Secoiridoid Glycosides from *Gentiana olivieri*

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From the whole plants of *Gentiana olivieri*, three new bitter secoiridoid glycosides, olivierosides A (1), B (2) and C (3) were isolated together with the known compounds, gentiopicroside, sweroside, 6′-*O*-β-D-glucosylgentiopicroside, swertiapunimarin, eustomoside, eustomorusside and septemfidoside, and the structures of the new compounds were elucidated based on spectroscopic and chemical evidence.

Key words Olivieroside; *Gentiana olivieri*; Gentianaceae; bitter secoiridoid glycoside

Gentiana olivieri GRIESEB.(Gentianaceae) is used as a bitter tonic, stomachic and stimulant of appetite in traditional medicine in Turkey.[1)] The plant is also used for diarrhea, common cold, stomachache, wound and ease of digestion in the Uzbekistan Republic.[2)] Several alkaloids,[3)] iridoid and secoiridoid glucosides,[4)] and flavonoid glycosides[4)] are known to be constituents. During the course of studies on the constituents of medicinal plants grown in the Uzbekistan Republic, we examined the glycosidic constituents of the title plant and isolated ten compounds, three of which are new bitter compounds termed olivierosides A (**1**), B (**2**) and C (**3**). This paper deals with the isolation and structural elucidation of these new compounds.

Compound **1** and **2** were isolated from the EtOAc soluble fraction and **3** was isolated from the water soluble fraction together with gentiopicro-side (**4**),[5)] 6′-*O*-β-D-glucosylgentiopicroside,[6)] sweroside (**5**),[7)] swertiapunimarin,[8)] eustomoside,[9)] eustomorusside[9)] and septemfidoside[10)] by combination of several chromatographies including highly porous synthetic resin Diaion HP-20, silica gel and reversed phase HPLC as described in the Experimental section.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D$ −224° (MeOH) and the molecular formula was determined as $C_{25}H_{26}O_{11}$ on the basis of its high resolution (HR) FAB-MS. The ^{1}H- and ^{13}C-(Table 1) NMR spectra of the aglycone part were essentially the same as those of **4**. The ^{1}H-NMR spectrum showed the signals due to *trans-p*-coumaroyl group [δ 6.19, 7.58 (each 1H, d, J=16.1 Hz), 6.82, 7.45 (each 2H, d, J=8.3 Hz)] and a methine proton [δ 4.77 (1H, br t, J=8.3 Hz)] on the carbon having an acyloxy group, which is one of the protons of the sugar moiety. Usual acetylation of **1** gave the tetraacetate (**1a**). Alkaline methanolysis of **1** followed by usual acetylation gave gentiopicroside tetraacetate (**4a**) [δ_H 1.95, 2.00, 2.03, 2.10 (each 3H, s)] and acetate of methyl *trans-p*-coumarate. This fact strongly suggested that olivieroside A is a *trans-p*-coumaroyl ester of **4**. The location of the acyl group was found to be at C-2′-*O* from comparisons of the ^{13}C-NMR spectra of **1** and **4**. The signal due to C-2′ underwent a downfield shift by 4 ppm and those of C-1′ and C-3′ suffered upfield shifts by 2.7 (or 2.3) and 2.4 ppm, respectively. Thus, the structure of **1** is represented as shown in the Formulae.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ −177° (MeOH) and the molecular formula was determined to be $C_{25}H_{28}O_{11}$, two mass units more than that of **1**, based on its HR-FAB-MS. In the ^{1}H-NMR spectrum of **2**, the proton signal at δ_H 5.59 which was observed in **1** disappeared and,

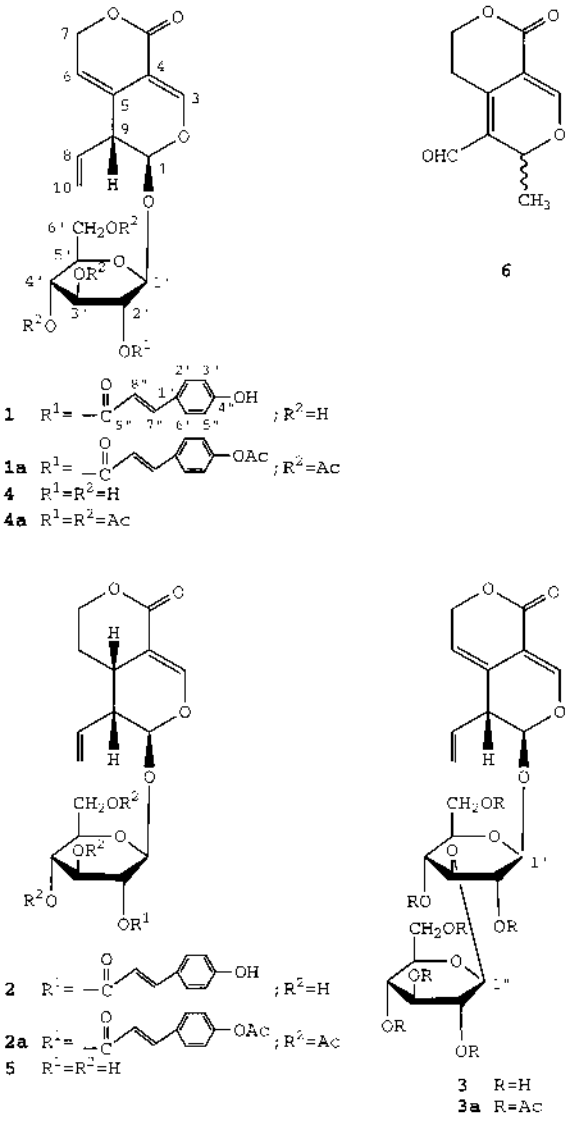

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Table 1. ^{13}C-NMR Data (δ) for Compounds **1**—**5** (100 MHz in CD_3OD)

C	1	2	3	4	5
1	97.9[a)]	97.8[a)]	98.6	98.5	98.0
3	149.4	153.5	150.7	150.6	154.0
4	105.2	106.0	104.9	104.9	106.0
5	127.1	28.8	127.0	127.0	28.4
6	118.1	25.7	117.2	117.2	25.9
7	70.6	69.6	70.9	70.9	69.7
8	134.6	132.9	134.9	135.0	133.3
9	46.3	43.4	46.6	46.6	43.8
10	118.3	121.0	118.6	118.5	120.9
11	165.8	168.5	166.3	166.3	168.5
1′	97.5[a)]	97.3[a)]	99.9	100.2	99.7
2′	78.5	78.6	73.8	74.5	74.7
3′	75.6	75.5	87.5	78.0	77.8
4′	71.6	71.7	69.9	71.5	71.5
5′	78.5	78.6	77.7	78.4	78.3
6′	62.6	62.6	62.6	62.8	62.6
1″	127.1	127.1	105.1		
2″	131.4	131.4	75.4		
3″	116.8	116.8	78.0[a)]		
4″	161.2	161.3	71.5		
5″	116.8	116.8	78.1[a)]		
6″	131.4	131.4	62.6		
7″	147.2	147.2			
8″	114.9	115.0			
9″	168.1	167.8			

a) Data in the same vertical column are interchangeable.

instead, a new methine signal (δ 2.88) and methylene signals (δ_H 1.61, 1.71) appeared. Thus, the aglycone portion was presumed to be the same as **5**. In fact, the ^{13}C-NMR signals of the aglycone part of **2** were essentially the same as those of **5**. Acetylation of **2** gave the tetraacetate (**2a**) [δ 1.99, 2.05, 2.12, 2.30 (each 3H, s)]. Since the carbon signals due to *trans-p*-coumaroyl moiety and glucopyranosyl moiety were essentially the same as those in **1**, the structure of olivieroside B was identified as 2′-*O*-*trans*-*p*-coumaroylsweroside (**2**).

Compound **3**, $[\alpha]_D$ −143° (MeOH), was obtained as an amorphous powder and the molecular formula, $C_{22}H_{30}O_{14}$ was determined based on its HR-FAB-MS. Hydrolysis of **3** with β-glucosidase from almond gave D-glucose as a sugar component. Although only D-glucose was obtained from enzymatic hydrolysis, observation of two anomeric protons [δ 4.56, 4.71 (each 1H, d, J=7.8, 8.3 Hz, respectively)] and two anomeric carbon signals (δ 99.9, 105.1) in the ^{1}H- and ^{13}C-NMR spectra strongly suggested the presence of two moles of β-D-glucopyranosyl units in the structure. Since the ^{1}H- and ^{13}C-NMR signals of the aglycone part were essentially the same as those of **1** and **4**, and gentiopicral (**6**)[11] was obtained on enzymatic hydrolysis, the structure was presumed to be β-D-glucopyranosylgentiopicroside. The connection of an additional β-D-glucopyranosyl unit to the original β-D-glucose unit of **4** was suggested to be on 3′-*O* by comparisons of ^{13}C-NMR data of **1** and β-laminaribiose[12] and was confirmed by interpretation of ^{1}H–^{1}H correlated spectroscopy (COSY) spectrum of the heptaacetate (**3a**) obtained by usual acetylation with acetic anhydride and pyridine. Thus, the signal [δ_H 3.89 (1H, t, J=9.6 Hz)] which suffered no downfield shift crossed peaks with the signal at δ_H 4.92 (H-2′) which then crossed peaks with an anomeric proton (δ_H 4.73). Based on the above findings, the structure of **3** was elucidated as 3′-*O*-β-D-glucopyranosylgentiopicroside.

Experimental

Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra (400 and 100 MHz, respectively) were recorded on a JEOL JNM EX-400 spectrometer, with tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS SX-102 spectrometer. FAB-MS were recorded using PEG-400 or *m*-nitrobenzyl alcohol as a matrix. UV spectra were recorded on a JASCO V-530SR spectrophotometer. IR spectra were taken on a Shimadzu IR-400 spectrophotometer or Perkin-Elmer 1720 IR FT spectrometer. Specific rotations were determined using a JASCO DIP-360 digital polarimeter. For purification, the following were used: the highly porous synthetic resin, Diaion HP-20 (Mitsubishi Chemical Co., Ltd., Tokyo), Kieselgel 60 Si gel (Merck, 230—400 mesh), packed column for HPLC [Cosmosil 10C_{18} (20×250 mm), detection, 230 nm; solvent, MeOH–H_2O (3 : 7) unless otherwise noted, 6 ml/min] and Silica gel 60 F_{254} TLC plates (Merck, 0.25 and 0.5 mm in thickness).

Plant Material The plants used were collected at Chimgan, Uzbekistan Republic, on June 9th, 1998 and identified as *Gentiana olivieri* GRIESEB. by Dr. F. O. Khassanof of the Institute of Botany, Academy of Sciences, Uzbekistan Republic. A voucher specimen (98 B 006) is deposited in the Herbarium of the Graduate School of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation Dried whole plants (111 g) were extracted with MeOH (1.8 l) at room temperature for two weeks and then refluxed twice with the same amount of MeOH. The combined MeOH extracts were concentrated *in vacuo*. The residue was dissolved in 90% MeOH (500 ml) and the solution was washed with *n*-hexane (500 ml×3). The 90% MeOH layer was concentrated *in vacuo*. The resultant residue was suspended in H_2O (300 ml) and the suspension was extracted with EtOAc (300 ml×3).

The aqueous layer was chromatographed on Diaion HP-20 (60 mm in diameter and 400 mm in length). Adsorbed materials were eluted with H_2O–MeOH with a stepwise increase of MeOH. Two and one-half l of 0%, 20% and 40% MeOH in H_2O, and MeOH were eluted, successively, and 500 ml fractions were collected. The residue (1.06 g) of fractions 9—12 was subjected to silica gel (55 g) column chromatography. Five hundred ml of each of $CHCl_3$ and $CHCl_3$–MeOH (9 : 1, 17 : 3, 4 : 1, 7 : 3) were eluted successively. From the $CHCl_3$–MeOH (17 : 3) eluate, 11 ml fractions were collected. Fractions 9—22 were combined and the residue (175.5 mg) was separated by preparative HPLC to give eustomorusside (7.7 mg)[9] and eustomoside (23.0 mg).[9] The residue (124.9 mg) of fractions 55—85 was separated by preparative HPLC to give 6′-*O*-β-D-glucopyranosylgentiopicroside (19.4 mg),[6] and swertiapunimarin (6.5 mg).[8] The residue (48.2 mg) from fractions 95—103 was separated by preparative HPLC to give septemfidoside (10.8 mg).[10]

The residue (7.85 g) of fractions 13—16 of Diaion HP-20 column chromatography was chromatographed over silica gel (150 g). $CHCl_3$ (1 l, fr. 1), $CHCl_3$–MeOH (19 : 1)(1 l, fr 2) and $CHCl_3$–MeOH (22 : 3) (300 ml, frs. 3—5) were first eluted and then $CHCl_3$–MeOH (22 : 3) (700 ml), $CHCl_3$– MeOH (17 : 3) (1 l) and $CHCl_3$–MeOH (4 : 1) (1 l) were eluted successively, collecting 11 ml fractions. An aliquot (104.8 mg) of the residue (4.02 g) from fractions 10—100 was separated by preparative HPLC to give gentiopicroside (**4**) (20.0 mg)[5] and sweroside (**5**) (28.1 mg).[7] The residue (521.2 mg) of fractions 137—160 was separated by preparative HPLC to give olivieroside C (**3**) (154.5 mg). The residue (526.5 mg) of fractions 179—213 was separated by preparative HPLC to give 6′-*O*-β-D-glucopyranosyl-gentiopicroside (115.0 mg)[6] and swertiapunimarin (47.9 mg).[8]

The EtOAc layer was concentrated *in vacuo* to give a residue (3.71 g) which was chromatographed over silica gel (150 g) with $CHCl_3$–MeOH with an increasing amount of MeOH. $CHCl_3$ (1 l), $CHCl_3$–MeOH (1 l, 19 : 1) and $CHCl_3$–MeOH (0.3 l, 9 : 1) were first eluted and then $CHCl_3$–MeOH (9 : 1, 700 ml), $CHCl_3$–MeOH (17 : 3, 1 l), $CHCl_3$–MeOH (4 : 1, 1 l) were eluted successively, collecting 11 ml fractions. The residues (145.5 mg and 101.6 mg) of fractions 23—40 and fractions 41—49 were separated by preparative HPLC (solvent, MeOH–H_2O, 2 : 3) to give olivieroside B (**2**) (12.1 mg) from the former and olivieroside A (**1**) (31.7 mg) from the latter.

The known compounds were identified by direct comparison of spectral data or by comparison of spectral data with those reported. The physical properties of the new compounds are as follows.

Olivieroside A (2′-*O*-*p*-Coumaroylgentiopicroside) (**1**): $[\alpha]_D^{23}$ −224° (c= 1.05, MeOH). UV (MeOH) λ_{max} (log ε): 233 (3.95), 315 (4.20). IR ν_{max} (film) cm^{-1}: 3385, 1699, 1516, 1270, 1207, 1171, 936, 836. ^{1}H-NMR (CD_3OD) δ: 3.28 (1H, br d, J=6.6 Hz, H-9), 3.35—3.42 (2H, m, H_a-4′, 5′), 3.63 (1H, t, J=8.3 Hz, H-3′), 3.70 (1H, dd, J=12.2, 5.9 Hz, H_a-6′), 3.94 (1H, br d, J=12.2 Hz, H_b-6′), 4 .77 (1H, br t, J=8.3 Hz, H-2′), *ca*. 4.8 (H′-1, overlapped), 5.15 (1H, br d, J=10.3 Hz, H_a-10), 5.18 (1H, br d, J=17.1 Hz,

H_b-10), 5.59 (1H, m, H-6), 5.66 (1H, d, J=2.0 Hz, H-1), 5.71 (1H, ddd, J=17.1, 10.3, 6.6 Hz, H-8), 6.19 (1H, d, J=16.1 Hz, H-8″), 6.82 (2H, d, J=8.3 Hz, H_2-3″, 5″), 7.31 (1H, s, H-3), 7.45 (2H, d, J=8.3 Hz, H_2-2″, 6″), 7.58 (1H, d, J=16.1 Hz, H-7″). ^{13}C-NMR: see Table 1. HR-FAB-MS (negative) *m/z*: 501.1432 $[M-H]^-$ (Calcd for $C_{25}H_{25}O_{11}$: 501.1397).

Olivieroside B (2′-*O*-*p*-Coumaroylsweroside) (**2**): $[\alpha]_D^{23}$ −177° (c=0.65, MeOH). UV (MeOH) λ_{max} (log ε): 235 (4.07), 316 (4.20). IR ν_{max} (film) cm^{-1}: 3385, 1699, 1607, 1516, 1270, 1207, 1171, 936, 836. ^{1}H-NMR (CD_3OD) δ: 1.61 (1H, m, H_a-6), 1.71 (1H, m, H_b-6), 2.66 (br dd, J=9.3, 6.8 Hz, H-9), 2.88 (1H, m, H-5), 3.38 (1H, m, H-5′), 3.38 (1H, t, J=9.8 Hz, H-4′), 3.65 (1H, t, J=9.8 Hz, H-3′), 3.70 (1H, dd, J=12.0, 5.6 Hz, H_a-6′), 3.93 (1H, dd, J=12.0, 1.5 Hz, H_b-6′), 4.16 (1H, br t, J=11.0 Hz, H_a-7), 4.32 (1H, br dd, J=11.0, 2.0 Hz, H_b-7), 4.83 (1H, dd, J=7.3, 9.8 Hz, H-2′), 4.93 (1H, d, J=7.3 Hz, H-1′), 5.25 (1H, dd, J=10.1, 2.0 Hz, H_a-10), 5.33 (1H, dd, J=17.1, 2.0 Hz, H_b-10), 5.48 (1H, ddd, J=17.1, 10.1, 6.8 Hz, H-8), 5.48 (1H, d, J=1.5 Hz, H-1), 6.24 (1H, d, J=15.4 Hz, H-8″), 6.80 (2H, d, J=8.8 Hz, H_2-3″, 5″), 7.43 (2H, d, J=8.8 Hz, H_2-2″, 6″), 7.48 (1H, d, J=2.4 Hz, H-3), 7.61 (1H, d, J=15.4 Hz, H-7″). ^{13}C-NMR: see Table 1. HR-FAB-MS (negative) *m/z*: 503.1523 $[M-H]^-$ (Calcd for $C_{25}H_{27}O_{11}$, 503.1553).

Olivieroside C (3′-*O*-*β*-D-Glucopyranoylgentiopicroside) (**3**): $[\alpha]_D^{22}$ −143° (c=1.20, MeOH). UV (MeOH) λ_{max} (log ε): 246 (sh) (3.68), 256 (sh) (3.71), 272 (3.77). IR ν_{max} (film) cm^{-1}: 3377, 1700, 1610, 1077, 936, 886, 843. ^{1}H-NMR (CD_3OD) δ: 3.24—3.29 (2H), 3.32—3.40 (5H), 3.55—3.69 (4H), 3.86—3.92 (2H, H_2-6′, 6″), 4.56 (1H, d, J=7.8 Hz, H-1′ or H-1″), 4.71 (1H, d, J=8.3 Hz, H-1′ or H-1″), 4.99 (1H, dd, J=17.6, 3.4 Hz, H_a-7), 5.08 (1H, br d, J=17.6 Hz, H_b-7), 5.21 (1H, dd, J=10.3, 1.5 Hz, H_a-10), 5.24 (1H, dd, J=17.1, 1.5 Hz, H_b-10), 5.62 (1H, m, H-6), 5.66 (1H, d, J=2.9 Hz, H-1), 5.75 (1H, ddd, J=17.1, 10.3, 6.8 Hz, H-8), 7.46 (1H, s, H-3). ^{13}C-NMR: see Table 1. HR-FAB-MS (negative) *m/z*: 517.1542 $[M-H]^-$ (Calcd for $C_{22}H_{29}O_{14}$: 517.1557).

Olivieroside A Tetraacetate (1a) **1** (3.0 mg) was dissolved in a mixture of pyridine (0.1 ml) and Ac_2O (0.1 ml) and the solution was kept overnight at room temperature. Excess MeOH was added to the solution and the solvent was removed *in vacuo*. The residue was purified by preparative TLC (solvent: Et_2O) to give **1a** (3.0 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.98, 2.04, 2.12, 2.31 (each 3H, s, 4×OAc), 3.29 (1H, br d, J=7.1 Hz, H-9), 3.79 (1H, m, H-5′), 4.18 (1H, dd, J=12.5, 2.2 Hz, H_a-6′), 4.30 (1H, dd, J=12.5, 4.7 Hz, H_b-6′), 4.83 (1H, dd, J=16.1, 3.9 Hz, H_a-7), 4.92 (1H, d, J=8.3 Hz, H-1′), 4.93 (1H, br d, J=16.1 Hz, H_b-7), 5.11 (1H, dd, J=8.3, 9.3 Hz, H-2′), 5.19 (1H, t, J=9.3 Hz, H-4′), 5.20 (1H, d, J=9.8 Hz, H_a-10), 5.21 (1H, d, J=17.6 Hz, H_b-10), 5.31 (1H, t, J=9.3 Hz, H-3′), 5.46 (1H, d, J=2.4 Hz, H-1), 5.54 (1H, m, H-6), 5.64 (1H, ddd, J=17.6, 9.8, 7.1 Hz, H-8), 6.21 (1H, d, J=15.8 Hz, H-8″), 7.15 (2H, d, J=8.3 Hz, H_2-3″ and 5″), 7.33 (1H, s, H-3), 7.56 (2H, d, J=8.3 Hz, H_2-2″ and 6″), 7.62 (1H, d, J=15.8 Hz, H-7″). HR-FAB-MS (positive) *m/z*: 671.2004 $[M+H]^+$ (Calcd for $C_{33}H_{35}O_{15}$: 671.1976).

Alkaline Methanolysis of Olivieroside A (1) **1** (13.6 mg) was dissolved in MeOH (2 ml), to which 1 N aqueous NaOH solution (0.2 ml) had been added. After being kept for 2 h at room temperature, the solution was neutralized with Amberlite IR-120B (H-form). The ion exchange resin was removed by filtration and the filtrate was concentrated *in vacuo* to give a residue which was acetylated with a mixture of pyridine (0.2 ml) and Ac_2O (0.2 ml) for 16 h. After working up as before, the product was separated by preparative TLC (solvent: Et_2O). The faster moving zone gave the acetate of methyl *p*-coumarate (4.8 mg) and the slower moving zone gave gentiopicroside tetraacetate (**4a**) (3.3 mg).

Methyl *p*-Coumarate Acetate: ^{1}H-NMR ($CDCl_3$) δ: 2.32 (3H, s, OAc), 3.81 (3H, s, $COOCH_3$), 6.40 (1H, d, J=15.9 Hz), 7.12 (2H, d, J=8.5 Hz), 7.54 (2H, d, J=8.5 Hz), 7.67 (1H, d, J=15.9 Hz). HR-EI-MS *m/z*: 220.0734 $[M]^+$ (Calcd for $C_{12}H_{12}O_4$: 220.0736).

4a: ^{1}H-NMR ($CDCl_3$) δ: 1.95, 2.00, 2.03, 2.10 (each 3H, 4×OAc), 3.30 (1H, br d, J=6.8 Hz, H-9), 3.74 (1H, m, H-5′), 4.16 (1H, dd, J=12.2, 2.2 Hz, H_a-6′), 4.28 (1H, dd, J=12.2 ,4.4 Hz, H_b-6′), 4.86 (1H, d, J=8.1 Hz, H-1), 4.94—5.00 (1H, H_a-7), 4.99 (1H, dd, J=9.6, 8,1 Hz, H-2′), 5.07—5.10 (1H, H_b-7), 5.09 (1H, t, J=9.6 Hz, H-4′), 5.19—5.25 (3H, H_2-10 and H-3′), 5.44 (1H, d, J=2.5 Hz, H-1), 5.73 (1H, m, H-6), 5.66 (1H, ddd, J=17.6, 10.3, 7.3 Hz, H-8), 7.40 (1H, s, H-3). HR-FAB-MS (positive, +NaI) *m/z*: 547.1400 $[M+Na]^+$ (Calcd for $C_{24}H_{28}O_{13}Na$: 547.1428).

Both compounds were identified by direct comparison of ^{1}H-NMR spectra with those of authentic samples.

Olivieroside B Tetraacetate (2a) **2** (2.2 mg) was dissolved in a mixture of pyridine (0.1 ml) and Ac_2O (0.1 ml) and the solution was kept overnight at room temperature. Work-up and purification as before gave **2a** (2.2 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.99, 2.05, 2.12, 2.30 (each 3H, s, 4×OAc), 2.67 (1H, m, H-9), 2.83 (1H, m, H-5), 3.80 (1H, m, H-5′), 4.13 and 4.32 (each 2H, m, H_2-7, H_2-6′), 4.98 (1H, d, J=7.8 Hz, H-1′), 5.13—5.18 (2H, H-2′, H-4′), 5.25—5.37 (4H, H-1, H-3′, H_2-10), 5.45 (1H, ddd, J=17.1, 9.8, 7.7 Hz, H-8), 6.25 (1H, d, J=15.9 Hz, H-8″), 7.14 (2H, d, J=8.5 Hz, H_2-3″, 5″), 7.50 (1H, d, J=2.5 Hz, H-3), 7.55 (2H, d, J=8.5 Hz, H_2-2″, 6″), 7.64 (1H, d, J=15.9 Hz, H-7″). HR-FAB-MS (positive) *m/z*: 673.2173 $[M+H]^+$ (Calcd for $C_{33}H_{37}O_{15}$: 673.2132).

Enzymatic Hydrolysis of Olivieroside C (3) **3** (41.2 mg) was dissolved in H_2O (5 ml), to which *β*-glucosidase from almond (To-yobo, Japan) (18.4 mg) had been added and the solution was kept at 37 °C for 22 h. After addition of H_2O (10 ml), the solution was extracted with EtOAc (20 ml) and the EtOAc extract was dried and evaporated *in vacuo* to give a residue which was purified by preparative TLC (solvent: Et_2O) to give gentiopicral (**6**) (3.6 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, d, J=6.4 Hz), 3.08 (2H, m), 4.41 (2H, m), 5.64 (1H, q, J=6.4 Hz), 7.95 (1H, s), 9.83 (1H, s). HR-EI-MS *m/z*: 194.0610 $[M]^+$ (Calcd for $C_{10}H_{10}O_4$, 194.0579). This compound was identified by comparison of ^{1}H-NMR spectrum with that of authentic sample.

The aqueous layer was concentrated *in vacuo* and the residue was purified by silica gel (3 g) chromatography with $CHCl_3$–MeOH with an increasing amount of MeOH content to give D-glucose (21.3 mg) which was identified by co-chromatography with authentic sample on silica gel TLC (solvent: *n*-BuOH : Me_2CO : H_2O 4 : 5 : 1, *Rf* 0.35). $[\alpha]_D$ +41.6° (c=1.07, H_2O).

Olivieroside C Heptaacetate (3a) **3** (15.3 mg) was acetylated with a mixture of Ac_2O (0.15 ml) and pyridine (0.15 ml) as described above. The reaction product was purified by preparative TLC (solvent: Et_2O, developed twice) to give **3a** (20.9 mg) which was recrystallized from EtOH to give colorless needles, mp 158—159 °C. IR ν_{max} ($CHCl_3$) cm^{-1}: 1745, 1610, 1365, 1230, 1200, 1065, 1035. ^{1}H-NMR ($CDCl_3$) δ: 1.96, 1.97, 2,01, 2,02, 2,03, 2,08, 2.10 (each 3H, s, 7×OAc), 3.30 (1H, br d, J=6.8 Hz, H-9), 3.69 (2H, m, H_2-5′, 5″), 3.89 (1H, t, J=9.6 Hz, H-3′), 4.04 (1H, dd, J=12.7, 2.2 Hz, H_a-6″), 4.17 (1H, dd, J=12,2 ,3.4 Hz, H_a-6′), 4.22 (1H, dd, J=12.2 ,4.4 Hz, H_b-6′), 4.36 (1H, dd, J=12.7, 4.4 Hz, H_b-6″), 4.57 (1H, d, J=8.3 Hz, H-1″), 4.73 (1H, d, J=8.3 Hz, H-1′), 4.88 (1H, t, J=8.3 Hz, H-2″), 4.92 (1H, t, J=8.3 Hz, H-2′), 4.95—5.00 (1H, H_a-7), 5.03—5.14 (4H, H_b-7, H-4′, H-3″ and H-4″), 5.21 (1H, d, J=10.3 Hz, H_a-10), 5.22 (1H, d, J=17.1 Hz, H_b-10), 5.43 (1H, d, J=2.0 Hz, H-1), 5.59 (1H, m, H-6), 5.65 (1H, ddd, J=17.1, 10.3, 6.8 Hz, H-8), 7.39 (1H, s, H-3). HR-FAB-MS (positive, +NaI) *m/z*: 835.2245 $[M+Na]^+$ (Calcd for $C_{36}H_{44}O_{21}Na$: 835.2273).

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