PREPARATION OF [7,7-$^2H_2$] LITHOCHOLIC ACID

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SUMMARY

Electrochemical deoxygenation of methyl 7-keto-lithocholate using deuterated reagents, followed by base hydrolysis is shown to give [7,7-$^2H_2$] lithocholic acid in 60% overall yield and 86% $^2H_2$ isotopic purity.

Key Words: Electrochemical reduction, deuterium labelling, bile acids, gas chromatography-mass spectrometry (GC/MS).

INTRODUCTION

The combination of deuterated bile acids and GC/MS has an important role both in the quantitation of bile acids (1-3) and the estimation of their pool size in humans (4). While several preparations of deuterated bile acids have been reported (3,5-7) they are generally unsatisfactory either due to the presence of endogenous unlabelled material or the location of label at a potentially enolizable position.

Recent work in the steroid field has shown that saturated 7-keto-steroids may be conveniently deuterated at C7 via electrochemical deoxygenation (8). We have extended this reaction to the readily available methyl 7-keto-lithocholate (methyl 3α-hydroxy-7-oxo-5β-cholanate), and report herein a simple two step preparation of [7,7-$^2H_2$] lithocholic acid.

EXPERIMENTAL

Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. 7-Keto-lithocholic acid was either purchased from Steraloids (Wilton, U.S.A.) or prepared from chenodeoxycholic acid (9). Thin-layer chromatography was carried out on Eastman precoated silica-gel sheets with visualization by conc. H_2SO_4/Vanillin spray and charring at 120°. GC/MS measurements were performed using a Hewlett-Packard 5992B system (column, 2 m x 2 mm i.d., 1.5% OV-101; T = 250°; helium, 25 ml min^{-1}).

Methyl 3α-hydroxy-5β- [7,7-$^2H_2$] -cholanate

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Methyl 7-keto-lithocholate (0.25 g, 0.63 mmol) was reacted at the cathode chamber of an electrochemical cell. The apparatus consisted of a 100 ml round-bottom flask, with a B34 glass stopper through which two holes were bored to accommodate the electrodes. The anode (lead, 2.3 cm by 4.4 cm) was defined by a dialysis bag. The cathode (lead, 2.7 cm x 6.0 cm) was defined by the flask volume. The solvent used was dioxan - 10% $^2H_2SO_4/^2H_2O$ (3:2 v/v). Reactions were conducted at approximately 500 mA and 3-5 V. When TLC ($CHCl_3$) indicated the reaction was complete, the catholyte was removed, neutralized with 10% sodium hydroxyide and extracted with ether. The ethereal layer was washed with water, brine, dried (Na_2SO_4) and then evaporated. The residue was then esterified with diazomethane. Chromatography on Sorbsil (10 g) and elution with ether/n-hexane (1:9, v.v) gave methyl [7,7-$^2H_2$] lithocholate (0.17 g, 70%) m.p. 129-130° (lit. (10) 126-127°), homogeneous by TLC ($CHCl_3$, Rf 0.8) and GC. The isotopic abundance was as shown for the corresponding acid in Table 1.

3α-Hydroxy-5β-[7,7-$^2H_2$]-cholanic acid

A mixture of methyl [7,7-$^2H_2$] lithocholate (0.15 g, 0.38 mmol) and potassium carbonate (0.5 g, 3.6 mmol) in methanol (10 ml) was refluxed overnight (16 h). The mixture was cooled, diluted with water (30 ml), extracted with chloroform (20 ml) and the organic layer discarded. The aqueous layer was then acidified with 10% hydrochloric acid, extracted with chloroform (6 x 15 ml) and the extract washed with water, brine and then dried (anhydrous Na_2SO_4). Removal of the solvent gave [7,7-$^2H_2$] lithocholic acid (0.13 g, 89%), which was recrystallized from methanol to have m.p. 190.5-191.5° (lit. (10) 184-186°). TLC analysis (isoamylacetate: isopropanol: ethyl acetate: acetic acid = 4:2:1:1; Rf = 0.85) showed it to be homogeneous. The isotopic abundance is given in Table 1.

Base-catalysed enolization:- Methyl 7-keto-lithocholate (0.1 g) was dissolved in $CH_3O^2H/^2H_2O$ (5:2, 7 ml) containing dissolved sodium (0.2 g) and heated under reflux for 3 days. The reaction was then cooled, neutralized with dil. hydrochloric acid and extracted with chloroform. The isolated compound (80%) was re-esterified with diazomethane prior to electrochemical reduction.

RESULTS AND DISCUSSION

Preliminary experiments with methyl 7-keto-lithocholate and 7-keto-lithocholic acid showed that both the carbomethoxy and carboxyl groups are not reduced under the conditions required for electrochemical deoxygenation at C7. Partial hydrolysis of the ester however, is noted. In the actual experiments methyl 7-keto-lithocholate rather than the corresponding acid, was used because of its greater solubility in the reaction solvent. Purification of the crude product after electrochemical reduction was more facile for the methyl ester than the corresponding acid, and accordingly the reaction mixture was re-esterified for this reason. Mild hydrolysis with K_2CO_3/MeOH then gave the required [7,7-$^2H_2$]-lithocholic acid.

Base-catalysed enolization of 7-keto-steroids prior to electrochemical reduction has allowed the ultimate synthesis of [6,6,7,7,8-$^2H_5$]-steroids (8). When a similar procedure was adopted with 7-keto-lithocholic acid, $^2H_7$-lithocholic acid was the final product, indicating extensive labelling at position C23 cf Baillie et al. (7). Labelling of the side-chain at C23 is unsatisfactory since this label has the potential to be lost via enolization.

TABLE 1

DEUTERIUM INCORPORATION INTO LITHOCHOLIC ACID

	Mass Fragments[a]	
Mode of Preparation	m/z 372 (I)	m/z 257 (II)
Electrochemical reduction	1.0% ^2H_o, 5.0% $^2H_1$, 86% $^2H_2$, 8% $^2H_3$,	2.0% ^2H_o, 7.0% $^2H_1$, 84% $^2H_2$, 8% $^2H_3$,
Base equilibration (13) + Electrochemical reduction	8.0% $^2H_4$, 40.2% $^2H_5$, 35% $^2H_6$, 16.8% $^2H_7$	17.8% $^2H_4$, 82.2% $^2H_5$,

[a] atoms % excess

The mass spectra (m/z 210-400) for ^2H_o, $^2H_2$ and $^2H_7$-lithocholic acids (Me ester-TMS derivatives) are summarized in Figure 1. The isotopic purities as determined at m/z 372 (M-90) and 257 (M-90-115) are given in Table 1. The structures for these ions may be represented as I and II.

Examination of the data in Table 1 establishes that for [7,7-$^2H_2$]-lithocholic acid, no label is located at position C23. The presence of a small amount of

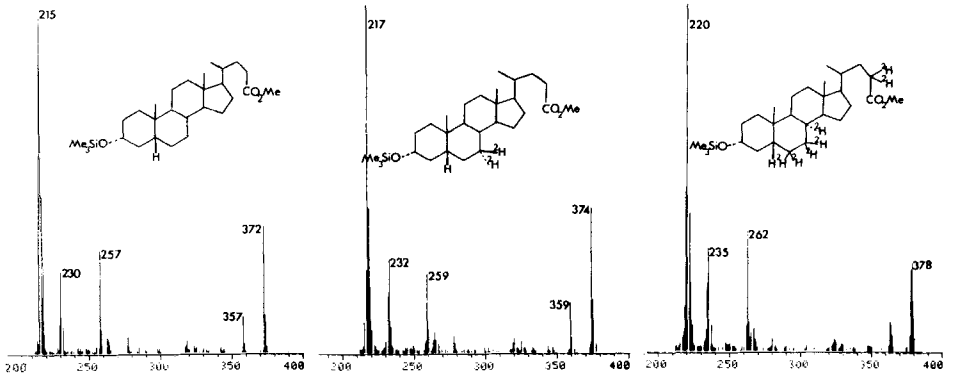
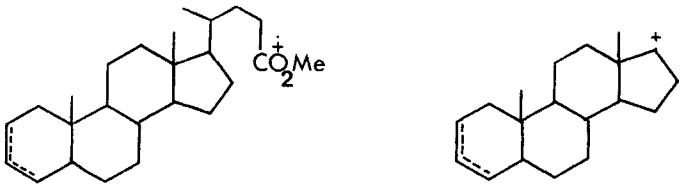


Figure 1: Partial electron-impact mass spectra of $^{2}H_{0}$, $^{2}H_{2}$ and $^{2}H_{7}$ - lithocholic acids as their methyl ester-trimethylsilyl ether derivatives.



$^{2}H_{3}$ may be attributed to exchange adjacent to the ketone prior to deoxygenation. Previously several workers have prepared deuterated bile acids by base-catalysed enolization (3,5,7). Only one group however, has acknowledged the presence of label at C23, and the difficulty in its removal (7). The mass spectra published by Angelin et al. (3) provide strong evidence for labelling at C23 since the deuterium content at the ions monitored (m/z 373, $^{2}H_{3}$-deoxycholic; m/z 373, $^{2}H_{5}$-cholic) is greater than that theoretically possible, based on the known mass fragmentation patterns (11). It is, therefore, apparent that preparation of deuterated bile acids using base-catalysed enolization must also invariably label the side-chain position C23.

Electrochemical reduction would also appear practical for the preparation of [7,7-$^{2}H_{2}$]deoxycholic acid from its respective 7-keto analogue. Extension to other acids, such as chenodeoxycholic, would seem unlikely since it has been reported (12) that the 12-keto group is unreactive towards electrochemical reduction.

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References

1.Miyazaki, H., Ishibashi, M., Inoue, M., Itoh, M. and Kubodera, T. - J. Chrom. 99: 553 (1974)

2.Eyem, J. - Adv. Mass Spectrom. 7:1534 (1978)

3.Angelin, B., Bjorkhem, I., and Einarsson, K. - J. Lipid Res. 19:527 (1978)

4.Hepner, G.W., Sturman, J.A., Hofmann, A.F. and Thomas, P.J. - J. Clin. Invest. 52:433 (1973)

5.Hachey, D.L., Szczepanik, P.A., Berngruber, O.W. and Klein, P.D. - J. Labelled Cpds. 9_:703 (1973)

6.Cowen, A.E., Hofmann, A.F., Hachey, D.L., Thomas, P.J., Belobaba, D.T.E., Klein, P.D. and Tokes, L. - J. Lipid Res. 17:231 (1976)

7.Baillie, T.A., Karls, M. and Sjovall, J. - J. Labelled Cpds. Radiopharm. 14: 849 (1978)

8.Phillipou, G., Seaborn, C.J. and Blair, I.A. - Aust. J. Chem. 32:2767 (1979)

9.Fieser, L.F. and Rajagopalan, S. - J. Amer. Chem. Soc. 71:3935 (1949)

10."Merck Index" 8th Edn., Merck Rahway, 1968, p. 624

11.Elliot, W.H. - In : "Biochemical Applications of Mass Spectrometry" (Waller, G.R. ed.) Wiley Interscience, New York, 1972, p.291

12.Muccino, R.R. and Djerassi, C. - J. Amer. Chem. Soc. 96:556 (1974)

13.Williams, D.H., Bhacca, N.S. and Djerassi, C. - J. Amer. Chem. Soc. 85:2810 (1963)