New Triterpenoids from *Gentiana lutea*

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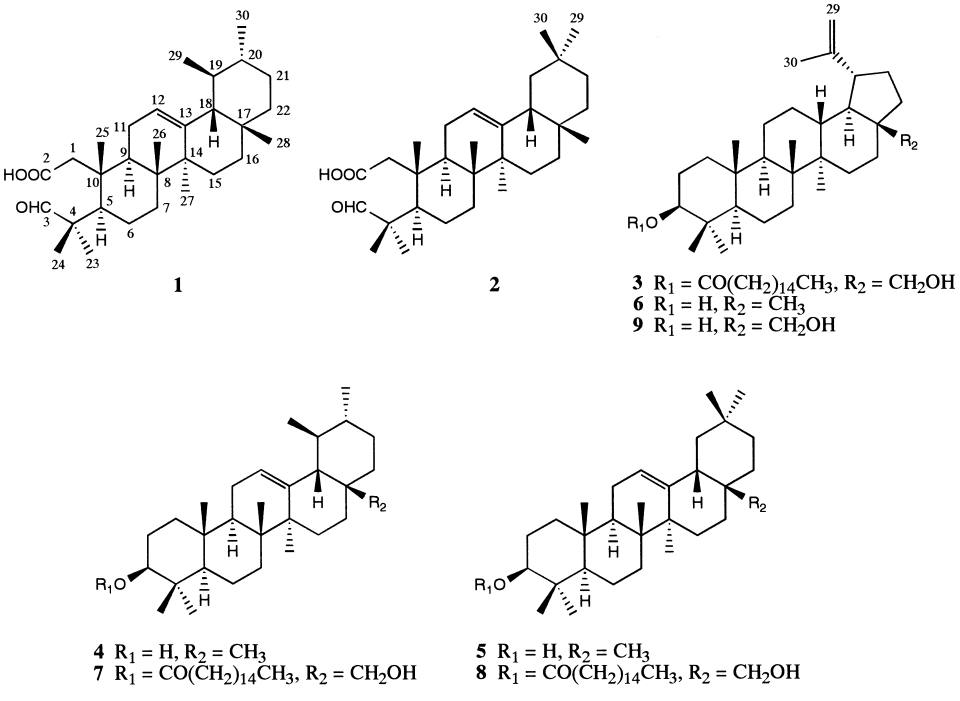
Three new triterpenoids, 2,3-*seco*-3-oxours-12-en-2-oic acid, 2,3-*seco*-3-oxoolean-12-en-2-oic acid, and betulin 3-*O*-palmitate, have been isolated from the rhizomes and roots of *Gentiana lutea*, together with five known ones. The structures of the new compounds were determined by spectral and chemical methods.

Key words *Gentiana lutea*; Gentianaceae; triterpenoid

The rhizomes and roots of *Gentiana lutea* L. (Gentianaceae) are the crude drug Gentianae Radix, used as an appetite stimulant.[1)] The constituents of this crude drug have been previously investigated and shown to contain secoiridoid glucosides[2)] and xanthones.[3)] In this paper, we describe the isolation and structural elucidation of three new triterpenoids, 2,3-*seco*-3-oxours-12-en-2-oic acid (**1**), 2,3-*seco*-3-oxoolean-12-en-2-oic acid (**2**) and betulin 3-*O*-palmitate (**3**), together with five known ones from the rhizomes and roots of *G. lutea*. The known compounds were identified as α-amyrin (**4**),[4)] β-amyrin (**5**),[4)] lupeol (**6**),[5)] uvaol 3-*O*-palmitate (**7**)[6)] and erythrodiol 3-*O*-palmitate (**8**),[7)] respectively, by comparison of their spectroscopic data with those previously described in the literature. To the best of our knowledge, this is the first report of the latter of these compounds from *G. lutea*. Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ +68.1°. The IR spectrum suggested the presence of a carbonyl group (1713 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O_3$ by high-resolution (HR)-MS and electron impact (EI)-MS gave fragment ion peaks at *m*/*z* 218, 203, and 189, diagnostic of triterpenoids having a Δ^{12}-double bond.[8)] The ^{1}H-NMR spectrum (Table 1) showed signals due to six tertiary methyl groups [δ_H 0.80 (3H, H_3-28), 1.01 (3H, H_3-25), 1.03 (3H, H_3-26), 1.05 (3H, H_3-27), 1.11 (3H, H_3-24), 1.17 (3H, H_3-23)], two secondary methyl groups [δ_H 0.77 (3H, H_3-29), 0.92 (3H, H_3-30)], a methylene group [δ_H 2.30 (1H, H-1a), 2.51 (1H, H-1b)], a trisubstituted olefinic proton [δ_H 5.16 (1H, H-12)], and an aldehyde proton [δ_H 9.78 (1H, H-3)]. The ^{13}C-NMR spectrum (Table 2) revealed 30 carbon signals that included two olefinic carbons [δ_C 124.2 (C-12), 139.4 (C-13)], a carboxyl carbon [δ_C 176.3 (C-2)], and an aldehyde carbon [δ_C 207.9 (C-3)]. The ^{1}H- and ^{13}C-NMR data for **1** were very similar to those of α-amyrin (**4**), except for the signals ascribed to ring A. The ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) correlations of H_2-1 to a carboxyl carbon (C-2) revealed that the carboxyl group was attached to C-2. The ^{1}H–^{13}C long-range correlations of H_3-23 and H_3-24 methyl groups to an aldehyde carbon (C-3) revealed that the aldehyde group was attached to C-3. In the nuclear Overhauser effect (NOE) difference spectra, NOEs were detected between H-5 and H_3-23 and between H_3-24 and H_3-25. These NOEs implied that H-5



1 **2**

3 $R_1 = CO(CH_2)_{14}CH_3$, $R_2 = CH_2OH$
6 $R_1 = H$, $R_2 = CH_3$
9 $R_1 = H$, $R_2 = CH_2OH$

4 $R_1 = H$, $R_2 = CH_3$
7 $R_1 = CO(CH_2)_{14}CH_3$, $R_2 = CH_2OH$

5 $R_1 = H$, $R_2 = CH_3$
8 $R_1 = CO(CH_2)_{14}CH_3$, $R_2 = CH_2OH$

Chart 1

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Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1**, **2**, and **4** (600 MHz, $CDCl_3$)

Hydrogen	1	2	4
1a	2.30 d (16.5)	2.28 d (16.5)	
1b	2.51 d (16.5)	2.49 d (16.5)	
3	9.78 s	9.76 s	3.23 dd (9.9, 5.1)
12	5.16 dd (4.4, 2.9)	5.20 dd (3.7, 3.7)	5.13 dd (3.6, 3.6)
23	1.17 s	1.16 s	1.00 s
24	1.11 s	1.10 s	0.79 s
25	1.01 s	1.00 s	0.96 s
26	1.03 s	0.99 s	1.01 s
27	1.05 s	1.12 s	1.07 s
28	0.80 s	0.83 s	0.80 s
29	0.77 d (5.9)	0.869 s[a)]	0.79 d (5.6)
30	0.92 d (6.2)	0.872 s[a)]	0.92 d (5.9)

Coupling constants (J in Hz) are given in parentheses. *a*) Assignments may be interchangeable.

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**, **2**, and **4** (150 MHz, $CDCl_3$)

Carbon	1	2	4
1	42.8	42.6	38.7[c)]
2	176.3	174.4	27.2
3	207.9	207.9	78.3
4	50.7	50.7	38.7[c)]
5	47.8	47.6	55.2
6	20.4	20.4	18.3
7	32.1	31.8	32.9
8	39.9	39.8	40.0
9	40.5	40.4	47.7
10	42.3	42.3	36.9
11	23.8	23.8	23.3
12	124.2	121.4	124.3
13	139.4	144.9	139.3
14	43.2	42.3	42.0
15	26.5	26.1	26.6
16	28.1	26.9	28.1
17	33.8	32.5	33.7
18	59.1	47.2	58.9
19	39.8	46.6	39.6[d)]
20	39.5	31.1	39.6[d)]
21	31.3	34.7	31.2
22	41.5	37.1	41.5
23	24.0	24.0	28.1
24	19.4[a)]	19.3[b)]	15.6[e)]
25	19.5[a)]	19.4[b)]	15.6[e)]
26	17.0	17.0	16.8
27	22.8	25.5	23.3
28	28.8	28.4	28.7
29	17.5	33.3	17.4
30	21.4	23.6	21.3

a, *b*) Assignments may be interchangeble. *c*—*e*) Signals were overlapped.

and H_3-23 methyl group occurred on the same face (α) of the molecule and H_3-24 and H_3-25 methyl groups occurred on the same face (β) of the molecule. Therefore **1** was determined to be 2,3-*seco*-3-oxours-12-en-2-oic acid.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ +52.7°. The IR spectrum suggested the presence of a carbonyl group (1724 cm^{-1}). The molecular formula was determined to be $C_{30}H_{48}O_3$ by HR-MS. The ^{1}H- and ^{13}C-NMR spectral data of **2** closely resembled those of **1**, except for the appearance of two tertiary methyl groups [δ_H 0.869 and 0.872 (each 3H, H_3-29, H_3-30); δ_C 23.6 (C-30), 33.3 (C-29)] instead of two secondary methyl groups in **1**. The HMBC correlations of H_3-29 and H_3-30 to C-19, C-20 and C-21 revealed that the H_3-29 and H_3-30 methyl groups were attached to C-20. Thus **2** was deduced to be 2,3-*seco*-3-oxoolean-12-en-2-oic acid.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ +34.9°. The molecular formula was determined to be $C_{46}H_{80}O_3$ by HR-MS. The ^{1}H-NMR spectral data of **3** closely resembled those of betulin (**9**), except for the presence of a saturated long-chain fatty acid ester group [δ_H 0.88 (3H, H_3-16′), 1.25 (CH_2), 2.28 (2H, H_2-2′)]. The alkaline hydrolysis of **3** in methanolic KOH yielded **9** and methyl palmitate. The ^{1}H-NMR chemical shift at H-3 of **3** was shifted downfield by +1.28 ppm compared with that of **9**, indicating that the palmitoyl group is located at the C-3 hydroxyl group. Thus **3** was determined to be betulin 3-*O*-palmitate.

Although most of the naturally occurring *seco*-triterpenoids are 3,4-*seco*-compounds,[9] there are some 2,3-*seco*-derivatives [*e.g.*, 2,3-*seco*-olean-12-ene-2,3,28-trioic acid from *Bursera graveolens* (Burseraceae),[10] 19α-hydroxy-2,3-*seco*-urs-12-ene-2,3,28-trioic acid,[11] 15α,19α-dihydroxy-2,3-*seco*-urs-12-ene-2,3,28-trioic acid and 19α,22α-dihydroxy-2,3-*seco*-urs-12-ene-2,3,28-trioic acid from *Musanga cecropioides* (Cecropiaceae),[12] and 2,3-*seco*-2-oxoolean-12-en-3-methylester-30-oic acid from *Dillenia papuana* (Dilleniaceae)[13]]. Compounds **1** and **2** are, to the best of our knowledge, the first 2,3-*seco*-triterpenoids from the genus *Gentiana* and the first 2,3-*seco*-triterpenoids with a C-2 carboxyl group and C-3 aldehyde group isolated from natural sources.

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM-LA 600 (600 and 150 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as an internal standard. EI- and HR-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPS; detector, RI-8020) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh). HPLC conditions: mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C. GC was carried out on a Shimadzu GC-7A gas chromatograph.

Plant Material The dried and powdered rhizomes and roots of *G. lutea* (from France) were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan, in 2001.

Extraction and Isolation The dried and powdered rhizomes and roots of *G. lutea* (1.5 kg) were extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure and the residue (160.0 g) was suspended in a small amount of water. This suspension was extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was concentrated under reduced pressure to afford a residue (67.0 g). Part of this residue (44.0 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1), and the eluate was separated into 35 fractions (frs. 1—35). Fraction 3 was purified by preparative HPLC to give **3** (1.1 mg), **7** (1.8 mg), and **8** (3.0 mg). Fraction 4 was purified by preparative HPLC to give **4** (4.1 mg), **5** (5.0 mg), and **6** (2.0 mg). Fraction 5 was purified by preparative HPLC to give **1** (7.9 mg) and **2** (3.6 mg).

2,3-*seco*-3-Oxours-12-en-2-oic Acid (**1**): Amorphous powder. $[\alpha]_D^{23}$ +68.1° (c=0.8, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3030, 2927, 2858, 1713, 1457, 1380. HR-MS *m/z*: 456.3600 (M^+, Calcd for $C_{30}H_{48}O_3$: 456.3604). EI-MS *m/z* (rel. int): 456 (M^+, 7), 218 (100), 203 (27), 189 (21). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

2,3-*seco*-3-Oxoolean-12-en-2-oic Acid (**2**): Amorphous powder. $[\alpha]_D^{23}$ +52.7° (c=0.4, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 3029, 2930, 2857, 1724, 1466, 1381. HR-MS *m/z*: 456.3625 (M^+, Calcd for $C_{30}H_{48}O_3$: 456.3604). EI-MS *m/z* (rel. int): 456 (M^+, 7), 218 (100), 203 (39), 189 (12). ^{1}H-NMR

(600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

Betulin 3-*O*-Palmitate (**3**): Amorphous powder. $[\alpha]_D^{23}$ +34.9° (*c*=0.1, $CHCl_3$). HR-MS *m/z*: 680.6125 (M^+, Calcd for $C_{46}H_{80}O_3$: 680.6107). EI-MS *m/z* (rel. int): 680 (M^+, 5), 662 (M^+-H_2O, 20), 647 ($M^+-H_2O-CH_3$, 16), 424 ($M^+-C_{16}H_{32}O_2$, 8), 234 (10), 218 (18), 203 (27), 189 (21). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.836 (3H, s, H_3-24), 0.841 (3H, s, H_3-23), 0.85 (3H, s, H_3-25), 0.88 (3H, t, *J*=7.3 Hz, H_3-16′), 0.98 (3H, s, H_3-27), 1.02 (3H, s, H_3-26), 1.25 (CH_2), 1.69 (3H, br s, H_3-30), 2.28 (2H, t, *J*=7.3 Hz, H_2-2′), 3.34 (1H, d, *J*=11.4 Hz, H-28a), 3.80 (1H, d, *J*=11.4 Hz, H-28b), 4.47 (1H, dd, *J*=11.0, 5.1 Hz, H-3), 4.58 (1H, d, *J*=1.5 Hz, H-29a), 4.68 (1H, d, *J*=2.2 Hz, H-29b).

Hydrolysis of 3 Compound **3** was refluxed with 5% methanolic KOH for 3 h. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was concentrated under reduced pressure to yield betulin and methyl palmitate. Each compound was identified by HPLC comparison with authentic samples. HPLC conditions: column, TSK gel ODS-120T (7.8 mm i.d.×30 cm, Tosoh); mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C. Betulin, t_R 14.0 min. Methyl palmitate, t_R 21.0 min. Methyl palmitate was also identified by GC comparison with authentic sample. GC conditions: column, 3% SE-52 on Chromosorb W (AW) (60—80 mesh), 3 mm i.d.×2 m; carrier gas, N_2; flow rate, 1.0 kg/cm^2; detector, FID; column temperature, 190 °C. Methyl palmitate, t_R 6.2 min.

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