Oxyfunctionalization Products of Terpenoids with Dimethyldioxirane and Their Biological Activity

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Oxyfunctionalization of the bioactive terpenoids, ursolic acid acetate (1), oleanolic acid acetate (5), lupeol acetate (12), and kaurenic acid (17), with dimethyldioxirane (DMDO) was investigated. Treatment of the terpenoids with DMDO under mild conditions afforded a variety of oxidation and oxydegradation products to yield naturally occurring and/or novel compounds in one step. After chromatographic separation, the structures of the individual isolated products were determined using spectroscopic methods including several homonuclear (^{1}H–^{1}H) and heteronuclear (^{1}H–^{13}C) shift-correlated 2D-NMR techniques. The inhibitory activity of the terpenoid derivatives against α-glucosidase was investigated and compounds 1, 3, 7, and 9 were found to exhibit potent activity.

Key words dimethyldioxirane; oxyfunctionalization; triterpenoid; diterpenoid; α-glucosidase

In analogy with enzyme-controlled reactions *in vivo*, such as cytochrome P-450 oxidase-dependent systems,[1] regio- and stereoselective oxyfunctionalization in substrates is a key transformation in the efficient and convenient synthesis of biologically active compounds, starting from abundantly available and less active terpenoids, steroids, and/or alkaloids occurring in nature. Hence various oxidants mimicking the P-450 enzymatic system have been developed by many groups.[2—4]

Recently, the successful use of dioxiranes, a new class of powerful and versatile cyclic peroxides with a three-membered ring, has been demonstrated to insert an oxygen atom into a variety of substrates.[5—7] Applications include the oxyfunctionalization of primary alcohols and aldehydes to the corresponding carboxylic acids, secondary alcohols to ketones, alkenes to epoxides, amines to nitro compounds, sulfides to sulfones or sulfoxides, and phosphines to phosphine oxides. Of further interest is that dioxiranes display high efficiency for the *O*-insertion reaction into "unactivated" tertiary and secondary C–H bonds of hydrocarbons.[8—10] Despite reports of such versatile reactivity and usefulness in organic synthesis, the further detailed, potential ability of dioxiranes remains to be explored.

The smallest (C_3) cyclic peroxide, dimethyldioxirane (DMDO), which is generated readily by the reaction of acetone with Oxone® ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$),[11,12] is a class of environmentally friendly oxidant that does not contain a toxic, hazardous heavy metal. DMDO was reported to display high efficiency in the oxyfunctionalization of various substrates.[5—7] DMDO also allowed the reaction to be performed under extremely mild, strictly neutral nonhydrolytic conditions; particularly noteworthy is the ease of handling and simplicity of workup procedures in experimental DMDO oxidation.

As a part of our continuing efforts directed toward the convenient, efficient synthesis of biologically active compounds, we became interested in the efficient utilization and development of DMDO oxidation. We report here the oxyfunctionalization of ursolic acid acetate (**1**), oleanolic acid acetate (**5**), lupeol acetate (**12**), and kaurenic acid (**17**) by DMDO to obtain more biologically active derivatives as well as to clarify the factors governing its reactivity and *O*-insertion position in oxidative transformations of the substrates. The biological activity of the oxidized products was also examined.

Results and Discussion

The substrates examined in this study are major bioactive triterpenoids (**1**, **5**, **12**) and a diterpenoid (**17**) occurring in a variety of plant species. To prevent the simultaneous oxidation of hydroxyl groups in the substrates to carbonyls, these compounds were protected as their acetyl derivatives prior to DMDO reaction. DMDO oxidation was carried out for 24—36 h at room temperature. All of the substrates were found to react readily with DMDO to form a variety of novel oxygenated products in one step. The reactivity and selectivity of the oxidation reactions depended significantly on the structures of the substrates.

The oxidation of ursolic acid acetate (**1**) with DMDO gave 11-oxo-ursolic acid acetate (**2**, 15% yield) and 11,12-dehydroursolic lactone (**3**, 11% yield) and its 20β-hydroxy derivative (**4**, 10% yield) (Fig. 1). In spite of the structural similarity of ursolic acid and oleanolic acid (positional isomer of a methyl group at C-19 and C-20), the DMDO oxidation of 3-*O*-acetyloleanolic acid (**5**) unexpectedly afforded 3-*O*-acetyl-12α-chloro-oleanolic lactone (**6**, 50% yield) as a major product together with 3-*O*-acetyl-9,11-dehydro-12α-hydroxy-oleanolic lactone (**7**, 20% yield) and its 12β-hydroxy derivative (**8**, 10% yield) and 3-*O*-acetyl-12-oxo-oleanolic lactone (**9**, 5% yield). As shown in Fig. 2, the chloride (**6**) may be produced by the action of hypochloric acid formed from $CHCl_3$ and DMDO during storage in a refrigerator. In the oxidation of **1**, it is considered that the double bond at C-12,13 is sterically hindered by the methyl group at C-19. Therefore hypochloric acid was not accessible to the double bond, and allylic oxidation occurred preferentially to give an allyl alcohol (**1′**), which in turn was converted to the enone (**2**) and lactone (**3**) by lactonization and dehydration. On the other hand, treatment of **5** with DMDO generated *in situ* in CH_2Cl_2 yielded only 2% of the chloride (**6**) together with 11-oxo-

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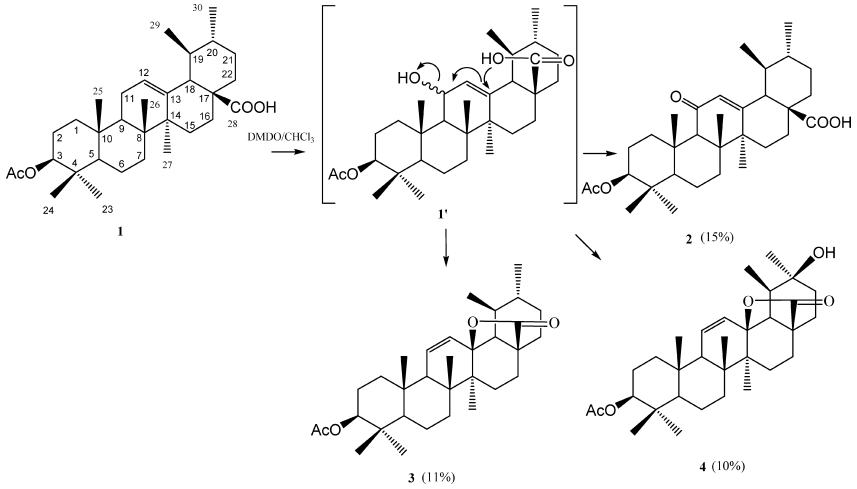

Fig. 1. Oxidation of Ursolic Acid Acetate with DMDO

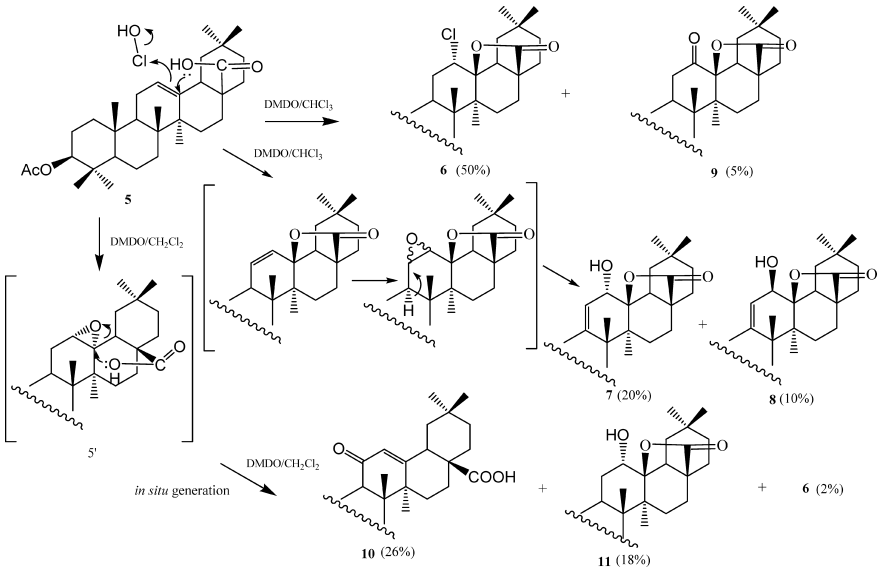

Fig. 2. Oxidation of Oleanolic Acid Acetate with DMDO

oleanolic acid acetate (**10**, 26% yield) and 3-*O*-acetyl-12α-hydroxyoleanolic lactone (**11**, 18% yield). These results suggest that CH_2Cl_2 was more stable against DMDO than $CHCl_3$, and that **10** and **11** may be produced through allylic oxidation and ring opening of the initially formed α-epoxide (**5′**), respectively (Fig. 2). Upon oxidation of lupeol acetate (**12**) with DMDO, 30-nor-20-oxo-lupeol acetate (**13**, 41% yield) and 30-oxo-lupeol acetate (**14**, 30% yield) were obtained as major products, along with two minor products (**15**, 5% yield, and **16**, 4% yield). The mechanism of the formation of major products from the initially formed epoxide (**12′**) of **12** can be rationalized as follows. Acid-catalyzed ring opening of the epoxide ring, followed by the addition of DMDO (path a) or by a proton loss from C-30 (path b), leads to the ketone (**13**) or α,β-unsaturated aldehyde (**14**) (Fig. 3).

Next, we examined the oxidation of kaurenic acid (**17**). Treatment of **17** with DMDO/$CHCl_3$ afforded 15,16-dehydro-17-hydroxykaurenic acid (**18**, 32% yield), 16,17-dihydroxykaurenic acid (**19**, 18% yield), 15,16α-epoxy-17-hydroxykaurenic acid (**20**, 16% yield), 17-chloro-16α-hydroxykaurenic acid (**21**, 3% yield), and dicarboxylic acid (**22**, 3% yield). The major products **18**, **19**, and **20** and the minor product **22** were produced *via* the initially formed epoxide **17′**, as shown in Fig. 4. The chlorohydrin (**21**) must be produced by the action of hypochloric acid produced during the storage of DMDO/$CHCl_3$. Each of the reaction products was separated on open column chromatography, followed by medium-pressure column chromatography (MPLC) or HPLC. The structures of the individually isolated products, particularly of the novel compounds **4**, **6**, **16**, and **21**, were determined on the basis of 2D homonuclear ^1H–^1H and heteronuclear ^1H–^{13}C shift-correlated 2D-NMR spectroscopy and MS spectral data.

Biological Activity The inhibitory activity of terpenoid derivatives synthesized in this study against α-glucosidase was evaluated, and the ursolic acid derivatives (**1**, **3**) and oleanolic acid derivatives (**7**, **8**, **9**) were found to exhibit more potent activity than the original terpenoids (Table 1).

Experimental

Melting points were determined on an electric micro hot stage and are uncorrected. IR spectra were obtained on a Bio Rad FTS-7 FT-IR spectrometer (Philadelphia, PA, U.S.A.) for samples in KBr pellets. ^1H- and ^{13}C-NMR

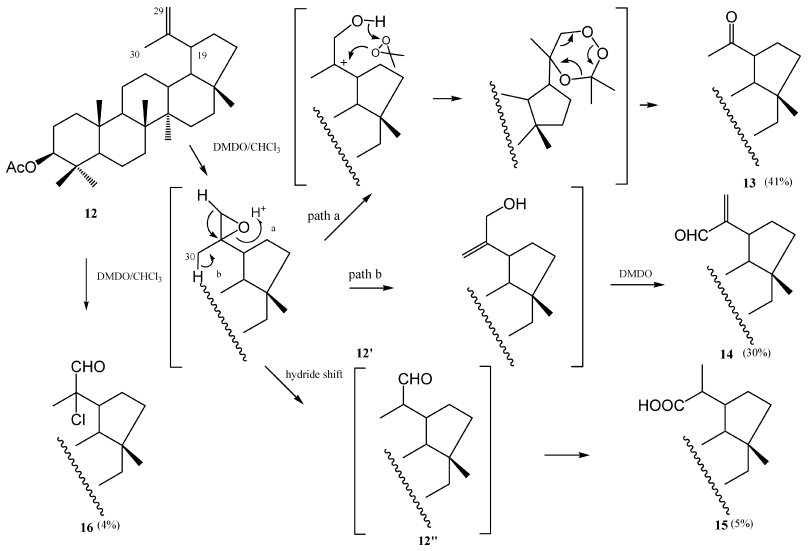

Fig. 3. Oxidation of Lupeol Acetate with DMDO

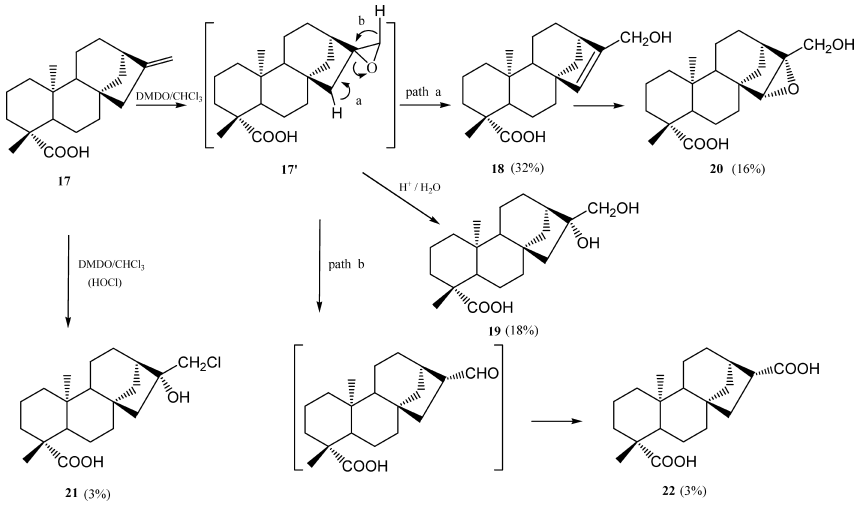

Fig. 4. Oxidation of Kaurenic Acid with DMDO

Table 1. Inhibitory Activity of Oxyfunctionalized Compounds (10 μM) against α-Glucosidase

Compound	Inhibition (%)	Compound	Inhibition (%)	Compound	Inhibition (%)
1	83.2	**9**	46.8	**17**	<10
2	<10	**10**	<10	**18**	<10
3	62.7	**11**	<10	**19**	24.5
4	<10	**12**	11.9	**20**	<10
5	<10	**13**	16.7	**21**	<10
6	20.9	**14**	31.6	**22**	<10
7	53.8	**15**	<10		
8	37.3	**16**	16.3		

spectra were measured on a JEOL JNM-EX 270 FT instrument (Tokyo, Japan) at 270 and 67.8 MHz, respectively, in $CDCl_3$ containing 0.1% Me_4Si as an internal standard. Chemical shifts are expressed as δ ppm relative to Me_4Si. ^{13}C-NMR signals corresponding to methyl (CH_3), methylene (CH_2), methine (CH), and quaternary (C) carbons were differentiated by means of distortionless enhancement by polarization transfer experiments. 2D-NMR spectra were measured on a JEOL GSX-400 spectrometer. Low-resolution electron-impact or fast atom bombardment mass spectra were recorded on a JEOL JMS-GC_{mate} mass spectrometer at 70 eV. High-resolution electron-impact or fast atom bombardment mass spectra were measured on a JEOL JMS-GC_{mate} mass spectrometer. A Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector was used isothermally at 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, Melbourne, Australia). The apparatus used for MPLC consisted of a YRD-880 RI-detector (Shimamura Tech. Co. Ltd., Tokyo, Japan) and a uf-3040s chromatographic pump using silica gel (230—400 mesh; Nacalai Tesque, Kyoto, Japan) as the adsorbent and benzene–EtOAc mixture as the eluent. The preparative normal-phase HPLC apparatus consisted of an Hitachi (Tokyo, Japan) L-7100 pump equipped with a Shodex RI detector (Tokyo, Japan) and a Kaseisorb LC 120-5 column (250 mm×10 mm i.d.; Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan) using benzene–EtOAc (9 : 1—5 : 5, v/v) mixtures as eluent. TLC was performed on precoated silica gel plates (0.25 mm layer thickness; E. Merck, Darmstadt, Germany) using hexane–EtOAc as the developing solvent.

A concentrated DMDO solution (0.17 mol) in $CHCl_3$ was prepared according to the previously reported method using Oxone® (caroate; $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), $NaHCO_3$, and acetone.[11,12] The substrates **1**, **5**, **12**, and **17** used in this study were from our laboratory collection, and the hydroxyl group at C-3 in **1**, **5**, and **12** was acetylated in the usual manner.

General Oxidation Procedure with DMDO To a solution of a terpenoid (500 mg) in CH_2Cl_2 (4.0 ml) was added a previously prepared solution of DMDO (0.17 mol, 8.0 ml) in $CHCl_3$. The mixture was left at room temperature for 12 h, and excess DMDO and solvent were evaporated off under reduced pressure. The above procedure was repeated several times, until the spot of the substrate disappeared with monitoring by TLC (total re-

action times, 24—36 h). After the reaction, the product was purified by passage through an open column on silica gel (70—230 mesh), followed by MPLC on silica gel or by HPLC.

Physicochemical Data for New Compounds 3-*O*-Acetyl-20β-hydroxy-urs-11-en-28,13-olide (**4**): Colorless amorphous solids crystallized from aqueous methanol, mp 276—278 °C. FAB-MS *m/z*: 512.3503 $[M]^+$ (Calcd for $C_{32}H_{48}O_5$: 512.3501). EI-MS *m/z*: 512 $[M]^+$ (10), 494 $[M-H_2O]^+$ (100), 484 $[M-COO]^+$ (4), 468 $[M-CH_3COOH]^+$ (26), 450 $[M-CH_3COOH-H_2O]^+$ (84). IR (KBr) cm^{-1}: 3495 (OH), 1734 (C=O). ^{1}H-NMR δ: 0.86 (3H, s, H-23), 0.87 (3H, s, H-27), 0.94 (3H, s, H-25), 1.03 (3H, d, *J*=8.6 Hz, H-29), 1.06 (3H, s, H-26), 1.16 (3H, s, H-24), 1.22 (3H, s, H-30), 2.06 (3H, s, C$\underline{H}_3$CO), 4.49 (1H, dd, *J*=5.6, 10.6 Hz, H-3), 5.54 (1H, dd, *J*=3.2, 10.5 Hz, H-11), 5.93 (1H, dd, *J*=1.5, 10.5 Hz, H-12). ^{13}C-NMR δ: 38.0 (C-1), 23.3 (C-2), 80.6 (C-3), 37.8 (C-4), 54.8 (C-5), 17.6 (C-6), 31.2 (C-7), 41.7 (C-8), 52.9 (C-9), 36.3 (C-10), 128.8 (C-11), 133.4 (C-12), 89.6 (C-13), 42.0 (C-14), 25.5 (C-15), 22.1 (C-16), 44.5 (C-17), 54.5 (C-18), 39.5 (C-19), 71.1 (C-20), 35.8 (C-21), 26.5 (C-22), 27.7 (C-23), 15.9 (C-24), 18.0 (C-25), 18.9 (C-26), 16.0 (C-27), 179.5 (C-28), 12.6 (C-29), 28.5 (C-30), 171.0 ($CH_3\underline{C}$=O), 21.3 ($\underline{C}H_3$CO).

3-*O*-Acetyl-12α-chloro-oleanan-28,13-olide (**6**): Colorless amorphous solids crystallized from aqueous methanol, mp 243—246 °C. FAB-MS *m/z*: 533.3396 $[M]^+$ (Calcd for $C_{32}H_{50}O_4Cl$: 533.3397). FAB-MS *m/z*: 533 $[M]^+$ (4), 487 $[M-HCOOH]^+$ (1), 473 $[M-CH_3COOH]^+$ (8), 437 $[M-CH_3COOH-HCl]^+$ (6) 154 (100), 136 (100). IR (KBr) cm^{-1}: 1724, 1729 (C=O). ^{1}H-NMR δ: 0.85 (3H, s, H-24), 0.88 (3H, s, H-23), 0.90 (3H, s, H-30), 0.91 (3H, s, H-25), 1.00 (3H, s, H-29), 1.20 (3H, s, H-27), 1.39 (3H, s, H-26), 2.05 (3H, s, C$\underline{H}_3$CO), 4.17 (1H, br s, H-12), 4.52 (1H, dd, *J*=5.2, 10.5 Hz, H-3). ^{13}C-NMR δ: 38.2 (C-1), 21.3 (C-2), 80.6 (C-3), 37.8 (C-4), 55.3 (C-5), 17.6 (C-6), 34.4 (C-7), 42.4 (C-8), 44.8 (C-9), 36.4 (C-10), 29.3 (C-11), 64.9 (C-12), 91.6 (C-13), 43.0 (C-14), 27.5 (C-15), 23.5 (C-16), 45.3 (C-17), 51.9 (C-18), 39.8 (C-19), 31.8 (C-20), 33.9 (C-21), 29.0 (C-22), 27.9 (C-23), 16.5 (C-24), 16.8 (C-25), 20.2 (C-26), 18.9 (C-27), 179.0 (C-28), 33.2 (C-29), 23.6 (C-30), 171.0 ($CH_3\underline{C}$=O), 21.3 ($CH_3\underline{C}$O).

3-*O*-Acetyl-12α-hydroxy-olean-9-en-28,13-olide (**7**): Colorless amorphous solids crystallized from aqueous methanol, mp 201—203 °C. EI-MS *m/z*: 512.3503 $[M]^+$ (Calcd for $C_{32}H_{48}O_5$: 512.3502). EI-MS *m/z*: 512 $[M]^+$ (10), 497 $[M-CH_3]^+$ (10), 452 $[M-CH_3COOH]^+$ (14), 437 $[M-CH_3COOH-H_2O]^+$ (5), 249 (100). IR (KBr) cm^{-1}: 2928 (OH), 1708, 1776 (C=O). ^{1}H-NMR δ: 0.88 (6H, s, H-23, H-24), 0.93 (3H, s, H-27), 0.99 (3H, s, H-30), 1.01 (3H, s, H-29), 1.22 (3H, s, H-25), 1.35 (3H, s, H-26), 2.06 (3H, s, C$\underline{H}_3$CO), 4.21 (1H, d, *J*=3.7 Hz, H-12), 4.47 (1H, dd, *J*=5.4, 10.5 Hz, H-3), 5.45 (1H, d, *J*=3.7 Hz, H-11). ^{13}C-NMR δ: 37.5 (C-1), 24.1 (C-2), 80.2 (C-3), 38.1 (C-4), 51.5 (C-5), 17.4 (C-6), 32.9 (C-7), 44.3 (C-8), 157.8 (C-9), 38.9 (C-10), 118.3 (C-11), 65.7 (C-12), 89.5 (C-13), 41.7 (C-14), 27.1 (C-15), 27.2 (C-16), 43.5 (C-17), 44.1 (C-18), 36.9 (C-19), 31.7 (C-20), 34.2 (C-21), 20.7 (C-22), 28.0 (C-23), 16.5 (C-24), 24.6 (C-25), 28.6 (C-26), 20.9 (C-27), 179.3 (C-28), 33.3 (C-29), 24.1 (C-30), 171.0 ($CH_3\underline{C}$=O), 21.3 ($\underline{C}H_3$CO).

3-*O*-Acetyl-28-chloro-29-oxolupeol (**16**): An amorphous powder. FAB-MS *m/z*: 518.3535 $[M]^+$ (Calcd for $C_{32}H_{51}O_3Cl$: 518.3527). EI-MS *m/z*: 518 $[M]^+$ (8), 503 $[M-CH_3]^+$ (7), 482 $[M-HCl]^+$ (34), 458 $[M-CH_3COOH]^+$ (19), 453 $[M-HCl-CHO]^+$ (11), 443 $[M-CH_3COOH-CH_3]^+$ (8), 426 $[M-SC]^+$ (25), 422 $[M-CH_3COOH-HCl]^+$ (10), 407 (4), 393 $[M-CH_3COOH-HCl-CHO]^+$ (6), 189 (100). IR (KBr) cm^{-1}: 1730 (C=O). ^{1}H-NMR δ: 0.81 (3H, s, H-28), 0.84 (3H, s, H-24), 0.85 (3H, s, H-25), 0.86 (3H, s, H-23), 0.95 (3H, s, H-27), 1.04 (3H, s, H-26), 1.56 (3H, s, H-30), 2.04 (3H, s, C$\underline{H}_3$CO), 2.22 (1H, m, H-19), 4.49 (1H, dd, *J*=5.2, 10.9 Hz, H-3), 9.43 (1H, s, H-29). ^{13}C-NMR δ: 38.5 (C-1), 23.7 (C-2), 80.9 (C-3), 37.8 (C-4), 55.3 (C-5), 18.2 (C-6), 34.4 (C-7), 41.3 (C-8), 50.1 (C-9), 37.0 (C-10), 21.3 (C-11), 27.8 (C-12), 37.0 (C-13), 43.5 (C-14), 27.3 (C-15), 35.4 (C-16), 44.5 (C-17), 48.5 (C-18), 46.4 (C-19), 79.7 (C-20), 29.2 (C-21), 39.7 (C-22), 28.0 (C-23), 16.5 (C-24), 16.2 (C-25), 16.1 (C-26), 14.7 (C-27), 19.4 (C-28), 195.7 (C-29), 24.3 (C-30), 171.0 ($CH_3\underline{C}$=O), 21.3 ($\underline{C}H_3$CO).

Ent-17-chloro-16-hydroxy-kauran-19-oic Acid (**21**): An amorphous powder. FAB-MS *m/z*: 354.1959 $[M]^+$ (Calcd for $C_{20}H_{31}O_3Cl$: 354.1962). EI-MS *m/z*: 354 $[M]^+$ (6), 336 $[M-H_2O]^+$ (21), 323 (55), 305 (100), 300 $[M-H_2O-HCl]^+$ (58). IR (KBr) cm^{-1}: 3200 (OH), 1700 (COOH). ^{1}H-NMR δ: 0.95 (3H, s, H-20), 1.24 (3H, s, H-18), 3.76, 3.84 (2H, ABq, *J*=11.0 Hz, H-17). ^{13}C-NMR δ: 40.6 (C-1), 19.0 (C-2), 37.0 (C-3), 43.7 (C-4), 56.8 (C-5), 21.9 (C-6), 41.7 (C-7), 45.2 (C-8), 55.7 (C-9), 39.7 (C-10), 18.6 (C-11), 26.0 (C-12), 46.2 (C-13), 37.8 (C-14), 51.8 (C-15), 80.7 (C-16), 53.5 (C-17), 28.9 (C-18), 183.5 (C-19), 15.5 (C-20).

Inhibition of α-Glucosidase[13] The inhibitory activity of all samples against α-glucosidase (Sigma G3651) was measured spectrophotometrically at pH 6.8 and 37 °C at 20 min using *p*-nitrophenyl α-D-glucopyranoside (PNP-G) 0.1 mM as a substrate and 0.017 units/ml of enzyme in 50 mM sodium phosphate buffer containing 100 mM NaCl. The activity was determined by measuring the librated *p*-nitrophenol at 405 nm due to the hydrolysis of PNP-G by α-glucosidase as measured on a microplate reader (Model 3550 Microplate Reader, Bio-Rad, Tokyo, Japan) at room temperature after the addition of 50 mM sodium bicarbonate (pH 10.0, 50 μM) to the reaction mixture.

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