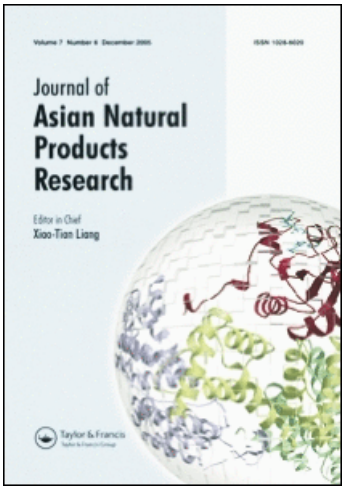

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An efficient semi-synthesis and structure revision of a cytotoxic triterpenoid 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid from *Liquidamber styraciflua*

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An efficient semi-synthesis and structure revision of a cytotoxic triterpenoid 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid from *Liquidamber styraciflua*

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An efficient partial synthesis of 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid (**1**), starting from oleanolic acid (**2**), has been developed in an efficient manner (13 steps, 21% yield), employing a photochemical reaction of nitrite **8** for the introduction of C-25 substituting group as the key step. Based on the comparison of its spectral data with those reported for compound **1** isolated from *Liquidamber styraciflua*, we found the structure in that paper was incorrectly assigned, and should be revised as 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid (**15**).

Keywords: Oleanolic acid; semi-synthesis; 25-Acetoxy-3α-hydroxyolean-12-en-28-oic acid; 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid

1. Introduction

25-Acetoxy-3α-hydroxyolean-12-en-28-oic acid (**1**) was reported as a new oleanane-type triterpenoid from the Cones of *Liquidamber styraciflua*, which showed moderate cytotoxicity against a disease-oriented panel of 39 human cancer cell lines, with tubulin as possible target.[1a]

Derivatives of 25-hydroxyl oleanolic acids are scarce in nature.[1a,1b,2,3] To further explore the structure activity relationship (SAR) of its anticancer activity as well as molecular target, we set out to prepare **1** from a naturally abundant triterpenoid, oleanolic acid (**2**) (Figure 1). Derivatisation of angular methyl group or "non-activated" positions is usually difficult to accomplish. However, Barton's protocol[4] by using a photochemical irridation of an adjacent nitrite has been successful in the semi-synthesis of an endothelin receptor antagonist myriceric acid A (27-caffeoyloxy-3-oxoolean-12-en -28-oic acid)[5] from **2**.

The structural modifications of oleanolic acid (**2**) have been explored widely in the last decades, but mainly focused on A-rings, C-rings, and C-28.[6–10] Here we will report the semi-synthesis of **1** from **2** in an efficient manner. Besides, we also found that the structure of 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid reported in the literature[1a,1b] has been incorrectly assigned, and should be revised as 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid (**15**).

2. Results and discussion

The synthesis of compound **1** is illustrated in Scheme 1. Jones' oxidation of **2** gave 3-oxooleanonic acid (**3**) quantitatively.[5] The 28-COOH in **3** was protected with benzyl by using benzyl chloride in the presence of K_2CO_3 in dioxane to afford **4** in 99% yield,[11] and 3-carbonyl group in **4** protected by using ethyleneglycol with catalytic amount of *p*-toluenesulphonic acid in benzene under azeotropic distillation condition in 96% yield.[12] $NaIO_4/RuCl_3/Bu_4NBr$ oxidation[13] of **5** to generate 11-carbonyl in **6** was more satisfactory (76% yield) than oxidations with Collins' reagent[14] or CrO_3 in AcOH, which needs a large excess of CrO_3, longer reaction time, and chromatographic purification of the crude product. C-11 carbonyl group in **6** was reduced stereoselectively into 11β-hydroxyl of compound **7** with sodium triethylborohydride in 96–99% yield. The stereochemistry of 11-OH was assigned on the basis of ^{1}H NMR spectrum of **7**, in which 11β-OH compounds usually exhibit H-11 in lower field (δ_H 4.33, dd, $J = 5.6$, 6.0 Hz for **7**) than 11α-OH compound (δ_H 4.08, m). 11β-OH of compound **7** was converted to nitrite **8** by the treatment with nitrosyl chloride in pyridine. The unstable nitrite **8** was washed with water, dried *in vacuo*, and dissolved in dry benzene, then irradiated under nitrogen with a high pressure mercury lamp (175 W). Removal of the solvent *in vacuo* left the unstable aldoxime **9**, which was dissolved in a mixture of dioxane, acetic acid and

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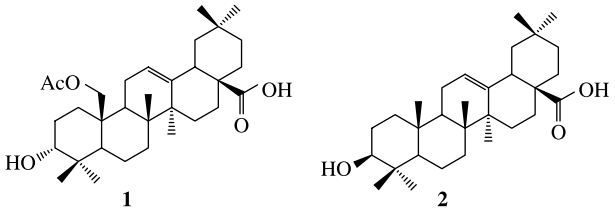
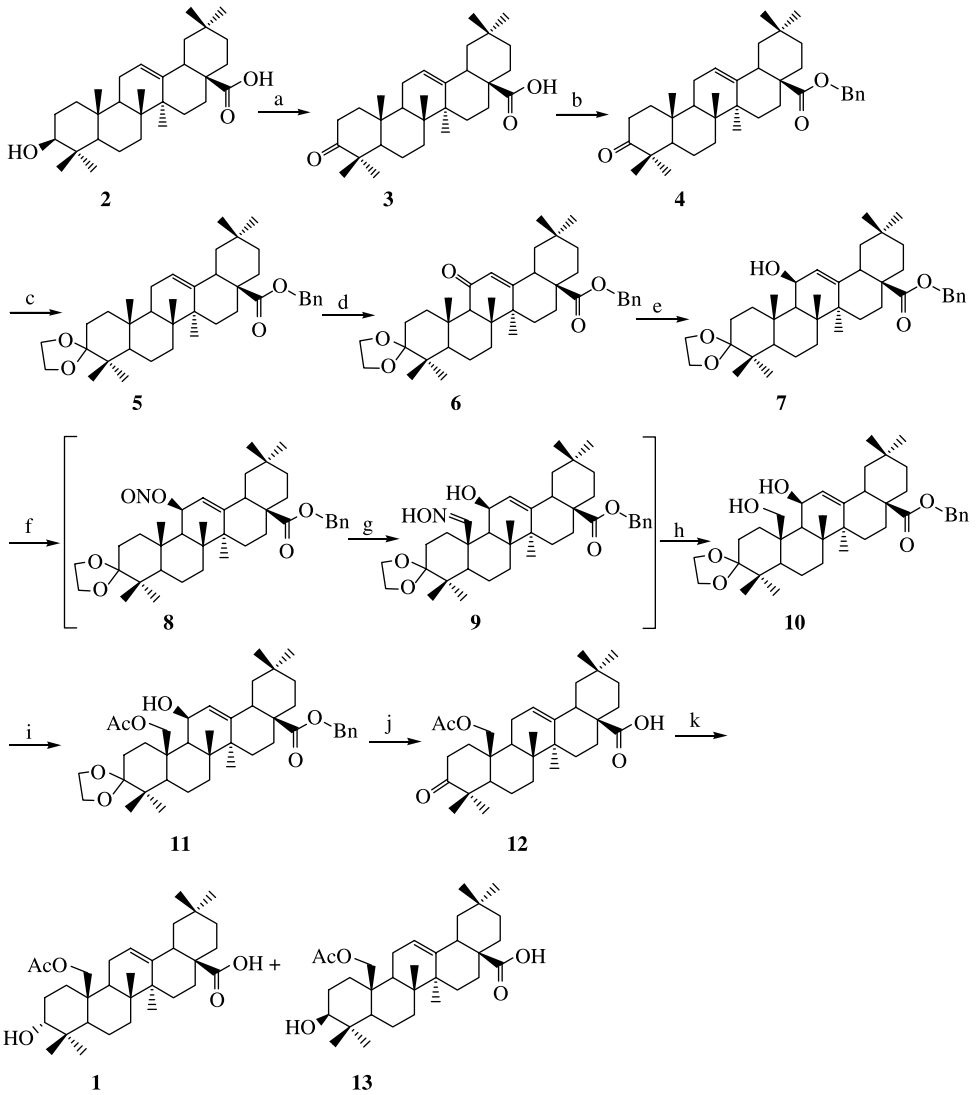
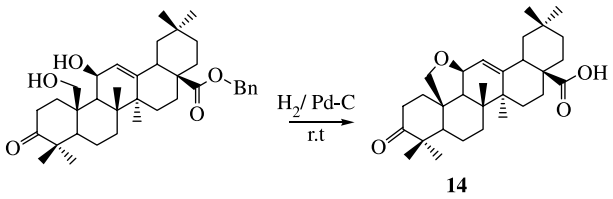


Figure 1. Structures of compounds **1** and **2**.

water and treated with sodium nitrite in water for 4 h. After usual workup the product was reduced by sodium borohydride in chloroform and ethanol to furnish **10**. As a whole, compound **10** can be prepared from **7** over four steps without chromatographic purification in 61.5% yield. The ^{1}H NMR spectrum of compound **10** exhibited six methyl signals, inferring one methyl in the starting material **7** was derivatised. Two proton signals due to –CH_2OH (δ 3.92, d; δ 4.11, d) as well as the carbon signal at δ_C 58.9 revealed an angular methyl group has been hydroxylated. Hydroxylation on 25-methyl group was assigned by analogy with the results for β-amyrin [4]. Next, 25-hydroxyl in **10** was acetylated selectively to afford **11**, with the characteristic proton signals for C_{25}-H (δ 4.46, d, $J = 12.0$ Hz; δ 4.89, d, $J = 12.0$ Hz) in ^{1}H NMR spectrum of **11**, which were shifted 0.3–0.6 ppm



Scheme 1. Synthesis of compound **1**. Reagents and conditions: (a) Jones' reagent, $CHCl_3$, CH_3COCH_3, r.t., 100%; (b) BnCl, K_2CO_3, dioxane, reflux, 99%; (c) ethyleneglycol, *p*-TsOH, benzene, reflux, 96%; (d) $NaIO_4/RuCl_3/Bu_4NBr$, CCl_4:H_2O = 1:1, r.t., 76%; (e) $NaBH(C_2H_5)_3$, THF, r.t., 96–99%; (f) NOCl, py; (g) hν, N_2, r.t.; (h) (π) AcOH, $NaNO_2$, H_2O, dioxane, r.t.; (θ) $NaBH_4$, $CHCl_3/C_2H_5OH$; [61% yield from (f) to (h)]; (π) Ac_2O, pyridine, r.t.. 56% (based on 44% recovery); (j) (π) H_2, Pd-C, EtOH, r.t., 96%; (θ) 30%TFA, CH_2Cl_2, r.t., 94%; (k) Al(*i*-PrO)$_3$, $AlCl_3$, *i*-PrOH, reflux, 80%.



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Scheme 2. Synthesis of compound **14**.

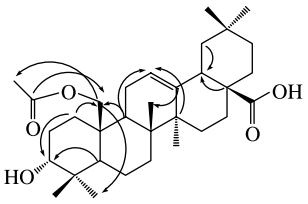

Figure 2. Key HMBC correlations of compound **1**.

downfield. During the deprotection of the benzyl protective group of C-28 in **11**, 11-allylic hydroxyl was also removed by catalytic hydrogenation over palladium on charcoal in 96% yield. In an attempt to remove 11-allylic hydroxyl before acetylation of 25-hydroxyl, ether **14** was obtained instead of 11-dehydroxyl product (Scheme 2). The dioxolane protective group of C-3 was removed by 30% TFA in CH_2Cl_2 in 94% yield to afford **12**, which was reduced by aluminium isopropoxide and isopropanol, to furnish final product **1** in 80%, and its isomer (25-acetoxyoleanolic acid, **13**) in 20%, respectively.

Surprisingly, the spectral data of compound **1** were not consistent with those in the literature.[1a] After reviewing the spectral data in the reference, we found that H-3 in the reported 25-acetoxy-3α-hydroxyolean-12-en-28-oic acid appeared in surprisingly low field (δ 4.99), compared to the NMR spectral data of the 3-acyloxy triterpenoids,[14] whereas the chemical shift of H-3 for our compound is δ 3.85, consistent with most 3α-hydroxyl triterpenoids.[16] Although in the literature,[1a] the authors supported the structure elucidation with HMBC spectrum, in which a long-range correlation between OCO*CH*$_3$ (δc 21.2) and H-3β was observed, but this should be more compatible for a 25-hydroxyl/3-acetoxy rather than a 25-acetoxy/3-hydroxyl structure. In addition, we observed our synthesised target compound **1** exhibited long-range correlation between O*CO*CH$_3$ (δc 171.0) and H-25 (δ 4.59 d; 4.79 d) (Figure 2). Therefore, we presumed the structure of the reported compound[1a] is actually 25-hydroxy-3α-acetoxyolean-12-en-28-oic acid (**15**, Figure 3), a regioisomer of **1**.

Our deduction can be strengthened by the identical spectral data of **15** with those of 25-hydroxy-3α-acetoxyolean-12-en-28-oic acid isolated from the fruits of *Liquidabar formosana*,[2] as well as those for the one erroneously assigned as **1** in 2004.[1a]

In conclusion, the target compound 25-acetoxyl-3α-hydroxyolean-12-en-28-oic acid (**1**) was prepared by partial synthesis in an efficient manner (13 steps, 21% yield). This not only provides a new example of derivatisation at the "non-activated" positions in the synthesis of triterpenoids, but also opens up new avenues to explore the pharmacological activities of the 25-OH triterpenoids.

3. Experimental

3.1 General experimental procedures

NMR spectra were determined with Mercury Plus 300 MHz, 400 MHz or INOVA 500 MHz spectrometers in $CDCl_3$. *J* and δ values were given in hertz and ppm, respectively. Mass spectra (ESI-MS) were recorded on Agilent LC-MSD-Trap-SL instrument. Melting points were determined with a Yanaco micrometer and are uncorrected.

Organic extracts were dried over anhydrous Na_2SO_4. TLC was performed with precoated TLC plate silica gel 60 F_{254}, and visualised after spraying with 10% H_2SO_4 in ethanol. Column chromatography was done with silica gel 60 (200–300 mesh).

3.2 3-Oxooleanolic acid (3)

A suspension of oleanolic acid (2.00 g, 4.4 mmol) in 100 ml of CH_2Cl_2/acetone (1:1) was cooled to 5°C, and a solution of Jones' reagent (2 ml, 1.2 equiv.) was added dropwise over 30 min maintaining the bath temperature at 5°C, and the mixture was stirred for an additional 15 min. To the reaction mixture *i*-PrOH (1 ml) and H_2O (4 ml) were added, and the resulting mixture was stirred at room temperature for 10 min. Water (30 ml) and CH_2Cl_2 (20 ml) were added to the mixture and organic layer was separated, which was then washed with saturated aq. NaCl (20 ml), dried, and concentrated *in vacuo*. Pure 3-oxo-oleanolic acid (**3**) was obtained by crystallisation in methanol. Compound **3**: mp 178–179°C. TLC (PE/Acetone (4:1)) R_f 0.33. ^{1}H NMR (300 MHz in $CDCl_3$): δ_H 0.80 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 1.02 (s, 3H, CH_3); 1.04 (s, 3H,

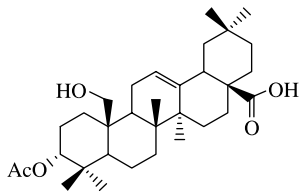

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Figure 3. Structure of 25-hydroxy-3α-acetoxylolean-12-en-28-oic acid (**15**).

CH_3); 1.08 (s, 3H, CH_3); 1.14 (s, 3H, CH_3); 2.36 (m, 1H, H-2); 2.54 (m, 1H, H-2); 2.68 (dd, 1H, $J = 4.5$, 13.8 Hz, H-18); 5.30 (t, 1H, $J = 3.3$ Hz, H-12). ^{13}C NMR (100 MHz in $CDCl_3$): δ_C 15.0, 16.9, 19.5, 21.4, 22.9, 23.5, 23.5, 25.8, 26.4, 27.7, 30.7, 32.1, 32.4, 33.0, 33.8, 34.1, 36.8, 39.1, 39.2, 41.0, 41.7, 45.8, 46.6, 46.9, 47.4, 55.3, 122.4, 143.6, 183.9, 217.7. ESI-MS (+): *m/z* 455.4 $[M + H]^+$. Its spectral data are identical to those reported in.[5]

3.3 3-Oxooleanano-28-benzyl ester (4)

To a solution of 3-oxooleanolic acid (2.00 g, 4.41 mmol) in 70 ml of dioxane, 2.8 ml of benzyl chloride (22.1 mmol) and 1.40 g of K_2CO_3 (10.14 mmol) were added. The mixture was refluxed for 20 h, and the solid was removed by filtration. The solvent was diluted with CH_2Cl_2 (150 ml). The organic layer was washed with saturated aq. NaCl (100 ml × 2), dried, and concentrated. The residue was crystallised in $CHCl_3$ and MeOH to give needle crystals (2.37 g, 99%). Compound **4**: mp 159–160°C. TLC (PE/Acetone (4:1)) R_f 0.51. ^{1}H NMR (500 MHz in $CDCl_3$): δ_H 0.65 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 1.01 (s, 3H, CH_3); 1.04 (s, 3H, CH_3); 1.08 (s, 3H, CH_3); 1.13 (s, 3H, CH_3); 2.35 (m, 1H, H-2); 2.53 (m, 1H, H-2); 2.91 (dd, 1H, $J = 4.0$, 13.5 Hz, H-18); 5.07 (ABq, 2H, $J = 13.0$ Hz, O*CH*$_2$Ph); 5.31 (br s, 1H, H-12); 7.35 (m, 5H, Ph). ^{13}C NMR (125 MHz in $CDCl_3$): δ_C 15.0, 16.8, 19.6, 21.5, 23.0, 23.5, 23.6, 25.8, 26.4, 27.6, 30.7, 32.2, 32.3, 33.1, 33.9, 34.1, 36.7, 39.1, 39.3, 41.5, 41.8, 45.8, 46.8, 46.9, 47.4, 55.3, 65.9, 122.3, 127.9, 128.0, 128.0, 128.4, 128.4, 136.4, 143.7, 177.4, 217.7. ESI-MS (+): *m/z* 545.3 $[M + H]^+$. Its spectral data are identical to those reported in.[15]

3.4 3-(1′, 3′-Dioxolane) oleanano-28-benzyl ester (5)

A suspension of **4** (893 mg, 1.642 mmol), toluene (40 ml), ethylene glycol (1.4 ml, 8.21 mmol), and TsOH (311 mg, 1.642 mmol) was refluxed for 10 h with azeotropic removal of water with a Dean-Stark apparatus. The resulting mixture was cooled and washed with saturated aq. $NaHCO_3$ (100 ml), and the aqueous layer was extracted with EtOAc (100 ml). The organic layers were combined and washed with saturated aq. NaCl, dried, concentrated to give crude **5**. Compound **5** (910 mg, 94.3%) was obtained by crystallisation in $CHCl_3$ and MeOH, mp 240–241°C. TLC (PE/Acetone (4:1)) R_f 0.71. ^{1}H NMR (400 MHz in $CDCl_3$): δ_H 0.61 (s, 3H, CH_3); 0.84 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.92 (s, 9H, CH_3 × 3); 1.13 (s, 3H, CH_3); 2.90 (dd, 1H, $J = 4.0$, 14.0 Hz; H-18); 3.90 (m, 4H, O*CH*$_2$*CH*$_2$O); 5.07 (ABq, 2H, $J = 12.4$ Hz; O*CH*$_2$Ph); 5.28 (t, 1H, $J = 3.2$ Hz, H-12); 7.34 (m, 5H, Ph). ^{13}C NMR (125 MHz in $CDCl_3$): δ_C 15.1, 16.9, 18.5, 20.2, 23.0, 23.0, 23.4, 23.7, 26.1, 26.7, 27.6, 30.7, 32.4, 32.5, 33.1, 33.9, 36.8, 36.9, 39.3, 41.3, 41.7, 42.0, 45.9, 46.7, 47.4, 53.3, 64.7, 64.9, 65.9, 113.3, 122.5, 127.9, 128.0, 128.0, 128.4, 128.4, 136.5, 143.7, 177.5. ESI-MS (+): *m/z* 589.4 $[M + H]^+$.

3.5 3-(1′,3′-Dioxolane) oleanano-11-oxo-28-benzyl ester (6)

Compound **5** (1.20 g, 2.04 mmol) was dissolved in CCl_4 (30 ml) and H_2O (30 ml), and $NaIO_4$ (2.18 g, 10.2 mmol), Bu_4NBr (1.31 g, 4.08 mmol) and $RuCl_3$ (84.87 mg, 0.41 mmol) were added. After stirring at room temperature for 24 h, the mixture was filtrated. The organic layer was separated, washed with saturated aq. NaCl (30 ml) and concentrated. Compound **6** was obtained by crystallisation in $CHCl_3$ and MeOH, mp 225–227°C. TLC (PE/Acetone (4:1)) R_f 0.52. ^{1}H NMR (400 MHz in $CDCl_3$): δ_H 0.74 (s, 3H, CH_3); 0.84 (s, 3H, CH_3); 0.92 (s, 6H, CH_3 × 2); 0.94 (s, 3H, CH_3); 1.10 (s, 3H, CH_3); 1.34 (s, 3H, CH_3); 2.36 (s, 1H, H-9); 3.02 (dd, 1H, $J = 4.0$, 14.0 Hz; H-18); 3.94 (m, 4H, O*CH*$_2$*CH*$_2$O); 5.07 (ABq, 2H, $J = 12.4$ Hz, O*CH*$_2$Ph); 5.63 (br s, 1H, H-12); 7.34 (m, 5H, Ph). ^{13}C NMR (125 MHz in $CDCl_3$): δ_C 16.1, 17.5, 18.9, 20.2, 22.8, 23.0, 23.4, 23.7, 26.7, 27.6, 29.7, 30.6, 31.6, 32.7, 32.8, 33.7, 37.2, 37.3, 41.6, 42.3, 43.5, 44.2, 44.9, 46.2, 53.0, 61.6, 64.6, 64.8, 66.3, 112.8, 128.0, 128.1, 128.1, 128.5, 128.5, 135.9, 168.4, 176.6, 200.3.

3.6 3-(1′,3′-Dioxolane) oleanano-11β-hydroxyl-28-benzyl ester (7)

Compound **6** (868 mg, 1.439 mmol) was dissolved in dry THF (40 ml) and cooled to −5°C, and $NaBH(C_2H_5)_3$ (7.2 ml, 7.2 mmol, 1 M in THF) was added dropwise. Reaction temperature was changed to room temperature after 30 min, and the mixture was stirred for an additional 1.5 h. 50 ml of H_2O was poured, and extracted with EtOAc (100 ml × 3). The organic layers were combined and washed with saturated aq. NaCl (80 ml), dried, and concentrated. The residue was purified by silica gel chromatography (PE/Acetone (20:1)) (96–99%).

Compound **7**: mp 183–185°C. TLC (PE/Acetone (4:1)) R_f 0.43. ^{1}H NMR (400 MHz in CD_3COCD_3): δ_H 0.80 (s, 3H, CH_3); 0.91 (s, 3H, CH_3); 0.92 (s, 6H, CH_3 × 2); 0.94 (s, 3H, CH_3); 1.11 (s, 3H, CH_3); 1.43 (s, 3H, CH_3); 2.84 (d, 1H, $J = 5.6$ Hz, 11-*OH*); 2.99 (dd, 1H, $J = 4.4$, 14.0 Hz, H-18); 3.90 (m, 4H, O*CH*$_2$*CH*$_2$O); 4.33 (dd, 1H, $J = 5.6$, 4.0 Hz, H-11); 5.08 (ABq, 2H, $J = 12.4$ Hz, O*CH*$_2$Ph); 5.33 (d, 1H, $J = 3.6$ Hz, H-12); 7.32 (m, 5H, Ph). ^{13}C NMR (100 MHz in CD_3COCD_3): δ_C 18.4, 19.5, 20.7, 23.6, 23.7, 23.8, 25.9, 27.1, 28.6, 30.0, 31.3, 33.2, 33.7, 34.3, 37.3, 38.9, 39.8, 42.0, 42.6,

43.0, 46.7, 47.1, 53.5, 54.7, 65.2, 65.4, 66.3, 66.5, 113.5, 128.2, 128.7, 128.8, 128.8, 129.3, 129.3, 137.6, 145.9, 177.3. ESI-MS (+): *m/z* 643.4 $[M + K]^+$.

A minor product **7a** (1–4%) was characterised as 3-(1′,3′-dioxolane) oleanano-11α-hydroxyl-28-benzyl ester, the 11-epimer of **7**.

Compound **7a**: TLC (PE/Acetone (4:1)) R_f 0.40. ^{1}H NMR (300 MHz in CD_3COCD_3): δ_H 0.65 (s, 3H, CH_3); 0.81 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.91 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.24 (s, 3H, CH_3); 2.89 (dd, 1H, $J = 4.2$, 13.2 Hz, H-18); 2.92 (d, 1H, $J = 7.2$ Hz, 11-*OH*); 3.88 (m, 4H, OCH_2CH_2O); 4.08 (m, 1H, H-11); 5.08 (br s, 2H, OCH_2Ph); 5.27 (d, 1H, $J = 3.9$ Hz, H-12); 7.37 (m, 5H, Ph).

3.7 3-(1′, 3′-Dioxolane) oleanano-11β, 25-dihydroxyl-28-benzyl ester (10)

A stream of NOCl, generated from $NaNO_2$ (87.4 g) and 35% HCl (509 ml), was introduced to a solution of **7** (100 mg, 0.166 mmol) in pyridine (5 ml) at −20°C. The mixture was stirred at −20°C for 30 min and poured into ice-water (10 ml). The resulting suspension was extracted with EtOAc (25 ml × 3), and the organic layers were combined and washed with saturated aq. NaCl (25 ml), dried, and concentrated. The crude nitrite was used for the next photochemical transformation without further purification.

The nitrite **8** (120 mg, 0.189 mmol) in dry toluene (12 ml) was irradiated with a 175 W high pressure lamp under nitrogen at room temperature for 24 h. The toluene was removed *in vacuo*, and the unstable residue in dioxane (12 ml), H_2O (4 ml), AcOH (8 ml) was treated with 75 mg/ml sodium nitrite in water (3.6 ml) at 0°C and left for 4 h. The mixture was extracted with EtOAc (25 ml × 3), and the organic layers were combined and washed with saturated aq. $NaHCO_3$ (25 ml × 3), and saturated aq. NaCl (25 ml × 3), dried, and concentrated. The residue was dissolved in $CHCl_3$ (3 ml) and MeOH (3 ml), 240 mg (6.488 mmol) of $NaBH_4$ was added and stirred for 6 h at room temperature. The solution was diluted with $CHCl_3$ (20 ml), the organic layer was washed with saturated aq. NaCl (10 ml × 3), dried, and concentrated. The residue was purified by silica gel chromatography (PE/Acetone (10:1)). Product **10** (54 mg) was afforded in 61% yield in four steps.

Compound **10**: mp 194–196°C. TLC (PE/Acetone (4:1)) R_f 0.24. ^{1}H NMR (500 MHz in $CDCl_3$): δ_H 0.87 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 0.94 (s, 3H, CH_3); 1.06 (s, 3H, CH_3); 1.12 (s, 3H, CH_3); 2.95 (dd, 1H, $J = 3.5$, 13.5 Hz, H-18); 3.92 (m, 5H, OCH_2CH_2O and H-25); 4.11 (d, 1H, $J = 13.0$ Hz, H-25); 4.18 (t, 1H, $J = 4.0$ Hz, H-11); 5.08 (ABq, 2H, $J = 12.5$ Hz, OCH_2Ph); 5.37 (d, 1H, $J = 4.0$ Hz, H-12); 7.34 (m, 5H, Ph). ^{13}C NMR (125 MHz in $CDCl_3$): δ_C 17.8, 20.8, 21.2, 23.1, 23.6, 24.1, 25.1, 26.7, 27.9, 30.7, 30.7, 32.1, 33.0, 33.8, 34.2, 39.3, 41.5, 42.0, 42.5, 43.3, 45.5, 46.6, 52.1, 54.8, 59.0, 64.5, 64.9, 65.3, 66.2, 113.0, 124.1, 128.3, 128.3, 128.3, 128.5, 128.5, 136.1, 148.5, 177.2. ESI-MS (+): *m/z* 643.3 $[M + Na]^+$; ESI-MS (−): *m/z* 655.3 $[M + Cl]^-$.

3.8 3-(1′, 3′-Dioxolane) oleanano-11β-hydroxyl-25-acetoxy-28-benzyl ester (11)

A solution of **10** (53 mg, 0.085 mmol) in $CHCl_3$ (1 ml) and pyridine (1.5 ml) was treated with Ac_2O (20 μl, 0.196 mmol) at room temperature for 13 h. The solution was diluted with $CHCl_3$ (20 ml), the organic layer was washed with saturated aq. NaCl (10 ml × 3), dried, and concentrated. Sample **11** (31 mg) was obtained by silica gel chromatography (PE/Acetone (20:1)) in 56% yield. 25 mg of starting material **10** was recycled in 44% yield.

Compound **11**: TLC (PE/Acetone (3:1)) R_f 0.68. ^{1}H NMR (300 MHz in $CDCl_3$): δ_H 0.83 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.93 (s, 3H, CH_3); 1.01 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 2.06 (s, 1H, OCOCH_3); 2.97 (dd, 1H, $J = 3.9$, 14.1 Hz, H-18); 3.93 (m, 4H, OCH_2CH_2O); 4.29 (br s, 1H, H-11); 4.46 (d, 1H, $J = 12.0$ Hz, H-25); 4.89 (d, 1H, $J = 12.0$ Hz, H-25); 5.07 (ABq, 2H, $J = 12.3$ Hz; OCH_2Ph); 5.33 (d, 1H, $J = 3.9$ Hz, H-12); 7.34 (m, 5H, Ph). ^{13}C NMR (150 MHz in $CDCl_3$): δ_C 18.1, 18.9, 19.6, 21.2, 22.9, 23.1, 23.6, 25.6, 27.8, 28.4, 30.7, 32.2, 32.4, 32.7, 33.0, 33.8, 39.0, 39.8, 41.2, 42.0, 42.4, 45.7, 46.4, 52.6, 53.8, 64.5, 64.8, 64.9, 65.4, 66.1, 112.8, 125.3, 128.2, 128.3, 128.3, 128.5, 128.5, 136.2, 147.0, 170.7, 177.1. ESI-MS (+): *m/z* 685.4 $[M + Na]^+$; ESI-MS (−): *m/z* 619.4 $[M - CH_3CO]^-$.

3.9 3-Oxo-25-acetoxy-oleanolic acid (12)

Compound **11** (20 mg, 0.030 mmol) dissolved in EtOH (1 ml, pretreated with $NaHCO_3$) was hydrogenated over 10% Pd-C (45 mg) at room temperature for 36 h. The reaction mixture was filtrated and concentrated. The residue (15 mg, 0.027 mmol) was treated with 30% TFA in CH_2Cl_2 (1 ml) at room temperature for 3 h. 10 ml of saturated aq. $NaHCO_3$ was poured into the reaction solution, and extracted with EtOAc (20 ml × 2). The organic layers were combined and washed with saturated aq. NaCl (20 ml), dried, and concentrated. The residue was purified by silica gel chromatography (PE/Acetone (10:1)). 13 mg of sample **12** was obtained in 94% yield.

Compound **12**: TLC (PE/Acetone (3:1)) R_f 0.26. ^{1}H NMR (600 MHz in $CDCl_3$): δ_H 0.74 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.93 (s, 3H, CH_3); 0.96 (s, 3H, CH_3); 1.08 (s, 3H, CH_3); 1.17 (s, 3H, CH_3); 1.96 (s, 1H, OCOCH_3);

2.35 (m, 1H, H-2); 2.54 (m, 1H, H-2); 2.83 (m, 1H, H-18); 4.25 (d, 1H, $J = 12.0$ Hz, H-25); 4.28 (d, 1H, $J = 12.0$ Hz, H-25); 5.28 (br s, 1H, H-12). ^{13}C NMR (150 MHz in $CDCl_3$): δ_C 17.3, 19.9, 20.0, 21.2, 23.1, 23.8, 23.9, 26.2, 28.0, 29.6, 29.9, 30.9, 31.9, 32.6, 33.3, 34.0, 34.7, 38.8, 39.3, 41.2, 41.9, 46.1, 46.1, 46.7, 48.8, 52.7, 64.0, 122.2, 143.8, 171.3, 183.7, 216.9.

Compound **14**: ^{1}H NMR (500 MHz in $CDCl_3$): δ_H 0.91 (s, 3H, CH_3); 0.94 (s, 3H, CH_3); 0.98 (s, 3H, CH_3); 1.06 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 1.10 (s, 3H, CH_3); 2.42 (m, 1H, H-2); 2.70 (m, 1H, H-2); 2.99 (m, 1H, H-18); 3.95 (d, 1H, $J = 9.0$ Hz, H-25); 4.19 (d, 1H, $J = 9.0$ Hz, H-25); 4.33 (br s, 1H, H-11); 5.62 (d, 1H, $J = 4.0$ Hz, H-12). ESI-MS (+): *m/z* 491.2 $[M + Na]^+$; ESI-MS (−): *m/z* 467.3 $[M - H]^-$.

3.10 3α-Hydroxyl-25-acetoxy-oleanolic acid (1)

A suspension of **12** (10 mg, 0.020 mmol), *i*-PrOH (0.5 ml), Al(*i*-PrO)$_3$ (43 mg, 0.215 mmol), and $AlCl_3$ (2 mg, 0.015 mmol) was refluxed for 1 h. 2 ml of 10% HCl was added and stirred for 10 min at room temperature. The reactive solution was extracted with EtOAc (20 ml × 3). The organic layers were combined and washed with saturated aq. NaCl (20 ml), dried, and concentrated. The residue was purified by silica gel chromatography (PE/Acetone (10:1)). Product **1** (8 mg, 80% yield) and its isomer **13** (2 mg, 20% yield) were afforded.

Compound **1**: TLC (PE/Acetone (3:1)), R_f 0.26. ^{1}H NMR (600 MHz in C_5D_5N): δ_H 0.90 (s, 3H, CH_3); 0.97 (s, 3H, CH_3); 0.99 (s, 3H, CH_3); 1.17 (s, 3H, CH_3); 1.18 (s, 3H, CH_3); 1.24 (s, 3H, CH_3); 2.02 (s, 1H, OCO*CH$_3$*); 2.81 (dd, 1H, $J = 4.2$, 13.8 Hz, H-18); 3.45 (br s, 1H, H-3); 4.59 (d, 1H, $J = 12.0$ Hz, H-25); 4.79 (d, 1H, $J = 12.6$ Hz, H-25); 5.25 (t, 1H, $J = 3.0$ Hz, H-12). ^{13}C NMR (150 MHz in C_5D_5N): δ_C 18.0, 18.6, 21.2, 22.7, 23.9, 23.9, 25.3, 26.7, 27.4, 28.5, 28.9, 29.9, 30.2, 31.1, 33.4, 33.5, 34.4, 37.9, 40.2, 41.0, 42.1, 42.7, 46.7, 46.8, 48.7, 49.4, 64.1, 74.9, 123.0, 145.1, 171.0, 180.4. HR-ESI-MS (+): *m/z* 537.3561 $[M + Na]^+$ (Calcd for $C_{32}H_{50}NaO_5$, 537.3556).

25-Acetoxy-oleanolic acid (**13**): TLC (PE/Acetone (3:1)) R_f 0.21. ^{1}H NMR (300 MHz in $CDCl_3$): δ_H 0.84 (s, 3H, CH_3); 0.85 (s, 3H, CH_3); 0.91 (s, 3H, CH_3); 0.93 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.14 (s, 3H, CH_3); 2.05 (s, 1H, OCO*CH$_3$*); 2.81 (dd, 1H, $J = 4.5$, 13.8 Hz, H-18); 3.25 (dd, 1H, $J = 4.8$, 4.8 Hz, H-3); 4.38 (d, 1H, $J = 12.6$ Hz, H-25); 4.46 (d, 1H, $J = 12.6$ Hz, H-25); 5.26 (t, 1H, $J = 3.6$ Hz, H-12). ESI-MS (+): *m/z* 471.3 $[M - CH_3CO^-]^+$.

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