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The Preparation of Some Carboxylabelled Bile Acids

Bile Acids and Steroids 2

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Lithocholic acid labelled with deuterium in position 3, 4 and 5 has been prepared by Pearlman *et al.*[1] and labelled cholic acid has been prepared biosynthetically by administering tritium water [2] or ^{14}C-labelled acetate [3] to rats and isolating cholic acid from the bile.

We have now prepared cholic, desoxycholic, chenodesoxycholic, lithocholic and cholanic acid labelled in the carboxylgroup with ^{14}C or ^{13}C for use in metabolic studies in progress in this institute.

They have all been prepared by bromine degradation of the silver salt of the acetylated acids, using the modified conditions described by Rottenberg [4]. The properties of the resulting norbromides are listed in Tables 1 and 2. Of these bromides only the one corresponding to desoxycholic acid has been prepared earlier by Brinck, Clark and Wallis [5]. The optical data in Table 2 show that the differences between the molecular rotation (M_D) of an acetylated norbromide and the corresponding acid is reasonably constant, varying between + 60° and + 82°.

The bromides have then been reconverted into the corresponding acids *via* a nitrile synthesis with labelled potassium cyanide prepared according to Belleau and Heard [6]. The yields calculated on the labelled cyanide have been 80—90 per cent.

The yield from the amorphous triacetoxynorbromide corresponding to cholic acid is as good as from the other crystalline bromides. An earlier report [4] that this bromide failed to yield a nitrile has been found to be a mistake.

From the labelled acids described here, the different, partially oxidized acids are easily available by existing methods (*cf.* esp. Fieser *et al.*[7]).

EXPERIMENTAL

23-Bromo-norcholane. 11.4 G of silver cholanate and 6.7 g of silver acetate were dried, finely powdered and treated with 3.4 ml of bromine in boiling ethyl bromide and worked up as described earlier [4]. The neutral product obtained weighed 9.3 g, *i. e.* 96.5 per cent crude yield. A small amount was sublimed at 130°/0.1 mm and recrystallized from ethanol, m.p. 93—94°. The main part (7.5 g) in light petroleum was filtered through a column of active alumina (30 g). Exhaustive extraction with petrol ether gave 6.9 g of bright yellow, wax-like material. A specimen, after two recrystallizations from acetone, melted at 93—94°. Analysis: see Table 1.

Table 1.

			M. p.	% C		% H		% Br	
				Calc.	Found	Calc.	Found	Calc.	Found
I	23-Bromonorcholane	$C_{23}H_{39}Br$	93—94	69.85	69.3	9.95	9.83	20.2	19.4
II	3(α)Acetoxy »	$C_{25}H_{41}O_2Br$	186—186.5	66.21	66.0	9.11	9.14	17.62	17.5
III	3(α)7(α)Diacetoxycholane	$C_{27}H_{43}O_4Br$	183—185	63.39	63.0	8.47	8.35	15.62	15.9
IV	3(α)12(α)Diacetoxycholane	$C_{27}H_{43}O_4Br$	133—134	63.39	63.2	8.47	8.30	15.62	15.4
V	3(α)7(α)12(α)Triacetoxycholane	$C_{29}H_{45}O_6Br$	amorph.						

Lithocholic acid. Lithocholic acid was prepared from desoxycholic acid according to Bergström and Haslewood [8] in 20 g batches in a steel bomb.

3(α)-acetoxycholanic acid. Lithocholic acid (m.p. 181—83°) was dissolved in 2 parts acetic anhydride and 1 part of pyridine and left on the steam bath for 3 hours. After cooling, water was slowly added with stirring when the acetylated acid crystallized. It was filtered off and washed thoroughly with dilute acetic acid, dried and recrystallized from aqueous acetone. Yield: 90 per cent of 3(α)-acetoxycholanic acid, m.p. 166—68°. (*Cf.* Reindel and Niederländer [9]).

23-Bromo-3(α)-acetoxy-norcholane. 6.4 G of the acetylated acid was dissolved in 100 ml ethanol and neutralized with sodium hydroxide (phenolphthalein). This solution was poured into a solution of 4 g silver nitrate and the silver salt was filtered off and washed with aqueous ethanol. The precipitate was dried overnight in a vacuum oven at 60°, pulverized and dried over phosphorus pentoxide *in vacuo*.

To 7.7 g of dry silver lithocholate was added 5.6 g of dry silver acetate and 200 ml of ethyl bromide (purified and freshly distilled over P_2O_5). To the boiling mixture (b.p. 38°) 2.6 ml of dry bromine was added, during 11 minutes, and boiling was continued for another 4 minutes, whereafter the mixture was allowed to cool.

The precipitated silver bromide was filtered off and washed with chloroform. The combined filtrates were washed successively with dilute aqueous solutions of potassium iodide and sodium thiosulphate and evaporated to dryness. The brownish crystalline product (7.3 g) was dissolved in 50 ml benzene and 25 ml light petroleum and filtered through a column of 50 g neutral alumina (activated 10 minutes at 180°).

The main fraction of colourless crystals weighed 4.0 g; m.p. ~180°. Recrystallizations from benzene-light petroleum gave an analytical sample, m.p. 185—88°. Analysis: see Table 1.

23-Bromo-3(α)-hydroxynorcholane was prepared by refluxing 500 mg of the acetate for 2 hours in 20 ml absolute ethanol containing 2.4 millimoles of hydrogen chloride. On

Table 2.

	Acetylated norbromide		Corresponding acetylated acid		M_D (acetyl.norbromide) − M_D (acetyl. acid)
	$[\alpha]_D$*	M_D	$[\alpha]_D$	M_D	
I	+35	+144	+18	+ 64	+80
II	+55	+250	+40	+168	+82
III	+26	+133	+15	+ 72	+61
IV	+97	+498	+92	+438	+60

* All rotations have been determined in chloroform ($c = 1$) at 24−25° in 1 dm micro tube.

careful addition of water the product crystallizes in long needles melting at 130−31°, unchanged on further recrystallization. (Found: Br 19.8. Calc. for $C_{23}H_{39}OBr$: Br 19.4.)

3(α)-hydroxycholanonitrile. 226 mg of the hydroxy bromide was refluxed with 223 mg potassium cyanide in 35 ml of absolute ethanol and 3 ml of water for 48 hours. The solution was then concentrated and the crystalline nitrile (194 mg) isolated. M.p. after recrystallization from aqueous ethanol 193−95°. (Found: C 80.7; H 10.9; N 3.9. Calc. for $C_{24}H_{39}ON$: C 80.61; H 10.99; N 3.91.)

Lithocholic acid. 130 mg of the nitrile was hydrolyzed in a mixture of 25 ml ethanol and 3 ml 30 per cent aqueous potassium hydroxide for 48 hours. The acid fraction weighed 108 mg. Crystallization from aqueous acetone yielded lithocholic acid, m.p. 180−81°.

In the synthesis with labelled potassium cyanide the same conditions were used except that about two moles of bromide was used per mole of cyanide. After refluxing for 48 hours the potassium hydroxide was added directly and the refluxing was continued for a further 48 hours. The overall yield of acid calculated on the labelled cyanide was 80−90 per cent.

Desoxycholic, chenodesoxycholic and cholic acid. These acids were degraded by essentially the same methods. The following simple procedure was found to yield crystalline acetylated acids from all three acids.

The acids were acetylated in an excess of acetic anhydride and pyridine by heating on the steam bath for 3 hours. After cooling, the reaction mixture was very slowly poured into ice cold dilute acetic acid with vigorous stirring when a crystalline precipitate of the acetoxy acids generally formed directly. The acid was filtered off, washed with water and dried *in vacuo* at 50°.

The silver salt was prepared and degraded as mentioned above. The acetylated norbromide was purified by recrystallization after filtering through aluminium oxide. In the isotopic nitrile syntheses from these bromides the acetylated bromide was used directly, as acid catalyzed deacetylation under the conditions used on the acetylated bromide from lithocholic acid apparently caused some dehydration.

In these syntheses with labelled cyanide the usual procedure was otherwise followed except that potassium hydroxide was added in an amount corresponding to the acetoxy groups. The same yields were obtained as reported above.

SUMMARY

Cholanic, lithocholic, desoxycholic, chenodesoxycholic and cholic acid labelled in the carboxyl groups have been prepared by degradation to corresponding norbromides followed by a nitrile synthesis with carbon labelled cyanide.

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