ARTICLE

OBC
www.rsc.org/obc

The remote-oxyfunctionalization of unactivated carbons in (5β)-3-oxobile acids by 2,6-dichloropyridine *N*-oxide catalyzed by ruthenium–porphyrin and HBr: a direct lactonization at C-20†

Shoujiro Ogawa,[a] Takashi Iida,*[a] Takaaki Goto,[b] Nariyasu Mano,[b] Junichi Goto[b] and Toshio Nambara[b]

[a] *Department of Chemistry, College of Humanities and Sciences, Nihon University, Sakurajousui, Setagaya, Tokyo 156-8550, Japan. E-mail: takaiida@chs.nihon-u.ac.jp*

[b] *Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Sendai 980-8578, Japan*

Received 21st November 2003, Accepted 2nd February 2004
First published as an Advance Article on the web 1st March 2004

Remote-oxyfunctionalization induced by 2,6-dichloropyridine *N*-oxide (DCP *N*-oxide) as an oxygen donor and a (5,10,15,20-tetramesitylporphyrinate) ruthenium(II) carbonyl complex (Ru-porphyrin) and HBr as catalysts was examined for a series of methyl ester-peracetylated derivatives of (5β)-3-oxobile acids. Using the DCP-*N*-oxide/Ru-porphyrin/HBr system, 5β-hydroxylation predominated for the substrates having a 12-acetoxyl substituent due to steric hindrance, but the presence of a 7-acetoxyl substituent decreased the reactivity of the 5β-position allowing for the competitive (20*S*)-20-oxyfunctionalization, subject to electronic constraints. A variety of novel 5β-hydroxylation and (20*S*)-24,20-γ-lactonization products, as well as their double-oxyfunctionalization and dehydration products, were obtained in one-step. The alkaline hydrolysis of the γ-lactones gave the corresponding stereoselective (20*S*)-20-hydroxy-carboxylic acids.

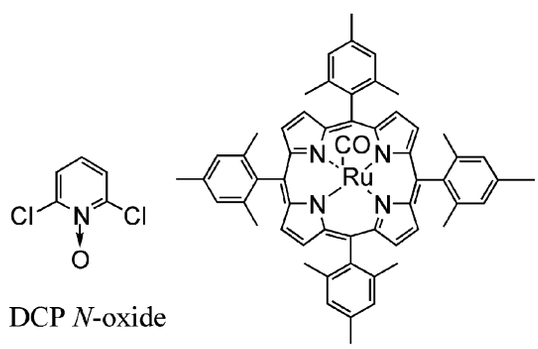
Introduction

Recent efforts in the organic synthesis of bioactive compounds have been focused on the development of so-called "biomimetic compounds", "artificial enzymes", "enzyme mimics" and/or the "remote-oxyfunctionalization" of unactivated hydrocarbons *via* non-microbial and non-enzymic methods.[1] For these purposes, a variety of versatile new oxygen-transfer reagents and/or catalysts have been reported by many groups of investigators.

The cytochrome P-450 oxidase-dependent system efficiently catalyzes the key transformation of unactivated carbon atoms of cholesterol to give steroid hormones and related biomolecules *in vivo*.[2] Therefore, the regio- and stereoselective remote-oxyfunctionalization (*e.g.*, hydroxylation and ketonization) of unactivated carbons, related to the cytochrome P-450 process, is of particular interest in obtaining bioactive steroids from abundantly available sterols or bile acid sources avoiding a multistep synthesis. Recent advances in the remote-oxyfunctionalization reactions of unactivated positions in steroids have been reviewed by Reese.[3] These include the radical relay reaction (Breslow reaction), the hypohalite reaction, nitrite photolysis (Barton reaction), ketone irradiations, the nitrene reaction, the Hofmann–Löffler–Freytag reaction, the lead tetraacetate reaction, dimethyldioxirane (DMDO), pentafluorodialkyloxaziridine, anodic oxidation, the free radical decomposition of peracids, ceric ammonium nitrate, peroxide reactions, chromic oxide, hypervalent organoiodine reagents, and porphyrins.

The remote-oxyfunctionalization of steroids carried out by cytochrome P-450 enzymes *in vivo*, makes use of a heme prosthetic group at the active site of the catalyst. As has been reviewed by Meunier[4a] and reported by other groups,[4b–4f] many attempts have been made to mimic the action of the cytochrome P-450 enzyme systems in the oxygenation and oxidation of various drugs and other biologically active compounds by using metalloporphyrin moieties.

The recent successful use of a powerful new oxidant system consisting of 2,6-dichloropyridine (DCP) *N*-oxide as an oxygen-donating reagent and a (5,10,15,20-tetramesitylporphyrinate) ruthenium(II) carbonyl complex [Ru-porphyrin, Ru(TMP)CO] and HBr as catalysts prompted us to apply this method to bile acids.[5] We describe here the remote-oxyfunctionalization of a series of the methyl ester-peracetylated derivatives of 3-oxbile acids with or without an acetoxyl substituent at the C-7 and/or C-12 positions in the 5β-nucleus using this DCP *N*-oxide/ruthenium porphyrin/HBr system.



DCP *N*-oxide

Ru(TMP)CO

Results and discussion

Almost all of the oxygenation reagents previously reported result predominantly in 5β-hydroxylation in 5β-steroids.[3] The use of DMDO as an oxygenation agent, however, results not only in 5β-hydroxylation, but also in 14α- and 17α-hydroxylations in the nucleus as well as their double-oxyfunctionalizations depending upon both the presence and the stereochemical nature of a 7-substituent in the substrate.[6] Nevertheless, direct remote-oxyfunctionalization of the unactivated cabons in the side-chain [-$CH(CH_3)CH_2CH_2COOH$] of

† Electronic supplementary information (ESI) available: ^{13}C-NMR chemical shifts for oxyfunctionalization products **6**–**23**, **26** and **27**. See http://www.rsc.org/suppdata/ob/b3/b314965j/

DOI: 10.1039/b314965j

Table 1 Oxidation products of (5β)-3-oxobile acid methyl ester-peracetates with DCP *N*-oxide/Ru(TMP)CO/HBr

	Substrate	Reaction time (*t*/h)	Products (yield, %)[a] Starting compound recovery	Products
	1	12	31	**6** (30), **7** (3), **8** (17), **9** (19)
	1	60	20	**6** (33), **7** (6), **8** (19), **9** (22)
	2	60	56	**10** (25), **11** (19)
	3	60	43	**12** (19), **13** (8), **14** (21), **15** (9)
	4	60	40	**16** (18), **17** (29), **18** (3), **19** (10)
	5	60	45	**20** (36), **21** (5), **22** (5), **23** (9)

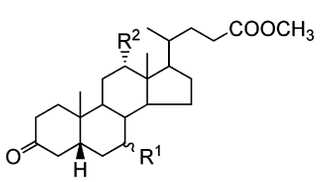
[a] Determined by capillary GC; for conditions, see Experimental section.

C_{24} (5β)-bile acids is very rare, though the C_8H_{17}-alkyl side-chain in the 5α-cholestane series of steroids is readily oxidized at the C-25 methine carbon to give the 25-hydroxylated derivatives exclusively.

An exploratory experiment revealed that remote-oxyfunctionalization with the DCP *N*-oxide/Ru-porphyrin/HBr system is more effective for methyl 3-oxo-5β-cholan-24-oate (**1**) than for methyl 3α-acetoxy-5β-cholan-24-oate under a prolonged reaction time (at 50 °C for 60 h), suggesting that the presence of the electron-withdrawing 3-oxo group interferes with the 5β-hydroxylation, compared with the 3α-acetoxyl group (see ref. 7). Based on this finding, a series of the methyl ester-peracetylated derivatives of (5β)-3-oxobile acids (**1**–**5**) with or without 7- and/or 12-substituents were used as substrates. The hydroxy groups at C-7 and C-12 were protected by the acetoxyl group to prevent simultaneous oxidation of the hydroxyls to ketones. The results are shown in Table 1. Treatment of **1**–**5** with DCP *N*-oxide/ruthenium porphyrin/HBr resulted in the formation of several monofunctionalized products, together with their double-functionalized ones. In addition, the regioselectivity of the *O*-insertion reaction was significantly influenced by the presence of a 7- or 12-acetoxyl group (see below).

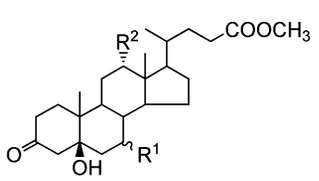
The oxidation of **1** with DCP *N*-oxide/Ru-porphyrin/HBr produced three major compounds, 5β-hydroxy-3-oxocholan-24-oate (**6**, 33%), (20*S*)-3-oxo-5β-cholan-*O*-24,20-lactone (**8**, 19%) and (20*S*)-5β-hydroxy-3-oxocholan-*O*-24,20-lactone (**9**, 22%), along with a small amount of methyl 3-oxo-4-cholen-24-oate (**7**, 6%).[7] The formation of the conjugated enone **7** may have been derived from the subsequent elimination of the 5β-hydroxy group in **6** under the reaction conditions used. These results for **1** differed from those observed by Shingaki *et al.*,[5b] who reported the formation of 5β-hydroxycholan-24-oic acid (70%) and (20*S*)-5β,20-dihydroxycholan-24-oic acid (3%) starting with 5β-cholan-24-oic acid using the same oxidizing system; (20*S*)-24,20-γ-lactonization products such as **8** and **9** were not detected at all. The presence of the electron-withdrawing 3-oxo group in **1**, therefore, seems to prevent the 5β-hydroxylation of the methine carbon at C-5, but accelerate the remote-oxyfunctionalization of the methine carbon at C-20. Nevertheless, the DCP *N*-oxide/Ru-porphyrin/HBr system is faster in adding an oxygen into the 5β C–H bond of **1** to give **6** as one of the main products.

A much different result was obtained for the DCP *N*-oxide/Ru-porphyrin/HBr oxidation of methyl 12α-acetoxy-3-oxo-5β-cholan-24-oate (**2**). After chromatographic purification of the reaction products, two major oxidized components were isolated and their structures identified as methyl 12α-acetoxy-5β-hydroxy-3-oxocholan-24-oate (**10**) and methyl 12α-acetoxy-

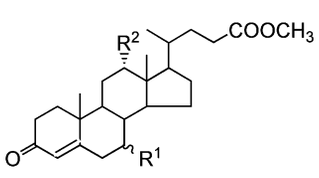


	R^1	R^2
1	H	H
2	H	α-OAc
3	α-OAc	H
4	β-OAc	H
5	α-OAc	α-OAc

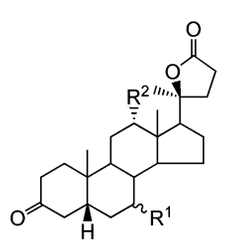
Ac = -$COCH_3$



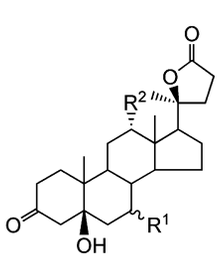
	R^1	R^2
6	H	H
10	H	α-OAc
12	α-OAc	H
16	β-OAc	H
20	α-OAc	α-OAc



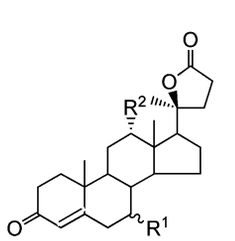
	R^1	R^2
7	H	H
11	H	α-OAc
13	α-OAc	H
21	α-OAc	α-OAc



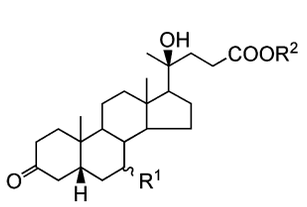
	R^1	R^2
8	H	H
14	α-OAc	H
17	β-OAc	H



	R^1	R^2
9	H	H
15	α-OAc	H
18	β-OAc	H
22	α-OAc	α-OAc



	R^1	R^2
19	β-OAc	H
23	α-OAc	α-OAc



	R^1	R^2
24	H	H
25	β-OH	H
26	H	CH_3
27	β-OH	CH_3

3-oxo-4-cholen-24-oate (**11**). No 20-oxygenated product was detected at all. The axial 12α-acetoxyl group in **2** may therefore prevent the attack of the bulky DCP *N*-oxide/Ru-porphyrin/HBr complex, like most other reagents sensitive to steric hindrance, on the methine carbon at C-20.

On the other hand, the addition of a 7-acetoxyl group in methyl 7α-acetoxy-3-oxo-5β-cholan-24-oate (**3**) and its 7β-isomer (**4**) substantially decreased the *O*-insertion rate at C-5 relative to **1** and **2**, while significantly increasing the insertion rate at C-20 regardless of the axial 7α- or equatorial 7β-configuration. Thus, when **3** was treated with the DCP *N*-oxide/Ru-porphyrin/HBr system, the successful formation of 20-oxygenated γ-lactones surpassed that of the 5β-hydroxylated compounds, yielding (20*S*)-7α-acetoxy-3-oxo-5β-cholan-*O*-24,20-lactone (**14**) and (20*S*)-7α-acetoxy-5β-hydroxy-3-oxocholan-*O*-24,20-lactone (**15**) as the major products (total yield, 30%) and methyl 7α-acetoxy-5β-hydroxy-3-oxocholan-24-oate (**12**) and methyl 7α-acetoxy-3-oxo-4-cholen-24-oate (**13**) as the minor components (total yield, 27%). Similarly, **4** produced three variants of 24,20-γ-lactones (total yield, 42%), *i.e.*, (20*S*)-7β-acetoxy-3-oxo-5β-cholan-*O*-24,20-lactone (**17**), (20*S*)-7β-acetoxy-5β-hydroxy-3-oxocholan-*O*-24,20-lactone (**18**) and (20*S*)-7β-acetoxy-3-oxo-4-cholen-*O*-24,20-lactone (**19**), together with methyl 7β-acetoxy-5β-hydroxy-3-oxo-cholan-24-oate (**16**, 18%). Since both the 7α- and 7β-acetoxyl groups of the 5β-steroid nucleus do not add significant steric hindrance to the β face of the bulky complex, the considerable variation in the ratio of the C-5 and C-20-oxidized products in **3** and **4** may be ascribed to the local electron density (electrostatic) of the two electron-withdrawing 3-oxo and 7-acetoxyl groups around C-5. The intriguing differences between the results of **2** and **3** (or **4**) can, therefore, be rationalized in terms of electronic effects rather than steric hindrance.[5,6] In all cases, the stereochemical configuration with respect to C-20 was retained during the oxyfunctionalization of the methine hydrogen (C-H) to the resulting γ-lactones.

Methyl 7α,12α-diacetoxy-3-oxo-5β-cholan-24-oate (**5**), having 7α- and 12α-acetoxyl substituents, afforded a 5β-hydroxy compound (**20**, 36%) and its dehydrated derivative (**21**) when subjected to DCP *N*-oxide/Ru-porphyrin/HBr oxidation. The oxidation of **5** was also accompanied by a reasonable amount (total yield, 14%) of the double-functionalized 5β-hydroxy-3-oxo-24,20-lactone (**22**) and 4-ene-3-oxo-24,20-lactone (**23**). The combined effect of the respective 7- and 12-acetoxyl groups on the oxidation at the C-5 and C-20 positions resulted in the competitive formation of both the 5β- and 20-oxygenated compounds, in analogy with **1**.

The formation of (20*S*)-24,20-γ-lactones (**8**, **9**, **14**, **15**, **17**, **18**, **19**, **22** and **23**) *via* the DCP *N*-oxide/Ru-porphyrin/HBr oxidation of **1** and **3**–**5** apparently involves an intramolecular esterification of the carboxyl group at C-24 with the newly inserted hydroxy group at C-20. Although the exact reaction mechanism is unclear, the fact that possible intermediary (20*S*)-20-hydroxy-24-methyl ester and/or (20*S*)-20-hydroxy-24-carboxylic acid derivatives were not isolated at all in any of the reactions implies that the reaction proceeds directly by a nucleophilic acyl substitution catalyzed by HBr.

Supporting evidence for the structures of the 24,20-γ-lactones (**8** and **17**) was achieved by alkaline hydrolysis with 10% methanolic KOH, followed by acidification with 10% H_2SO_4 to give the (20*S*)-20-hydroxy-carboxylic acids (**24** and **25**). The free acids **24** and **25** were then converted into the corresponding C-24 methyl esters (**26** and **27**) with diazomethane, while esterification with *p*-toluenesulfonic acid in methanol reproduced the original γ-lactones (**8** and **17**), demonstrated in the ^{1}H- and ^{13}C-NMR and GC-MS analyses.[6a,8] Of particular interest is the appearance of a singlet signal occurring at *ca*. 1.47 (or 1.38) ppm due to the 21-methyl protons and the absence of a singlet signal at *ca*. 3.65 ppm in the ^{1}H-NMR of the C-24 methyl ester. In the ^{13}C-NMR, a characteristic signal due to C-20 appears in the low field region of *ca.* 88.5 ppm, while the C-25 signal of the methyl ester at *ca*. 51.5 ppm is absent. The GC-MS of (20*S*)-24,20-γ-lactones exhibited intense signals consistent with the sequential loss of side-chains (S.C.) of the $C_5H_7O_2$ γ-lactone ring (99 a.m.u) from the respective M, (M − AcOH), (M − 2AcOH) and/or related ions, instead of $C_6H_{11}O_2$ (112 a.m.u.) from the C-24 methyl ester.

According to previous findings, the regioselectivity of the *O*-insertion reaction using this reagent system depends upon the steric and electronic effects of the electron-withdrawing substituents situated in the vicinity of the methine carbon under consideration.[5] Electron-withdrawing 3-oxo and 7-acetoxyl substituents destabilize the transition state for the DCP *N*-oxide/Ru-porphyrin/HBr complex insertion in the nearby methine carbon at C-5 through unfavorable electrostatic interactions, and, as a consequence, insertion occurs at C-20. The interplay of these and perhaps other factors may be more subtle: the oxyfunctionalization of C-20 in **1** and **3**–**5** requires that the 3-oxo and 7-acetoxyl groups cause a large decrease in the reaction rate at C-5 compared with C-20, either by decreasing the electron density in the equatorial 5β C–H bond more than it does on the pseudo-axially oriented 20β C–H bond, or by destabilizing the transition state leading to insertion at C-5 more than the transition state leading to C-20 insertion.

In conclusion, the remote-oxyfunctionalization of the methyl ester-peracetylated derivatives of 3-oxobile acids with a 12α-acetoxyl group *via* DCP *N*-oxide/Ru-porphyrin/HBr system resulted in primarily 5β-hydroxylation, while (20*S*)-24,20-lactonization predominanted for 3-oxobile acids having 3-oxo and 7-acetoxyl substituents. A direct, one-step synthesis of the (20*S*)-24,20-γ-lactones and their hydrolysis products, (20*S*)-20-hydroxylated 3-oxobile acids, from abundant and common bile acids may be useful for the easy, efficient synthesis of bioactive steroids such as steroid hormones and cardiotonic steroids.

Experimental

Materials and methods

Melting points (mp) were determined on an electric micro hot stage and are uncorrected. Infrared (IR) spectra were obtained on a Bio Rad FTS-7 FT-IR spectrometer (Philadelphia, USA) for samples in KBr tablets. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM-EX 270 FT NMR instrument (Tokyo, Japan) at 270 and 67.8 MHz, respectively, with $CDCl_3$ containing 0.1% Me_4Si as the solvent. Chemical shifts are expressed as δ (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 experiments. Low-resolution mass (LR-MS) spectra were recorded on a JEOL JMC-Automass 150 gas chromatography-mass spectrometry (GC-MS) at 70 eV with an electron ionization (EI) probe using the positive ion mode (PIM). High-resolution mass (HR-MS) spectra were performed using JEOL JMS-LCmate and JMS-700 double-focusing magnetic mass spectrometers equipped with an electrospray ionization (ESI) probe using the PIM and with the EI probe under the PIM, respectively. The resolution of the ESI mass spectrometer was set at 3000, and the voltages for the electrospray, orifice and ring lens were 2.5 kV, 30 V and 100 V, respectively. The temperatures of the orifice and desolvating plate were 150 and 250 °C, respectively. HR-MS spectra were also recorded on a JEOL DX-303 (JMS-AX500) mass spectrometer equipped with an EI probe under the PIM. A Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector was used isothermally at 270 or 300 °C fitted with a chemically bonded fused silica capillary column (25QC3/BPX5; 25 m × 0.32 mm i.d.; film thickness, 0.25 μm; SGE). The apparatus used for medium-pressure liquid chromatography (MPLC) consisted of a Shimamura YRD-880

RI-detector (Tokyo, Japan) and a uf-3040s chromatographic pump using silica gel 60 (230–400 mesh; Nacalai Tesque, Kyoto, Japan) as the adsorbent and benzene–EtOAc mixtures as the eluent. Thin-layer chromatography (TLC) was performed on precoated silica gel plates (0.25 mm layer thickness; E.Merck, Darmstadt, Germany) using hexane–EtOAc–acetic acid mixtures (80 : 20 : 1–20 : 80 : 1, v/v/v) as the developing solvent.

DCP *N*-oxide was prepared according to the procedure of Rousseau and Robins.[9] The method of Lindsey *et al*. was used for the preparation of 5,10,15,20-tetrakis-(2,4,6-trimethylphenyl)-porphyrin.[10] The ruthenium porphyrin complex, Ru(TMP)CO, was prepared by a slight modification of the method of Rillema *et al*.[11]

General procedure for the oxidation using DCP *N*-oxide/Ru-porphyrin/HBr

To a magnetically stirred benzene solution (3 ml) of bile acid (1.3 mmol) and molecular sieves (850 mg, 4 Å), DCP *N*-oxide (630 mg, 3.8 mmol), Ru(TMP)CO (5 mg, 5.5 μmol) and HBr (50 μl) were successively added, and the mixture was stirred at 50 °C for 60 h for **1**–**5** (the reaction was monitored by TLC). The reaction product was extracted with benzene, and the combined extracts were washed with water, dried with Drierite, and evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel (60 g) eluting with benzene–EtOAc (8 : 2–5 : 5, v/v) mixtures and then by MPLC on silica gel (230–400 mesh, 21 g) eluting with benzene–EtOAc (9 : 1, v/v).

Oxidation products of methyl 3-oxo-5β-cholan-24-oate 1

Methyl 5β-hydroxy-3-oxocholan-24-oate 6. Isolated from the reaction product of **1** as a colorless amorphous solid (Fr. 2); crystallized from aq. acetone; mp 170–172 °C; IR ν_{max}/cm^{-1} 1702, 1741 (C=O), 3382 (OH); ^{1}H-NMR δ 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, $COOCH_3$); LR-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); HR-MS (ESI-PIM), Calc. for $C_{25}H_{40}O_4Na$ [M + Na]$^+$: 427.2824. Found: *m*/*z*, 427.2810.

Methyl 3-oxo-4-cholen-24-oate 7. Isolated from the reaction product of **1** as a colorless amorphous solid (Fr. 1); crystallized from aq. acetone; mp 124–126 °C [lit.[12] mp, 125–126 °C]; IR ν_{max}/cm^{-1} 1677, 1736 (C=O), 1616, 3008 (C=C); ^{1}H-NMR δ 0.71 (3H, s, 18-CH_3), 0.91 (3H, s, *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-CH); LR-MS *m*/*z* 386 (M, 24%), 371 (M − CH_3, 5), 344 (M − CH_2CO, 8), 329 (M − CH_3 − CH_2CO, 8), 313 (4), 271 (M − S.C. − ring D, 14), 229 (M − S.C. − ring D, 100), 211 (M − H_2O − S.C. − ring D, 21).

(20*S*)-3-Oxo-5β-cholan-*O*-24,20-lactone 8. Isolated from the reaction product of **1** as a noncrystalline substance (Fr. 3); IR ν_{max}/cm^{-1} 1735, 1771 (C=O); ^{1}H-NMR δ 0.84 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.46 (3H, s, 21-CH_3); LR-MS *m*/*z* 372 (M, 37%), 354 (M − H_2O, 24), 339 (M − H_2O − CH_3, 63), 321 (M − $2H_2O$ − CH_3, 8), 302 (87), 273 (M − S.C., 51), 246 (M − S.C. − part of ring D, 95), 231 (M − S.C. − ring D, 95), 213 (M − H_2O − S.C. − ring D, 100); HR-MS (EI-PIM), Calc. for $C_{24}H_{36}O_3$ [M]$^+$: 372.2664. Found: *m*/*z*, 372.2642.

(20*S*)-5β-Hydroxy-3-oxocholan-*O*-24,20-lactone 9. Isolated from the reaction product of **1** as a colorless amorphous solid (Fr. 4); crystallized from acetone–hexane; mp 164–166 °C; IR ν_{max}/cm^{-1} 1736, 1773 (C=O), 3414 (OH); ^{1}H-NMR δ 0.84 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.47 (3H, s, 21-CH_3); LR-MS *m*/*z* 370 (M − H_2O, 47%), 355 (M − H_2O − CH_3, 13), 328 (M − H_2O − CH_2CO, 43), 313 (M − H_2O − CH_3 − CH_2CO, 8), 271 (M − H_2O − S.C., 71), 244 (M − H_2O − S.C. − part of ring D, 60), 229 (M − H_2O − S.C. − ring D, 100), 201 (31); HR-MS (EI-PIM), Calc. for $C_{24}H_{36}O_4$ [M]$^+$: 388.2614. Found: *m*/*z*, 388.2592.

Oxidation products of methyl 12α-acetoxy-3-oxo-5β-cholan-24-oate 2

Methyl 12α-acetoxy-5β-hydroxy-3-oxocholan-24-oate 10. Isolated from the reaction product of **2** as a colorless amorphous solid (Fr. 2); crystallized from toluene–hexane; mp 129–133 °C; IR ν_{max}/cm^{-1} 1723 (C=O), 3505 (OH); ^{1}H-NMR δ 0.77 (3H, s, 18-H_3), 0.80 (3H, d, *J* 6.2 Hz, 21-H_3), 0.98 (3H, s, 19-H_3), 2.07 (3H, s, $COCH_3$), 3.66 (3H, s, $COOCH_3$), 5.14 (1H, br m, 12β-H); LR-MS *m*/*z* 462 (M$^+$, <1%), 419 (6), 402 (M − AcOH, 3), 387 (M − AcOH − CH_3, 18), 384 (M − AcOH − H_2O, 11), 332 (100), 287 (M − AcOH − S.C., 7), 269 (M − AcOH − H_2O − S.C., 21), 251, (M − AcOH − $2H_2O$ − S.C., 8), 227 (M − AcOH − H_2O − S.C. − ring D, 10), 217 (35), 209 (M − AcOH − $2H_2O$ − S.C. − ring D, 26); HR-MS (ESI-PIM), Calc. for $C_{27}H_{42}O_6Na$ [M + Na]$^+$: 485.2879. Found: *m*/*z*, 485.2848.

Methyl 12α-acetoxy-3-oxo-4-cholen-24-oate 11. Isolated from the reaction product of **2** as a colorless amorphous solid (Fr. 1); crystallized from ethanol–hexane; mp 120–123 °C [lit.[13a] mp, 124–126 °C]; IR ν_{max}/cm^{-1} 1711, 1753 (C=O), 1654, 2965 (C=C); ^{1}H-NMR δ 0.79 (3H, s, 18-CH_3), 0.80 (3H, s, *J* 6.5 Hz, 21-CH_3), 1.16 (3H, s, 19-CH_3), 3.66 (3H, s, $COOCH_3$), 5.12 (1H, m, 12β-H), 5.74 (1H, s, 4-CH); LR-MS *m*/*z* 444 (M$^+$, 1%), 402 (1), 384 (M − AcOH, 32), 369 (M − AcOH − CH_3, 11), 269 (M − AcOH − S.C., 100), 261 (26), 251, (M − AcOH − H_2O − S.C., 20), 231 (24), 227 (M − AcOH − S.C. − ring D, 25), 211 (29), 209 (M − AcOH − H_2O − S.C. − ring D, 26).

Oxidation products of methyl 7α-acetoxy-3-oxo-5β-cholan-24-oate 3

Methyl 7α-acetoxy-5β-hydroxy-3-oxocholan-24-oate 12. Isolated from the reaction product of **3** as a colorless amorphous solid (Fr. 2); crystallized from EtOAc–hexane; mp 177–180 °C; IR ν_{max}/cm^{-1} 1732, 1719 (C=O), 3348 (OH); ^{1}H-NMR δ 0.69 (3H, s, 18-H_3), 0.93 (3H, d, *J* 6.2 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 2.05 (3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 5.01 (1H, m, 7β-H); LR-MS *m*/*z* 384 (M − AcOH − H_2O, 16%), 269 (M − AcOH − H_2O − S.C., 38), 249 (M − AcOH − $2H_2O$ − S.C., 12), 227 (M − AcOH − H_2O − S.C. − ring D, 14); HR-MS (ESI-PIM), Calc. for $C_{27}H_{42}O_6Na$ [M + Na]$^+$: 485.2879. Found: *m*/*z*, 485.2891.

Methyl 7α-acetoxy-3-oxo-4-cholen-24-oate 13. Isolated from the reaction product of **3** as colorless prisms (Fr. 1); crystallized from aq. methanol; mp 207–208 °C [lit.[13b] mp, 206–208 °C]; IR ν_{max}/cm^{-1} 1732 (C=O), 1663, 3069 (C=C); ^{1}H-NMR δ 0.71 (3H, s, 18-H_3), 0.92 (3H, d, *J* 6.2 Hz, 21-H_3), 1.20 (3H, s, 19-H_3), 2.03 (3H, s, $COCH_3$), 3.66 (3H, s, $COOCH_3$), 5.00 (1H, m, 7β-H), 5.69 (1H, s, 4-CH); LR-MS *m*/*z* 429 (M − CH_3, 2%), 384 (M − AcOH, 16), 369 (M − AcOH − CH_3, 5), 269 (M − AcOH − S.C., 30), 227 (M − AcOH − S.C. − ring D, 20).

(20*S*)-7α-Acetoxy-3-oxo-5β-cholan-*O*-24,20-lactone 14. Isolated from the reaction product of **3** as a colorless amorphous solid (Fr. 3); crystallized from aq.methanol; mp 187–190 °C; IR ν_{max}/cm^{-1}; ^{1}H-NMR δ 0.84 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.47 (3H, s, 21-H_3), 2.04 (3H, s, $COCH_3$), 4.98 (1H, m, 7β-H); LR-MS *m*/*z* 430 (M$^+$, 2%), 370 (M − AcOH, 4), 337 (M − AcOH − H_2O − CH_3, 38), 299 (32), 271 (M − AcOH − S.C., 20), 229 (M − AcOH − S.C. − ring D, 15), 211 (M − AcOH − H_2O − S.C. − ring D, 36); HR-MS (EI-PIM), Calc. for $C_{26}H_{38}O_5$ [M]$^+$: 430.2719. Found: *m*/*z*, 430.2716.

(20*S*)-7α-Acetoxy-5β-hydroxy-3-oxocholan-*O*-24,20-lactone 15. Isolated from the reaction product of **3** as a colorless amorphous solid (Fr. 4); crystallized from EtOAc–hexane; mp 232–234 °C; IR ν_{max}/cm^{-1} 1762, 1715 (C=O), 3449 (OH); ^{1}H-NMR δ 0.85 (3H, s, 18-H$_3$), 1.03 (3H, s, 19-H$_3$), 1.47 (3H, s, 21-H$_3$), 2.05 (3H, s, COCH$_3$), 5.02 (1H, m, 7β-H); LR-MS *m*/*z* 368 (M − AcOH − H$_2$O, 7%), 353 (M − AcOH − H$_2$O − CH$_3$, 10), 335 (M − AcOH − 2H$_2$O − CH$_3$, 6), 281 (74), 269 (M − AcOH − H$_2$O − S.C., 32), 255 (32), 227 (M − AcOH–H$_2$O − S.C. − ring D, 7); HR-MS (ESI-PIM), Calc. for $C_{26}H_{38}O_6Na$ [M + Na]$^+$: 469.2566. Found: *m*/*z*, 469.2567.

Oxidation products of methyl 7β-acetoxy-3-oxo-5β-cholan-24-oate 4

Methyl 7β-acetoxy-5β-hydroxy-3-oxocholan-24-oate 16. Isolated from the reaction product of **4** as colorless thin plates (Fr. 1); crystallized from EtOAc–hexane; mp 169–171 °C; IR ν_{max}/cm^{-1} 1731, 1756 (C=O), 3423 (OH); ^{1}H-NMR δ 0.72 (3H, s, 18-H$_3$), 0.92 (3H, d, *J* 6.5 Hz,21-H$_3$), 1.04 (3H, s, 19-H$_3$), 2.00 (3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 4.62 (1H, br m, 7α-H); LR-MS *m*/*z* 384 (M − AcOH − H$_2$O, 5%), 281 (15), 267 (M − AcOH − H$_2$O − S.C., 13), 227 (M − AcOH − H$_2$O − S.C. − ring D, 5), 207 (100); HR-MS (ESI-PIM), Calc. for $C_{27}H_{42}O_6Na$ [M + Na]$^+$: 485.2879. Found: *m*/*z*, 485.2865.

(20*S*)-7β-Acetoxy-3-oxo-5β-cholan-*O*-24,20-lactone 17. Isolated from the reaction product of **4** as a noncrystalline substance (Fr. 2); IR ν_{max}/cm^{-1} 1680, 1762 (C=O); ^{1}H-NMR δ 0.88 (3H, s, 18-H$_3$), 1.08 (3H, s, 19-H$_3$), 1.46 (3H, s, 21-H$_3$), 2.00 (3H, s, COCH$_3$), 4.97 (1H, br m, 7α-H); LR-MS *m*/*z* 370 (M − AcOH, 21%), 355 (M − AcOH − CH$_3$, 17), 300 (58), 281 (66), 271 (M − AcOH − S.C., 60), 221 (M − AcOH − H$_2$O − S.C. − ring D, 30); HR-MS (ESI-PIM), Calc. for $C_{26}H_{38}O_5Na$ [M + Na]$^+$: 453.2617. Found: *m*/*z*, 453.2604.

(20*S*)-7β-Acetoxy-5β-hydroxy-3-oxocholan-*O*-24,20-lactone 18. Isolated from the reaction product of **4** as a colorless amorphous solid (Fr. 4); crystallized from EtOAc–hexane; mp 233–236 °C; IR ν_{max}/cm^{-1} 1745, 1779 (C=O), 3442 (OH); ^{1}H-NMR δ 0.90 (3H, s, 18-H$_3$), 1.24 (3H, s, 19-H$_3$), 1.46 (3H, s, 21-H$_3$), 2.04 (3H, s, COCH$_3$), 4.62 (1H, br m, 7α-H), 5.78 (1H, s, 4-CH); LR-MS *m*/*z* 368 (M − AcOH − H$_2$O, 17%), 353 (M − AcOH − H$_2$O − CH$_3$, 15), 335 (M − AcOH − 2H$_2$O − CH$_3$, 10), 281 (27), 269 (M − AcOH − H$_2$O − S.C., 32), 255 (32), 227 (M − AcOH − H$_2$O − S.C. − ring D, 17); HR-MS (ESI-PIM), Calc. for $C_{26}H_{38}O_6Na$ [M + Na]$^+$: 469.2566. Found: *m*/*z*, 469.2563.

(20*S*)-7β-Acetoxy-3-oxo-4-cholen-*O*-24,20-lactone 19. Isolated from the reaction product of **4** as a colorless amorphous solid (Fr. 3); crystallized from EtOAc–hexane; mp 222–226 °C; IR ν_{max}/cm^{-1} 1701, 1742 (C=O), 1680, 2965 (C=C); ^{1}H-NMR δ 0.88 (3H, s, 18-H$_3$), 1.05 (3H, s, 19-H$_3$), 1.47 (3H, s, 21-H$_3$), 2.01 (3H, s, COCH$_3$), 4.64 (1H, br m, 7α-H); LR-MS *m*/*z* 368 (M − AcOH, 16%), 353 (M − AcOH − CH$_3$, 10), 335 (M − AcOH − H$_2$O − CH$_3$, 5), 281 (17), 269 (M − AcOH − S.C., 17), 255 (15), 227 (M − AcOH − S.C. − ring D, 17); HR-MS (ESI-PIM), Calc. for $C_{26}H_{36}O_5Na$ [M+Na]$^+$: 451.2460. Found: *m*/*z*, 451.2438.

Oxidation products of methyl 7α,12α-diacetoxy-3-oxo-5β-cholan-24-oate 5

Methyl 7α,12α-diacetoxy-5β-hydroxy-3-oxocholan-24-oate 20. Isolated from the reaction product of **5** as a colorless amorphous solid (Fr. 2); crystallized from EtOAc–hexane; mp 175–177 °C; IR ν_{max}/cm^{-1} 1772, 1680 (C=O), 3399 (OH); ^{1}H-NMR δ 0.77 (3H, s, 18-H$_3$), 0.82 (3H, d, *J* 5.4 Hz, 21-H$_3$), 1.00 (3H, s, 19-H$_3$), 2.09, 2.11 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 5.02 (1H, m, 7β-H), 5.16 (1H, m, 12β-H); LR-MS *m*/*z* 382 (M − 2AcOH − H$_2$O, 7%), 330 (53), 267 (M − 2AcOH − H$_2$O − S.C., 10), 249 (M − 2AcOH − H$_2$O − S.C., 5), 240 (M − 2AcOH – H$_2$O − S.C. − part of ring D, 8), 225 (M − 2AcOH − H$_2$O − S.C. − ring D, 10), 207 (100); HR-MS (ESI-PIM), Calc. for $C_{29}H_{44}O_8Na$ [M + Na]$^+$: 543.2934. Found: *m*/*z*, 543.2963.

Methyl 7α,12α-diacetoxy-3-oxo-4-cholen-24-oate 21. Isolated from the reaction product of **5** as a colorless amorphous solid (Fr. 1); crystallized from aq. methanol; mp 186–189 °C [lit.[13c] mp, 183–186 °C]; IR ν_{max}/cm^{-1} 1744 (C=O), 1690, 2977 (C=C); ^{1}H-NMR δ 0.79 (3H, s, 18-H$_3$), 0.81 (3H, d, *J* 6.2 Hz, 21-H$_3$), 1.19 (3H, s, 19-H$_3$), 2.06, 2.09 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 5.02 (1H, m, 7β-H), 5.11 (1H, m, 12β-H), 5.70 (1H, s, 4-CH); LR-MS *m*/*z* 382 (M − 2AcOH, 7%), 267 (M − 2AcOH − S.C., 100), 249 (M − 2AcOH − H$_2$O − S.C., 11), 240 (M − 2AcOH − S.C. − part of ring D, 8), 225 (M − 2AcOH − S.C. − ring D, 18), 207 (35).

(20*S*)-7α,12α-Diacetoxy-5β-hydroxy-3-oxocholan-*O*-24,20-lactone 22. Isolated from the reaction product of **5** as a noncrystalline substrate (Fr. 4); IR ν_{max}/cm^{-1} 1688, 1719 (C=O), 3402 (OH); ^{1}H-NMR δ 0.88 (3H, s, 18-H$_3$), 1.01 (3H, s, 19-H$_3$), 1.38 (3H, s, 21-H$_3$), 2.08, 2.14 (each 3H, s, COCH$_3$), 5.05 (1H, m, 7β-H), 5.27 (1H, m, 12β-H); LR-MS *m*/*z* 426 (M − AcOH − H$_2$O, 3), 366 (M − 2AcOH − H$_2$O, 44), 267 (M − 2AcOH − H$_2$O − S.C., 100), 243 (19), 240 (M − 2AcOH − H$_2$O − S.C. − part of ring D, 79), 225 (M − 2AcOH − H$_2$O − S.C. − ring D, 66); HR-MS (ESI-PIM), Calc. for $C_{28}H_{40}O_8Na$ [M + Na]$^+$: 527.2621. Found: *m*/*z*, 527.2652.

(20*S*)-7α,12α-Diacetoxy-3-oxo-4-cholen-*O*-24,20-lactone 23. Isolated from the reaction product of **5** as colorless prisms (Fr. 3); crystallized from aq. methanol; mp 251–254 °C; IR ν_{max}/cm^{-1} 1711 (C=O), 1630, 3000 (C=C); ^{1}H-NMR δ 0.94 (3H, s, 18-H$_3$), 1.04 (3H, s, 19-H$_3$), 1.38 (3H, s, 21-H$_3$), 2.07, 2.14 (each 3H, s, COCH$_3$), 5.00 (1H, m, 7β-H), 5.22 (1H, m, 12β-H), 5.71 (1H, s, 4-CH); LR-MS *m*/*z* 366 (M − 2AcOH, 22%), 335 (13), 298 (72), 267 (M − 2AcOH − S.C., 100), 240 (M − 2AcOH − S.C. − part of ring D, 81), 225 (M − 2AcOH − S.C. − ring D, 30), 221 (14); HR-MS (EI-PIM), Calc. for $C_{28}H_{38}O_7$ [M]$^+$: 486.2618. Found: *m*/*z*, 486.2591.

(20*S*)-20-Hydroxy-3-oxo-5β-cholan-24-oic acid 24 and its C-24 methyl ester 26

A 5% methanolic KOH solution of the lactone **8** (30 mg) was refluxed for 2 h. Most of the solvent was evaporated under reduced pressure. The residual oil dissolved in water was carefully neutralized with 5% H_2SO_4 while cooling in an ice bath. The precipitate was filtered, washed with water, and recrystallized from EtOAc–hexane to give the title compound **24** as a colorless amorphous solid; mp 158–162 °C; yield, 25 mg, 70%; IR ν_{max}/cm^{-1} 1735, 1771 (C=O), 3410 (OH); ^{1}H-NMR (as the C-24 methyl ester **26** prepared by treating **24** with diazomethane) δ 0.82 (3H, s, 18-CH$_3$), 1.25 (3H, s, 19-CH$_3$), 1.25 (3H, s, 21-CH$_3$), 3.67 (3H, s, COOCH$_3$); LR-MS *m*/*z* (as the methyl ester **26**) 384 (M − H$_2$O, 35%), 371 (M − H$_2$O − CH$_3$, 35), 368 (M − 2H$_2$O, 10), 281 (70), 273 (M − H$_2$O − S.C., 3), 255 (M − 2H$_2$O − S.C., 7), 221, (100); HR-MS (EI-PIM) (as the methyl ester **26**), Calc. for $C_{25}H_{40}O_4$ [M]$^+$: 404.2927. Found: *m*/*z*, 404.2941.

(20*S*)-7β,20-Dihydroxy-3-oxo-5β-cholan-24-oic acid 25 and its C-24 methyl ester 27

Alkaline hydrolysis of the lactone **17** (30 mg) with 5% methanolic KOH followed by acidification with 5% H_2SO_4, as described for the preparation of **24**, gave the free acid **25**, which was recrystallized from EtOAc–hexane as a colorless amorphous solid; mp 174–176 °C; yield, 18 mg, 66%; IR ν_{max}/cm^{-1}

1735, 1771 (C=O), 3433, 3445 (OH); ^{1}H-NMR (as the C-24 methyl ester **27** prepared by treating **25** with diazomethane) δ 0.85 (3H, s, 18-CH_3), 1.25 (3H, s, 19-CH_3), 1.25 (3H, s, 21-CH_3), 3.47 (1H, dt, J_1 12.2 Hz, J_2 5.4 Hz, 7α-H), 3.67 (3H, s, $COOCH_3$); LR-MS *m*/*z* (as the methyl ester **27**) 420 (M^+, 4%), 402 (M − H_2O, 10), 384 (M − $2H_2O$, 10), 287 (M − H_2O − S.C., 18), 272 (M − $2H_2O$ − S.C., 100), 211 (47); HR-MS (EI-PIM) (as the methyl ester **27**), Calc. for $C_{25}H_{40}O_5$ $[M]^+$: 420.2876. Found: *m*/*z*, 420.2861.

Acknowledgements

We would like to express our thanks to Mr. Daniel C. Aspleaf for his helpful comments. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1 B. M. Trost, (ed.), in *Comprehensive Organic Synthesis: Selectivity, Strategy & Efficiency in Modern Organic Chemistry*, Pergamon Press, Oxford, 1999, vol. 7 *(Oxidation)—Oxidation of Unactivated C–H Bonds*, pp. 1–82.
2 E. Jonson and M. Waterman, (ed.), in *Cytochrome P450 (Part B)*, Academic Press, San Diego, 1996.
3 P. B. Reese, *Steroids*, 2001, **66**, 481.
4 (*a*) B. Meunier, *Chem. Rev.*, 1992, **92**, 1411; (*b*) R. Breslow, X. Zhang and Y. Huang, *J. Am. Chem. Soc.*, 1997, **119**, 4535; (*c*) J. Yang, R. Weinberg and R. Breslow, *Chem. Commun.*, 2000, 531; (*d*) R. Breslow, B. Gabriele and J. Yang, *Tetrahedron Lett.*, 1998, **39**, 2887; (*e*) M. D. Kaufmann, P. A. Grieco and D. W. Bougie, *J. Am. Chem. Soc.*, 1993, **115**, 11648; (*f*) A. M. S. Chauhan, A. S. Kandadai, N. Jain and A. Kumar, *Chem. Pharm, Bull.*, 2003, **51**, 1345.
5 (*a*) H. Ohtake, T. Higuchi and M. Hirobe, *Heterocycles*, 1995, **40**, 867; (*b*) T. Shingaki, K. Miura, T. Higuchi, M. Hirobe and T. Nagano, *Chem. Commun.*, 1997, 861; (*c*) H. Ohtake, T. Higuchi and M. Hirobe, *J. Am. Chem. Soc.*, 1992, **114**, 10660.
6 (*a*) C. Cerrè, A. F. Hofmann, C. D. Schteingart, W. Jia and D. Maltby, *Tetrahedron*, 1997, **53**, 435; (*b*) T. Iida, T. Yamaguchi, R. Nakamori, M. Hikosaka, N. Mano, J. Goto and T. Nambara, *J. Chem. Soc., Perkin Trans. 1*, 2001, 2229.
7 T. Iida, S. Ogawa, K. Shiraishi, G. Kakiyama, T. Goto, N. Mano and J. Goto, *ARKIVOC*, 2003(viii), 170.
8 T. Iida, M. Hikosaka, G. Kakiyama, K. Shiraishi, C. D. Schteingart, L. R. Hagey, H-T. Ton-Nu, A. F. Hofmann, N. Mano, J. Goto and T. Nambara, *Chem. Pharm. Bull.*, 2002, **50**, 1327.
9 R. J. Rousseau and R. K. Robins, *J. Heterocycl. Chem.*, 1965, **2**, 196.
10 J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *J. Org. Chem.*, 1987, **52**, 827.
11 (*a*) D. P. Rillema, J. K. Nagle, L. F. Barringer, Jr. and T. J. Meyer, *J. Am. Chem. Soc.*, 1981, **103**, 56; (*b*) J. T. Groves and R. Quinn, *Inorg. Chem.*, 1984, **23**, 3844.
12 G. Aranda, M. Fetizon and N. Tayeb, *Tetrahedron*, 1987, **43**, 4147.
13 Authentic compounds **11**, **13** and **21** were prepared by acylation of the corresponding 3-oxobile acid methyl esters (see references cited below) with acetic anhydride in pyridine using standard procedures. (*a*) T. Iida, T. Tamura, T. Matsumoto and F. C. Chang, *J. Lipid Res.*, 1985, **26**, 874; (*b*) T. Iida, T. Momose, T. Nambara and F. C. Chang, *Chem. Pharm. Bull.*, 1986, **34**, 1929; (*c*) T. Iida, T. Momose, F. C. Chang and T. Nambara, *Chem. Pharm. Bull.*, 1986, **34**, 1934.