SYNTHESIS OF HOMOURSODEOXYCHOLIC ACID AND [11,12-^{3}H]HOMOURSODEOXYCHOLIC ACID

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ABSTRACT

Homoursodeoxycholic acid and [11,12-^{3}H]homoursodeoxycholic acid were synthesized from ursodeoxycholic acid and homocholic acid, respectively. Ursodeoxycholic acid (Ia) was converted to 3α,7β-diformoxy-5β-cholan-24-oic acid (Ib) using formic acid. Reaction of the diformoxy derivative (Ib) with thionyl chloride yielded the acid chloride (II) which was treated with diazomethane to produce 3α,7β-diformoxy-25-diazo-25-homo-5β-cholan-24-one (III). Homoursodeoxycholic acid (IV) was formed from the diazoketone (III) by means of the Wolff rearrangement of the Arndt-Eistert synthesis.

N-Bromosuccinimide oxidation of homocholic acid (V), which was prepared from cholic acid by the same procedure described above, afforded 3α,12α-dihydroxy-7-oxo-25-homo-5β-cholan-25-oic acid (VI). Reduction of the 7-ketohomodeoxycholic acid (VI) with sodium in 1-propanol gave 3α,7β,12α-trihydroxy-25-homo-5β-cholan-25-oic acid (VII). The methyl ester of 7-epihomocholic acid (VII) was partially acetylated to give methyl 3α,7β-diacetoxy-12α-hydroxy-25-homo-5β-cholan-25-oate (VIII) using a mixture of acetic anhydride, pyridine and benzene. Dehydration of the diacetoxy derivative (VIII) with phosphorus oxychloride yielded methyl 3α,7β-diacetoxy-25-homo-5β-chol-11-en-25-oate (IX). Reduction of the unsaturated ester (IX) with tritium gas in the presence of platinum oxide catalyst followed by alkaline hydrolysis gave [11,12-^{3}H]homoursodeoxycholic acid.

INTRODUCTION

Chenodeoxycholic acid (CDC) and ursodeoxycholic acid (UDC) have been used as gallstone-dissolving drugs in man (1,2). It was, however, shown that either CDC or UDC is efficiently converted into lithocholic acid (LC) by the action of intestinal microorganisms during their enterohepatic circulation (3-5), and that the resulting monohydroxylated bile acid, LC, is a potential hepatotoxic compound (6,7). Thus, there exists a need to develop more efficacious cholelitholytic drugs with a

reduced potential for hepatotoxity. Therefore, it would be desirable to design bile acid analogs with increased resistance to bacterial degradation, especially 7-dehydroxylation.

Since differential length of the side chain of bile acids has been shown to cause striking changes in their hepatic metabolism (8,9), we postulate that the extent of bacterial degradation of higher or lower homologs of CDC and UDC would be different from that of these C_{24} bile acids themselves. In testing the possibility that the homologs of CDC and UDC are resistant to bacterial 7-dehydroxylation, it was necessary to prepare such compounds with radioisotope labels. The present paper describes the synthesis of the C_{25} homolog of UDC, homoursodeoxycholic acid (HUDC) and [11,12-^{3}H]HUDC.

MATERIALS AND METHODS

General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer as KBr discs. Proton nuclear magnetic resonance (PMR) spectra were measured at 90 MHz on a Hitachi R-40 spectrometer using pyridine-d_5 as the solvent. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane internal standard. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A gas chromatograph using a glass column (2 m x 3 mm) packed with 3% OV-17, 3% QF-1, or 2% Poly I-110 on 80/100 mesh Gas Chrom Q. Preparation of methyl esters and trimethylsilyl (TMS) ethers was carried out as described previously (10). Acetate derivatives of bile acid methyl esters were prepared by the method reported previously (11). Mass spectra of free bile acids were obtained by direct inlet insertion technique with a JEOL Model D-300 mass spectrometer under conditions of ion source temperature, 150° C and ionization voltage, 70 eV. Gas-liquid chromatography-mass spectrometry (GLC-MS) was carried out on a Shimadzu QP-1000 gas chromatograph-mass spectrometer. The following operating conditions were employed: column, SE-30 capillary column (0.25 mm x 25 m); column temperature 270°C; ionization current 300 μA; ionization voltage 70 eV. Thin-layer chromatography (TLC) was carried out on precoated silica gel G plates (250 μm thickness, Merck) with a 10% solution of phosphomolybdic acid in ethanol as the detection reagent. For TLC of labeled compounds, samples were applied to the plate as a streak (1-3 cm wide). Reference spots of known compounds were applied on either site of the streak. Pertinent bands were scraped from the developed plate according to the positions of the reference spots which were made

visible by means of the phosphomolybdate reagent, and transferred to scintillation vials together with scintillation solution to measure radioactivity. In addition, the radioactivity of 5 mm sections between the origin and solvent front was assayed. Radioactivity was measured using a toluene-based scintillation solution in a Packard Tricarb model 3320 liquid scintillation spectrometer. Suitable corrections were made for background and quenching.

Synthesis of homoursodeoxycholic acid (HUDC)

3α,7β-Diformoxy-5β-cholan-24-oic acid (Ib)

Formylation of ursodeoxycholic acid (UDC) (Ia) was carried out by dissolving 30 g of UDC in 60 mL of formic acid and heating the solution at 55°C for 6 hr. The reaction mixture was poured into 5 L of water and the precipitate was collected by filtration. Crystallization from ethanol gave 27.5 g of colorless crystals (Ib) with the following properties: mp 174-175°C; PMR (δ): 0.60 (3H, s, 18-CH_3), 0.85 (3H, s, 19-CH_3), 0.97 (3H, d, J=6 Hz, 21-CH_3), 4.8-5.1 (2H, m, 3β- and 7α-H), 8.23 (1H, s, 3α- or 7β-OOCH), 8.28 (1H, s, 7β- or 3α-OOCH).

3α,7β-Diformoxy-25-diazo-25-homo-5β-cholan-24-one (III)

To 11 g of Ib was added 38 mL of thionyl chloride and the reaction was allowed to proceed at room temperature for 2 hr. The excess of thionyl chloride was completely removed at room temperature *in vacuo* followed by repeated evaporation with benzene and the resulting acid chloride was used without further purification. The acid chloride was dissolved in dry benzene (100 mL) and added dropwise to a freshly prepared ethereal diazomethane solution at 0°C (12). The mixture was allowed to stand at room temperature for 18 hr. Evaporation of the solvents gave an oily residue (12 g). The residue was chromatographed on a silica gel (250 g) column using benzene-ethyl acetate (9:1, by vol) as the eluting solvent to give 3.0 g of III with following properties: amorphous powder; TLC (Rf): 0.56 (solvent system, benzene-ethyl acetate, 4:1); PMR (δ): 0.60 (3H, s, 18-CH_3), 0.86 (3H, s, 19-CH_3), 0.92 (3H, d, J=6 Hz, 21-CH_3), 4.8-5.0 (2H, m, 3β- and 7α-H), 8.27 (1H, s, 3α- or 7β-OOCH), 8.32 (1H, s, 7β- or 3α-OOCH).

Homoursodeoxycholic acid (HUDC) (IV)

A solution containing 21.6 g of III, 48 mL of benzyl alcohol and 48 mL of freshly-distilled collidine was added to a preheated flask (200°C) and the temperature was maintained at 180-200°C for 15 min. The reaction mixture was cooled to room temperature, 200 mL of water was added and the solution was extracted with two 200 mL portions of ether. The ethereal extracts were washed successively with water (200 mL), 0.1 N HCl (200 mL), water (200 mL), 5% $NaHCO_3$ (200 mL), then with water to neutrality. The ether solution was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The resulting gum was dissolved in 50 mL of 10% methanolic KOH and refluxed for 1 hr. The hydrolysate was diluted with 300 mL of water and extracted with ether in order to remove neutral material. After acidification with HCl, the aqueous layer was extracted with ether. Evaporation of the solvent followed by recrystallization from methanol-ethyl acetate gave rods (4.5 g) of IV; mp 174-175°C; PMR (δ): 0.71 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 1.01 (3H, d, J=6

Hz, 21-CH_3), 3.6-4.0 (2H, m, 3β- and 7α-H).

Synthesis of [11,12-^{3}H]homoursodeoxycholic acid

3α,12α-Dihydroxy-7-oxo-25-homo-5β-cholan-25-oic acid (VI)

To a solution of homocholic acid (HC) (V) (2.1 g) dissolved in 100 mL of acetone was added a solution of N-bromosuccinimide (1.3 g) in 20 mL of water at room temperature for 2 hr. The reaction mixture was diluted with 500 mL of water and extracted with ethyl acetate. Evaporation of the solvent followed by recrystallization from acetone gave 1.5 g of colorless crystals of VI: mp 213-214°; PMR (δ): 0.75 (3H, s, 18-CH_3), 1.15 (3H, d, J=6 Hz, 21-CH_3), 1.20 (3H, s, 19-CH_3), 3.80 (1H, m, 3β-H), 4.20 (1H, m, 12β-H).

3α,7β,12α-Trihydroxy-25-homo-5β-cholan-25-oic acid (VII)

To a solution of VI (2.0 g) in 75 mL of dry 1-propanol was added 7.5 g of Na. After refluxing for 3 hr, the reaction mixture was diluted with water (100 mL), acidified with HCl and extracted with ethyl acetate. Evaporation of the solvent gave 1.8 g of an oily residue. The residue was methylated with ethereal diazomethane solution, and purified on a silica gel (50 g) column eluting with increasing amounts of acetone in benzene. Fractions eluted with 30% acetone were concentrated to dryness and the residue was hydrolysed with 5% methanolic KOH. Extraction followed by recrystallization from ethyl acetate gave needles (426 mg) of VII; mp 185.5-186.0°C; PMR (δ): 0.85 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.20 (3H, d, J=6 Hz, 21-CH_3), 3.70-4.00 (2H, m, 3β- and 7α-H), 4.23 (1H, m, 12β-H).

Methyl 3α,7β-diacetoxy-12α-hydroxy-25-homo-5β-cholan-25-oate (VIII)

Six hundred milligrams of VII was methylated by the usual manner with diazomethane. A solution of the methyl ester in a mixture of acetic anhydride (3.0 mL), pyridine (2.0 mL) and benzene (2.0 mL) was allowed to stand at room temperature for 3 hr. The reaction mixture was then poured into water and extracted with ether. The extract was washed with 0.2 N HCl , water, 5% $NaHCO_3$, and water, then evaporated to dryness. The residue (0.97 g) was chromatographed on a column of silica gel (25 g). Elution with a 4:1 mixture of benzene and ethyl acetate followed by recrystallization from methanol-water gave 282 mg of colorless crystals (VIII) with the following properties: mp 124.5-125°C; PMR (δ): 0.85 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 1.15 (3H, d, J=6 Hz, 21-CH_3), 2.01 (3H, s, 3α- or 7β-$OCOCH_3$), 2.06 (3H, s, 7β- or 3α-$OCOCH_3$), 3.65 (3H, s, -$COOCH_3$),4.17 (1H, m, 12β-H), 4.70-5.10 (2H, m, 3β- and 7α-H).

Methyl 3α,7β-diacetoxy-25-homo-5β-chol-11-en-25-oate (IX)

A solution of VIII (710 mg) in pyridine (9 mL) and $POCl_3$ (1.3 mL) was warmed at 55°C for 24 hr. The reaction mixture was poured into ice cold 0.1 N HCl and extracted with ether. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (25 g). Elution with a 19:1 mixture of benzene and ethyl acetate gave 121 mg of IX as amorphous powder: PMR (δ): 0.72 (3H, s, 18-CH_3), 0.85 (3H, s, 19-CH_3), 0.96 (3H, d, J=6 Hz, 21-CH_3), 2.02 (6H, s, 3α- and 7β-$OCOCH_3$), 3.62 (3H, s, -$COOCH_3$), 4.6-5.0 (2H, m, 3β- and 7α-H),

5.35 (1H, d, J=10 Hz, 12-H), 6.15 (1H, dd, J_1=10 Hz, J_2≈3 Hz, 11-H).

[11,12-^{3}H]Homoursodeoxycholic acid (X)

Reduction of IX (79 mg) with tritium gas in the presence of platinum oxide catalyst was performed at New England Nuclear Co.. The product was dissolved in 5% methanolic KOH and refluxed for 2 hr. The hydrolysate was diluted with water, acidified with HCl and extracted with ether. After evaporation of the solvent, the residue was subjected to reversed phase partition column chromatography (13) using Hostalene (polyethylene powder, Farbwerke Hoechst, A.G., Germany) as the supporting material, 55% aqueous methanol as the moving phase, and chloroform:heptane 9:1 (v/v) as the stationary phase. The column fractions were monitored by TLC. The effluents containing X were combined and evaporated to dryness. Recrystallization from ethyl acetate gave 57 mg of X with mp 174-175°C. The labeled HUDC (X) was identical with unlabeled HUDC (IV) by comparison of TLC, GLC, IR, PMR, and mass spectra. The specific radioactivity of the labeled HUDC (X) was 4.0 Ci/mol and its radiopurity was shown to be greater than 99% checked by radio-TLC.

RESULTS AND DISCUSSION

This paper describes the synthesis and physical properties of HUDC and [11,12-^{3}H]HUDC. The synthetic route to HUDC is shown in Chart 1.

Protection of the free hydroxyl groups of UDC (Ia) was accomplished by preparation of the formate ester using formic acid. The diformoxy derivative (Ib) was easily converted to the corresponding acid chloride (II) using thionyl chloride. This compound was not isolated but was freed of any residual thionyl chloride by repeated evaporation with benzene. The acid chloride (II) was added to an ethereal solution of diazomethane to form the diazoketone (III). The diazoketone (III) had strong infrared absorption at 2082 cm^{-1} which is characteristic of the $CH_2N{=}N$ functional group. HUDC (IV) was formed from the diazoketone (III) using benzyl alcohol and collidine with subsequent heating of the reaction mixture to 180-200°C. Results of GLC analysis of HUDC (Table 1) are in accord with the proposed structure. The C_{25}-bile acid had retention times of 1.17, 1.25, and 1.29 relative to UDC on QF-1, OV-17

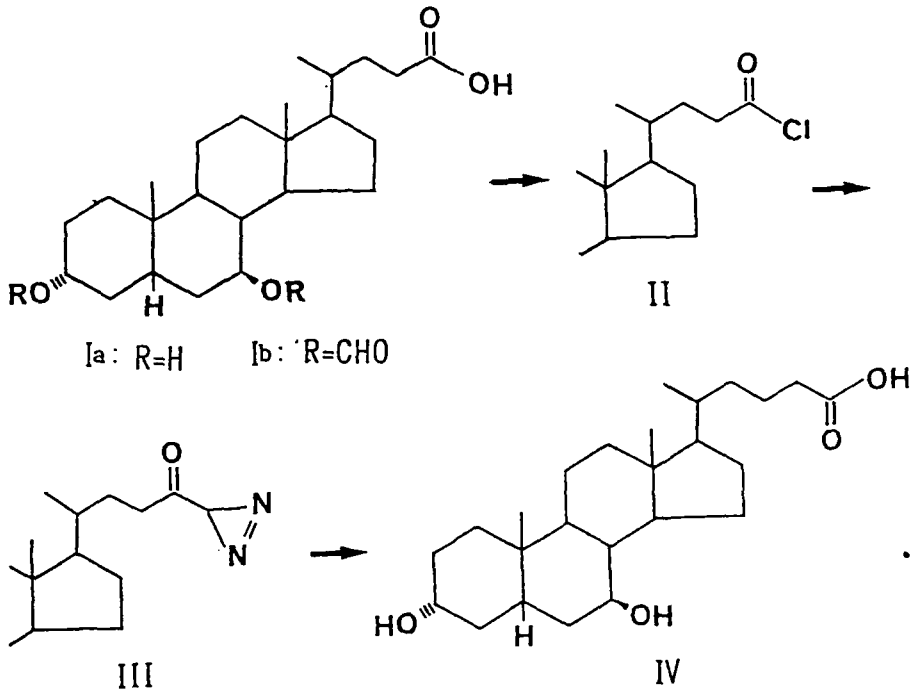


CHART 1

Table 1. Relative retention times on GLC of HUDC, UDC, HC, and cholic acid

Bile acid	Relative retention times[a]		
	QF-1	OV-17	Poly I-110
HUDC (C_{25})	1.40	1.38	1.92
UDC (C_{24})	1.20	1.10	1.49
Ratio C_{25}/C_{24}	*1.17*	*1.25*	*1.29*
HC (C_{25})	1.24	1.14	0.99
Cholic acid (C_{24})	1.04	0.90	0.78
Ratio C_{25}/C_{24}	*1.19*	*1.26*	*1.27*

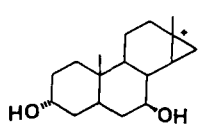
Bile acids were chromatographed as their TMS ether-methyl ester derivatives.
a; Relative to TMS ether of methyl deoxycholate.

and Poly I-110 columns, respectively. These ratios were in good agreement with those of retention times between HC and cholic acid. The IR spectrum of HUDC was similar to that of UDC with peaks at 3330, 1715, 1450, 1380, 1045, and 905 cm^{-1}. PMR spectrum of HUDC closely resembled that of UDC with signals due to the C-18, C-19, and C-21 methyl groups and signals due to the 3β- and 7α-protons. The mass spectra of HUDC and the TMS ether derivative of methyl HUDC were identical to those of UDC and the TMS ether derivative of methyl UDC, respectively, with respect to peak intensities and fragmentation patterns (Table 2). The only difference was that the peaks in the spectra of the C_{25} bile acid and its methyl ester-TMS ether derivative were shifted 14 mass units upfield because of the additional CH_2

Table 2. Mass fragment ions and relative intensities of HUDC and UDC

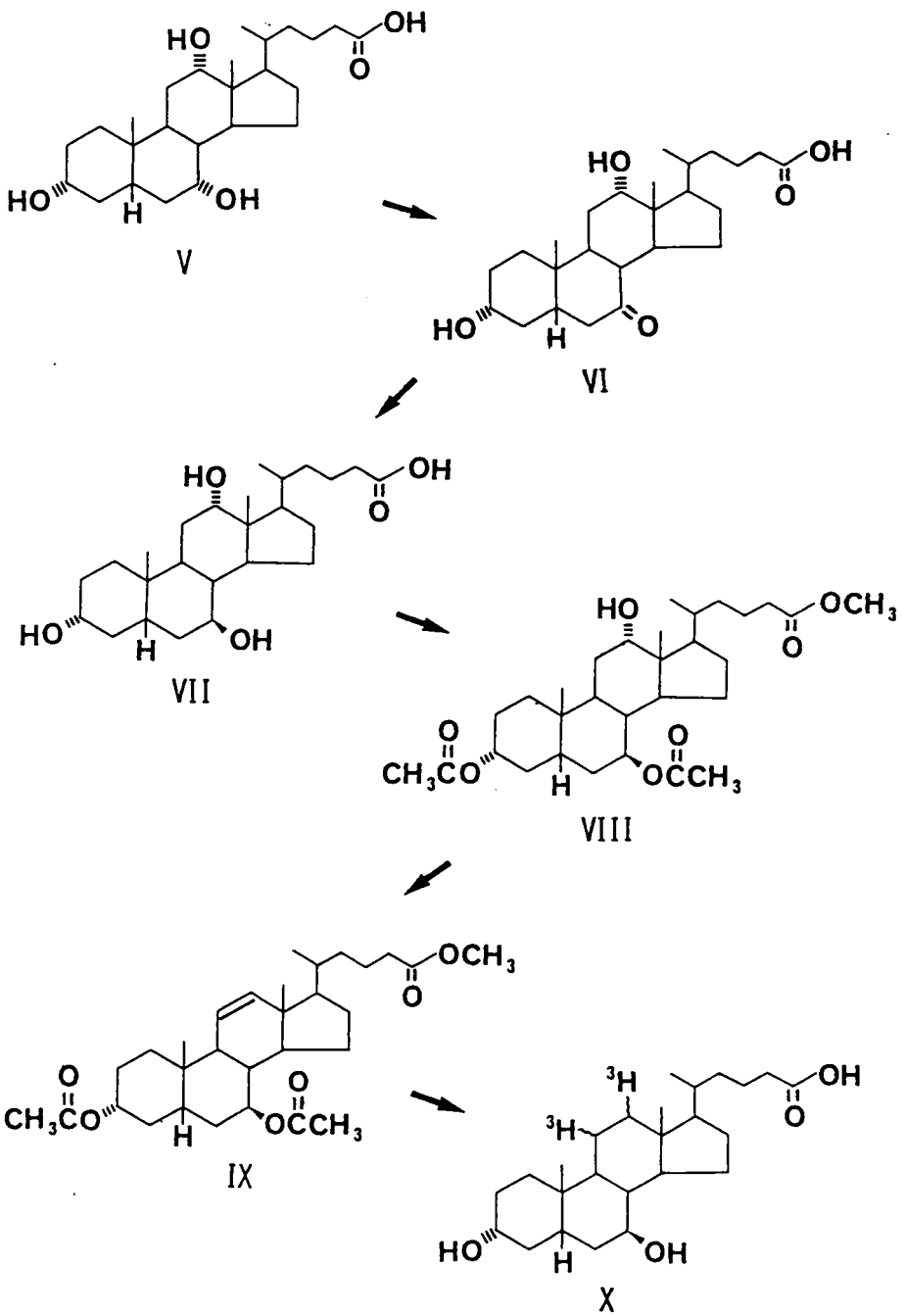
Fragment ion	Free acid		Fragment ion	TMS ether-methyl ester	
	HUDC	UDC		HUDC	UDC
	m/z(relative intensity)			m/z(relative intensity)	
[M]	406 (24)	392 (50)	[M]	564 (5)	550 (3)
[M-18]	388 (50)	374 (74)	[M-15]	549 (6)	535 (8)
[M-2x18]	370 (100)	356 (100)	[M-90]	474 (100)	460 (100)
[M-(2x18+15)]	355 (30)	341 (37)	[M-(90+15)]	459 (2)	445 (4)
[M-(18+72)]	316 (23)	302 (18)	[M-2x90]	384 (45)	370 (66)
[M-(18+SC)]	273 (18)	273 (15)	[M-(2x90+15)]	369 (32)	355 (17)
Fragment A	264 (39)	264 (46)	[M-(90+SC)]	345 (5)	345 (9)
[M-(2x18+SC)]	255 (61)	255 (41)	[M-(2x90+SC)]	255 (34)	255 (44)
[A-18]	246 (15)	246 (13)			

SC: side chain. Fragment A:



moiety present in the bile acid side chain. The mass spectrum of HUDC showed a molecular ion at m/z 406, fragments at m/z 388 and 370 (base peak) corresponding to the loss of one and two hydroxyl groups, respectively, and a peak at m/z 255 which was characteristic of a dihydroxy bile acid (14). In the mass spectrum of the TMS ether derivative of methyl HUDC, major fragments at m/z 474 (base peak) and 384 corresponded to the loss of one and two TMS-OH from the steroid nucleus. Fragments were also observed for the molecular ion (m/z 564), the loss of one TMS-OH + the side chain (m/z 345) and the loss of both TMS-OH + the side chain (m/z 255). On the whole, the spectral properties of HUDC were similar to those of UDC.

The synthetic route of [11,12-^{3}H]HUDC (X) is shown in Chart 2. HC (V), which was prepared from cholic acid by the method previously reported (15), was oxidized with N-bromosuccinimide in aqueous acetone to give 3α,12α-dihydroxy-7-oxo-25-homo-5β-cholan-25-oic acid (VI). The 7-oxo bile acid (VI) was treated with sodium in 1-propanol to give 3α,7β,12α-trihydroxy-25-homo-5β-cholan-25-oic acid (VII). Methylation followed by partial acetylation of the trihydroxy acid (VII) afforded methyl 3α,7β-diacetoxy-12α-hydroxy-25-homo-5β-cholan-25-oate (VIII). Dehydration of the 12α-hydroxy group of the diacetate (VIII) with phosphorus oxychloride in pyridine gave methyl 3α,7β-diacetoxy-25-homo-5β-chol-11-en-25-oate (IX). The PMR spectrum of the unsaturated ester (IX) was in full agreement with the assigned structure, exhibiting two olefinic proton resonances centered at δ 6.15 and δ 5.35 for the C-11 and C-12 hydrogens, respectively. The mass fragmentation pattern of IX was identical to that of the acetate derivative of methyl HUDC (Table 3). The only difference was that the peaks in the spectrum of IX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OCCH3
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IX
3H
OH
O
3H
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OH
H
X

CHART 2

Table 3. Mass fragment ions and relative intensities of methyl 3α,7β-diacetoxy-25-homo-5β-chol-11-en-25-oate (IX) and the corresponding derivative of HUDC

Fragment ion	IX	Diacetyl derivative of methyl HUDC
	m/z (relative intensity)	
[M-60]	442 (62)	444 (2)
[M-(60+15)]	427 (15)	429 (2)
[M-2x60]	382 (100)	384 (90)
[M-(2x60+15)]	367 (29)	369 (36)
[M-(2x60+31)]	351 (4)	353 (9)
[M-(2x60+54)]	328 (36)	330 (7)
[M-(60+SC)]	313 (12)	315 (9)
[M-(2x60+SC)]	253 (90)	255 (100)

SC: side chain.

were shifted 2 mass units downfield because of the double bond present in the steroid nucleus. Major fragments at m/z 442 and 382 (base peak) corresponded to the loss of one and two CH_3COOH groups from the steroid nucleus, respectively. Fragments were also observed for the loss of one CH_3COOH + the side chain (m/z 313) and the loss of both CH_3COOH + the side chain (m/z 253).

Catalytic reduction of IX with tritium gas, followed by alkaline hydrolysis gave [11,12-^{3}H]HUDC (X). After purification by reversed phase partition column chromatography followed by recrystallization, the final specific radioactivity of the [11,12-^{3}H]HUDC was 4.0 Ci/mol and its radiopurity checked by radio-TLC was shown to be greater than 99 %. The radioactive compound should be useful for studying the metabolism of the C_{25} bile acid.

APPENDIX

The following trivial names and abbreviations have been used in this papers:
Lithocholic acid(LC) : 3α-hydroxy-5β-cholan-24-oic acid;
Chenodeoxycholic acid(CDC) : 3α,7α-dihydroxy-5β-cholan-24-oic acid;
Ursodeoxycholic acid(UDC) : 3α,7β-dihydroxy-5β-cholan-24-oic acid;
Cholic acid : 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid;
Homoursodeoxycholic acid(HUDC) : 3α,7β-dihydroxy-25-homo-5β-cholan-25-oic acid;
Homocholic acid(HC) : 3α,7α,12α-trihydroxy-25-homo-5β-cholan-25-oic acid.

REFERENCES

* To whom correspondence should be addressed.

1. Danzinger, R.G., Hofmann, A.F., Schoenfield L.J., and Thistle, J.L., N. ENG. J. MED., **286**, 1 (1972).
2. Nakagawa, S., Makino, I., Ishizuka, T., and Doki, I., LANCET, 2, 367 (1977).
3. Danielsson, H., Eneroth, P., Hellström, K., Lindstedt, S., and Sjövall, J., J. BIOL. CHEM., 255, 2299 (1963).
4. Fedorowski, T., Salen, G., Tint, G.S., and Mosbach, E.H., GASTRO-ENTEROLOGY, 77, 1068 (1979).
5. Bazzoli, F., Fromm, H., Sarva, R.P., Sembrat, R.F., and Geryak, S., GASTROENTEROLOGY, 83, 753 (1982).
6. Fisher, C.D., Cooper, N.S., Rothschild, M.A., and Mosbach, E.H., AM. J. DIG. DIS., **19**, 877 (1974).
7. Dyrszka, H., Salen, G., Zaki, F.G., Chen, T., and Mosbach, E.H., GASTROENTEROLOGY, **70**, 93 (1976).
8. Lindstedt, S., and Tryding, N., ARKIV KEMI, **11**, 137 (1957).
9. Czuba, B., and Vessey, D.A., J. BIOL. CHEM., **257**, 8761 (1982).
10. Une, M., Matsumoto, N., Kihira, K., Yasuhara, M., Kuramoto, T., and Hoshita, T., J. LIPID RES., **21**, 269 (1980).
11. Roovers, J., Evrard. E., and Vanderhaeghe, H., CLIN. CHIM. ACTA, **19**, 449 (1968).
12. Ruzicka, L., Plattner, P.A., and Heusser, H., HELV. CHIM. ACTA, **27**, 186 (1944).
13. Norman, A., ACTA CHEM. SCAND., **7**, 1413 (1953).
14. Elliot, W.H., in: Biochemical Applications of Mass Spectrometry (G.R. Waller, Editor), John Wiley and Sons, New York, (1971), pp. 291-312.
15. Pearlman W.H., J. AM. CHEM. SOC., **69**, 1475 (1947).