Studies on the Constituents of *Gentiana* Species. II.[1] A New Triterpenoid, and (*S*)-(+)- and (*R*)-(−)-Gentiolactones from *Gentiana lutea*

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A new triterpenoid, 12-ursene-3β, 11α-diol 3-*O*-palmitate (1), has been isolated from the rhizomes and roots of *Gentiana lutea*, together with the artificial diene derivative, 9 (11), 12-ursadien-3β-ol 3-*O*-palmitate (1a) and five known compounds (3—7). Their structures were established on the basis of spectral analysis. In addition, (±)-gentiolactone [(±)-2], isolated from this plant, was successfully separated into its enantiomers [(+)-2, (−)-2] for the first time, and the absolute configurations at C-9 of (+)-2, (−)-2 were assigned as *S* and *R*, respectively, from the optical rotations and the circular dichroism (CD) spectral data.

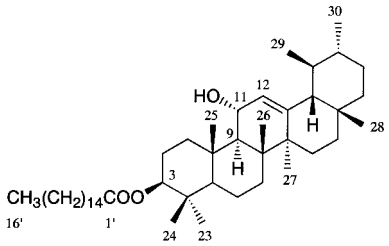
Key words *Gentiana lutea*; Gentianaceae; triterpenoid; (*S*)-(+)-gentiolactone; (*R*)-(−)-gentiolactone

We recently reported the isolation of 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, along with five known ones from the rhizomes and roots of *Gentiana lutea* L. (Gentianaceae).[1] In the course of further studies on the constituents of this plant, a new triterpenoid, 12-ursene-3β, 11α-diol 3-*O*-palmitate (**1**), the artificial diene derivative , 9 (11), 12-ursadien-3β-ol 3-*O*-palmitate (**1a**) and five known compounds (**3**—**7**) were isolated. In addition, (±)-gentiolactone [(±)-**2**], isolated from this plant, was successfully separated into its enantiomers [(+)-**2**, (−)-**2**] for the first time, and the absolute configurations at C-9 of (+)-**2** and (−)-**2** were assigned as *S* and *R*, respectively, from the optical rotations and the circular dichroism (CD) spectral data. This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the experimental section. Five known compounds were identified as squalene (**3**), stigmasterol (**4**), campesterol (**5**), β-sitosterol (**6**) and β-sitosterol β-D-glucopyranoside (**7**), by direct comparison with authentic samples.[2]

Compound **1** was obtained as an amorphous powder, $[\alpha]_D^{25}$ +31.1° ($CHCl_3$). The molecular formula was determined to be $C_{46}H_{80}O_3$ by high-resolution (HR)-electron impact (EI)-MS. The ^{1}H- and ^{13}C-NMR spectral data of **1** closely resembled that of α-amyrin isolated from this plant,[1] except for the presence of an oxygenated methine moiety [δ_H 4.25 (1H, H-11), δ_C 68.4 (d, C-11)] and a saturated long-chain fatty acid ester group [δ_H 0.88 (3H, H_3-16′), 1.25 (20H, H_2-4′—13′), 2.29 (2H, H_2-2′), δ_C 173.7 (s, C-1′)] in **1**. Furthermore, ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) of **1** implied connectivity for H-12 to the oxygenated methine proton described above. The alkaline hydrolysis of **1** in methanolic KOH yielded methyl palmitate. The ^{1}H-NMR chemical shift at H-3 [δ_H 4.53 (1H, dd, J=8.9, 7.6 Hz)] of **1** was shifted downfield by +1.31 ppm compared with that of α-amyrin, indicating that the palmitoyl group is located at the C-3 hydroxyl group of 11-hydroxy-α-amyrin. This deduction was supported by ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) correlations between H-3 and C-1′. From the above data, the coupling constant of H-9 ($J_{9,11}$=8.8 Hz) and the nuclear Overhauser enhancement spectroscopy (NOESY) correlations (H-11/H_3-25, 26), the structure of **1** was determined to be 12-ursene-3β, 11α-diol 3-*O*-palmitate.

When compound **1** was dissolved in $CDCl_3$, and was left in a refrigerator (12 °C) for 2 months, an allylic hydroxyl group at C-11 in **1** completely eliminated the hydroxyl group to form a 9(11), 12-diene conjugated system (UV λ_{max} 275 nm), resulting in the artificial diene derivative (**1a**). The structure of **1a** was determined to be 9(11), 12-ursadien-3β-ol 3-*O*-palmitate by spectral analysis, and was further confirmed by comparison of the spectroscopic data with those of 9(11), 12-ursadien-3β-ol 3-*O*-acetate.[3] Ito and Lai reported that urs-9(11), 12-diene derivatives were easily obtained by the photolysis of urs-12-en-11-ol derivatives,[4] however, it is likely that the trace HCl in the NMR solvent ($CDCl_3$) catalyzed the reaction.

Compound **2**, $[\alpha]_D^{25}$ −2.3° (MeOH), was identified as (±)-



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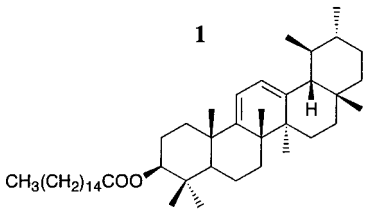
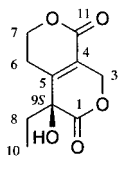
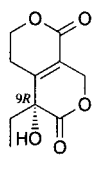


1a



(*S*)-(+)-2



(*R*)-(−)-2

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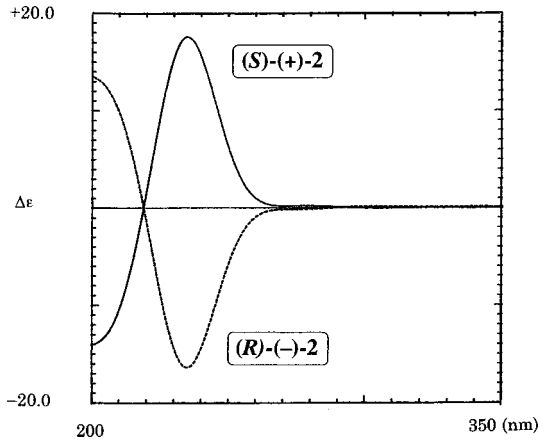

Fig. 1. The CD Spectra of (*S*)-(+)-**2** and (*R*)-(−)-**2** (MeOH)

gentiolactone [(±)-**2**] by comparison of the spectral data with those reported in literature.[5,6] This compound has already been isolated from the dried roots of *G. purpurea*, however, the authors of the paper reported that the compound which shows no optical activity is a racemate according to X-ray diffraction analysis.[5] (±)-Gentiolactone [(±)-**2**], isolated from this plant, was successfully separated into its enantiomers [(+)-**2**: ($[\alpha]_D^{25}$ +111.0°, (−)-**2**: $[\alpha]_D^{25}$ −110.6°] by HPLC using a chiral column. The CD curves of (+)-**2** and (−)-**2** were also symmetricall opposites (Fig. 1) [(+)-**2**: 234.5 nm ($\Delta\varepsilon$ +17.5), (−)-**2**: 235.0 nm ($\Delta\varepsilon$ −16.4)]. The absolute configuration at C-9 of (+)-**2** was assigned as *S* from the signs of the optical rotation and the Cotton effect of the CD spectrum. That is, the optical rotation of (+)-**2** exhibited the same sign when compared with that of (4*S*)-4-ethyl-4-hydroxy-1*H*-pyrano[3,4-c]pyridine-3,8 (4*H*,7*H*)-dione ($[\alpha]_D$ +117.0°),[7] which was an important precursor for the synthesis of (*S*)-camptothecin.[8,9] Furthermore, the CD spectrum of (+)-**2** showed a positive Cotton effect, which is the same sign of the Cotton effect on that of 9-β-D-glucosyl-(*S*)-camptothecin.[10] Therefore, the absolute configuration at C-9 of (−)-**2** was assigned as *R*, and this conclusion was further supported by comparing the signs of the optical rotation of (−)-**2** and (4*R*)-4-ethyl-1,4-dihydro-4-hydroxy-3*H*-2-benzopyrane-3-one ($[\alpha]_D$ −48.3°).[11] Accordingly, compounds (+)-**2** and (−)-**2** were concluded to be (*S*)-(+)- and (*R*)-(−)-gentiolactone, respectively. This is the first example of the separation of (±)-gentiolactone into its enantiomers, and is the assignment of their absolute configurations at C-9.

Experimental

The CD spectra were obtained with a JASCO J-720 spectropolarimeter. The instruments, materials and experimental conditions were the same as in our previous paper.[1]

Extraction and Isolation The extraction and isolation procedures were as described in our previous paper.[1] Fraction 3, obtained from the $CHCl_3$-soluble portion of the dried and powdered rhizomes and roots of *G. lutea*, was purified by prep. HPLC [column, ODS-120T (7.8 mm i.d.×30 cm, Tosoh); mobile phase, MeOH; RI detector; column temp., 40 °C; flow rate, 1.5 ml/min] to give **1** (4.8 mg), (±)-**2** (17.0 mg), **3** (3.8 mg), **4** (5.5 mg), **5** (3.0 mg) and **6** (6.0 mg). The separation of (±)-**2** into its enantiomers [(*S*)-(+)-**2** (5.7 mg, t_R 40.8 min), (*R*)-(−)-**2** (6.5 mg, t_R 46.0 min)] was achieved by HPLC [column, Chiralcel OD column (4.6 mm i.d.×25 cm, Daicel Chemical Ltd.); mobile phase, *n*-hexane–iso-PrOH (9 : 1); UV detector, 206 nm; column temp., 26 °C; flow rate, 1.0 ml/min]. Fraction 8 was chromatographed on a silica gel column using $CHCl_3$–MeOH (19 : 1) to give **7** (3.8 mg). Compounds **3**—**7** were identified as squalene, stigmasterol, campesterol, β-sitosterol and β-sitosterol β-D-glucopyranoside, respectively, by direct comparison with authentic samples.[2]

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

Hydrogen	**1**	**1a**
3	4.53 dd (8.9, 7.6)	4.52 dd (11.7, 4.8)
9	1.52 d (8.8)	
11	4.25 m	5.59 d (5.9)
12	5.19 d (3.3)	5.45 d (5.9)
23	0.88 s	0.89 s
24	0.89 s	0.90 s
25	1.12 s	1.24 s
26	1.06 s	1.17 s
27	1.16 s	0.89 s
28	0.80 s	0.86 s
29	0.86 d (7.0)	0.81 d (6.6)
30	0.93 br s	0.93 br s
2′	2.29 t (7.8)	2.30 m
4′—13′	1.25 m	1.25 m
16′	0.88 t (7.0)	0.88 t (7.3)

Coupling constants (*J* in Hz) are given in parentheses.

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

Carbon		**1**		**1a**	
1	1′	40.5	173.7	37.0	173.7
2	2′	23.8	34.9	24.3	34.9
3	3′	80.3	25.2	80.3	25.2
4	4′	38.03	29.71[a)]	38.5	29.71[b)]
5	5′	55.4	29.70[a)]	51.2	29.7[b)]
6	6′	18.2	29.68[a)]	18.2	29.68[b)]
7	7′	33.58	29.67[a)]	32.0	29.67[b)]
8	8′	42.1	29.65[a)]	43.1	29.65[b)]
9	9′	55.8	29.61[a)]	154.2	29.6[b)]
10	10′	38.0	29.5[a)]	37.9	29.5[b)]
11	11′	68.4	29.4[a)]	115.5	29.4[b)]
12	12′	128.7	29.3[a)]	123.0	29.3[b)]
13	13′	143.0	29.2[a)]	141.4	29.2[b)]
14	14′	43.3	31.9	40.7	31.9
15	15′	26.7	22.7	28.2	22.7
16	16′	27.9	14.1	26.1	14.1
17		33.64		33.7	
18		58.1		57.3	
19		39.3		39.0	
20		39.4		39.4	
21		31.1		31.2	
22		41.3		41.1	
23		28.2		28.16	
24		16.8		17.4	
25		16.9		17.5	
26		18.0		22.1	
27		23.1		25.5	
28		28.7		28.7	
29		17.6		16.8	
30		21.3		21.5	

a, *b*) Assignments may be interchangeble.

12-Ursene-3β, 11α-diol 3-*O*-Palmitate (1) Amorphous. $[\alpha]_D^{25}$ +31.1° (c=0.27, $CHCl_3$). EI-MS *m/z* (rel. int): 680 (M^+, 2) 662 (M^+-H_2O, 100), 647 ($M^+-H_2O-CH_3$, 41), 255 (10). CI-MS *m/z* (rel. int): 681 (M^++H, 19), 663 (M^++H−H_2O, 100). HR-EI-MS *m/z*: 680.6085 (M^+, Calcd for $C_{46}H_{80}O_3$: 680.6108). ^{1}H-NMR (600 MHz, $CDCl_3$): Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): Table 2.

9(11), 12-Ursadien-3β-ol 3-*O*-Palmitate (1a) Amorphous. $[\alpha]_D^{25}$ +150.2° (c=0.36, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 213.0 (4.72), 275 (4.04). EI-MS *m/z* (rel. int): 662 (M^+, 100), 407 (17), 255 (29). HR-EI-MS *m/z*: 662.5989 (M^+, Calcd for $C_{46}H_{78}O_2$: 662.6002). ^{1}H-NMR (600 MHz,

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

(±)-Gentiolactone [(±-2)] Amorphous. $[\alpha]_D^{25}$ −2.3° (c=1.3, MeOH). CD (c=3.29×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): −0.26 (238.0).

(*S*)-(+)-Gentiolactone [(*S*)-(+)-2] Amorphous. $[\alpha]_D^{25}$ +111.0° (c=0.20, MeOH). CD (c=2.81×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): +17.5 (234.5). UV λ_{max} (MeOH) nm (log ε): 206 (3.78), 222 (3.76). EI-MS m/z (rel. int): 212 (M^+, 0.3), 183 (15), 168 (66). 139 (100). HR-EI-MS m/z: 212.0712 (M^+, Calcd for $C_{10}H_{12}O_5$: 212.0685). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.26 (1H, br dt, J=16.3, 1.7 Hz, H-3$_B$), 5.02 (1H, ddd, J=16.3, 3.4, 2.2 Hz, H-3$_A$), 4.52 (1H, br dt, J=11.2, 5.6 Hz, H-7$_B$), 4.47 (1H, ddd, J=11.2, 9.0, 4.7 Hz, H-7$_A$), 3.46 (1H, s, OH-9), 2.82, 2.65 (each 1H, m, H_2-6), 1.86, 1.72 (each 1H, dq, J=14.6, 7.4 Hz, H_2-8), 1.01 (3H, t, J=7.4 Hz, H_3-10). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 172.6 (C-1), 161.5 (C-11), 153.4 (C-4), 119.8 (C-5), 72.1 (C-9), 66.7 (C-3), 66.6 (C-7), 30.8 (C-8), 22.6 (C-6), 7.7 (C-10).

(*R*)-(−)-Gentiolactone [(*R*)-(−)-2] Amorphous. $[\alpha]_D^{25}$ −110.6° (c=0.26, MeOH). CD (c=2.78×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): −16.4 (235.0). UV λ_{max} (MeOH) nm (log ε): 206 (3.78), 222 (3.76). EI-MS m/z (rel. int): 212 (M^+, 0.4), 183 (24), 168 (53). 139 (100). HR-EI-MS m/z: 212.0714 (M^+, Calcd for $C_{10}H_{12}O_5$: 212.0685). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.26 (1H, br dt, J=16.3, 1.7 Hz, H-3$_B$), 5.02 (1H, ddd, J=16.3, 3.4, 2.2 Hz, H-3$_A$), 4.52 (1H, br dt, J=11.2, 5.6 Hz, H-7$_B$), 4.47 (1H, ddd, J=11.2, 9.0, 4.7 Hz, H-7$_A$), 3.46 (1H, s, OH-9), 2.82, 2.65 (each 1H, m, H_2-6), 1.86, 1.72 (each 1H, dq, J=14.6, 7.4 Hz, H_2-8), 1.01 (3H, t, J=7.4 Hz, H_3-10). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 172.6 (C-1), 161.5 (C-11), 153.4 (C-4), 119.8 (C-5), 72.1 (C-9), 66.7 (C-3), 66.6 (C-7), 30.8 (C-8), 22.6 (C-6), 7.7 (C-10).

Hydrolysis of 1 Compound **1** (*ca.* 1 mg) 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 methyl palmitate. This compound was identified by GC comparison with the 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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References and Notes

1) Part 2 in the series "Studies on the Constituents of *Gentiana* Species," for part 1: Toriumi Y., Kakuda R., Kikuchi Masaf., Yaoita Y., Kikuchi Masao, *Chem. Pharm. Bull.*, **51**, 89—91 (2003).
2) Kakuda R., Yaoita Y., Machida K., Kikuchi M., *J. Tohoku Pharm. University*, **47**, 55—60 (2000).
3) Matsunaga S., Tanaka R., Akagi M., *Phytochemistry*, **27**, 535—537 (1988).
4) Ito K., Lai J., *Chem. Pharm. Bull.*, **27**, 210—214 (1979).
5) Suhr I. H., Arends P., Jensen B., *Phytochemistry*, **17**, 135—138 (1978).
6) Tan R. X., Kong L. D., *Phytochemistry*, **46**, 1035—1038 (1997).
7) Comins D. L., Nolan J. M., *Org. Lett.*, **3**, 4255—4257 (2001).
8) Comins D. L., Saha J. K., *Tetrahedron Lett.*, **36**, 7995—7998 (1995).
9) Comins D. L., Baevsky M. F., Hong H., *J. Am. Chem. Soc.*, **114**, 10971—10972 (1992).
10) Kitajima M., Nakamura M., Watanabe A., Takayama H., Aimi N., *J. Chem. Soc., Perkin Trans. 1*, **1998**, 389—390 (1998).
11) Curran D. P., Ko S.-B., *J. Org. Chem.*, **59**, 6139—6141 (1994).