benzene washed-Unisil. The 2 β -methoxy compound was eluted from the column with benzene-chloroform (1:1). A formate derivative of XII was eluted with chloroform, and unreacted XII was eluted with chloroform-methanol (9:1). Recrystallization of the 2 β -methoxy compound (XXVI) from dilute methanol yielded 90 mg of rectangular plates, m.p. 170-171°, α_D +66°, λ max in methanol 244 μ , ϵ 12,700, NMR one methoxy group at 3.33 ppm.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 74.98; H, 10.18.

2 β ,14 α -Dihydroxy-3 β -methoxy-5 β -cholest-7-en-6-one (XXVII) -- A mixture of 500 mg of 2 β ,3 β ,14 α -trihydroxy-5 α -cholest-7-en-6-one (XI), 25 ml of dimethylformamide, 3 ml of dimethyl sulfate, and 6 g of lithium carbonate was reacted and processed as in the preparation of XXVI. Recrystallization from dilute methanol gave 190 mg of the 3 β -methoxy derivative of XI, m.p. 188-191°, α_D +48°. The 190 mg of material was isomerized by heating at 50° in 15 ml of methanol, 1.5 ml of water, and 300 mg of potassium carbonate for 30 min. The solution was diluted with water, and the precipitate was collected, dried and separated by preparative TLC (solvent system, benzene-ethyl acetate (1:1)). The upper zone that moved faster than the 3 β -methoxy derivative of XI was separated and recrystallized from dilute acetone to give 80 mg of the 3 β -methoxy-5 β -compound (XXVII), m.p. 190-193° λ max 245 μ in methanol, ϵ 12,100, ν max in CS_2 3595, and 3475 cm^{-1} (hydroxyl) 1670 (carbonyl) and 1618 cm^{-1} (double bond). The 2 β -methoxy-5 β -compound (XXVI) and the 3 β -methoxy-5 β -derivative (XXVII) had similar R_f values and infrared spectra.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38. Found: C, 75.03; H, 10.00.

2 β ,3 β ,14 α -Trihydroxy-5 β -cholest-7-en-6-one 2 β ,3 β -dimethanesulfonate (XXVIII) --To a solution of 200 mg of XII and 10 ml of pyridine at 0° was added 2 ml of methanesulfonyl chloride. The mixture was allowed to stand at 0° for 2 hr and was poured into ice and water. The precipitate was collected, washed with water, and dried. Recrystallization from acetone-hexane gave 185 mg of XXVIII, m.p. 153-155°, α_D +73°, λ max in methanol 245 μ , ϵ 12,800, ν max in Nujol 3475 cm^{-1} (hydroxyl), 1655 (carbonyl), and 1618 cm^{-1} (double bond).

Anal. Calcd. for $C_{29}H_{48}O_8S_2$: C, 62.56; H, 8.69. Found: C, 62.40; H, 8.46.

2 β ,3 β -14 α -Trihydroxy-5 β -cholest-7-en-6-one 2 β ,3 β -acetonide (XXIX) - A mixture of 200 mg of XII, 20 mg of p-toluenesulfonic acid, and 25 ml of acetone was refluxed for 1 hr. The solution was poured into an ice cold 2% solution of sodium bicarbonate, and the precipitate was collected, and dried. Recrystallization from hexane gave 160 mg of XXIX, m.p. 184-186°, λ max 242 μ in methanol, ϵ 12,100. (Lit. (3) m.p. 182-184.5°, ϵ 12,600).

5 β -cholest-7-en-2 β ,3 β ,6 β ,14 α -tetrol (XXX) --A suspension of 1 g of lithium aluminum hydride, 200 mg of XII, and 75 ml of tetrahydrofuran was refluxed for 2 hr. The excess lithium aluminium hydride was destroyed by treating the mixture with ethyl acetate, and then with water. Dilute hydrochloric acid was added until the solution was slightly acidic. The solution was filtered, and the filtrate was reduced in volume and diluted with water, and the precipitate was collected. TLC analyses showed some faster moving material and, two components (probably the 6 α and 6 β -hydroxy isomers) that showed very little difference in Rf values but had lower Rf values than XII. The compounds were separated on a Unisil column and the lower zone (the major component) was obtained pure by eluting with chloroform - 95% ethanol (10:1). Recrystallization from acetone gave fine needles, m.p. 133-135°, α_D +20.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.38; H, 10.70.

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11. Carbon and hydrogen analyses were obtained on all of the intermediates and final products and were in agreement with the theoretical calculated values.
12. Chromatography of mother liquors of intermediates VI, VIIa, IX, and X improved the overall yield by only 2%.

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