PREPARATION OF PREGNANE DERIVATIVES FROM BILE ACIDS

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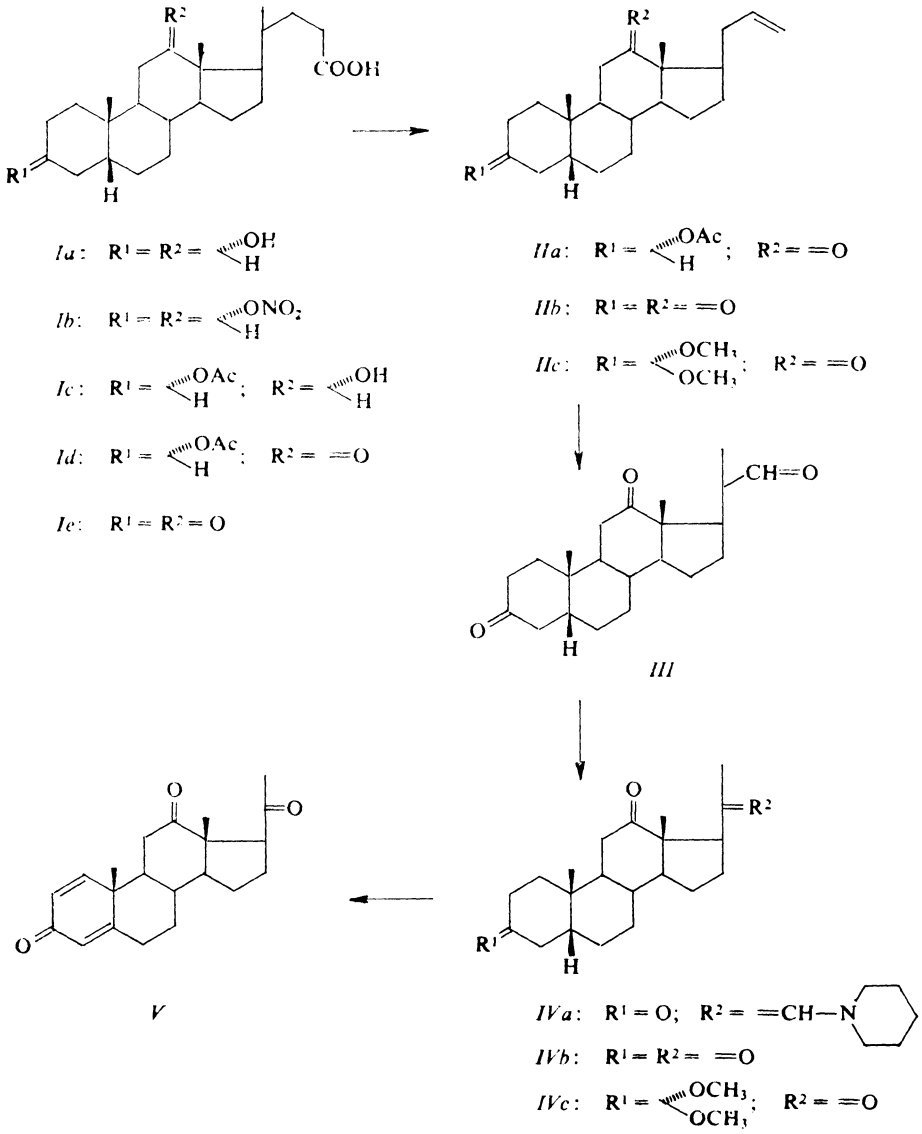
The paper describes decarboxylation of derivatives of 3α,12α-dihydroxy-5β-cholanic acid to 24-norchol-22-ene, and conversion of this key intermediate to pregnane derivatives.

As part of our search for raw materials for the production of steroid hormones we also focussed our attention on the bile acids. We chose decarboxylation of the free acids with lead tetraacetate in the presence of pyridine and cupric salts, which produces a hydrocarbon one carbon shorter and having a terminal double bond; the method had been worked out for decarboxylation of aliphatic acids and used in the chemistry of steroids and terpenes[1-4].

The starting materials were 3α,12α-dihydroxy-5β-cholanic acid (*Ia*) and some of its derivatives, the desired products being the corresponding 24-norchol-22-enes. The acid *Ia* was converted into its 3α,12α-dinitrooxy derivative *Ib* (ref.[5]), 3α-acetoxy ester *Ic* (Jenloz's method[6] for acetylation of ethiocholenic acid), 3α-acetoxy-12-one *Id* and 3,12-dione *Ie*. The derivatives *Ia* and *Ic*, containing a free hydroxy group, gave no norcholene at all, the derivative *Ib* gave it in a very poor yield (detection by TLC). Good starting compounds proved to be the 3α-acetoxy-12-one *Id*, and especially the 3,12-dione *Ie*, whose decarboxylation afforded 24-norchol-22-enes *IIa* (yield 48%) and *IIb* (73–85%), respectively.

24-Norchol-22-ene-3,12-dione (*IIb*) was used for further degradation in the sequence of reactions that had been applied to stigmasterol and ergosterol. Ozonization of the terminal double bond in *IIb* gave 22-oxo-23,24-bisnorcholane-3,12-dione (*III*, yield 76%), which was converted by piperidine to enamine *IVa*, which was directly oxidized by sodium chromate in acetic acid[7] to pregnane-3,12,20-trione (*IVb*) in a yield of 50%. Use of dimethyl ketal *IIc* for this sequence of reactions proved impossible since *IIc* was unstable even under the conditions of the ozonization.

The trione *IVb*, in the form of its dimethyl ketal *IVc*, was dehydrogenated by selenium dioxide under the conditions described for dehydrogenation of the 12-oxo derivatives, such as hecogenine[8]. However, the only product to be isolated was pregna-1,4-diene-3,12,20-trione (*V*). Not even prolonged reaction time and higher doses of selenium dioxide led to a product with the Δ^{11}-12-oxo grouping. Dehydrogenation of the free trione *IVb* gave the same result.



EXPERIMENTAL

The melting points were determined on the Kofler stage. The optical rotations were measured in chloroform with an accuracy of $\pm 3°$. The analytical samples were dried over phosphorus pentoxide at room temperature for 16 h. The ultraviolet spectra were measured in methanol, using a spectrophotometer Zeiss VSU-1. The ^{1}H NMR spectra were measured in deuteriochloroform with a spectrometer Tesla BS 487 C (80 Hz), the internal standard being tetramethylsilane. The chemical shifts are given in ppm. Thin-layer chromatography was carried out on Silufol

UV 254 (Kavalier, Votice) in benzene containing dioxan (10%) and n-butyl acetate (2%). The spots were detected with concentrated sulphuric acid and by heating to *c.* 100°C. Column chromatography ran on silica gel Silpearl Kavalier (Votice). Gas chromatography was carried out in an apparatus Labora Chrom III IKZ. Identity of products was confirmed by mixed melting points, TLC, gas chromatography and ^{1}H NMR spectra.

3α,12α-Dinitrooxycholanic Acid (*Ib*)

Three g of 2α,12α-dihydroxycholanic acid (*Ia*) was added in the course of 2 min to a solution of 30 ml of 70% nitric acid in 120 ml of acetanhydride, cooled to −5°C. After 30 min the mixture was poured into 800 ml of ice-cold water under stirring. Then precipitate was collected on a filter and washed with water until it was neutral; yield 3·3 g (92·6%) of *Ib* dinitrate, m.p. 195−199°C (decomposition), $[\alpha]_D^{21} = +95{\cdot}3°$ (*c* 1·2), a single product by TLC. Reported[6] m.p. 199°C, $[\alpha]_D^{25} = +96{\cdot}1°$ ($CHCl_3$).

3α-Acetoxy-12α-hydroxycholanic Acid (*Ic*)

Ten g of *Ia* was dissolved in 100 ml of pyridine, 10 ml of acetanhydride was added and the mixture was allowed to react for 15 h at room temperature. Then 4 ml of water and 5 ml of acetic acid were added and the mixture was heated to 100°C for 60 min, cooled and distilled to remove the solvents. The oily residue was dissolved in 300 ml of ether and the solution was shaken with 5% hydrochloric acid, pre-cooled to 0°C. At this temperature the acid was transferred by 5% sodium carbonate (100 ml) to the aqueous phase, which was then brought to pH 2 with hydrochloric acid and shaken with 400 ml of ether. The combined organic layers were washed with water, dried ($MgSO_4$) and taken to dryness. The residue was a single product (TLC). The analytical sample was obtained by crystallization from ether–hexane: m.p. 151−162°C (80−100°C after recrystallization); $[\alpha]_D = +51{\cdot}3°$ (*c* 1·9); ^{1}H NMR spectrum: 0·64 (3 H, s, 18-H), 0·90 (3 H, s, 19-H), 4·60 (1 H, bm, 12 β-H), 5·31 (1 H, bs, 12-OH).

3α-Acetoxy-12-oxocholanic Acid (*Id*)

To a solution of 13 g of *Ic* in 130 ml of acetone was gradually added, during 90 min, 16 ml of the Kiliani agent[9] under stirring at room temperature. After another 30 min the mixture was poured into 1 300 ml of ice-cold water. The precipitate was collected on a filter and washed with water until the water was neutral; yield 10 g, of *Id* m.p. 179−183°C, a single compound by TLC. The analytical sample was obtained by recrystallization (ether–hexane): m.p. 180 to 185°C, $[\alpha]_D^{23} = +107{\cdot}9°$ (*c* 1·02); ^{1}H NMR spectrum: 0·89 (3 H, d, 21-H), 0·98 (6 H, s, 18-H, 19-H), 1·95 (3 H, (3 H, s, CH_3COO—), 4·60 (1 H, bm, 3β-H), 9·60 (1 H, bs, —COOH). Reported[10] m.p. 199−200°C, $[\alpha]_D^{20} = +102 \pm 2$ (dioxane).

3,12-*Dioxocholanic acid* (Ie): a suspension of 10 g of *Ia* in 100 ml of acetone was oxidized with 40 ml of the Kiliani agent as in the case of *Id* (the acid completely dissolved in the course of the reaction); yield 10 g of the dioxo acid *Ie* (a single compound by TLC), m.p. 179−181°C; $[\alpha]_D^{23} = 94{\cdot}8°$ (dioxan, *c* 0·6). Reported[11] m.p. 180°C, $[\alpha]_D = 94 \pm 2°$ (dioxan, *c* 2·5).

3α-Acetoxy-24-norchol-22-en-12-one (*IIa*)

A solution of *Id* (4·3 g, 0·01 mol) in benzene (300 ml) was dried by distilling off azeotropically 50 ml of the solvent. After cooling, lead tetraacetate (6·4 g, 0·0145 mol), cupric acetate (0·4 g, 0·002 mol) and pyridine (3·2 ml, 0·004 mol) were added. The stirred mixture was heated to 90°C for 30 min, then to 110°C for 90 min. After cooling, the insoluble portion was removed by filtration, the filtrate was washed with water till it was neutral, then it was dried with magnesium

sulphate and taken to dryness. The residue was purified by chromatography on silica gel (120 g). Elution with benzene–dichloromethane (2 : 1) gave the norcholene *IIa* (1·85 g, 47·9%), practically pure by TLC. The analytical sample was obtained by crystallization from methanol: m.p. 117 to 121°C, $[\alpha]_D^{21} = +117{\cdot}4°$ (*c* 1·2). ^{1}H NMR spectrum: 0·92 (3 H, d, 21-H), 0·98 (6 H, s, 18-H, 19-H), 1·98 (3 H, s, CH_3CO—), 4·60 (1 H, bm, 3 β-H) 4·88 (1 H, dd, 23-*cis* H), 5·58 (1 H, m, 22-H), 5·82 (1 H, dd, 23-*trans* H). For $C_{25}H_{38}O_3$ (386·5) calculated: 77·67% C, 9·91% H; found: 77·53% C, 9·80% H.

24-*Norchol*-22-en-3,12-*dione* (IIb): 9·3 g of the acid *Ie* in 90 ml of benzene was mixed with 5·0 ml of pyridine, 40 g of lead tetraacetate and 0·9 g of cupric acetate. The mixture was heated under nitrogen to 90°C for 2 h, to 110°C for another 2 h, cooled and worked up as *IIa*. The residue (7·28 g), 80% of which was the norcholene *IIb* (GLC), was crystallized from aceteone–n-heptane; yield 6·06 g (73%) of *IIb*, m.p. 168—172°C. The analytical sample was obtained by recrystallization from acetone: m.p. 170—172°C, $[\alpha]_D^{24} = +76{\cdot}5°$ (*c* 1·2). ^{1}H NMR spectrum: 0·98 (3 H, d, 21-H), 1·05 (3 H, s, 18-H), 1·10 (3 H, s, 19-H), 4·90 (2 H, m, 23-H), 5·70 (1 H, m, 22-H). For $C_{23}H_{34}O_2$ (342·5) calculated: 80·65% C, 10·01% H; found 79·88% C, 9·99% H. Repeated experiments yielded as much as 85% of the norcholene *IIb*.

3,3-Dimethoxy-24-norchol-22-ene-12-one (*IIc*)

Six g of *IIb* was dissolved in 125 ml of methanol containing 100 mg of *p*-toluenesulphonic acid. The mixture was boiled for 2 h, cooled down, concentrated to a volume of 30 ml and allowed to crystallize; yield 4·8 g (91%) of *IIc*, m.p. 130—137°C, pure by TLC. The analytical sample was obtained by crystallization from methanol with a trace of pyridine: m.p. 139—141·5°C, $[\alpha]_D^{26} = +89{\cdot}6°$ (*c* 2·2). ^{1}H NMR spectrum: 0·95 (3 H, d, 21-H), 1·01 (6 H, s, 18-H, 19-H), 3·10, 3·15 (6 H, s, CH_3O), 4·90 (1 H, dd, 23-H, J = 18·0, 2·0 Hz), 4·85 (1 H, dd, 23-H, J = 9·0, 2·0 Hz), 5·70 (1 H, m, 22-H). For $C_{25}H_{40}O_3$ (376·6) calculated: 77·27% C, 10·38% H; found: 77·58% C, 10·31% H.

22-Oxo-23,24-dinorcholane-3,12-dione (*III*)

Six g of *IIb* was dissolved in 250 ml of dichloromethane containing 1·7 ml of pyridine. The solution was chilled to —75°C and ozonized for 2 h (the total amount of O_3 was 2 mol equivalents). The ozonide was decomposed with 8·2 g of zinc dust and 37 ml of acetic acid. The insoluble portion was removed by filtration. The filtrate was shaken with water, a 3% solution of sodium hydrogen carbonate and water until it was neutral; then it was dried with calcium chloride and taken to dryness; yield 4·6 g of *III* (75%), pure by TLC. The analytical sample was obtained by crystallization from dichloromethane–n-heptane: m.p. 143—147°C, $[\alpha]_D^{24} = +72°$ (*c* 1·2). ^{1}H NMR spectrum: 1·04 (3 H, s, 18-H), 1·10 (3 H, s, 19-H), 1·08 (3 H, d, 21-H), 9·58 (1 H, d, 22-H). For $C_{22}H_{32}O_3$ (344·5) calculated: 76·70% C, 9·36% H; found: 76·68% C, 9·30% H.

5-β-Pregnane-3,12,20-trione (*IVb*)

Fifteen g of the aldehyde *III* was converted into the enamine *IVa* by 15 hours' boiling with 50 ml of piperidine in 150 ml of toluene. No unreacted aldehyde *III* was detected in the reaction mixture by TLC. After cooling to 5+°C the mixture was added during 60 min to a solution of 20 g of sodium dichromate in 100 ml of acetic acid and 70 ml of toluene at 5°C. After stirring for 2 h at 5°C, the excess of the oxidizing agent was removed by adding 20 ml of methanol and the mixture was diluted with 500ml of water. The aqueous layer was shaken with toluene. The toluene extract was combined with the organic layer. The solution was washed with water till it was neutral, dried with magnesium sulphate and taken to dryness. The residue (9·5 g) was crystal-

lized from benzene; yield 7·2 g (50%) of *IVb*, m.p. 188—195°C, pure by TLC. Recrystallization from the same solvent gave the analytical sample: m.p. 198—202°C, $[\alpha]_D^{24} = +182°$ (*c* 1·1). Reported[12] m.p. 201—202°C, $[\alpha]_D^{17} = +182{\cdot}1 \pm 7°$ (acetone, *c* 0·55).

Pregna-1,4-diene-3,12,20-trione (*V*)

a) 2·4 g of *IVb* was converted into its dimethyl ketal by boiling with 2 mg of *p*-toluenesulphonic acid and 50 ml of methanol. After cooling the mixture was diluted with benzene and washed with 10% sodium carbonate and water till it was neutral. The solution was dried with magnesium sulphate and the solvent was removed by distillation. The residue (3·0 g, a single compound by TLC) was dissolved in 210 ml of t-butanol and 5 ml of pyridine. After the addition of 2·0 g of selenium dioxide the stirred mixture was boiled under an inert atmosphere for 36 h and cooled down. The insoluble portion was removed by filtration and the filtrate was taken to dryness. The residue was dissolved in 20 ml of acetic acid and 2 ml of a solution of chromium trioxide (25 g of CrO_3 in 100 ml of water) was added. The mixture was stirred for 4 h at room temperature and poured into 500 ml of water. The precipitate was collected on a filter. The crude *V* (2 g) was purified by chromatography on silica gel (60 g), with benzene–dichloromethane (2 : 1) as eluant; yield 1·02 g of pure *V* (43%), m.p. 177—180°C, $[\alpha]_D^{24} = +208°$ (*c* 1·0). UV spectrum: $\lambda_{max} = 244$ nm (log = 4·163). ^{1}H NMR spectrum: 1·01 (3 H, s, 18-H), 1·30 (3 H, s,. 19-H), 2·20 (3 H, s, CH_3CO—), 6·08 (1 H, bs, 4-H), 6·21 (1 H, med, 2-H), 6·80 (1 H, d, 1-H) For $C_{21}H_{26}O_3$ (326·4) calculated: 77·27% C, 8·03% H; found 77·12% C, 7·95% H.

b) 3 g of *IVb* in 150 ml of t-butanol was dehydrogenated by boiling with 3 g of selenium dioxide under nitrogen for 48 h. The mixture was worked up as described under *a*); yield 0·89 g of *V* (29·7%), identical with that prepared by procedure *a*).

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