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Two New 19-Hydroxyursolic Acid-type Triterpenes from Peruvian 'Uña de Gato' (*Uncaria tomentosa*)

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Abstract—Two new triterpenes having a 19-hydroxyursolic acid skeleton were isolated from 'Uña de Gato' (*Uncaria tomentosa*), a traditional herbal medicine in Peru. Their structures were determined by spectroscopic analyses and chemical conversion.

Introduction

Uña de Gato (Cat's claw)[1] is a Peruvian traditional herbal medicine for the treatment of various ailments including arthritics, inflammation and cancer. The original plants of Uña de Gato are known to be *Uncaria tomentosa* and *U. guianensis* (Rubiaceae) that are distributed in the South American continent. Chemical studies on the constituents of these *Uncaria* species have been done during the last two decades. From *U. tomentosa*, oxindole and indole alkaloids,[2–5] quinovic acid glycosides,[4,6–8] polyoxygenated triterpenes,[8,9] catechins[10] and sterols[11] were isolated. From *U. guianensis*, oxindole and indole alkaloids[2] and quinovic acid glycosides[12] were isolated. Recently, pharmacologically significant activities such as antiinflammatory,[4,13] antioxidant,[14] anti-HIV[15] and anticancer effects[16] have been reported. We studied the constituents of Peruvian 'Uña de Gato' (*U. tomentosa*) as an extension of our chemical studies on the constituents of *Uncaria* species of Rubiaceae.[17] We report here the isolation and structure elucidation of new triterpenes from Peruvian 'Uña de Gato' (*U. tomentosa*).

Results and Discussion

From the MeOH extract of Peruvian 'Uña de Gato' (*U. tomentosa*), two new triterpenes (**1**, **2**) were isolated together with two known triterpenes [3β, 6β, 19α-trihydroxy-urs-12-en-23-al-28-oic acid (**3**), 3β, 6β, 19α-trihydroxy-urs-12-en-28-oic acid (**4**)]. The structures of the known compounds (**3**, **4**) were deduced from the spectroscopic data and were confirmed by comparison with the reported data (Fig. 1).[9,17g]

The HRFABMS spectrum of **1** under the negative ion mode gave a quasi molecular ion peak at *m/z* 499.3058 ($[M-H]^-$) corresponding to the molecular formula $C_{30}H_{43}O_6$ (*m/z* 499.3059). The 1H NMR spectrum (CD_3OD) showed one singlet peak of aldehyde at δ 9.57, five singlet peaks of methyl groups at δ 1.49, 1.47, 1.21, 0.97 and 0.84, one doublet peak of a methyl group at δ 0.93 (J=6.6 Hz), and a peak of an olefinic proton at δ 5.35 (br-t). In the ^{13}C NMR spectrum (CD_3OD), one aldehyde carbon at δ 210.6, one carboxyl carbon at δ 182.3, six methyl carbons, and two sp^2 carbons of a tri-substituted olefin at δ 139.7 and 128.9 were observed. Therefore, **1** was deduced to be a 19-substituted ursan-type triterpenoid possessing both an aldehyde and a carboxyl group. Furthermore, a signal at δ 213.5 due to a ketone carbon was observed in the ^{13}C NMR spectrum. In the HMBC spectrum (Fig. 2), the two-bond C–H couplings were observed between the ketone carbon (δ 213.5) and H-5 (δ 2.69), and between the same ketone carbon and H_2-7 (δ 2.57 and 1.89, each d), indicating that the ketone was located at C-6 in the ursan skeleton. Cross-peaks between H_3-24 (δ 1.49) and both C-3 (δ 75.5) and an aldehyde carbon (δ 210.6) indicated that the aldehyde group was located on C-4. In the differential NOE experiment, irradiation of the aldehyde proton (δ 9.57) led to the enhancement of the signal intensity of H-5 (3%), indicating that the aldehyde is at the C-23 position. HMBC cross-peaks between H-18 (δ 2.53) and a carboxyl carbon (δ 182.3) and between H-18 and olefinic carbons (δ 139.7 and 128.9) demonstrated that C-28 is oxidized to a carboxylic acid. Irradiation of the olefinic proton (δ 5.35) led to 3% enhancement of the signal intensity of H_3-29 in the differential NOE experiment, indicating that the hydroxyl group on C19 is α-orientated. From

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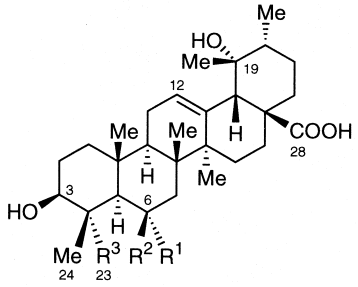


1 : R^1, R^2=O, R^3=CHO
2 : R^1, R^2=O, R^3=CH_2OH

3 : R^1=H, R^2=OH, R^3=CHO
4 : R^1=H, R^2=OH, R^3=Me

Figure 1.

the above data, the structure of **1** was deduced to be 3β, 19α-dihydroxy-6-oxo-urs-12-en-23-al-28-oic acid.

In 1989,[17g] we reported our observation that a 19α-hydroxy-ursan-type triterpene shows a characteristic proton signal in a region around δ 2.6 ppm as a double triplet with coupling constants of J_1=J_2=~13 Hz and J_3=~4.5 Hz; this deshielded signal was observed well separated from other ordinal methylene proton signals. We assigned this peak to 16α-H (axial) on the basis of the peak shape with three coupling constants, $J_{16\alpha,16\beta}$=$J_{15\beta,16\alpha}$=~13 Hz and $J_{15\alpha,16\alpha}$=~4.5 Hz. We believed that the characteristic shift was reasonably explained in terms of a deshielding anisotropic effect due to a 19α-hydroxy group in addition to the same effect caused by a $\Delta_{12,13}$-double bond. This time again, as expected, we noticed that compound **1** showed the corresponding signal at δ 2.60 with coupling constants of $J_{16\alpha,16\beta}$=$J_{15\beta,16\alpha}$=13.3 Hz and $J_{15\alpha,16\alpha}$=4.6 Hz; this observation fully proved the useful diagnostic value of the corresponding peak. We then precisely proved the correctness of the signal assignment by extensive use of NMR measurements as follows. In the HMBC spectrum, a cross-peak between H-18 (δ 2.53) and a methylene carbon (δ 26.4) which is correlated with the double triplet proton at δ 2.60 in the HMQC spectrum was observed, suggesting that the proton at δ 2.60 could be assigned as H-16 or H-22. Furthermore, an HMBC cross-peak between the proton at δ 2.60 and C-15 methylene carbon (δ 29.5), whose assignment was established by the HMBC correlation between H_3-27 (δ 1.47), was observed. Therefore, the proton at δ 2.60 was confirmed to be H-16.

To confirm the proposed structure, a chemical correlation of **1** with a molecule with established chemical structure was carried out. **1** was treated with CH_2N_2 in MeOH, and **5** was produced instead of the expected methyl ester (**6**). FABMS of **5** revealed a quasi molecular ion peak at *m/z* 529 ($[M+H]^+$). In the ^{1}H NMR spectrum, a singlet peak due to a methyl ester at C-28 was observed at δ 3.60. Furthermore, a characteristic signal due to aldehyde in the starting material disappeared, and signals at δ 4.26 (H-23), δ 4.31 (dd, H-31), δ 3.55 (dd, H-31) and signals due to C-23 (δ 77) and C-31 (δ 75.7) were observed, indicating that a tetrahydrofurane ring moiety fused to the A ring was formed. The configuration of the 23-hydroxy group was confirmed to be *S* by the differential NOE experiment; irradiation of the methyne proton (δ 4.26) led to the enhancement of the intensity of H_3-24 (5.4%). In 1989, compound **3** was found in *U. florida* in this laboratory, and was converted to compound **7**.[17g] We now employed this compound **7**. The hydroxyl group of **7** was oxidized with pyridinium dichromate in CH_2Cl_2 to produce the ketone (**8**). Subsequent deacetylation of **8** with NaOMe in MeOH gave **6**. The expected tetrahydrofurane ring formation took place when **6** was treated with CH_2N_2 in MeOH, and the resulting material was proved to be completely identical with **5** derived from natural **1**. Thus, the structure of **1** was precisely established. In the above reaction of **6** with CH_2N_2 a minor product (**9**) was obtained and its structure was determined by the following spectroscopic observations. In the 1H and ^{13}C NMR spectra, a singlet 3H-signal at δ 2.34 in the 1H NMR spectrum and carbon signals at δ 217.1 and 29.8 in the ^{13}C NMR spectrum were observed instead of signals due to the aldehyde of the starting material, indicating the existence of an acetyl group. An HMBC cross-peak between an acetyl methyl (δ 2.34) and C-4 (δ 53.6) indicated that the acetyl group was positioned on C-4. The formation of compound **9** can be considered to take place through an intermediary oxirane (Schemes 1 and 2).

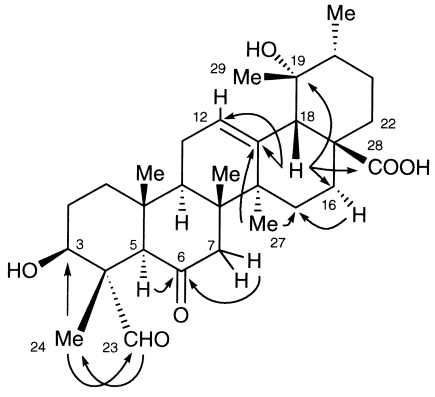
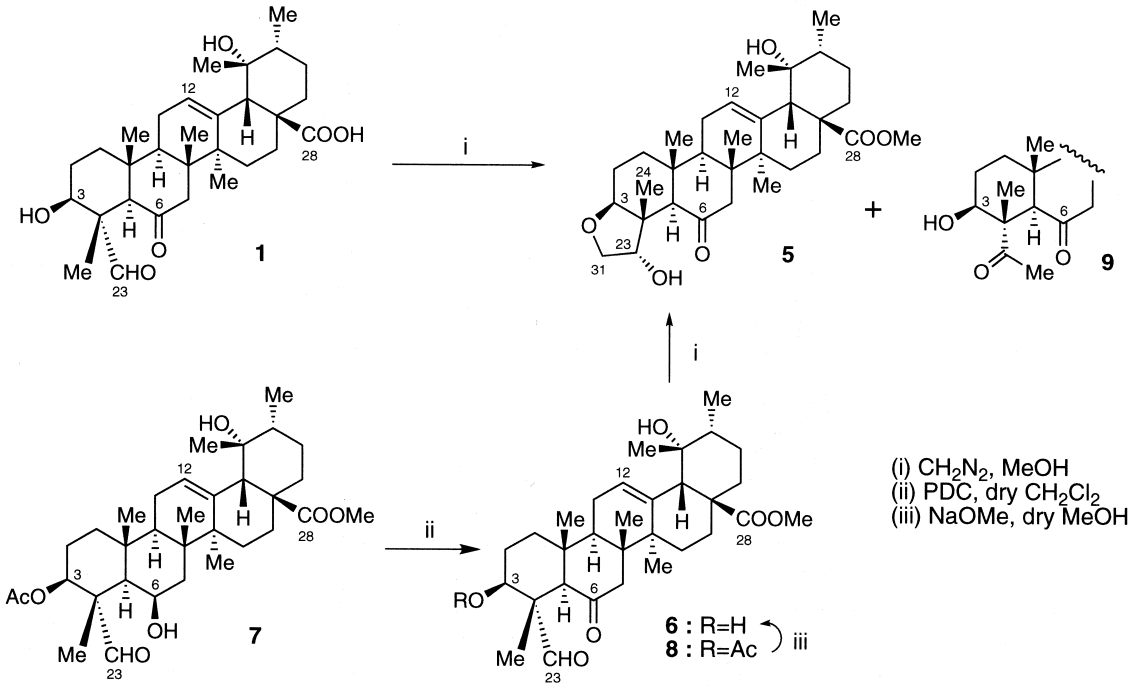
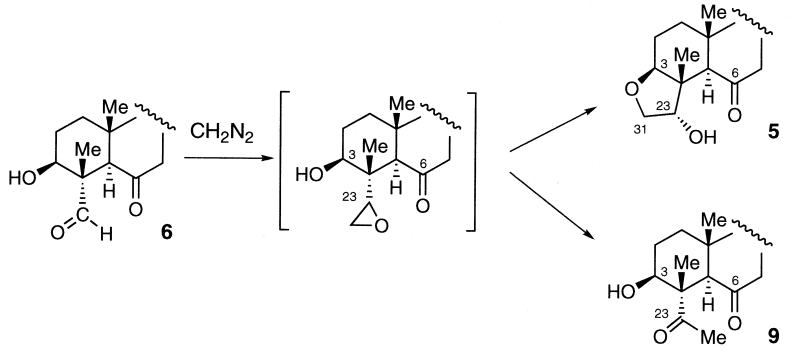


Figure 2. Selected HMBC of **1**.

The HRFABMS spectrum of **2** under the negative ion mode gave a quasi molecular ion peak at *m/z* 501.3214 ($[M-H]^-$) corresponding to the molecular formula $C_{30}H_{45}O_6$ (*m/z* 501.3216). The 1H NMR spectrum (CD_3OD) showed five singlet peaks of methyl groups at δ 1.48, 1.21, 1.06, 0.96 and 0.81, one doublet peak of a methyl group at δ 0.93 (*J*=6.6 Hz), a pair of doublet peaks due to a hydroxymethyl group at δ 3.51 and 3.38 (each d, *J*=10.7 Hz) and an olefinic proton at δ 5.34 (m). In the ^{13}C NMR spectrum (CD_3OD), six methyl carbons, one hydroxymethyl carbon at δ 66.0, one carboxyl carbon at δ 182.0 and two sp^2 carbons of a trisubstituted olefin at δ 139.6 and 129.0 were observed. The NMR spectra of **2** were similar to those of **1** except for the



Scheme 1.



Scheme 2.

existence of one hydroxymethyl group in place of an aldehyde group in **2**. In the HMBC spectrum, cross-peaks between H-5 (δ 2.62), H_2-7 (δ 2.73 and 1.79, each d) and a carbonyl carbon (δ 215.8) and between the hydroxymethyl protons and C-3 (δ 72.3), C-4 (δ 42.4), C-5 (δ 58.9) and C-24 (δ 12.8) were observed, indicating that a ketone and a hydroxymethyl group were located on C-6 and C-4, respectively. In the differential NOE experiment, irradiation of the hydroxymethyl proton (δ 3.38) led to the enhancement of the signal intensity of H-5 (2%) and H_3-24 (3%) indicating that 23-methyl carbon is oxidized to a hydroxymethyl group. Irradiation of the olefinic proton (δ 5.34) led to 3% enhancement of the signal intensity of H_3-29, indicating the hydroxyl group on C19 is α-orientated. From the above data, the structure of **2** was deduced to be 3β, 19α-dihydroxy-6-oxo-urs-12-en-23-ol-28-oic acid.

Further investigation including isolation of alkaloids, triterpene glycosides and other type compounds is in progress.

Experimental

General

1H and ^{13}C NMR spectra: recorded at 500 and 125.65 MHz, respectively. JEOL JNM A-500. EIMS: direct probe insertion at 70 eV. JEOL JMS-AM20. HREIMS: Hitachi RMU-7M. FABMS and HRFABMS: JEOL JMS-HX110. TLC: precoated Kieselgel 60 F254 plates (Merck, 0.25 mm thick). Column Chromatography: Kieselgel 60 [Merck, 70–230 mesh (for open chromatography) and 230–400 mesh (for flash chromatography)], DIAION HP20 [Mitsubishikasei]. MPLC: C. I. G. prepacked column CPS-HS-221-05 (SiO_2) and CPO-HS-221-20 (ODS) [Kusano Kagakukikai].

Plant material

'Uña de Gato' that was imported from Peru was used. The

Table 1. ^{13}C NMR Data for compounds **1** and **2** (Data were recorded in CD_3OD at 125 MHz)

C	1	2
1	39.3	39.9
2	27.4[a]	27.1[a]
3	75.5	72.3
4	53.0	42.4
5	62.2	58.9
6	213.5	215.8
7	50.4	51.7
8	46.6	48.1
9	48.0	48.6
10	42.1	44.0
11	24.8	25.0
12	128.9	129.0
13	139.7	139.6
14	43.12	43.0
15	29.5	29.5
16	26.4	26.4
17	[b]	[b]
18	55.0	54.9
19	73.6	73.5
20	43.06	43.1
21	27.3[a]	27.2[a]
22	38.9	38.9
23	210.6	66.0
24	12.1	12.8
25	17.9	17.4
26	18.8	18.0
27	25.6	25.5
28	182.3	182.0
29	27.0	27.0
30	16.5	16.6

[a] Interchangeable.
[b] Under CD_3OD signal.

original plant of Peruvian 'Uña de Gato' used in this study was *U. tomentosa* (stem, stem bark).

Extraction and Isolation

Peruvian 'Uña de Gato' (835 g dried weight) was extracted with hot MeOH six times (1 L×6) to give the MeOH extract (total 90 g). 50 g of the MeOH extract (1st, 2nd and 3rd extracts were combined) was subjected to column chromatography on DIAION HP20 (2.1 cm×85 cm) to give seven fractions; fr. A MeOH–H_2O=3:7 (500 mL) 12.80 g, fr. B MeOH–H_2O=1:1 (1 L) 8.22 g, fr. C MeOH–H_2O=7:3 (1 L) 13.44 g, fr. D MeOH (1 L) 12.44 g, fr. E MeOH–acetone=1:1 (1 L) 0.11 g, fr. F acetone (1 L) 0.60 g, insoluble (0.27 g). Fr. D (12.44 g) was purified by SiO_2 gel open column chromatography, flash column chromatography and MPLC (SiO_2, ODS) to give two known triterpenes (3β, 6β, 19α-trihydroxy-urs-12-en-23-al-28-oic acid (**3**, 4.9 mg), 3β, 6β, 19α-trihydroxy-urs-12-en-28-oic acid (**4**, 106.1 mg)) and two new triterpenes (**1**, **2**). New compounds were purified as follows. Fr. D (MeOH fraction) was subjected to SiO_2 gel open column chromatography using MeOH–$CHCl_3$. After evaporation of the fractions eluted with 35–65% MeOH–$CHCl_3$ (4263 mg), solid (837 mg) was filtered. The residue was then separated by SiO_2 gel open column chromatography using MeOH–$CHCl_3$. The fractions eluted with 7% MeOH–$CHCl_3$ were purified by MPLC (SiO_2, AcOEt–*n*-Hexane=4:1) to give **1** (7.7 mg). The fractions eluted with 5% MeOH–$CHCl_3$ were purified by SiO_2 gel flash column chromatography (AcOEt–*n*-Hexane=4:1) and MPLC (ODS, H_2O–MeOH=1:4) to give **2** (8.4 mg).

3β, 19α-Dihydroxy-6-oxo-urs-12-en-23-al-28-oic acid (1). Colorless amorphous powder: $[\alpha]_D^{25}$ +48.0 (*c* 0.38, MeOH); 1H NMR (CD_3OD, 500 MHz) δ 9.57 (1H, s, H-23), 5.35 (1H, br-t, *J*=3.7 Hz, H-12), 3.56 (1H, dd, *J*=11.4 and 4.5 Hz, H-3), 2.69 (1H, s, H-5), 2.60 (1H, ddd, *J*=13.3, 13.3 and 4.6 Hz, H-16α), 2.57 (1H, d, *J*=14.4 Hz, H-7), 2.53 (1H, s, H-18), 2.37 (1H, dd, *J*=11.2 and 6.6 Hz, H-9), 2.18 (1H, m, H-11), 2.06 (1H, ddd, *J*=18.3, 11.2 and 3.7 Hz, H-11), 1.89 (1H, d, *J*=14.4 Hz, H-7), 1.49 (3H, s, H_3-24), 1.47 (3H, s, H_3-27), 1.21 (3H, s, H_3-29), 0.97 (3H, s, H_3-25), 0.93 (3H, d, *J*=6.6 Hz, H_3-30), 0.84 (3H, s, H_3-26); ^{13}C NMR: Table 1; FABMS (negative, NBA) *m/z* 499 $[M-H]^-$; HRFABMS (negative, NBA) *m/z* 499.3058 $[M-H]^-$ (calcd for $C_{30}H_{43}O_6$, 499.3059).

3β, 19α-Dihydroxy-6-oxo-urs-12-en-23-ol-28-oic acid (2). Colorless amorphous powder: $[\alpha]_D^{24}$ +20.9 (*c* 0.37, MeOH); 1H NMR (CD_3OD, 500 MHz) δ 5.34 (1H, m, H-12), 3.54 (1H, dd, *J*=11.3 and 4.3 Hz, H-3), 3.51 and 3.38 (each 1H, d, *J*=10.7 Hz, H-23), 2.73 (1H, d, *J*=12.6 Hz, H-7), 2.62 (1H, s, H-5), 2.61 (1H, ddd, *J*=13.4, 13.4, 4.6 Hz, H-16α), 2.51 (1H, s, H-18), 2.33 (1H, br-dd, *J*=11.0 and 6.6 Hz, H-9), 2.16 and 2.01 (each 1H, m, H-11), 1.79 (1H, d, *J*=12.6 Hz, H-7), 1.48 (3H, s, H_3-27), 1.21 (3H, s, H_3-29), 1.06 (3H, s, H_3-24), 0.96 (3H, s, H_3-25), 0.93 (3H, d, *J*=6.6 Hz, H_3-30), 0.81 (3H, s, H_3-26); ^{13}C NMR: Table 1; FABMS (negative, NBA) *m/z* 501 $[M-H]^-$; HRFABMS (negative, NBA) *m/z* 501.3214 $[M-H]^-$ (calcd for $C_{30}H_{45}O_6$, 501.3216).

Reaction of natural 1 with CH_2N_2

An ether solution of freshly prepared CH_2N_2 was added to a solution of natural **1** (4.0 mg, 0.008 mmol) in MeOH (1 mL). After 15 min, the solvent was removed under reduced pressure. The residue was purified by HPLC (AcOEt–*n*-Hexane=1:1) to give **5** (1.0 mg, y. 24%).

Compound 5: 1H NMR ($CDCl_3$, 500 MHz) δ 5.42 (1H, br-t, *J*=3.3 Hz, H-12), 4.31 (1H, dd, *J*=10.4, 5.2 Hz, H-31), 4.26 (1H, m, H-23), 3.60 (3H, s, $COOCH_3$), 3.55 (1H, dd, *J*=10.4, 1.2 Hz, H-31), 3.30 (1H, dd, *J*=12.1, 3.5 Hz, H-3), 2.62 (1H, s, H-18), 2.54 (1H, m, H-16α), 2.52 (1H, d, *J*=14.7 Hz, H-7), 2.51 (1H, s, H-5), 2.30 (1H, dd, *J*=11.1, 6.6 Hz, H-9), 1.99 (1H, d, *J*=14.7 Hz, H-7), 1.38 (3H, s, H_3-27), 1.23 (3H, s, H_3-29), 1.01 (3H, s, H_3-25), 0.98 (3H, s, H_3-24), 0.95 (3H, d, *J*=6.7 Hz, H_3-30), 0.80 (3H, s, H_3-26); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 212.8 (C-6), 178.1 (C-28), 137.9 (C-13), 128.7 (C-12), 82.4 (C-3), 75.7 (C-31), 73.2 (C-19), 58.7 (C-5), 53.2 (C-18), 51.6 ($COOCH_3$), 49.0 (C-7), 47.9 (C-17), 46.9 (C-9), 45.3 and 45.2 (C-4 and C-8), 41.94 and 41.87 (C-10 and C-14), 41.1 (C-20), 38.7 (C-1), 37.2 (C-22), 28.1 (C-15), 27.4 (C-29), 25.9 (C-21), 25.5 (C-27), 25.2 (C-16), 24.3 (C-11), 21.6 (C-2), 19.1 (C-25), 18.3 (C-26), 16.0 (C-30), 14.7 (C-24), C-23 was under $CDCl_3$ signals; FABMS (NBA) *m/z* (%) 529 $[M+H]^+$, 511; HRFABMS (NBA) *m/z* 529.3518 $[M+H]^+$ (calcd for $C_{32}H_{49}O_6$, 529.3529).

Preparation of 6-oxo compound 8

A solution of **7** (10.0 mg, 0.018 mmol) in dry CH_2Cl_2 (1 mL) was added to a solution of pyridinium dichromate (9.1 mg, 0.024 mmol) in dry CH_2Cl_2 (2 mL) under argon at 0°C. The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was subjected to SiO_2 column chromatography using 10% MeOH–$CHCl_3$ and then AcOEt–*n*-hexane=1:1 to give 6-oxo compound **8** (10.0 mg, y. quant.).

*Compound **8**:* 1H NMR ($CDCl_3$, 500 MHz) δ 9.56 (1H, s, H-23), 5.41 (1H, m, H-12), 4.75 (1H, dd, *J*=11.4, 4.4 Hz, H-3), 3.59 (3H, s, COOCH_3), 2.67 (1H, s, H-5), 2.61 (1H, s, H-18), 2.54 (1H, m, H-16α), 2.48 (1H, d, *J*=14.7 Hz, H-7), 2.00 (3H, s, COCH_3), 1.99 (1H, d, *J*=14.7 Hz, H-7), 1.62 (3H, s, H_3-24), 1.38 (3H, s, H_3-27), 1.24 (3H, s, H_3-29), 1.00 (3H, s, H_3-25), 0.94 (3H, d, *J*=6.7 Hz, H_3-30), 0.76 (3H, s, H_3-26); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 210.0 (C-6), 206.7 (C-23), 178.1 (C-28), 170.0 (*C*OCH$_3$), 137.9 (C-13), 128.1 (C-12), 76.0 (C-3), 73.1 (C-19), 62.0 (C-5), 53.0, 51.6, 50.0, 49.1, 47.8, 46.6, 45.1, 41.7, 41.1, 40.9, 37.7, 37.2, 28.1, 27.4, 25.9, 25.2, 25.1, 23.7, 22.8, 21.0, 18.0, 17.4, 16.0, 12.6; EIMS *m/z* (%) 556 (M^+, 8), 496 (26), 424 (25), 219 (13), 201 (28), 179 (100), 146 (71); HREIMS *m/z* 556.3407 (calcd for $C_{33}H_{48}O_7$, 556.3401).

Deacetylation of 6-oxo compound 8

1 M NaOMe in MeOH solution (9 μL) was added to a solution of **8** (10.0 mg, 0.018 mmol) in dry MeOH (1 mL) under argon at room temperature and the mixture was stirred at the same temperature for 3 h. Cold water was added to the reaction mixture and the whole was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by MPLC (AcOEt–*n*-hexane=1:1) to give the alcohol **6** (6.8 mg, y. 74%).

*Compound **6**:* 1H NMR ($CDCl_3$, 500 MHz) δ 9.72 (1H, s, H-23), 5.41 (1H, m, H-12), 3.67 (1H, m, H-3), 3.59 (3H, s, COOCH_3), 2.62 (1H, s, H-5), 2.56 (1H, s, H-18), 2.54 (1H, ddd, *J*=11.0, 11.0, 4.6 Hz, H-16α), 2.49 (1H, d, *J*=14.7 Hz, H-7), 2.00 (1H, d, *J*=14.7 Hz, H-7), 1.56 (3H, s, H_3-24), 1.39 (3H, s, H_3-27), 1.23 (3H, s, H_3-29), 0.98 (3H, s, H_3-25), 0.94 (3H, d, *J*=6.7 Hz, H_3-30), 0.77 (3H, s, H_3-26); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 210.5 (C-6), 209.5 (C-23), 178.1 (C-28), 137.9 (C-13), 128.2 (C-12), 74.6 (C-3), 73.1 (C-19), 61.5 (C-5), 53.1, 51.7, 51.4, 49.1, 47.8, 46.7, 45.1, 41.7, 41.1, 38.0, 37.2, 28.1, 27.4, 26.5, 25.9, 25.2, 25.1, 23.8, 18.0, 17.4, 16.0, 11.7; EIMS *m/z* (%) 514 (M^+, 7), 454 (42), 408 (82), 382 (30), 336 (51), 249 (63), 219 (34), 201 (100); HRFABMS (NBA) *m/z* 515.3361 $[M+H]^+$ (calcd for $C_{31}H_{47}O_6$, 515.3372).

Reaction of 6 with CH_2N_2

An ether solution of freshly prepared CH_2N_2 was added to a solution of **6** (6.8 mg, 0.013 mmol) in MeOH (1 mL). After overnight, the solvent was removed under reduced pressure. The residue was purified by MPLC (AcOEt–*n*-Hexane=3:2) to give **5** (2.8 mg, y. 40%) and **9** (1.4 mg, y. 20%).

*Compound **9**:* 1H NMR ($CDCl_3$, 500 MHz) δ 5.40 (1H, br-t, *J*=3.8 Hz, H-12), 3.63 (1H, m, H-3), 3.59 (3H, s, COOCH_3), 2.88 (1H, s, H-5), 2.62 (1H, s, H-18), 2.54 (1H, m, H-16α), 2.49 (1H, d, *J*=14.7 Hz, H-7), 2.34 (3H, s, COCH_3), 1.93 (1H, d, *J*=14.7 Hz, H-7), 1.60 (3H, s, H_3-24), 1.39 (3H, s, H_3-27), 1.23 (3H, s, H_3-29), 0.96 (3H, s, H_3-25), 0.94 (1H, d, *J*=6.7 Hz, H-30), 0.73 (3H, s, H_3-26); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 217.1 (*C*OCH$_3$), 211.4 (C-6), 178.1 (C-28), 137.9 (C-13), 128.3 (C-12), 73.2 (C-19), 62.7 (C-5), 53.6 (C-4), 53.1 (C-18), 51.6 (COO*C*H$_3$), 29.8 (CO*C*H$_3$), 27.4 (C-29), 25.3 (C-27), 18.0 (C-26), 17.6 (C-25), 16.0 (C-30), 12.3 (C-24), 49.3, 47.8, 47.0, 45.2, 41.6, 41.1, 40.8, 38.3, 37.2, 28.1, 27.2, 25.9, 25.2, 23.8, C-3 was under $CDCl_3$ signals; FABMS (NBA) *m/z* (%) 529 $[M+H]^+$. HRFABMS (NBA) *m/z* 529.3506 $[M+H]^+$ (calcd for $C_{32}H_{49}O_6$, 529.3529).

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