461. *Basic Derivatives of Steroids. 3-Amino-7 : 12-dihydroxy- and 3-Amino-12-hydroxy-cholanic Acid.*

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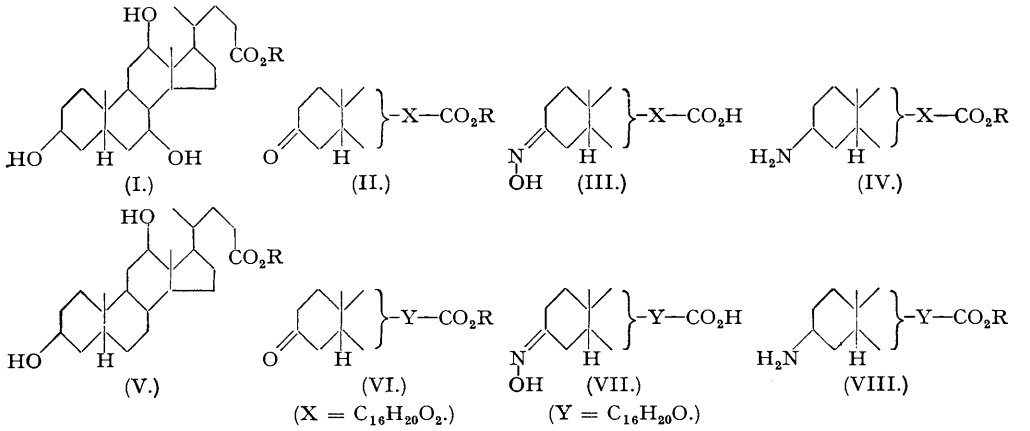
Oxidation of methyl cholate (I; R = Me) and methyl deoxycholate (V; R = Me) with aluminium *tert.*-butoxide effects preferential oxidation to the corresponding 3-keto-compounds. These keto-derivatives (II; R = Me) and (VI; R = Me) have been converted into oximes (III) and (VII), and these in turn have been transformed into 3-amino-7 : 12-dihydroxycholanic acid (IV; R = H) and 3-amino-12-hydroxycholanic acid (VIII; R = H), respectively. The bacteriostatic activities of these amino-acids and some of their esters against *Staph. aureus* and *Aerobacter aerogenes* have been determined; the *iso*amyl ester of 3-amino-7 : 12-dihydroxycholanic acid proved to be the most active.

Previous investigations of basic derivatives of cholesterol (Barnett, Ryman, and Smith, *J.*, 1946, 524, 528) and of cholane and norcholane (James, Smith, Stacey, and Webb, *J.*, 1946, 665) indicated that these substances have bacteriostatic activity. We have now extended this work to derivatives of hydroxycholanic acids, which possess an amino-group at $C_{(3)}$, namely, 3-amino-7 : 12-dihydroxycholanic acid (IV; R = H) and 3-amino-12-hydroxycholanic acid (VIII; R = H).

These amino-compounds were prepared by reduction of the oximes of the 3-keto-acids, the latter being derived by preferential oxidation of the 3-hydroxyl group with a mixture of aluminium *tert.*-butoxide and acetone (Oppenauer, *Rec. Trav. chim.*, 1937, **56**, 137). Thus methyl cholate (I; R = Me) and methyl deoxycholate (V; R = Me) yielded methyl 7 : 12-dihydroxy-3-keto-cholanate (II; R = Me) (cf. Kuwada and Morimoto, *Bull. Chem. Soc. Japan*, 1942, **17**, 147) and

methyl 12-hydroxy-3-ketocholanate (VI; R = Me) (cf. Riegel and McIntosh, *J. Amer. Chem. Soc.*, 1944, **66**, 1099), respectively, as the main products of the reaction. In each case, the oxidation was accompanied by the partial hydrolysis of the ester grouping which gave rise to a variable yield of the corresponding 3-keto-acid.



Although oxidation at $C_{(3)}$ may be facilitated by a Δ^5-ethenoid linkage (Barton and Jones, *J.*, 1943, 599; Inhoffen *et al.*, *Ber.*, 1938, **71**, 1024; Miescher and Klarer, *Helv. Chim. Acta*, 1939, **22**, 962; Butenandt and Schmidt-Thomé, *Ber.*, 1939, **72**, 182) this linkage appears not to be essential as oxidation of the saturated compounds (I; R = Me) and (V; R = Me) proceeds smoothly and appears to be confined to the 3-position (see Ehrenstein and Stevens, *J. Org. Chem.*, 1940, **5**, 660; Fuchs and Reichstein, *Helv. Chim. Acta*, 1943, **26**, 511; Gallagher and Xenos, *J. Biol. Chem.*, 1946, **165**, 365). Moreover, the product is easy to isolate (cf. Gallagher, *ibid.*, 1940, **133**, xxxvi).

The identity of the methyl 7 : 12-dihydroxy-3-ketocholanate (II; R = Me) was established as described in the experimental section. Its hydrolysis afforded the *acid* (II; R = H), which crystallised from aqueous ethyl alcohol with or without alcohol of crystallisation, according to the concentration of ethyl alcohol in the solvent mixture.

The methyl 12-hydroxy-3-ketocholanate (VI; R = Me) was characterised by its m. p., the m. p. of the derived acid (VI; R = H), and the smooth conversion of the latter into the crystalline oxime (VII).

Reduction of the *oximes* (III) and (VII) with sodium in boiling amyl alcohol gave the corresponding 3-amino-compounds (IV; R = H) and (VIII; R = H), respectively, which were isolated as crystalline amino-acid *hydrochlorides*. The stereochemical arrangement of the group at $C_{(3)}$ in each case was not ascertained.

In an attempt to prepare 3-amino-7 : 12-dihydroxycholanic acid, 3-chloro-7 : 12-dihydroxycholanic acid was treated with ammonia (cf. Windaus and Adamla, *Ber.*, 1911, **44**, 3051). The experiment failed, however, and 7 : 12-dihydroxy-Δ^3-cholenic acid (Wieland, Honold, and Pascual-Vila, *Z. physiol. Chem.*, 1923, **130**, 326) was the main product of the reaction.

The *methyl* and the iso*amyl* ester (IV; R = Me and *iso*-C_5H_{11}) and the iso*amyl* ester (VIII; R = *iso*-C_5H_{11}) were prepared in the usual way, and their bacteriostatic activities against *Staph. aureus* and *Aerobact. aerogenes* were determined. In view of the marked activity of the iso*amyl ester* (IV; R = *iso*-C_5H_{11}) a further study of related substances is being pursued.

The bacteriostatic activities of the basic derivatives against *Staph. aureus* and *Aerobact. aerogenes*, determined by the serial dilution method in a glucose–peptone broth medium, were as follows:

Hydrochloride of:	Limiting dilution inhibiting growth after 24 hours. *Staph. aureus.*	*Aerobact. aerogenes.*
3-Amino-7 : 12-dihydroxycholanic acid	1 : 4,000	1 : 4,000
Methyl 3-amino-7 : 12-dihydroxycholanate	1 : 4,000	1 : 4,000
*iso*Amyl 3-amino-7 : 12-dihydroxycholanate	1 : 512,000	1 : 64,000
3-Amino-12-hydroxycholanic acid	1 : 16,000	1 : 8,000
*iso*Amyl 3-amino-12-hydroxycholanate	1 : 32,000	1 : 16,000

Experimental.

(A) *Oxidation of Methyl* 3 : 7 : 12-*Trihydroxycholanate* (*Methyl Cholate*) *with Oppenauer's Reagent.*—A solution of dry methyl cholate (30 g.) and aluminium *tert.*-butoxide (36 g.) in a mixture of dry benzene (900 c.c.) and dry acetone (375 c.c.) was boiled under reflux for 18 hours. The turbid reaction product was cooled and poured with stirring into 2N-sulphuric acid (600 c.c.). The benzene layer was separated and washed with 2N-sulphuric acid (twice), water (twice), sodium hydrogen carbonate solution (twice), and again with water. After drying (Na_2SO_4), the solvent was removed under reduced pressure, and the residue recrystallised from benzene–light petroleum (1 : 1). After recrystallisation from aqueous methyl alcohol, the methyl 7 : 12-dihydroxy-3-ketocholanate (18·7 g.) formed small prisms, m. p. 166°. From methyl alcohol it separated as large colourless prisms, m. p. 171—172°, $[\alpha]_D^{15}$ +36·5° in methyl alcohol (*c*, 2·5) (Found : C, 71·4; H, 9·6. Calc. for $C_{25}H_{40}O_5$: C, 71·4; H, 9·5%). There was no depression of m. p. in admixture with a specimen, m. p. 172—172·5°, $[\alpha]_D^{20}$ +36·1° in methyl alcohol (*c*, 1·0), prepared by Haslewood's method (*Biochem. J.*, 1944, **38**, 108).

The sodium hydrogen carbonate extract of the benzene solution (see above) was acidified with dilute sulphuric acid, and the resulting precipitate filtered off after 18 hours. The dried solid (1·5 g.) was extracted with light petroleum, and the residue crystallised from acetone–light petroleum. The product separated as short white needles (0·75 g.), m. p. 182—184° (sintering at 118—123°). After recrystallisation from aqueous alcohol, the product had m. p. 186—188°, $[\alpha]_D^{20}$ +36·9° in ethyl alcohol (*c*, 1·0) [cf. 7 : 12-dihydroxy-3-ketocholanic acid : m. p. 123°, $[\alpha]_D^{16}$ +37·5° (*c*, 3·7) in ethyl alcohol] (Found : C, 71·4; H, 9·3. Calc. for $C_{24}H_{38}O_5$: C, 70·9; H, 9·4%).

The Hammarsten reaction (see Haslewood, *Biochem. J.*, 1946, **40**, 52) of this compound was identical with that of 7 : 12-dihydroxy-3-ketocholanic acid (*i.e.*, yellow-green–violet) and different from that of both 3 : 12-dihydroxy-7-ketocholanic acid and 3 : 7-dihydroxy-12-ketocholanic acid.

Methyl 12-*Hydroxy*-3-*keto*-7-*acetoxycholanate.*—A solution of methyl 7 : 12-dihydroxy-3-ketocholanate (0·55 g.) in glacial acetic acid (5·5 c.c.) and acetyl chloride (0·42 g.) was kept at room temperature for 3 days and then poured with stirring into water (100 c.c.). The precipitated solid was collected after 15 minutes, washed with water, and crystallised from aqueous methyl alcohol. Recrystallisation from methyl alcohol gave *methyl* 12-*hydroxy*-3-*keto*-7-*acetoxycholanate* (0·4 g.) as colourless needles (Found : C, 70·1; H, 8·75. $C_{27}H_{42}O_6$ requires C, 70·1; H, 9·1%), $[\alpha]_D^{17}$ +59·4° in chloroform (*c*, 1·0), m. p. 193—195° alone and in admixture with a specimen of methyl 12-hydroxy-3-keto-7-acetoxycholanate {m. p. 193–195°, $[\alpha]_D^{17}$ +59·3° in chloroform (*c*, 2·5)} prepared from methyl 7 : 12-dihydroxy-3-ketocholanate obtained by Haslewood's method. The ester reacted with 2 equivs. of sodium hydroxide (0·1354 g. required 5·65 c.c. of 0·103N-sodium hydroxide for hydrolysis. $C_{27}H_{42}O_6$ requires 5·69 c.c.).

Methyl 3-*Keto*-7 : 12-*diacetoxycholanate.*—A solution of methyl 7 : 12-dihydroxy-3-ketocholanate (1·0 g.) in dry pyridine (4·0 c.c.) and acetic anhydride (3 c.c.) was heated at 100° for 30 hours. After cooling, the product was poured with stirring into cold water (200 c.c.), and the solid which separated was collected after 15 minutes, washed with water, and dissolved in ether (50 c.c.). The ethereal solution was washed with dilute hydrochloric acid (twice), water (twice), sodium hydrogen carbonate solution (twice), and again with water. After drying ($CaCl_2$), the solution was evaporated, and the residue crystallised from ethyl alcohol. A further crystallisation from aqueous ethyl alcohol gave methyl 7 : 12-diacetoxy-3-ketocholanate (0·6 g.) as fine needles, m. p. 186—189° (Found : C, 68·7; H, 8·8. Calc. for $C_{29}H_{44}O_7$: C, 69·0; H, 8·8%). After recrystallisation from benzene–light petroleum (1 : 1) it had m. p. 190—191°, $[\alpha]_D^{15}$ +56·8° in chloroform (*c*, 3·3). The m. p. was not depressed on admixture with an authentic specimen of methyl 7 : 12-diacetoxy-3-ketocholanate, m. p. 190—191°, $[\alpha]_D^{15}$ +57·3° in chloroform (*c*, 3·3).

Ethyl 7 : 12-*Dihydroxy*-3-*ketocholanate.*—A solution of methyl 7 : 12-dihydroxy-3-ketocholanate (1·5 g.) in ethyl alcohol (45 c.c.) containing concentrated sulphuric acid (1·0 c.c.) was boiled under reflux for 6 hours, cooled, and poured with stirring into water (30 c.c.). After 24 hours the precipitated solid was filtered off, washed with water, and crystallised from aqueous ethyl alcohol. Ethyl 7 : 12-dihydroxy-3-ketocholanate (1·1 g.) separated as fine colourless needles, m. p. 168—169°, and after recrystallisation from benzene containing a little light petroleum it (Found : C, 71·45; H, 9·85. Calc. for $C_{26}H_{42}O_5$: C, 71·85; H, 9·7%) had $[\alpha]_D^{16}$ +32·0° in chloroform (*c*, 4·8), m. p. 179—181° alone or in admixture with an authentic specimen of m. p. 179—181°, $[\alpha]_D^{16}$ +31·6° in chloroform (*c*, 4·0), prepared according to Haslewood's method (*loc. cit.*).

7 : 12-*Dihydroxy*-3-*ketocholanic Acid.*—The foregoing methyl ester (1·4 g.) was hydrolysed by boiling it for 4 hours under reflux with 0·96N-sodium hydroxide (15 c.c.) in ethyl alcohol (20 c.c.). The solution was then diluted with water (50 c.c.) and concentrated under reduced pressure to remove ethyl alcohol. After cooling, the solution was further diluted with water (50 c.c.) and acidified with dilute hydrochloric acid. After 16 hours the partly crystalline product which separated was filtered off, washed with water, and crystallised from aqueous ethyl alcohol. The dry compound was washed with ether–light petroleum (1 : 1) and recrystallised from aqueous ethyl alcohol. This afforded 7 : 12-*dihydroxy*-3-*ketocholanic acid* (0·8 g.) (Found : C, 68·7; H, 9·7; OEt, 9·8. $C_{24}H_{38}O_5,C_2H_5{\cdot}OH$ requires C, 69·0; H, 9·8; OEt, 9·95%) as tetrahedra, $[\alpha]_D^{16}$ +37·9° in ethyl alcohol (*c*, 4·0), m. p. 121—123° (cf. Sihn, *J. Biochem. Japan*, 1938, **27**, 425) alone or in admixture with an authentic specimen which had $[\alpha]_D^{16}$ +37·5° in ethyl alcohol (*c*, 3·7), m. p. 121—123°.

In order to prove that the ethoxyl content of the crystals (m. p. 121—123°) was due to solvation the following experiment was carried out. The crystals were heated *in vacuo* at 140° for 2 hours, and an ethoxyl determination was carried out on the glassy residue (Found : OEt, nil). When a portion of this glassy residue was crystallised from aqueous alcohol as before, crystals separated, m. p. 120—122° alone or in admixture with the original crystals. Crystallisation of the residue from a large volume of aqueous alcohol gave fine needles, m. p. 181—182° (cf. Kuwada and Morimoto, *loc. cit.*), which, when recrystallised from a small volume of aqueous alcohol, formed tetrahedra which first melted at 120°, then resolidified at 130—140°, and finally melted at 181—182°.

3-*Keto*-7 : 12-*diformoxycholanic Acid.*—A mixture of 7 : 12-dihydroxy-3-ketocholanic acid (0·45 g.),

sodium formate (0·15 g.), and formic acid (1·5 c.c.) was heated to 70—80° for 5 hours. The cooled solution was poured into water (100 c.c.), and the resulting precipitate collected after 1 hour, washed with water, dried *in vacuo* over phosphoric oxide, and crystallised from aqueous alcohol. The product (0·33 g.) separated as small white needles (m. p. 170° with previous sintering at 155°) which showed $[\alpha]_D^{18}$ +83·6° in ethyl alcohol (*c*, 1·0) (Found : C, 67·2; H, 8·1. $C_{26}H_{38}O_7$ requires C, 67·5; H, 8·3%). The *diformoxy*-compound was deformylated at room temperature with dilute aqueous sodium hydroxide (0·1377 g. required 6·60 c.c. of 0·1297N-sodium hydroxide. 7 : 12-Diformoxy-3-ketocholanic acid requires 6·87 c.c. for removal of the formyl groups and neutralisation of the carboxyl group). The deformylated product which separated upon acidification was filtered off, washed with water, and crystallised from aqueous ethyl alcohol. It separated as white needles, m. p. 190—192°. Thus, formylation and deformylation of the acid appear to effect its purification.

Oxime of 7 : 12-*Dihydroxy*-3-*ketocholanic Acid.*—A solution of the acid (10 g.) in the minimum of absolute alcohol was boiled under reflux for 3 hours with hydroxylamine hydrochloride (5 g.) and aqueous 5N-sodium hydroxide (12 c.c.). The cooled mixture was acidified with glacial acetic acid, poured into water (600 c.c.), and set aside for 18 hours. The resulting precipitate was collected, dried, and dissolved by prolonged boiling with absolute ethyl alcohol (250 c.c.). On cooling, small white prisms of the *oxime* separated (6·3 g.), m. p. 232° (decomp.), $[\alpha]_D^{21}$ +51·2° in ethyl alcohol (*c*, 1·0) (Found : C, 69·0; H, 9·4; N, 3·5. $C_{24}H_{39}O_5N$ requires C, 68·4; H, 9·3; N, 3·3%). A further 1·2 g. of oxime were recovered from the mother-liquors upon concentration.

The oxime formed from the acid fraction, m. p. 186—188° (0·1 g.), in the above manner showed $[\alpha]_D^{18}$ +52° in ethyl alcohol (*c*, 0·8) and m. p. 235° (decomp.).

3-*Amino*-7 : 12-*dihydroxycholanic Acid Hydrochloride.*—A solution of the oxime (7·5 g.) in boiling amyl alcohol (375 c.c.) was treated with sodium (30 g.), added in small pieces during 4·5 hours. The cooled solution was neutralised with dilute hydrochloric acid and evaporated under reduced pressure. The last traces of amyl alcohol were removed by simultaneous addition and distillation of water under reduced pressure. The residue was dissolved in 5N-sodium hydroxide, and the alkaline solution acidified with dilute hydrochloric acid, saturated with sodium chloride, and kept for 18 hours. The resulting precipitate was filtered off, dried *in vacuo* over phosphoric oxide, and dissolved in absolute alcohol (50 c.c.). The alcoholic solution was filtered, and evaporated to dryness. The resulting light-brown syrup upon crystallisation from alcohol and acetone (1 : 4) yielded 3-*amino*-7 : 12-*dihydroxycholanic acid hydrochloride* (4 g.) as small white needles. After recrystallisation from alcohol and acetone it showed m. p. 255° (decomp.), $[\alpha]_D^{25}$ +35·6° in ethyl alcohol (*c*, 1·0) (Found : C, 62·0; H, 9·55; N, 3·45; Cl, 8·5; H_2O, 3·8. $C_{24}H_{42}O_4NCl,H_2O$ requires C, 62·4; H, 9·5; N, 3·2; Cl, 8·0; H_2O, 3·9%).

Methyl ester. A solution of the above amino-acid hydrochloride (0·5 g.) in 1% methanolic hydrogen chloride (10 c.c.) was boiled under reflux for 3 hours. The solution was evaporated under reduced pressure and kept *in vacuo* over solid sodium hydroxide for 24 hours. Two crystallisations of the residue from alcohol and acetone (1 : 4) yielded the *methyl* ester *hydrochloride* (0·4 g.), m. p. 274° (decomp.), $[\alpha]_D^{18}$ +35·9° in ethyl alcohol (*c*, 1·0) (Found : N, 3·15; OMe, 6·4. $C_{25}H_{44}O_4NCl$ requires N, 3·0; OMe, 6·8%).

iso*Amyl ester.* A mixture of 3-amino-7 : 12-dihydroxycholanic acid hydrochloride (0·5 g.), *iso*amyl alcohol (30 c.c.), and 10N-hydrochloric acid (2 c.c.) was heated on a steam-bath for 3 hours. The solvents were removed by distillation under reduced pressure, and the residue crystallised from alcohol and acetone. The iso*amyl* ester *hydrochloride* thus obtained (0·45 g.), on drying *in vacuo* over phosphoric oxide and sodium hydroxide, had m. p. 260° (decomp.), $[\alpha]_D^{25}$ +29·7° in ethyl alcohol (*c*, 1·0) (Found : C, 66·75; H, 10·0; N, 2·7; Cl, 7·4; H_2O, 1·9. $C_{29}H_{52}O_4NCl,\frac{1}{2}H_2O$ requires C, 66·6; H, 10·2; N, 2·7; Cl, 6·9; H_2O, 1·72%).

(B) *Oxidation of Methyl Deoxycholate with Aluminium* tert.-*Butoxide.*—A solution of methyl deoxycholate (14·7 g.) and aluminium *tert.*-butoxide (15·5 g.) in a mixture of dry benzene (400 c.c.) and dry acetone (160 c.c.) was boiled for 18 hours under reflux. The solution was cooled and poured with stirring into 2N-sulphuric acid (500 c.c.). The benzene layer was separated, washed with 2N-sulphuric acid (twice), sodium hydrogen carbonate (twice), and water (once). After drying ($CaCl_2$), the solution was evaporated to give a pale yellow syrup which readily crystallised. Recrystallisation from aqueous ethyl alcohol gave material, m. p. 134—138°. Further crystallisation of this product from light petroleum afforded methyl 12-hydroxy-3-ketocholanate (8·3 g., 57%), m. p. 140—142°, $[\alpha]_D^{23}$ +51° in ethyl alcohol (*c*, 0·5) (Found : C, 74·25; H, 10·05. Calc. for $C_{25}H_{40}O_4$: C, 74·2; H, 10·0%).

The sodium hydrogen carbonate extract of the benzene solution was acidified with dilute sulphuric acid, and the resulting precipitate collected after 18 hours, washed with water, and dried. Crystallisation of the crude solid (2·3 g.) from aqueous acetic acid yielded white plates (1·8 g.), m. p. 105—110°. Recrystallisation from acetone and light petroleum yielded 12-hydroxy-3-ketocholanic acid, $[\alpha]_D^{18}$ ++51° in ethyl alcohol (*c*, 1·0), m. p. and mixed m. p. 155—158°. The compound was converted into its oxime (see below), m. p. 143—144°, $[\alpha]_D^{21}$ +60·6° in ethyl alcohol (*c*, 1·0), in the usual way.

12-*Hydroxy*-3-*ketocholanic Acid.*—A solution of methyl 12-hydroxy-3-ketocholanate (1·3 g.) in absolute alcohol (20 c.c.) and 30% sodium hydroxide (2·5 c.c.) was kept at room temperature for 20 hours, poured into water (600 c.c.), and acidified, and the resulting precipitate was filtered off and washed with water. Crystallisation from dilute acetic acid gave a product, m. p. 105°. Recrystallisation from aqueous alcohol yielded 12-hydroxy-3-ketocholanic acid (1·05 g.), m. p. 156—158°, $[\alpha]_D^{18}$ +52° in ethyl alcohol (*c*, 1·0).

Oxime. A mixture of this acid (0·95 g.), absolute alcohol (4 c.c.), hydroxylamine hydrochloride (0·5 g.), and 5N-sodium hydroxide (2 c.c.) was boiled under reflux for 3 hours. The cooled solution was acidified with glacial acetic acid, poured into water, and kept for 18 hours. The resulting crystalline solid was filtered off, washed, dried *in vacuo* over phosphoric oxide, and recrystallised first from absolute alcohol and then from aqueous methyl alcohol. The *oxime* (0·8 g.) had m. p. 144—145°, $[\alpha]_D^{21}$ +60·9° in ethyl alcohol (*c*, 1·0) (Found : C, 67·95; H, 9·6; N, 3·4; H_2O, 4·2. $C_{24}H_{39}O_4N,H_2O$ requires C, 68·0; H, 9·75; N, 3·3; H_2O, 4·25%).

Reduction of the Oxime of 12-*Hydroxy*-3-*ketocholanic Acid.*—A solution of the oxime (1 g.) in boiling

amyl alcohol (50 c.c.) was treated with sodium (5 g.) added portionwise during 4—5 hours. The cooled solution was neutralised with dilute hydrochloric acid and evaporated to dryness under reduced pressure. The residue was dissolved in 2N-sodium hydroxide, acidified with 2N-hydrochloric acid, saturated with sodium chloride, and set aside for 18 hours. The resulting precipitate was filtered off, dried *in vacuo* over phosphoric oxide, and dissolved in absolute alcohol. The alcoholic solution was filtered, the filtrate concentrated to dryness, and the residue crystallised from alcohol–acetone. Recrystallisation from alcohol–acetone gave 3-*amino*-12-*hydroxycholanic acid hydrochloride* (0·25 g.), $[\alpha]_D^{21}$ +50·8° in ethyl alcohol (*c*, 1·0), m. p. 239° (sintering at 185°) (Found, after drying at 200° : C, 67·3; H, 9·8; N, 3·4. $C_{24}H_{42}O_3NCl$ requires C, 67·3; H, 9·9; N, 3·3%).

The residue from the mother-liquors of the first crystallisation was dissolved in dilute aqueous sodium hydroxide, and the slightly cloudy solution extracted with ether. The aqueous solution was acidified with dilute hydrochloric acid, and the resulting precipitate filtered off. Crystallisation from aqueous acetone yielded slightly coloured needles, m. p. 175—180° (0·1 g.). The compound was dissolved in aqueous alcohol, and the solution treated with charcoal, filtered, and allowed to crystallise. The white needles thus produced had m. p. 175—180°, raised by further crystallisation from aqueous methyl alcohol to 180—182°. This product, which did not contain nitrogen, was not investigated further.

iso*Amyl* 3-*Amino*-12-*hydroxycholanate Hydrochloride*.—A solution of 3-amino-12-hydroxycholanic acid hydrochloride (0·1 g.) in *iso*amyl alcohol (6 c.c.) and 10N-hydrochloric acid (0·4 c.c.) was heated on a steam-bath for 6 hours. The solution was evaporated to dryness under reduced pressure, and the residue crystallised from alcohol–acetone. The iso*amyl* ester *hydrochloride* separated as white prisms, m. p. 242—245° (decomp.), $[\alpha]_D^{18}$ +35·3° in ethyl alcohol (*c*, 1·0) (Found.: N, 2·7. $C_{29}H_{52}O_3NCl$ requires N, 2·8%).

Treatment of 3-*Chloro*-7 : 12-*dihydroxycholanic Acid with Ammonia*.—A suspension of 3-chloro-7 : 12-dihydroxycholanic acid (1·2 g.; Wieland, Honold, and Pascual-Vila, *loc. cit.*) in alcoholic ammonia (15 c.c.) containing ammonium iodide (0·3 g.) was heated at 180° for 8 hours. The product was extracted with ethyl alcohol, and the combined extracts evaporated under reduced pressure. The residual solid was suspended in hot water (25 c.c.), and N-sodium hydroxide added to complete dissolution (ammonia evolved). Separation of the sodium salt of an unsaturated acid occurred on cooling the solution. The solid was collected at the pump, and the filtrate acidified with acetic acid; the precipitate thus formed was filtered off, washed with water, and dried. The filtrate was free from bile acid derivatives. Recrystallisation of the product (0·5 g.) from ethyl acetate and from ethyl alcohol gave small prisms, m. p. 214—215°. The product was unsaturated, decolorised a solution of bromine in carbon tetrachloride, and did not contain nitrogen. It appeared to be 7 : 12-dihydroxy-Δ^3-cholenic acid, m. p. 215—217° (Wieland, Honold, and Pascual-Vila, *loc. cit.*), in admixture with which it gave no depression of the m.p. It was noted that the unsaturated acid of Wieland *et al.* (*loc. cit.*) formed a sparingly soluble sodium salt.

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