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Preparation of 3β-Hydroxychol-5-enic Acid from Hyodesoxycholic Acid and Corresponding 24-^{14}C-labelled Acids. Bile Acids and Steroids 24

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The preparation of 3β-hydroxychol-5-enic acid-24-^{14}C *via* the dimethanesulphonylderivative of hyodesoxycholic acid-24-^{14}C is described. The preparation of the latter compound is also described.

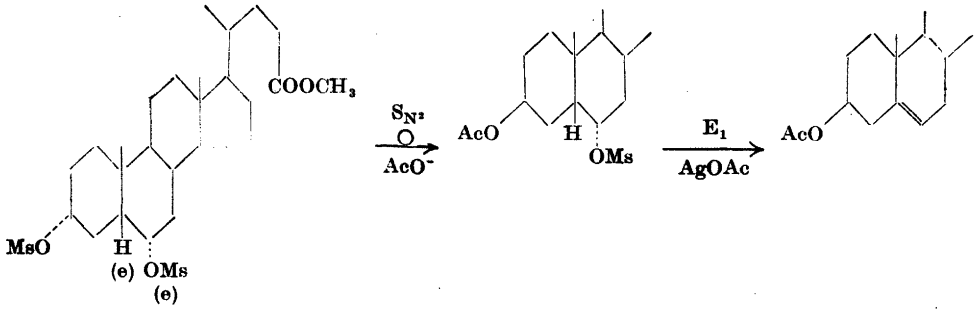
In connection with metabolic studies we needed some carbon labelled 3β-hydroxychol-5-enic acid. As the silver salt-bromine degradation [1] of the acetylated dibromide of this acid yielded mostly secondary products, the convenient nitrile-synthesis with labelled cyanide could not be utilized and other methods had to be sought. Methods for the preparation of 3β-hydroxychol-5-enic acid from hyodesoxycholic acid have recently been described. Yamataki and Ushizawa [2] treated methyl hyodesoxycholate with phosphorus oxychloride in pyridine and replaced the halogen in the 3β-chlorochol-5-enate so obtained with acetate. Shimitzu [3] transformed hyodesoxycholic acid into 3β,6β-dihydroxy *allo*cholanic acid according to Windaus [4] and the 6β-hydroxyl was then eliminated after protection of the 3β-hydroxyl through succinoylation.

We have worked out another procedure for this conversion based on earlier work by Lardon [5]. He found that a 3β,6β-dimethansulphonoxyandrostane derivative by treatment with silver acetate in boiling acetic acid yielded the corresponding 3β-acetoxyandrost-5-ene derivative, indicating an elimination reaction followed by acetolysis with retention of the configuration at C_3. However, the isomeric 3α,6β-dimethansulphonoxycoprostane derivative yielded under the same conditions the same 3β-acetoxycoprost-5-ene derivative, *i. e.* an indication that in this case the first reaction is an $S_{N'}$ reaction at C_3 with inversion followed by an elimination. Shoppee [6] has suggested that these observations indicate that the 6-methansulphonyl group in both these compounds are β-orientated. The *trans*-configuration would explain the more facile elimination in the former case, *cf.* also [7].

When we applied this method [5] to hyodesoxycholic acid (3α,6α-dihydroxycholanic acid), *i. e.* treated the dimesylate of methyl hyodesoxycholate with

silver acetate in boiling acetic acid, an acceptable yield (27 %) of 3β-acetoxy-cholen-5-ate could be isolated by chromatography of the reaction product. A considerable amount of a product with conjugated double bonds ($\varepsilon_{234} \sim 15\,000$) was also obtained but not characterized.

Concerning the mechanism of this reaction the conformation of A and B rings has to be considered. The occurrence of inversion of configuration at C_3 indicates that a normal S_{N^2} reaction with inversion has taken place before the elimination. Had the 5:6 double bond been formed first, a retention of configuration should have been expected. The reason for the less facile elimination of the 5-hydrogen and the 6-methane-sulphonoxy group in our case than in



Lardon's androstane derivative where the groups are presumable also *trans* might be that both groups are equatorial in hyodesoxycholic acid in relation to ring B whereas in the latter case they are both axial. The formation of products absorbing at 234 mμ indicates, that a double elimination also is taking place presumably with the formation of cholan-3,5-dienic acid.

The preparation of carboxy labelled hyodesoxycholic acid with the method of Bergström, Rottenberg and Voltz [1] is also described in the experimental part. From this material 3β-hydroxy-chol-5-enic acid-24-^{14}C has been prepared with the method outlined above.

EXPERIMENTAL

3α,6α-Diacetoxy norcholanylbromide. A mixture of hyodesoxycholic acid (10 g), pyridine (25 ml) and acetic anhydride (30 ml) was heated on the boiling water bath for one hour, then water (5 ml) was added slowly with cooling and the solution was then very slowly poured into about one litre of vigorously stirred ice water containing some ice. The acetylated acid then separated in a crystalline condition. The product was filtered and washed with cold water containing a little acetic acid until free from pyridine. After drying at room temperature the product melted at 95–98° C.

This product (10.5 g) was dissolved in 30 ml of ethanol and exactly neutralized with sodium hydroxide (phenolphthalein). A solution of silver nitrite (246 ml; 0.1 *M*) was then added slowly with shaking and the precipitated silver salt was isolated by filtration and washed with hot water. It was thoroughly dried over phosphorus pentoxide *in vacuo*. Yield 12.1 g.

Decarboxylation: About 200 ml of ethyl bromide was distilled from P_2O_5 onto a mixture of the finely prowdered silver salt (12.1 g) and silver acetate (3.5 g) in a system protected from the atmospheric moisture. Dry bromine (2.6 ml) was then slowly added to the refluxing suspension. The neutral reaction product was then isolated as described earlier. Yield 8.6 g.

The crude product was then chromatographed on alumina (50 g) in benzene-light petroleum (40°–60°) 4/6 (v/v). The material in the first 300 ml of eluate was combined and crystallized twice from light petroleum. Yield 3.2 g, M. p. 141–42° unchanged on further recrystallizations. (Found: C 63.41; H 8.36; Br 15.1. $C_{27}H_{43}O_4$ requires C 63.3; H 8.47; Br 15.6.)

Hyodesoxycholic acid-24-^{14}C. A mixture of 3α,6α-diacetoxy*nor*cholanylbromide (200 mg), $K^{14}CN$ (9.5 mg; 1 mC) and KOH (44.5 mg) in 5 ml of 80 % (v/v) ethanol/water was kept in a sealed tube in a water bath at 95° for 48 hours. The contents of the tube were then directly hydrolyzed in a steel tube at 140° for 7 hours after addition of 25 ml of 10 % (v/v) potassium hydroxide in 80 % ethanol. After evaporation of the ethanol the reaction mixture was acidified and extracted with ether. 68 mg of acidic products was extracted from the ether phase with dilute sodium hydroxide. An ether solution of the acidic products was treated with diazomethane and chromatographed on a column prepared from silicic acid (2 g) and Super-Cel (1 g) in methylene chloride. Methyl hyodesoxycholate was eluted with methylene chloride containing 2 % of methanol. Yield 40 mg, M. p. 75°.

Methyl 3α,6α-dimethanesulfonoxycholanate. Methyl hyodesoxycholate-24-^{14}C was dissolved in dry pyridine (1 ml) and methanesulpnonylchloride (0.15 ml) was added at 0°. The mixture was left at room temperature for 24 hours. The product was extracted with ether after dilution with water at 0° and the ether solution was washed successively with hydrochloric acid, water, carbonate and water. The dry product was a yellow amorphous foam (92 mg). (Found: S 11.0. Calc. for $C_{27}H_{46}O_8S_2$: S 11.4.)

Methyl 3β-acetoxychol-5-enate. A mixture of the dimethanesulfonylderivative of methyl hyodesoxycholanate (100 mg) and silver acetate (100 mg) was refluxed 2 hours in glacial acetic acid (3 ml). The mixture was evaporated to dryness *in vacuo* and the residue extracted with ether. This solution was washed with aqueous carbonate and water, dried over sodium sulphate. After evaporation to dryness, the residue (72 mg) was chromatographed on alumina (5 g). Each fraction = 30 ml.

1	Light petroleum/benzene	4:1	31.9 mg	oil, $\varepsilon_{234} \simeq 15\,000$ (M = 370)
2			62.4 »	
3			1.9 »	
4	»	2:1	18.0 »	crystalline, m. p. ~ 150°
5			17.5 »	
6	Benzene		16.5 »	
7			7.5 »	
8			2.3 »	
9	Benzene + 2 % MeOH		7.0 »	oil, S-containing
10			0.3 »	

The crystalline fractions (4–8) were combined and crystallized from methylene chloride-methanol. M. p. 154–55°, unchanged on further crystallization. (Found: C 75.0; H 9.8. Calc. for $C_{27}H_{42}O_4$: C 75.4; H 9.8.) The m. p. was not depressed by admixture of authentic methyl 3β-acetoxychol-5-enate (m. p. 155°). (Obtained from *Ciba Ltd*, Basel, through the courtesy of Dr. Wettstein.) The IR-spectrum and powder diffraction patterns of these preparations were also found to be identical. We are grateful to Prof. Stenhagen and Dr. Skogh at Uppsala University for these determinations.

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