98. *The Synthesis of 5 : 6-Dimethyl-1 : 2-benzanthraquinone, a Degradation Product of Deoxycholic Acid.*

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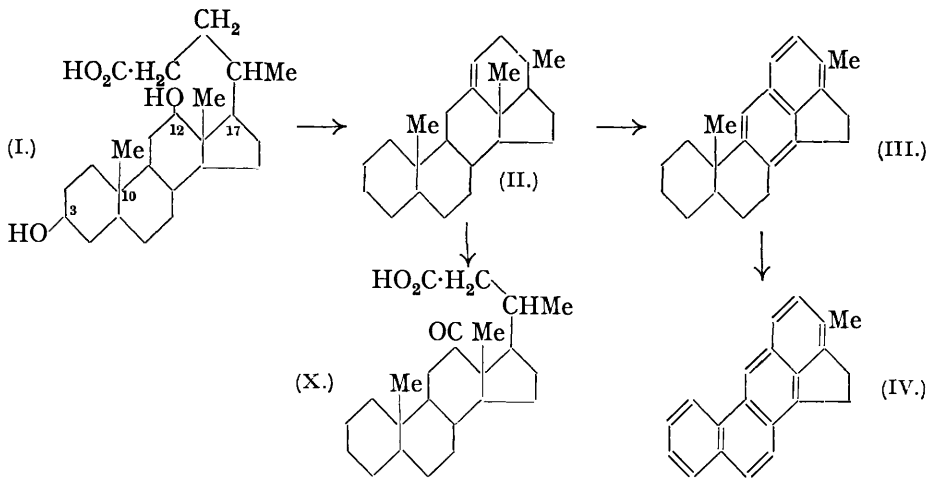
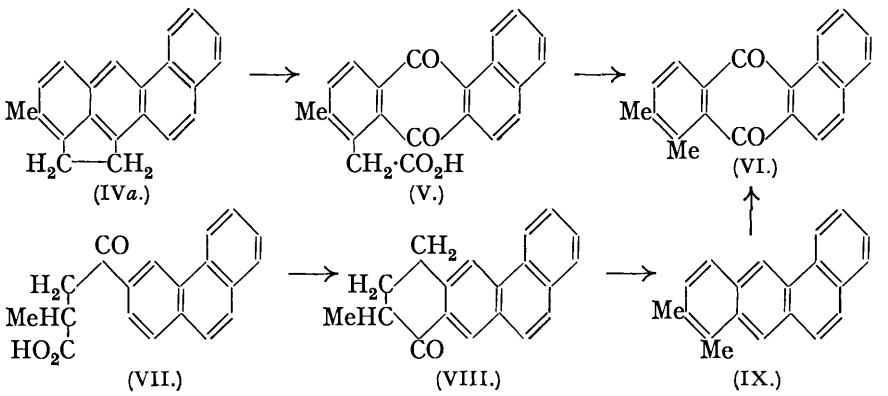
Selenium dehydrogenation of dehydronorcholene (II), the pyrolysis product of 12-ketocholanic acid (Wieland and Schlichting, *Z. physiol. Chem.*, 1925, **150**, 273), gave a 30% yield of a yellow hydrocarbon which was readily obtained pure through its purplish-black, sparingly soluble *picrate*. This hydrocarbon was oxidised by chromic acid to a quinone-acid which on decarboxylation passed into a compound having the properties of a homologue of 1 : 2-benzanthraquinone.



The appearance of a paper by Wieland and Dane (*ibid.*, 1933, **219**, 240) describing the selenium dehydrogenation of dehydronorcholene necessitated the publication of a preliminary note concerning these experiments (*Chem. and Ind.*, 1933, **52**, 758). Wieland and Dane attributed structure (IV) to the dehydrogenation product, which was clearly

identical with our compound, and termed it methylcholanthrene, a convenient name which we propose to retain. They merely recorded the formation of methylcholanthrene and its reduction to a hexahydro-derivative (we regard the constitution which they assigned to this as improbable), and we therefore continued our investigation of the structure of methylcholanthrene, which has now been completed.

We have shown conclusively that the decarboxylated quinone from methylcholanthrene is 5 : 6-*dimethyl*-1 : 2-*benzanthraquinone* (VI), for esterification of the acid arising from its oxidation with permanganic acid gave methyl anthraquinone-1 : 2 : 5 : 6-tetracarboxylate, identical with that previously obtained by similar oxidation of 1 : 2 : 5 : 6-dibenzanthraquinone (Cook, J., 1931, 2531), and the same 5 : 6-dimethyl-1 : 2-benzanthraquinone was then synthesised by a method which established its constitution. The molecular formula of methylcholanthrene was shown definitely to be $C_{21}H_{16}$ by analyses of the pure hydrocarbon and its picrate, and in the light of the chemistry of the sterols and bile acids the reactions by which it is converted into the quinone-acid, $C_{21}H_{14}O_4$, and thence into 5 : 6-dimethyl-1 : 2-benzanthraquinone, admit of no other interpretation than that methylcholanthrene is correctly represented by formula (IV) * and that its oxidation product is 6-*methyl*-1 : 2-*benzanthraquinonyl*-5-*acetic acid* (V).



In our first dehydrogenation experiment with dehydronorcholene we also isolated a *hydrocarbon* which, after purification through its picrate, gave analytical figures in agreement with the formula $C_{22}H_{26}$. This was evidently a partially dehydrogenated compound (probably III), but we did not isolate it in subsequent experiments. A study of this intermediate compound would probably provide direct evidence of the position of the quaternary methyl group placed at C_{10}, and we are therefore investigating the partial dehydrogenation of dehydronorcholene with a view to obtaining more material.

Of the variety of synthetic methods now available for the production of the 1 : 2-benzanthracene ring system, the most suitable one for our purpose was that due to Haworth and Mavin (J., 1933, 1012), which one of us (*ibid.*, p. 1592) has already used for the synthesis of 5-methyl-1 : 2-benzanthracene. Condensation of phenanthrene with methylsuccinic anhydride led to a mixture of keto-acids, separated by fractional crystallisation of their *methyl* esters into β-2-*phenanthroyl*iso*butyric acid* (the less soluble isomeride) and β-3-*phenanthroyl*iso*butyric acid* (VII) (the predominating isomeride). The constitutions of these two acids were established by independent syntheses from 2- and 3-bromoacetylphenanthrenes and ethyl sodiomethylmalonate (compare Haworth and Mavin, *loc. cit.*). Reduction of methyl β-3-phenanthroyl*iso*butyrate by Clemmensen's method was unsatisfactory owing to the large proportion of material converted into polymeric reduction products. By the Kishner–Wolff method, however, the *semicarbazone* of the acid was readily converted into γ-3-*phenanthryl*-α-*methylbutyric acid*, which by dehydration with stannic chloride gave 5-*keto*-6-*methyl*-5 : 6 : 7 : 8-*tetrahydro*-1 : 2-*benzanthracene* (VIII).

* For the sake of clarity this formula is reproduced in formula (IV*a*) with the more familiar arrangement of the benzanthracene ring system.

This ketone was treated with methylmagnesium iodide, and the resinous carbinol heated with selenium, the resulting 5 : 6-*dimethyl*-1 : 2-*benzanthracene* (IX) being finally oxidised to the quinone (VI). This was identical with the quinone prepared from methylcholanthrene, and we have therefore synthesised a degradation product of deoxycholic acid (3 : 12-dihydroxycholanic acid; I) which still contains 20 of the 24 carbon atoms present in the molecule of the bile acid.

These experiments provide entirely independent confirmation of several important features in regard to the constitution of the sterols and bile acids :—

(a) The presence of a hydrogenated phenanthrene ring system with an additional ring attached at the 1 : 2-positions receives direct experimental support.

(b) The five-membered nature of ring IV is confirmed, a six-membered ring being completely incompatible with our results.

(c) The side chain must be attached to position 17 of the ring system (steroï system of numbering). Wieland and Dane (*Z. physiol. Chem.*, 1933, **219**, 240) inferred this from the fact that dehydronorcholene could be dehydrogenated to a hydrocarbon $C_{21}H_{16}$, but cited no evidence of the structure of this product.

(d) The position of the keto-group in 12-ketocholanic acid is defined, and hence that of one of the hydroxyl groups of deoxycholic and cholic acids.

A possible criticism of the validity of some of these conclusions would lie in the argument that the prolonged heating above 300°, necessary for the conversion of 12-ketocholanic acid into dehydronorcholene, may have resulted in structural change. This is unlikely, but in any case we have carried out a simple experiment which completely disposes of the possibility. Wieland and Wiedersheim (*Z. physiol. Chem.*, 1930, **186**, 233) oxidised dehydronorcholene to a keto-acid which, on the modern formulation, should be 12-keto-norcholanic acid (X). That this is indeed the case we showed by reducing this acid by the Kishner–Wolff method to norcholanic acid, identical with a specimen prepared by degradation of cholanic acid by the method of Wieland, Schlichting, and Jacobi (*Z. physiol. Chem.*, 1926, **161**, 93).

The suggestion that the molecular structure of methylcholanthrene might be expected to be associated with cancer-producing properties (Cook, *Proc. Roy. Soc.*, 1933, *B*, **113**, 277) has received verification by animal experiments, for which we are indebted to Professor E. L. Kennaway. A solution of methylcholanthrene in benzene (0·3%) has now been applied twice a week to a series of 20 mice for 150 days. This has given epitheliomas in 8 mice, and all of the 9 mice still alive bear tumours. With this particular series of mice the appearance of tumours has been more rapid than in the case of any other carcinogenic compound hitherto examined (the first two tumours had appeared on the 75th day).

The experiments which we record thus show how it is possible for a natural product of animal metabolism to be converted into a carcinogenic compound by simple transformations of a biochemical character (oxidation, reduction, dehydration, decarboxylation, dehydrogenation). The essential features of this conversion are (a) cyclisation of the side chain and (b) dehydrogenation of the ring system. There is as yet no evidence that (a) occurs in the body; that (b) may occur in normal circumstances is rendered likely by the increasing amount of evidence that the œstrus-producing hormones are related chemically, and probably biologically, to the sterols. Hence in all probability dehydrogenating enzymes are responsible for the formation of ketohydroxyœstrin, containing one aromatic ring, and equilenin, containing two aromatic rings (Girard *et al.*, *Compt. rend.*, 1932, **195**, 981; 1933, **196**, 137). Unpublished experiments by one of us (J. W. C.) have shown conclusively that equilenin contains the same ring system as œstrin.

Experimental.

(The analyses were all carried out by Dr. A. Schoeller.)

Methylcholanthrene and its Degradation Products. Dehydronorcholene.—Dehydrodeoxycholic acid (3 : 12-diketocholanic acid) (Wieland and Sorge, *Z. physiol. Chem.*, 1916, **97**, 18) was obtained free from chromium by crystallisation from dilute alcohol containing a little acetic acid, and was reduced to 12-ketocholanic acid in alcoholic solution with pure zinc sticks and

hydrochloric acid, by the method recommended by Wieland, Dane, and Kraft (*ibid.*, 1932, **210**, 281) for the reduction of dehydrocholic acid to 7 : 12-diketocholanic acid.

For thermal decomposition to dehydronorcholene (compare Wieland and Schlichting, *loc. cit.*), 12-ketocholanic acid (8 g.) was heated at 320—345° for 8 hours in an atmosphere of carbon dioxide, and the resinous product distilled in a vacuum. The fraction, b. p. 200—215°/4 mm., was crystallised from alcohol, giving long colourless needles (1·6—2 g.) of dehydronorcholene (II).

Methylcholanthrene (compare Wieland and Dane, *ibid.*, 1933, **219**, 243).—Dehydronorcholene (5 g.) was heated with selenium (15 g.) at 320—340° for 47 hours (after 28 hours the product crystallised on cooling). The whole was extracted with benzene and the combined extract from two such batches was freed from benzene and distilled in a vacuum. The first fraction, b. p. approx. 222—227°/3—4 mm., formed an oil which did not crystallise, but from which 50 mg. of methylcholanthrene were isolated as picrate, by treatment with alcoholic picric acid. The fraction, b. p. 240—260°/3—4 mm., formed an orange oil which crystallised in part. Recrystallisation from benzene–alcohol gave 0·35 g. of crude methylcholanthrene, m. p. 163—167°. The residue in the distillation flask was then sublimed at 240°/4 mm., and the sublimate was recrystallised from benzene–alcohol, giving a further 1·65 g. of methylcholanthrene. By fresh treatment with selenium (8 g.) at 320—340° for 24 hours of all of the liquid fractions from which the methylcholanthrene had been separated there was obtained an additional 0·55 g. of methylcholanthrene. The total yield was thus 2·6 g. or 30%.

For purification, methylcholanthrene was treated in benzene solution with an equal weight of picric acid, and the *picrate*, which formed purplish-black needles, was recrystallised from benzene until it melted constantly at 177—178° (Found : C, 65·2; H, 3·8. $C_{21}H_{16},C_6H_3O_7N_3$ requires C, 65·15; H, 3·85%). Methylcholanthrene (IV), regenerated from the pure picrate, crystallised from benzene in straw-yellow needles, m. p. 176·5—177·5° (Found : C, 93·8; H, 6·0. Calc. for $C_{21}H_{16}$: C, 94·0; H, 6·0%). This hydrocarbon did not react with maleic anhydride in boiling xylene (1 hour's heating), nor was its yellow colour removed by this treatment.

In a preliminary experiment, dehydrogenation of dehydronorcholene (1 g.) by heating with selenium (3 g.) at 325—340° for 26 hours gave a product from which methylcholanthrene was recrystallised (from alcohol after vacuum distillation). The alcoholic mother-liquors were treated with picric acid and gave a red picrate, m. p. 163—166°, after recrystallisation from alcohol. The *hydrocarbon* (probably III), obtained from this, crystallised from methyl alcohol in colourless plates, m. p. 132—134° (Found : C, 90·6; H, 9·0. $C_{22}H_{26}$ requires C, 91·0; H, 9·0%).

6-*Methyl*-1 : 2-*benzanthraquinonyl*-5-*acetic Acid* (V).—When sodium dichromate (5 g.) was added to a cold suspension of powdered methylcholanthrene (1 g.) in acetic acid (20 c.c.) and the whole shaken at room temperature for 15 minutes, oxidation took place, the methylcholanthrene being replaced by a substance of different crystalline form. A sample of this was collected, extracted with dilute sodium carbonate solution (which removed a trace of acidic substance), and recrystallised from acetic acid. This substance, m. p. 228—229° (decomp.), probably an intermediate ketone, was not purified on account of its instability. The remainder of the original suspension was boiled for ½ hour and cooled, and the product collected and extracted with boiling dilute sodium carbonate solution. Acidification of the filtrate gave a good yield of a gelatinous yellow precipitate of 6-*methyl*-1 : 2-*benzanthraquinonyl*-5-*acetic acid* (V), which was dried and recrystallised from acetic acid and then from xylene (Found : C, 76·4; H, 4·45. $C_{21}H_{14}O_4$ requires C, 76·35; H, 4·3%). This acid formed an orange-yellow crystalline powder which gave the Liebermann anthraquinol reaction with zinc dust and alkali, and had m. p. 250—260° (decomp.), depending upon the rate of heating.

5 : 6-*Dimethyl*-1 : 2-*benzanthraquinone* (VI).—The aforesaid acid (0·4 g.) was heated at 400° in an atmosphere of carbon dioxide for 3—4 minutes. A vacuum was then applied, the resulting sublimate was dissolved in benzene, and the benzene solution, after extraction with sodium carbonate to ensure removal of unchanged acid, was concentrated. The resulting *quinone* (VI) crystallised from acetic acid in long orange-yellow needles, m. p. 229—230° (Found : C, 83·8, 83·75; H, 4·9, 4·9. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%). The yield obtained in this operation was extremely small on account of extensive charring. Decarboxylation was smoothly effected, however, by boiling a solution of the quinone-acid (50 mg.) in quinoline (5 c.c.) with a little copper-bronze for 3 hours. The product was isolated and recrystallised from acetic acid, yielding 30 mg. of the non-acidic quinone.

Methyl Anthraquinone-1 : 2 : 5 : 6-*tetracarboxylate*.—The oxidation of the foregoing quinone (VI) (0·24 g.) with potassium permanganate and sulphuric acid was carried out in the usual way (compare Cook, J., 1931, 2531; 1933, 1595), and the silver salt (70 mg.) of the resulting

acid esterified with methyl iodide. Crystallisation of the product from xylene and then acetic acid gave methyl anthraquinone-1 : 2 : 5 : 6-tetracarboxylate, m. p. 292—294°, alone or mixed with an authentic sample prepared from 1 : 2 : 5 : 6-dibenzanthraquinone (Found : C, 59·8; H, 3·8. Calc. : C, 60·0; H, 3·7%).

Synthesis of 5 : 6-*Dimethyl*-1 : 2-*benzanthraquinone.* β-2 (*and* 3)-*Phenanthroyl*iso*butyric Acids.*—(a) Methylsuccinic anhydride (43 g.) and then powdered phenanthrene (74 g.) were gradually introduced into an ice-cold solution of anhydrous aluminium chloride (125 g.) in nitrobenzene (500 c.c.). The whole was kept at room temperature for 19 hours with occasional shaking, the product decomposed with ice and hydrochloric acid, and the nitrobenzene removed in steam. The residue was extracted with dilute sodium carbonate solution, and the solution filtered to free it from unchanged phenanthrene. The crude mixture of acids, precipitated from the cold filtrate, was crystallised from acetic acid, dried, and esterified with methyl alcohol–hydrogen chloride. The mixture of esters was dissolved in alcohol (1 l.), and the solution well cooled. Methyl β-2-phenanthroyl*iso*butyrate separated and was recrystallised from alcohol–*cyclo*hexane (yield, 3·6 g.), forming almost colourless plates, m. p. 133—134°. Hydrolysis of this ester gave β-2-phenanthroyl*iso*butyric acid, m. p. 224—226°.

Concentration of the alcoholic mother-liquors of the above methyl ester, and recrystallisation from *cyclo*hexane of the material which separated, gave 35 g. of *methyl* β-3-*phenanthroyl*iso*butyrate*, m. p. 79—83°. Further crystallisation from *cyclo*hexane gave small colourless needles, m. p. 88—89° (Found : C, 78·3; H, 5·9. $C_{20}H_{18}O_3$ requires C, 78·4; H, 5·9%). β-3-*Phenanthroyl*iso*butyric acid* (VII), formed from this ester by hydrolysis, crystallised from methyl alcohol or benzene in small colourless crystals, m. p. 179—180·5° (Found : C, 77·9; H, 5·5. $C_{19}H_{16}O_3$ requires C, 78·05; H, 5·5%).

(b) 2-Bromoacetylphenanthrene (3·3 g.) was condensed with ethyl sodiomethylmalonate, and the product hydrolysed and decarboxylated, exactly as described by Haworth and Mavin (*loc. cit.*) for the analogous condensation with ethyl sodiomalonate. The resulting β-2-*phenanthroyl*iso*butyric acid* crystallised from benzene in colourless microscopic needles, m. p. 225—227° (Found : C, 78·5; H, 5·7%), and gave a *methyl* ester, m. p. 134·5—135·5° (Found : C, 78·3; H, 5·9%). This acid and its ester did not depress the m. p.'s of the corresponding compounds prepared as described under (a).

Similar condensation between the crude bromination product of 3-acetylphenanthrene and ethyl sodiomethylmalonate yielded β-3-phenanthroyl*iso*butyric acid, m. p. 175—178°, which gave a methyl ester, m. p. 85—86°. These two compounds were identical with the corresponding substances obtained as under (a).

γ-3-*Phenanthryl*-α-*methylbutyric Acid.*—The *semicarbazone* of β-3-phenanthroyl*iso*butyric acid crystallised from alcohol in colourless plates, m. p. 193—195° (with gas) (Found : N, 11·4. $C_{20}H_{19}O_3N_3$ requires N, 12·0%). This semicarbazone (2 g.) was heated at 195—200° for 6 hours with a solution of sodium ethoxide (2 g. of sodium in 24 c.c. of alcohol), and the product diluted with water, extracted with ether, and acidified. The crude acid (4·7 g. from 6 g. of semicarbazone) was crystallised from a small volume of methyl alcohol and then from light petroleum, from which γ-3-*phenanthryl*-α-*methylbutyric acid* crystallised as clusters of microscopic colourless needles, m. p. 119—121° (Found : C, 81·9; H, 6·7. $C_{19}H_{18}O_2$ requires C, 82·0; H, 6·5%).

5-*Keto*-6-*methyl*-5 : 6 : 7 : 8-*tetrahydro*-1 : 2-*benzanthracene* (VIII).—A mixture of γ-3-phenanthryl-α-methylbutyric acid (3·1 g.) and anhydrous stannic chloride (4 c.c.) was heated for an hour at 120°. The cooled solution was treated with ice and hydrochloric acid and extracted with ether, and the ethereal extract washed with dilute hydrochloric acid and then with dilute sodium carbonate solution. The cyclic *ketone* which remained after removal of the ether was recrystallised from methyl alcohol (yield, 0·85 g.). A pure sample from *cyclo*hexane formed lemon-yellow leaflets, m. p. 137—138·5° (Found : C, 87·7; H, 6·1. $C_{19}H_{16}O$ requires C, 87·6; H, 6·2%).

5 : 6-*Dimethyl*-1 : 2-*benzanthracene* (IX).—Powdered 5-keto-6-methyl-5 : 6 : 7 : 8-tetrahydro-1 : 2-benzanthracene (3 g.) was slowly added to an ice-cold Grignard solution prepared from methyl iodide (1·5 c.c.), magnesium turnings (0·6 g.), and anhydrous ether (20 c.c.). After being kept at room temperature for 1½ hours, the suspension was treated with ice and ammonium chloride, the ethereal solution washed and dried (sodium sulphate), and the ether removed. The residual resin was heated at 300—310° for 10 hours with selenium (2 g.). The product was extracted with benzene, and the extract evaporated to small bulk and diluted with alcohol. The resulting crystals (2·2 g.) were sublimed at 220°/4 mm., and the sublimate treated with an equal weight of picric acid in benzene. After recrystallisation from benzene, 5 : 6-*dimethyl*-1 : 2-*benzanthracene picrate* formed dark red needles, m. p. 191—193° (Found :

C, 64·4; H, 4·1. $C_{20}H_{16},C_6H_3O_7N_3$ requires C, 64·3; H, 3·95%). Decomposition of this picrate with sodium carbonate yielded 5 : 6-*dimethyl*-1 : 2-*benzanthracene* (IX), which crystallised from alcohol in almost colourless plates (1·1 g.), m. p. 187—188° [Found : C, 93·4; H, 6·4; *M* (Rast), 257, 260. $C_{20}H_{16}$ requires C, 93·7; H, 6·3%; *M*, 256].

Reduction of the dimethyl-1 : 2-benzanthraquinone obtained from methylcholanthrene, by the two-stage method previously described for such quinones (Cook, J., 1932, 468), yielded a product, m. p. 182—185°, not depressed by this synthetic sample of 5 : 6-dimethyl-1 : 2-benzanthracene. There was insufficient material for complete purification.

5 : 6-*Dimethyl*-1 : 2-*benzanthraquinone* (VI).—This separated when a solution of 5 : 6-dimethyl-1 : 2-benzanthracene (0·1 g.) in acetic acid (4 c.c.) was boiled for ½ hour with sodium dichromate (0·2 g.). After recrystallisation from acetic acid the quinone formed long orange-yellow needles, m. p. 232—234°, which did not depress the m. p. of the quinone obtained from methylcholanthrene (Found : C, 83·9; H, 4·9. Calc. : C, 83·9; H, 4·9%).

Norcholanic Acid from Dehydronorcholene.

12-*Ketonorcholanic Acid* (X).—This was obtained by oxidation of dehydronorcholene with potassium permanganate in acetic acid (Wieland and Wiedersheim, *Z. physiol. Chem.*, 1930, **186**, 233), and formed colourless plates, m. p. 185·5—186·5° * (Found : C, 76·45; H, 9·9. Calc. : C, 76·6; H, 10·1%).

Norcholanic Acid.—A solution of 12-ketonorcholanic acid (0·4 g.) in alcohol (10 c.c.) was heated for 3 hours with a concentrated aqueous solution of semicarbazide hydrochloride (0·4 g.) and sodium acetate (0·4 g.). The *semicarbazone* separated from alcohol, in which it was very sparingly soluble, in colourless crystals, m. p. 235° (with gas) (Found : N, 9·8. $C_{24}H_{39}O_3N_3$ requires N, 10·1%).

This semicarbazone (0·4 g.) was heated at 170—175° for 3 hours with sodium ethoxide (0·4 g. of sodium in 6 c.c. of alcohol). The whole was diluted with water and the sparingly soluble sodium salt was collected and shaken with ether and dilute hydrochloric acid. The acid obtained from the ethereal extract was recrystallised from acetic acid, and had m. p. 175·5—176·5°, alone or mixed with a specimen of norcholanic acid prepared by degradation of cholanic acid as described by Wieland, Schlichting, and Jacobi (*loc. cit.*). Esterification with ethyl alcohol–hydrogen chloride gave ethyl norcholanate, m. p. 65—66° (lit., 66—67°).

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