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OLEANANE-TYPE TRITERPENES FROM *VIBURNUM AWABUKI*

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Key Word Index—*Viburnum awabuki*; Caprifoliaceae; wood; oleanane-type triterpene; erythrodiol; 1-oxo-erythrodiol; 11-oxo-erythrodiol; 13,28-epoxy-11-oleanene-3-one; castanopsone.

Abstract—Three new oleanane-type triterpenes have been isolated from the wood of *Viburnum awabuki*. Their structures have been elucidated as 3β,28-dihydroxy-12-oleanene-1-one, 3β,28-dihydroxy-12-oleanene-11-one and 13,28-epoxy-11-oleanene-3-one, respectively, by extensive analysis of spectroscopic data including comparison of ^{13}C NMR data with those of erythrodiol and some chemical transformations. The structure of 1-oxo-erythrodiol has been confirmed by X-ray crystallographic analysis.

INTRODUCTION

The leaves of *Viburnum awabuki* elaborates a number of vibsane-type diterpenes with novel 11- and seven-membered carbon skeletons [1–3], whereas no diterpenes have been so far found in its wood and fruit [4]. As a part of our phytochemical studies on *V. awabuki*, we have continued to examine chemical components in its wood. As a result, we could isolate three new oleanane-type triterpenes **1**, **2**, and **4** along with known castanopsone (**3**) [5] from the methanol extract of the wood. In this paper, we report the isolation and structural elucidation of these new triterpenes.

RESULTS AND DISCUSSION

The wood of *V. awabuki* was extracted with methanol and the methanol extract partitioned between ethyl acetate and water. The ethyl acetate soluble portion was fractionated by repeated column chromatography on silica gel and Sephadex LH-20 to give three new oleanane-type triterpenes named 1-oxo-erythrodiol (**1**), 11-oxo-erythrodiol (**2**) and 13,28-epoxy-11-oleanene-3-one (**4**).

Compound **1** had a molecular formula of $C_{30}H_{48}O_3$, established by the HR-EI mass spectrum (*m/z* 456.3606 $[M]^+$). The IR spectrum of **1** showed the presence of a hydroxyl group at 3393 cm^{-1} and a carbonyl group at 1698 cm^{-1}. Acetylation of **1** with Ac_2O-pyridine yielded the diacetate **1a** proving the presence of two hydroxy groups in the molecule of **1**. The ^{1}H NMR spectrum of **1** revealed the presence of seven tertiary methyl groups [δ_H 0.87, 0.89, 0.99, 1.02, 1.05, 1.19, and 1.30 (each 3H, *s*)], an isolated oxymethylene group at δ_H 3.21 and 3.55 (each 1H, *d*, J = 11.0 Hz) as well as of an oxygen-bearing methine at δ_H 3.49 (1H, *dd*, J = 12.2, 4.9 Hz). Additionally, the EI mass spectrum showed a typical fragment ion peak at *m/z* 234 due to a retro-Diels–Alder cleavage occurring on the C ring of a 12-olenanene-type triterpene. This spectral feature indicates that compound **1** belongs to an oleanane-type triterpene having a ketone function. In fact, the ^{13}C NMR data (Table 1) of **1** were very similar to those of erythrodiol (**5**) [5] except for the presence of the carbonyl resonance at δ_C 212.4. The sole carbonyl group should be placed at the C-1 position according to the HMBC correlations, as shown in Fig. 1. Thus, 1-oxo-erythrodiol (**1**) was elucidated to be 3β,28-dihydroxy-12-oleanene-1-one, which has not been found in the literatures. Finally, the proposed structure for **1** was confirmed by X-ray crystallographic analysis of the diacetate **1a**, as depicted in Fig. 2.

Compound **2** was found to have the same molecule formula $C_{30}H_{48}O_3$ as **1** on the HR-EI mass spectrum (*m/z* 456.3601 $[M]^+$) and the ^{1}H NMR spectrum of **2** resembled to that of **1** except for H-9 and H-12 olefinic proton signals appeared as singlet at δ_H 2.34 and 5.57, respectively. The presence of two hydroxy groups were substantiated by converting **2** into the diacetate **2a**, and also the spectral data [245 nm; 1651 cm^{-1}; δ_H 5.75

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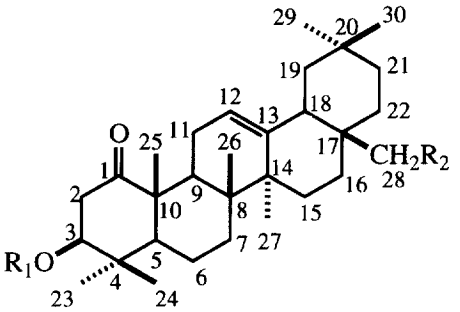
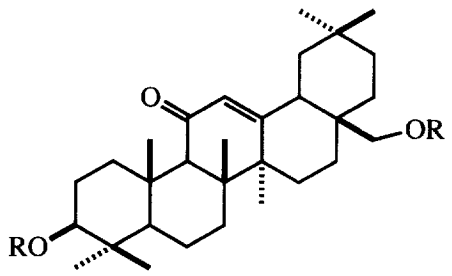
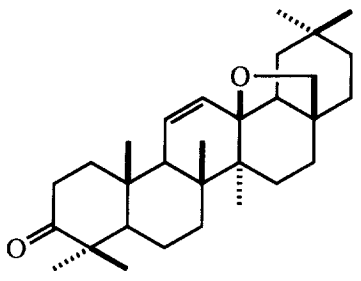


1 $R_1 = H, R_2 = OH$
1a $R_1 = Ac, R_2 = OAc$
3 $R_1 = R_2 = H$



2 R = H
2a R = Ac



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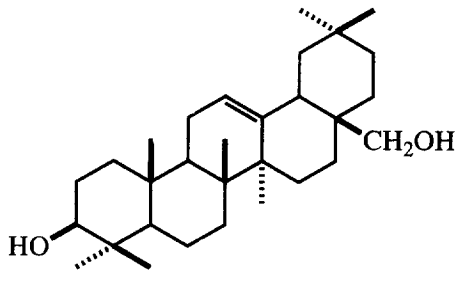

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(1H, *s*, H-12); δ_C 200.1 (C-11)] of **2** suggested that a sole carbonyl group involved in a conjugated system. In the HMBC experiment as summarized in Fig. 1, the H-9 singlet signal showed a long-range correlation with the sole carbonyl signal. This means that the ketone function should be located on the C-11 position in a 12-oleanene skeleton. Thus, 11-oxo-erythrodiol (**2**) represents as 3β,28-dihydroxy-12-oleanene-11-one, which is the first isolation as aglycone though its glycosides have been known [6].

Compound **4** had a molecular formula of $C_{30}H_{46}O_2$, established by the HR-EI mass spectrum (*m/z* 438.3504 $[M]^+$), and its IR spectrum showed the presence of a carbonyl group (1703 cm^{-1}) which was also suggested by the ^{13}C NMR data at δ_C 217.4, but displayed no absorptions due to hydroxy group. The NMR spectra (Table 1) of **4** disclosed the presence of seven tertiary methyl groups at δ_H 0.88, 0.95, 0.96, 1.03, 1.04, 1.09 and 1.13 (each 3H, *s*), a disubstituted double bond [δ_H 5.42 (1H, *dd*, J = 10.4, 3.2 Hz, H-12) and 5.84 (1H, *dd*, J = 10.4, 1.4 Hz, H-11); δ_C 131.4 and 131.6] as well as of an isolated oxymethylene [δ_H 3.27 and 3.72; δ_C 77.3]. The sole carbonyl signal had distinct HMBC correlations with the H_3-23 (δ_H 1.09) and the H_3-24 (δ_H 1.04) signals. These spectra data indicate that **4** is 3-oxo-oleanane-type triterpene. In the HMBC experiment as summarized in Fig. 1, the oxymethylene signals showed cross peaks to the oxygen-bearing quaternary carbon signal at δ_C 84.7, which in turn correlated to the olefinic proton signal at δ_H 5.42. The other olefinic signal at δ_H 5.84 coupled to the vicinal H-9. Thereby, the disubstituted double bond must be placed at the $\Delta^{11,12}$ position on the C-ring of the oleanane framework, and also in consideration of 8 degrees of unsaturation, the 6-membered ring should be formed via an ether linkage between C-28 and C-13. Finally, **4** was clarified on the basis of NOEs to adopt the relative sterochemistry as shown in Fig. 3. Thus, the structure of compound **4** was assigned as 13,28-epoxy-11-oleanene-3-one.

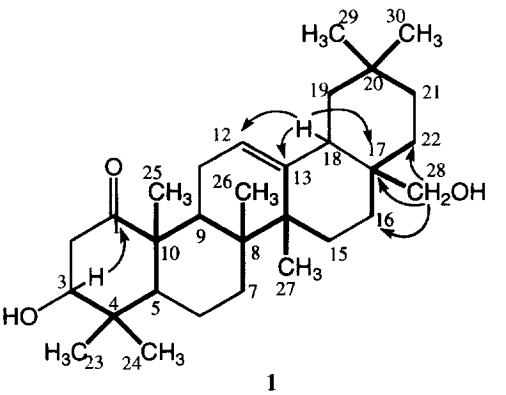
In the leaves of *V. awabuki*, vibsane-type diterpenes, lupane-type triterpenes, coumarin glucosides [7], and flavonoid glycosides [8] have been biosynthesized, but no oleanane-type triterpenoids isolated in the present study on its wood have not been found. It should be noted that a number of rearranged dammarane triterpenes were isolated from the leaves of *Viburnum dilatatum* [9, 10]. On the other hand, from the wood and the fruits of *V. awabuki* benzofuran-type lignans [4], guaiane-type sesquiterpenoids [11], and oleanane-type and lupane-type triterpenoids have been isolated, but no vibsane-type diterpenoid characteristic of the chemical components of *V. awabuki* has been detected.

EXPERIMENTAL

General. ^1H and ^{13}C NMR: TMS as int. standard. CC: silica gel (Merck, 230–400 mesh and Wakogel C-300) and Sephadex LH-20 (Pharmacia). TLC: pre-

Table 1. ^{13}C NMR data of **1–5** (at 100 MHz, δ in $CDCl_3$)

C	1	2	3	4	5
1	212.4	39.2	211.7	39.0	38.6
2	44.1	27.3	45.8	34.0	27.2
3	78.6	78.8	78.0	217.4	79.0
4	39.3	39.2	38.7	47.7	38.8
5	54.0	55.0	53.4	54.7	55.2
6	17.8	17.5	17.2	18.9	18.4
7	32.5	32.7	32.0	30.7	32.6
8	42.0	45.4	41.3	41.5	39.8
9	39.1	61.8	38.5	52.6	47.6
10	52.3	37.0	51.7	36.2	36.9
11	25.3	200.1	25.2	131.6	23.6
12	123.0	128.3	121.1	131.4	122.3
13	143.2	169.4	143.5	84.7	144.2
14	39.7	43.4	39.1	43.8	41.7
15	25.5	25.8	25.5	25.3	25.6
16	22.0	21.6	23.1	25.6	22.0
17	37.0	37.1	36.5	41.5	36.9
18	42.5	42.7	43.5	51.1	42.3
19	46.1	44.9	46.8	37.1	46.5
20	30.9	31.1	27.9	31.7	31.0
21	34.1	32.9	34.2	34.9	34.1
22	31.0	30.1	30.5	30.8	31.0
23	16.0	15.6	15.4	26.1	15.5
24	28.5	28.1	27.8	20.8	28.1
25	15.0	16.4	14.4	17.2	15.5
26	17.5	18.6	17.0	19.0	16.7
27	25.7	23.4	26.3	19.3	25.9
28	69.8	69.7	23.1	77.3	69.7
29	33.2	32.9	32.7	33.6	33.2
30	23.6	23.4	24.7	23.6	23.6



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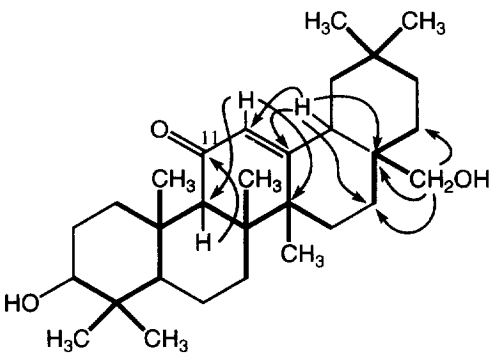

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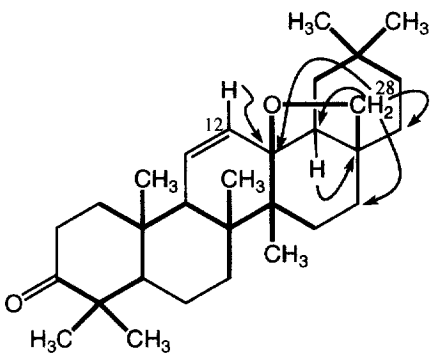

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Fig. 1. Partial structures (bold lines) obtained by HMBC correlations from methyl proton signals and representative HMBC correlations (arrows) from particular proton signals of compounds **1**, **2** and **4**.

coated silica gel F254 (Merck). Spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4.

Plant material. *V. awabuki* was collected in Tokushima, Japan. A voucher specimen has been deposited in this institute.

Extraction and isolation. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc soluble portion (50 g) was sepd by CC on silica gel (Merck) alternately with *n*-hexane, *n*-hexane–EtOAc (9:1; 7:3; 2:3), EtOAc and EtOAc–MeOH (9:1) to give 6 frs (1–6). Fr. 4 (6.5 g) was again sepd by CC on silica gel (Merck) with CH_2Cl_2–EtOAc (1:1) to give 4 frs (7–10). Fr. 7 (1.5 g) was purified by repeated CC on silica gel (C-300) with CH_2Cl_2–EtOAc (3:1) followed by CC on LH-20 with MeOH to give compounds **1** (18 mg) and **2** (6.2 mg). Fr. 3 (8.6 g) was sepd by CC on silica gel (C-300) with CH_2Cl_2–EtOAc (10:1) to give 7 frs (11–17). Fr. 16 (332 mg) was filtered to remove the ppt. The filtrate was again sepd by CC on silica gel with CH_2Cl_2–EtOAc (4:1) to give 7 frs (18–24). Fr. 22 (49 mg) was purified by recycling HPLC [recycled × 6; JAIGEL-1H (20 × 600 mm i.d.). $CHCl_3$ (3.5 ml min^{-1})] followed by CC on LH-20 with $CHCl_3$–*n*-hexane (3:2) to afford castanopsone **3** (9 mg) and **4** (5 mg).

1-*Oxo-erythrodiol* (**1**). Colourless prisms. mp 218–221°. $[\alpha]_D^{24}$ +84.3° (*c* 1.55, $CHCl_3$). EIMS *m/z* (rel. int.): 456.3606 $[M]^+$ (calcd 456.3603 for $C_{30}H_{48}O_3$) (12), 426 $[M-30]^+$ (25), 234 $[M-C_{14}H_{22}O_2]^+$ (10). IR ν_{max}^{FT} cm^{-1}: 3393 (OH), 1698 (C=O). ^1H (400 MHz, $CDCl_3$): δ 0.87 (3H, *s*, H_3-30), 0.89 (3H, *s*, H_3-29), 0.90 (1H, *dd*, *J* = 12.7, 2.0 Hz, H-5), 0.99 (3H, *s*, H_3-26), 1.02 (3H, *s*, H_3-23), 1.05 (3H, *s*, H_3-24), 1.06 (1H, *m*, H-15), 1.13 (1H, *dd*, *J* = 13.2, 3.9 Hz, H-19), 1.19 (3H, *s*, H_3-27), 1.30 (3H, *s*, H_3-25), 1.44 (1H, *ddd*, *J* = 12.5, 12.5, 3.7 Hz, H-7), 1.50 (1H, *ddd*, *J* = 13.9, 13.9, 4.6 Hz, H-16), 1.73 (1H, *dd*, *J* = 13.4, 13.2 Hz, H-19), 1.83 (1H, *ddd*, *J* = 18.1, 6.8, 2.9 Hz, H-11), 1.89 (1H, *ddd*, *J* = 13.9, 13.9, 4.4 Hz, H-22), 1.98 (1H, *dd*,

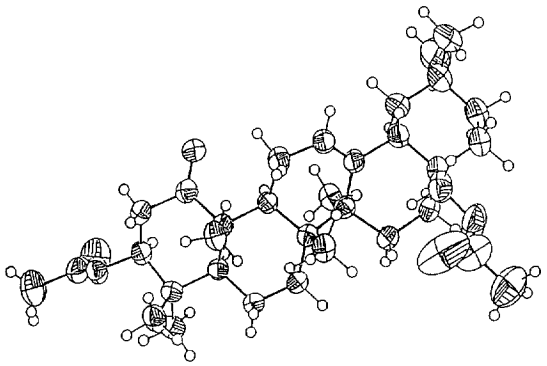

Fig. 2. The ORTEP drawing of 1-oxo-erythrodiol diacetate (**1a**).

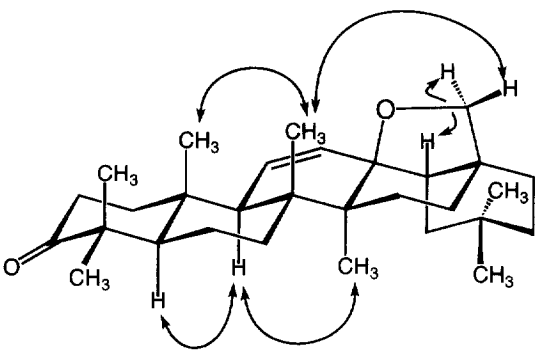

Fig. 3. Relative stereochemistry for **4** based on NOEs indicated by arrows.

J = 13.2, 3.9 Hz, H-18), 2.23 (1H, *dd*, J = 6.8, 4.6 Hz, H-9), 2.32 (1H, *ddd*, J = 18.1, 4.9, 4.6 Hz, H-11), 2.37 (1H, *dd*, J = 12.0, 4.9 Hz, H-2), 3.03 (1H, *dd*, J = 12.2, 12.0 Hz, H-2), 3.21 (1H, *d*, J = 11.0 Hz, H-28), 3.49 (1H, *dd*, J = 12.2, 4.9 Hz, H-3), 3.55 (1H, *d*, J = 11.0 Hz, H-28), 5.20 (1H, *dd*, J = 4.9, 2.9 Hz, H-12). ^{13}C NMR: Table 1.

Acetylation of **1**. A mixt. of **1** (5 mg), Ac_2O (0.4 ml) and pyridine (0.6 ml) was stood at room temp overnight. The reaction mixt. was evapd *in vacuo*. The residue was chromatographed on silica gel [CH_2Cl_2–EtOAc (10:1)] to yield the diacetate **1a** (5.5 mg) as colourless crystals, mp 195–197° (from MeOH). HR-EIMS *m/z* (rel. int.): 540.3807 $[M]^+$ (8) (calcd 540.3815 for $C_{34}H_{52}O_5$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, *s*, H_3-30), 0.89 (3H, *s*, H_3-29), 0.96 (3H, *s*, H_3-26), 1.00 (1H, *s*, H_3-23), 1.05 (1H, *s*, H_3-24), 1.17 (3H, *s*, H_3-27), 1.30 (3H, *s*, H_3-25), 2.05 (6H, *s*, OAc), 2.47 (1H, *dd*, J = 12.4, 4.6 Hz, H-2α), 2.93 (1H, *dd*, J = 12.4, 12.0 Hz, H-2β), 3.68 (1H, *d*, J = 11.0 Hz, H-28), 4.00 (1H, *d*, J = 11.0 Hz, H-28), 4.75 (1H, *dd*, J = 12.0, 4.6 Hz, H-3), 5.21 (1H, *t*, J = 3.5 Hz, H-12).

11-*Oxo-erythrodiol* (**2**). Colourless prisms, mp 145–147°. $[\alpha]_D^{24}$ +60.9° (*c* 0.77, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε) 245 (3.88). EIMS *m/z* (rel. int.): 456.3601 $[M]^+$ (calcd 456.3603 for $C_{30}H_{48}O_3$), 441 $[M-15]^+$ (71), 248 $[M-C_{14}H_{24}O]^+$ (100). IR ν_{max}^{FT} cm^{-1}: 3406 (OH), 1651 (C=O). ^{1}H (400 MHz, $CDCl_3$): δ 0.69 (1H, *dd*, J = 11.5, 2.5 Hz, H-5), 0.81 (3H, *s*, H_3-24), 0.89 (3H, *s*, H_3-29), 0.92 (3H, *s*, H_3-30), 0.98 (1H, *ddd*, J = 13.4, 13.4, 4.1 Hz, H-1), 1.00 (3H, *s*, H_3-23), 1.11 (3H, *s*, H_3-26), 1.13 (3H, *s*, H_3-25), 1.15 (1H, *dd*, J = 13.4, 4.2 Hz, H-19), 1.18 (1H, *ddd*, J = 13.9, 4.4, 2.2 Hz, H-15), 1.33 (1H, *dd*, J = 13.9, 3.9 Hz, H-21), 1.35 (1H, *ddd*, J = 13.9, 4.4, 4.4 Hz, H-16), 1.39 (3H, *s*, H_3-27), 1.65 (2H, *m*, H-2), 1.73 (1H, *dd*, J = 13.9, 13.4 Hz, H-19), 1.77 (1H, *ddd*, J = 13.9, 13.9, 4.4 Hz, H-15), 2.15 (1H, *dd*, J = 13.9, 4.2 Hz, H-18), 2.34 (1H, *s*, H-9), 2.78 (1H, *ddd*, J = 13.4, 3.7, 3.7 Hz, H-1), 3.22 (1H, *d*, J = 11.0 Hz, H-28), 3.23 (1H, *dd*, J = 10.5, 5.5 Hz, H-3), 3.47 (1H, *d*, J = 11.0 Hz, H-28), 5.57 (1H, *s*, H-12). ^{13}C NMR: Table 1.

Acetylation of **2**. A mixt. of **2** (3 mg), Ac_2O (0.2 ml) and pyridine (0.5 ml) was stood overnight. The reaction mixt. was evapd *in vacuo*. The residue was chromatographed on silica gel [CH_2Cl_2–EtOAc (3:1)] to yield the diacetate **2a** (2.5 mg) as amorphous powders. EIMS *m/z* (rel. int.): 540.3818 $[M]^+$ (26) (calcd 540.3815 for $C_{34}H_{52}O_5$), 481 $[M-OAc]^+$ (25), 271 (100). IR ν_{max}^{FT} cm^{-1}: 1738 (C=O), 1660 (conj. C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, *s*), 0.88 (6H, *s*), 0.90 (3H, *s*), 0.92 (3H, *s*), 1.04 (1H, *dddd*, J = 13.7, 13.7, 3.7, 3.7 Hz, H-1α), 1.12 (3H, *s*), 1.15 (3H, *s*), 1.38 (3H, *s*), 2.01 (1H, *ddd*, J = 13.7, 13.7, 4.4 Hz, H-16), 2.05 (3H, *s*, OAc), 2.06 (3H, *s*, OAc), 3.69 (1H, *d*, J = 11.2 Hz, H-28), 3.95 (1H, *d*, J = 11.2 Hz, H-28), 4.51 (1H, *dd*, J = 11.7, 4.9 Hz, H-3), 5.57 (1H, *s*, H-12).

13,28-*Epoxy*-11-*oleanene*-3-*one* (**4**). Colourless prisms, mp 200–203°. $[\alpha]_D^{24}$ +105.6° (*c* 0.20, $CHCl_3$). EIMS *m/z* (rel. int.): 438.3504 $[M]^+$ (22) (calcd 438.3498 for $C_{30}H_{46}O_2$), 420 $[M-18]^+$ (100), 407 $[M-OMe]^+$ (59), 392 $[M-C_2H_6O]^+$ (13). IR ν_{max}^{FT} cm^{-1}: 1703 (C=O). ^{1}H (400 MHz, $CDCl_3$): δ 0.88 (3H, *s*, H_3-30), 0.95 (3H, *s*, H_3-27), 0.96 (3H, *s*, H_3-29), 1.03 (3H, *s*, H_3-25), 1.04 (3H, *s*, H_3-24), 1.09 (3H, *s*, H_3-23), 1.11 (1H, *ddd*, J = 13.2, 12.9, 6.0 Hz, H-16), 1.13 (1H, *s*, H_3-26), 1.21 (1H, *ddd*, J = 13.6, 4.1, 2.5 Hz, H-21), 1.27 (1H, *m*, H-19), 1.31 (1H, *dd*, J = 12.6, 3.0 Hz, H-5), 1.35 (1H, *dd*, J = 13.6, 4.4 Hz, H-21), 1.39 (1H, *m*, H-1), 1.43 (1H, *m*, H-22), 1.49 (2H, *m*, H-6), 1.52 (1H, *m*, H-22), 1.66 (1H, *m*, H-18), 1.73 (1H, *d*, J = 12.6 Hz, H-19), 1.80 (1H, *ddd*, J = 13.2, 13.2, 5.8 Hz, H-15), 1.93 (1H, *m*, H-9), 2.01 (1H, *m*, H-16), 2.07 (1H, *ddd*, J = 13.2, 7.3, 3.8 Hz, H-1), 2.40 (1H, *ddd*, J = 16.0, 6.9, 3.8 Hz, H-2), 2.60 (1H, *ddd*, J = 16.0, 11.0, 7.3 Hz, H-2), 3.27 (1H, *dd*, J = 6.9, 2.1 Hz, H-28), 3.72 (1H, *d*, J = 6.9 Hz, H-28), 5.42 (1H, *dd*, J = 10.4, 3.2 Hz, H-12), 5.84 (1H, *dd*, J = 10.4, 1.4 Hz, H-11). ^{13}C NMR: Table 1.

X-ray crystallographic analysis of **1a**. An X-ray analysis was performed on a Mac Sciences MXC 18 diffractometer with Cu Kα (λ = 1.54178) radiation. The structure of **1a** was solved by direct method using CRYSTAN SIR 92 and refined by full-matrix least-squares using CRYSATN. The crystal data: $C_{34}H_{52}O_5$: monoclinic; space group $P21$ (#4); a = 18.551 (4),

$b = 12.212$ (4), $c = 6.974$ (2) Å, $\beta = 94.49$ (2)°; $Dx = 1.14$ gcm^{-3}; $z = 2$; μ(Cu Kα) = 5.13 cm^{-1}; final $R = 0.050$.

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