Stereospecific synthesis of 3β-hydroxylated bile alcohols[1]

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Summary This paper describes the synthesis of 5β-cholestane-3β,7α,25-triol and 5β-cholestane-3β,7α,12α,25-tetrol from their corresponding 3α-analogs. The method consists of refluxing a mixture of a steroid alcohol, triphenylphosphine, and diethyl azodicarboxylate in benzene solution with an acid such as formic acid. The sterically pure ester (3β-formate) so formed after saponification then allows an easy access to the epimer of the starting alcohol. Differentiation of these 3β-hydroxy bile alcohols from their corresponding 3α-epimeric analogs was made possible on the basis of proton, ^{13}C-NMR, and mass spectra as well as chromatographic mobility. Steric requirements of sterols and nucleophilicity of attacking acidic components played an important role for the success of this synthesis. Only equatorial hydroxyl groups in these bile alcohols reacted under mild conditions and epimerization, as well as protection of the alcoholic group, was achieved in one step. Formic acid was the acid of choice since the axial formate ester formed is sufficiently reactive to be hydrolyzed ($KHCO_3$/aq · MeOH) under mild conditions.—**Dayal, B., D. N. Greeley, T. H. Williams, G. S. Tint, and G. Salen.** Stereospecific synthesis of 3β-hydroxylated bile alcohols. *J. Lipid Res.* 1984. **25:** 646–650.

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In 1971, Salen (1) reported that the rare inherited lipid storage disease cerebrotendinous xanthomatosis (CTX) was associated with defective bile acid synthesis. Specifically, patients with this disorder excrete very little chenodeoxycholic acid in their bile but large amounts of bile alcohols (2). Subsequent studies demonstrated that these bile alcohols were 5β-cholestanes hydroxylated at C-3α, C-7α, C-12α, C-25, and elsewhere in the side chain (3–6). The possibility that some of these compounds might be bile alcohols carrying a 3β-hydroxyl group was suggested by their gas–liquid chromatographic properties. To test this assumption it was necessary to synthesize some representative 3β-hydroxylated bile alcohols. This report describes the synthesis of the 3β-epimers of 5β-cholestane-3α,7α,25-triol and 5β-cholestane-3α,7α,12α,25-tetrol.

Conventional syntheses of the known isomeric bile alcohols at carbon 3 involve reduction of the appropriate ketones, produced by selective oxidation, to the corresponding epimeric alcohols which are usually separated by preparative thin-layer or column chromatography and yields are low (7, 8).

Recent use (9–11) of diethyl azodicarboxylate-triphenylphosphine-formic acid for inverting alcohols suggested that a direct inversion of a 3α-hydroxy bile alcohol to its epimer without passing through an intermediary ketone might be feasible. This is a report of the successful application of the method, as exemplified by conversion of 5β-cholestane-3α,7α,25-triol and 5β-cholestane-3α,7α,12α,25-tetrol to their corresponding 3β-epimers (**Fig. 1**).

EXPERIMENTAL

Methods

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, IA), model MP-126000, and are uncorrected.

TLC. The bile alcohols (triols and tetrols) epimeric at carbon-3 were separated on silica gel G plates (Brinkmann Instruments, Westbury, NJ, 0.25 mm thickness) with the solvent systems: chloroform–acetone–methanol 70:20:5.5 (v/v) and chloroform–acetone–methanol 70:50:10 (v/v), respectively. The spots were made visible with phosphomolybdic and sulfuric acid.

^{1}H- and ^{13}C-NMR spectroscopy. Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded on a Varian Associates XL-200 spectrometer equipped with Fourier transform mode. All NMR spectra were taken in $CDCL_3$ solution with Me_4Si as the internal standard. All the CMR spectra were recorded in the proton noise-decoupled mode to obtain exact chemical shifts, and the degree of substitution at each carbon was determined by experiments in the single-frequency off-resonance decoupled mode.

GLC. The bile alcohols, as the TMS derivatives, were analyzed on a 180 cm × 4 mm column packed with 3% OV-17, 3% QF-1, or 1% HI-EEF-8BP on 80/100 mesh Gas Chrome Q; column temperatures 270°C (3% OV-17) (N_2 flow 40 ml/min), 235°C (3% QF-1) (N_2 flow 25 ml/min), and 240°C (for 1% HI-EEF-8BP) (N_2 flow 40 ml/min) (Hewlett-Packard model 7610 gas chromatograph, Palo Alto, CA).

Mass spectra of the epimeric bile alcohols were obtained

Abbreviations: CTX, cerebrotendinous xanthomatosis; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMS, trimethylsilyl; NMR, nuclear magnetic resonance; RRT, relative retention time; EI-MS, electron impact–mass spectrum; RT, retention time; S.C., side chain.

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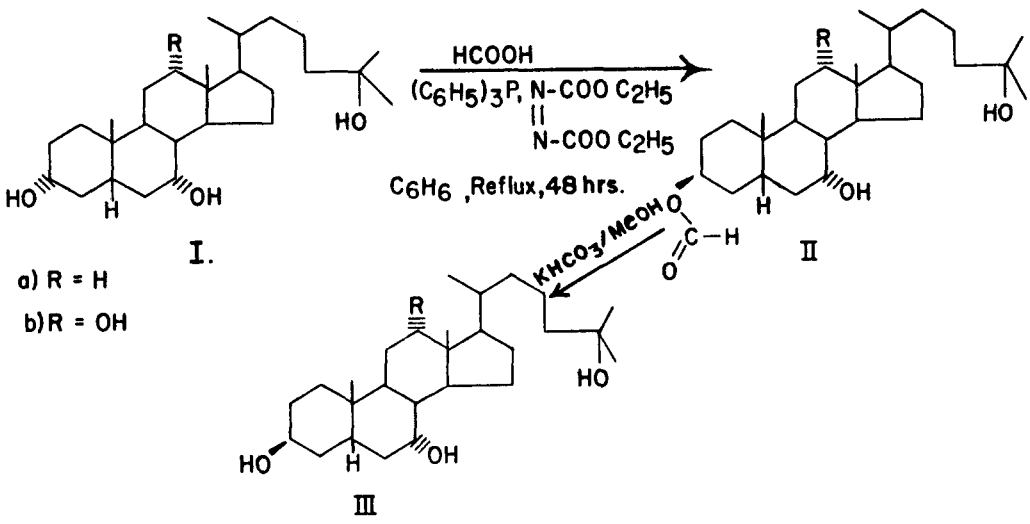

Fig. 1. Synthesis of 5β-cholestane-3β,7α,25-triol (IIIa) and 5β-cholestane-3β,7α,12α,25-tetrol (IIIb). Ia, 5β-cholestane-3α,7α,25-triol; IIa, 5β-cholestane-3β,7α,25-triol-3-formate; IIIa, 5β-cholestane-3β,7α,25-triol; Ib, 5β-cholestane-3α,7α,12α,25-tetrol; IIb, 5β-cholestane-3β,7α,12α,25-tetrol-3-formate; IIIb, 5β-cholestane-3β,7α,12α,25-tetrol.

with a Varian MAT-5 spectrometer (Varian Associates, Palo Alto, CA).

Optical rotations were determined in methanol on a Perkin-Elmer (Norwalk, CT) model 141 polarimeter.

RESULTS

Preparation of 5β-cholestane-3β,7α,25-triol (Fig. I, IIIa)

5β-Cholestane-3α,7α,25-triol [(Ia, 62.0 mg (0.15 mmol) mp 184–187°C, $[\alpha]_D^{25}$ + 15.6°)] (12) and triphenylphosphine (118 mg, 0.45 mmol) were dissolved in dry benzene (5.0 ml) containing formic acid (20.7 mg, 0.45 mmol). To this solution was added dropwise diethyl azodicarboxylate (78.4 mg, 0.45 mmol) dissolved in dry benzene (3.5 ml). A moderately exothermic reaction took place. The contents were then refluxed and the progress of the reaction was monitored by TLC. As estimated by TLC monitoring, complete esterification at C-3 took place in about 48 hr. The reaction mixture was then evaporated to dryness and the residue was stirred twice with diethyl ether; a white precipitate due to triphenyl phosphine oxide formed and was filtered. After evaporating the ether solution, the residue was stirred twice with cyclohexane–chloroform 1:1 and the solution was filtered from the crystalline diethyl hydrazodicarboxylate ($C_2H_5O_2C$-NH · NH-$CO_2C_2H_5$). Removal of solvent under reduced pressure afforded a syrupy product which was chromatographed over a Florisil column using increasing amounts (5–10%) of ethyl acetate in benzene as solvent. Last four fractions (50 ml each) eluted with benzene–ethyl acetate 90:10 gave 5β-cholestane-3β,7α,25-triol 3-formate (36.0 mg) as a waxy low-melting solid. The formate ester (36.0 mg) and potassium hydrogen carbonate (15 mg) were placed in a flask closed with a serum cap and the flask was flushed out with argon. Tetrahydrofuran (900 μl), methanol (2.0 ml), and water (200 μl) were injected, and the mixture was stirred at room temperature (about 20°C). When the hydrolysis was complete (2–3 hr) as shown by TLC, water (1 ml) was added and the mixture was extracted with ethyl acetate. Removal of ethyl acetate under reduced pressure afforded 32.0 mg of an amorphous powder. Purification by column chromatography on neutral alumina (grade IV) with ethyl acetate gave 24.0 mg of 5β-cholestane-3β,7α,25-triol, mp 157–160°C. This compound had lower retention time on GLC and higher TLC mobility (R_f) compared to the corresponding 3α-analog and showed identical mass spectrum except for a few changes in its intensity. A comparison of TLC, GLC, NMR (^1H and ^{13}C) characteristics of 3β- and 3α-triols is given in **Table 1** and **Table 2.** The mass spectrum (EI-MS) of the underivatized 5β-cholestane-3β,7α,25-triol exhibited a base peak at m/z 59(100% intensity), and prominent peaks at 402(56%, M − H_2O), 387(8%, M − H_2O − CH_3), 384(73%, M − $2H_2O$), 369(40%, M − $2H_2O$ − CH_3), 366(9%, M − $3H_2O$), 351(11%, M − $3H_2O$ − CH_3), 289(19%, M − S.C. − 2H), 273(28%, M − S.C. − H_2O), 271(40%, M − S.C. − H_2O − 2H), 255(13%, M − S.C. − $2H_2O$), 253(10%, M − S.C. − $2H_2O$ − 2H).

[Under identical conditions, significant peaks in the mass spectrum (EI-MS) of the 3α-epimer were observed at m/z 402(26%, M − H_2O), 384(100%, M − $2H_2O$), 369(40%, M − $2H_2O$ − CH_3), 366(35%, M − $3H_2O$), 351(25%, M − $3H_2O$ − CH_3), 289(15%, M − S.C.

TABLE 1. Physical characteristics of 5β-cholestane-triols and tetrols isomeric at carbon-3

Sterol	mp, °C	$[\alpha]_D^{25}$ CH_3OH deg	TLC R_f	GLC Retention Time of TMS Ethers Relative to 5α-Cholestane[a] 3% QF-1	3% OV-17	1% HI-EFF-8BP
5β-Cholestane-3α,7α,25-triol	184–187	+15.6	0.55[b]		2.82	1.72
5β-Cholestane-3β,7α,25-triol	157–160		0.70[b]		2.62	1.34
5β-Cholestane-3α,7α,12α,25-tetrol	188–190	+36.8	0.28[c]	2.91	2.58	
5β-Cholestane-3β,7α,12α,25-tetrol			0.35[c]	2.78	2.26	

[a] Retention time of 5α-cholestane on 1% HI-EFF-8BP, 4.97 min; column temp., 240°C; N_2 flow 40 ml/min; on 3% QF-1, 2.65 min; column temp., 235°C, N_2 flow 25 ml/min; on 3% OV-17, 6.55 min; column temp., 270°C, N_2 flow 40 ml/min.
[b] Solvent system: chloroform–acetate–methanol 70:20:5.5 (v/v/v).
[c] Solvent system: chloroform–acetone–methanol 70:50:10 (v/v/v). Silica gel G plates; 0.25-mm thickness.

− 2H), 273(16%, M − S.C. − H_2O), 271(40%, M − S.C. − H_2O − 2H), 255(36%, M − S.C. − $2H_2O$), 253(34%, M − S.C. − $2H_2O$ − 2H).]

Epimerization of 5β-cholestane-3α,7α,12α,25-tetrol to 5β-cholestane-3β,7α,12α,25-tetrol (Fig. I, IIIb)

5β-Cholestane-3α,7α,12α,25-tetrol [(Ib, 31.0 mg, (0.07 mmol), mp 188–189°C, $[\alpha]_D^{25}$, + 36.8°)] (3, 4, 13) and triphenylphosphine (56 mg, 0.213 mmol) were dissolved in dry benzene (3.5 ml) containing formic acid (10 mg, 0.213 mmol). Diethylazodicarboxylate (37.1 mg, 0.213 mmol) dissolved in dry benzene (2 ml) was added dropwise and the mixture was refluxed for 24 hr. The reaction was worked up in the usual way as described for 5β-cholestane-3β,7α,25-triol and the product was chromatographed on silica gel to afford a noncrystalline foam (22.0 mg) consisting mainly of 5β-cholestane-3β,7α,12α,25-tetrol 3-formate. Hydrolysis of the formate (22.0 mg) was accomplished as before by means of aqueous methanolic potassium hydrogen carbonate. The pale yel-

TABLE 2. Proton (200 MHz) and ^{13}C-NMR spectral data[a] ($CDCl_3$) of isomeric 5β-cholestane-triols and tetrols

Carbon	5β-Cholestane-3α,7α,25-triol ^{13}C-NMR	^{1}H-NMR	5β-Cholestane-3β-7α,25-triol ^{13}C-NMR	^{1}H-NMR	5β-Cholestane-3α,7α,12α,25-tetrol ^{13}C-NMR	^{1}H-NMR	5β-Cholestane-3β,7α,12α,25-tetrol ^{13}C-NMR	^{1}H-NMR
C-1	35.34		29.82		35.37		29.71	
C-2	30.68		27.78		30.09		27.56	
C-3	72.02	3.47 (3βH axial)[b]	67.00	4.08 (3αH eq)[b]	71.72	3.39 (3βH ax)[b]	66.93	4.09 (3αH eq)[b]
C-4	39.67		36.70		39.46		36.60	
C-5	41.49		36.00		41.56		35.98	
C-6	34.58		34.19		34.58		34.07	
C-7	68.58	3.86 (7βH eq)[b]	68.80	3.87 (7βH eq)[b]	68.46	3.84 (7βH eq)[b]	68.57	4.03 (7βH eq)[b]
C-8	38.45		39.38		39.46		39.60	
C-9	32.86		32.15		26.33		26.05	
C-10	35.06		35.62		34.82		35.20	
C-11	20.75		20.91		28.04		28.55	
C-12	39.91		39.72		73.18	3.98 (12βH eq)[b]	73.05	3.88 (12βH eq)[b]
C-13	42.67		42.70		46.37		46.59	
C-14	50.50		50.50		41.56		42.12	
C-15	23.73		23.76		23.29		23.17	
C-16	28.31		28.31		27.72		27.68	
C-17	56.05		56.04		47.24		47.61	
C-18	11.77	0.66	11.76	0.66	12.47		12.59	
C-19	22.79	0.91	23.24	0.95	22.48	0.70	22.71	0.71
C-20	35.77		35.76		35.80	0.91	35.45	0.95
C-21	18.65	0.93	18.64	0.93	17.20	1.00	17.75	0.99
C-22	36.42		36.40		36.44		36.30	
C-23	20.59		20.74		20.79		20.78	
C-24	44.42		44.39		44.37		44.35	
C-25	71.10		71.13		71.05		71.13	
C-26	29.65	1.22	29.28	1.21	29.01	1.19	29.29	1.22
C-27	29.65	1.22	29.71	1.21	29.01	1.19	29.71	1.22

[a] The ^{13}C-NMR data are for the carbons indicated and the ^{1}H-NMR data are for the corresponding hydrogens on these carbons.
[b] Conformation of H geminal to substituent: axial and equatorial.

low semi-solid (18 mg) obtained was subjected to column chromatography on neutral alumina (grade IV). Elution with ethyl acetate–methanol 97:3 gave pure 5β-cholestane-3β,7α,12α,25-tetrol (13 mg). This compound gave a single spot on TLC; it had a higher R_f and a lower RT than the corresponding 3α-analog. The TLC, GLC, and NMR [^{1}H and ^{13}C] characteristics are given in Tables 1 and 2. Significant peaks in the mass spectrum (EI-MS) of the free 3β-hydroxy tetrol were observed at m/z 436(16%, M^+), 418(5%, $M^+ - H_2O$), 400(7%, $M^+ - 2H_2O$), 382(19%, $M^+ - 3H_2O$), 364(10%, $M^+ - 4H_2O$), 271(55%, $M^+ - 2H_2O$ − S.C.), 253(100%, $M^+ - 3H_2O$ − S.C.).

[Under identical conditions significant peaks in the mass spectrum (EI-MS) of 3α-epimer were observed at m/z 418(14%, $M^+ - H_2O$), 400(43%, $M^+ - 2H_2O$), 385(18%, $M^+ - 2H_2O - CH_3$), 382(100%, $M^+ - 3H_2O$), 367(33%, $M^+ - 3H_2O - CH_3$), 364(30%, $M^+ - 4H_2O$), 271(78%, $M^+ - 2H_2O$ − S.C.), 253(63%, $M^+ - 3H_2O$ − S.C.).]

DISCUSSION

This report describes a convenient and stereospecific synthetic procedure for the preparation of the following C_{27}-steroid bile alcohols: 5β-cholestane-3β,7α,25-triol and 5β-cholestane-3β,7α,12α,25-tetrol (Fig. 1). These bile alcohols will be useful both as substrates and as reference compounds in studies dealing with the hepatic inversion of the hydroxyl group at carbon-3.

The overall scheme for alcohol inversion from compound I to compound III is given in Fig. 1. This approach complements two other frequently used methods, namely the reaction of the alcohol involving tosylation (TsCL/Pyr.) and inversion of C-3 tosylates with DMF/HO^- or treatment of the 3α-mesylate (or 3α-tosylate) with KO_2/crown ether (14–19).

The inversion of 5β-cholestane-3α,7α,25-triol and 5β-cholestane-3α,7α,12α,25-tetrol to the 3β-epimers in the presence of $RO_2CN{=}NCO_2R/(C_6H_5)_3P/HCOOH$ proceeded smoothly and sterically pure formate ester was formed in good yield without detectable amounts of elimination. Saponification of the ester then allowed an easy access to the epimer of the starting alcohol as revealed by the ^{1}H-NMR spectra of IIIa and IIIb, (Table 2) in which the 3β-H signal at δ3.47 moved to δ4.08 after inversion which indicated equatorial disposition of H geminal to substituent. The configuration of the epimeric carbons at position 3 of the steroidal alcohols was also confirmed by their characteristic ^{13}C-NMR spectra. The axial configuration of isobile alcohols at position 3 was exhibited by an upfield shift of 3.8 to 5.0 ppm as compared to their equatorial analogs (20) (Table 2).

In contrast to the diagnostic NMR data in (Table 2) which provide the most secure means of differentiation, the physical constants collected in Table 1 demonstrate more practical useful information especially when dealing with very small quantities. Differences in chromatographic mobilities (TLC and GLC) certainly distinguished the C-3 epimers.

The mass spectra of the 3β-hydroxy triol and tetrol (TMS derivative) were quite similar to that of the 3α-analogs, particularly with regard to the base peak, m/z 131, characteristic of the TMS ether of 25-hydroxy sterols (21). Examination of the mass spectrum of the underivatized 5β-cholestane-3β,7α,25-triol failed to provide a molecular ion (m/z 420), but did show the ions m/z, 402, 384, and 366 which represent loss of one, two, and three molecules of water. Dehydration and loss of the side chain was indicated by the ions m/z 273, [M − (111 + $2H_2O$)] and 255[M − (111 + $3H_2O$)] and the peaks at m/z 271 and 253 corresponded to dehydration and loss of the side chain together with two hydrogen atoms from the steroid nucleus (22). On the other hand, the fragmentation pattern of the free 5β-cholestane-3β,7α,12α,25-tetrol provided a molecular ion (m/z 436) and a series of ions m/z 418, 400, and 382 corresponding to the loss of one, two, and three molecules of water. Abundances of the ions namely 253(100%) and 271(55%) in 3β-hydroxy tetrol indicated the generation of a third nuclear double bond due to transfer of hydrogen from the nucleus to a newly generated double bond in the side chain, probably at position 24–25 (22, 23).[2]

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