SHORT REPORT

ACG publications

Rec. Nat. Prod. 16:6 (2022) 651-656

records of natural products

Three Rare Furan Derivatives Transformed from Secoiridoids were Isolated from the Roots of *Gentiana macrophylla*

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(Received January 26, 2022; Revised March 29, 2022; Accepted April 17, 2022)

Abstract: Three furan derivatives (**1-3**) were isolated from the methanol extract of *Gentiana macrophylla* Pall. (Gentianaceae). Their structures were determined on the basis of spectroscopic analysis including HR-MS, 1D and 2D NMR. Genfurtate (**1**) was identified as a new compound, while, swertianglide (**2**) was firstly reported in genus *Gentiana*, and anglelone (**3**) was firstly reported in the plant *G. macrophylla*. Accordingly, their biosynthesis pathways transformed from secologanin in *G. macrophylla* was deduced.

Keywords: *Gentiana macrophylla*; Gentianaceae; furan derivatives; structural elucidation; biosynthesis; secoiridoid glycosides.

1. Plant Source

Gentiana macrophylla Pall., a perennial herb, with ovate-elliptic or narrowly elliptic rosette leaves, clusters of purple flowers and kink cylindrical roots, is widely distributed in 400-2400 meters altitude mountainous region in Northwest of China[1]. In this study, the roots of *Gentiana macrophylla* Pall. (Gentianaceae) was collected from Fengxian country in Shaanxi Province of China in October 2019, and identified by Prof. Wei Wang (Shaanxi University of Chinese Medicine). A voucher specimen (No. GM-20191020) has been deposited in the Herbarium of Shaanxi University of Chinese Medicine (herbarium code: SNTCM).

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products November-December 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.322.2201.2333
Available online: April 26, 2022

2. Previous Studies

Gentiana macrophylla Pall. is one the herbaceous plant in genus *Gentian* of Gentianaceae. The roots of it, as well as three other *Gentiana* species, *Gentiana straminea* Maxim., *Gentiana crassicaulis* Duthie ex Burk. and *Gentiana dahurica* Fisch. are prescribed as the original sources of Radix Gentianae Macrophyllae (RGM) in China Pharmacopoeia [2], used for the treatment of rheumatism, arthralgia, apoplexy, jaundice, and diabetes. Phytochemical studies revealed that the constituents in *G. macrophylla* were monoterpenes (iridoids, secoiridoids, and their glycosides), flavones, lignans and triterpenes [3]. Among them, secoiridoid glycosides were determined as the main active ingredients [4-6, 11]. Moreover, the biosynthesis pathways of secoiridoids have been investigated extensively, in which, one of their components, secologanin was determined as the key biosynthesis precursor of monoterpenoid alkaloids, used for antineoplastic drugs, such as camptothecin, vincristine and vinblastine [7-9]. Besides, secologanin was also considered to be the precursor of diversity of secoiridoids in further downstream [10].

3. Present Study

Our group is engaged in discovering diversity structures, revealing their biosynthesis *in vivo*, then using molecular biology techniques to produce them for further pharmacological research [11-15]. In this study, three rare furan derivatives (**1-3**) from the roots of *G. macrophylla* were reported (Figure 1). Accordingly, their biosynthesis pathways transformed from secoiridoids were deduced (Scheme 1).

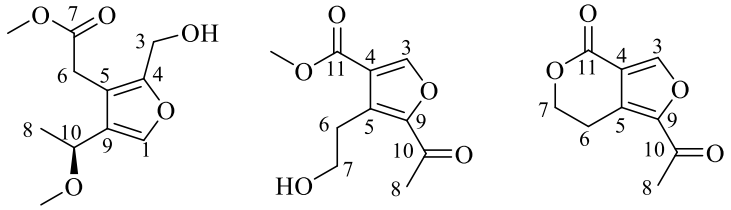


Figure 1. Structures of compounds **1–3**

The extraction and separation procedures were described as follows. The roots of *G. macrophylla* (40 kg) were extracted with MeOH at 55℃ for two times (each time 80 L for 1.5 h). After removing the solvent under reduced pressure, the crude extracts (5 kg) were obtained, redissolved in water and subjected to macroporous resin column chromatography eluted with (H_2O/MeOH, 1:0 to 0:1) to obtain four fractions (A1-A4). The A3 (370 g) fraction was separated to afford six fractions (Fr.1-Fr.6) with pre-parative HPLC (DAC-50 60 mm × 600 mm, C_{18} 75 μm particles; UV detector, 240 nm; flow rate: 13 mL/min) using gradient solvent system MeOH/H_2O (5:95 to 95:5) as the mobile phase. Fr.3 (20 g) was further subjected to silica gel CC, eluted with (CH_2Cl_2/CH_3OH, 20:1 to 0:1) to give five subfractions (Fr.3.1-Fr.3.5). Fr.3.2 (2.2 g) was subjected to Sephadex LH-20 CC, eluted with CH_2Cl_2/CH_3OH (1:1) to obtain six fractions (Fr.3.2.1-Fr.3.2.6). Fr.3.2.3 (225 mg) was separated by Sephadex LH-20 CC with MeOH, further purified by semipreparative HPLC (YMC-Pack ODS-A, 10 mm × 250 mm, 5 μm particles, UV detector, 240 nm, flow rate: 3 mL/min) with ACN/H_2O (22:74) as the mobile phase to yield **1** (4 mg; t_R=12 min). Fr.3.2.5 (40 mg) was separated by Sephadex LH-20 CC with CH_2Cl_2/MeOH (1:1), further purified by semipreparative HPLC (Kromasil 100-5-C18, 10 mm × 250 mm, 5 μm particles; UV detector, 240 nm; flow rate: 3 mL/min) with MeOH/H_2O (42:58) as the mobile phase to yield **2** (3 mg, t_R=23 min) and **3** (2.8 mg, t_R=25 min).

*Genfurtate (**1**)*: a colorless oil; $[\alpha]_D^{20}$ ﹣25.0 (c 0.1, CH_3OH); HR-ESI-MS: *m/z* 227.0918 $[M-H]^-$ (calcd. for $C_{11}H_{15}O_5$, 227.0919); IR ν_{max} (KBr): 3433 (OH), 2930 (CH), 1740 (C=O), 1441,1350 and

1330 (C=C) cm^{-1}; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data: see Table 1.

*Swertianglide (**2**)*: a white powder; HR-ESI-MS: *m/z* 235.0569 $[M+Na]^+$ (calcd. for $C_{10}H_{12}O_5$, 235.0582); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data: see Table 1.

*Anglelone (**3**)*: a white powder; HR-ESI-MS: *m/z* 203.0328 $[M+Na]^+$ (calcd. for $C_{11}H_{16}O_5$, 203.0320); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data: see Table 1.

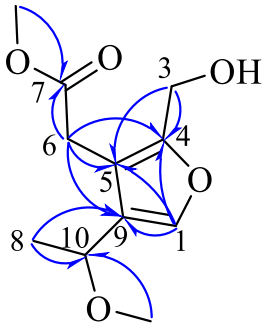


Figure 2. Key HMBC (H→C) correlations of compound **1**

Genfurtate (**1**) was proposed with a molecular formula of $C_{11}H_{16}O_5$ by HR-ESI-MS at *m/z* 227.0918 $[M - H]^-$ (calcd. for $C_{11}H_{15}O_5$, 227.0919), indicating four degrees of unsaturation. The correlative signals of the ^1H NMR and ^{13}C NMR were assigned by HSQC experiments (Table 1), exhibiting 11 carbon resonance signals due to one methyl [δ_H 1.41 (3H, d, J = 6.5 Hz, H-8) and δ_C 20.8 (C-8)], two methoxy groups [δ_H 3.21 (3H, s, OMe) and δ_C 55.8 (OMe)] and [δ_H 3.68 (3H, s, OMe) and δ_C 52.5 (OMe)], two methylenes [δ_H 4.25 (2H, s, H-3) and δ_C 55.5 (C-3); δ_H 3.58 (2H, s, H-6) and δ_C 20.8 (C-6)], one methine [δ_H 4.38 (1H, q, J = 13.2, 6.6 Hz, H-10) and δ_C 72.5 (C-10)], one trisubstituted double bone [δ_H 7.38 (1H, s, H-1) and δ_C 140.4 (C-1); δ_C 128.8 (C-9)], one tetrasubstituted double bone [δ_C 154.2 (C-4); δ_C 115.3 (C-5)], and one ester carbonyl carbon [δ_C 173.6 (C-7)]. In HMBC spectrum (Figure 2), correlations of H-1 to C-4, C-5, C-9 indicated the presence of a trisubstituted furan moiety; correlations of OMe at δ_H 3.68 to C-7, H-6 to C-7, C-5, C-4 and C-9 suggested one substituted moiety as an acetoxyl group; correlations of OMe at δ_H 3.21 to C-10, H-8 to C-10 and C-9, H-10 to C-9, C-5 and C-1 suggested one substituted moiety as a methoxyethyl group; correlations of H-3 to C-4 and C-5 suggested one substituted moiety as hydroxymethyl group.

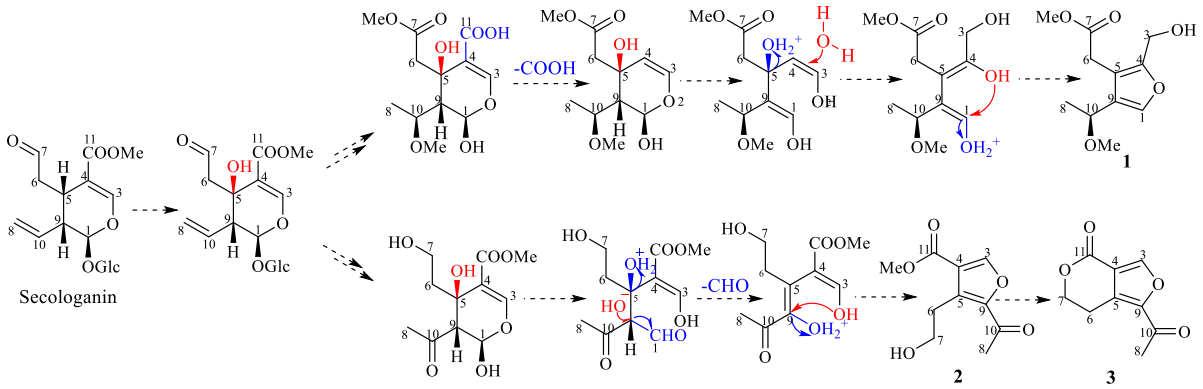
Table 1. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of compounds **1–3** in CD_3OD

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	140.4	7.38 (s)				
2						
3	55.5	4.50 (s)	151.7	8.28 (s)	148.9	8.17 (s)
4	154.0		121.8		118.2	
5	115.3		131.2		128.6	
6	29.7	3.58 (s)	28.3	3.37 (t, 7.2)	22.3	3.31 (t, 6.2)
7	173.6		62.4	3.70 (t, 7.6)	66.8	4.62 (t, 6.2)
8	20.8	1.41 (d, 6.6)	27.3	2.51(s)	27.2	2.48 (s)
9	128.8		151.5		147.5	
10	72.5	4.38 (dd, 13.1, 6.6)	190.8		188.8	
11			164.4		162.2	
7-OMe	52.5	3.68 (s)				
11-OMe	55.8	3.21 (s)	52.1	3.87 (s)		

Thus, the planar structure of **1** was determined as 4-hydroxymethyl-5-acetoxyl-9-methoxyethylfuran refer to the precursor secologanin (Scheme 1). In addition, only one chiral carbon was present at C-10, and its configuration was identified as *S* refer to opposite optical orientation with (*R*)-(+)-2-(1′-methoxy)ethylfuran [16], accordingly. Therefore, **1** was determined as (S)-(-)-4-hydroxymethyl-5-acetoxyl-9-methoxyethylfuran.

Two other known trisubstituted furan derivatives were also identified as swertianglide (**2**), once isolated from *Swertia angustifolia* of Gentianaceae [17], and anglelone (**3**), once isolated from *Fagraea fragrans* [18, 19], *Tachiadenus longiflorus* [20] and *Gentiana veitchiorum* of Gentianaceae [21]. Thus, **2** was firstly reported from the genus *Gentiana* and **3** was firstly reported from the plant *G. macrophylla*.

Furan moiety as one part of compounds was formed depending on structural feature of α-isopropylidene ketone unit [22] or succinaldehyde unit (or its aldehyde/ketones-enol tautomerism unit) [23]. Thus, iridoids and secoiridoids had the potential to form furan derivatives, however, so far, only several furan derivatives such as anglelone and swertianglide were separated from the plants of Gentianaceae. The plausible biosynthesis pathways of them were proposed in Scheme 1. Secologanin, a crucial intermediate to form a large and still expanding group of monoterpenoids such as gentiopicroside, sweroside, swertiamarin, *etc*, was also deduced as the precursor to form furan derivatives in the plants of Gentianaceae. Its biosynthesis pathway was initiated from MVA or MEP pathway to generate IPP and GPPS, then, under the geraniol synthase GES, geraniol 8-oxidase G8O, iridoid synthase IS, glucosyltransferase 7-DLGT, hydroxytransferase 7-DLH and loganic acid *O*-methyltransferase LAMT to successively catalytic generate geraniol, 8-oxogeranial, 7-deoxyloganetic acid, 7-deoxyloganic acid, loganic acid and loganin, and finally under secologanin synthase SLS to secologanin [13]. During the formation of furan derivatives, secologanin was first hydroxylated at C-5 to active the internal impetus for the transformation. Although 5-hydroxysecologanin was not isolated from the plants so far, we deduced this reaction based on two aspects: one as the occurrence from sweroside to swertiamarin, the other as the incapable rearrangement to generate the key succinaldehyde unit for the formation of furan moiety. Moreover, the decarboxylation at C-4 or C-9 was also occurred for the penta-cyclization to form furan ring. Thus, the biosynthesis pathways for genfurtate (**1**), swertianglide (**2**) and anglelone (**3**) transformed from secologanin in *G. macrophylla* were deduced (Scheme 1).



Scheme 1. Putative biosynthesis pathways of **1**–**3** transformed from secologanin in *G. macrophylla*

Acknowledgments

This work was partially supported by the Natural Science Foundation of China (No. 81903753, 81503237), the Key Research and Development Program of Shaanxi Province (No. 2022SF-089), Project on Collaborative In-novation of Shaanxi Provincial Department of Education (No. 21JY011), Key Program (A) of Shaanxi Provincial Health Commission (No. 2021A008), the Natural Science Foundation of Qinghai (No. 2022-ZJ-917), the Fundamental Research Funds for the

Shaanxi University of Chinese Medicine (No.123010203), Subject Innovation Team of Shaanxi University of Chinese Medicine (No. 2019 YS01).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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