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The application of dimethyldioxirane for the selective oxidation of polyfunctional steroids

Tomoaki Sasaki [a], Ryusei Nakamori [a], Takeru Yamaguchi [a], Yuka Kasuga [a], Takashi Iida [a,*], Toshio Nambara [b]

[a] *Department of Chemistry, College of Humanities and Sciences, Nihon University, Setagaya, Sakurajousui, Tokyo 156-8550, Japan*
[b] *Hoshi University, Shinagawa, Ebara, Tokyo 147-8501, Japan*

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Abstract

Oxidation and epoxidation reactions of a series of structurally different steroids related to methyl 5β-cholanoates having hydroxyl groups and/or double bonds by treatment with dimethyldioxirane (DMDO) are described. Steroidal alcohols, olefines, and unsaturated alcohols and conjugated enones with DMDO were transformed into ketones, epoxides, and epoxy-ketones, respectively, in good isolated yields. The regio- and stereoselectivities for DMDO reaction differing from those observed for organic peracids, *tert*-butyl hydroperoxide and alkaline hydrogen peroxide are also discussed.

Keywords: Dimethyldioxirane; Oxidation; Epoxidation; Steroidal alcohol; Unsaturated steroid; Steroidal conjugated enone; Regioselectivity; Stereoselectivity

1. Introduction

Recently, two groups of investigators (Curci et al., 1995; Murray, 1989) have reviewed the characteristics and usefulness of a new class of powerful oxidants and/or oxygen-transfer reagents, dimethyldioxirane (DMDO) and its some analogs, in organic syntheses. Despite a variety of versatile reactivities and chemical transformations such reactions as primary alcohols to carboxylic acids, secondary alcohols to ketones, alkenes to epoxides, amines to nitro compounds, sulfides to sulfones or sulfoxides, and tertiary carbon atoms to tertiary alcohols have been known (Murray and Jeyaraman, 1985; Adam et al., 1991; Singh and Murray, 1992), a further detailed and potential abilities of DMDO in organic syntheses remain still unclear. The major reason may be ascribed to its treatment and commercial unavailability, since the generation and stability of DMDO are somewhat troublesome which limits the wide application of the reagent for routine synthetic work. However, DMDO as an oxidant on various substrates appears to be also attractive from the

* Corresponding author. Tel.: +81-3-33291151; fax: +81-3-33039899.
E-mail address: takaiida@chem.chs.nihon-u.ac.jp (T. Iida).

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viewpoint of so-called 'green chemistry' (Fernandez-Escobar et al., 1998), because it consists of only carbon, hydrogen and oxygen atoms, and without any toxic and hazardous heavy metals such as chromium (VI) or lead, and decomposed to methyl acetate in a relatively short period of time at room temperature (Singh and Murray, 1992).

As part of our ongoing programme of synthesis of biologically and physiologically important steroids and related compounds, we were interested in efficient utilization and development of the unique oxidant for a convenient preparation of synthetically useful key intermediates. To our knowledge, reaction of polyfunctional steroids with DMDO has not yet been reported systematically. We describe here the reactivity and chemical transformations of DMDO towards a wide variety of structurally different steroids related to methyl 5β-cholanoates having hydroxyl and/or oxo functions as well as double bonds in the steroid nucleus.

Table 1
Reactions of polyfunctional methyl 5β-cholonates with DMDO

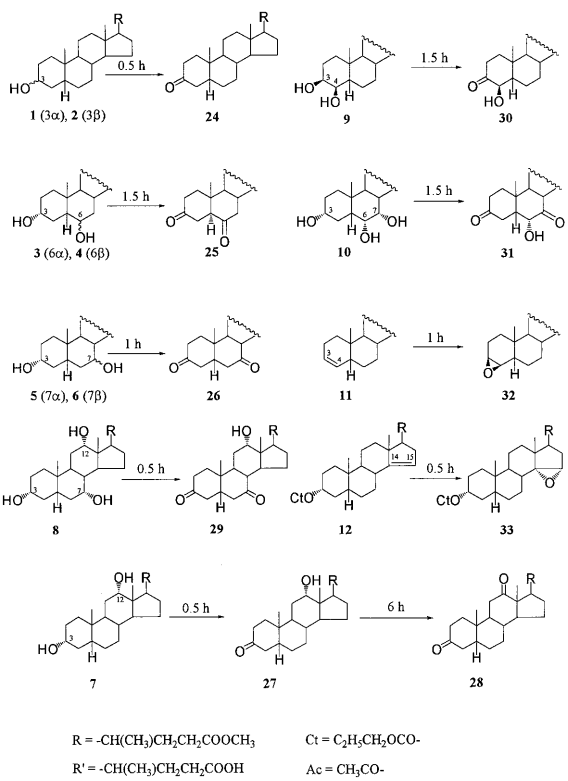
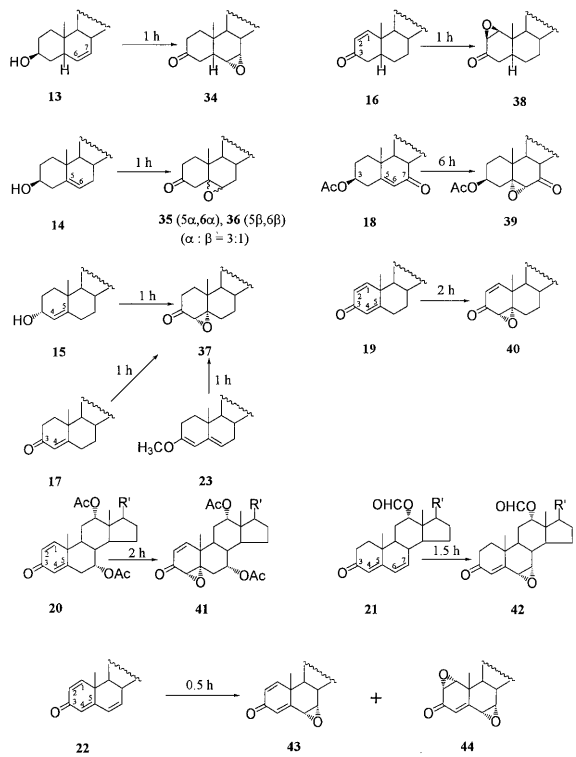


Table 1 (*Continued*)



2. Results and discussion

The experimental procedure is very simple. Thus a mixture of steroid (1.0 mmol) and a concentrated DMDO (2.0 mmol) solution in $CHCl_3$ (see Experimental section) was allowed to stand at room temperature for a certain period of time (0.5–6 h), until completion of the reaction (monitored by TLC). The subsequent work-up procedure was also especially easy: after the reaction, an excess amount of DMDO and the solvent were evaporated and the residue was purified by a direct crystallization or by passing through a column of silica gel.

Our preliminary experiment revealed that vari-

ous alkali- and acid-sensitive functional and protecting groups are essentially inert under the mild and neutral experimental conditions used in this study. So far examined, formyl, acetyl, methanesulfonyl, *p*-toluenesulfonyl, and carbethoxy groups, tetrahydropyranyl and *t*-butyldimethylsilyl ethers, dimethylacetal, and methyl ester proved to be completely stable on exposure to a DMDO solution.

The results are shown in Table 1, together with the structures of compounds examined and their major isolated reaction products. Irrespective of the position and stereochemical configuration of secondary hydroxyl groups in the 5β-steroid nucleus, DMDO oxidized rapidly (0.5–1 h) isolated hydroxyls at the 3α-, 3β-, 6α-, 6β-, 7α-, and 7β-positions in saturated methyl 5β-cholanoates (**1–6**) to afford the corresponding ketones (**24–26**) in excellent isolated yields (80–90%). On the other hand, when 12α-hydroxylated compounds (**7** and **8**) were subjected to the DMDO reaction for 0.5 h, the sole isolated products were the corresponding 12α-hydroxy-ketones (**27** and **29**); the prolonged exposure (6 h) of **7** to a DMDO solution resulted in the formation of the completely oxidized ketones (**28**). Since the regiospecificity of DMDO on steroid molecules differs from those observed for other oxidizing agents such as chromic acid, chromium VI oxide–pyridine complexes (e.g. pyridinium chlorochromate), manganese IV oxide or silver carbonate (Carruthers, 1986; Tserng, 1978), DMDO is, therefore, usefully utilized as an alternative site-selective oxidizing agent in steroid syntheses.

Of further interest was that under a strictly neutral experimental condition, treatment of methyl 3,6-dihydroxy-5β-cholanoates (**3** and **4**) with DMDO brought about the formation of allomerized 3,6-dioxo 5α-compound (**25**), as evidenced by ^{13}C-NMR (see Experimental section). The unexpected observation can be interpreted as a result of enolization from C-5 of intermediary 6-oxo-5β-steroids (A/B-*cis*) to the more stable 5α-isomers (A/B-*trans*) (Haslewood, 1967). In addition, when methyl 5β-cholanoates having a vicinal *cis* glycol structure (**9** and **10**) were subjected to the DMDO reaction, only an axially-oriented hydroxyl group was oxidized preferentially to give monooxidized α-hydroxy-ketones (**30** and **31**) in good isolated yields (69–73%) without oxidative glycol cleavage of the C_1–C_2 bond.

In contrast to the conventionally employed epoxidizing reagents of simple alkenes with organic peracids (e.g. *m*-chloroperbenzoic acid) (Matthews and Hassner, 1972), allylic alcohols with *tert*-butyl hydroperoxide (TBHP) (Sharpless and Michaelson, 1973) or conjugated enones with alkaline hydrogen peroxide (e.g. H_2O_2–NaOH) (Matthews and Hassner, 1972), DMDO epoxidized all of the three variants of the double bonds regio- and stereoselectively without any difficulty. The observations show that uncojugated and conjugated carbon–carbon double bonds as well as homoallylic and allylic alcohols succeed to react with DMDO. Thus, DMDO reaction of unsaturated steroidal alcohols caused simultaneous oxidation–epoxidation of both the hydroxyl group and double bond in the same molecules, resulting in the formation of the corresponding epoxy-ketones in one-step. Assignment of the stereochemistry to the epoxides was determined on the basis of the ^{1}H- and/or ^{13}C-NMR signals in comparison with those of analogous steroids reported previously (Cross, 1962; Tori et al., 1964; Mihailovic et al., 1969; Glotter et al., 1970; Holland et al., 1982).

Oxidation of 5β-steroidal 3-ene (**11**) and 14-ene (**12**) with DMDO afforded exclusively the corresponding 3β,4β- and 14α,15α-epoxides (**32** and **33**), respectively, indicating that the reagent attacks the less hindered β-face of the ring A and the α-face of the rings B, C and D in the *cis*-fused 5β-steroid molecules, in analogy with those observed for organic peracid epoxidation (Matthews and Hassner, 1972). Treatment of 6-en-3β-ol (**13**) with DMDO lead to the formation of the 6α,7α-epoxy-3-ketone (**34**) in one-step and in a good yield (72%), while that of homoallylic 5-en-3β-ol (**14**) produced a mixture of the 5α,6α- and 5β,6β-epoxy-3-ketones (**35** and **36**) in the ratio of ca. 3:1 (evidenced by ^{1}H-NMR). Furthermore, allylic 4-en-3α-ol (**15**) was transformed directly into the 4α,5α-epoxy-3-ketone (**37**) in 58% isolated yield. According to the previous findings with organic peracids or TBHP (Henbest and Wilson, 1957; Sharpless and Michaelson, 1973), epoxidation of

cyclic allylic alcohols occurs principally *cis* to the hydroxyl and yields the corresponding *syn*-epoxy-alcohols via a hydrogen-bonded transition state. This generalization was consistent with the present result with DMDO, though the hydroxyl function was oxidized simultaneously. A similar result was also obtained in the epoxidation of 4β-hydroxycholesterol by the dioxirane intermediate generated in the reaction of potassium caroate with acetone to give a ca. 3:1 mixture of the 5β,6β-and 5α,6α-epoxides (Cicala et al., 1982).

Traditionally, alkaline hydrogen peroxide has long been used as a sole epoxidizing agent of α,β-unsaturated ketones to the corresponding epoxy-ketones, which involves nucleophilic attack of hydroperoxide ion on the conjugated double bond, as opposed to the electrophilic addition mechanism of organic peracids on an isolated carbon–carbon double bond (Matthews and Hassner, 1972); recently, TBHP in the presence of lithium hydroxide has also been employed for the purpose (Holland et al., 1982). The alkaline peroxide epoxidations are, however, unsuitable for conjugated enones possessing alkali-sensitive functional and protecting groups such as esters or ketols in the same molecules. The use of DMDO eliminates such a drawback, because those groups are insensitive to a neutral DMDO epoxidizing condition. In fact, epoxidation of conjugated 4-en-3-one (**17**) and 3β-acetoxy-5-en-7-one esters (**18**) with DMDO produced the corresponding α-epoxides (**37** and **39**) stereoselectively without simultaneous hydrolysis of C-24 ester and C-3 acetoxy group, whereas that of 1-en-3-one ester (**16**) in the normal *cis* 5β-series afforded the β-epoxide (**38**) predominantly. It is also worth noting here to compare the stereoselectivity of the DMDO with that of alkaline peroxides: thus, **17** and its analogous steroidal 4-en-3-ones are usually oxidized to the corresponding 3-oxo-4β,5β-epoxides predominantly with alkaline peroxides (Holland, et al., 1982; Henbest and Jackson, 1967); the reverse was true with DMDO.

When serial conjugated double bonds are present in steroidal ketones, a specific double bond was oxidized first by DMDO to afford unsaturated monoepoxy-ketones site-selectively. In the case of 1,4-diene-3-ketones (**19** and **20**), epoxidation with DMDO for 2 h gave only the corresponding unsaturated 4α,5α-epoxy-3-ketones (**40** and **41**) in good yields (71–76%); on the other hand, prolonged treatment of analogous cholest-1,4-dien-3-one with perbenzoic acid in refluxing benzene yielded the 4β,5β-epoxycholest-1-en-3-one (Burgess, 1962) in a low yield; in addition, 21-acetoxy-17α-hydroxy-1,4-pregnadiene-3,11,12-trione with DMDO has been converted to the corresponding 1α,2α-epoxide, probably owing to the steric effect of 19-methyl and a dipole interaction with the C-11 carbonyl (Bovicelli, et al., 1992).

It has been also previously found that oxidation of methyl 3-oxochola-1,4,6-trien-24-oate (**22**) with alkaline hydrogen peroxide yields methyl 1α,2α-epoxy-3-oxochola-4,6-dien-24-oate as the major product, in which the double bond nearest to the C-3 carbonyl function is epoxidized (Herz and Ocampo, 1982). With DMDO, however, the 6,7-double bond of analogous 12α-formyloxy-3-oxochola-4,6-dien-24-oic acid (**21**) and compound **22**, which is more remote from the C-3 carbonyl function than the other double bonds, was epoxidized first, to give the respective unsaturated 6α,7α-epoxy-3-ketones (**42** and **43**) preferentially. The observed difference between DMDO and alkaline hydrogen peroxide epoxidations can be rationally interpreted in terms of the electrophilicity of DMDO, in analogy with organic peracids (Matthews and Hassner, 1972), since the 6,7-double bond should be more electron rich than the 1,2- or 4,6-double bond and therefore susceptible to electrophilic attack of DMDO (Cicala et al., 1982). An another interesting result observed was that when methyl 3-methoxychola-3,5-dien-24-oate (**23**) was subjected to the DMDO reaction, the 4α,5α-epoxy-3-ketone (**37**) is isolated in a reasonable yield (44%).

Since the regio- and stereoselectivities of DMDO often differ from those of organic peracids, TBHP, and/or alkaline hydrogen peroxide, DMDO is of particular importance for preparing useful key intermediates and may entry a new class of versatile oxidizing and/or

oxygen-transfer reagents in the synthesis of bioactive steroids.

3. Materials and methods

Melting points (m.p.) 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) as KBr tablets. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a JEOL JNM-EX270 FT NMR instrument (Tokyo, Japan) with $CDCl_3$ containing 0.1% Me_4Si as the solvent; chemical shifts are expressed in δ ppm relative to Me_4Si. Mass spectra (MS) were recorded on a JEOL JMC-Automass 150 gas chromatograph-mass spectrometer at 70 eV. Thin-layer chromatography (TLC) was performed on a pre-coated silica gel 60 plate (Merck, Darmstadt, Germany) using hexane-ethyl acetate (EtOAc) or hexane-EtOAc-acetic acid mixtures as the developing solvents.

Monopersulfate compound ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$; oxone®) was available from Aldrich, Milwaukee, WI. The following 23 bile acid methyl esters or free acids were used as substrates: methyl 3α-hydroxy-5β-cholan-24-oate (**1**); methyl 3β-hydroxy-5β-cholan-24-oate (**2**); methyl 3α,6α-dihydroxy-5β-cholan-24-oate (**3**); methyl 3α,6β-dihydroxy-5β-cholan-24-oate (**4**); methyl 3α,7α-dihydroxy-5β-cholan-24-oate (**5**); methyl 3α,7β-dihydroxy-5β-cholan-24-oate (**6**); methyl 3α,12α-dihydroxy-5β-cholan-24-oate (**7**); methyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (**8**); methyl 3β,4β,-dihydroxy-5β-cholan-24-oate (**9**); methyl 3α,6α,7α-trihydroxy-5β-cholan-24-oate (**10**); methyl 5β-chol-3-en-24-oate (**11**); methyl 3α-cathyloxy-5β-chol-14-en-24-oate (**12**); methyl 3β-hydroxy-5β-chol-6-en-24-oate (**13**); methyl 3β-hydroxychol-5-en-24-oate (**14**); methyl 3α-hydroxychol-4-en-24-oate (**15**); methyl 3-oxo-5β-chol-1-en-24-oate (**16**); methyl 3-oxochol-4-en-24-oate (**17**); methyl 3β-acetoxy-7-oxochol-5-en-24-oate (**18**); methyl 3-oxochola-1,4-dien-24-oate (**19**); methyl 7α,12α-diacetoxy-3-oxochola-1,4-dien-24-oate (**20**); 12α-formyloxy-3-oxochola-4,6-dien-24-oic acid (**21**); methyl 3-oxochola-1,4,6-trien-24-oate (**22**); methyl 3-methoxychola-3,5-dien-24-oate (**23**). Almost all of the steroid samples were from our laboratory collection.

3.1. Preparation of DMDO in $CHCl_3$ solution

DMDO was generated by the reaction of oxone® and acetone in the presence of sodium hydrogen carbonate and recovered as an acetone solution, according to the literature method described in detail (Murray, 1989; Adam et al., 1991; Singh and Murray, 1992). The DMDO solution in acetone was then concentrated by extracting with an approximate amount of $CHCl_3$ (Cerre et al., 1997). The concentration of the resulting DMDO solution in $CHCl_3$ thus obtained was determined by iodometric titration (Adam et al., 1987).

3.2. General procedure for the oxidation by DMDO

To a solution of steroid (1 mmol) in CH_2Cl_2 (10 ml) was added a freshly prepared solution of DMDO (0.20 M, 10 ml; 2 mmol) in $CHCl_3$, and the mixture was allowed to stand at room temperature for a certain period of times, until completion of the reaction (monitored by TLC). After the reaction, an excess amount of DMDO and solvents were evaporated under a reduced pressure and the residue was crystallized directly from an appropriate solvent. Otherwise, the reaction product was purified by passing through a column of silica gel eluting with toluene-EtOAc mixtures.

3.3. Methyl 3-oxo-5β-cholan-24-oate (24)

Obtained from **1** and **2** in 90 and 83% yields, respectively; m.p., 113–116°C (aq. methanol), (Hill et al., 1991), m.p., 119–120°C). IR ν_{max} cm^{-1}: 1715, 1735 (C = O). ^{1}H-NMR: 0.68 (3H, s, 18-CH_3), 0.92 (3H, d, J = 6.2 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 3.67 (3H, s, $COOCH_3$). MS *m*/z (relative intensity); 388 (M^+, 97%), 356 (M^+-32, 85%), 273 (M^+-side chain (S.C.; 115), 100%), 231 (M^+-115–ring D (42), 30%).

3.4. Methyl 3,6-dioxo-5α-cholan-24-oate (25)

Obtained from **3** and **4** in 87 and 84% yields, respectively; m.p., 147–149°C (EtOAc-hexane) (Hill et al., 1991, m.p., 149–151°C). IR ν_{max} cm^{-1}: 1705, 1743 (C = O). ^{1}H-NMR δ: 0.69 (3H, s, 18-H), 0.93 (3H, d, J = 6.5 Hz, 21-H), 0.96 (3H, s, 19-H), 3.67 (3H, s, $COOCH_3$). ^{13}C-NMR δ ; 12.0 (C-18), 12.5 (C-19), 18.2 (C-21), 21.6 (C-11), 23.9 (C-15), 27.9 (C-16), 30.8 (C-23), 31.0 (C-22), 35.2 (C-20), 36.9 and 37.3 (C-2,7), 37.9 (C-8), 38.0 (C-1), 39.3 (C-12), 41.2 (C-10), 43.0 (C-13), 46.5 (C-4), 51.5 (C-25), 53.4 (C-9), 55.7 (C-17), 56.5 (C-14), 57.5 (C-5), 174.6 (C-24), 209.0 (C-6), 211.2 (C-3). MS *m*/z (relative intensity); 402 (M^+, 86%), 384 (M^+-H_2O (18), 85%), 329 (100%), 287 (M^+-115, 35%), 245 (M^+-115-42, 42%).

3.5. Methyl 3,7-dioxo-5β-cholan-24-oate (26)

Obtained from **5** and **6** in 80 and 84% yields, respectively; m.p., 150–152°C (aq. methanol), (Hill, et al., 1991), m.p., 152–155°C). IR ν_{max} cm^{-1}: 1701, 1738 (C = O). ^{1}H-NMR δ: 0.70 (3H, s, 18-CH_3), 0.93 (3H, d, J = 6.5 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 3.67 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 402 (M^+, 16%), 384 (M^+-18, 8%), 370 (M^+-32, 9%), 287 (M^+-115, 100%), 269 (M^+-115-18, 60%), 245 (M^+-115-42, 18%).

3.6. Methyl 12α-hydroxy-3-oxo-5β-cholan-24-oate (27)

Obtained from **7** in 87% yield; m.p., 145–147°C (EtOAc-hexane) (Tohma et al., 1986), m.p. , 148–149°C). IR ν_{max} cm^{-1}: 1720 (C = O), 3521 (OH). ^{1}H-NMR: 0.72 (3H, s, 18-CH_3), 0.98 (3H, d, J = 5.9 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 3.67 (3H, s, $COOCH_3$), 4.04 (1H, m, 12β-H). MS *m*/z (relative intensity): 404 (M^+, 3%), 386 (M^+-18, 19%), 271 (M^+-115-18, 100%).

3.7. Methyl 3,12-dioxo-5β-cholan-24-oate (28)

Obtained from **7** in 84% yield; m.p., 130–132°C (aq. ethanol) (Hill, et al., 1991, m.p., 133–134°C). IR ν_{max} cm^{-1}: 1708, 1735 (C = O). ^{1}H-NMR: 0.86 (3H, d, J = 6.5 Hz, 21-CH_3), 1.06 (3H, s, 18-CH_3), 1.11 (3H, s, 19-CH_3), 3.66 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 402 (M^+, 100%), 384 (M^+-18, 23%), 287 (M^+-115, 13%), 269 (M^+-115-18, 13%).

3.8. Methyl 12α-hydroxy-3,7-dioxo-5β-cholan-24-oate (29)

Obtained from **8** in 72% yield; m.p., 190–192°C (aq. methanol) (Hill, et al., 1991, m.p., 194.5°C). IR ν_{max} cm^{-1}: 1703, 1735 (C = O), 3502 (OH). ^{1}H-NMR: 0.72 (3H, s, 18-CH_3), 0.84 (3H, d, J = 6.2 Hz, 21-CH_3), 1.28 (3H, s, 19-CH_3), 3.66 (3H, s, $COOCH_3$), 4.05 (1H, m, 12β-H). MS *m*/z (relative intensity): 418 (M^+, 2%), 400 (M^+-18, 13%), 286 (100%), 285 (M^+-115-18, 89%).

3.9. Methyl 4β-hydroxy-3-oxo-5β-cholan-24-oate (30)

Obtained from **9** in 69% yield; m.p., 113–116°C (aq. methanol). IR ν_{max} cm^{-1}: 1721, 1741 (C = O), 3503 (OH). ^{1}H-NMR δ: 0.69 (3H, s, 18-CH_3), 0.92 (3H, d, J = 6.2 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 3.67 (3H, s, $COOCH_3$), 4.38 (1H, d, J = 11.3 Hz, 4α-H). MS *m*/z (relative intensity): 404 (M^+, 41%), 389 (M^+-CH_3 (15), 93%), 386 (M^+-18, 100%), 262 (M^+-115-part of D (27), 50%), 247 (M^+-115-27-15, 50%).

3.10. Methyl 6α-hydroxy-3,7-dioxo-5β-cholan-24-oate (31)

Obtained from **10** in 83% yield; viscous oil. IR ν_{max} cm^{-1}: 1711, 1741 (C = O). ^{1}H-NMR δ: 0.71 (3H, s, 18-CH_3), 0.94 (3H, d, J = 6.2 Hz, 21-CH_3), 1.35 (3H, s, 19-CH_3), 3.67 (3H, s, $COOCH_3$) 4.55 (1H, m, 6β-H). MS *m*/z (relative intensity): 418 (M^+, 25%), 400 (M^+-18, 12%), 386 (M^+-32, 24%), 345 (M^+-73, 10%), 303 (M^+-115, 14%), 285 (M^+-115-18, 18%), 134 (100%).

3.11. Methyl 3β,4β-epoxy-5β-cholan-24-oate (32)

Obtained from **11** in 64% yield; m.p., 105–107°C (methanol). IR ν_{max} cm^{-1}: 1732 (C = O). ^{1}H-NMR: 0.66 (3H, s, 18-CH_3), 0.87 (3H, s,

19-CH_3), 0.91 (3H, d, J = 6.2 Hz, 21-CH_3), 2.86 (1H, d, J = 2.7 Hz, 4α-H), 3.22 (1H, m, 3α-H), 3.66 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 388 (M^+, 86%), 355 (M^+-18-15, 100%), 273 (M^+-115, 16%), 231 (M^+-115-42, 72%).

3.12. Methyl 3α-cathyloxy-14α,15α-epoxy-5β-cholan-24-oate (33)

Obtained from **12** in 72% yield; m.p., 143–146°C (methanol). IR ν_{max} cm^{-1}: 1737 (C = O). ^{1}H-NMR: 0.84 (3H, s, 18-CH_3), 0.85 (3H, d, J = 6.2 Hz, 21-CH_3), 0.95 (3H, s, 19-CH_3), 1.29(3H, t, J = 7.0 Hz, $\underline{CH_3}CH_2OCO$), 3.32 (H, s, 15β-H), 3.66 (3H, s, $\overline{COOCH_3}$), 4.16 (2H, q, J = 7.0 Hz, $CH_3\underline{CH_2}OCO$), 4.55 (1H, brm, 3β-H). MS *m*/z (relative intensity): 460 (M^+, 1%), 386 (M^+-C_2H_5COOH (74), 33%), 368 (M^+-74-18, 100%).

3.13. Methyl 6α,7α-epoxy-3-oxo-5β-cholan-24-oate (34)

Obtained from **13** in 55% yield; m.p., 133–135°C (EtOAc-hexane). IR ν_{max} cm^{-1}: 1716, 1743 (C = O). ^{1}H-NMR δ: 0.73 (3H, s, 18-CH_3), 0.93 (3H, d, J = 5.4 Hz, 21-CH_3), 0.95 (3H, s, 19-CH_3), 3.13 (2H, m, 6β- and 7β-H), 3.67 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 402 (M^+, 3%), 384 (M^+-18, 5%), 369 (M^+-18-15, 13%), 287 (M^+-115, 21%), 269 (M^+-115-18, 91%).

3.14. Methyl 5ξ,6ξ-epoxy-3-oxo-5β-cholan-24-oate (35 and 36)

Obtained from **14** in 83% yield as a mixture of the **α**- and **β**- epoxides (ratio, 3:1); m.p., 108–110°C (aq. methanol). IR ν_{max} cm^{-1}: 1669, 1743 (C = O). ^{1}H-NMR δ: (5α,6α-epoxide), 0.61 (3H, s, 18-CH_3), 0.74 (3H, s, 19-CH_3), 2.90 (1H, d, J = 4.3 Hz, 6β-H), 3.66 (3H, s, $COOCH_3$); (5β,6β-epoxide), 0.64 (3H, s, 18-CH_3), 0.74 (3H, s, 19-CH_3), 3.09 (H, d, J = 4.3 Hz, 6α-H), 3.66 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 402 (M^+, 35%), 387 (M^+-15, 11%), 269 (M^+-115-18, 6%), 227 (M^+-115-27-18-15, 19%).

3.15. Methyl 4α,5α-epoxy-3-oxocholan-24-oate (37)

Obtained from **15**, **17**, and **23** in 58, 80, and 44% yields, respectively; m.p., 138–140°C (methanol). IR ν_{max} cm^{-1}: 1701, 1740 (C = O). ^{1}H-NMR δ: 0.70 (3H, s, 18-CH_3), 0.92 (3H, d, J = 6.2 Hz, 21-CH_3), 1.06 (3H, s, 19-CH_3), 3.03 (1H, s, 4β-H), 3.67 (3H, s, $COOCH_3$). ^{13}C-NMR δ: 12.0 (C-18), 16.5 (C-19), 18.2 (C-21), 21.4 (C-11), 24.2 (C-15), 28.0 (C-16), 28.9 and 29.1 (C-6,7), 29.7 (C-1), 30.9 (C-23), 31.0 (C-22), 33.1 (C-2), 35.3 and 35.4 (C-8,20), 36.7 (C-10), 39.6 (C-12), 42.5 (C-13), 50.6 (C-9), 51.5 (C-25), 55.6 (C-14), 55.8 (C-17), 62.9 (C-4), 70.2 (C-5), 174.7 (C-24), 207.1 (C-3). MS *m*/z (relative intensity): 402 (M^+, 9%), 384 (M^+-18, 16%), 287 (M^+-115, 40%), 269 (M^+-115-18, 27%), 245 (M^+-115-42, 9%).

3.16. 1β,2β-epoxy-3-oxo-5β-cholan-24-oate (38)

Obtained from **16** in 84% yield; m.p., 120–122°C (methanol) (Tohma, et al., 1986, m.p. , 123–124°C). IR ν_{max} cm^{-1}: 1713, 1737 (C = O). ^{1}H-NMR δ: 0.67 (3H, s, 18-CH_3), 0.91 (3H, d, J = 6.2 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 3.28 (1H, d, J = 4.1 Hz, 1α-H), 3.42 (1H, d, J = 4.1 Hz, 2α-H), 3.66 (3H, s, $COOCH_3$). MS *m*/z (relative intensity): 402 (M^+, 2%), 316 (M^+-86, 14%), 287 (M^+-115, 8%), 260 (M^+-115-27, 6%), 245 (M^+-115-42, 8%), 227 (M^+-115-42-18, 7%), 201 (100%).

3.17. Methyl 3β-acetoxy-5α,6α-epoxy-7-oxocholan-24-oate (39)

Obtained from **18** in 72% yield; m.p., 133–135°C (methanol). IR ν_{max} cm^{-1}: 1694, 1731 (C = O). ^{1}H-NMR δ: 0.66 (3H, s, 18-CH_3), 0.91 (3H, d, J = 6.2 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 2.03 (3H, s, 3-$COCH_3$), 3.04 (1H, s, 6β-H), 3.66 (3H, s, $COOCH_3$), 4.93 (1H, brm, 3α-H). MS *m*/z (relative intensity): 400 (M^+-CH_3COOH (60), 4%), 382 (M^+-60-18, 7%), 372 (25%), 345 (M^+-115, 14%), 327 (M^+-115-18, 15%), 285 (M^+-115-42-18, 19%), 267 (M^+-115-60-18, 22%).

3.18. Methyl 4α,5α-epoxy-3-oxochol-1-en-24-oate (40)

Obtained from **19** in 71% yield; m.p., 103–106°C (methanol). IR ν_{max} cm^{-1}: 1681, 1731 (C = O). ^{1}H-NMR δ: 0.71 (3H, s, 18-CH_3), 0.91 (3H, d, J = 6.2 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 3.21 (1H, s, 4β-H), 3.66 (3H, s, $COOCH_3$), 5.85 (1H, d, J = 10.8 Hz, 2-H), 6.52 (1H, d, J = 10.8 Hz, 1-H). MS *m*/z (relative intensity): 400 (M^+, 7%), 385 (M^+-15, 16%), 327 (M^+-73, 10%), 285 (M^+-115, 64%), 267 (M^+-115-18, 39%), 258 (M^+-115-27, 9%).

3.19. Methyl 7α,12α-diacetoxy-4α,5α-epoxy-3-oxochol-1-en-24-oate (41)

Obtained from **20** in 76% yield; m.p., 172–175°C (EtOAc-hexane). IR ν_{max} cm^{-1}: 1681,1736 (C = O). ^{1}H-NMR δ: 0.79 (3H, s, 18-CH_3), 0.80 (3H, d, J = 5.4 Hz, 21-CH_3), 1.30 (3H, s, 19-CH_3), 2.12 (6H, s, 7- and 12-$COCH_3$), 3.11 (1H, s, 4β-H), 3.66 (3H, s, $COOCH_3$), 5.06 (1H, brs, 7β-H), 5.12 (1H, brs, 12β-H), 5.87 (1H, d, J = 10.8 Hz, 2-H), 6.43 (1H, d, J = 10.8 Hz, 1-H). MS *m*/z (relative intensity): 341 (M^+-115-60, 30%), 323 (M^+-115-18-60, 7%), 281 (M^+-115-60-27-18-15, 100%), 263 (M^+-115-60-60-18, 20%).

3.20. 12α-Formyloxy-6α,7α-epoxy-3-oxochol-4-en-24-oic acid (42)

Obtained from **21** in 61% yield; m.p., 201–203°C (methanol). IR ν_{max} cm^{-1}: 1685 (C = O). ^{1}H-NMR δ: 0.84 (3H, s, 18-CH_3), 0.86 (3H, d, J = 6.5 Hz, 21-CH_3), 1.09 (3H, s, 19-CH_3), 3.38 (1H, d, J = 5.4 Hz, 7β-H), 3.48 (1H, d, J = 5.4 Hz, 6β-H), 5.27 (1H, brs, 12β-H), 6.13 (1H, s, 4-H), 8.07 (1H, s, 12α-OCHO). MS (as the methyl ester) *m*/z (relative intensity): 383 (M^+-HCOOH (46)-15, 23%), 382 (39%), 268 (M^+-115-46-15, 60%), 267 (100%).

3.21. Methyl 6α,7α-epoxy-3-oxochola-1,4-dien-24-oate (43)

Obtained from **22** in 58% yield; m.p., 160–163°C (methanol). IR ν_{max} cm^{-1}: 1724 (C = O). ^{1}H-NMR δ: 0.77 (3H, s, 18-CH_3), 0.93 (3H, d, J = 6.2 Hz, 21-CH_3), 1.20 (3H, s, 19-CH_3), 3.35 (1H, m, 7β-H), 3.63 (1H, d, J = 3.8 Hz, 6β-H), 3.67 (3H, s, $COOCH_3$), 6.22 (1H, d, J = 10.3 Hz, 2-H), 6.47 (1H, s, 4-H), 7.00 (H, d, J = 10.3 Hz, 1-H). MS *m*/z (relative intensity): 398 (M^+, 3%), 383 (M^+-15, 1%), 380 (M^+-18, 2%), 265 (M^+-115-18, 13%), 238 (M^+-115-27-18, 10%), 223 (M^+-115-27-18-15, 11%), 147 (100%).

Compound **44** was obtained in 20% yield; m.p., 203–205°C (methanol). IR ν_{max} cm^{-1}: 1697, 1735 (C = O). ^{1}H-NMR: 0.76 (3H, s, 18-CH_3), 0.94 (3H, d, J = 6.2 Hz, 21-CH_3), 1.14 (3H, s, 19-CH_3), 3.31 (1H, m, 7β-H), 3.45 (2H, m, 1β- and 6β-H), 3.51 (1H, d, J = 3.5 Hz, 2β-H), 3.67 (3H, s, $COOCH_3$), 6.11 (1H, s, 4H). MS *m*/z (relative intensity): 399 (M^+-15, 38%), 382 (M^+-32, 61%), 299 (M^+-115, 12%), 281 (M^+-115-18, 100%).

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