Tetrahedron Letters 58 (2017) 1459-1461

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Rigenolides B and C, conjugates of norsecoiridoid and secoiridoid glucoside from *Gentiana rigescens* Franch.



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ARTICLE INFO

Article history: Received 25 January 2017 Revised 21 February 2017 Accepted 24 February 2017 Available online 27 February 2017

Keywords: Norsecoiridoid Secoiridoid Rigenolides B and C Gentiana rigescens Gentianaceae

ABSTRACT

Two new conjugates of norsecoiridoid and secoiridoid glucoside, rigenolides B (1) and C (2), were isolated from a Chinese herbal medicine *Gentiana rigescens* Franch. Their structures were established by comprehensive spectroscopic analysis. The absolute configurations of 1 and 2 were elucidated by comparison of the ECD spectra with TDDFT calculated spectra.

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The genus Gentiana, the largest member of Gentianaceae, consists of about 400 species. Various Gentiana plants have been included in herbal remedies for poor appetite and digestive problems worldwide.¹ A number of iridoids, secoiridoids, and their glycosides have been isolated from Gentiana plants to date. Gentiana rigescens Franch. is a perennial herb, whose roots are used to treat hepatitis and cholecystis by the Yi ethnic minority group living in Yunnan province, China. As part of our study for traditional herbal medicines used by ethnic minority groups in Yunnan province,² aimed at searching for new drug leads, we have investigated the extract from the aerial parts of G. rigescens and reported the isolation of secoiridoids and norsecoiridoids.³ Among others, rigenolide A³ is a structurally unique secoiridoid glucoside possessing a cyclobutane moiety. Further investigation of the extract resulted in the isolation of two new conjugates of norsecoiridoid and secoiridoid glucoside, rigenolides B (1) and C (2). In this paper, we describe the isolation and structure elucidation of 1 and 2.

The MeOH extract from the aerial parts of *G. rigescens* (1.9 kg, dry) was partitioned with EtOAc and H_2O . Chromatographic separation of the H_2O -soluble materials using Diaion HP-20 column,

* Corresponding author. E-mail address: kasiwada@tokushima-u.ac.jp (Y. Kashiwada). ODS column, silica gel column, and reversed phase HPLC afforded rigenolides B (1, 3 mg) and C (2, 1 mg) (Chart 1).

Rigenolide B $(1)^4$ was obtained as an optically active white amorphous powder $\{[\alpha]_D^{20} - 72.0(c0.31, \text{ MeOH})\}$. The molecular formula of $C_{25}H_{30}O_{12}$ was established by the HRESIMS (m/z545.1617 [M+Na]⁺, Δ – 1.8 mmu). Comparison of the ¹H and ¹³C NMR spectra (Table 1) with the literature data for known secoiridoids indicated that 1 had a secoiridoid glucoside moiety (unit A, C-1-C-11 and C-1'-C-6') and unit B (C-3"-C-11"). Unit A of 1 was deduced to be gentiopicroside⁵ by resemblance of their NMR data. In contrast, the 1D NMR spectra suggested that unit B was comprised of nine carbons including one α,β -unsaturated carboxy moiety, one acetal carbon, one oxygenated sp³ methine, three sp³ methylenes, including one oxygenated, and one secondary methyl (Table 1). ¹H-¹H COSY cross-peaks of oxygenated sp³ methylene (H_2-7'') with H_2-6'' and HMBC correlations for H_2-7'' with C-5'' and C-11" and for H2-6" with C-4" revealed the presence of an $\alpha,$ β -unsaturated- δ -lactone moiety (C-4"-C-7" and C-11") (Fig. 1). The ¹H-¹H COSY spectrum also suggested the presence of a 2-oxygenated propyl group (C-8"-C-10"), while HMBC cross-peaks of H-9"/C-4", H-9"/C-5", and H-3"/C-4" indicated the connectivity of C-5" to C-9" and of C-4" to the acetal carbon (C-3"). The ether linkage between C-3" and C-8" was disclosed by an HMBC correlation for H-3" with C-8". The syn relationship for H-3" and H-8" was





Chart 1. The structures of rigenolides B (1) and C (2).

indicated by a NOESY correlation between these protons. The structure of unit B, thus assigned (Fig. 1), corresponds to *epi*-swercinctolide B,³ previously isolated from the same plant origin by our group. These units were shown to be linked through an ether linkage between C-3" (unit B) and C-6' (unit A) by an HMBC cross-peak of H-3" to C-6'. Therefore, the gross structure of rigeno-lide B (1) was assigned as shown in Fig. 1.

Rigenolide C (**2**)⁶ was isolated as an optically active white amorphous powder { $[\alpha]_D^{20} - 96.6(c\,0.10, MeOH)$ }, and its molecular formula, C₂₅H₃₀O₁₂, determined by the HRESIMS (*m*/*z* 545.1653 [M +Na]⁺, Δ + 1.8 mmu) was identical to that of **1**. Though the ¹H and ¹³C NMR spectra of **2** and **1** were almost superimposable (Table 1), subtle differences were found for the chemical shifts of C-5' and C-3". These observations suggested their diastereomeric relationship, whereas the H-3"/H-8"-syn relationship for **2** was revealed by a NOESY correlation for H-3"/H-8" (Fig. 1).

The stereochemistry of rigenolides B (1) and C (2) were assigned as follows. Acid hydrolysis⁷ of 1 gave a sugar moiety, which was treated successively with L-cysteine methyl ester hydrochloride and phenylisothiocyanate.^{8,9} HPLC analysis of the reaction mixture revealed the sugar moiety of 1 to be D-glucose.¹⁰ Similarly, the sugar moiety of 2 was assigned as D-glucose. The H-1/H-9-*anti*

Table 1



Fig. 1. Key 2D NMR correlations of rigenolides B (1) and C (2).

relationships for 1 and 2 were indicated by resemblance of their ${}^{3}J_{\text{H-1/H-9}}$ values (Table 1) with the literature value (J = 3.0 Hz).⁵ Therefore, taking the H-3"/H-8"-syn relationships in 1 and 2 assigned by NOESY analysis into account, the ECD spectra of their four possible diastereomers (**3a**: 1S.9R.3"R.8"R: **3b**: 1S.9R.3"S.8"S: **3c**: 1*R*,9*S*,3"*R*,8"*R*; **3d**: 1*R*,9*S*,3"*S*,8"*S*) were calculated¹¹ to compare with those experimental spectra of **1** and **2**. The experimental ECD spectrum of gentiopicroside was also compared with the calculated spectra (Fig. 2). In the calculated ECD spectra of 1S,9R isomers (3a and 3b), negative Cotton effects were found around 270 nm, whereas 1*R*,9*S* isomers (**3c** and **3d**) showed positive Cotton effects around 270 nm. These observations suggested that the sign of Cotton effect around 270 nm reflects the absolute configuration at C-1 and C-9. A negative Cotton effect at 277 nm observed in gentiopicroside possessing the 1S,9R configuration was well consistent with the observation described above. Rigenolides B(1) and C(2)also showed negative Cotton effects at 278 and 273 nm, respectively (Fig. 3), suggesting the 1S,9R configurations of 1 and 2. In contrast, the 3"R,8"R configurations of 1 and the 3"S,8"S configuration of **2** were concluded by resemblance of the

Position	1		2	
	δ _H (J in Hz)	δ _C	δ _H (J in Hz)	δ _C
1	5.62 (1H, d, 3.1)	98.8	5.60 (1H, d, 3.1)	98.9
3	7.44 (1H, d, 1.1)	150.7	7.45 (1H, d, 1.1)	150.9
4	=	104.9	-	104.8
5	-	127.1	-	127.2
6	5.60 (1H, m)	117.2	5.59 (1H, m)	117.0
7	5.06 (1H, dd, 17.7, 1.3)	70.9	5.06 (1H, dd, 17.7, 1.3)	70.9
	4.98 (1H, dd, 17.7, 3.5)		4.98 (1H, dd, 17.7, 3.5)	
8	5.74 (1H, ddd, 17.2, 10.2, 6.9)	135.0	5.71 (1H, ddd, 17.4, 10.4, 6.9)	135.0
9	3.28 (1H, m)	46.7	3.28 (1H, m)	46.7
10	5.20 (1H, ddd, 17.2, 1.3, 1.3)	118.5	5.22 (1H, ddd, 17.4, 1.3, 1.3)	118.6
	5.18 (1H, ddd, 10.2, 1.3, 1.3)		5.19 (1H, ddd, 10.4, 1.3, 1.3)	
11	-	166.3	-	166.3
1′	4.63 (1H, d, 8.0)	100.5	4.63 (1H, d, 8.0)	100.6
2′	3.14 (1H, dd, 9.0, 8.0)	74.5	3.17 (1H, dd, 9.0, 8.0)	74.5
3′	3.34 (1H, dd, 9.0, 9.0)	77.9	3.35 (1H, dd, 9.0, 9.0)	78.2
4′	3.27 (1H, m)	71.5	3.24 (1H, dd, 9.0, 9.0)	71.9
5′	3.48 (ddd, 9.0, 6.5, 1.7)	77.7	3.54 (1H, ddd, 9.0, 6.8, 1.9)	76.6
6′	4.05 (1H, dd, 11.7, 1.7)	68.6	4.01 (1H, dd, 11.5, 1.9)	68.6
	3.77 (1H, dd, 11.7, 6.5)		3.81 (1H, dd, 11.5, 6.8)	
3″	5.39 (1H, s)	95.0	5.36 (1H, s)	93.9
4″	-	123.7	-	123.7
5″	-	157.1	-	156.8
6″	2.59 (1H, ddd, 18.0, 9.7, 6.0)	29.3	2.60 (1H, ddd, 17.0, 10.0, 6.0)	29.3
	2.39 (1H, ddd, 18.0, 4.8, 4.8)		2.36 (1H, ddd, 17.0, 4.6, 4.6)	
7″	4.38 (2H, m)	67.0	4.38 (2H, m)	66.9
8″	4.20 (1H, m)	63.0	4.21 (1H, m)	63.2
9″	2.29 (1H, dd, 19.0, 3.9)	37.5	2.27 (1H, dd, 18.9, 4.1)	37.5
	2.21 (1H, dd, 19.0, 10.6)		2.21 (1H, dd, 18.9, 10.3)	
10″	1.27 (d, 6.3)	21.0	1.26 (1H, d, 6.3)	21.0
11″	-	165.0	-	165.0



Fig. 2. Experimental ECD spectrum of gentiopicroside and calculated spectra of possible diastereomers (3a-3d) of rigenolides B (1) and C (2).



Fig. 3. Experimental ECD spectra of rigenolides B (1) and C (2) and calculated spectra of their possible diastereomers (3a-3d).

experimental ECD spectra of 1 and 2 with those calculated spectra of **3a** and **3b**, respectively (Fig. 3). Consequently, the absolute stereochemistry of rigenolides B (1) and C (2) were assigned as shown in Chart 1.

The investigation of the aerial parts of Gentiana rigescens resulted in the isolation of two new conjugates of norsecoiridoid and secoiridoid glucoside, regenolides B (1) and C (2). Although some conjugates structurally similar to 1 and 2 have been reported to date,¹⁴ their absolute stereochemistry remains to be assigned. In this study, the structures of rigenolides B (1) and C (2) including the absolute stereochemistry were elucidated on the basis spectroscopic analysis, chemical conversion, and TDDFT ECD calculation.

Acknowledgments

This investigation was partly supported by Grant-in-Aid for Scientific Research (B. No. 18406028) for the Japan Society for the Promotion of Science.

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- Rigenolides B (1, 1.0 mg) and C (2, 0.8 mg) was separately treated with 5% H₂SO₄ (2 mL) at 70 °C for 3 h. After neutralization by anion-exchange resin (IRA-400, ORGANO Co.), solvent was evaporated to give the residue containing the sugar moiety of 1 and 2, respectively.
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- The residue containing the sugar moiety of rigenolide B (1) and L-cysteine methyl ester hydrochloride (0.5 mg) was dissolved in pyridine (100 μ L) and the mixture was heated at 60 °C for 1 h, and then phenylisothiocyanate (10 μ L) was added to the mixture and heated at 60 °C for 1h
- 10. HPLC analysis of the reaction mixture Cosmosil 5C18 AR-II, Nacalai Tesque, 4.6×250 mm, flow rate 0.8 mL/min, UV detection at 250 nm, eluent CH₂CN/50 mM H₃PO₄ ag. 1:3, column temperature 35 °C gave a peak at 17.2 min. The retention time was identical to that of the derivative from authentic p-glucose.
- Conformational searches and DFT calculations were carried out on Spartan 14 (Wavefunction, Irvine, CA) and Gaussian 09¹² programs, respectively. Possible four diastereomers [3a: 15,9R,3"R,8"R; 3b: 15,9R,3"S,8"S; 3c: 1R,9S,3"R,8"R; 3d: 1R,9S,3"S,8"S] of rigenolides B (1) and C (2) were subjected to conformational search using MMFF94s as the force field. The initial stable conformers with Boltzmann distributions over 1% (6, 8, 9, and 11 conformers, respectively) were further optimized by DFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a polarizable continuum model (PCM). The stable conformers with Boltzmann distributions over 1% were subjected to TDDFT calculations at the B3LYP/6-31G(d) level in the presence of MeOH with a PCM. The resultant rotatory strengths of the lowest 30 excited states for each conformer were converted into Gaussian-type curves with half-bands (0.3 eV for **3a** and **3b**; 0.2 eV for **3c** and **3d**) using SpecDis v1.61.¹³ The calculated CD spectra were composed after correction based on the Boltzmann distributions of the stable conformers. The calculated ECD spectrum of 3a was red-shifted by 10 nm.
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