43. *Derivatives of* cis-3-*Hydroxy-*Δ^5*-cholenic Acid.*

By G. A. D. Haslewood.

cis-3-Hydroxy-$\Delta^{5:7}$-choladienic acid, the "cholanic acid analogue of ergosterol," has been obtained by the action of dimethylaniline on methyl 3 : 7-dibenzoyloxy-Δ^5-cholenate. The last substance was prepared from methyl *cis*-7-keto-3-acetoxy-Δ^5-cholenate, obtained (together with methyl 6-keto-3 : 5-diacetoxycholanate) by chromic acid oxidation of methyl *cis*-3-acetoxy-Δ^5-cholenate. The dimethylaniline method of introducing a C_{7-8} double bond into the sterol nucleus is here definitely superior to

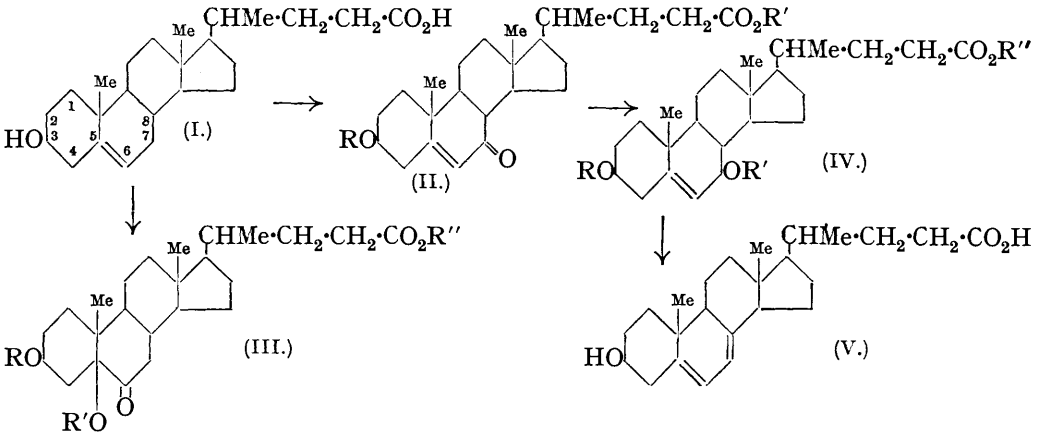
pyrolysis, which chiefly yielded crystalline substances of unknown constitution. Acids corresponding to the above esters have been prepared and characterised. *cis*-3-Hydroxy-$\Delta^{5:7}$-choladienic acid and its water-soluble salts will be tested for antirachitic provitamin activity.

THE acetate of the methyl ester of *cis*-3-hydroxy-Δ^5-cholenic acid (I), obtained from cholesteryl acetate dibromide (Wallis and Fernholz, *J. Amer. Chem. Soc.*, 1935, **57**, 1504; Ruzicka and Wettstein, *Helv. Chim. Acta*, 1935, **18**, 986; Butenandt, Dannenbaum, and Hanisch, *Z. physiol. Chem.*, 1935, **237**, 57), has been oxidised by the chromic acid method employed by German workers for the acetates of cholesterol (Windaus, Lettré, and Schenck, *Annalen*, 1935, **520**, 98), stigmasterol (Linsert, *Z. physiol. Chem.*, 1936, **241**, 125), and sitosterol (Wunderlich, *ibid.*, p. 116).

In addition to the expected *methyl* cis-7-*keto*-3-*acetoxy*-Δ^5-*cholenate* * (II; R = CO·CH_3, R′ = CH_3), there was obtained a saturated ketodiacetoxy-ester, which is probably a *methyl* 6-*keto*-3 : 5-*diacetoxycholanate* (III; R = R′ = CO·CH_3, R″ = CH_3). This substance on hydrolysis gave (probably) a 6-*keto*-3 : 5-*dihydroxycholanic acid* (III; R = R′ = R″ = H), characterised by a *monoacetyl methyl* ester and a *monosemicarbazone*. The corresponding cholestane ketodiacetoxy-compound, hydrolysable to a dihydroxy-ketone, was obtained in an analogous manner by Schenck (*Z. physiol. Chem.*, 1936, **243**, 119) from cholesteryl acetate, and was shown by him to be 6-keto-3 : 5-diacetoxy-cholestane.

Methyl *cis*-7-keto-3-acetoxy-Δ^5-cholenate [which on hydrolysis gave cis-3-*hydroxy*-7-*keto*-Δ^5-*cholenic acid* (II; R = R′ = H), characterised by a *semicarbazone*] was converted by aluminium *iso*propoxide into a *methyl* 3 : 7-*dihydroxy*-Δ^5-*cholenate* (IV; R = R′ = H, R″ = CH_3), hydrolysable to a 3 : 7-*dihydroxy*-Δ^5-*cholenic acid* (IV; R = R′ = R″ = H). Benzoylation of the crude, partly hydrolysed reduction product gave the *dibenzoate* (IV; R = R′ = CO·C_6H_5, R″ = CH_3) of methyl 3 : 7-dihydroxy-Δ^5-cholenate.

Pyrolysis of this dibenzoate, by the method used by the German workers for the corresponding derivatives of cholesterol, stigmasterol, and sitosterol (Windaus *et al.*, Linsert, Wunderlich, *locc. cit.*), gave chiefly a crystalline *substance*, hydrolysed to an *acid*, $C_{24}H_{38\ (or\ 40)}O_4$, which was precipitable by digitonin. This acid and the parent substance showed no sterol colour reactions. The products of the pyrolysis, after saponification, yielded, further, a dihydroxy-acid, apparently impure 3 : 7-dihydroxy-Δ^5-cholenic acid, and also a very small amount of *cis*-3-hydroxy-$\Delta^{5:7}$-choladienic acid (V).



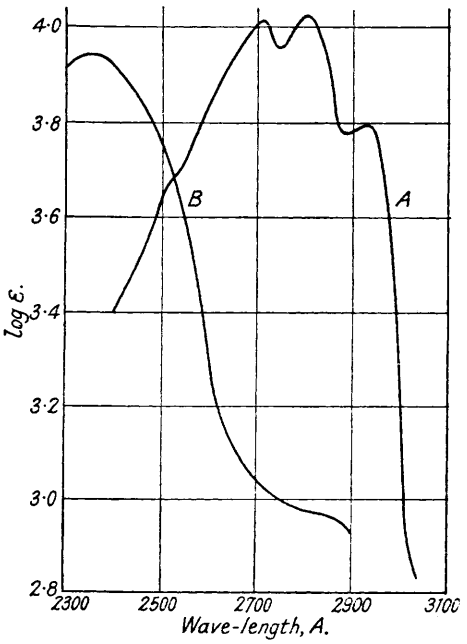
When, however, the dibenzoate of methyl 3 : 7-dihydroxy-Δ^5-cholenate was refluxed with dimethylaniline, the crystalline product obtained, which was readily isolated,

* At about the time that this substance and the corresponding acid were obtained, the author learned privately that they had been independently prepared by F. S. Spring and E. R. H. Jones, in collaboration with Professor I. M. Heilbron. The author wishes to express his appreciation of the open discussion which then took place with these workers on the problems connected with this research.

yielded on hydrolysis only cis-3-*hydroxy-*$\Delta^{5:7}$*-choladienic acid* (V), which was characterised by its *acetyl methyl* ester. The dimethylaniline method was found to be applicable also to the preparation of 7-dehydrocholesterol from the dibenzoate of 7-hydroxycholesterol.

The acid (V), which was the substance chiefly sought in this research, showed the colour reactions characteristic of ergosterol. Its absorption spectrum (curve *A*, for which, as also for curve *B*, the author is indebted to Professor C. W. Small) is very similar to that of ergosterol and shows maxima at 2710, 2810, and 2935 A.

Curve *B* shows the absorption spectrum of *cis*-3-hydroxy-7-keto-Δ^5-cholenic acid.



A. cis-3-*Hydroxy-*$\Delta^{5:7}$*-choladienic acid.*
B. cis-3-*Hydroxy-7-keto-*Δ^5*-cholenic acid.*
Ethyl alcohol solutions.

Experimental.

All melting points are uncorrected. * denotes micro-analysis by Dr. I. A. Schoeller. Optical rotations were carried out in a 0·5 dm. Fischer micro-tube. Ethereal solutions were dried with anhydrous sodium sulphate. Compounds marked † were precipitable by digitonin.

Purification of cis-3-*Hydroxy-*Δ^5*-cholenic Acid* (I).—Insoluble sodium salts from the debrominated acid fraction of the oxidation of cholesteryl acetate dibromide were hydrolysed with boiling alcoholic sodium hydroxide. The washed and dried ethereal extract of the diluted acidified product was evaporated, and the residue dissolved in a little fresh ether. The crystals separating were washed with ether and recrystallised from dilute alcohol containing benzene. This product (20 g.) in alcohol (1 l.) was treated with an excess of diazomethane, and the solvent evaporated. The residue, in pyridine (50 ml.), was heated at 100° for 30 minutes with acetic anhydride (50 ml.), and the product precipitated with water. Methyl *cis*-3-acetoxy-Δ^5-cholenate formed white needles (21 g.), m. p. 154—157°, from dilute alcohol.

cis-3-*Hydroxy-7-keto-*Δ^5*-cholenic Acid and Derivatives* (II).—The above acetoxy methyl ester (20 g.) in acetic acid (500 ml.) was stirred at 60° and treated with a solution of chromic oxide (16 g.) in acetic acid (180 ml.) and water (20 ml.), added during 1 hour. The temperature was kept at 55—60° for a further 3 hours, and the mixture then poured into much water and kept overnight. A solution of the precipitate in ether was washed with water, dilute sodium carbonate solution, and water, dried, and evaporated. From ethyl alcohol (200 ml.) the residue deposited crystals (4·8 g.) of *methyl* cis-*7-keto-3-acetoxy-*Δ^5*-cholenate* (II; R = $CO{\cdot}CH_3$, R′ = CH_3), which on recrystallisation from ethyl alcohol formed long colourless needles, m. p. 177—178° (Found: C, 72·9; H, 9·1.* $C_{27}H_{40}O_5$ requires C, 72·9; H, 9·1%). This product (0·2 g.) in alcohol (15 ml.) and 2N-sodium hydroxide (5 ml.) was kept overnight. Dilution of the acidified solution then gave cis-3-*hydroxy-7-keto-*Δ^5*-cholenic acid* † (II; R = R′ = H), which after crystallisation from benzene and dilute alcohol formed small colourless plates, m. p. 223—225° (Found: C, 74·0; H, 9·2.* $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·35%). $[\alpha]_D^{19°}$ − 115° (*c* = 0·812, in ethyl alcohol). The acid gave a yellow colour in the Liebermann–Burchard reaction. The *semicarbazone* formed white crystals, decomp. 267—269°, from dilute alcohol (Found: N, 9·3. $C_{25}H_{39}O_4N_3$ requires N, 9·4%).

3 : 5-*Dihydroxy-6-ketocholanic Acid and Derivatives* (III).—The alcoholic liquors from the first crystallisation of methyl *cis*-7-keto-3-acetoxy-Δ^5-cholenate were diluted with water and filtered from the first gummy precipitate. The filtrate, on further dilution, gave crystals (1·2 g.), m. p. 161—168°, of *methyl 6-keto-*3 : 5-*diacetoxycholanate* (III; R = R′ = $CO{\cdot}CH_3$, R″ = CH_3), which, recrystallised from methyl alcohol, benzene–light petroleum, and ethyl alcohol, gave colourless needles of constant m. p. 172—173° (Found: C, 69·2; H, 8·8.* $C_{29}H_{44}O_7$ requires C, 69·0; H, 8·9%). When the crude product (0·2 g.) was kept in solution

in alcohol (6 ml.) and 2N-sodium hydroxide (5 ml.), crystals of sodium salt appeared. After 5 hours, these were collected, washed with alcoholic sodium hydroxide, and dissolved in water. Acidification gave 3 : 5-*dihydroxy*-6-*ketocholanic acid* † (III; R = R′ = R″ = H), which formed long colourless needles, m. p. 278—279°, from dilute alcohol (Found : C, 71·1, 70·9; H, 9·3, 9·4.* $C_{24}H_{38}O_5$ requires C, 70·9; H, 9·4%). $[\alpha]_D^{19°}$ − 38° (*c* = 0·648, in ethyl alcohol). The acid gave a deep red colour in the Liebermann–Burchard reaction, and did not decolourise bromine in chloroform. The acid (0·3 g.) in alcohol was treated with an excess of diazomethane, and the solvent evaporated. The residue was heated at 100° in acetic anhydride (1 ml.) for 20 minutes. Dilution with water precipitated *methyl* 5-*hydroxy*-6-*keto*-3-*acetoxycholanate*, which crystallised from alcohol in colourless needles, m. p. 203—204° (Found : C, 69·9; H, 9·1.* $C_{27}H_{42}O_6$ requires C, 70·1; H, 9·2%). 3 : 5-*Dihydroxy*-6-*ketocholanic acid monosemicarbazone* crystallised from alcohol in glistening needles, decomp. 267—269° (Found : N, 8·8.* $C_{25}H_{41}O_5N_3$ requires N, 9·1%).

3 : 7-*Dihydroxy*-Δ⁵-*cholenic Acid and Derivatives* (IV).—A mixture of methyl *cis*-7-keto-3-acetoxy-Δ⁵-cholenate (1·8 g.), *iso*propyl alcohol (12 ml.), and freshly distilled aluminium *iso*propoxide (0·8 g.) was partly distilled during 8 hours. The yellow solution was cooled and treated with a solution of potassium hydroxide (1 g.) in methyl alcohol (15 ml.). After 30 minutes, the mixture was poured into water (200 ml.), and the precipitate collected and washed. (The filtrate on acidification gave a small quantity of crude acid, which was methylated with diazomethane, dissolved in ether, and precipitated with light petroleum as for the main fraction.) An ethereal solution of the precipitate was washed with water, dried, filtered, and evaporated to small volume. On addition of light petroleum, there was formed a precipitate (1 g.) of *methyl* 3 : 7-*dihydroxy*-Δ⁵-*cholenate* † (IV; R = R′ = H; R″ = CH_3), which crystallised from dilute alcohol in long white needles, m. p. 142—144° (Found : C, 74·6; H, 9·7.* $C_{25}H_{40}O_4$ requires C, 74·2; H, 10·0%). The crude light petroleum precipitate (1 g.), in pyridine (6 ml.), was treated gradually with benzoyl chloride (3 ml.) and kept at 0° for 24 hours. The mixture was diluted with water, and a washed and dried ethereal extract evaporated. With methyl alcohol, the oily residue gave crystals (0·8 g.) of the *dibenzoate* of methyl 3 : 7-dihydroxy-Δ⁵-cholenate. Recrystallisation from benzene–light petroleum and methyl alcohol–acetone gave long white needles, m. p. 165—166° (Found : C, 76·4; H, 7·9.* $C_{39}H_{48}O_6$ requires C, 76·4; H, 7·9%). Hydrolysis of this compound yielded 3 : 7-*dihydroxy*-Δ⁵-*cholenic acid* † (IV; R = R′ = R″ = H), which crystallised from dilute alcohol in small white needles, m. p. 188—190° (decomp.) (Found : C, 73·55; H, 9·7.* $C_{24}H_{38}O_4$ requires C, 73·8; H, 9·8%). $[\alpha]_D^{19°}$ − 54° (*c* = 0·768, in ethyl alcohol). This acid and its derivatives gave a blue colour, changing to purple, in the Liebermann–Burchard reaction, and an intense blue colour with antimony trichloride in chloroform. Treatment of the above methyl ester with acetic anhydride in pyridine gave white needles, m. p. 161—164°, which showed no colour reactions.

Pyrolysis of the Dibenzoate.—The dibenzoate was heated at 195—200°/0·1 mm. for 1 hour. The following were representative experiments :—

(a) The pure dibenzoate (80 mg., m. p. 164—166°) gave a crystalline *product* (70 mg.), which was recrystallised from benzene–light petroleum and ethyl alcohol, forming white needles, m. p. 165—166° (Found : C, 75·7, 75·8; H, 8·4, 8·1%*). Treatment of this substance for 24 hours at room temperature with ethyl alcohol (10 ml.) and N-sodium hydroxide (1 ml.) gave on acidification colourless needles,† m. p. 214—216°, which, like the parent compound, showed no colour reactions (Found : C, 73·6; H, 9·3.* $C_{24}H_{38}O_4$ requires C, 73·8; H, 9·8%).

(b) From the crude dibenzoate (0·3 g.) was obtained, after purification from light petroleum, a product (0·15 g.), m. p. 136—145°, which was dissolved in alcohol (10 ml.) and left for 16 hours with 2N-sodium hydroxide (1 ml.). The mixture was diluted, acidified (acetic acid), and ether-extracted. The insoluble salts obtained on washing the ether with 2N-sodium hydroxide were collected and dissolved in water, and the acidified solution re-extracted with ether. The residue from the evaporation of the washed and dried extract gave, with a little benzene, long needles (80 mg.), m. p. 141—143° after recrystallisation from dilute alcohol. The substance † gave blue —→ purple Liebermann–Burchard, and blue antimony trichloride reactions (Found : C, 73·4; H, 8·6%*).

(c) In another experiment, the final product from the above treatment of the crude dibenzoate (0·4 g.) was a few mg. of (V), m. p. 205—210°.

cis-3-*Hydroxy*-Δ⁵·⁷-*choladienic Acid* (V).—The dibenzoate (0·2 g.) was refluxed for 8 hours with freshly distilled dimethylaniline (5 ml.). The cooled solution was poured into dilute hydrochloric acid and extracted with ether, and the washed and dried extract evaporated.

The residue separated from methyl alcohol in white crystals (80 mg.), which were refluxed for 15 minutes with alcohol (10 ml.) and 2N-sodium hydroxide (1 ml.). The cooled diluted acidified mixture was ether-extracted, and the extract washed with sodium hydroxide solution and with water. Evaporation of the washed and dried ethereal extract of the acidified washings gave white crystals, which were recrystallised from alcohol–benzene and from dilute alcohol, from which cis-3-*hydroxy*-$\Delta^{5:7}$-*choladienic acid* † (V) formed long white needles, m. p. 214—216° (decomp.) (Found : C, 77·1; H, 9·6.* $C_{24}H_{36}O_3$ requires C, 77·4; H, 9·75%). $[\alpha]_D^{19°}$ — 69° (c = 0·679, in ethyl alcohol). This substance (30 mg.), in alcohol, was treated with an excess of diazomethane, and the solvent removed. The residue, in pyridine (0·5 ml.), was heated with acetic anhydride (0·5 ml.) for 20 minutes at 100°. Precipitation of the cooled mixture with water gave *methyl* cis-3-*acetoxy*-$\Delta^{5:7}$-*choladienate*, which crystallised from methyl alcohol in white needles, m. p. 125—127° (Found : C, 75·2; H, 9·35.* $C_{27}H_{40}O_4$ requires C, 75·65; H, 9·4%). The choladienic acid and its acetyl methyl ester gave a deep blue —→ purple colour in the Liebermann–Burchard reaction, a bluish-green colour with antimony trichloride in chloroform, and an intense Tortelli–Jaffé reaction.

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