

## Comparative Studies of 'Bile Salts'

### 6. PARTIAL SYNTHESIS OF COPROSTANIC ACID, A 'STEM ACID' OF PRIMITIVE BILE SALTS

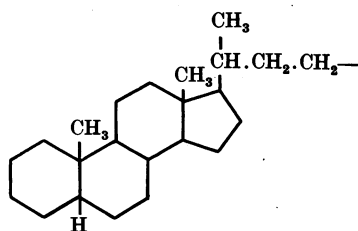
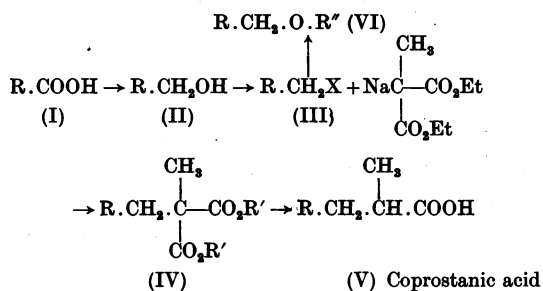
BY R. J. BRIDGWATER AND G. A. D. HASLEWOOD

*Guy's Hospital Medical School, London, S.E. 1*

(Received 19 April 1952)

A fundamental problem in studying the bile salts of primitive animals is to provide proof of the nature of the carbon skeleton of biliary alcohols or acids whose molecules probably contain twenty-seven or twenty-eight carbon atoms. Such substances have frequently been degraded to known  $C_{24}$  bile acids or their lower homologues, but until recently no unequivocal evidence concerning a complete carbon skeleton had been published. The present authors (Bridgwater & Haslewood, 1952), in a preliminary note, reported the partial synthesis from cholanic acid of a  $C_{27}$  acid ('coprostanic acid'; coprostan-26-oic acid), also derived without loss of carbon atoms from the trihydroxycoprostanic acid isolated from the bile of Crocodylidae (Haslewood, 1952). This work is now described in detail.

(III, X=I). The iodide (III, X=I) was condensed with diethyl sodiomethylmalonate to give the substituted malonic ester (IV,  $R'=Et$ ) which on hydrolysis yielded the acid (IV,  $R'=H$ ). Decarboxylation of this *in vacuo* gave coprostanic acid (coprostan-26-oic acid, V), which was identical in chemical and physical properties with the stem acid,  $C_{27}H_{46}O_2$ , already obtained from trihydroxycoprostanic acid. The infrared spectra of the partially synthetic and 'natural' coprostanic acids were determined for us by Dr A. R. H. Cole, and curves kindly supplied by him are shown in Fig. 1. They leave no doubt about the identity of the two compounds examined. As by-products, the action of alkaline methanol or ethanol on unchanged halide (III) gave cholanyl methyl ether (VI,  $R'=CH_3$ ) and cholanyl ethyl ether (VI,  $R'=C_2H_5$ ).



### RESULTS

Cholanic acid (I) was converted by lithium aluminium hydride reduction of the ethyl ester to cholane-24-ol (II) which was converted into the corresponding chloride (III, X=Cl; Wessely & Swoboda, 1951), bromide (III, X=Br) and iodide

### EXPERIMENTAL

Melting points are uncorrected. Micro-analyses were done by Weiler and Strauss, Oxford. Optical rotations were measured in a 1 dm. steel microtube.  $Al_2O_3$  was 'Type H', supplied by Peter Spence and Sons, Widnes. Pentane, b.p. 34–35°, was purified by repeated shaking with  $H_2SO_4$ , followed by washing with water and aqueous KOH: the material was then dried over KOH and distilled over fresh KOH.

*Cholan-24-ol* (II) (compare Wessely & Swoboda, 1951). To a solution of lithium aluminium hydride (7.5 g.) in dry ether (200 ml.) was added during 10 min. a solution of ethyl cholanoate (17.5 g.) in dry ether (200 ml.) whilst the reaction flask was cooled with ice water. After 1 hr. at 20° the reaction mixture was slowly poured on to a mixture of crushed ice and 2N- $H_2SO_4$  (excess). The ether layer was then isolated and washed with aqueous  $H_2SO_4$ , water, aqueous  $Na_2CO_3$  (10%, w/v) and water. The ethereal extract was dried ( $Na_2SO_4$ ) and evaporated to yield cholane-24-ol (9.0 g.), which on recrystallization from acetone gave needles of m.p. 128–129.5°;  $[\alpha]_D^{21} + 21 \pm 2^\circ$  in  $CHCl_3$  (c, 3.7). (Found: C, 83.0; H, 12.1. Calc. for  $C_{24}H_{44}O$ : C, 83.2; H, 12.2%). A further crop of cholane-24-ol (1.5 g.), m.p. 125–126°, was obtained from the mother liquors. A mixture of cholane-24-ol (75 mg.) in acetic acid (0.75 ml.), acetic anhydride (0.25 ml.) and 6N- $HClO_4$  (0.3 ml.) was kept at about 20° for 0.5 hr. The solution was poured into water and the precipitated solid was crystallized from acetone, from which 24-acetoxycholane formed colourless needles of m.p. 85.5–86.5°. (Found: C, 80.3; H, 11.0.  $C_{26}H_{44}O_2$  requires C, 80.4; H, 11.4%.)

**24-Chlorocholane** (III, X=Cl). Cholan-24-ol (7.5 g.) was ground with  $\text{PCl}_5$  (5 g.) and, after 1 hr., the resulting mass was treated with water and extracted with ether. After filtration of a certain amount of ether-insoluble material, evaporation of the ether gave a brown oil which was dissolved in pentane. The solution was passed through a short column of  $\text{Al}_2\text{O}_3$  (approx. 25 g.). Evaporation of the combined pentane eluates (about 400 ml.) yielded a white crystalline compound, recrystallization of which from acetone gave colourless prisms of 24-chlorocholane, m.p. 74–75°. (Found: C, 78.9; H, 10.7; Cl, 10.3. Calc. for  $\text{C}_{24}\text{H}_{41}\text{Cl}$ : C, 79.0; H, 11.3; Cl, 9.7%.)

was apparently complete (approx. 3 hr.). 24-Iodocholane (5.0 g., 0.011 mole) in dry toluene (30 ml.) was then slowly added from a dropping funnel whilst the mixture was boiled under reflux, addition taking place during 0.5 hr. After boiling for a further 7 hr. the reaction mixture was treated with water and extracted with ether. The extract, after washing with water, drying with  $\text{Na}_2\text{SO}_4$  and evaporation, gave an oil. This oil was dissolved in a small quantity of pentane and adsorbed on to a column of  $\text{Al}_2\text{O}_3$  (120 g.). Elution of the column with pentane (400 ml.) resulted in the recovery of crystalline material (2.88 g.) which was composed chiefly of unchanged iodocholane. Elution with

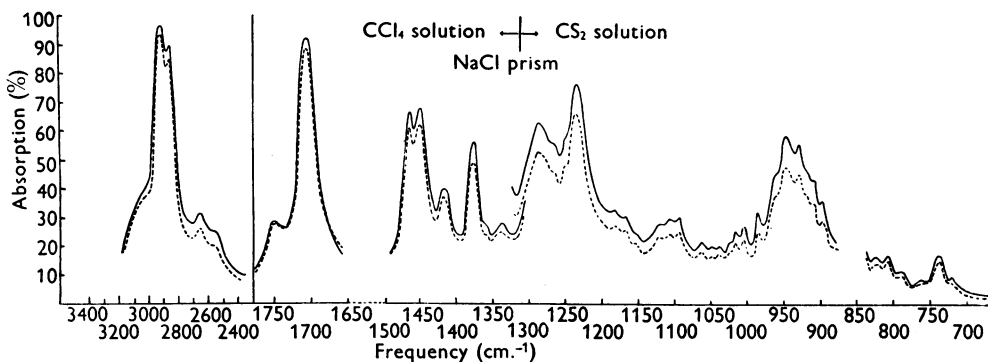


Fig. 1. Infrared spectra of partially synthetic coprostanic acid, —; and of alligator stem acid,  $\text{C}_{27}\text{H}_{46}\text{O}_3$ , - - -. For experimental details see text.

**24-Bromocholane** (III, X=Br). A procedure identical with the above, with cholan-24-ol (523 mg.) and  $\text{PBr}_5$  (approx. 1 g.) yielded, after chromatography, crystals (397 mg.) which on recrystallization from acetone gave 24-bromocholane (324 mg.) as long plates, m.p. 86–86.5°. (Found: Br, 19.7.  $\text{C}_{24}\text{H}_{41}\text{Br}$  requires Br, 19.5%.)

**24-Iodocholane** (III, X=I). Cholan-24-ol (830 mg.) was refluxed for 2 hr. with red P (90 mg.) and  $\text{I}_2$  (1.14 g.) in toluene (15 ml.). The reaction mixture was treated with water and ether. The ether layer was washed with water, aqueous  $\text{Na}_2\text{S}_2\text{O}_3$ , water, aqueous  $\text{NaHCO}_3$  and water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give a yellow crystalline solid, which was purified by chromatography in pentane on  $\text{Al}_2\text{O}_3$ . This yielded white crystals (953 mg.; 87%), recrystallization of which from acetone gave white needles of 24-iodocholane (705 mg.), m.p. 101–102°;  $[\alpha]_D^{25} + 23 \pm 2^\circ$  in  $\text{CHCl}_3$  (c, 2.8). (Found: I, 27.7.  $\text{C}_{24}\text{H}_{41}\text{I}$  requires I, 27.8%.) A further crop (128 mg., m.p. 100.5–101.5°) was obtained from the mother liquors.

In a larger scale preparation, cholic acid (10 g.) was esterified by slow dissolution in ethanol (200 ml.) containing  $\text{H}_2\text{SO}_4$  (2%, v/v) and leaving at 20° for 5 hr. The reaction mixture was poured into water and the precipitated ethyl cholanoate filtered off, washed and dried. This crude ester was reduced directly with lithium aluminium hydride as described and the resulting crude alcohol converted into the iodide, which was then purified by chromatography to give 24-iodocholane (10 g.). Overall yield, 79%.

**25-Methyl-25:25-dicarboxy-24-homocholane** (IV, R'=Et). A mixture of Na (0.9 g.; 0.039 mole) and diethyl methylmalonate (7.5 ml.; 0.044 mole) was refluxed with dry toluene (50 ml.) until the formation of the sodio derivative

pentane/benzene (80% v/v) gave crystalline material (2.04 g.; 37%), recrystallization of which from a little acetone gave 25-methyl-25:25-dicarboxy-24-homocholane, m.p. 64–66°. (Found: C, 76.9; H, 10.6.  $\text{C}_{32}\text{H}_{54}\text{O}_4$  requires C, 76.4; H, 10.8%.)

**24-Ethoxycholane** (VI, R'=C<sub>2</sub>H<sub>5</sub>). In an initial condensation, Na (92 mg.) was dissolved in a mixture of diethyl methylmalonate (0.6 ml.) and ethanol (3 ml.). 24-Chlorocholane (1.43 g.) was then added over a period of 15 min., followed by further ethanol (7 ml.). After a total refluxing time of 2.5 hr., treatment with water and ether, followed by washing, drying and evaporation of the ether gave an oil (1.6 g.) which after chromatography in pentane (approx. 200 ml.) on  $\text{Al}_2\text{O}_3$  (60 g.) gave unchanged chlorocholane (1.0 g.) in the pentane eluates and a second crystalline compound (0.14 g.) in the pentane/benzene (80%, v/v) eluates. Recrystallization of the latter (0.14 g.) from acetone yielded (probably) 24-ethoxycholane, m.p. 88–90°. (Found: C, 83.4; H, 12.2.  $\text{C}_{26}\text{H}_{46}\text{O}$  requires C, 83.4; H, 12.4%.)

In another experiment, condensation of 24-chlorocholane (365 mg.) with diethyl methylmalonate (0.15 ml.) gave, after purification, 25-methyl-25:25-dicarboxy-24-homocholane (25 mg.; 5%) as well as unchanged chlorocholane.

**24-Methoxycholane** (VI, R'=CH<sub>3</sub>). A procedure similar to that described above, but with methanol instead of ethanol, resulted in the isolation of a compound of m.p. 70.5–71.5° which was thought to be 24-methoxycholane (see below).

24-Iodocholane (100 mg.) was refluxed for 2 hr. with methanolic KOH (10 ml. of 4% w/v). Treatment of the reaction product with water, followed by ether extraction and evaporation of the washed and dried ether, gave an oil. Chromatography of this on  $\text{Al}_2\text{O}_3$  resulted in a recovery of

the iodocholane (32 mg.; 32%) from the pentane eluates and a compound (46 mg.; 58%) from the pentane/benzene (80%, v/v) eluates, which on recrystallization from acetone gave crystals of 24-methoxycholane, m.p. 71–71.5°. (Found: C, 82.9; H, 12.5.  $C_{25}H_{44}O$  requires C, 83.2; H, 12.3%.) The mixed melting point with the product described above was 71–72°.

*Hydrolysis of the ester* (IV, R=Et). The above ester (0.6 g.) with ethanol (12 ml.) and aqueous KOH (1.2 ml. of 40%, w/v) was heated in a metal bomb for 2.5 hr. at 120–125°. Dilution with water of the contents of the cooled bomb gave a clear solution which on acidification with HCl yielded a gelatinous solid. Before collection of this the mixture was treated with excess NaCl to ensure complete precipitation. The precipitate was collected by suction on a filter, washed with water and squeezed as dry as possible; it was then dried *in vacuo* over  $H_2SO_4$ . The total yield of dried product (IV, R=H) from 2.1 g. of ester was 1.84 g.: no attempt was made at further purification.

*Coprostanic acid* (V). The above product (50 mg.) was heated in a sublimation apparatus at 0.05–0.02 mm. Hg. At about 165°, decarboxylation occurred and the product changed into a brown oil. This was distilled up to about 200°, forming a nearly colourless 'glass', which was collected and dissolved in methanol. Careful dilution of the warm solution, followed by standing, caused the crystallization of coprostanic acid as long colourless needles (36 mg.; 86% calculated from IV, R'=Et), which had m.p. 106–108°. The mixed melting point with coprostanic acid (m.p. 103.5–105.5°) from bile salts of Crocodylidae (Haslewood, 1952) was 104–106.5°.  $[\alpha]_D^{21} + 28 \pm 1^\circ$  in  $CHCl_3$  (c, 1.5). (Found: C, 80.6; H, 11.9. Calc. for  $C_{27}H_{46}O_2$ : C, 80.5; H, 11.5%.) On a larger scale, decarboxylation followed by distillation at up to 280°/0.05 mm. Hg. of the acid (IV, R'=H) (1.84 g.) finally gave coprostanic acid, crystallizing as long white needles (1.25 g.) from aqueous acetone.

The above-mentioned 'glass' (0.1 g.) formed by distillation of IV (R=H) in methanol was treated with excess diazomethane. The solvent was evaporated and the residue, in pentane, was poured on to  $Al_2O_3$  (5 g.). Elution with 8% (v/v) benzene/pentane gave a total of 68 mg. of crystalline material, apparently a single substance. This was *methyle coprostanate*, which crystallized from methanol as colourless needles, m.p. 56–57°. (Found: C, 80.6; H, 11.7.  $C_{25}H_{46}O_2$  requires C, 80.7; H, 11.6%.)

#### *Infrared spectra*

The infrared spectra were measured by Dr A. R. H. Cole with a Perkin-Elmer Spectrometer, model 12c, equipped with a sodium chloride prism, kindly made available by the Chester Beatty Research Institute. A 1 mm. cell was used. Solvents were: 650–1310  $cm^{-1}$ ,  $CS_2$ ; 1310–3200  $cm^{-1}$ ,  $CCl_4$ . Concentrations in  $CS_2$  were: coprostanic acid, 16.44 mg./ml.; alligator stem acid, 12.64 mg./ml. The  $CCl_4$  solutions were made up using recovered material; the concentrations were not known accurately. The region between 840 and 880  $cm^{-1}$  was partly obscured by an absorption band of  $CS_2$ , and this region is omitted in view of the doubtful accuracy of the spectra.

## DISCUSSION

The present work makes clear beyond reasonable doubt the nature of the carbon skeleton and the position of the carboxyl group in derivatives of coprostanic acid. It provides a substance which should be useful as a reference compound in the  $C_{27}$  series of bile acids and alcohols.

It might be expected that a malonic ester synthesis of the type described would lead to a mixture racemic with respect to the new asymmetric centre introduced at  $C_{(25)}$ . Such, however, did not appear to be the case, for the 'natural' and partially synthetic coprostanic acids seemed to be identical in all properties, including optical rotation, which we examined. It is, perhaps, difficult to be absolutely certain on this point, because of the very small quantity of the 'natural' material available for rotation measurements. We have, of course, no direct experience to guide us as to what the general properties of a mixture of substances isomeric at  $C_{(25)}$  would be. Comparison with cholanic acid ( $[M]_D + 79^\circ$ ) suggests that in our coprostanic acid ( $[M]_D + 113^\circ$ ), the contribution to  $[M]_D$  of the new centre at  $C_{(25)}$  is about  $+34^\circ$ : a negative value for this in the 25-epimer should have been detected when the rotations were examined.

The method of partial synthesis seems to us to be of special value for the investigation of higher homologues of the  $C_{24}$  bile acids and we hope to apply it in other cases.

## SUMMARY

1. Cholanic acid ( $C_{24}H_{40}O_2$ , side chain;  $-CH(CH_3).CH_2.CH_2.COOH$ ) has been converted by a malonic ester synthesis to coprostan-26-oic acid (coprostanic acid,  $C_{27}H_{46}O_2$ ; side chain  $-CH(CH_3).(CH_2)_3.CH(CH_3)COOH$ ). The intermediates and by-products of this partial synthesis have been isolated and characterized.

2. Coprostanic acid has been shown to be identical with the  $C_{27}$  stem acid previously made from the bile salts of Crocodylidae; it should be a useful reference compound.

The authors wish to express their gratitude to Dr A. R. H. Cole (University of Western Australia) for the infrared measurements. They also thank Dr Norman Evers and Messrs Allen and Hanburys Ltd. for a generous gift of cholic acid.

## REFERENCES

- Bridgwater, R. J. & Haslewood, G. A. D. (1952). *Biochem. J.* **51**, xxiv.  
 Haslewood, G. A. D. (1952). *Biochem. J.* **52**, 583.  
 Wessely, F. & Swoboda, W. (1951). *Mh. Chem.* **82**, 437.