The Preparation of 3α,12α-Dihydroxy-25-methylcoprostane Bile Acids and Steroids 56

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The compound 3α,12α-dihydroxy-25-methyl-coprostan, which does not seem to have been described in literature before, was needed for metabolic studies. We have prepared it through anodic (Kolbe) synthesis from desoxycholic acid and *t*-butylacetic acid [1]. In order to promote mixed coupling, a 20 to 22 fold excess of *t*-butylacetic acid has been used resulting in a 17 % yield of the compound calculated on the basis of the bile acid used.

The pure substance was isolated from the neutral product of the electrolysis through chromatography on silicic acid. Reversed phase partition chromatography on hydrophobic Supercel according to the method of Danielsson [2] has also been used.

The molecular rotation of our compound has been found to be the same as that of coprostan-3α,12α-diol [3].

Experimental. All melting points were taken on electrically heated aluminium block and are corrected. Desoxycholic acid, crystallised from ethanol-water, was used without further drying.

A mixture of 504 mg of desoxycholic acid, 3 ml (23 mmole) of *t*-butylacetic acid [1] and 56 mg (2.42 mmole) of sodium was dissolved in 50 ml of methanol. This solution was electrolysed using the procedure reported before. Temperature of the electrolyte 25°–40°.

The solution was then taken to dryness in vacuum, water (40 ml) was added and it was extracted with ether (5 × 40 ml). The ethereal solution was washed with 10 % sodium hydroxide solution, water, 1 N hydrochloric acid and then water and then evaporated to dryness.

Adsorption chromatography on silicic acid. The residue (500 mg) was chromatographed on a column (i.d. of tube 24 mm) made with a mixture of silicic acid (16 g) (Silicic Acid powder, Baker-analysed-reagent; dried at 120° for 30 h) and Hyflo Supercel (8 g). Effluent-fractions 50 ml.

Fraction	*Eluent*	*Eluate*
1	methylenechloride	2 mg oil
2—5	Methylenechloride/ methanol (99:1 v/v)	0.5—2 mg oil in each;
6	—»—	74.0 mg, crystallised on addition of methanol
7	—»—	165.7 mg, crystallised on addition of methanol
8, 9, 10	—»—	20.2, 9.7 and 6 mg, respectively, oil, did not crystallise as above.

Several crystallisations from methanol and from acetone yielded 68 mg of white needles, m.p. 131.3°—132.3° from fractions 6 and 7.

Reversed phase partition chromatography. The combined mother liquors were taken to dryness and then crystallised once from methanol. The crystals (76 mg) were subjected to reversed phase partition chromatography [3] on hydrophobic Supercel (13.5 g) with 55 % *iso*propanol in water as moving phase and chloroform/heptane (1:4) (12 ml) as stationary phase. Fractions of about 3 ml were collected and the solvent was removed from them in a vacuum oven at about 55°.

The main band of crystalline material appeared in the fractions corresponding to 120—142 ml effluent. After two crystallisations of the material from these fractions from methanol 14 mg of white needles, m.p. 131.3°—132.3°, were obtained from them.

Total yield 82 mg (17 % calculated on the amount of bile acid used). Further crystallisations did not raise the melting point. (Found: C 80.2; H 12.1. Calc. for $C_{28}H_{50}O_2$: C 80.4; H 12.0).

$[\alpha]_D^{20} = +43.7° \pm 0.4°$ (in chloroform, $c = 0.0230$ g/ml solution). $M_D = +182$.

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