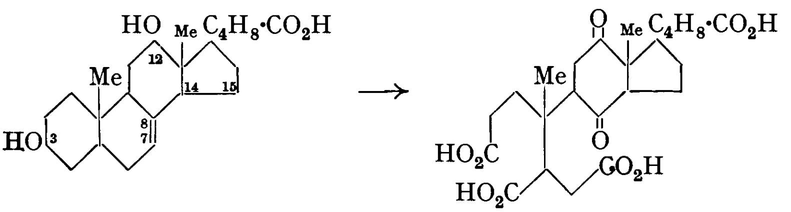
111. *The Action of Selenium Dioxide on Sterols and Bile Acids. Part II. The Formation of Isomeric Dihydroxycholadienic Acids from* apo*Cholic Acid and Dihydroxycholenic Acid and a Note on the Oxidation Products of Dihydroergosterol.*

By R. K. CALLOW.

THE dehydration of cholic acid by zinc chloride in acetic acid yields a mixture of two isomeric acids, $C_{24}H_{38}O_4$, dihydroxycholenic acid and *apo*cholic acid (Boedecker, *Ber.*, 1920, **53**, 1852; Boedecker and Volk, *Ber.*, 1921, **54**, 2489). Dihydroxycholenic acid is catalytically reduced to deoxycholic acid (Boedecker and Volk, *Ber.*, 1922, **55**, 2302), and its oxidative degradation (Borsche and Todd, *Z. physiol. Chem.*, 1931, **197**, 173; Wieland and Dane, *ibid.*, 1932, **206**, 243; **212**, 263) yields a diketo-tetracarboxylic acid, $C_{24}H_{34}O_{10}$. This degradation was interpreted by formulæ (I) and (II), on the assumption that the original acid was 3 : 12-dihydroxy-$\Delta^{7:8}$-cholenic acid (I), though a $\Delta^{14:15}$-acid also would give a diketo-tetracarboxylic acid.

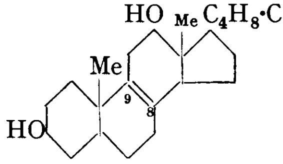


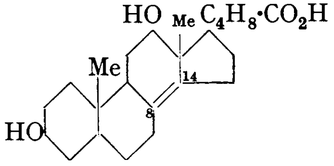
(I.) (II.)

*apo*Cholic acid is not affected by catalytic hydrogenation, but its close relation to dihydroxycholenic acid followed from the observation of Boedecker and Volk (*Ber.*, 1922, **55**, 2302) that either acid, when treated with bromine, was dehydrogenated (by the loss of hydrogen bromide from the dibromide) to give dihydroxycholadienic acids, m. p. 247°, $[\alpha]_D$ + 20·3° and 19·6° in alcohol, respectively, which were reduced catalytically to *apo*cholic acids (acetic acid compounds, $[\alpha]_D$ + 55·9° and + 52·04° in alcohol, respectively; acetic acid compound of original *apo*cholic acid, $[\alpha]_D$ + 45·35°). These results were fully confirmed by Wieland and Deulofeu (*Z. physiol. Chem.*, 1931, **198**, 127), who, however, thought that the discrepancy between the optical rotations was worth noting, and found that the product giving the acetic acid compound of $[\alpha]_D$ + 52°, which they named β-*apo*cholic acid, yielded a dihydroxycholadienic acid of $[\alpha]_D$ − 1°. Dihydroxycholadienic acid can also be obtained by oxidation of *apo*cholic acid with permanganate, or with perbenzoic acid, or by decomposition of *apo*cholic acid oxide, or by oxidation of dihydroxycholenic acid with perbenzoic acid (Borsche and Todd, *Z. physiol. Chem.*, 1931, **197**, 173). No attention was paid in the last investigation to optical rotations, either of the dihydroxycholadienic acids or of the *apo*cholic acids derived from them, and the assumptions of identity were based solely upon melting points. Wieland and Deulofeu (*loc. cit.*) also obtained dihydroxycholadienic acid from *apo*cholic acid and perbenzoic acid, and by hydrolysis of dihydroxycholenic acid oxide (cf. Wieland and Dane, *Z. physiol. Chem.*, 1932, **206**, 243). Wieland and Dane (*ibid.*, 1932, **212**, 263) attempted to determine the position of the ethylenic linkage in *apo*cholanic acid by the oxidation methods which had been applied to dihydroxycholenic acid. The product of oxidation by permanganate was a monocarboxylic acid, having the composition of a diketonic compound, consistent with either of the formulæ (III) and (IV) for *apo*cholic acid. The former was preferred, on account of an analogy with the formation of $\Delta^{9:10}$-octalin from α-decalol, although the oxidation product in this case would, presumably, be a compound with a ten-membered ring.

A third isomeric acid, *iso*dihydroxycholenic acid, was isolated by Yamasaki (*Z. physiol. Chem.*, 1933, **220**, 42) from the product of treatment of cholic acid with hydrochloric acid. It was also obtained by isomerisation of *apo*cholic acid with hydrochloric acid. To this isomeride, which was not reducible catalytically, Yamasaki assigned the available constitution (IV). Later (*ibid.*, 1935, **233**, 10) he demonstrated the interconvertibility of *apo*cholic and dihydroxycholenic acids under the influence of hydrogen chloride (cf. unpublished work

(III.)



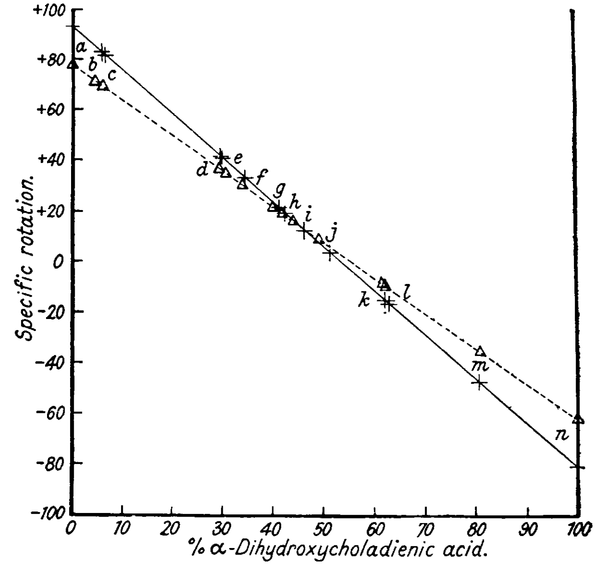


(IV.)

in this laboratory by Rosenheim and Starling, referred to by Rosenheim and King, *Chem. and Ind.*, 1932, 954).

The present investigation is a continuation of the work described in Part I on the oxidation of steroids by selenium dioxide (Callow and Rosenheim, J., 1933, 387). It was

FIG. 1.



Optical rotation and assumed composition of mixtures of α- *and* β-*dihydroxycholadienic acids.*

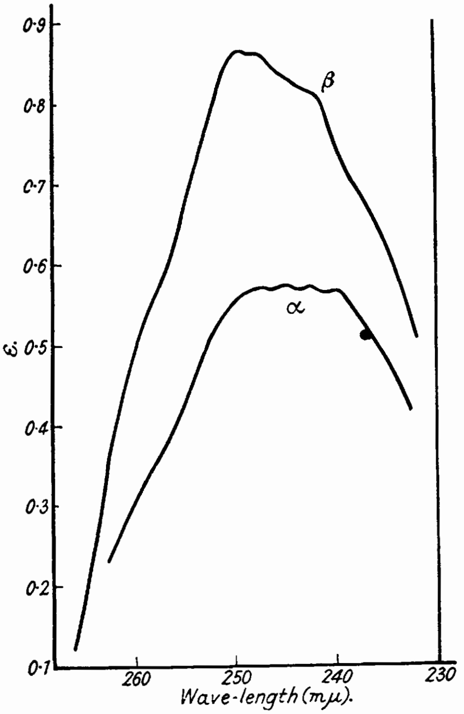
Δ $[\alpha]_D$; + $[\alpha]_{5461}$.

Source of acid : a, β-apo*Cholic acid + selenium dioxide.* b, c, apo*Cholic acid + selenium dioxide.* d, β-apo*Cholic acid + perbenzoic acid.* e, *Decomposition of dihydroxycholenic acid oxide.* f, *Decomposition of* apo*cholic acid oxide.* g, *Methyl dihydroxycholenate + selenium dioxide.* h, *Acid from Dr. Boedecker.* i, *Methyl dihydroxycholenate + perbenzoic acid.* j, *Methyl dihydroxycholenate + selenium dioxide.* k, apo*Cholic acid + bromine.* l, *Dihydroxycholenic acid + bromine.* m, apo-*Cholic acid + perbenzoic acid, prep.* (b). n, apo*Cholic acid + perbenzoic acid, prep.* (a).

found that *apo*cholic acid reacted readily with selenium dioxide in aqueous-alcoholic solution to give a product having the composition of dihydroxycholadienic acid and not depressing the melting point of an authentic specimen kindly sent by Dr. Boedecker. The new acid, however, had $[\alpha]_D$ +71·0° and $[\alpha]_{5461}$ +82·1° in alcohol, and Dr. Boedecker's acid, in accord with published data, had $[\alpha]_D$ + 18·9° and $[\alpha]_{5461}$ + 19·5°. Since figures for the optical rotation of " dihydroxycholadienic acids " prepared by other methods were not available, a number of these preparations were repeated. A highly lævorotatory acid, $[\alpha]_D$ − 62° and $[\alpha]_{5461}$ − 81°, was obtained from *apo*cholic acid and perbenzoic acid by the method of Borsche and Todd (*loc. cit.*). On the other hand, *apo*cholic acid oxide, formed in the same reaction, decomposed to give an acid with $[\alpha]_D$ + 30° and $[\alpha]_{5461}$ + 33°. Optical rotations between those of the two extreme lævo- and dextro-rotatory acids were observed for the acids prepared from *apo*cholic acid and perbenzoic acid by the method of Wieland and Deulofeu (*loc. cit.*), from *apo*cholic acid and bromine, from dihydroxycholenic acid and bromine, from methyl dihydroxycholenate and perbenzoic acid or selenium dioxide, and from the decomposition of dihydroxycholenic acid oxide. It being assumed that these extremes corresponded to pure lævo- and pure dextro-rotatory acid (which it is proposed to call α- and β-*dihydroxycholadienic acid*, respectively), a graph was constructed showing the theoretical rotations of mixtures of these two acids, which will lie along the straight lines joining the values of $[\alpha]_D$ and $[\alpha]_{5461}$ for 100% α-acid and 100% β-acid (Fig. 1). (The values $[\alpha]_D$ + 77·7° and $[\alpha]_{5461}$ +92·8° are taken for the β-acid; see below.) If the values of $[\alpha]_D$ and $[\alpha]_{5461}$ for other products are plotted on these lines, the compositions will be indicated by the abscissæ, and if the points for $[\alpha]_D$ and $[\alpha]_{5461}$ for a given product fall one above the other at the same composition, evidence will be afforded that the product is a mixture of α- and β-dihydroxycholadienic acid. Actually, there are small discrepancies, particularly for the products from methyl dihydroxycholenate. These may be accounted for by the difficulty of purifying the materials from unchanged dihydroxycholenic acid. A further check on the assumptions which have been made is afforded by plotting the values of α_{5461}/α_D against composition. The value of this ratio is of the order of 1·2 for steroids. From the value 1·20 for the β-acid it falls through 1·00 at 47% β-acid, to $-\infty$, and the curve reappears at the top of the diagram to fall to 1·33 for the α-acid. It would therefore seem that the latter is not pure, and that the pure α-acid is yet more highly lævorotatory. The determination of the rotatory dispersion, in cases of this kind, can afford valuable evidence as to homogeneity.

The two dihydroxycholadienic acids have different absorption spectra (Fig. 2). The curve for the β-acid shows a fairly sharp maximum at 249 mμ, but that for the α-acid is much lower and shows a broad maximum between 240 mμ and 250 mμ. Absorption curves of intermediate type are given by materials which are assumed to be mixtures of these two acids, and there is a fair correlation between the absorption and the composition deduced from the optical activity.

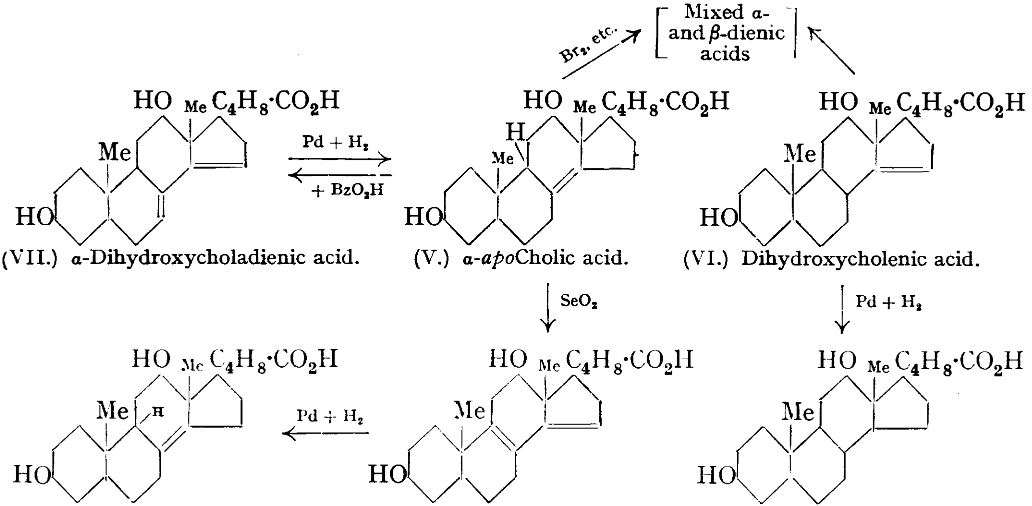
FIG. 2.



Absorption spectra of α- and β-dihydroxycholadienic acids in alcoholic solution (0·002%).

Both α- and β-dihydroxycholadienic acid take up one molecule only of hydrogen when catalytically reduced. The α-acid yields *apo*cholic acid, the acetic acid compound of which has $[\alpha]_D$ +42·3°, in fair agreement with the value, + 45·35°, given by Boedecker (*loc. cit.*). The β-acid yields an isomeride of *apo*cholic acid, which it is proposed to call β-apo*cholic acid*. β-*apo*Cholic acid and its acetic acid and xylene compounds melt at slightly lower temperatures than the α-compounds; the optical rotation, $[\alpha]_D$ + 73·5°, is considerably higher than that of the known *apo*cholic acid. It would appear that the material which Wieland and Deulofeu called β-*apo*cholic acid was a mixture of the ordinary α-isomeride and the β-acid now described. β-*apo*Cholic acid reacted with selenium dioxide to give a β-dihydroxycholadienic acid with high dextrorotation. With perbenzoic acid a mixture containing a relatively high proportion of β-dihydroxycholadienic acid was obtained. Adam, Askew, and Danielli (*Biochem. J.*, **1935**, **29**, 1799) examined surface films of α- and β-*apo*cholic acid and found that they formed gaseous films with identical surface pressure and surface potential, indicating that the isomerism does not involve any serious change in the orientation of the polar groups relative to the ring system.

The interpretation of these reactions makes it necessary to reconsider the formulæ ascribed to dihydroxycholenic and *apo*cholic acids. Rosenheim and King (*Chem. and Ind.*, 1932, **464**, 954) and Morrison and Simpson (J., 1932, 1710) have stressed the analogy between α- and β-ergostenol and *apo*cholic and dihydroxycholenic acid. With the further work of Achtermann (*Z. physiol. Chem.*, 1934, **225**, 141) and of Laucht (*ibid.*, 1935, **237**, 236) on the constitution of α($\Delta^{8:14}$)-ergostenol and β($\Delta^{14:15}$)-ergostenol, more certain data are now available, and our knowledge of the ergostenol group can be applied to the acids. The formula proposed by Wieland and Dane (*loc. cit.*) for dihydroxycholenic acid with the ethylenic linkage at positions 7 : 8 is similar to the formula for γ-ergostenol, but the latter substance (Windaus and Langer, *Annalen*, 1933, **508**, 105) is isomerised by finely divided platinum to α-ergostenol, which is not reducible. Since dihydroxycholenic acid is reduced normally to deoxycholic acid, it is better that the $\Delta^{14:15}$-constitution (VI), analogous to that of β-ergostenol, should be assigned to it; the behaviour on oxidation can, as we have seen, be reconciled with this. Further, the direct interconvertibility of dihydroxycholenic and *apo*cholic acid suggests for the latter the $\Delta^{8:14}$-constitution (V), which is also in accord with other evidence. The relations of the isomeric dihydroxycholenic and dihydroxycholadienic acids can then be formulated :



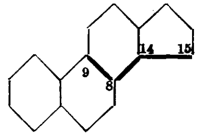
(VII.) α-Dihydroxycholadienic acid. (V.) α-*apo*Cholic acid. (VI.) Dihydroxycholenic acid.

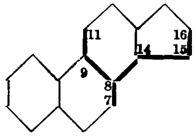
(IX.) β-*apo*Cholic acid. (VIII.) β-Dihydroxycholadienic acid. (X.) Deoxycholic acid.

These formulæ are supported by the following considerations. The methods of formation of α- and β-dihydroxycholadienic acid indicate that they contain conjugated ethylenic linkages. The marked selective absorption in the ultra-violet region confirms the existence of conjugation, and the fact that the absorption of the β-acid is greater and at a longer wave-length than that of the α-acid suggests that in the β-acid there is an enhancing factor such as proximity of a hydroxyl group or presence of the two ethylenic linkages in the same ring. Both acids reacted rather slowly with maleic anhydride in boiling xylene, but no characterisable products could be separated.

On the assumption that the singly unsaturated acids may contain the ethylenic linkage in positions 8 : 9, 8 : 14, or 14 : 15, as shown by the heavy lines in (XI), the doubly unsaturated acids may conceivably contain the ethylenic linkages in the positions shown by heavy lines in (XII). It is possible to eliminate some of the possible formulæ on more or less convincing grounds. The presence of ethylenic linkages at positions 11 : 9-8 : 14, in the same ring as the 12-hydroxyl group, would certainly lead to high absorption at a

(XI.)

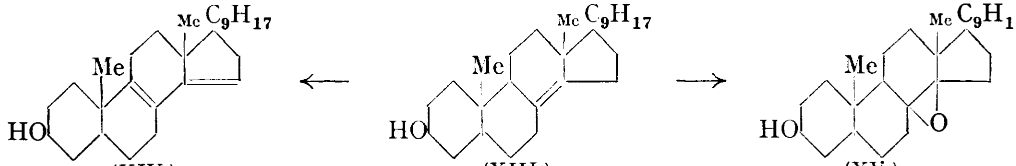




(XII.)

longer wave-length than is actually observed. It may even be doubted whether 7 : 8-9 : 11 ethylenic linkages are admissible, but direct evidence on this point is lacking. On chemical grounds the mechanism of the formation of an ethylenic linkage at positions 15 : 16 presents difficulties. If these restrictions are accepted, we are limited to the formulæ (VII) and (VIII) for the two dihydroxycholadienic acids. Of these two structures, the $\Delta^{9:8-14:15}$-compound, with two ethylenic linkages in adjacent rings, will be expected to have the higher absorption at longer wave-lengths, and this structure is therefore assigned to β-dihydroxycholadienic acid. Further, the formation of a stereoisomeric *apo*cholic acid from β-dihydroxycholadienic acid, but not from α-dihydroxycholadienic acid, is simply explained, for 1 : 4 addition of hydrogen in the former case creates an asymmetric carbon atom at position 9, whereas no asymmetry is introduced in the latter case. The formation of a dibromide from *apo*cholic acid or of an 8 : 14-glycol from an 8 : 14-oxide leads directly to the two dienic acids by elimination of hydrogen bromide or of water from positions 14 : 15 on the one side and from either positions 8 : 9 or 8 : 7 on the other. It must be assumed that, when an oxide or bromide is not the intermediate compound, selenium dioxide attacks the tertiary carbon atom 9, whereas perbenzoic acid attacks the secondary carbon atom 7. The formation of the dienic acids from dihydroxycholenic acid or the oxide is explicable by attack on the tertiary carbon atom 8, but the decomposition of the oxide is not simple; however, since this decomposition is carried out in presence of acid, it is not unreasonable to assume that migration of the ethylenic linkages takes place.

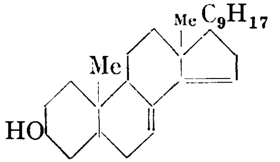
It is now possible to explain the reaction of selenium dioxide with dihydroergosterol described in Part I (*loc. cit.*). Ergosterol-*D* and ergosterol-B_3 are, respectively, the products from the immediate action of selenium dioxide and of the decomposition of the oxide. Ergosterol-*D*, $[\alpha]_D + 17°$ in chloroform, absorption maximum at 242 mμ, $E^{1\%}_{1\,\mathrm{cm.}} = 540$, is considered to be the analogue of β-dihydroxycholadienic acid, $[\alpha]_D + 78°$ in alcohol, absorption maximum at 248 mμ, $E^{1\%}_{1\,\mathrm{cm.}} = 430$; and ergosterol-$B_3$, $[\alpha]_D - 207°$ in chloroform, absorption maximum at 242 mμ, $E^{1\%}_{1\,\mathrm{cm.}} = 255$, is the analogue of α-dihydroxycholadienic acid, $[\alpha]_D - 62°$ in alcohol (probably greater for the pure acid, see above), absorption maximum at 240—248 mμ, $E^{1\%}_{1\,\mathrm{cm.}} = 240$. The reactions are, therefore, to be formulated :



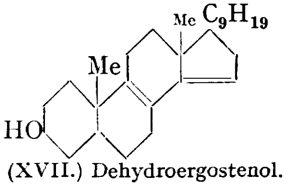
(XIV.) Ergosterol-*D*.

(XIII.) Dihydroergosterol.

(XV.) Dihydroergosterol oxide.



(XVI.) Ergosterol-B_3.



(XVII.) Dehydroergostenol.

Preliminary experiments only have been carried out with α-ergostenol. Under the conditions employed, only one product was isolated, namely, dehydroergostenol, with the physical properties recorded in the literature, to which the formula (XVII) is now assigned. The possible formation of isomeric compounds should be worth investigation, particularly in view of the experiences of Windaus and Lüttringhaus (*Annalen*, 1930, **481**, 119) and of Laucht (*loc. cit.*), who obtained complex products by treatment of α-ergostenol with perbenzoic acid and with ozone, respectively.

EXPERIMENTAL.

apo*Cholic Acid and Selenium Dioxide.*—Solutions of *apo*cholic acid (20 g.) in absolute alcohol (250 c.c.) and selenium dioxide (16 g., 3 mols.) in water (150 c.c.) were mixed and kept at 37° for

16 hours. After cooling, the mixture of red selenium and white crystalline material was collected, washed with water, and stirred with sufficient sodium carbonate solution to dissolve the acid and after saturation with sodium sulphate and addition of a little charcoal the liquid was filtered and acidified with hydrochloric acid, yielding 12·1 g. of β-*dihydroxycholadienic acid*, m. p. 232—238°. This was recrystallised four times alternately from dilute acetic acid and glacial acetic acid, and finally crystallised by solution in hot alcohol containing a little ammonia and precipitation by the cautious addition of acetic acid. The m. p. was now constant at 253—255°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 71·0°, $[\alpha]_{5461}^{20^\circ}$ + 82·1° (*c*, 0·476); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·16. The absorption spectrum showed a maximum at 249 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 430) with a subsidiary protuberance at about 242 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 400) (Fig. 2).

The yield was lower (2 g. of crude product from 5 g.) when the reaction was carried out for 10 minutes on the water-bath; the product, recrystallised from methyl alcohol and dried over phosphoric oxide, had m. p. 251—252·5°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 69°, $[\alpha]_{5461}^{20^\circ}$ + 81·5° (*c*, 0·493); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·18 [Found (micro): C, 73·7; H, 9·2. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·3%].

Methyl apo*Cholate and Selenium Dioxide.*—Methyl *apo*cholate (Boedecker, *loc. cit.*), recrystallised from methyl alcohol, had m. p. 85—94° (Found: loss at 100°/0·1 mm., 7·4. $C_{25}H_{40}O_4$,MeOH requires loss 7·3%). The solvent-free substance had m. p. 112—115° [Found (micro): C, 73·7; H, 9·7. Calc. for $C_{25}H_{40}O_4$: C, 74·2; H, 10·0%]. A solution of the ester (1 g.) in alcohol (13·3 c.c.) was mixed with selenium dioxide (1 g.) in water (6·7 c.c.) and kept at 37° for 24 hours, the selenium (0·15 g.) removed, and the filtrate diluted with water. Ether extracted a gum, which was hydrolysed with alcoholic sodium hydroxide. The free acid, twice recrystallised from alcoholic ammonia by addition of acetic acid, had m. p. 254—257° and $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 74° (*c*, 0·196). The absorption maximum was at 248 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 480).

Methyl β-*dihydroxycholadienate* was prepared by refluxing the silver salt for 3 hours with methyl iodide. Extraction of the product with methyl alcohol and evaporation to dryness yielded a gum, which was crystallised from dilute acetic acid (1 : 1); after separation of a small amount of acid the ester crystallised; it was recrystallised and then had m. p. 87—89°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 62·5°, $[\alpha]_{5461}^{20^\circ}$ + 71° (*c*, 0·489); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·13. The absorption maximum was at 249 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 377) [Found: (micro) C, 70·0; H, 9·1; (macro) loss at 95°/12 mm., 12·3. $C_{25}H_{38}O_4$,$C_2H_4O_2$ requires C, 70·1; H, 9·15; loss, 12·1%].

apo*Cholic Acid and Perbenzoic Acid.*—(*a*) The method of Wieland and Deulofeu (*loc. cit.*) was slightly modified. The solution resulting from the reaction of 10 g. of *apo*cholic acid was extracted with aqueous sodium hydroxide, the extract acidified with hydrochloric acid, and the oily precipitate washed with a little light petroleum and then with ether. Crystallisation from dilute acetic acid yielded 4 g. of crude acid, m. p. 228—237°. After three recrystallisations from dilute acetic acid and finally from alcoholic ammonia by addition of acetic acid, the dihydroxycholadienic acid had m. p. 252—255°, not depressed by the acid prepared with selenium dioxide, and $[\alpha]_{\mathrm{D}}^{20^\circ}$ − 35·3°, $[\alpha]_{5461}^{20^\circ}$ − 47·7° (*c*, 0·457); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·35. The absorption spectrum showed a broad maximum at 243 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 300).

The acid was converted through the silver salt into the methyl ester, an uncrystallisable gum being obtained. This was dried at 100°/10 mm. and treated with 3 : 5-dinitrobenzoyl chloride in pyridine; methyl α-bis-3′ : 5′-dinitrobenzoyloxycholadienate, isolated with some difficulty, was obtained in needles, m. p. 165—166·5°, after recrystallisation from ethyl acetate.

(*b*) By the method of Borsche and Todd (*loc. cit.*), interaction of *apo*cholic acid and perbenzoic acid in acetone and chloroform yielded *apo*cholic acid oxide, m. p. 201—205°, $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 42°, $[\alpha]_{5461}^{20^\circ}$ + 49° (*c*, 0·524); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·16; it showed no selective absorption in 0·5% alcoholic solution. The mother-liquor of the crude oxide yielded α-dihydroxycholadienic acid, m. p. 252—254°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ − 62°, $[\alpha]_{5461}^{20^\circ}$ − 81° (*c*, 0·229); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·33. The absorption spectrum showed a broad maximum at 240—248 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 240) (Fig. 2).

Decomposition of apo*Cholic Acid Oxide.*—By the method of Borsche and Todd (*loc. cit.*), an acid was obtained, m. p. 248—251°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 30°, $[\alpha]_{5461}^{20^\circ}$ + 33° (*c*, 0·23); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·09. The absorption spectrum showed a fairly sharp maximum at 249 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 420).

apo*Cholic Acid and Bromine.*—The method of Boedecker and Volk (*Ber.*, 1922, **55**, 2302) gave a 50% yield of a crude product, m. p. 230—236°, which, after crystallisation from dilute acetic acid, followed by alcoholic ammonia and acetic acid, had m. p. 248—251°, $[\alpha]_{\mathrm{D}}^{28^\circ}$ − 8·4°, $[\alpha]_{5461}^{28^\circ}$ − 15·4° (*c*, 0·357); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·44. The absorption spectrum showed a broad maximum in the region of 245 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 325).

Dihydroxycholadienic acid obtained from Dr. Boedecker.—This had m. p. 250—254°; $[\alpha]_{\mathrm{D}}^{20^\circ}$ + 18·9°, $[\alpha]_{5461}^{20^\circ}$ + 19·5° (*c*, 0·424); $\alpha_{5461}/\alpha_{\mathrm{D}}$ = 1·03; broad absorption band with maximum at 248 mμ ($E^{1\%}_{1\,\mathrm{cm.}}$ = 330).

Methyl Dihydroxycholenate.—The acid used was obtained from the J.D. Riedel-E. de Haen A.G., Berlin. It had m. p. 265—268°; $[\alpha]_{\mathrm{D}}^{25^\circ} + 64^\circ$, $[\alpha]_{\mathrm{D}}^{25^\circ} + 71^\circ$ (*c*, 0·276); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}11$. As its sparing solubility was an inconvenience, it was converted into the methyl ester by way of the silver salt. As a control of its purity, the ester was converted into the **3** : 5-dinitrobenzoate and recovered. After removal of solvent of crystallisation at 100°/10 mm., the ester was treated with 3 : 5-dinitrobenzoyl chloride in pyridine on the water-bath. *Methyl bis-3′ : 5′-dinitrobenzoyloxycholenate* formed pale yellow needles from ethyl acetate, m. p. 209—210·5° [Found (micro) : C, 59·0; H, 5·8. $C_{39}H_{44}O_{14}N_4$ requires C, 59·1; H, 5·6%]. Hydrolysis with alcoholic sodium hydroxide and two recrystallisations from alcoholic ammonia and acetic acid (charcoal) yielded dihydroxycholenic acid, m. p. 269—271°; $[\alpha]_{\mathrm{D}}^{25^\circ} + 63^\circ$, $[\alpha]_{5461}^{25^\circ} + 73^\circ$ (*c*, 0·323); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}16$. Reduction of the methyl ester in methyl alcohol with hydrogen and palladised charcoal, and hydrolysis, yielded deoxycholic acid, isolated as the acetic acid compound, m. p. 139—143°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 48{\cdot}3^\circ$, $[\alpha]_{5461}^{20^\circ} + 58{\cdot}0^\circ$ (*c*, 0·404); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}20$.

Methyl Dihydroxycholenate and Selenium Dioxide.—In the oxidation (ester 1 g., alcohol 13·3 c.c.; selenium dioxide 1 g., water 6·7 c.c.; kept at 37° for 24 hours) the precipitation of selenium began more slowly than with *apo*cholic acid. After removal of selenium (0·85 g.) the product was hydrolysed and worked up in the usual way. The recrystallised acid had m. p. 259—261°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 21{\cdot}7^\circ$, $[\alpha]_{5461}^{20^\circ} + 21{\cdot}1^\circ$ (*c*, 0·196); $\alpha_{5461}/\alpha_{\mathrm{D}} = 0{\cdot}975$. The absorption spectrum showed a broad maximum at 242—249 mμ ($E_{1\,\mathrm{cm.}}^{1\%} = 260$). Another preparation (yield, 0·4 g. from 2 g.) had m. p. 259—262°; $[\alpha]_{\mathrm{D}}^{27^\circ} + 8{\cdot}8^\circ$, $[\alpha]_{5461}^{27^\circ} + 3{\cdot}8^\circ$ (*c*, 0·404); $\alpha_{5461}/\alpha_{\mathrm{D}} = 0{\cdot}375$. The absorption spectrum showed a broad maximum at 240—250 mμ ($E_{1\,\mathrm{cm.}}^{1\%} = 343$).

Methyl Dihydroxycholenate and Perbenzoic Acid.—The ester (2 g.) in chloroform (5 c.c.) was treated with 20 c.c. of a solution of perbenzoic acid in chloroform (0·006 g. of active oxygen per c.c.). The mixture became slightly warm. After 4 hours the solution was washed with aqueous sodium carbonate and evaporated to dryness, and the residue hydrolysed with alcoholic sodium hydroxide. After recrystallisation from alcoholic ammonia and acetic acid and again from dilute acetic acid, the product had m. p. 250—252°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 16^\circ$, $[\alpha]_{5461}^{20^\circ} + 12{\cdot}5^\circ$ (*c*, 0·30); $\alpha_{5461}/\alpha_{\mathrm{D}} = 0{\cdot}78$. The absorption spectrum showed a broad maximum at 245—250 mμ ($E_{1\,\mathrm{cm.}}^{1\%} = 370$).

Decomposition of Dihydroxycholenic Acid Oxide.—The oxide (Wieland and Deulofeu, *loc. cit.*), crystallised from ethyl acetate, had m. p. 180—181°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 50^\circ$, $[\alpha]_{5461}^{20^\circ} + 60^\circ$ (*c*, 0·238); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}20$. A 0·1% solution in alcohol showed no selective absorption, but when diluted to 0·002% with dilute sulphuric acid and kept for 24 hours it produced a band with a broad maximum at 240—250 mμ ($E_{1\,\mathrm{cm.}}^{1\%} = 220$). Decomposition of the oxide by warming in dilute aqueous-alcoholic sulphuric acid yielded a product, which, recrystallised from alcoholic ammonia and acetic acid, had m. p. 240—244°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 34{\cdot}5^\circ$, $[\alpha]_{5461}^{20^\circ} + 41^\circ$ (*c*, 0·315); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}18$. The absorption spectrum showed a fairly sharp maximum at 249 mμ ($E_{1\,\mathrm{cm.}}^{1\%} = 400$).

Catalytic Reduction of β-Dihydroxycholadienic Acid.—The acid (3 g.) was reduced in glacial acetic acid solution (150 c.c.) with hydrogen and palladised charcoal (0·5 g. of palladous chloride on 5 g.); the volume of hydrogen absorbed in 2 hours was 180 c.c. (calc., 172·5 c.c.). The product was precipitated with water and recrystallised, first from alcohol (charcoal) containing xylene, and finally twice from acetic acid. The *acetic*-β-apo*cholic acid* obtained had m. p. 150—155°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 73{\cdot}5^\circ$, $[\alpha]_{5461}^{20^\circ} + 86{\cdot}5^\circ$ (*c*, 0·435); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}18$. The absorption spectrum showed slight absorption at 240—250 mμ corresponding to the presence of about 0·3% of unreduced β-dihydroxycholadienic acid ($E_{1\,\mathrm{cm.}}^{1\%} = 1{\cdot}3$) [Found (micro) : C, 69·9; H, 9·5. $C_{24}H_{38}O_4,C_2H_4O_2$ requires C, 69·3; H, 9·4%]. Freed from acetic acid by recrystallisation from aqueous alcohol, and dried at 120° in a vacuum, the free β-apo*cholic acid* had m. p. 163—165°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 78{\cdot}5^\circ$, $[\alpha]_{5461}^{20^\circ} + 92{\cdot}4^\circ$ (*c*, 0·361); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}18$. Another specimen of the acetic acid compound, prepared in the same way, had $[\alpha]_{\mathrm{D}}^{20^\circ} + 74{\cdot}8^\circ$, $[\alpha]_{5461}^{20^\circ} + 88{\cdot}4^\circ$ (*c*, 0·477); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}18$; the absorption corresponded to 0·5% of unreduced acid. *Xylene*-β-apo*cholic acid*, prepared by crystallisation from alcohol containing a little xylene, and dried in a vacuum over phosphoric oxide, had m. p. 156·5—161°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 69{\cdot}7^\circ$, $[\alpha]_{5461}^{20^\circ} + 82{\cdot}5^\circ$ (*c*, 0·665); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}18$ [Found (micro) : C, 75·7; H, 9·9. $C_{24}H_{38}O_4,0{\cdot}5C_8H_{10}$ requires C, 75·8; H, 9·8%]. *Methyl* β-apo*cholate*, prepared from the silver salt and methyl iodide and recrystallised from aqueous methyl alcohol, had m. p. 84—87°; $[\alpha]_{\mathrm{D}}^{20^\circ} + 79^\circ$, $[\alpha]_{5461}^{20^\circ} + 90^\circ$ (*c*, 0·246); $\alpha_{5461}/\alpha_{\mathrm{D}} = 1{\cdot}14$ [Found, in air-dried material: (micro) C, 70·0; H, 10·2; loss at 80°/0·1 mm. over phosphoric oxide (macro), 6·2. Found for solvent-free material (micro) : C, 73·7; H, 10·0. $C_{25}H_{40}O_4,1{\cdot}5H_2O$ requires C, 69·6; H, 10·0; loss, 6·3%. $C_{25}H_{40}O_4$ requires C, 74·2; H, 10·0%].

β-apo*Cholic Acid and Selenium Dioxide.*—Acetic-β-*apo*cholic acid (0·5 g.) was treated with selenium dioxide in the usual way. The product was not exhaustively purified, owing to its small amount. Recrystallised from dilute acetic acid, it had m. p. 233—240°; $[\alpha]_D^{20°} + 77{\cdot}7°$, $[\alpha]_{5461}^{20°} + 92{\cdot}8°$ (*c*, 0·285); $\alpha_{5461}/\alpha_D = 1{\cdot}20$. The absorption spectrum showed a maximum at 250 mμ ($E_{1\,cm.}^{1\%} = 310$).

β-*apoCholic Acid and Perbenzoic Acid.*—The recrystallised product had m. p. 246—250°; $[\alpha]_D^{20°} + 36{\cdot}5°$, $[\alpha]_{5461}^{20°} + 41{\cdot}5°$ (*c*, 0·278); $\alpha_{5461}/\alpha_D = 1{\cdot}14$; absorption maximum at 248 mμ ($E_{1\,cm.}^{1\%} = 395$).

Catalytic Reduction of α-Dihydroxycholadienic Acid.—The acid (2 g.) in acetic acid (100 c.c.), with palladised charcoal (0·5 g. of palladous chloride on 5 g.), absorbed 125 c.c. of hydrogen in 1½ hours (calc., 115 c.c.). The acetic-*apo*cholic acid, precipitated by water and recrystallised from acetic acid, had m. p. 140—155°; $[\alpha]_D^{20°} + 42{\cdot}3°$, $[\alpha]_{5461}^{20°} + 50{\cdot}4°$ (*c*, 0·479); $\alpha_{5461}/\alpha_D = 1{\cdot}19$ [Found (micro): C, 69·1; H, 9·2. Calc. for $C_{24}H_{38}O_4,C_2H_4O_2$: C, 69·3; H, 9·4%]. It was converted into the xylene compound, m. p. 165—169°; $[\alpha]_D^{20°} + 39{\cdot}6°$, $[\alpha]_{5461}^{20°} + 46{\cdot}3°$ (*c*, 0·6295); $\alpha_{5461}/\alpha_D = 1{\cdot}17$ [Found (micro): C, 75·3; H, 9·8. Calc. for $C_{24}H_{38}O_4,0{\cdot}5C_8H_{10}$: C, 75·8; H, 9·8%].

α-Ergostenol and Selenium Dioxide.—α-Ergostenol (1 g.), m. p. 130—133°, and selenium dioxide (1 g.) in 75% aqueous alcohol (200 c.c.) were kept for 16 hours; little reaction occurred. The mixture was refluxed for 1 hour, and the selenium (0·1 g.) removed by filtration. The product (0·8 g.) separated on cooling; m. p. 123—127°. It was acetylated by boiling with acetic anhydride for 15 minutes. After three recrystallisations from alcohol and finally from ethyl acetate, dehydroergostenyl acetate was obtained, m. p. 136—137°; $[\alpha]_D^{20°} - 34{\cdot}3°$, $[\alpha]_{5461}^{20°} - 43{\cdot}6°$ in chloroform (*c*, 0·45); $\alpha_{5461}/\alpha_D = 1{\cdot}28$. Hydrolysis and recrystallisation from alcohol yielded dehydroergostenol, m. p. 139·5—141°; $[\alpha]_D^{20°} - 17{\cdot}5°$, $[\alpha]_{5461}^{20°} - 23°$ in chloroform (*c*, 0·2575); $\alpha_{5461}/\alpha_D = 1{\cdot}32$. Another preparation was converted into the 3 : 5-*dinitrobenzoate*, which formed yellow prisms from ethyl acetate, m. p. 163—166°; $[\alpha]_D^{20°} - 3{\cdot}0°$, $[\alpha]_{5461}^{20°} - 5{\cdot}7°$ in benzene (*c*, 0·437) [Found (micro): C, 70·8; H, 8·2. $C_{35}H_{48}O_6N_2$ requires C, 70·9; H, 8·2%].

Note on Physical Measurements.—The m. p.'s were all observed in an electrically heated block with short-stem thermometers, and are, therefore, "corrected." The optical rotations were measured in a 4 dm. tube; all solutions were in absolute alcohol unless otherwise stated. I am indebted to Dr. F. A. Askew, formerly of this Institute, and to E. H. Pitte, our technical assistant, for most of the absorption measurements, which were made in 0·002% or 0·003% alcoholic solution by the method described by Philpot and Schuster (*Medical Research Council, Special Report Series*, 1933, No. 177).

SUMMARY.

The action of selenium dioxide on *apo*cholic acid yields the dextrorotatory β-dihydroxycholadienic acid, and the action of perbenzoic acid yields the lævorotatory α-dihydroxycholadienic acid. The "dihydroxycholadienic acids" described in the literature, formed by other methods, are mixtures of these two isomerides, as are also the products obtained from dihydroxycholenic acid. The α- and the β-dienic acid have identical m. p.'s, but different absorptions in the ultra-violet region. The α-acid is catalytically reduced to *apo*cholic acid, and the β-acid yields β-*apo*cholic acid, distinguished by its higher dextrorotation. It is concluded that *apo*cholic acid and dihydroxycholenic acids are $\Delta^{8:14}$- and $\Delta^{14:15}$-compounds, respectively, and that α- and β-dihydroxycholadienic acid are $\Delta^{7:8-14:15}$- and $\Delta^{9:8-14:15}$-compounds, respectively. The two *apo*cholic acids differ in the configuration of carbon atom 9.

Further consideration of the results described in Part I (Callow and Rosenheim, J., 1933, 387) indicates that ergosterol-*D*, formed by the interaction of dihydroergosterol and selenium dioxide, is the $\Delta^{9:8-14:15-22:23}$-compound, and the oxide formed at the same time is the $\Delta^{8:14}$-compound and yields the $\Delta^{7:8-14:15-22:23}$-compound, ergosterol-B_3, on decomposition.

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