Synthesis of four diastereoisomers at carbons 24 and 25 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid, intermediates of bile acid biosynthesis[1]

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Abstract The synthesis of four stereoisomers at C-24 and C-25 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid is described. Pyridium chlorochromate oxidation of 3α,7α,12α-triacetoxy-5β-cholan-24-ol (II) prepared from cholic acid (I) afforded 3α,7α,12α-triacetoxy-5β-cholan-24-al (III) which was converted to a mixture of the four stereoisomers (IV–VII) by a Reformatsky reaction with ethyl DL-α-bromopropionate followed by alkaline hydrolysis. Separation of these isomers (IV–VII) was achieved by silica gel column chromatography, and subsequent reversed-phase partition column chromatography. The configurations at C-24 were elucidated by conversion of each isomer into (24R)- or (24S)-5β-cholestane-3α,7α,12α,24-tetrol (XII or XI) by Kolbe electric coupling, the C-24 configurations of which were determined by modified Horeau's method and ^{13}C-nuclear magnetic resonance spectroscopy. The stereochemistries at C-25 were deduced by comparison of IV–VII with the products of the hydroboration followed by oxidation with alkaline hydrogen peroxide of (24E)-3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acid (XIII).— **Une, M., F. Nagai, K. Kihira, T. Kuramoto, and T. Hoshita.** Synthesis of four diastereoisomers at carbons 24 and 25 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid, intermediates of bile acid biosynthesis. *J. Lipid Res.* 1983. **24:** 924–929.

Supplementary key words bile acids • high performance liquid chromatography

In the formation of cholic acid from cholesterol in mammalian liver (1), the steroid nucleus undergoes change first, forming 5β-cholestane-3α,7α,12α-triol. Side chain degradation is initiated by a 26-hydroxylation of the triol to give 5β-cholestane-3α,7α,12α,26-tetrol, followed by a further oxidation to give 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA). The most commonly accepted mechanism whereby THCA is converted into cholic acid involves Coenzyme A and the formation of propionic acid. Hence, cleavage of the side chain of THCA takes place by β-oxidation of the CoA ester, a process analogous to the β-oxidation of long-chain fatty acids, and 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid (TeHCA) would be an intermediate in this biosynthetic pathway. The postulated intermediate, TeHCA, was prepared from 3α,7α,12α-trihydroxy-5β-cholan-24-al by a Reformatsky reaction with ethyl DL-α-bromopropionate (2) and its conversion to cholic acid in bile fistula guinea pigs was confirmed (3). In addition, Masui and Staple (4) have shown that the mitochondrial fraction of rat liver homogenate supplemented with 100,000 *g* supernatant fluid catalyzes the conversion of THCA into a more polar metabolite. From comparison with the compound prepared by the above method, the metabolite was identified as TeHCA. However, the stereochemistry at C-24 and C-25 of the biosynthetic TeHCA has remained obscure, because reference standards of known absolute configuration have not been available. The TeHCA prepared by the above method was a mixture of four isomers at C-24 and C-25 and no studies have yet been carried out to isolate each TeHCA and to determine its stereochemistry. In order to have a better understanding of the mechanism of bile acid biosynthesis, we have now directed our attention to the synthesis of the four stereoisomers of TeHCA.

EXPERIMENTAL

General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in methanol on a Union Giken model PM-101 automatic polarimeter at room temperature. Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer as KBr discs. ^{1}H-Nuclear magnetic reso-

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; TeHCA, 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; IR, infrared; PMR, proton nuclear magnetic resonance; CMR, carbon-13 nuclear magnetic resonance; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; HPLC, high performance liquid chromatography; RRT, relative retention time; TMS, trimethylsilyl.

[1] This paper is part XXIII of a series entitled "Comparative biochemical studies of bile acids and bile alcohols." Part XXII. T. Kuramoto, Y. Noma, and T. Hoshita. 1983. *Chem. Pharm. Bull. (Tokyo).* **31:** 1330–1334.

nance (PMR) and ^{13}C-nuclear magnetic resonance (CMR) spectra were measured at 90 MHz on a Hitachi R-40 spectrometer and at 100 MHz on a JEOL PFT-100 spectrometer equipped with an EC-6 computer, respectively. Chemical shifts (δ) are given in ppm downfield from internal tetramethylsilane. Thin-layer chromatography (TLC) was carried out on precoated silica gel G plates (Merck) or octadecyldimethylsilyl silica gel TLC plates (RP-18, Merck) with a 10% solution of phosphomolybdic acid in methanol as the detection reagent. Gas–liquid chromatography (GLC) was run on a Shimadzu GC-6A gas chromatograph using a glass column (2 m × 3 mm) packed with 3% OV-17 on 80/100 mesh Gas-Chrom Q. Bile acids to be analyzed were converted to the methyl ester–TMS ether derivatives and injected to the gas chromatograph. All retention times are given relative to the TMS ether derivative of methyl cholate (RRT = 1.00). High performance liquid chromatography (HPLC) was carried out with a Shimadzu 830 liquid chromatograph equipped with a single-wavelength (254 nm) UV detector. The column was of a reversed-phase type (TSK Gel LS-410, 4 mm ID × 30 cm, Toyo Soda Co. Ltd). The moving phase was 80% methanol, and its flow rate was 0.7 ml/min. All samples were analyzed as the *p*-bromophenacyl esters which were prepared as described previously (5). 'The usual workup' refers to extraction with organic solvent, washing to neutrality, drying over Na_2SO_4, filtration, and evaporation under reduced pressure.

Synthesis and isolation of four stereoisomers of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid

3α,7α,12α-Triacetoxy-5β-cholan-24-al (III) (**Fig. 1**). To a solution of 3α,7α,12α-triacetoxy-5β-cholan-24-ol, II (20 g) in freshly distilled dichloromethane (300 ml), pyridium chlorochromate (15 g), and sodium acetate (1.1 g) were added with stirring at room temperature. After standing for 4 hr at room temperature followed by the usual workup (ether), the crude product was chromatographed on a silica gel column (200 g) to give III (12.0 g), PMR (pyridine-d_5) δ ppm: 0.67 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3), 0.84 (3H, d, J = 6 Hz, 21-CH_3), 1.97, (6H, s, 2 × -$OCOCH_3$), 2.03 (3H, s, -$OCOCH_3$), 4.70 (1H, m, 3β-H), 5.05 (1H, m, 7β-H), 5.23 (1H, m, 12β-H), 9.83 (1H, t, J = 3 Hz, -CHO).

3α,7α,12α,24-Tetrahydroxy-5β-cholestan-26-oic acids (IV–VII). A solution of III (12 g) in dry benzene (100 ml) was added to a mixture of ethyl DL-α-bromopropionate (20 ml), dry benzene (100 ml), granulated zinc (30 g), a few crystals of iodine, and a small amount of powdered copper. When the mixture was refluxed on a steam bath, a vigorous reaction occurred and the solution became cloudy. Gently refluxing was continued for 1.5 hr with rapid stirring. The reaction mixture was then cooled, poured into ice water, acidified with 10% H_2SO_4, and extracted with ether (300 ml × 3). After the usual workup, the product was dissolved in 200 ml of 5% KOH–methanol and the solution was allowed to reflux for 2 hr. The hydrolyzate was extracted with ethyl acetate (300 ml × 3) after dilution with water and acidification with dilute HCl. The usual workup afforded a mixture (8.0 g) of IV–VII. The mixture was methylated with freshly prepared ethereal diazomethane solution and the resulting methylated mixture was chromatographed on a silica gel column (500 g) using a solvent system of acetone graded into ethyl acetate. The fractions were monitored by TLC (silica gel G, solvent system, ethyl acetate–acetone 7:3). Elution with 20% acetone in ethyl acetate gave two fractions, containing the methylated TeHCA. The material (200 mg) in the more rapidly eluted fraction was hydrolyzed with 5% KOH–methanol (100 ml) for 2.5 hr at reflux temperature. After the usual workup (ethyl acetate), the product was chromatographed on a reversed-phase partition column (Lober RP-8, 2.5 cm × 31 cm, Merck) using 80% methanol as the moving phase to give two compounds, IV and V: IV, (57 mg), mp 170–171°C (from ethyl acetate); $[\alpha]_D$: +23.5° (c = 1.14, methanol); IR ν_{max}^{KBr} cm^{-1}: 1715, 3390; PMR (pyridine-d_5) δ ppm: 0.83 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.24 (3H, d, J = 6 Hz, 21-CH_3), 1.24 (3H, d, J = 6 Hz, 27-CH_3), 3.70 (1H, m, 3β-H), 4.10 (1H, m, 7β-H), 4.27 (1H, m, 12β-H), 4.35 (1H, m, 24-H); compound V (28 mg), mp 173–175°C (from ethyl acetate); $[\alpha]_D$: +26.7° (c = 0.72, methanol); IR ν_{max}^{KBr} cm^{-1}: 1715, 3390; PMR (pyridine-d_5) δ ppm: 0.82 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 1.25 (3H, d, J = 6 Hz, 21-CH_3), 1.25 (3H, d, J = 6 Hz, 27-CH_3), 3.70 (1H, m, 3β-H), 4.09 (1H, m, 7β-H), 4.25 (1H, m, 12β-H), 4.29 (1H, m, 24-H). By the same procedure, the slower eluted fraction gave compound VI (21 mg), mp 217–220°C (from ethyl acetate); $[\alpha]_D$: +61.7° (c = 0.78, methanol); IR ν_{max}^{KBr} cm^{-1}: 1715, 3390; PMR (pyridine-d_5) δ ppm: 0.81 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.25 (3H, d, J = 6 Hz, 21-CH_3), 1.25 (3H, d, J = 6 Hz, 27-CH_3), 3.70 (1H, m, 3β-H), 4.07 (1H, m, 7β-H), 4.25 (1H, m, 12β-H), 4.30 (1H, m, 24-H), and compound VII (49 mg), mp 149–150°C (from ethyl acetate); $[\alpha]_D$: +44.6° (c = 0.52, methanol); IR ν_{max}^{KBr} cm^{-1}: 1715, 3390; PMR (pyridine-d_5) δ ppm: 0.81 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.25 (3H, d, J = 6 Hz, 21-CH_3), 1.25 (3H, d, J = 6 Hz, 27-CH_3), 3.70 (1H, m, 3β-H), 4.08 (1H, m, 7β-H), 4.25 (1H, m, 12β-H), 4.30 (1H, m, 24-H).

Determination of absolute configuration at C-24 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid

(24S)- and (24R)-3α,7α,12α-Triacetoxy-5β-cholestan-24-ols (IX and X). To a solution of 3α,7α,12α-triacetoxy-

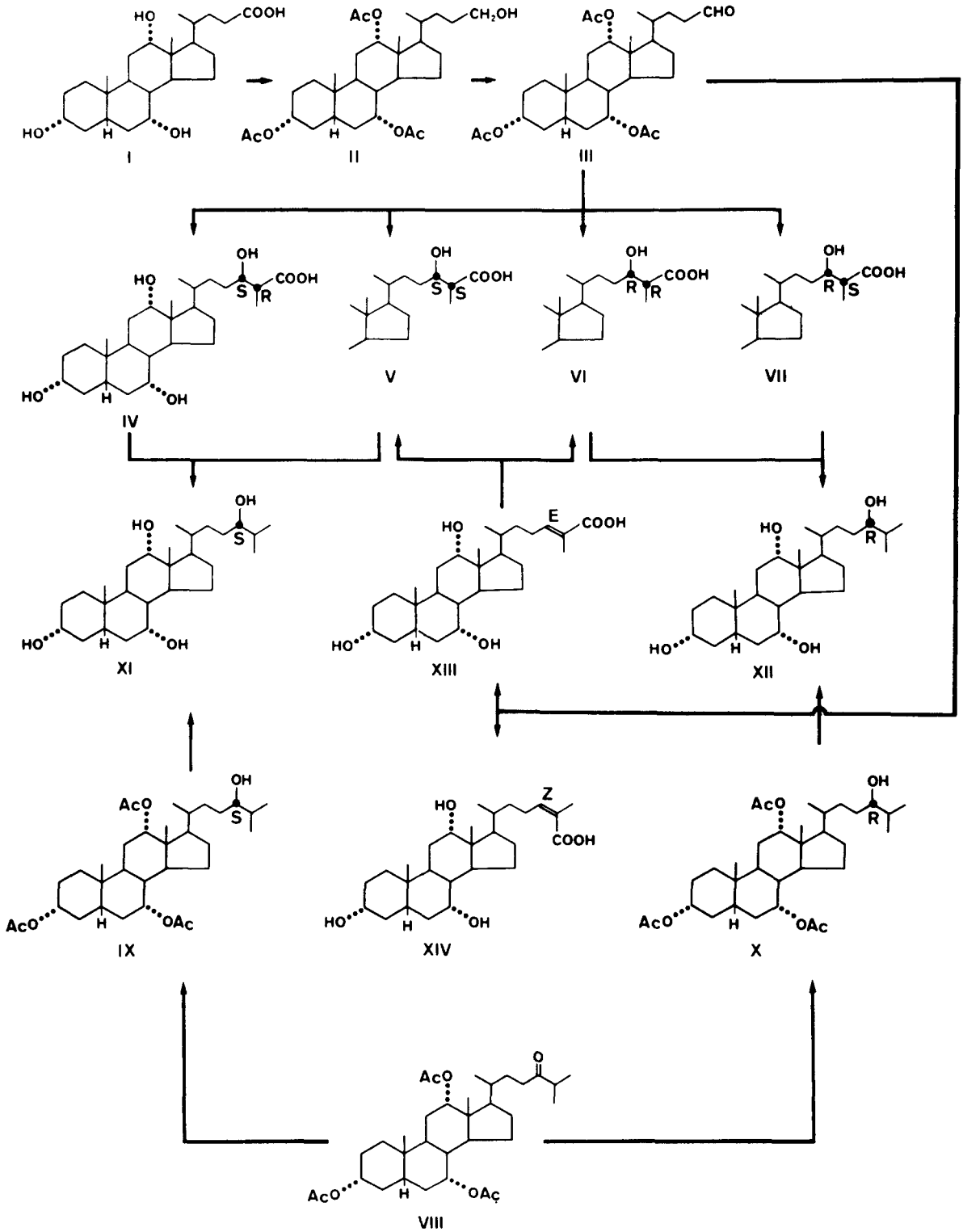

Fig. 1. Synthesis of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid. I, cholic acid; II, 3α,7α,12α-triacetoxy-5β-cholan-24-ol; III, 3α,7α,12α-triacetoxy-5β-cholan-24-al; IV, (24S,25R)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; V, (24S,25S)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; VI, (24R,25R)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; VII, (24R,25S)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; VIII, 3α,7α,12α-triacetoxy-5β-cholestan-24-one; IX, (24S)-3α,7α,12α-triacetoxy-5β-cholestan-24-ol; X, (24R)-3α,7α,12α-triacetoxy-5β-cholestan-24-ol; XI, (24S)-5β-cholestane-3α,7α,12α,24-tetrol; XII, (24R)-5β-cholestane-3α,7α,12α,24-tetrol; XIII, (24E)-3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acid; XIV, (24Z)-3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acid.

5β-cholestan-24-one (VIII, 900 mg) in methanol (50 ml) was added $NaBH_4$ (800 mg). The reaction mixture was allowed to stand at room temperature for 2 hr. After the usual workup (ether), silica gel column chromatography of the crude reduction products using benzene–ethyl acetate 8:2 as eluting solvent gave 301 mg of IX (more rapidly eluted); TLC (silica gel G, benzene–ethyl acetate 7:3), R_f = 0.43; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 2930: PMR (pyridine-d_5) δ ppm: 0.72 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 0.98 (6H, d, J = 6 Hz, 26 and 27-CH_3), 1.07 (3H, d, J = 6 Hz, 21-CH_3), 1.95, 1.98, and 2.03 (9H, s, 3-, 7-, and 12-$OCOCH_3$), 3.50 (1H, m, 24-H), 4.75 (1H, m, 3β-H), 5.07 (1H, m, 7β-H), 5.29 (1H, m, 12β-H); and 322 mg of X (slowly eluted); TLC (silica gel G, benzene–ethyl acetate 7:3), R_f = 0.39; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 2930; PMR (pyridine-d_5) δ ppm: 0.72 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 0.98 (6H, d, J = 6 Hz, 26 and 27-CH_3), 1.07 (3H, d, J = 6 Hz, 21-CH_3), 1.95, 1.98, and 2.03 (9H, s, 3-, 7-, and 12-$OCOCH_3$), 3.50 (1H, m, 24-H), 4.75 (1H, m, 3β-H), 5.07 (1H, m, 7β-H), 5.29 (1H, m, 12β-H).

Modified Horeau's method. Four mg of IX or X was dissolved in 12 μl of dry pyridine and 12 μl of (+)-α-phenylbutyric anhydride. The reaction mixture was kept at 40°C. After 2 hr, 12 μl of (+)-(R)-α-phenylethylamine was added and the reaction mixture was stirred. After standing at room temperature for 15 min, 400 μl of ethyl acetate was added and a 1-μl aliquot of ethyl acetate was subjected to GLC analysis (column, OV-17, column temperature 215°C). Measurements of the peak areas of (+)-(R)-α-phenylethylamides of (−)-(R)- and (+)-(S)-α-phenylbutyric acids (retention times, 7.8 and 8.8 min, respectively) were accomplished with a Shimadzu model EIA automatic integrator.

(24S)- and (24R)-5β-Cholestane-3α,7α,12α,24-tetrols (XI and XII). The triacetoxycholestanetetrol (IX, 100 mg) was hydrolyzed with 5% KOH-methanol (50 ml) for 1 hr at reflux. The usual workup (ethyl acetate) gave XI, mp 186–187°C [reported, 186–186.5°C (6)]; TLC (silica gel G, ethyl acetate–acetone 7:3), R_f = 0.28. By the same procedure, X gave XII, mp 185–186°C [reported, 185–185.5°C (6)]; TLC (silica gel G, ethyl acetate–acetone 7:3), R_f = 0.20.

Electrolysis of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid. Acetic acid (3 ml) and 50 mg each of TeHCA, IV, V, VI, or VII were added to a solution of Na (500 mg) in methanol (150 ml). The solution was electrolyzed for 3 hr in a 200-ml beaker with two platinum electrodes, using direct current from a 30 V source. The current was maintained at 1 A and the current polarity was reversed every 15 min. The solution was stirred constantly and kept at 15–25°C by external cooling. After the usual workup (ethyl acetate), the product was analyzed by TLC with solvent system, ethyl acetate–acetone 7:3. The product from IV or V was identified as XI, and the product from VI or VII was XII.

Determination of absolute configuration at C-25 of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid

(24E)-3α,7α,12α-Trihydroxy-5β-cholest-24-en-26-oic acid (XIII). 3α,7α,12α-Triacetoxy-5β-cholan-24-al (III, 5.0 g) and α-carbetoxy-ethyl-phosphorane (10.0 g) were dissolved in dry benzene (200 ml) and the solution was refluxed for 6 hr in an atmosphere of nitrogen. The usual workup (benzene) gave a crude product (4.5 g) that was hydrolyzed with 5% KOH-methanol (200 ml) for 2 hr at reflux temperature. The usual workup (ethyl acetate) gave a syrup that showed two peaks (RRT, 1.71 and 2.30) on GLC (the methyl ester-TMS ether, column, OV-17, column temperature, 270°C) in a ratio of ca 7:3. Recrystallization from ethyl acetate–acetone gave the major product, XIII (2.5 g), mp 205–206°C, IR ν_{max}^{KBr} cm^{-1}: 1645, 1682, 3400; PMR (pyridine-d_5) δ ppm: 0.78 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.19 (3H, d, J = 6 Hz, 21-CH_3), 1.80 (3H, s, 27-CH_3), 3.65 (1H, m, 3β-H), 4.05 (1H, m, 7β-H), 4.20 (1H, m, 12β-H), 7.13 (1H, t, J = 6 Hz, 24-H). Concentration of the mother liquor afforded a second crop of crystals of XIII. After removal of the crystals by filtration, the filtrate was concentrated to give a residue that could not be recrystallized. GLC analysis revealed that the residue consisted mainly of the minor product, XIV, PMR (pyridine-d_5) δ ppm: 0.78 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.19 (3H, d, J = 6 Hz, 21-CH_3), 1.80 (3H, s, 27-CH_3), 3.65 (1H, m, 3β-H), 4.05 (1H, m, 7β-H), 4.20 (1H, m, 12β-H), 5.85 (1H, t, J = 6 Hz, 24-H).

Hydroboration of (24E)-3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acid (XIII). In a dry three-neck flask equipped with a condenser, a thermometer, and a pressure-equilibrated dropping funnel were placed tetrahydrofuran (20 ml), 2-methyl-2-butene (1.5 ml), and sodium borohydride (120 mg). The flask was immersed in an ice-bath and boron-trifluoride ethereate (1.0 ml) was added dropwise to the well-stirred reaction mixture over a period of 10 min. The reaction mixture was permitted to remain an additional 2 hr at 0–5°C, then XIII (40 mg) was added to the solution. After 30 min, the reaction mixture was treated with 3 N KOH (5 ml) and 30% H_2O_2 (5 ml); it was then allowed to stand for 30 min at room temperature. After the usual workup (ethyl acetate), the products were converted to the *p*-bromophenacyl esters and analyzed by HPLC. The products showed two peaks on HPLC; the retention times were identical with those of V and VI.

RESULTS AND DISCUSSION

The synthetic route to the four isomers at C-24 and C-25 of TeHCA is shown in Fig. 1. 3α,7α,12α-Tri-

TABLE 1. Physical and chromatographic data of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid

	IV	V	VI	VII
mp, °C	170–171	173–175	219–220	149–150
$[\alpha]_D$	+23.5	+26.7	+61.7	+44.6
R_f Value on silica gel G TLC[a]	0.34	0.30	0.27	0.26
R_f Value on reversed-phase TLC[b]	0.46	0.42	0.43	0.41
RRT on GLC[c]	2.13	2.13	2.13	2.13
RRT[d] on HPLC[e]	0.90	0.98	1.00	1.08
Chemical shift at C-24 in CMR	73.2	74.6	72.9	73.5

[a] Solvent system, isopropanol–benzene–acetic acid 30:10:1 (v/v/v).
[b] Solvent system, methanol–water 9:1 (v/v).
[c] Bile acids were chromatographed on 3% OV-17 column as their methyl ester–TMS-ether derivatives.
[d] Relative to the *p*-bromophenacyl ester of cholic acid (RRT = 1.00).
[e] Bile acids were chromatographed on a reversed-phase column (TSK Gel LS-410) as their *p*-bromophenacyl ester derivatives.

acetoxy-5β-cholan-24-ol (II) was prepared from cholic acid (I) according to the published method (7). Pyridium chlorochromate oxidation of the alcohol (II) afforded 3α,7α,12α-triacetoxy-5β-cholan-24-al (III). Treatment of III with an excess of ethyl DL-α-bromopropionate and zinc followed by alkaline hydrolysis provided a mixture of the four C-24,25 isomers of TeHCA, IV–VII. The mixture was treated with diazomethane and the resulting methylated mixture was chromatographed on a silica gel column using a solvent system of acetone graded into ethyl acetate to get two fractions. After alkaline hydrolysis of the material in the more rapidly eluted fraction, the product was subjected to reversed-phase partition column chromatography on a highly porous polymer with 80% methanol as an eluant, yielding two isomers of TeHCA, IV and V, in pure form. By the same procedure, the other two isomers of TeHCA, VI and VII, were obtained from the slowly eluted fraction. Physical and chromatographic data of IV–VII are shown in **Table 1.** These four isomers can be distinguished by high performance liquid chromatography (HPLC).

The stereochemistries at C-24 of IV–VII were elucidated as follows. 3α,7α,12α-Triacetoxy-5β-cholestan-24-one (VIII) was prepared as described previously (8). By sodium borohydride reduction, the ketone (VIII) was converted into a mixture of two epimeric 3α,7α,12α-triacetoxy-5β-cholestan-24-ols (IX and X), which was separated by silica gel column chromatography. For elucidation of the configuration at C-24, application of the modified Horeau's method (9) of IX and X was undertaken. As shown in **Table 2,** in the GLC analysis of the amide derivatives of (+)-(R)-α-phenylethylamine, the peak area for (−)-(R)-α-phenylbutyric acid compared to that for (+)-(S)-α-phenylbutyric acid was larger with the less polar alcohol (IX) than the more polar isomer (X). This result indicated that IX can be assigned the 24S configuration and its counterpart, X, the 24R configuration. This assignment is further supported by measurement of CMR spectra of two 24-epimeric 5β-cholestane-3α,7α,12α,24-tetrols (XI and XII) that were prepared from IX and X, respectively, by alkaline hydrolysis. On comparing the CMR spectra of XI and XII, small differences were observed for C-20 (36.7 ppm for XI, 36.2 ppm for XII) and C-24 (76.5 ppm for XI, 76.1 ppm for XII); these signals appeared slightly downfield in XI compared to XII. The same stereochemical effects have been reported in epimeric pairs of 24-substituted steroids. These resonances in the 24R compounds were always slightly more upfield than the 24S isomers (10, 11). Thus, it can be concluded that XI is (24S)-5β-cholestane-3α,7α,12α,24-tetrol, and its counterpart, XII, is the 24R-isomer. The same configurational assignments at C-24 of XI and XII have tentatively been made

TABLE 2. Modified Horeau's method

Compounds[a]	Ratio of Peak Areas (%) (+)-(R)-α-Phenylethylamide of (−)-(R)-α-Phenylbutyric acid	Ratio of Peak Areas (%) (+)-(R)-α-Phenylethylamide of (+)-(S)-α-Phenylbutyric acid	Configuration[b]
IX	53	47	S
X	51	49	R

[a] IX, (24S)-3α,7α,12α-Triacetoxy-5β-cholestan-24-ol; X, (24R)-3α,7α,12α-triacetoxy-5β-cholestan-24-ol.
[b] See text.

by Masui and Staple (6) on the basis of optical rotation differences. The configurations at C-24 of IV–VII were deduced by the conversion of each TeHCA into the (24R)- or (24S)-5β-cholestane-3α,7α,12α,24-tetrol (XII or XI). Kolbe electric coupling with acetic acid of IV or V gave the 24S-tetrol (XI). By the same procedure, VI or VII gave the 24R-epimer (XII). It can, therefore, be concluded that IV and V have the 24S configuration, while VI and VII have the 24R configuration.

The stereochemistries at C-25 of IV–VII were deduced as follows. When the Wittig reagent prepared from ethyl DL-α-bromopropionate was reacted with the aldehyde (III), two isomeric 3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acids (XIII and XIV) were obtained in a ratio of ca 7:3. Fractional recrystallization from ethyl acetate–acetone gave the major product (XIII) in pure form and the minor product (XIV) in slightly impure form. In their PMR spectra, the C-24 proton signals of XIII appeared at 7.13 ppm, and those of XIV appeared at 5.85 ppm, indicating that the geometry of the former could be assigned as the 24E form, and of the latter as the 24Z form (12). Hydroboration of the 24E-isomer (XIII) with disiamylborane at 0°C and subsequent oxidation with 30% hydrogen peroxide gave a mixture of two TeHCAs in an almost equimolar ratio. The two TeHCAs were identical with V and VI but not with IV and VII as regards chromatographic behaviors on HPLC. From the known mechanism (*cis*-addition) of hydroboration (13), the established geometry (24E) of the α,β-unsaturated acid (XIII), and the established configurations at C-24 of V(24S) and VI(24R), the configurations at C-25 of V and VI were determined as 25S and 25R, respectively. Consequently, the other two TeHCAs, IV and VII should have the configurations of 25R and 25S, respectively.

It has now been established that IV is (24S,25R)-TeHCA; V is (24S,25S); VI is (24R,25R); and VII is (24R,25S).

Finally, the availability of all four C-24,25 isomers of TeHCA permits us to elucidate the stereochemistry of the biosynthetic TeHCA formed from THCA by a rat liver preparation. The detail of such a study will be reported in a later paper of this series.

Manuscript received 26 October 1982 and in revised form 10 March 1983.

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