yield 5.3 g. (65%). Authentic Δ^4-menthenone prepared from Δ^3-menthene nitrosochloride[12] has the following properties: n_D^{23} 1.4710, λ_{max} 236 mμ (log ϵ 3.97).

The dibenzal derivative was prepared by allowing the cleavage product to react with benzaldehyde in absolute ethanol in the presence of sodium ethoxide. The product was recrystallized from ethanol and sublimed, m.p. 138–139°, λ_{max} 275 mμ (log ϵ 4.35) [lit.[13] 140–141°].

Anal. Calcd. for $C_{24}H_{24}O$: C, 87.76; H, 7.36. Found: C, 87.86; H, 7.31.

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Contribution to the Study of Marine Products. XLVI. 24- and 25-Dehydrocholesterol

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24- and 25-dehydrocholesterol have been prepared from 25-ketonorcholesterol. The identity of desmosterol and 24-dehydrocholesterol has been substantiated.

Desmosterol is one of the two new sterols which Stokes, Fish, and Hickey[1] have recently isolated from chick embryos. It shows some superficial resemblance to 25-dehydrocholesterol[2,3] (IIa) but the absence in its infrared spectrum of a strong band at 11.3μ indicated a lack of terminal unsaturation and consequently a difference in the two compounds. On the basis of other chemical evidence, and on good biogenetic grounds, the authors drew the significant conclusion that desmosterol is 24-dehydrocholesterol (IVa), a compound assumed to be one of the final steps in the biosynthesis of cholesterol.

Since it is to be expected that desmosterol will be encountered in many other natural sterol mixtures, its preparation and that of its 25-dehydro-isomer have been included in the program of synthesis of natural sterol now in progress in this laboratory.[4] The 25-dehydrocholesterol (IIa) may readily be prepared from 25-ketonorcholesterol by means of the Wittig reaction. This very useful method was first applied to sterols by Barton, Campos-Neves, and Cookson[5] in their preparation of 3-methylsterols, and more extensively also by Sondheimer and Mechoulian.[6] More recently the method has been used in the synthesis of 24-methylenecholesterol by the present authors[7] and Idler and Fagerlund.[8] The latter authors largely anticipated our own observations on the Wittig-type synthesis of 25-dehydrocholesterol (IIa). In the present approach 25-ketonorcholesterol (Ia) was first converted by a transpyranylation reaction into the ether (Ic) which afforded the corresponding 25-dehydrocholesterol derivative (IIc) when treated with the required Wittig reagent. To prove its structure, the sterol (IIa) was converted to cholestanol and to 25-ketonorcholesterol (Ia). In the latter conversion the double bond of the sidechain was first selectively hydroxylated with osmium tetroxide, and the resulting glycol cleaved with periodic acid according to procedures previously described.[7] The properties of 25-dehydrocholesterol are in close agreement with those reported by Idler and Fagerlund.[8]

Compounds assigned the structure of 25-dehydrocholesterol had first been prepared by the direct or indirect dehydration of 25-hydroxycholesterol (IIIa).[2,3] The presence in these preparations of terminal unsaturation was well substantiated through spectrographic evidence by the original authors and subsequently by Stokes.[1] Idler and Fagerlund,[8] however, did not observe the characteristic infrared band at 11.3 μ, and concluded that such preparations contained little if any of 25-dehydrocholesterol (IIa), and consisted essentially of the 24-dehydroisomer (IVa). We have reinvestigated the dehydration of the tertiary alcohol (III) in the hope of finding a method leading mainly if not exclusively to the 24-isomer, *i.e.*, desmosterol (IVa). An analogous elimination has recently been used in the synthesis of lanosterol.[9] When the monoacetate of the tertiary al-

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cohol (IIIb) is treated with phosphorus oxychloride in pyridine, or refluxed with glacial acetic acid, there is obtained a mixture of compounds among which the 25-dehydrocholesterol (IIa) represents at least twenty-five per cent. Hydrolysis of this mixture, and recrystallization of the resulting sterols results in products in which the 25-dehydro-isomer has been enriched. Ozonolysis of such mixtures affords both formaldehyde and acetone, indicating the presence of both the 25- and 24-isomer.

If the dehydration of IIIb is carried out in dioxane containing sulfuric acid a product is obtained which no longer shows absorption at 11.3 μ, and which is therefore devoid of significant amounts of the 25-dehydroisomer (IIa). The recrystallized product was shown to be 24-dehydrocholesterol (IVa) by its hydrogenation to cholestanol and its ozonolysis, which afforded acetone, identified as its 2, 4-dinitrophenylhydrazone. A direct comparison of the melting points and infrared spectra of synthetic 24-dehydrocholesterol and desmosterol from chick embryos proved the identity of the two compounds.

EXPERIMENTAL

All melting points were taken in open capillary tubes with Anschutz thermometers. Optical rotations were determined in a 1-dm. tube with a Rudolph photoelectric polarimeter. The samples were dissolved in 2.0 ml. of chloroform. The infrared spectra were determined in potassium bromide pellets with a Perkin-Elmer Model 21 spectrophotometer. The values were corrected against the spectrum of the atmosphere.

25-Norcholestene-3β-ol-25-one (Ia) (*25-ketonorcholesterol*). A generous sample of this material was obtained through the courtesy of the Schering Corp. Recrystallization afforded large plates, m.p. 115–116° with clearing at 127°; $[\alpha]_D^{25}$ −44.4° (c = 0.81); λ_{max} 2.96, 5.83, 6.00μ; lit., m.p. 126–127° with sintering at 110°;[10] 117–127° and 127–129°.[11]

The *acetate* (Ib) m.p. 140.5–142°; $[\alpha]_D^{27}$ −43.6° (c = 2.09); λ_{max} 5.77, 5.84, 6.00, 7.98μ (lit. m.p. 141.5–142°,[9] m.p. 137.5–138.5°[10]).

25-Norcholesten-25-one-3β-(2′-tetrahydropyranyl)-ether (Ic). A mixture of 25-ketonorcholesterol (Ia) (2.0 g.), 2-methoxytetrahydropyran[12] (20 ml.), and Dowex-50 (H-form, dried at 70° for 24 hr.) (2.0 g.) was kept in a flask protected with a calcium chloride tube. The resin was collected by filtration and washed with ether. The combined solvents were evaporated to dryness, and the residue dissolved in a small amount of hexane and chromatographed on neutral alumina (activity VI). The hexane eluate was evaporated to dryness and the residue recrystallized from methanol; 1.91 g., m.p. 104–106°; $[\alpha]_D^{23}$ −29.7° (c = 1.18); λ_{max} 5.83, 6.00μ (lit. m.p. 104.9–106.2°; $[\alpha]_D^{28}$ −28.6°[13]). Additional material was obtained from the mother liquors.

Δ5,25-Cholestadiene-3β-(2′-tetrahydropyranyl)-ether (IIc). The following operations were carried out in an atmosphere of purified nitrogen and under exclusion of moisture. In a pressure flask was placed triphenylmethylphosphonium bromide (1.31 g.) and anhydrous ether (25 ml.). To this suspension was added 3.9 ml. of a 0.95N butyllithium solution in ether, and the mixture was stirred magnetically until a clear, yellow-orange solution had been obtained. Under suitable precautions,[7] this solution was mixed with a solution of Ic (1.72 g.) in anhydrous ether (50 ml.) which resulted in the immediate formation of a voluminous precipitate. An additional quantity of ether (25 ml.) was added, the mixture stirred for 1 hr., and finally heated under pressure in an oil bath at 65° for 10 hr.

The flask was cooled and the excess reagent destroyed by addition of ordinary ether (U.S.P.), and the suspension filtered through a pad of Celite. The ether was evaporated and the residue dissolved in hexane and chromatographed on a silicic acid–Celite 535 (2:1) column. The pyranyl ether was eluted by benzene-hexane (1:1). Evaporation of the solvent gave the crystalline pyranyl ether (IIc) (1.67 g.); m.p. 124–125°. After several recrystallizations from ethanol it afforded large plates, m.p. 126–127°; $[\alpha]_D^{27}$ −21.4° (c = 1.38); λ_{max} 3.27, 11.30μ.

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.75; H, 10.95.

5,25-Dehydrocholesterol (25-dehydrocholesterol). The pyranyl ether (IIc)(1.67 g.) was dissolved in hexane (75 ml.), and to this solution was added methanol (75 ml.) containing six drops of concentrated hydrochloric acid. The mixture was kept at room temperature with occasional stirring for one hour and then evaporated to dryness *in vacuo*. The crystalline residue was recrystallized from methanol; long needles (1.0 g.), m.p. 132–133.5°; $[\alpha]_D^{24}$ −42.9° (c = 1.42); λ_{max} 2.96, 3.26, 5.65, 6.09, 11.30 μ. Idler and Fagerlund[8] have reported m.p. 132.5°; $[\alpha]_D^{22}$ −40.7°. The acetate was prepared by treatment of the sterol with acetic anhydride in pyridine; m.p. 110.5–112°; $[\alpha]_D^{25}$ −44.5° (c = 2.08); λ_{max} 3.27, 5.75, 6.07, 8.05, 11.27 μ. Idler and Fagerlund[8] have reported m.p. 112°; $[\alpha]_D^{22}$ −44.4°.

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.38; H, 10.73.

Oxidation of 25-dehydrocholesterol. To a solution of 25-dehydrocholesteryl acetate (0.335 g.) in benzene (5 ml.) and a few drops of pyridine was added osmium tetroxide (0.2 g.) in benzene (20 ml.). The black solution was stirred for 5 hr. and then mixed with a solution of sodium sulfite (2 g.) in ethanol (90 ml.) and water (12 ml.). The stirring was continued overnight, the black precipitate was collected on a Celite pad and washed with ethanol. The clear solution was reduced in volume *in vacuo*, poured into water, and the precipitate was extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The white amorphous residue was dissolved in ethanol (20 ml.) and pyridine (4 ml.) and treated with a solution of periodic acid (0.275 g.) in water (1 ml.). After 10 hr. the solution was poured into water, the resulting precipitate extracted with chloroform, and the extract was washed with sodium bicarbonate solution, water, and finally dried over anhydrous sodium sulfate. The extract was evaporated and the residue dissolved in hexane and chromatographed on neutral alumina (activity 1). The fraction eluted with benzene-ether (9:1) was recrystallized from methanol, 0.175 g., m.p. 140–142° which did not change upon further recrystallization; $[\alpha]_D^{25}$ −42.0° (c = 1.14). The material proved to be identical in all respects with the 25-ketonorcholesteryl acetate (Ib) described above. The sterol obtained by hydrolysis of the acetate, m.p. 114–116°, clearing at 127°; $[\alpha]_D^{27}$ −42.4° (c = 1.49) proved to be identical with 25-ketonorcholesterol (Ia).

Hydrogenation of 25-dehydrocholesterol. Catalytic hydrogenation of 25-dehydrocholesterol with a 5% palladium on carbon catalyst in absolute ethanol gave cholestanol, m.p. 141–142°; $[\alpha]_D^{24}$ +20.6° (c = 1.36); acetate, m.p. 109–110°; $[\alpha]_D^{24}$ +11.8 (c = 0.64).

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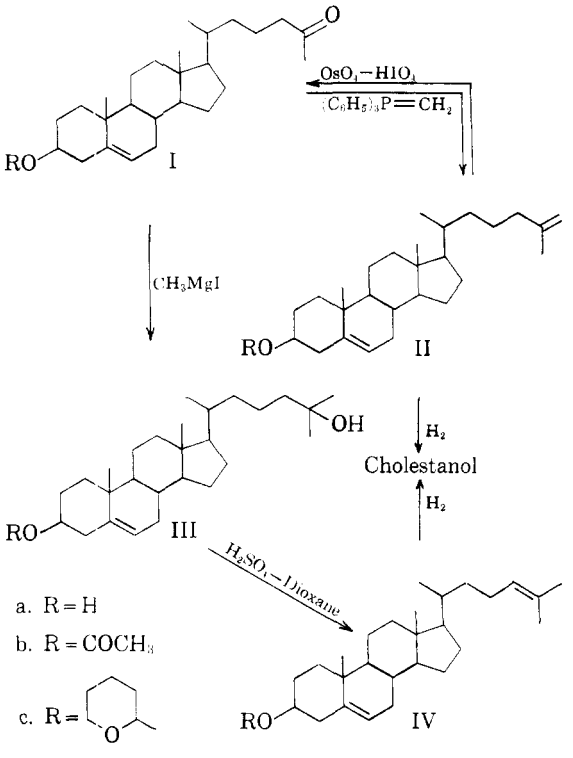

Δ⁵-Cholestene-3β,25-diol (25-hydroxycholesterol) (IIIa). This sterol was prepared by the action of methylmagnesium iodide on either 25-ketonorcholesterol (Ia) or its acetate (Ib) as previously described;[2,3] m.p. 179–180° (lit. m.p. 181.8–182.5°[2]); acetate, m.p. 139.5–140.5°, clearing at 143°; $[\alpha]_D^{25}$ −39.7° (*c* = 1.20); λ_{max} 3.00, 5.76, 8.03, 12.47μ; (lit. m.p. 142–142.8°[2]; $[\alpha]_D^{20}$ −40.4°).

Δ⁵,²⁴-Cholestadiene-3β-ol (24-dehydrocholesterol, desmosterol) (IVa). A solution of 25-hydroxycholesteryl acetate (IIIb) (1.00 g.) in 10% sulfuric acid–dioxane (wt./wt.) (225 ml.) was allowed to stand overnight at room temperature. The solution was then poured into water, the acid neutralized by addition of solid sodium bicarbonate, and the precipitate extracted with ether. The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude residue was reacetylated with acetic anhydride (10 ml.) and pyridine (10 ml.) at 100° for one hour, and the crude acetate dissolved in hexane and chromatographed on neutral alumina (activity II). The fraction eluted by hexane-benzene (9:1) was recrystallized several times from methanol; 0.35 g. of large plates, m.p. 92.5–93°;[14] $[\alpha]_D^{25}$ −40.6° (*c* = 0.9); λ_{max} 5.76, 7.32, 8.02, 12.45μ.

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.51; H, 10.95.

The acetate was hydrolyzed by aqueous alcoholic potassium hydroxide to afford 24-dehydrocholesterol (desmosterol), m.p. 120.5–121°; $[\alpha]_D^{25}$ −39.2 (*c* = 1.71); λ_{max} 2.96, 7.28, 12.51μ. Desmosterol,[1] m.p. 120.8–121.2°, $[\alpha]_D^{27}$ −40.2°.

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.12; H, 11.56.

The compound proved identical with the desmosterol isolated from chick embryos.[1]

Hydrogenation of 24-dehydrocholesterol acetate. Hydrogenation of IVb with a platinum oxide catalyst in glacial acetic acid gave cholestanyl acetate, m.p. 108–109°, which afforded cholestanol upon hydrolysis, m.p. 139–140°. The compounds gave no depression of melting points with authentic samples and showed identical infrared spectra.

Ozonolysis of 24-dehydrocholesteryl acetate. A stream of ozone (6%) was passed for 15 min. through a solution of the acetate (0.15 g.) in highly purified glacial acetic acid (20 ml.). The gases leaving the solution were passed through a scrubber containing water (50 ml.). At the end of the reaction the water and acetic acid solution were combined, diluted with more water (50 ml.), and subjected to steam distillation. The distillate (50 ml.) was added to a solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. After standing overnight the precipitate (38 mg.) was collected, dissolved in benzene, and the solution passed through an alumina column. Evaporation of the solvent and recrystallization of the residue from ethanol gave acetone 2,4-dinitrophenylhydrazone, m.p. 124–125°, which did not depress the melting point of authentic material and gave the same infrared spectrum as the reference compound.

Other dehydrations of 25-hydroxycholesteryl acetate. A solution of 25-hydroxycholesteryl acetate (0.51 g.) in pyridine (15 ml.) was refluxed with freshly distilled phosphorus oxychloride for 0.5 hr., the solution cooled, poured on ice, and extracted with ether. The extract was washed with dilute hydrochloric acid, water, dried, and evaporated to dryness. The residue was dissolved in hexane, the solution passed through a neutral alumina column (activity VI) and the hexane eluate recrystallized from methanol; 0.345 g. of plates, m.p. 91–93° $[\alpha]_D^{25}$ −42.6° (*c* = 1.05). The infrared spectrum of this material indicated the presence of at least 25% of 25-dehydrocholesteryl acetate (IIb). Hydrolysis of the acetate gave a sterol mixture, m.p. 121.5–122°; $[\alpha]_D^{25}$ −40.9° (*c* = 1.08) in which the 25-isomer had been enriched to more than 50%. A similar mixture was obtained when 25-hydroxycholesteryl acetate was refluxed for 12 hr. with glacial acetic acid.

The mixed acetates were ozonized by the same procedure described above. The 2,4-dinitrophenylhydrazones were extracted with ether, the extract washed thoroughly with water, dried, and evaporated. The residue was dissolved in chloroform and chromatographed on a bentonite-Celite 535 (3:1) column.[15] The first zone eluted by chloroform-ethanol (20:1) was formaldehyde 2,4-dinitrophenylhydrazone. After two recrystallizations from methanol it afforded nice needles, m.p. 165°. The second zone eluted with chloroform-ethanol (10:1) proved to be acetone 2,4-dinitrophenylhydrazone, m.p. 125°. Both compounds did not depress the melting points of authentic samples and gave the same infrared spectra as the reference compounds.

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(14) It has been pointed out to us by W. M. Stokes in a private communication that on a Kofler block the melting point of this acetate is 98°.

(15) J. W. White, *Anal. Chem.*, **20**, 725 (1948); J. A. Elvidge and M. Whalley, *Chem. and Ind. (London)*, **1955**, 589.