Partial Synthesis of the Two 3α:7α:12α-Trihydroxycoprostanic Acids and of Similar Bile Acids with Extended Side Chains

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Recent work has emphasized the importance of C_{27} bile acids both as evolutionary precursors of the C_{24} bile acids and as likely intermediates in the biosynthesis of the latter compounds from cholesterol (for reviews see Bergström, 1955; Haslewood, 1955).

Much of the early work on the C_{27} and C_{28} bile acids and alcohols was carried out in Japan, but an acid $C_{27}H_{46}O_5$ was isolated in this laboratory from the bile of various members of the Crocodylidae (Haslewood, 1952*a*). This was thought to be 3α:7α:12α-trihydroxycoprostanic acid, and was later proved to be a C_{27} acid by synthesis of the 'stem acid' coprostanic acid, $C_{27}H_{46}O_2$, from cholanic acid (Bridgwater & Haslewood, 1952). An acid named 'α-trihydroxybisnorsterocholanic' acid, m.p. 172°, was isolated from the bile of the frog *Rana catesbiana* (Kurauti & Kazuno, 1939), and 'β-trihydroxybisnorsterocholanic acid', m.p. 190–195°, was later isolated from the same source (Mabuti, 1941). Both these acids were also isolated from the bile of *Rana nigromaculata nigromaculata* (Komatsubara, 1954). The 'α-trihydroxybisnorsterocholanic acid', m.p. 172°, has been shown to be identical with trihydroxycoprostanic acid isolated from the bile of *Alligator mississippiensis* (Haslewood, 1952*a*) and the terms 'α-' and 'β-trihydroxybisnorsterocholanic acid' are now replaced by 'α-' and 'β'-trihydroxycoprostanic acid respectively.

This earlier work left two further aspects of the problem to be elucidated, namely the position and configuration of the three hydroxyl groups in the trihydroxycoprostanic acids and, more important, the question of isomerism at C-25, since, with the conversion *in vivo* of the terminal *iso*propyl group of the cholesterol side chain into $-CHMe.CO_2H$, a new asymmetric centre is introduced. It was therefore decided to attempt the partial synthesis of the two 3α:7α:12α-trihydroxycoprostanic acids and similar acids with extended side chains (i.e. bile acids in which the $-CHMe.CH_2.CH_2.CO_2H$ chain is lengthened).

Some extensions of the side chains of bile acids have been previously reported, thus, β-3α:7α:12α-trihydroxycoprostanic acid, m.p. 195° (Komatsubara, 1954; Bridgwater, 1955), and bishomocholic acid (Kazuno, Komatsubara & Baba, 1954; Kazuno, Mori & Goto, 1955) have both been synthesized.

The lengthening of the bile acid side chain in the presence of ring hydroxyl groups presents certain difficulties with the more usual methods, e.g. the use of the Arndt–Eistert synthesis

$$[R.CO_2H \rightarrow R.COCl \rightarrow R.CO.CHN_2 \rightarrow R.CH_2.CO_2Et \rightarrow R.CH_2.CO_2H]$$

(cf. Pearlman, 1947) is laborious since it extends the side chain by only one carbon atom at a time. The application of the Kolbe electrolytic synthesis to the preparation of acids, by employing the half-ester of a dibasic acid, has been exploited in another field (Bounds, Linstead & Weedon, 1953, and previous papers), and the subject has been reviewed (Weedon, 1952). The use of this method for the extension of the side chain of the bile acids has not yet been fully explored, and the investigation of the cross-coupling reactions which can occur by the electrolysis of cholic acid in the presence of some simple half-esters is now reported.

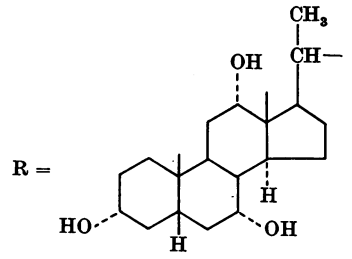
RESULTS

The product resulting from an electrolysis using cholic acid and a simple half-ester is a complex mixture. From theoretical considerations, the norcholyl radical (II) arising from the cholate ion (I) can undergo a coupling, with the radical resulting from the half-ester employed to form an ester, e.g. (III), or with another norcholyl radical to form a dimer (IV); a further possibility is a disproportionation reaction to give an unsaturated compound (V), together with the corresponding saturated compound (VI).

In the cross-coupling electrolysis of cholic acid with methyl hydrogen succinate the compounds corresponding to (III, $n=4$), (IV) and (V) were all isolated by chromatography of the product on alumina.

The products arising from the coupling of radicals from the half-ester molecules with themselves, e.g. dimethyl adipate, were eluted from the alumina with ether. Acetone removed a compound regarded as 3α:7α:12α-trihydroxy-23-norchol-22-ene (V) together with the 25:26-bishomocholyl ester (III, $n=4$); the last substance was separated after

hydrolysis to the corresponding acid. An amorphous product of high melting point, regarded as 23:23′-bisnorcholyl (IV), was eluted with methanol. Hydrolysis of the methyl bishomocholate (III, $n=4$) gave 25:26-bishomocholic acid, which was shown by an examination of the infrared spectrum to be different from cholic acid. Chromic oxidation of this acid gave dehydro-25:26-bishomocholic acid (3:7:12-trioxo-25:26-bishomocholanic acid) which on Wolff–Kishner reduction (Huang–Minlon modification) gave 25:26-bishomocholanic acid, isolated as the ethyl ester.



$$\underset{(I)}{R.CH_2.CH_2.CO_2^-} \rightarrow \underset{(II)}{R.CH_2.CH_2.} \rightarrow \begin{cases} R.[CH_2]_n.CO_2Me & (III) \\ (R.CH_2.CH_2.)_2 & (IV) \\ R.CH{:}CH_2 & (V) \\ R.CH_2.CH_3 & (VI) \end{cases}$$

A similar cross-coupling of cholic acid with ethyl hydrogen malonate allowed the preparation of 25-homocholic acid (from III, $n=3$) in about 27% yield. This acid was identical with 25-homocholic acid prepared by the Arndt–Eistert method (Pearlman, 1947).

Methylsuccinic anhydride was resolved into its D- and L-forms, and each of these was treated with an equivalent amount of methanol to give a product consisting of a mixture of

$HO_2C.CH_2.CH(CH_3).CO_2Me$ (A)
and $MeO_2C.CH_2.CH(CH_3).CO_2H$ (B)

(both D- or both L-). Since compound (B) is an α-substituted acid, normal coupling should be largely or totally suppressed (see Weedon, 1952). On electrolysis of the half-ester (A+B) from L-methylsuccinic anhydride and cholic acid, an acid, m.p. 195–196°, was obtained which was identical with a specimen of partially synthetic 'β'-trihydroxycoprostanic acid (Komatsubara, 1954) kindly supplied by Professor T. Kazuno and which had been shown to be identical with the acid occurring in the bile of *Rana nigromaculata nigromaculata*. Use of the half-ester (A+B) from D-methylsuccinic anhydride gave an acid, m.p. 160°, with softening at 95–105°, identical with that obtained by using the half-ester from DL-methylsuccinic anhydride.

A similar series of experiments were carried out with cholic acid and DL-, D- and L-methyl hydrogen β-methylglutarate

$(HO_2C.CH_2.CHMe.CH_2.CO_2Me;$

for preparation and resolution see Linstead, Lunt & Weedon, 1950). The DL-half-ester gave two acids (trihydroxyhomocoprostanic acids), separated by chromatography and crystallization, which could be prepared separately by employing the individual D- or L- half-esters (see Table 1 for physical data). The acid prepared from the L-β-methylglutaric half-ester was degraded by the Wieland–Barbier method to give an acid which on hydrolysis yielded 3α:7α:12α-trihydroxy-25-D-coprostanic acid, m.p. 180–182°, identical with the naturally occurring acid (trihydroxycoprostanic acid, Haslewood, 1952*a*; 'α'-trihydroxycoprostanic acid, Komatsubara, 1954).

A similar degradation of the ethyl ester of the acid prepared from D-β-methylglutaric half-ester gave 3α:7α:12α-trihydroxy-25-L-coprostanic acid, m.p. 195–196°, which was identical with the 'β' acid and with the acid prepared from L-methylsuccinic half-ester (see above).

DISCUSSION

Chemical. *dextro*Methyl hydrogen β-methylglutarate has been related to L-glyceraldehyde, and *laevo*methyl hydrogen β-methylglutarate has been

Table 1. *Preparation and properties of extended-chain bile acids*

Half-ester used	Acid formed on electrolysis with cholic acid: Name	M.p.	$[\alpha]_D$
$HO_2C.CH_2.CO_2Me$	25-Homocholic	202°	
$MeO_2C.[CH_2]_2.CO_2H$	25:26-Bishomocholic	195	+34°
L-$MeO_2C.CHMe.CH_2.CO_2H$	3α:7α:12α-Trihydroxy-25-L-coprostanic	195–196	+43
L-$MeO_2C.CH_2.CHMe.CH_2.CO_2H$	3α:7α:12α-Trihydroxy-25-D-homocoprostanic	205–207	+37
D-$MeO_2C.CH_2.CHMe.CH_2.CO_2H$	3α:7α:12α-Trihydroxy-25-L-homocoprostanic	173–176	+29
(For comparison	3α:7α:12α-Trihydroxy-25-D-coprostanic	180–182	+27)

related to D-glyceraldehyde (Ställberg & Stenhagen, 1947); so that the two trihydroxyhomocoprostanic acids can be related to the two glyceraldehydes: further, since the two homo acids have been degraded to the corresponding trihydroxycoprostanic acids, the relationship of these latter two acids to the glyceraldehydes can also be established. These relationships are shown more clearly in Fig. 1.

The two naturally occurring trihydroxycoprostanic acids are therefore isomeric at C-25. The acid of m.p. 182° (Japanese 'α') has already been correctly formulated as trihydroxycoprostan-26-oic acid (Haslewood, 1952*a*) and the acid of m.p. 195° (Japanese 'β') would therefore be trihydroxycoprostan-27-oic acid. However, to conform with the nomenclature in the steroid field these acids could be termed 25-D- and 25-L-trihydroxycoprostanic acids. The homo acids will therefore be called homocoprostanic acids, i.e. 25-D- or 25-L-.

The use of the half-esters from D- and L-methylsuccinic anhydrides gave anomalous results. The product from D-methylsuccinic anhydride and methanol should consist of a mixture of the D-enantiomorphs of both $HO_2C.CHMe.CH_2.CO_2Me$ and $MeO_2C.CHMe.CH_2.CO_2H$, of which only the latter would be expected to undergo normal anodic coupling to any extent. The fact that the D-anhydride and the DL-anhydride both gave half-esters which yielded an identical acid (but one not identical with either of the trihydroxycoprostanic acids), on coupling with cholic acid, suggests that racemization may have occurred at some stage or that some coupling of the substance

$$HO_2C.CHMe.CH_2.CO_2Me$$

may, in fact, have taken place, in either case yielding a mixture. L-Methylsuccinic anhydride gave a product from which the trihydroxycoprostanic acid, m.p. 195–196°, was isolated after three crystallizations. Since the coupling of the L-enantiomorph

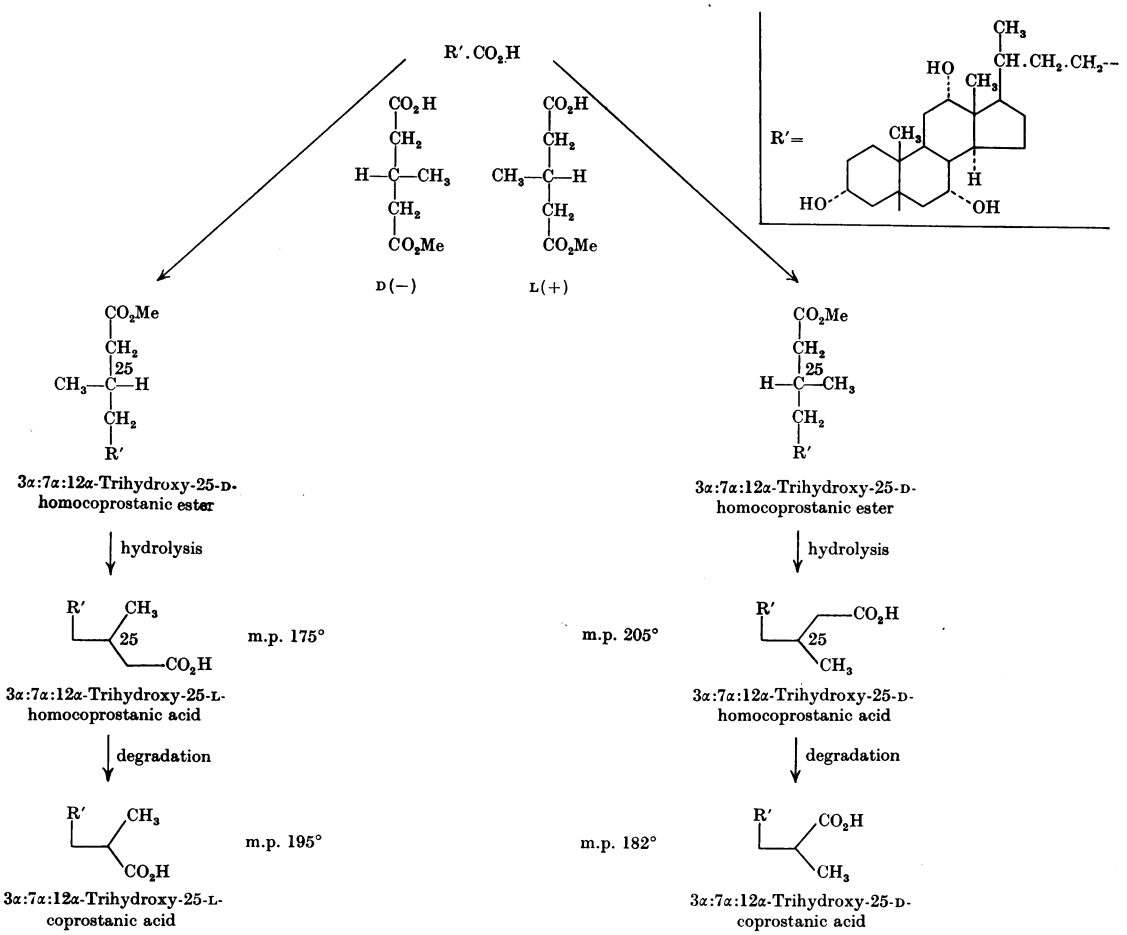


Fig. 1

should produce the 25-D-acid (trihydroxycoprostan-26-oic acid, m.p. 182°), the result of this coupling is clearly misleading, for the acid of m.p. 195° is the 25-L-(trihydroxycoprostan-26-oic acid) isomer. Thus the use of the optical enantiomorphs of methylsuccinic acid half-esters cannot be regarded as reliable, and indeed the same difficulties will possibly also be encountered with other unsymmetrical half-esters in the Kolbe electrolysis.

Biological. The demonstration that the two trihydroxycoprostanic acids are C-25 isomers, together with the fact that both compounds occur together in the biles of at least two species, namely *Rana catesbiana* and *R. nigromaculata nigromaculata*, suggests that, in the initial oxidation *in vivo* of the terminal *iso*propyl group of cholesterol, the enzyme responsible is not selective for either C-26 or C-27, so that initially a mixture of alcohols would result, namely 25-D-3α:7α:12α-26- and 25-L-3α:7α:12α-26-tetrahydroxycoprostanes, these two compounds then being oxidized to the two trihydroxycoprostanic acids respectively.

The occurrence of C_{27} acids in some species of the Ranidae, whereas in others, e.g. *Rana temporaria*, the chief constituents are alcohol sulphates, e.g. ranol sulphate (Haslewood, 1952*b*), is an interesting species difference. Two pathways (*A* or *B*) are available to the C_{27} alcohols after their formation; thus they may be either oxidized or conjugated as the next step:

$$\begin{array}{l} -CH_2\ OH \xrightarrow{A} -CH_2.O.SO_3Na \\ \quad\quad\downarrow B \\ -CO_2H \end{array}$$

If route *A* is followed by a species, then production of acid may be difficult, if not impossible, further oxidation leading to a greater degree of hydroxylation, to produce substances such as ranol. Route *B* will of course lead to an acidic substance, which may then be conjugated (e.g. with taurine) or may be further oxidized to the C_{24} acid.

Isomerism at C-25 in steroids has been detected in natural products from plant sources, e.g. sarsasapogenin and smilagenin (Scheer, Kostic & Mosettig, 1953). Cholegenin and *iso*cholegenin have also been shown to be isomeric at C-25 (Mazur & Spring, 1954).

EXPERIMENTAL

General. Melting points are uncorrected. Microanalyses were done by Weiler and Strauss, Oxford.

Reagents. Al_2O_3 was 200-mesh Type H (Peter Spence and Sons, Widnes). Ethanolic KOH was approx. N-KOH in 50% (v/v) ethanol–water. CrO_3 (20%) was a solution of about 20 g. of CrO_3 in a minimal volume of water and made up to 100 ml. with acetic acid.

Electrolyses. Electrolyses with large electrodes (5 cm. × 6 cm. and 5 cm. × 5 cm.) were carried out in a flat-bottomed cylindrical vessel, diameter 10 cm. and height 30 cm., with internal cooling (two glass coils containing circulating tap water, or circulating cold acetone which was cooled in a copper coil surrounded by acetone–solid CO_2, the circulation being maintained by means of a small electric pump). External cooling was also applied which consisted of ice–water or solid CO_2–cooled acetone. Electrolyses employing smaller electrodes (1 cm. × 3 cm.) were carried out in a boiling tube (diameter 3·5 cm., height 20 cm.) with external cooling only. In all cases the electrodes were reversed periodically to minimize coating. Occasionally it was necessary to stop the electrolysis, remove the electrodes and wipe off the gummy material; this was not necessary after the introduction of the very cold acetone cooling system. The d.c. potential was 110 v.

Isolation of products. Procedure A involved ether–ethyl acetate (1:1) extraction, washing the ether with saturated $NaHCO_3$ (twice) and water (three times) and drying with Na_2SO_4.

Procedure B. To the methanolic solution after electrolysis was added 2M acetic acid in slight excess (approx. 1·1 mol./mol. of Na used in the electrolysis). Most of the solvent was then removed by distillation, the last traces being removed with the aid of a water pump and the residue subjected to procedure *A*. Evaporation of the organic solvent gave a neutral fraction (1 × *n* g.) and acidification (2M-HCl) of the $NaHCO_3$ solution yielded an insoluble acid fraction, which was collected and washed with water. After drying (vacuum desiccator 1–2 days) the neutral fraction was dissolved in ether and run on a column of Al_2O_3 (approx. 30 × *n* g.) prepared from a slurry in ether. The column was then eluted in turn with ether, 1:1 ether–acetone, and finally methanol.

Procedure C. This consisted in refluxing the appropriate product with ethanolic KOH (10 ml./g. of product) for approx. 1 hr. The saponified solution was diluted to about 10 times its volume with water, and filtered. The residue was washed with water to give a neutral residue. The filtrate, together with the washings, was acidified to give an insoluble acid.

Preparation of half-esters

Methyl hydrogen DL-*methylsuccinate.* DL-Methylsuccinic acid (Brown, 1946) was converted into the anhydride (Naps & Johns, 1940), and this (1 mol.) was refluxed on a water bath with methanol (1·04 mol.) for 2 hr. The resulting mixture of half-esters was used without further purification.

D- *and* L-*Methylsuccinic anhydrides.* The DL-half-ester was resolved by means of the strychnine salt (Berner, Leonardsen, Grøntoft & Dahl, 1939), the L-anhydride being crystallized three times from benzene until complete optical purity was obtained. The anhydrides were then treated with methanol as described above to give the corresponding half-esters.

Methyl hydrogen β-methylglutarate. This compound was prepared and resolved (Linstead *et al.* 1950).

Preparation of bile acids with extended side chains

25:26-*Bishomocholic acid.* Cholic acid (20·4 g.) and methyl hydrogen succinate (19·8 g.) were added to a solution of Na (0·20 g.) in methanol (600 ml.). The solution was electrolysed with two platinum electrodes (5·0 cm. × 6·0 cm.) at a distance apart of about 2–3 mm. The current varied from 6 to 4A during the first 1·5 hr., thereafter dropping off rapidly. After a total electrolysis time of 2·5 hr. the reaction was stopped and the product submitted to procedure *B*,

yielding a neutral fraction (approx. 21 g.), together with an acidic fraction (8 g.) which on drying and crystallization from ethyl acetate yielded cholic acid, m.p. and mixed m.p. 198–200°. The neutral fraction was chromatographed on Al_2O_3 eluates of 1 l. each being collected and evaporated, giving fractions 1–18. Combined fractions 1–2 (eluent, ether) were oils (total 5·5 g.). Fractions 3–7 [eluent, ether/ether–acetone (1:1)] were oils (1·0 g.) which gave a negative Hammarsten HCl test. Combined fractions 8 and 9 (eluent, acetone) furnished an oil (4·8 g.), which after procedure *C* yielded chiefly neutral material, and an insoluble acid (0·10 g.) which was collected and washed with water. Fractions 10–12 (eluent, acetone) gave crystals (3·1 g.), which on similar hydrolysis (procedure *C*) yielded very little neutral material and an acid (2·8 g.) similarly treated. The two samples of acid were combined, dried and recrystallized twice from ethyl acetate to give small platelets of 25:26-bishomocholic acid, m.p. 194–195°, mixed m.p. with cholic acid (m.p. 199–200°), 173–195°; $[\alpha]_D^{22} + 34 \pm 2°$ in ethanol (*c*, 2·0). Yield, 22%. (Found: C, 69·8; H, 10·25. Calc. for $C_{26}H_{44}O_5, \frac{1}{2}H_2O$: C, 70·05; H, 10·20.) A small quantity of this acid in methanol was treated with diazomethane (excess). Removal of the solvent and chromatography of the product on alumina yielded an acetone fraction, which on recrystallization from benzene–pentane afforded long needles of *methyl* 25:26-*bishomocholate*, m.p. 148–150°. mixed m.p. with methyl cholate (m.p. 147°) 130–135°. (Found: C, 71·6; H, 10·25. $C_{27}H_{46}O_5$ requires C, 71·95; H, 10·3%.) This ester was also obtained by direct recrystallization of the crystalline material from fractions 10–12 (above). Both bishomocholic acid and its methyl ester gave a blue colour in the Hammarsten HCl test.

Fractions 13–18 (eluents, acetone for 13–16 and methanol for 17–18) did not yield any acidic material on hydrolysis. The material from fraction 17, on crystallization from methanol, afforded needle-like crystals of (?)3α,3′α:7α,7′α:12α,12′α-hexahydroxybis-23:23′-norcholanyl, m.p. 312–313° (shrinking at 180°).

A similar electrolysis employing cholic acid (20·4 g.), methyl hydrogen succinate (26·4 g.), and Na (0·2 g.) in methanol (650 ml.) with Pt electrodes (5 cm. × 6 cm.) (current 8–6A, time 3 hr.) gave a neutral product (36 g.) which was separated on Al_2O_3 (600 g.) as described above, eluates of 1 l. each being collected. Fraction 1 (eluent, ether) was an oil (23 g.). Fractions 2–6 (eluent, 1:1 ether–acetone) were oils (1·6 g.). Fraction 7 (eluent, acetone) was an oil (0·6 g.) which gave a deep-yellow colour with tetranitromethane in $CHCl_3$. Fraction 8 (eluent, acetone) was an oil (1·1 g.) which gave a faint-yellow colour with tetranitromethane in $CHCl_3$. Fractions 9–14 (eluents, acetone) were crystalline (total 4·2 g.). Each fraction was separately subjected to procedure *C*, to yield in every case a neutral residue together with an insoluble acid which was filtered off, washed and dried *in vacuo*. Three crystallizations of the combined acids from ethyl acetate yielded 25:26-bishomocholic acid (2·7 g.), m.p. 195°. The neutral material from fraction 9 on crystallization from a small quantity of acetone gave crystals of (?)3α:7α:12α-*trihydroxy*-23-*norcholene*, m.p. 179–181°. (Found: C, 75·2; H, 10·9. $C_{23}H_{40}O_3$ requires C, 75·75: H, 11·05.)

Dehydrobishomocholic acid (3:7:12-*trioxo*-25:26-*bishomocholic acid*). 25:26-Bishomocholic acid (890 mg.) in acetic acid (10 ml.) was treated with 20% CrO_3 (2·7 ml.) with cooling. After 10 min. at 20°, the mixture was poured into water, which was then saturated with NaCl and allowed to stand overnight. The resulting solid was filtered off, dissolved in excess of warm 2N-NaOH and the solution cooled and filtered. The filtrate was acidified with excess of 10N-HCl, the precipitated acid filtered off, washed with water and recrystallized from methanol–water (3:1), to yield small needle-like crystals of *dehydrobishomocholic acid* (3:7:12-*trioxo*-25:26-*bishomocholanic acid*), m.p. 220·5–221·5° (decomp.), with darkening at 217°; mixed m.p. with dehydrocholic acid (m.p. 232–234°), 218–230°. (Found: C, 72·6; H, 9·05. $C_{26}H_{38}O_5$ requires C, 72·5; H, 8·9%.)

Ethyl 25:26-*bishomocholanate*. The dehydro acid (190 mg.) was added to a solution of Na (0·10 g.), 90% hydrazine hydrate (0·20 ml.) and diethylene glycol (8·0 ml.). The mixture was refluxed for 1·25 hr., after which time the temperature was raised to 208° by removal of the condenser, and refluxed for a further 3 hr. The reaction mixture was cooled, treated with water and acidified with 10N-HCl. The resulting precipitate was collected, washed, dissolved in ethanol and treated with diazoethane to give a product, which, after removal of the solvent, was adsorbed on Al_2O_3 (10 g.) and eluted with pentane–benzene (1:1) (approx. 200 ml.). Evaporation of the eluate left crystals which, on recrystallization from 90% ethanol, gave *ethyl* 25:26-*bishomocholanate*, m.p. 59·5–60·5°. (Found: C, 80·0; H, 11·5. $C_{28}H_{48}O_2$ requires C, 80·55; H, 11·5%.)

25-*Homocholic acid*. A solution of cholic acid (10·2 g.), and Na (0·10 g.) in methanol (450 ml.) was electrolysed while ethyl hydrogen malonate was added in the following manner: 0 min., 7·0 g.; 50 and 100 min., 3·5 g. in methanol (25 ml.) (total, 14·0 g.). Two Pt electrodes (5 cm. × 5 cm.) were used at a distance apart of 2–3 mm. The current varied from 4 to 2A and the duration of the electrolysis was 4 hr. Procedure *B* yielded cholic acid (8·9 g.), together with a neutral fraction. Chromatography of the latter in the manner described above gave, after initial elution with ether, oils from the acetone eluates which were combined and subjected to procedure *C*. The insoluble acid so obtained was extracted by procedure *A* to yield an oil (0·2 g.), which was combined with a similarly obtained oil (0·3 g.) resulting from elution of the column with acetone–methanol (1:1). The combined product, on standing with a little acetone overnight, gave crystals which on recrystallization from acetone yielded 25-homocholic acid (m.p. 215–216°) [mixed m.p. with an authentic sample (m.p. 212–214°), 214–216°; mixed m.p. with cholic acid (m.p. 198–200°), 187–201°]. Yield, 37%.

(?)3α:7α:12α-*Trihydroxy*-25-DL-*coprostanic acid*. Cholic acid (5·1 g.) and methyl hydrogen-DL-methylsuccinate (10·95 g.) were added to a solution of Na (0·05 g.) in methanol (70 ml.). The mixture was electrolysed with Pt electrodes (1 cm. × 3 cm.). The current was about 1A and the reaction time was 3·5 hr. Procedure *B* gave recovered cholic acid (3·7 g.) and a neutral fraction (5·0 g.). The latter on chromatography (250 ml. eluates) gave fractions 1–2 (eluent, ether; oil, 3·7 g.); fractions 3 [eluent, ether–acetone (1:1)] and 4 (eluent, acetone), traces of oil; fraction 5 (eluent, acetone; oil, 0·15 g.); fractions 6–10 (eluent, acetone; oils, combined wt. 0·885 g.). This last oil (0·885 g.) by procedure *C* formed an insoluble acid (0·6 g.) giving a weak (blue) Hammarsten HCl test. The acid on recrystallization twice from ethyl acetate yielded needles of (?)3α:7α:12α-*trihydroxy*-25-DL-*coprostanic acid*, m.p. 164–166°, with some softening at 92°; $[\alpha]_D^{28} + 31·2 \pm 2°$ in ethanol (*c*, 2·1). Total yield of crude

acid, 46%. (Found: C, 69·2; H, 10·2. $C_{27}H_{46}O_5,H_2O$ requires C, 69·2; H, 10·3%.) This acid (48 mg.) was esterified with 2·5% (v/v) H_2SO_4–ethanol (1 ml.) for 64 hr. at room temp. The product was poured into aqueous $NaHCO_3$ and extracted with ether. Evaporation of the washed ether furnished (?)*ethyl* 3α:7α:12α-*trihydroxy*-25-DL-*coprostanate*, m.p. 122–125°.

An electrolysis in which identical quantities with the above were used, but with methyl hydrogen D-methylsuccinate (prepared from the D-anhydride) in place of the DL-isomer, yielded a final acid product which was identical in every way with the product described above as (?)3α:7α:12α-trihydroxy-25-DL-coprostanic acid.

3α:7α:12α-*Trihydroxy*-25-L-*coprostanic acid*. A similar electrolysis (1A/7 hr.) with cholic acid (10·2 g.) methyl hydrogen-L-methylsuccinate (29·34 g.) and Na (0·1 g.) in methanol (100 ml.) resulted, after procedure *B*, in a recovered cholic acid fraction of 5·0 g. and a neutral product (10 g.), which was chromatographed. Ether (500 ml.) and ether–acetone (1:1, 250 ml.) were passed through the column and the eluates discarded. Elution with acetone (2 l.) gave an oil which by procedure *C* yielded an insoluble acid; after four crystallizations from ethyl acetate the acid yielded 3α:7α:12α-trihydroxy-25-L-coprostanic acid, m.p. 194–196°; $[\alpha]_D^{22}+42·6\pm2°$ in ethanol (*c*, 2·13), mixed m.p. with Japanese β acid (m.p. 195–197°, kindly supplied by Professor T. Kazuno), 192–195°. (Found: C, 72·0; H, 10·4; calc. for $C_{27}H_{46}O_5$, C, 72·0; H, 10·3%.) The infrared spectra of the two acids were compared by Dr I. D. P. Wootton and found to be identical.

3α:7α:12α-*Trihydroxy*-25-D- *and* -25-L-26-*homocoprostanic acids*. A solution of cholic acid (8·16 g.), methyl hydrogen DL-β-methylglutarate (12·8 g.), and Na (0·075 g.) in methanol (80 ml.) was electrolysed for 4·5 hr. with Pt electrodes (1 cm. × 3 cm.) (current = 2–1A). Working up by procedure *B* gave recovered cholic acid (5·5 g.) and a neutral product (9 g.). This latter was then chromatographed, eluates of 300 ml. being collected. Combined fractions 1–2 (eluent, ether) gave an oil (5·1 g.). Fraction 3 (eluent, ether–acetone 1:1) and fractions 4–5 (eluent, acetone) gave traces of oil, which on procedure *C* gave an acid product, from which crystals of 3α:7α:12α-*trihydroxy*-25-L-26-*homocoprostanic acid*, m.p. 166–168° (see below), were obtained. Fraction 6 (eluent, acetone) furnished an oil (0·22 g.) which on procedure *C* yielded an acid (0·155 g.). Fraction 7 (eluent acetone–methanol 4:1) gave an oil (1·44 g.) which on procedure *C* furnished an acid (0·805 g.). This acid on crystallization from acetone and recrystallization from ethyl acetate gave fine crystals of 3α:7α:12α-*trihydroxy*-25-D-26-*homocoprostanic acid*, m.p. 197–200° (see below); $[\alpha]_D^{23}+33·9°\pm2°$ in ethanol (*c*, 1·0). (Found: C, 72·15; H, 10·05. $C_{28}H_{48}O_5$ requires C, 72·35; H, 10·4%.) This acid gave a yellow colour, finally developing to a greenish shade after several hours, in the Hammarsten HCl test.

3α:7α:12α-*Trihydroxy*-25-D-*homocoprostanic acid*. A similar electrolysis (current 9–5A; temp. 25–30°; time 3 hr.) with L-methyl hydrogen β-methylglutarate (58 g.), cholic acid (30 g.) and Na (0·9 g.) in methanol (600 ml.) resulted, after procedure *A* and chromatography of the neutral fraction, in the isolation from the combined acetone fractions of an oil, which was hydrolysed by procedure *C* to give an acid, recrystallization of which three times from ethyl acetate gave 3α:7α:12α-*trihydroxy*-25-D-*homocoprostanic acid* (1·93 g.), m.p. 205–207°; $[\alpha]_D^{19}+36·9\pm2°$ in ethanol (*c*, 2·1). (Found: C, 72·8; H, 10·3. Calc. for $C_{28}H_{48}O_5$: C, 72·35; H, 10·4%.) Esterification of this acid (4% H_2SO_4 in absolute ethanol) and two crystallizations from C_6H_6–light petroleum (b.p. 60–80°) yielded *ethyl* 3α:7α:12α-*trihydroxy*-25-D-*homocoprostanate*, m.p. 137–139°. (Found: C, 73·0; H, 10·85. $C_{30}H_{52}O_5$ requires C, 73·1; H, 10·6%.)

3α:7α:12α-*Trihydroxy*-25-L-*homocoprostanic acid*. A similar electrolysis (current 4–2A; temp. approx. 30°; time 3·25 hr.), using D-methyl hydrogen β-methylglutarate (17·5 g.), cholic acid (9·0 g.) and Na (0·3 g.) in methanol (200 ml.) likewise gave, after procedure *B*, chromatography and hydrolysis by procedure *C*, 3α:7α:12α-*trihydroxy*-25-L-*homocoprostanic acid*, m.p. 173–176°, $[\alpha]_D^{21}+28·9\pm2°$ in ethanol (*c*, 0·8). (Found: C, 72·4; H, 10·2. $C_{28}H_{48}O_5$ requires C, 72·35; H, 10·4.)

3α:7α:12α-*Trihydroxy*-25-D-*coprostanic acid*. 3α:7α:12α-Trihydroxy-25-D-homocoprostanic acid (2·1 g.) was esterified with diazomethane and the resulting ester in benzene (20 ml.) was added over a period of 10 min. to a Grignard complex prepared from Mg (2·1 g.), bromobenzene (9·6 ml.) and ether (30 ml.). The mixture was warmed on a water bath for 1 hr. and left at room temp. overnight. This product was treated with 2M-H_2SO_4 and subjected to procedure *A* to give a gum; this was refluxed with aq. ethanolic (1:1) KOH (2M) in order to hydrolyse unchanged ethyl ester, and the neutral fraction extracted into ethyl acetate. This was washed once with water, twice with saturated $NaHCO_3$ and twice with water, the ethyl acetate solution dried (Na_2SO_4) and evaporated to give a gum. This, after drying for 2 days *in vacuo*, was acetylated for 15 min. with acetic acid (35 ml.), acetic anhydride (30 ml.) and 8·5N perchloric acid (0·3 ml.). This mixture was poured into water (100 ml.) and extracted with ether, the ether washed twice with aqueous $NaHCO_3$, twice with water, dried (Na_2SO_4) and evaporated. The crude acetate was then dissolved in benzene and chromatographed on Al_2O_3 (90 g.). The column was developed with benzene (200 ml.), benzene–ether (1:1) (50 ml.) and ether (800 ml.), the material eluted by the ether being isolated as a gum. This was oxidized by dissolving in $CHCl_3$ (6 ml.), acetic acid (24 ml.) and treating over 10 min. with CrO_3 (1·0 g.) in water (3 ml.)–acetic acid (21 ml.) at 40°. After keeping this mixture at 40° for a further 0·5 hr., methanol (1·0 ml.) was added and the mixture poured into water (approx. 150 ml.). The aqueous phase was subjected to procedure *A*, followed by hydrolysis according to procedure *C*. This yielded a brown gum which crystallized from ethyl acetate to give short rod-like crystals, m.p. 174–176° (50 mg.), recrystallization of which (ethyl acetate) gave 3α:7α:12α-trihydroxy-25-D-coprostanic acid, m.p. 180–182°; $[\alpha]_D+27·4\pm2°$ in ethanol (*c*, 0·7). (Found: C, 71·7, H, 10·2. Calc. for $C_{27}H_{46}O_5$: C, 72·0; H, 10·3%.) The infrared spectrum of this acid was identical with that of the natural acid isolated from alligator bile (Haslewood, 1952*b*).

3α:7α:12α-*Trihydroxy*-25-L-*coprostanic acid*. The trihydroxy-25-L-homocoprostanic acid (110 mg.) was esterified with diazomethane and the resulting ester in benzene (3 ml.) was added to a Grignard complex prepared from Mg (100 mg.) and bromobenzene (0·5 ml.) in ether (10 ml.). After 1 hr. refluxing on a water bath the mixture was left overnight, after which time it was treated with 2N-H_2SO_4 (approx. 2 ml.) and subjected to procedure *A*. The crude (neutral) carbinol was refluxed with ethanolic KOH (4 ml.) and re-extracted by procedure *A*. The resulting carbinol was

refluxed with acetic acid (3 ml.) and acetic anhydride (1·5 ml.) for 2 hr., after which the product was again extracted by procedure *A* to yield an oil (150 mg.). This oil in benzene was chromatographed on Al_2O_3 (3 g.), when the diphenyl was eluted with benzene. Ether eluted an oil (60 mg.) which was dissolved in $CHCl_3$ (1·0 ml.) with acetic acid (2·0 ml.) and treated with CrO_3 (40 mg.) in water (0·4 ml.)–acetic acid (1·5 ml.) for 0·75 hr. in a water bath maintained at 50°. After cooling and the addition of methanol (0·5 ml.) and water (5 ml.), the product was isolated by procedure *A* and hydrolysed by refluxing (0·5 hr.) with ethanolic KOH (3 ml.). This hydrolysate was filtered, the filtrate acidified with aqueous HCl and the precipitated acid removed by filtration. After drying, the acid was recrystallized twice from ethyl acetate to yield crystals (7 mg.) of 3α:7α:12α-trihydroxy-25-L-coprostanic acid, m.p. 194–196°, mixed m.p. with the Japanese 'β' acid (above) 194–196°.

SUMMARY

1. The Kolbe electrolytic cross-coupling synthesis has been employed for the synthesis of homocholic, bishomocholic, 3α:7α:12α-trihydroxy-25-L-coprostanic and the two (25-L and 25-D)-3α:7α:12α-trihydroxyhomocoprostanic acids, the latter two compounds being thus related to L- and D-β-methylglutaric acids and hence to L- and D-glyceraldehyde.

2. The two (25-L and 25-D)-3α:7α:12α-trihydroxyhomocoprostanic acids were degraded by the Wieland–Barbier method into the two corresponding (25-L and 25-D)-3α:7α:12α-trihydroxycoprostanic acids. These latter two acids were identical with the two naturally occurring 3α:7α:12α-trihydroxycoprostan-26-oic acids (Japanese 'α-' and 'β-' trihydroxycoprostanic acids respectively), which have been isolated from the biles of various species.

3. The formation of a new asymmetric centre by the oxidation *in vivo* of a methyl group in cholesterol to give bile alcohols or bile acids may be considered to be non-specific, in the sense that both optical isomers are obtained.

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The Isolation of Cytidine Diphosphate Glycerol, Cytidine Diphosphate Ribitol and Mannitol 1-Phosphate from *Lactobacillus arabinosus*

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It was shown earlier (Baddiley & Mathias, 1954) that hot aqueous ethanolic extracts of *Lactobacillus arabinosus* contain, in addition to many of the known nucleotide coenzymes, two hitherto undescribed cytosine nucleotides. When the work was started the only known natural cytosine nucleotides were those obtained by chemical or enzymic hydrolysis of nucleic acids. More recently the presence of cytidine 5′-mono-, -di- and -tri-phosphates has been demonstrated in various animal tissue extracts (Hurlbert, Schmitz, Brumm & Potter, 1954). The natural occurrence of these simple cytosine nucleotides suggests that derivatives of cytidine 5′-pyrophosphate with coenzyme function might also be found. This is supported by the recent discovery that cytidine