136. *Basic Derivatives of Cholane and Norcholane.*

By Sybil P. James, F. Smith, M. Stacey, and M. Webb.

The synthesis of certain basic nitrogen derivatives of cholane and norcholane is described. The hydrochlorides of 23-amino-3 : 7 : 12-trihydroxynorcholane (I) and 23-amino-3 : 12-dihydroxynorcholane (II) were made by the method of Caldwell and 23-*amino*-3 : 12-*dihydroxy*-Δ^8-*norcholene* (III) was similarly prepared from 3 : 12-*dihydroxy*-Δ^8-*cholenylhydrazine* while the decomposition of 3 : 12-*dihydroxy*-7-*ketocholanylhydrazine* 7-*hydrazone* (IV) afforded 23-*amino*-3 : 12-*dihydroxy*-7-*ketonorcholane hydrochloride* (V). Dehydration of triformylcholylamide gave 3 : 7 : 12-*triformyl*-23-*cyanonorcholane* from which 3 : 7 : 12-*trihydroxy*-23-*cyanonorcholane* (VII) was obtained. This was converted into 23-*amidino*-3 : 7 : 12-*trihydroxynorcholane hydrochloride* (VIII), 24-*amino*-3 : 7 : 12-*trihydroxycholane hydrochloride* (IX), and 3 : 7 : 12-triketo-23-cyanonorcholane (XI) which gave a *trioxime*. (I) and (IX) furnished the corresponding guanido-derivatives (VI) and (X). 7-Amino-3 : 12-dihydroxycholanic acid was obtained on reduction of the *oxime* of ethyl 3 : 12-dihydroxy-7-ketocholanate and was isolated as the *hydrochloride* (XII) and as the N-*benzoyl* derivative. Reduction of the trioxime derived from dehydrocholic acid and subsequent removal of the residual oxime group gave a *diamino-keto-acid dihydrochloride* (presumably 3 : 7-diamino-12-ketocholanic acid dihydrochloride, XIII).

The *in vitro* antibacterial action of the bile acids against certain Gram-positive micro-organisms is well known and the literature has been adequately reviewed by Sobotka (" Physiological Chemistry of the Bile," Baillière, Tindall, and Cox, 1937, 125). Attempts to use the bile acids for chemotherapeutic purposes, principally in the case of pneumococcus (White, " The Biology of Pneumococcus," New York, 1938, 507), have, however, been complicated by the toxicity, hæmolytic action, and destructive effect on leucocytes produced by these acids. With the object of preparing arsenical therapeutics of greater lipoid solubility than that of compounds previously employed, Lieb, Verdino, and Schadendorff (*Annalen*, 1934, **512**, 89) formed condensation

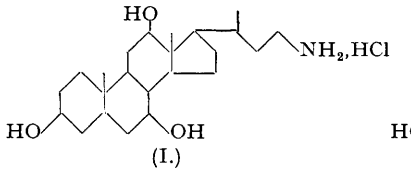
products of *p*-arsanilic acid with the 3-chlorocarboxyl derivatives of cholesterol and cholic acid. The compounds, however, exhibited no activity against *Spirillæ*.

Some derivatives of sulphanilamide with cholic acid were made by Haslewood (*Biochem. J.*, 1941, **35**, 1307). It was found (Barber, Dible, and Haslewood, *Biochem. J.*, 1943, **37**, VI) that the compound $R{\cdot}CO{\cdot}NH{\cdot}NH{\cdot}SO_2{-}C_6H_4{-}NH_2$ (R = cholyl) had considerable bacteriostatic activity against *Streptococcus hæmolyticus* and *Streptococcus pneumoniæ* but had no action on the coliform group of organisms.

Bile salts have a marked action in removing the ribonucleic acid and other constituents from the surface of certain Gram-positive micro-organisms (Henry and Stacey, *Nature*, 1943, 157, 671) so that it was deemed desirable to attempt the synthesis of some basic derivatives of sterols and bile acids in order to examine their bacteriostatic power. Moreover, the occurrence of steroid substances throughout the animal body suggested that this type of molecule might form a vehicle for the conveyance of groups such as amino-, amidino-, and guanidino-, known to have anti-bacterial power, to the most deeply seated tissues. Further, the possibility that some basic steroid derivatives might show anti-carcinogenic activity was envisaged.

23-Amino-3 : 7 : 12-trihydroxynorcholane and 23-amino-3 : 12-dihydroxynorcholane have been described by Caldwell (*J. Amer. Chem. Soc.*, 1938, **60**, 991; 1939, **61**, 3584) and the cyano-group was introduced into the sterol nucleus by Butenandt (*Ber.*, 1938, **71**, 1487; 1939, **72**, 182). 7-Aminocholestane was prepared by Eckhardt (*Ber.*, 1938, **71**, 461) and the preparation of new amino-derivatives of cholestane was carried out side by side with the present work (see Barnett, Ryman, and Smith, this vol., pp. 524, 528).

The present communication deals with some basic derivatives of cholane and norcholane. These may be conveniently divided into two groups, those with the basic group in the side chain and those with basic groups in the nucleus.



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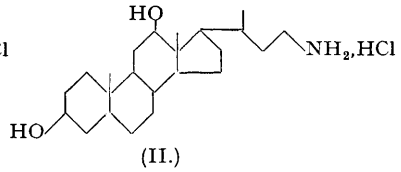

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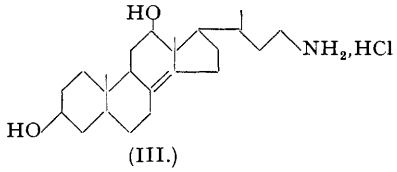

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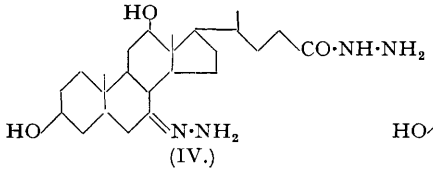

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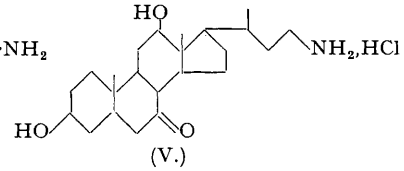

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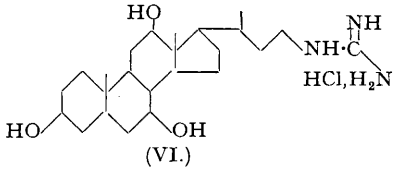

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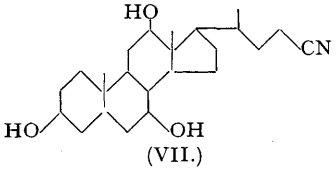

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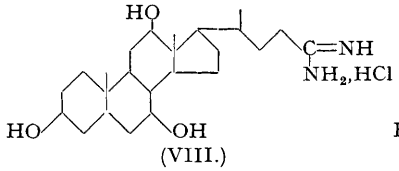

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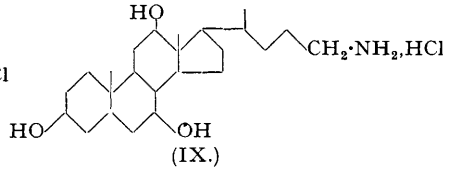

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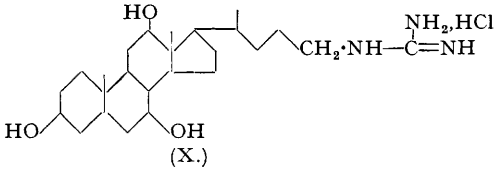

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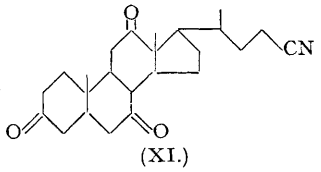


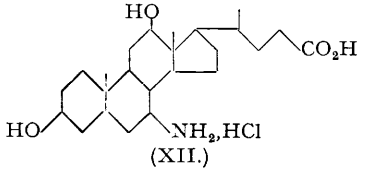
(XI.)

Of the first group the hydrochlorides of 23-amino-3 : 7 : 12-trihydroxynorcholane (I) and 23-amino-3 : 12-dihydroxynorcholane (II) were prepared in crystalline form by the method of Caldwell (*loc. cit.*). 23-*Amino*-3 : 12-*dihydroxy*-Δ^8-*norcholene* (III) was made by a similar method involving the decomposition, *via* the corresponding azide, of 3 : 12-*dihydroxy*-Δ^8-*cholenylhydrazine*. 23-*Amino*-3 : 12-*dihydroxy*-7-*ketonorcholane hydrochloride* (V) was also prepared by a similar series of reactions. Ethyl 3 : 12-dihydroxy-7-ketocholanate (Haslewood, *Biochem. J.*, 1944, **38**, 108) on treatment with hydrazine in aqueous alcohol afforded 3 : 12-*dihydroxy*-7-*ketocholanylhydrazine* 7-*hydrazone* (IV); this was converted, by the action of nitrous acid, into the azide which was subsequently decomposed by glacial acetic acid to give 23-amino-3 : 12-dihydroxy-7-ketonorcholane, isolated as the *hydrochloride* (V). 23-*Guanido*-3 : 7 : 12-*trihydroxynorcholane hydrochloride* (VI) was produced by the interaction of cyanamide and (I) in absolute alcohol at 130°.

The synthesis of 3 : 7 : 12-*trihydroxy*-23-*cyanonorcholane* (VII), which was used for the preparation of 23-*amidino*-3 : 7 : 12-*trihydroxynorcholane hydrochloride* (VIII) and 24-*amino*-3 : 7 : 12-*trihydroxycholane hydrochloride* (IX), was achieved in the following way. Methyl cholate was smoothly converted by alcoholic ammonia into cholylamide which afforded triformylcholylamide on being heated with formic acid. This product

was identical with a sample prepared by the method of Cortese and Bauman (*J. Amer. Chem. Soc.*, 1935, **57**, 1393) though our method of synthesis appeared to provide an easier route. Dehydration of triformylcholylamide by thionyl chloride gave 3 : 7 : 12-*triformyl*-23-*cyanonorcholane*. Ready removal of the formyl groups from this was effected by treatment with dilute sodium hydroxide and there resulted 3 : 7 : 12-trihydroxy-23-cyanonorcholane (VII). Alkaline hydrolysis of (VII) gave cholic acid, so that the treatment with thionyl chloride had affected the amide group only. Reduction of (VII) with sodium and amyl alcohol gave 24-amino-3 : 7 : 12-trihydroxycholane hydrochloride and the same product was obtained from (VII), though in comparatively poor yield, by catalytic hydrogenation. Cholylamidine hydrochloride was prepared by the method of Pinner ("Die Imido Äther") though without the intermediate isolation of the imino ether. 24-*Guanido*-3 : 7 : 12-*trihydroxycholane hydrochloride* (X) was obtained by treatment of (IX) with cyanamide.

Two compounds containing basic groups in the nucleus were prepared. These were 7-*amino*-3 : 12-*dihydroxycholanic acid hydrochloride* (XII) and a *diamino-keto-acid*, presumably 3 : 7-diamino-12-ketocholanic acid dihydrochloride (XIII). No attempt was made to separate the α- and β-forms of nuclear substituted derivatives.



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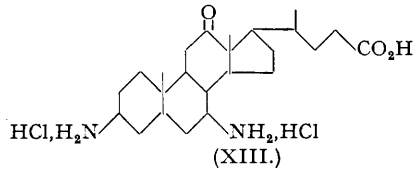

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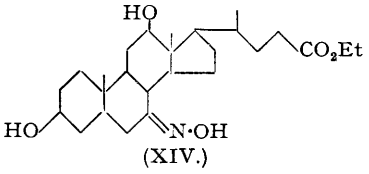

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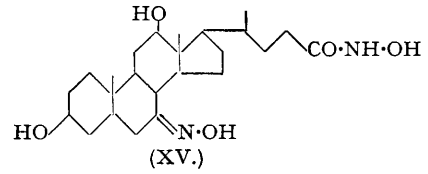

(XV.)

Ethyl 3 : 12-dihydroxy-7-ketocholanate on treatment with hydroxylamine at 80° for 3 hours gave the corresponding *oxime* (XIV). Reaction at higher temperatures and for a longer period resulted in the formation of the *oxime hydroxamic acid* (XV). Reduction of the oxime (XIV) by the method of Eckhardt (*loc. cit.*) gave 7-amino-3 : 12-dihydroxycholanic acid, isolated either as the crystalline hydrochloride above or as the 7-*benzamido*-derivative. The diamino-keto-acid was prepared by reduction, using sodium in amyl alcohol, of the trioxime derived from dehydrocholic acid followed by the removal of the residual oxime residue presumably attached to C_{12}. The product was isolated as the dihydrochloride (XIII).

No success was achieved in the introduction of cyano-groups into the nucleus. Pirronne (*Gazzetta*, 1932, **62**, 1101) reported the preparation of dicyanocholesterol by the action of anhydrous hydrogen cyanide upon cholesterol dibromide. We were unable to repeat this work and similar treatment of various halogen derivatives of steroids failed to give products containing nitrogen. The preparation of a cyanohydrin from dehydrocholic acid was attempted. While there was some indication that such a compound was formed efforts to isolate it failed and attempted dehydration of the crude material by the method of Butenandt (*Ber., loc. cit.*) did not give the required cyanide.

An attempt was made to prepare 3 : 7 : 24-triamino-12-ketocholane trihydrochloride. Oxidation of (VII) by the method of Hammarsten (*Ber.*, 1881, **14**, 71) gave 3 : 7 : 12-*triketo*-23-*cyanocholane* (XI) from which a *trioxime* was obtained. Reduction of the trioxime gave a product containing the triamine, but an acidic compound, probably (XIII), was also present.

Nuclear amines were found to have little or no *in vitro* bacteriostatic activity against *Staph. aureus* and *Lactobacillus helveticus*. Of the basic cholane and norcholane derivatives 23-guanido-3 : 7 : 12-trihydroxynorcholane had as shown below the highest *in vitro* bacteriostatic activity. This compound inhibited the growth of Gram-positive organisms at dilutions up to 1 : 64,000 but was only weakly bacteriostatic (1 : 4,000) for the Gram-negative bacillus, *Lactis ærogenes*.

The bacteriostatic activities of the basic derivatives were determined against *Staph. aureus*, *Lactobacillus helveticus*, and *B. lactis ærogenes* by the serial dilution method in glucose–peptone broth medium.

Hydrochlorides of :	Limiting dilution inhibiting growth after 24 hours at 37°. *Staph. aureus.*	*L. helveticus.*	*B. lactis ærogenes.*
23-Amino-3 : 7 : 12-trihydroxynorcholane	1 : 16,000	1 : 16,000	1 : 2000
23-Amino-3 : 12-dihydroxynorcholane	inactive	1 : 8,000	inactive
23-Amino-3 : 12-dihydroxy-7-ketonorcholane	inactive	1 : 1,000	inactive
Cholylamidine	1 : 2,000	1 : 2,000	1 : 1000
23-Guanido-3 : 7 : 12-trihydroxynorcholane	1 : 64,000	1 : 64,000	1 : 4000
24-Amino-3 : 7 : 12-trihydroxycholane	1 : 8,000	1 : 8,000	1 : 2000
23-Amino-3 : 12-dihydroxy-Δ^8-norcholene	1 : 8,000	1 : 8,000	1 : 1000
3 : 7-Diamino-12-ketocholanic acid	inactive	inactive	inactive
7-Amino-3 : 12-dihydroxycholanic acid	inactive	inactive	inactive

Experimental.

23-*Amino*-3 : 7 : 12-*trihydroxynorcholane Hydrochloride* (*Cholamine Hydrochloride*).—This compound was prepared by the method of Caldwell (1938, *loc. cit.*) and had $[\alpha]_D^{21°}$ + 32·7° in water (*c*, 0·6), m. p. 315° (decomp.) (Found : Cl, 7·5. Calc. for $C_{23}H_{42}O_3NCl$: Cl, 8·5%). " Cholamine hydrochloride " was obtained in crystalline form by the addition of a few drops of 5N-hydrochloric acid to a solution of the free base in acetone. The needle-like crystals obtained showed $[\alpha]_D^{18°}$ + 32·2° in water (*c*, 1·0) (Found : N, 4·0. Calc. for $C_{23}H_{42}O_3NCl$: N, 3·4%).

23-*Amino*-3 : 12-*dihydroxynorcholane*.—This compound was obtained by the method of Caldwell (1939, *loc. cit.*). The crude base had m. p. 127—129°, $[\alpha]_D^{18°}$ + 45·9° in alcohol (*c*, 1·8). The addition of ethereal hydrogen chloride to a solution of the dry base in absolute alcohol resulted in the separation of the *hydrochloride*, which was collected at the pump and washed with ether. On recrystallisation from aqueous acetone small prisms were obtained having m. p. 283° and $[\alpha]_D^{18°}$ + 52·9° in alcohol (*c*, 0·3) (Found : N, 4·0. $C_{23}H_{42}O_2NCl$ requires N, 3·5%).

Apocholylhydrazine.—Methyl apocholate (4 g.) (Boedecker, *Ber.*, 1920, **53**, 1852) was boiled under reflux for 10 hours with ethyl alcohol (14 c.c.) and an aqueous solution of hydrazine hydrate (8 c.c. of 50%). The solution was then concentrated under reduced pressure and finally evaporated to dryness in a vacuum over concentrated sulphuric acid. Recrystallisation of the residue from ethyl acetate gave white needles (2·9 g.) of *apocholylhydrazine*, m. p. 191—192°, $[\alpha]_D^{21°}$ + 35·6° in alcohol (*c*, 1·5) (Found : N, 6·9. $C_{24}H_{40}O_3N_2$ requires N, 6·9%).

23-*Amino*-3 : 12-*dihydroxy*-Δ^8-*norcholene*.—Sodium nitrite solution (0·5N) was slowly added to apocholylhydrazine (2·5 g.), dissolved in hydrochloric acid (400 c.c., 0·1N) at 0°, until the presence of a slight excess of nitrous acid was detected with starch–iodide paper. The mixture was kept at 0° for ½ hour and then the precipitated azide was collected at the pump, washed with water, and transferred while still moist into glacial acetic acid (30 c.c.). The mixture was heated at 100° for about 1 hour when the evolution of gas ceased, and was then cooled to 0° and made alkaline to litmus by the addition of sodium hydroxide solution. The precipitated *amine* was collected in a sintered glass funnel, washed with water, dried in a vacuum over phosphorus pentoxide and sodium hydroxide, and recrystallised from toluene. Crystals were obtained having m. p. 97—100° (Found : N, 4·3. $C_{23}H_{39}O_2N$ requires N, 3·9%).

23-*Amino*-3 : 12-*dihydroxy*-Δ^8-*norcholene Hydrochloride* (" *Apocholamine Hydrochloride* ") (III).—Dry hydrogen chloride was led into a solution of the crude amine in chloroform until the precipitation of the hydrochloride was complete. The supernatant liquid was decanted and the residual gum was triturated with acetone until it became solid. The *hydrochloride* was recrystallised from aqueous acetone containing a few drops of hydrochloric acid. Colourless needles were obtained, m. p. >290°, which showed $[\alpha]_D^{18°}$ + 39·3° in alcohol (*c*, 2·0) (Found : N, 3·7. $C_{23}H_{40}O_2NCl$ requires N, 3·5%).

Hydrazone of 3 : 12-*Dihydroxy*-7-*ketocholanylhydrazine* (IV).—Ethyl 3 : 12-dihydroxy-7-ketocholanate (10 g.) in ethyl alcohol (50 c.c.) was boiled under reflux for 30 hours with aqueous hydrazine hydrate (10 c.c. of 50%) and the solution was concentrated to about 20 c.c. under reduced pressure and finally evaporated to dryness in a vacuum desiccator over concentrated sulphuric acid. The residue (5·8 g.) was finely powdered, dried in a vacuum at 100°, and recrystallised from ethyl acetate to give colourless needles of the *hydrazone*, $[\alpha]_D^{18°}$ − 92·3° in alcohol (*c*, 1·3), m. p. >300° (Found : N, 12·9. $C_{24}H_{42}O_3N_4$ requires N, 12·9%).

23-*Amino*-3 : 12-*dihydroxy*-7-*ketonorcholane* (V).—3 : 12-Dihydroxy-7-ketocholanylhydrazine hydrazone (3·6 g.) was treated with sodium nitrite in acid solution as described for the preparation of apocholamine (see above) except that the decomposition of the azide was carried out at 60—70°. The free base finally obtained was dried in a vacuum over phosphorus pentoxide and sodium hydroxide and was then exhaustively extracted with boiling toluene. The extract was dried by slow distillation of the toluene during 4 hours, filtered, and allowed to cool. The amine separated in small needles (m. p. 123—127°) which were washed by decantation with toluene, dried in a vacuum, and dissolved in absolute ethyl alcohol. A stream of dry hydrogen chloride was led into the cooled alcoholic solution and then the amine hydrochloride was precipitated by the addition of dry ether. The solid was washed by decantation with ether, dried in a vacuum, and crystallised from aqueous acetone containing a little hydrochloric acid. The *product* (0·8 g.) formed small prisms, m. p. 263° (decomp.), $[\alpha]_D^{18°}$ ± 0° in alcohol (*c*, 0·43) (Found : Cl, 8·8; N, 3·4. $C_{23}H_{40}O_3NCl$ requires Cl, 8·6; N, 3·4%).

23-*Guanido*-3 : 7 : 12-*trihydroxynorcholane Hydrochloride* (VI).—A sample of the crystalline " cholamine hydrochloride " (1 g.) was heated with cyanamide (1 g.) in sodium-dry alcohol (25 c.c.) at 130° for 17 hours. The solution was filtered and concentrated to small bulk. Crystals of dicyanamide then separated; these were removed and the mother liquor was poured into dry acetone (200 c.c.) with stirring. The white solid *hydrochloride* (0·45 g.) which was precipitated was separated on the centrifuge and washed with acetone. It gave a positive Sakaguchi test and after recrystallisation from aqueous acetone showed $[\alpha]_D^{23°}$ + 41° in 50% aqueous alcohol (*c*, 0·5), m. p. 246° (Found : N, 9·1; Cl, 7·2. $C_{24}H_{44}O_3N_3Cl$ requires N, 9·3; Cl, 7·8%).

Cholylamide.—Methyl cholate (30 g.) was heated in the autoclave for 12 hours at 130° and 30 atmospheres' pressure with methyl alcohol (300 c.c.) which had been saturated at 0° with ammonia. The solution was evaporated to dryness and the residue recrystallised from 30% aqueous alcohol (charcoal). The crystals (21·7 g.; 75% of the theory) had m. p. 105° alone and in admixture with a specimen of cholylamide prepared by the method of Cortese and Baumann (*loc. cit.*).

Triformylcholylamide.—Cholylamide (6·7 g.) was heated at 55—60° with freshly distilled formic acid (75 c.c.) for 5 hours and then kept overnight. The excess of acid was removed under diminished pressure and the residue dissolved in acetone–benzene. The addition of light petroleum to the solution resulted in the separation of crystals of triformylcholylamide which, after a short time, were filtered off and washed with light petroleum. The crystals (3 g.) had m. p. 187° in agreement with Cortese and Baumann (*loc. cit.*). A further crop of crystals (1·2 g.) was obtained from the mother liquor.

Triformylcholyl Cyanide.—Triformylcholylamide (3 g.) was boiled under reflux for 3½ hours with thionyl chloride (50 c.c.) and then the thionyl chloride was removed under reduced pressure. The glass-like residue was taken up in acetone and the solution slowly poured into water with stirring. The solid which separated was filtered at the pump and washed with water. It was combined with the product from a second experiment in which the same amounts were used, and recrystallised from aqueous acetone to give crystals of *triformylcholyl cyanide* (3·5 g.), m. p. 188° depressed in admixture with triformylcholylamide (Found : N, 3·2. $C_{27}H_{39}O_6N$ requires N, 3·0%).

Cholyl Cyanide.—Triformylcholyl cyanide (3·54 g.) was dissolved in alcohol and sodium hydroxide solution (25 c.c., 1 N) was added. The solution was heated at 50—60° for 1 hour and was then diluted with water. Needle-like crystals immediately separated. These were filtered off, washed with water, and dried in a vacuum desiccator (2·3 g.), m. p. 216—218°. After recrystallisation from aqueous alcohol crystals of *cholyl cyanide* (VII), m. p. 218°, $[\alpha]_D^{18°}$ + 38·7° in alcohol (*c*, 0·9), were obtained (Found : C, 73·6; H, 10·2; N, 3·6. $C_{24}H_{39}O_3N$ requires C, 73·96; H, 10·1; N, 3·64%).

Hydrolysis of Cholyl Cyanide.—Cholyl cyanide (0·14 g.) was boiled under reflux for 9 hours with potassium hydroxide solution (1 c.c., 40%). The solution was cooled and acidified with dilute hydrochloric acid. A crystalline solid (0·12 g.)

separated which, after recrystallisation from ethyl alcohol, had m. p. 196° alone and in admixture with an authentic specimen of cholic acid.

Cholylamidine Hydrochloride (VIII).—Cholyl cyanide (2·3 g.) was dissolved in sodium-dry ethyl alcohol (45 c.c.) and sodium-dry ethyl alcohol (15 c.c.) saturated at 0° with dry hydrogen chloride was added. The solution was kept at 0° for 68 hours and then as much as possible of the excess hydrogen chloride was removed by means of a current of dry air. An alcoholic solution (75 c.c.) of ammonia (saturated at 0°) was then carefully added to the cooled solution and the mixture was kept overnight at 0°. The ammonium chloride which separated was filtered off and the filtrate evaporated to dryness. The residue was extracted with cold water (100 c.c.). The insoluble material was recrystallised from aqueous alcohol to give crystals (1·12 g.) of cholyl cyanide, m. p. and mixed m. p. 218°. Evaporation of the mother-liquor gave a residue which was recrystallised from alcohol, ether, and light petroleum. Clusters of needle-like crystals (0·194 g.) of *cholylamidine hydrochloride* separated, m. p. 295°, $[\alpha]_D^{16°}$ + 27° in water (*c*, 0·9) (Found : C, 65·3; H, 9·9; N, 6·2; Cl, 7·3. $C_{24}H_{43}O_3N_2Cl$ requires C, 65·0; H, 9·8; N, 6·3; Cl, 8·0%). The aqueous extract was evaporated to dryness and re-extracted with water (75 c.c.). The crystalline material (0·47 g.) which did not dissolve had m. p. 290° alone and in admixture with the cholylamidine hydrochloride previously isolated. A further quantity of (VIII) was obtained by salting out from the aqueous extract. It was freed from sodium chloride by extraction with alcohol. Evaporation of the extract gave a residue which was recrystallised from alcohol, ether, and light petroleum to give crystals (0·1 g.), m. p. 290°. Thus the total yield of cholylamidine hydrochloride was 0·76 g.

24-*Amino*-3 : 7 : 12-*trihydroxycholane Hydrochloride* (IX).—(*a*) Cholyl cyanide (1·75 g.) was dissolved in amyl alcohol (200 c.c.) and the solution was boiled under reflux during the gradual addition of sodium (20 g.) over 3½ hours. Towards the end of the reaction a further amount of amyl alcohol (50 c.c.) was added. The solution was cooled and diluted with water (300 c.c.). The alcoholic layer was separated, washed repeatedly with water, and evaporated to dryness under diminished pressure. The residue was digested overnight with dilute hydrochloric acid (470 c.c., 0·04N); a small amount of syrupy material did not dissolve. Addition of sodium hydroxide solution in excess to the aqueous extract brought about the separation of a white solid which was quickly filtered on a glass sintered funnel, washed with water, and dried in a vacuum over soda lime. Yield, 1·12 g. The free base (1·02 g.) was dissolved in dry chloroform and dry hydrogen chloride was led into the solution. The hydrochloride (0·62 g.) which separated was obtained as a white powder on trituration with ether. It was recrystallised from aqueous acetone to which a drop of very dilute hydrochloric acid was added when small needle-like crystals of 24-*amino*-3 : 7 : 12-*trihydroxycholane hydrochloride* were obtained, m. p. 267° (decomp.), $[\alpha]_D^{21°}$ + 34° in alcohol (*c*, 0·9) (Found : N, 3·9; Cl, 8·2. $C_{24}H_{44}O_3NCl$ requires N, 3·3; Cl, 8·3%).

(*b*) Cholyl cyanide (2·5 g.), purified by sublimation in a vacuum, was ground to a fine powder and hydrogenated in ether (600 c.c.) in the presence of Raney nickel at 115—120° and 160 atmospheres for 3 hours with continuous stirring. After allowing to cool overnight the mixture was evaporated in nitrogen to give a syrupy product which was alkaline in reaction. It was dissolved in alcohol and a drop of concentrated hydrochloric acid was added and then acetone and light petroleum until a faint turbidity developed. Crystals (0·13 g.) quickly separated and were filtered off, m. p. 263° (decomp.). Evaporation of the mother liquor at 40° (bath temp.) under reduced pressure gave a crystalline residue which was extracted with cold water. Recrystallisation of the insoluble material from aqueous alcohol gave crystals (1·52 g.) of cholyl cyanide, m. p. and mixed m. p. 215°. The aqueous extract was neutralised (phenolphthalein) and evaporated to dryness to give a residue which was extracted first with acetone to remove unchanged cholyl cyanide and then with ethyl alcohol to remove sodium chloride. Evaporation of the filtered extract gave a residue consisting of (IX) which was recrystallised from ethyl alcohol and acetone containing a little hydrochloric acid. Crystals (0·1 g.) were obtained, m. p. 267° (decomp.), $[\alpha]_D^{18°}$ + 30° in water (*c*, 1·0). The total yield of (IX) was 0·23 g. (9%).

24-*Guanido*-3 : 7 : 12-*trihydroxycholane Hydrochloride* (X).—24-Amino-3 : 7 : 12-trihydroxycholane hydrochloride (0·3 g.) was heated with cyanamide (0·3 g.) in sodium-dry alcohol (40 c.c.) in a sealed tube at 130° for 17 hours. The solution, which gave a positive Sakaguchi test, was concentrated to small bulk and a solid (0·1 g.) precipitated by the addition of acetone. This was dissolved in a few drops of dilute hydrochloric acid and acetone was added. The product (27 mg.) was separated in the centrifuge, washed with acetone, and dried in a vacuum. It had $[\alpha]_D^{14°}$ + 23° in alcohol (*c*, 0·9) (Found : N, 9·0. $C_{25}H_{46}ONCl$ requires N, 8·9%).

Dehydrocholyl Cyanide (3 : 7 : 12-*Triketo*-23-*cyanonorcholane*) (XI).—Cholyl cyanide (2·1 g.) was dissolved in glacial acetic acid (5 c.c.) and a 10% solution of chromic acid in glacial acetic acid (35 c.c.) was added in small portions. The temperature was not allowed to rise above 40°. When all the oxidising mixture had been added the solution was allowed to stand for 15 minutes and was then diluted with water (500 c.c.). The insoluble product was filtered off and washed with water. Recrystallisation from ethyl alcohol afforded crystals (1·15 g.) of *dehydrocholyl cyanide*, m. p. 242° (Found : C, 75·5; H, 8·6; N, 3·85. $C_{24}H_{33}O_3N$ requires C, 75·2; H, 8·7; N, 3·65%).

Trioxime of Dehydrocholyl Cyanide.—Dehydrocholyl cyanide (1·15 g.) was dissolved in ethyl alcohol (105 c.c.) and boiled under reflux with hydroxylamine hydrochloride (0·58 g. in 1·25 c.c. of water) and sodium acetate (1·25 g. in 2·5 c.c. water) for 10 hours. No separation of an oxime occurred and after standing overnight the clear solution was poured into water. The white solid which separated was filtered off, washed with water, and recrystallised from aqueous alcohol, m. p. 225° (decomp.) (Found : N, 13·1. $C_{24}H_{36}O_3N_4$ requires N, 13·3%).

Oxime of Ethyl 3 : 12-*Dihydroxy*-7-*ketocholanate*.—Hydroxylamine hydrochloride (4·8 g.), dissolved in the minimum amount of water, was mixed with a solution of sodium hydroxide (12 c.c., 5N) and alcohol (50 c.c.) was added. The precipitate of sodium chloride was filtered off and ethyl 3 : 12-dihydroxy-7-ketocholanate (10 g.), dissolved in absolute alcohol (50 c.c.), was added to the filtrate and the solution was heated at 70—80° under reflux for 4 hours. Subsequent removal of the solvent under reduced pressure afforded a residue which was extracted with ether. The extract was dried ($MgSO_4$), filtered, and evaporated to dryness to give the *oxime* (XIV) (6·2 g.). On recrystallisation from methyl alcohol large rectangular plates were obtained, m. p. 93—94°, $[\alpha]_D^{14°}$ − 44·3° in alcohol (*c*, 5·9) (Found : N, 3·1. $C_{26}H_{43}O_5N$ requires N, 3·1%). A further quantity of oxime (1·2 g.), m. p. 91°, was obtained from the mother liquors.

Oxime of the Hydroxamic Acid (XV).—Ethyl 3 : 12-dihydroxy-7-ketocholanate (1·2 g.), hydroxylamine hydrochloride (0·58 g.), and sodium hydroxide (7·5 c.c., 1N) in ethyl alcohol (10 c.c.) were boiled under reflux for 5 hours. The solvents were distilled off under diminished pressure and the residue extracted three times with methyl alcohol (20 c.c.). The combined extracts were concentrated to half volume under reduced pressure and kept for 16 hours. The crystals which separated were collected, washed with methyl alcohol, and recrystallised from the same solvent. Large plates (0·5 g.) were obtained, m. p. 214—215° (decomp.), $[\alpha]_D^{22°}$ − 51·4° in alcohol (*c*, 1·9) (Found : C, 66·1; H, 8·95; N, 6·6. $C_{24}H_{40}O_5N_2$ requires C, 66·1; H, 9·2; N, 6·4%).

The *oxime* was acid to litmus, and dissolved in dilute sodium hydroxide and was precipitated on acidification of the alkaline solution. It was readily soluble in methyl and ethyl alcohols, soluble in ethyl acetate, sparingly soluble in ether, chloroform, and benzene, and insoluble in water.

7-*Amino*-3 : 12-*dihydroxycholanic Acid*.—Sodium (30 g.) was added in small quantities over a period of 4 hours to a solution of (XIV) (10 g.) in amyl alcohol (500 c.c.) at 100°. An additional 50 c.c. of amyl alcohol were required to dissolve the last traces of sodium. The cooled solution was made neutral to litmus by the addition of sulphuric acid

(5N) and was kept for 14 hours. The sodium sulphate which separated was filtered off and washed with ethyl alcohol. The filtrate and washings were evaporated to dryness under reduced pressure and the residue, dried in a vacuum at 100°, was exhaustively extracted with absolute alcohol. Evaporation of the extract gave crude sodium 7-amino-3 : 12-dihydroxycholanate (9·4 g.) as an amorphous, water-soluble yellow solid.

Hydrochloride of 7-Amino-3 : 12-dihydroxycholanic Acid (XII).—A solution of crude sodium 7-amino-3 : 12-dihydroxycholanate (3 g.) in absolute alcohol (15 c.c.) was treated with a slow stream of dry hydrogen chloride until no further separation of sodium chloride occurred. The solution was filtered and the residue washed with ethyl alcohol. The filtrate and washings were concentrated under diminished pressure, and the amine hydrochloride precipitated by the addition of dry ether, washed by decantation with anhydrous ether, and dried in a vacuum. The white solid (2·0 g.) crystallised from 50% aqueous ethyl alcohol after long standing, forming large colourless prisms (0·5 g.), m. p. 265—267° (decomp.) (Found : N, 3·2; Cl, 7·7. $C_{24}H_{42}O_4NCl$ requires N, 3·2; Cl, 8·0%). The *compound* was readily soluble in water, soluble in ethyl and methyl alcohols, insoluble in other organic solvents.

7-Benzamido-3 : 12-dihydroxycholanic Acid.—Sodium 7-amino-3 : 12-dihydroxycholanate (0·9 g.) in water (25 c.c.) containing sodium hydroxide (0·4 g.) was treated with benzoyl chloride (0·36 c.c.) added dropwise with shaking over a period of 5 minutes. The solution was kept at room temperature for a further 15 minutes with frequent agitation and was then warmed to 50—60° for a short time. The cooled solution was acidified with dilute hydrochloric acid and the precipitate filtered off after 16 hours and washed with water until free from acid and then with ether. The residue was dissolved in methyl alcohol and the filtered solution evaporated under reduced pressure to give a solid which crystallised on trituration with benzene. Recrystallisation from a large volume of benzene afforded the *benzoyl* derivative of (XII) as colourless plates (0·4 g.), m. p. 185°, $[\alpha]_D^{18°}$ + 39·4° in alcohol (*c*, 1·3) (Found : N, 2·65. $C_{31}H_{45}O_5N$ requires N, 2·7%).

Reduction of 3 : 7 : 12-Trioximinocholanic Acid.—The trioxime (3·1 g.), derived from dehydrocholic acid prepared according to the method of Schenck and Kirchhof (*Z. physiol. Chem.*, 1929, **181**, 193), was boiled under reflux with amyl alcohol (750 c.c.) for 5½ hours during the gradual addition of sodium (40 g.). The cooled solution was poured into water and the alcoholic layer was separated and repeatedly washed with water and then evaporated to dryness under reduced pressure. The residue was dissolved in the minimum amount of water and hydrochloric acid was added until the solution was just acid to litmus. The volume was then made up to 100 c.c. and the acidity adjusted to 1N, and the solution was boiled for 1 hour to ensure the elimination of the oxime group from C_{12}. The solution was filtered, diluted with water (200 c.c.), and evaporated to dryness under reduced pressure at 40° to give a residue which was extracted with alcohol. Removal of solvent from the filtered extract gave a resinous product (1·49 g.) which was purified by extracting with cold water and filtering from some insoluble material (0·27 g.). Evaporation of the filtrate gave a colourless residue which was dissolved in ethyl alcohol; ether was then added to the clear solution. A white precipitate was produced and was separated on the centrifuge, washed with ether, and dried in the vacuum desiccator. This *product* (XIII) (1·06 g.) was readily soluble in water and showed $[\alpha]_D^{16°}$ + 42° in water (*c*, 0·7). A 2% solution had pH 3·1 (Found : N, 5·3; Cl, 14·84. $C_{24}H_{42}O_3N_2Cl_2$ requires N, 5·9; Cl, 14·88%). The aqueous washings from the amyl alcohol were examined and a further amount (0·85 g.) of the dihydrochloride of the diamino-acid was obtained.

Reduction of Trioximinocholyl Cyanide.—The trioxime (0·95 g.), derived from 3 : 7 : 12-triketo-23-cyanonorcholane prepared as described above, was boiled under reflux with amyl alcohol (250 c.c.) for 3 hours during the gradual addition of sodium (15 g.). The product was isolated as described in the previous experiment. A white powder (0·3 g.) was obtained which was probably a mixture of the dihydrochloride of the diamino-acid and the trihydrochloride of the triamino-monoketo-derivative of cholane, since it dissolved in water to give an acid solution and contained 17·6% Cl (Calc. for $C_{24}H_{42}O_3N_2Cl_2$: Cl, 14·88%. Calc. for $C_{24}H_{47}ON_3Cl_3$: Cl, 21·0%).

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