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Osmiumporphyrin-Catalyzed Oxyfunctionalization and Isomerization of Natural (5β)-Bile Acids with *tert*-Butyl Hydroperoxide

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tert-Butyl hydroperoxide catalyzed by (*meso*-5,10,15,20-tetramesitylporphyrinate)osmium(II) carbonyl [Os(TMP)CO] was shown to be an efficient, versatile oxyfunctionalization system for the methyl ester peracetate derivatives of a series of common, natural (5β)-bile acids. Hydroxylation at C-5 and C-14, ketonization at C-15 and C-16, and isomerization at C-5 and C-14 in the nucleus were all attained in one step. Factors governing the regioselectivity as well as the mechanism of formation of these compounds are discussed.

Introduction

Metalloporphyrin-catalyzed oxyfunctionalization of unactivated C–H bonds in hydrocarbons by a single oxygen atom transfer reagent (oxygen donor) is a versatile oxidation procedure and is analogous to hydroxylation mediated by cytochrome P-450 enzymes in vivo.[1] Therefore, a variety of synthetic metalloporphyrin catalysts were developed to mimic the action of the P-450 enzymes. These catalysts differ in their structures at the *meso* position and in the metal of the central ligands; they are used in combination with a suitable oxygen donor.[2] Some of the metalloporphyrin/oxygen donor systems are now known to efficiently catalyze alkane or aromatic hydroxylation and alkene or arene epoxidation.[1]

In a previous paper,[3] we reported the development of a new and powerful oxidant system consisting of (*meso*-5,10,15,20-tetramesitylporphyrinate)osmium(II) carbonyl [Os(TMP)CO] as a precatalyst and *tert*-butyl hydroperoxide (TBHP) as an oxygen donor. The oxidant system is thought to proceed through an active *trans*-dioxoosmiumporphyrin intermediate with a high turnover rate (200:1) as a catalyst. The reactivity and regioselectivity of hydroxylation/epoxidation on unactivated C–H bonds in substrates differed considerably from those reported previously for other metalloporphyrin/oxygen donor systems.[4] In continuation of our program of synthesis of bioactive and uncommon steroids from abundantly available natural bile acids or steroids, we describe here the oxidation of the methyl ester peracetate derivatives of a series of (5β)-bile acids with the Os(TMP)CO/TBHP system. Factors governing the regioselectivity of oxyfunctionalization of the resulting hydroxy compounds, as well as the mechanism of isomerization at C-5 and C-14, are discussed.

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Results and Discussion

Substrates examined in this study were five common, natural (5β)-bile acids, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid; **1a**), ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid; **2a**), deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid; **3a**), hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid; **4a**), and cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; **5a**). These bile acids differ from one another in the number, position, and stereochemical configuration of the hydroxy groups at the C-3, C-6, C-7, and/or C-12 positions. To prevent the simultaneous oxidation of the hydroxy groups and to increase the solubility in the reaction solvent (i.e. benzene), the bile acids were converted into their methyl ester peracetate derivatives before they were subjected to the oxidation procedure.

The oxidation reaction was carried out under mild conditions by heating a solution of each of the substrates (**1a**–**5a**; 1.1 mmol) and anhydrous *tert*-butyl hydroperoxide (TBHP) (20 equiv.) in benzene at reflux (96 h) in the presence of a catalytic amount of O_S(TMP)CO (0.005 equiv.). The use of anhydrous TBHP as an oxygen donor is essential to increase the reactivity, and the compound is readily obtainable from commercially available 70% THBP by CH_2Cl_2 extraction. Prolonged heating at reflux caused the forma-

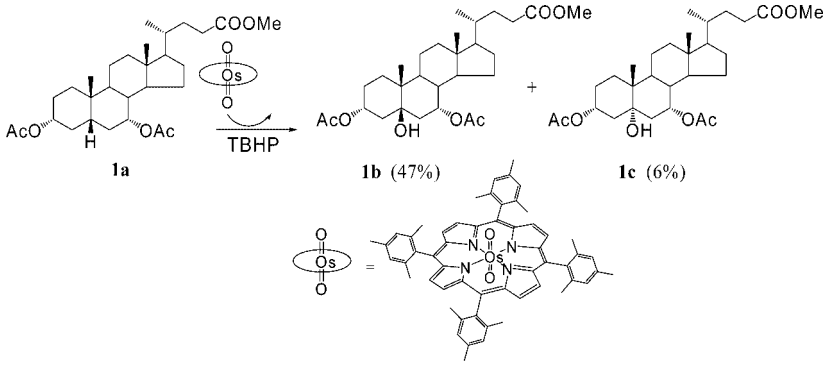
tion of increasing amounts of unknown components. These compounds showed longer retention times by capillary GC analysis, which could be due to the formation of multioxyfunctionalized products. After each reaction, the major oxygenated products, which usually showed lower R_f values on normal phase TLC, were isolated by open column chromatography, followed by medium-pressure liquid chromatography (MPLC) or preparative HPLC.

The oxidant system showed a new reactivity and regioselectivity in the oxyfunctionalization of unactivated C–H bonds in the substrates and produced a variety of novel oxygenated derivatives in one step. Total conversion of the respective parent compounds (**1a**–**5a**) to the corresponding oxygenated derivatives was in a range of 44–53%, which indicates the efficient turnover of an active *trans*-dioxoosmiumporphyrin catalyst derived directly from Os(TMP)CO and TBHP in the reaction system.[4]

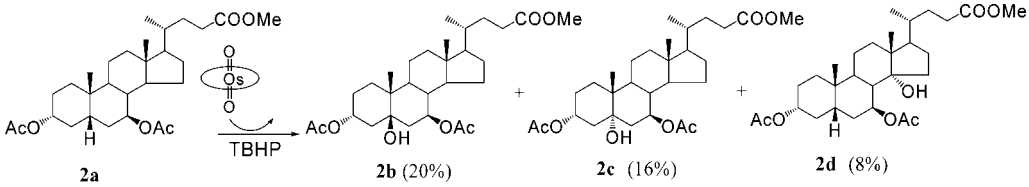
When methyl chenodeoxycholate diacetate (**1a**) having an axially oriented 7α-acetoxyl group was subjected to the Os(TMP)CO/TBHP oxidation, methyl 3α,7α-diacetoxy-5β-hydroxycholanoate (**1b**)[5] was obtained as a main product (47% yield), accompanied by a small amount of 5α-hydroxy isomer **1c** (6% yield; Scheme 1). The predominant insertion of the 5β-hydroxy group into (5β)-bile acids is known to be catalyzed by various oxygen-atom donating reagents such as di-*tert*-butyl diperoxycarbonate,[6] silver hexafluoroantimonate(V) ($AgSbF_6$),[7] dimethyldioxirane,[8] perfluorodialkyloxazirines,[9] or rutheniumporphyrin/2,6-dichloropyridine *N*-oxide/HBr.[10] The easy attack on the electron-enriched tertiary methine 5β-H suggests that the Os–porphyrin catalyst is electrophilic. Furthermore, because the 5β-H in **1a** with a *cis* A/B ring fusion is sterically less hindered, the oxidation would preferentially occur at the C-5 position (Scheme 1).

Under the identical reaction conditions, methyl ursodeoxycholate diacetate (**2a**), which has an equatorial 7β-acetoxyl group, yielded three major hydroxylation products (Scheme 2). The products were characterized as methyl 3α,7β-diacetoxy-5β-hydroxycholanoate (**2b**; 20%), methyl 3α,7β-diacetoxy-5α-hydroxycholanoate (**2c**; 16%), and methyl 3α,7β-diacetoxy-14α-hydroxy-5β-cholanoate (**2d**; 8%). The ^{1}H and ^{13}C NMR and LRMS spectra of **2b** and **2d** were in good agreement with those reported in the literature.[5] The difference in the regioselectivity between **1a** and **2a** towards the oxidation with Os(TMP)CO/TBHP can probably be attributed to steric environments. The 7α-acetoxyl group in **1a** has a *syn* diaxial relationship with respect to the tertiary methine 14α-H. In contrast, the 7β-acetoxyl group in **2a** has a less-crowded gauche conformation, which permits more facile access of an active *trans*-dioxoosmiumporphyrin species to the 14α-H position to yield **2d**.

5α-Hydroxylation in **1a** and **2a** is of particular interest because it renders the inversion of the A/B ring junction from the *cis* 5β-steroids to the *trans* 5α-hydroxy derivatives (**1c** and **2c**). In the ^{1}H NMR spectrum, the 3β-H signal (br. m) in **1a** and **2a** was shifted downfield by 0.43 ppm by 5β-hydroxylation and resonated at δ = 5.02–5.10 ppm in **1b** and **2b**. The corresponding 3β-H (m) was deshielded by 0.53 ppm by 5α-hydroxylation (**1c** and **2c**) and appeared at δ = 5.13–5.20 ppm. Both the 7β-H (m) in **1c** and the 7α-H (br. m) in **2c** also caused a downfield shift of 0.09–0.25 ppm and occurred at δ = 4.97 ppm. In the ^{13}C NMR spectrum, the chemical shifts of the α carbon at C-3 (δ = 69.8 and 70.5 ppm) and C-5 (δ = 73.4 and 74.2 ppm) for **1c** and **2c** agreed well with those (δ = 70.8 and 73.0 ppm, respectively) reported for analogous cholestan-5α-ol and cholestane-3α,5α-diol 3-acetate.[11] The β carbon signals at C-4 and C-6 in **1b** and **1c** occurred at δ = 40.6 and 40.8 ppm (or vice



Scheme 1.



Scheme 2.

versa), respectively,[5] whereas they appeared at δ = 36.9 ppm in **1c**. Similarly, the chemical shifts of the C-4 (δ =36.8 ppm) and C-6 (δ =39.2 ppm) signals in **2c** differed markedly from those observed for **2b** (39.1 and 42.0 ppm, respectively). The above ^{1}H and ^{13}C NMR spectroscopic data for **1c** and **2c** are consistent with the assignment of 5α-hydroxy configuration.

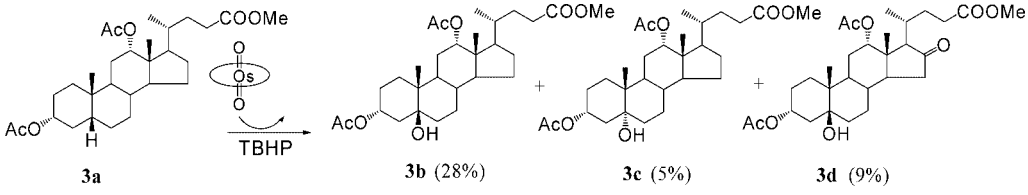
As shown in Scheme 3, treatment of methyl deoxycholate diacetate (**3a**) possessing an axial 12α-acetoxyl group with the Os(TMP)CO/TBHP system resulted in simultaneous hydroxylation at C-5, hydroxylation–isomerization at C-5, and ketonization at C-16 to give methyl 3α,12α-diacetoxy-5β-hydroxycholanoate (**3b**; 28%), methyl 3α,12α-diacetoxy-5α-hydroxycholanoate (**3c**; 5%), and methyl 3α,12α-diacetoxy-16-oxo-5β-cholanoate (**3d**; 9%). The ^{1}H and ^{13}C NMR spectra of **3b**[5,12] and **3d**[12] agreed completely with those reported previously. Again, the 3β-H signal (br. m) of **3b** in the ^{1}H NMR spectrum resonated at δ = 5.05 ppm, whereas the corresponding signal (m) of **3c** appeared at δ = 5.20 ppm. In addition, the ^{13}C NMR spectroscopic chemical shifts of the C-3 (δ =70.8 ppm), C-4 (δ =37.4 ppm), and C-5 (δ =73.1 ppm) signals for **3c** were very similar to those observed for **1c** and **2c**. Ketonization at C-16, instead of hydroxylation at the axially oriented methine 14α- or 17α-H, is also ascribed to steric effects of the axial 12α-acetoxyl group (Scheme 3).

When methyl cholate triacetate (**5a**) having both 7α- and 12α-acetoxyl groups was treated with Os(TMP)CO/TBHP, four hydroxylation and ketonization products were isolated and their structures were identified as follows (Scheme 4): methyl 3α,7α,12α-triacetoxy-5β-hydroxycholanoate (**5b**; 27%), methyl 3α,7α,12α-triacetoxy-15-oxo-5β-cholanoate (**5c**; 15%), 3α,7α,12α-triacetoxy-16-oxo-5β-cholanoate (**5d**; 7%), and 3α,7α,12α-triacetoxy-5β-hydroxy-16-oxocholanoate (**5e**; 4%).

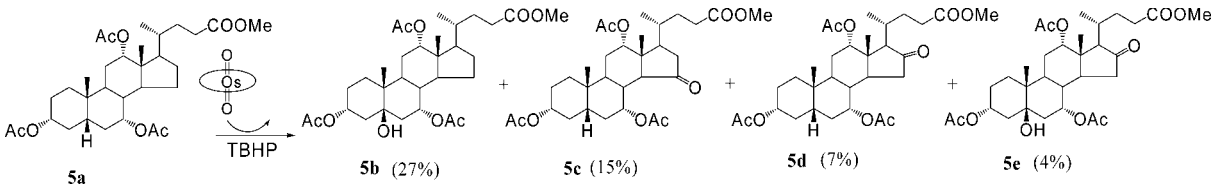
Although the formation of **5b**, **5d**, and **5e** is similar to the result reported for the oxidation of **5a** with dimethyldioxirane,[5,12] 15-ketonization (**5c**) is specific for the Os(TMP)CO/TBHP oxidation. The ^{1}H and ^{13}C NMR spectroscopic data for **5c** provided confirmatory evidence for the structure. The ^{1}H NMR spectrum of **5c** showed an appreciable downfield shift (0.85 ppm) of the 7β-H (m) and resonated at δ = 5.76 ppm relative to that (δ =4.91 ppm) of **5a**. The large deshielding is probably due to spatial proximity of the 7β-H with a carbonyl group. In the ^{13}C NMR spectrum, **5c** showed the presence of a carbonyl group at δ = 213.3 ppm, which is similar to that (δ =215.1 ppm) reported for 15-oxosteroids.[13] As expected, the β-carbons at C-14 and C-16 were deshielded to a large extent (10.7 and 13.6 ppm) and resonated at δ = 54.0 and 40.7 ppm, respectively, whereas the γ-carbon at C-17 was shifted upfield by 4.1 ppm and occurred at δ = 43.2 ppm.

A much different regioselectivity was observed for methyl hyodeoxycholate diacetate (**4a**), which has an equatorially oriented 6α-acetoxyl group (Scheme 5). The oxygenation of **4a** with the Os(TMP)CO/TBHP system occurred preferentially at the C-14 position to afford methyl 3α,6α-diacetoxy-14α-hydroxy-5β-cholanoate (**4b**; 28%)[14] and methyl 3α,6α-diacetoxy-14β-hydroxy-5β-cholanoate (**4c**; 11%), along with methyl 3α,6α-diacetoxy-14α,15α-epoxy-5β-cholanoate (**4d**; 7%). The expected 5β-hydroxylation did not occur at all, probably because the 6α-acetoxyl group in **4a** has a gauche conformation with respect to the adjacent 5β-H. The presence of the 6α-acetoxyl group, therefore, completely shields the attack of an active *trans*-dioxoosmiumporphyrin species on the 5β-H, which allows competitive 14-hydroxylation subject to steric and electronic constraints. Again, the *trans* C/D ring junction in **4b** was isomerized to the *cis* form of **4c** (see below). Epoxide **4d** is probably formed by elimination of a 14-hydroxy group in **4b** or **4c** and subsequent epoxidation of the resulting Δ^{14}-unsaturated intermediate.

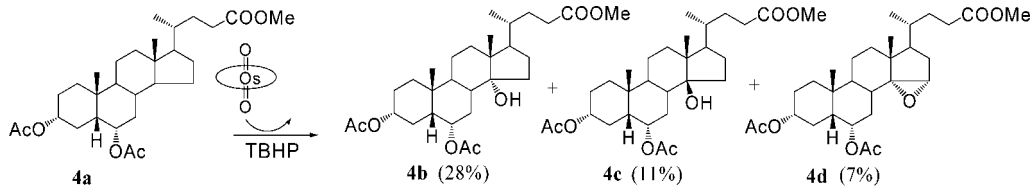
The position and stereochemistry of a newly inserted oxygen function for **4b**–**4d** were determined by measuring the ^{1}H–^{1}H and ^{1}H–^{13}C shift-correlated 2D NMR spectra, which included ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{1}H–^{13}C HMQC, and ^{1}H–^{13}C HMBC as well as DEPT measurements. Table 1 shows the complete ^{1}H and ^{13}C spectroscopic resonance assignments of **4b**–**4d**. In the ^{1}H NMR spectrum of **4b**, the 19- and 21-methyl protons were barely



Scheme 3.



Scheme 4.



Scheme 5.

shifted (0.98 and 0.89 ppm, respectively) relative to those of parent compound **4a**, whereas the 18-methyl signal (δ = 0.64 ppm in **4a**) was shifted downfield by 0.14 ppm and resonated at δ = 0.78 ppm. In the ^{13}C NMR spectrum of **4b**, the α carbon at C-14 exhibited a large downfield shift of 29.2 ppm and appeared at δ = 85.3 ppm. Similarly, expected downfield shifts (3.0–8.9 ppm) and upfield shifts (7.6–7.7 ppm) were observed for the β carbons (C-8, C-13, and C-15) and the γ carbons (C-9 and C-12), respectively. These ^{1}H and ^{13}C chemical shift values in **4b** were consistent with those reported in the literature.[14]

In contrast, a comparison of the ^{1}H NMR spectra of **4a** and **4c** indicated that the 18-methyl signal in **4c** was shifted further downfield by 0.34 ppm and occurred at δ = 0.98 ppm, though the 19- and 21-methyls (0.96 and 0.91 ppm, respectively) were not shifted at all. The chemical shifts of the 18- and 19-methyl protons were in good agreement with those (δ =0.97 ppm) reported for 3β-acetoxy-14β-hydroxy-5β-pregnan-20-one.[15] As expected in the ^{13}C NMR spectrum of **4c**, the α (C-14) and β carbons (C-8, C-13, and C-15) were shifted downfield by 28.7 and 4.2–8.0 ppm, respectively, whereas the γ carbons (C-9 and C-16) were shifted upfield by 4.0 ppm. Of note is that the C-22 and C-23 signals have essentially the same ^{13}C NMR chemical shift (ca. 31.0 ppm) in various C_{24} (5β)-bile acid derivatives, and resonate at δ = 28.5 and 32.4 ppm, respectively, which suggests the presence of a hydroxy substituent on the β-face of the steroid nucleus. To confirm the stereochemical configuration at C-14 in **4c**, the NOE spectra were measured. In the NOESY, a correlation peak was observed between 7α-H and 9α-H. Irradiation of the 7α-H in the difference NOE showed a correlation with 4α-H (δ =1.75 ppm) and 15α-H (δ =1.53 ppm), indicating their spatial proximity. Thus, the hydroxy group at C-14 in **4c** was assigned to the β configuration. In addition, the HMBC spectrum of **4c** showed correlation peaks between a carbon signal at δ =

Table 1. Complete ^{1}H and ^{13}C NMR chemical shifts of 14-oxygenated compounds **4b**, **4c**, and **4d**.[a]

	4b				**4c**				**4d**			
Carbon no.	Type	^{13}C	^{1}H		Type	^{13}C	^{1}H		Type	^{13}C	^{1}H	
			α	ß			α	ß			α	ß
1	CH_2	35.1	1.82	1.17	CH_2	34.9	1.82	1.16	CH_2	34.9	1.85	1.15
2	CH_2	26.1	1.66	1.85	CH_2	26.3	1.56	1.82	CH_2	25.9	1.64	1.82
3	CH	73.6		4.70 (br-m)	CH	73.6		4.70 (br-m)	CH	73.5		4.68 (br-m)
4	CH_2	26.3	1.73	1.52	CH_2	26.6	1.75	1.47	CH_2	26.4	1.70	1.49
5	CH	45.4		1.75	CH	45.1		1.79	CH	45.3		1.74
6	CH	71.0		5.18 (m)	CH	71.1		5.14 (m)	CH	70.6		5.15 (m)
7	CH_2	26.5	1.57	1.65	CH_2	27.0	1.40	2.00	CH_2	25.3	1.52	1.40
8	CH	37.6		1.88	CH	41.2		1.70	CH	31.7		2.28
9	CH	32.2	1.98		CH	35.8	1.66		CH	36.4	1.86	
10	C	35.9			C	36.2			C	36.1		
11	CH_2	19.6	1.40	1.25	CH_2	21.0	1.26	1.14	CH_2	20.6	1.50	1.31
12	CH_2	32.1	2.05	1.70	CH_2	43.3	1.25	1.52	CH_2	35.7	1.87	1.55
13	C	46.7			C	47.0			C	41.3		
14	C	85.3			C	84.9			C	73.6		
15	CH_2	32.9	2.42	1.38	CH_2	32.0	1.53	1.87	CH	58.4		3.35 (s)
16	CH_2	26.9	2.02	1.40	CH_2	24.3	1.86	1.65	CH_2	31.9	2.13	1.25
17	CH	50.6	1.77		CH	56.8	1.54		CH	48.4	1.25	
18	CH_3	15.7	0.78 (s)		CH_3	15.5	0.98 (s)		CH_3	14.6	0.84 (s)	
19	CH_3	22.9	0.98 (s)		CH_3	23.2	0.96 (s)		CH_3	23.0	0.99 (s)	
20	CH	35.1	1.48		CH	33.8	1.54		CH	33.2	1.46	
21	CH_3	18.1	0.89 (d, 6.2)		CH_3	20.5	0.91 (d, 6.4)		CH_3	18.4	0.86 (d, 6.8)	
22	CH_2	31.0	1.37, 1.83 (each, m)		CH_2	28.6	1.30, 1.75 (each, m)		CH_2	30.6	1.37, 1.78 (each, m)	
23	CH_2	31.0	2.26, 2.38 (each, m)		CH_2	32.5	2.23, 2.32 (each, m)		CH_2	30.8	2.21, 2.36 (each, m)	
24	C	174.6			C	174.6			C	174.5		
$COO\underline{C}H_3$	CH_3	51.5	3.66		CH_3	51.5	3.65		CH_3	51.5	3.66	
$O\underline{C}OCH_3$	C	170.5			C	170.3, 170.4			C	170.6		
$OCO\underline{C}H_3$	CH_3	21.3, 21.4	2.01, 2.03		CH_3	21.3, 21.4	2.01, 2.04		CH_3	21.3, 21.4	2.00, 2.02	

[a] Measured in $CDCl_3$ at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR; chemical shifts are expressed as δ [ppm] relative to Me_4Si; abbreviations used: s, singlet; d, doublet; m, multiplet; br. m, broad multiplet; values in parentheses refer to signal multiplicity and coupling constant (J in Hz).

84.8 ppm and 18-CH_3 (δ =0.98 ppm) and between a carbon signal at δ = 56.6 ppm and 18- and 21-CH_3. The results indicate that the ^{13}C NMR signals that appear at δ = 84.8 and 56.6 ppm should be assigned to C-14 and C-17, respectively.

For **4d**, the 18-methyl proton was deshielded by 0.2 ppm relative to that of **4a** and appeared at δ = 0.84 ppm. The appearance at δ = 3.35 ppm of a proton signal (s) arising from 15β-H (see below) would be strong evidence for the 14α,15α-epoxide because the ^1H NMR chemical shift is in agreement with that observed for methyl 3α-cathyloxy-14α,15α-epoxy-5β-cholan-24-oic acid.[16] A comparison of the ^{13}C NMR spectrum of **4d** with that of **4a** revealed that α epoxidation at C-14 caused an appreciable downfield shift of C-14 by 17.5 ppm and of C-15 by 34.4 ppm; they resonated at δ = 73.6 and 58.4 ppm, respectively. A correlation peak between 15β-H (δ =3.35 ppm) and 7β-H (1.41) was observed in the NOESY spectrum of **4d**, which is consistent with the epoxide ring having an α configuration. Further evidence for the 14α,15α-epoxide was confirmed by the appearance of a correlation peak between C-14 and 18-CH_3 in the HMBC spectrum and the presence of a peak, at δ = 3.35 ppm, arising from 15β-H in the HMQC spectrum.

As mentioned above, a remarkable feature of the Os(TMP)CO/TBHP oxidation was simultaneous formation of isomeric 5β- and 5α-hydroxy derivatives (**1b** vs. **1c**, **2b** vs. **2c**, and **3b** vs. **3c**) from **1a**–**3a** and of 14α- and 14β-hydroxy isomers (**4b** vs. **4c**) from **4a**. It is generally accepted that 5β-steroids with a *cis* A/B ring fusion are easily hydroxylated at the 5β-position by many oxidants,[5–10] whereas analogous 5α-hydroxylation is severely limited for sterically more crowded 5α-steroids with a *trans* A/B ring fusion.[9,17,18] Rotman and Mazur[19] previously reported that the photoirradiation of 5α-androstane-3β,17β-diol diacetate in *tert*-butyl alcohol in the presence of peracetic acid results in both the 5α- and 14β-hydroxylations in one step.

The possible mechanism of the simultaneous formation of 5β- and 5α-hydroxy derivatives can be rationalized by the following two pathways as outlined in Figure 1. According to the previous findings of Gorodetsky et el.,[20] one-step epimerization at unactivated tertiary carbon atoms in saturated cyclohexane derivatives, including steroid derivatives, by irradiation in the presence of mercuric bromide or *N*-bromosuccinimide in hydrocarbon solution proceeds through free radical intermediates. On this basis, a metalloporphyrin peroxy radical attacks the tertiary methine carbon at C-5 in substrates **1a**–**3a**, which generates an osmium-porphyrin hydroperoxide and C-5 alkyl radicals (pathway A).[3,21] As can been seen in route A-1, attack of the hydroperoxide on the free radicals gives 5β-alcohols (**1b**–**3b**) exclusively. Alternatively, electron transfer of a fraction of the free radicals to osmium metal[22] generates the carbocations (route A-2). Hydration of the carbocations by nucleophilic attack of water on both the α- and β-faces affords 5α-alcohols (**1c**–**3c**), together with **1b**–**3b** in a competing reaction. In agreement with this possibility, Waters et al.[23] reported one-step 5α- and 5β-hydroxylations of 4- or 5-cholestene in *tert*-butyl alcohol, water, and *o*-xylene by photosensitized isomerization–hydroxylation, which proceeds through carbocation intermediates. In this case (pathway B), 5β-hydroxy compounds **1b**–**3b** are dehydrated by *trans* diaxial elimination to afford respective Δ^4-unsaturated intermediates, which in turn undergo protonation to yield stable tertiary C-5 carbocations.[24] Nucleophilic attack of water on the carbocations gives stereoisomeric 5β- and 5α-alcohols in a competing reaction.

To confirm which of the proposed mechanisms (pathway A or B) was more favorable, isolated 5β-hydroxy esters **1b**–**3b** were subjected to the Os(TMP)CO/TBHP oxidation under the same reaction conditions to see if corresponding 5α-hydroxy isomers **1c**–**3c** could be formed by **1b**–**3b**. The time-course of the formation of **1c**–**3c** was followed by capillary

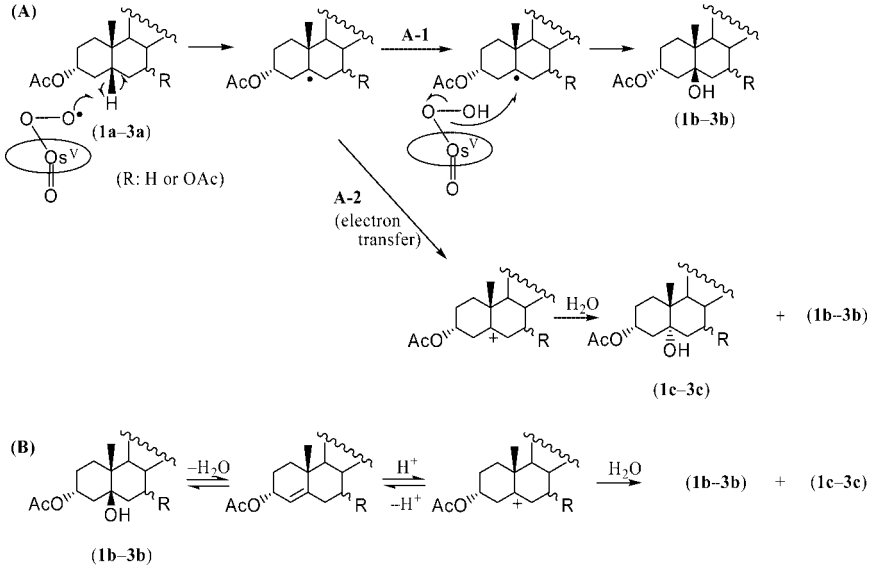


Figure 1. Possible mechanisms of simultaneous formation of isomeric 5α- and 5β-hydroxylated derivatives.

GC analysis. The result showed that although **1b**–**3b** was transformed into **1c**–**3c**, each of the isomerization ratios was much smaller (0.5–1.6%) than the expected value (5–16%), which suggested a minor route of pathway B. Therefore, major routes controlling the simultaneous formation of the 5β- and 5α-hydroxy derivatives would be involved in the mechanism shown in pathway A. The predominant formation of **1b**–**3b**, compared to the corresponding 5α-hydroxylation (**1c**–**3c**), supports the above hypothesis. Similarly, concurrent occurrence of 14α- and 14β-hydroxy isomers **4b** and **4c**, respectively, from **4a** could also take place through both C-14 free radical and C-14 carbocation routes.

In conclusion, the methyl ester peracetate derivatives of a series of common, natural (5β)-bile acids were effectively oxyfunctionalized with anhydrous TBHP catalyzed by Os(TMP)CO to cause 5β- and 14α-hydroxylations and/or ketonization at C-15 and C-16 regioselectively. Of further interest was that the oxidation system caused isomerization of the A/B or C/D ring junction to give the corresponding 5α- or 14β-hydroxy isomers, respectively, in one step. The reactivity of unactivated C–H bonds toward Os(TMP)CO/TBHP depends significantly on both their electronegativity and steric availability. The oxidant system reported here appears to be of considerable utility in achieving remote oxyfunctionalization of substrates whose synthesis in the past was quite cumbersome. Work on further applications and on the mechanism of the Os(TMP)CO/TBHP reaction is now in progress.

Experimental Section

Materials and Methods: Melting points (m.p.) were determined with an electric micro hot stage and are uncorrected. IR spectra were obtained with a JASCO FT-IR 4100 spectrometer (Tokyo, Japan) for samples in KBr tablets. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL JNM-EX 270 instrument (Tokyo, Japan) and $CDCl_3$ containing 0.1% Me_4Si as the solvent; chemical shifts are expressed as δ (ppm) relative to Me_4Si. Homonuclear (^{1}H–^{1}H) and heteronuclear (^{1}H–^{13}C) shift-correlated 2D NMR spectra (^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{1}H–^{13}C HMQC, and ^{1}H–^{13}C HMBC) were measured with a JEOL GSX-400 spectrometer by using a standard pulse sequence and parameters recommended by the manufacturer. ^{13}C NMR spectroscopic signals corresponding to the methyl (CH_3), methylene (CH_2), methine (CH), and quaternary (C) carbons were differentiated by means of DEPT experiment. Low-resolution mass (LRMS) spectra were recorded with a JEOL-GCmate gas chromatography/mass spectrometry at 70 eV with an electron ionization (EI) probe by using the positive ion mode (PIM). High-resolution mass (HRMS) spectra were performed with a JEOL-GCmate with an electron ionization (EI) probe in the PIM. A Shimadzu GC-2010 gas chromatograph (GC) equipped with a flame ionization detector was used isothermally at 280 °C or 300 °C or with temperature programming (260 to 300 °C at 2 °C min^{-1}) fitted with a chemically bonded, fused-silica capillary column (25QC3/BPX5; 25 m X 0.32 mm i.d.; film thickness, 0.25 μm; SGE, Yokohama, Japan). Preparative HPLC was carried out on an apparatus consisting of a Hitachi L-7100 pump (Tokyo, Japan) and a Shodex RI-102 detector (Tokyo, Japan) with a Senshu Pak PEGASIL ODS column (250 mm X 10 mm i.d., Tokyo, Japan); a mixture of methanol/water (9:1 to 4:1) was used as the mobile phase. The apparatus used for normal phase (NP) MPLC consisted of a Shimamura YRD-880 RI-detector (Tokyo, Japan) and uf-3040 chromatographic pump with silica gel 60 (230–400 mesh; Nacalai Tesque, Inc., Kyoto, Japan) as adsorbent and hexane/EtOAc (9:1 to 4:1) mixtures as eluent. Reverse-phase (RP) MPLC was carried out by using Cosmosil 75C_{18}-PREP (Nacalai Tesque) as adsorbent and methanol/water (4:1) or acetonitrile/water (7:3 to 13:7) as the eluent. NP-TLC was performed on precoated silica gel 60F_{254} plates (0.25 mm layer thickness; Merck, Darmstadt, Germany) with hexane/EtOAc (7:3 to 2:3) mixtures as the developing solvent. RP-TLC was carried out on precoated RP-18$_{F254S}$ plates (Merck) using methanol/water (9:1 to 4:1) as the developing solvent.

Substrates (**1a**–**5a**) used in this study were from our laboratory collection. TBHP (70%) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan); it was extracted with CH_2Cl_2 and the organic layer was evaporated at below 25 °C under reduced pressure prior to use. *meso*-Tetramesitylporphyrin was prepared by a slight modification of the procedure of Lindsey et al.[25] Os(TMP)CO complex was prepared from the tetramesitylporphyrin and $Os_3(CO)_{12}$ by a literature method of Che et al.[26]

General Procedure for the Oxyfunctionalization of Bile Acid Derivatives by Os(TMP)CO/TBHP: To a solution of bile acid methyl ester peracetate derivative (1.1 mmol) and molecular sieves (250 mg; 4 Å) in benzene (5 mL) was successively added Os(TMP)CO (6 mg, 5.5 μmol) and anhydrous TBHP (1.9 mL, 22 mmol), and the mixture was heated at reflux for 96 h; the reaction was monitored by TLC. After completion of the reaction, each of the products was isolated by a combined use of open column chromatography, NP- or RP-MPLC and/or HPLC.

Methyl 3α,7α-Diacetoxy-5β-hydroxycholan-24-oate (1b): Isolated from the reaction product of **1a** by open column chromatography (EtOAc/hexane, 2:3) as a colorless amorphous solid (fraction 1, 47% yield) crystallized from EtOAc/hexane. M.p. 155–157 °C (ref.[5] 158–159 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.65 (s, 3 H, 18-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.92 (d, J = 7.3 Hz, 3 H, 21-CH_3), 2.03, 2.07 (s, each 3 H, -$COCH_3$), 3.67 (s, 3 H, -$COOCH_3$), 4.92 (m, 1 H, 7β-H), 5.02 (br. m, 1 H, 3β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 11.6 (C-18), 15.8 (C-19), 18.2 (C-21), 20.8 (C-11), 21.4 ($OCOCH_3$), 21.4 ($OCOCH_3$), 23.6 (C-15), 26.2 (C-2), 27.9 (C-16), 29.3 (C-1), 30.9 (C-22), 31.1 (C-23), 35.2 (C-20), 36.9, 37.2 (C-8, C-9), 39.1 (C-12), 39.8 (C-10), 40.6, 40.8 (C-4, C-6), 42.5 (C-13), 50.2 (C-14), 51.5 ($COOCH_3$), 55.6 (C-17), 70.9 (C-3 and C-7), 74.4 (C-5), 170.2 ($OCOCH_3$), 170.5 ($OCOCH_3$), 174.6 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3463 (OH), 1734, 1714 (C=O) cm^{-1}. LRMS (EI): *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,7α-Diacetoxy-5α-hydroxycholan-24-oate (1c): Isolated from the reaction product of **1a** by RP-MPLC (acetonitrile/water; 7:3) as a colorless amorphous solid (fraction 2, 6% yield) crystallized from methanol/water. M.p. 122–125 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.66 (s, 3 H, 18-CH_3), 0.93 (d, J = 6.2 Hz, 3 H, 21-CH_3), 0.96 (s, 3 H, 19-CH_3), 2.07, 2.09 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.97 (m, 1 H, 7β-H) 5.13 (m, 1 H, 3β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 11.8 (C-18),

15.5 (C-19), 18.2 (C-21), 20.7 (C-11), 21.5 ($OCOCH_3$), 21.6 ($OCOCH_3$), 23.5 (C-15), 25.6 (C-1), 26.3 (C-16), 27.9 (C-2), 30.9, 31.0 (C-22, C-23), 35.3 (C-20), 36.9 (C-4 and C-6), 37.6 (C-8), 39.2 (C-9), 39.3 (C-12), 39.6 (C-10), 42.8 (C-13), 50.1 (C-14), 51.5 ($COOCH_3$), 55.5 (C-17), 69.8, 71.3 (C-7 or 3), 73.4 (C-5), 170.0 ($OCOCH_3$), 170.4 ($OCOCH_3$), 174.7 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3450 (OH), 1734 (C=O) cm^{-1}. LRMS (EI): *m*/*z* (%) = 446 (2) [M – AcOH], 428 (23) [M – AcOH – H_2O], 386 (53) [M – 2AcOH], 368 (100) [M – 2AcOH – H_2O], 353 (40) [M – 2AcOH – H_2O – CH_3], 332 (48), 313 (8) [M – AcOH – H_2O – S.C.], 253 (28) [M – 2AcOH – H_2O – S.C.]. HRMS (FAB): calcd. for $C_{29}H_{46}O_7Na$ [M + Na]$^+$ 529.3142; found 529.3147.

Methyl 3α,7β-Diacetoxy-5β-hydroxycholan-24-oate (2b): Isolated from the reaction product of **2a** by open column chromatography (EtOAc/hexane, 3:2) as colorless thin plates (fraction 3, 20% yield) crystallized from methanol/water. M.p. 152–154 °C (ref.[5] 148–149 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.68 (s, 3 H, 18-CH_3), 0.93 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.94 (s, 3 H, 19-CH_3), 1.99, 2.02 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.65 (m, 1 H, 7α-H), 5.10 (br. m, 1 H, 3β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.0 (C-18), 16.2 (C-19), 18.3 (C-21), 21.2 (C-11), 21.3 ($OCOCH_3$), 21.7 ($OCOCH_3$), 25.5 (C-15), 25.9 (C-2), 28.4 (C-16), 29.1 (C-1), 30.9, 31.0 (C-22, C-23), 35.2 (C-20), 39.0 (C-10), 39.1 (C-4), 39.2 (C-8), 39.6 (C-12), 41.4 (C-9), 42.0 (C-6), 43.3 (C-13), 51.5 ($COOCH_3$), 54.9 (C-17), 55.3 (C-14), 70.5 (C-3), 73.9 (C-7), 74.4 (C-5), 170.4 ($OCOCH_3$), 170.4 ($OCOCH_3$), 174.6 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3487 (OH), 1736, 1712 (C=O) cm^{-1}. LRMS: *m*/*z* (%) = 386 (16) [M – 2AcOH], 368 (36) [M – 2AcOH – H_2O], 332 (51), 271 (64) [M – 2AcOH – S.C.], 253 (78) [M – 2AcOH – H_2O – S.C.], 110 (100).

Methyl 3α,7β-Diacetoxy-5α-hydroxycholan-24-oate (2c): Isolated from the reaction product of **2a** by RP-MPLC (acetonitrile/water, 7:3) as a colorless amorphous solid (fraction 1, 16% yield) crystallized from methanol/water. M.p. 144–147 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.69 (s, 3 H, 18-CH_3), 0.92 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.99 (s, 3 H, 19-CH_3), 1.99, 2.06 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.97 (br. m, 1 H, 7α-H) 5.20 (m, 1 H, 3β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.1 (C-18), 16.2 (C-19), 18.3 (C-21), 21.0 (C-11), 21.4 ($OCOCH_3$), 21.8 ($OCOCH_3$), 25.4, 25.5, 26.7 (C-1, C-2, C-15), 28.4 (C-16), 30.9, 30.0 (C-22, C-23), 35.3 (C-20), 36.8 (C-4), 38.9 (C-10), 39.2 (C-6), 39.4 (C-8), 39.8 (C-12), 43.5 (C-13), 44.3 (C-9), 51.5 ($COOCH_3$), 54.8 (C-17), 55.0 (C-14), 70.5 (C-3), 73.7 (C-7), 74.2 (C-5), 169.2 ($OCOCH_3$) 170.5 ($OCOCH_3$) 174.7 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3449 (OH), 1735 (C=O) cm^{-1}. LRMS (EI): *m*/*z* (%) = 446 (1) [M – AcOH], 428 (31) [M – AcOH – H_2O], 386 (14) [M – 2AcOH], 368 (100) [M – 2AcOH – H_2O], 353 (29) [M – 2AcOH – H_2O – CH_3], 332 (22), 313 (20) [M – AcOH – H_2O – S.C.], 253 (24) [M – 2AcOH – H_2O – S.C.]. HRMS (FAB): calcd. for $C_{29}H_{46}O_7Na$ [M + Na]$^+$ 529.3142; found 529.3141.

Methyl 3α,7β-Diacetoxy-14α-hydroxy-5β-cholan-24-oate (2d): Isolated from the reaction product of **2a** by RP-MPLC (acetonitrile/water, 7:3) as a noncrystalline substance (fraction 2, 8% yield) (ref.[5] viscous oil). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.79 (s, 3 H, 18-CH_3), 0.90 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.98 (s, 3 H, 19-CH_3), 2.00, 2.02 (s, each 3 H, -$COCH_3$), 3.67 (s, 3 H, -$COOCH_3$), 4.66 (br. m, 1 H, 3β-H), 5.15 (br. m, 1 H, 7α-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 15.6 (C-18), 18.3 (C-21), 20.0 (C-11), 21.4 ($OCOCH_3$), 21.9 ($OCOCH_3$), 22.8 (C-19), 26.3 (C-2), 27.3 (C-16), 31.1, 31.2 (C-22, C-23), 32.0 (C-12), 32.3 (C-9), 32.8, 33.0 (C-4, C-6), 34.1 (C-10), 34.7 (C-1), 35.0 (C-15 and C-20), 42.0 (C-5), 43.4 (C-8), 47.4 (C-13), 49.5 (C-17), 51.5 ($COOCH_3$) 69.4 (C-7), 73.6 (C-3), 84.0 (C-14) 170.6 ($OCOCH_3$), 170.8 ($OCOCH_3$), 174.6 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3518 (OH), 1735 (C=O) cm^{-1}. LRMS: *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).

Methyl 3α,12α-Diacetoxy-5β-hydroxycholan-24-oate (3b): Isolated from the reaction product of **3a** by open column chromatography (EtOAc/hexane, 1:1) as colorless needles (fraction 3, 28% yield) crystallized from Et_2O/hexane. M.p. 129–130 °C (ref.[5] 127–128 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.72 (s, 3 H, 18-CH_3), 0.81 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.87 (s, 3 H, 19-CH_3), 2.02, 2.10 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 5.05 (br. m, 1 H, 3β-H), 5.10 (m, 1 H, 12β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.2 (C-18), 15.9 (C-19), 17.4 (C-21), 21.2 ($OCOCH_3$), 21.3 ($OCOCH_3$), 23.3 (C-15), 25.8 (C-16), 26.0 (C-2), 27.2 (C-11), 28.1 (C-7), 29.0 (C-1), 30.7, 30.8 (C-22, C-23), 34.6 (C-20), 34.7 (C-8), 36.5 (C-6), 37.0 (C-9), 38.0 (C-4), 38.9 (C-10), 44.7 (C-13), 47.4 (C-17), 49.3 (C-14), 51.4 ($COOCH_3$), 71.2 (C-3), 75.0 (C-5), 75.5 (C-12), 170.3 ($OCOCH_3$), 170.4 ($OCOCH_3$), 174.5 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3475 (OH), 1730 (C=O) cm^{-1}. LRMS (EI): *m*/*z* (%) = 428 (7) [M – AcOH – H_2O], 386 (3) [M – 2AcOH], 368 (31) [M – 2AcOH – H_2O], 332 (22), 331 (17) [M – AcOH – S.C.], 313 (18) [M – AcOH – H_2O – S.C.], 271 (23) [M – 2AcOH – S.C.], 253 (100) [M – 2AcOH – H_2O – S.C.], 211 (18) [M – 2AcOH – H_2O – S.C. – ring D].

Methyl 3α,12α-Diacetoxy-5α-hydroxycholan-24-oate (3c): Isolated from the reaction product of **3a** by RP-MPLC (acetonitrile/water, 13:7) as colorless thin plates (fraction 2, 5% yield) crystallized from methanol/water. M.p. 159–160 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.73 (s, 3 H, 18-CH_3), 0.80 (d, *J* = 5.9 Hz, 3 H, 21-CH_3), 0.94 (s, 3 H, 19-CH_3), 2.07, 2.08 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 5.07 (m, 1 H, 12β-H), 5.20 (m, 1 H, 3β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.4 (C-18), 15.7 (C-19), 17.5 (C-21), 21.4 ($OCOCH_3$), 21.5 ($OCOCH_3$), 23.3 (C-15), 25.2 (C-1), 25.5 (C-2), 25.6 (C-16), 26.7 (C-11), 27.2 (C-7), 30.8, 31.0 (C-22, C-23), 33.5 (C-6), 34.7 (C-8 and C-20), 37.4 (C-4), 39.1 (C-10), 39.3 (C-9), 45.0 (C-13), 47.4 (C-17), 48.9 (C-14), 51.5 ($COOCH_3$), 70.8 (C-3), 73.1 (C-5), 76.0 (C-12), 169.2 ($OCOCH_3$), 170.6 ($OCOCH_3$), 174.6 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3590 (OH), 1738 (C=O) cm^{-1}. LRMS (EI): *m*/*z* (%) = 446 (4) [M – AcOH], 428 (100) [M – AcOH – H_2O], 386 (6) [M – 2AcOH], 368 (91) [M – 2AcOH – H_2O], 353 (54) [M – 2AcOH – H_2O – CH_3], 332 (51) [M – $2CH_3$ – S.C. – part of ring D], 313 (29) [M – AcOH – H_2O – S.C.], 253 (86) [M – 2AcOH – H_2O – S.C.]. HRMS (FAB): calcd. for $C_{29}H_{46}O_7Na$ [M + Na]$^+$ 529.3142; found 529.3141.

Methyl 3α,12α-Diacetoxy-16-oxo-5β-cholan-24-oate (3d): Isolated from the reaction product of **3a** by RP-MPLC (acetonitrile/water, 13:7) as a noncrystalline substance (fraction 1, 9% yield) (ref.[12] viscous oil). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.89 (s, 3 H, 18-CH_3), 0.93 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.94 (s, 3 H, 19-CH_3), 2.02, 2.04 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.72 (br. m, 1 H, 3β-H), 5.10 (m, 1 H, 12β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 14.0 (C-18), 17.9 (C-21), 21.3 ($OCOCH_3$), 21.4 ($OCOCH_3$), 23.0 (C-19), 24.9 (C-7), 26.0 (C-11), 26.6 (C-2), 26.7 (C-6), 30.1 (C-20), 30.2 (C-23), 31.8 (C-22), 32.2 (C-4), 34.2 (C-10), 34.4 (C-1), 34.5, 34.7 (C-8, C-9), 38.2 (C-15), 41.6 (C-5), 43.7 (C-14), 45.7 (C-13), 51.5 ($COOCH_3$), 61.3 (C-17), 73.9 (C-12), 74.0 (C-3), 170.1 ($OCOCH_3$), 170.5 ($OCOCH_3$), 174.2 (C-24), 216.9 (C-16) ppm. IR (KBr): $\tilde{\nu}$ = 1738 (C=O) cm^{-1}. LRMS (EI): *m*/*z* (%) = 444 (5) [M – AcOH], 429 (10) [M – AcOH – CH_3], 390 (9) [M – S.C. + H], 384 (10) [M – 2AcOH]. 375 (15) [M – CH_3 – S.C. + H], 371 (57), 352 (25), 330 (16) [M – AcOH – S.C. + H], 315 (28) [M –

AcOH – CH_3 – S.C. + H], 311 (54), 270 (27) [M – 2AcOH – S.C. + H], 255 (66) [M – 2AcOH – CH_3 – S.C. + H], 170 (100).

Methyl 3α,6α-Diacetoxy-14α-hydroxy-5β-cholan-24-oate (4b): Isolated from the reaction product of **4a** by NP-MPLC (EtOAc/hexane, 1:9) as colorless needles (fraction 2, 28% yield) recrystallized from methanol/water. M.p. 163–165 °C (ref.[14] 163–165 °C). IR (KBr): $\tilde{\nu}$ = 3578 (OH), 1734 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 506 (<1) [M], 428 (3) [M – AcOH – H_2O], 368 (8) [M – 2AcOH – H_2O], 313 (13), 281 (8), 253 (100) [M – 2AcOH – H_2O – S.C.], 211 (26) [M – 2AcOH – H_2O – S.C. – ring D].

Methyl 3α,6α-Diacetoxy-14β-hydroxy-5β-cholan-24-oate (4c): Isolated from the reaction product of **4a** by NP-MPLC (EtOAc/hexane, 1:9) as a noncrystalline substance (fraction 3, 11% yield). IR (KBr): $\tilde{\nu}$ = 3528 (OH), 1738 (C=O) cm^{-1}. LRMS (FAB): *m/z* (%) = 529 (7) [M + Na], 489 (2) [M – H_2O + H], 429 (3) [M – AcOH – H_2O + H], 413 (4) [M – AcOH – CH_3 – H_2O], 369 (17) [M – 2AcOH – H_2O + H]. HRMS (FAB): calcd. for $C_{29}H_{46}O_7Na$ [M + Na]$^+$ 529.3142; found 529.3142.

Methyl 3α,6α-Diacetoxy-14α,15α-epoxy-5β-cholan-24-oate (4d): Isolated from the reaction product of **4a** by RP-HPLC (methanol/water, 4:1) as a noncrystalline substance (fraction 1, 7% yield). IR (KBr): $\tilde{\nu}$ = 2949, 2878 (C–H), 1736, 1698 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 504 (7) [M]$^+$, 486 (14) [M – H_2O], 444 (28) [M – AcOH], 426 (43) [M – AcOH – H_2O], 411 (10) [M – AcOH – CH_3 – H_2O], 389 (100) [M – S.C.], 384 (72) [M – 2AcOH], 366 (64) [M – 2AcOH – H_2O], 351 (64) [M – 2AcOH – CH_3 – H_2O], 311 [M – 2AcOH – S.C. 45], 269 [M – AcOH – H_2O – S.C. 46], 251 (61) [M – 2AcOH – H_2O – S.C.]. HRMS (EI): calcd. for $C_{29}H_{44}O_7$ [M]$^+$ 504.3087; found 504.3087.

Methyl 3α,7α,12α-Triacetoxy-5β-hydroxycholan-24-oate (5b): Isolated from the reaction product of **5a** by open column chromatography (EtOAc/hexane, 1:1) as a noncrystalline substance (fraction 3, 27% yield) (ref.[5] m.p. 87–89 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.73 (s, 3 H, 18-CH_3), 0.82 (d, *J* = 6.2 Hz, 3 H, 21-CH_3), 0.89 (s, 3 H, 19-CH_3), 2.04, 2.08, 2.10 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.95 (m, 1 H, 7β-H), 5.02 (br. m, 1 H, 3β-H), 5.10 (m, 1 H, 12β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.1 (C-18), 15.6 (C-19), 17.4 (C-21), 21.3 ($OCOCH_3$), 21.4 ($OCOCH_3$), 21.4 ($OCOCH_3$), 22.8 (C-15), 25.8 (C-11), 26.3 (C-2), 27.1 (C-16), 29.1 (C-1), 30.7, 30.8 (C-22, C-23), 31.7 (C-9), 34.5 (C-20), 37.0 (C-8), 39.3 (C-10), 40.6, 40.7 (C-4, C-6), 43.1 (C-14), 44.8 (C-13), 47.2 (C-17), 51.5 ($COOCH_3$), 70.5 (C-7), 70.8 (C-3), 74.1 (C-5), 75.0 (C-12), 170.1 ($OCOCH_3$), 170.4 ($OCOCH_3$), 170.5 ($OCOCH_3$), 174.5 (C-24) ppm. IR (KBr): $\tilde{\nu}$ = 3526 (OH), 1736 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 444 (6) [M – 2AcOH], 426 (18) [M – 2AcOH – H_2O], 384 (48) [M – 3AcOH], 366 (71) [M – 3AcOH – H_2O], 351 (36) [M – 3AcOH – H_2O – CH_3], 330 (29) [M – AcOH – H_2O – S.C. – ring D + H], 329 (72) [M – 2AcOH – S.C.], 311 (29) [M – 2AcOH – H_2O – S.C.], 269 (72) [M – 3AcOH – S.C.], 251 (100) [M – 3AcOH – H_2O – S.C.].

Methyl 3α,7α,12α-Triacetoxy-15-oxo-5β-cholan-24-oate (5c): Isolated from the reaction product of **5a** by RP-MPLC (acetonitrile/water, 13:7) as a noncrystalline substance (fraction 2, 15% yield). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.85 (s, 3 H, 18-CH_3), 0.90 (d, *J* = 7.0 Hz, 3 H, 21-CH_3), 0.92 (s, 3 H, 19-CH_3), 2.02, 2.04, 2.20 (s, each 3 H, -$COCH_3$), 3.67 (s, 3 H, -$COOCH_3$), 4.57 (br. m, 1 H, 3β-H), 5.20 (m, 1 H, 12β-H), 5.76 (m, 1 H, 7β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 13.3 (C-18), 17.7 (C-21), 21.3 ($OCOCH_3$), 21.4 ($OCOCH_3$), 21.5 ($OCOCH_3$), 22.3 (C-19), 25.2 (C-11), 26.8 (C-2), 28.3 (C-9), 29.2 (C-4), 30.3 (C-6), 30.6 (C-22), 30.8 (C-23), 34.0 (C-8), 34.1 (C-10), 34.2 (C-20), 34.6 (C-1), 34.7 (C-7), 40.5 (C-5), 40.7 (C-16), 43.2 (C-17), 44.3 (C-13), 51.6 ($COOCH_3$), 54.0 (C-14), 69.3 (C-7), 73.8 (C-3), 74.2 (C-12), 169.7 ($OCOCH_3$), 170.0 ($OCOCH_3$), 170.5 ($OCOCH_3$), 174.0 (C-24), 213.3 (C-15) ppm. IR (KBr): $\tilde{\nu}$ = 1730 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 562 (18) [M]$^+$, 519 (100) [M – $COCH_3$], 502 (13) [M – AcOH], 484 (14) [M – AcOH – H_2O], 459 (50) [M – AcOH – $COCH_3$], 442 (28) [M – 2AcOH], 424 (24) [M – 2AcOH – H_2O], 399 (41) [M – 2AcOH – $COCH_3$], 382 (44) [M – 3AcOH], 367 (32) [M – 3AcOH – CH_3], 267 (48) [M – 3AcOH – S.C.]. HRMS (EI): calcd. for $C_{31}H_{46}O_9$ [M]$^+$ 562.3142; found 562.3140.

Methyl 3α,7α,12α-Triacetoxy-16-oxo-5β-cholan-24-oate (5d): Isolated from the reaction product of **5a** by RP-MPLC (acetonitrile/water, 13:7) as a noncrystalline substance (fraction 1, 7% yield) (ref.[12] viscous oil). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.91 (s, 3 H, 18-CH_3), 0.92 (d, *J* = 5.1 Hz, 3 H, 21-CH_3), 0.95 (s, 3 H, 19-CH_3), 2.05, 2.07, 2.10 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.60 (br. m, 1 H, 3β-H), 4.88 (m, 1 H, 7β-H), 5.11 (m, 1 H, 12β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 13.8 (C-18), 18.0 (C-21), 21.3 ($OCOCH_3$), 21.4 ($OCOCH_3$), 21.5 ($OCOCH_3$), 22.4 (C-19), 24.8 (C-11), 26.8 (C-2), 29.1 (C-9), 30.0 (C-20 and C-23), 31.0 (C-6), 31.7 (C-22), 34.2 (C-4), 34.4 (C-10), 34.6 (C-1), 36.6 (C-14), 37.6 (C-15), 38.1 (C-8), 40.7 (C-5), 45.7 (C-13), 51.5 ($COOCH_3$), 60.9 (C-17), 70.4 (C-7), 73.5 (C-12), 73.8 (C-3), 170.0 ($OCOCH_3$), 170.0 ($OCOCH_3$), 170.4 ($OCOCH_3$), 174.1 (C-24), 216.0 (C-16) ppm. IR (KBr): $\tilde{\nu}$ = 1732 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 487 (78) [M – AcOH – CH_3], 442 (9) [M – 2AcOH], 433 (9) [M – CH_3 – S.C. + H], 382 (9) [M – 3AcOH], 351 (28) [M – 3AcOH – OCH_3], 333 (28), 309 (66), 295 (26), 268 (31) [M – 3AcOH – S.C. + H], 267 (34) [M – 3AcOH – S.C.], 253 (72) [M – 3AcOH – CH_3 – S.C. + H], 170 (100).

Methyl 3α,7α,12α-Triacetoxy-5β-hydroxy-16-oxocholan-24-oate (5e): Isolated from the reaction product of **5a** by open column chromatography (EtOAc/hexane, 1:1) as a noncrystalline substance (fraction 4, 4% yield) (ref.[12] viscous oil). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.91 (s, 3 H, 18-CH_3), 0.92 (d, *J* = 6.5 Hz, 3 H, 21-CH_3), 0.93 (s, 3 H, 19-CH_3), 2.05, 2.11, 2.18 (s, each 3 H, -$COCH_3$), 3.66 (s, 3 H, -$COOCH_3$), 4.92 (m, 1 H, 7β-H), 5.02 (br. m, 1 H, 3β-H), 5.12 (m, 1 H, 12β-H) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 13.8 (C-18), 15.6 (C-19), 18.0 (C-21), 21.2 ($OCOCH_3$), 21.4 ($OCOCH_3$), 21.4 ($OCOCH_3$), 25.1 (C-11), 26.3 (C-2), 28.9 (C-1), 30.0 (C-20 and C-23), 31.7 (C-22), 31.9 (C-9), 35.9 (C-14), 37.6 (C-15), 37.8 (C-8), 39.5 (C-10), 40.5, 40.6 (C-4, C-6), 45.5 (C-13), 51.5 ($COOCH_3$), 60.8 (C-17), 70.3 (C-7), 70.6 (C-3), 73.1 (C-12), 73.9 (C-5), 169.9 ($OCOCH_3$), 170.0 ($OCOCH_3$), 170.5 ($OCOCH_3$), 174.1 (C-24) 215.8 (C-16) ppm. IR (KBr): $\tilde{\nu}$ = 3538 (OH), 1738 (C=O) cm^{-1}. LRMS (EI): *m/z* (%) = 578 (5) [M$^+$], 503 (14) [M – AcOH – CH_3], 464 (9) [M – S.C. + H], 449 (9) [M – CH_3 – S.C. + H], 401 (9), 380 (14) [M – 3AcOH – H_2O], 365 (22) [M – 3AcOH – H_2O – CH_3], 349 (33), 325 (58) [M – 2AcOH – H_2O – S.C.], 311 (36) [M – 2AcOH – H_2O – CH_3 – S.C. + H], 283 (25) [M – 3AcOH – S.C.], 269 (49) [M – 2AcOH – H_2O – CH_3 – S.C. – ring D], 251 (100) [M – 3AcOH – H_2O – CH_3 – S.C. + H], 207 (85).

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