[Chem. Pharm. Bull. 36(1) 134—141 (1988)]

Synthesis and Determination of Stereochemistry of Four Diastereoisomers at the C-24 and C-25 Positions of 3α,7α,12α,24-Tetrahydroxy-5β-cholestan-26-oic Acid

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(Received July 7, 1987)

Four diastereoisomers at the C-24 and C-25 positions of 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid (varanic acid) were synthesized in a stereochemically defined manner and also by a non-stereoselective route, followed by chromatographic separation. Their stereochemistry at the C-24 and C-25 positions was established on the basis of the known stereochemical course of the reactions employed for the synthesis, and ^{1}H and ^{13}C-nuclear magnetic resonance spectroscopic data of these isomers. It is concluded from the present work that the previous stereochemical assignment for the (24*R*,25*S*) and (24*R*,25*R*) isomers must be revised.

Keywords——3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid; cholic acid; chiral imide enolate; Mitsunobu inversion; varanic acid; HPLC; bile acid

Introduction

In the major pathway for the biosynthesis of cholic acid from cholesterol, the latter compound is first converted into 3α,7α,12α-trihydroxy-5β-cholestane and then ω-oxidation takes place at the side chain terminal leading to 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid. Side chain cleavage of this acid is thought to proceed in a similar manner to the β-oxidation of long chain fatty acids. Thus, 3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid (**1**) (varanic acid) is considered to be one of the intermediates in this process, and some evidence for the putative role of the tetrahydroxy acid intermediate has been reported.[2)] However, it is not known which of the four stereoisomers **1a**—**d** at the C-24 and C-25 positions is the true intermediate. To solve this problem, we required the stereochemically defined isomers of this intermediate. In this paper we would like to describe the synthesis and structure determination of the four stereoisomers of the tetrahydroxy acid. On the basis of the evidence presented here it is concluded that the previous assignment for (24*R*,25*S*) and (24*R*,25*R*) isomers made by Hoshita and co-workers[3)] must be revised.

Results and Discussion

A diastereoisomeric mixture (four isomers) of the tetrahydroxy acid (**1**) has previously been prepared,[4)] and was separated into two components (24*S*-isomers *vs.* 24*R*-isomers) by silica gel chromatography.[2a)] Recently the four stereoisomers have been obtained in a stereochemically pure form by way of chromatographic separation.[3)] In the present experiments we intended to synthesize these isomers in a stereoselective manner as outlined in Chart

1. The synthetic route shown in the chart has the advantage that the stereochemistry at the C-24 and C-25 positions of the products is predictable from the known stereochemical course of the two key reactions: aldol condensation using a chiral imide enolate developed by Evans *et al.*,[5] and Mitsunobu inversion reaction.[6] The aldehyde **2**,[3] obtained from cholic acid triacetate in two steps [(i) ethyl chloroformate/triethylamine and then sodium borohydride, (ii) Swern oxidation], was reacted with the boron enolate **3** generated from (+)-(4*R*,5*R*)-4-methyl-5-phenyloxazolid-2-one according to the published procedure.[5] The reaction afforded the coupling product **5** (24*S*,25*R*-isomer) in 86% yield with concomitant formation of the (24*S*,25*S*)-isomer in 8% yield. The major product **5** was proved to be stereochemically homogeneous on the bases of thin layer chromatography (TLC) and ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) analyses. Similarly the chiral aldol reaction of the aldehyde **2** with the boron enolate **4** of (+)-(4*S*)-*N*-propionyl-4-isopropyloxazolid-2-one afforded the coupling product **6** (24*R*,25*S*-isomer) in 83% yield. In this case formation of stereoisomers of **6** was not detected. Stereochemical homogeneity of the imide **6** was confirmed in the same manner as described above. The relatively small value of J_{24-25} (**5**: 2.3 Hz, **6**: 2.9 Hz) in the ^{1}H-NMR of the imides supported the *syn* relationship at the C-24 and C-25 chiral centers (*vide infra*).[7]

To obtain the other two stereoisomers, the imides **5** and **6** were each submitted to the Mitsunobu inversion reaction using benzoic acid, triphenylphosphine and diethyl azodicarboxylate. The results varied somewhat from run to run (a typical run is described in Experimental). However, the benzoates **7** and **8**, in amounts sufficient to yield stereochemical

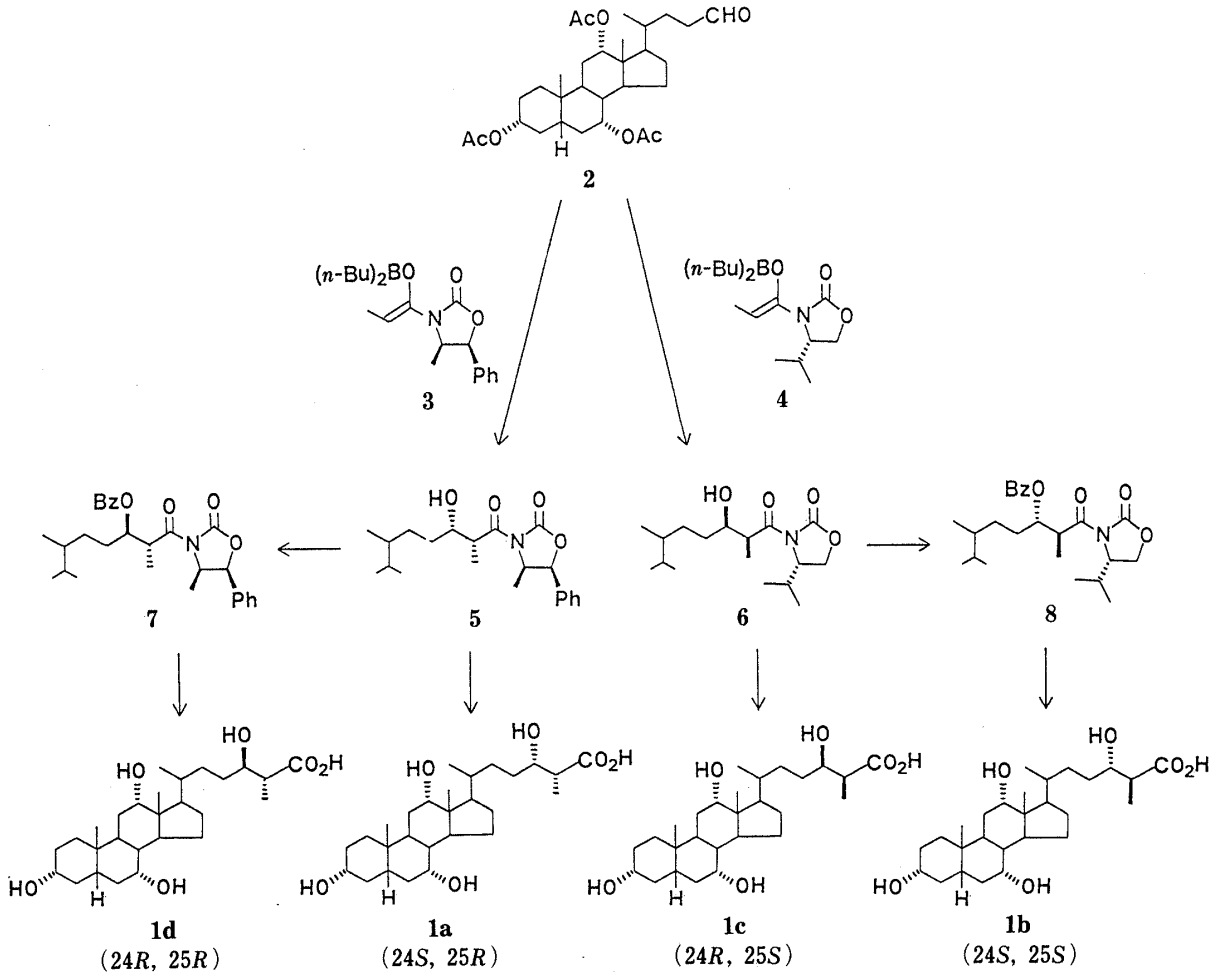


Chart 1

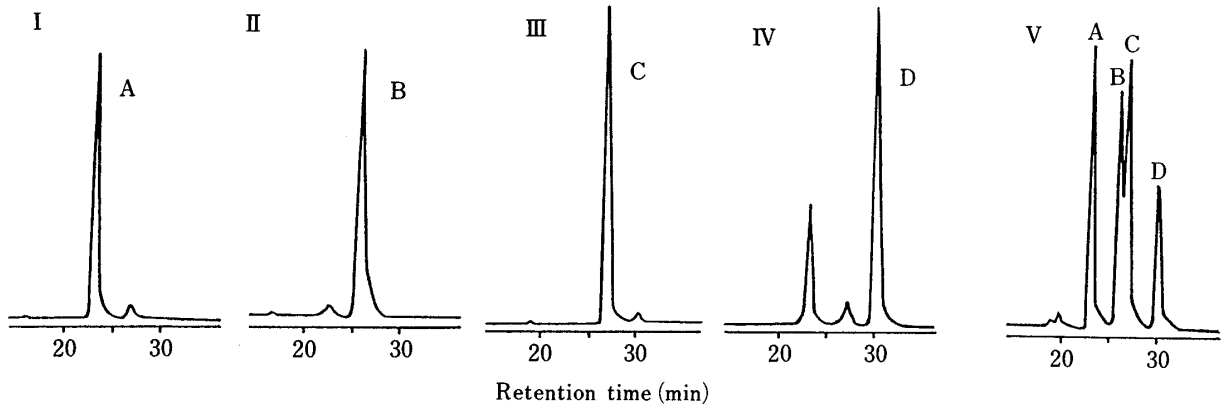
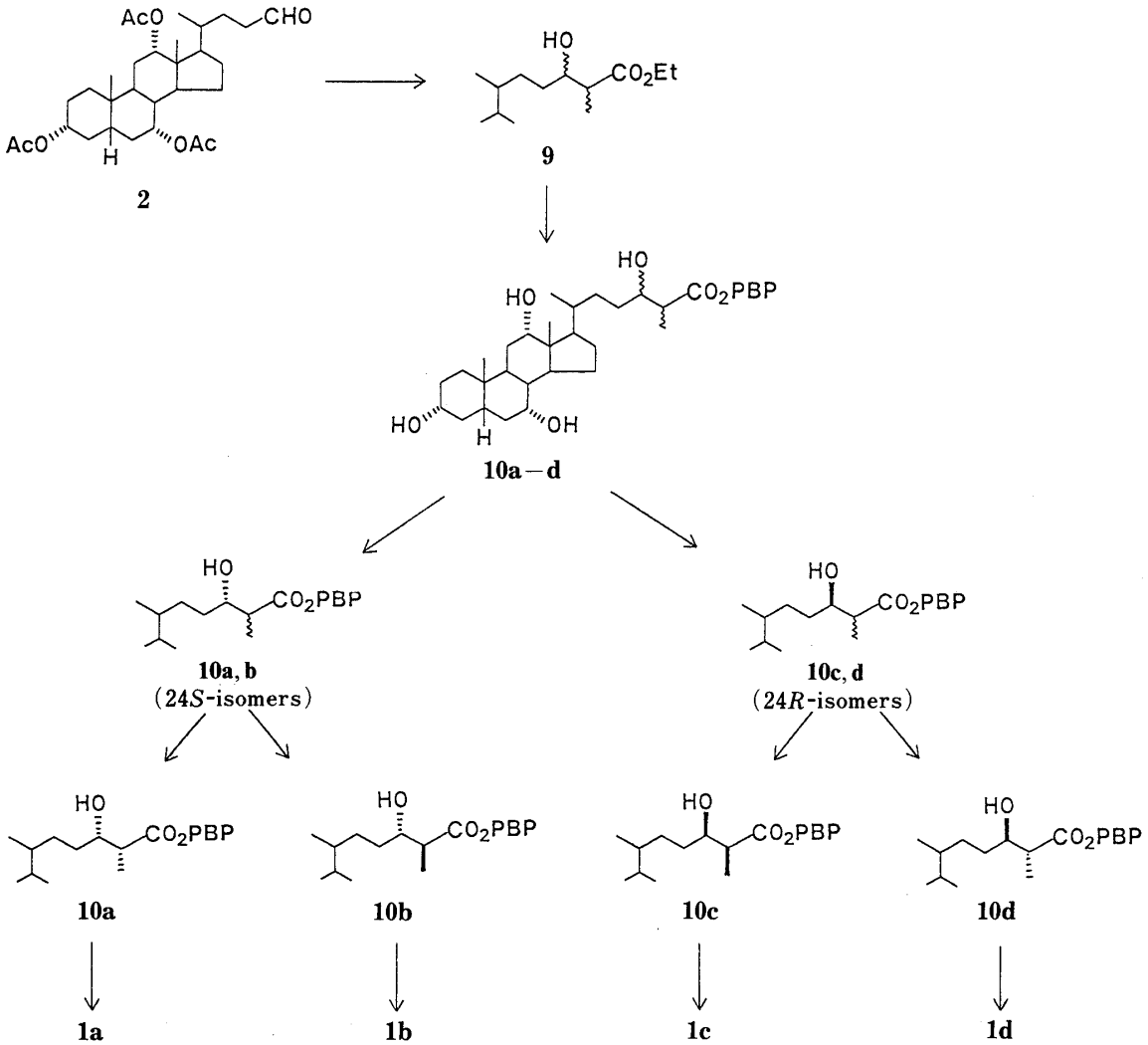


Fig. 1. HPLC Profile of the *p*-Bromophenacyl Ester Derivatives of the Tetrahydroxy Acids **1a—d**

I: **1a** obtained from the imide **5**. II: **1b** obtained from the benzoate **8**. III: **1c** obtained from the imide **6**. IV: **1d** obtained from the benzoate **7**. V: **1a—d** obtained *via* Reformatsky reaction.

In IV, the peak ascribable to the peak A could be derived from the benzoate ester (no inversion at C-24) formed in the Mitsunobu reaction.



PBP refers *p*-bromophenacyl (ester)

Chart 2

information, were obtained from **5** and **6**, respectively. Alkaline hydrolysis of the benzoates **7** and **8** gave the acids **1d** and **1b**, respectively, which were analyzed, after conversion into the *p*-bromophenacyl esters, by high-performance liquid chromatography (HPLC) using a reversed-phase ODS column. The imides **5** and **6** were also hydrolyzed to give the acids **1a** and **1c**, respectively. Figure 1 illustrates the elution profile of the four *p*-bromophenacyl esters **10**. Consideration of the established stereochemical course[5,6] of the reactions employed for the synthesis of those isomers allowed us to determine the configurations at the C-24 and C-25 positions. Thus, the peaks A, B, C, and D could be assigned as those of the (24*S*,25*R*), (24*S*,25*S*), (24*R*,25*S*), and (24*R*,25*R*) isomers, respectively. The present assignment is not consistent with that made by Hoshita *et al.*,[3] who gave a reversed assignment of the last two isomers.[8] Since the analytical conditions were quite similar in the two cases, the discrepancy suggested erroneous assignments of the two isomers.

To confirm the present assignment and to obtain larger amounts of pure samples of the four isomers, we then prepared[3] and separated a diastereoisomeric mixture, and investigated the ^{1}H- and ^{13}C-NMR spectra of the separated samples. The separation of the isomers was effectively achieved by a modification of the method of Hoshita's group[3] (Chart 2). A diastereoisomeric mixture of the ethyl ester **9**, obtained by the Reformatsky reaction of the aldehyde **2** and ethyl bromopropionate, was hydrolyzed to the acid, which was then converted into the *p*-bromophenacyl ester **10**. The ester **10** exhibited two spots on silica gel TLC, as in the case of the corresponding methyl ester. Flash column chromatography on silica gel effected separation into the less polar (24*S*)-component **10a, b** and the more polar (24*R*)-component **10c, d**. Further separation into single isomers was performed by preparation HPLC (a reversed-phase ODS column): **10a, b** afforded a more mobile isomer **10a** and a less mobile isomer **10b**; **10c, d** similarly afforded a more mobile isomer **10c** and a less mobile isomer **10d**. Stereochemical purity of the separated isomers was confirmed to be at least 98% by HPLC. The isomers **10a**, **10b**, **10c**, and **10d** obtained in this way corresponded to the peaks A, B, C, and D, respectively (see Fig. 1). The *p*-bromophenacyl esters **10a—d** were each hydrolyzed to the corresponding acids **1a—d**. It was confirmed that epimerization did not take place during the hydrolysis by conversion of each acid into the respective *p*-bromophenacyl ester, followed by HPLC analysis.

TABLE I. ^{1}H-NMR Data (in Part) for the *p*-Bromophenacyl Esters **10a—d** (in $CDCl_3$)

Isomers	24-H	25-H	27-CH_3	$COOCH_2CO$
10a	3.22 (m)	2.75 (dq, 3.1, 7.1)	1.23 (d, 7.3)	5.26, 5.52 (d, 16, 0.26[a])
10b	3.69 (m)	2.71 (quintet, 6.9)	1.28 (d, 7.2)	5.32, 5.46 (d, 16, 0.14)
10c	3.20 (m)	2.76 (dq, 3.1, 7.1)	1.24 (d, 6.8)	5.25, 5.53 (d, 16, 0.28)
10d	3.71 (m)	2.70 (quintet, 6.9)	1.27 (d, 6.8)	5.33, 5.45 (d, 16, 0.12)

a) ΔAB in ppm.

TABLE II. ^{13}C-NMR Data for the Side Chain of the *p*-Bromophenacyl Esters **10a—d** (in $CDCl_3$)

Isomers	C-20	C-21	C-22[a]	C-23[a]	C-24	C-25	C-26	C-27
10a	35.8	17.7	30.3	32.3	72.6	44.4	174.8	9.4
10b	35.8	17.7	30.9	31.5	74.1	45.9	174.6	12.4
10c	35.7	17.6	30.7	32.3	72.5	45.0	174.6	10.0
10d	35.7	17.7	31.0	31.5	73.8	46.4	174.8	12.5

a) Assignments may be interchanged.

TABLE III. ^{13}C-NMR Data for the Side Chain of the Tetrahydroxy Acids **1a**—**d** (in d_5-Pyridine)

Isomers	C-20	C-21	C-22[a)]	C-23[a)]	C-24	C-25	C-26	C-27
1a	36.5	18.0	32.5	33.1	73.0	46.2	178.2	11.9
1b	36.6	18.1	31.6	32.8	73.9	47.0	178.1	14.1
1c	36.2	17.9	32.5	32.9	72.5	46.8	178.1	12.6
1d	36.1	17.9	31.5	32.0	73.4	47.2	178.6	14.4

a) Assignments may be interchanged.

TABLE IV. Selected ^{1}H-NMR Data for the Tetrahydroxy Acids **1a**—**d** (in d_5-Pyridine)

Isomers	24-H	27-H_3[a)]	Isomers	24-H	27-H_3[a)]
1a	4.44 (m)	1.58 (d, 6.8)	**1c**	4.40 (m)	1.59 (d, 6.8)
1b	4.18 (m)	1.44 (d, 7.3)	**1d**	4.20 (m)	1.45 (d, 7.1)

a) The chemical shifts reported in Ref. 3 are apparently incorrect.

^{1}H- and ^{13}C-NMR analyses of the ester **10a**—**d** and the acid **1a**—**d** unequivocally established the correctness of the stereochemical determinations mentioned above. It is generally expected[7)] that in ^{1}H-NMR the vicinal coupling constant of β-hydroxycarbonyl compounds, *e.g.* J_{24-25} of **10a**—**d** or **1a**—**d**, is smaller for the *syn* isomers (2—6 Hz) than for the *anti* isomers (7—10 Hz) and that the methyl (*e.g.*, C-27) resonance in ^{13}C-NMR is observed in the range 9—13 ppm for the *syn* isomers and 12—18 ppm for the *anti* isomers. Indeed, the most diagnostic difference in the ^{1}H-NMR of **10a**—**d** was found in the value of J_{24-25}: a small value for the *syn* isomers **10a** and **10c** (3.1 Hz) in contrast to a large value for the *anti* isomer **10b** and **10d** (6.9 Hz) (Table I). Further, regularity in the chemical shifts of the C-24 methine, C-27 methyl, and methylene protons of the *p*-bromophenacyl moiety was also noted (Table I). In the ^{13}C-NMR of **10a**—**d** the C-27 methyl signals of the *syn* isomers were observed at higher field (**10a**, 9.4 ppm and **10c**, 10.0 ppm) whereas those of the *anti* isomers were found at lower field (**10b**, 12.4 ppm and **10d**, 12.5 ppm) (Table II). Analogously the C-27 carbons of the *syn* isomers **1a** and **1c** were observed at higher field than those of the *anti* isomers **1b** and **1d** (Table III). Although the chemical shifts of the C-24 carbons were not very different from each other, the (24*R*,25*S*) and (24*R*,25*R*) isomers reported by Hoshita's group were identified as our (24*R*,25*R*) and (24*R*,25*S*) isomers, respectively, based on the reported chemical shifts of the C-24 carbons.[3)] Further, this identification was substantiated by the mobility in reversed-phase TLC, where the (24*R*,25*R*)-isomer was more mobile than the (24*R*,25*S*)-isomer. It can be seen from Table IV that the ^{1}H-NMR signals of the C-27 methyl and C-24 methine protons of **1a**—**d** show a diagnostic difference.

We conclude from the evidence presented here that the previous stereochemical assignment[3)] for the (24*R*,25*S*) and (24*R*,25*R*) isomers should be reversed and the elution order of the *p*-bromophenacyl ester of the four isomers both in our and their HPLC columns is as shown in Fig. 1.

Recently Hoshita and co-workers have demonstrated stereospecific formation of (24*R*,25*S*)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-26-oic acid (corresponds to peak D in Fig. 1) from (25*R*)- and (25*S*)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acids by rat liver homogenate.[9)] The stereochemistry of the isomer formed in their work should be read as (24*R*,25*R*) according to the present assignment. In addition, the tetrahydroxy acid isolated from the frog, *Bombina orientalis*, should be the (24*R*,25*R*)-isomer.[10)] In order to examine the

stereochemical specificity in the side chain cleavage process, incubation of the four diastereoisomers obtained in the present work with rat liver homogenate is being carried out.

Experimental

Melting points were determined on a hot-stage microscope and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-200 (200 MHz for ^{1}H-NMR) spectrometer in a given solvent with tetramethylsilane as an internal reference. Routine column chromatography was performed with Kiesel gel 60 (70—230 mesh, Merck) and flash column chromatography was done with Kiesel gel 60 (mesh 230—400, Merck). Analytical HPLC was performed on a Shim-pack CLC ODS column (15 × 0.6 cm i.d.) with ultraviolet (UV) monitoring at 254 nm. Preparative HPLC was performed with a YMC-Pack S-343 column (25 × 2 cm i.d.).

(4*R*,5*R*,24′*S*,25′*R*)-*N*-3′α,7′α,12′α-Triacetoxy-24′-hydroxy-5′β-cholestan-26′-oyl-4-methyl-5-phenyloxazolid-2-one (5)——(n-Bu)$_2$BOTf (1 M solution in CH_2Cl_2, 1.9 ml) and ethyldiisopropylamine (0.37 ml, 2.13 mmol) were added to a solution of *N*-propionyl-4-methyl-5-phenyloxazolid-2-one (447 mg, 1.92 mmol) in CH_2Cl_2 (3.8 ml) at 0 °C under argon. The solution was stirred for 30 min at 0 °C and then cooled to −60 °C. The aldehyde **2** (698 mg, 1.35 mmol) in CH_2Cl_2 (12 ml) was added and the mixture was stirred for 30 min at −60 °C and then for 1.5 h at room temperature. After the addition of phosphate buffer (pH 7) the whole mixture was extracted with ether. The extract was washed with 2 N HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel (40 g) column chromatography with hexane–ethyl acetate (1 : 1) as an eluting solvent to give the amorphous imide **5** (869 mg, 86%) and a slightly less polar by-product (82 mg, 8%) which was characterized as the (24*S*,25*R*)-isomer by conversion into the *p*-bromophenacyl ester. Compound **5**: ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s, 18-H$_3$), 0.84 (3H, d, J=6.4 Hz, 21-H$_3$), 0.89 (3H, d, J=6.4 Hz, NCHC$\underline{H}_3$), 0.92 (3H, s, 19-H$_3$), 1.23 (3H, d, J=6.8 Hz, 27-H$_3$), 2.05, 2.10, 2.16 (3H each, s, CH_3CO), 3.77 (1H, dq, J=2.3, 6.8 Hz, 25-H), 3.90 (1H, m, 24-H), 4.57 (1H, m, 3-H), 4.80 (1H, quintet, J=6.9 Hz, NC$\underline{H}$CH$_3$), 4.90 (1H, m, 7-H), 5.10 (1H, m, 12-H), 5.70 (1H, d, J=7.1 Hz, OC$\underline{H}$Ph), 7.3—7.5 (5H, m, Ph). *Anal.* Calcd for $C_{43}H_{61}NO_{10}$: C, 68.68; H, 8.18; N, 1.86. Found: C, 68.75; H, 8.26; N, 1.81.

(4*S*,24′*R*,25′*S*)-*N*-3′α,7′α,12′α-Triacetoxy-24′-hydroxy-5′β-cholestan-26′-oyl-4-isopropyloxazolid-2-one (6)——The amorphous imide **6** (660 mg, 83%) was obtained from the aldehyde **2** (581 mg) and *N*-propionyl-4-isopropyloxazolid-2-one (264 mg) in the same manner as described above. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, 18-H$_3$), 0.82 (3H, d, J=5.9 Hz, 21-H$_3$), 0.89, 0.92 (3H each, d, J=6.8 Hz, (C$\underline{H}_3$)$_2$CH), 0.92 (3H, s, 19-H$_3$), 1.24 (3H, d, J=7.3 Hz, 27-H$_3$), 2.05, 2.10, 2.13 (3H each, s, CH_3CO), 3.77 (1H, dq, J=2.9, 6.9 Hz, 25-H), 3.86 (1H, m, 24-H), 4.22 (1H, dd, J=3.5, 8.0 Hz, OC$\underline{H}_2$CH), 4.29 (1H, t, J=7.0 Hz, OC$\underline{H}_2$CH), 4.47 (1H, dt, J=3.5, 7.0 Hz, CHN), 4.57 (1H, m, 3-H), 4.88 (1H, m, 7-H), 5.07 (1H, m, 12-H). *Anal.* Calcd for $C_{43}H_{61}NO_{10}$: C, 66.55; H, 8.73; N, 1.99. Found: C, 66.48; H, 8.80; N, 2.18.

(24*S*,25*R*)-3α,7α,12α,24-Tetrahydroxy-5β-cholestan-26-oic Acid (1a)——A mixture of the imide **5** (91 mg, 0.12 mmol) in MeOH (4.5 ml) and 5% aqueous KOH (4.5 ml) was stirred at room temperature for 2 h and at 80 °C for 5 h. The reaction mixture was cooled, acidified with 2 N HCl, and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was purified on a Lobar column (Merck, RP-8, methanol as an eluting solvent) to give the acid **1a** (36 mg, 64%). The spectroscopic data are essentially identical to those described later.

(24*R*,25*S*)-3α,7α,12α,24-Tetrahydroxy-5β-cholestan-26-oic Acid (1c)——The acid **1c** (32 mg, 70%) was obtained from the imide **6** (69 mg) in the same manner as described above. The spectroscopic data are essentially identical to those described later.

Mitsunobu Inversion of the (24*S*,25*R*)-Imide 5 Followed by Alkaline Hydrolysis——Diethyl azodicarboxylate (0.13 ml, 0.82 mmol) and benzoic acid (102 mg, 0.83 mmol) were added to a stirred solution of the imide **5** (418 mg, 0.56 mmol) and Ph_3P (218 mg, 0.83 mmol) in benzene (3.5 ml), and the mixture was stirred for 3 d. Extractive work-up (ethyl acetate) afforded the residue, which was purified by silica gel (25 g) chromatography with hexane–ethyl acetate (2 : 1) as an eluting solvent to give the benzoate **7** (174 mg, 37%). A mixture of the benzoate and 5% KOH/MeOH (3 ml) and H_2O (0.5 ml) was heated at reflux for 2 d. Acidification with 2 N HCl and extractive work-up (ethyl acetate) afforded the acid **1d** (52 mg, no purification).

Mitsunobu Inversion of the (24*R*,25*S*)-Imide 6 Followed by Alkaline Hydrolysis——The benzoate **8** (260 mg, 64%) was obtained from the imide **6** (353 mg) as described above. The benzoate was similarly hydrolyzed to give the acid **1b** (118 mg, no purification).

***p*-Bromophenacyl Ester Formation of 1a—d for HPLC Analysis**——A portion of each acid (11 mg, 0.024 mmol) was added to a solution of *p*-bromophenacyl bromide (27 mg, 0.097 mmol) and ethyldiisopropylamine (50 μl, 0.29 mmol) in acetonitrile–methanol (9 : 1, 5 ml), and the mixture was stirred at room temperature for 3 h, then concentrated. The residue was purified by preparative TLC (Merck, Kiesel gel 60 precoated plate, 0.5 mm thickness, developed with acetone–ethyl acetate (1 : 2)). The *p*-bromophenacyl ester was analyzed by HPLC with methanol–water (83 : 17) as an eluting solvent at a flow rate of 0.7 ml/min (results are shown in Fig. 1).

Diastereoisomeric Mixture of *p*-Bromophenacyl 3α,7α,12α,24-Tetrahydroxy-5β-cholestan-26-oate (10a—d)——

The stereoisomeric mixture of **1a—d** was prepared by the Reformatsky reaction of 3α,7α,12α-triacetoxy-5β-cholan-24-al with ethyl bromopropionate followed by hydrolysis according to the published method.[3] The acid **1a—d** (2.62 g) was converted into the *p*-bromophenacyl ester **10a—d** (2.67 g, 75%) as described above.

Separation of 10a—d by Silica Gel Flash Chromatography——The ester (2.0 g) was separated into a less polar component **10a, b** (591 mg) and a more polar component **10c, d** (471 mg) by silica gel (150 g) flash chromatography with benzene–acetone (4 : 1) as an eluting solvent.

Preparative HPLC Separation of 10a, b/10c, d into the Isomers 10a/10b and 10c/10d——The less polar component **10a, b** was separated by preparative HPLC (methanol–H_2O (4 : 1) as an eluting solvent, flow rate 10 ml/min, monitored by UV absorption measurement at 254 nm) into **10a** (shorter retention time) (216 mg) and **10b** (longer retention time) (166 mg). Similarly, the more polar component **10c, d** (471 mg) was separated into **10c** (shorter retention time) (176 mg) and **10d** (longer retention time) (122 mg).

10a: mp 155—156 °C (from acetone–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 1.02 (3H, d, J=6.4 Hz, 21-H_3), 1.23 (3H, d, J=7.3 Hz, 27-H_3), 2.75 (1H, dq, J=3.1, 7.1 Hz, 25-H), 3.22 (1H, m, 24-H), 3.45 (1H, m, 3-H), 3.85 (1H, m, 7-H), 4.00 (1H, m, 12-H), 5.26, 5.52 (1H each, d, J=16.0 Hz, $COOCH_2CO$), 7.67, 7.80 (2H each, d, J=10.0 Hz, Ph). *Anal.* Calcd for $C_{35}H_{52}BrO_7$: C, 63.25; H, 7.88. Found: C, 63.35; H, 7.78.

10b: Amorphous. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 1.02 (3H, d, J=6.4 Hz, 21-H_3), 1.28 (3H, d, J=7.2 Hz, 27-H_3), 2.70 (1H, quintet, J=6.9 Hz, 25-H), 3.41 (1H, m, 3-H), 3.69 (1H, m, 24-H), 3.86 (1H, m, 7-H), 4.00 (1H, m, 12-H), 5.32, 5.46 (1H each, d, J=16.0 Hz, $COOCH_2CO$), 7.66, 7.80 (2H each, d, J=10.0 Hz, Ph). *Anal.* Calcd for $C_{35}H_{52}BrO_7$: C, 63.25; H, 7.88. Found: C, 63.42; H, 7.73.

10c: Amorphous. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 1.02 (3H, d, J=5.4 Hz, 21-H_3), 1.24 (3H, d, J=6.8 Hz, 27-H_3), 2.76 (1H, dq, J=3.1, 7.1 Hz, 25-H), 3.20 (1H, m, 24-H), 3.45 (1H, m, 3-H), 3.86 (1H, m, 7-H), 4.00 (1H, m, 12-H), 5.25, 5.53 (1H each, d, J=16.0 Hz, $COOCH_2CO$), 7.67, 7.81 (2H each, d, J=9.5 Hz, Ph). *Anal.* Calcd for $C_{35}H_{52}BrO_7$: C, 63.25; H, 7.88. Found: C, 63.39; H, 7.90.

10d: mp 141—142 °C (from acetone–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-H_3), 0.90 (3H, s, 19-H_3), 1.01 (3H, d, J=5.9 Hz, 21-H_3), 1.27 (3H, d, J=6.8 Hz, 27-H_3), 2.70 (1H, quintet, J=6.9 Hz, 25-H), 3.42 (1H, m, 3-H), 3.71 (1H, m, 24-H), 3.85 (1H, m, 7-H), 4.00 (1H, m, 12-H), 5.33, 5.45 (1H each, d, J=16.0 Hz, $COOCH_2CO$), 7.67, 7.81 (2H each, d, J=9.5 Hz, Ph). *Anal.* Calcd for $C_{35}H_{52}BrO_7$: C, 63.25; H, 7.88. Found: C, 63.32; H, 7.83.

Alkaline Hydrolysis of the *p*-Bromophenacyl Ester into the Corresponding Acids——A mixture of 5% aqueous KOH (2 ml) and a solution of the ester **10a** (30 mg) in methanol (4 ml) was stirred at 0 °C for 2 h. Acidification with 2 N HCl and extractive work-up (ethyl acetate) gave a residue, which was purified on a Lobar column as described above to give the acid **1a** (20 mg, 95%). The other isomers **1b—d** were obtained in the same manner from the corresponding esters **10b—d**. These acids were crystallized from ethyl acetate and obtained as an amorphous solid. The homogeneity of the solid was confirmed by ^{1}H-NMR, ^{13}C-NMR, and reversed-phase TLC (see below). However, the solid samples of **1a—d** showed a rather broad melting point at *ca.* 115 °C (measured after drying *in vacuo* at 50 °C for 2 h). Further purification of **1a—d** using reversed-phase (ODS) and/or Sephadex LH-20 column chromatography followed by crystallization from ethyl acetate did not increase the melting point. *Rf* values of **1a—d** on reversed-phase TLC (Merck, RP-18 F_{254S} HPTLC plate, developed with methanol–H_2O (9 : 1)) are 0.54 (**1a**), 0.51 (**1b**), 0.51 (**1c**), and 0.49 (**1d**).

1a: ^{1}H-NMR (d_5-pyridine) δ: 0.81 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.26 (3H, d, J=6.3 Hz, 21-H_3), 1.58 (3H, d, J=6.8 Hz, 27-H_3), 3.75 (1H, m, 3-H), 4.09 (1H, m, 7-H), 4.25 (1H, m, 12-H), 4.44 (1H, m, 24-H); (CD_3OD) δ: 0.71 (3H, s, 18-H_3), 0.92 (3H, s, 19-H_3), 1.02 (3H, d, J=6.3 Hz, 21-H_3), 1.12 (3H, d, J=7.4 Hz, 27-H_3), 3.80 (1H, m, 7-H), 3.95 (1H, m, 12-H).

1b: ^{1}H-NMR (d_5-pyridine) δ: 0.81 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.26 (3H, d, J=6.3 Hz, 21-H_3), 1.44 (3H, d, J=7.3 Hz, 27-H_3), 3.75 (1H, m, 3-H), 4.09 (1H, m, 7-H), 4.18 (1H, m, 24-H), 4.25 (1H, m, 12-H); (CD_3OD) δ: 0.71 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 0.99 (3H, d, J=6.4 Hz, 21-H_3), 1.27 (3H, d, J=7.2 Hz, 27-H_3), 3.82 (1H, m, 7-H), 3.95 (1H, m, 12-H).

1c: ^{1}H-NMR (d_5-pyridine) δ: 0.81 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.26 (3H, d, J=6.3 Hz, 21-H_3), 1.59 (3H, d, J=6.8 Hz, 27-H_3), 3.75 (1H, m, 3-H), 4.09 (1H, m, 7-H), 4.25 (1H, m, 12-H), 4.40 (1H, m, 24-H); (CD_3OD) δ: 0.71 (3H, s, 18-H_3), 0.92 (3H, s, 19-H_3), 1.01 (3H, d, J=5.9 Hz, 21-H_3), 1.13 (3H, d, J=7.1 Hz, 27-H_3), 3.78 (1H, m, 7-H), 3.95 (1H, m, 12-H).

1d: ^{1}H-NMR (d_5-pyridine) δ: 0.81 (3H, s, 18-H_3), 1.01 (3H, s, 19-H_3), 1.26 (3H, d, J=6.3 Hz, 21-H_3), 1.45 (3H, d, J=7.1 Hz, 27-H_3), 3.75 (1H, m, 3-H), 4.09 (1H, m, 7-H), 4.20 (1H, m, 24-H), 4.25 (1H, m, 12-H); (CD_3OD) δ: 0.71 (3H, s, 18-H_3), 0.92 (3H, s, 19-H_3), 1.01 (3H, d, J=6.2 Hz, 21-H_3), 1.16 (3H, d, J=7.1 Hz, 27-H_3), 3.79 (1H, m, 7-H), 3.95 (1H, m, 12-H).

Acknowledgement The authors wish to thank Dr. Yasuaki Ogawa, Pharmaceutical Development Laboratory, Meiji Seika Kaisha Ltd., for the preparative HPLC facilities.

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