Potential Bile Acid Metabolites. 25. Synthesis and Chemical Properties of Stereoisomeric 3\(\alpha\),7\(\alpha\),16- and 3\(\alpha\),7\(\alpha\),15-Trihydroxy-5\(\beta\)-cholan-24-oic Acids

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Epimeric 3\(\alpha\),7\(\alpha\),16- and 3\(\alpha\),7\(\alpha\),15-trihydroxy-5\(\beta\)-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 17\(\alpha\)- and 14\(\alpha\)-hydroxy derivatives with POCl3 or conc. H2SO4, the respective 16\(\alpha\)- and 15\(\alpha\)-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-butyllamine-borane complex afforded the epimeric 3\(\alpha\),7\(\alpha\),16- or 3\(\alpha\),7\(\alpha\),15-trihydroxy derivatives exclusively. A facile formation of the corresponding \(\varepsilon\)-lactones between the side chain carboxyl group at C-24 and the 16\(\alpha\)- (or 16\(\beta\))-hydroxyl group in bile acids is also clarified.

Key words remote oxyfunctionalization; dimethyldioxirane; 3\(\alpha\),7\(\alpha\),16-trihydroxy-5\(\beta\)-cholan-24-oic acid; 3\(\alpha\),7\(\alpha\),15-trihydroxy-5\(\beta\)-cholan-24-oic acid; \(\varepsilon\)-lactone

The 16- and 15-hydroxy derivatives of chenodeoxycholic acid (3\(\alpha\),7\(\alpha\)-dihydroxy-5\(\beta\)-cholan-24-oic acid: CDCA; 1), and deoxycholic acid (3\(\alpha\),12\(\alpha\)-dihydroxy-5\(\beta\)-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. 3\(\alpha\),12\(\alpha\),16\(\alpha\)-Trihydroxy-5\(\beta\)-cholan-24-oic acid, named as “pythocholic acid”, was isolated some years ago by Haslewood and Woolton from certain primitive snakes (boas and pythons), including Cylindrophis. 2,3 Hagey et al. have recently reported the existence of “avicholic acid”, 3\(\alpha\),7\(\alpha\),16\(\alpha\)-trihydroxy acid, as a primary bile acid in many birds such as herons (Ardeidae), pelicans (Pelecanidae), and owls (Tytonidae). 4–8 Meanwhile, the 15\(\alpha\)-hydroxy derivative of CDCA (1) and its C-24 sulfonate analogue have also been shown to be major bile acids in marsupials9 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 peroxo 5\(\beta\)-cholanoic acid in n-octane gives a mixture of 16\(\alpha\)- and 16\(\beta\)-hydroxy-5\(\beta\)-cholanes. Kimura et al.12,13 have reported chemical synthesis of 15\(\alpha\)-hydroxy lithocholic acid (3\(\alpha\)-hydroxy-5\(\beta\)-cholan-24-oic acid: LCA) and DCA using a ferrous ion-molecular oxygen system. In more recent works, the 15\(\beta\)-hydroxylation of LCA and DCA has been attained by microbiological transformation employing Penicillium species, Rhizoctonia solani and Absidia coerulea14 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 3\(\alpha\),7\(\alpha\),16- (3, 4) and 3\(\alpha\),7\(\alpha\),15-trihydroxy-5\(\beta\)-cholan-24-oic acids (5, 6), as well as a related stereoisomer (18), starting from 1 or ursodeoxycholic acid (3\(\alpha\),7\(\alpha\)-dihydroxy-5\(\beta\)-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 \(\beta\)-attached \(\mathrm{CH}_3\) alkyl side chain, but also by the axially-oriented 7\(\alpha\)-hydroxyl group.

As outlined in Charts 2 and 3, a key strategic point in the present work was to make use of 17\(\alpha\)- and 14\(\alpha\)-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 works17,18 involving a highly efficient, stereoselective remote oxyfunctionalization of unactivated methine carbons in steroids with a powerful oxidant, dimethyldioxirane (DMDO), has led to direct inser-
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 CHCl₃ 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 CHCl₃–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 (POCl₃) 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–Et₂O (9 : 1, v/v). The less polar, major component (41% isolated yield) was characterized as the D₁₆-ester (11a),¹⁹) and the more polar, minor component (30%) as the D₁₇(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 ¹H-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 ¹H-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 ¹³C-NMR. Elimination of the 14α-hydroxyl group in the ester 10a was successfully achieved by treating with conc. H₂SO₄ in dioxane at room temperature overnight to give the Δ¹⁴-ester (13a) exclusively (73% isolated yield).

Hydroboration, followed by oxidation with alkaline hydrogen peroxide of the Δ₁₆- and Δ¹⁴-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 (B₂H₆),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 ¹H-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
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. 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. NH₃/Li, Zn(BH₄)₂, NaBH₄/PdCl₂, 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-
Analogously, reduction of 3,7,15-triketone droxy esters (13) with tert-C₄H₉NH₂·BH₃, respectively. Usual alkaline hydrolysis of the individual 3α,7α,16- and 3α,7α,15-trihydroxy esters (3a—6a) with methanolic potassium hydroxide, followed by acidification with H₂SO₄ 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.²,⁶

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 ¹H- and ¹³C-NMR spectral data for 3a—6a, respectively. Differentiation between the 16α- and 16β-epimers (3a vs. 4a) was made by the ¹H-NMR spectra, in which the 18-methyl signal in 4a resonates at lower-field by 0.20 ppm than that of 3a²⁸ 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 ¹H-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-H¹0.¹⁵ [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 ¹³C-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-oates.²⁹ However, the C-16 in 3a (76.7 ppm) and 4a (72.1 ppm) and the C-15 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.
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 16α- and 15β-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-5β-cholestone-3β,5α-diol\(^{11}\) and methyl 3α-cathoxy-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., pyrocholic acid, 3α,12α,16α-trihydroxy-5β-cholane-24-oic acid, forms readily the corresponding pyrocholic lactone by dissolving the free acid in NaOH solution, acidified with H\(_2\)SO\(_4\), 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 \(\alpha\)-lactones, 3α,7α-dihydroxy-5β-cholane-24,16α-lactone (22)\(^{31}\) and 3α,7α-dihydroxy-5β-cholane-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 occurs 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\(^{31}\) originating from the loss of one and two trimethylsilyl 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\(_2\)H\(_5\)O\(_2\)) 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 occurring by treating 17α-hydroxy acid (21), derived from 9a by alkaline hydrolysis, with \(\pi\)-tolenesulfonic acid in EtOAc to give the corresponding \(\delta\)-lactone, 3α,7α-dihydroxy-5β-cholane-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. \(\text{H}^-\) and \(\text{C}^-\)-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 \(\delta\) 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 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–Et\(_2\)O–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 oxene\(^{17}\) and acetone.\(^{18}\) All compounds were dried by azetropic distillation before use in reaction.

### Table 3. Mass Spectral Fragment Ions of Stereoisomeric Methyl 3α,7α,16α- and 3α,7α,15α-Trihydroxy-5β-cholane-24-oates (3a—6a)\(^{a}\)

<table>
<thead>
<tr>
<th>Major ions</th>
<th>Relative intensity (%)</th>
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<tbody>
<tr>
<td></td>
<td>3a</td>
</tr>
<tr>
<td>638</td>
<td>M</td>
</tr>
<tr>
<td>623</td>
<td>M–CH(_3)</td>
</tr>
<tr>
<td>548</td>
<td>M–TMSOH</td>
</tr>
<tr>
<td>533</td>
<td>M–TMSOH–CH(_3)</td>
</tr>
<tr>
<td>458</td>
<td>M–2TMSOH</td>
</tr>
<tr>
<td>443</td>
<td>M–2TMSOH–CH(_3)</td>
</tr>
<tr>
<td>368</td>
<td>M–3TMSOH</td>
</tr>
<tr>
<td>364</td>
<td>M–3TMSOH</td>
</tr>
<tr>
<td>353</td>
<td>M–3TMSOH–CH(_3)</td>
</tr>
<tr>
<td>329</td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>M–3TMSOH–S.C.</td>
</tr>
</tbody>
</table>

\(^{a}\) Measured as the trimethylsilyl ether derivatives; TMSOH, trimethylsilanol; S.C., side chain.
which was recrystallized from EtOAc–hexane as colorless prisms: mp, 157–160°C (lit., 15 mp, 157–160°C). IR, ν\(_\text{max}\) cm\(^{-1}\): 1728 (ester C=O), 3545 (OH). H-NMR, δ: 0.74 (s, 3H, 18-CH₃), 0.91 (d, 3H, J=7.0 Hz, 21-CH₃), 0.94 (s, 3H, 19-CH₃), 2.02 and 2.06 (each 3H, 3\(_6\)-7r-2,7r-OCOCH₃), 3.67 (s, 3H, COOH), 4.59 (br m, 1H, 7β-H), 4.90 (br d, 1H, J=2.7 Hz, 7β-H). LR-MS, m/z: 446 (4%, M\(^+\)-AcOH), 428 (19%, M\(^+\)-AcOH–H₂O), 386 (100%), M\(^+\)-AcOH. 368 (82%, M\(^+\)-AcOH–H₂O–CH₃), 332 (91%, M\(^+\)-AcOH–H₂O–S–C ring D), 226 (40%, M\(^+\)-AcOH–H₂O–S–C–part of ring D), 211 (30%, M\(^+\)-AcOH–H₂O–S–C ring D).

The more polar compound was recrystallized from EtOAc–hexane to give methyl 3α,7α-dihydroxy-5β-hydroxycyclododeca-24-oate as a colorless amorphous solid: yield, 550 mg. 27D mp, 156–160°C (lit., 15 mp, 158–160°C). 1H-NMR, δ: 0.75 (s, 3H, 18-CH₃), 0.89 (s, 3H, 19-CH₃), 1.21 (s, 3H, 17-CH₃), 3.66 (s, 3H, COOH), 4.37 (br m, 1H, 6r, 6s-2H), 2.00 and 2.02 (each 3H, 3\(_6\), 3\(_8\)-7r-OCO), 3.67 (s, 3H, COOCH₃), 4.92 (br m, 1H, J=2.7 Hz, 7β-H). LR-MS, m/z: 528 (4%, M\(^+\)-AcOH), 410 (25%, M\(^+\)-AcOH–H₂O–CH₃), 368 (19%, M\(^+\)-AcOH–H₂O–S–C ring D). 353 (27%, M\(^+\)-AcOH–H₂O–S–C–part of ring D), 226 (40%, M\(^+\)-AcOH–H₂O–S–C–part of ring D), 211 (30%, M\(^+\)-AcOH–H₂O–S–C ring D).

Methyl 3α,7β-Diacytio-14α-hydroxy-5β-bolchol-24-oate (10a) UDCA methyl ester-diacytio-8a (2.0 g) was subjected to the remote-oxygenfunctionalization 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 a silica gel (70-230 mesh, 60 g). Elution was conducted under a stream of nitrogen. The elution with hexane–benzene–EtOAc (9:1, v/v) afforded the least polar 8a and then methyl 3α,7β-Diacytio-16-oxo-5β-bolchol-24-oate. 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) provided a less polar component, which was characterized as the described 14α-hydroxy ester 10a. Although this compound was homogeneous according to TLC and GC, it resisted crystallization: yield, 278 mg (13%). IR, ν\(_\text{max}\) cm\(^{-1}\): 1737 (ester C=O), 3518 (OH). H-NMR, δ: 0.79 (s, 3H, 18-CH₃), 0.90 (s, 3H, 19-CH₃), 1.45 (s, 3H, 21-CH₃), 2.03 and 2.05 (each 3H, 3\(_6\), 3\(_8\)-7r-OCOCH₃), 4.68 (br m, 1H, 3\(_6\)-7r, 6r-CH), 4.76 (br m, 1H, 7β-H). LR-MS, m/z: 428 (8%, M\(^+\)-AcOH–H₂O), 368 (24%, M\(^+\)-AcOH–H₂O–CH₃), 314 (9%), 281 (at 9%), 253 (100%, M\(^+\)-AcOH–H₂O–S–C ring D), 211 (10%, 212 (25%).

A more polar component eluting with hexane–benzene–EtOAc (5:1:4, v/v) afforded the compound (170 mg), which was recrystallized from aqueous methanol as colorless amorphous solid: yield, 190 mg (10%); mp, 202–204°C (lit., 15 mp, 202–204°C). IR, ν\(_\text{max}\) cm\(^{-1}\): 1743, 1711 (C=O). H-NMR, δ: 0.84 (s, 3H, 18-CH₃), 0.98 (s, 3H, 19-CH₃), 1.45 (s, 3H, 21-CH₃), 2.03 and 2.05 (each 3H, 3\(_6\), 3\(_8\)-7r-OCOCH₃), 4.68 (br m, 1H, 3\(_6\)-7r, 6r-CH), 4.76 (br m, 1H, 7β-H). LR-MS, m/z: 414 (14%, M\(^+\)-AcOH), 354 (85%, M\(^+\)-AcOH–H₂O), 339 (41%, M\(^+\)-AcOH–CH₃), 313 (10%), 300 (100%, M\(^+\)-AcOH–S–C ring D), 288 (88%, M\(^+\)-AcOH–S–C–part of ring D), 281 (7%), 253 (56%), 241 (19%), 228 (94%, M\(^+\)-AcOH–S–C–part of ring D), 227 (100%), 213 (75%, M\(^+\)-AcOH–S–C ring D).
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, \(\nu_{\text{max}}\) cm\(^{-1}\): 1712 (ketone C=O), 1739 (ester C=O). \(^1\)H-NMR, \(\delta\): 0.75 (3 H, 18-CH\(_3\)), 1.01 (3 H, J=6.5 Hz, 21-CH\(_3\)), 1.31 (3 H, 19-CH\(_3\)), 3.68 (3 H, 18-OCH\(_3\)), LR-MS, m/z: 416 (37%), M\(^+\) 401 (100%, M–CH\(_3\)), 303 (21%). HR-MS (ESI-PIM) Caled for C\(_{24}\)H\(_{39}\)NO\(_5\) [M+Na\(^+\)]: 449.2482. Found: m/z, 449.2484.

Methyl 3\(^\beta\),7\(^\alpha\),15\(^\alpha\)-Trihydroxy-5\(^\beta\)-cholan-24-oate (3a) and Methyl 3\(^\beta\),7\(^\alpha\),16\(^\beta\)-Trihydroxy-5\(^\beta\)-cholan-24-oate (4a) tert-Butylamine–borane complex (100 mg) was added to a stirred solution of the 3,7,16-triketo ester (4a), which resisted crystallization: yield, 44 mg (43%); viscous oil. IR, \(\nu_{\text{max}}\) cm\(^{-1}\): 1712 (ester C=O), 1739, 1533 (OH). \(^1\)H-NMR, \(\delta\): 0.94 (3 H, 18-CH\(_3\)), 0.98 (3 H, 19-CH\(_3\)), 1.36 (brm, 1 H, 19\(\alpha\)-CH\(_3\)), 3.67 (s, 3 H, COOCH\(_3\)), 3.76 (brm, 1 H, 7\(\alpha\)-CH\(_3\)), 4.29 (t, 1 H, J=5.4 Hz, 15\(\alpha\)-CH\(_3\)), \(^1\)C-NMR, \(\delta\): 14.5 (C-18), 18.4 (C-21), 21.1 (C-11), 23.3 (C-19), 30.2 (C-22), 30.9 (C-23), 31.1 (C-24), 34.1 (C-10), 34.9 (C-14), 37.5 (C-5), 37.6 (C-4), 37.8 (C-17), 39.1 (C-3), 39.4 (C-18), 41.2, 42.1, 42.3 (C-4), 43.3 (C-13), 51.5 (COOCH\(_3\)), 55.8, 61.7 (C-14), 61.2 (C-15), 71.1 (C-17), 71.3 (C-3), 174.7 (C-24), LR-MS (as the Me-TMS ether), m/z: 533 (11%, M+TMS–CH\(_3\)), 417 (19, 2TMSOH), 443 (11%, M+2TMS–CH\(_3\)), 431 (83%, M+TMS–S, C–S), 368 (44%, M+3TMS–S), 353 (6%, M+2TMS–SO\(_2\)), 343 (13%, M+2TMS–SO\(_2\)–S, C–S), 283 (100%), 253 (53%, M+TMS–S–C). HR-MS (ESI-PIM) Caled for C\(_{24}\)H\(_{37}\)O\(_6\)Na\(_2\) [M+Na\(^+\)]: 445.2930. Found: m/z, 445.2930.

Fr. 1. 3\(^\alpha\),7\(^\alpha\),15\(^\alpha\)-Trihydroxy ester (6a): yield, 30 mg (21%); viscous oil. IR, \(\nu_{\text{max}}\) cm\(^{-1}\): 1728 (ester C=O), 3350 (OH). \(^1\)C-NMR and LR-MS, see Tables 1–3. HR-MS (ESI-PIM) Caled for C\(_{24}\)H\(_{39}\)Na\(_2\)O\(_5\) [M+Na\(^+\)]: 449.2430. Found: m/z, 449.2398.

Fr. 2. Recrystallization of the eluate from acetone–hexane gave 3\(^\beta\),7\(^\alpha\),15\(^\alpha\)-Trihydroxy ester (18a) as colorless needles: yield, 26 mg (18%); mp, 203—205°C. IR, \(\nu_{\text{max}}\) cm\(^{-1}\): 1738 (ester C=O), 3271 (OH). \(^1\)H-NMR, \(\delta\): 0.94 (3 H, J=6.8 Hz, 21-CH\(_3\)), 0.98 (3 H, 19-CH\(_3\)), 1.36 (brm, 1 H, 19\(\alpha\)-CH\(_3\)), 3.67 (s, 3 H, COOCH\(_3\)), 3.76 (brm, 1 H, 7\(\alpha\)-CH\(_3\)), 4.29 (t, 1 H, J=5.4 Hz, 15\(\alpha\)-CH\(_3\)), \(^1\)C-NMR, \(\delta\): 14.5 (C-18), 18.4 (C-21), 21.1 (C-11), 23.3 (C-19), 30.2 (C-22), 30.9 (C-23), 31.1 (C-24), 34.1 (C-10), 34.9 (C-14), 37.5 (C-5), 37.6 (C-4), 37.8 (C-17), 39.1 (C-3), 39.4 (C-18), 41.2, 42.1, 42.3 (C-4), 43.3 (C-13), 51.5 (COOCH\(_3\)), 55.8, 61.7 (C-14), 61.2 (C-15), 71.1 (C-17), 71.3 (C-3), 174.7 (C-24), LR-MS (as the Me-TMS ether), m/z: 533 (11%, M+TMS–CH\(_3\)), 417 (19, 2TMSOH), 443 (11%, M+2TMS–CH\(_3\)), 431 (83%, M+TMS–S, C–S), 368 (44%, M+3TMS–S), 353 (6%, M+2TMS–SO\(_2\)), 343 (13%, M+2TMS–SO\(_2\)–S, C–S), 283 (100%), 253 (53%, M+TMS–S–C). HR-MS (ESI-PIM) Caled for C\(_{24}\)H\(_{37}\)O\(_6\)Na\(_2\) [M+Na\(^+\)]: 445.2930. Found: m/z, 445.2930.

Fr. 3. 3\(^\alpha\),7\(^\alpha\),15\(^\alpha\)-Trihydroxy ester (5a): yield, 38 mg (27%); viscous oil. IR, \(\nu_{\text{max}}\) cm\(^{-1}\): 1739 (ester C=O), 3229 (OH). \(^1\)C-NMR and LR-MS, see Tables 1—3. HR-MS (ESI-PIM) Caled for C\(_{24}\)H\(_{39}\)Na\(_2\O\(_5\) [M+Na\(^+\)]: 449.2430. Found: m/z, 449.2498.

Methyl 3\(^\beta\),7\(^\alpha\),16\(^\beta\)-Trihydroxy-5\(^\beta\)-cholan-24-oic Acid (3) The ester 3a (40 mg) was refluxed in 5% methanolic KOH (50 ml) for 1. Most of the solvent was evaporated off, and the residue was dissolved in water and acidified with 10% H\(_2\)SO\(_4\) 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: yield, 38 mg (98%); mp, 131—134°C. IR, \(\nu_{\text{max}}\) cm\(^{-1}\): 1709 (ester C=O), 3278 (OH). \(^1\)H-NMR (CDCl\(_3\)+10% DMSO-d\(_6\)), \(\delta\): 0.67 (3 H, 18-CH\(_3\)), 0.89 (3 H, 19-CH\(_3\)), 0.95 (3 H, J=5.9 Hz, 21-CH\(_3\)), 3.44 (brm, 1 H, 3\(\beta\)-OH), 3.81 (m, 1 H, 7\(\beta\)-OH), 4.00 (t, 1 H, J=5.4 Hz, 16-HR). HR-MS (ESI-NIM) Caled for C\(_{23}\)H\(_{31}\)O\(_4\) [M+H\(^+\)]: 407.2977. Found: m/z, 407.2970.
described in the preparation 22: yield, 16 mg (82%); mp, 128—131 °C (colorless amorphous solid from acetone–hexane). IR, νmax cm⁻¹: 1711 (ester C=O), 3456 (OH). ¹H-NMR, δ: 0.84 (s, 3H, 18-CH₃), 0.91 (s, 3H, 19-CH₃), 1.09 (d, 3H, J=6.8 Hz, 21-CH₃), 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–CH₃), 354 (47%, M–2TMSOH), 343 (31%), 253 (100%). HR-MS (APCI-PIM) Calcd for C₂₄H₃₅O₂ [M+H–2H₂O]⁺: 355.2637. Found: m/z, 355.2651.

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References and Notes

8) Since submission of this manuscript, Hagey et al.⁷ have recently reported the isolation and structural determination of 3 as a novel primary bile acid in the Shoebill stork (Balaeniceps rex) and herons: the ¹H- and ¹³C-NMR and mass spectral data reported therein for the natural 3 agreed nearly completely with those for the synthetic one.