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A new depside and a new secoiridoid from the aerial parts of *Gentiana olivieri* from flora of Turkey

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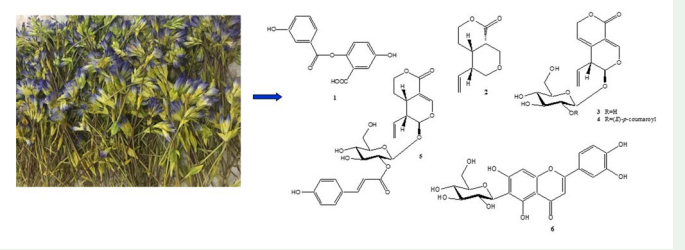
ABSTRACT

A new depside, olivieridepside (**1**), and a new secoiridoid, olivierigenin (**2**) were isolated from the aerial parts of *Gentiana olivieri* Griseb. along with four known compounds, gentiopicroside (**3**), olivierosides A (**4**) and B (**5**) and isoorientin (**6**). The structures of the isolates were determined by extensive 1 D and 2 D NMR spectroscopy and HR-MS analysis. This is the first report on the occurrence of a depside structure in the genus *Gentiana*. Moreover, a rare type of non-glycosidic secoiridoid (**2**) lacking an oxygenated group at C-1 is also being reported for the first time from this genus. The chemotaxonomic importance of the isolates was discussed in detail.



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1. Introduction

The genus *Gentiana* (Gentianaceae) contains around 400 species mainly distributed in Asia, Europe and North America (Pan et al. 2016). Some of these species have long been used in different traditional medicines for the treatment of a wide variety of diseases. To illustrate, the underground parts of *G. lutea*, an official drug listed in many Pharmacopoeias, are mainly used to treat digestive problems, to enhance appetite and to strengthen immune system in some European countries (Cvetković et al. 2020; Jarić et al. 2015). *G. cruciata* is utilized to lower cholesterol, as an antidiabetic agent

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and to improve digestion and appetite in Serbia (Jarić et al. 2015). *G. rhodantha* is indicated for the treatment of hepatitis, jaundice and cough, while *G. farreri* is used against pneumonia and fever in the traditional Tibetan medicine (Pan et al. 2016). There are 12 *Gentiana* species growing wild in the flora of Turkey (Pritchard 1978). Among these species, *G. olivieri* is well known and it is particularly abundant in the southeastern part of Turkey. The flowering aerial parts of this species is used as bitter tonic, stomachic, to treat diabetes as well as to combat some mental diseases (Baytop 1999; Sezik et al. 2005). In Uzbekistan, the same species is used as laxative, against common cold and stomachache and to aid digestion (Takeda et al. 1999). The extracts prepared from the aerial part of *G. olivieri* showed antidiabetic (Sezik et al. 2005), hepatoprotective (Deliorman Orhan et al. 2003), anticonvulsant (Aslan et al. 2011), antidepressant (Berk et al. 2020) and immunomodulatory (Singh et al. 2012) activities. Despite the widely usage in traditional medicine and diverse biological activities of its extract, the phytochemical composition of *G. olivieri* was not studied in detail. Few previous phytochemical studies on this species showed that it contains secoiridoids (Takeda et al. 1999), flavonoids (Ersöz and Çalış 1991) and alkaloids (Mansoor et al. 2002). Therefore, we aimed to obtain the secondary metabolites from the aerial parts of *G. olivieri* that might be responsible for the bioactivities of this species as well as to contribute to chemotaxonomic evaluation of *G. olivieri* and the genus *Gentiana*.

2. Results and discussion

The flowering aerial parts of *G. olivieri* were extracted with MeOH. The crude MeOH extract was suspended in H_2O and partitioned with $CHCl_3$ and EtOAc, respectively. Chromatographic separations of the EtOAc fraction yielded a new depside (**1**), four secoiridoids (**2**–**5**) including a new one (**2**) and a flavone-*C*-glycoside (**6**) (Figure 1).

Compound **1** was isolated as a pale yellowish amorphous powder. Its molecular formula was established as $C_{14}H_{10}O_6$ by HRESIMS (*m/z* 273.0391 $[M\text{-}H]^-$, calcd for $C_{14}H_9O_6$, 273.0393) and NMR analyses. Its UV spectrum displayed maxima at 226 and 302 nm indicating its aromatic nature. The IR spectrum showed absorption bands characteristic for hydroxyl (3318 cm^{-1}), ester carbonyl (1735 cm^{-1}), carboxyl (1699 cm^{-1}) and aromatic (1578, 1541 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table S1) only consisted of seven aromatic proton signals which were observed as an *ABX* [δ_H 7.63 (d, $J = 3.0$ Hz), 7.12 (dd, $J = 8.7$, 3.0 Hz), 6.84 (d, $J = 8.7$ Hz)] and *ABCD* [δ_H 7.62 (m), 7.55 dd ($J = 2.4$, 1.7 Hz), 7.35 (t, $J = 7.9$ Hz), 7.07 (dddd, $J = 8.2$, 3.5, 2.4, 0.9 Hz] systems by the help of COSY and HMBC spectra (Figure S14). The ^{13}C-JMOD NMR spectrum (Table S1) spectrum exhibited 12 aromatic signals between 117.3 and 160.5 ppm as well as two carbonyl signals at δ_C 175.0 and 167.2. Detailed analysis of COSY (Figure S3), HSQC (Figure S4) and HMBC (Figure S5) spectra led the structure to be composed of two aromatic subunits. The cross-peak between δ_C 175.0 (C-7) and δ_H 7.63 (H-6) determined the carboxyl group to be located at C-1. Similarly, the long-range coupling of the carbonyl group at δ_C 167.2 (C-7′) with the proton signals at δ_H 7.62 (H-6′) and 7.55 (H-2′) positioned carbonyl group at C-1′. Based on these data, the aromatic units were found to be linked to each other through a depside bond established between C-7′

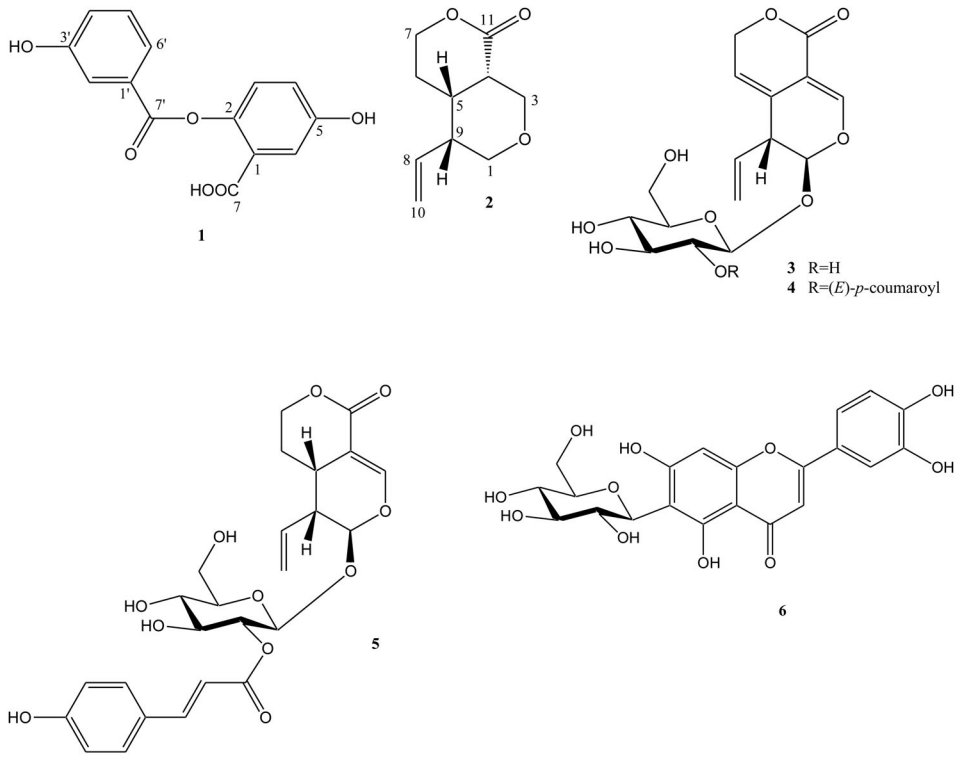

Figure 1. Chemical structures of compounds **1**–**6** isolated from *G. olivieri*.

and C-2. Accordingly, the chemical structure of **1** was proposed as depicted and given the trivial name olivieridepside based on the species name of *G. olivieri*.

Compound **2** was obtained as a colourless oil. The HRESIMS data (*m/z* 183.1019 ($[M+H]^+$ calcd 183.1015 for $C_{10}H_{15}O_3$) supported a molecular formula of $C_{10}H_{14}O_3$. The UV spectrum showed a maximum at 232 nm. The ^{1}H NMR spectrum of **2** (Table S2) contained signals for one terminal vinyl [δ_H 5.79 dddd ($J = 17.1$, 10.5, 8.4, 2.0 Hz), 5.19 dd ($J = 10.5$, 2.0 Hz), 5.16 dd ($J = 17.1$, 2.0 Hz)], three methines (δ_H 2.72 m and 2.30 m, 2H), three non-equivalent oxymethylenes [δ_H 4.36 (m), 4.33 (m), 3.97 dd ($J = 10.8$, 3.9 Hz), 3.75 dd ($J = 10.8$, 3.9 Hz), 3.65 (m)] as well as one non-equivalent methylene (δ_H 2.00 and 1.75 each m) groups which were observed in a spin system in the COSY spectrum (Figures S9 and S14). The ^{13}C-JMOD NMR spectrum (Table S2) showed 10 signals which were characteristic for a secoiridoid nucleus bearing a lactone ring formed between C-7 and C-4 (Takeda et al. 1999). However, the absence hydroxyl group at C-1 and the double bond between C-3 and C-4 were evident in the structure of **2**. A detailed analysis of COSY (Figure S9), HSQC (Figure S10) and HMBC (Figure S11) spectra suggested that **2** is a non-glycosidic secoiridoid lacking a double bond between C-3 and C-4 and a hydroxyl group at C-1 which are very common in secoiridoids (Dinda et al. 2007). The key HMBC correlations (Figures S11 and 14) between C-11 (δ_C 176.3) and H_2-3/H-5/H_2-7, between C-1 (δ_C 63.3) and H-5/H-8, between C-4 (δ_C 48.2) and H-9, between C-5 (δ_C 35.3) and H_2-7/H-8, between C-9 (δ_C 51.2) and H_2-10 confirmed the depicted structure. Compound **2** showed structural

resemblance to secostrychnosin that was isolated from *Strychnos cathayensis* (Cheng et al. 2001). However, there is no unsaturation in the pyran ring of **2**. The relative configuration of **2** was determined by NOESY experiment (Figure S12). NOESY correlations of H-4/H-8, H-4/H_2-10, H-5/H-8, H-9/H_2-10 indicated the β-orientation of H-5, H-9, and H-4. Based on the above findings, compound **2** was identified as a new non-glycosidic secoiridoid and named as olivierigenin.

Some structurally related secoiridoids were reported to possess antidiabetic, antimicrobial, antispasmodic and antiinflammatory activities (Dinda et al. 2007; Selvam et al. 2018). Thus, olivierigenin **(2)** might show similar activities. Furthermore, it could be one of the antidiabetic principles of *G. olivieri* whose flowering aerial parts were shown experimentally to have *in vivo* antidiabetic activity (Sezik et al. 2005).

The known compounds were identified as gentiopicroside (**3**), olivierosides A (**4**) and B (**5**) (Takeda et al. 1999) and isoorientin (**6**) (Çalış et al. 2006) by comparing their NMR data with those reported in the literature.

In this study a new depside was reported for the first time from the genus *Gentiana*, while a similar structure was obtained previously from the genus *Lomatogonium* which is also a member of Gentianaceae family (Li et al. 2008). Isoorientin, isolated from several *Gentiana* species including this study, was also reported from the genus *Lomatogonium*. These findings may imply a close chemotaxomic relationship between the genera *Gentiana* and *Lomatogonium*. This assumption was also corroborated by other phytochemical reports on the genus *Lomatogonium* indicating the presence of secoiridoids and xanthones that also constitute the major secondary metabolite classes of *Gentiana* species (Li et al. 2015; Jia et al. 2011; He et al. 2015). A new non-glycosidic secoiridoid as well as three known secoiridoid glycosides were obtained in our study. Iridoids and secoiridoids are regarded as useful chemotaxonomic markers particularly in dicotyledonous angiosperm. Among the isolated secoiridoids, olivierigenin (**2**) possess a unique structure as it lacks an oxygenated group at C-1. This type of secoiridoids are rarely encountered in nature (Dinda et al. 2007). Besides **2**, two secoirioid glucosides esterifed with *p*-coumaric acid namely olivierosides A (**4**) and B (**5**) are also reported in the current study. These two ester secoirioid glucosides were only isolated from *G. olivieri* previously (Takeda et al. 1999). Thus, compounds **2**, **4** and **5** could be used as valuable chemotaxonomic marker for *G. olivieri* within the genus *Gentiana* in future studies.

3. Experimental

3.1. General

HRESIMS data were measured on a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer equipped with ESI ion source in positive and negative ionization mode. UV and IR spectra were recorded on a HP Agilent 8453 spectrophotometer and a Perkin-Elmer 2000 FT-IR spectrometer, respectively. NMR spectra were acquired in CD_3OD on a Bruker Avance DRX 500 spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C. The signals of the deuterated solvents were taken as references. The chemical shift values (δ) were presented in ppm and coupling constants (*J*) are in Hz. Fractions were monitored on silica gel 60 F_{254} precoated TLC plates. The spots were visualized

under UV light (254/366 nm) or by spraying with 1% vanillin/H_2SO_4 followed by heating for 2-3 min. For medium-pressure liquid chromatographic (MPLC) separations, the Sepacore® Flash Systems X10/X50 (Büchi) system was used with Redi sep columns (LiChroprep C_{18}: 130, and 30 g; SiO_2: 40 g, Teledyne Isco). Sephadex LH-20 (Fluka) was used for gel filtration chromatography. Column chromatography (CC) was performed with SiO_2 (Merck). The solvent used for chromatographic separations were of analytical grade.

3.2. Plant material

The aerial parts of *G. olivieri* Griseb. were collected from Nizip, Gaziantep, Turkey (37°09′24″N 37°74′35″E) in May 2019. The plant material was identified by one of us (HK). A voucher specimen (YEF 19002) has been deposited at the Herbarium of the Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey.

3.3. Extraction and isolation

The air-dried aerial parts of *G. olivieri* (410 g) were powdered and extracted two times with MeOH (4 L) at 45 °C for 4 h. The organic solvent was evaporated under vacuum to give crude MeOH extract (120.3 g, yield 29.3%). The methanolic extract was dispersed in H_2O (200 mL) and then successively partitioned with $CHCl_3$ (3 × 200 mL) and EtOAc (3 × 200 mL). The EtOAc fraction (2.2 g) was separated by Sephadex LH-20 (200 g) column eluting with MeOH to give seven main fractions, frs. A-G. Fraction B (1.231 g) was subjected to C_{18}-Medium Pressure Liquid Chromatography (C_{18}-MPLC, 130 g) using gradient mixture of H_2O-MeOH (15 to 55% MeOH) as mobile phase to yield frs. $B_{1\text{-}8}$. Fraction B_1 (357 mg) was chromatographed on SiO_2-MPLC (40 g) eluting with CH_2Cl_2-MeOH (0 to 30% MeOH) gradient system to give **3** (142 mg) and **2** (7 mg). Fraction B_5 (87 mg) was separated by SiO_2 (10 g) CC using a gradient of CH_2Cl_2-MeOH (2.5 to 15% MeOH) to afford frs. $B_{5a\text{-}5e}$. Compounds **4** and **5** (4 mg) was obtained as an inseparable (near 1:1 ratio) mixture from fr. B_{5a} (23 mg) by SiO_2 (6 g) CC using the gradient mixture of CH_2Cl_2-MeOH (95:5 to 90:10). Separation of fr. F (136 mg) was done by C_{18}-MPLC (30 g) with a gradient of H_2O-MeOH (20 to 50% MeOH) to give **6** (25 mg) along with seven subfractions, frs. $F_{1\text{-}7}$. Fraction F_6 (28 mg) was loaded onto Sephadex LH-20 (10 g) CC eluting with MeOH yielded **1** (2.5 mg).

3.3.1. Olivieridepside (1)

Amorphous powder; UV (MeOH): λ_{max} = 226,302 nm; IR (KBr): ν_{max} = 3318, 1735, 1699, 1578, 1541 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 6.84 (1H, d, J = 8.7 Hz, H-3), 7.12 (1H, dd, J = 8.7, 3.0 Hz, H-4), 7.63 (1H, d, J = 3.0 Hz, H-6), 7.55 (1H, dd, J = 2.4, 1.7 Hz, H-2′), 7.07 (1H, dddd, J = 8.2, 3.5, 2.4, 0.9 Hz, H-4′), 7.35 (1H, t, J = 7.9 Hz, H-5′), 7.62 (1H, m, H-6′). ^{13}C NMR (125 MHz, MeOD-d_4): δ 120.7 (C-1), 143.4 (C-2), 117.6 (C-3), 127.0 (C-4), 160.5 (C-5), 123.9 (C-6), 175.0 (C-7), 132.2 (C-1′), 117.3 (C-2′), 159.0 (C-3′), 121.8 (C-4′), 130.7 (C-5′), 122.0 (C-6′), 167.2 (C-7′); HRESIMS *m/z* 273.0391 $[M–H]^-$ (calcd. for $C_{14}H_9O_6$, 273.0393).

3.3.2. Olivierigenin (2)

Colorless oil; $[\alpha]_D^{28} = -10.8$ ($c = 0.35$, MeOH); UV (MeOH): $\lambda_{max} = 232$ nm; IR (KBr): $\nu_{max} = 1714, 1405, 1072\,cm^{-1}$; ^1H NMR (500 MHz, CD_3OD): δ 3.65 (2H, m, H-1), 3.97 (1H, dd, $J = 10.8, 3.9$ Hz, H-3), 3.75 (1H, dd, $J = 10.8, 3.9$ Hz, H-3), 2.72 (1H, m, H-4), 2.30 (1H, m, H-5), 2.00 (1H, m, H-6), 1.75 (1H, m, H-6), 4.36 (1H, m, H-7), 4.33 (1H, m, H-7), 5.79 (1H, dddd, $J = 17.1, 10.5, 8.4, 2.0$ Hz, H-8), 2.30 (1H, m, H-9), 5.19 (1H, dd, $J = 10.5, 2.0$ Hz, H-10), 5.16 (1H, dd, $J = 17.1, 2.0$ Hz, H-10). ^{13}C NMR (125 MHz, MeOD-d_4): δ 63.3 (C-1), 63.9 (C-3), 48.2 (C-4), 35.3 (C-5), 27.2 (C-6), 69.0 (C-7), 138.8 (C-8), 51.2 (C-9), 118.4 (C-10), 176.3 (C-11); HRESIMS *m/z* 183.1019 $[M + H]^+$ (calcd. for $C_{10}H_{15}O_3$, 183.1015).

4. Conclusion

Chemical study of the EtOAc fraction of the MeOH extract from *G. olivieri* yielded a new depside (**1**), and a new secoiridoid (**2**) together with four known compounds. Their structures were elucidated by means of NMR and HR-MS techniques. This is the first report on the occurrence of a depside and a rare type of non-glycosidic secoiridoid in the genus *Gentiana*. Among the isolates, compounds **2**, **4** and **5** could be useful markers for the chemotaxonomy of *G. olivieri* within the genus *Gentiana* in future studies. *G. olivieri* deserves to be deeply investigated to discover new potential bioactive metabolites.

5. Supplementary material

NMR and HRMS spectra of new compounds (**1** and **2**) can be found online.

Conflict of interest

The authors declare no conflict of interest.

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