THE CONVERSION OF HYODESOXYCHOLIC ACID TO PROGESTERONE[1]

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ABSTRACT

A novel transformation of 3α,6α-dihydroxy steroids to their 3β-hydroxy-Δ⁵ analogues was developed and applied to the preparation of progesterone from hyodesoxycholic acid.

Hyodesoxycholic acid was first isolated from hog bile by Windaus (17) who showed it to be a 3,6-dihydroxycholanic acid. The configurations of the two hydroxyl groups however were not elucidated until recently when they were assigned the α-positions (10). Since then this bile acid has not received a great deal of attention despite the fact that it represents a by-product of packing house operations. One of the reasons for this lack of interest has been undoubtedly the difficulty of its isolation from the crude animal bile, and only recently have adequate isolation procedures been worked out (2, 14). As a result, hyodesoxycholic acid is now a readily accessible material and it was the object of our work to utilize this bile acid for the preparation of physiologically active steroids.

Two major changes must be performed in order to convert methyl hyodesoxycholate (I*a*) to progesterone (XII). Firstly the side chain has to be degraded with loss of three carbon atoms and secondly the 3α,6α-dihydroxy grouping has to be transformed to the 3-keto-Δ⁴ structure.

The side chain of I has previously been degraded by the classical Barbier–Wieland method to provide 3,6-dihydroxy-pregnan-20-one (7, 9). Later on, the procedure of Meystre and Miescher was applied to the same problem and resulted in greatly improved yields (6, 11). Our experiments were designed to improve this latter procedure, and at the same time the intermediate products were isolated and characterized. One of the modifications developed in the course of this work concerned the reaction of III*b* with N-bromosuccinimide which in the past was carried out in carbon tetrachloride with a light catalyst. This is hazardous on a large scale and also requires the installation of expensive equipment. It was found that a chemical free radical promoter such as diacetyl peroxide or α,α′-azo-diisobutyronitrile could be used instead of the light catalyst. The use of hydrocarbon solvents was found to be much superior to carbon tetrachloride since the brominated product was quite stable in hexane or commercial hydrocarbon mixtures in contrast to the chlorinated solvents. Various other modifications, of a minor nature, are described in the experimental section but it may be pointed out that these have significantly contributed to greatly enhanced yields (55% of VIII*a* from I*a*).

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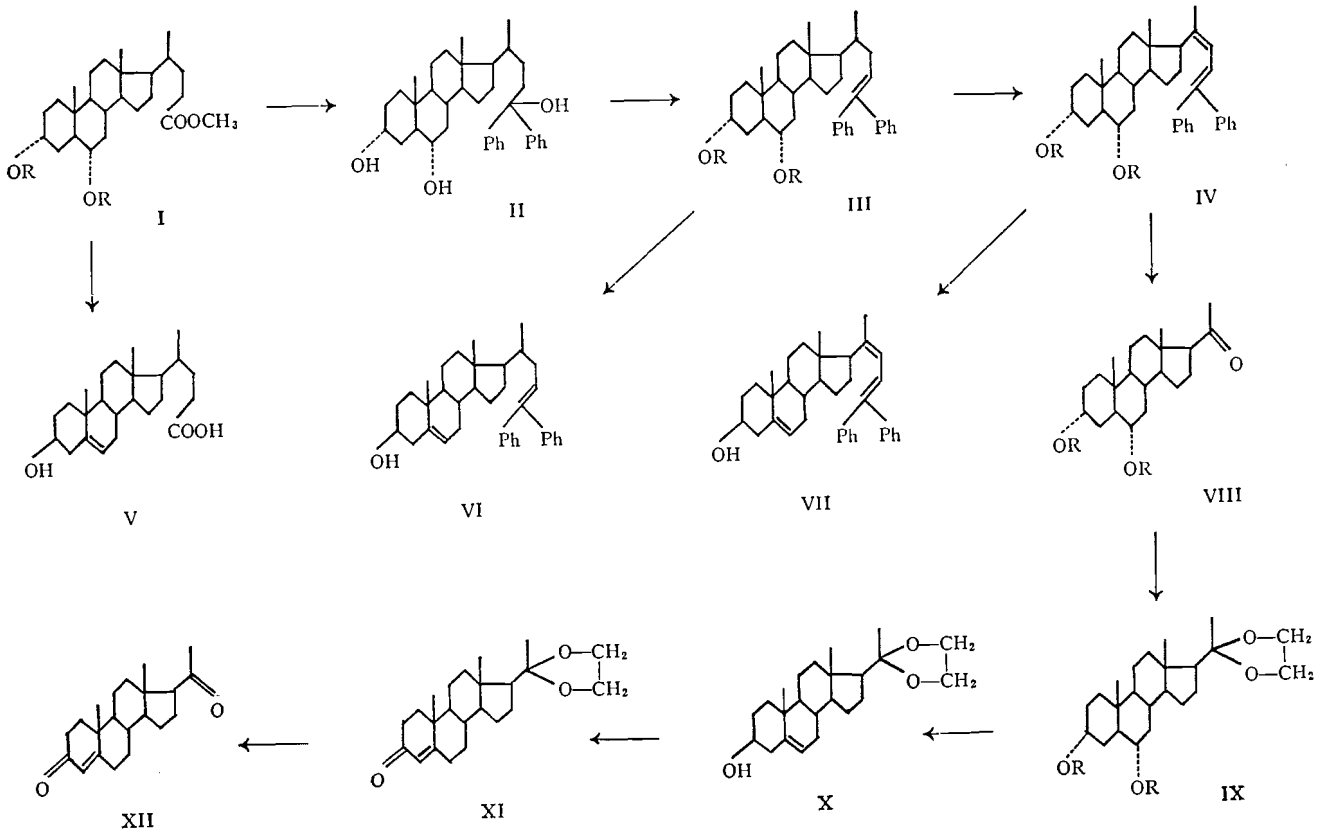

a, R = H *b*, R = Ac *c*, R = CH_3—C$_6$H$_4$—SO_2—

It then remained to develop a method whereby the 3α,6α-dihydroxy grouping could be converted in good yield to the 3-keto-Δ^4 analogue. The earliest attempts in this direction had been made by Marker (8, 9) who obtained progesterone by preferential hydrolysis of 3α,6α-diacetoxy-pregnan-20-one (VIII*b*) to the corresponding 3α-hydroxy-6α-acetoxy compound, followed by oxidation and dehydration. This was confirmed (4) when a small amount of 3-keto-6α-hydroxycholanic acid was isolated from I*b* by partial hydrolysis and subsequent oxidation. Several investigators then turned their attention to the use of preferential oxidations which would directly give 3-keto-6α-hydroxy steroids which in turn could be dehydrated readily. These oxidations were carried out by using Raney nickel with cyclohexanone (5, 6), aluminum tertiary butoxide and acetone (4), aluminum phenylate and acetone (5, 16), and N-bromosuccinimide in aqueous acetone (12, 13). Without exception the 3-keto-6-hydroxy derivative was accompanied by 3,6-diketo compounds and by unreacted starting material, and in most cases chromatography had to be resorted to in order to isolate the desired product. The 3-keto-6-hydroxy steroid was then transformed to the corresponding 3-keto-Δ^4 analogue by tosylation and dehydrotosylation (4, 5, 6) or by treatment with phosphorus oxychloride in pyridine (12, 13). Yamasaki (18, 19) then discovered that methyl hyodesoxycholate could be dehydrated to 3β-chloro-Δ^5-cholenic acid by the action of phosphorus oxychloride; treatment with potassium acetate, followed by saponification, provided 3β-hydroxy-Δ^5-cholenic acid. Two alternative methods for the preparation of 3β-acetoxy-Δ^5 steroids from the corresponding 3α,6α-ditosylates by treatment with silver acetate in acetic acid (1) or potassium acetate in acetic anhydride (15) have recently been reported. The yields are 30% and 45% respectively.

The approach that was used in our laboratories was similar to the methods referred to above (1, 15). We first tosylated methyl hyodesoxycholate to give quantitatively the corresponding ditosylate (I*c*). When the latter was refluxed with metal acetates in acetic acid it yielded, after saponification, 30% of V. When the dehydrotosylation was carried out in a neutral solvent such as acetic anhydride or aqueous acetone the yield of V increased to 50%. In these reactions there was invariably obtained a large amount of heteroannular dienes as shown by their ultraviolet absorption at 235 mμ. An improved procedure which we reported in an earlier note (20) employed a reaction medium of potassium acetate in aqueous dimethylformamide and in this way a marked increase in yield (70–75%) was achieved. The same results were obtained when N-methyl-pyrrolidone or N,N-dimethylacetamide was used. This method was then applied successfully to the products derived from the side-chain degradation of hyodesoxycholic acid and the desired 3β-hydroxy-Δ^5 steroids were isolated by crystallization. However, when 3α,6α-ditosyloxy-pregnan-20-one (VIII*c*) was treated accordingly the products had to be chromatographed to provide pure pregnenolone and besides the yield was lower than expected. This was due to the alkaline conditions prevailing during the dehydrotosylation reaction which probably cause racemization at C-17 of the steroid nucleus. To overcome this difficulty the keto group in VIII*a* was protected by formation

of the ethylene ketal IX*a*; the latter was then tosylated and dehydrotosylated in the usual manner, the 20-ketone group being regenerated by mild acid hydrolysis either before or after the Oppenauer oxidation. In this fashion progesterone was obtained from hyodesoxycholic acid in 30–35% yield.

During the dehydrotosylation of 3α,6α-ditosyloxy steroids, having a ring A/B *cis* junction, several reactions take place. There is an S_N2 mechanism operative at C-3 as evidenced by complete inversion at this location—a careful chromatography of the products failed to reveal any traces of the 3α-epimer. The formation of the double bond in ring B is due to an elimination reaction of the E1 type. As stated before there is always obtained a quantity of heteroannular diene which results from elimination both at C-6 and at C-3. It is interesting to observe that as the reaction medium is changed from acetic acid to acetic anhydride to aqueous dimethylformamide, the amount of 3β-hydroxy-Δ^5 steroid increases while the yield of diene decreases. The ratio of these products depends entirely on the relative rates of substitution and elimination. Since the 3α-tosyloxy group in compounds such as *Ic* is equatorial and therefore not inclined to ionic elimination, the S_N2 mechanism at C-3 prevails as long as ring B remains saturated. If, however, elimination at C-6 is the first event leading to formation of the double bond between C-5 and C-6, a 3α-tosyloxy-Δ^5 compound is produced as an intermediate. The latter has the substituent at C-3 in the axial configuration and is therefore readily converted to the heteroannular diene. This has been clearly demonstrated in the case of epicholesterol tosylate (3) which on acetolysis affords quantitatively cholestadiene. It is thus obvious that increased yields of 3β-hydroxy-Δ^5 compounds must be due to an increased rate of S_N2 at C-3 or, in other words, due to a relative decreased rate of E1 at C-6. Changing the solvents as indicated above provides a reaction medium that becomes progressively more alkaline; it is well known that unimolecular eliminations proceed more readily in acid solution mainly because ionization or carbonium ion formation is the result of electrophilic attack. The increased yield of 3β-hydroxy-Δ^5 steroid is therefore ascribed to a suppression in the rate of elimination at C-6 in the strongly basic medium of aqueous dimethylformamide containing potassium acetate. Other factors such as dielectric constant of the solvent, temperature, and steric considerations enter undoubtedly into the mechanism of the reaction but it is believed that the pH of the medium is the most important contribution.

EXPERIMENTAL[2]

3α,6α,24-Trihydroxy-24,24-diphenyl-cholane (II)

A Grignard reagent was prepared from magnesium turnings (19.2 gm.) and bromobenzene (85 ml.) in tetrahydrofuran (150 ml.). There was then added a solution of methyl hyodesoxycholate benzene complex (25 gm.) in tetrahydrofuran (175 ml.) and the mixture was refluxed for 15 hr. with constant stirring. The solution was poured into ice-cold, dilute acid and the resulting precipitate was filtered off, washed with water and toluene to yield 26.2 gm. of material,

[2]*The microanalyses were kindly performed by Mr. E. Thommen, Basel, Switzerland.*

m.p. 194–201°C. The toluene extract was evaporated to dryness and the residue crystallized from acetone to provide an additional 3.2 gm. of product, m.p. 133° and 204°C. Recrystallization from benzene gave II, m.p. 204.5–206.5°C.

3α,6α-Diacetoxy-24,24-diphenyl-Δ²³-cholene (IIIb)

Compound II (8 gm.) was refluxed for five hours with acetic acid (40 ml.) and acetic anhydride (8 ml.). The solution was then poured into water and the precipitate was filtered off, washed, and dried to provide III*b* (8.93 gm.). This material was recrystallized first from aqueous acetone then from methanol to give crystals, m.p. 122–124°C. and $[\alpha]_D^{26}$ +43.6° (*c*, 1.334, dioxane).

3α,6α-Dihydroxy-24,24-diphenyl-Δ²³-cholene (IIIa)

Hydrolysis of III*b* was carried out by refluxing with 4% methanolic potassium hydroxide for two hours. The solution was poured into water and the precipitate, after being washed and dried, was recrystallized from aqueous methanol to yield nearly quantitatively III*a*, m.p. 184–186°C., $[\alpha]_D^{26}$ +36.8° (*c*, 0.891, dioxane) and $E_{1cm}^{1\%}$ 305 (λ_{max} 250 mμ).

3α,6α-Ditosyloxy-24,24-diphenyl-Δ²³-cholene (IIIc)

Compound III*a* (5 gm.) was dissolved in pyridine (20 ml.) and tosyl chloride (5.7 gm.) was added. After 48 hr. at 20°C. the excess tosyl chloride was decomposed by addition of water (2 ml.), the solution was then poured into dilute acid and extracted with ether. The solvent extract was washed with dilute acid, then with water, dried over sodium sulphate, and evaporated to a small volume. Crystallization provided 7.4 gm. of III*c*, m.p. 130–132°C. Three crystallizations from ether and iso-octane gave material, m.p. 133–135°C. and $[\alpha]_D^{23}$ +30.6° (*c*, 1.425, dioxane). Analysis: Calcd. for $C_{50}H_{60}O_6S_2$: C, 73.13; H, 7.37; S, 7.81. Found: C, 72.98, 73.00; H, 7.46, 7.52; S, 8.04, 7.99.

3α,6α-Diacetoxy-24,24-diphenyl-Δ²⁰,²³-choladiene (IVb)

Compound III*b* (5 gm.) was dissolved in hexane (60 ml.) and there was then added sodium bicarbonate (0.9 gm.), N-bromosuccinimide (1.8 gm.), and α,α′-azo-diisobutyronitrile (150 mgm.) or a 25% solution of diacetyl peroxide in dimethyl phthalate (0.2 ml.). This mixture was refluxed for one hour while being stirred vigorously. The precipitated succinimide was then removed by filtration and the filtrate added to acetic acid (55 ml.) containing sodium acetate (5 gm.). After removal of hexane by distillation, the solution was refluxed for one-half hour and finally poured into water to provide, after filtration and drying, 4.96 gm. of crude IV*b*. This material had $E_{1cm}^{1\%}$ 398 (λ_{max} 306 mμ), indicating a yield of 85.6%. Repeated recrystallization from methanol and ethyl acetate afforded pure IV*b*, m.p. 125–126°C., $[\alpha]_D^{24}$ +49.2° (*c*, 2.344, chloroform), $[\alpha]_D^{24}$ +35.1° (*c*, 3.126, dioxane), and $E_{1cm}^{1\%}$ 462 (λ_{max} 306 mμ). This compound gave a green color with trichloroacetic acid, a deep brown with tetranitromethane, and a wine-red in the Liebermann–Burchard reaction. Analysis: Calcd. for $C_{40}H_{50}O_4$: C, 80.77; H, 8.47. Found: C, 80.85, 80.83; H, 8.79, 8.72.

3α,6α-Dihydroxy-24,24-diphenyl-Δ^{20,23}-choladiene (*IVa*)

The diacetate (IV*b*) was saponified by refluxing with 4% methanolic potassium hydroxide. Repeated alternate recrystallization from benzene and aqueous methanol yielded IV*a*, m.p. 211.5–216°C., $[\alpha]_D^{24}$ +48.5° (*c*, 2.579, chloroform) and $E_{1cm}^{1\%}$ 555 (λ_{max} 306 mμ). Analysis: Calcd. for $C_{36}H_{46}O_2$: C, 84.66; H, 9.08. Found: C, 84.71, 84.70; H, 9.08, 9.10.

3α,6α-Ditosyloxy-24,24-diphenyl-Δ^{20,23}-choladiene (*IVc*)

Compound IV*a* (2 gm.) dissolved in pyridine (10 ml.) was treated with tosyl chloride (1.8 gm.). After 48 hr. at 20°C., the excess reagent was decomposed by addition of ice, the mixture was poured into dilute acid and extracted with chloroform. The solvent extract was washed, dried, and evaporated leaving a residue which crystallized from ether to give 2.9 gm. (91%) of IV*c*, m.p. 149–151°C. Two further recrystallizations from ether gave material, m.p. 156–157°C. (decomp.) and $[\alpha]_D^{20}$ +26.2° (*c*, 1.44, dioxane). Analysis: Calcd. for $C_{50}H_{58}O_6S_2$: C, 73.31; H, 7.14; S, 7.83. Found: C, 73.26, 73.20; H, 7.19, 7.23; S, 8.05, 7.99.

3α,6α-Dihydroxy-pregnan-20-one (*VIIIa*)

A solution of IV*b* (17.7 gm.) in chloroform (200 ml.) and 80% acetic acid (220 ml.) was cooled to 0°C. and there was then added within one-half hour 80% acetic acid (200 ml.) containing chromic acid (12.3 gm.). The solution was stirred at 0°C. for four hours, the excess oxidizing agent was destroyed by addition of sodium bisulphite, and the solvents were evaporated *in vacuo*. The residue was diluted with water and extracted with ether, the solvent extract was washed and taken to dryness.

Hydrolysis of the residue with methanol (300 ml.) and concentrated hydrochloric acid (5 ml.) at 20°C. for 14 hr. was followed by neutralization with sodium bicarbonate. The methanol was evaporated and the residue taken up in ether–chloroform and saturated salt solution. The solvent extract was washed once more with salt solution, dried, and evaporated. The residue was dissolved in hot benzene (100 ml.), the solution cooled, the precipitate filtered off and dried to provide 7.82 gm. (78%) of crystals, m.p. 90–95° and 179–181°C. Recrystallization from ethyl acetate afforded VIII*a*, m.p. 192–193°C. and $[\alpha]_D^{24}$ +69.7° (*c*, 1.597, dioxane). Analysis: Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.26, 75.23; H, 10.30, 10.37.

3α,6α-Ditosyloxy-pregnan-20-one (*VIIIc*)

Compound VIII*a* (2.5 gm.) was treated in the usual manner with pyridine (10 ml.) and tosyl chloride (4.2 gm.). The reaction mixture, after addition of dilute acid, was extracted with ether and the solvent extract worked up to give a residue which crystallized from ether to afford VIII*c* (3.93 gm.; 82%), m.p. 140–142°C. Two further recrystallizations from ether gave material, m.p. 147–148°C. and $[\alpha]_D^{24}$ +34° (*c*, 1.342, dioxane). Analysis: Calcd. for $C_{35}H_{46}O_7S_2$: C, 65.39; H, 7.21; S, 9.98. Found: C, 65.45, 65.43; H, 7.37, 7.32; S, 9.69, 9.75.

3α,6α-Dihydroxy-pregnan-20-one Ethylene Ketal (IXa)

The ketone VIII*a* (3.06 gm.) was dissolved in dry benzene (100 ml.) and ethylene glycol (6 ml.). After addition of *p*-toluenesulphonic acid monohydrate (150 mgm.), the mixture was stirred and refluxed for four hours, during which time water was continually removed by the use of a Dean-Stark trap. On cooling, saturated sodium bicarbonate solution (2 ml.) was added and the solvents were evaporated *in vacuo*. The solid residue was shaken with saturated salt solution, filtered off, washed with water, and dried to furnish 3.34 gm. (98%) of material, m.p. 218–228°C. Recrystallization from methanol afforded pure IX*a*, m.p. 227–228°C. and $[\alpha]_D^{26}$ +6.44° (*c*, 1.63, pyridine). Analysis: Calcd. for $C_{23}H_{38}O_4$: C, 72.97; H, 10.11. Found: C, 72.81, 72.86; H, 10.07, 10.03.

Acetylation of IX*a* provided the corresponding diacetate (IX*b*), crystallized from aqueous methanol, m.p. 125–132°C. Two recrystallizations from the same solvent yielded material, m.p. 139–140°C. and $[\alpha]_D^{24}$ +15.03° (*c*, 1.55, pyridine). Analysis: Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.84, 70.04; H, 9.32, 9.40.

3α,6α-Ditosyloxy-pregnan-20-one Ethylene Ketal (IXc)

Tosylation of IX*a* (0.9 gm.) with pyridine (6 ml.) and tosyl chloride (1.4 gm.) was carried out as described above. The products were taken up in ethylene dichloride and the solvent extract was washed, dried, and evaporated. From ether, crystals of IX*c* (1.42 gm.; 87%), m.p. 147–148°C., were deposited. Crystallization from benzene–hexane raised the m.p. to 151.5–152°C. and this sample then had $[\alpha]_D^{24}$ +5.21° (*c*, 1.515, pyridine). Analysis: Calcd. for $C_{37}H_{50}O_8S_2$: C, 64.72; H, 7.28; S, 9.32. Found: C, 64.60, 64.59; H, 7.58, 7.35; S, 9.16.

Pregnenolone Ethylene Ketal (X)

Compound IX*c* (8 gm.) was dissolved in dimethylformamide (85 ml.) and treated with a solution of potassium acetate (12 gm.) in water (20 ml.). The solution was kept at 95°C. for four hours. After complete removal of the solvents *in vacuo*, the residue was saponified by refluxing with methanolic potassium hydroxide. Part of the methanol was evaporated, the residue was diluted with water, cooled, and the resulting precipitate was filtered off. Thorough washing and drying gave a residue (4.38 gm.), m.p. 107–125°C. This was recrystallized repeatedly from methanol containing a few drops of pyridine to yield a pure sample of X, m.p. 164–167°C. and $[\alpha]_D^{24}$ −30.6° (*c*, 1.86, pyridine). Analysis: Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.62, 76.54; H, 10.16, 10.17.

Progesterone 20-Ethylene Ketal (XI)

A solution of X (3.5 gm.) in toluene (100 ml.) and cyclohexanone (30 ml.) was distilled briefly to remove traces of moisture and, after addition of aluminum isopropoxide (2 gm.), the solution was refluxed for one hour. The mixture was treated with water (20 ml.), the solvents were removed *in vacuo*, and the residue was taken up in benzene. The solvent extract was filtered and

evaporated to give a solid which was leached with hexane and filtered off to yield 3.25 gm. (93%) of crude XI, m.p. 184–189°C. Recrystallization from ethyl acetate provided material, m.p. 189–191°C., $[\alpha]_D^{24}$ +97.5° (*c*, 1.13, pyridine), and $E_{1cm}^{1\%}$ 447 (λ_{max} 242 mμ). Analysis: Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.04, 77.11; H, 9.61, 9.59.

Progesterone (XII)

Compound XI (2 gm.) was dissolved in acetone (30 ml.) and water (3 ml.) by warming briefly and, after addition of *p*-toluenesulphonic acid (100 mgm.), the solution was kept at 20°C. for 15 hr. The acetone was then evaporated, the residue was treated with a dilute solution of sodium bicarbonate, and the precipitate was filtered off, washed with water, and dried. This product (1.82 gm.), m.p. 117–128°C., was crystallized from aqueous acetone to provide progesterone, m.p. 131–133°C., $[\alpha]_D^{24}$ +177.2° (*c*, 2.5, dioxane).

Methyl Hyodesoxycholate Ditosylate (Ic)

Methyl hyodesoxycholate (25 gm.) was dissolved in dry pyridine (50 ml.) and tosyl chloride (28.8 gm.) was added. The solution was kept at room temperature for two days. Subsequently, the mixture was cooled, the excess reagent was decomposed by ice, and the solution was poured into ice-cold, dilute hydrochloric acid. After the mixture was stirred for one-half hour, the precipitate was filtered, washed with water, and dried to yield 43.9 gm. of material. Recrystallization from ethyl acetate afforded 41.6 gm. (95%) of I*c*, m.p. 165–167°C. and $[\alpha]_D^{24}$ +9.8° (*c*, 1.016, dioxane). Analysis: Calcd. for $C_{39}H_{54}O_8S_2$: C, 65.53; H, 7.61; S, 8.97; OCH_3, 4.34. Found: C, 65.60, 65.55; H, 7.68, 7.65; S, 9.19; OCH_3, 4.50.

3β-Hydroxy-Δ⁵-cholenic Acid (V)

A solution of potassium acetate (7.3 gm.) in water (3.5 ml.) and dimethylformamide (40 ml.) was heated to 100–105°C. After addition of I*c* (5 gm.), the solution was kept at the above temperature for five hours. It was then poured into cold, dilute hydrochloric acid and the resulting precipitate was filtered off and washed with water. The wet solid was saponified by refluxing with 4% methanolic potassium hydroxide (70 ml.) for two hours. The solution was poured into dilute acid, the precipitate was filtered, washed, and dried. Recrystallization from ethyl acetate yielded 1.92 gm. (73%) of product, m.p. 220–227°C. Two crystallizations from acetic acid gave pure V, m.p. 230–233°C., giving an insoluble digitonide and a yellow coloration with tetranitromethane. The identity of V was conclusively established by melting point and mixed melting point of the corresponding methyl ester and methyl ester acetate.

The filtrate was evaporated to dryness to leave a residue (0.71 gm.) which was found, by ultraviolet absorption measurement at 235 mμ, to contain 0.3 gm. of choladienic acid.

3β-Hydroxy-24,24-diphenyl-Δ⁵,²³-choladiene (VI)

The dehydrotosylation of III*c* (5 gm.) was carried out under the conditions described in the preceding experiment. After saponification and crystallization of the residue from acetone, there was obtained 1.95 gm. of crystals, m.p.

164–170°C. Recrystallization from the same solvent gave a pure sample of VI, m.p. 176–179°C.

3β-Hydroxy-24,24-diphenyl-$\Delta^{5,20,23}$-cholatriene (VII)

Compound IV*c* (81.5 gm.) was dehydrotosylated in the manner set forth for the preparation of V. Following saponification, the reaction products were crystallized from ethanol to give 38.5 gm. (78%) of VII, m.p. 130–137°C. Recrystallization from hexane, then from ethanol, gave m.p. 157–161°C.

A sample of VII (1 gm.) dissolved in ethyl acetate (15 ml.) was added to a warm solution of anhydrous oxalic acid (0.2 gm.) in ethyl acetate (2 ml.). A precipitate formed at once; after refrigeration, the crystals were filtered off, washed with ethyl acetate, and dried to yield 0.95 gm. of the adduct. Three recrystallizations from ethyl acetate provided a pure sample of the adduct, m.p. 192–195°C. and $[\alpha]_D^{20}$ −14.2° (*c*, 1.01, dioxane). Analysis: Calcd. for $C_{36}H_{44}O.\frac{1}{2}C_2H_2O_4$: C, 82.64; H, 8.44. Found: C, 82.53, 82.63; H, 8.56, 8.63.

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