JOC Article

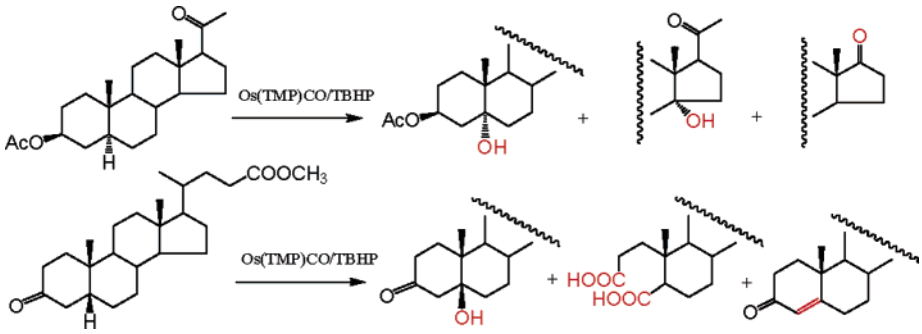
# Regioselective Oxyfunctionalization of Unactivated Carbons in Steroids by a Model of Cytochrome P-450: Osmiumporphyrin Complex/*tert*-Butyl Hydroperoxide System

Takashi Iida,*[,†] Shoujiro Ogawa,[†] Keiji Hosoi,[†] Mitsuko Makino,[‡] Yasuo Fujimoto,[‡] Takaaki Goto,[§] Nariyasu Mano,[§] Junichi Goto,[§,||] and Alan F. Hofmann[⊥]

*Departments of Chemistry and General Studies, College of Humanities and Sciences, Nihon University, Sakurajousui, Setagaya, Tokyo 156-8550, Japan, Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai 980-8578, Japan, Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan, and Department of Medicine, University of California, San Diego, La Jolla, California 92093-0813*

*takaiida@chs.nihon-u.ac.jp*

*Received August 31, 2006*




*tert*-Butyl hydroperoxide catalyzed by (5,10,15,20-tetramesitylporphyrinate) osmium(II) carbonyl [Os(TMP)CO] complex was found to be a highly efficient versatile oxidant for C−H carbons in steroid substrates. When reacted with representative steroids with an estrane, pregnane, 5$\beta$-cholane, or 5$\alpha$-cholestane structure, regioselective oxyfunctionalization and/or oxidative degradation occurred to give a variety of novel and uncommon derivatives in one step.

## Introduction

Oxyfunctionalizations of unactivated C−H bonds in hydrocarbons are efficiently performed *in vivo* by cytochrome P-450 enzymes, which carry a heme prosthetic group in the active site.[1] Such oxidations are of great interest from a mechanistic standpoint and are also important biologically as they are often the initial step in many detoxification pathways. Therefore, a number of metalloporphyrin/oxygen transfer reagent systems mimicking the P-450 enzymes have been developed for the oxygenation of alkane, alkene, arene, and aromatic hydrocarbons.[2,3]

Recently, Higuchi *et al.*[4] and other groups[5,6] have reported a powerful oxidant system consisting of 2,6-dichloropyridine *N*-oxide (DCP *N*-oxide) as a single oxygen atom source, (*meso*-5,10,15,20-tetramesitylporphyrinate) ruthenium(II) carbonyl [Ru(TMP)CO] complex (or its analogues) as a precatalyst, and HBr as a cocatalyst. The system promotes highly efficient, stereo-

* Corresponding author. Telephone: 81-3-3329-1151. Fax: 81-3-3303-9899.
† Department of Chemistry, Nihon University.
‡ Department of General Studies, Nihon University.
§ Tohoku University.
|| Tohoku University Hospital.
⊥ University of California, San Diego.

(1) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841.
(2) Meunier, B. *Chem. Rev.* **1992**, *92*, 1411.
(3) Reese, P. B. *Steroids* **2001**, *66*, 481.
(4) (a) Ohtake, H.; Higuchi, T.; Hirobe, M. *J. Am. Chem. Soc.* **1992**, *114*, 10660. (b) Ohtake, H.; Higuchi, T.; Hirobe, M. *Heterocycles* **1995**, *40*, 867. (c) Higuchi, T.; Satake, C.; Hirobe, M. *J. Am. Chem. Soc.* **1995**, *117*, 8879. (d) Shingaki, T.; Miura, K.; Higuchi, T.; Hirobe, M.; Nagano, T. *Chem. Commun.* **1997**, 861.
(5) (a) Iida, T.; Ogawa, S.; Shiraishi, K.; Kakiyama, G.; Goto, T.; Mano, N.; Goto, J. *ARKIVOC* **2003**, *viii*, 171. (b) Iida, T.; Ogawa, S.; Miyata, S.; Goto, T.; Mano, N.; Goto, J.; Nambara, T. *Lipids* **2004**, *39*, 873. (c) Ogawa, S.; Iida, T.; Goto, T.; Mano, N.; Goto, J.; Nambara, T. *Org. Biomol. Chem.* **2004**, *2*, 1013.
(6) Zhang, R.; Yu, W.-Y.; Che, C-H. *Tetrahedron: Asymmetry* **2005**, *16*, 3520.

10.1021/jo061800g CCC: $37.00 
Published on Web 01/10/2007

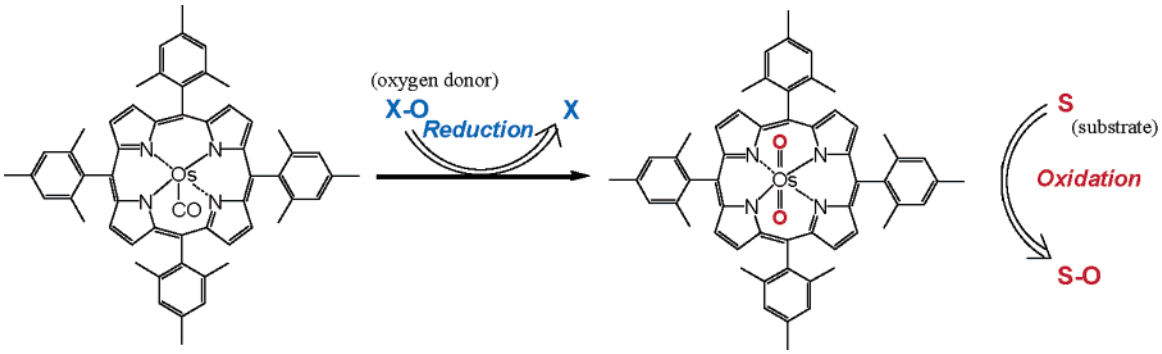



**FIGURE 1.** Os(TMP)CO/TBHP oxidant system.

**TABLE 1. Effects of a Combination of Metalloporphyrin-Catalyzed Oxyfunctionalizations on the Reactivity and Regioselectivity of Substrate 3a**

| | | | | | | products, yield (%)[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| run[a] | precatalyst | oxygen donor | solvent | temp | reaction time (h) | **3b** | **3c** | **3d** | 6-oxo | 6α-hydroxy | 5β-hydroxy-15-oxo | 5β-hydroxy-24,20-lactone |
| 1 | Fe(TPFP)Cl | PhIO | $CH_2Cl_2$ | rt | 96 | 8 | | | 8 | 3 | | |
| 2 | Mn(TPFP)Cl | PhIO | $CH_2Cl_2$ | rt | 96 | 5 | | | 6 | 2 | | |
| 3 | Os(TMP)CO | PhIO | $CH_2Cl_2$ | rt | 96 | 7 | | | | | | |
| 4 | Ru(TMP)CO | DCP *N*-oxide/HBr | benzene | 50 °C | 48 | 48 | | | | | 11 | 11 |
| 5 | Os(TMP)CO | DCP *N*-oxide/HBr | benzene | 50 °C | 96 | | | | | | | |
| 6 | Os(TMP)CO | 70% TBHP | benzene | 80 °C | 96 | 10 | 3 | 2 | | | | |
| 7 | Os(TPP)CO | TBHP | benzene | 80 °C | 96 | 25 | 7 | 8 | | | | |
| 8 | Os(TMP)CO | TBHP | benzene | 80 °C | 96 | 32 | 9 | 9 | | | | |
| 9 | Os(TPFP)CO | TBHP | benzene | 80 °C | 96 | 30 | 5 | 9 | | | | |

[a] For runs 1−3, the reaction mixtures contained substrate (5 mg, 11.4 μmol), PhIO (20 equiv), and precatalyst (0.008 equiv). For runs 4 and 5, the reaction mixtures contained substrate (5 mg, 11.4 μmol), DCP *N*-oxide (3 equiv), precatalyst (0.004 equiv), 48% HBr (0.5 μl), and 4 Å molecular sieves (10 mg). For runs 6−9, the reaction mixtures contained substrate (5 mg, 11.4 μmol), oxidant (20 equiv), precatalyst (0.004 equiv), and 4 Å molecular sieves (5 mg). [b] Determined by capillary GC.

selective oxidation of olefins, alcohols, allylic alcohols, sulfides, aromatic hydrocarbons, and alkanes with an excellent turnover of the catalyst.

As part of our effort to develop an effective metalloporphyrin together with an oxygen donor for the oxyfunctionalization of inactive C−H bonds in steroid substrates, we have developed and report here a new powerful, unique oxidant, which involves the combination of (*meso*-5,10,15,20-tetramesitylporphyrinate) osmium(II) carbonyl [Os(TMP)CO] and *tert*-butyl hydroperoxide (TBHP) without a cocatalyst (Figure 1). Thus, the structure on the left in Figure 1 should have two axial oxygen ligands attached to the osmium atom; therefore, its oxidized analogue (right) would possess one "O" and one "$O_2$" axial ligand. The way it is drawn now suggests that the dioxo complex is the active oxidant. The system had regioselectivity differing from that of the Ru(TMP)CO/DCP *N*-oxide/HBr system.[4,5] We describe the use of this system on compounds of the estrane (**1a**), pregnane (**2a**), 5β-cholane (**3a** and **4a**), and 5α-cholestane (**5a**) series of steroids to yield a variety of novel and scarce derivatives in one step. We also discuss factors governing the O-insertion position, oxidative degradation, and regioselectivity of the oxidant system.

## Results and Discussion

Table 1 shows the effects of a combination of metalloporphyrins/oxygen donors on the reactivity and regioselectivity of the oxygenation of methyl 3α-acetoxy-5β-cholanoate (**3a**) as a substrate. Our preliminary work was directed toward identification of the optimal metalloporphyrin complex as a precatalyst as well as the preferred oxygen-donating reagent. For the metal of the metalloporphyrin, Fe,[7] Mn,[7e,8] Ru,[4−6,9] and Os[10] were compared, with aryl groups being the *meso*-substituents. On the basis of previous findings,[7−10] we examined the following precatalysts: (5,10,15,20-tetrapentafluorophenylporphyrinate) iron(III) chloride [Fe(TPFP)Cl]; (5,10,15,20-tetrapentafluorophenylporphyrinate) manganese(III) chloride [Mn(TPFP)Cl]; Ru(TMP)CO; (5,10,15,20-tetraphenylporphyrinate) osmium(II) carbonyl [Os(TPP)CO]; Os(TMP)CO; and (5,10,15,20-tetrapentafluorophenylporphyrinate) osmium(II) carbonyl [Os(TPFP)CO]. We found that varying the electron-donating and electron-withdrawing substituents on the aromatic rings attached at the *meso*-position on the porphyrin nucleus had little effect

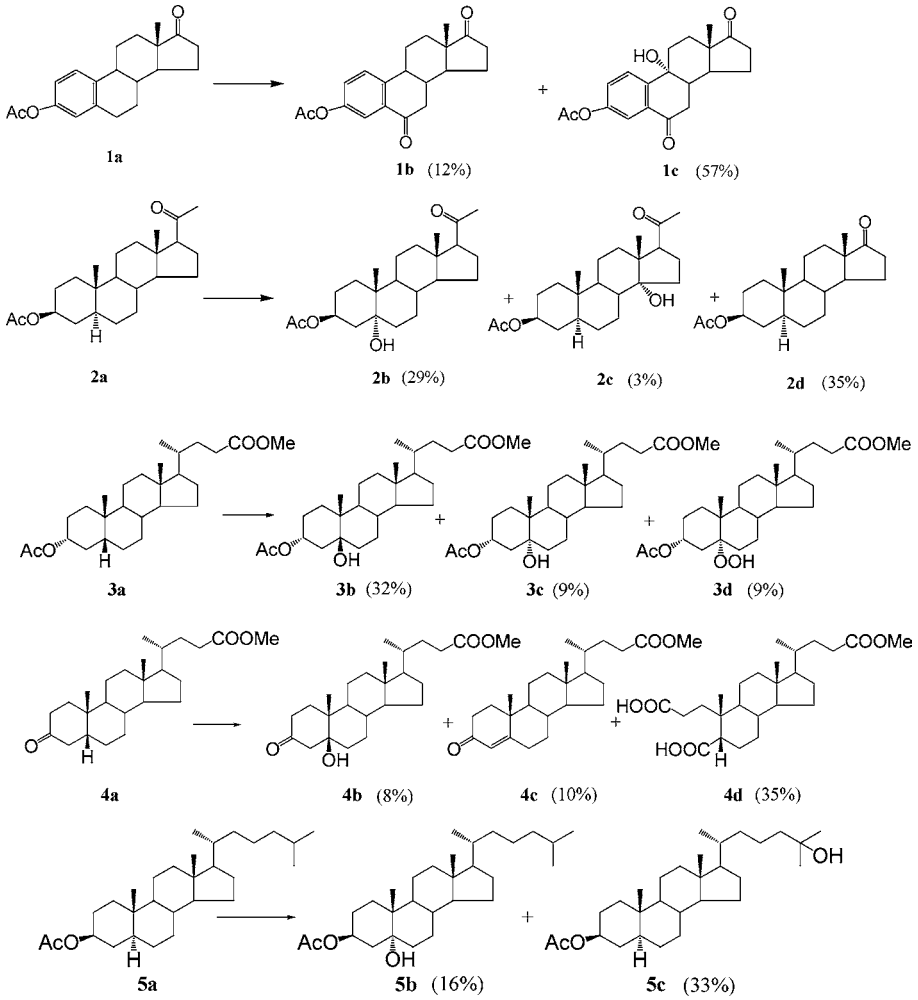
(7) (a) Paulson, D. R.; Ullman, R.; Sloane, R. B.; Closs, G. L. *J. Chem. Soc., Chem. Commun.* **1974**, 186. (b) Mansuy, D.; Bartoli, J.-F.; Chottard, J.-C.; Lange, M. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 909. (c) Chauhan, S. M. S.; Kandadai, A. S.; Jain, N.; Kumar, A. *Chem. Pharm. Bull.* **2003**, *51*, 1345. (d) Constantino, L.; Iley, J. *Org. Biomol. Chem.* **2004**, *2*, 1894. (e) Rebelo, S. L. H.; Pereira, M. M.; Simões, M. M. Q.; Neves, M. G. P. M. S.; Cavaleiro, J. A. S. *J. Catal.* **2005**, *234*, 76.

(8) (a) Battioni, P.; Renaud, J. P.; Bartoni, J. F.; Reina-Artiles, M.; Fort, M.; Mansuy, D. *J. Am. Chem. Soc.* **1988**, *110*, 8462. (b) Collman, J. P.; Tanaka, H.; Hembre, R. T.; Brauman, J. I. *J. Am. Chem. Soc.* **1990**, *112*, 3689. (c) Banfi, S.; Maiocchi, A.; Moggi, A.; Montanari, F.; Quici, S. *J. Chem. Soc., Chem. Commun.* **1990**, 1794. (d) Breslow, R.; Huang, Y.; Zhang, X.; Yang, J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11156. (e) Gross, Z.; Simkhovich, L.; Galili, N. *Chem. Commun.* **1999**, 599. (f) Yang, J.; Weinberg, R.; Breslow, R. *Chem. Commun.* **2000**, 531. (g) Belvedere, S.; Breslow, R. *Bioorg. Chem.* **2001**, *29*, 321. (h) Collman, J. P.; Zeng, L.; Decréau, R. A. *Chem. Commun.* **2003**, 2974. (i) Smith, J. R. L.; Iamamoto, Y.; Vinhalo, F. S. *J. Mol. Catal. A: Chem.* **2006**, *252*, 23.

(9) Groves, J. T.; Quinn, R. *J. Am. Chem. Soc.* **1985**, *107*, 5790.

(10) (a) Che, C.-M.; Chung, W.-C. *J. Chem. Soc., Chem. Commun.* **1986**, 386. (b) Gross, Z.; Mahammed, A. *J. Mol. Catal. A: Chem.* **1999**, *142*, 367. (c) Li, Y.; Huang, J.-S.; Zhou, Z.-Y.; Che, C.-M. *J. Am. Chem. Soc.* **2001**, *123*, 4843.

**SCHEME 1**




on the reactivity and selectivity. On the contrary, changing the central metal ligand influenced strongly the efficiency of total yields of each oxidized product.

The choice of oxygen-donating reagents such as iodosobenzene (PhIO),[8d−i,10a,b] DCP *N*-oxide,[4−6] and TBHP[7b,d] was also important, depending upon the accompanying precatalyst. The combined use of PhIO or DCP *N*-oxide and Os(TMP)CO was found to be less effective (see below). According to the previous findings, DCP *N*-oxide is a specific oxygen donor against Ru(TMP)CO precatalyst,[11] while PhIO itself is easily converted into less active iodoxybenzene ($PhIO_2$).[12] In addition, the presence of water in the reaction system would also accelerate decomposition of an active *trans*-dioxoosmiumporphyrin intermediate (see below).[13] In this study, commercially available 70% TBHP,[7b,d] instead of 30% $H_2O_2$,[7c,8a,c] was employed as a peroxide because of its high active oxygen content as well as easy handling. A comparative study (runs 6 versus 8 in Table 1) revealed that the use of anhydrous TBHP, which is easily obtainable by organic solvent extraction (e.g., $CH_2Cl_2$) prior to use, increased the yield about three times compared to that obtained using 70% TBHP under the same reaction conditions. Therefore, all of the oxidation reactions were carried out under anhydrous conditions.

The Fe(TPFP)Cl/PhIO, Mn(TPFP)Cl/PhIO, and Os(TMP)CO/PhIO systems were unstable or inactive in oxyfunctionalization of the substrate **3a** (runs 1−3). Although the oxidation of **3a** with the Ru(TMP)CO/DCP *N*-oxide/HBr system (run 4) gave the 5$\beta$-hydroxy, 5$\beta$-hydroxy-15-oxo, and 5$\beta$-hydroxy-24,-20-lactone derivatives in good yield,[5] the analogous Os(TMP)CO/DCP *N*-oxide/HBr system (run 5) was inactive. All of the TBHP oxidations catalyzed by (*meso*-tetraarylporphyrinate) osmium(II) carbonyl [Os(TAP)CO] complexes (runs 7−9) in the absence of water showed high reactivity and essentially identical regioselectivity. In addition, the Os(TMP)CO/TBHP system had a regioselectivity different from the Ru(TMP)CO/DCP *N*-oxide/HBr one (run 4 versus 7). Thus, both the reactivity and regioselectivity depend markedly upon a combination of the central metal of the metalloporphyrins and the oxygen donor counterparts. Of various combinations examined, the Os(TMP)CO/TBHP system was the most active. Its high oxidizing ability seems to be closely related to the chemical nature of the Os atom (e.g., $OsO_4$).

Substrates examined in this study were five typical steroids (**1a**−**5a**) belonging to the estrane, pregnane, 5$\beta$-cholane, and 5$\alpha$-cholestane series. These compounds differed in the structures of the A/B-ring and/or the side chains at C-17. To prevent the simultaneous oxidation of hydroxyl groups in the substrates **1a**,

(11) Higuchi, T.; Ohtake, H.; Hirobe, M. *Tetrahedron Lett.* **1989**, *30*, 6545.
(12) Nappa, M. J.; Tolman, C. A. *Inorg. Chem.* **1985**, *24*, 4711.
(13) Kuroda, Y.; Hiroshige, T.; Ogoshi, H. *J. Chem. Soc., Chem. Commun.* **1990**, 1594.

**2a**, **3a**, and **5a** to carbonyls, these compounds were protected as their acetyl derivatives. All of the substrates were found to react readily with the Os(TMP)CO/TBHP system to form a variety of oxygenated compounds in one step. As shown in Scheme 1, oxidation of estrone 3-acetate (**1a**) with Os(TMP)CO (0.004 equiv) and TBHP (20 equiv) in refluxing benzene for 12 h resulted in regioselective ketonization at C-6 and hydroxylation at C-9 to give the corresponding 6-oxo (**1b**, 12% yield) and 9α-hydroxy-6-oxo (**1c**, 57%) derivatives, respectively, with a total of 69% conversion. The predominant benzylic oxygenation of **1a** is similar to the results reported for the oxidation of analogous estrane derivatives with Ru(TMP)CO/DCP *N*-oxide/HBr,[5b] dimethyldioxirane,[14] or TBHP/cobalt acetate.[15] When 3*β*-acetoxy-5α-pregnan-20-one (**2a**) was subjected to the Os(TMP)CO/TBHP oxidation for 96 h, 3*β*-acetoxy-5α-hydroxypregnan-20-one (**2b**, 29%) and 3*β*-acetoxy-5α-androstan-17-one (**2d**, 35%) were isolated as the main products, accompanied by a small amount of 3*β*-acetoxy-14α-hydroxy-5α-pregnan-20-one (**2c**, 3%). Both the 5α- and 14α-hydroxylations proceeded with the complete retention of the configuration at C-5 and C-14, as determined by the C-5 signal in **2b** appearing at 74.9 ppm[16] and the C-14 signal in **2c** occurring at 84.8 ppm[17] in the $^{13}$C NMR spectra. These results suggest that the electron-enriched C-5 and C-14 tertiary methine carbons are more reactive than secondary methylene carbons, the latter having electronic constraints. As a result, an oxygen atom is preferentially inserted on the methine protons. A similar electrophilic hydroxylation has also been reported for other oxygen atom transfer reagents.[5c,18,19] Furthermore, the insertion of a 5*β*-hydroxyl group into less sterically hindered 5*β*-steroids with *cis* A/B-ring fusion is generally accepted,[18−21] whereas 5α-hydroxylation is uncommon for more sterically crowded trans 5α-steroids. Therefore, the predominant formation in **2b** is particularly noteworthy, in analogy with the result reported for the photooxygenation of 5α-androstane-3*β*,17*β*-diol diacetate with peracetic acid[22] or the reaction of palladium(II), thallium(III), and lead(IV) trifluoroacetates with 3*β*-acetoxyandrost-5-en-7-one.[23] Of additional interest was the formation of an appreciable amount of **2d** by the oxidative degradation of the pregnane side chain in **2a**.

Numerous methods have been reported for the degradation of the $C_{21}$ 20-ketopregnane type of steroids to the $C_{19}$ 17-ketoandrostane derivatives.[24] In the present case, **2d** is probably formed by the Baeyer−Villiger reaction at the 20-oxo group in **2a** to give an ester intermediate, which in turn is hydrolyzed and then oxidized as shown in Figure 2. The MS and $^1$H NMR spectral data for **2d** agreed completely with those of an authentic specimen. The facile transformation, therefore, allows a new entry to the synthesis of androstane-type compounds from the 20-ketopregnane derivatives.

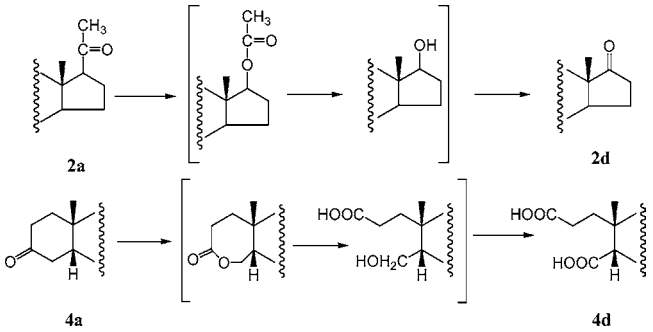



**FIGURE 2.** Oxidative degradation mechanism of ketosteroids by Baeyer−Villiger reaction.

We have previously reported[5a] that the oxidation of methyl 3α-acetoxy-5*β*-cholanoate (**3a**) with dimethyldioxirane or Ru(TMP)CO/DCP *N*-oxide/HBr affords methyl 3α-acetoxy-5*β*-hydroxycholanoate (**3b**) and its dioxyfunctionalized analogues, because of the favored steric and electron-enriched environments at C-5 as mentioned above. However, when **3a** was subjected to the Os(TMP)CO/TBHP oxidation, methyl 3α-acetoxy-5α-hydroxycholan-24-oate (**3c**, 9%) and methyl 3α-acetoxy-5α-hydroperoxycholan-24-oate (**3d**, 9%) were obtained unexpectedly in moderate yield, along with the expected **3b** (32%). The similarity of *cis* 5*β*- and *trans* 5α-steroids (**3a** and **2a**, respectively) toward 5-hydroxylation (**3b**, 32% and **2b**, 29%) would suggest that steric effects of the substrates on the Os(TMP)CO/TBHP oxidation are less than those for Ru(TMP)CO/DCP *N*-oxide/HBr or dimethyldioxirane.[4d,5a] Although the reason for the inversion of the A/B-ring fusion in the 5α-oxygenated products (**3c** and **3d**) is unclear, a similar 5α-oxyfunctionalization with the oxidant was also observed for other 5*β*-bile acids (unpublished results). To confirm the presence of the hydroperoxy group, reduction of the hydroperoxide **3d** with triphenylphosphine[25] gave rise to the deoxygenation product, the chromatographic data of which were consistent with those for **3c**. Further confirmatory evidence for the structures of **3c** and **3d** was supported by measuring the several $^1$H−$^1$H and $^1$H−$^{13}$C shift-correlated 2D NMR spectra.

Table 2 shows the complete $^1$H and $^{13}$C resonance assignments of **3c** and **3d**. In the $^{13}$C NMR spectra, the α-carbon atoms having acetoxy and hydroxyl groups resonated at 71.0 and 73.2 ppm in **3c** and at 70.0 and 83.6 ppm in **3d**, indicating that one hydroxyl function was inserted into **3a**. The C-3 signal appearing at 74.3 ppm in **3a** was shifted upfield by 3.3 ppm and resonated at 71.0 ppm in **3c** and at 70.0 ppm in **3d** in the $^1$H−$^{13}$C HETCOR spectra, probably owing to the *γ*-effect of the α-carbon at C-5. On the other hand, the 3*β*-H signal at 4.72 ppm as a multiplet in **3a** was deshielded by 0.47 ppm in **3c** (0.32 ppm in **3d**) and occurred at 5.19 ppm (5.04 ppm). Although the $^{13}$C chemical shifts of the C-1 to C-7 in **3c** and **3d** agreed very well with those reported for 5α-cholestan-3α,5α-diol 3-acetate,[26] the remaining signals were almost unchanged

(14) Brown, D. S.; Marples, B. A.; Muxworthy, J. P.; Baggaley, K. H. *J. Chem. Res., Synop.* **1992**, 28.
(15) Modica, E.; Bombieri, G.; Colombo, D.; Marchini, N.; Ronchetti, F.; Scala, A.; Toma, L. *Eur. J. Org. Chem.* **2003**, 2964.
(16) (a) Blunt, J. W. *Aust. J. Chem.* **1975**, *28*, 1017. (b) Eggert, H.; VanAntwerp, C. L.; Bhacca, N. S.; Djerassi, C. *J. Org. Chem.* **1976**, *41*, 71.
(17) Iida, T.; Shiraishi, K.; Ogawa, S.; Goto, T.; Mano, N.; Goto, J.; Nambara, T. *Lipids* **2003**, *38*, 281.
(18) Cerrè, C.; Hofmann, A. F.; Schteingart, C. D.; Jia, W.; Maltby, D. *Tetrahedron* **1997**, *53*, 435.
(19) Iida, T.; Yamaguchi, T.; Nakamori, R.; Hikosaka, M.; Mano, N.; Goto, J.; Nambara, T. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2229.
(20) Arnone, A.; Cavicchioli, M.; Montanari, V.; Resnati, G. *J. Org. Chem.* **1994**, *59*, 5511.
(21) Bégué, J.-P. *J. Org. Chem.* **1982**, *47*, 4268.
(22) Rotmann, A.; Mazur, Y. *J. Am. Chem. Soc.* **1972**, *94*, 6228.
(23) Ruddock, P. L.; Williams, D. J.; Reese, P. B. *Steroids* **2004**, *69*, 193.
(24) Oliveto, E. P. In *Organic Reactions in Steroid Chemistry: Synthesis and Degradation of the Pregnane Side-Chain*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, 1971; Vol. II, pp 127−236.
(25) Fukuyama, Y.; Kubo, M.; Minami, H.; Yuasa, H.; Matsuo, A.; Fujii, T.; Morisaki, M.; Harada, K. *Chem. Pharm. Bull.* **2005**, *53*, 72−80.
(26) VanAntwerp, C. L.; Eggert, H.; Meakins, G. D.; Miners, J. O.; Djerassi, C. *J. Org. Chem.* **1977**, *42*, 789.

**TABLE 2. Complete $^1$H and $^{13}$C Chemical Shifts of 5α-Oxygenated Compounds 3c and 3d[a]**

| | 3c | | | | 3d | | | | 3a | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| carbon No. | type | $^{13}$C | $^1$H | | type | $^{13}$C | $^1$H | | type | $^{13}$C[b] | $^1$H[c] | |
| | | | α | β | | | α | β | | | α | β |
| 1 | $CH_2$ | 25.6 | 1.77 | 1.39 | $CH_2$ | 26.6 | 1.73 | 1.21 | $CH_2$ | 35.0 | 1.75 | 0.94 |
| 2 | $CH_2$ | 25.6 | 1.39 | 1.78 | $CH_2$ | 25.5 | 1.32 | 1.74 | $CH_2$ | 26.3 | 1.29 | 1.60 |
| 3 | CH | 71.0 | | 5.19 (m) | CH | 70.0 | | 5.04 (m) | CH | 74.3 | | 3.51 |
| 4 | $CH_2$ | 37.4 | 1.61 | 1.86 | $CH_2$ | 31.6 | 2.08 | 1.65 | $CH_2$ | 32.2 | 1.71 | 1.45 |
| 5 | C | 73.2 | | | C | 83.6 | | | CH | 41.9 | | 1.35 |
| 6 | $CH_2$ | 33.6 | 1.31 | 1.49 | $CH_2$ | 28.0 | 1.31 | 1.73 | $CH_2$ | 27.0 | 1.23 | 1.83 |
| 7 | $CH_2$ | 26.8 | 1.56 | 1.33 | $CH_2$ | 25.3 | 1.39 | 1.85 | $CH_2$ | 26.6 | 1.09 | 1.39 |
| 8 | CH | 34.9 | | 1.37 | CH | 35.0 | | 1.38 | CH | 35.7 | | 1.38 |
| 9 | CH | 45.2 | 1.56 | | CH | 45.0 | 1.43 | | CH | 40.4 | 1.41 | |
| 10 | C | 39.5 | | | C | 39.4 | | | C | 34.5 | | |
| 11 | $CH_2$ | 20.9 | 1.35 | 1.19 | $CH_2$ | 20.9 | 1.32 | 1.18 | $CH_2$ | 20.8 | 1.38 | 1.23 |
| 12 | $CH_2$ | 40.0 | 1.16 | 1.94 | $CH_2$ | 39.9 | 1.10 | 1.91 | $CH_2$ | 40.1 | 1.14 | 1.96 |
| 13 | C | 42.7 | | | C | 42.7 | | | C | 42.7 | | |
| 14 | CH | 56.1 | 1.14 | | CH | 56.1 | 1.05 | | CH | 56.4 | 1.05 | |
| 15 | $CH_2$ | 24.0 | 1.01 | 1.56 | $CH_2$ | 24.0 | 1.01 | 1.57 | $CH_2$ | 24.1 | 1.04 | 1.56 |
| 16 | $CH_2$ | 28.1 | 1.85 | 1.24 | $CH_2$ | 28.1 | 1.84 | 1.24 | $CH_2$ | 28.1 | 1.85 | 1.27 |
| 17 | CH | 55.8 | 1.10 | | CH | 55.8 | 1.09 | | CH | 56.0 | 1.10 | |
| 18 | $CH_3$ | 12.1 | 0.64 (s) | | $CH_3$ | 12.1 | 0.63 (s) | | $CH_3$ | 12.0 | 0.64 (s) | |
| 19 | $CH_3$ | 15.9 | 0.93 (s) | | $CH_3$ | 16.1 | 0.98 (s) | | $CH_3$ | 23.3 | 0.91 (s) | |
| 20 | CH | 35.4 | 1.38 | | CH | 35.4 | 1.39 | | CH | 35.3 | 1.41 | |
| 21 | $CH_3$ | 18.2 | 0.89 (d, 6.5) | | $CH_3$ | 18.2 | 0.88 (d, 6.5) | | $CH_3$ | 18.2 | 0.92 (d) | |
| 22 | $CH_2$ | 31.0 | 1.30, 1.77 (each, m) | | $CH_2$ | 31.0 | l.30, 1.75 (each, m) | | $CH_2$ | 31.0 | 1.29, 1.75 (each, m) | |
| 23 | $CH_2$ | 31.1 | 2.20, 2.33 (each, m) | | $CH_2$ | 31.1 | 2.06, 2.33 (each, m) | | $CH_2$ | 31.0 | 2.12, 2.32 (each, m) | |
| 24 | C | 174.8 | | | C | 174.8 | | | C | 174.3 | | |
| $COOCH_3$ | $CH_3$ | 51.5 | 3.66 | | $CH_3$ | 51.5 | 3.66 | | $CH_3$ | 51.4 | | |
| $OCOCH_3$ | C | 169.3 | | | C | 170.1 | | | C | 170.6 | | |
| $OCOCH_3$ | $CH_3$ | 21.5 | 2.04 | | $CH_3$ | 21.7 | 2.09 | | $CH_3$ | 21.4 | | |

[a] Measured in $CDCl_3$. Chemical shifts were expressed as $\delta$ ppm relative to $Me_4Si$. Abbreviations used: s, singlet; d, doublet; m, multiplet. Values in parentheses refer to signal multiplicity and coupling constant ($J$ in Hz). [b] Measured in $CDCl_3$ at 270 MHz. [c] As the C-3 free alcohol (ref 34).

from **3a**. These findings indicate that the C-8 and C-9 tertiary methine carbons are excluded from hydroxylation, whereas hydroxylation occurs readily at the C-5 methine carbon situated in the vicinity of the 3α-acetoxyl group. The supporting evidence for **3c** and **3d** was the presence of a correlation peak between the 19-methyl protons and the C-5 quaternary carbon in the heteronuclear multiple bond connectivity (HMBC) spectra owing to the long-range $^1$H−$^{13}$C coupling. In contrast, in each of the ROESY spectra the 19-methyl signal was correlated with the 2β-H and 4β-H owing to the 1,3-diaxial relationship, suggesting the *trans* A/B-ring fusion in **3c** and **3d**. The molecular ion $M^+$ in the low-resolution mass spectrometry (LRMS) and the ion-adducted molecule $[M + Na]^+$ in the high-resolution mass spectrometry (HRMS) (see Experimental Section) also supported each of the proposed structures of **3c** and **3d**.

A much different result was obtained when methyl 3-oxo-5β-cholan-24-oate (**4a**), differing only in the C-3 function from **3a**, was treated with Os(TMP)CO/TBHP. The isolated products were identified as methyl 3,4-seco-5β-cholan-24-oate-3,4-dioic acid (**4d**, 35%), accompanied by two minor components, methyl 3-oxo-5β-hydroxycholan-24-oate (**4b**, 8%) and methyl 3-oxo-4-cholen-24-oate (**4c**, 10%). The conjugated enone **4c** would be derived by the subsequent elimination of the 5β-hydroxyl group from **4b**. As mentioned above, the oxidative cleavage of **4a** by Baeyer−Villiger reaction[24] would preferentially occur at the C-3 position to give an intermediary lactone derivative, which on hydrolysis and subsequent oxidation yields the 3,4-seco dicarboxylic acid **4d** (Figure 2). To confirm the structure of **4d**, methyl 3β,4β-dihydroxy-5β-cholanoate, prepared from lithocholic acid in several steps,[27] was oxidized with Jones reagent to yield a single component, which agreed completely with **4d**, according to their chromatographic and spectral comparisons.

The oxygenation of 5α-cholestan-3β-yl acetate (**5a**) with Os(TMP)CO/TBHP resulted in the formation of 3β-acetoxycholestan-5α-ol (**5b**, 16%) and 3β-acetoxy-5α-cholestan-25-ol (**5c**, 33%) in an approximate ratio of 1:2. Again, a direct 5α-hydroxylation was attained for **5a** to produce **5b**. For **5c**, the easy attack on the electron-enriched C-25 isopropylic methine carbon in **5a** is common for oxygen transfer reagents[5,18,19] and supports the electrophilicity of the osmiumporphyrin complex. Our previous study[19] revealed that the oxidation product of **5a** with dimethyldioxirane is a mixture of four components consisting of **5b** and its dioxygenated derivatives at the C-14, C-17, and C-20 positions. The oxyfunctionalization of **5a** with Os(TMP)CO/TBHP is, therefore, more regioselective than that observed with dimethyldioxirane.

Although the mechanism for a single atom oxygen activation and transfer, generated by the Os(TMP)CO/TBHP system, is not yet certain, a possible pathway would proceed *via* an active *trans*-dioxoosmiumporphyrin intermediate from the precatalyst,[8i,10b,28] as outlined by the insertion of an oxygen atom into a methine carbon in Figure 3. The results suggest that the oxidized osmium complex reacts with the substrates to generate a radical with a lifetime long enough for inversion to occur (see products from **3a**). Possibly a metalloporphyrin peroxy radical attacks the C−H bond, generating an osmiumporphyrin hydroperoxide and alkyl radical. Particularly noteworthy is a direct 5-hydroxylation on both 5α- and 5β-steroids and the Baeyer−Villiger reaction of ketosteroids as well as the oxyfunctionalization at methine and benzylic carbons. Therefore, the Os(TMP)CO/TBHP system

(27) Iida, T.; Momose, T.; Chang, F. C.; Goto, J.; Nambara, T. *Chem. Pharm. Bull.* **1989**, *37*, 3323.

(28) Groves, J. T. *Inorg. Biochem.* **2006**, *100*, 434.

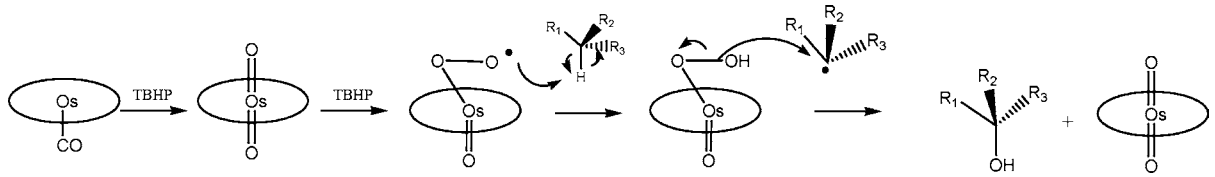



**FIGURE 3.** Possible mechanism of Os(TMP)CO-catalyzed hydroxylation of unactivated C−H saturated carbons by TBHP.

promises to be a useful tool for selective, efficient synthesis of bioactive, hydrophilic steroids from less active, lipophilic resources, which are abundantly available in nature. Further exploration of the scope of the Os(TMP)CO/TBHP system based on oxyfunctionalization will be reported in due course.

## Experimental Section

**Materials and Methods.** Melting points (mp) were determined on an electric micro hot stage and were uncorrected. Infrared (IR) spectra were obtained on a spectrometer for samples in KBr tablets. Proton ($^1$H) and carbon ($^{13}$C) were obtained on an NMR instrument at 270 and 67.8 MHz, respectively, with $CDCl_3$ containing 0.1% $Me_4Si$ as the solvent; chemical shifts are expressed as $\delta$ (ppm) relative to $Me_4Si$. $^{13}$C NMR signals corresponding to the methyl ($CH_3$), methylene ($CH_2$), methine (CH), and quaternary (C) carbons were differentiated by means of DEPT experiment. LRMS spectra were recorded by gas chromatography−mass spectrometry (GC−MS) at 70 eV with an electron ionization (EI) probe using the positive ion mode (PIM). HRMS spectra were performed using a mass spectrometer equipped with an electrospray ionization (ESI) probe using the PIM. A gas chromatograph equipped with a flame ionization detector was used isothermally at 280 °C fitted with a chemically bonded fused silica capillary column (25 m × 0.32 mm i.d.; film thickness, 0.25 $\mu$m). Preparative high-performance liquid chromatography (HPLC) apparatus consisted of a pump equipped with a RI detector and an ODSPH column (250 mm × 10 mm i.d.) using methanol/water (9:1−8:2, v/v) as eluent. The apparatus used for normal-phase (NP) medium-pressure liquid chromatography (MPLC) consisted of an RI detector and uf-3040 chromatographic pump using silica gel 60 (230−400 mesh) as adsorbent and hexane/EtOAc (9:1−8:2, v/v) mixtures as eluent. Reversed-phase (RP) MPLC was carried out by using Cosmosil 75$C_{18}$-PREP as adsorbent and methanol/water (9:1−8:2, v/v) as eluent. Thin layer chromatography (TLC) was performed on precoated silica gel 60$F_{254}$ plates (0.25-mm layer thickness) using hexane/EtOAc (8:2−1:1, v/v) mixtures as developing solvent. RP-TLC was carried out on precoated RP-18$F_{254S}$ plates (Merck) using methanol/water (9:1−8:2, v/v) as developing solvent.

2D NMR spectra were recorded at 23 °C for ca. 0.05 mM solutions in $CDCl_3$ in a 5-mm tube on an instrument (600 and 149.4 MHz for $^1$H and $^{13}$C, respectively). $^1$H and $^{13}$C signal assignments were made using a combination of 2D homonuclear ($^1$H−$^1$H) and heteronuclear ($^1$H−$^{13}$C) shift-correlated techniques, which included $^1$H−$^1$H COSY, long-range $^1$H−$^1$H COSY, $^1$H−$^1$H rotational nuclear Overhauser effect (ROESY), $^1$H−$^{13}$C HETCOR, $^1$H detected heteronuclear multiple quantum coherence (HMQC), and $^1$H detected HMBC experiments. These 2D NMR spectra were recorded by using standard pulse sequence and parameters recommended by the manufacturer.

Estrone 3-acetate (**1a**) was purchased from a commercial supplier. 5α-Pregnan-3$\beta$-ol-20-one 3-acetate (**2a**) was prepared from 5-pregnen-3$\beta$-ol-20-one (pregnenolone) by the usual manner. The remaining steroid substrates (**3a**−**5a**) were from our laboratory collection. 70% TBHP was purchased from a commercial supplier. It was extracted with $CH_2Cl_2$, and the organic layer was evaporated under reduced pressure prior to use. *meso*-Tetraarylporphyrins were prepared by a slight modification of the procedure of Lindsey *et al*.[29] The Os(TAP)CO complexes were prepared from the tetraarylporphyrins and $Os_3(CO)_{12}$ by the method reported by Che *et al*.[30]

**General Procedure for the Oxyfunctionalization by Os(TMP)CO/TBHP.** To a solution of steroid (1.4 mmol) and molecular sieves (250 mg; 4 Å) in benzene (5 mL), Os(TMP)CO (6 mg, 5.5 $\mu$mol) and anhydrous TBHP (1.9 mL, 28 mmol) were successively added, and the mixture was refluxed for 12−96 h. The reaction was monitored by TLC. After the reaction, each of the products was isolated by passage through an open column chromatography on silica gel, followed by NP- (or RP-) MPLC or by preparative RP-HPLC.

**Oxyfunctionalization Products of Estrone 3-Acetate (1a). 3-Acetoxyestra-1,3,5(10)-triene-6,17-dione (1b). 1b** was isolated from the reaction product of **1a** as colorless thin plates [fraction (Fr. 1)] crystallized from EtOAc; mp 186−189 °C (lit.,[5b] mp 186−189 °C); IR $\nu_{max}$/cm$^{-1}$ 1767, 1738, 1680 (C=O); $^1$H NMR ($CDCl_3$) $\delta$: 0.92 (3H, s, 18-$CH_3$), 2.32 (3H, s, −$COCH_3$), 7.27 (1H, dd, $J_1$ = 8.3, $J_2$ = 2.4 Hz, 2-H), 7.48 (1H, d, $J$ = 8.1 Hz, 1-H), 7.77 (1H, d, $J$ = 2.4 Hz, 4-H); $^{13}$C NMR $\delta$: 13.6 (C-18), 20.9 (OCO*C*H$_3$), 21.3 (C-15), 25.0 (C-11), 31.1 (C-12), 35.6 (C-16), 39.1 (C-8), 43.0 (C-7 and C-9), 47.5 (C-13), 50.2 (C-14), 120.1 (C-4), 126.6 (C-2), 127.1 (C-1), 133.5 (C-5), 143.3 (C-10), 149.3 (C-3), 169.3 (O*C*OCH$_3$), 196.3 (C-6), 219.5 (C-17); LR-EI-MS *m*/*z* 326 (M$^+$, 18%), 284 (M − part of ring D, 100), 266 (M−AcOH, 5), 240 (12), 227 (14).

**3-Acetoxy-9α-hydroxyestra-1,3,5(10)-triene-6,17-dione (1c). 1c** was isolated from the reaction product of **1a** as colorless needles (Fr. 2) crystallized from EtOAc; mp 216−219 °C (lit.,[5b] mp 216−219 °C); IR $\nu_{max}$/cm$^{-1}$ 3519 (OH), 1760, 1745 (C=O); $^1$H NMR ($CDCl_3$) $\delta$: 0.91 (3H, s, 18-$CH_3$), 2.32 (3H, s, −$COCH_3$), 7.22 (1H, dd, $J_1$ = 8.8, $J_2$ = 2.4 Hz, 2-H), 7.41 (1H, d, $J$ = 8.3 Hz, 1-H), 7.77 (1H, d, $J$ = 2.4 Hz, 4-H); $^{13}$C NMR $\delta$: 12.7 (C-18), 20.9 (C-15), 21.1 (OCO*C*H$_3$), 27.3 (C-11), 31.9 (C-12), 35.6 (C-16), 36.6 (C-7), 40.8 (C-8), 43.4 (C-14), 47.4 (C-13), 69.3 (C-9), 120.8 (C-4), 125.6 (C-2), 127.3 (C-1), 134.0 (C-5), 144.4 (C-10), 150.7 (C-3), 169.3 (O*C*OCH$_3$), 196.6 (C-6), 219.4 (C-17); LR-EI-MS *m*/*z* 342 (M$^+$, 35%), 324 (M − $H_2O$, 1), 300 (M-part of ring D, 100), 282 (M − $H_2O$ − part of ring D, 19), 243 (7), 215 (23).

**Oxyfunctionalization Products of 3$\beta$-Acetoxy-5α-pregnane-20-one (2a). 3$\beta$-Acetoxy-5α-androstan-17-one (2d). 2d** was isolated from the reaction product of **2a** as a colorless amorphous solid (Fr. 1) crystallized from petroleum ether; mp 103−104 °C (lit.,[31] mp 103−104 °C); IR $\nu_{max}$/cm$^{-1}$ 1737 (C=O); $^1$H NMR ($CDCl_3$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 0.86 (3H, s, 19-$CH_3$), 2.02 (3H, s, −$COCH_3$), 4.69 (1H, brm, 3α-H); $^{13}$C NMR $\delta$: 12.2 (C-19), 13.8 (C-18), 20.4 (C-11), 21.4 (OCO*C*H$_3$), 21.8 (C-15), 27.4 (C-2), 28.2 (C-6), 30.8 (C-4), 31.5 (C-7), 33.9 (C-1), 35.0 (C-8), 35.6 (C-10), 35.8 (C-16), 36.7 (C-12), 44.6 (C-5), 47.8 (C-13), 51.3 (C-9), 54.3 (C-14), 73.5 (C-3), 170.7 (O*C*OCH$_3$), 221.2 (C-17); LR-EI-MS *m*/*z* 332 (M$^+$, 39%), 314 (M − $H_2O$, 4), 288 (15), 272 (M − AcOH, 100), 257 (M − AcOH − $CH_3$, 39), 239 (M − AcOH −

(29) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Margurettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827.
(30) Che, C.-M.; Poon, C.-K.; Chung, W.-C.; Gray, H. B. *Inorg. Chem.* **1985**, *24*, 1277.
(31) Cardwell, H. M. E.; Cornforth, J. W.; Duff, S. R.; Holtermann, H.; Robinson, R. *J. Chem. Soc.* **1953**, 361.

$H_2O - CH_3$, 13), 228 (15), 218 (M − AcOH − $H_2O$ − ring D, 59), 201 (42), 108 (51).

**3β-Acetoxy-5α-hydroxypregnane-20-one (2b). 2b** was isolated from the reaction product of **2a** as a colorless amorphous solid (Fr. 3) crystallized from aqueous methanol; mp 179−183 °C; IR $\nu_{max}$/$cm^{-1}$ 3422 (OH), 1729, 1685 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.60 (3H, s, 18-$CH_3$), 1.00 (3H, s, 19-$CH_3$), 2.02 (3H, s, −$COCH_3$), 2.12 (3H, s, 21-$CH_3$), 5.16 (1H, brm, 3α-H); $^{13}$C NMR $\delta$: 13.5 (C-18), 16.0 (C-19), 21.2 (C-11), 21.4 (OCO*C*$H_3$), 22.8 (C-16), 24.3 (C-15), 25.8 (C-2), 26.7 (C-7), 30.5 (C-1), 31.5 (C-21), 34.5 (C-6), 34.7 (C-8), 38.8 (C-10), 39.0 (C-4), 40.1 (C-12), 44.3 (C-13), 45.4 (C-9), 56.3 (C-14), 63.7 (C-17), 70.7 (C-3), 74.9 (C-5), 170.7 (O*C*OCH$_3$), 209.7 (C-20); LR-EI-MS *m*/*z* 376 ($M^+$, 3%), 358 (M − $H_2O$, 33), 340 (M − $2H_2O$, 2), 316 (M − AcOH, 14), 298 (M − AcOH − $H_2O$, 100), 283 (M − AcOH − $H_2O$ − $CH_3$, 38), 262 (91), 255 (M − AcOH − $H_2O$ − ring D, 25), 213 (39); HR-EI-MS, calcd for $C_{23}H_{36}O_4$ $[M]^+$, 376.2614; found, *m*/*z*, 376.2600.

**3β-Acetoxy-14α-hydroxy-5α-pregnane-20-one (2c). 2c** was isolated from the reaction product of **2a** as colorless amorphous solid (Fr. 2) crystallized from aqueous methanol; mp 164−168 °C; IR $\nu_{max}$/$cm^{-1}$ 3424 (OH), 1733, 1687 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.82 (3H, s, 18-$CH_3$), 0.97 (3H, s, 19-$CH_3$), 2.02 (3H, s, −$COCH_3$), 2.23 (3H, s, 21-$CH_3$), 4.68 (1H, brm, 3α-H); $^{13}$C NMR $\delta$: 12.1 (C-19), 15.3 (C-18), 20.8 (C-11), 21.4 (OCO*C*$H_3$), 24.9 (C-16), 27.4 (C-7), 27.6 (C-2), 28.5 (C-6), 33.4 (C-12 and C-21), 33.8 (C-4), 35.7 (C-10), 36.9 (C-1), 39.0 (C-15), 39.8 (C-8), 44.3 (C-5), 49.2 (C-9 and C-13), 62.3 (C-17), 73.6 (C-3), 84.8 (C-14), 170.7 (O*C*OCH$_3$), 217.9 (C-20); LR-EI-MS *m*/*z* 376 ($M^+$, 19%), 358 (M − $H_2O$, 30), 348 (88), 333 (M − S.C., 10), 315 (M − $H_2O$ − S.C., 7), 290 (62), 283 (M − AcOH − $H_2O$ − $CH_3$, 3), 272 (100), 255 (M − AcOH − $H_2O$ − ring D, 10); HR-EI-MS, calcd for $C_{23}H_{36}O_4$ $[M]^+$, 376.2613; found, *m*/*z*, 376.2616.

**Oxyfunctionalization Products of Methyl 3α-Acetoxy-5β-cholan-24-oate (3a). Methyl 3α-Acetoxy-5α-hydroxycholan-24-oate (3c). 3c** was isolated from the reaction product of **3a** as colorless needles (Fr. 1) crystallized from EtOAc/hexane; mp 123−127 °C; IR $\nu_{max}$/$cm^{-1}$ 3449 (OH), 1735 (C═O); LR-EI-MS *m*/*z* 448 ($M^+$, 1%), 430 (M − $H_2O$, 28), 415 (M − $H_2O$ − $CH_3$, 3), 388 (M − AcOH, 5), 370 (M − AcOH − $H_2O$, 100), 355 (M − AcOH − $H_2O$ − $CH_3$, 35), 334 (66), 273 (M − AcOH − S.C., 12), 255 (M − AcOH − $H_2O$ − S.C., 20), 213 (M − AcOH − $H_2O$ − S.C. − ring D, 46); HR-ESI-MS, calcd for $C_{27}H_{44}O_5Na$ $[M + Na]^+$, 471.3086; found, *m*/*z*, 471.3051.

**Methyl 3α-Acetoxy-5α-hydroperoxycholan-24-oate (3d). 3d** was isolated from the reaction product of **3a** as colorless needles (Fr. 2) crystallized from EtOAc/hexane; mp 153−156 °C; IR $\nu_{max}$/$cm^{-1}$ 3449 (OH), 1736 (C═O); LR-EI-MS *m*/*z* 464 ($M^+$, 1%), 446 (M − $H_2O$, 7), 430 (M − $H_2O_2$, 30), 388 (35), 370 (M − AcOH − $H_2O_2$, 100), 355 (M − AcOH − $H_2O$ − $CH_3$, 24), 334 (75), 273 (M − AcOH − S.C., 19), 255 (M − AcOH − $H_2O_2$ − S.C., 27), 213 (M − AcOH − $H_2O_2$ − S.C. − ring D, 35); HR-ESI-MS, calcd for $C_{27}H_{44}O_6Na$ $[M + Na]^+$, 487.3036; found, *m*/*z*, 487.3078.

**Methyl 3α-Acetoxy-5β-hydroxycholan-24-oate (3b). 3b** was isolated from the reaction product of **3a** as colorless needles (Fr. 3) crystallized from benzene/hexane; mp 175−177 °C (lit.,[19] mp 175−177 °C); IR $\nu_{max}$/$cm^{-1}$ 3454 (OH), 1735, 1708 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.64 (3H, s, 18-$CH_3$), 0.90 (3H, s, 19-$CH_3$), 0.91 (3H, d, $J$ = 7.3 Hz, 21-$CH_3$), 2.02 (3H, s, −$COCH_3$), 3.67 (3H, s, −$COOCH_3$), 5.08 (1H, brm, 3β-H); $^{13}$C NMR $\delta$: 12.0 (C-18), 16.3 (C-19), 18.2 (C-21), 21.1 (C-11), 21.4 (OCO*C*$H_3$), 24.2 (C-15), 26.1 (C-2), 28.1 (C-16), 28.6 (C-7), 29.4 (C-1), 31.0 (C-22 and C-23), 34.9 (C-8), 35.3 (C-20), 36.9 (C-6), 38.1 (C-4), 39.6 (C-10), 39.8 (C-12), 42.5 (C-13), 43.2 (C-9), 51.5 (COO*C*$H_3$), 55.8 (C-17), 56.5 (C-14), 71.4 (C-3), 75.4 (C-5), 170.5 (O*C*OCH$_3$), 174.7 (C-24); LR-EI-MS *m*/*z* 430 (M − $H_2O$, 8), 370 (M − AcOH − $H_2O$, 16), 334 (38), 273 (M − AcOH − S.C., 23), 255 (M − AcOH − $H_2O$ − S.C., 56), 228 (M − AcOH − $H_2O$ − S.C. − part of ring D, 33), 213 (M − AcOH − $H_2O$ − S.C. − ring D, 100).

**Oxyfunctionalization Products of Methyl 3-Oxo-5β-cholan-24-oate (4a). Methyl 3-Oxo-4-cholene-24-oate (4c). 4c** was isolated from the reaction product of **4a** as a colorless amorphous solid (Fr. 1) crystallized from aqueous acetone; mp 124−126 °C (lit.,[32] mp 126−127 °C); IR $\nu_{max}$/$cm^{-1}$ 1616, 3008 (C═O) 1736, 1677 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.71 (3H, s, 18-$CH_3$), 0.91 (3H, d, $J$ = 6.2 Hz, 21-$CH_3$), 1.18 (3H, s, 19-$CH_3$), 3.67 (3H, s, −$COOCH_3$), 5.73 (1H, s, 4-H); $^{13}$C NMR $\delta$: 11.9 (C-18), 17.3 (C-19), 18.2 (C-21), 21.0 (C-11), 24.1 (C-15), 28.0 (C-16), 30.9, 31.0 (C-22, C-23), 32.0 (C-7), 32.9 (C-6), 33.9 (C-2), 35.3 (C-20), 35.6 (C-8), 35.7 (C-1), 38.6 (C-10), 39.6 (C-12), 42.4 (C-13), 51.1 (COO*C*$H_3$), 53.7 (C-9), 55.7, 55.8 (C-14, C-17), 123.7 (C-4), 171.6 (C-5), 174.7 (C-24), 199.6 (C-3); LR-EI-MS *m*/*z* 386 ($M^+$, 24), 371 (M − $CH_3$, 5), 344 (M − $CH_2CO$, 8), 329 (M − $CH_3$ − $CH_2$-CO, 8), 313 (4), 271 (M − S.C., 14), 229 (M − S.C. − ring D, 100), 211 (M − $H_2O$ − S.C. − ring D, 21).

**Methyl 5β-Hydroxy-3-oxocholan-24-oate (4b). 4b** was isolated from the reaction product of **4a** as a colorless amorphous solid (Fr. 2) crystallized from aqueous acetone; mp 170−172 °C (lit.,[5a] mp 170−172 °C); IR $\nu_{max}$/$cm^{-1}$ 3382 (OH), 1741, 1702 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.68 (3H, s, 18-$CH_3$), 0.91 (3H, d, $J$ = 6.5 Hz, 21-$CH_3$), 1.00 (3H, s, 19-$CH_3$), 3.67 (3H, s, −$COOCH_3$); $^{13}$C NMR $\delta$: 12.0 (C-18), 16.1 (C-19), 18.2 (C-21), 21.6 (C-11), 24.0 (C-15), 28.0 (C-16), 29.0 (C-7), 30.9 (C-22 and C-23), 31.1 (C-1), 34.7 (C-8), 35.2 (C-20), 36.1 (C-6), 37.3 (C-2), 39.7 (C-12), 40.1 (C-10), 42.5 (C-13), 43.6 (C-9), 49.3 (C-4), 51.4 (COO*C*$H_3$), 55.7 (C-17), 56.4 (C-14), 78.4 (C-5), 174.6 (C-24), 211.6 (C-3); LR-EI-MS *m*/*z* 386 (M − $H_2O$, 23), 355 (M − $2H_2O$ − $CH_3$, 3), 329 (M − $H_2O$ − $CH_3$ − $CH_2CO$, 6), 229 (M − $H_2O$ − S.C. − ring D, 100), 211 (M − $2H_2O$ − S.C. − ring D, 24).

**Methyl 3,4-Seco-5β-cholan-24-oate-3,4-dioic Acid (4d). 4d** was isolated from the reaction product of **4a** as colorless needles (Fr. 3) crystallized from acetone; mp 242−245 °C; IR $\nu_{max}$/$cm^{-1}$ 3449 (OH), 1739, 1703 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.66 (3H, s, 18-$CH_3$), 0.91 (3H, d, $J$ = 6.2 Hz, 21-$CH_3$), 0.96 (3H, s, 19-$CH_3$), 3.66 (3H, s, −$COOCH_3$); $^{13}$C NMR $\delta$: 12.1 (C-18), 18.2 (C-21), 19.0 (C-19), 21.5 (C-11), 24.1 (C-15), 24.6 (C-6), 26.3 (C-7), 28.1 (C-16), 28.8 (C-2), 31.0 (C-22 and C-23), 33.8 (C-1), 35.3 (C-8), 35.4 (C-20), 36.7 (C-10), 39.9 (C-12), 42.5 (C-13), 45.6 (C-9), 48.1 (C-5), 51.5 (COO*C*$H_3$), 55.8 (C-14 and C-17), 174.8 (C-24), 180.7, 181.3 (C-3, C-4); LR-EI-MS *m*/*z* 436 ($M^+$, 5%), 418 (M − $H_2O$, 100), 400 (M − $2H_2O$, 9), 390 (53), 345 (45), 335 (35), 317 (42), 276 (54), 275 (M − $2H_2O$ − S.C., 48), 221 (60).

**Oxyfunctionalization Products of 5α-Cholestan-3β-yl-acetate (5a). 3β-Acetoxycholestan-5α-ol (5b). 5b** was isolated from the reaction product of **5a** as a colorless amorphous solid (Fr. 1) crystallized from aqueous methanol; mp 180−182 °C (lit.,[33] mp 185−186 °C); IR $\nu_{max}$/$cm^{-1}$ 3449 (OH), 1736, 1704 (C═O); $^1$H NMR ($CDCl_3$) $\delta$: 0.65 (3H, s, 18-$CH_3$), 0.86 (6H, d, $J$ = 6.5 Hz, 26- and 27-$CH_3$), 0.90 (3H, d, $J$ = 6.5 Hz, 21-$CH_3$), 1.00 (3H, s, 19-$CH_3$), 2.02 (3H, s, −$COCH_3$), 5.16 (1H, brm, 3α-H); $^{13}$C NMR $\delta$: 12.1 (C-18), 16.1 (C-19), 18.6 (C-21), 21.3 (C-11), 21.5 (OCO*C*$H_3$), 22.6 (C-26), 22.8 (C-27), 23.9 (C-23), 24.1 (C-15), 25.9 (C-7), 26.8 (C-2), 28.0 (C-25), 28.2 (C-16), 30.5 (C-1), 34.5 (C-6), 34.7 (C-8), 35.8 (C-20), 36.2 (C-22), 38.8 (C-10), 39.5 (C-24), 40.0 (C-4 and C-12), 42.7 (C-13), 45.6 (C-9), 56.1 (C-14), 56.2 (C-17), 70.9 (C-3), 75.0 (C-5), 170.7 (O*C*OCH$_3$); LR-EI-MS *m*/*z* 446 ($M^+$, 4%), 428 (M − $H_2O$, 66), 413 (M − $H_2O$ − $CH_3$, 4), 386 (M − AcOH, 9), 368 (M − AcOH − $H_2O$, 100), 353 (M − AcOH − $H_2O$ − $CH_3$, 37), 332 (45), 274 (31), 228 (36), 213 (54).

**3β-Acetoxy-5α-cholestan-25-ol (5c). 5c** was isolated from the reaction product of **5a** as a colorless amorphous solid (Fr. 2) crystallized from aqueous methanol; mp 124−126 °C (lit.,[19] mp 124−126 °C); IR $\nu_{max}$/$cm^{-1}$ 3392 (OH), 1728 (C═O); $^1$H NMR

(32) Issidorides, C. H.; Fieser, M.; Fieser, L. F. *J. Am. Chem. Soc.* **1960**, *82*, 2002.

(33) Dalton, F.; McDougall, J. I.; Meakins, G. D. *J. Chem. Soc.* **1963**, 4068.

(34) David, N. K.; Harold, C. T.; Christal, D.; Karen, A. W.; Kelvin, E. S.; Shahid, L.; Robert, W. P. H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1567.

($CDCl_3$) $\delta$: 0.65 (3H, s, 18-$CH_3$), 0.82 (3H, s, 19-$CH_3$), 0.91 (3H, d, $J$ = 6.8 Hz, 21-$CH_3$), 1.21 (6H, s, 26- and 27-$CH_3$), 2.02 (3H, s, $-COCH_3$), 4.68 (1H, brm, 3α-H); $^{13}$C NMR $\delta$: 12.0 (C-18), 12.1 (C-19), 18.6 (C-21), 20.8 (C-23), 21.1 (C-11), 21.4 (OCO*C*$H_3$), 24.1 (C-15), 27.4 (C-2), 28.2 (C-16), 28.5 (C-6), 29.1 (C-26), 29.3 (C-27), 31.9 (C-7), 34.0 (C-4), 35.4 (C-8 and C-10), 35.7 (C-20), 36.4 (C-22), 36.7 (C-1), 39.9 (C-12), 42.5 (C-13), 44.4 (C-24), 44.6 (C-5), 54.2 (C-9), 56.2 (C-17), 56.4 (C-14), 71.0 (C-25), 73.7 (C-3), 170.6 (O*C*OC$H_3$); LR-EI-MS *m*/*z* 428 (M $-$ $H_2O$, 14), 413 (M $-$ $H_2O$ $-$ $CH_3$, 8), 353 (M $-$ AcOH $-$ $H_2O$ $-$ $CH_3$, 15), 315 (44), 255 (44), 215 (M $-$ AcOH $-$ S.C. $-$ ring D, 43), 95 (100).

**Acknowledgment.** We thank Dr. Toshio Nambara, Professor Emeritus, Tohoku University, for critically reading the manuscript and providing useful comments. We also thank the Nihon University Joint Research Subsidy for financial support in 2006.

**Supporting Information Available:** General experimental information, materials, and methods. Analytical data for all products. Table showing effects of a combination of metalloporphyrin-catalyzed oxyfunctionalizations on the reactivity and regioselectivity of substrate **3a**. Table showing complete $^1$H and $^{13}$C chemical shifts of 5α-oxygenated compounds **3c** and **3d**. Copies of $^1$H and $^{13}$C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO061800G