Chemical studies on Mongolian and Japanese medicinal plants
— Constituents of *Gentianella amarella* ssp. *acuta*, *Lomatogonium carinthiacum*, *Ligularia sibirica*, *Caragana jubata*, and *Hypericum erectum* —

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General introduction

As the dramatic development of scientific technologies, world health has been improved to a great extent, including the successful control of many infectious disorders such as smallpox and plague, and the major declines in infant, child, and maternal mortality. Global life expectancy at birth increased from under 40 years in the first decade of the twentieth century to some 65 years now. In many economically advanced countries, average life expectancy at birth is up to 80 years. Nowadays, lots of diseases including angiocardiopathy, hypertention, diabetes mellitus, and cancer are coming to the fore and new curative drugs are urgently needed to be developed [1].

Natural resources, including plants, microbes, and marine organisms have been recognized to be a rich source of secondary metabolites with diverse structures and biological activities. Since the early 1800s, pure bioactive compounds such as colchicine, morphine, quinine, and atropine were isolated, which was regarded as a starting of a new pharmaceutical epoch based on the natural products [2]. Nowadays, nature products are also an indispensable part in pharmaceutical industry. Of 1562 drugs approved by the U.S. Food and Drug Administration from 1981 to 2014, more than 50% drugs possessing the structures derived from natural resources. Additionally, compounds arising from nature plant occupy 65% in 1211 approved small-molecule drugs from 1981 to 2014 [3]. Thus, natural products served as a source of novel drug entities, are playing a significant role in the discovery of new treatments for the modern pharmaceutical care.

Our laboratory has been investigating the constituents of medicinal plants used in diverse areas in East and Central Asia such as Mongolia, Japan, China, and Bangladesh, designated to identify natural products-based lead compounds for new therapeutic agents. As part of this study, phytochemical investigations on four Mongolian medicinal plants, *Gentianella amarella* ssp. *acuta*, *Lomatogonium carinthiacum*, *Ligularia sibirica*, and *Caragana jubata*, as well as a Japanese medicinal plant, *Hypericum erectum*, were taken out to isolate new secondary metabolites.

Chapter 1

Study on the constituents of Mongolian medicinal plants

1.1. Mongolian medicinal plants

Mongolia lies in the northern part of the Central Asian plateau, which is situated between China and Russia. The landlocked country occupies a differential ecological transition zone, where the Gobi Desert, the Central Asian Steppe, the Siberian Taiga forest, and the Altai Mountains meet together. The extreme harsh climate with long and cold winters and short summers offers a variety of plant species, some of which are globally endangered [4].

Mongolians used traditional medicines to prevent and cure the diseases of human and animals, and to improve animal productivity and fertility from ancient times. About 72% of traditional Mongolian medicines were developed from medicinal plants, and over 28% were from animal or mineral sources [4].

Although approximately 3000 species of plants are existing in the Mongolian flora, only about 100 species are being used for medicinal treatment at present. Consequently, most of them remain to be evaluated for their medicinal usages and bioactive components [4].

1.2. Chemical studies on *Gentianella amarella* ssp. *acuta*

1.2.1. Introduction

The genus *Gentianella* Moench (Gentianaceae), famous for its bitter taste, contains about 250 species of annual, biennial, or perennial plants, distributed in a wide range from the mountains of temperature regions to the Arctic Pole. Previous studies on the *Gentianella* plants led to the isolation of plenty of secondary metabolites such as xanthones, secoiridoids, flavone glycosides, and sesterterpenoids [5].

Gentianella amarella ssp. *acuta* (synonym *Gentiana acuta*) is an annual herb distributed in East Asia, Siberia, and North America, and has been used as a traditional herbal medicine for the treatment of hepatitis, fever, headache, and gallbladder disorders in Mongolia. Xanthones, steroids, triterpenoids, flavonoids, and iridoids have been isolated from this species previously (Fig. 1.2.1) [6,7]. As part of our research on Mongolian traditional medicines [9], aiming at searching natural product-based lead compounds, the constituents of the aerial parts of *G. amarella* ssp. *acuta* were investigated.

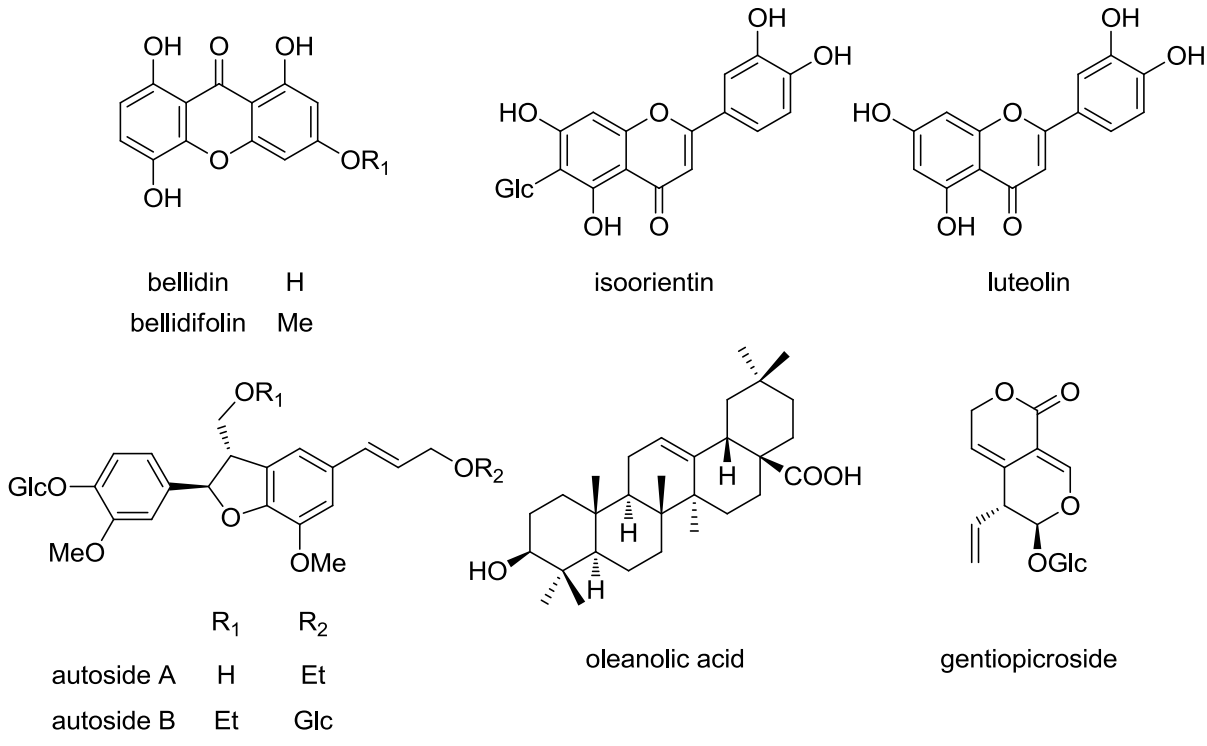
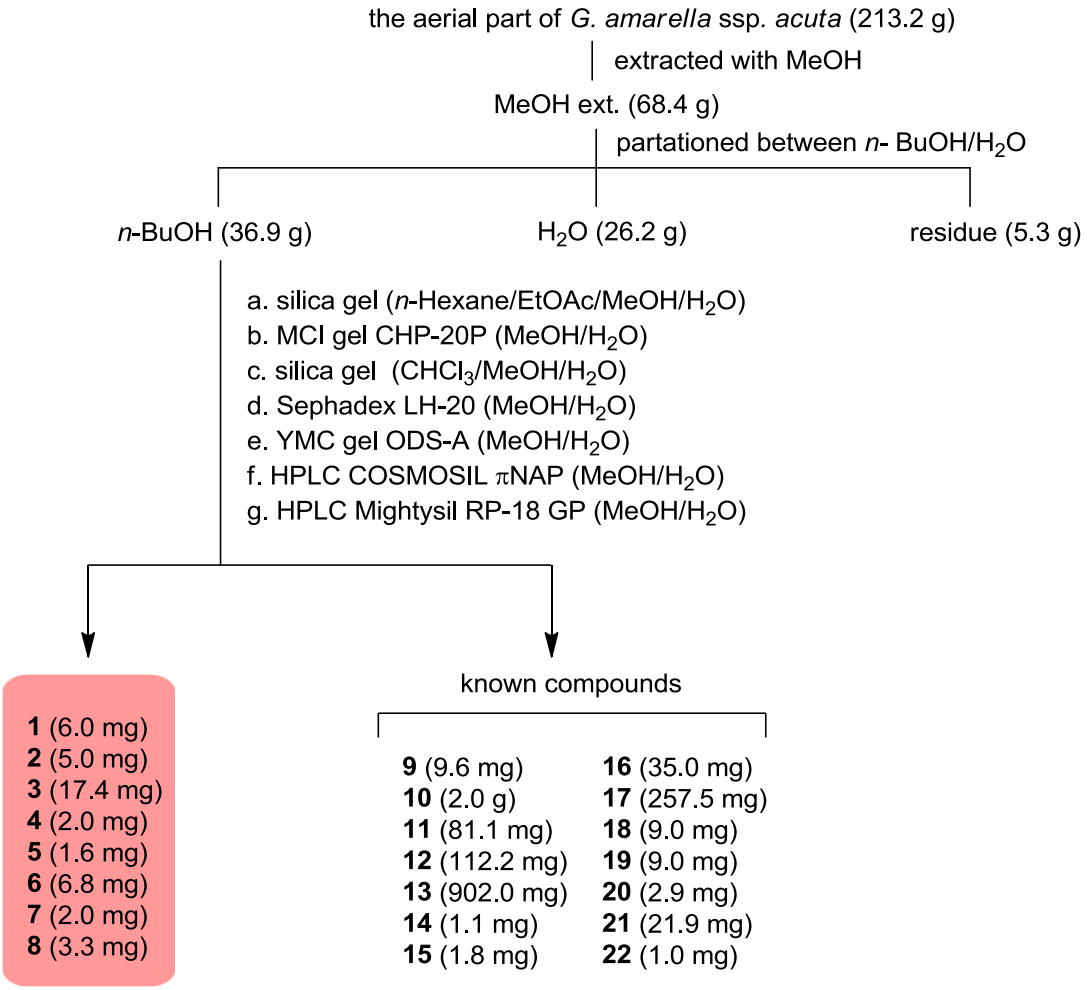


Figure 1.2.1. Previously isolated compounds from *G. amarella* ssp. *acuta*.

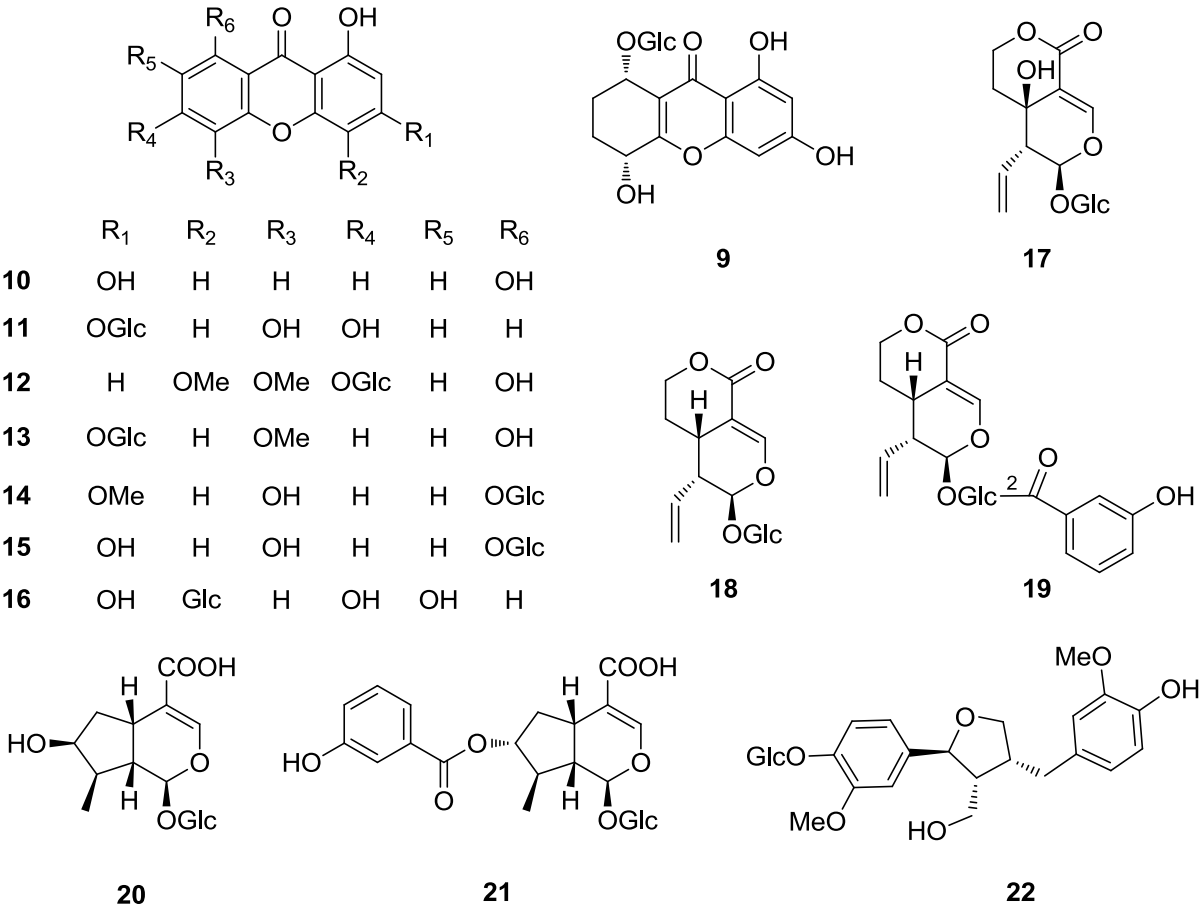
1.2.2. Extraction and isolation



Scheme 1. Isolation procedures for compounds **1**–**22**.

The aerial part of *G. amarella* ssp. *acuta* (213.2 g), collected Hosvsgol province, Mongolia in August 2011, was air-dried and extracted with MeOH. The MeOH extract (68.4 g) was partitioned between *n*-BuOH and H_2O. Repeated chromatographic separations of the *n*-BuOH-soluble material (36.9 g) gave two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**), as well as 14 known compounds (**9**–**22**) (Scheme 1). The known compounds were identified to be campestroside (**9**) [9], bellidin (**10**) [10], patuloside A (**11**) [11], corymbiferin 3-*O*-β-D-glucoside (**12**) [7], 3-*O*-β-D-glucosyl-1,8-dihydroxy-5-methoxyxanthone (**13**) [12], swertianolin (**14**) [13], norswertianolin (**15**) [14], isomangiferin (**16**) [15], swertiamarin (**17**) [16], sweroside A (**18**) [16], desacetylcentapicrin (**19**) [17], loganic acid (**20**) [18], swertiaside A (**21**) [19], and lariciresinol 4'-*O*-β-D-glucoside

(**22**) [20], by comparison of their spectroscopic data with the literature data (Fig. 1.2.2).



	R_1	R_2	R_3	R_4	R_5	R_6
10	OH	H	H	H	H	OH
11	OGlc	H	OH	OH	H	H
12	H	OMe	OMe	OGlc	H	OH
13	OGlc	H	OMe	H	H	OH
14	OMe	H	OH	H	H	OGlc
15	OH	H	OH	H	H	OGlc
16	OH	Glc	H	OH	OH	H

Figure 1.2.2. Known compounds (**9**–**22**) from the aerial parts of *G. amarella* ssp. *acuta*.

1.2.3. Structure elucidation

1.2.3.1. Structures for 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**)

Compounds **1** and **2** were obtained individually as an optically active pale yellow amorphous solids {$[\alpha]^{20}_D$ +78.1 (*c* 0.34, MeOH) for **1**; $[\alpha]^{20}_D$ +9.1 (*c* 0.15, MeOH) for **2**}. The HRESIMS revealed that **1** and **2** had the same molecular formula of $C_{13}H_{12}O_6$ (*m/z* 287.0526 $[M+Na]^+$, Δ –0.6 mmu for **1**; 287.0528 $[M+Na]^+$ Δ –0.4 mmu for **2**). The ^{1}H NMR spectrum of **1** measured in CD_3OD showed the signals of two *meta*-coupled aromatic protons, two oxygenated sp^3 methines, and two sp^3 methylenes (Fig. 1.2.3 and Table 1.2.1), while the resonance due to a hydrogen-bonded hydroxy proton (δ_H 12.83, s) was observed in the ^{1}H NMR spectrum in DMSO-d_6. The ^{13}C NMR spectrum of **1** revealed the presence of 13 carbons including one carbonyl carbon, five aromatic carbons, and two olefinic carbons. Though the feature of the 1D NMR spectra of **2** was similar to that of **1**, subtle differences were found for the chemical shifts of H-5, H_2-6, and H_2-7. The 1D NMR spectra of **2** also resembled those of tetrahydrobellidifolin (1,5*R*,8*S*-tetrahydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone) [21] except for the absence of the methoxy signal. These observations implied **1** and **2** to be demethxyl derivatives of tetrahydrobellidifolin, and a diastereomeric relationship between **1** and **2**.

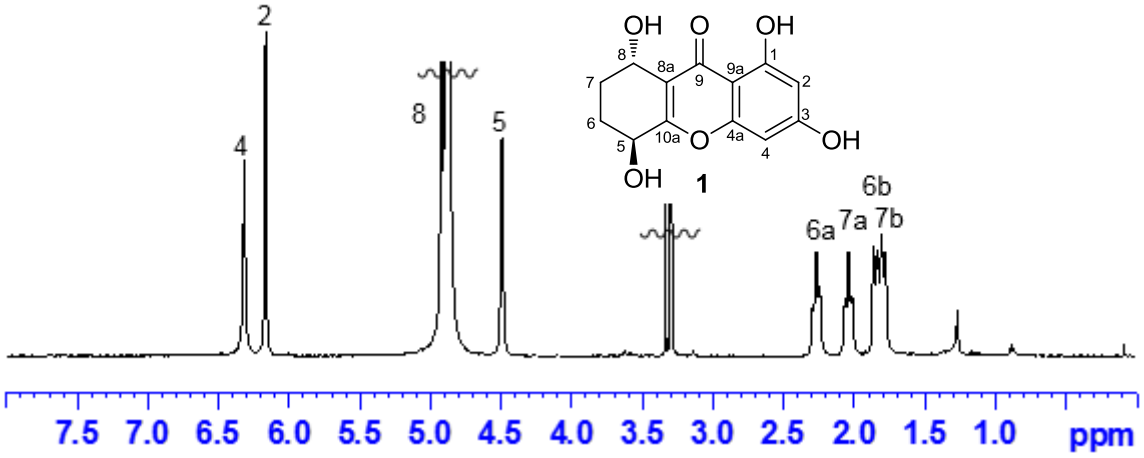


Figure 1.2.3. ^{1}H NMR spectrum of **1** in CD_3OD (500 MHz).

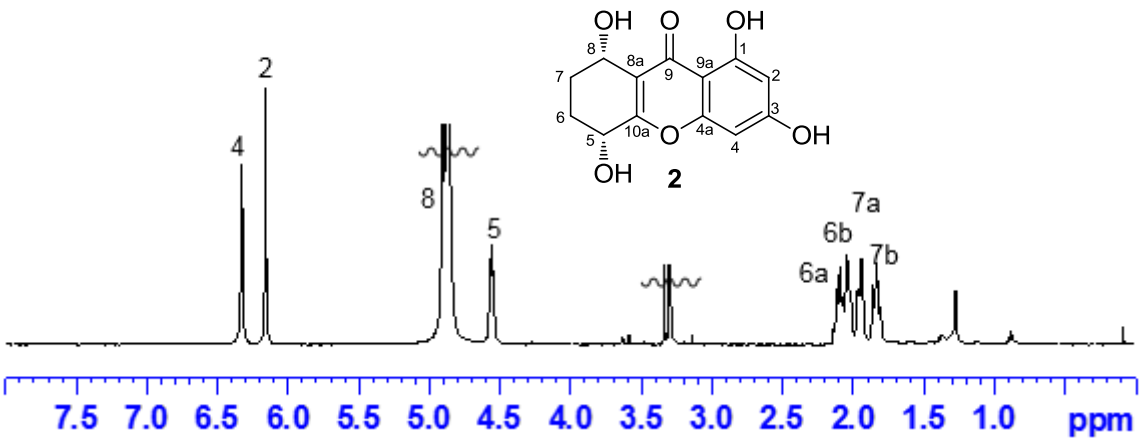


Figure 1.2.4. ^{1}H NMR spectrum of **2** in CD_3OD (500 MHz).

The planer structures of **1** and **2** were confirmed to be 1,3,5,8-tetrahydroxy-5,6,7,8-tetrahydroxanthone by analysis of the ^{1}H-^{1}H COSY and HMBC spectra (Fig. 1.2.5). Though 1,3,5,8-tetrahydroxy-5,6,7,8-tetrahydroxanthone was reported as a constituent of Gentianaceous plants, *Swertia punicea* [22] and *Lomatogonium carinthiacum* [23], the reported 1D NMR data [23] resembled neither those of **1** nor **2**.

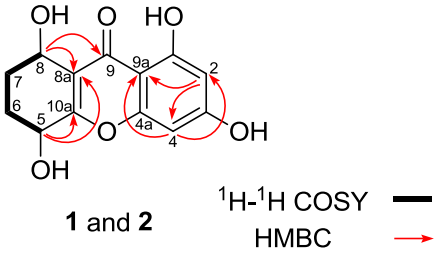


Figure 1.2.5. Selected ^{1}H-^{1}H COSY and HMBC correlations for **1** and **2**.

The relative and absolute configurations of **1** and **2** were assigned as follows. The pseudochair conformation of the cyclohexene ring (C-8a, C-5–C-8, and C-10a) and the H-5/H-8-*anti* relationship for **1** was suggested by NOESY correlations for H-8/H_2-7 and H-5/H_2-6 (Fig. 1.2.6**A**) and small ^{3}J values of the vicinal couplings for H-8/H_2-7 and H-5/H_2-6. NOESY correlations of H-8/H_2-7 and H-5/H-7β for **2** indicated the orientations of H-8 and H-5 to be pseudoequatorial and pseudoaxial, respectively (Fig. 1.2.6**B**). Thus, the H-5/H-8-*syn* relationship for **2** was assigned. This assignment was supported by resemblance of the chemical shifts of H-5, H_2-6, H_2-7, and H-8 for **2** with those for tetrahydrobellidifolin [21].

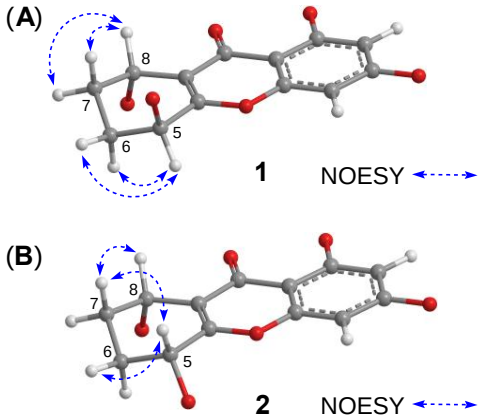


Figure 1.2.6. Selected NOESY correlations and relative stereochemistry for (**A**) 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and for (**B**) 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**) (protons of hydroxy groups are omitted).

Since **1** displayed only a quite weak cotton effect in the ECD spectrum, **1** was converted into the 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**1b**). The ECD spectrum of **1b** was similar to the calculated spectrum of (5*S*,8*S*)-**1b** rather than that of (5*R*,8*R*)-**1b** (Fig. 1.2.7**A**), indicating the 5*S* and 8*S* configurations of **1**. Confirmation of the 5*R* and 8*S* configurations of **2** was also obtained by comparison of the ECD spectrum of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**2b**) of **2** with the calculated spectra for a pair of enantiomers in the same manner (Fig. 1.2.7**B**).

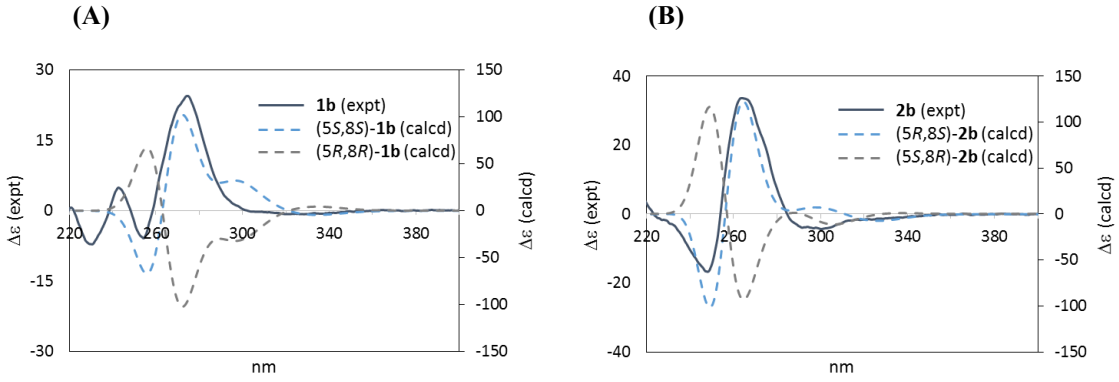


Figure 1.2.7. Experimental and calculated ECD spectra of (**A**) 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**1b**) of (5*S*,8*S*)-5,6,7,8-tetrahydro-1,3,5,8-tetrahydroxyxanthone (**1**) and (**B**) 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (**2b**) of (5*R*,8*S*)-5,6,7,8-tetrahydro-1,3,5,8-tetrahydroxyxanthone (**2**).

1.2.3.2. Gross structure for amarellin A (**3**)

Amarellin A (**3**) was isolated as an optically active pale yellow amorphous solid {$[\alpha]^{20}_{D}$ + 34.4 (*c* 0.15, MeOH)}. The molecular formula of **3** was assigned as $C_{19}H_{22}O_{11}$ by the HRESIMS (*m/z* 449.1076 $[M+Na]^+$, Δ +1.6 mmu). The ^{1}H NMR spectrum displayed the resonances due to a tetrahydroxanthone moiety similar to those of **1**, along with the signals arising from a sugar moiety (Fig. 1.2.8 and Table 1.2.2). The sugar moiety was deduced to be glucose based on the ^{1}H NMR coupling patterns and the ^{13}C chemical shifts. The glycosidic linkage at C-8 was revealed by HMBC analysis (Fig. 1.2.9).

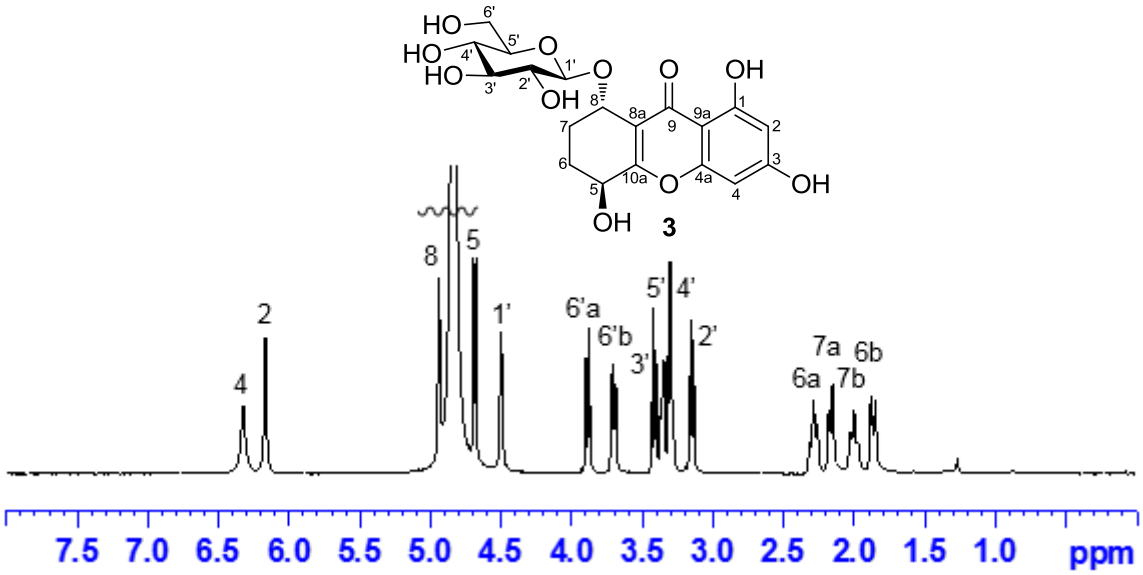


Figure 1.2.8. ^{1}H NMR spectrum of amarellin A (**3**) in CD_3OD (500 MHz).

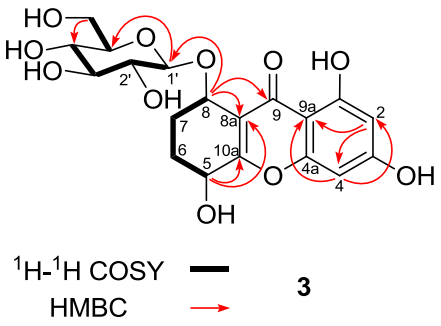


Figure 1.2.9. Selected ^{1}H-^{1}H COSY and HMBC correlations for amarellin A (**3**).

1.2.3.3. Gross structures for amarellins B (**4**) and C (**5**)

The HRESIMS suggested that amarellins B (**4**) and C (**5**) had the molecular formulae $C_{18}H_{20}O_{10}$ and $C_{19}H_{22}O_{11}$, respectively (*m/z* 419.0946 $[M+Na]^+$, Δ –0.8 mmu for **4**; *m/z* 449.1061 $[M+Na]^+$, Δ +0.1 mmu for **5**). Interpretations of the 2D NMR spectra indicated that **4** and **5** were 8-*O*-β-xyloside and 1-*O*-β-glucoside of **1**, respectively. In the ^{1}H NMR spectrum of **5** measured in C_5D_5N, the characteristic signal due to a hydrogen-bonded hydroxy proton was absent, which supported the connectivity of C-1 to the glucose for **5**.

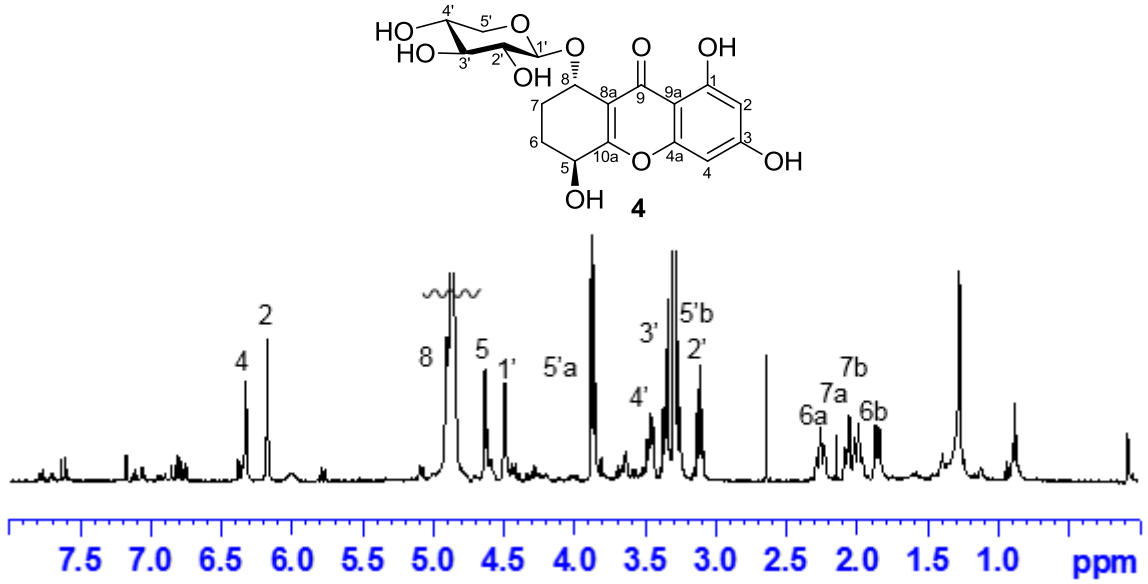


Figure 1.2.10. ^{1}H NMR spectrum of amarellin B (**4**) in CD_3OD (500 MHz).

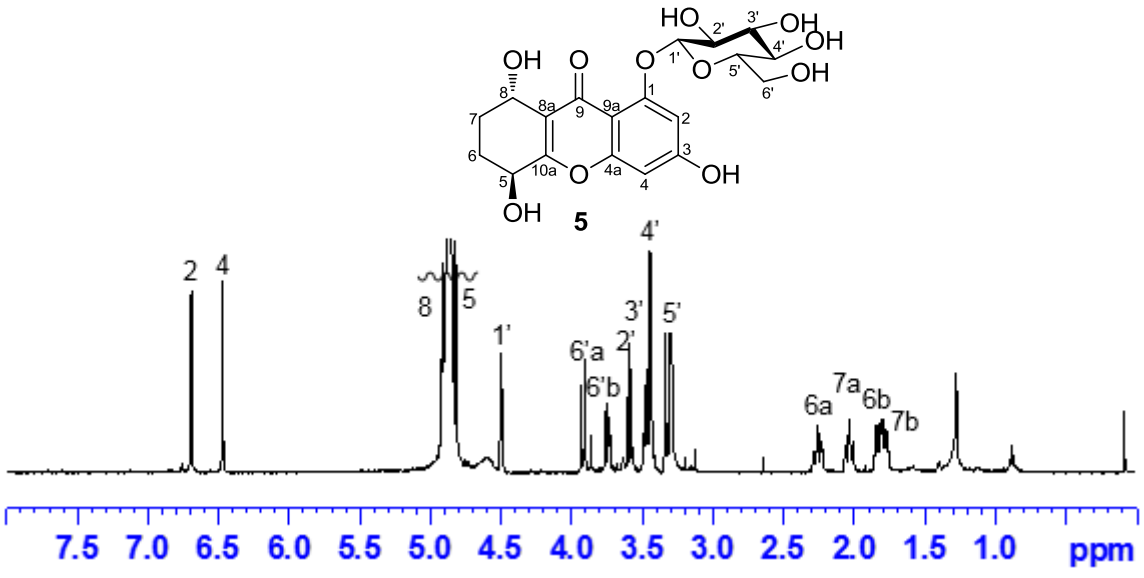


Figure 1.2.11. ^{1}H NMR spectrum of amarellin C (**5**) in CD_3OD (500 MHz).

1.2.3.4. Gross structures for amarellins D–F (**6**–**8**)

Analysis of the HRESIMS and 1D NMR spectra (Table 1.2.3) showed that amarellins D–F (**6**–**8**) were also glycosides of tetrahydroxanthone. The 1D NMR data suggested that the sugar moiety of **6** was xylose, while those of **7** and **8** were glucose. In contrast, the ^{1}H signals of the aglycone moieties of **6**–**8** resembled that of **2**. The locations of the sugar moieties in **6**–**8** were assigned by HMBC analysis.

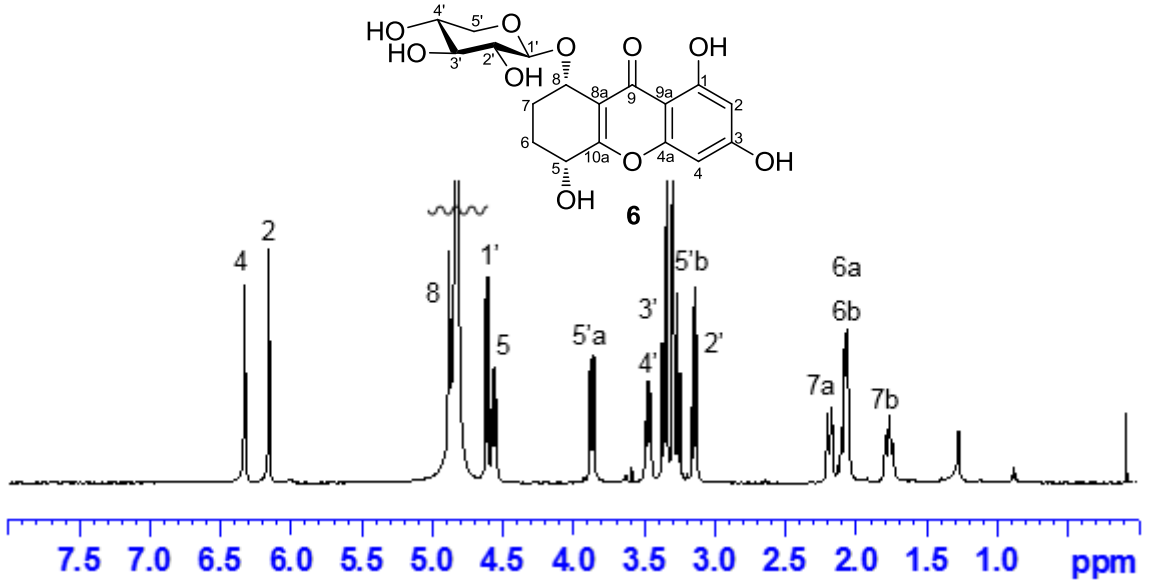


Figure 1.2.12. ^{1}H NMR spectrum of amarellin D (**6**) in CD_3OD (500 MHz).

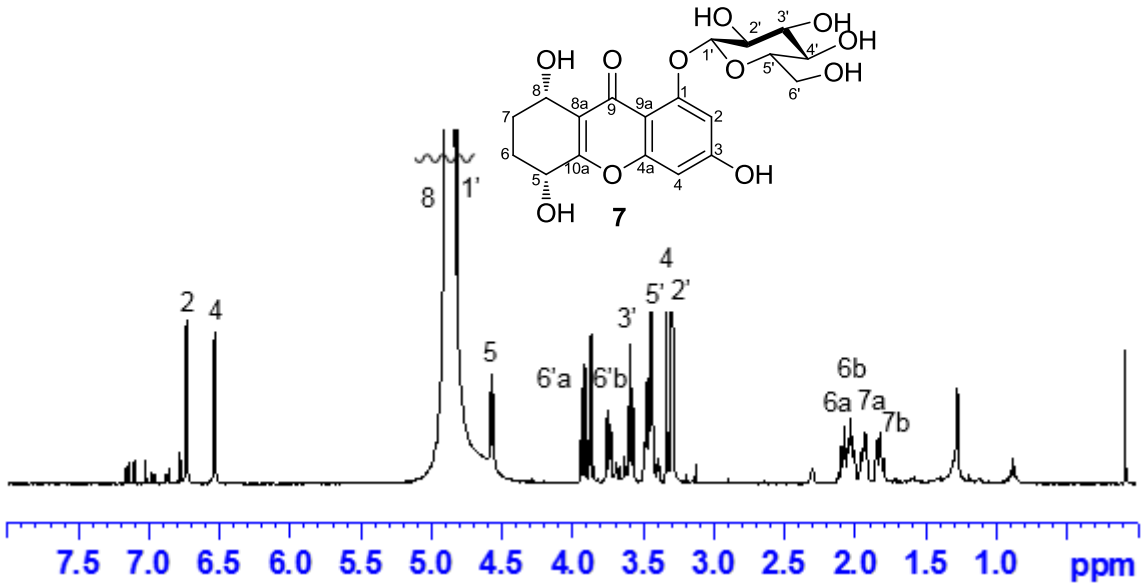


Figure 1.2.13. ^{1}H NMR spectrum of amarellin E (**7**) in CD_3OD (500 MHz).

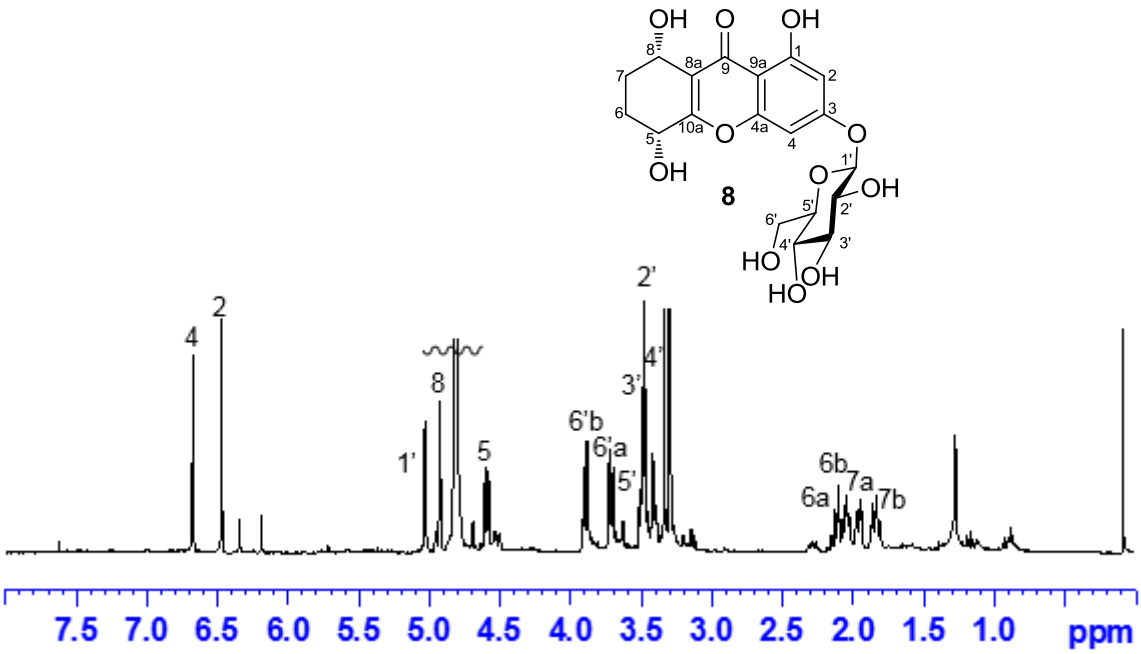


Figure 1.2.14. ^{1}H NMR spectrum of amarellin F (**8**) in CD_3OD (500 MHz).

1.2.3.5. Idenditication of the sugar moieties for amarellins A–F (3–8)

Acid hydrolysis of amarellin A (**3**) gave the sugar moiety, which was treated with L-cysteine methyl ester and followed by reacted with *o*-tolylisothiocyanate [24]. HPLC analysis of the reaction mixture showed a peak, whose retention time was identical to that of the derivative of authentic D-glucose prepared in the same procedure, suugesting the sugar moiety of **3** to be D-glucose. In the same way, the sugar moieties of amarellins C (**5**), E (**7**), and F (**8**) were also assigned as D-glucose, whereas those of amarellins B (**4**) and D (**6**) were elucidated to be D-xylose. The β-glycosidic linkages between the sugar moietyies of **3**–**8** and respective aglycones were concluded based on the coupling constants of their anomeric protons (Table 1.2.2 and 1.2.3).

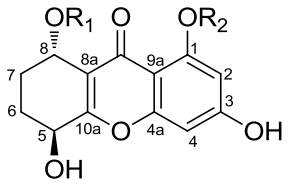
1.2.3.6. Absolute configurations for amarellins A–F (3–8)

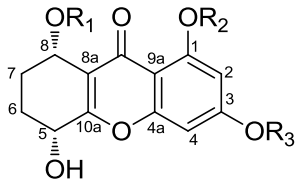
Enzymatic hydrolyses of amarellins A (**3**), C (**5**), E (**7**), and F (**8**) with β-glucosidase were carried out to give their aglycones (**3a**, **5a**, **7a**, and **8a**, respectively), while amarellins B (**4**) and

D (**6**) were hydrolyzed with cellulase to afford the aglycones **4a** and **6a**, respectively. The spectroscopic data of **3a**, **4a**, and **5a** including the HRSEIMS data, ^{1}H NMR spectra, and optical rotations were identical to those of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), whereas **6a**, **7a**, and **8a** were assigned as 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**). Consequently, the structures of amarellins A–F (**3**–**8**) were assigned as shown in Chart 1.

1.2.4. Summary

Though a variety of plant-derived xanthones have been reported so far, 5,6,7,8-tetrahydroxanthones have been isolated limited from Gentianaceous plants [9, 21–23,25,26]. In this research, the aerial parts of a Mongolian medicinal plant *Gentianella amarella* ssp. *acuta* were investigated to give two tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and six new 5,6,7,8-tetrahydroxanthone glycosides, amarellins A–F (**3**–**8**). On the basis of spectroscopic analysis, chemical conversion, and ECD calculation, the structures of amarellins A–C (**3**–**5**) were assigned as 8-*O*-β-D-glucoside, 8-*O*-β-D-xyloside, and 1-*O*-β-D-glucoside of **1**, respectively, while amarellins D–F (**6**–**8**) were elucidated to be 8-*O*-β-D-xyloside, 1-*O*-β-D-glucoside, and 3-*O*-β-D-glucoside of 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), respectively.





	R_1	R_2
1	H	H
3	Glc	H
4	Xyl	H
5	H	Glc

	R_1	R_2	R_3
2	H	H	H
6	Xyl	H	H
7	H	Glc	H
8	H	H	Glc

Chart 1. Structures of tetrahydroxanthones, 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**), and amarellins A–F (**3**–**8**).

Table 1.2.1 ^{1}H and ^{13}C NMR data for 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**) and 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**) in CD_3OD.

Position	**1**		**2**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	163.5	–	163.4	–
2	100.0	6.17 (1H, brs)	100.1	6.16 (1H, brs)
3	166.3	–	166.6	–
4	95.0	6.32 (1H, brs)	95.1	6.33 (1H, brs)
4a	159.4	–	159.3	–
10a	165.9	–	167.1	–
5	65.5	4.49 (1H, brs)	67.3	4.56 (1H, brt, 7.4)
6	27.0	2.27 (1H, brt, 13.1)	27.4	2.09, 2.03 (each 1H, m)
		1.85 (1H, brd, 13.1)		
7	26.5	2.04 (1H, brt, 13.4)	28.8	1.95 (1H, m)
		1.80 (1H, brd, 13.4)		1.83 (1H, brt, 13.3)
8	61.4	4.92 (1H, brs)	62.2	4.91 (1H, t, 3.9)
8a	119.1	–	119.2	–
9	183.0	–	183.0	–
9a	105.6	–	105.3	–

Table 1.2.2 ^{1}H and ^{13}C NMR data for amarellins A–C (**3**–**5**) in CD_3OD.

Position	**3**		**4**		**5**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	163.3	–	162.2[a]	–	160.2	–
2	100.1	6.16 (1H, brs)	100.3[a]	6.18 (1H, brs)	106.0	6.69 (1H, d, 2.2)
3	166.3	–	165.6[a]	–	168.2[a]	–
4	95.1	6.32 (1H, brs)	95.1[a]	6.33 (1H, brs)	99.8	6.47 (1H, d, 2.2)
4a	159.3	–	159.6[a]	–	160.9	–
10a	166.8	–	167.1[a]	–	163.9	–
5	65.3	4.50 (1H, brs)	65.3	4.49 (1H, dd, 3.5, 1.8)	65.5	4.50 (1H, t, 4.1)
6	26.9	2.28 (1H, brt, 14.2)	26.8	2.27 (1H, tt, 14.1, 3.5)	27.2	2.26 (1H, tt, 14.0, 4.1)
		1.87 (1H, brd, 14.2)		1.86 (1H, dd, 14.1, 1.8)		1.83 (1H, m)
7	24.7	2.17 (1H, brd, 14.2)	24.7	2.08 (1H, dd, 14.2, 3.1)	26.6	2.04 (1H, dd, 14.0, 3.6)
		2.00 (1H, brt, 14.2)		1.99 (1H, tt, 14.2, 2.3)		1.78 (1H, ,m)
8	71.1	4.93 (1H, brs)	71.2	4.91 (1H, brs)	62.5	4.91 (1H, t, 3.6)
8a	117.4	–	117.4	–	120.5	–
9	183.1	–	183.1[a]	–	179.3	–
9a	105.7	–	105.9[a]	–	108.4[a]	–
1'	105.2	4.69 (1H, d, 7.8)	104.8	4.63 (1H, d, 7.9)	104.8	4.82 (1H, d, 7.8)
2'	75.7	3.13 (1H, dd, 9.0, 7.8)	75.6	3.11 (1H, dd, 9.6, 7.9)	74.8	3.59 (1H, dd, 9.0, 7.8)
3'	77.8	3.42 (1H, t, 9.0)	77.7	3.36 (1H, t, 9.6)	77.4	3.48 (1H, m)
4'	71.5	3.30 (1H, m)	71.2	3.46 (1H, m)	71.2	3.44 (1H, m)
5'	78.1	3.35 (1H, m)	67.2	3.87 (1H, dd, 11.1, 4.7)	78.5	3.44 (1H, m)
				3.28 (1H, m)		
6'	62.7	3.70 (1H, dd, 12.0, 5.5)			62.5	3.75 (1H, dd, 12.0, 3.5)
		3.89 (1H, dd, 12.0, 2.1)				3.92 (1H, brd, 12.0)

[a] Signal was detected by the HMBC spectrum

Table 1.2.3 ^{1}H and ^{13}C NMR data for amarellins D–F (**6**–**8**) in CD_3OD.

Position	**6**		**7**		**8**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	163.7[a]	–	160.2	–	163.1	–
2	100.3	6.16 (1H, brs)	105.3	6.73 (1H, brs)	101.0	6.47 (1H, d, 2.1)
3	166.8	–	166.9[a]	–	164.9	–
4	95.1	6.33 (1H, brs)	99.4	6.54 (1H, brs)	95.9	6.68 (1H, d, 2.1)
4a	159.3	–	160.6	–	158.8	–
10a	168.2	–	165.2	–	167.9	–
5	67.5	4.56 (1H, dd, 9.3, 7.3)	67.2	4.57 (1H, dd, 8.7, 6.0)	67.4	4.59 (1H, dd, 8.9, 6.2)
6	27.3	2.08, 2.04 (each 1H, m)	27.5	2.08, 2.03 (each 1H, m)	27.3	2.11, 2.05 (each 1H, m)
7	27.8	2.19 (1H, brd, 15.0)	28.7	1.95 (1H, brd, 14.1)	28.8	1.97, 1.84 (each 1H, m)
		1.77 (1H, brt, 15.0)		1.83 (1H, brt, 14.1)		
8	71.3	4.88 (1H, brs)	63.1	4.90 (1H, m)[b]	61.9	4.93 (1H, t, 3.5)
8a	117.6	–	120.9	–	119.7	–
9	182.8	–	179.3	–	183.2	–
9a	105.3[a]	–	108.7[a]	–	107.1	–
1'	105.9	4.61 (1H, d, 7.6)	105.3	4.82 (1H, d, 7.7)	101.6	5.03 (1H, d, 7.3)
2'	75.6	3.14 (1H, dd, 9.0, 7.6)	74.7	3.59 (1H, dd, 8.9, 7.7)	74.7	3.47 (1H, m)
3'	77.7	3.36 (1H, t, 9.0)	77.3	3.47 (1H, m)	77.8	3.47 (1H, m)
4'	71.2	3.48 (1H, m)	71.2	3.45 (1H, m)	71.1	3.41 (1H, m)
5'	67.2	3.87 (1H, dd, 11.4, 5.4)	78.5	3.45 (1H, m)	78.3	3.50 (1H, m)
		3.26 (1H, m)				
6'			62.5	3.75 (1H, dd, 11.5, 3.8)	62.4	3.71 (1H, dd, 12.2, 5.5)
				3.93 (1H, brd, 11.5)		3.90 (1H, dd, 12.2, 2.2)

[a] Signal was detected by the HMBC spectrum, [b] Signal was overlapped with that of HOD

1.3. Chemical studies on *Lomatogonium carinthiacum*

1.3.1. Introduction

The genus *Lomatogoniu* (Gentianaceae), distributed in Asia, North America, and Europe, contains 18 species of plants. *Lomatogoniu carinthiacum*, an annual medicinal remedy, has been used in Russian medicinal system for the treatment of disorders of gallbladder and liver as a Transbaikalian folk, and to treat infuluenza, liver diseases, jaundice, and typhoid in Mongolia [27–29]. Previous chemical research showed the presence of flavonoids, iridoids, xanthones, and triterpenoids in this plant [23, 28–31]. In continuation of our investigation on Mongolian traditional herbal medicines, the constituents of the aerial parts of *L. carinthiacum* were investigated.

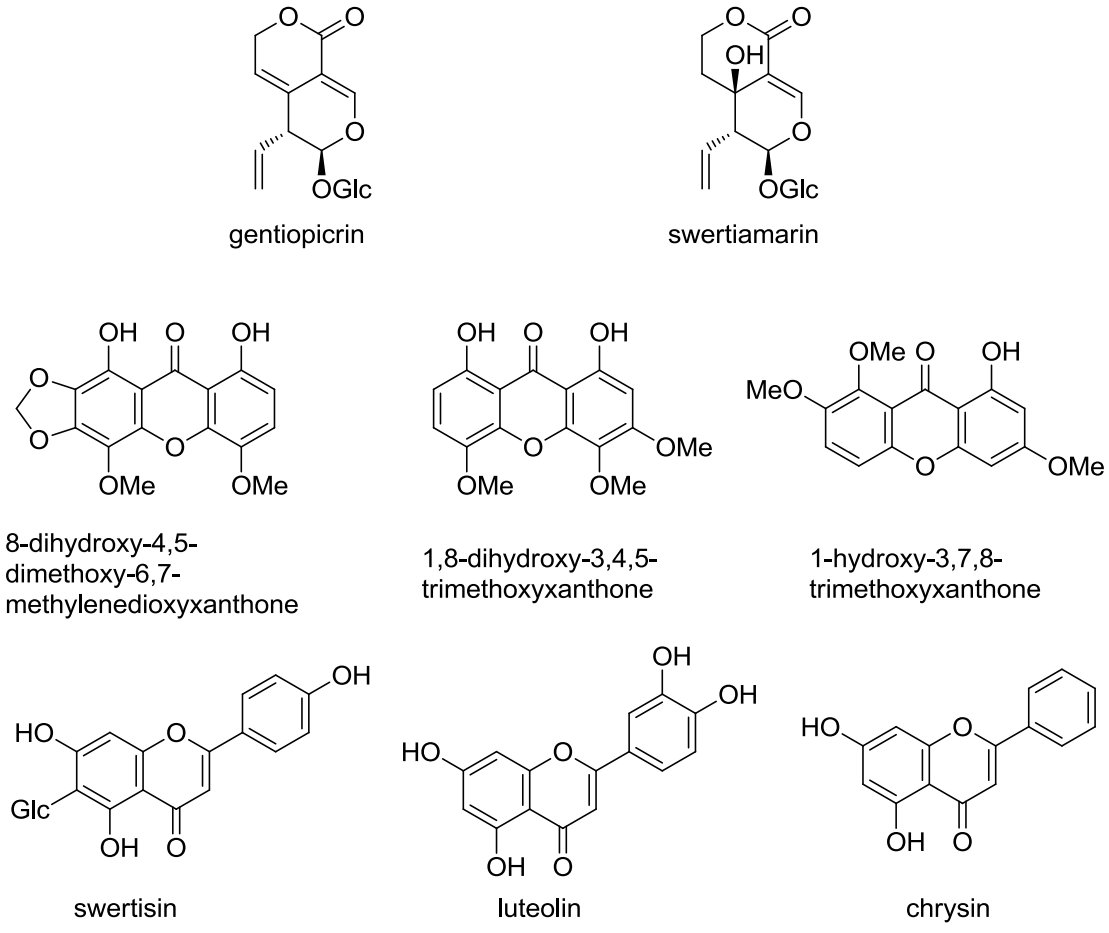
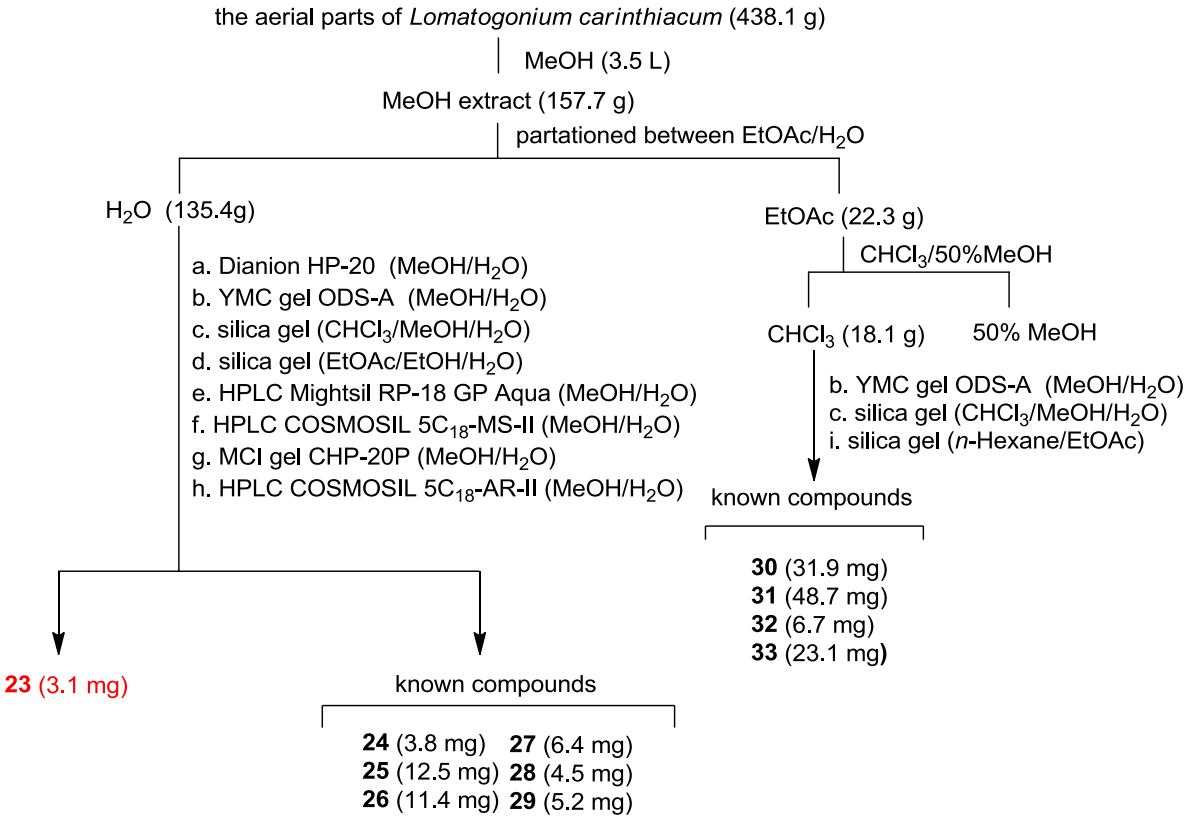


Figure 1.3.1. Previously isolated compounds from *L. carinthiacum*.

1.3.2. Extraction and isolation



Scheme 2. Isolation procedures for compounds **23**–**33**.

The dried aerial parts of *L. carinthiacum* (438.1 g), collected in August 2011 in Mongolia, were extracted with MeOH to give the extract (157.7 g), which was partitioned between EtOAc and H_2O. Seven compounds (**23**–**29**), including one new compound (**23**) were isolated from the H_2O-soluble material (22.3 g), and four compounds (**30**–**33**) were isolated from the EtOAc-soluble material (135.4 g).

The structures of compounds **24**–**33** were determined as swertiajaposide D (**24**) [32], adoxosidic acid (**25**) [33], secologanoside (**26**) [34], β-D-glucosyl 4-*O*-β-D-glucosyl caffeate (**27**) [35], 6'-*O*-β-D-glucosylswertiamarin (**28**) [36], luteolin 5-*O*-β-D-glucoside (**29**) [37], 1-hydroxy-3,7-dimethoxyxanthone (**30**) [38], decussatin (**31**) [39], epoxyconiferyl alcohol (**32**) [40], luteolin (**33**) [41], by comparison of their spectroscopic data with the literature data (Fig. 1.3.2).

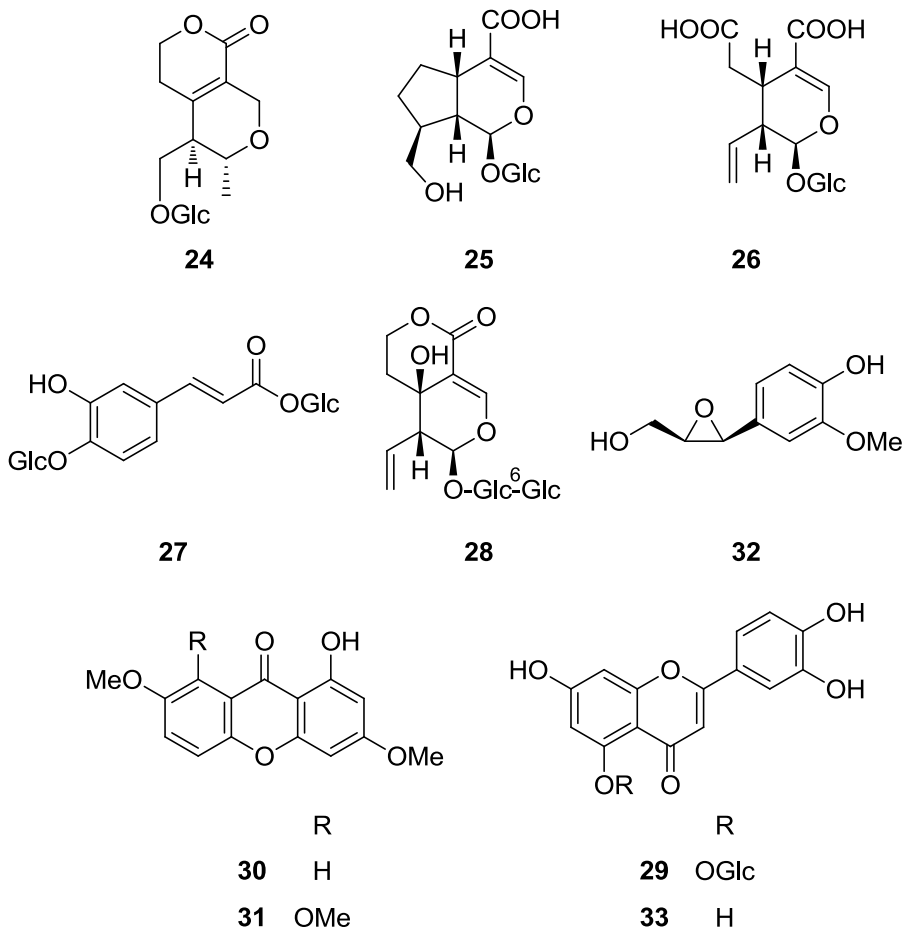


Figure 1.3.2. Known compounds (**24**–**33**) from the aerial parts of *L. carinthiacum*.

1.3.3. Structure elucidation for compound 23

The ^{1}H spectrum of **23** (Fig. 1.3.3) was quite similar to that of literature data for (3-*O*-β-D-glucosyl)-feruloyl-*O*-β-D-glucoside [42], except for the geometry for C-7/C-8. The geometory in **23** was assigned to be *Z* by a NOESY correlation for H-7 with H-8 (Fig. 1.3.4). Therefore, the structure of **23** was elucidated as shown (Fig. 1.3.3).

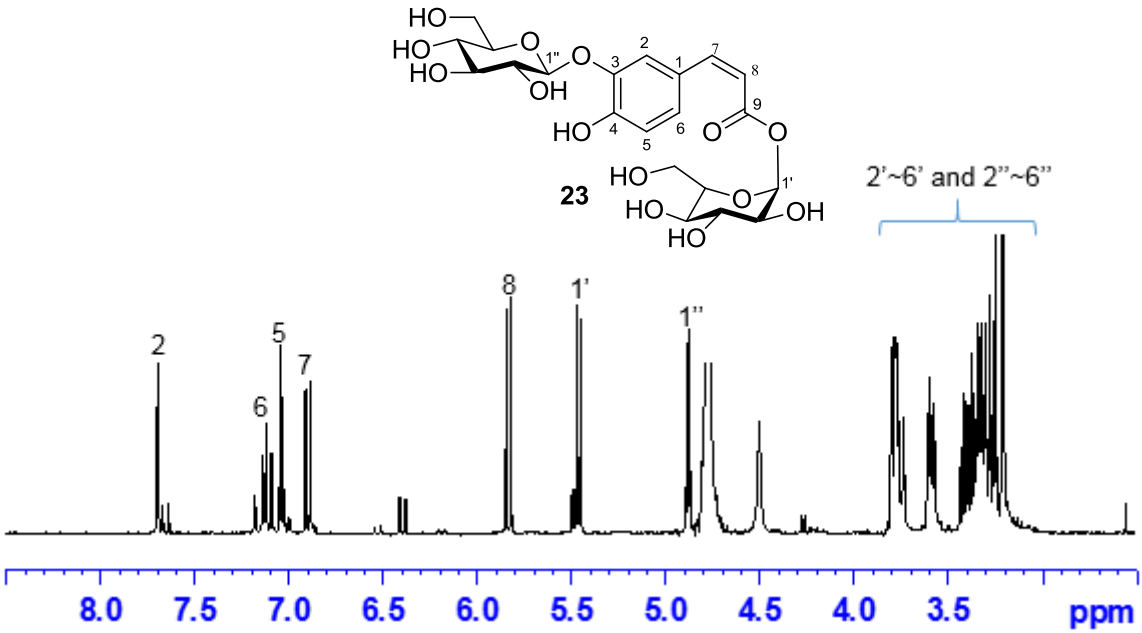


Figure 1.3.3. ^{1}H NMR spectrum of compound **23** in CD_3OD.

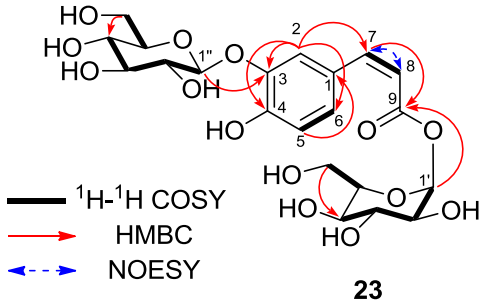


Figure 1.3.4. Selected 2D NMR correlations for **23**.

Table 1.3. ^{1}H and ^{13}C NMR data for compound **23** in CD_3OD.

Position	δ_C	δ_H (*J* in Hz)
1	130.6	–
2	115.7	7.70 (1H, brd, 2.0)
3	149.3	–
4	150.0	–
5	116.6	7.04 (1H, d, 8.5)
6	126.3	7.13 (1H, dd, 8.5, 2.0)
7	146.4	6.90 (1H, d, 13.0)
8	116.6	5.84 (1H, d, 13.0)
9	166.4	–
Sugar 1		
1’	95.7	5.48 (1H, d, 8.2)
2’	74.0	3.27 (1H, m)
3’	77.9[a)]	3.37 (1H, m)
4’	71.1[b)]	3.32 (1H, m)
5’	78.3[c)]	3.32 (1H, m)
6’	62.4[d)]	3.78 (1H, m)
		3.60 (1H, m)
Sugar 2		
1’’	102.2	4.81 (1H, d, 7.8)
2’’	74.8	3.43 (1H, m)
3’’	78.1[a)]	3.37 (1H, m)
4’’	71.3[b)]	3.32 (1H, m)
5’’	78.9[c)]	3.32 (1H, m)
6’’	62.4[d)]	3.78 (1H, m)
		3.60 (1H, m)

a–d) signals may be interchangeable.

1.3.4. Summary

Investigation for the aerial parts of *L. carinthiacum* resulted in the isolation of 11 compounds (**23**–**33**), including one new phenolic glycoside (**23**). The structures of known compounds (**24**–**33**) were assigned by comparison of their spectral data with the literature data, while the structure of new phenolic glycoside (**23**) was elucidated by spectroscopic analysis. This is the first example for the isolation of a phenolic glycoside from this species.

1.4. Chemical studies on *Ligularia sibirica*

1.4.1. Introduction

The genus *Ligularia* (Asteraceae) comprises about 150 species, which are mainly distributed in Eastern and Central Asian regions. The *Ligularia* plants have long been used as herbal remedies for the treatment of bronchitis, hemoptysis, phthisis, hepatitis, rheumatalgia, and asthma [43,44]. A variety of sesquiterpenoids including eremophilanes, eudesmanes, bisabolanes, benzofurans, and gemacrenes possessing antibacterial activity and cytotoxicity have been isolated from these species [45–49]. *Ligularia sibirica* has a wide distribution range from East Asia to the European part of Russia. Since there are not so many chemical reports for *L. sibirica* [50–52], phytochemical investigation on the roots and rhizomes of *L. sibirica*, collected in Mongolia, was taken out, resulting in the isolation of 11 sesquiterpenes (**34**–**44**).

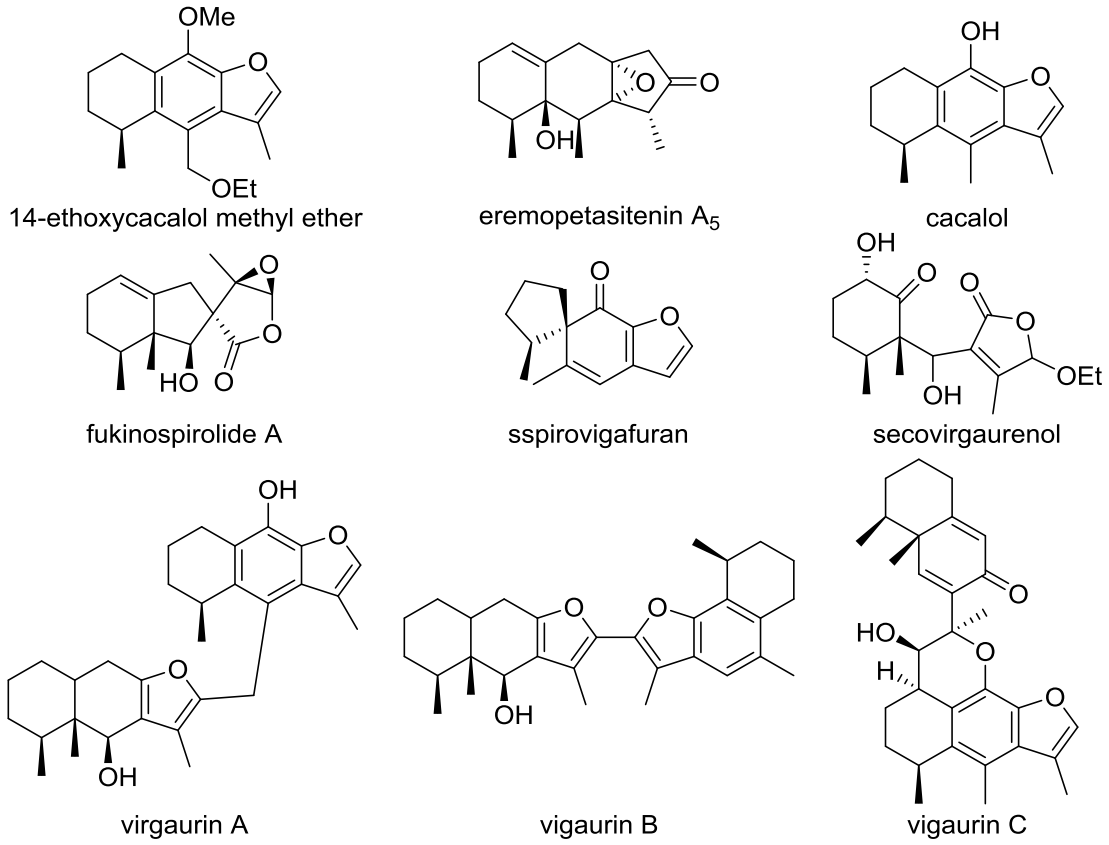
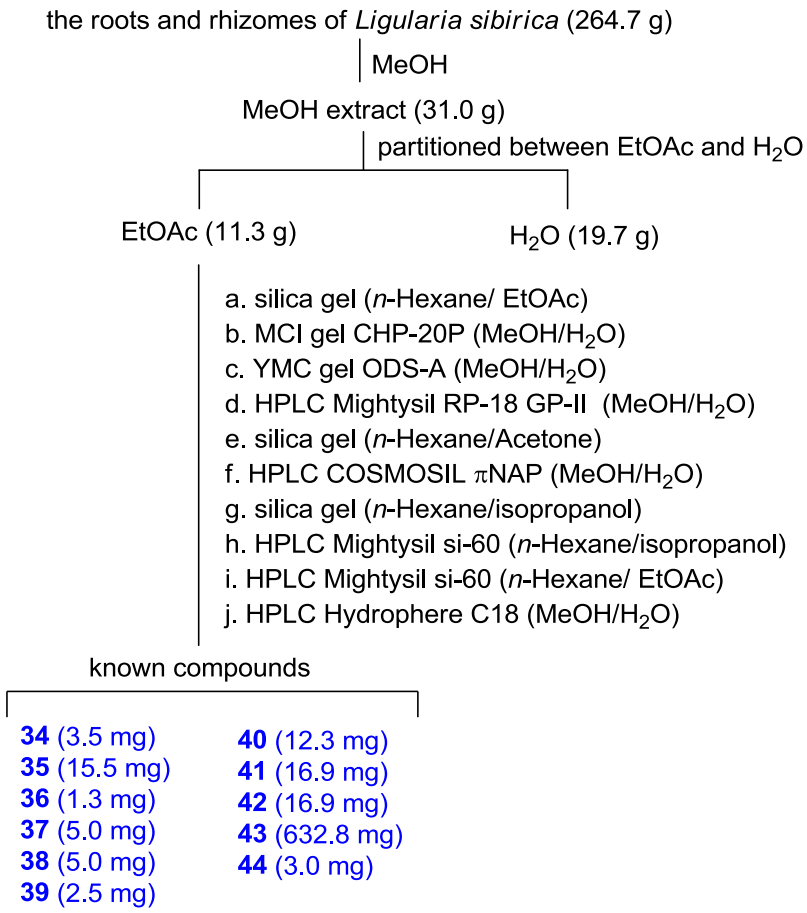


Figure 1.4.1. Previously isolated compounds from *Ligularia* plants.

1.4.2. Extraction and isolation



Scheme 3. Isolation procedures for compounds **34**–**44**.

The MeOH extract (31.0 g) of the roots and rhizomes of *Ligularia sibirica* (264.7 g), collected in August 2015 in Mongolia, was partitioned with EtOAc and H_2O. Compounds **34**–**44** were isolated from the EtOAc-soluble material (11.3 g) (Scheme 3).

Compounds **34**–**44** were identified as 6β-isobutyryloxy-1β,10β-epoxyeuryopsin (**34**) [53], 6β-angeloyloxy-1β,10β-epoxyeuryopsin (**35**) [54], 1β,10β-epoxy-6-oxofuranoeremophilane (**36**) [55], 1β,10β-epoxy-3β-(2-methylacryloyloxy)-6β-acetyloxy-furanoeremophilane (**37**) [56], 1β,10β-epoxy-3β-angeloyloxy-6β-acetyloxy-furanoeremophilane (**38**) [57], guai-6-en-10β-ol (**39**) [58], 1β,10β-epoxyfuranoeremophilan-6β-ol (**40**) [59], 11(*R*,*S*)-1β-hydroxy-8-oxoeremophil-6,9-diene-12-al (**41**) [59], (11*S*)-1β-hydroxy-8-oxoeremophil-6,9-diene-12-nor-11-ol (**42**) [60], 1β,10β-epoxyfuranoeremophilan-6β-yl 2-hydroxymethylprop-2-enoate (**43**) [61], and 1β-hydroxy-8,11-dioxoeremophil-12-nor-6,9-diene (**44**) [62] by comparison of their spectra with the literature ones.

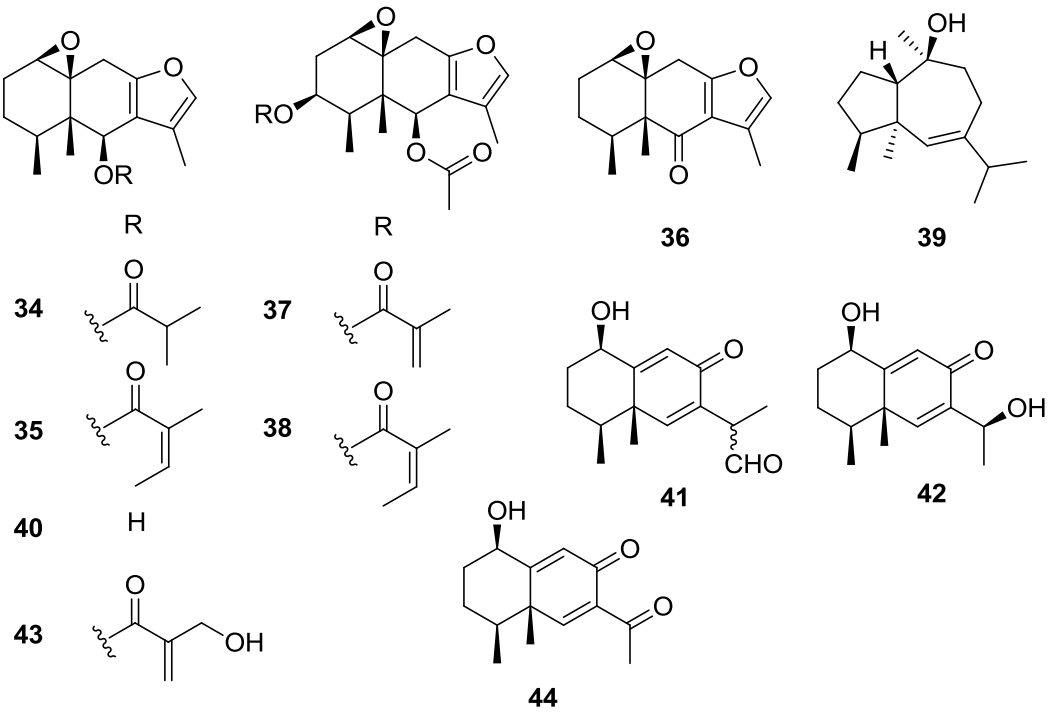


Figure 1.4.2. Known compounds (**34**–**44**) from the roots and rhizomes of *L. sibirica*.

1.4.3. Summary

Chemical investigation for the roots and rhizomes of *L. sibirica* was carried to give 10 eremophilane sesquiterpenes (**34**–**35**, **40**–**43**) and one guaine sesquiterpenes (**39**). This research suggested the eremopilane sesquiterpene is the main component of this species. A guaine sesquiterpene (**39**) was isolated for the first time from *L. sibirica*.

1.5. Chemical studies on *Caragana jubata*

1.5.1. Introduction

Caragana jubata, belonging to the family of Fabaceae, is a perennial woody deciduous shrub distributed widely throughout the Euro-Asia continent, eastwards to Russian, Siberia and Korea, and south to Nepal, Bhutan, Sikkim and northern India [63]. In the course of our ethnopharmacological study, we learned that the barks and twigs of this plant have been used in Mongolian traditional medicine for the treatment of gynecological problems, plethora and hypertension. In addition, the extract of this plant has been reported to exhibit anti-tumor, anti-inflammatory and anti-fungal activities. Although previous research on *C. jubata* revealed the presences of flavones and pterocarpan glycosides [63–65], not many phytochemical studies on triterpene saponins of this plant were taken out. In our continuing studies on Mongolian medicinal plants, the composition of the stems of *C. jubata* was investigated.

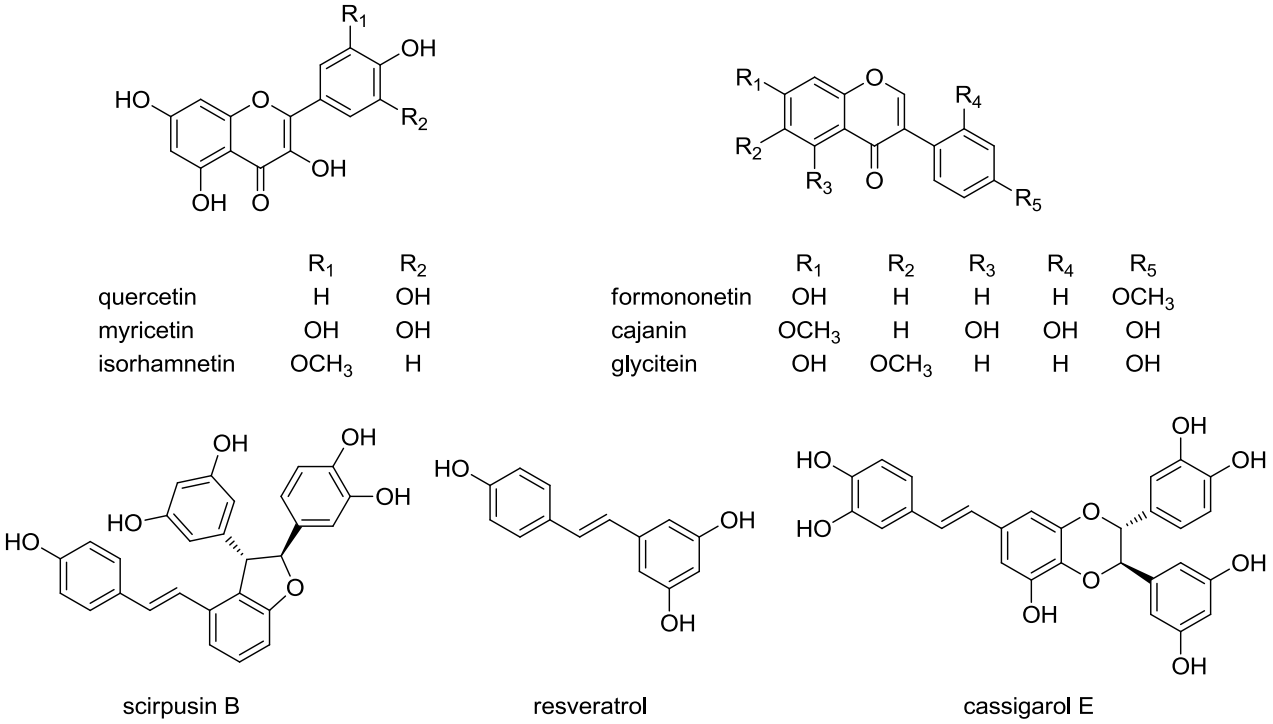
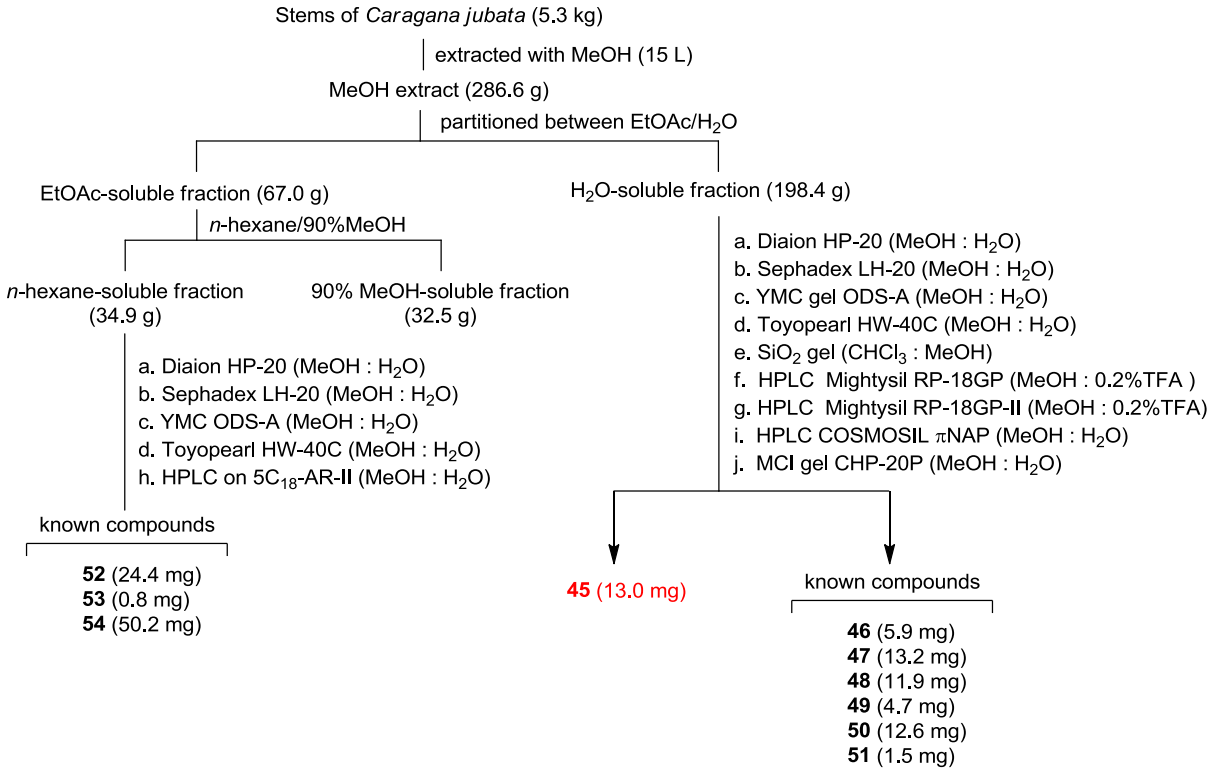


Figure 1.5.1 Compounds previously isolated from the *C. jubata.*

1.5.2. Extraction and isolation



Scheme 4. Isolation procedures for compounds **45**–**54**.

The air-dried stems of *Caragana jubata* (5.3 kg), collected in August 2011 in Mongolia, were extracted with MeOH. The concentrated extract (282.6 g) was then partitioned with H_2O and EtOAc. The EtOAc-soluble fraction was further partitioned between *n*-hexane and 90% MeOH. The H_2O-soluble fraction (198.4 g) was subjected to column chromatographies repeatedly to give seven triterpenoid saponins (**45**–**51**). In contrast, three flavonoids (**52**–**54**) were isolated from the 90% MeOH-soluble fraction (32.5 g).

Structures of compounds **47**–**50**, **52**–**54** were identified as caraganin A (**47**), caraganin B (**48**) [66], myrioside B (**49**) [67], 3β-[(β-D-glucosyl)oxy]olean-12-en-30-oic acid (**50**) [68], 7,8,3'-trihydroxy-4'-methoxyisoflavone (**52**) [69], 7,4',6'-trihydroxyisoflavone (**53**) [70], and butin (**54**) [71], by comparison of their spectra data with those described in the literature. Based on spectroscopic analysis compounds **46** and **51** were elucidated to be 29-methoxyl caraganin B and 6'-methoxyl myrioside B, which might be artifacts, a methyl ester group was introduced during the isolation procedure.

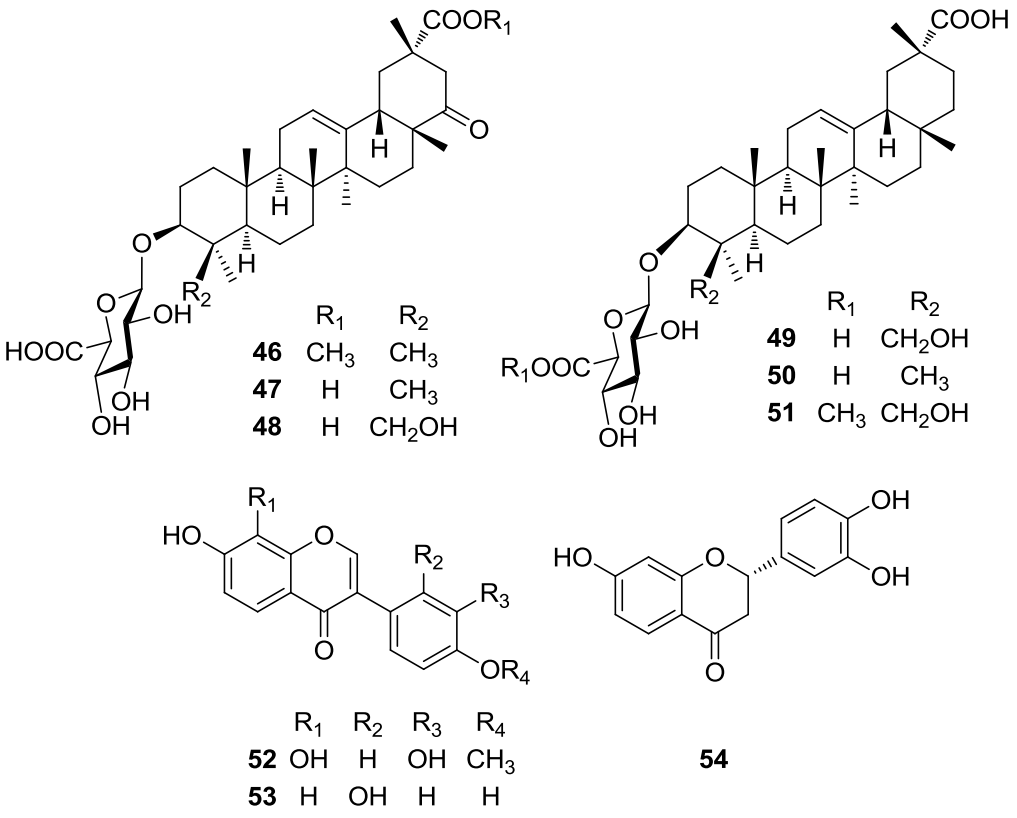


Figure 1.5.2. Known compounds (**47**–**50**, **52**–**54**), and artifacts (**46** and **51**) from the stems of *C. jubata*.

1.5.3. Structure elucidation for compound 45

Compound **45** was obtained as a white amorphous powder, and its molecule formula was determined as $C_{36}H_{52}O_{11}$ by HRESIMS (*m/z* 683.3466, $[M+Na]^+$, Δ –4.1 mmu). The ^{1}H-NMR spectrum showed an olefinic proton [δ_H 5.84 (1H, s)], seven tertiary methyl [δ_H 1.32 (3H, s); δ_H 1.03 (3H, s); δ_H 1.23 (3H, s); δ_H 1.06 (3H, s); δ_H 1.42 (3H, s); δ_H 1.14 (3H, s); δ_H 1.32 (3H, s)], and an oxygen-bearing methine [δ_H 3.41 (1H, dd, *J* =14.0, 4.0 Hz)], together with one anomeric proton [δ_H 5.03 (1H, d, *J* = 7.8 Hz)] (Fig. 1.4.3). The ^{13}C-NMR and DEPT spectrums of compound **45** showed 36 carbon resonances, including two carbonyl carbons (δ_C 198.7; δ_C 213.1), two carboxyl carbons (δ_C 177.9; δ_C 172.2), and six quartenary carbons (δ_C 39.3; δ_C 44.1; δ_C 36.7; δ_C 43.0; δ_C 46.9; δ_C 44.7). These spectral data suggested **45** to be an olean or urs-type triterpenoid glycoside. The H-18 signal pattern in the ^{1}H NMR spectrum indicated the presence of the olean-12-ene type triterpene as an aglycone.

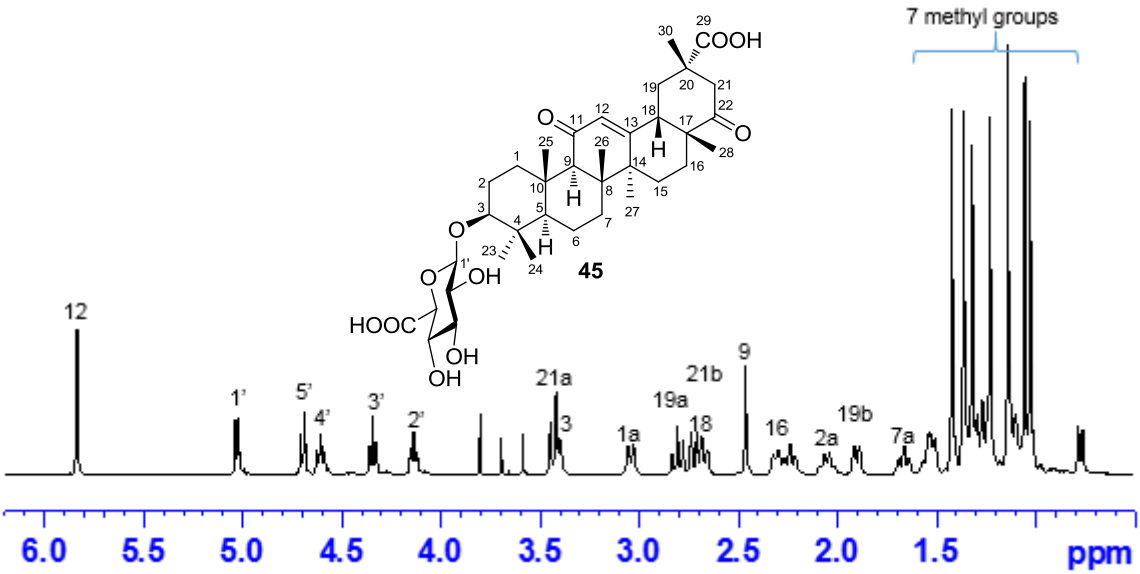


Figure 1.5.3. ^{1}H NMR spectrum of compound **45** in C_5D_5N.

The planar structure of **45** was elucidated by 2D NMR spectroscopic analysis. The HMBC correlation of Me-23 and Me-24 with an oxygen-bearing methine indicated the presence of the oxygen-function at C-3. The signal at δ_H 2.46 (H-9) showed the HMBC correlation with a carbonyl carbon indicated the presence of the carbonyl group at C-11. The position of another carbonyl group could be assigned at C-22 by the HMBC correlation with Me-28. The HMBC correlation of the methyl signal [δ_H 1.32 (3H, s)] with C-20 and a carboxyl resonance indicated the carboxyl group either at C-29 or C-30.

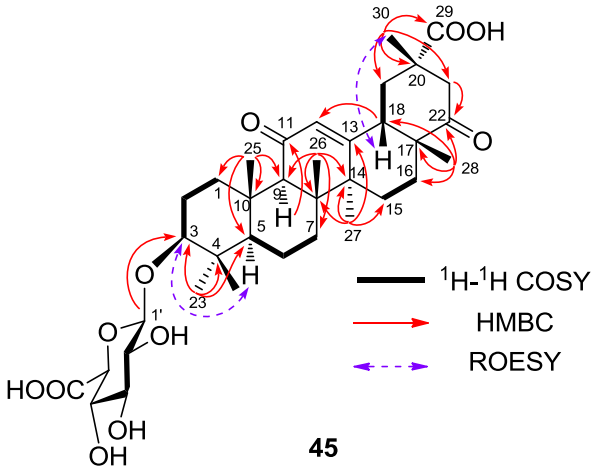


Figure 1.5.4. Selected 2D NMR correlations for **45**.

The remaining five carbon resonances, including a carboxyl carbon (δ_C 172.2), and four oxymethine carbons (δ_C 74.9; δ_C 77.5; δ_C 72.8; δ_C 77.3), together with an anomeric carbon (δ_C

106.6), were considered to be arising from a sugar moiety. Since the proton signals resonated with these oxymethines had a large coupling constant, the presence of a glucuronyl group was indicated as a sugar moiety. This was further confirmed by the acid hydrolysis of **1**, which liberated a glucuronic acid. The location of the glucuronyl group was assigned at C-3 by the HMBC correlation of the anomeric proton signal with C-3, and its β-linkage was elucidated from the *J*-value (7.8 Hz) of the anomeric proton signal.

The configuration of the C-3 oxygen function was elucidated as β from the ROESY correlation of H-3 and H-5. The position of the carboxyl group at C-20 was assigned to be C-29 by the ROESY correlation of H-18 with methyl signal at C-30. Based on these evidences, the structure of compound **45** was elucidated as shown in Fig. 1.5.4.

Table 1.5.1 ^{1}H and ^{13}C NMR data for compound **45** in C_5D_5N.

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	3.04 (1H, br d, 13.0)	38.8	17	—	46.9
	1.10 (1H, m)		18	2.67 (1H, dd, 13.5, 3.5)	46.2
2	2.05 (1H, dd, 12.8, 11.0)	26.1	19	1.90 (1H, br d, 13.0)	39.5
	1.18 (1H, br d, 11.5)			2.81 (1H, t, 13.5)	
3	3.40 (1H, dd, 11.0, 4.0)	88.1	20	—	44.7
4	—	39.3	21	2.72 (1H, d, 15.0)	45.7
5	0.78 (1H, d, 12.0)	54.6		3.43 (1H, d, 15.0)	
6	1.34 (1H, m)	17.0	22	—	213.1
	1.52 (1H, d, 11.5)		23	1.32 (3H, s)	27.5
7	1.27 (1H, m)	32.3	24	1.03 (3H, s)	16.4
	1.56 (1H, d, 13.0)		25	1.23 (3H, s)	16.2
8	—	44.1	26	1.06 (3H, s)	18.1
9	2.46 (1H, s)	61.4	27	1.42 (3H, s)	22.2
10	—	36.7	28	1.14 (3H, s)	20.8
11	—	198.7	29	—	177.9
12	5.84 (1H, s)	128.9	30	1.32 (3H, s)	21.0
13	—	164.5	1’	5.03 (1H, d, 7.8)	106.6
14	—	43.0	2’	4.14 (1H, br dd, 9.0, 7.8)	74.9
15	1.13 (1H, m)	32.2	3’	4.34 (1H, t, 9.0)	77.5
	1.67 (1H, td, 13.6, 3.7)		4’	4.61 (1H, br dd, 9.5, 9.0)	72.8
16	2.24 (1H, td, 13.6, 4.0)	26.1	5’	4.70 (1H, d, 9.5)	77.3
	1.28 (1H, m)		6’	—	172.2

1.5.4. Summary

Seven olean-type triterpene saponins (**45-51**) and three flavonoids (**52-54**) were isolated from the stems of *Caragana jubata*, which was used medicinally in Mongolia. Compounds **45**, **46**, and **51** have not been described previously in the literature. However, compounds **46** and **51** might be artifacts, in which a methyl ester group was introduced during the isolation procedure. In contrast, compound **50** was isolated for the first time as a natural product, while the isolation of two isoflavonoid derivatives (**53**, **54**) was also the first example from this genus.

This study showed that saponins were major constituents of this plant. Aglycone moiety of these saponins was oleanene-type triterpene, in which several oxygen functions in C- and E-rings, while their sugar moiety was glucuronic acid.

Since saponins isolated from this plant are structurally related to the oleanene glucuronides, such as glycyrrhizin [72,73], having hepatoprotective activity, similar activity was also expected. Thus, evaluation of hepatoprotective activity of the isolated compounds is under progress.

Chapter 2

Study on the constituents of Japanese medicinal plant, *Hypericum erectum*

2.1. Introduction

Japan is an island nation located in the Pacific Ocean in East Asia. The islands of Japan stretch a long distance from north to south and cover a wide range of climatic zones, resulting in a high diversity of plants [74].

The genus *Hypericum* (Hypericaceae) comprises almost 500 species germinating as herbs, shrubs, and trees, which are growing in temperate sections and mountains in the tropical areas [75]. *Hypericum* plants have been used as herbal remedies for the treatment of cuts, burns, melancholia, anxiety, abdominal, and urogenital pains [76–79]. A variety of prenylated acylphloroglucinols with interesting biological activities such as antibacterial, antiviral, antiproliferative, antioxidative, and antidepressant activities have been isolated from *Hypericum* plants to date [78–80]. *Hypericum erectum* is distributed in the mountains and hills in Japan, China, and Korea. The aerial parts of *H. erectum* are of medicinal values to heal hurts, sooth bruises, and malarial fever [76]. Some antibacterial and antihemorrhagic prenylated phloroglucinols have been isolated from this species [81–83]. In our continuing research on the constituents of *Hypericum* plants [80,84–86], the roots of *H. erectum* were investigated.

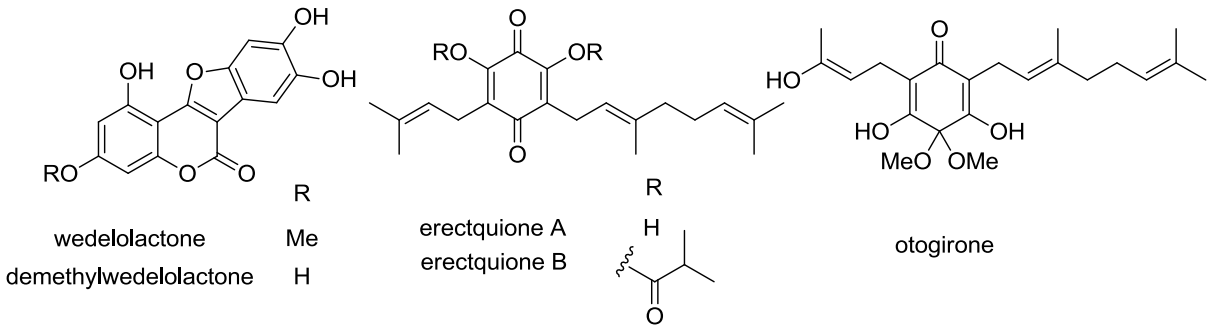
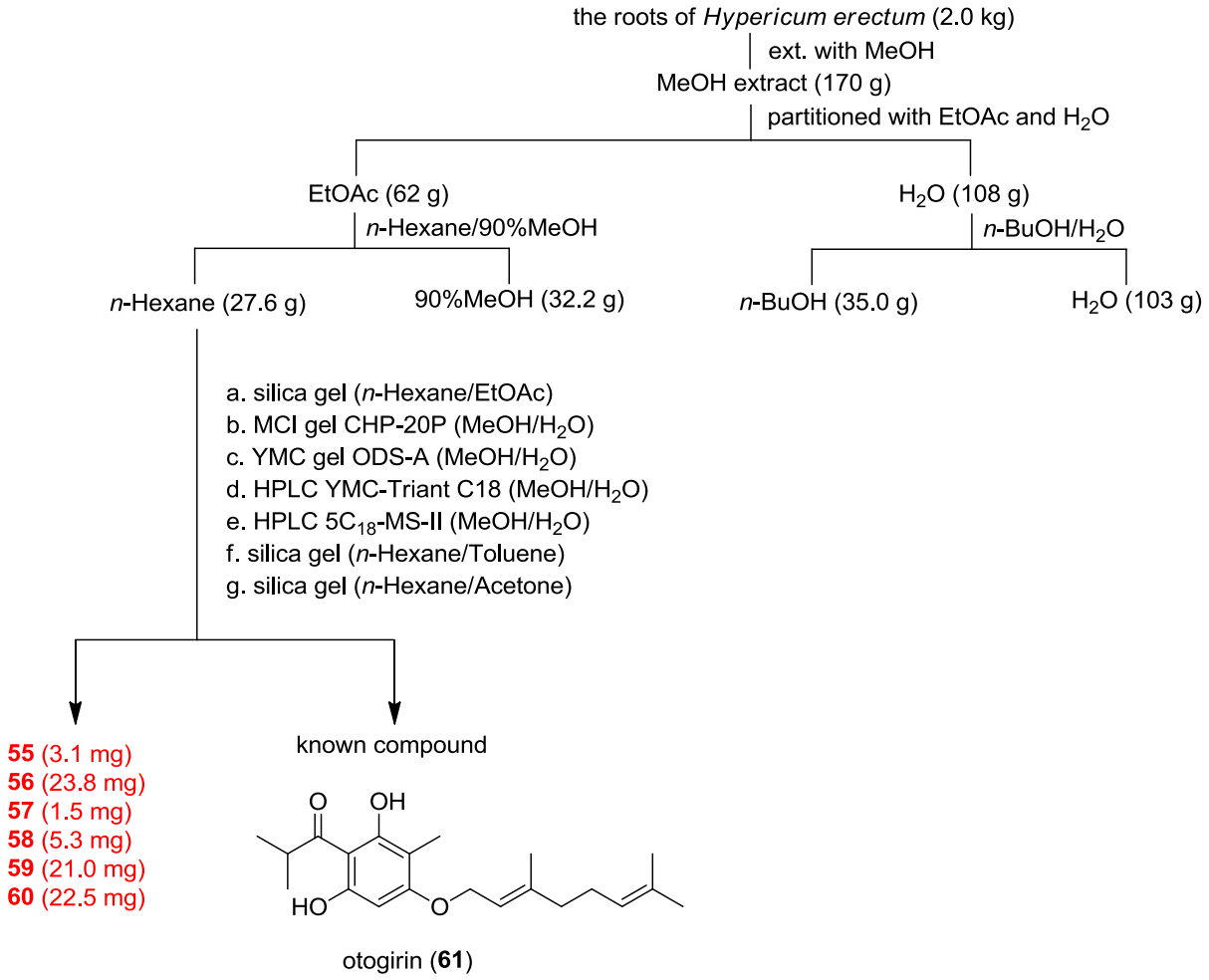


Figure 2.1. Previously isolated compounds from *H. erectum*.

2.2. Extraction and isolation

The MeOH extract of *H. erectum* roots was partitioned with EtOAc and water. The EtOAc-soluble material was further partitioned with 90% MeOH aq. and *n*-hexane. Repeated chromatographic separations of the *n*-hexane-soluble material gave six new prenylated acylphloroglucinols, erecricins A–E (**55**–**59**) and adotogirin (**60**), together with one known prenylated acylphloroglucinol (**61**) which was identified as otogirin by comparison of the spectroscopic data with the literature data [81].



Scheme 5. Isolation procedures for compounds **55**–**61**.

2.3. Structure elucidation

2.3.1. Structure elucidation for erecricin A (55)

Erecricin A (**55**) was obtained as an optical active colorless oil {$[\alpha]_D$ +58.9 (*c* 0.34, $CHCl_3$)}, and its molecular formula was established as $C_{31}H_{44}O_4$ by the HRESIMS (*m/z* 479.3151 $[M-H]^-$, Δ –1.0 mmu). The ^1H NMR spectrum showed the signals of five olefinic protons, three sp^3 methylenes, two sp^3 methines, and ten methyls as well as the characteristic down-field shifted signal (δ_H 19.04, s) due to a hydrogen-bonded hydroxy group (Table 2.1), indicating the existence of a β-diketone moiety with an enol form (Fig. 2.2) [84].

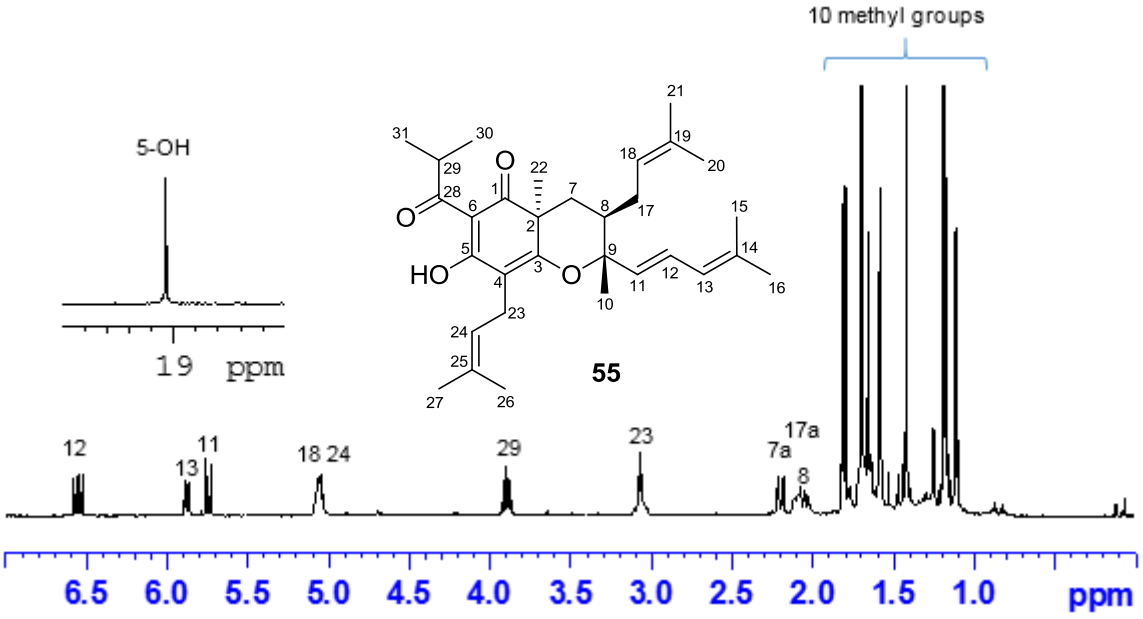


Figure 2.2. ^1H NMR spectrum of erecricin A (**55**) in $CDCl_3$ (500MHz).

The ^{13}C NMR and DEPT spectra suggested the presence of 31 carbons including two enols, four olefins, two ketone carbonyl carbons, one quaternary carbon, and one oxygenated tertiary carbon (Table 2.1). From these observations, erecricin A (**55**) was deduced to be a prenylated acylphloroglucinol with four isoprene units. Comparison of the 1D NMR spectra of **55** with the literature data implied that **55** had a structure similar to hypelodin A [84], a prenylated acylphloroglucinol possessing the chromane core, but had different substituents at C-2 and C-6. The substituent at C-2 in **55** was assigned as a methyl group by HMBC interpretaitions of H_3-22 with C-1, C-2, C-3, and C-7 (Fig. 2.3), while a 2-methyl propanoyl group at C-6 was revelaed by ^1H-^1H COSY cross-peaks of H-29 with H_3-30 and H_3-31 and HMBC correlations for H_3-30 with C-28, OH-5 with C-4, C-5, C-6 and C-28. Therefore, the gross structure of **55**

was elucidated as shown in Fig. 2.3.

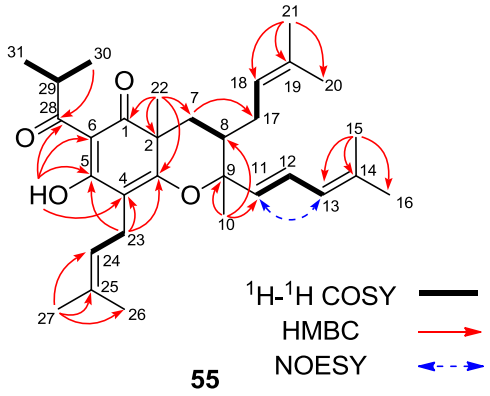


Figure 2.3. Selected 2D NMR correlations of erecricin A (**55**).

NOESY correlations for H-8/H$_3$-22, H-7β/H$_3$-10, and H-17b/H$_3$-10 suggested the pseudochair conformation of the tetrahydropyran ring (C-2, C-3, and C-7–C-9) and the pseudoaxial orientations for H-7β, H-8, 10-Me, and 22-Me (Fig. 2.4). This was underpinned by a large ^3J value of the vicinal coupling H-7β/H-8 (J = 14.3 Hz) (Table 2.1). Thus, the relative stereochemistry for **55** was assigned as shown in Chart 2.

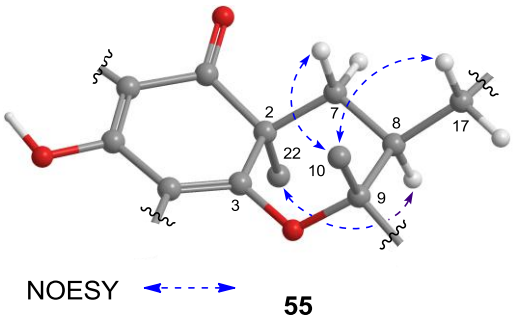


Figure 2.4. Selected NOESY correlations and relative stereochemistry for the chromane core of erecricin A (**55**) (protons of methyl groups were omitted).

2.3.2. Structure elucidation for erecricin B (56)

The HRESIMS revealed the molecular formula of erecricin B (**56**) to be $C_{32}H_{46}O_4$ {*m/z* 517.3317 $[M+Na]^+$, Δ +2.3 mmu}, larger by 14 mass units than that of **55**. Though the 1D NMR spectra of **56** (Fig. 2.5 and Table 2.2) were similar to those of **55**, the resonances of a 2-methylbutanoyl group were observed for **56** in place of the signals of the 2-methylpropanyl moiety for **55**. The presence of the 2-methylbutanoyl group at C-6 in **56** was confirmed by

HMBC analysis. The relative configuration of C-29 remains to be assigned, since any NOESY correlations were not observed. Thus, erecricin B was elucidated to be **56** (Chart 2).

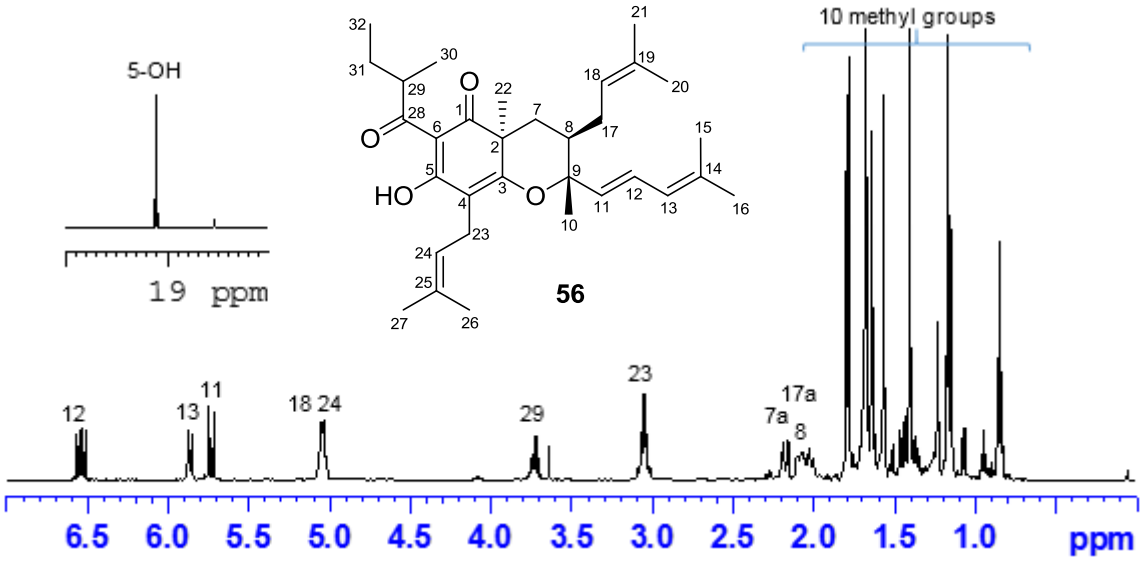


Figure 2.5. ^{1}H NMR spectrum of erecricin B (**56**) in $CDCl_3$ (500MHz).

2.3.3. Structure elucidation for erecricin C (57)

The molecular formula of erecricin C (**57**) was assigned as $C_{32}H_{46}O_4$ by the HRESIMS {*m/z* 517.3269 $[M+Na]^+$, Δ –2.5 mmu}, which was identical to that of **56**. Analysis of the 1D NMR spectra (Fig. 2.6 and Table 2.2) indicated **57** to be a diastereomer of **56** at the tetrahydropyran moiety. NOESY correlations of H_3-22/H-8 and H-7β/H-11 revealed the pseudoaxial orientations of H-7β, 22-Me, and the substituent at C-9 (Fig. 2.7). Therefore, the structure of **57** was assigned as shown in Chart 2.

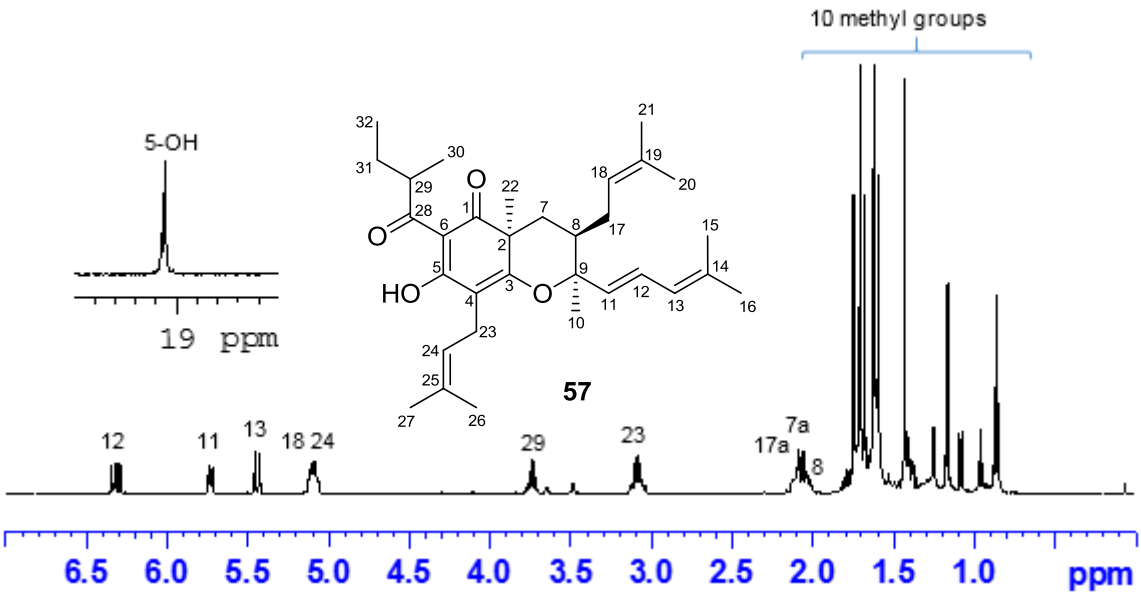


Figure 2.6. ^{1}H NMR spectrum of erecricin C (**57**) in $CDCl_3$ (500MHz).

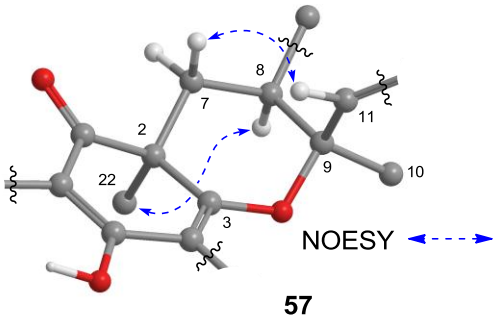


Figure 2.7. Selected NOESY correlations and relative stereochemistry for the chromane core of erecricin C (**57**) (protons of methyl groups were omitted).

2.3.4. Structure elucidation of erecricins D (58) and E (59)

Erecricins D (**58**) and E (**59**) were individually isolated as optical active colorless oil {$[\alpha]_D$ –25.2 (*c* 0.44, $CHCl_3$) for **58**; $[\alpha]_D$ –3.8 (*c* 2.17, $CHCl_3$) for **59**}. The molecular formula of **58** was assigned to be $C_{31}H_{44}O_4$ in light of the HRESIMS (*m/z* 479.3151 $[M–H]^-$, Δ –1.0 mmu). In the ^{1}H NMR spectrum of **58** (Fig. 2.8), a pair of down-field shifted hydroxy proton signals (δ_H 19.08 and 18.66) were observed in a ratio of ca. 3:1, indicating the presence of two tautomers (**58a** and **58b**).

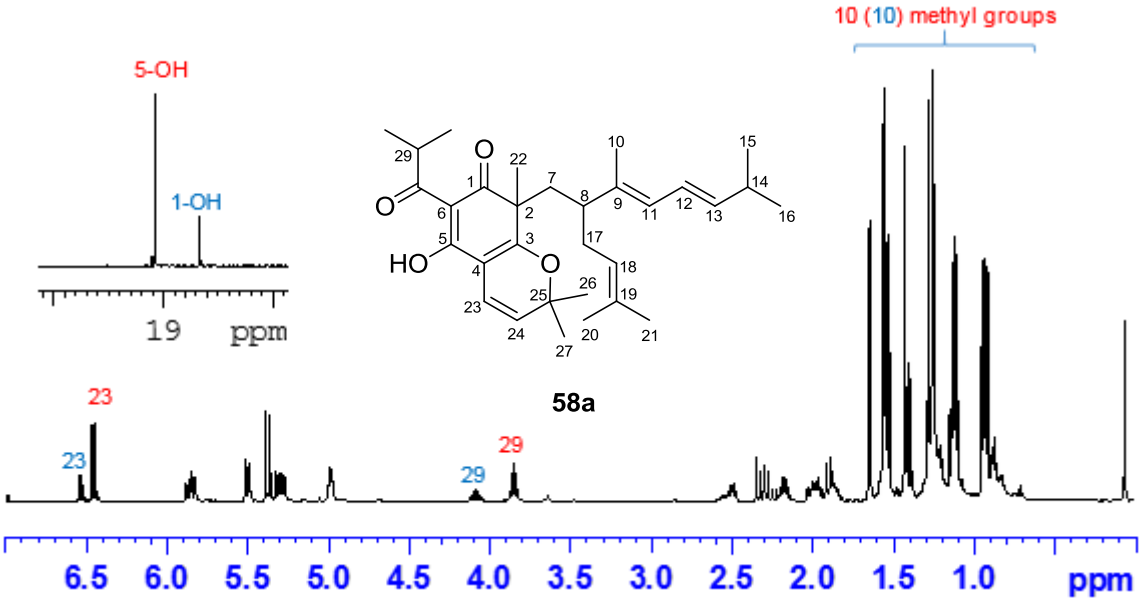


Figure 2.8. ^{1}H NMR spectrum of erecricin D (**58**) in $CDCl_3$ (500MHz).

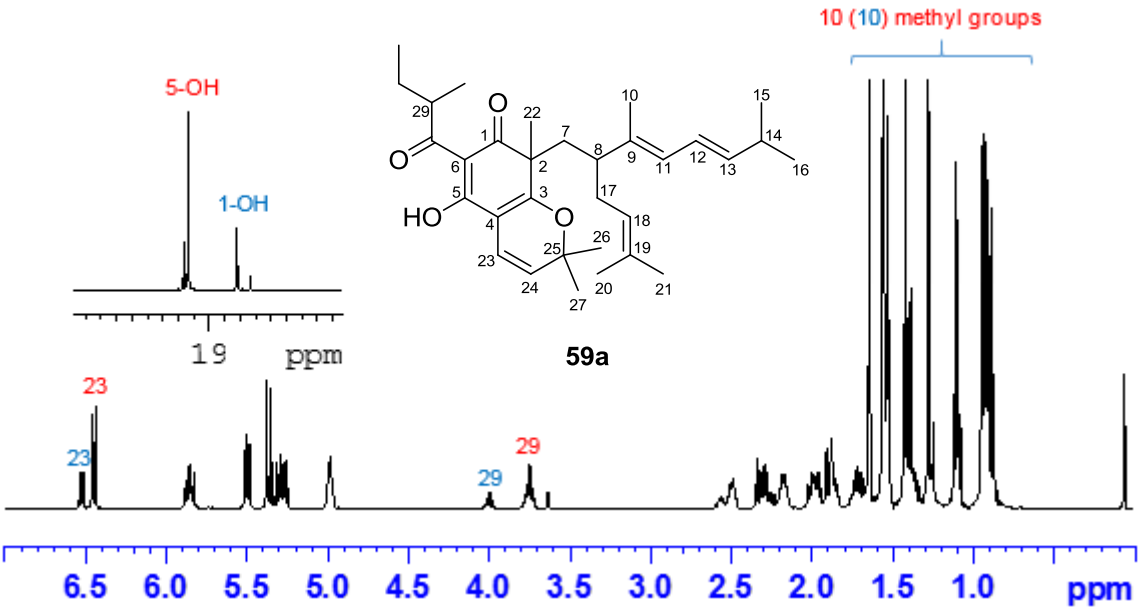


Figure 2.9. ^{1}H NMR spectrum of erecricin E (**59**) in $CDCl_3$ (500MHz).

The feature of the 1D NMR spectra of **58** were similar to those of hyperguinone B [87], a prenylated acylphlorglucinol with a chromene core, whereas the signals of the different substituent consisting of 15 carbons were observed for **58** in place of the resonances due to the prenyl group at C-2 in hyperguinone B. The substituent was elucidated by 2D NMR analysis (Fig. 2.10). The ^{1}H-^{1}H COSY spectrum indicated the connectivities of C-7–C-8, C-11–C-14,

C-14–C-15, and C-14–C-16, while the connectivities among C-8, C-10, and C-11 via C-9 were suggested by HMBC correlations for H_3-10 with C-8, C-9, and C-11. HMBC correlations for H_3-21 with C-18, C-19, and C-20 and ^{1}H-^{1}H cross-peaks of H-8/H_2-17 and H_2-17/H-18 revealed the presence of a prenyl group at C-8. Similarly, ericricin E (**59**) was elucidated to be a prenylated acylphloroglucinol possessing the same chromene core as seen in **58** with a 2-methylbutanoyl group at C-6. Thus, the planer structures of ericricins D (**58**) and E (**59**) were assigned as shown in Chart 2, while the stereochemistries for **58** and **59** were not elucidated.

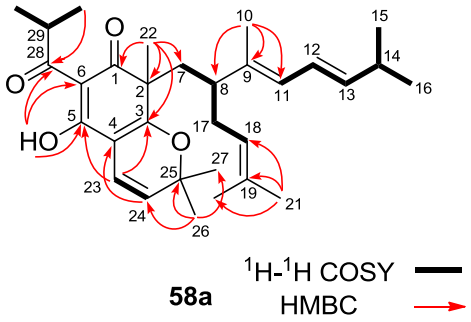


Figure 2.10. Selected 2D NMR correlations for major tautomer **58a** of erecricin D (**58**).

Table 2.1 ^{1}H and ^{13}C NMR data for erecricins A (**55**) in $CDCl_3$

Position	δ_C	δ_H (*J* in Hz)
1	197.7	–
2	48.0	–
3	171.6	–
4	115.6	–
5	189.3	–
6	105.4	–
7	31.0	2.20 (1H, dd, 14.3, 3.8)
		1.46 (1H, d, 14.3)
8	40.3	2.04 (1H, m)
9	86.0	–
10	19.1	1.19 (3H, s)
11	133.2	5.74 (1H, d, 15.3)
12	126.7	6.65 (1H, dd, 15.3, 10.9)
13	124.3	5.88 (1H, d, 10.9)
14	137.0	–
15	18.4	1.80 (3H, s)
16	26.0	1.82 (3H, s)
17	29.5	2.10 (1H, m)
		1.72 (1H, m)
18	121.5	5.07 (1H, m)
19	133.7	–
20	17.9	1.58 (3H, s)
21	25.8	1.70 (3H, s)
22	28.5	1.42 (3H, s)
23	21.2	3.07 (2H, m)
24	121.9	5.05 (1H, m)
25	131.7	–
26	17.9	1.70 (3H, s)
27	25.7	1.66 (3H, s)
28	208.0	–
29	35.4	3.90 (1H, sept, 6.8)
30	18.8	1.11 (3H, d, 6.8)
31	18.9	1.18 (3H, d, 6.8)
5-OH		19.04 (1H, s)

Table 2.2 ^{1}H and ^{13}C NMR data for erecricins B (**56**) and C (**57**) in $CDCl_3$

	56		**57**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	197.8	–	197.8	–
2	48.0	–	48.5	–
3	171.5	–	172.6	–
4	115.7	–	114.4	–
5	189.3	–	189.4	–
6	106.3	–	106.3	–
7	30.8	2.18 (1H, dd, 14.4, 3.9)	30.7	2.05 (1H, m)
		1.44 (1H, d, 14.4)		1.41 (1H, m)
8	40.3	2.03 (1H, m)	41.7	2.04 (1H, m)
9	85.9	–	86.5	–
10	18.9	1.17 (3H, s)	25.8	1.62 (3H, s)
11	133.1	5.73 (1H, d, 15.3)	128.9	5.44 (1H, d, 15.3)
12	126.7	6.54 (1H, dd, 15.3, 10.9)	127.8	6.32 (1H, dd, 15.3, 10.8)
13	124.3	5.86 (1H, d, 10.9)	124.4	5.73 (1H, d, 10.8)
14	137.0	–	136.9	–
15	18.3	1.80 (3H, s)	18.3	1.71 (3H, s)
16	26.1	1.78 (3H, s)	26.0	1.75 (3H, s)
17	29.5	2.07 (1H, m)	29.9	2.13 (1H, m)
		1.69 (1H, m)		1.74 (1H, m)
18	121.5	5.05 (1H, m)	121.6	5.11 (1H, m)
19	133.7	–	133.7	–
20	17.9	1.57 (3H, s)	17.9	1.60 (3H, s)
21	25.8	1.68 (3H, s)	25.9	1.71 (3H, s)
22	28.4	1.41 (3H, s)	28.9	1.43 (3H, s)
23	21.2	3.05 (2H, m)	21.3	3.09 (2H, m)
24	121.9	5.04 (1H, m)	121.6	5.08 (1H, m)
25	131.7	–	131.8	–
26	17.9	1.68 (3H, s)	17.9	1.68 (3H, s)
27	25.7	1.64 (3H, s)	25.6	1.62 (3H, s)
28	207.2	–	206.9	–
29	41.7	3.73 (1H, m)	41.6	3.75 (1H, m)
30	17.0	1.16 (1H, d, 6.8)	17.1	1.17 (1H, d, 6.8)
31	26.6	1.71 (1H, m)	26.7	1.72 (1H, m)
		1.38 (1H, m)		1.39 (1H, m)
32	11.9	0.85 (3H, t, 7.4)	11.8	0.86 (3H, t, 7.4)
5-OH		19.10 (1H, s)		19.06 (1H, s)

Table 2.3. ^{1}H and ^{13}C NMR data for major tautomers (**58a** and **59a**) of erecricins D (**58**) and E (**59**) in $CDCl_3$

	58a		**59a**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	193.9	–	193.9	–
2	51.5	–	51.4	–
3	172.6	–	172.2	–
4	105.1	–	104.8	–
5	185.9	–	185.9	–
6	105.8	–	106.4	–
7	40.1	2.32, 1.90 (each 1H, m)	39.9	2.31, 1.90 (each 1H, m)
8	38.4	2.50 (1H, m)	38.3	2.50 (1H, m)
9	137.1	–	137.0	–
10	18.5	1.54 (3H, d, 10.9)	18.4	1.55 (3H, d, 10.9)
11	129.0	5.50 (1H, d, 10.9)	128.9	5.49 (1H, d, 10.9)
12	122.7	5.86 (1H, dd, 15.5, 10.9)	122.6	5.86 (1H, dd, 14.9, 10.8)
13	139.9	5.28 (1H, dd, 15.5, 6.6)	139.8	5.28 (1H, dd, 14.9, 6.8)
14	31.3	2.18 (1H, m)	31.1	2.18 (1H, m)
15	22.1	0.95 (3H, d, 7.0)	22.0	0.94 (3H, d, 6.7)
16	22.4	0.92 (3H, d, 7.0)	22.4	0.92 (3H, d, 6.7)
17	33.3	1.97 (1H, m)	33.1	1.99 (1H, m)
		1.87 (1H, m)		1.86 (1H, m)
18	122.5	4.99 (1H, brt, 7.6)	122.4	4.99 (1H, brs)
19	132.4	–	132.1	–
20	17.9	1.56 (3H, s)	17.8	1.55 (3H, s)
21	25.7	1.65 (3H, s)	25.6	1.65 (3H, s)
22	27.5	1.29 (3H, s)	27.3	1.28 (3H, s)
23	115.0	6.45 (1H, d, 10.1)	115.0	6.45 (1H, d, 10.1)
24	123.3	5.38 (1H, d, 10.1)	123.2	5.37 (1H, d, 10.1)
25	80.7	–	80.7	–
26	28.3	1.43 (3H, s)	28.3	1.43 (3H, s)
27	29.3	1.56 (3H, s)	29.2	1.56 (3H, s)
28	207.7	–	207.1	–
29	35.4	3.86 (1H, sept, 6.9)	41.7	3.75 (1H, m)
30	18.9	1.13 (3H, d, 6.9)	11.9	1.10 (1H, d, 6.9)
31	18.9	1.12 (3H, d, 6.9)	26.3	1.71 (1H, m)
				1.36 (1H, m)
32			11.7	0.89 (3H, t, 7.8)
5-OH		19.08 (1H, s)		19.01 (1H, s)

2.3.5. Structure elucidation of adotogirin (60)

The 1D NMR spectra of adotogirin (**60**) (Fig. 2.11 and Table 2.4), $C_{22}H_{32}O_4$, resembled to those of a known acylphloroglucinol otogirin (**61**), except for the presence of the signals due to a *sec*-butyl group in place of those due to the isopropyl group in **61**. These observations implied that **60** is an prenylated acylphloroglucinol with a 2-methylbutanoyl group, a methyl group, and an *O*-geranyl group. The structure of adotogirin (**60**) was confirmed by analysis of the 2D NMR spectra (Fig. 2.12).

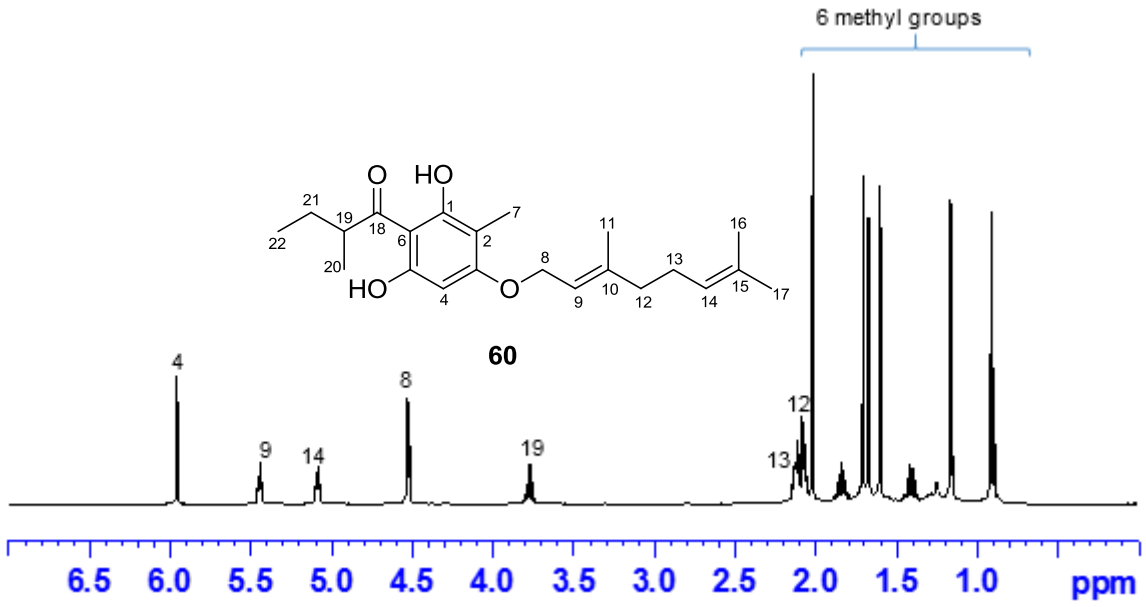


Fig. 2.11. ^{1}H NMR spectrum of adotogirin (**60**) in $CDCl_3$ (500MHz).

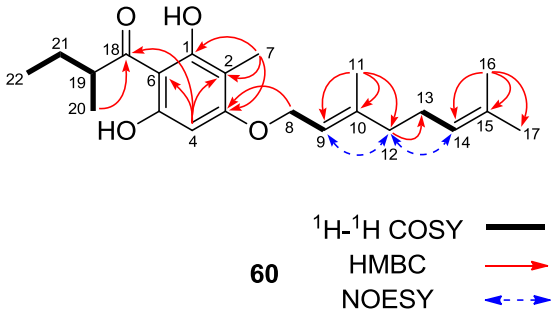


Fig. 2.12. Selected ^{1}H-^{1}H COSY and HMBC correlations for adotogirin (**60**).

Table 2.4 ^{1}H and ^{13}C NMR data for adotogirin (**60**) in $CDCl_3$

Position	δ_C	δ_H (*J* in Hz)
1	160.2[a)]	–
2	103.9[b)]	–
3	162.5	–
4	92.5	5.96 (1H, s)
5	160.8[a)]	–
6	104.3[b)]	–
7	7.2	2.02 (3H, s)
8	65.3	4.52 (2H, d, 6.3)
9	118.9	5.44 (1H, t, 6.3)
10	141.4	–
11	16.7	1.71 (3H, s)
12	39.4	2.08 (2H, m)
13	26.2	2.12 (2H, m)
14	123.6	5.08 (1H, t, 6.6)
15	131.9	–
16	17.7	1.60 (3H, s)
17	25.6	1.67 (3H, s)
18	210.4	–
19	45.9	3.77 (1H, q, 6.8)
20	16.7	1.16 (3H, d, 6.8)
21	27.0	1.84 (1H, m)
		1.41 (1H, m)
22	11.9	0.91 (3H, t, 7.4)

a,b) Signals may be interchangeable.

2.4. Bioassy for compounds 55–61

Erecricins A–E (**55**–**59**), adotogirin (**60**) and otogirin (**61**) were evaluated for their antimicrobial activities on strains of *Staphylococcus aureus* (MRSA and MSSA), *Bacillus subtilis*, and *Escherichia coli*. Among the tested compounds, adotogirin (**60**) and otogirin (**61**) exhibited an antimicrobial activity against MRSA {**60**: MIC range 0.5–4.0 μg/mL (MIC_{50} 1.0 μg/mL), **61**: MIC 0.5–8.0 μg/mL (MIC_{50} 0.5 μg/mL)}, MSSA {**60**: MICs 1.0 μg/mL for all strains; **61**: MIC range 0.5–1.0 μg/mL (MIC_{50} 0.5 μg/mL)}, and *Bacillus subtilis* (MIC each 2.0 μg/mL), while antiplasmodial activity, cytotoxic activity, and antagonistic activity against thromboxane A_2 and leukotriene D_4 of **61** have been reported [84,86]. In contrast, ericricins A–E (**55**–**59**) did not show any antimicrobial activity.

2.5. Summary

Investigation of the MeOH extract from the roots of *Hypericum erectum* afforded five new bicyclic prenylated achylphloroglucinols, erecricins A–E (**55**–**59**), and one prenylated achylphloroglucinols with an *O*-geranyl moiety, adotogirin (**60**), together with a known prenylated acylphlotoglucinol, otogirin (**61**), whose structures were elucidated by spectroscopic analysis. Adotogirin (**60**) and otogirin (**61**) showed antimicrobial activities against *Staphylococcus aureus* and *Bacillus subtilis*.

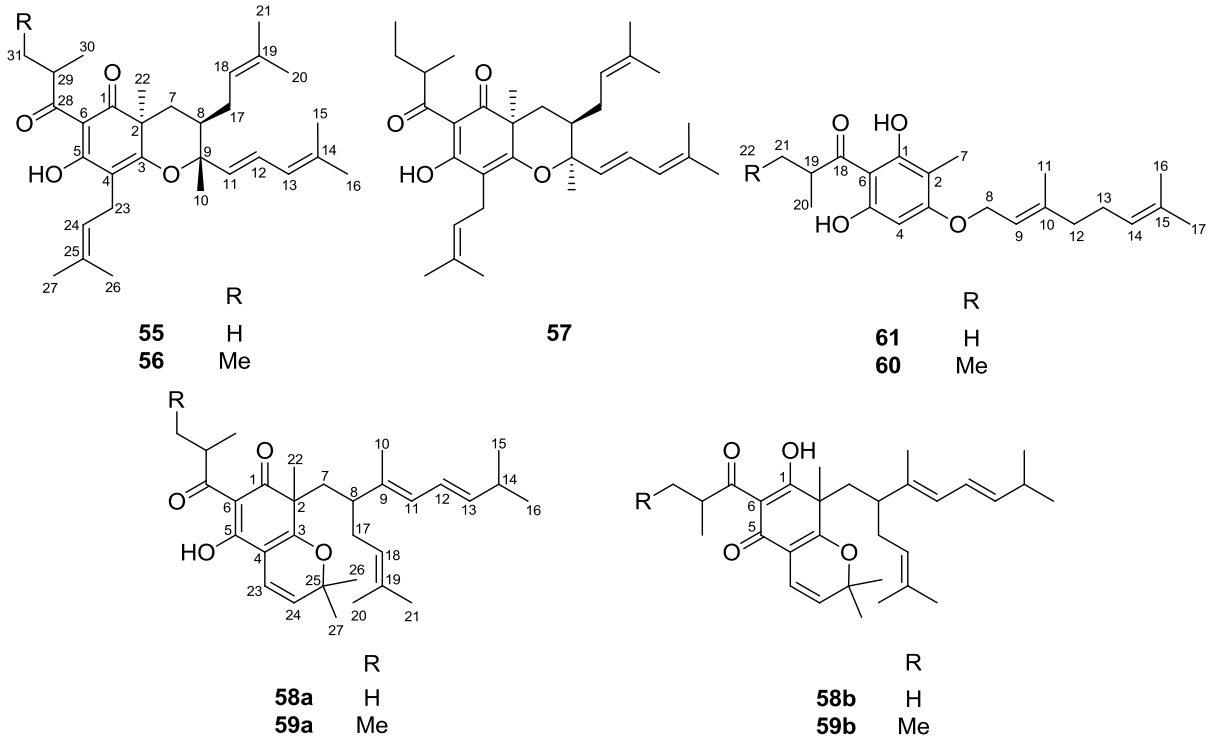


Chart 2. Structures of erecricins A–E (**55**–**59**), adotogirin (**60**), and otogirin (**61**) (**58a**/**59a** and **58b**/**59b** are major and minor tautomers of **58** and **59**, respectively).

Chapter 3

Experimental Section

3.1. General experimental procedures

Optical rotations were measured by a JASCO P-2200 digital polarimeter. UV, ECD, and IR spectra were recorded on a Hitachi U-3900H, a JASCO J-1500, and a JASCO FT-IR-6200 spectrophotometers, respectively. MS were obtained on a Waters LCT PREMIER 2695. NMR spectra were measured by a Bruker AVANCE-500 instrument using tetramethylsilane as an internal standard. Column chromatography was performed with silica gel 60N (63-210 μm, Kanto Chemical), MCI gel CHP-20P (75-150 μm, Mitsubishi Chemical), Dianion HP-20 (Mitsubishi Chemical), YMC gel ODS-A (S-50 μm, YMC Co., Ltd.), Toyopearl HW-40C (TOSOH Corporation), and Sephadex LH-20 (25-100 μm, GE Health Care). HPLC was performed on YMC-Triant C18 (5 μm, 20×250 mm; YMC Co., Ltd.), Hydrosphere C18 (5 μm, 20×250 mm; YMC Co., Ltd.), Mightysil RP-18 GP (5 μm, 20×250 mm; Kanto Chemical), Mightysil RP-18 GP-II (5 μm, 20×250 mm), Mightysil Si 60 (5 μm, 20×250 mm; Kanto Chemical), COSMOSIL πNAP (5 μm, 20×250 mm, Nacalai Tesque), COSMOSIL 5C_{18}-AR-II (5 μm, 20×250 mm), and COSMOSIL 5C_{18}-MS-II (5 μm, 20×250 mm).

3.2. Experimental procedure of chapter 1.2

3.2.1. Plant material

Gentianella amarella ssp. *acuta* was collected at Hosvsgol province, Mongolia in August 2011. A voucher specimen (11JM0077) was deposited on the herbarium of Tokushima University.

3.2.2. Extraction and isolation

The air-dried aerial parts of *G. amarella* ssp. *acuta* (213.3 g) were extracted with MeOH at room temperature to give the extract (68.4 g), which was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble-material (36.9 g) was subjected to repeated column chromatographies as shown in Fig. 3.1, resulting in the isolation of 22 compounds (**1**–**22**).

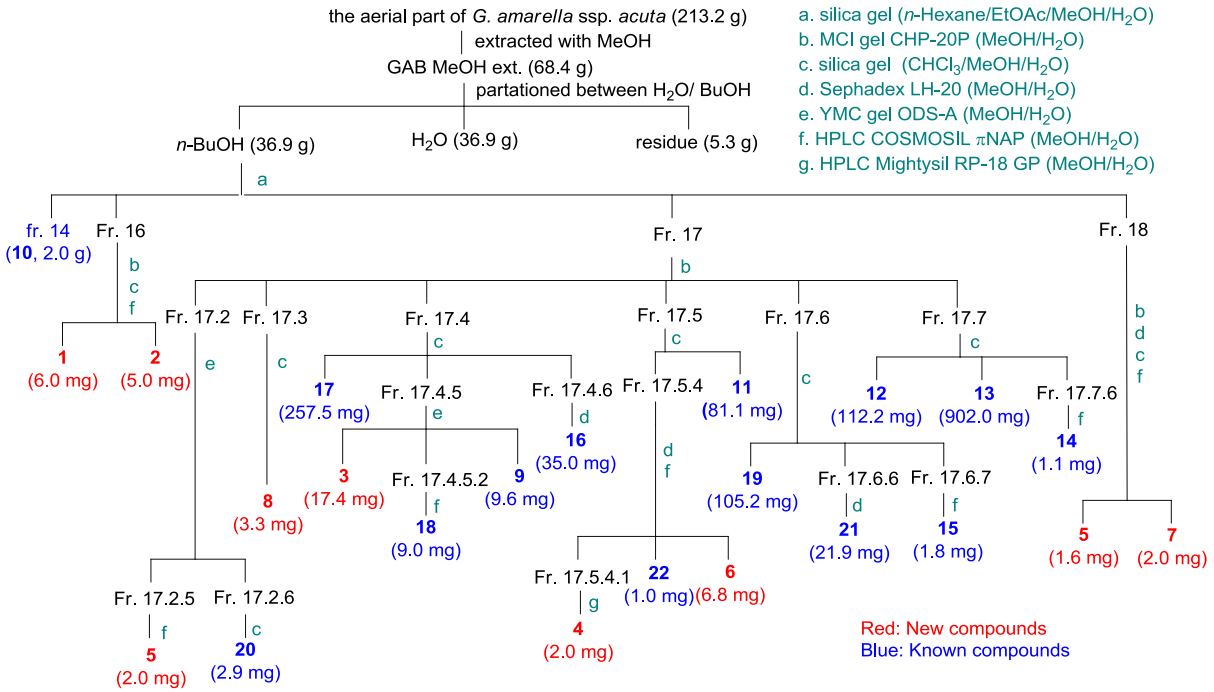


Figure 3.1. Isolation scheme for the aerial parts of *G. amarella* ssp. *acuta*.

3.2.3. 1,3,5*S*,8*S*-Tetrahydroxy-5,6,7,8-tetrahydroxanthone (1)

Pale yellow solid; $[\alpha]^{20}_{D}$ +78.1 (*c* 0.34, MeOH); HRESIMS *m/z* 287.0526 $[M+Na]^+$ (calcd for $C_{13}H_{12}O_6Na$, 287.0532); UV (MeOH) λ_{max} 260 (ε 13000) and 317 (ε 5400) nm; IR (KBr) ν_{max} 3392, 2360, 2335, and 1653 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) (Table 1.2.1); ^1H NMR (DMSO-d_6) δ_H 12.83 (1H, s, 1-OH), 6.29 (1H, brs, H-4), 6.13 (1H, brs, H-2), 4.73 (1H, brs, H-8), 4.37 (1H, brs, H-5), 2.16 (1H, brt, *J* = 13.9 Hz, H-6a), 1.87 (1H, brt, *J* = 13.9 Hz, H-7a), 1.70 (1H, brd, *J* = 13.9 Hz, H-6b), and 1.63 (1H, brd, *J* = 13.9 Hz, H-7b); ^1H NMR (C_5D_5N) δ_H 13.46 (1H, s, 1-OH), 6.69 (1H, brs, H-4), 6.54 (1H, brs, H-2), 5.40 (1H, brs, H-8), 4.80 (1H, brs, H-5), 2.68 (1H, brt, *J* = 13.4 Hz, H-6a), 2.42 (1H, brt, *J* = 13.4 Hz, H-7a), 2.16 (1H, m, H-6b), 2.11 (1H, m, H-7b).

3.2.4. 1,3,5*R*,8*S*-Tetrahydroxy-5,6,7,8-tetrahydroxanthone (2)

Pale yellow solid; $[\alpha]^{20}_{D}$ +9.1 (*c* 0.15, MeOH); HRESIMS *m/z* 287.0528 $[M+Na]^+$ (calcd for $C_{13}H_{12}O_6Na$, 287.0532); UV (MeOH) λ_{max} 259 (ε 12000) and 318 (ε 4700) nm; IR (KBr) ν_{max} 3414, 2357, and 1659 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) (Table 1.2.1); ^1H NMR (DMSO-d_6) δ_H 12.84 (1H, s, 1-OH), 6.29 (1H, brs, H-4), 6.13 (1H, brs, H-2), 4.73 (1H, brs, H-8) 4.47 (1H, dd, *J* = 8.5, 7.4 Hz, H-5), 2.01 (1H, m, H-6a), 1.90 (1H, m, H-6b), 1.75 (1H, m, H-7a), and 1.65 (1H, m, H-7b); ^1H NMR (C_5D_5N) δ_H 13.44 (1H, s, 1-OH), 6.67 (1H, d, *J* = 2.7 Hz, H-4), 6.54 (1H, d, *J* = 2.7 Hz, H-2), 5.35 (1H, t, d, *J* = 3.3 Hz, H-8), 4.89 (1H, dd, *J* = 9.1, 5.8 Hz, H-5), 2.71 (1H, m, H-6a), 2.27 (1H, m, H-6b), 2.25 (1H, m, H-7a), 1.93 (1H, tt, *J* = 13.3, 1.9 Hz, H-7b).

3.2.5. Preparation of 3-*O*-methyl-1,5,8-tris-4-methoxybenzoate (1b and 2b) of 1 and 2

A mixture of 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**, 2.0 mg), CH_3I (100 μL), and K_2CO_3 (65 mg) in dry acetone (2.0 mL) was stirred at room temperature for 2 h. After removal of inorganic salts by filtration, the filtrate was concentrated under reduced pressure to give 1,5*S*,8*S*-trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**1a**). To a solution of **1a**, 4-dimethylaminopyridine (23.8 mg), and triethylamine (70 μL) in CH_2Cl_2 (5 mL) was added 4-methoxybenzoyl chloride (19.6 mg), and the mixture was stirred at room temperature for 4 h. After addition of water (10 mL), the mixture was extracted with EtOAc (10 mL×3). The EtOAc-soluble material was purified by a silica gel column (*n*-hexane/EtOAc, 8:2 to 7:3) to afford 3-

O-methyl-1,5,8-tris-4-methoxybenzoate (**1b**, 2.3 mg) of **1**. Methylation of 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**, 1.5 mg) as for **1** gave 1,5*R*,8*S*-trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**2a**), whose 1,5,8-tris-4-methoxybenzoate (**2b**, 2.1 mg) was obtained by the same procedure as described above.

3.2.5.1. 1,5*S*,8*S*-Trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**1a**)

White amorphous solid; HRESIMS *m/z* 301.0679 $[M+Na]^+$ (calcd for $C_{14}H_{14}O_6Na$, 361.0688); ^1H NMR (CD_3OD) δ_H 6.53 (1H, d, *J* = 2.0 Hz, H-4), 6.33 (1H, d, *J* = 2.0 Hz, H-2), 4.94 (1H, t, *J* = 3.1 Hz, H-8), 4.52 (1H, dd, *J* = 4.4, 3.3 Hz, H-5), 3.86 (3H, s, 3-OMe), 2.28 (1H, tt, *J* = 13.6, 3.1 Hz, H-6a), 2.05 (1H, tt, *J* = 13.6, 3.3 Hz, H-7a), 1.87 (1H, m, H-6b), and 1.81 (1H, m, H-7b).

3.2.5.2. 3-*O*-Methyl-1,5,8-tris-4-methoxybenzoate of **1** (**1b**)

White amorphous solid; HRESIMS *m/z* 703.1799 $[M+Na]^+$ (calcd for $C_{38}H_{32}O_{12}Na$, 703.1791); ^1H NMR ($CDCl_3$) δ_H 8.11 (2H, d, *J* = 8.7 Hz, Bz), 8.01 (2H, d, *J* = 8.7 Hz, Bz), 7.90 (1H, d, *J* = 8.7 Hz, Bz), 6.94 (2H, d, *J* = 8.7 Hz, Bz), 6.93 (2H, d, *J* = 8.7 Hz, Bz), 6.86 (2H, d, *J* = 8.7 Hz, Bz), 6.77 (1H, d, *J* = 2.5 Hz, H-4), 6.70 (1H, d, *J* = 2.5 Hz, H-2), 6.31 (1H, t, *J* = 2.8 Hz, H-8), 6.12 (1H, dd, *J* = 3.8, 1.6 Hz, H-5), 3.87 × 3 (each 3H, s, OMe × 3), 3.84 (3H, s, 3-OMe), 2.36 (1H, tt, *J* = 14.4, 3.8 Hz, H-6a), 2.22 (1H, dd, *J* = 14.4, 2.8 Hz, H-7a), 2.11 (1H, m, H-6b), and 2.10 (1H, m, H-7b); ECD (MeOH) Δε (nm) –7.2 (230), +4.9 (243), –5.9 (254), and +24.5 (275).

3.2.5.3. 1,5*R*,8*S*-Trihydroxy-3-methoxy-5,6,7,8-tetrahydroxanthone (**2a**)

White amorphous solid; HRESIMS *m/z* 301.0688 $[M+Na]^+$ (calcd for $C_{14}H_{14}O_6Na$, 301.0688); ^1H NMR (CD_3OD) δ_H 6.54 (1H, d, *J* = 2.3 Hz, H-4), 6.33 (1H, d, *J* = 2.3 Hz, H-2), 4.92 (1H, t, *J* = 4.6 Hz, H-8), 4.60 (1H, dd, *J* = 9.0, 6.4 Hz, H-5), 3.86 (3H, s, 3-OMe), 2.12 (1H, m, H-6a), 2.05 (1H, m, H-6b), 1.96 (1H, m, H-7a), and 1.87 (1H, m, H-7b).

3.2.5.4. 3-*O*-Methyl-1,5,8-tris-4-methoxybenzoate of **2** (**2b**)

White amorphous solid; HRESIMS *m/z* 703.1790 $[M+Na]^+$ (calcd for $C_{38}H_{32}O_{12}Na$, 703.1791); ^1H NMR ($CDCl_3$) δ_H 8.12 (2H, d, *J* = 9.1 Hz, Bz), 8.08 (2H, d, *J* = 9.1 Hz, Bz), 7.95

(1H, d, *J* = 9.1 Hz, Bz), 6.99 (2H, d, *J* = 9.1 Hz, Bz), 6.90 (2H, d, *J* = 9.1 Hz, Bz), 6.88 (2H, d, *J* = 9.1 Hz, Bz), 6.70 (1H, d, *J* = 2.4 Hz, H-2), 6.69 (1H, d, *J* = 2.4 Hz, H-4), 6.22 (1H, t, *J* = 3.6 Hz, H-8), 6.14 (1H, dd, *J* = 9.1, 6.7 Hz, H-5), 3.90 (3H, s, 3-OMe), 3.85 × 3 (each 3H, s, OMe × 3), 2.32 (1H, m, H-7a), 2.27 (1H, m, H-6a), 2.25 (1H, m, H-6b), and 2.00 (1H, m, H-7b); ECD (MeOH) Δε (nm) –16.7 (248), +33.6 (264), and –4.3 (301).

3.2.6. Calculation of ECD spectra of 1b and 2b

Conformational searches and DFT calculations were carried out on Spartan 10 [88] and Gaussian 09 [89], respectively. The enantiomers (5*S*,8*S*-**1b**, 5*R*,8*R*-**1b**, 5*R*,8*S*-**2b**, and 5*S*8*R*-**2b**) were separately subjected to conformational searches using MMFFaq as the force field. The initial low-energy conformers with Boltzmann distributions over 1% 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 low-energy conformers for 5*S*,8*S*-**1b**, 5*R*,8*R*-**1b**, 5*R*,8*S*-**2b**, and 5*S*8*R*-**2b** with Boltzmann distributions over 1% (16, 16, 13, and 15 conformers, respectively) 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 (0.2 eV) using SpecDis v1.61 [90]. The calculated ECD spectra were composed after correction based on the Boltzmann distribution of the stable conformers. The calculated ECD spectra of 5*S*,8*S*-**1b** and 5*R*,8*R*-**1b** were red-shifted by 15 nm, respectively.

3.2.7. Amarellin A (3)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +34.4 (*c* 0.15, MeOH); HRESIMS *m/z* 449.1076 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) λ_{max} 220 (ε 15700), 258 (ε 13000), and 300 (ε 5200) nm; IR (KBr) ν_{max} 3393, 2360, 2342, and 1659 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.2).

3.2.8. Amarellin B (4)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +25.7 (*c* 0.09, MeOH); HRESIMS *m/z* 419.0946 $[M+Na]^+$ (calcd for $C_{18}H_{20}O_{10}Na$, 419.0954); UV (MeOH) λ_{max} 260 (ε 10300) and 299 (ε 4400) nm; IR (KBr) ν_{max} 3407, 2361, 2337, and 1638 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.2).

3.2.9. Amarellin C (5)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ –14.4 (*c* 0.14, MeOH); HRESIMS *m/z* 449.1061 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) λ_{max} 226 (ε 12800), 254 (ε 11300), and 298 (ε 5500) nm; IR (KBr) ν_{max} 3384, 2361, 2337, and 1651 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.2).

3.2.10. Amarellin D (6)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ +7.7 (*c* 0.14, MeOH); HRESIMS *m/z* 419.0963 $[M+Na]^+$ (calcd for $C_{18}H_{20}O_{10}Na$, 419.0954); UV (MeOH) λ_{max} 252 (ε 16800), 260 (ε 17800), and 298 (ε 6300) nm; IR (KBr) ν_{max} 3408, 2361, 2336, and 1654 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.3).

3.2.11. Amarellin E (7)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ –51.8 (*c* 0.22, MeOH); HRESIMS *m/z* 449.1066 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) λ_{max} 227 (ε 13700), 254 (ε 11000), and 297 (ε 4500) nm; IR (KBr) ν_{max} 3410, 2361, 2336, and 1638 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.3).

3.2.12. Amarellin F (8)

Pale yellow amorphous solid; $[\alpha]^{20}_{D}$ –30.5 (*c* 0.14, MeOH); HRESIMS *m/z* 449.1066 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_{11}Na$, 449.1060); UV (MeOH) λ_{max} 231 (ε 11500), 245 (ε 12100), 250 (ε 12300), and 289 (ε 4500) nm; IR (KBr) ν_{max} 3400, 2360, 2336, and 1646 cm^{-1}; ^1H and ^{13}C NMR (Table 1.2.3).

3.2.13. Acid hydrolysis of amarellins A–F (3–8)

Amarellin A (**3**, 0.5 mg) was treated with 1M HCl (3.0 mL) at 80 °C for 2 h. The reaction mixture was neutralized with an anion-exchange resin (IRA-400, ORGANO Co.), and evaporated to give a residue. The residue and L-cysteine methyl ester hydrochloride (0.5 mg) was dissolved in pyridine (0.1 mL) and heated at 60 °C for 1h, and then *o*-tolylisothiocyanate (10 μL) was added to the mixture and heated at 60 °C for 1 h. The reaction mixture was analyzed by ODS HPLC [COSMOSIL 5C_{18}-AR-II (ϕ 4.6 × 250 mm); solvent, CH_3CN/50 mM H_3PO_4 aq. (25:75); flow rate, 0.8 mL/min; UV detection, 250 nm; column temperature, 35 °C] to give a

peak at 17.5 min. The retention time was identical to that of the derivative of authentic D-glucose prepared by the same procedure as described above. Acid hydrolysis followed by HPLC analyses for amarellins C (**5**), E (**7**), and F (**8**) were carried out in the same manner as for **3**, suggesting the sugar moieties of **5**, **7**, and **8** to be D-glucose. Similarly, the sugar moieties of amarellins B (**4**) and D (**6**) were assigned as D-xylose by comparison of the retention time for the derivatives of **4** and **6** with that for the derivative of anthentic D-xylose [t_R 20.4 min].

3.2.14. Enzymatic hydrolysis of amarellins A–F (3–8)

Amarellin A (**3**, 1.5 mg) was treated with β-glucosidase (from almonds, 1.5 mg, Sigma Chemical Co.) in water (2 mL) at 38 ºC for 2h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL × 3). The EtOAc-soluble material was purified by HPLC on COSMOSIL πNAP (5 μm, ϕ10 × 250 mm; MeOH/H_2O, 38:62) to furnish an aglycone (**3a**, 0.4 mg) of **3**. Similarly, amarellins C (**5**), E (**7**), and F (**8**) were separately hydrolyzed by β-glucosidase to give their aglycones (**5a**, **7a**, and **8a**, respectively). A mixture of amarellin B (**4**, 1.5 mg) and cellulase (from *Trichoderma longibrachiatum*, 9.0 mg, Sigma Chemical Co.) in water (2 mL) was kept standing at 38 ºC for 24h. After dilution with water (10 mL), the reaction mixture was extracted with EtOAc (10 mL × 3), and the EtOAc-soluble material was purified by HPLC on COSMOSIL πNAP (5 μm, ϕ10 × 250 mm; MeOH/H_2O, 45:55) to afford an aglycone (**4a**) of **4**. Enzymatic hydrolysis for amarellin D (**6**) was carried out in the same manner as described for **4** to give an aglycone (**6a**) of **6**. Spectroscopic data including the HRESIMS data, ^{1}H NMR spectra, and optical rotations for the aglycones **3a**, **4a**, and **5a** were identical to those for 1,3,5*S*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**1**), while spectroscopic data for the aglycones **6a**, **7a**, and **8a** were coincident with those for 1,3,5*R*,8*S*-tetrahydroxy-5,6,7,8-tetrahydroxanthone (**2**).

3.3. Experimental procedure of chapter 1.3

3.3.1. Plant material

Lomatogoniu carinthiacum was collected at Hosvsgol province, Mongolia in August 2011. A voucher specimen (11JM0072) was deposited on the herbarium of Tokushima University.

3.3.2. Extraction and isolation

The air-dried aerial parts of *L. carinthiacum* (438.1 g) were extracted with MeOH to give the extract (157.7 g), which was partitioned between EtOAc and H_2O. The EtOAc soluble material was partitioned with $CHCl_3$ and 50% MeOH aq. The H_2O-soluble-material (135.4 g) and $CHCl_3$-soluble-material were subjected to repeated column ch`romatographies as shown in Fig. 3.2, resulting in the isolation of 11 compounds (**23**–**33**).

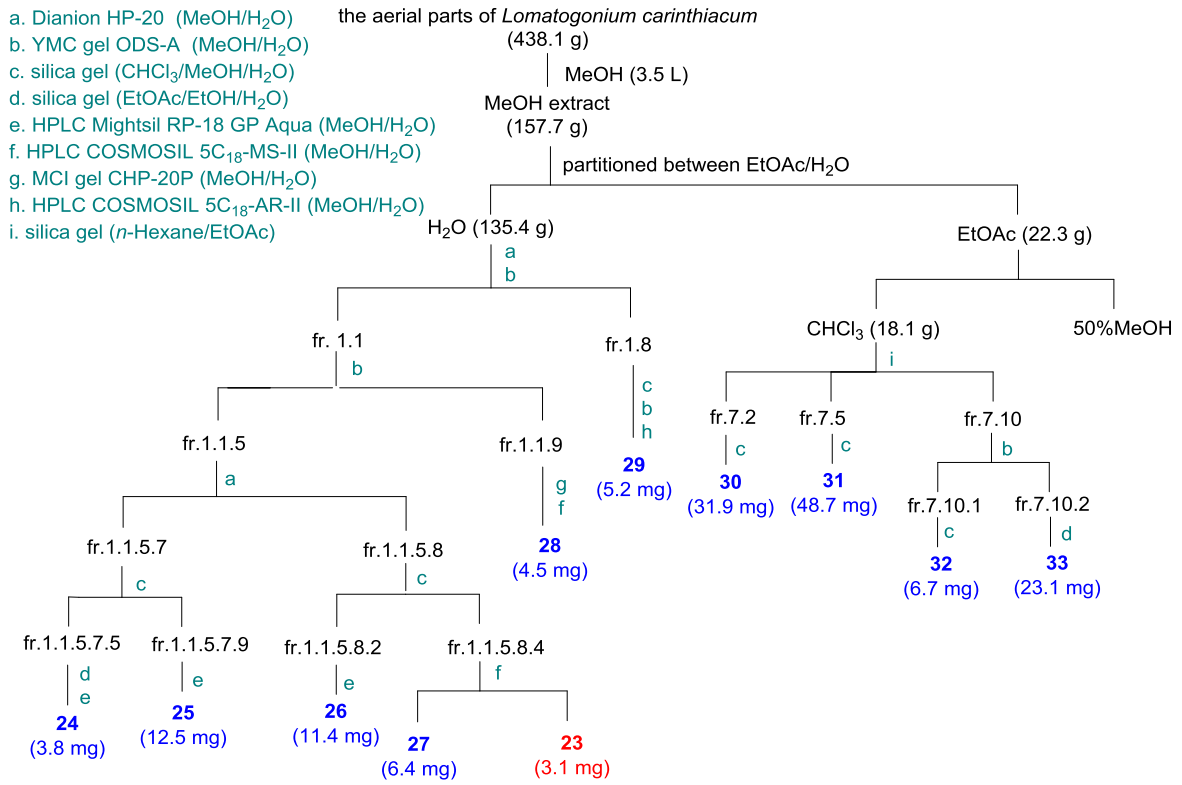


Figure 3.2. Isolation scheme for the aerial parts of *L. carinthiacum*.

3.4. Experimental procedure of chapter 1.4

3.4.1. Plant material

Ligularia sibirica was collected at Hentiy Province, Mongolia in August 2015. A voucher specimen (15JM0016) was deposited on the herbarium of Tokushima University.

3.4.2. Extraction and isolation

The roots and rhizomes of *L. sibirica* (264.7 g) were air-dried and extracted with MeOH at room temperature to give the extract (31.0 g), which was partitioned between EtOAc and H_2O. The EtOAc-soluble-material (11.3 g) was subjected to repeated column chromatographies as shown in Fig. 3.3, resulting in the isolation of 11 sesquiterpenes (**34**–**44**).

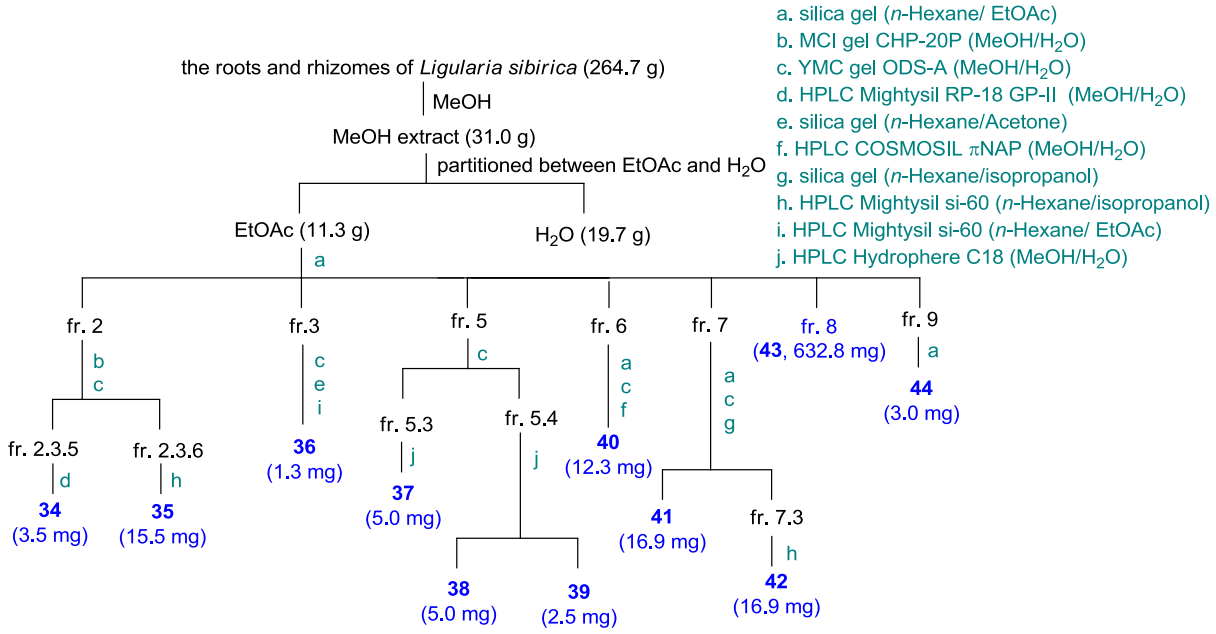


Figure 3.3. Isolation scheme for the aerial parts of *L. sibirica*.

3.5. Experimental procedure of chapter 1.5

3.5.1. Plant material

Caragana jubata was collected at Arhangay Province, Mongolia in August 2011. A voucher specimen (11JM0001) was deposited on the herbarium of Tokushima University.

3.5.2. Extraction and isolation

The MeOH extract (282.6 g) of air-dried stems of *C. jubata* (5.3 kg) was partitioned with EtOAc and H_2O. The EtOAc-soluble material was further partitioned between *n*-hexane and 90% MeOH aq. The H_2O-soluble material (198.4 g) was subjected to column chromatographies (Fig. 3.4) to give seven triterpenoid saponins (**45**–**51**), while three flavonoids (**52**–**54**) were isolated from the 90% MeOH-soluble material (32.5 g).

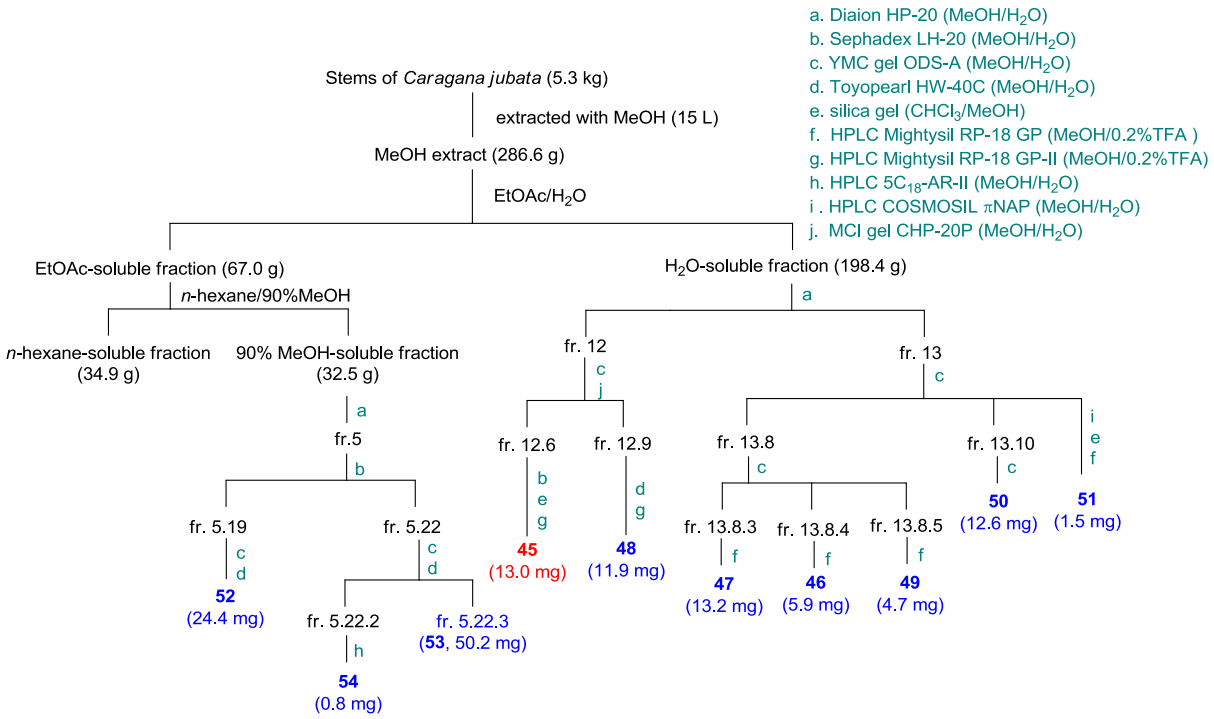


Figure 3.4. Isolation scheme for the aerial parts of *C. jubata*.

3.5.3. Compound 45

White amorphous powder; $[\alpha]_D$ –7.4 (*c* 0.13, MeOH); HRESIMS: *m/z* 683.3466, $[M+Na]^+$ (calcd for $C_{36}H_{52}O_{11}Na$, 683.3407) ; ^1H and ^{13}C NMR (Table 1.5.1).

3.6. Experimental procedure of chapter 2

3.6.1. Plant material

Hypericum erectum was cultivated at the herbarium of Tokushima University, was collected in July 2014. A voucher specimen (HYE201407) was deposited in the herbarium.

3.6.2. Extraction and isolation

The dried roots (2.0 kg) of *H. erectum* were extracted with methanol at room temperature to give the extract (170.7 g), which was partitioned with EtOAc and water. The EtOAc-soluble material (62.0 g) was partitioned with *n*-hexane and 90% MeOH aq. The *n*-hexane-soluble

material (27.6 g) was subjected to column chromatographies as shown in Fig. 3.5 to give six new compounds (**55**–**60**), as well as one known acylphloroglucinol, otogirin (**61**).

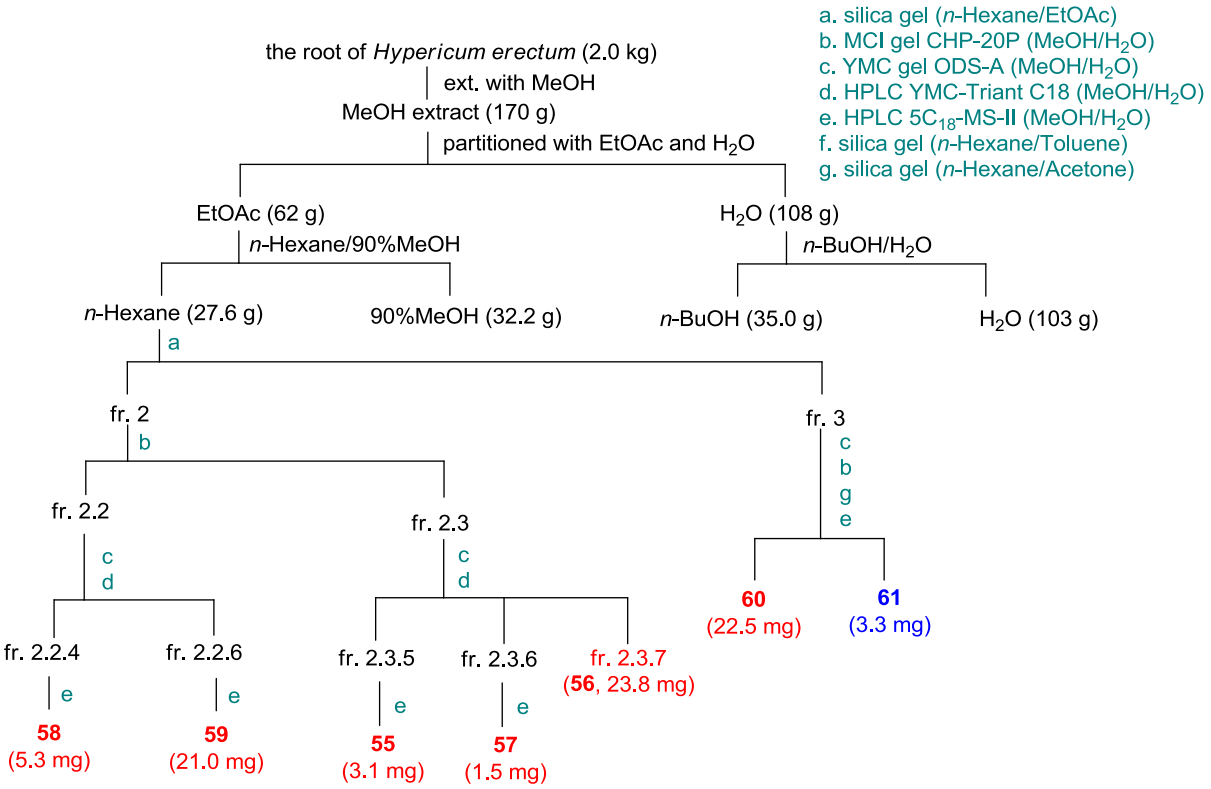


Figure 3.5. Isolation scheme for the roots of *H. erectum*.

3.6.3. Erecricin A (55)

Colorless oil; $[\alpha]_D$ +58.9 (c 0.34, $CHCl_3$); HRESIMS *m/z* 479.3151 $[M+H]^+$ (calcd for $C_{31}H_{43}O_4$, 479.3161); ^1H and ^{13}C NMR (Table 2.1).

3.6.4. Erecricin B (56)

Colorless oil; $[\alpha]_D$ +69.4 (*c* 2.70, $CHCl_3$); HRESIMS *m/z* 517.3317 $[M+Na]^+$ (calcd for $C_{32}H_{46}O_4Na$, 517.3294); ^1H and ^{13}C NMR (Table 2.2).

3.6.5. Erecricin C (57)

Colorless oil; $[\alpha]_D$ –60.0 (*c* 0.18, $CHCl_3$); HRESIMS *m/z* 517.3269 $[M+Na]^+$ (calcd for

$C_{32}H_{46}O_4Na$, 517.3294); ^1H and ^{13}C NMR (Table 2.2).

3.6.6. Erecricin D (58)

Colorless oil; $[\alpha]_D$ –25.2 (*c* 0.44, $CHCl_3$); HRESIMS *m/z* 479.3151 $[M–H]^-$ (calcd for $C_{31}H_{43}O_4$, 479.3161); ^1H and ^{13}C NMR for major tautomer **58a** (Table 2.3); ^1H NMR for minor tautomer **58b**: δ_H 18.66 (1H, s, 1-OH), 6.53 (1H, d, *J* = 10.1 Hz, H-23), 5.86 (1H, dd, *J* = 15.5, 10.9 Hz, H-12), 5.50 (1H, d, *J* = 10.9 Hz, H-11), 5.33 (1H, d, *J* = 10.1 Hz, H-24), 5.28 (1H, m, H-13), 4.99 (1H, m, H-18), 3.86 (1H, sept, *J* = 6.8 Hz, H-29), 2.50 (1H, m, H-8), 2.32 (1H, m, H-7a), 2.18 (1H, m, H-14), 1.97 (1H, m, H-17a), 1.90 (1H, m, H-7b), 1.87 (1H, m, H-17b), 1.65 (3H, s, H_3-21), 1.56 (6H, s, H_3-20 and H_3-27), 1.54 (3H, d, *J* = 10.9 Hz, H_3-10), 1.43 (3H, s, H_3-26), 1.40 (3H, s, H_3-22), 1.15 (3H, d, *J* = 6.8 Hz, H_3-30), 1.11 (3H, d, *J* = 6.8 Hz, H_3-31), 0.94 (3H, d, *J* = 6.9 Hz, H_3-15), and 0.92 (3H, d, *J* = 6.9 Hz, H_3-16); ^{13}C NMR for minor tautomer **58b**: δ_C 209.1 (C-28), 197.5 (C-1), 179.7 (C-5), 165.0 (C-3), 139.9 (C-13), 137.1 (C-9), 132.4 (C-19), 129.0 (C-11), 123.7 (C-24), 122.7 (C-12), 122.5 (C-18), 116.4 (C-23), 109.9 (C-4), 109.6 (C-6), 79.1 (C-25), 47.0 (C-2), 40.1 (C-7), 38.4 (C-8), 35.4 (C-29), 33.3 (C-17), 31.3 (C-14), 29.3 (C-27), 28.3 (C-26), 26.9 (C-22), 25.7 (C-21), 22.1 (C-15), 22.4 (C-16), 18.9 x 2 (C-30 and C-31), 18.4 (C-10), and 17.9 (C-20).

3.6.7. Erecricin E (59)

Colorless oil; $[\alpha]_D$ –3.8 (*c* 2.17, $CHCl_3$); HRESIMS *m/z* 533.3223 $[M+Na]^+$ (calcd for $C_{32}H_{46}O_5Na$, 533.3243); ^1H and ^{13}C NMR for major tautomer **59a** (Table 2.3); ^1H NMR for minor tautomer **59b**: δ_H 18.82 (1H, s, 1-OH), 6.52 (1H, d, *J* = 10.0 Hz, H-23), 5.86 (1H, dd, *J* = 14.9, 10.9 Hz, H-12), 5.49 (1H, d, *J* = 10.9 Hz, H-11), 5.33 (1H, d, *J* = 10.0 Hz, H-24), 5.28 (1H, m, H-13), 4.99 (1H, m, H-18), 4.00 (1H, sept, *J* = 6.9 Hz, H-29), 2.57 (1H, m, H-8), 2.31 (1H, m, H-7a), 2.18 (1H, m, H-14), 1.99 (1H, m, H-17a), 1.90 (1H, m, H-7b), 1.86 (1H, m, H-17b), 1.71 (1H, m H-31a), 1.65 (3H, s, H_3-21), 1.56 (3H, s, H_3-27), 1.55 (3H, s, H_3-20), 1.53 (3H, d, *J* = 10.9 Hz, H_3-10), 1.43 (3H, s, H_3-26), 1.40 (3H, s, H_3-22), 1.36 (1H, m H-31b), 1.10 (3H, d, *J* = 6.9 Hz, H_3-30), 0.94 (3H, d, *J* = 6.6 Hz, H_3-15), 0.92 (3H, d, *J* = 6.6 Hz, H_3-16), and 0.89 (3H, dd, *J* = 12.7, 6.9 Hz, H_3-32); ^{13}C NMR for minor tautomer **59b**: δ_C 208.6 (C-28), 198.2 (C-1), 180.4 (C-5), 164.9 (C-3), 139.8 (C-13), 137.0 (C-9), 132.1 (C-19), 128.9 (C-11),

123.4 (C-24), 122.6 (C-12), 122.4 (C-18), 116.3 (C-23), 109.6 (C-6), 109.3 (C-4), 79.1 (C-25), 47.0 (C-2), 42.0 (C-29), 39.9 (C-7), 38.3 (C-8), 33.1 (C-17), 31.1 (C-14), 29.2 (C-27), 28.3 (C-26), 27.3 (C-22), 25.6 (C-21), 22.4 (C-16), 22.3 (C-15), 17.8 (C-20), and 11.7 (C-32).

3.6.8. Adotogirin (60)

Pale yellow amorphous solid; $[\alpha]_D$ +7.7 (*c* 0.21, MeOH); HRESIMS *m/z* 383.2199 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_4Na$, 383.2198); ^1H and ^{13}C NMR (Table 2.4).

3.6.9. Antimicrobial assay

Test microorganisms. Seven clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains, three clinical isolates of methicillin-sensitive *S. aureus* (MSSA) strains, *S. aureus* 209P and Smith, one *Bacillus subtilis* strain, and four *Escherichia coli* strains were used in this study. All the strains of microorganisms used in this work were kindly supplied by Dr. C. Sano, the School of Medicine, Schimane University (Shimane, Japan).

Susceptibility testing. The MICs were determined by broth microdilution method in 96-well microtiter plates with cation-supplemented Mueller-Hinton broth (CAMHB; Ca^{2+}, 25 μg/mL; Mg^{2+}, 12.5 μg/mL; Becton Dickinson, Sparks, MD) according to the current guidelines of the Clinical and Laboratory Standards Institute (CLSI). All the strains of microorganisms were inoculated at a final bacterial density of about 5×10^5 CFU/ml. Then, *S. aureus* strains were incubated at 35 °C for 20 h, and *B. subtilis* and *E. coli* strains were incubated at 37 °C for 20 h before the MICs were determined. The MICs of the test compounds were reported as an MIC, MIC range, and MIC_{50}.

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