Potential Bile Acid Metabolites. 25. Synthesis and Chemical Properties of Stereoisomeric 3α,7α,16- and 3α,7α,15-Trihydroxy-5β-cholan-24-oic Acids[1)]

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Epimeric 3α,7α,16- and 3α,7α,15-trihydroxy-5β-cholan-24-oic acids and some related compounds were synthesized from chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), respectively. The key reaction involved one-step remote oxyfunctionalization of unactivated methine carbons at C-17 of CDCA and at C-14 of UDCA as their methyl ester-peracetate derivatives with dimethyldioxirane (DMDO). After dehydration of the resulting 17α- and 14α-hydroxy derivatives with $POCl_3$ or conc. H_2SO_4, the respective Δ^{16}- and Δ^{14}-unsaturated products were subjected to hydration *via* hydroboration followed by oxidation to yield the 3,7,16- and 3,7,15-triketones, respectively. Stereoselective reduction of the respective triketones with *tert*-butylamine-borane complex afforded the epimeric 3α,7α,16- or 3α,7α,15-trihydroxy derivatives exclusively. A facile formation of the corresponding ε-lactones between the side chain carboxyl group at C-24 and the 16α- (or 16β-) hydroxyl group in bile acids is also clarified.

Key words remote oxyfunctionalization; dimethyldioxirane; 3α,7α,16-trihydroxy-5β-cholan-24-oic acid; 3α,7α,15-trihydroxy-5β-cholan-24-oic acid; ε-lactone

The 16- and 15-hydroxy derivatives of chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid: CDCA; **1**), and deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid: DCA), are of sustained interest in synthetic, biological, physiological, and metabolic studies, because they are major bile acids in some vertebrates. 3α,12α,16α-Trihydroxy-5β-cholan-24-oic acid, named as "pythocholic acid", was isolated some years ago by Haslewood and Wootton from certain primitive snakes (boas and pythons), including *Cylindrophis*.[2,3)] Hagey *et al.* have recently reported the existence of "avicholic acid", 3α,7α,16α-trihydroxy acid, as a primary bile acid in many birds such as herons (*Ardeidae*), pelicans (*Pelecanidae*), and owls (*Tytonidae*).[4—8)] Meanwhile, the 15α-hydroxy derivative of CDCA (**1**) and its C-24 sulfonate analogue have also been shown to be major bile acids in marsupials[9)] and in the liver of hamsters,[10)] respectively.

However, a definitive structural determination of the 16- and 15-hydroxy bile acids, particularly for the stereochemical configuration of a hydroxyl group at the C-16 and -15 positions, remains uncertain, due to the unavailability of authentic reference compounds. Unequivocal proof of their identification requires chemical synthesis and demonstration of identity of the isolated compounds with synthetic ones. The availability of these uncommon bile acids as authentic specimens also provides an unique opportunity to study the chemical, biological, and physicochemical properties of such organic molecules.

Chemical and/or microbiological syntheses of some of 16- and 15-hydroxy bile acids have been reported by several groups of workers. Beque *et al.*[11)] have reported that the thermolysis of peroxy 5β-cholanoic acid in *n*-octane gives a mixture of 16α- and 16β-hydroxy-5β-cholanes. Kimura *et al.*[12,13)] have reported chemical synthesis of 15α-hydroxy lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid: LCA) and DCA using a ferrous ion-molecular oxygen system. In more recent works, the 15β-hydroxylation of LCA and DCA has been attained by microbiological transformation employing *Penicillium* species, *Rhizoctonia solani* and *Absidia coerulea*[14)] or *Cunninghamella blakesleena* St-22.[15,16)]

To our knowledge, however, chemical and/or microbiological syntheses of 16- and 15-hydroxy derivatives of a primary bile acid, CDCA, have hitherto been unreported. For our series of studies on new and uncommon potential bile acids and their metabolites, we report here an effective, short step synthesis of epimeric 3α,7α,16- (**3**, **4**) and 3α,7α,15-trihydroxy-5β-cholan-24-oic acids (**5**, **6**), as well as a related stereoisomer (**18**), starting from **1** or ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid: UDCA; **2**), respectively (Chart 1). In addition, the differences in the chemical properties between the 16- and 15-hydroxy bile acids were also clarified.

Results and Discussion

Introduction of an oxygen-containing function at the C-16 and -15 positions of the five-membered D-ring in CDCA (**1**) has not yet been accomplished, probably due to strong shieldings not only by the β-attached C_5 alkyl side chain, but also by the axially-oriented 7α-hydroxyl group.

As outlined in Charts 2 and 3, a key strategic point in the present work was to make use of 17α- and 14α-hydroxy intermediates (**9a**, **10a**), which were attained in one-step from the methyl ester-diacetate derivatives (**7a**, **8a**) of **1** and **2**, respectively. Application of our recent works[17,18)] involving a highly efficient, stereoselective remote oxyfunctionalization of unactivated methine carbons in steroids with a powerful oxidant, dimethyldioxirane (DMDO), has led to direct inser-

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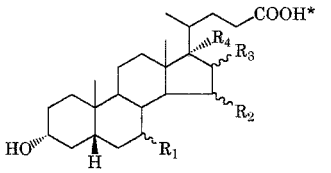
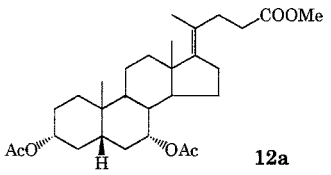
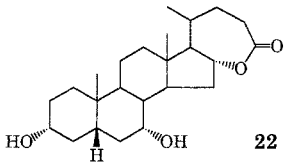


	R_1	R_2	R_3	R_4
1	α-OH	H	H	H
2	β-OH	H	H	H
3	α-OH	H	α-OH	H
4	α-OH	H	β-OH	H
5	α-OH	α-OH	H	H
6	α-OH	β-OH	H	H
18	β-OH	β-OH	H	H
21	α-OH	H	H	OH

* The corresponding C-24 methyl esters were designated "a".



12a



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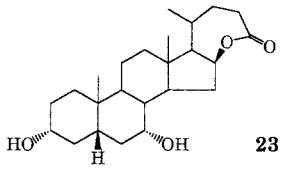

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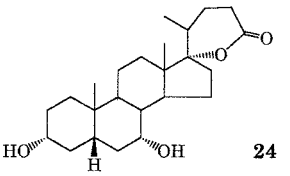

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Chart 1

tion of an oxygen atom at the methine protons of C-17 in **7a** and of C-14 in **8a**. Thus, when **7a** was treated with a freshly prepared solution of DMDO (0.32 mol) in $CHCl_3$ for 24 h at room temperature, the desired 17α-hydroxy ester (**9a**) was isolated in 13% yield, after careful purification of the oxidation product by normal-phase (NP) medium-pressure liquid chromatography (MPLC) on silica gel, eluting with $CHCl_3$–methanol (99 : 1, v/v). Both the experimental and work-up procedures for the DMDO reaction were very simple and straightforward as described in detail in the Experimental section. A similar remote oxyfunctionalization was also attained, when the ester-peracetate (**8a**) was subjected to the DMDO oxidation to give the 14α-hydroxy ester (**10a**) in 12% isolated yield. The observed difference in the regioselectivity of oxyfunctionalization between **7a** and **8a** was explained in terms of the steric environment as well as the degree of electron density of the target methine carbons in the substrates.[17,18]

Subsequent treatment of the ester (**9a**) in pyridine with phosphoryl chloride ($POCl_3$) at 65 °C for 24 h led to elimination of the 17α-hydroxyl group. The resulting dehydration product, which consisted of a mixture of two components having essentially identical mobility on TLC, was efficiently separated by NP-MPLC on a column of silica gel, eluting with a mixture of toluene–Et_2O (9 : 1, v/v). The less polar, major component (41% isolated yield) was characterized as the Δ^{16}-ester (**11a**),[19] and the more polar, minor component (30%) as the $\Delta^{17(20)}$-isomers (**12a**). The assignment of the (*E*)-configuration (*i.e.*, *cis* relationship between 18- and 21-methyl groups) to **12a** followed from a previous ^{1}H-NMR finding[20]; the 21-methyl proton in the (*E*)-isomer of 17(20)-dehydrocholesterol appears at 1.68 ppm, whereas that in the (*Z*)-isomer occurs at 1.55 ppm. For **12a** the ^{1}H-NMR spectrum showed the 21-methyl proton at 1.69 ppm as a singlet, while two olefinic quaternary carbon signals were observed at 122.6 and 144.2 ppm in the ^{13}C-NMR. Elimination of the 14α-hydroxyl group in the ester **10a** was successfully achieved by treating with conc. H_2SO_4 in dioxane at room temperature overnight to give the Δ^{14}-ester (**13a**) exclusively (73% isolated yield).

Hydroboration, followed by oxidation with alkaline hydrogen peroxide of the Δ^{16}- and Δ^{14}-esters (**11a**, **13a**) would be expected to yield the corresponding 16α- and 15α-hydroxy products, respectively.[21] In preliminary experiments, when dicyclohexylborane, disiamylborane or 9-borabicyclo[3.3.1]-nonanone (9-BBN) was employed as a hydroborating reagent to avoid simultaneous reduction of the methyl ester at C-24,[12,13,22] each reagent was completely inert at 0 °C and/or room temperature against **11a** and **13a**, probably because of a steric hindrance of the bulky dialkylboranes. By changing the reagent to less bulky diborane (B_2H_6),[23] however, the hydroboration-oxidation reaction of **11a** and **13a** proceeded smoothly at an elevated temperature of 50 °C and/or room temperature for 2 h,[24,25] but was accompanied by simultaneous reduction of the methyl ester at C-24 as well as hydrolysis of the acetoxyl groups at C-3 and C-7, to produce the 3α,7α,16α,24- and 3α,7β,15α,24-tetrols (**14**, 90% and **15**, 72%) in good isolated yields, respectively. The α-configuration of a hydroxyl group at C-16 in **14** and at C-15 in **15** was based on the comparison of their ^{1}H-NMR chemical shifts and signal multiplicity with those of related compounds (*i.e.*, **3a**, **5a**) as described below in detail.

Eventually, the desired stereoisomeric 3α,7α,16- and 3α,7α,15-trihydroxy esters (**3a**—**6a**) were prepared from the tetrols **14** and **15**, *via* the intermediates, 3,7,16- and 3,7,15-trioxo esters (**16a**, **17a**), respectively. Thus, the preparation of

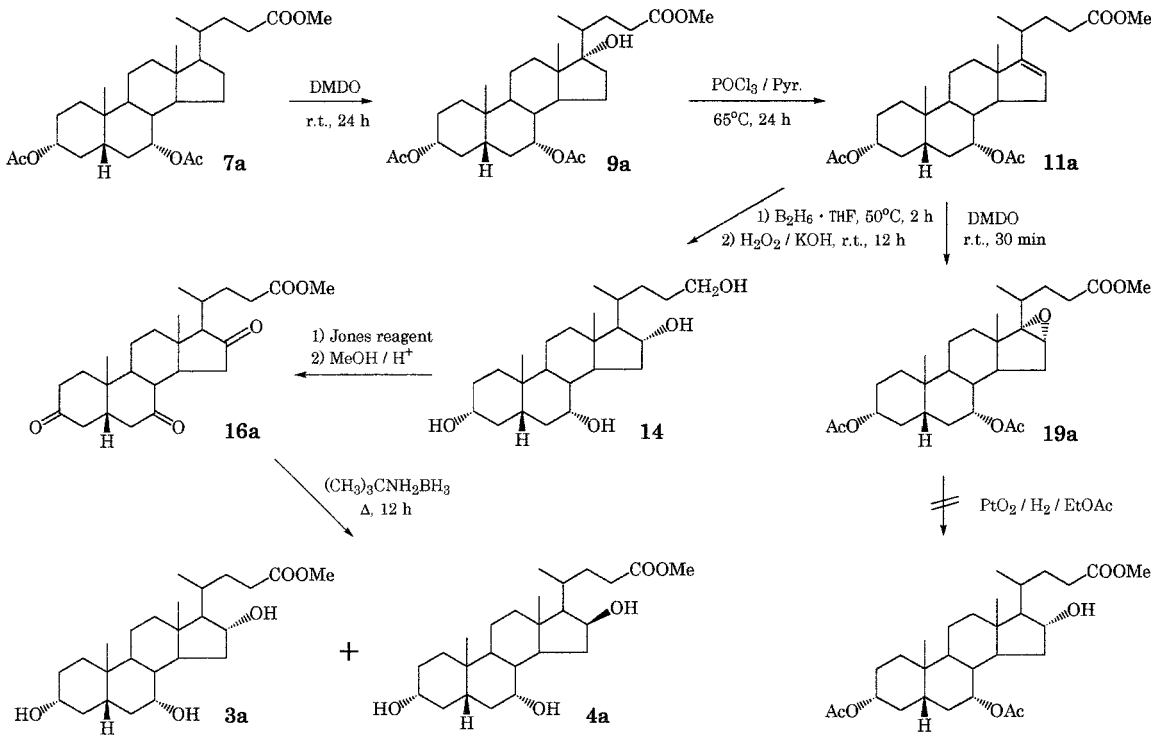

Chart 2

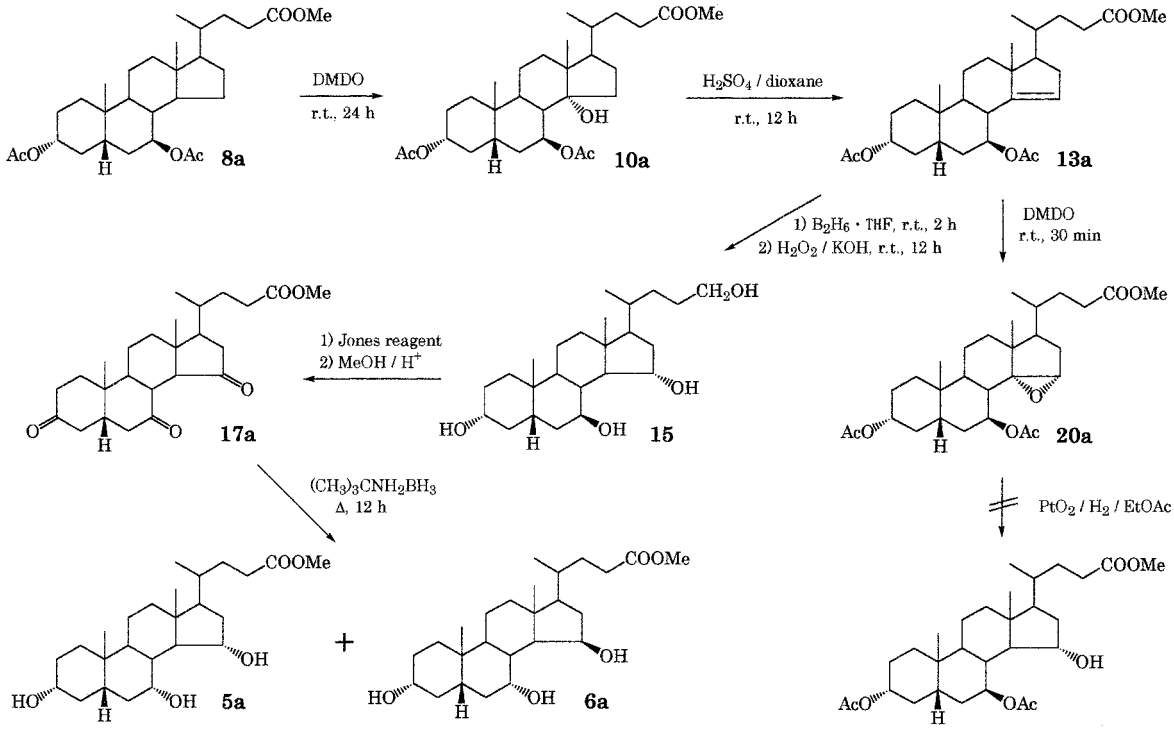

Chart 3

16a (or **17a**) was carried out by oxidation of **14** (or **15**) with Jones reagent at room temperature for 30 min and subsequent re-esterification of the resulting 3,7,16- (or 3,7,15-) trioxo acid.

Stereoselective reduction of an oxo group to a hydroxyl in steroids has been studied extensively by many investigators.[26,27] Depending on both the reducing agents and reaction conditions employed, either α- or β-alcohols, or the epimeric mixture may be obtained. Our exploratory experiments in which reduction of the 3,7,16-triketone (**16a**) with various reducing agents such as liq. NH_3/Li, $Zn(BH_4)_2$, $NaBH_4$/$PdCl_2$, *tert*-amyl alchohol/K, or *K*-Selectride for preparing methyl 3α,7α,16-trihydroxy-5β-cholan-24-oate stereoselectively, gave an unsatisfactory result, *i.e.*, a complicated mix-

Table 1. ^{1}H-NMR Chemical Shifts of Stereoisomeric Methyl 3α,7α,16- and 3α,7α,15-Trihydroxy-5β-cholan-24-oates (**3a**—**6a**)

	18-CH_3	19-CH_3	21-CH_3	3β-H	7β-H	15-H	16-H	$COOCH_3$
3a	0.67 (s)	0.89 (s)	0.95 (d, 5.4)	3.43 (br m)	3.84 (m)		4.01 (d, 2.7)	3.66 (s)
4a	0.87 (s)	0.91 (s)	0.97 (d, 6.3)	3.46 (br m)	3.88 (m)		4.50 (br m)	3.70 (s)
$\Delta\delta$	0.20	0.02	0.02	0.03	0.04		0.50	0.04
5a	0.67 (s)	0.90 (s)	0.91 (d, 5.6)	3.46 (br m)	4.03 (m)	4.03 (m)		3.67 (s)
6a	0.95 (s)	0.95 (s)	0.94 (d, 5.2)	3.48 (br m)	4.18 (m)	4.31 (t, 5.4)		3.67 (s)
$\Delta\delta$	0.28	0.05	0.03	0.02	0.15	0.28		0.00

s=singlet, d=doublet, t=triplet, m=multiplet, br m=broad multiplet; values in parentheses refer to coupling constant (J in Hz); $\Delta\delta$ value means the difference in the chemical shifts between **4a** *vs.* **3a** and **6a** *vs.* **5a**.

Table 2. ^{13}C-NMR Chemical Shifts of Stereoisomeric Methyl 3α,7α,16- and 3α,7α,15-Trihydroxy-5β-cholan-24-oates (**3a**—**6a**)

Carbon	**3a**	**4a**	$\Delta\delta^{a)}$	**5a**	**6a**	$\Delta\delta^{a)}$
1	35.3	35.2	−0.1	35.3	35.2	−0.1
2	30.6	30.6	0	30.5	30.7	0.2
3	72.1	71.9	−0.2	71.9	72.0	0.1
4	39.4	39.7	0.3	40.1	39.9	−0.2
5	41.6	41.4	−0.2	41.4	41.4	0
6	34.1	34.8	0.7	33.4	35.4	2.0
7	68.3	68.3	0	67.7	68.0	0.3
8	38.8	39.0	0.2	39.0	35.2	−3.8
9	32.7	32.8	0.1	33.8	33.1	−0.7
10	35.0	35.0	0	34.8	35.2	0.4
11	20.3	20.2	−0.1	20.6	20.5	−0.1
12	40.0	39.8	−0.2	40.3	41.0	0.7
13	43.9	42.4	−1.5	43.8	42.3	−1.5
14	47.5	48.3	0.8	58.0	56.0	−2.0
15	36.2	34.8	−1.4	72.4	70.5	−1.9
16	76.7	72.1	−4.6	39.3	41.8	2.5
17	66.3	61.9	−4.4	54.1	54.8	0.7
18	13.1	12.8	−0.3	13.1	14.5	1.4
19	22.8	22.8	0	22.8	22.7	−0.1
20	33.8	29.3	−4.5	34.8	35.1	0.3
21	18.6	17.9	−0.7	18.0	18.3	0.3
22	30.8	30.2	−0.6	30.8	30.9	0.1
23	31.0	30.2	−0.8	30.8	30.9	0.1
24	175.0	175.8	0.8	174.7	174.7	0
$COO\underline{C}H_3$	51.5	51.9	0.4	51.5	51.5	0

a) Difference in the chemical shifts between **4a** *vs.* **3a** and **6a** *vs.* **5a**.

ture. However, when *tert*-butylamine–borane complex (*tert*-$C_4H_9NH_2\cdot BH_3$)[27] was applied for the reduction of **16a**, the reduction reaction proceeded cleanly in refluxing CH_2Cl_2 for 12 h to give a mixture of 3α,7α,16α- and 3α,7α,16β-trihydroxy esters (**3a**, **4a**) in an approximate ratio of 1 : 2. The two epimers could be separated cleanly by NP-MPLC on silica gel, eluting with EtOAc–methanol (95 : 5, v/v). The above finding may suggest that the reagent attacks from a less hindered α-side of the D-ring in **16a** to yield **4a** as the major product. Analogously, reduction of 3,7,15-triketone **17a** with *tert*-$C_4H_9NH_2\cdot BH_3$ also afforded a similar epimeric mixture of 3α,7α,15α- and 3α,7α,15β-trihydroxy esters (**5a**, **6a**), accompanied by the simultaneous formation of 3α,7β,15β-trihydroxy ester (**18a**) in an approximate ratio of 1 : 1 : 1. The three diastereomers were successfully resolved by reversed-phase (RP)-MPLC on a C_{18}-bonded silica gel, eluting with methanol–water (8 : 2—7 : 3, v/v). Thus, of the eight possible stereoisomeric 3,7,16- or 3,7,15-trihydroxy compounds, essentially only the two or three desired 16- or 15-epimeric mixtures were efficiently obtained by reduction of **16a** or **17a** with *tert*-$C_4H_9NH_2\cdot BH_3$, respectively. Usual alkaline hydrolysis of the individual 3α,7α,16- and 3α,7,15-trihydroxy esters (**3a**—**6a**, **18a**) with methanolic potassium hydroxide, followed by acidification with H_2SO_4 afforded the corresponding free acids (**3**—**6**, **18**) nearly quantitatively. The physical and spectral properties of 3α,7α,16α-triols (**3a**) were virtually consistent with those reported recently.[7,8]

Determination of the position and stereochemistry of a 16- and 15-hydroxyl group in five-membered D-ring in bile acids has been unclear for a long time, probably owing to the unavailability of authentic reference compounds. We have now in hand a complete set of the four possible stereoisomeric 3α,7α,16- and 3α,7α,15-trihydroxy compounds (**3a**—**6a**). Tables 1 and 2 show the ^{1}H- and ^{13}C-NMR spectral data for **3a**—**6a**, respectively. Differentiation between the 16α- and 16β-epimers (**3a** *vs.* **4a**) was made by the ^{1}H-NMR spectra, in which the 18-methyl signal in **4a** resonates at lower-field by 0.20 ppm than that of **3a**,[28] due to the pseudo-1,3-diaxial relationship. Also, the 16α-H (br m) in **4a** appeared at much lower field by 0.50 ppm than the corresponding 16β-H (d) in **3a**. Similar ^{1}H-NMR differences in the chemical shifts and signal multiplicity between the 15α- and 15β-epimers (**5a**, **6a**) were observed for the 18-methyl[12—14] [0.67 ppm (s) in **5a** and 0.95 ppm (s) in **6a**], 15-H[10,14] [4.03 ppm (m) in **5a** and 4.31 ppm (t) in **6a**] and 7β-H [4.03 ppm (m) in **5a** and 4.18 ppm (m) in **6a**] resonances.

In the ^{13}C-NMR spectra, the C-3 (71.9—72.1 ppm) and C-7 (67.7—68.3 ppm) carbon signals in **3a**—**6a** had essentially identical chemical shifts and the values were found to be very similar to those of the parent methyl 3α,7α-dihydroxy-5β-cholan-24-oate.[29] However, the C-16 in **3a** (76.7 ppm) and **4a** (72.1 ppm) and the C-15[10,16] in **5a** (72.4 ppm) and **6a** (70.5 ppm) much differed from one another, depending upon the position and stereochemistry of the hydroxyl groups. In this connection, differences in the chemical shifts of the neighboring β-carbons (C-15, -17 in **3a** and **4a**; C-14, -16 in **5a** and **6a**) and γ-carbons (C-13, -20 in **3a** and **4a**; C-8, -13 in **5a** and **6a**) between the 16- or 15-epimeric pairs are also noteworthy.

Meanwhile, the mass spectra of **3a**—**6a** measured as their trimethylsilyl (TMS) ether derivatives are shown in Table 3. The spectral pattern of each of the epimeric pairs was very similar to each other, but it much differed between 3α,7α,16- (**3a**, **4a**) and 3α,7α,15-triols (**5a**, **6a**). For example, the base peak constituted of an ion at m/z 368 (M−3TMSOH) in the 3α,7α,16-triols which is absent in the spectra of 3α,7α,15-ones and of an ion at m/z 458 (M−2TMSOH) in the 3α,7α,15-triols.

Table 3. Mass Spectral Fragment Ions of Stereoisomeric Methyl 3α,7α,16- and 3α,7α,15-Trihydroxy-5β-cholan-24-oates (**3a**—**6a**)[a)]

Major ions		Relative intensity (%)			
		3a	**4a**	**5a**	**6a**
638	M	3	3	1	
623	M−CH_3	14	11		
548	M−TMSOH	16	17	21	3
533	M−TMSOH−CH_3				4
458	M−2TMSOH	44	44	100	100
443	M−2TMSOH−CH_3			20	17
368	M−3TMSOH	100	100		
364				18	16
353	M−3TMSOH−CH_3	35	30		9
329		34	35		
253	M−3TMSOH−S.C.	22	20		68

a) Measured as the trimethylsilyl ether derivatives; TMSOH, trimethylsilanol; S.C., side chain.

DMDO, a powerful oxidant, which was successfully used for one-step remote-oxyfunctionalization of **7a** and **8a** to **9a** and **10a**, respectively, was also an attractive agent for the stereoselective epoxidation of a double bond.[30)] By treatment of Δ^{16}- and Δ^{15}-esters (**11a**, **13a**) with a solution of DMDO in $CHCl_3$, epoxidation reaction proceeded rapidly under mild conditions (30 min at room temperature) to give the corresponding 16α,17α- and 14α,15α-epoxides (**19a**, **20a**), respectively, in a high isolated yield of 92—94%. The stereochemistry of the resulting epoxides was determined on the basis of the ^{1}H chemical shifts and signal patterns of the 16β-H (3.32 ppm as s) in **19a** and 15β-H (3.20 ppm as s) in **20a**, in comparison with those reported for analogous 16α,17α-epoxy-5α-cholestane-3β,5α-diol[31)] and methyl 3α-cathyloxy-14α,15α-epoxy-5β-cholan-24-oate.[30)] Subsequent hydrogenolysis of **19a** and **20a** would be expected to yield 3α,7α-diacetoxy-16α-hydroxy and 3α,7α-diacetoxy-15α-hydroxy esters, respectively.[32)] However, attempted reductive cleavage of **19a** and **20a** with H_2 in the presence of PtO_2 in EtOAc under atmospheric pressure was unsuccessful and found to be completely inert against the conditions employed.

As expected, esterification of 16α- and 16β-hydroxy acids (**3**, **4**) dissolved in methanol with a solution of diazomethane in ether afforded the corresponding methyl esters (**3a**, **4a**) quantitatively, according to chromatographic and ^{1}H-NMR analyses. However, conventional treatment of the same acid **3** (or **4**) with *p*-toluenesulfonic acid or conc. HCl in methanol always produced a mixture of two components, one (a more polar component) of which was in accord with the methyl ester **3a** (or **4a**). According to the finding of Haslewood *et al.*,[2,33)] pythocholic acid, 3α,12α,16α-trihydroxy-5β-cholan-24-oic acid, forms readily the corresponding pythocholic lactone by dissolving the free acid in NaOH solution, acidified with H_2SO_4, and then warmed to 70 °C for 15 h. When each of the acids **3** and **4** dissolved in EtOAc was treated with a catalytic amount of *p*-toluenesulfonic acid or conc. HCl for 2 h at room temperature, a single reaction product, which was identical with the respective less polar component mentioned above, was formed, and their structures were characterized as the corresponding ε-lactones, 3α,7α-dihydroxy-5β-cholane *O*-24,16α-lactone (**22**)[7)] and 3α,7α-dihydroxy-5β-cholane *O*-24,16β-lactone (**23**), respectively, based on the chromatographic behaviors, ^{1}H-NMR and mass fragmentation patterns. The above result indicates that, under the usual acid-catalyzed conditions, intramolecular esterification between the side chain carboxyl group at C-24 and the hydroxyl group at C-16 in **3** and **4** occurred preferentially. In the ^{1}H-NMR spectra of **22** and **23**, no methyl ester signal occurrs at *ca.* 3.65 ppm. On the other hand, the mass spectra of the trimethylsilyl (TMS) ether derivatives of **22** and **23** showed intense fragments at *m*/*z* 444 and 354[7)] originating from the loss of one and two trimethylsilanol molecules (TMSOH) from the molecular ion (M^+, *m*/*z* 534, <1%), and a diagnostic peak at *m*/*z* 253 originating from the further loss of the lactone ring ($C_5H_9O_2$) from the ion at *m*/*z* 354. In addition, ions at *m*/*z* 429 and 339 arising from the sequential loss of CH_3 from the ions at *m*/*z* 444 and 354 always accompanied in the mass spectra. A similar lactonization of the side chain carboxyl group was also occurred by treating 17α-hydroxy acid (**21**), derived from **9a** by alkaline hydrolysis, with *p*-toluenesulfonic acid in EtOAc to give the corresponding δ-lactone, 3α,7α-dihydroxy-5β-cholane *O*-24,17α-lactone (**24**).

On the contrary, 15α- and 15β-hydroxy acids (**5**, **6**, **18**) were found to be completely insensitive to lactonization and afforded the expected methyl esters with methanol-conc. HCl (**5a**, **6a**, **18a**). Since there was no evidence for differentiating between 16- and 15-hydroxy bile acids, the above observation may be usefully utilized for their characterization.

Experimental

Melting points (mp) were determined on a micro hot-stage apparatus and are uncorrected. IR spectra were obtained on a Bio Rad FTS-7 FT-IR spectrometer (Philadelphia, U.S.A.) as KBr tablets. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM-EX 270 FT NMR instrument (Tokyo, Japan) at 270 and 68.80 MHz, respectively, with $CDCl_3$ or $CDCl_3$ containing 10—30% DMSO-d_6 as the solvent; chemical shifts are expressed as δ ppm relative to tetramethylsilane. Electron impact low-resolution mass spectra (LR-MS) were recorded on a JEOL JMC-Automass 150 gas chromatograph-mass spectrometer at 70 eV. High-resolution mass spectra (HR-MS) was obtained on a JEOL JMS-LCmate double focusing magnetic mass spectrometer equipped with an electrospray ionization (ESI) or an atmospheric pressure chemical ionization (APCI) under the positive ion mode (PIM) or the negative ion mode (NIM). The apparatus used for MPLC consisted of a Shimamura YRD-880 RI-detector (Tokyo, Japan) and an uf-3040S chromatographic pump using silica gel 60 (230—400 mesh, Nakalai Tesque, Kyoto, Japan) as the NP adsorbent or ODS-AM 120-S50 as the RP adsorbent (YMC Co. Ltd., Kyoto, Japan). TLC was performed on pre-coated silica gel (0.25 mm layer thickness; E. Merck, Germany) using hexane–EtOAc–acetic acid mixtures (80 : 20 : 1—20 : 80 : 1, v/v/v) as the developing solvent.

A solution (0.32 mol/l) of DMDO in $CHCl_3$ was prepared according to literature method using oxone® and acetone.[17,18)] All compounds were dried by azeotropic distillation before use in reactions.

Methyl 3α,7α-Diacetoxy-17α-hydroxy-5β-cholan-24-oate (9a) To a solution of CDCA methyl ester–diacetate (**7a**) (2.0 g) in CH_2Cl_2 (10 ml) was added a freshly prepared solution of DMDO (0.35 mol/l; 24 ml) in $CHCl_3$ with an ice-bath cooling. The mixture was left to stand at room temperature for 12 h, and excess amounts of the reagent and solvent were evaporated under reduced pressure. The above procedure was repeated (two runs, total reaction time, 24 h), and the reaction product, which consisted of four major components (**9a** and two monohydroxy and one dihydroxy compounds), was poured into a column of silica gel (70—230 mesh, 60 g). Elution with benzene–EtOAc (4 : 1, v/v) afforded the starting compound **7a**; 790 mg (40%). Continued elution with the same solvent system gave a mixture of two monohydroxy components (750 mg).

The monohydroxy fraction (see above) was then subjected to NP-MPLC on silica gel (230—400 mesh, 40 g). Elution with $CHCl_3$–methanol mixture (99 : 1, v/v) provided two well-separated components. The less polar compound (314 mg, 15%) was identified as the desired 17α-hydroxy ester **9a**,

which was recrystallized from EtOAc–hexane as colorless prisms: mp, 157—160 °C (lit.,[17] mp, 157—160 °C). IR, ν_{max} cm^{-1}: 1728 (ester C=O), 3545 (OH). ^{1}H-NMR, δ: 0.74 (s, 3H, 18-CH_3), 0.91 (d, 3H, J=7.0 Hz, 21-CH_3), 0.94 (s, 3H, 19-CH_3), 2.02 and 2.06 (s, each 3H, 3α-, 7α-OCOCH_3), 3.67 (s, 3H, COOCH_3), 4.59 (br m, 1H, 3β-H), 4.90 (br d, 1H, J=2.7 Hz, 7β-H). LR-MS, *m/z*: 446 (4%, M−AcOH), 428 (19%, M−AcOH−H_2O), 386 (100%, M−2AcOH), 368 (82%, M−2AcOH−H_2O), 353 (18%, M−2AcOH−H_2O−CH_3), 332 (91%), 313 [35%, M−AcOH−H_2O−side chain (S.C.)], 286 (12%, M−AcOH−H_2O−S.C.−ring D.), 253 (37%, M−2AcOH−H_2O−S.C.), 226 (40%, M−2AcOH−H_2O−S.C.−part of ring D.), 211 (30%, M−2AcOH−H_2O−S.C.−ring D.).

The more polar compound was recrystallized from EtOAc–hexane to give methyl 3α,7α-dihydroxy-5β-hydroxycholan-24-oate as a colorless amorphous solid: yield, 550 mg, 27%; mp, 156—158 °C (lit.,[18] mp, 158—159 °C). IR, ν_{max} cm^{-1}: 1735 (ester C=O), 3475 (OH). ^{1}H-NMR, δ: 0.65 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 0.92 (d, 3H, J=7.3 Hz, 21-CH_3), 2.03 and 2.07 (s, each 3H, 3α-, 7α-OCOCH_3), 3.67 (s, 3H, COOCH_3), 4.92 (br d, 1H, J=2.4 Hz, 7β-H), 5.02 (br m, 1H, 3β-H). LR-MS, *m/z*: 428 (19%, M−AcOH−H_2O), 386 (100%, M−2AcOH), 368 (81%, M−2AcOH−H_2O), 353 (17%, M−AcOH−H_2O−CH_3), 332 (91%), 313 (35%, M−AcOH−H_2O−S.C.), 286 (13%, M−AcOH−H_2O−S.C.−part of ring D.), 271 (92%, M−2AcOH−S.C.), 253 (37%, M−2AcOH−H_2O−S.C.), 226 (40%, M−2AcOH−H_2O−S.C.−part of ring D.), 211 (30%, M−2AcOH−H_2O−S.C.−ring D.).

Methyl 3α,7β-Diacetoxy-14α-hydroxy-5β-cholan-24-oate (10a) UDCA methyl ester-diacetate **8a** (2.0 g) was subjected to the remote-oxyfunctionalization with DMDO and processed as described for the preparation of **9a** to yield an oily residue, which was consisted of seven components by GC. The oil was chromatographed on a column of silica gel (70—230 mesh, 60 g). Elution with benzene–EtOAc (9 : 1, v/v) afforded the least polar **8a** and then methyl 3α,7β-diacetoxy-16-oxo-5β-cholan-24-oate.[17] Continued elution with benzene–EtOAc (7 : 3, v/v) gave a mixture of two monohydroxy compounds (470 mg), which were then purified by NP-MPLC on silica gel (230—400 mesh, 20 g). Elution with hexane–benzene–EtOAc (5 : 3 : 2, v/v/v) provided a less polar component, which was characterized as the desired 14α-hydroxy ester **10a**. Although this compound was homogeneous according to TLC and GC, it resisted crystallization: yield, 278 mg (13%). IR, ν_{max} cm^{-1}: 1737 (ester C=O), 3518 (OH). ^{1}H-NMR, δ: 0.79 (s, 3H, 18-CH_3), 0.90 (d, 3H, J=6.2 Hz, 21-CH_3), 0.98 (s, 3H, 19-CH_3), 2.00 and 2.02 (s, each 3H, 3α-, 7β-OCOCH_3), 3.67 (s, 3H, COOCH_3), 4.66 (br m, 1H, 3β-H), 5.15 (br m, 1H, 7α-H). LR-MS, *m/z*: 428 (8%, M−AcOH−H_2O), 368 (24%, M−2AcOH−H_2O), 353 (9%, M−2AcOH−H_2O−CH_3), 314 (9%), 281 (5%), 253 (100%, M−2AcOH−H_2O−S.C.), 239 (10%), 212 (25%).

A more polar component eluting with hexane–benzene–EtOAc (5 : 1 : 4, v/v/v) afforded (20*S*)-3α,7β-diacetoxy-5β-cholane *O*-24,20-lactone, which was recrystallized from aqueous methanol as colorless amorphous solid: yield, 190 mg (10%); mp, 202—204 °C (lit.,[17] mp, 202—204 °C). IR, ν_{max} cm^{-1}: 1743, 1771 (C=O). ^{1}H-NMR, δ: 0.84 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 1.45 (s, 3H, 21-CH_3), 2.03 and 2.05 (s, each 3H, 3α-, 7β-OCOCH_3), 4.68 (br m, 1H, 3β-H), 4.76 (br m, 1H, 7α-H). LR-MS, *m/z*: 414 (14%, M−AcOH), 354 (85%, M−2AcOH), 339 (41%, M−2AcOH−CH_3), 313 (10%), 300 (10%, M−AcOH−S.C.−CH_3), 288 (8%, M−AcOH−S.C.−part of ring D.), 281 (7%), 253 (56%), 241 (19%), 228 (94%, M−2AcOH−S.C.−part of ring D.), 227 (100%), 213 (75%, M−2AcOH−S.C.−ring D.).

Methyl 3α,7α-Diacetoxy-5β-chol-16-en-24-oate (11a) To the 17α-hydroxy ester (**9a**; 120 mg) dissolved in dry pyridine (3 ml) $POCl_3$ (1 ml) was added dropwise. After the mixture was stirred at 65 °C for 24 h, it was cooled in an ice-bath, ice chips were added gradually, and the reaction product was extracted with CH_2Cl_2. The combined extract was washed with 10% HCl and water, dried over Drierite, and evaporated. The oily residue, which consisted of two components as evidenced by TLC (four developments by benzene–Et_2O; 9 : 1, v/v), was subjected to NP-MPLC on a column of silica gel (230—400 mesh, 12 g) and elution with toluene–Et_2O (9 : 1, v/v) gave a less polar component, which was recrystallized from methanol as colorless needles and characterized as the $\Delta^{17(20)}$-ester **12a**: yield, 35 mg (30%); mp, 119—121 °C. IR, ν_{max} cm^{-1}: 1737 (ester C=O). ^{1}H-NMR, δ: 0.82 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 1.69 (s, 3H, 21-CH_3), 2.03 and 2.04 (s, each 3H, 3α-, 7α-OCOCH_3), 3.66 (s, 3H, COOCH_3), 4.59 (br m, 1H, 3β-H), 4.94 (br d, 1H, J=2.4 Hz, 7β-H). LR-MS, *m/z*: 428 (10%, M−AcOH), 413 (15%, M−AcOH−CH_3), 368 (17%, M−2AcOH), 353 (26%, M−2AcOH−CH_3), 313 (26%, M−AcOH−S.C.), 281 (100%), 253 (66%, M−2AcOH−S.C.). HR-MS (ESI-PIM) Calcd for $C_{29}H_{44}NaO_6$ [M+Na]$^+$: 511.3036. Found: *m/z*, 511.3034.

A more polar component was recrystallized from aqueous methanol as colorless thin plates and identified as the desired Δ^{16}-ester **11a**: yield, 48 mg (42%); mp, 109—111 °C (lit.,[19] mp, 109—110 °C). IR, ν_{max} cm^{-1}: 1735 (ester C=O). ^{1}H-NMR, δ: 0.73 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 1.03 (d, 3H, J=7.0 Hz, 21-CH_3), 2.02 and 2.05 (s, each 3H, 3α-, 7β-OCOCH_3), 3.66 (s, 3H, COOCH_3), 4.60 (br m, 1H, 3β-H), 4.97 (br d, 1H, J=2.7 Hz, 7β-H), 5.28 (br s, 1H, 16-H). LR-MS, *m/z*: 428 (9%, M−AcOH), 413 (15%, M−AcOH−CH_3), 368 (19%, M−2AcOH), 353 (27%, M−2AcOH−CH_3), 313 (34%, M−AcOH−S.C.), 281 (34%), 253 (100%, M−2AcOH−S.C.). HR-MS (ESI-PIM) Calcd for $C_{29}H_{44}NaO_6$ [M+Na]$^+$: 511.3036. Found: *m/z*, 511.3038.

Methyl 3α,7β-Diacetoxy-5β-chol-14-en-24-oate (13a) A solution of the 14α-hydroxy ester **10a** (250 mg) in 5% conc. H_2SO_4-dioxane (w/w, 50 ml) was allowed to stand overnight at room temperature. The reaction product was extracted with Et_2O, and the combined extract was washed successively with water, 5% $NaHCO_3$, and saturated brine, dried over Drierite, and evaporated. The oily residue was chromatographed on a column of silica gel (70—230 mesh, 20 g). The fraction eluted by benzene–EtOAc (7 : 3, v/v) afforded the title compound which resisted crystallization: yield, 175 mg (73%). IR, ν_{max} cm^{-1}: 1739 (ester C=O). ^{1}H-NMR δ: 0.92 (s, 3H, 18-CH_3), 0.93 (d, 3H, J=5.9 Hz, 21-CH_3), 0.97 (s, 3H, 19-CH_3), 2.00 and 2.01 (s, each 3H, 3α-, 7β-OCOCH_3), 3.67 (s, 3H, COOCH_3), 4.67 (br m, 1H, 3β-H), 5.01 (br s, 1H, 15-H), 5.05 (br m, 1H, 7α-H). LR-MS, *m/z*: 428 (9%, M−AcOH), 368 (62%, M−2AcOH), 353 (42%, M−2AcOH−CH_3), 314 (43%), 253 (100%, M−2AcOH−S.C.). HR-MS (ESI-PIM) Calcd for $C_{29}H_{44}NaO_6$ [M+Na]$^+$: 511.3036. Found: *m/z*, 511.3035.

3α,7α,16α,24-Tetrahydroxy-5β-cholane (14) To a stirred solution of the Δ^{16}-ester **11a** (100 mg) in dry THF (3 ml) a solution of BH_3·THF (1.0 mol; 1.3 ml) was added gradually, and the mixture was stirred at 50 °C for 2 h under a stream of N_2. After cooling the solution, 3 N-NaOH (1 ml) and then 30% H_2O_2 (1 ml) were added with an ice-bath cooling, and the mixture was stirred overnight at room temperature. The resulting solution was acidified with 10% HCl and the reaction product extracted with EtOAc. The combined EtOAc layer was washed with saturated brine and evaporated. Recrystallization of the product from EtOAc–hexane gave the 3α,7α,16α,24-tetrahydroxy-5β-cholane **14** as a colorless amorphous solid: yield, 72 mg (90%); mp, 184—187 °C. IR, ν_{max} cm^{-1}: 3332 (OH). ^{1}H-NMR ($CDCl_3$+CD_3OD, 1 : 1, v/v), δ: 0.69 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 3.46 (1H, br m, 3β-H), 3.54 (2H, br m, 24-H_2), 3.79 (m, 1H, 7β-H), 3.94 (m, 1H, 16β-H). LR-MS (as the TMS ether), *m/z*: 682 (<1%, M), 592 (19%, M−TMSOH), 577 (9%, M−TMSOH−CH_3), 565 (10%), 502 (33%, M−2TMSOH), 455 (15%), 433 (12%, M−TMSOH−S.C.), 412 (42%, M−2TMSOH), 343 (23%, M−2TMSOH−S.C.), 329 (26%), 281 (28%), 253 (100%, M−3TMSOH−S.C.), 239 (49%). HR-MS (ESI-NIM) Calcd for $C_{24}H_{41}O_4$ [M−H]$^-$: 393.3005. Found: *m/z*, 393.2997.

3α,7β,15α,24-Tetrahydroxy-5β-cholane (15) The Δ^{14}-ester **13a** (150 mg) was treated with BH_3–THF, followed by alkaline hydrogen peroxide as described for the preparation of **14**. Recrystallization of the product from EtOAc gave the 3α,7β,15α,24-tetrahydroxy-5β-cholane **15** as a colorless amorphous solid: yield, 87 mg (72%); mp, 172—174 °C. IR, ν_{max} cm^{-1}: 3251 (OH). ^{1}H-NMR, δ: 0.72 (s, 3H, 18-CH_3), 0.94 (d, 3H, J=5.6 Hz, 21-CH_3), 0.96 (s, 3H, 19-CH_3), 3.60 (br m, 4H, 3β-, 7α-, 24-H_2), 4.07 (br m, 1H, 15β-H). LR-MS (as the TMS ether), *m/z*: 682 (<1%, M), 592 (4%, M−TMSOH), 577 (15%, M−TMSOH−CH_3), 502 (25%, M−2TMSOH), 487 (7%, M−2TMSOH−CH_3), 433 (100%, M−TMSOH−S.C.), 327 (64%), 253 (19%, M−3TMSOH−S.C.), 207 (32%). HR-MS (ESI-NIM) Calcd for $C_{24}H_{41}O_4$ [M−H]$^-$: 393.3005. Found: *m/z*, 393.2994.

Methyl 3,7,16-Trioxo-5β-cholan-24-oate (16a) Jones reagent (1 ml) was added dropwise to a stirred solution of the 3α,7α,16α,25-tetrol **14** (100 mg) in acetone (13 ml) under 10 °C, and the mixture was stirred for 30 min at room temperature. Methanol (2 ml) was added, and the oxidation product extracted with EtOAc. The combined EtOAc layer was washed with saturated brine, dried over Drierite, and evaporated. After re-esterification of the residue with methanol and *p*-toluenesulfonic acid, the product was chromatographed on a column of silica gel (70—230 mesh, 10 g). Elution with benzene–EtOAc (3 : 2, v/v) and recrystallization of the eluate from EtOAc–hexane gave the title compound **16a** as colorless thin plates: yield, 99 mg (94%); mp, 148—150 °C. IR, ν_{max} cm^{-1}: 1709 (ketone C=O), 1735 (ester C=O). ^{1}H-NMR, δ: 0.85 (s, 3H, 18-CH_3), 1.00 (d, 3H, J=6.2 Hz, 21-CH_3), 1.34 (s, 3H, 19-CH_3), 3.67 (s, 3H, COOCH_3). LR-MS, *m/z*: 416 (3%, M), 401 (38%, M−CH_3), 369 (35%), 329 (22%), 287 (100%). HR-MS (ESI-PIM) Calcd for $C_{25}H_{36}NaO_5$ [M+Na]$^+$: 439.2460. Found: *m/z*, 439.2469.

Methyl 3,7,15-Trioxo-5β-cholan-24-oate (17a) The 3α,7β,15α,24-tetrol **15** (110 mg) was converted to the 3,7,15-trioxo ester **17a** by the

method as described for the preparation of **16a**. Recrystallization of the product from EtOAc–hexane gave **17a** as colorless amorphous solid: yield, 101 mg (87%); mp, 178—180 °C. IR, ν_{max} cm^{-1}: 1712 (ketone C=O), 1739 (ester C=O). ^{1}H-NMR, δ: 0.75 (s, 3H, 18-CH$_3$), 1.01 (d, 3H, J=6.5 Hz, 21-CH$_3$), 1.31 (s, 3H, 19-CH$_3$), 3.68 (s, 3H, COOCH$_3$). LR-MS, *m/z*: 416 (37%, M), 401 (100%, M−CH$_3$), 301 (23%). HR-MS (ESI-PIM) Calcd for $C_{25}H_{36}NaO_5$ [M+Na]$^+$: 439.2460. Found: *m/z*, 439.2448.

Methyl 3α,7α,16α-Trihydroxy-5β-cholan-24-oate (3a) and Methyl 3α,7α,16β-Trihydroxy-5β-cholan-24-oate (4a) *tert*-Butylamine–borane complex (100 mg) was added to a stirred solution of the 3,7,16-triketone (100 mg) in CH_2Cl_2 (8 ml), and the mixture was refluxed overnight. After cooling the mixture, 10% HCl (3 ml) added and the solution stirred at room temperature for 30 min. The CH_2Cl_2 layer was washed with 5% $NaHCO_3$ and water, dried over Drierite, and evaporated. The oily residue, which consisted essentially of two components on TLC, was chromatographed on a column of silica gel (70—230 mesh, 80 g). Elution with EtOAc–methanol (19 : 1, v/v) provided two well-separated fractions. The less polar fraction was identified as the 3α,7α,16β-trihydroxy ester (**4a**), which resisted crystallization: yield, 44 mg (43%); viscous oil. IR, ν_{max} cm^{-1}: 1721 (ester C=O), 3358 (OH). (^{1}H-, ^{13}C-NMR and LR-MS, see Tables 1—3). HR-MS (ESI-PIM) Calcd for $C_{25}H_{42}NaO_5$ [M+Na]$^+$: 445.2930. Found: *m/z*, 445.2925.

The more polar fraction was characterized as the 16α-epimer (**3a**) of **4a**: yield, 16 mg (16%); viscous oil. IR, ν_{max} cm^{-1}: 1739 (ester C=O), 3271 (OH). (^{1}H-, ^{13}C-NMR and LR-MS, see Tables 1—3). HR-MS (ESI-PIM) Calcd for $C_{25}H_{42}NaO_5$ [M+Na]$^+$: 445.2930. Found: *m/z*, 445.2928.

Methyl 3α,7α,15α-Trihydroxy-5β-cholan-24-oate (5a) and Methyl 3α,7α,15β-Trihydroxy-5β-cholan-24-oate (6a) The 3,7,15-trioxo ester **17a** (140 mg) was treated with *tert*-$C_4H_9NH_2\cdot BH_3$ as described for the preparation of **3a** and **4a**. After being processed analogously, the oily product was purified by RP-MPLC on a column of C_{18}-bonded silica gel (16 g). Elution with methanol–H_2O (8 : 2—7 : 3, v/v) afforded the following three well-separated fractions.

Fr. 1. 3α,7α,15β-Trihydroxy ester (**6a**): yield, 30 mg (21%); viscous oil. IR, ν_{max} cm^{-1}: 1728 (ester C=O), 3350 (OH). (^{1}H-, ^{13}C-NMR and LR-MS, see Tables 1—3). HR-MS (ESI-PIM) Calcd for $C_{25}H_{42}NaO_5$ [M+Na]$^+$: 445.2930. Found: *m/z*, 445.2900.

Fr. 2. Recrystallization of the eluate from acetone–hexane gave 3α,7β,15β-trihydroxy ester (**18a**) as colorless needles: yield, 26 mg (18%); mp, 203—205 °C. IR, ν_{max} cm^{-1}: 1738 (ester C=O), 3271 (OH). ^{1}H-NMR, δ: 0.94 (d, 3H, J=6.8 Hz, 21-CH$_3$), 0.96 (s, 3H, 18-CH$_3$), 0.98 (s, 3H, 19-CH$_3$), 3.60 (br m, 1H, 3β-H), 3.67 (s, 3H, COOCH$_3$), 3.76 (br m, 1H, 7α-H), 4.29 (t, 1H, J=5.4 Hz, 15α-H). ^{13}C-NMR, δ: 14.5 (C-18), 18.4 (C-21), 21.1 (C-11), 23.3 (C-19), 30.2 (C-2), 30.9 (C-22), 31.1 (C-23), 34.1 (C-10), 34.9 (C-1), 34.9 (C-20), 37.2 (C-6), 37.5 (C-4), 38.7 (C-12), 39.1 (C-9), 39.4 (C-8), 41.2 (C-16), 42.3 (C-5), 43.0 (C-13), 51.5 (COOCH$_3$), 55.8 (C-17), 61.1 (C-14), 71.1 (C-15), 71.2 (C-7), 71.3 (C-3), 174.7 (C-24). LR-MS (as the Me-TMS ether), *m/z*: 533 (11%, M−TMSOH−CH$_3$), 458 (7%, M−2TMSOH), 443 (11%, M−2TMSOH−CH$_3$), 433 (81%, M−TMSOH−S.C.), 368 (4%, M−3TMSOH), 353 (6%, M−3TMSOH−CH$_3$), 343 (13%, M−2TMSOH−S.C.), 314 (13%), 283 (100%), 253 (55%, M−3TMSOH−S.C.). HR-MS (ESI-PIM) Calcd for $C_{25}H_{42}NaO_5$ [M+Na]$^+$: 445.2930. Found: *m/z*, 445.2900.

Fr. 3. 3α,7α,15α-Trihydroxy ester (**5a**): yield, 38 mg (27%); viscous oil. IR, ν_{max} cm^{-1}: 1739 (ester C=O), 3229 (OH). (^{1}H-, ^{13}C-NMR and LR-MS, see Tables 1—3). HR-MS (ESI-PIM) Calcd for $C_{25}H_{42}NaO_5$ [M+Na]$^+$: 445.2930. Found: *m/z*, 445.2948.

3α,7α,16α-Trihydroxy-5β-cholan-24-oic Acid (3) The ester **3a** (40 mg) was refluxed in 5% methanolic KOH (5.0 ml) for 1 h. Most of the solvent was evaporated off, and the residue was dissolved in water and acidified with 10% H_2SO_4 with stirring and ice-bath cooling. The precipitate was collected by filtration, washed with water, and recrystallized from EtOAc–hexane as a colorless amorphous solid of **3**: yield, 38 mg (98%); mp, 131—134 °C. IR, ν_{max} cm^{-1}: 1709 (ester C=O), 3278 (OH). ^{1}H-NMR ($CDCl_3$+10% DMSO-d_6), δ: 0.67 (s, 3H, 18-CH$_3$), 0.89 (s, 3H, 19-CH$_3$), 0.95 (d, 3H, J=5.9 Hz, 21-CH$_3$), 3.44 (br m, 1H, 3β-H), 3.81 (m, 1H, 7β-H), 4.00 (t, 1H, J=5.4 Hz, 16β-H). HR-MS (ESI-NIM) Calcd for $C_{24}H_{39}O_5$ [M−H]$^-$: 407.2797. Found: *m/z*, 407.2807.

3α,7α,16β-Trihydroxy-5β-cholan-24-oic Acid (4) The ester **4a** (50 mg), hydrolyzed with 5% methanolic KOH and processed as described for the preparation of **3**, yielded the crude acid. Recrystallization from EtOAc–hexane gave **4** as a colorless amorphous solid: yield, 42 mg (86%); mp, 148—150 °C. IR, ν_{max} cm^{-1}: 1712 (ester C=O), 3356 (OH). ^{1}H-NMR ($CDCl_3$+10% DMSO-d_6), δ: 0.86 (s, 3H, 18-CH$_3$), 0.91 (s, 3H, 19-CH$_3$), 0.97 (d, 3H, J=5.9 Hz, 21-CH$_3$), 3.43 (br m, 1H, 3β-H), 3.84 (m, 1H, 7β-H), 4.49 (br m, 1H, 16α-H). HR-MS (ESI-NIM) Calcd for $C_{24}H_{39}O_5$ [M−H]$^-$: 407.2797. Found: *m/z*, 407.2827.

3α,7α,15α-Trihydroxy-5β-cholan-24-oic Acid (5) The ester **5a** (50 mg), hydrolyzed by the usual manner, recrystallized from acetone–hexane as a colorless amorphous solid of **5**: yield, 42 mg (86%); mp, 203—205 °C. IR, ν_{max} cm^{-1}: 1705 (ester C=O), 3209 (OH). ^{1}H-NMR ($CDCl_3$+10% DMSO-d_6), δ: 0.72 (s, 3H, 18-CH$_3$), 0.94 (s, 3H, 19-CH$_3$), 0.96 (d, 3H, J=5.4 Hz, 21-CH$_3$), 3.49 (br m, 1H, 3β-H), 4.06 (m, 2H, 7β-, 15β-H). HR-MS (ESI-NIM) Calcd for $C_{24}H_{39}O_5$ [M−H]$^-$: 407.2797. Found: *m/z*, 407.2819.

3α,7α,15β-Trihydroxy-5β-cholan-24-oic Acid (6) The ester **6a** (50 mg), hydrolyzed by the usual manner, recrystallized from EtOAc as colorless needles of **6**: yield, 40 mg (82%); mp, 130—132 °C. IR, ν_{max} cm^{-1}: 1705 (ester C=O), 3337 (OH). ^{1}H-NMR ($CDCl_3$+30% DMSO-d_6), δ: 0.94 (br s, 3H, 18-CH$_3$), 0.94 (br s, 3H, 19-CH$_3$), 0.94 (br s, 3H, 21-CH$_3$), 3.43 (br m, 1H, 3β-H), 4.11 (m, 1H, 7β-H), 4.27 (br m, 1H, 15α-H). HR-MS (ESI-NIM) Calcd for $C_{24}H_{39}O_5$ [M−H]$^-$: 407.2797. Found: *m/z*, 407.2801.

3α,7β,15β-Trihydroxy-5β-cholan-24-oic Acid (18) The ester **18a** (50 mg), hydrolyzed by the usual manner, recrystallized from aqueous methanol as a colorless amorphous solid: yield, 39 mg (81%); mp, 126—128 °C. IR, ν_{max} cm^{-1}: 1703 (ester C=O), 3279 (OH). ^{1}H-NMR ($CDCl_3$+30% DMSO-d_6), δ: 0.97 (br s, 3H, 18-CH$_3$), 0.97 (br s, 3H, 19-CH$_3$), 0.97 (br s, 3H, 21-CH$_3$), 3.60 (br m, 1H, 3β-H), 3.73 (br m, 1H, 7α-H), 4.29 (m, 1H, 15α-H). HR-MS (ESI-NIM) Calcd for $C_{24}H_{39}O_5$ [M−H]$^-$: 407.2797. Found: *m/z*, 407.2781.

Methyl 3α,7α-Diacetoxy-16α,17α-epoxy-5β-cholan-24-oate (19a) To a solution of the Δ^{16}-ester (**11a**; 100 mg) in CH_2Cl_2 (1.0 ml) was added a DMDO solution in $CHCl_3$ (0.32 mol/l, 1.5 ml), and the mixture was allowed to stand at room temperature for 30 min. Excess amounts of the DMDO and solvent were evaporated under reduced pressure, and the residue was recrystallized directly from hexane to give an analytically pure sample of the title compound (**19a**): yield, 95 mg (92%); mp, 111—114 °C. IR, ν_{max} cm^{-1}: 1724 (ester C=O). ^{1}H-NMR, δ: 0.79 (s, 3H, 18-CH$_3$), 0.94 (s, 3H, 19-CH$_3$), 1.01 (d, 3H, J=6.8 Hz, 21-CH$_3$), 2.03 and 2.05 (each s, 6H, 3α-, 7α-OCOCH$_3$), 3.32 (s, 1H, 16β-H), 3.67 (s, 3H, COOCH$_3$), 4.57 (br m, 1H, 3β-H), 4.87 (m, 1H, 7β-H). LR-MS, *m/z*: 504 (3%, M), 444 (5%, M−AcOH), 429 (11%, M−2AcOH−CH$_3$), 329 (39%, M−AcOH−S.C.), 269 (39%, M−2AcOH−S.C.), 224 (100%). HR-MS (ESI-PIM) Calcd for $C_{29}H_{44}NaO_7$ [M+Na]$^+$: 527.2985. Found: *m/z*, 527.2973.

Methyl 3α,7β-Diacetoxy-14α,15α-epoxy-5β-cholan-24-oate (20a) This compound was prepared from the Δ^{14} ester **13a** (100 mg) by the epoxidation method as described in the preparation of **19a**: yield, 97 mg (94%); viscous oil. IR, ν_{max} cm^{-1}: 1732 (ester C=O). ^{1}H-NMR, δ: 0.87 (d, 3H, J=8.1 Hz, 21-CH$_3$), 0.88 (s, 3H, 18-CH$_3$), 0.98 (s, 3H, 19-CH$_3$), 1.99 and 2.01 (each s, 6H, 3α-, 7β-OCOCH$_3$), 3.20 (s, 1H, 15β-H), 3.67 (s, 3H, COOCH$_3$), 4.65 (br m, 1H, 3β-H), 4.71 (br m, 1H, 7α-H). LR-MS, *m/z*: 444 (36%, M−AcOH), 429 (11%, M−2AcOH−CH$_3$), 389 (43%, M−S.C.), 384 (10%, M−2AcOH), 329 (76%, M−AcOH−S.C.), 290 (42%), 269 (100%, M−2AcOH−S.C.). HR-MS (ESI-PIM) Calcd for $C_{29}H_{44}NaO_7$ [M+Na]$^+$: 527.2985. Found: *m/z*, 527.2979.

3α,7α-Dihydroxy-5β-cholane *O*-24,16α-Lactone (22) A solution of the 3α,7α,16α-trihydroxy acid (**3**: 20 mg) in EtOAc (2 ml) containing *p*-toluenesulfonic acid (2 mg) or conc. HCl (one drop) was left to stand at room temperatrure for 2 h. The organic layer was washed with saturated brine, dried over Drierite, and evaporated to give the title compound **22**: yield, 16 mg (81%); viscous oil. IR, ν_{max} cm^{-1}: 1727 (ester C=O), 3456 (OH). ^{1}H-NMR, δ: 0.75 (s, 3H, 18-CH$_3$), 0.90 (s, 3H, 19-CH$_3$), 1.03 (d, 3H, J=6.5 Hz, 21-CH$_3$), 3.46 (br m, 1H, 3β-H), 3.84 (d, 1H, J=2.7 Hz, 7β-H), 4.65 (t, 1H, J=8.1 Hz, 16β-H). LR-MS (as the TMS ether), *m/z*: 444 (4%, M−TMSOH), 429 (3%, M−TMSOH−CH$_3$), 354 (68%, M−2TMSOH), 339 (13%, M−2TMSOH−CH$_3$), 253 (100%). HR-MS (APCI-PIM) Calcd for $C_{24}H_{35}O_2$ [M+H−2H$_2$O]$^+$: 355.2637. Found: *m/z*, 355.2653.

3α,7α-Dihydroxy-5β-cholane *O*-24,16β-Lactone (23) This compound was prepared from the 3α,7α,16β-trihydroxy acid (**4**) by the procedure as described in the preparation of **22**: yield, 15 mg (80%); viscous oil. IR, ν_{max} cm^{-1}: 1720 (ester C=O), 3429 (OH). ^{1}H-NMR, δ: 0.85 (s, 3H, 18-CH$_3$), 0.92 (s, 3H, 19-CH$_3$), 1.02 (d, 3H, J=6.8 Hz, 21-CH$_3$), 3.48 (br m, 1H, 3β-H), 3.87 (br d, 1H, J=2.7 Hz, 7β-H), 4.88 (br m, 1H, 16α-H). LR-MS (as the TMS ether), *m/z*: 444 (11%, M−TMSOH), 429 (15%, M−TMSOH−CH$_3$), 411 (5%), 354 (100%, M−2TMSOH), 339 (16%, M−2TMSOH−CH$_3$), 253 (60%). HR-MS (ACPI-PIM) Calcd for $C_{24}H_{35}O_2$ [M+H−2H$_2$O]$^+$: 355.2637. Found: *m/z*, 355.2655.

3α,7α-Dihydroxy-5β-cholane *O*-24,17α-Lactone (24) This compound was prepared from the 3α,7α,17α-trihydroxy acid (**21**) by the procedure as

described in the preparation **22**: yield, 16 mg (82%); mp, 128—131 °C (colorless amorphous solid from acetone–hexane). IR, ν_{max} cm^{-1}: 1711 (ester C=O), 3456 (OH). ^{1}H-NMR, δ: 0.84 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 1.09 (d, 3H, J=6.8 Hz, 21-CH_3), 3.46 (br m, 1H, 3β-H), 3.85 (d, 1H, J=2.7 Hz, 7β-H). LR-MS (as the TMS ether), *m*/*z*: 444 (4%, M−TMSOH), 429 (32%, M−TMSOH−CH_3), 354 (47%, M−2TMSOH), 343 (31%), 253 (100%). HR-MS (APCI-PIM) Calcd for $C_{24}H_{35}O_2$ $[M+H-2H_2O]^+$: 355.2637. Found: *m*/*z*, 355.2651.

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References and Notes

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