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Chemical Constituents of the Aerial Parts of *Chloranthus japonicus* Sieb.

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Abstract – Chemical investigation of the aerial parts of *Chloranthus japonicus* Sieb. led to the isolation a new compound, 9-hydroxy heterogorgiolide (**1**) and isofraxidin-7-*O*-β-D-glucopyranoside (**2**), the isolation of which is reported for the first time from this plant, along with the known components, β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, palmitic acid and octacosanoic acid. The structures of compound **1** and **2** were determined on the basis of spectroscopic data including two dimensional NMR and high resolution MS.
Keywords – *Chloranthus japonicus* Sieb., 9-hydroxy heterogorgiolide, isofraxidin-7-*O*-β-D-glucopyranoside

Introduction

Chloranthus japonicus Sieb. (Chloranthaceae) is a perennial herb growing in Korea, Japan and northeastern China. The Chloranthaceae is a small family with three genera and around seventy species occurring in tropical America, East Asia and the Pacific region with one genera and three species known to grow in Korea. The aerial parts and the roots of *C. japonicus* have been used for boils, dermatopathia, enteric fever, detoxification, and diseases caused by blood stagnation in Korea as a folk remedy. (Bae, 2000; Chung 1968)

The isolation of chloranthalactone C, D and E, chloranthalactone C epoxide, atracthyenolide I and III (Uchida *et al.*, 1980), shizukanolide and glechomanolid (Kawabata *et al.*, 1981) were reported from the aerial parts of this plant. Among them, atracthyenolide III was reported to show an antiinflamatory effect and shizukanolide and glechomanolid were to have mild effect against microbes.

In the course of our searching for anti-platelet and anti-oxidant plant components, six compounds were isolated from *C. japonicus*. Compound **1**, 9-hydroxy heterogorgiolide, was a new compound and compound **2**, isofraxidin-7-*O*-β-D-glucopyranoside (eleutheroside B_1), was isolated for the first time from this plant for the best of our knowledge. The other four compounds were β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, palmitic acid and octacosanoic acid. Their chemical structures were determined on the basis of their spectroscopic parameters of EI-MS, FAB-MS, ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H COSY, HETCOR, HMBC and NOESY spectral data.

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Experimental

Plant material – The plant material was collected at Mt. Baekam in June 2002 and identified by Prof. Ki Hwan Bae, College of Pharmacy, Chungnam National University. The voucher specimen (JE200206-1) has been deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Korea.

General experimental procedures – ^{1}H-NMR (300 MHz), ^{13}C-NMR (75.5 MHz) and two dimensional (^{1}H-^{1}H COSY and HETCOR) spectra were measured on Varian Gemini 2000 spectrometer (U.S.A.) using TMS as an internal standard. HMBC and NOESY-NMR spectra were recorded on UI 500 FT-NMR spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR (Varian, Inc. USA). IR spectra were obtained with a Jasco FT/IR-3300 spectrometer on KBr plate. UV spectra were recorded with a Hitachi U-3210 UV-VIS spectrometer. EI-MS spectrum was obtained on a Hewlett Packard 5989B mass spectrometer (U.S.A.). FAB-MS spectrum was measured on JMS-700 Mstation mass spectrometer (JEOL Ltd, Japan). Melting point was determined on a Büchi B-540 melting point apparatus and uncorrected. $[\alpha]_D$ was measured on a Jasco P-1020 autopolarimeter. FAB-MS, HMBC and NOESY spectra were measured in Seoul Branch, Korea Basic Science Institute.

Extraction and isolation – The dried aerial parts of *C.*

japonicus (5 kg) were percolated with methanol for two months at room temperature. The MeOH extract concentrated *in vacuo* (472 g) was partitioned with $CHCl_3$ and H_2O, and the $CHCl_3$ layer (146 g) after concentration was partitioned with *n*-hexane and 90% MeOH to obtain *n*-hexane fraction (fr. I, 44 g) and 90% MeOH fraction (fr. II, 102 g). H_2O layer was extracted with EtOAc to obtain EtOAc soluble fraction (fr. III, 20 g) and then with BuOH to obtain BuOH soluble fraction (fr. IV, 35 g). Fr. II (80 g) was subjected to a silica gel (2.0 kg) column chromathgraphy eluting with $CHCl_3$:MeOH (10 : 0 → 2 : 8) to give sub-fractions II-1~II-28. Sub-fraction II-14 (5 g) [$CHCl_3$: MeOH (9.9 : 0.1)] was re-chromatographed on a silica gel (700 g) column eluting with hexane : EtOAc (9 : 1) to give compounds **1** (210 mg), **3** (115 mg), **5** (4 mg) and **6** (40 mg). Fr. IV (30 g) was applied to a silica gel (1.4 kg) column and eluted with a gradient solvent mixture of $CHCl_3$: MeOH (10 : 0→3 : 7) yielding nine sub-fractions IV-1~IV-9. Compound **2** (102 mg) was obtained from the sub-fraction IV-3. Compound **4** (14 mg) was obtained from IV-2 upon silica gel (400 g) column chromatography eluting with $CHCl_3$: MeOH (9 : 1).

Compound 1 – white amorphous powder, mp 238~239°C, R*f* = 0.53 on silica gel (Hexane-EtOAc, 7:3), $[\alpha]_D^{23}$ = −185.2° (c 0.5, $CHCl_3$); HR-FAB-MS : $C_{16}H_{21}O_4$, *m/z* 277.1417 $[M+H]^+$ calcd : *m/z* 277.1440; UV (MeOH) λ_{max} (log ε) : 213 nm (4.01); IR ν_{max} cm^{-1} (KBr) : 3518, 1778 (α, β unsaturated γ-lactone), 1693, 1655 (C=C), 1132 (C-O); ^{1}H-NMR ($CDCl_3$ and CD_3OD, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75.5 MHz) : see Table **1**.

Compound 2 – colorless crystalline powder, mp 202~203°C, R*f* = 0.35 on silica gel ($CHCl_3$-MeOH, 8:2), $[\alpha]_D^{25}$ = +44.1° (c 0.5, MeOH); EI-MS *m/z* 222 [M-($C_6H_{12}O_6$)]$^+$; UV (MeOH) λ_{max} (log ε) : 340 (4.24), 289 (4.26), 224 (4.29); IR ν_{max} cm^{-1} (KBr) : 3458 (OH), 1718 (C=O), 1574, 1485 (aromatic C=C); ^{1}H-NMR (300 MHz, pyridine-d_5) : δ 3.74 (3H, *s*, 6-OCH_3), 4.0~4.5 (10H, *m*), 4.14 (3H, *s*, 8-OCH_3), 6.08 (1H, *d*, *J* = 7.8 Hz), 6.39 (1H, *d*, *J* = 9.6 Hz, H-3), 6.83 (1H, *s*, H-5), 7.64 (1H, *d*, *J* = 9.6 Hz, H-4); ^{13}C-NMR (75.5 MHz, pyridine- d_5) : δ 56.6 (6-OCH_3), 61.8 (8-OCH_3), 62.4 (C-6`), 71.4 (C-4`), 75.8 (C-2`), 79.1 (C-5`), 103.9 (C-1`), 105.5 (C-5), 115.0 (C-4a), 115.2 (C-3), 141.4 (C-8), 143.0 (C-7), 143.5 (C-8a), 144.0 (C-4), 150.3 (C-6), 160.5 (C-2)

Results and discussion

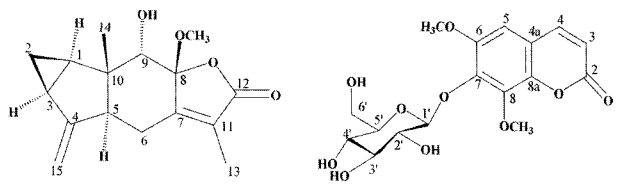
The MeOH extract of the aerial parts of *C. japonicus* was fractionated into several solvent fractions. The 90% MeOH fraction (fr. II) and the BuOH fraction (fr. IV) were repeatedly chromatographed over silica gel to yield compounds **1~6**. Compounds **3**, **4**, **5** and **6** were identified, by the direct comparison of the spectral data with the literature values, as β-sitosterol (Flamini *et al.*, 2001; Ju *et al.*, 2000; Yun-Choi *et al.*, 2003), β-sitosterol-3-*O*-β-D-glucopyranoside (Chang *et al.*, 1981; Gohar *et al.*, 2000), palmitic acid (Ashworth *et al.*, 1985) and octacosanoic acid (Lee *et al.*, 1995) respectively, which are occurring ubiquitously in higher plants and also have previously been isolated from this genus. (Gao *et al*, 1987)

Compound **1** was isolated as an amorphous white powder. The molecular formula of **1** was determined as $C_{16}H_{21}O_4$ on the basis of HR-FAB-MS and ^{13}C-NMR data (Table. 1). The IR spectrum of **1** revealed absorptions assigned to α,β-unsaturated γ-lactone (3518 and 1778 cm^{-1}) and double bond (1693 and 1655 cm^{-1}) functionalities. ^{13}C-NMR experiments, including DEPT analysis, indicated the presence of three methyl groups, three methylene carbons, four methines and six quaternary carbons. The presence of one tetrasubstituted double bond and one exocyclic methylene suggested by ^{13}C-NMR signals at δ 106.0, 128.0, 152.1 and 156.3 and by two signals (1H each) at δ 4.70 and 5.00 in the ^{1}H-NMR spectrum. The presence of a carbonyl signal at δ 171.0 in ^{13}C-NMR, an infrared absorption at 1778 cm^{-1} and an UV absorbance at 213nm illustrated that **1** possesses an α,β-unsaturated γ-lactone. The presence of a methoxyl group was indicated by ^{13}C- and ^{1}H-NMR signals at δ 50.3 and δ 3.21, respectively. The presence of a hydroxyl proton signal at δ 3.41 (OH, *d*, *J* = 7.5 Hz), which disappeared with D_2O was observed. The presence of a cyclopropane ring was strongly suggested by the shielded signals at δ 0.67 (1H, *dt*, *J* = 3.9 and 5.1 Hz) and 0.82 (1H, *dt*, *J* = 5.1 and 8.1 Hz). The remaining five- and six- membered rings were subsequently confidently assigned by ^{1}H-^{1}H COSY and HMBC correlation data (Table. 1, Fig. 2). These data allowed the complete planar structure of 9-hydroxy heterogorgiolide to be assigned to compound **1** (Fig. 1). The stereochemistry of 9-hydroxy heterogorgiolide was assigned by the interpretation of ^{1}H-NMR coupling and the NOESY spectral data. (Fig. 3) NOE enhancement experiments were also performed to confirm the stereochemistry. When the methyl protons at δ 0.50 (3H, *s*) were irradiated, NOE enhancements were observed for H-2β, H-6β and H-9β suggesting the bridgehead methyl group at the β-axial position and the hydroxyl group (9-OH) at the α-axial position. The stereochemistry of all substituents are identical to those of other linderane sesquiterpenes previously isolated from terrestrial plants and marine organisms. (Kawabata *et al.*, 1979; Kawabata

Table 1. NMR data for 9-hydroxy heterogorgiolide

C#/H#	δ ^{13}C (DEPT)[a]	δ ^{1}H (m, *J* in Hz)[a]	δ ^{1}H (m, *J* in Hz)[b]	^{1}H-^{1}H COSY (H#)[a]	HMBC correlation (H#)	
					^{2}J	^{3}J
1	22.8 (CH)	2.11 (*br dt*, 3.9, 8.1)	2.06 (*dt*, 3.9, 8,1)	2ab, 3	3	14
2a	15.7 (CH_2)	0.67 (*dt*, 3.9, 5.1)	0.69 (*dt*, 3.9, 5.1)	1, 2b, 3		
2b		0.82 (*dt*, 5.1, 8.1)	0.82 (*ddd*, 5.1, 8.4, 8.1)	1, 2a, 3		
3	23.6 (CH)	1.95 (*m*)	1.96 (*m*)	1, 2ab, 15ab	1, 2b	15ab
4	152.1 (C)				5, 15ab	2ab
5	51.8 (CH)	3.32 (*m*)	3.47 (*m*)	6ab, 15ab	6ab	9, 14, 15ab
6a	22.5 (CH_2)	2.29 (*m*)	2.32 (*ddd*, 0.9, 12.3, 18)	5, 6b, 13		
6b			2.43 (*ddd*, 1.8, 6.6, 18)	5, 6a, 13		
7	156.3 (C)				6ab	13
8	105.8 (C)				9	6ab, C9-OH, 16
9	79.6 (CH)	3.83 (*d*, 7.5)	3.71 (*s*)	C9-OH	C9-OH	1, 5, 14
		3.41 (OH, *d*, 7.5)				
10	43.8 (C)				5, 9, 14	2b, 6ab, C9-OH
11	128.0 (C)				13	6ab
12	171.0 (C=O)					13
13	8.4 (CH_3)	1.87 (*t*, 1.2)	1.83 (*dd*, 0.9, 1.8)	6ab		
14	20.1 (CH_3)	0.50 (*s*)	0.51 (*s*)	9		5, 9
15a	106.0 (CH_2)	4.70 (*br t*)	4.76 (*br t*)	3, 5, 15b		
15b		5.00 (*m*)	4.97 (*m*)	3, 5, 15a		
OCH_3(16)	50.3 (OCH_3)	3.21 (*s*)	3.17 (*s*)			

[a] ^{1}H and ^{13}C assignment made on the basis of HETCOR ($CDCl_3$, 300 and 75.5 MHz), [b] CD_3OD



1 2

Fig. 1. Compounds **1** and **2**.

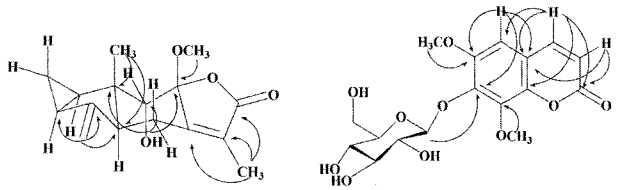


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Fig. 2. Selected HMBC correlations of compounds **1** and **2**.

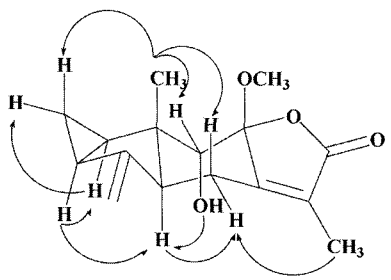

Fig. 3. NOSEY correlation of compound **1**.

et al., 1981; Kawabata *et al.*, 1995; Maia *et al.*, 1999; Tahara *et al.*, 1981; Uchida *et al.*, 1980)

Compound **2** was obtained as colorless crystalline powder from MeOH. The IR spectrum showed presence of hydroxyl group at 3458 cm^{-1}, α-pyrone C=O at 1718 cm^{-1}, and aromatic C=C at 1574, 1485 cm^{-1}. In the ^{1}H-NMR spectrum, compound **2** revealed one aromatic singlet and, two doublets at δ 6.39 (1H, *d*, *J* = 9.6 Hz) and δ 7.64 (1H, *d*, *J* = 9.6 Hz) ascribable to the α-pyrone moiety. Two methoxy signals appeared at δ 3.74 (3H, *s*) and δ 4.14 (3H, *s*). The spectrum further showed an anomeric carbon signal at δ 6.08 (1H, *d*, *J*=7.8 Hz), indicating the presence of a sugar moiety in this compound. The ^{13}C-NMR spectrum with DEPT showed the presence of six non-protonated carbons at δ 115.0 (C-4a), 141.4 (C-8), 143.0 (C-7), 143.5 (C-8a), 150.3 (C-6), 160.5 (C-2) and one methylene carbon at δ 62.4 (C-6`), two methyl carbons at δ 56.6 (6-OCH_3), 61.8 (8-OCH_3), and eight methine carbons. The signals for sugar moiety at δ 62.4, 71.4, 75.8, 78.4, 79.1, 103.9 were confirmed the presence of β-D-glucose. With the above spectral data, the structure of compound **2** was identified as isofraxidin-7-*O*-β-D-glucopyranoside (eleutheroside B_1) by the comparison of the spectral data with the literature values. (Kozawa *et al.*, 1983)

9-hydroxy heterogorgiolide (**1**) is a new compound and this is the first report of isofraxidin-7-*O*-β-D-

glucopyranoside (**2**) from this plant for the best of our knowledge.

Compound **1** and **2** showed only very mild inhibitory effects on collagen, U46619, AA and epinephrine induced platelet aggregation. Compound **3** and **4** showed 2~6-fold higher potency than ASA on U46619 induced rat platelet aggregation. Compound **4** was equally potent as ASA on epinephrine and AA induced platelet aggregation. (Jin *et al.*, 2004) Compound **3** showed weak DPPH radical scavenging activity.

The isolated compounds demonstrated only milder activities than expected from the anti-aggregatory and anti-oxidative inhibitory activities of the solvent fractions. The above findings suggested the possibilities of the presence of other potential active components in this plant besides the presently separated compounds.

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