Triterpenes from Natural and Transformed Roots of *Plocama pendula*

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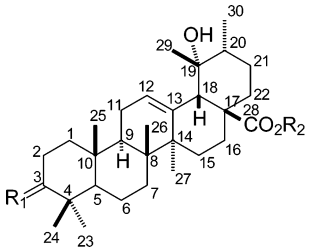
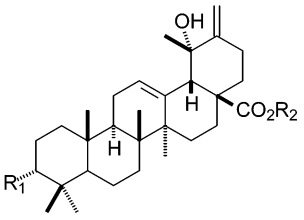
Plocama pendula root cultures transformed by *Agrobacterium rhizogenes* were established. From these cultures two new pentacyclic triterpenes, 3-*epi*-pomolic acid 3α-acetate (3α-acetoxy-19α-hydroxyursa-12-en-28-oic acid) (**1**) and baloic acid (3α-acetoxy-19α-hydroxyursa-12,20(30)-dien-28-oic acid) (**9**), were isolated. The former was also obtained from *P. pendula* natural roots, together with another two new pentacyclic triterpenes, 3-*epi*-pomolic acid (3α,19α-dihydroxyursa-12-en-28-oic acid) (**3**) and 19α-hydroxyoleanonic acid (19α-hydroxy-3-oxo-olean-12-en-28-oic acid) (**12**).

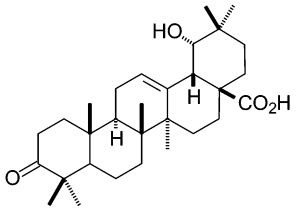
Plocama pendula Ait. (Rubiaceae) is an endemic plant of the Canary Islands. As with other species of this family, it is characterized phytochemically by its content of anthraquinones.[1,2] In this paper we describe the isolation of two new triterpenes, 3-*epi*-pomolic acid 3α-acetate (**1**) and baloic acid (**9**), from transformed root cultures by *Agrobacterium rhizogenes* and another two new compounds, 3-*epi*-pomolic acid (**3**) and 19α-hydroxyoleanonic acid (**12**), from the natural roots of *P. pendula*.



1 R_1 = αOAc, H R_2 = H
2 R_1 = αOAc, H R_2 = Me
3 R_1 = αOH, H R_2 = H
4 R_1 = αOH, H R_2 = Me
5 R_1 = O R_2 = H
6 R_1 = O R_2 = Me
7 R_1 = βOH, H R_2 = H
8 R_1 = βOAc, H R_2 = H



9 R_1 = OAc R_2 = H
10 R_1 = OAc R_2 = Me
11 R_1 = OH R_2 = H



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The HRMS of **1** showed a molecular ion at *m*/*z* 514.3655, corresponding to the molecular formula, $C_{32}H_{50}O_5$, and the loss of acetic acid at *m*/*z* 454. The ^{1}H NMR spectrum showed the signals of six angular methyls, the C-30 secondary methyl at δ 0.96 (d, J = 7 Hz), the methyl of an acetate group, the H-18 singlet at δ 2.55, the H-3 singlet at 4.63, geminal to the acetoxy group, and the H-12 triplet at δ 5.37 (J = 3.5 Hz). The resonances of H-18, the C-29 methyl (δ 1.24), geminal to a hydroxyl group, and the C-30 methyl were characteristic of pomolic acid (**7**) derivatives,[3,4] which possess an α-alcoholic group at C-19. The location of the acetoxy group at C-3 was determined considering the correlation of the two methyls at C-4 (H-23 and H-24) with C-3 in the HMBC spectrum. The α-axial orientation of this acetoxy group was established by the form of the signal of its geminal proton observed at δ 4.63 (br s)[5] and the high-field shift of the resonances of C-1 and C-5, due to the γ-gauche effect.[6] The ^{13}C NMR spectrum (Table 1), which was assigned unambiguously using 2D NMR data (COSY, HSQC, and HMBC), was in accordance with this structure. This was also confirmed chemically. Methylation of **1** with diazomethane afforded the acetate methyl ester **2**. Hydrolysis of this led to the 3α-alcohol, **4**, which was oxidized with Jones reagent to afford the 3-oxo derivative, **6**. This was identical with the methyl ester of pomonic acid.[7] Accordingly, the structure of compound **1** was determined as 3-*epi*-pomolic acid 3α-acetate (3α-acetoxy-19α-hydroxyursa-12-en-28-oic acid).

Table 1. ^{13}C NMR Data of **1**, **4**, **8**, **9**, and **12**

carbon	**1**	**4**	**8**	**9**	**12**
1	33.4	32.8	38.1	33.6	38.9
2	22.6	25.1	23.6	22.7	34.2
3	78.2	76.1	80.9	78.2	217.4
4	39.9	40.0	37.7	39.7	47.5
5	49.9	48.8	55.2	50.1	55.4
6	18.1	18.3	18.2	18.1	19.7
7	32.4	32.6	32.6	32.9	32.1
8	36.4	37.0	39.9	36.5	39.6
9	46.9	46.9	47.1	47.0	47.2
10	36.8	37.2	36.9	37.0	37.0
11	23.5	23.5	23.5	23.4	23.8
12	129.4	129.3	129.3	129.8	124.9
13	137.7	137.9	137.9	137.8	142.7
14	41.1	41.1	41.0	41.5	41.4
15	28.1	28.1	28.2	27.8	27.9
16	25.3	25.9	25.3	25.5	27.5
17	47.6	47.8	47.8	47.7	45.3
18	52.8	53.2	52.8	54.1	43.6
19	73.0	73.1	73.1	72.5	81.6
20	41.0	41.1	41.0	152.3	34.6
21	25.9	25.4	25.9	27.4	28.1
22	37.3	37.4	37.1	35.8	32.5
23	27.4	27.4	27.4	27.8	26.3
24	21.8	22.2	16.6	21.9	21.4
25	15.0	14.9	15.3	15.2	14.7
26	16.9	16.6	16.9	17.1	17.0
27	24.6	24.6	24.4	23.7	24.4
28	183.2	178.3	183.6	181.6	183.0
29	27.4	28.2	28.0	29.9	28.1
30	16.1	16.1	16.1	107.3	24.9

A second new triterpene obtained from the hairy roots of *P. pendula* was named baloic acid (**9**). The HRMS showed the molecular formula, $C_{32}H_{48}O_5$. Its ^{1}H NMR spectrum showed six angular methyls, an acetate group, a proton geminal to an axial acetoxy group (δ 4.64, br s), an exocyclic methylene (δ 4.79, s and 5.03, s), and a vinylic proton (δ 5.44, t). This spectroscopic behavior was similar to that of **1**, except for an exocyclic double bond instead of a secondary methyl group. The ^{13}C NMR spectrum (Table 1) showed the resonance of the carbons bearing oxygens, C-3, C-19, and C-28, at δ 78.2, 72.5, and 181.6, respectively, while the vinylic carbons C-12, C-13, C-20, and C-30 appeared at δ 129.8,

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137.8, 152.3, and 107.3, respectively. The structure was confirmed by 2D NMR spectroscopy. Thus, in the HMBC spectrum, correlations were observed of H-18 with C-12, C-13, C-14, C-16, C-17, C-19, C-22, C-28, and C-29, of H-23 and H-24 with C-3, C-4, and C-5, of H-25 with C-1, C-5, C-9, and C-10, of H-26 with C-7, C-9, and C-14, of H-27 with C-14, of H-29 with C-18, C-19, and C-20, and of H-30 with C-19 and C-21. Thus, the structure of baloic acid was determined as 3α-acetoxy-19α-hydroxyursa-12,20(30)-dien-28-oic acid (**9**). The corresponding alcohol **11** is unknown, but a 24-hydroxy derivative of this, coussaric acid, has been obtained from another plant in the Rubiaceae, *Coussarea brevicaulis*.[8]

Known sterols such as campesterol, stigmasterol, and β-sitosterol, and triterpenes such as oleanolic and ursolic acids, and the acetates of these acids, were also isolated from the hairy roots of *P. pendula*.

From the natural roots of *P. pendula* another two new triterpenes were obtained and characterized as 3-*epi*-pomolic acid (**3**) and 19α-hydroxyoleanonic acid (**12**), respectively. Compound **3** was obtained in a very small amount and identified only by considering its ^{1}H NMR spectroscopic and MS data. The former were very similar to that of the corresponding alcohol **1**, but showing now in **3** the H-3β signal at δ 3.41, while this was δ 4.63 in **1**. The resonances of H-16, H-18, and the methyl groups could also be assigned by comparison with those observed for **1**. Thus, the structure 3α,16α-dihydroxyursa-12-en-28-oic acid (3α-*epi*-pomolic acid) (**3**) was given to this new triterpene. A compound analogous to **3**, but hydroxylated at C-24 and named barbinervic acid, has been isolated from *Clethra barbinervis*[9] and *Petiveria alliacea*.[10]

The structure of the final new triterpene was determined as **12**. The HREIMS showed the molecular formula, $C_{30}H_{46}O_4$. The ^{1}H NMR spectrum displayed characteristic signals of seven angular methyl groups, a proton geminal to an oxygen function (δ 3.35, br s), and an olefinic hydrogen (δ 5.46, t). The ^{13}C NMR spectrum showed signals of seven methyls, nine methylenes, three methines, an oxygenated methine (δ 81.6), six quaternary carbons, a double bond (δ 124.9 and 142.7, C-12 and C-13), an oxo group (δ 217.4), and a carbonyl of an acid group (δ 183.0). Consequently, compound **12** must be a triterpene acid of the oleanane type containing an oxo group and a secondary alcohol. Correlations of H-23 and H-24 with the oxo group were observed in the HMBC spectrum, which indicated that this function was at C-3, while correlations of H-29 and H-30 with C-19 indicated that the secondary alcohol was at C-19. An α-configuration was assigned to this group considering the pattern of resonance of H-19, a broad singlet. Other cross-peaks displayed were those of H-18 (δ 3.11) with C-12 and C-13. The assignment of the ^{13}C NMR spectrum is given in Table 1. Consequently, the structure of this new compound was determined as 19α-hydroxy-3-oxo-olean-12-en-28-oic acid (19α-hydroxyoleanonic acid) (**12**). The corresponding C-3β acetate, rubiprasin C, has been isolated from *Rubia cordifolia*.[11]

The known triterpenoids and sterols isolated from the hairy roots were also obtained from the natural roots. Moreover, from the latter, a mixture of the sterols campest-4-en-3-one, β-sitost-4-en-3-one, and stigmast-4-en-3-one, and the triterpenes 3β-hydroxyolean-11,-13(18)-dien-28-oic acid (**13**),[12,13] pomolic acid 3β-acetate[14] (**8**), and pomonic acid (**5**),[3,15] were also obtained.

Experimental Section

General Experimental Procedures. Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1600 FT spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution at 500.13 MHz with a Bruker AMX2-500 spectrometer. ^{13}C NMR spectra were run in $CDCl_3$ at 50.32 and 125.13 MHz with a Bruker AC-200 or a Bruker AMX2-500 spectrometer, respectively. Chemical shifts are given in ppm (δ). Mass spectra and HRMS were taken at 70 eV in a Micromass Autospec spectrometer. Dry column chromatography was performed on Merck silica gel 0.02−0.063 mm. Semipreparative HPLC with a Beckman System Gold 125P and a Beckman ultrasphere column (Si 1 × 25 cm, 5 μm) was used. Conformations of minimum energy were determined by computational methods employing the Hyperchem 7.1 program of Hypercube.

Plant Material. The plant *P. pendula*, commonly known as "balo", was collected in January 2004, at the coast of Chío, and the seeds in July 2001, at the coast of Barranco Hondo (Tenerife Island). Both were identified by Prof. P. Pérez de Paz, Department of Plant Biology, Botanical Section, University of La Laguna, Tenerife.

Sterile plantlets of *P. pendula* were obtained by seed germination in aseptic conditions. *Agrobacterium rhizogenes* ATCC-15834 was directly inoculated by a needle to the stem of the plantlets, cultured on agar medium containing 30 g L^{-1} sucrose and half-strength Murashige and Skoog salts.[16] Roots appeared at the inoculation site after 4 weeks. The induced hairy roots were excised and cultured on hormone-free 1/2 Gamborg B5 solid medium[17] supplemented with 30 g L^{-1} sucrose and 0.5 mg mL^{-1} ampicillin to eliminate the bacteria. The axenic hairy roots thus obtained were subcultured in the dark at 25 °C on the same solid medium without antibiotics every 25−30 days.

DNA Extraction and Analysis. Total genomic DNA was extracted from transformed root tissue and from the untransformed root plants using a GenElute plant genomic DNA miniprep kit (Sigma). Plasmid DNA from *A. rhizogenes* strain ATCC-15834 was used as a positive control. Polymerase chain reaction was performed using REDExtract-N-Amp plant PCR kit (Sigma) to detect the insertion of T_L-DNA and T_R-DNA of *A. rhizogenes* ATCC 15834 in the transformed roots. The oligonucleotide primers for T_L-DNA were 5′-ATGGATCCCAAATTGCTATTCCTTCCA-3′ and 5′-TTAGGCTTCTTTCTTCAGG TTTA-3′, which amplify a segment complementary to the 5′ coding sequence of *rol* B to the 3′ coding sequence of *rol* C on the T_L-DNA region, and 5′-CGGAAATTGTGGCTCGTTGTGGAC-3′ and 5′-AATCGTTCAGAGAGCGTCCGAAGTT-3′, which amplify the *ags* gene of the T_R-DNA region. PCR amplification was performed in a DNA thermal cycler (Applied Biosystems 2700, Foster City, CA) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 55 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR reaction mixture was electrophoresed on a 1.2% agarose gel using Tris-acetate−EDTA buffer and visualized by ethidium bromide staining under ultraviolet light at 260 nm.

Culture Methods. The hairy roots were cultivated in the dark at 25 °C in 250 mL Erlenmeyer flasks containing 100 mL of hormone-free 1/2 Gamborg B5 liquid medium supplemented with 30 g L^{-1} sucrose and shaken on a rotary shaker at 80 rpm. After 4 weeks, the hairy roots (581 g) were harvested and separated from the culture medium by filtration through filter paper under vacuum.

Extraction and Isolation. The hairy roots were freeze-dried (45.3 g), powdered, and extracted with ethanol in a Soxhlet. The ethanolic extract was evaporated to dryness under reduced pressure, affording a syrupy extract (11.7 g). Then this extract was separated into six fractions by vacuum liquid chromatography (VLC) using an *n*-hexane−EtOAc gradient. All fractions were further chromatographed on a silica gel column and/or by preparative normal-phase HPLC on an Inertsil Prep-sil (Gasukuro Kogyo) 25 × 2 i.d. column and Ultrasphere Si (Beckman) 25 × 1 i.d. to give in order of polarity a mixture of campesterol, β-sitosterol, and stigmasterol (380 mg), 3-*epi*-pomolic acid 3α-acetate (**1**) (8 mg), baloic acid (**9**) (6 mg), the acetates of oleanolic and ursolic acids (8 mg), and ursolic and oleanolic acids (40 mg).

The dry crust from roots of *P. pendula* (1.8 kg) was finely cut and extracted with ethanol. In this way, a 120 g extract was obtained, which was chromatographed using the same methods employed for the extract of the hairy roots, giving a mixture of campest-4-en-3-one, β-sitost-4-en-3-one, and stigmast-4-en-3-one (16 mg), campesterol, β-sitosterol, and stigmasterol (650 mg), pomonic acid (**5**) (2 mg), 3-*epi*-pomolic acid 3α-acetate (**1**) (2 mg), pomolic acid 3β-acetate (**8**) (10 mg), a mixture of the acetates of oleanolic and ursolic acid (120 mg), oleanolic acid and ursolic acid (42 mg), 3β-hydroxyolean-11,12(13)-dien-28-oic acid (**13**) (1 mg), 3-*epi*-pomolic acid (**3**) (0.8 mg), and 19α-hydroxyoleanonic acid (**12**) (3 mg).

3-*epi*-Pomolic acid 3α-acetate (1): $[\alpha]_D$ +31.7 (*c* 0.06, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.74 (3H, s, H-26), 0.85 (3H, s, H-23), 0.88 (3H, s, H-24), 0.94 (3H, s, H-25), 0.96 (3H, d, J = 7 Hz, H-30), 1.06 (1H, br d, J = 14 Hz, H-15β), 1.21 (3H, s, H-29), 1.31 (3H, s, H-27), 2.07 (3H, s, −OAc), 2.54 (1H, m, H-16), 2.55 (1H, s, H-18),

4.63 (1H, br s, H-3), 5.37 (1H, t, $J = 3.5$ Hz, H-12); EIMS *m*/*z* 514 $[M]^+$ (1), 496 (1), 468 (17), 454 (12), 396 (14), 336 (4), 264 (8), 248 (54), 246 (23), 218 (16), 203 (56), 202 (25), 201 (34), 200 (20), 190 (100), 189 (52), 187 (28), 185 (22), 175 (46), 173 (23), 171 (14), 161 (17), 159 (18), 157 (13), 149 (19), 146 (83), 133 (43); HREIMS *m*/*z* 514.3655 (calcd for $C_{32}H_{50}O_5$, 514.3658).

3α-Acetate methyl ester of 3-*epi*-pomolic acid (2): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71 (3H, s, H-26), 0.87 (3H, s, H-23), 0.91 (3H, s, H-24), 0.95 (3H, s, H-25), 0.97 (3H, d, $J = 7$ Hz, H-30), 1.23 (3H, s, H-29), 1.33 (3H, s, H-27), 2.09 (3H, s, −OAc), 2.54 (1H, m, H-16), 2.62 (1H, s, H-18), 3.62 (3H, s), 4.65 (1H, br s, H-3), 5.39 (1H, t, $J =$ 3.5 Hz, H-12); EIMS *m*/*z* 528 $[M]^+$ (2), 468 (38), 453 (7), 260 (12), 218 (16), 201 (34), 190 (60), 189 (28), 179 (100), 175 (25), 146 (57); HREIMS *m*/*z* 528.3801 (calcd for $C_{33}H_{52}O_5$, 528.3814).

3-*epi*-Pomolic acid (3): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.75 (3H, s, H-26), 0.84 (3H, s, H-24), 0.92 (3H, s, H-25), 0.96 (3H, s, H-23), 0.96 (3H, d, $J = 7$ Hz, H-30), 1.06 (1H, td, $J = 14$ and 5 Hz, H-15β), 1.22 (3H, s, H-29), 1.28 (3H, s, H-27), 2.54 (1H, m, H-16), 2.55 (1H, s, H-18), 3.41 (1H, t, $J = 2.8$ Hz, H-3), 5.38 (1H, t, $J = 3.5$ Hz, H-12); EIMS *m*/*z* 472 $[M]^+$ (3), 454 (12), 439 (7), 426 (13), 264 (32), 246 (53), 231 (29), 218 (20), 207 (28), 201 (74), 190 (100), 175 (51) 146 (93); HREIMS *m*/*z* 472.3571 (calcd for $C_{30}H_{48}O_4$, 472.3553).

Hydrolysis of 2. A solution of the acetate methyl ester **2** (4 mg) in methanol (1 mL) was treated with 5% methanolic KOH (3 mL) at room temperature for 96 h. The usual workup afforded 3-*epi*-pomolic acid methyl ester (**4**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.68 (3H, s, H-26), 0.84 (3H, s, H-24), 0.92 (3H, s, H-25), 0.94 (3H, d, $J = 7$ Hz; H-30), 0.95 (3H, s, H-23), 1.24 (3H, s, H-29), 1.28 (3H, s, H-27), 2.51 (1H, m, H-16), 2.60 (1H, s, H-18), 3.41 (1H, t, $J = 2.8$ Hz, H-3), 3.60 (3H, s, −OMe), 5.4 (1H, t, $J = 3.6$ Hz, H-12); EIMS *m*/*z* 486 $[M]^+$ (3), 468 (21), 450 (19), 426 (12), 408 (14), 391 (13), 390 (4), 351 (24), 333 (25), 260 (20), 247 (15), 219 (20), 207 (26), 201 (57), 190 (74), 179 (100), 175 (42), 146 (57); HREIMS *m*/*z* 486.3708 (calcd for $C_{31}H_{50}O_4$, 486.3709).

Oxidation of 4. A solution of the alcohol **4** (3.1 mg) in acetone (2 mL) was treated with Jones reagent (0.25 mL) at 0 °C for 1 h. The excess chromic acid was destroyed with 2-propanol, after which the solvent was partially evaporated and the residue diluted with water and extracted with $CHCl_3$. Usual workup and methylation of the residue with diazomethane afforded pomonic acid methyl ester (**6**). This compound was identical with that obtained by methylation of pomonic acid (**5**) with diazomethane.

Pomolic acid 3β-acetate (8): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.73 (3H, s, H-26), 0.85 (3H, s, H-24), 0.88 (3H, s, H-23), 0.94 (3H, s, H-25), 0.95 (3H, d, $J = 6.5$ Hz, H-30), 1.21 (3H, s, H-29), 1.25 (3H, s, H-27), 2.04 (3H, s), 2.53 (1H, s, H-18), 4.50 (1H, dd, $J = 10$ and 6 Hz, H-3), 5.34 (1H, t, $J = 3.6$ Hz, H-12); EIMS *m*/*z* 514 $[M]^+$ (3), 468 (31), 454 (24), 439 (9), 396 (10), 246 (25), 218 (22), 203 (16), 201 (27), 190 (80), 187 (16), 185 (13), 175 (22), 173 (16), 165 (12), 159 (15), 146 (100), 133 (26); HREIMS *m*/*z* 514.3620 (calcd for $C_{32}H_{50}O_5$, 514.3658).

Baloic acid (9): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (3H, s, H-26), 0.86 (3H, s, H-23), 0.89 (3H, s, H-24), 0.94 (3H, s, H-25), 1.26 (3H, s, H-27), 1.41 (3H, s, H-29), 2.08 (3H, s, −OAc), 2.24 (1H, ddd, $J =$ 13, 6 and 2 Hz, H-21), 2.48 (1H, td, $J = 13$ and 5 Hz, H-16), 2.71 (1H, td, $J = 13$ and 6 Hz, H-21), 2.85 (1H, s, H-18), 4.64 (1H, br s, H-3), 4.79 and 5.03 (each 1H, s, H-30), 5.44 (1H, t, $J = 3.5$ Hz, H-12); EIMS *m*/*z* 434 $[M - AcOH - H_2O]^+$ (3), 393 (14), 322 (100), 250 (18), 210 (22), 149 (15).

Methyl ester of baloic acid (10): $[\alpha]_D$ +12 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.74 (3H, s, H-26), 0.85 (3H, s, H-23), 0.89 (3H, s, H-24), 0.94 (3H, s, H-25), 1.25 (3H, s, H-27), 1.41 (3H, s, H-29), 1.90 (1H, m, H-11), 2.07 (3H, s, −OAc), 2.24 (1H, br d, $J = 13$ Hz, H-21), 2.48 (1H, td, $J = 13$ and 5 Hz, H-16), 2.71 (1H, td, $J = 13$ and 6 Hz, H-21), 2.91 (1H, s, H-18), 3.62 (3H, s), 4.64 (1H, t, $J = 2.5$ Hz, H-3), 4.79 and 5.03 (each 1H, s, H-30), 5.44 (1H, t, $J = 3.5$ Hz, H-12); EIMS *m*/*z* 526 $[M]^+$ (8), 508 (12), 466 (61), 451 (7), 424 (8), 276 (18), 263 (17), 244 (12), 217 (30), 203 (26), 190 (100), 187 (29), 179 (38), 175 (32), 173 (47); HREIMS *m*/*z* 526.3623 (calcd for $C_{33}H_{50}O_5$, 528.3658).

19α-Hydroxyoleanonic acid (12): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80 (3H, s, H-26), 0.98 (3H, s, H-29), 1.00 (3H, s, H-30), 1.06 (3H, s, H-24), 1.07 (3H, s, H-25), 1.10 (3H, s, H-23), 1.29 (3H, s, H-27), 3.11 (1H, br s, H-18), 3.35 (1H, br s, H-19), 5.46 (1H, t, $J = 3.5$ Hz, H-12); EIMS *m*/*z* 470 $[M]^+$ (13), 455 (3), 452 (7), 437 (4), 424 (48), 352 (23), 264 (73), 246 (87), 231 (42), 205 (40), 201 (100), 146 (87); HREIMS *m*/*z* 470.3406 (calcd for $C_{30}H_{46}O_4$, 470.3396).

3β-Hydroxyolean-11,13(18)-dien-28-oic acid (13): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78, 0.79, 0.80, 0.91, 0.95, 0.98 and 0.99 (each 3H, s), 1.68 (1H, d, $J = 14$ Hz, H-19), 2.54 (1H, dd, $J = 14$ and 2 Hz, H-19), 3.24 (1H, dd, $J = 11.5$ and 5 Hz; H-3), 5.66 (1H, br d, $J =$ 10.5 Hz, H-12) and 6.43 (1H, dd, $J = 10.5$ and 3 Hz, H-11); EIMS *m*/*z* 454 $[M]^+$ (77), 436 (8), 421 (7), 409 (27), 391 (7), 300 (13), 255 (29), 234 (25), 207 (38), 203 (48), 201 (23), 189 (100), 187 (42), 175 (34), 173 (28), 163 (29), 147 (25), 145 (31); HREIMS *m*/*z* 454.3433 (calcd for $C_{30}H_{50}O_3$, 454.3447).

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