Comparative study on chemical constituents of three *Gentiana* drugs, Gentianae Scabrae Radix, Gentianae Macrophyllae Radix and Gentianae Radix, and their anti-inflammatory activity

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Contents

Abstract

The genus *Gentiana* (Gentianaceae) consists of approximately 360 species. Many plants from this genus have been widely used in traditional medicine. There are three *Gentiana* drugs recorded in the Chinese Pharmacopoeia (CP), Japanese Pharmacopoeia (JP) and Japanese standards for non-pharmacepoeial crude drugs (Non-JPS), namely Gentianae Scabrae Radix (GSR), Gentianae Macrophyllae Radix (GMR) and Gentianae Radix (GR). GSR is prescribed as the root and rhizome of *G. scabra*, *G. manshurica*, *G. triflora* and *G. rigescens* in CP mainly for the treatment of hepatitis, cholecystitis and allergic inflammations. The former three are also recorded in the JP. GMR is prescribed as the root of *G. macrophylla*, *G. straminea*, *G. crassicaulis* and *G. dahurica* in CP and Non-JPS, mainly used for treatment of various inflammatory diseases such as rheumatoid arthritis. GR is prescribed as the root and rhizome of *G. lutea* in JP and European Pharmacopoeia, mainly used for the treatment of insufficient gastric secretions, intestinal and gastric inflammation, *etc*. The different uses of each *Gentiana* drug also show up in traditional medicine for the preparation, *e.g.* GSR being included in heat-clearing prescription “Longdan-Xiegan Tang”, GMR being included in damp-clearing prescription “Duhuo-Jisheng Tang”, and GR being used in the preparation of bitter tonic. GSR sometimes used as a substitution of GR is also recorded.

Previous studies on *Gentiana* drugs from different species revealed a series of iridoid and secoiridoid glycosides, with common principles as gentiopicroside, sweroside and swertiamarin. However, the variation in their chemical compositions, especially the species-specific components of them related to therapeutic effects

remains unclear to date. Therefore it is necessary to explore deeply for the sub-major or minor constituents, which might be species-specific to be responsible for bio-activities and/or used as markers for identification. From a phytochemical viewpoint, chemical constituents with anti-inflammatory activity in *Gentiana* drugs are obscure and detailed phytochemical comparison among them is lacking. This study aims to investigate the chemical constituents of the three *Gentiana* drug respectively, and to comparatively analyze their chemical composition, as well as to evaluate anti-inflammatory activity *in vitro* for the isolated compounds.

1. Phytochemical investigation and anti-inflammatory activity of GSR, the root and rhizome of *G. scabra*

The botanical source of used GSR was identified as *G. scabra* by genetic analysis of nucleotide sequence of rDNA ITS region. Crude chloroform and methanol extracts of the identified GSR were priorly screened for their inhibitory effect against LPS-induced NO, IL-6 and TNF-α productions *in vitro*. The chloroform extract was found to possess potential anti-inflammatory activity (IC_{50} 112.80 μg/mL of NO inhibition and 176.81 μg/mL of IL-6 inhibition, respectively), which was therefore taken for further investigation. The phytochemical investigation on this bioactive extract led to isolation of 19 secoiridoid glycosides (**1**-**19**), including seven new compounds (**1**-**5**, **7**, **10**); as well as two lignans (**20**, **21**), three triterpenoids (**22**-**24**) and four compounds of other types (**25**-**28**). Among the known compounds, nine compounds (**6**, **13**, **15**, **17**, **18**, **22**-**25**) are isolated from this plant for the first time. The secoiridoids as the representative constituents were taken for assay of inhibitory effect on LPS-induced NO, IL-6 and TNF-*α* productions in RAW264 cells. 8-*epi*-kingiside derivatives **1**-**3**;

kingiside derivatives **4**-**6**; and sweroside derivative **10** showed inhibition activity against IL-6 production with IC_{50} of 51.70-63.80 μM, whereas sweroside derivatives **12** and **15**-**19** and one swertiamarin derivative **13** showed inhibition effects on both of NO and IL-6 productions with IC_{50} of 60.55-94.95 μM and 48.91-75.45 μM, respectively. All the test compounds exhibited weak inhibitory activity (IC_{50} > 100 μM) in the case of TNF-*α* bioassay.

2. Phytochemical investigation and anti-inflammatory activity of GMR, the root of *G. crassicaulis*

The botanical source of used GMR was identified as *G. crassicaulis* by genetic analysis of nucleotide sequence of rDNA ITS region. Crude chloroform and methanol extracts of the identified GMR, as well as the water-soluble fraction and 30%, 60%, 90% methanol eluate fractions obtained from the methanol extract through a macroporous resin fractionation procedure were priorly screened for their inhibitory effect against LPS-induced NO and IL-6 productions *in vitro*. Among the extracts and fractions, 30% and 60% methanol eluate fractions were found to possess the most promising anti-inflammatory activity, which were therefore taken for further investigation. The phytochemical investigation on these two bioactive fractions led to isolation of 20 secoiridoid glycosides (**7**-**9**, **29**-**45**), including five new compounds (**29**-**31**, **41**, **42**); as well as four lignans (**21** and **46**-**48**), one *C*-glucoflavonoid (**49**) and seven compounds of other types (**50**-**56**). Among the isolated compounds, gentiananosides A (**41**) and B (**42**) were concluded to be novel secoiridoid glycosides with an ether linkage between C-2' of the sugar moiety and C-3 of the aglycone. 18 known compounds (**32**, **34**, **35**, **38**-**40**, **43**-**47**, **49**-**55**) are isolated from this plant for

the first time. Compounds **29**-**33**, **36**, **39** and **40** exhibited inhibitory effects on both NO and IL-6 productions with IC_{50} of 79.88-95.02 μM and 70.62-77.42 μM, whereas **37**, **41** and **LA** exhibited inhibitory effects against only IL-6 production with IC_{50} of 75.35, 88.09 and 51.28 μM, respectively. All the test compounds exhibited weak inhibitory activity (IC_{50} > 100 μM) in the case of TNF-*α* bioassay.

3. Chemical constituents of GR, the root of *G. lutea*, and comparison of chemical composition among GSR, GMR and GR

The botanical source of used GR was morphologically identified as *G. lutea*. The chemical investigation of the methanol extract of GR led to isolation of 20 compounds, including 11 secoiridoids (**9**, **36**, **37**, **43**, **44**, **57**-**62**), six xanthones (**63**-**68**), one *C*-glucoflavonoid (**69**), one lignan (**21**) and one methyl benzoate derivative (**27**).

All the isolated compounds from the three identified *Gentiana* drugs above were used for comparative analysis to explore their potential chemical marker(s) by HPLC method. As a result, HPLC profiles of GSR, GMR and GR revealed six major common peaks which were identified as loganic acid (**LA**), gentiopicroside (**9**), 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), swertiamarin (**36**) and sweroside (**37**), respectively. The chemical composition of GR obviously differed from those of GSR and GMR in containing xanthones (**63**-**68**), which could be probably used as chemical markers. GMR containing macrophylloside D (**54**), a 2-methoxyanofinic acid derivative belonging to chromenes, clearly differed from GSR containing a group of acetylated and/or benzoylated secoiridoid glycosides (**11**-**17**). Besides, some identified common compounds from GSR and GMR also differed in their contents.

4. Conclusion

In the current study, 70 compounds, including 12 new ones, have been isolated from the identified GSR, GMR and GR. They are mainly the secoiridoids, as well as xanthones, *C*-glucoflavonoids, lignans, triterpenoids and compounds of other types. Secoiridoids as the representative constituents from GSR and GMR exhibited moderate inhibitory effects against LPS-induced NO and IL-6 productions *in vitro*, however they are attractive in their structural diversity. The HPLC profiles of the three *Gentiana* drugs showed high similarities, whereas phytochemical characterstics of each drug was observed, such as the acetylated and/or benzoylated secoiridoids in GSR, 2-methoxyanofinic acid derivatives in GMR, and xanones in GR, which could be used as candidate markers for authentication and standardization of *Gentiana* drugs. Additionally, the chemical diversity elucidated by this study suggests that these three *Gentiana* drugs might have their own pharmacological effects, which imply that the substitution among them should be paid attention.

General Introduction

The genus *Gentiana* of family Gentianaceae consists of approximately 360 species mainly distributed in Eurasia, regions from southwest of China to Himalaya, and Europe (Alps) [Ho *et al.*, 1995; The Asahi Shimbun Company, 1994]. More than 30 species have been widely used in traditional medicine. There are three *Gentiana* drugs recorded in the Chinese Pharmacopoeia (CP), Japanese Pharmacopoeia (JP) and Japanese standards for non-pharmacepoeial crude drugs (Non-JPS), namely Gentianae Scabrae Radix (GSR), Gentianae Macrophyllae Radix (GMR) and Gentianae Radix (GR). GSR is prescribed as the root and rhizome of *Gentiana scabra* Bunge, *G. manshurica* Kitagawa, *G. triflora* Pallas and *G. rigescens* Franchet in CP mainly for the treatment of hepatitis, cholecystitis and allergic inflammations [The State Pharmacopoeia Commission of P. R. China, 2011]. The former three are also recorded in the JP [The Ministry of Health, Labour and Welfare, 2011]. GMR is prescribed as the root of *G. macrophylla* Pallas, *G. straminea* Maximowicz, *G. crassicaulis* Duthie ex Burkill and *G. dahurica* Fischer in CP and Non-JPS, mainly used for treatment of various inflammatory diseases such as rheumatoid arthritis [The State Pharmacopoeia Commission of P. R. China, 2011; non-JPS 1012, 2012]. GR is prescribed as the root and rhizome of *G. lutea* in JP and European Pharmacopoeia (EP) [The Ministry of Health, Labour and Welfare, 2011; Council of Europe, 2004], mainly used for the treatment of insufficient gastric secretions, intestinal and gastric inflammation, *etc*. [Kumar *et al.*, 2005]. The different uses of each *Gentiana* drug also show up in traditional medicine prescription. For examples, GSR is included in heat-clearing prescription “Longdan Xiegan Tang” (竜胆瀉肝湯) mainly for cases with

flaming up of sthenia fire in the liver and gallbladder, manifested as headache, hypochondriac pain, congestion of the conjunctiva and deafness, *etc.* [Ou *et al.*, 1991], while GMR is included in damp-clearing prescription “Duhuo Jisheng Tang” (獨活寄生湯) mainly used for prolonged arthralgia, hypofunction of liver and kidney and insufficiency of vital energy and blood, *etc.* [Ou *et al.*, 1991]. In Japan, GR is often included in the prescription of laxative [Kuwano *et al*., 1984], and sometimes it is substituted by other *Gentiana* drugs such as GR [Grieve, 1995].

Previous studies on these *Gentiana* drugs have revealed a series of iridoids (mainly secoiridoid glycosides) with representative of gentiopicroside, sweroside and swertiamarin [Cao *et al.*, 2010; Liu *et al.*, 2014], as well as triterpenes [Kakuda *et al.*, 2002 and 2003; Fan *et al.*, 2010], lignans [Ando *et al.*, 2007; Wang *et al.*, 2013], flavonoids [Yamada *et al.*, 2005; Khan *et al.*, 1997], xanthones [Hayashi *et al.*, 1988] and compounds of other types [Wang *et al.*, 2013; Huh *et al.*, 1998]. Some specific activities have been reported for these metabolites. For example, gentiopicroside can suppress xylene-induced mouse ear edema and albumin-induced rat paw edema [Chen *et al.*, 2008]; sweroside exhibits anti-osteoporotic effect [Sun *et al.*, 2013], and swertiamarin has anti-diabetic properties [Vaidya *et al.*, 2013]; all the three compounds have the hepatoprotective [Hase *et al.*, 1997; Jaishree *et al.*, 2010] and wound healing activities [Ozturk *et al.*, 2006]. Accordingly, the chemical constituents with promising bioactivities could justify the traditional uses of *Gentiana* drugs. On the other hand, structural diversity and distribution of secoiridoids in different *Gentiana* species and drugs exhibited interesting characteristics. For example, the genus *Gentiana* is apparently characterized by the universal occurrence of iridoids and secoiridoids with ten-carbon skeleton, whereas some derivatives, *e.g.* esterified with acetyl and/or benzoyl moieties, are unique to certain *Gentiana* species and drugs

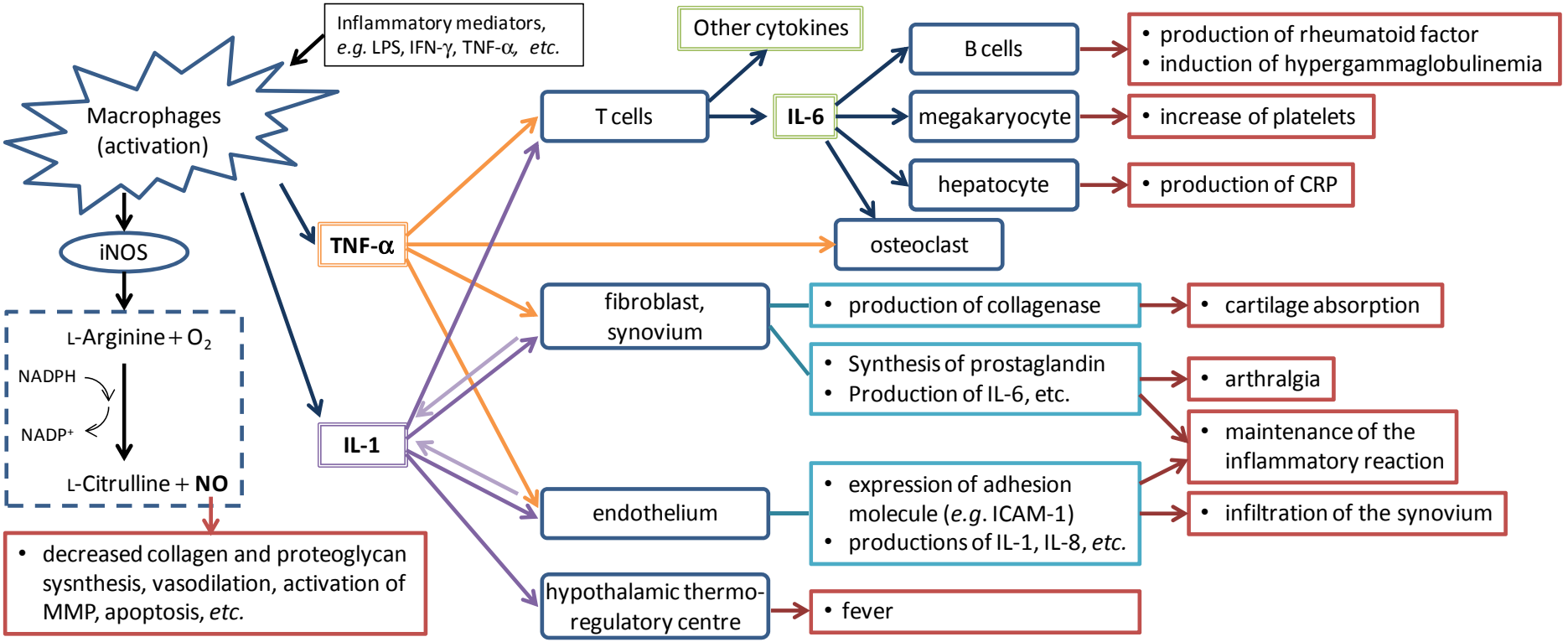
[Jensen *et al.*, 2002; Kumar *et al.*, 2005]. Other characteristically informative types of compounds such as *C*-glucoxanthone and *C*-glucoflavones also have been recorded and are likely to be served as important chemical markers for their identification [Jensen *et al.*, 2002]. All these findings implied that such species-specific chemical compositions might be responsible for the differences in the therapeutic effects and/or used as markers for identification or standardization of *Gentiana* drugs.

However, chemical characteristics of each *Gentiana* drugs that could be used for identification and discrimination remains unclear. As anti-inflammatory agent, the representative chemical constituents with anti-inflammatory activity are obscure. Furthermore, detailed phytochemical comparison among the three *Gentiana* drugs to reveal the differences in their chemical composition is lacking. This study aims to investigate the chemical constituents of each *Gentiana* drug, then to evaluate anti-inflammatory activity of the isolated compounds, and finally to comparatively analyze their chemical composition.

The first, different crude extracts and/or fractions obtained from GSR, the root and rhizome of *G. scabra*, and GMR, the root of *G. crassicaulis*, were screened for their anti-inflammatory activity priorly, then the bioactive ones were taken for further phytochemical investigation to afford compounds, followed by evaluation of anti-inflammatory activity, respectively (Sections 1 and 2). The second, phytochemical investigation on GR derived from *G. lutea* was carried out to explore its chemical composition (Section 3). The third, using the isolated compounds as references, HPLC comparison of chemical composition among GSR, GMR and GR was carried out to characterize the chemical composition of each *Gentiana* drug and to establish their differences and/or similarities (Section 3).

As for evaluation of anti-inflammatory activity, biomarker interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and nitric oxide (NO) play important roles in the pathogenesis of inflammatory disease such rheumatoid arthritis (RA) (Figure 2). Therefore, the bioassay in this study has been focused on the IL-6, TNF-α and NO inhibitions in macrophage cell experiments.

Figure 1 Three *Gentiana* drugs (a, GSR, Gentianae Scabare Radix, the root and rhizome of *G. scabra*; b, GMR, Gentianae Macrophyllae Radix, the root of *G. crassicaulis*,; c, GR, Gentianae Radix, the root and rhizome of *G. lutea*)



Abbreviations: TNF-α: Tumor Necrosis Factor; IL: Interleukin; MMP: matrix metalloproteinase; CRP: C-reactive protein; iNOS: Inducible nitric oxide synthase

Figure 2 Role of biomarkers IL-6, TNF-α and NO in the pathogenesis of inflammatory disease (rheumatoid arthritis)

1. Phytochemical investigation and anti-inflammatory activity of Gentianae Scabrae Radix: the root and rhizome of *Gentiana scabra*

1.1 Introduction

Previous phytochemical studies on Gentianae scabrae Radix (GSR) revealed a series of compounds including iridoids (mainly secoiridoid glycosides) [Jiang *et al.*, 2005], triterpenes [Kakuda, *et al.*, 2002], lignans and flavonoids [Xu, *et al.*, 2009]. The different botanical sources are found to contain the common principal constituents as gentiopicroside, sweroside and swertiamarin [Jiang *et al.*, 2005; Gao *et al.*, 2006]. Moreover, some sub-major or minor constituents were continually reported, which essentially exhibit the diversity of their chemical composition. *G. scabra* is one of the dominated species available in the crude drug markets. Therefore it is necessary to elucidate its chemical constituents more deeply. Secoiridoids as the representative constituents are chiefly evidenced to possess multiple-effect such as anti-inflammation [Park *et al.*, 2010; Saravanan *et al.*, 2014]. To date, over 20 secoiridoids have been identified in *G. scabra*, however there are still some minor secoiridoids have been detected in our preliminary research, and few reports regarding their anti-inflammatory activity. With the objective to enrich the chemical information of *G. scabra* and to explore bio-active secoiridoids, this section mainly focuses on the isolation and identification of chemical constitutions from GSR identified as *G. scabra*. Furthermore, the inhibitory effects of isolated secoiridoids against LPS-induced NO, IL-6 and TNF-α productions in RAW264 cells were evaluated because the induction of nitric oxide (NO) synthesis has been suggested as one of the major responses to inflammatory stimuli in macrophages [Nathan *et al.*, 1991; Kobayashi, 2010] and some pro-inflammatory cytokines, such as IL-6 and TNF-α, are considered as major mediators, which play an important role in various inflammatory diseases [Scheller *et al.*, 2011; Bauqh *et al.*, 2001].

1.2 Materials

Gentianae Scabrae Radix (GSR, 竜胆) (LOT no. 1201C010901, produced in Liaoning, China) was purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan) on 21 June, 2012. The botanical source of the material was identified as *G. scabra* by genetic analysis of internal transcribed spacer (ITS) sequence of nuclear ribosomal DNA (rDNA) which was completely identical to those registered in GenBank (JQ890595, AB564723, GQ864015, GQ864016). A voucher sample (TMPW No. 27499) has been deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama, Japan.

1.3 Isolation and structure determination

The $CHCl_3$ extract of identified GSR, which was found to possess potential anti-inflammatory activity (IC_{50} 112.80 μg/mL of NO inhibition and 176.81 μg/mL of IL-6 inhibition) in the preliminary screening, was subjected to a series of column chromatography (CC) over normal and reverse phase silica gel, and further purified by preparative HPLC to afford mainly secoiridoids (**1**-**19**), including seven new ones (**1**-**5**, **7**, **10**). Lignans (**20**, **21**), triterpenoids (**22**-**24**) and compounds of other types (**25**-**28**) were also isolated and identified. The isolation procedure was illustrated in Chart 1.1 and the structures of the isolated compounds were shown in Figure 1.1.

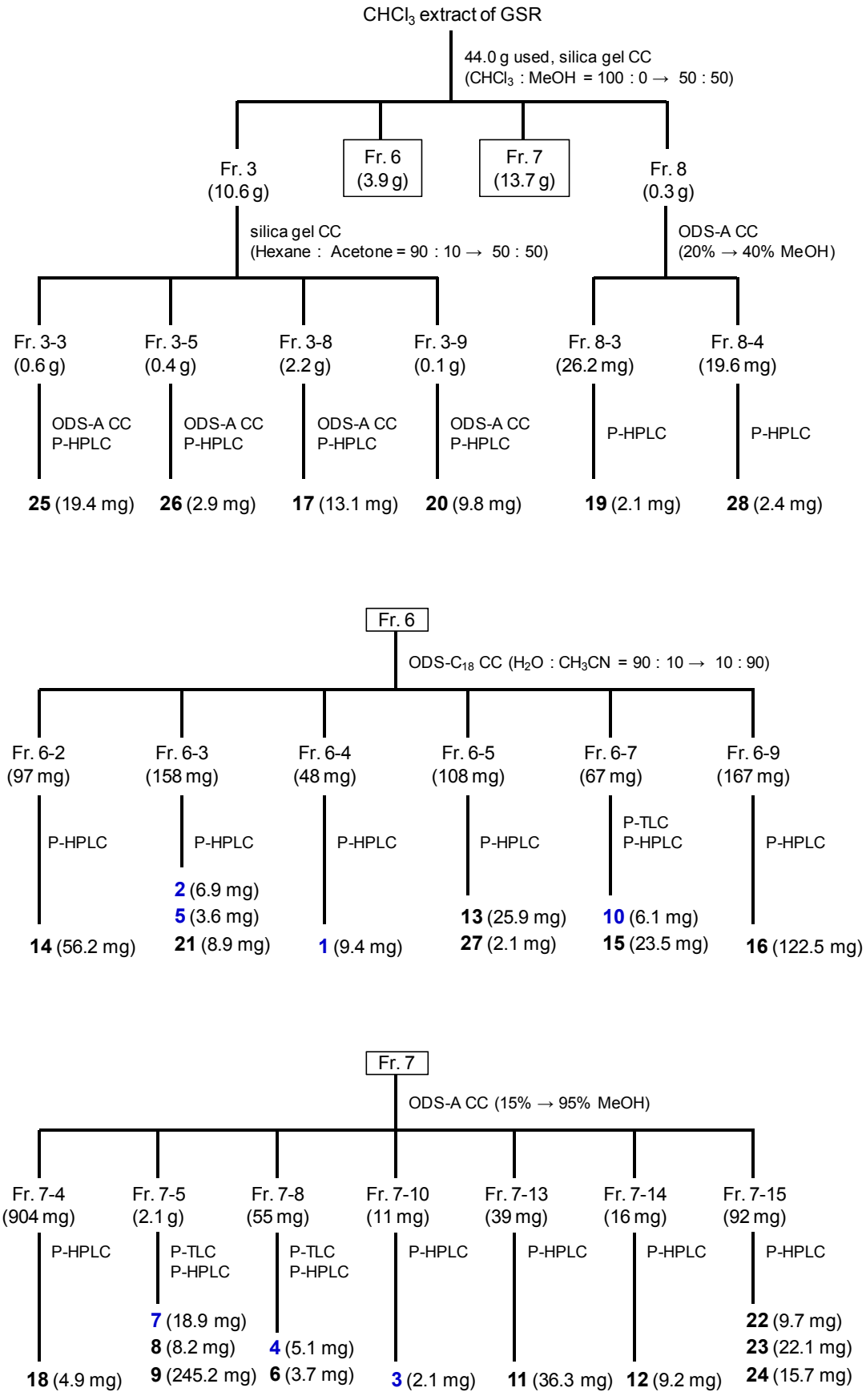


Chart 1.1 Isolation of Gentianae Scabrae Radix (竜胆) derived from *G. scabra*

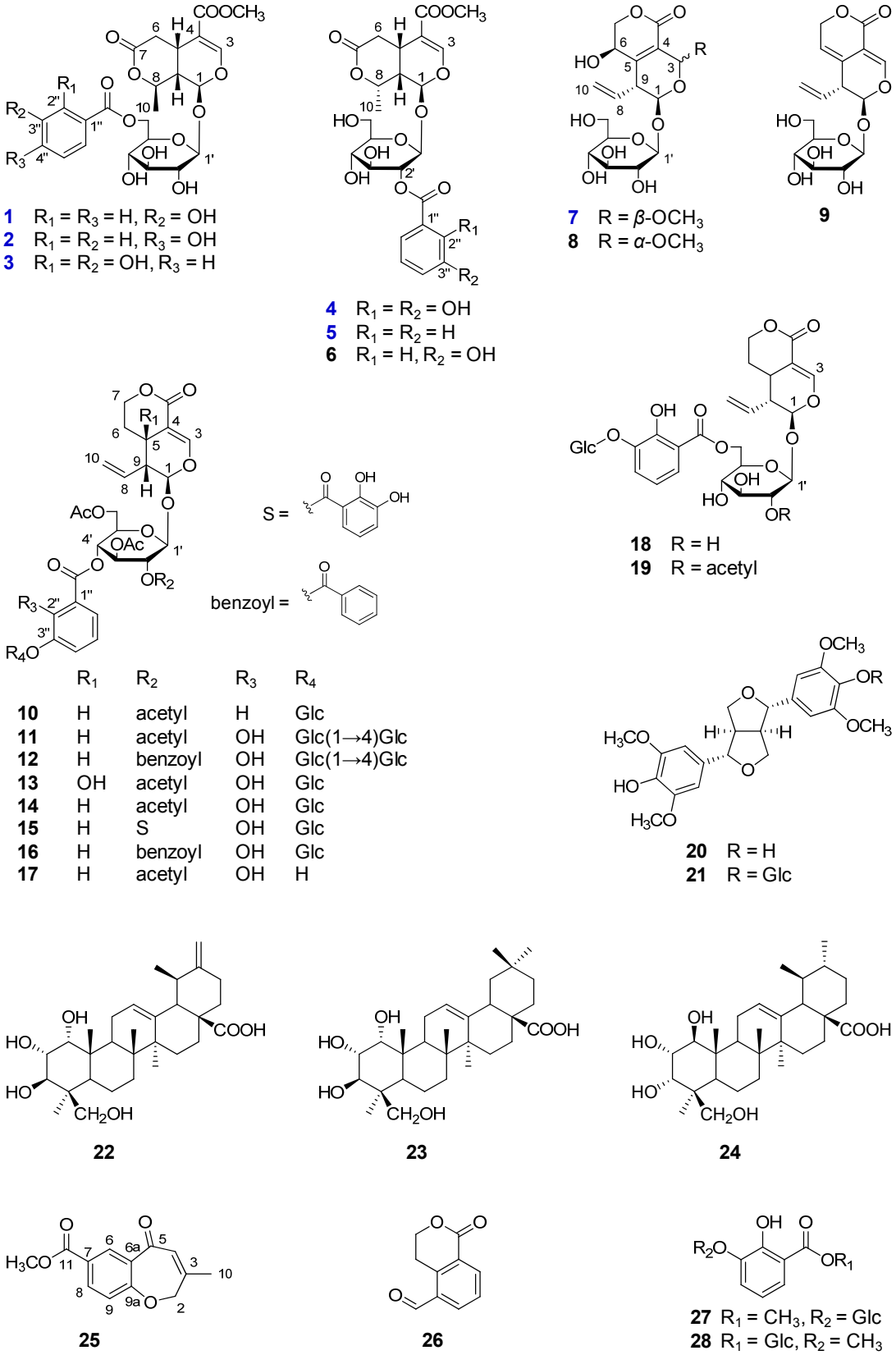


Figure 1.1 Structures of compounds isolated from Gentianae Scabrae Radix (竜胆) derived from *G. scabra*

6′-*O*-(3″-Hydroxybenzoyl)-8-*epi*-kingiside (**1**): white, amorphous solid; $[\alpha]_D^{25}$ –28.72° (*c* 0.05, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m/z* 525.1596 $[M+H]^+$, indicating the molecular formula of $C_{24}H_{28}O_{13}$. The ^{1}H-NMR spectrum (Table 1.1) showed doublets at δ_H 7.51 (d, *J* = 0.8 Hz) and 5.23 (d, *J* = 7.6 Hz) which were attributed to H-3 and H-1, being indicative of an iridoid nucleus. A set of aromatic proton signals at δ_H 7.46 (ddd, *J* = 8.0, 1.6, 1.6 Hz), 7.40 (dd, *J* = 2.8, 1.6 Hz), 7.26 (t, *J* = 8.0 Hz) and 7.01 (ddd, *J* = 8.0, 2.8, 1.6 Hz) suggested the presence of a *m*-disubstituted aromatic ring. Moreover, one methoxyl proton signal at δ_H 3.70 (3H, s), one methyl proton signal at δ_H 1.34 (3H, d, *J* = 6.4 Hz) and one anomeric proton signal at δ_H 4.71 (1H, d, *J* = 8.4 Hz) were also recognized. The coupling constant of the anomeric proton signal indicated the linkage is of *β*-configuration. The ^{13}C-NMR spectrum (Table 1.1) revealed 24 carbon signals, including signals at δ_C 154.4 (C-3) and 96.5 (C-1) assignable to the iridoid nucleus, as well as signals from three carbonyl groups (δ_C 174.8, 168.2, 167.8), one *β*-D-glucopyranosyl group (δ_C 100.9, 77.7, 75.8, 74.6, 71.6 and 64.2), one methoxyl group (δ_C 51.9) and one methyl group (δ_C 21.5). The 2D-NMR (^{1}H-^{1}H COSY, HMQC and HMBC) data, as well as the characteristic signal of methyl group in the ^{13}C-NMR spectrum clearly indicated an 8-*epi*-kingiside structure in **1**. As reported in previous literatures, chemical shifts of C-10 are indicatively different in kingiside (*ca.* δ_C 18.0) and 8-*epi*-kingiside (*ca.* δ_C 21.5) types of secoiridoids [Garcia *et al.*, 1989 and 1990]. Furthermore, NOE correlations of H-10 with H-9 and of H-8 with H-1 (Figure 1.2) revealed a *β*-orientation of methyl group (C-10) at C-8, which was in agreement with the previously described configuration of an 8-*epi*-kingiside skeleton [Xu *et al.*, 2008]. In addition to the carbon signals belonging to 8-*epi*-kingiside moiety, seven of the remaining carbon signals were assigned to *m*-hydroxybenzoyl group by analyses of the 2D-NMR data. The linkage of *m*-hydroxybenzoyl group to C-6' of the sugar unit in 8-*epi*-kingiside moiety

Table 1.1 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of compounds **1**-**5** (δ in ppm, J in Hz)

position	6′-*O*-(3″-hydroxybenzoyl)-8-*epi*-kingiside **(1)**		6′-*O*-(4″-hydroxybenzoyl)-8-*epi*-kingiside (**2**)		6′-*O*-(2″,3″-dihydroxybenzoyl)-8-*epi*-kingiside (**3**)		2′-*O*-(2″,3″-dihydroxybenzoyl) kingiside (**4**)		2′-*O*-benzoyl kingiside (**5**)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.23, d (7.6)	96.5	5.21, d (7.6)	96.5	5.27, d (7.6)	96.6	5.67, d (2.8)	94.3	5.69, d (3.2)	93.9
3	7.51, d (0.8)	154.4	7.52, d (1.2)	154.4	7.52, d (1.2)	154.3	7.09, s	153.7	7.18, s	153.8
4		109.3		109.3		109.5		111.9		111.9
5	2.97, m	28.5	2.97, m	28.7	3.02, m	28.2	3.14, m	26.6	3.13, m	26.7
6a	2.13, dd (16.4, 11.2)	34.7	2.10, dd (16.4, 12.0)	34.8	2.21, dd (16.4, 11.2)	34.7	2.72, dd (16.0, 4.8)	33.8	2.70, dd (16.0, 4.4)	33.8
6b	2.75, dd (16.4, 4.0)		2.74, dd (16.4, 3.6)		2.80 , dd (16.4, 4.0)		2.88, dd (16.0, 5.6)		2.88, dd (16.0, 6.0)	
7		174.8		174.7		174.5		174.9		174.9
8	4.23, dq (6.4, 7.2)	75.7	4.23, dq (6.4, 7.2)	75.7	4.30, dq (6.4, 7.2)	75.5	4.69, dq (7.2, 6.4)	75.6	4.70, dq (6.4, 6.0)	75.4
9	2.07, ddd (7.6, 7.6, 7.2)	41.8	2.06, ddd (8.0, 7.6, 7.2)	41.8	2.09, ddd (7.6, 7.6, 7.2)	41.9	2.55, ddd (8.8, 6.4, 6.0)	40.1	2.51, ddd (8.4, 6.4, 6.0)	40.1
10	1.34, d (6.4)	21.5	1.34, d (6.4)	21.6	1.38, d (6.4)	21.4	1.45, d (7.2)	17.2	1.45, d (6.4)	17.4
11		168.2		168.2		168.2		167.2		167.2
11-O$\underline{CH_3}$	3.70, s	51.9	3.71, s	52.0	3.72, s	51.9	3.36, s	51.8	3.26, s	51.7
1-*O*-glucosyl										
1'	4.71, d (8.4)	100.9	4.70, d (8.0)	100.9	4.73, d (8.0)	101.1	4.99, d (7.6)	98.0	4.98, d (8.0)	97.9
2'	3.23, dd (9.2, 8.4)	74.6	3.22, dd (9.2, 8.0)	74.7	3.24, dd (9.2, 8.0)	74.6	4.98, dd (8.0, 7.6)	75.1	4.94, dd (8.4, 8.0)	75.3
3'	3.42, m	77.7	3.42, m	77.7	3.42, m	77.7	3.69, dd (8.0, 8.8)	75.4	3.71, (8.4, 8.8)	75.9
4'	3.44, m	71.6	3.43, m	71.7	3.44, m	71.6	3.40, dd (8.4, 8.8)	71.7	3.39, dd (9.6, 8.8)	71.8
5'	3.62, m	75.8	3.60, m	75.9	3.66, m	75.8	3.46, m	78.6	3.45, m	78.7
6'a	4.52, dd (11.2, 6.0)	64.2	4.50, dd (12.0, 6.0)	63.7	4.55, dd (11.6, 6.0)	64.7	3.72, dd (12.0, 6.0)	62.7	3.72, dd (12.0, 6.4)	62.8
6'b	4.71, dd (11.2, 2.8)		4.68, dd (12.0, 2.0)		4.77, dd (11.6, 2.4)		3.96, dd (12.0, 2.0)		3.96, dd (12.0, 2.0)	
O-benzoyl										
1"		132.5		122.0		113.9		113.6		131.3
2"	7.40, dd (2.8, 1.6)	117.1	7.86, dd (7.2, 2.0)	116.4		151.2		151.5	7.99, dd (8.0, 1.2)	129.6
3"		158.9	6.81, dd (7.2, 2.0)	132.9		147.3		147.1	7.47, t (8.0)	130.8
4"	7.01, ddd (8.0, 2.8, 1.6)	121.6		163.9	7.01, dd (8.0, 1.6)	121.9	7.03, dd (8.0, 1.6)	121.9	7.61, t (8.0)	134.3
5"	7.26, t (8.0)	130.8	6.81, dd (7.2, 2.0)	132.9	6.75, t (8.0)	120.3	6.76, t (8.0)	120.1	7.47, t (8.0)	130.8
6"	7.46, ddd (8.0, 1.6, 1.6)	121.4	7.86, dd (7.2, 2.0)	116.4	7.35, dd (8.0, 1.6)	121.2	7.26, dd (8.0, 1.6)	121.3	7.99, dd (8.0, 1.2)	129.6
7"		167.8		167.9		171.3		170.6		167.0

was deduced from the proton signals of H_a-6' (δ_H 4.52) and H_b-6' (δ_H 4.71), which were down shift by 0.90 and 0.80 ppm compared with 8-*epi*-kingiside [Garcia *et al.*, 1989] and confirmed by the observation of HMBC correlations from H-6' to C-7". The (1*S*, 5*S*, 8*R*, 9*S*) absolute configuration of **1** could be assigned on the basis of the relative configuration deduced from the typical ^{13}C-NMR chemical shifts and NOE data, as well as the specific rotation ($[\alpha]_D$ –28.72°) which was close to that of 8-*epi*-kingiside tetraacetate with unequivocally absolute structure [Inouye *et al.*, 1970 and 1974]. From the data above, the structure of **1**, including absolute stereochemistry, was established and found to be a new compound.

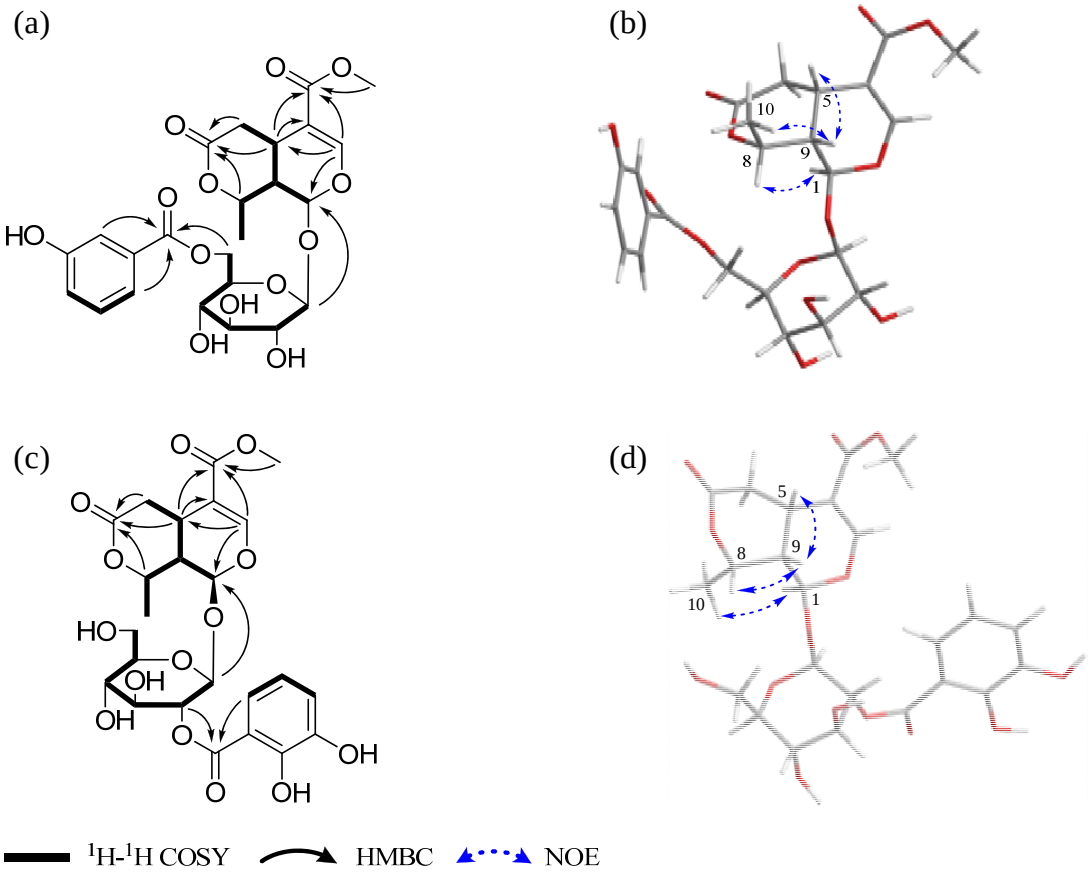


Figure 1.2 Key ^{1}H-^{1}H COSY, HMBC and NOEs s for compounds **1** (a, b) and **4** (c, d)

6′-*O*-(4″-Hydroxybenzoyl)-8-*epi*-kingiside (**2**): white, amorphous solid; $[\alpha]_D^{25}$ –28.20° (*c* 0.03, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m*/*z* 525.1603 $[M + H]^+$, indicating the molecular formula of $C_{24}H_{28}O_{13}$. The ^{1}H- and

^{13}C-NMR data (Table 1.1) revealed considerable similarity to those of **1**. However, the appearance of *p*-disubstituted aromatic signals at δ_H 6.81 (2H, dd, *J* = 7.2, 2.0 Hz, H-3″, 5″) and 7.86 (2H, dd, *J* = 7.2, 2.0 Hz, H-2″, 6″) in the ^{1}H-NMR data of **2** completely differed from a *m*-disubstituted aromatic signals detected in **1**. The ^{13}C- and 2D-NMR data also supported the above observation. The location of the *p*-hydroxybenzoyl group was determined at C-6' of the sugar unit in 8-*epi*-kingiside moiety by the proton signal values of H_a-6' and H_b-6' (δ_H 4.50 and 4.68). The HMBC correlations of H-6′ with C-7″completely supported the deduced connections. Furthermore, the specific rotation was similar to that of **1**, suggesting that **2** had the same stereochemistry as **1**. Thus, the structure of **2** was established and found to be a new compound.

6′-*O*-(2″,3″-Dihydroxybenzoyl)-8-*epi*-kingiside (**3**): white, amorphous solid; $[\alpha]_D^{25}$ −21.32° (*c* 0.05, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m*/*z* 541.1548 $[M + H]^+$, indicating the molecular formula of $C_{24}H_{28}O_{14}$. The ^{1}H- and ^{13}C-NMR data (Table 1.1) revealed considerable similarity to those of **1** and **2**. However, the appearance of *o*,*m*-trisubstituted aromatic signals at δ_H 7.01 (dd, *J* = 8.4, 1.6 Hz, H-4″), 6.75 (t, *J* = 8.0 Hz, H-5″), 7.35 (dd, *J* = 8.0, 1.6 Hz, H-6″) in the ^{1}H-NMR data completely differed from a *m*-disubstituted aromatic signals detected in **1** and *p*-disubstituted aromatic signals in **2**. The ^{13}C- and 2D-NMR data also supported the above observation. The location of the *o*,*m*-dihydroxybenzoyl group were determined at C-6' of the sugar unit in 8-*epi*-kingiside moiety by the proton signal values of H_a-6' and H_b-6' (δ_H 4.55 and 4.77). The HMBC correlations of H-6′ with C-7″ completely supported the deduced connections. Furthermore, the specific rotation was close to that of **1** and **2**, suggesting that **3** had the same stereochemistry as **1** and **2**. Thus, the structure of **3** was established and found to be a new compound.

2′-*O*-(2″,3″-Dihydroxybenzoyl) kingiside (**4**): white, amorphous solid; $[\alpha]_D^{25}$ −87.76° (*c* 0.05, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m*/*z* 541.1549 $[M + H]^+$, indicating the molecular formula of $C_{24}H_{28}O_{14}$. The ^{1}H-, ^{13}C- and 2D-NMR data (Table 1.1) revealed a common kingiside moiety. The carbon signals of C-10 at δ_C 17.2, similar to that of kingiside (δ_C 18.4) [Garcia *et al.*, 1990], confirmed the presence of this moiety. The NOE correlation between H-10 and H-1 was also indicative of *α*-orientation of methyl group (C-10) at C-8 in the kingiside moiety (Figure 1.2) [Xu *et al.*, 2008]. Moreover, proton signals at δ_H 7.03 (1H, dd, *J* = 8.0, 1.6 Hz), 6.76 (1H, t, *J* = 8.0 Hz) and 7.26 (1H, dd, *J* = 8.0, 1.6 Hz) indicated the presence of *o*,*m*-dihydroxybenzoyl group. These findings were supported by the corresponding carbon signals and the 2D-NMR data. Compared with **1**, the proton signal of H-2' was obviously deshielded from δ_H 3.23 to 4.98, as well as the upfield shift of H-6', suggesting that the *o*,*m*-dihydroxybenzoyl moiety was located at C-2' of sugar unit of the kingiside moiety. It was further confirmed by the HMBC correlations of H-2′ with C-7″. The absolute configuration was deduced to be (1*S*, 5*S*, 8*S*, 9*S*) based on the above mentioned relative configurations and the specific rotation which was close to that of kingiside tetraacetate with unequivocally absolute structure [Inouye *et al.*, 1970 and 1974]. Thus, the structure of **4** was established and found to be a new compound.

2′-*O*-Benzoyl kingiside (**5**): white, amorphous solid; $[\alpha]_D^{25}$ −88.27° (*c* 0.03, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m*/*z* 509.1650 $[M + H]^+$, indicating the molecular formula of $C_{24}H_{28}O_{12}$. Interpretation of the ^{1}H-, ^{13}C- and 2D-NMR data (Table 1.1) revealed structural similarity to those of **4**. However, the differences observed in their ^{1}H-NMR data, that is the appearance of proton signals of benzoyl group at δ_H 7.99 (2H, dd, *J* = 8.0, 1.2 Hz), 7.47 (2H, t, *J* = 8.0 Hz) and 7.61

(1H, t, *J* = 8.0 Hz), differed from the *o*,*m*-dihydroxybenzoyl signals detected in **4**, suggesting the presence of a benzoyl group in **5**. It was supported by the corresponding carbon signals and the 2D-NMR data. The HMBC correlations of H-2′ with C-7″ indicated the connection of the benzoyl group at C-2' of sugar unit of the kingiside moiety. The absolute configuration was deduced to be the same as **4** on basis of its relative configuration and specific rotation close to that of **4** and kingiside tetraacetate with unequivocally absolute structure [Inouye *et al.*, 1970 and 1974]. Accordingly, the structure of **5** was established and found to be a new compound.

2′-*O*-(3″-Hydroxybenzoyl)-kingiside (**6**): white, amorphous solid with the molecular formula of $C_{24}H_{28}O_{13}$ (524 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.45 (1H, dd, *J* = 8.0, 2.8 Hz, H-6''), 7.38 (1H, d, *J* = 2.8 Hz, H-2''), 7.27 (1H, t, *J* = 8.0 Hz, H-5''), 7.19 (1H, s, H-3), 7.01 (1H, dd, *J* = 8.0, 2.8 Hz, H-4''), 5.68 (1H, d, *J* = 3.2 Hz, H-1), 4.93 (1H, d, *J* = 8.8 Hz, H-1'), 4.69 (1H, m, H-8), 3.95 (1H, dd, *J* = 10.4, 2.0 Hz, H_b-6'), 3.71 (1H, dd, *J* = 10.4, 4.8 Hz, H_a-6'), 3.14 (1H, m, H-5), 2.88 (1H, dd, *J* = 15.6, 5.2 Hz, H_b-6), 2.71 (1H, dd, *J* = 15.6, 4.8 Hz, H_a-6), 2.51 (1H, m, H-9), 1.45 (1H, d, *J* = 6.8 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 174.9 (C-7), 167.3 (C-11), 166.9 (C-7''), 158.7 (C-3''), 153.8 (C-3), 132.4 (C-1''), 130.5 (C-5''), 121.9 (C-6''), 117.4 (C-2''), 111.9 (C-4), 98.0 (C-1'), 93.9 (C-1), 78.7 (C-5'), 75.9 (C-8), 75.3 (C-3'), 75.2 (C-2'), 71.8 (C-4'), 62.8 (C-6'), 51.7 (OCH_3), 40.1 (C-9), 33.8 (C-6), 26.7 (C-5), 17.3 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Xu *et al.*, 2008]. It is the first isolation of 2′-*O*-(3″-hydroxybenzoyl)-kingiside from this plant so far known.

6*β*-Hydroxy-3-*epi*-swertiajaposide A (**7**): white, amorphous solid; $[\alpha]_D^{25}$ −79.34° (*c* 0.20, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m/z*

405.1396 $[M+H]^+$ corresponding to the molecular formula of $C_{17}H_{24}O_{11}$. Analyses of the ^1H-, ^{13}C- and 2D-NMR data (Table 1.2) revealed that the planar structure of **7** was identical to 6*β*-hydroxyswertiajaposide A (**8**) [Kikuchi *et al.*, 2005]. However, the differences in the proton signals of H-3 (**7**: δ_H 5.23, d, J = 2.4 Hz, *vs.* **8**: δ_H 5.54, d, J = 1.2 Hz), H-6 (**7**: δ_H 4.45, ddd, J = 9.2, 5.6, 2.4 Hz, *vs.* **8**: δ_H 4.15, ddd, J = 2.4, 1.6, 1.2 Hz), H_a-7 (**7**: δ_H 4.13, dd, J = 10.4, 9.2 Hz, *vs.* **8**: δ_H 4.41, dd, J = 10.8, 2.4 Hz) and H_b-7 (**7**: δ_H 4.39, dd, J = 10.4, 5.6 Hz, *vs.* **8**: δ_H 4.43, dd, J = 10.8, 1.6 Hz), as well as the carbon signals of C-4 (**7**: δ_C 121.8, *vs.* **8**: δ_C 125.3), C-5 (**7**: δ_C 156.2, *vs.* **8**: δ_C 152.9), C-7 (**7**: δ_C 70.8, *vs.* **8**: δ_C 73.4) and C-9 (**7**: δ_C 43.8, *vs.* **8**: δ_C 46.8) indicated that **7** was a diastereomer of **8**. The correlation of H-1 with H-3, H-1 with H-8 and H-8 with H-6 (Figure 1.3) observed in the NOE experiments indicated that H-1, H-3 and H-8 were all cofacial (*α*-orientation), thus allowed to establish both of the hydroxyl group at C-6 and the methoxyl group at C-3 to be *β*-configuration in **7**. Conversely, the NOESY spectrum of **8** showed cross-peaks of H-1 with the methoxyl group at C-3 and H-8 with both H-1 and H-6, indicating the hydroxyl group at C-6 to be *β*-configuration and the methoxyl group at C-3 to be *α*-configuration in **8**, which is in agreement with the previously report [Kikuchi *et al.*, 2005]. Thus, the structure of **7** was established and found to be a new compound.

Notably, **7** was once reported as “qinjiaoside A” [Liu *et al.*, 1994], with the methoxyl group at C-3 was incorrectly determined as *β*-configuration by the evidence of predicting that the splitting of H-3 was caused by a “W or M” coupling with H-1(*α*-orientation). In the present research, the ^1H-^1H COSY spectrum of **7** showed significant cross-peak between H-3 and H-6 (Figure. 1.3), rather than between H-3 and H-1. This fact indicated that the splitting of H-3 was caused by a homoallylic coupling with H-6. The homoallylic coupling occurs over five bonds (^5J) in allylic

systems and is generally observed when both C-H σ bonds are parallel aligned with π-orbital of an intervening double bond. It was supported by an inspection of a Dreiding model of **7** (Figure 1.3), in which both of H-3 and H-6 are axially positioned with the intervening double bond of C-4 and C-5. Therefore, it is misleading to determine the configuration of C-3 relying on the coupling constant of H-3 and configuration of H-1. The relative configuration of **7** was finally determined by the NOE analysis in the present study. Accordingly, the "qinjiaoside A" was suggested to be 6*β*-hydroxyswertiajaposide A (**8**) but not the structure reported by comparing their NMR data comprehensively.

Table 1.2 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of 6*β*-hydroxy-3-*epi*-swertiajaposide A (**7**) (*δ* in ppm, *J* in Hz)

position	δ_H	δ_C
1	5.47, s	96.5
3	5.23, d (2.4)	94.4
4		121.8
5		156.2
6	4.45, ddd (9.2, 5.6, 2.4)	61.9
7a	4.13, dd (10.4, 9.2)	70.8
7b	4.39, dd (10.4, 5.6)	
8	5.72, ddd (16.8, 10.4, 8.4)	134.1
9	3.54, d (8.4)	43.8
10a	5.28, dd (10.4, 0.8)	120.3
10b	5.29, dd (16.8, 0.8)	
11		163.2
1-*O*-glucosyl		
1'	4.72, d (7.6)	99.4
2'	3.22, dd (9.2, 7.6)	74.9
3'	3.42, t (9.2)	78.1
4'	3.28, t, (9.2)	71.4
5'	3.32, m	78.5
6'a	3.67, dd (12.0, 6.0)	62.6
6'b	3.89, dd (12.0, 2.4)	
3-OCH_3	3.58, s	58.2

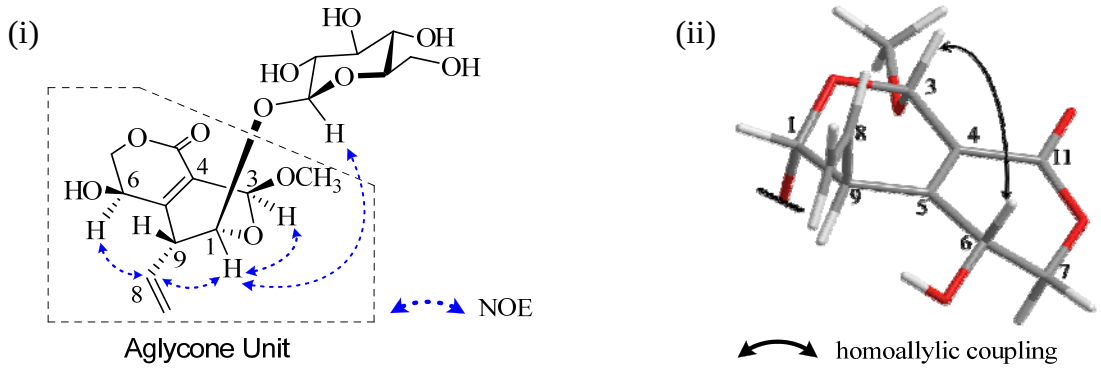


Figure 1.3 Key NOEs for 6*β*-hydroxy-3-*epi*-swertiajaposide A (**7**) (i) and the homoallylic coupling between H-3 and H-6 based on the Dreiding model of aglycone (ii)

6*β*-Hydroxyswertiajaposide A (**8**): white, amorphous solid with the molecular formula of $C_{17}H_{24}O_{11}$ (404 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 5.78 (1H, ddd, *J* = 16.8, 10.8, 8.4 Hz, H-8), 5.54 (1H, d, *J* = 1.2 Hz, H-3), 5.43 (1H, d, *J* = 4.4 Hz, H-1), 5.35 (1H, dd, *J* = 10.8, 1.6 Hz, H_b-10), 5.34 (1H, dd, *J* = 16.8, 1.6 Hz, H_a-10), 4.69 (1H, d, *J* = 7.6 Hz, H-1'), 4.43 (1H, dd, *J* = 10.8, 1.6 Hz, H_b-7), 4.41 (1H, dd, *J* = 10.8, 2.4 Hz, H_a-7), 4.15 (1H, ddd, *J* = 2.4, 1.6, 1.2 Hz, H-6), 3.88 (1H, dd, *J* = 12.0, 1.6 Hz, H_b-6'), 3.66 (1H, dd, *J* = 12.0, 5.6 Hz, H_a-6'), 3.51 (3H, s, OCH_3), 3.34 (1H, t, *J* = 9.2 Hz, H-3'), 3.31 (1H, m, H-5'), 3.28 (1H, m, H-4'), 3.25 (1H, dd, *J* = 8.4, 4.4 Hz, H-9), 3.18 (1H, dd, *J* = 9.2, 7.6 Hz, H-2'); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 163.2 (C-11), 152.9 (C-5), 134.5 (C-8), 125.3 (C-4), 121.6 (C-10), 99.2 (C-1'), 95.5 (C-3), 95.3 (C-1), 78.4 (C-5'), 77.9 (C-3'), 74.7 (C-2'), 73.4 (C-7), 71.6 (C-4'), 62.7 (C-6'), 61.8 (C-6), 56.6 (OCH_3), 46.8 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikuchi *et al.*, 2005].

Gentiopicroside (**9**): white, amorphous solid with the molecular formula of $C_{16}H_{20}O_9$ (356 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.45 (1H, d, *J* = 1.2 Hz, H-3), 5.76 (1H, ddd, *J* = 16.8, 10.0, 6.8 Hz, H-8), 5.66 (1H, d, *J* = 2.8 Hz, H-1), 5.61 (1H, m, H-6), 5.24 (1H, ddd, *J* = 16.8, 1.6, 1.6 Hz, H_b-10), 5.20 (1H, ddd, *J* = 10.1, 1.6, 1.6 Hz,

H_a-10), 5.05 (1H, m, H_b-7), 5.01 (1H, m, H_a-7), 4.65 (1H, d, *J* = 7.6 Hz, H-1'), 3.89 (1H, dd, *J* = 12.0, 1.6 Hz, H_b-6'), 3.65 (1H, dd, *J* = 12.0, 6.0 Hz, H_a-6'), 3.35 (1H, t, *J* = 8.8 Hz, H-3'), 3.32 (1H, m, H-5'), 3.30 (1H, ddd, *J* = 6.8, 2.8, 1.6 Hz, H-9), 3.25 (1H, dd, *J* = 9.6, 8.8 Hz, H-4'), 3.15 (1H, dd, *J* = 8.8, 7.6 Hz, H-2'); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 166.3 (C-11), 150.6 (C-3), 135.1 (C-8), 127.1 (C-5), 118.5 (C-10), 117.2 (C-6), 105.0 (C-4), 100.2 (C-1'), 98.5 (C-1), 78.5 (C-5'), 78.0 (C-3'), 74.6 (C-2'), 71.6 (C-4'), 70.9 (C-7), 62.8 (C-6'), 46.7 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Mpondo *et al.*, 1988].

2″-Dehydroxytrifloroside (**10**): white, amorphous solid; $[\alpha]_D^{25}$ −160.45° (*c* 0.02, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at *m*/*z* 767.2378 $[M + H]^+$ corresponding to the molecular formula of $C_{35}H_{42}O_{19}$. The ^{1}H-NMR spectrum (Table 1.3) showed a set of characteristic signals at δ_H 5.31 (d, *J* = 2.0 Hz, H-1), 7.51 (d, *J* = 2.8 Hz, H-3), 2.82 (m, H-5), 5.45 (ddd, *J* = 17.2, 10.0, 8.0 Hz, H-8), 5.21 (dd, *J* = 10.0, 2.0 Hz, H_a-10) and 5.27 (dd, *J* = 17.2, 2.0 Hz, H_b-10), assignable to a secoiridoid moiety. Two anomeric proton signals at δ_H 5.11 (d, *J* = 8.4 Hz, H-1′) and 4.87 (d, *J* = 7.6 Hz, H-1‴), as well as signals of three acetyl methyl groups at δ_H 1.79, 1.88 and 1.93 (each 3H and s) were also recognized. The above proton signal pattern was quite similar to that of trifloroside (**14**) [Ikeshiro *et al.*, 1983]. However, three aromatic protons (δ_H 7.47, 7.45 and 6.87) of the benzoyl group in **14** were replaced by a set of signals at δ_H 7.25 (ddd, *J* = 8.0, 2.4, 1.6 Hz), 7.32 (t, *J* = 8.0 Hz), 7.55 (ddd, *J* = 8.0, 2.4, 1.6 Hz) and 7.62 (t, *J* = 1.6 Hz) in **10**, which indicated the presence of a *m*-hydroxybenzoyl group. The ^{13}C-NMR data (Table 1.3) were in good agreement with those of **14**, except for the aromatic carbon signals from the *m*-hydroxybenzoyl group at δ_C 131.5 (C-1″), 118.4 (C-2″), 159.2 (C-3″), 123.5 (C-4″), 130.8 (C-5″), 124.7 (C-6″) and 166.3 (C-7″) in **10**. The HMBC correlations of H-1′ with C-1, H-2′ (δ_H 4.90)

Table 1.3 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopic data of 2″-dehydroxy-trifloroside (**10**) (δ in ppm, J in Hz)

position	δ_H	δ_C
1	5.31, d (2.0)	98.6
3	7.51, d (2.8)	153.4
4		106.7
5	2.82, m	28.7
6a	1.61, m	25.8
6b	1.72, m	
7a	4.26, td (11.6, 2.4)	70.0
7b	4.37, dq (11.6, 2.0)	
8	5.45, ddd (17.2, 10.0, 8.0)	132.8
9	2.65, ddd (8.0, 6.0, 2.0)	43.4
10a	5.21, dd (10.0, 2.0)	121.3
10b	5.27, dd (17.2, 2.0)	
11		168.0
1-*O*-glucosyl		
1'	5.11, d (8.4)	97.6
2'	4.90, dd (8.4, 10.0)	72.2
3'	5.45, dd (10.0, 8.0)	73.2
4'	5.20, m	70.6
5'	4.00, m	73.4
6'a	4.11, dd (12.4, 2.8)	63.2
6'b	4.18, dd (12.4, 3.2)	
O-benzoyl		
1"		131.5
2"	7.62, t (1.6)	118.4
3"		159.2
4"	7.25, ddd (8.0, 2.4, 1.6)	123.5
5"	7.32, t (8.0)	130.8
6"	7.55, ddd (8.0, 2.4, 1.6)	124.7
7"		166.3
3"-*O*-glucosyl		
1"'	4.87, d (7.6)	102.2
2"'	3.38, m	74.8
3"'	3.40, m	78.3
4"'	3.28, m	71.5
5"'	3.51, m	78.0
6"'a	3.61, dd (12.0, 6.0)	62.7
6"'b	3.86, dd (12.0, 2.4)	
O<u>C</u>OCH$_3$		171.0
		171.4
		172.3
OCO<u>CH$_3$</u>	1.79, s	20.5
	1.88, s	20.6
	1.93, s	20.7

with OCO-2′ (ester carbonyl carbon, δ_C 171.0), H-3′ (δ_H 5.45) with OCO-3′ (δ_C 171.4), H-6′ (δ_H 4.11, 4.18) with OCO-6′ (δ_C 172.3), H-4′ (δ_H 5.20) with C-7″, and H-1‴ with C-3″ completely supported the deduced planar structure. The absolute configuration was deduced to be (1*S*, 5*S*, 9*R*) from the same chiral centres as sweroside with unequivocally absolute structure [Inouye *et al.*, 1970]. From the data above, the structure of **10** was established and found to be a new compound.

4‴-*O*-*β*-D-Glucopyranosyltrifloroside (**11**): white, amorphous solid with the molecular formula of $C_{41}H_{52}O_{25}$ (944 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.59 (1H, d, *J* = 2.0 Hz, H-3), 7.49 (1H, dd, *J* = 8.4, 2.0 Hz, H-4"), 7.43 (1H, dd, *J* = 8.4, 2.0 Hz, H-6"), 6.87 (1H, t, *J* = 8.4 Hz, H-5"), 5.53 (1H, ddd, *J* = 16.8, 9.2, 7.2 Hz, H-8), 5.52 (1H, m, H-3'), 5.40 (1H, d, *J* = 1.6 Hz, H-1), 5.34 (1H, dd, *J* = 16.8, 2.4 Hz, H_b-10), 5.31 (1H, m, H-4'), 5.28 (1H, dd, *J* = 10.6, 2.4 Hz, H_a-10), 5.19 (1H, d, *J* = 8.4 Hz, H-1'), 5.00 (1H, dd, *J* = 8.4, 8.0 Hz, H-2'), 4.94 (1H, d, *J* = 7.2 Hz, H-1'"), 4.46 (1H, m, H_b-7), 4.44 (1H, d, *J* = 8.0 Hz, H-1""), 4.33 (1H, m, H_a-7), 2.89 (1H, m, H-5), 2.75 (1H, m, H-9), 2.00 (3H, s, CH_3), 1.96 (3H, s, CH_3), 1.90 (3H, s, CH_3), 1.79 (1H, m, H_b-6), 1.71 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 169.4 (C-7"), 167.9 (C-11), 153.3 (C-3), 152.7 (C-2"), 147.2 (C-3"), 132.7 (C-8), 125.0 (C-4"), 124.3 (C-6"), 121.4 (C-10), 120.2 (C-5"), 114.6 (C-1"), 106.7 (C-4), 104.6 (C-1""), 103.0 (C-1'"), 98.6 (C-1), 97.6 (C-1'), 80.1 (C-4'"), 78.1 (C-5""), 77.9 (C-3""), 76.8 (C-5'"), 76.1 (C-3'"), 74.9 (C-2'"), 74.6 (C-2""), 73.3 (C-5'), 72.9 (H-3'), 72.2 (C-2'), 70.9 (C-4', C-4""), 70.0 (C-7), 63.3 (C-6'), 62.4 (C-6'"), 61.64 (C-6""), 43.4 (C-9), 28.7 (C-5), 25.8 (C-6), 172.2, 171.4, 171.0, 20.7, 20.5, 20.5 (3 × $OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kim *et al.*, 2009]

4‴-*O*-*β*-D-Glucopyranosylscabraside (**12**): white, amorphous solid with the

molecular formula of $C_{45}H_{52}O_{26}$ (1008 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.93 (2H, d, J = 6.8 Hz, H-2''', 6'''), 7.61 (1H, t, J = 6.8 Hz, H-4'''), 7.51 (1H, dd, J = 8.0, 1.6 Hz, H-6''), 7.46 (2H, t, J = 6.8 Hz, H-3''', 5'''), 7.43 (1H, dd, J = 8.0, 1.6 Hz, H-4''), 7.28 (1H, d, J = 1.6 Hz, H-3), 6.88 (1H, t, J = 8.0 Hz, H-5''), 5.76 (1H, dd, J = 9.2, 8.0 Hz, H-3'), 5.41 (1H, s, H-1), 5.34 (1H, d, J = 8.0 Hz, H-1'), 5.32 (1H, m, H-8), 5.27 (1H, dd, J = 15.2, 1.6 Hz, H_b-10), 5.24 (1H, dd, J = 9.6, 1.6 Hz, H_a-10), 4.94 (1H, d, J = 8.4 Hz, H-1''''), 4.44 (1H, d, J = 8.0 Hz, H-1''''), 4.26 (1H, m, H_b-7), 3.87 (1H, m, H_a-7), 2.70 (1H, m, H-5), 2.68 (1H, m, H-9), 2.02 (3H, s, CH_3), 1.85 (3H, s, CH_3), 1.67 (1H, m, H_b-6), 1.53 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) δ 169.4 (C-7''), 167.1 (C-11), 166.7 (C-7'''), 153.2 (C-3), 152.8 (C-2''), 147.3 (C-3''), 135.1 (C-4'''), 132.6 (C-8), 130.6 (C-2''', C-6'''), 130.2 (C-1'''), 130.0 (C-3''', 5'''), 125.1 (C-4''), 124.4 (C-6''), 121.4 (C-10), 120.3 (C-5''), 114.7 (C-1''), 106.2 (C-4), 104.6 (C-1''''), 103.1 (C-1''''), 97.8 (C-1), 96.9 (C-1'), 80.1 (C-4''''), 78.2 (C-5''''), 77.9 (C-3''''), 76.8 (C-5''''), 76.1 (C-3''''), 74.9 (C-2''''), 74.6 (C-2''''), 73.2 (C-5'), 73.1 (H-3'), 72.8 (C-2'), 71.4 (C-4''''), 71.0 (C-4'), 69.6 (C-7), 63.3 (C-6'), 62.5 (C-6''''), 61.6 (C-6''''), 43.9 (C-9), 28.8 (C-5), 25.6 (C-6), 172.3, 171.4, 20.6, 20.5 (2 $\times$ $OCOCH_3$).The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kim *et al.*, 2009].

Gelidoside (**13**): white, amorphous solid with the molecular formula of $C_{35}H_{42}O_{21}$ (798 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.56 (1H, s, H-3), 7.47 (1H, dd, J = 8.0, 1.6 Hz, H-6''), 7.43 (1H, dd, J = 8.0, 1.6 Hz, H-4''), 6.87 (1H, t, J = 8.0 Hz, H-5''), 5.57 (1H, s, H-1), 5.41 (1H, m, H-8), 5.36 (1H, dd, J = 15.2, 2.0 Hz, H_b-10), 5.33 (1H, dd, J = 10.0, 2.0 Hz, H_a-10), 5.14 (1H, d, J = 8.4 Hz, H-1'), 5.04 (1H, dd, J = 9.6, 8.4 Hz, H-2'), 4.88 (1H, d, J = 7.6 Hz, H-1'''), 4.74 (1H, m, H_b-7), 4.33 (1H, m, H_a-7), 2.97 (1H, m, H-9), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.92 (3H, s, CH_3),1.87 (1H, m, H_b-6), 1.79 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) δ 169.3 (C-7''), 167.6 (C-11), 153.0 (C-2''),

152.6 (C-3), 147.3 (C-3"), 133.1 (C-8), 124.9 (C-4"), 124.2 (C-6"), 121.7 (C-10), 120.3 (C-5"), 114.5 (C-1"), 110.5 (C-4), 103.3 (C-1'"), 99.7 (C-1), 98.5 (C-1'), 78.3 (C-5'"), 77.7 (C-3'"), 74.8 (C-2'"), 73.0 (C-5'), 72.6 (H-3'), 72.3 (C-2'), 71.2 (C-4'"), 70.8 (C-4'), 66.2 (C-7), 64.2 (C-5), 63.1 (C-6'), 62.4 (C-6'"), 52.1 (C-9), 33.4 (C-6), 172.4, 172.2, 171.3, 20.7, 20.6, 20.5 ($3 \times OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Caliş *et al.*, 1990]. It is the first isolation of gelidoside from this plant so far known.

Trifloroside (**14**): white, amorphous solid with the molecular formula of $C_{17}H_{24}O_{11}$ (404 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.58 (1H, d, J = 2.0 Hz, H-3), 7.47 (1H, dd, J = 8.4, 1.6 Hz, H-6"), 7.45 (1H, dd, J = 8.4, 1.6 Hz, H-4"), 6.87 (1H, t, J = 8.4 Hz, H-5"), 5.53 (1H, ddd, J = 17.2, 10.0, 7.6 Hz, H-8), 5.52 (1H, m, H-3'), 5.40 (1H, d, J = 2.0 Hz, H-1), 5.35 (1H, dd, J = 17.2, 2.0 Hz, H_b-10), 5.31 (1H, m, H-4'), 5.29 (1H, dd, J = 10.2, 2.0 Hz, H_a-10), 5.19 (1H, d, J = 8.0 Hz, H-1'), 4.99 (1H, dd, J = 8.0, 10.0 Hz, H-2'), 4.88 (1H, d, J = 7.6 Hz, H-1'"), 4.47 (1H, dq, J = 12.0, 2.0 Hz, H_b-7), 4.33 (1H, dt, J = 12.0, 2.8 Hz, H_a-7), 4.31 (1H, dd, J = 12.4, 3.6 Hz, H_b-6'), 4.23 (1H, dd, J = 12.4, 3.2 Hz, H_a-6'), 4.15 (1H, m, H-5'), 3.88 (1H, dd, J = 12.4, 1.6 Hz, H_b-6'"), 3.67 (1H, dd, J = 12.4, 5.2 Hz, H_a-6'"), 2.89 (1H, m, H-5), 2.74 (1H, m, H-9), 2.00 (3H, s, CH_3), 1.95 (3H, s, CH_3), 1.90 (3H, s, CH_3), 1.79 (1H, m, H_b-6), 1.71 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) δ 169.4 (C-7"), 168.0 (C-11), 153.3 (C-3), 152.6 (C-2"), 147.3 (C-3"), 132.7 (C-8), 124.9 (C-4"), 124.2 (C-6"), 121.3 (C-10), 120.3 (C-5"), 114.5 (C-1"), 106.7 (C-4), 103.3 (C-1'"), 98.6 (C-1'), 97.6 (C-1), 78.3 (C-3'"), 77.7 (C-5'"), 74.8 (C-2'"), 73.3 (C-5'), 72.9 (C-3'), 72.2 (C-2'), 71.3 (C-4'"), 70.9 (C-4'), 70.0 (C-7), 63.2 (C-6'), 62.4 (C-6'"), 43.4 (C-9), 28.7 (C-5), 25.7 (C-6), 172.2, 171.3, 171.0, 20.7, 20.5, 20.4 ($3 \times OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ikeshiro *et al.*, 1983].

Macrophylloside A (**15**): white, amorphous solid with the molecular formula of $C_{40}H_{44}O_{22}$ (876 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.52 (1H, dd, J = 8.4, 1.6 Hz, H-6'''), 7.45 (1H, dd, J = 8.4, 1.6 Hz, H-4'''), 7.26 (1H, br s, H-3), 7.23 (1H, dd, J = 8.0, 1.2 Hz, H-6''), 7.02 (1H, dd, J = 8.0, 1.2 Hz, H-4''), 6.88 (1H, t, J = 8.4 Hz, H-5'''), 6.72 (1H, t, J = 8.0 Hz, H-5''), 5.78 (1H, m, H-3'), 5.46 (1H, m, H-8), 5.42 (1H, s, H-1), 5.35 (1H, d, J = 8.0 Hz, H-1'), 5.27 (1H, dd, J = 16.0, 1.6 Hz, H_b-10), 5.24 (1H, dd, J = 10.4, 1.6 Hz, H_a-10), 4.89 (1H, d, J = 7.6 Hz, H-1''''), 4.23 (1H, m, H_b-7),4.06 (1H, m, H_a-7), 2.70 (1H, m, H-5), 2.68 (1H, m, H-9), 2.02 (3H, s, CH_3), 1.88 (3H, s, CH_3), 1.67 (1H, m, H_b-6), 1.54 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) δ 170.5 (C-7'''), 169.4 (C-7''), 167.0 (C-11), 153.1 (C-3), 152.6 (C-2''), 151.3 (C-2'''), 147.3 (C-3''), 147.2 (C-3'''), 132.5 (C-8), 125.0 (C-4''), 124.2 (C-6''), 122.7 (C-6'''), 121.4 (C-10), 121.0 (C-4'''), 120.7 (C-5'''), 120.3 (C-5''), 114.6 (C-1''), 112.9 (C-1'''), 106.0 (C-4), 103.3 (C-1''''), 97.7 (C-1), 96.7 (C-1'), 78.3 (C-5''''), 77.7 (C-3''''), 74.8 (C-2''''), 73.2 (C-5'), 72.8 (C-2'), 72.7 (C-3'), 71.3 (C-4'), 71.0 (C-4''''), 69.4 (C-7), 63.3 (C-6'), 62.4 (C-6''''), 43.2 (C-9), 28.7 (C-5), 25.7 (C-6), 172.2, 171.3, 20.6, 20.4 ($2 \times OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Tan *et al.*, 1996]. It is the first isolation of macrophylloside A from this plant so far known.

Scabraside (**16**): white, amorphous solid with the molecular formula of $C_{40}H_{44}O_{20}$ (844 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.93 (2H, d, J = 7.2 Hz, H-2''', 6'''), 7.59 (1H, t, J = 7.2 Hz, H-4'''), 7.44 (2H, t, J = 6.8 Hz, H-3''', 5'''), 7.39 (1H, d, J = 8.0 Hz, H-6''), 7.34 (1H, d, J = 1.6 Hz, H-3), 7.25 (1H, d, J = 8.0 Hz, H-4''), 6.75 (1H, t, J = 8.0 Hz, H-5''), 5.65 (1H, dd, J = 9.6, 8.0 Hz, H-3'), 5.39 (1H, m, H-8), 5.38 (1H, s, H-1), 5.33 (1H, dd, J = 15.2, 2.0 Hz, H_b-10), 5.24 (1H, dd, J = 9.6, 1.6 Hz, H_a-10), 5.14 (1H, d, J = 7.6 Hz, H-1'), 4.85 (1H, d, J = 6.8 Hz, H-1''''), 4.21 (1H, m, H_b-7), 3.74 (1H, m, H_a-7), 2.63 (1H, m, H-5), 2.61 (1H, m, H-9), 2.01 (3H, s, CH_3), 1.85 (3H, s, CH_3), 1.54 (2H, m,

H-6); ^{13}C-NMR (CD_3OD, 100 MHz) δ 169.4 (C-7"), 167.1 (C-11), 166.7 (C-7'"), 153.1 (C-3), 152.6 (C-2"), 147.3 (C-3"), 135.0 (C-4'"), 132.6 (C-8), 130.5 (C-6'", 2'"), 130.1 (C-1'"), 129.9 (C-5'", 3'"), 124.9 (C-4"), 124.2 (C-6"), 121.3 (C-10), 120.3 (C-5"), 114.6 (C-1"), 106.2 (C-4), 103.3 (C-1""), 97.8 (C-1), 96.9 (C-1'), 78.3 (C-5""), 77.7 (C-3""), 74.8 (C-2""), 73.2 (C-5'), 73.1 (H-3'), 72.8 (C-2'), 71.3 (C-4""), 71.0 (C-4'), 69.5 (C-7), 63.3 (C-6'), 62.4 (C-6""), 43.2 (C-9), 28.7 (C-5), 25.6 (C-6), 172.2, 171.4, 20.6, 20.4 (2 $\times$ $OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kim *et al.*, 2009].

Deglucosyltrifloroside (**17**): white, amorphous solid with the molecular formula of $C_{40}H_{44}O_{20}$ (844 $[M]^+$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.59 (1H, d, J = 2.4 Hz, H-3), 7.28 (1H, d, J = 8.0 Hz, H-6"), 7.13 (1H, d, J = 8.0 Hz, H-4"), 6.81 (1H, t, J = 8.0 Hz, H-5"), 5.48 (1H, m, H-8), 5.35 (1H, dd, J = 16.8, 2.4 Hz, H_b-10), 5.33 (1H, br s, H-1), 5.32 (1H, dd, J = 10.0, 2.4 Hz, H_a-10), 5.09 (1H, d, J = 8.4 Hz, H-1'), 5.03 (1H, dd, J = 8.4, 8.0 Hz, H-2'), 4.46 (1H, m, H_b-7), 4.32 (1H, m, H_a-7), 3.95 (1H, m, H-5'), 2.87 (1H, m, H-5), 2.70 (1H, m, H-9), 2.03 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.93 (3H, s, CH_3), 1.72 (2H, m, H-6); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.8 (C-7"), 165.0 (C-11), 151.3 (C-3), 149.1 (C-2"), 145.1 (C-3"), 130.9 (C-8), 121.0 (C-4"), 120.9 (C-10), 120.4 (C-6"), 119.6 (C-5"), 111.0 (C-1"), 105.2 (C-4), 96.4 (C-1), 95.9 (C-1'), 71.8 (C-5'), 71.4 (H-3'), 70.4 (C-2'), 69.3 (C-4'), 68.1 (C-7), 62.0 (C-6'), 41.9 (C-9), 27.3 (C-5), 24.5 (C-6), 170.4, 169.6, 169.3, 20.5, 20.4, 20.3 (3 $\times$ $OCOCH_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Inouye *et al.*, 1974]. It is the first isolation of deglucosyltrifloroside from this plant so far known.

6'-*O*-(2-Hydroxyl-3-*O*-*β*-D-glucopyranosyl-benzoyl)-sweroside (**18**): white, amorphous solid with the molecular formula of $C_{29}H_{36}O_{17}$ (656 $[M]^+$). ^{1}H-NMR (DMSO

400 MHz) δ 7.48 (1H, d, J = 2.4 Hz, H-3), 7.39 (1H, d, J = 8.0 Hz, H-6''), 7.36 (1H, d, J = 8.0 Hz, H-4''), 6.84 (1H, t, J = 8.0 Hz, H-5''), 5.42 (1H, m, H-8), 5.34 (1H, br s, H-1), 5.22 (1H, dd, J = 16.8, 2.0 Hz, H_b-10), 5.12 (1H, dd, J = 10.2, 2.0 Hz, H_a-10), 5.09 (1H, d, J = 8.0 Hz, H-1'), 4.85 (1H, d, J = 6.8 Hz, H-1'''), 4.38 (1H, m, H_b-7), 4.26 (1H, m, H_a-7), 2.64 (1H, m, H-5), 2.50 (1H, m, H-9), 1.73 (1H, m, H_b-6), 1.49 (1H, m, H_a-6); ^{13}C-NMR (DMSO, 100MHz) δ 167.9 (C-7''), 164.6 (C-11), 151.4 (C-3), 150.0 (C-2''), 146.0 (C-3''), 132.2 (C-8), 122.7 (C-6''), 120.8 (C-4''), 120.3 (C-10), 118.7 (C-5''), 114.5 (C-1''), 104.8 (C-4), 101.1 (C-1'''), 98.8 (C-1'), 96.5 (C-1), 77.2 (C-5'''), 76.4 (C-3'''), 76.1 (C-5'), 73.7 (C-3'), 73.2 (C-2'''), 73.0 (C-2'), 70.0 (C-4'), 69.7 (C-4'''), 67.7 (C-7), 64.2 (C-6'), 60.7 (C-6'''), 41.6 (C-9), 26.8 (C-5), 24.2 (C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Chen *et al.*, 2009]. It is the first isolation of 6'-*O*-(2-hydroxyl-3-*O*-*β*-D-glucopyranosyl-benzoyl)-sweroside from this plant so far known.

Verbenoside B (**19**): white, amorphous solid with the molecular formula of $C_{29}H_{36}O_{17}$ (656 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.59 (1H, dd, J = 8.4, 1.6 Hz, H-6''), 7.57 (1H, d, J = 2.4 Hz, H-3), 7.42 (1H, dd, J = 8.4, 1.6 Hz, H-4''), 6.88 (1H, t, J = 8.4 Hz, H-5''), 5.45 (1H, m, H-8), 5.31 (1H, br s, H-1), 5.30 (1H, dd, J = 16.8, 2.4 Hz, H_b-10), 5.24 (1H, dd, J = 10.2, 2.4 Hz, H_a-10), 4.93 (1H, d, J = 8.0 Hz, H-1'), 4.91 (1H, d, J = 7.6 Hz, H-1'''), 4.44 (1H, m, H_b-7), 4.32 (1H, m, H_a-7), 2.88 (1H, m, H-5), 2.66 (1H, m, H-9), 2.00 (3H, s, CH_3), 1.72 (1H, m, H_b-6), 1.65 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100MHz) δ 170.9 (C-7''), 168.1 (C-11), 153.5 (C-3), 152.8 (C-2''), 147.3 (C-3''), 132.8 (C-8), 124.6 (C-6''), 123.8 (C-4''), 121.3 (C-10), 120.2 (C-5''), 114.7 (C-1''), 106.6 (C-4), 103.0 (C-1'''), 98.6 (C-1), 97.9 (C-1'), 78.3 (C-5'''), 77.7 (C-3'''), 75.5 (C-3'), 75.3 (C-5'), 74.8 (C-2'''), 74.7 (C-2'), 71.3 (C-4'), 71.3 (C-4'''), 70.0 (C-7), 65.1 (C-6'), 62.5 (C-6'''), 43.4 (C-9), 28.7 (C-5), 25.7 (C-6), 171.8, 21.0 ($OCOCH_3$).

The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Xu *et al.*, 2010]. It is the first isolation of verbenoside B from this genus so far known.

(–)-Syringaresinol (**20**): colorless needles with the molecular formula of $C_{22}H_{26}O_8$ (418 $[M]^+$); $[\alpha]_D^{25}$ –27.73° (*c* 0.03, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 6.65 (4H, s, H-2, 6, 2', 6'), 4.71 (2H, d, *J* = 4.0 Hz, H-7, 7'), 4.26 (2H, dd, *J* = 9.2, 6.8 Hz, H_b-8, 8'), 3.87 (2H, dd, *J* = 9.2, 4.0 Hz, H_a-8, 8'), 3.84 (12H, s, 4 × OCH_3), 3.14 (2H, m, H-9, 9'); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 149.3 (C-3, 3'), 149.3 (C-5, 5'), 136.2 (C-4, 4'), 133.1 (C-1, 1'), 104.5 (C-2, 2'), 104.5 (C-6, 6'), 87.6 (C-7, 7'), 72.8 (C-8, 8'), 56.7 (4 × OCH_3), 55.5 (C-9, 9'). The above spectroscopic data were the same with those of the reported values [Abe *et al.*, 1988].

(–)-Syringaresinol-4'-*O*-*β*-D-glucopyranoside (**21**): white, amorphous solid with the molecular formula of $C_{28}H_{36}O_{13}$ (580 $[M]^+$); $[\alpha]_D^{25}$ –27.81° (*c* 0.08, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 6.71 (2H, s, H-2, 6), 6.64 (2H, s, H-2', 6'), 4.86 (1H, d, *J* = 7.6 Hz, H-1'''), 4.76 (1H, d, *J* = 4.0 Hz, H-7), 4.71 (1H, d, *J* = 4.0 Hz, H-7'), 4.28 (2H, m, H_b-9, 9'), 3.91 (1H, d, *J* = 3.2 Hz, H_a-9), 3.89 (1H, d, *J* = 3.2 Hz, H_a-9'), 3.85 (3H, s, 2 × OCH_3), 3.84 (6H, s, 2 × OCH_3), 3.76 (1H, dd, *J* = 8.0, 2.0 Hz, H_b-6''), 3.65 (1H, dd, *J* = 8.0, 4.8 Hz, H_a-6''), 3.45 (1H, m, H-2''), 3.40 (2H, m, H-3'', H-4''), 3.18 (1H, m, H-5''), 3.12 (2H, m, H-8, 8'); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 154.4 (C-3, 5), 149.4 (C-3', 5'), 139.5 (C-1), 136.2 (C-4'), 135.6 (C-4), 133.1 (C-1'), 105.3 (C-1''), 104.8 (C-2, 6), 104.5 (C-2', 6'), 87.6 (C-7'), 87.2 (C-7), 78.3 (C-5''), 77.8 (C-3''), 75.7 (C-2''), 72.9 (C-9, 9'), 71.3 (C-4''), 62.6 (C-6''), 57.0 (2 × OCH_3), 56.8 (2 × OCH_3), 55.7 (C-8'), 55.5 (C-8). The above spectroscopic data were the same with those of the reported values [Kobayashi *et al.*, 1985].

1*α*,2*α*,3*β*,24-Tetrahydroxyursa-12,20(30)-dien-28-oic acid (**22**): white, amorphous solid with the molecular formula of $C_{30}H_{46}O_6$ (502 $[M]^+$). ^{1}H-NMR (pyriding-D_5, 400 MHz) δ 5.58 (1H, s, H-12), 4.80 (1H, s, H_b-30), 4.75 (1H, s, H_a-30), 4.72 (1H, d, J = 2.4 Hz, H-1), 4.33 (1H, dd, J = 9.6, 2.4 Hz, H-2), 4.23 (1H, d, J = 9.6 Hz, H-3), 4.21 (1H, d, J = 10.8 Hz, H_b-24), 3.89 (1H, d, J = 10.8 Hz, H_a-24), 2.74 (1H, dd, J = 11.2 Hz, H-18), 1.69 (3H, s, H-23), 1.31 (3H, s, H-25), 1.16 (3H, s, H-26), 1.13 (3H, s, H-29), 1.09 (3H, d, J = 6.8 Hz, H-29); ^{13}C-NMR (pyriding-D_5, 100 MHz) δ 179.3 (C-28), 153.9 (C-20), 137.9 (C-13), 127.4 (C-12), 105.0 (C-30), 81.1 (C-3), 74.6 (C-1), 71.6 (C-2), 65.2 (C-24), 55.6 (C-18), 49.3 (C-5), 49.2 (C-9), 48.3 (C-17), 44.9 (C-4), 43.8 (C-10), 42.5 (C-14), 40.8 (C-8), 39.7 (C-22), 37.7 (C-19), 34.3 (C-7), 32.8 (C-21), 28.7 (C-15), 27.7 (C-11), 24.9 (C-16), 23.9 (C-23), 23.7 (C-27), 19.0 (C-6), 17.8 (C-29), 16.6 (C-26), 13.6 (C-25). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Zhang *et al.*, 1994]. It is the first isolation of 1*α*,2*α*,3*β*,24-tetrahydroxyursa-12,20(30)-dien-28-oic acid from this plant so far known.

1*α*,2*α*,3*β*,24-Tetrahydroxyolean-12-en-28-oic acid (**23**): white, amorphous solid with the molecular formula of $C_{30}H_{48}O_6$ (504 $[M]^+$). ^{1}H-NMR (pyriding-D_5, 400 MHz) δ 5.63 (1H, s, H-12), 4.72 (1H, br s, H-1), 4.33 (1H, dd, J = 8.8, 2.8 Hz, H-2), 4.23 (1H, d, J = 8.8 Hz, H-3), 4.20 (1H, d, J = 10.4 Hz, H_b-24), 3.89 (1H, d, J = 10.4 Hz, H_a-24), 3.31 (1H, dd, J = 13.6, 4.0 Hz, H-18), 2.29 (1H, m, H-9), 1.79 (2H, m, H-19), 1.66 (3H, s, H-23), 1.40 (3H, s, H-27), 1.32 (3H, s, H-25), 1.14 (3H, s, H-26), 0.98 (3H, s, H-30), 0.92 (3H, s, H-29); ^{13}C-NMR (pyriding-D_5, 100 MHz) δ 180.3 (C-28), 143.8 (C-13), 124.0 (C-12), 81.0 (C-3), 74.7 (C-1), 71.7 (C-2), 65.2 (C-24), 49.4 (C-5), 49.3 (C-9), 46.7 (C-17), 46.4 (C-19), 44.9 (C-4), 43.8 (C-10), 42.2 (C-14), 42.0 (C-18), 40.6 (C-8), 34.2 (C-21), 34.0 (C-7), 33.2 (C-22, 29), 30.9 (C-20), 28.4 (C-15), 28.0 (C-11), 26.1 (C-27), 23.9 (C-16), 23.8 (C-23, 30), 19.1 (C-6), 17.8 (C-26), 13.5 (C-25). The above

^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Zhang *et al.*, 1994]. It is the first isolation of 1*α*,2*α*,3*β*,24-tetrahydroxyolean-12-en-28-oic acid from this plant so far known.

1*β*,2*α*,3*α*,24-Tetrahydroxyurs-12-en-28-oic acid (**24**): white, amorphous solid with the molecular formula of $C_{30}H_{48}O_6$ (504 $[M]^+$). ^{1}H-NMR (pyriding-D_5, 400 MHz) *δ* 5.60 (1H, s, H-12), 4.72 (1H, d, *J* = 2.4 Hz, H-3), 4.33 (1H, dd, *J* = 9.6, 2.4 Hz, H-2), 4.25 (1H, s, H-1), 4.21 (1H, d, *J* = 11.2 Hz, H_b-24), 3.89 (1H, d, *J* = 11.2 Hz, H_a-24), 2.63 (1H, d, *J* = 10.8 Hz, H-18), 1.69 (3H, s, H-23), 1.32 (3H, s, H-25), 1.20 (3H, s, H-27), 1.16 (3H, s, H-26), 0.98 (3H, d, *J* = 6.4 Hz, H-30), 0.93 (3H, d, *J* = 5.6 Hz, H-29); ^{13}C-NMR (pyriding-D_5, 100 MHz) *δ* 180.0 (C-28), 138.3 (C-13), 127.0 (C-12), 81.1 (C-1), 74.6 (C-3), 71.6 (C-2), 65.2 (C-24), 53.5 (C-18), 49.4 (C-5), 49.2 (C-9), 48.1 (C-17), 44.9 (C-4), 43.8 (C-10), 42.5 (C-14), 40.8 (C-8), 39.4 (C-19, 20), 37.5 (C-7), 34.3 (C-22), 31.1 (C-21), 28.7 (C-15), 27.8 (C-11), 25.0 (C-16), 23.9 (C-23, 27), 21.4 (C-29), 19.0 (C-6), 17.9 (C-26), 17.5 (C-30), 13.6 (C-25). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Lahlou *et al.*, 1999]. It is the first isolation of 1*β*,2*α*,3*α*,24-tetrahydroxyurs-12-en-28-oic acid from this plant so far known.

Gentioxepine (**25**): yellowish, amorphous solid with the molecular formula of $C_{13}H_{12}O_4$ (232 $[M]^+$). ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ* 8.68 (1H, d, *J* = 2.4 Hz, H-6), 8.11 (1H, dd, *J* = 8.4, 2.4 Hz, H-8), 7.11 (1H, d, *J* = 8.4 Hz, H-9), 6.35 (1H, d, *J* = 0.8 Hz, H-4), 4.68 (1H, s, H-2), 3.91 (3H, s, OCH_3), 2.07 (1H, d, *J* = 1.2 Hz, H-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ* 187.4 (C-5), 166.0 (C-11), 162.5 (C-10a), 152.7 (C-3), 135.3 (C-8), 133.6 (C-6), 131.5 (C-4), 128.3 (C-6a), 125.7 (C-7), 121.4 (C-9), 72.9 (C-2), 52.2 (OCH_3), 22.9 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the

same with those of the reported values [Wang *et al.*, 2013]. It is the first isolation of gentioxepine from this plant so far known.

Erythrocentaurin (**26**): colorless needles with the molecular formula of $C_{10}H_8O_3$ (176 $[M]^+$). ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ* 10.22 (1H, s, H-11), 8.40 (1H, d, *J* = 8.0 Hz, H-6), 8.06 (1H, d, *J* = 8.0 Hz, H-8), 7.63 (1H, t, *J* = 8.0 Hz, H-7), 4.56 (2H, dd, *J* = 6.4, 6.0 Hz, H-4), 3.59 (2H, dd, *J* = 6.4, 6.0 Hz, H-3); ^{13}C-NMR ($CDCl_3$, 100MHz) *δ* 191.9 (C-11), 164.2 (C-1), 141.1 (C-10), 138.5 (C-6), 135.7 (C-8), 132.6 (C-5), 127.9 (C-7), 127.0 (C-9), 66.8 (C-3), 24.6 (C-4). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Chen *et al.*, 2006]. It has been reported as one of the metabolites of gentiopicroside and swertiamarin *in vivo* and *in vitro* [el-Sedawy *et al.*, 1989], and it is also found as naturally occurring compounds.

2-Hydroxy-3-*O*-*β*-D-glucosylbenzoic acid methyl ester (**27**): white, amorphous solid with the molecular formula of $C_{14}H_{18}O_9$ (330 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) *δ* 7.40 (1H, d, *J* = 8.0 Hz, H-6), 7.35 (1H, d, *J* = 8.0 Hz, H-4), 6.85 (1H, t, *J* = 8.0 Hz, H-5), 4.85 (1H, d, *J* =6.8 Hz, H-1'), 3.87 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100MHz) *δ* 168.5 (C-7), 149.7 (C-2), 145.8 (C-3), 122.7 (C-6), 120.6 (C-4), 118.6 (C-5), 114.4 (C-1), 101.0 (C-1'), 77.0 (C-5'), 76.2 (C-3'), 73.1 (C-2'), 69.5 (C-4'), 60.5 (C-6'), 52.2 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ikeshiro *et al.*, 1990]. It could be obtained through alkaline methanolysis of gelidoside (**13**) [Ikeshiro *et al.*, 1990].

2-Hydroxy-3-methoxybenzoic acid glucose ester (**28**): white, amorphous solid with the molecular formula of $C_{14}H_{18}O_9$ (330 $[M]^+$). ^{1}H-NMR (CD_3OD 400 MHz) *δ* 7.54 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 7.41 (1H, dd, *J* = 8.0, 1.2 Hz, H-4), 6.86 (1H, t, *J* = 8.0 Hz, H-5), 4.91 (1H, d, *J* =7.6 Hz, H-1'), 3.95 (3H, s, OCH_3); ^{13}C-NMR (CD_3OD, 100MHz) *δ*

171.1 (C-7), 152.8 (C-3), 147.2 (C-2), 124.5 (C-4), 123.6 (C-6), 120.1 (C-5), 114.7 (C-1), 103.0 (C-1'), 78.3 (C-5'), 77.7 (C-3'), 74.8 (C-2'), 71.3 (C-4'), 62.5 (C-6'), 53.0 (OCH_3). The above ^1H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Huh *et al.*, 1998].

1.4 Inhibitory activity against LPS-induced NO, IL-6 and TNF-α productions

All the isolated secoiridoids were priorly evaluated for the cell viability under stimulation by the CCK-8 assay. As a result, all the test compounds at a dose level up to 100.00 μM or parthenolide at a dose level up to 10.00 μM did not exhibit obvious cytotoxicity. Four doses of test compounds (12.50-100.00 μM) or parthenolide (1.25 - 10.00 μM) in the presence of LPS (0.1 μg/ml) for 24 h were taken for the assay of inhibitory effect against NO, IL-6 and TNF-*α* productions in RAW264 cells (Table 1.4).

Table 1.4 Inhibitory effect of compounds **1**-**19** on LPS-induced NO and IL-6 productions in RAW264 cells

compound	inhibition of NO	inhibition of IL-6	compound	inhibition of NO	inhibition of IL-6
	IC_{50} (μM)[a]	IC_{50} (μM)		IC_{50} (μM)	IC_{50} (μM)
1	NA[b]	51.70 ± 1.32	**13**	90.49 ± 2.99	67.91 ± 0.61
2	NA	58.11 ± 1.34	**15**	64.74 ± 6.74	48.91 ± 3.59
3	NA	57.55 ± 3.09	**16**	60.55 ± 2.09	75.45 ± 2.84
4	NA	61.10 ± 1.86	**17**	79.10 ± 4.65	62.08 ± 1.54
5	NA	58.63 ± 2.53	**18**	76.88 ± 7.21	59.07 ± 4.82
6	NA	63.80 ± 2.38	**19**	84.67 ± 5.34	66.11 ± 4.96
10	NA	58.19 ± 4.90	**7**-**9**, **11**, **14**	NA	NA
12	94.95 ± 3.04	51.36 ± 0.28	parthenolide	3.10 ± 0.14	1.93 ± 0.20

[a] The IC_{50} values were calculated using SigmaPlot (Version 11.5) with a four parameter logistic nonlinear regression model with the sample absorbencies over the log concentrations of the test samples. The results are expressed as the mean ± SD of three independent experiments. [b] NA: test compounds having IC_{50} values more than 100 μM.

From Table 1.4, compounds **1**-**6** and **10** showed inhibitory activity on IL-6 production with IC_{50} values of 51.70-63.80 μM, whereas **12**, **13**, **15**-**19** showed inhibition effects on both of NO and IL-6 productions with IC_{50} values of 60.55-94.95 μM and 48.91-75.45 μM, respectively. All the test compounds exhibited weak inhibition (IC_{50} >100 μM) in the case of TNF-*α* assay (data not listed).

1.5 Discussions

Phytochemical investigation of $CHCl_3$ extract of GSR derived from *G. scabra* led to the isolation of mostly secoiridoid glycosides, with majority of acetylated and/or benzoylated gentiopicroside and sweroside derivatives. Compounds of this type were mainly reported from the plants of GSR origins such as *G. scabra* and *G. triflora* [Kim *et al.*, 2009; Wang *et al.*, 2013], therefore it could be the typical chemical composition of GSR. Several 8-*epi*-kingiside and kingiside derivatives were also found in small amount, which were seldom reported from the plants of GSR origins, suggested that compounds of this type had a limited distribution in recorded GSR origins. These finding could be considered as important features of GSR derived from *G. scabra*.

Bioactivity assay showed gentiopicroside (**9**), the predominant component in GSR, and other secoiridoid glycosides exhibited relatively weak anti-inflammatory activity *in vitro* when compared with the positive control parthenolide. However, gentiopicroside has already been proven to be active in anti-inflammatory studies *in vivo*, and some *in vitro* metabolites of gentiopicroside such as dihydroisocoumarin exhibited promising activity [Park *et al.*, 2010], which suggested that the secoiridoid glycosides are likely precursors for anti-inflammatory and other activities [Wang *et al.*, 2014].

By comparing the IC_{50} value of test compounds, the substitution of benzoyl moiety at C-2' in **12** (*vs.* **11**) and **16** (*vs.* **14**), as well as the presence of hydroxyl group at C-5 in **13** (*vs.* **14**) enhanced the inhibitory effect against both NO and IL-6 productions, which was agreed well with previously reported results [Wang *et al.*, 2013; Lv *et al.*, 2012]. The absence of glucose unit at C-3'' in **17** (*vs.* **11** and **14)** resulted in obvious increase of both NO and IL-6 inhibitory effects, however the opposite effect on inhibitory activity against IL-6 production when the C-2' substituted with a benzoyl moiety in **12** (*vs.* **16**) was observed. Furthermore, the hydroxyl group at C-2'' in **14** (*vs.* **10**) triggered obvious activity decrease. Additionally, 8-*epi*-kingiside derivatives **1**-**3** showed more potent inhibitory activity than kingiside derivatives **4**-**6** in the assay of inhibitory effect against IL-6 production, which might be another important factor for optimum anti-inflammation through inhibition of IL-6 production.

Summary of Section I

1) The $CHCl_3$ extract of GSR identified as *G. scabra* was found to possess potential anti-inflammatory activity (IC_{50} 112.80 μg/mL of NO inhibition and 176.81 μg/mL of IL-6 inhibition, respectively) in the preliminary screening, which was therefore taken for further investigation.

2) Phytochemical investigation led to the isolation of 19 secoiridoid glycosides (**1**-**19**), including 7 new ones (**1**-**5**, **7**, **10**); as well as two lignans (**20**, **21**), three triterpenoids (**22**-**24**) and four compounds of other types (**25**-**28**). Among them, gentiopicroside and sweroside derivatives were the major components, and five kingiside and 8-*epi*-kingiside derivatives were also found in small amount. Nine known compounds (**6**, **13**, **15**, **17**, **18**, **22**-**25**) are isolated from this plant for the first time

3) In the assay of inhibitory effect of compounds **1**-**19** on LPS-induced NO, IL-6 and TNF-α productions in macrophages, 8-*epi*-kingiside derivatives **1**-**3**; kingside derivatives **4**-**6**; and sweroside derivative **10** showed inhibition activity against IL-6 production with IC_{50} of 51.70-63.80 μM, whereas sweroside derivatives **12** and **15**-**19** and one swertiamarin derivative **13** showed inhibition effects on both of NO and IL-6 productions with IC_{50} of 60.55-94.95 μM and 48.91-75.45 μM, respectively. All the test compounds exhibited weak inhibitory activity (IC_{50} >100 μM) in the case of TNF-α bioassay.

2. Phytochemical investigation and anti-inflammatory activity of Gentianae Macrophyllae Radix: the root of *Gentiana crassicaulis*

2.1 Introduction

Previous studies on the multiple sources of Gentianae Macrophyllae Radix (GMR) revealed the chemical similarities with that of Gentianae Scabrae Radix (GSR), yet the sub-major or minor constituents essentially exhibit the chemical and biological characteristics of their own. *G. crassicaulis* is one of the principal sources of GMR available in the crude drugs markets, but there are few reports regarding the chemical constituents and their anti-inflammatory activity [Lv *et al.*, 2012; Liang *et al.*, 2013]. This study aims to investigate the chemical constituents of GMR and to evaluate the *in vitro* anti-inflammatory activity of the isolated compounds.

2.2 Materials

Gentianae Macrophyllae Radix (GMR, 秦艽) (produced in Sichuan Province, China, Lot no. 034312002) was purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan) on 21 June, 2012. The botanical source of the material was identified as *G. crassicaulis* by genetic analysis of nucleotide sequence of rDNA ITS region. A voucher sample (TMPW No. 27500) has been deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama, Japan.

2.3 Isolation and structure determination

Crude chloroform and methanol extracts of the identified GMR, as well as the water-soluble, 30%, 60% and 90% aqueous methanol eluates obtained from the methanol extract through a macroporous resin fractionation procedure were priorly

screened for their inhibitory effect against LPS-induced NO and IL-6 productions in RAW264 cells. Among them, 30% and 60% aqueous methanol eluates were found to possess the most promising anti-inflammatory activity (30% methanol eluates: IC_{50} of 91.90 μg/mL for NO inhibition and 85.73 μg/mL for IL-6 inhibition; 60% methanol eluates: IC_{50} of 103.36 μg/mL for NO inhibition and 87.41 μg/mL for IL-6 inhibition). Subsequently, the two bioactive fractions were subjected to a series of chromatographic separation steps to afford 20 secoiridoid glycosides (**7**-**9**, **29**-**45**), including five new compounds (**29**-**31**, **41**, **42**); as well as four lignans (**21** and **46**-**48**), one *C*-glucoflavonoid (**49**) and seven compounds of other types (**50**-**56**). The isolation procedure was illustrated in Chart 2.1 and the structures of the isolated compounds were shown in Figure 2.1.

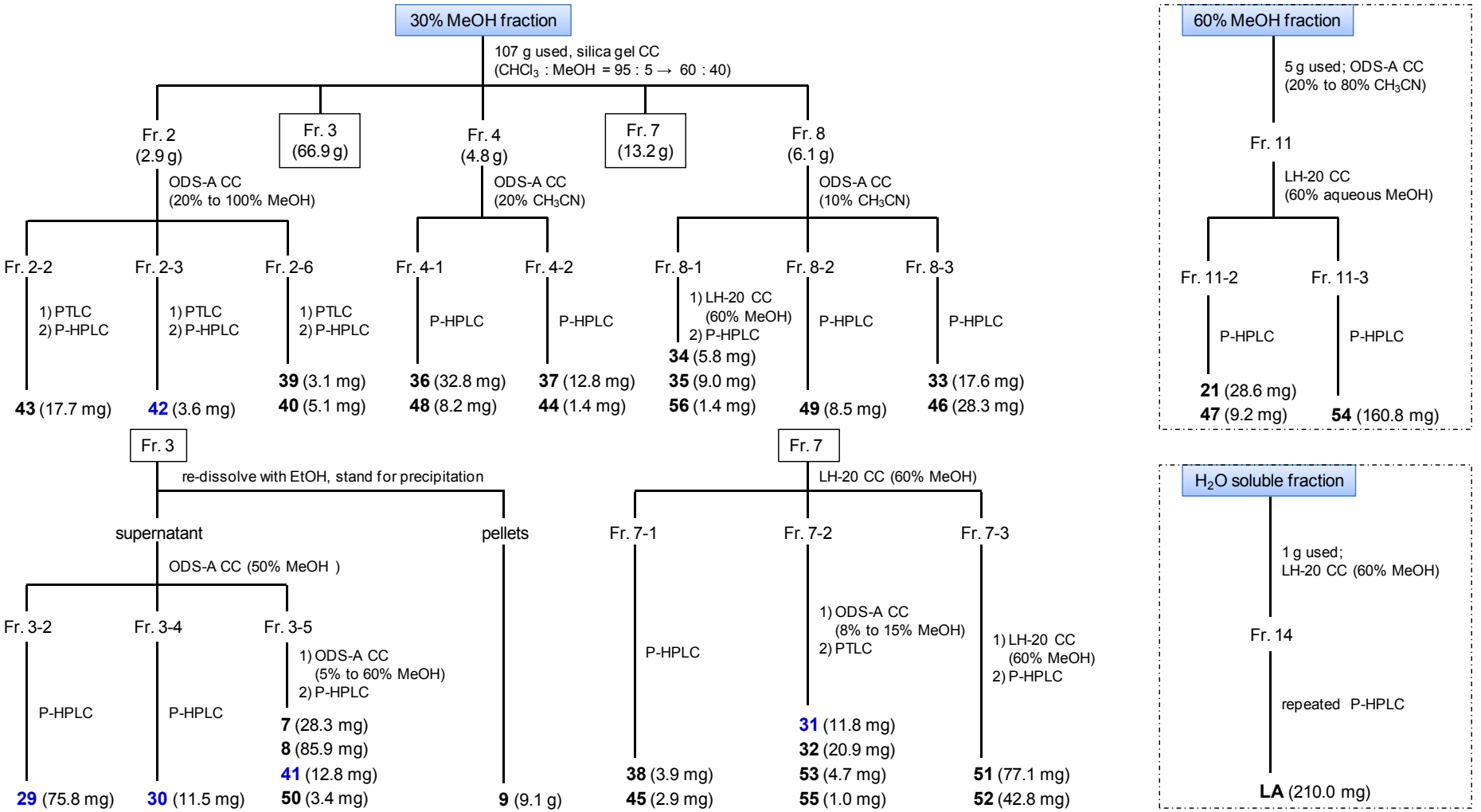


Chart 2.1 Isolation of Gentianae Macrophyllae Radix (秦艽) derived from *G. crassicaulis*

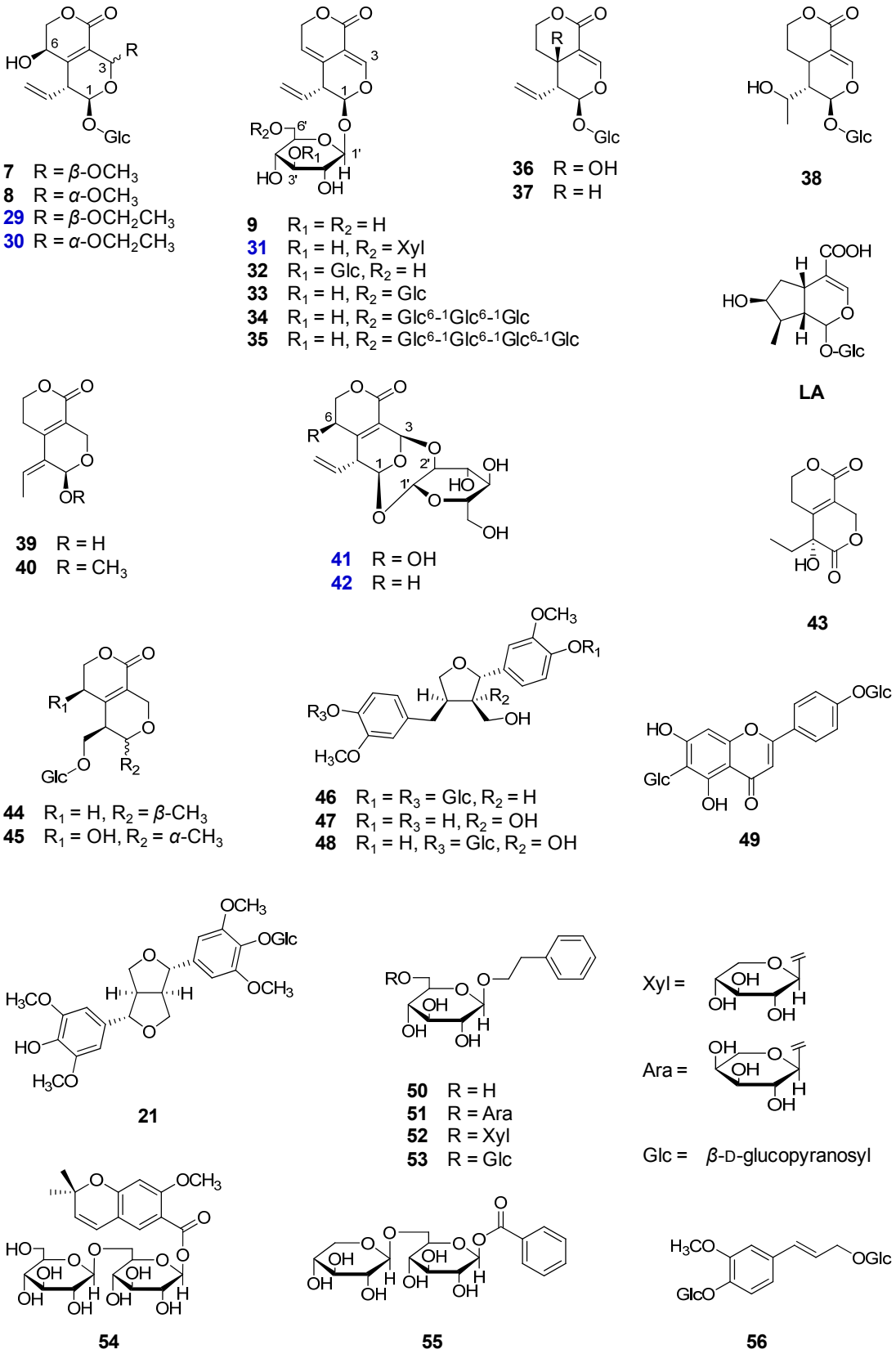


Figure 2.1 Structures of compounds isolated from Gentianae Macrophyllae Radix (秦艽) derived from *G. crassicaulis*

Gentiananoside D (**29**): white, amorphous solid; $[\alpha]_D^{25}$ −100.89° (*c* 0.10, MeOH). The HR-ESI-MS displayed pseudomolecular ion peak at 441.1364 $[M+Na]^+$ corresponding to the molecular formula $C_{18}H_{26}O_{11}$. The ^{1}H- and ^{13}C-NMR data (Tables 2.1 and 2.2) revealed considerable similarity to those of 6*β*-hydroxyswertiajaposide A (**8**), except for a series of signals assignable to an ethoxyl group at δ_H 3.77 and 4.03, δ_C 68.1, CH_2-12; δ_H 1.27, δ_C 15.3, CH_3-13, which were differed from the methoxyl signals (δ_H 3.51, δ_C 56.6) in **8**. The ^{1}H-^{1}H COSY correlation of H_2-12 with H_3-13 and HMBC correlation from H-12 to C-3 (Figure 2.2) confirmed the presence of ethoxyl group at C-3. Subsequently, the planar structure was deduced to be a 3-demethoxy-3-ethoxy derivative of **8**. The NOE correlations between H-1 and H-3, H-1 and H-8, H-8 and H-6, as well as H-3 and H-8 (Figure 2.2) indicated that H-1, H-3, H-6 and H-8 in **29** were all cofacial (*α*-orientation), ascertaining the hydroxyl group at C-6 and ethoxyl group at C-3 were both of *β*-configuration. Accordingly, the structure of **29** was established and found to be a new compound.

Gentiananoside C (**30**): white, amorphous solid; $[\alpha]_D^{25}$ −164.03° (*c* 0.10, MeOH). The HR-ESI-MS displayed pseudomolecular ion peak at 441.1366 $[M+Na]^+$ corresponding to the molecular formula $C_{18}H_{26}O_{11}$, which is identical to that of **29**. Further analyses of the ^{1}H-, ^{13}C- and 2D-NMR data revealed the same planar structure as **29**. However, the differences in the proton signals of H-1 (**30**: δ_H 5.43, d, *J* = 4.4 Hz, *vs.* **29**: δ_H 5.48, s), H-3 (**30**: δ_H 5.64, d, *J* = 0.8 Hz, *vs.* **29**: δ_H 5.34, d, *J* = 2.0 Hz), H-6 (**30**: δ_H 4.15, m, *vs.* **29**: δ_H 4.46, ddd, *J* = 10.0, 5.6, 2.0 Hz), H_a-7 (**30**: δ_H 4.40, dd, *J* = 12.8, 2.4 Hz, *vs.* **29**: δ_H 4.13, dd, *J* = 10.4, 10.0 Hz), and H_b-7 (**30**: δ_H 4.44, dd, *J* = 12.8, 2.0 Hz, *vs.* **29**: δ_H 4.38, dd, *J* = 10.4, 5.6 Hz) suggested that **30** to be a diastereomer of **29**. The stereochemistry at C-3 and C-6 was deduced to be of *α*- and

Table 2.1 ^{1}H-NMR spectroscopic data of compounds **29**-**31**, **41** and **42** in CD_3OD (400 MHz, δ in ppm, J in Hz)

position	gentiananoside D (**29**) (mult.; J_{HH})	gentiananoside C (**30**)	6'-O-β-D-xylopyranoysl gentiopicroside (**31**)	gentiananoside A (**41**)	gentiananoside B (**42**)
1	5.48 (s)	5.43 (d; 4.4)	5.64 (d; 3.2)	5.40 (s)	5.33 (s)
3	5.34 (d; 2.0)	5.64 (d; 0.8)	7.45 (br s)	5.74 (d; 2.4)	5.76 (d; 2.4)
6a	4.46 (ddd; 10.0, 5.6, 2.0)	4.15 (m)	5.62 (m)	4.40 (m)	2.39 (ddd; 18.4, 4.0, 3.6)
6b					2.74 (m)
7a	4.13 (dd; 10.4, 10.0)	4.40 (dd; 12.8, 2.4)	4.99 (dd; 18.0, 3.6)	4.19 (dd; 12.4, 10.0)	4.43 (dd; 3.6, 1.6)
7b	4.38 (dd; 10.4, 5.6)	4.44 (dd; 12.8, 2.0)	5.05 (br d; 18.0)	4.41 (dd; 12.4, 7.6)	4.45 (br d; 3.6)
8	5.71 (ddd; 17.6, 10.0, 8.4)	5.78 (ddd; 18.0, 10.8, 9.2)	5.76 (ddd; 16.8, 10.4, 6.8)	5.70 (ddd; 18.8, 10.4, 8.0)	5.67 (ddd; 18.4, 10.0, 8.4)
9	3.54 (br d; 8.4)	3.26 (dd; 9.2, 4.4)	3.28 [a]	3.53 (br d; 8.0)	3.11 (br d; 8.4)
10a	5.27 (br d; 10.0)	5.35 (br d; 18.0)	5.22 (br d; 10.4)	5.32 (dt; 10.4, 1.2)	5.31 (br d; 10.0)
10b	5.28 (br d; 17.6)	5.36 (dd; 10.8, 0.8)	5.24 (br d; 16.8)	5.33 (dt; 18.8, 1.2)	5.33 (dt; 18.4, 0.8)
12a	3.77 (dq; 9.6, 7.6)	3.72 (dq; 9.6, 7.6)			
12b	4.03 (dq; 9.6, 6.8)	3.89 (dq; 9.6, 6.8)			
13	1.27 (dd; 7.6, 6.8)	1.23 (dd; 7.6, 6.8)			
1-*O*-glucosyl					
1'	4.74 (d; 8.0)	4.68 (d; 8.0)	4.64 (d; 7.6)	4.97 (d; 6.8)	4.96 (d; 6.8)
2'	3.22 (dd; 9.2, 8.0)	3.18 (dd; 8.8, 8.0)	3.16 (dd; 8.0, 7.6)	3.36 (m)	3.34 [a]
3'	3.41 (dd; 9.2, 8.4)	3.39 (dd; 8.8, 8.4)	3.36 (m)	3.56 (dd; 8.8, 8.4)	3.54 (dd; 8.8, 8.4)
4'	3.31 (m)	3.28 (m)	3.31 [a]	3.30 (m)	3.31 [a]
5'	3.33 (m)	3.31 (m)	3.48 (m)	3.33 (m)	3.33 [a]
6'a	3.67 (dd; 11.6, 6.0)	3.65 (dd; 12.0, 5.6)	3.72 (dd; 11.6, 6.0)	3.65 (dd; 12.0, 5.6)	3.64 (dd; 12.0, 5.6)
6'b	3.89 (dd; 11.6, 2.4)	3.86 (dd; 12.0, 2.4)	4.12 (dd; 11.6, 2.0)	3.86 (dd; 12.0, 2.0)	3.86 (dd; 12.0, 1.6)
O-xylosyl					
1"			4.31 (d; 7.6)		
2"			3.18 (m)		
3"			3.32 [a]		
4"			3.50 (m)		
5"a			3.20 (t; 11.6)		
5"b			3.86 (dd; 11.6, 6.4)		

[a] Overlapped with solvent signals.

Table 2.2 ^{13}C-NMR spectroscopic data of compounds **29**-**31**, **41** and **42** in CD_3OD (100 MHz, δ in ppm)

position	gentiananoside D (**29**) (type)		gentiananoside C (**30**)		6'-O-β-D-xylopyranoysl gentiopicroside (**31**)		gentiananoside A (**41**)		gentiananoside B (**42**)	
1	96.2	(CH)	95.2	(CH)	98.8	(CH)	95.5	(CH)	95.4	(CH)
3	93.3	(CH)	94.4	(CH)	150.7	(CH)	91.1	(CH)	91.3	(CH)
4	122.0	(C)	125.6	(C)	104.9	(C)	120.9	(C)	121.5	(C)
5	156.1	(C)	152.6	(C)	127.1	(C)	160.5	(C)	158.6	(C)
6	62.0	(CH)	61.8	(CH)	117.2	(CH)	62.3	(CH)	28.7	(CH_2)
7	70.8	(CH_2)	73.4	(CH_2)	70.9	(CH_2)	71.2	(CH_2)	66.7	(CH_2)
8	134.2	(CH)	134.6	(CH)	134.9	(CH)	132.9	(CH)	132.9	(CH)
9	43.7	(CH)	46.9	(CH)	46.6	(CH)	45.2	(CH)	50.8	(CH)
10	120.3	(CH_2)	121.7	(CH_2)	118.7	(CH_2)	121.3	(CH_2)	121.3	(CH_2)
11	163.2	(C)	163.3	(C)	166.3	(C)	163.0	(C)	163.9	(C)
12	68.1	(CH_2)	66.0	(CH_2)						
13	15.3	(CH_3)	15.6	(CH_3)						
1-*O*-glucosyl										
1'	99.2	(CH)	99.1	(CH)	100.4	(CH)	98.9	(CH)	99.0	(CH)
2'	75.0	(CH)	74.7	(CH)	74.5	(CH)	81.6	(CH)	81.7	(CH)
3'	78.3	(CH)	78.0	(CH)	77.9	(CH)	76.2	(CH)	76.3	(CH)
4'	71.4	(CH)	71.6	(CH)	71.3	(CH)	70.6	(CH)	70.7	(CH)
5'	78.5	(CH)	78.5	(CH)	77.3	(CH)	79.0	(CH)	79.1	(CH)
6'	62.7	(CH_2)	62.7	(CH_2)	69.9	(CH_2)	62.5	(CH_2)	62.6	(CH_2)
O-xylosyl										
1"					105.7	(CH)				
2"					74.9	(CH)				
3"					77.9	(CH)				
4"					71.1	(CH)				
5"					67.0	(CH_2)				

β-configuration by the observation of NOE correlations between H-1 and H-8, as well as H-8 and H-6, but not between H-1 and H-3. Thus, the structure of **30** was established and found to be a new compound.

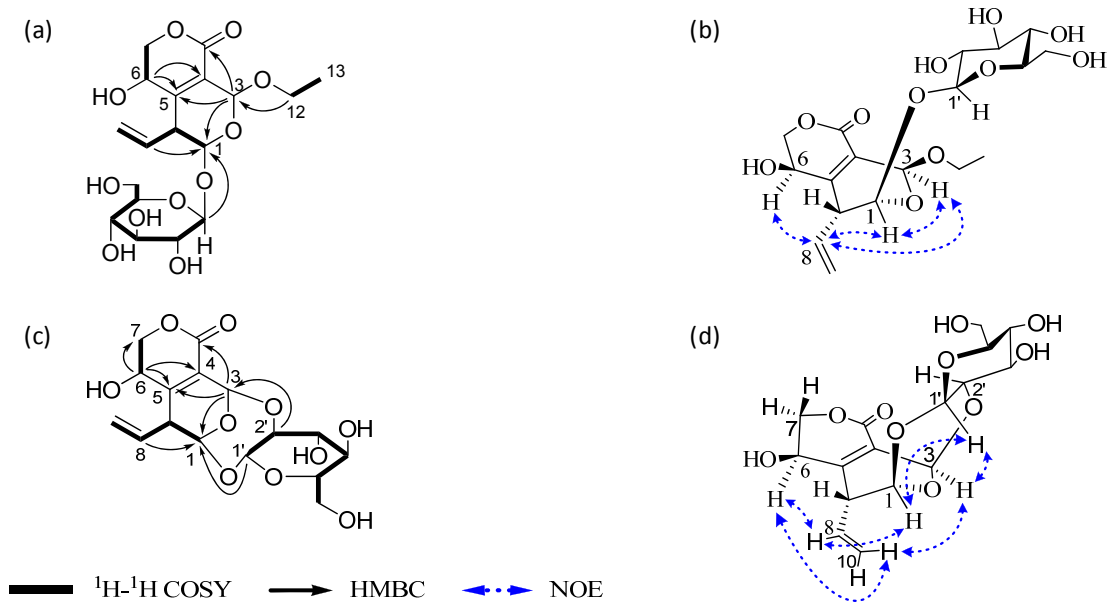


Figure 2.2 The key ^{1}H-^{1}H COSY, HMBC and NOEs of gentiananoside D (**29**) (a and b, both in CD_3OD) and gentiananoside A (**41**) (c, in CD_3OD; d, in D_2O)

6'-*O*-*β*-D-Xylopyranosylgentiopicroside (**31**): white, amorphous solid; $[\alpha]_D^{25}$ −67.22° (*c* 0.05, MeOH). The HR-ESI-MS displayed a pseudomolecular ion peak at 511.1415 $[M+Na]^+$ corresponding to the molecular formula $C_{21}H_{28}O_{13}$. The ^{1}H-NMR spectrum (Table 2.1) displayed signals of one acetal methine at δ_H 5.64 (d, *J* = 3.2 Hz, H-1) and one oxyolefinic methine at δ_H 7.45 (br s, H-3) as characteristics of an iridoid nucleus, as well as signals from a terminal vinyl group at δ_H 5.76 (ddd, *J* = 16.8, 10.4, 6.8 Hz, H-8), 5.22 (br d, *J* = 10.4 Hz, H_a-10) and 5.24 (br d, *J* = 16.8 Hz, H_b-10), an unsaturated methine at δ_H 5.62 (m, H-6) and an oxygenated methylene at δ_H 4.99 (dd, *J* = 18.0, 3.6 Hz, H_a-7), 5.05 (br d, *J* = 18.0 Hz, H_b-7). These proton signals strongly suggested that the aglycone moiety was essentially the same as that of gentiopicroside (**9**). Moreover, two anomeric proton signals at δ_H 4.64 (d, *J* = 7.6 Hz,

H-1') and 4.31 (d, *J* = 7.6 Hz, H-1") were recognized, and the coupling constants of which indicated that the glycosyl linkages were of *β*-configuration. The ^{13}C-NMR spectrum (Table 2.2) exhibited close similarity to that of **9**, except for a set of additional signals at δ_C 105.7 (C-1"), 74.9 (C-2"), 77.9 (C-3"), 71.1 (C-4") and 67.0 (C-5") which were assigned to a terminal *β*-D-xylopyranosyl group by comparison with the reported data [Bock *et al.*, 1983]. Acidic hydrolysis of **31** yielded D-xylose and D-glucose accordingly. The terminal *β*-D-xylopyranosyl group was involved in a glycosyl linkage at C-6' of the inner *β*-D-glucopyranosyl group, which was deduced from the downfield carbon signal at δ_C 69.9 (+7.1 ppm), when comparing the ^{13}C-NMR data of **31** with that of **9**. It was confirmed by the HMBC correlation from H-1" to C-6'. Thus, the structure of **31** was established and found to be a new compound.

Olivieroside C (**32**): white, amorphous solid with the molecular formula of $C_{22}H_{30}O_{14}$ (518 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.45 (1H, s, H-3), 5.75 (1H, ddd, *J* = 17.2, 10.4, 3.6 Hz, H-8), 5.66 (1H, d, *J* = 2.8 Hz, H-1), 5.62 (1H, br s, H-6), 5.24 (1H, ddd, *J* = 17.2, 1.2, 1.2 Hz, H_b-10), 5.22 (1H, ddd, *J* = 8.0, 1.2, 1.2 Hz, H_a-10), 5.08 (2H, m, H-7), 4.71 (1H, d, *J* = 7.6 Hz, H-1'), 4.56 (1H, d, *J* = 7.6 Hz, H-1"); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 166.3 (C-11), 150.7 (C-3), 134.9 (C-8), 130.0 (C-5), 118.6 (C-10), 117.2 (C-6), 105.1 (C-1"), 104.9 (C-4), 99.9 (C-1'), 98.6 (C-1), 87.5 (C-3'), 78.1 (C-5"), 78.1 (C-3"), 77.8 (C-5'), 75.4 (C-2"), 73.9 (C-2'), 71.5 (C-4"), 70.9 (C-7), 69.9 (C-4'), 62.6 (C-6"), 62.6 (C-6'), 46.6 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Takeda *et al.*, 1999]. It is the first isolation of olivieroside C from this plant so far known.

6'-*O*-*β*-D-Glucopyranosylgentiopicroside (**33**): white, amorphous solid with the molecular formula of $C_{22}H_{30}O_{14}$ (518 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.46 (1H, s,

H-3), 5.76 (1H, ddd, *J* = 17.2, 10.4, 3.2 Hz, H-8), 5.64 (1H, d, *J* = 3.2 Hz, H-1), 5.62 (1H, br s, H-6), 5.25 (1H, ddd, *J* = 17.2, 1.6, 1.6 Hz, H_b-10), 5.21 (1H, ddd, *J* = 9.6, 1.6, 1.6 Hz, H_a-10), 5.03 (2H, m, H-7), 4.67 (1H, d, *J* = 8.0 Hz, H-1'), 4.38 (1H, d, *J* = 8.0 Hz, H-1''); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 166.4 (C-11), 150.8 (C-3), 134.9 (C-8), 127.0 (C-5), 118.8 (C-10), 117.2 (C-6), 105.0 (C-1''), 104.9 (C-4), 100.4 (C-1'), 98.8 (C-1), 78.1 (C-3'), 78.0 (C-5''), 77.9 (C-3''), 77.4 (C-5'), 75.1 (C-2''), 74.5 (C-2'), 71.6 (C-4''), 71.4 (C-4'), 71.0 (C-7), 70.0 (C-6'), 62.8 (C-6''), 46.6 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kakuda *et al.*, 2001].

Scabran G_3 (**34**): white, amorphous solid with the molecular formula of $C_{28}H_{40}O_{19}$ (680 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.47 (1H, br s, H-3), 5.77 (1H, ddd, *J* = 17.6, 10.4, 3.6 Hz, H-8), 5.64 (1H, d, *J* = 3.2 Hz, H-1), 5.62 (1H, br s, H-6), 5.25 (1H, ddd, *J* = 17.6, 1.6, 1.6 Hz, H_b-10), 5.22 (1H, ddd, *J* = 10.0, 1.6, 1.6 Hz, H_a-10), 5.02 (2H, m, H-7), 4.69 (1H, d, *J* = 8.4 Hz, H-1'), 4.39 (1H, d, *J* = 7.6 Hz, H-1''), 4.38 (1H, d, *J* = 8.0 Hz, H-1'''); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 166.3 (C-11), 150.8 (C-3), 134.9 (C-8), 127.0 (C-5), 118.8 (C-10), 117.1 (C-6), 105.1 (C-1''), 104.9 (C-4), 104.8 (C-1'''), 100.4 (C-1'), 98.9 (C-1), 77.9 (C-3''), 77.9 (C-3'), 77.9 (C-5''''), 77.8 (C-3'''), 77.2 (C-5'), 77.0 (C-5''), 75.0 (C-2''), 75.0 (C-2'''), 74.4 (C-2'), 71.5 (C-4'), 71.4 (C-4''), 71.4 (C-4'''), 70.9 (C-7), 70.4 (C-6'), 70.0 (C-6''), 62.7 (C-6'''), 46.5 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikichi *et al.*, 2005]. It is the first isolation of scabran G_3 from this plant so far known.

Scabran G_4 (**35**): white, amorphous solid with the molecular formula of $C_{34}H_{50}O_{24}$ (842 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.48 (1H, br s, H-3), 5.75 (1H, ddd, *J* = 17.6, 10.0, 2.8 Hz, H-8), 5.64 (1H, d, *J* = 3.2 Hz, H-1), 5.62 (1H, br s, H-6), 5.24 (1H, dd, *J* =

17.6, 1.6, Hz, H_b-10), 5.22 (1H, ddd, *J* = 10.0, 1.6, Hz, H_a-10), 5.04 (2H, m, H-7), 4.68 (1H, d, *J* = 8.0 Hz, H-1'), 4.39 (1H, d, *J* = 7.6 Hz, H-1''), 4.38 (1H, d, *J* = 7.6 Hz, H-1'''), 4.37 (1H, d, *J* = 8.0 Hz, H-1''''); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 167.2 (C-11), 151.7 (C-3), 135.7 (C-8), 127.8 (C-5), 119.4 (C-10), 117.8 (C-6), 105.6 (C-4), 105.5 (C-1''), 105.4 (C-1'''), 105.4 (C-1''''), 101.0 (C-1'), 99.4 (C-1), 78.0 (C-3'), 78.0 (C-3''), 78.0 (C-3'''), 78.0 (C-5''''), 77.8 (C-3''''), 77.2 (C-5'), 77.1 (C-5''), 76.9 (C-5'''), 75.1 (C-2''), 75.1 (C-2'''), 75.1 (C-2''''), 74.5 (C-2'), 71.6 (C-4'), 71.6 (C-4''), 71.6 (C-4'''), 71.6 (C-4''''), 70.9 (C-7), 70.6 (C-6'), 70.5 (C-6''), 70.0 (C-6'''), 60.0 (C-6''''), 46.9 (C-9). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikichi *et al.*, 2005]. It is the first isolation of scabran G_4 from this plant so far known.

Swertiamarin (**36**): white, amorphous solid with the molecular formula of $C_{16}H_{22}O_{10}$ (374 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.64 (1H, s, H-3), 5.72 (1H, d, *J* = 1.6 Hz, H-1), 5.42 (1H, m, H-8), 5.35 (1H, dd, *J* = 18.4, 2.8 Hz, H_b-10), 5.20 (1H, dd, *J* = 9.6, 2.8 Hz, H_a-10), 4.75 (1H, m, H_b-7), 4.64 (1H, d, *J* = 8.0 Hz, H-1'), 4.35 (1H, m, H_a-7), 3.89 (1H, dd, *J* = 12.4, 2.4 Hz, H_b-6'), 3.66 (1H, dd, *J* = 12.4, 5.6 Hz, H_a-6'), 3.21 (1H, dd, *J* = 9.2, 8.0 Hz, H-2'), 2.93 (1H, dd, *J* = 9.2, 0.8 Hz, H-9), 1.92 (1H, m, H_b-6), 1.74 (1H, br d, *J* = 14.4 Hz, H_a-6); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 167.9 (C-11), 154.8 (C-3), 133.8 (C-8), 121.2 (C-10), 108.8 (C-4), 100.2 (C-1'), 99.1 (C-1), 78.5 (C-5'), 77.8 (C-3'), 74.4 (C-2'), 71.4 (C-4'), 65.9 (C-7), 64.3 (C-5), 62.5 (C-6'), 51.9 (C-9), 33.7(C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Hidehiro *et al.*, 2007].

Sweroside (**37**): white, amorphous solid with the molecular formula of $C_{16}H_{22}O_9$ (358 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.59 (1H, d, *J* = 2.4 Hz, H-3), 5.55 (1H, d, *J*

= 2.8 Hz, H-1), 5.53 (1H, m, H-8), 5.30 (1H, dd, *J* = 18.4, 1.6 Hz, H_b-10), 5.27 (1H, dd, *J* = 10.0, 1.6 Hz, H_a-10), 4.68 (1H, d, *J* = 8.0 Hz, H-1'), 4.44 (1H, m, H_b-7), 4.36 (1H, m, H_a-7), 3.89 (1H, dd, *J* = 12.0, 2.0 Hz, H_b-6'), 3.66 (1H, dd, *J* = 12.0, 6.0 Hz, H_a-6'), 3.13-3.19 (2H, m, H-5, 2'), 2.69 (1H, m H-9), 1.72 (1H, m, H_b-6), 1.69 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 168.5 (C-11), 153.9 (C-3), 133.3 (C-8), 120.8 (C-10), 106.0 (C-4), 99.7 (C-1'), 97.9 (C-1), 78.4 (C-3'), 77.8 (C-5'), 74.7 (C-2'), 71.5 (C-4'), 69.7 (C-7), 62.7 (C-6'), 43.8 (C-9), 28.4 (C-5), 25.9 (C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Cambie *et al.*, 1990].

8-Hydroxy-10-hydrosweroside (**38**): white, amorphous solid with the molecular formula of $C_{16}H_{24}O_{10}$ (376 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 7.59 (1H, d, *J* = 2.4 Hz, H-3), 5.91 (1H, d, *J* = 1.2 Hz, H-1), 4.67 (1H, d, *J* = 8.4 Hz, H-1'), 4.48 (1H, m, H_b-7), 4.32 (1H, m, H_a-7), 3.88 (1H, dd, *J* = 12.0, 2.4 Hz, H_b-6'), 3.76 (1H, m, H-8), 3.68 (1H, dd, *J* = 12.0, 5.6 Hz, H_a-6'), 3.22 (1H, m, H-5), 1.99 (1H, m, H-9), 1.89 (2H, m, H-6), 1.29 (3H, d, *J* = 6.0 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 168.7 (C-11), 154.8 (C-3), 106.6 (C-4), 99.9 (C-1'), 95.8 (C-1), 78.3 (C-3'), 77.9 (C-5'), 74.7 (C-2'), 71.5 (C-4'), 70.0 (C-8), 65.2 (C-7), 62.6 (C-6'), 44.5 (C-9), 28.7 (C-5), 26.3 (C-6), 24.2 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Tan *et al.*, 1998]. It is the first isolation of 8-hydroxy-10-hydrosweroside from this plant so far known.

Swerimilegenin H (**39**): white, amorphous solid with the molecular formula of $C_{10}H_{12}O_4$ (196 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 6.20 (1H, q, *J* = 7.2 Hz, H-8), 5.87 (1H, s, H-1), 4.52 (1H, d, *J* = 16.8 Hz, H_b-3), 4.42 (2H, m, H-7), 4.31 (1H, d, *J* = 16.4 Hz, H_a-3), 2.63 (2H, m, H-6), 1.92 (3H, d, *J* = 7.2 Hz, H-10); ^{13}C-NMR (CD_3OD,

100MHz) *δ* 166.4 (C-11), 145.4 (C-5), 133.3 (C-9), 131.8 (C-8), 121.3 (C-4), 89.1 (C-1), 67.3 (C-7), 57.3 (C-3), 23.5 (C-6), 13.9 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Geng *et al.*, 2013]. It is the first isolation of swerimilegenin H from this plant so far known.

Swerimilegenin I (**40**): white, amorphous solid with the molecular formula of $C_{11}H_{14}O_4$ (210 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 6.22 (1H, q, *J* = 7.6 Hz, H-8), 5.46 (1H, s, H-1), 4.43 (2H, m, H-7), 4.32 (2H, br s, H-3), 3.49 (3H, s, OCH_3), 2.61 (2H, m, H-6), 1.89 (3H, d, *J* = 7.6 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 166.3 (C-11), 145.3 (C-5), 132.8 (C-8), 132.1 (C-9), 121.1 (C-4), 96.2 (C-1), 67.2 (C-7), 57.5 (C-3), 55.2 (OCH_3), 23.4 (C-6), 13.9 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Geng *et al.*, 2013]. It is the first isolation of swerimilegenin I from this plant so far known.

Gentiananoside A (**41**): white, amorphous solid; $[\alpha]_D^{25}$ −91.85° (*c* 0.10, MeOH). The HR-ESI-MS spectra displayed pseudomolecular ion peak at 395.0946 $[M+Na]^+$ corresponding to the molecular formula $C_{16}H_{20}O_{10}$. The ^{1}H- and ^{13}C-NMR spectra (Tables 2.1, 2.2) were similar to those of 6*β*-hydroxyswertiajaposide A (**8**), except for the absence of a methoxyl group, as well as the obviously downfield shift of C-5 and C-2' from δ_C 152.9 and 74.6 in **8** to δ_C 160.5 and 81.6 in **41**, and the upfield shift of C-3 and C-4 from δ_C 95.4 and 125.2 in **8** to δ_C 91.1 and 120.9 in **41**, respectively. HMBC correlations of H-1' with C-1 and H-2' with C-3 (Figure 2.2) indicated the linkages of C(1')-O-C(1) and C(2')-O-C(3). Therefore, the planar structure was deduced as shown in Figure 2.1. The NOE correlations between H-6 and H-8, H-8 and H-1, as well as H-1 and H-3 (Figure 2.2) indicated that H-6, H-1 and H-3 were all cofacial (*α*-orientation), which ascertained *β*-configuration of both the hydroxyl group at C-6

and the ether linkage at C-3 bonded to C-2' of sugar moiety. In addition, NOE correlation between H-6 and H_a-10, H_a-10 and H-3, H-1 and H-1', as well as H-1' and H-3 were also observed (Figure 2.2). From the above data, the structure of **41** was deduced to be 3-demethoxy-3,2'-cyclic ether derivative of compound **8** and found to be a new one. It is noteworthy that **41** is a novel secoiridoid glycoside with an additional ether linkage between C-2' of the sugar moiety and C-3 of the aglycone, which may be formed by the introduction of hydroxyl group at C-3 followed by dehydration-cyclization of C(3)-C(2') via an ether linkage.

Gentiananoside B (**42**): white, amorphous solid; $[\alpha]_D^{25}$ −98.69° (*c* 0.10, MeOH). The HR-ESI-MS spectra displayed pseudomolecular ion peak at 379.0997 $[M+Na]^+$ corresponding to the molecular formula $C_{16}H_{20}O_9$. The ^{1}H- and ^{13}C-NMR data (Tables 2.1, 2.2) exhibited a similar signal pattern to that of **41**, however, two methylene proton signals were observed in **42** instead of an oxymethine signal in **41**. Moreover, the oxymethine carbon signal at δ_C 62.3 assignable to C-6 in **41** was largely shifted upfield to δ_C 28.7 in **42**. These facts suggested that **42** was a 6-dehydroxyl analogue of **41**. It was confirmed by the ^{1}H-^{1}H COSY spectrum, in which cross-peaks were observed between H_a-6 and H_b-6, and between H-6 (2H) and H-7 (2H). The same *β*-configuration of the ether linkage at C-3 bonded to C-2' of sugar moiety was determined by the NOE correlation between H-1 and H-3. From the above data, the structure of **42** were established and found to be a new compound. It is also a novel secoiridoid glycoside with an additional ether linkage between C-2' of the sugar moiety and C-3 of the aglycone.

(*R*)-(−)-Gentiolactone (**43**): white, amorphous solid with the molecular formula of $C_{10}H_{12}O_5$ (212 $[M]^+$); $[\alpha]_D^{25}$ −100.50° (*c* 0.008, MeOH). ^{1}H-NMR (CD_3OD, 400MHz) *δ*

5.15 (1H, dt, *J* = 17.2, 2.4 Hz, H_b-3), 5.04 (1H, ddd, *J* = 17.2, 3.2, 2.8 Hz, H_a-3), 4.48 (2H, m, H-7), 4.68 (2H, m, H-6), 1.93 (1H, dq, *J* = 14.4, 8.0 Hz, H_b-3), 1.78 (1H, dq, *J* = 14.4, 8.0 Hz, H_a-3), 0.96 (3H, t, *J* = 7.6 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 173.8 (C-1), 163.9 (C-11), 155.5 (C-5), 121.4 (C-4), 73.6 (C-9), 68.2 (C-7), 67.4 (C-3), 31.5 (C-8), 23.7 (C-6), 8.29 (C-10). The above spectroscopic data were the same with those of the reported values [Kakuda *et al.*, 2003]. It is the first isolation of (*R*)-(–)-gentiolactone from this plant so far known.

Swertiajaposide E (**44**): white, amorphous solid with the molecular formula of $C_{16}H_{24}O_9$ (360 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 4.42 (1H, m, H_b-7), 4.39 (1H, br d, *J* = 15.6 Hz, H_b-3), 4.35 (1H, m, H_a-7), 4.29 (1H, dd, *J* = 15.6, 3.2 Hz, H_a-3), 4.28 (1H, d, *J* = 5.6 Hz, H_b-1), 4.25 (1H, d, *J* = 8.0 Hz, H-1'), 3.78 (1H, dq, *J* = 6.4, 2.8 Hz, H-8), 3.65 (1H, dd, *J* = 5.6, 2.0 Hz, H_a-1), 3.14 (1H, dd, *J* = 9.2, 8.0 Hz, H-2'), 2.83 (1H, m, H_b-6), 2.46 (1H, m, H_a-6), 2.45 (1H, m, H-9), 1.29 (3H, d, *J* = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 165.7 (C-11), 155.1 (C-5), 124.4 (C-4), 104.5 (C-1'), 78.1 (C-3'), 78.1(C-5'), 75.0 (C-2'), 72.7 (C-8), 71.7 (C-4'), 68.2 (C-1), 67.8 (C-7), 65.6 (C-3), 62.8 (C-6'), 43.8 (C-9'), 28.8 (C-6), 18.0 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikuchi *et al.*, 2008]. It is the first isolation of swertiajaposide E from this plant so far known.

Swertiajaposide F (**45**): white, amorphous solid with the molecular formula of $C_{16}H_{24}O_{10}$ (376 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 4.49 (1H, dd, *J* = 11.6, 3.6 Hz, H_b-7), 4.46 (1H, m, H-6), 4.37 (1H, dd, *J* = 16.4, 1.6 Hz, H_b-3), 4.31 (1H, dd, *J* = 11.6, 8.0 Hz, H_a-7), 4.25 (1H, d, *J* = 7.6 Hz, H-1'), 4.16 (1H, dt, *J* = 16.4, 1.6 Hz, H_a-3), 4.07 (1H, dd, *J* = 10.8, 4.0 Hz, H_b-1), 3.96 (1H, m, H-8), 3.86 (1H, dd, *J* = 10.8, 2.0 Hz, H_a-1), 2.57 (1H, m, H-9), 1.29 (3H, d, *J* = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) *δ*

164.5 (C-11), 152.4 (C-5), 126.3 (C-4), 104.7 (C-1'), 78.2 (C-3'), 78.1(C-5'), 75.0 (C-2'), 73.0 (C-7), 71.6 (C-4'), 71.0 (C-8), 69.2 (C-1), 62.8 (C-6'), 62.1 (C-6), 62.0 (C-3), 43.0 (C-9), 18.7 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikuchi *et al.*, 2008]. It is the first isolation of swertiajaposide F from this plant so far known.

Clemastanin B (**46**): white, amorphous solid with the molecular formula of $C_{32}H_{44}O_{16}$ (684 $[M]^{+}$). ^{1}H-NMR (CD_3OD, 400MHz) δ 7.13 (1H, d, J = 8.4 Hz, H-5), 7.08 (1H, d, J = 8.4 Hz, H-5'), 6.98 (1H, d, J = 1.6 Hz, H-2), 6.88 (1H, d, J = 2.0 Hz, H-2'), 6.87 (1H, dd, J = 8.4, 1.6 Hz, H-6), 6.76 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 4.86 (1H, d, J = 8.0 Hz, H-1'''), 4.85 (1H, s, H-7), 4.84 (1H, d, J = 7.6 Hz, H-1''), 4.00 (1H, dd, J = 8.4, 6.4 Hz, H_b-9'), 3.85 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.84 (1H, m, H_b-9), 3.72 (1H, dd, J = 8.4, 5.6 Hz, H_a-9'), 3.64 (1H, m, H_a-9), 2.94 (1H, dd, J = 13.2, 4.4 Hz, H_b-7'), 2.72 (1H, m, H-8'), 2.54 (1H, dd, J = 13.2, 11.2 Hz, H_a-7'), 2.35 (1H, m, H-8); ^{13}C-NMR (CD_3OD, 100MHz) δ 150.8 (C-3'), 150.8 (C-3), 147.3 (C-4), 146.4 (C-4'), 139.4 (C-1), 137.1 (C-1'), 122.2 (C-6'), 119.5 (C-6), 118.2 (C-5), 117.9 (C-5'), 114.3 (C-2'), 111.3 (C-2), 103.0 (C-1''), 102.9 (C-1'''), 83.8 (C-7), 78.2 (C-5'', 5'''), 77.8 (C-3'', 3'''), 74.9 (C-2'', 2'''), 73.6 (C-9'), 71.3 (C-4'', 4'''), 62.5 (C-6'', 6'''), 60.5 (C-9), 56.7 (OCH_3), 54.1 (C-8), 43.7 (C-8'), 33.6 (C-7'). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Gamal *et al.*, 1997]. It is the first isolation of clemastanin B from this plant so far known.

(–)-Berchemol (**47**): colorless needles with the molecular formula of $C_{20}H_{24}O_7$ (376 $[M]^{+}$). ^{1}H-NMR (CD_3OD, 400MHz) δ 6.96 (1H, br s, H-2), 6.81 (1H, d, J = 1.6 Hz, H-2'), 6.76 (1H, d, J = 7.6 Hz, H-5), 6.70 (1H, d, J = 7.6 Hz, H-5'), 6.66 (1H, dd, J = 7.6, 1.6 Hz, H-6), 6.64 (1H, dd, J = 7.6, 1.6 Hz, H-6'), 4.82 (1H, s, H-7), 4.06 (1H, dd, J = 8.4,

6.4 Hz, H_b-9'), 3.85 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.79 (1H, dd, *J* = 11.2, 6.0 Hz, H_b-9), 3.65 (1H, dd, *J* = 8.4, 5.6 Hz, H_a-9'), 3.60 (1H, br d, *J* = 11.2 Hz, H_a-9), 3.09 (1H, dd, *J* = 12.8, 1.6 Hz, H_b-7'), 2.58 (1H, m, H-8'), 2.47 (1H, dd, *J* = 12.8, 8.4 Hz, H_a-7'), 2.35 (1H, m, H-8); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 149.0 (C-3'), 148.6 (C-3), 147.2 (C-4), 145.8 (C-4'), 133.2 (C-1), 130.7 (C-1'), 122.2 (C-6'), 121.5 (C-6), 116.2 (C-5'), 115.5 (C-5), 113.4 (C-2'), 112.7 (C-2), 85.6 (C-7), 83.2 (C-8), 71.9 (C-9'), 64.5 (C-9), 56.3 (2 × OCH_3), 51.9 (C-8'), 35.1 (C-7'). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Sakurai *et al.*, 1989]. It is the first isolation of (–)-berchemol from this plant so far known.

(–)-Berchemol-4'-*O*-*β*-D-glucoside (**48**): white, amorphous solid with the molecular formula of $C_{26}H_{34}O_{11}$ (522 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 7.09 (1H, d, *J* = 8.4 Hz, H-5'), 6.96 (1H, br s, H-2), 6.90 (1H, d, *J* = 2.0 Hz, H-2'), 6.77 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 6.76 (2H, m, H-5, 6), 4.84 (1H, d, *J* = 7.6 Hz, H-1''), 4.82 (1H, s, H-7), 4.06 (1H, dd, *J* = 8.4, 6.4 Hz, H_b-9'), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.85 (1H, m, H_b-9), 3.67 (1H, m, H_a-9'), 3.63 (1H, m, H_a-9), 3.13 (1H, dd, *J* = 12.8, 3.2 Hz, H_b-7'), 2.59 (1H, m, H-8'), 2.53 (1H, dd, *J* = 12.8, 11.2 Hz, H_a-7'), 2.35 (1H, m, H-8); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 150.9 (C-3'), 148.6 (C-3), 147.2 (C-4), 146.4 (C-4'), 137.0 (C-1'), 130.7 (C-1), 122.3 (C-6'), 121.5 (C-6), 118.2 (C-5'), 115.5 (C-5), 114.3 (C-2'), 112.7 (C-2), 103.0 (C-1''), 85.6 (C-7), 83.2 (C-8), 78.2 (C-5''), 77.8 (C-3''), 74.9 (C-2''), 71.8 (C-9'), 71.3 (C-4''), 64.5 (C-9), 62.5 (C-6''), 56.7 (OCH_3), 56.3 (OCH_3), 51.7 (C-8'), 35.1 (C-7'). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Schumacher *et al.*, 2002].

Isosaponarin (**49**): yellowish, amorphous solid with the molecular formula of $C_{27}H_{30}O_{15}$ (594 $[M]^+$). ^{1}H-NMR (DMSO, 400MHz) *δ* 8.04 (2H, d, *J* = 8.8 Hz, H-2', 6'),

7.19 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.90 (1H, s, H-3), 6.54 (1H, s, H-8), 5.03 (1H, d, *J* = 6.8 Hz, H-1'''), 4.59 (1H, d, *J* = 10.0 Hz, H-1''); ^{13}C-NMR (DMSO, 100MHz) *δ* 181.9 (C-4), 163.5 (C-7), 162.7 (C-2), 160.5 (C-5), 160.1 (C-4'), 156.2 (C-9), 128.0 (C-2', 6'), 123.8 (C-1'), 116.5 (C-3', 5'), 108.9 (C-6), 103.7 (C-3), 103.3 (C-10), 99.7 (C-1'''), 93.6 (C-8), 81.5 (C-5''), 78.8 (C-3''), 77.0 (C-5'''), 76.4 (C-3'''), 73.0 (C-2'''), 72.9 (C-1''), 70.5 (C-4''), 70.1 (C-1''), 69.5 (C-4'''), 61.4 (C-6''), 60.5 (C-6'''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Hosoya *et al.*, 2005]. It is the first isolation of isosaponarin from this plant so far known.

2-Phenylethyl-*O*-*β*-D-glucopyranoside (**50**): white, amorphous solid with the molecular formula of $C_{14}H_{20}O_6$ (284 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 7.25 (4H, m, H-2, 3, 5, 6), 7.16 (1H, m, H-4), 4.30 (1H, d, *J* = 7.6 Hz, H-1'), 4.09 (1H, m, H_b-8), 3.85 (1H, dd, *J* = 11.2, 1.6 Hz, H_b-6'), 3.76 (1H, m, H_a-8), 3.66 (1H, dd, *J* = 11.2, 5.2 Hz, H_b-6'), 3.18 (1H, dd, *J* = 8.0, 7.6 Hz, H-2'), 2.93 (2H, m, H-7); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 140.0 (C-1), 130.0 (C-2, 6), 129.3 (C-3, 5), 127.2 (C-4), 104.4 (C-1'), 78.1 (C-3'), 78.0 (C-5'), 75.1 (C-2'), 71.7 (C-8), 71.6 (C-4'), 62.7 (C-6'), 37.2 (C-7). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Yoneda *et al.*, 2008]. It is the first isolation of 2-phenylethyl-*O*-*β*-D-glucopyranoside from this plant so far known.

2-Phenylethyl-6-*O*-(*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside (**51**): white amorphous solid with the molecular formula of $C_{19}H_{28}O_{10}$ (416 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 7.25 (4H, m, H-2, 3, 5, 6), 7.16 (1H, m, H-4), 4.30 (1H, d, *J* = 8.0 Hz, H-1'), 4.29 (1H, d, *J* = 5.8 Hz, H-1''), 4.08 (1H, m, H_b-8), 4.07 (1H, dd, *J* = 11.2, 2.0 Hz, H_b-6'), 3.84 (1H, dd, *J* = 12.4, 3.2 Hz, H_b-5''), 3.78 (1H, m, H_a-8), 3.72 (1H, dd, *J* = 11.2, 5.2 Hz, H_a-6'), 3.57 (1H, dd, *J* = 12.4, 5.8 Hz, H-4''), 3.19 (1H, m, H_a-5''), 2.93 (2H, m,

H-7); ^{13}C-NMR (CD_3OD, 100MHz) δ 140.1 (C-1), 130.1 (C-2, 6), 129.3 (C-3, 5), 127.2 (C-4), 105.2 (C-1''), 104.4 (C-1'), 77.9 (C-3'), 76.9 (C-5'), 75.1 (C-2'), 74.2 (C-3''), 72.4 (C-2''), 71.9 (C-8), 71.6 (C-4'), 69.5 (C-4''), 69.5 (C-6'), 66.8 (C-5''), 37.3 (C-7). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Yoshikawa *et al.*, 1997]. It is the first isolation of 2-phenylethyl-6-*O*-(*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside from this plant so far known.

2-Phenylethyl-6-*O*-(*β*-D-xylopyranosyl)-*β*-D-glucopyranoside (**52**): white, amorphous solid with the molecular formula of $C_{19}H_{28}O_{10}$ (416 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) δ 7.25 (4H, m, H-2, 3, 5, 6), 7.16 (1H, m, H-4), 4.31 (1H, d, *J* = 7.2 Hz, H-1'), 4.29 (1H, d, *J* = 7.6 Hz, H-1''), 4.08 (1H, dd, *J* = 11.2, 2.0 Hz, H_b-6'), 4.06 (1H, m, H_b-8), 3.85 (1H, dd, *J* = 11.6, 4.8 Hz, H_b-5''), 3.71-3.78 (2H, m, H_a-8, H_a-6'), 3.15-3.22 (3H, m, H-2', 2'', H_a-5''), 2.93 (2H, m, H-7); ^{13}C-NMR (CD_3OD, 100MHz) δ 140.1 (C-1), 130.1 (C-2, 6), 129.4 (C-3, 5), 105.5 (C-1''), 104.4 (C-1'), 99.0 (C-1'), 77.9 (C-3'), 77.7 (C-5'), 77.0 (C-3''), 75.0 (C-2''), 74.9 (C-2'), 71.9 (C-8), 71.4 (C-4''), 71.2 (C-4'), 69.8 (C-6'), 66.9 (C-5''), 37.3 (C-7). The above ^{1}H- and ^{13}C-NMR data were the same with those of the reported values [Otsuka *et al.*, 1990]. It is the first isolation of 2-phenylethyl-6-*O*-(*β*-D-xylopyranosyl)-*β*-D-glucopyranoside from this plant so far known.

2-Phenylethyl-6-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranoside (**53**): white, amorphous solid with the molecular formula of $C_{20}H_{30}O_{11}$ (446 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) δ 7.25 (4H, m, H-2, 3, 5, 6), 7.16 (1H, m, H-4), 4.37 (1H, d, *J* = 8.0 Hz, H-1'), 4.31 (1H, d, *J* = 7.6 Hz, H-1''), 4.14 (1H, dd, *J* = 11.2, 2.0 Hz, H_b-6'), 4.07 (1H, m, H_b-8), 3.85 (1H, dd, *J* = 12.0, 2.0 Hz, H_b-6''), 3.78 (1H, m, H_a-8), 3.76 (1H, dd, *J* = 11.2, 4.8 Hz, H_a-6'), 3.65 (1H, *J* = 12.0, 5.6 Hz, H_a-6''), 3.44 (1H, m, H-5'), 3.16-3.23 (2H, m, H-2', 2''), 2.93 (2H, m, H-7); ^{13}C-NMR (CD_3OD, 100MHz) δ 140.1 (C-1), 130.1 (C-2, 6),

129.4 (C-3, 5), 127.2 (C-4), 104.9 (C-1'), 104.5 (C-1''), 78.1 (C-3'), 78.0 (C-3''), 77.1 (C-5'), 77.1 (C-5''), 75.1 (C-2'), 75.1 (C-2''), 71.9 (C-8), 71.6 (C-4'), 71.5 (C-4''), 69.8 (C-6'), 62.8 (C-6''), 37.3 (C-7). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Araya *et al.*, 2005]. It is the first isolation of 2-phenylethyl-6-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranoside from this plant so far known.

Macrophylloside D (**54**): white, amorphous solid with the molecular formula of $C_{25}H_{34}O_{14}$ (558 $[M]^{+}$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 7.69 (1H, s, H-6), 6.48 (1H, s, H-3), 6.34 (1H, d, *J* = 9.6 Hz, H-1'), 6.22 (1H, m, H-2'), 6.21 (1H, d, *J* = 7.6 Hz, H-1''), 4.35 (1H, d, *J* = 7.6 Hz, H-1'''), 4.18 (1H, dd, *J* = 11.6, 2.0 Hz, H_b-6'''), 3.84 (3H, s, OCH_3), 3.82 (1H, dd, *J* = 12.0, 2.0 Hz, H_b-6''), 3.79 (1H, dd, *J* = 11.6, 5.2 Hz, H_a-6'''), 3.65 (1H, dd, *J* = 12.0, 5.6 Hz, H_a-6''), 1.43 (6H, d, *J* = 0.8 Hz, 2 × CH_3); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 165.1 (C-7), 163.6 (C-2), 160.4 (C-4), 131.7 (C-6), 129.8 (C-2'), 121.9 (C-1'), 114.9 (C-1), 111.2 (C-5), 104.4 (C-1'''), 101.3 (C-3), 95.6 (C-1''), 78.9 (C-3'), 77.9 (C-3'''), 77.9 (C-5'''), 77.9 (C-3''), 77.8 (C-5''), 75.0 (C-2'''), 73.9 (C-2''), 71.4 (C-4'''), 70.9 (C-4''), 69.4 (C-6''), 62.6 (C-6'''), 56.5 (OCH_3), 28.7 (C-4', 5'). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Tan *et al.*, 1996]. It was reported in *G. macrophylla*, *G. straminea*, *G. dahurica* and *G. lutea*, and this is the first isolation of macrophylloside D from this species.

Poacynose (**55**): white, amorphous solid with the molecular formula of $C_{18}H_{24}O_{11}$ (416 $[M]^{+}$). ^{1}H-NMR (CD_3OD, 400MHz) *δ* 8.09 (2H, d, *J* = 7.6 Hz, H-2, 6), 7.63 (1H, t, *J* = 7.6 Hz, H-4), 7.50 (1H, d, *J* = 9.6 Hz, H-1'), 6.22 (1H, m, H-2'), 6.21 (1H, d, *J* = 7.6 Hz, H-1''), 4.35 (2H, t, *J* = 7.6 Hz, H-3, 5), 5.70 (1H, d, *J* = 7.6 Hz, H-1'), 4.28 (1H, d, *J* = 7.2 Hz, H-1''); ^{13}C-NMR (CD_3OD, 100MHz) *δ* 166.7 (C-7), 134.8 (C-4), 131.0 (C-2,

6), 130.8 (C-1), 129.6 (C-3, 5), 105.2 (C-1''), 96.3 (C-1'), 77.9 (C-3'), 77.8 (C-5'), 77.7 (C-3''), 74.8 (C-2''), 74.0 (C-2'), 71.1 (C-4'), 71.0 (C-4''), 69.5 (C-6'), 66.9 (C-5''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Morikawa *et al.*, 2012]. It is the first isolation of poacynose from this plant.

Isoconiferinoside (**56**): white, amorphous solid with the molecular formula of $C_{22}H_{32}O_{13}$ (504 $[M]^+$). ^{1}H-NMR (CD_3OD, 400MHz) δ 7.10 (1H, d, J = 8.0 Hz, H-6), 7.09 (1H, d, J = 2.4 Hz, H-3), 6.95 (1H, dd, J = 8.0, 2.4 Hz, H-5), 6.62 (1H, d, J = 16.0 Hz, H-1'), 6.28 (1H, dt, J = 16.0, 6.4 Hz, H-2'), 4.89 (1H, d, J = 7.6 Hz, H-1''), 4.36 (1H, d, J = 8.0 Hz, H-1'''), 3.86 (3H, s, OCH_3); ^{13}C-NMR (CD_3OD, 100MHz) δ 150.9 (C-2), 147.8 (C-1), 133.4 (C-4), 133.4 (C-1'), 125.6 (C-2'), 120.9 (C-5), 117.8 (C-6), 111.4 (C-3), 103.3 (C-1'''), 102.7 (C-1''), 78.2 (C-3''),78.1 (C-3'''), 78.0 (C-5'''), 77.9 (C-5''), 75.2 (C-2'''), 74.9 (C-2''), 71.7 (C-4'''), 71.3 (C-4''), 70.8 (C-3'), 62.8 (C-6'''), 62.5 (C-6''), 56.7 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Sugiyama *et al.*, 1993].

Loganic acid (**LA**): white, amorphous solid with the molecular formula of $C_{16}H_{24}O_{10}$ (376 $[M]^+$). δ 7.35 (1H, d, J = 1.2 Hz, H-3), 5.22 (1H, d, J = 4.0 Hz, H-1), 4.61 (1H, d, J = 8.4 Hz, H-1'), 4.00 (1H, dd, J = 4.8, 3.6 Hz, H-7), 3.85 (1H, dd, J = 12.0, 2.0 Hz, H_b-6'), 3.62 (1H, dd, J = 12.0, 5.6 Hz, H_a-6'), 3.15 (1H, dd, J = 8.4, 8.0 Hz, H-2'), 3.05 (1H, m, H-5), 2.18 (1H, m, H-9), 1.98 (1H, m, H_b-6), 1.82 (1H, m, H-8), 1.61 (1H, m, H_a-6), 1.05 (3H, d, J = 6.8 Hz, H-10); ^{13}C-NMR (CD_3OD, 100MHz) δ 170.9 (C-11), 152.1 (C-3), 114.1 (C-4), 100.0 (C-1'), 97.6 (C-1), 78.3 (C-5'), 77.9 (C-3'), 75.1 (C-7), 74.7 (C-2'), 71.5 (C-4'), 62.7 (C-6'), 46.5 (C-9), 42.6 (C-6), 42.1 (C-8), 32.1 (C-5), 13.4 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Calis *et al.*, 1984].

2.4 Inhibitory activity against LPS-induced NO, IL-6 and TNF-α productions

Among the isolated secoiridoids, **44** and **45** were not tested for their inhibitory effect against IL-6, TNF-α and NO productions in LPS-induced RAW264 cells due to the low amounts. The prior cell viability assay showed that all the test compounds at a dose level up to 100.00 μM or parthenolide at a dose level up to 10.00 μM did not exhibit obvious cytotoxicity. Four doses of test compounds (12.50-100.00 μM) or parthenolide (1.25-10.00 μM) in the presence of LPS (0.1 μg/ml) for 24 h were taken for the assay of inhibitory effect against IL-6, TNF-α and NO productions. As a result (Table 2.3), compounds **29**-**33**, **36**, **38**-**40** exhibited inhibitory effects on both NO and IL-6 productions with IC_{50} of 79.88-95.02 μM and 70.62-77.42 μM, whereas **37**, **41** and **LA** exhibited inhibitory effects against only IL-6 production with IC_{50} of 75.35, 88.09 and 51.28 μM, respectively. All the test compounds exhibited weak inhibition (IC_{50} >100 μM) in the case of TNF-*α* assay (data not listed).

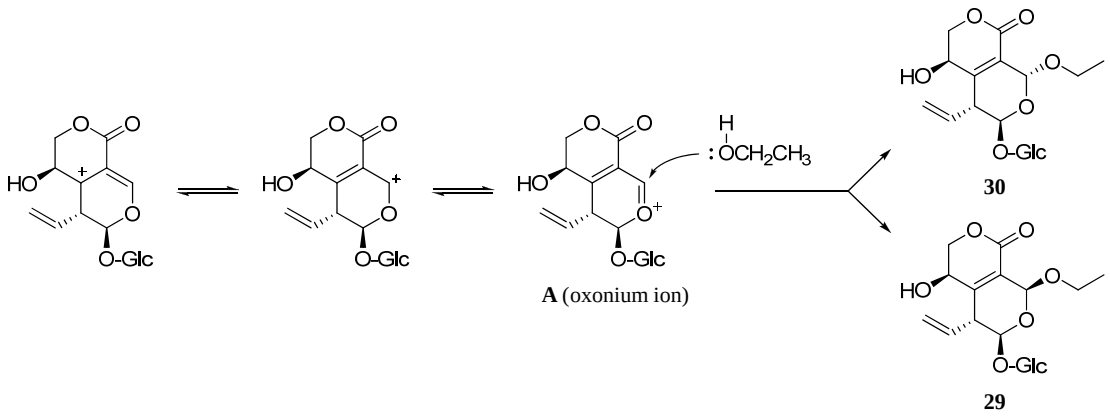
Table 2.3 Inhibitory effects of compounds **LA** and **29**-**43** on LPS-induced NO and IL-6 productions in RAW264 cells

compound	inhibition of NO	inhibition of IL-6	compound	inhibition of NO	inhibition of IL-6
	IC_{50} (μM)[a]	IC_{50} (μM)		IC_{50} (μM)	IC_{50} (μM)
29	89.71 ± 0.60	73.99 ± 1.87	**38**	81.45 ± 3.27	73.56 ± 4.01
30	87.37 ± 0.66	76.93 ± 0.65	**39**	93.41 ± 1.77	77.19 ± 2.37
31	79.88 ± 2.01	70.62 ± 0.34	**40**	90.69 ± 1.74	77.29 ± 1.85
32	89.51 ± 1.58	71.56 ± 2.11	**41**	NA	88.09 ± 2.27
33	94.21 ± 1.57	77.42 ± 2.54	**LA**	NA	51.28 ± 3.20
36	95.02 ± 2.53	74.06 ± 3.69	**34, 35, 42, 43**	NA	NA
37	NA[b]	75.35 ± 2.36	parthenolide	3.10 ± 0.14	1.93 ± 0.20

[a] The IC_{50} values were calculated using SigmaPlot (Version 11.5) with a four parameter logistic nonlinear regression model with the sample absorbencies over the log concentrations of the test samples. The results are expressed as the mean ± SD of three independent experiments. [b] NA: test compounds having IC_{50} values more than 100 μM.

2.5 Discussions

Phytochemical investigation of the MeOH extract of GMR derived from *G. crassicaulis* led to the isolation of mostly secoiridoids (glycosides and aglycones). Among them, two novel secoiridoid glycosides (**41** and **42**) with additional ether linkage between sugar moiety and aglycon were found. Compounds of this type in the plant kingdom are rare, which could be important chemical characteristics of GMR derived from *G. crassicaulis*. Besides, compounds **29** and **30** were supposed to be artifacts because the two could not be detected in H_2O or MeOH extract of dried roots of *G. crassicaulis*. These two compounds might be produced during separation processes in which EtOH was used as solvent for re-dissolution. The occurrence of both C-3 epimers **29** and **30** suggests that they might be formed through an oxonium ion intermediate **A** (Scheme 2.1) [Boros *et al.*, 1990].



Scheme 2.1 Proposed pathway of formation of gentiananosides C (**30**) and D (**29**)

Bioactivity assay showed that all the isolated secoiridoid glycosides exhibited relatively weak anti-inflammatory activity *in vitro* when compared with the positive control parthenolide, which was similar with the previous results of GSR. Herein, the predominant components swertiamarin (**36**) and loganic acid (**LA**) found in GMR exhibited moderate activity but have already been proven to be active in

anti-inflammatory studies *in vivo*, and some *in vitro* metabolites of which exhibited promising activity [Park *et al.*, 2010; Wang *et al.*, 2014]. The present and previous results implied that the secoiridoid glycosides might be precursors for a series of anti-inflammatory metabolites and the conversion to its real active metabolites are essential for their pharmaceutical actions.

By comparing the IC_{50} value of test compounds, the presence of hydroxyl group at C-5 in **36** (*vs.* **37**) enhanced the inhibitory effect against IL-6 production, which was agreed well with the previously reported results [Wang *et al.*, 2013; Lv *et al.*, 2012]. The presence of hydroxyl group at C-6 in **41** (*vs.* **42**) also enhanced the inhibitory effect against IL-6 production. Moreover, secoiridoid glycosides containing chains of disaccharide units in **31**, **32** and **33** exhibited stronger IL-6 and NO inhibitions than that of monosaccharide unit in **9** or polysaccharide units in **34** and **35**. In the case of chains of disaccharide units, the presence of primeverosyl unit composed of one xylose and one glucose joined with a β(1→6) glycosidic linkage in **31** exhibited stronger IL-6 and NO inhibitions than that of gentiobiosyl unit composed of two glucoses joined with the same glycosidic linkage in **33**. Further in the case of chains of disaccharide composed of two glucoses, the presence of a β(1→3) glycosidic linkage in **32** exhibited stronger IL-6 and NO inhibitions than that that of a β(1→6) glycosidic linkage in **33**. These finding implied that number, type and connectivity of sugar moieties in secoiridoid glycosides could also affect their anti-inflammatory activity.

Summary of Section II

1) The 30% and 60% MeOH eluates obtained from the crude extract of identified GMR through a macroporous resin fractionation procedure were found to possess the most promising anti-inflammatory activity, which was therefore taken for further investigation.

2) Phytochemical investigation of the above two bioactive factions led to the isolation of 20 secoiridoid glycosides (**7**-**9**, **29**-**45**), including five new compounds (**29**-**31**, **41**, **42**); as well as four lignans (**21**, **46**-**48**), one *C*-glucoflavonoid (**49**) and seven compounds of other types (**50**-**56**). Among the isolated compounds, gentiananosides A (**41**) and B (**42**) were concluded to be novel secoiridoid glycosides with an ether linkage between C-2' of the sugar moiety and C-3 of the aglycone. Among the known compounds, 18 compounds (**32**, **34**, **35**, **38**-**40**, **43**-**47**, **49**-**55**) are isolated from this plant for the first time.

3) *In vitro* anti-inflammatory bioassay showed that compounds **29**-**33**, **36**, **38**-**40** exhibited moderate NO and IL-6 inhibition with IC_{50} values of 79.88-95.02 μM, and 70.62-77.42 μM, respectively, while compounds **37**, **41** and **LA** exhibited only moderate IL-6 inhibition with IC_{50} values 75.35, 88.09 and 51.28 μM, respectively. All the test compounds exhibited weak inhibitory activity (IC_{50} >100 μM) in the case of TNF-α bioassay.

3. Phytochemical investigation of Gentianae Radix, the root and rhizome of *G. lutea*, and comparison of chemical composition among Gentianae Scabrae Radix, Gentianae Macrophyllae Radix and Gentianae Radix

3.1 Introduction

Gentianae Radix (GR) is commonly known as gentian or yellow gentian due to the yellow color of the root. GR is very well known for the bitter taste, and often used for bitter tonics and to flavor alcoholic drinks (bitters). Previous studies reported the bitter principles of secoiridoid types such as amarogentin, the most bitter compound known. It is also reported that the presence of xanthones resulted in the yellow color. Because of its market demand and none of available natural resource in Japan, the imported GR is sometimes substituted by other *Gentiana* drugs such as GSR, but the chemical and biological evidences are lacking. From a phytochemical viewpoint, this study aims to investigate the chemical constituents of GR.

To further clarify the phytochemical characteristics of the three *Gentiana* drugs may contribute to justify their different functions and therapies, and to achieve their standardization and quality assurance. However, detailed phytochemical comparison among them is lacking, and phytochemical characteristics of each *Gentiana* drug are obscure. Therefore, it is necessary to analyze the chemical composition of these *Gentiana* drugs comparatively. One of the feasible approaches is chromatographic fingerprinting analysis, which has been accepted as a strategy for identification and/or quality assessment of crude drugs by the WHO [WHO, 1991], the FDA [U.S. Department of Health and Human Services, CDEF, 2004], the European Agency for the Evaluation of Medicinal Products (EMEA) [EMEA, 2006] and the China Food and Drug Administration (CFDA) [Drug Administration Bureau of China, 2002]. Even though HPLC fingerprinting analysis has been used for identification and assessment of the stability of GSR and GMR derived from each species [Jiang *et al.*, 2005; Cao *et al.*, 2009; Liu *et al.*, 2014], only the common and principal components such as

loganic acid, gentiopicroside, swertiamarin, and sweroside were used as markers for overall evaluation. The variation in their chemical composition, especially the distinctive component in each of them remains unclear. Using the isolated compounds from GSR, GMR and GR as references, comparison of chemical composition in the three *Gentiana* drugs, as well as quantitative analysis of the referring compounds were performed to establish the differences and similarities.

3.2 Materials

Gentianae Radix (GR, ゲンチアナ) (produced in France, LOT No. 36-4704) was purchased from Maetyuu Co., Ltd. (Nara, Japan) on 12 February, 2014. It was morphologically identified as *G. lutea*. A voucher sample (TMPW No. 28010) has been deposited in the Museum of Materia Medica, Institute of Natural Medicine, University of Toyama, Japan.

3.3 Isolation and structure determination

The crude drug (100 g) was extracted with MeOH, followed by solvent-solvent partition to afford $CHCl_3$ and *n*-BuOH fractions. HPLC-guided isolation of these two fractions led to isolation of 20 compounds, including 11 secoiridoids (**9**, **36**, **37**, **43**, **44**, **57**-**62**), six xanthones (**63**-**68**), one *C*-glucoflavonoid (**69**), one lignan (**21**) and one methyl benzoate derivative (**27**). The isolation procedure was illustrated in Chart 3.1 and the structures of the isolated compounds were shown in Figure 3.1.

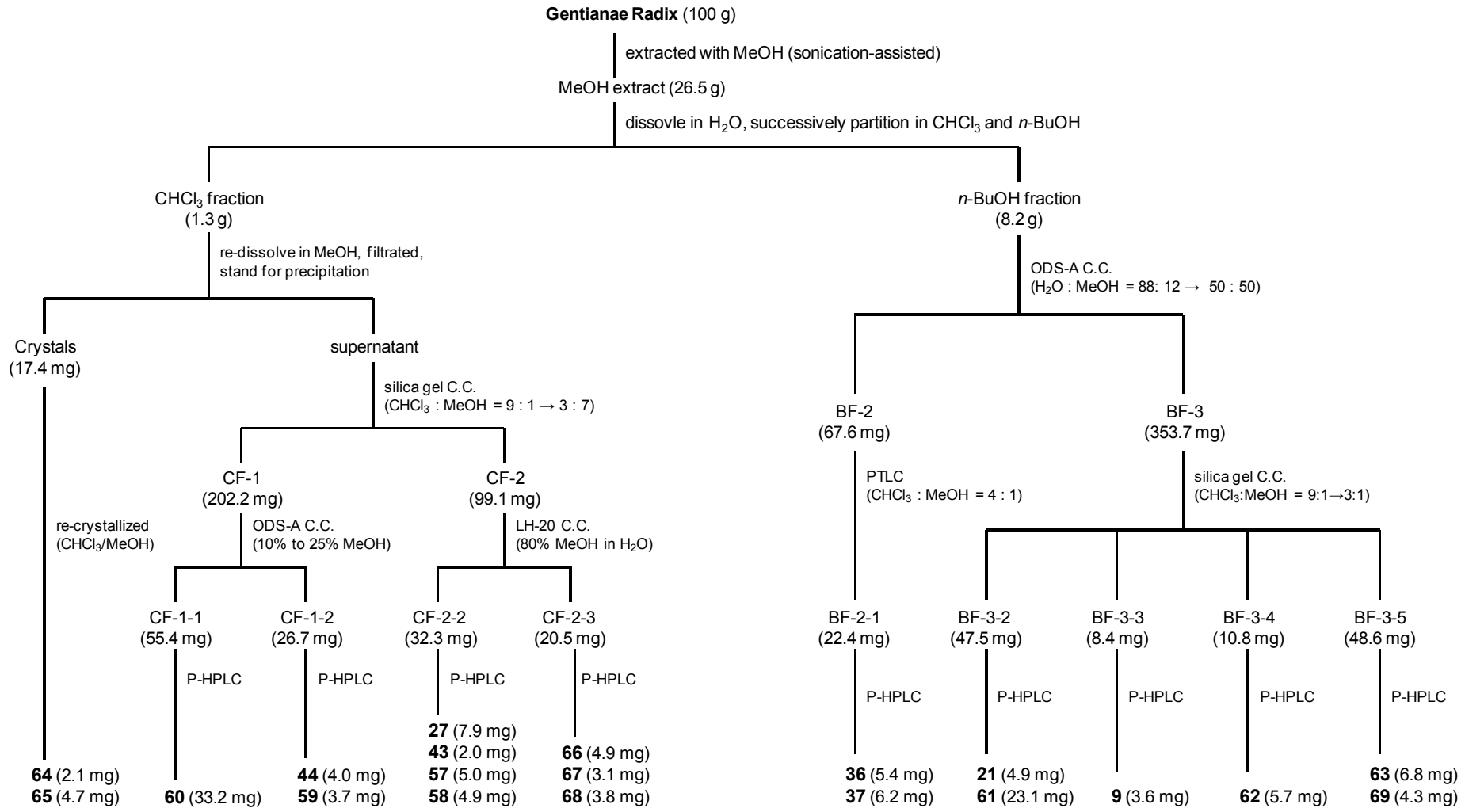


Chart 3.1 Isolation of Gentianae Radix (ゲンチアナ) derived from *G. lutea*

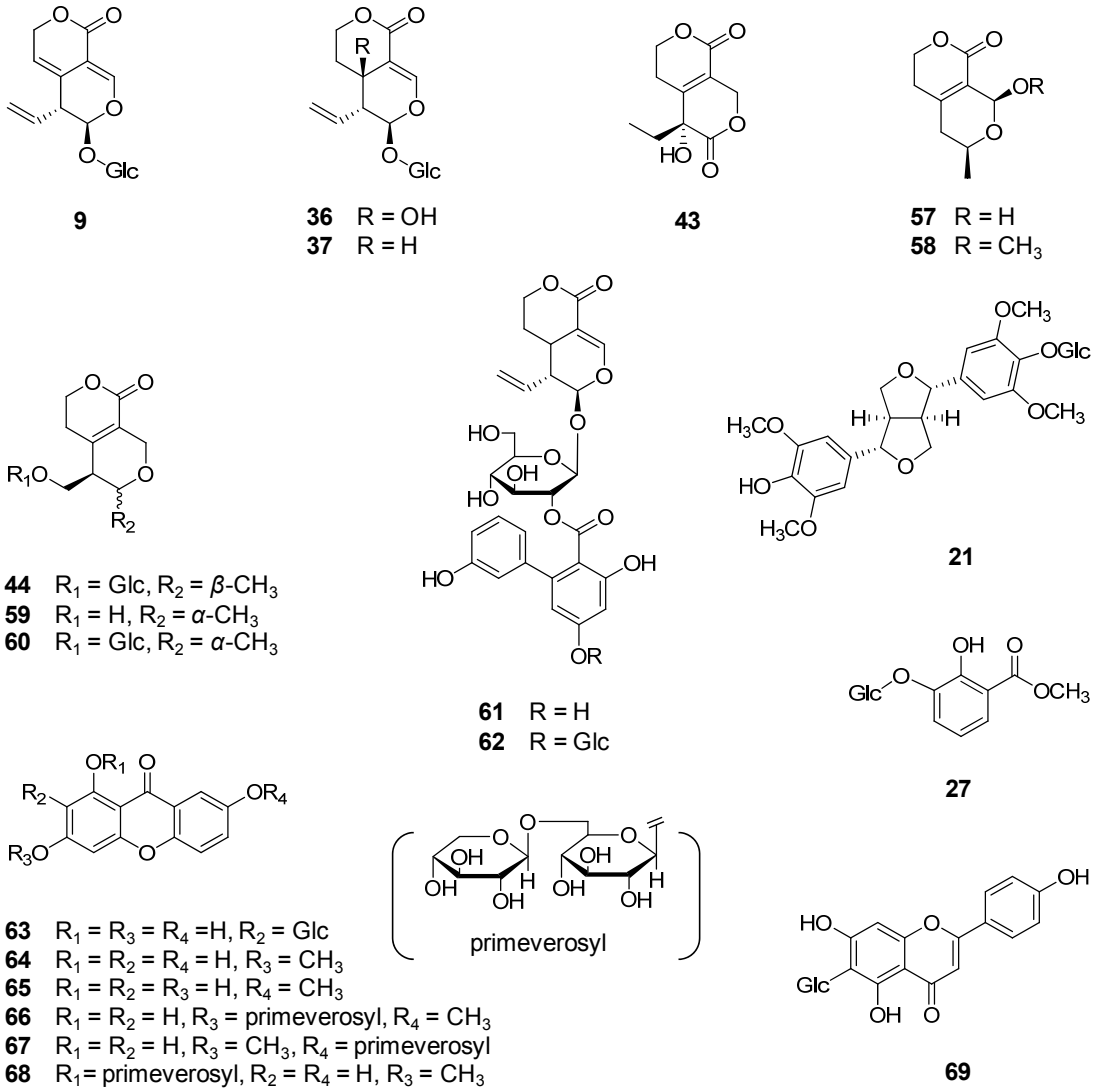


Figure 3.1 Structures of compounds isolated from Gentianae Radix (ゲンチアナ) derived from *G. lutea*

Swercinctolide B (**57**): colourless gum with the molecular formula of $C_9H_{12}O_4$ (184 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 5.58 (1H, s, H-3), 4.39 (2H, m, H-7), 4.26 (1H, m, H-8), 2.58 (1H, m, H_b-6), 2.38 (1H, ddd, J = 18.0, 5.2, 4.4 Hz, H_a-6), 2.27 (1H, dd, J = 19.2, 4.0 Hz, H_b-9), 2.19 (1H, dd, J = 19.2, 6.4 Hz, H_a-9),1.25 (3H, d, J = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz) δ 165.3 (C-11), 156.3 (C-5), 125.1 (C-4), 88.1 (C-3), 67.0 (C-7), 62.6 (C-8), 37.7 (C-9), 29.4 (C-6), 21.1 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Yang *et al.*, 2012]. It is the first isolation of swercinctolide B from this plant so far known.

Swerimilegenin E (**58**): colourless gum with the molecular formula of $C_{10}H_{14}O_4$ (198 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 5.14 (1H, s, H-3), 4.38 (2H, m, H-7), 4.14 (1H, m, H-8), 3.43 (3H, s, OCH_3), 2.58 (1H, m, H_b-6), 2.37 (1H, ddd, J = 18.0, 4.8, 4.4 Hz, H_a-6), 2.27 (1H, dd, J = 19.2, 4.0 Hz, H_b-9), 2.19 (1H, dd, J = 19.2, 10.4 Hz, H_a-9),1.27 (3H, d, J = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz) δ 165.1 (C-11), 157.0 (C-5), 123.7 (C-4), 95.5 (C-3), 66.9 (C-7), 62.9 (C-8), 55.8 (OCH_3), 37.5 (C-9), 29.3 (C-6), 20.9 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Geng *et al.*, 2013]. It is the first isolation of swerimilegenin E from this plant so far known.

Deglucosyl swertiajaposide D (**59**): colourless gum with the molecular formula of $C_{10}H_{14}O_4$ (198 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 4.44 (1H, m, H_b-7), 4.37 (1H, m, H_a-7), 4.29 (1H, m, H_b-3), 4.19 (1H, m, H_a-3), 3.84 (1H, dd, J = 12.0, 5.6 Hz, H_b-1), 3.80 (1H, m, H-8), 3.71 (1H, dd, J = 12.0, 3.6 Hz, H_a-1), 2.72 (1H, m, H_b-6), 2.37 (1H, m, H_a-6), 2.18 (1H, m, H-9), 1.30 (3H, d, J = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz) δ 165.9 (C-11), 153.4 (C-5), 125.4 (C-4), 71.6 (C-8), 67.5 (C-7), 63.5 (C-3), 60.2 (C-1), 48.4 (C-9), 27.0 (C-6), 19.3 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikuchi *et al.*, 2008]. It is the first isolation of deglucosyl swertiajaposide D from this plant so far known.

Swertiajaposide D (**60**): white, amorphous solid with the molecular formula of $C_{16}H_{24}O_9$ (360 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 4.43 (1H, dd, J = 10.8, 5.6 Hz, H_b-7), 4.38 (1H, dd, J = 10.8, 4.8 Hz, H_a-7), 4.30 (1H, m, H_b-3), 4.22 (1H, d, J = 8.0 Hz, H-1'), 4.21 (1H, m, H_a-3), 4.09 (1H, dd, J = 10.4, 3.2 Hz, H_b-1), 3.87 (1H, dd, J = 11.2, 5.6 Hz, H_b-6'), 3.84 (1H, m, H-8), 3.78 (1H, dd, J = 10.4, 4.0 Hz, H_a-1), 3.66 (1H, dd, J = 11.2, 2.8 Hz, H_a-6'), 3.13 (1H, dd, J = 8.8, 8.0 Hz, H-2'), 2.81 (1H, m, H_b-6), 2.41 (1H,

m, H_a-6), 2.36 (1H, m, H-9), 1.31 (3H, d, *J* = 6.4 Hz, H-10); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 166.0 (C-11), 153.5 (C-5), 125.0 (C-4), 104.6 (C-1'), 78.2 (C-3'), 78.1 (C-5'), 74.9 (C-2'), 71.8 (C-8), 71.6 (C-4'), 67.8 (C-7), 67.7 (C-1), 63.7 (C-3), 62.8 (C-6'), 46.5 (C-9), 26.8 (C-6), 19.3 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kikuchi *et al.*, 2008]. It is the first isolation of swertiajaposide D from this plant so far known.

Amarogentin (**61**): white, amorphous solid with the molecular formula of $C_{29}H_{30}O_{13}$ (586 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.43 (1H, d, *J* = 2.8 Hz, H-3), 7.17 (1H, t, *J* = 8.4 Hz, H-5'''), 6.78 (1H, dd, *J* = 8.4, 2.4 Hz, H-4'''), 6.72 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'''), 6.71 (1H, s, H-2'''), 6.29 (1H, d, *J* = 2.8 Hz, H-4''), 6.16 (1H, d, *J* = 2.8 Hz, H-6''), 5.42 (1H, m, H-8), 5.39 (1H, d, *J* = 1.2 Hz, H-1), 5.26 (1H, dd, *J* = 15.2, 2.0 Hz, H_b-10), 5.22 (1H, dd, *J* = 10.0, 2.0 Hz, H_a-10), 4.73 (1H, dd, *J* = 9.6, 7.6 Hz, H-2'), 4.37 (1H, ddd, *J* = 11.2, 4.0, 2.0 Hz, H_b-7), 4.29 (1H, d, *J* = 7.6 Hz, H-1'), 4.25 (1H, m, H_a-7), 3.84 (1H, dd, *J* = 12.0, 2.0 Hz, H_b-6'), 3.60 (1H, dd, *J* = 12.0, 6.0 Hz, H_a-6'), 3.23 (1H, t, *J* = 7.2 Hz, H-4'), 3.09 (1H, m, H-5'), 2.81 (1H, dd, *J* = 9.6, 7.2 Hz, H-3'), 2.74 (1H, m, H-5), 2.58 (1H, m, H-9), 1.69 (1H, m, H_b-6), 1.57 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 171.5 (C-7''), 167.6 (C-11), 166.0 (C-3''), 163.9 (C-5''), 157.4 (C-3'''), 153.7 (C-3), 148.6 (C-1''), 146.5 (C-1'''), 132.8 (C-8), 129.3 (C-5'''), 121.2 (C-6'''), 121.0 (C-10), 116.5 (C-2'''), 114.5 (C-4'''), 112.8 (C-6''), 105.6 (C-4), 104.1 (C-2''), 103.1 (C-4''), 97.2 (C-1), 96.8 (C-1'), 78.3 (C-5'), 74.8 (C-3'), 74.6 (C-2'), 71.6 (C-4'), 69.5 (C-7), 62.4 (C-6'), 43.4 (C-9), 28.7 (C-5), 25.8 (C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Wang *et al.*, 2001].

5"-*O*-*β*-D-Glucopyranosyl amarogentin (**62**): white, amorphous solid with the molecular formula of $C_{35}H_{40}O_{18}$ (748 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.29 (1H, d, *J* = 2.8 Hz, H-3), 7.17 (1H, t, *J* = 8.0 Hz, H-5'''), 6.79 (1H, dd, *J* = 8.0, 2.4 Hz, H-4'''), 6.73 (1H, dd, *J* = 8.0, 2.4 Hz, H-6'''), 6.72 (1H, s, H-2'''), 6.59 (1H, d, *J* = 2.4 Hz, H-4''), 6.44 (1H, d, *J* = 2.4 Hz, H-6''), 5.41 (1H, m, H-8), 5.37 (1H, d, *J* = 2.0 Hz, H-1), 5.25 (1H, dd, *J* = 15.2, 1.2 Hz, H_b-10), 5.22 (1H, dd, *J* = 11.6, 1.2 Hz, H_a-10), 5.05 (1H, d, *J* = 7.2 Hz, H-1''''), 4.73 (1H, dd, *J* = 9.6, 8.4 Hz, H-2'), 4.36 (1H, m, H_b-7), 4.34 (1H, d, *J* = 8.4 Hz, H-1'), 4.29 (1H, m, H_a-7), 3.87 (1H, dd, *J* = 12.4, 2.4 Hz, H_b-6'), 3.84 (1H, dd, *J* = 12.0, 2.4 Hz, H_b-6''''), 3.66 (1H, dd, *J* = 12.4, 6.4 Hz, H_a-6'), 3.60 (1H, dd, *J* = 12.0, 6.4 Hz, H_a-6''''), 3.23 (1H, t, *J* = 8.8 Hz, H-4'), 3.09 (1H, m, H-5'), 2.79 (1H, dd, *J* = 9.6, 8.8 Hz, H-3'), 2.75 (1H, m, H-5), 2.58 (1H, m, H-9), 1.71 (1H, m, H_b-6), 1.58 (1H, m, H_a-6); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 171.1 (C-7''), 167.7 (C-11), 165.4 (C-3''), 162.6 (C-5''), 157.6 (C-3'''), 153.7 (C-3), 148.1 (C-1''), 146.0 (C-1'''), 132.8 (C-8), 129.5 (C-5'''), 121.1 (C-6'''), 121.0 (C-10), 116.5 (C-2'''), 114.8 (C-4'''), 113.3 (C-6''), 106.6 (C-2''), 105.5 (C-4), 104.2 (C-4''), 100.9 (C-1''''), 97.7 (C-1), 97.3 (C-1'), 78.4 (C-5', C-5''''), 77.8 (C-3''''), 75.0 (C-3'), 74.8 (C-2', C-2''''), 71.6 (C-4'), 71.3 (C-4''''), 69.6 (C-7), 62.6 (C-6''''), 62.4 (C-6'), 43.5 (C-9), 28.6 (C-5), 25.8 (C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ando *et al.*, 2002]. It is the first isolation of 5"-*O*-*β*-D-glucopyranosyl amarogentin from this plant so far known.

Neolancerin (**63**): yellow, amorphous solid with the molecular formula of $C_{19}H_{18}O_{10}$ (406 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) *δ* 7.48 (1H, d, *J* = 3.2 Hz, H-8), 7.37 (1H, d, *J* = 7.2 Hz, H-5), 7.25 (1H, dd, *J* = 7.2, 3.2 Hz, H-6), 6.38 (1H, s, H-4), 4.91 (1H, d, *J* = 9.6 Hz, H-1'), 4.19 (1H, dd, *J* = 9.6, 9.2 Hz, H-2'), 3.88 (1H, dd, *J* = 12.4, 2.4 Hz, H_b-6'), 3.73 (1H, dd, *J* = 12.4, 5.6 Hz, H_a-6'); ^{13}C-NMR (CD_3OD, 100 MHz) *δ* 182.0 (C-9),

166.1 (C-3), 163.5 (C-1), 158.9 (C-11), 155.4 (C-7), 151.1 (C-12), 125.4 (C-6), 122.1 (C-13), 119.8 (C-5), 109.4 (C-8), 107.9 (C-2), 103.6 (C-10), 94.9 (C-4), 82.6 (C-5'''), 80.2 (C-3'''), 75.3 (C-1'''), 72.5 (C-2'''), 71.8 (C-4'''), 62.9 (C-6'''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Li *et al.*, 1998]. It is the first isolation of neolancerin from this plant so far known.

Gentisin (**64**): yellow needles with the molecular formula of $C_{14}H_{10}O_5$ (258 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) δ 7.48 (1H, d, J = 9.2 Hz, H-5), 7.41 (1H, d, J = 3.2 Hz, H-8), 7.30 (1H, dd, J = 9.2, 3.2 Hz, H-6), 6.59 (1H, d, J = 1.2 Hz, H-4), 6.36 (1H, d, J = 1.2 Hz, H-2), 3.87 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100 MHz) δ 180.0 (C-9), 166.4 (C-3), 162.4 (C-1), 157.4 (C-11), 154.1 (C-7), 149.1 (C-12), 124.8 (C-6), 120.4 (C-13), 119.0 (C-5), 107.9 (C-8), 102.8 (C-10), 96.8 (C-2), 92.5 (C-4), 56.1 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Takaaki *et al.*, 1988].

Isogentisin (**65**): yellow needles with the molecular formula of $C_{14}H_{10}O_5$ (258 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) δ 7.49 (1H, d, J = 9.2 Hz, H-5), 7.41 (1H, d, J = 2.8 Hz, H-8), 7.30 (1H, dd, J = 9.2, 2.8 Hz, H-6), 6.32 (1H, d, J = 2.0 Hz, H-4), 6.17 (1H, d, J = 2.0 Hz, H-2), 3.83 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100 MHz) δ 179.4 (C-9), 165.7 (C-3), 162.7 (C-1), 157.4 (C-11), 155.6 (C-7), 150.0 (C-12), 124.4 (C-6), 120.1 (C-13), 119.2 (C-5), 105.2 (C-8), 101.9 (C-10), 98.0 (C-2), 92.8 (C-4), 56.7 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ando *et al.*, 2007].

Gentioside (**66**): colourless needles with the molecular formula of $C_{25}H_{28}O_{14}$ (552 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) δ 7.65 (1H, d, J = 9.2 Hz, H-5), 7.51 (1H, d, J = 2.8 Hz, H-8), 7.47 (1H, dd, J = 9.2, 2.8 Hz, H-6), 6.76 (1H, d, J = 2.0 Hz, H-4), 6.46 (1H, d,

J = 2.0 Hz, H-2), 5.03 (1H, d, *J* = 6.8 Hz, H-1'), 4.17 (1H, d, *J* = 7.6 Hz, H-1"), 3.87 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100 MHz) *δ* 180.1 (C-9), 164.2 (C-3), 162.2 (C-1), 157.2 (C-11), 155.8 (C-7), 150.3 (C-12), 125.0 (C-6), 120.2 (C-13), 119.6 (C-5), 105.2 (C-8), 104.2 (C-1"), 103.6 (C-10), 99.8 (C-1'), 98.7 (C-2), 94.6 (C-4), 55.8 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Takaaki *et al.*, 1988].

1-Hydroxy-3-methoxy-7-*O*-primeverosylxanthone (**67**): colourless needles with the molecular formula of $C_{25}H_{28}O_{14}$ (552 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) *δ* 7.69 (1H, s, H-8), 7.67 (1H, dd, *J* = 10.0, 2.4 Hz, H-6), 7.61 (1H, d, *J* = 10.0 Hz, H-5), 6.65 (1H, d, *J* = 1.6 Hz, H-4), 6.42 (1H, d, *J* = 1.6 Hz, H-2), 4.92 (1H, d, *J* = 6.8 Hz, H-1'), 4.17 (1H, d, *J* = 7.6 Hz, H-1"), 3.90 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100 MHz) *δ* 179.8 (C-9), 166.5 (C-3), 162.4 (C-1), 157.3 (C-11), 153.8 (C-7), 150.7 (C-12), 125.5 (C-6), 120.2 (C-13), 119.2 (C-5), 110.5 (C-8), 103.9 (C-1"), 102.8 (C-10), 101.2 (C-1'), 96.9 (C-2), 92.6 (C-4), 56.1 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Takaaki *et al.*, 1988].

7-Hydroxy-3-methoxy-*O*-primeverosylxanthone (**68**): colourless needles with the molecular formula of $C_{25}H_{28}O_{14}$ (552 $[M]^+$). ^{1}H-NMR (DMSO, 400 MHz) *δ* 7.42 (1H, d, *J* = 8.8 Hz, H-5), 7.41 (1H, s, H-8), 7.23 (1H, dd, *J* = 8.8, 3.2 Hz, H-6), 6.80 (2H, s, H-4, H-2), 4.92 (1H, d, *J* = 8.0 Hz, H-1'), 4.18 (1H, d, *J* = 7.2 Hz, H-1"), 3.90 (3H, s, OCH_3); ^{13}C-NMR (DMSO, 100 MHz) *δ* 174.5 (C-9), 164.5 (C-3), 159.2 (C-1), 158.6 (C-11), 153.9 (C-7), 148.1 (C-12), 123.5 (C-6), 122.7 (C-13), 118.5 (C-5), 108.9 (C-8), 106.5 (10), 104.1 (C-1"), 102.5 (C-1'), 100.3 (C-2), 95.4 (C-4), 68.6 (C-6'), 65.6 (C-6"), 56.1 (OCH_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Takaaki *et al.*, 1988].

Isovitexin (**69**): yellow, amorphous solid with the molecular formula of $C_{21}H_{20}O_{10}$ (432 $[M]^+$). ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.80 (2H, d, J = 9.2 Hz, H-2', H-6'), 6.90 (1H, d, J = 8.8 Hz, H-3', H-5'), 6.55 (1H, s, H-3), 6.46 (1H, s, H-8), 4.90 (1H, d, J = 7.2 Hz, H-1'), 4.18 (1H, dd, J = 8.0, 7.2 Hz, H-2'), 3.89 (1H, dd, J = 10.4, 2.4 Hz, H_b-6'), 3.75 (1H, dd, J = 10.4, 5.2 Hz, H_a-6'); ^{13}C-NMR (CD_3OD, 100 MHz) δ 184.0 (C-4), 166.1 (C-2), 164.9 (C-7), 162.8 (C-4'), 162.0 (C-5), 158.7 (C-9), 129.4 (C-2', C-6'), 123.1 (C-1'), 117.0 (C-3', C-5'), 109.2 (C-6), 105.2 (C-10), 103.8 (C-3), 95.2 (C-8), 82.6 (C-5'''), 80.1 (C-3'''), 75.3 (C-1'''), 72.6 (C-2'''), 71.8 (C-4'''), 62.9 (C-6'''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Rayyan *et al.*, 2005].

3.4 HPLC comparison of chemical composition among GSR, GMR and GR

3.4.1 Selections of extractions and HPLC conditions

Based on the previously known procedure [Wu, 2014], extraction with MeOH under ultra-sonication was adopted and 0.1% formic acid in H_2O (A)-acetonitrile (B) was selected as mobile phase in this study. A gradient elution (0 min, 10% B; 2 min, 10% B; 15 min, 15% B; 40 min, 40% B; 50 min, 95% B; 58min, 95% B) with flow rate at 1.0 mL/min showed good resolution and separation of the mostly peaks detected in the HPLC system.

An ideal fingerprint chromatogram should contain sufficient information about the constituents and represent the characteristics of the samples, *e.g.* separation of characteristic peaks, appropriate retention time and steady baseline. In order to achieve an optimized chromatogram, UV spectra were recorded from 190 to 400 nm

and multiple chromatograms at different wavelengths were investigated. The wavelength 254 nm was selected for detection, at which both the resolution of detectable peaks and the baseline reach an optimization in chromatograms. This wavelength also provided the best resolution of gentiopicroside (**9**), 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), swertiamarin (**36**), sweroside (**37**) and macrophylloside D (**54**) in the MeOH extract of the samples, therefore it was chosen for their quantitative analyses. Other quantitative constituents (**LA**, **11**-**14**, **16**, **17**, **61**, **64**, **66**, **67**, and **69**) showed the maximum UV absorption at nearly 210 nm, at which peak areas were used for quantitative analyses.

3.4.2 Determination of relative retention times (*RRT*) and identification of peaks

Gentiopicroside (gps, **9**) was chosen as a reference due to its relatively high content, easy accessibility and suitable retention time, then the *RRT*s of the detected peaks with orders (1, 2, …, n) were calculated following the formula:

$RRT_{(x)} = t_{R(x)} / t_{R(gps)}$, x = 1, 2, …, n.

For compounds **LA**, **34**, **33**, **36**, **9**, **37**, **63**, **69**, **62**, **68**, **54**, **61**, **66**, **67**, **13**, **11**, **14** (**15**), **12**, **16**, **17** and **64** (**65**), the *RRT*s are 0.541, 0.572, 0.774, 0.794, 1.000, 1.042, 1.358, 1.484, 1.499, 1.701, 1.706, 1.868, 1.894, 1.921, 2.202, 2.223, 2.302, 2.419, 2.460, 2.576, 2.627, respectively. These values were stable on different samples. By comparing the *RRT*s, peak orders and spectroscopic data with those of reference compounds, the detected peaks in the MeOH extract of the samples were identified.

3.4.3 Comparative analysis of the samples

The representative HPLC chromatograms of GSR, GMR and GR were shown in Figure 3.2. Five common peaks were shown up in all the samples, which were identified as loganic acid (**LA**), 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), swertiamarin (**36**), gentiopicroside (**9**) and sweroside (**37**). Some distinctive peaks in each sample were also observed, which could be used as potential chemical markers. In detail, the chemical composition of GR obviously differed from those of GSR and GMR in containing xanthones (**63**-**68**). GMR containing macrophylloside D (**54**) belonging to 2-methoxyanofinic acid derivative clearly differed from GSR containing a group of acetylated and/or benzoylated secoiridoid glycosides (**11**-**17**). Besides, some identified common compounds such as loganic acid (**LA**) and gentiopicroside (**9**) apparently differed in their contents.

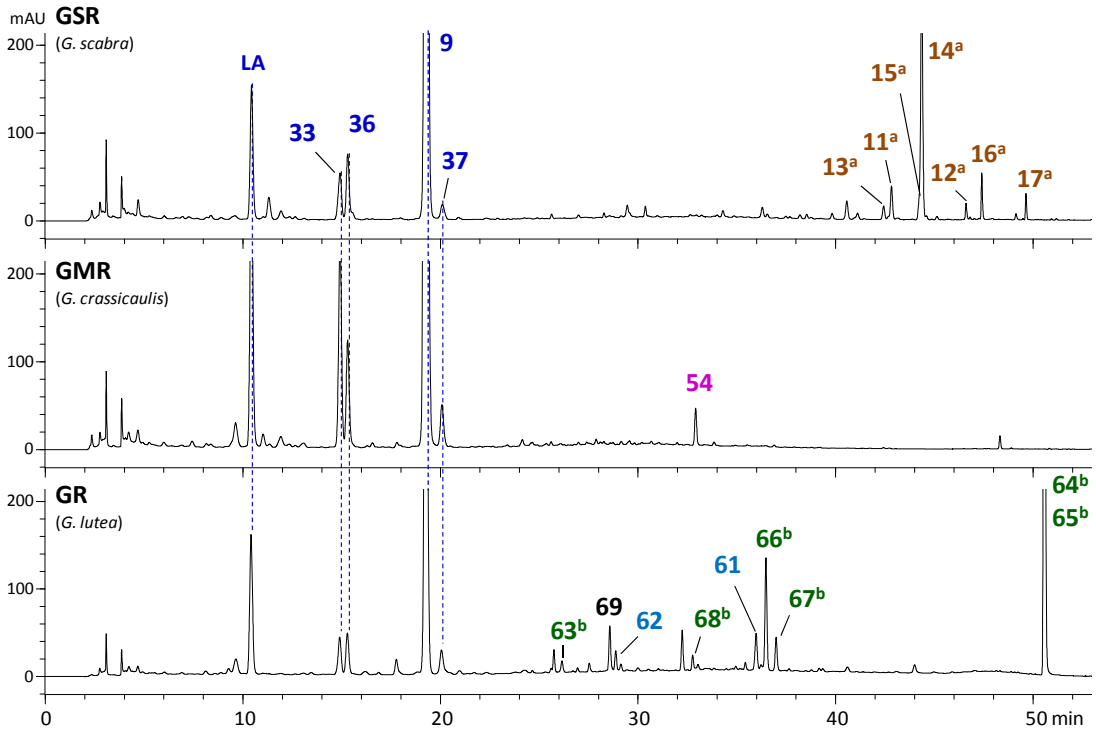


Figure 3.2 Representative HPLC chromatograms of GSR, GMR and GR (254 nm). a, acetylated and/or benzoylated secoiridoid glycosides; b, xanthones.

3.4.4 Calibration curves and contents of quantitative compounds

Using the available methods, total 17 identified constituents of GSR, GMR and GR were simultaneously measured. The result was shown in Figure 3.3.

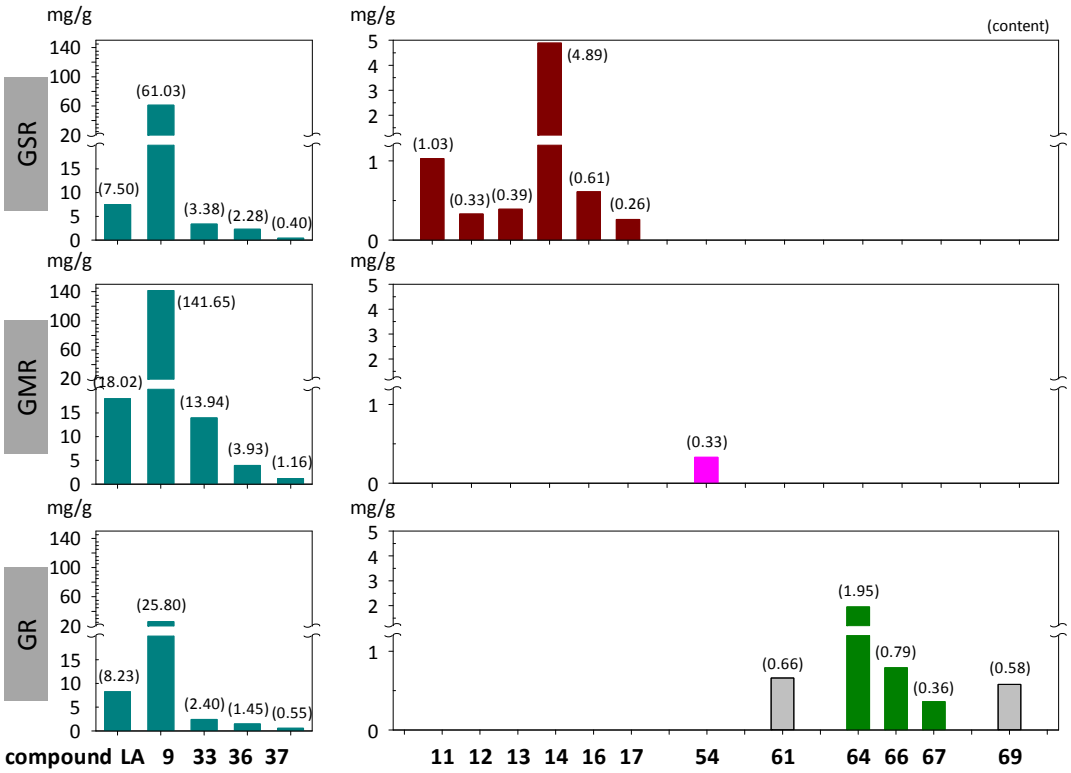


Figure 3.3 Contents of 17 compounds in GSR, GMR and GR

Loganic aicd (**LA**), gentiopicroside (**9**), 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), swertiamarin (**36**), and sweroside (**37**) as the common principles of the three *Gentiana* drugs, they showed the similar content pattern with the most high content of gentiopicroside (**9**), followed by loganic acid (**LA**), 6'-*O*-*β*-D-glucopyranosyl-gentiopicroside (**33**), swertiamarin (**36**) and sweroside (**37**) in sequence. On the other hand, the contents of them varied largely in each *Gentiana* drug. GMR containing the most high contents of the five common components among the three *Gentiana* drugs, with loganic acid (**LA**) of 18.02 mg/g, gentiopicroside (**9**) of 141.65 mg/g, 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**) of 13.94 mg/g, swertiamarin (**36**) of 3.93 mg/g,

and sweroside (**37**) of 1.16 mg/g. 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**) showed the maximum content variation from 2.40 mg/g in GR to 13.94 mg/g in GMR. These finding could be considered as important chemical characteristics of the three *Gentiana* drugs. The contents of distinctive compounds in each *Gentiana* drug were also determined. Among the acetylated and/or benzoylated derivates of secoiridoid glycosides in GSR, trifloroside (**14**) showed the highest content of 4.89 mg/g. GR contained a series of xanthones, with the highest content of gentisin (**64**) 1.95 mg/g. GMR contained macrophylloside D (**54**) of 0.33 mg/g.

3.5 Discussions

Phytochemical investigation of the MeOH extract of GR derived from *G. lutea* led to the isolation of a series of secoiridoids (glycosides and aglycones), xanthones and compounds of other types. Among the secoiridoids, compounds **57** and **58** with 9-*C* skeleton are isolated from GR derived from *G. lutea* for the first time. Compounds of this type were seldom reported from the recorded GSR and GMR origins, therefore they could be used as potential marker for identification.

Comparative study on the three *Gentiana* drugs revealed the similarities and differences among them. Some distinctive peaks in each sample were also observed, which could be used as potential chemical markers. For example, xanthones as the distinctive components clearly differed from the chemical composition of GSR and GMR. Moreover, some common principles of each *Gentiana* drugs also differed in their content. These findings could be used for the identification and standardization of *Gentiana* drug.

For the content determination, the gentiopicroside content prescribed in CP should be not less than 30 mg/g in GSR, while the sum of gentiopicroside and loganic acid should be not less than 25 mg/g in GMR. From the results above, both the employed GSR and GMR qualified the requirements of quality standards. On the other hand, the current quality control strategy for determining a single constituent as gentiopicroside or few constituents seems to be not reliable because of the idea that the therapeutic effect of *Gentiana* drugs originates from the comprehensive activities of the multiple constituents. The present study for simultaneous determination of 17 compounds in *Gentiana* drugs denote a major improvement, as it enables the determination of known major, sub-major and minor compounds, which might related to the therapeutic effects. Quantitative analysis of the three *Gentiana* drugs revealed the content of common principles varied largely in each drug, which could be another important chemical characteristics of the three *Gentiana* drugs. All the results above have been confirmed by further analyses on more samples of *Gentiana* species and drugs

Summary of Section III

1) Chemical investigation on the MeOH extract of GR led to isolation and identification of 20 compounds, including 11 secoiridoids (**9**, **36**, **37**, **43**, **44**, **57**-**62**), six xanthones (**63**-**68**), one *C*-glucoflavonoid (**69**), one lignan (**21**) and one methyl benzoate derivative (**27**). Six known compounds (**57**-**60**, **62**, **63**) are isolated from this plant for the first time.

2) HPLC profiles of the three *Gentiana* drugs revealed five major common peaks identified as loganic acid (**LA**), gentiopicroside (**7**), sweroside (**8**), swertiamarin (**9**), and 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**).

3) The chemical composition of GR obviously differed from those of GSR and GMR in containing xanthones (**63**-**68**). GMR cotaining macrophylloside D (**54**) belonging to chromenes clearly differed from GSR containing a group of acetylated and/or benzoylated secoiridoid glycosides (**11**-**17**). Besides, the contents of 17 compounds (**LA**, **9**, **11**-**14**, **16**, **17**, **33**, **36**, **37**, **54**, **61**, **64**, **66**, **67**, **69**) in the three used crude drugs were quantitatively analyzed, and some identified common compounds from GSR and GMR such as loganic aicd (**LA**) and gentiopicroside (**9**) apparently differed in their contents.

General Discussion

Phytochemical investigation on the three *Gentiana* drugs revealed a series of compounds including iridoids and secoiridoids, lignans, triterpenoids, *C*-gluco-flavonoids and compounds of other types, as well as xanthones found only in GR derived from *G. lutea*. As the representative components, the isolated iridoids and secoiridoids could be classified into eight groups: (1) major iridoid and secoiridoid glycosides as loganic acid (**LA**), gentiopicroside (**9**), 6'-*O*-*β*-D-glucopyranosyl gentiopicroside (**33**), swertiamarin (**36**) and sweroside (**37**); (2) aglycon adducts of the major secoiridoid glycosides with representative of 6*β*-hydroxyswertiajaposide A (**8**); (3) acetylated and/or benzoylated secoiridoid glycosides with representative of trifloroside (**14**); (4) phenylbenzoylated secoiridoid glycosides as **61** and **62**; (5) polyglucosylated secoiridoid glycosides with representatives of scabrans G_3 (**34**) and G_4 (**35**); (6) 8-*epi*-kingiside and kingiside derivatives; (7) non-glycosidic secoiridoids; (8) exceptional secoiridoids and their derivatives including two novel secoiridoid glycosides **41** and **42** with an additional ether linkage between C-2' of the sugar moiety and C-3 of the aglycon, as well as compounds **57** and **58** with 9-*C* skeleton. Among them, the major secoiridoid glycosides can be found in all the three *Gentiana* drugs in large amount. Group of aglycon adducts of secoiridoid glycosides were mainly found in GSR and GMR in large amount, however further analysis showed some of them such as **29** and **30** might be artifacts. The acetylated and/or benzoylated secoiridoid glycosides were mainly found in the $CHCl_3$ extract of GSR derived from *G. scabra*, while the phenylbenzoylated secoiridoid glycosides were only found in the MeOH extract of GR derived from *G. lutea*, and the polyglucosylated

secoiridoid glycosides were mainly found in the MeOH extract of GMR derived from *G. crassicaulis*. The 8-*epi*-kingiside and kingiside derivatives were only found in $CHCl_3$ extract of GSR derived from *G. scabra*, which was seldom reported from the officially-listed botanical sources of GSR or GMR, suggested that compounds of this group had a limited distribution in these plants. The non-glycosidic secoiridoids can be found in the MeOH extract of both GMR and GR. The exceptional secoiridoids **41** and **42** were found in GMR derived from *G. crassicaulis*, while **57** and **58** were found in GR derived from *G. lutea*, all of which could be important chemical characteristics of their respective species. All the isolated compounds from the identified GSR, GMR and GR provided the fundamental information benefiting bioactivity evaluation and comparative analysis in different *Gentiana* species and drugs.

In vitro bioassay showed the isolated secoiridoids (mainly glycosides) exhibited moderate to weak anti-inflammatory activity. In particular, most of the secoiridoids have moderate inhibitory effect against LPS-induced IL-6 production, but not TNF-α production. Structural factors such as the presence of hydroxyl group at C-5 of the aglycon moiety and benzoyl group at C-2' of the sugar moiety, as well as the sugar chains of the secoiridoid glycosides might affect their *in vitro* anti-inflammatory activity. It is notable that some major components in the three *Gentiana* drugs such as loganic acid (**LA**) and gentiopicroside (**9**) have been proven to be active in anti-inflammatory studies *in vivo* [Chen *et al.*, 2008; Recio *et al*., 1994], while some metabolites such as gentianine produced from these secoiridoid glycosides exhibited strong anti-inflammatory activity *in vivo* and *in vitro* [Yang et al., 2000; Wang *et al.*, 2014]. These findings implied that secoiridoid glycosides might be precursors for a series of anti-inflammatory metabolites and the conversion to its real active metabolites was essential for their pharmaceutical actions.

From the current result, distribution of five major components, loganic acid (**LA**), gentiopicroside (**9**), 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), swertiamarin (**36**) and sweroside (**37**) were found to be their common feature, while the presence of acetylated and/or benzoylated secoiridoid glycosides in GSR derived from *G. scabra*, and xanthones in GR derived from *G. lutea*, as well as the absence of compounds of the above two types and the presence of non-secoiridoids macrophylloside D (**54**) in GMR derived from *G. crassicaulis* clearly differed to each other. Furthermore, quantitative analysis of the three *Gentiana* drugs revealed another important chemical characteristics with content variables of the five common principles. All the current results have been confirmed by further analyses on more samples of *Gentiana* species and drugs. In addition, some minor components such as 8-*epi*-kingiside and kingiside derivatives, novel secoiridoid glycosides **41** and **42**, secoiridoids **57** and **58** with *9*-C skeleton, and polyglucosylated secoiridoid glycosides could also be potential markers for identification and discrimination, but they were not concluded in this study because of the low content. By using more sensitive chemical profiling approach such as LC-MS, and combining with multivariate statistical techniques, the more details of these structural diversity and distribution of chemical constituents in different *Gentiana* species and drugs would be elucidated, while the current HPLC-DAD analysis has great potential for guiding future studies to examine the chemical composition and content variation of *Gentiana* drugs and to promote their standardization.

General Conclusion

1) In the current study, 70 compounds, including 12 new ones (**1**-**5**, **7**, **10**, **29**-**31**, **41**, **42**), have been isolated from the identified GSR, GMR and GR. They are mainly secoiridoids, including two novel secoiridoid glycosides (**41**, **42**) with an ether linkage between C-2' of the sugar moiety and C-3 of the aglycone, as well as xanthones, lignans, triterpenoids, *C*-glucoflavonoids and compounds of other types. Nine known compounds (**6**, **13**, **15**, **17**, **18**, **22**-**25**) from GSR derived from *G. scabra*, 18 known compounds (**32**, **34**, **35**, **38**-**40**, **43**-**47**, **49**-**55**) from GSR derived from *G. crassicaulis*, and 6 known compounds (**57**-**60**, **62**, **63**) from GR derived from *G. lutea* are isolated for the first time.

2) Most of the secoiridoids isolated from GSR and GMR, as the representative constituents in *Gentiana* drugs, exhibited moderate inhibitory effects against LPS-induced NO and IL-6 productions in macrophages, whereas only showed weak inhibitory effects in the case of TNF-α assay.

3) Comparative analysis of the three *Gentiana* drugs showed high similarities in their chemical composition. Meanwhile, some chemical characteristics of each drug was observed, which could be used as candidate markers for identification and standardization of *Gentiana* drugs.

4) The observed chemical diversity suggests that the three *Gentiana* drugs might have their own pharmacological effects, which imply that their uses in substitution should be taken for further investigation.

4. Experimental

4.1 Experimental instrumentation and chemicals

Optical rotations were measured on a JASCO P2100 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-460 Plus spectrophotometer. ^{1}H-, ^{13}C- and 2D-NMR spectra were recorded on a JEOL ECX400 Delta spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm and *J* values are given in Hz. EI-MS spectra were measured on JEOL JMS-GC mate II mass spectrometer. HR-ESI-MS measurements were carried out on a Accella HPLC system equipped with an Orbitrap-EX mass spectrometer (Thermofisher Scientific) and on LTQ-Orbitrap XL mass spectrometer with electron transfer dissociation (ETD) capability (Thermo Fischer Scientific). Column chromatography (CC) was performed with normal-phase silica gel (C-200 or C-300 HG, Wako), reverse-phase silica gel (ODS-A, 75 μm, YMC), Diaion HP-21 resin (Mitsubishi) and Sephadex LH-20 gel (GE Healthcare Bio-Science AB). Preparative HPLC was performed on a Waters HPLC system equipped with a Delta 600 pump and 2489 UV/Visible detector on YMC-pack R&D ODS-A column (250 × 20 mm i.d., 5 μm). Pre-coated silica gel 60 F_{254} plates (0.25 mm or 1.00 mm thickness; Merck, Germany) were used in analytical and preparative TLC for monitoring and purifying the fractions, respectively. All solvents used in extraction and isolation were of analytical grade and purchased from Wako. To work with sugar analysis, D-(+)-glucose (purity ≥ 98%) and D-(+)-xylose (extra pure grade) were obtained from Wako, L-(−)-glucose (purity ≥ 99%) were obtained from Sigma-Aldrich, and L-(−)-xylose (purity ≥ 97%) was obtained from TCI. To work with the cell cultures, Minimum Essential Medium (MEM), PBS and 0.05% trypsin-EDTA

were obtained from Gibco. FBS was obtained from HyClone®. LPS, $NaNO_2$, *N*-1-naphthylethylene diamine dihydrochloride, sulfanilamide and DMSO were obtained from Wako. Parthenolide (purity ≥ 90%) was obtained from Sigma-Aldrich. Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Lab and commercially available ELISA kits were obtained from Pepro Tech.

For quantitative analysis, a Shimadzu HPLC system consisting of a LC-10AD pump, an on-line DGU-20A degasser, a SIL-10AD auto-injector, a CTO-10-AS column oven and a SPD-M20A diode array detector (DAD) was employed. HPLC grade acetonitrile, ultrapure water and formic acid (abt. 99%) were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Compounds **LA**, **9**, **11**, **12**, **13**, **14**, **16**, **17**, **33**, **36**, **37**, **54**, **61**, **64**, **66**, **67** and **69** were tested the purity to be higher than 94%, 94%, 85%, 95%, 98%, 95%, 99%, 95%, 95%, 99%, 98%, 95%, 99%, 96%, 90%, 96% and 99%, respectively.

4.2 Extraction and isolation of compounds from GSR

GSR (1.5 kg) was extracted successively with $CHCl_3$ (9 L × 4, 1.5 h each time) and MeOH (9 L × 4, 1.5 h each time) under sonication at room temperature. The solvents were removed *in vacuo* to afford $CHCl_3$ extract (49 g) and MeOH extract (527 g). $CHCl_3$ extract (44 g used), which possessed relatively strong anti-inflammatory activity, was then separated over a silica gel column (75 × 6 cm i.d.) eluting with a gradient solvent condition of $CHCl_3$/MeOH (0:1 → 1:1, v/v) to give eight fractions F1-F8. F3 (10.6 g) was subjected to silica gel CC (60 × 4 cm i.d.) eluting with hexane/acetone (9:1 → 1:1, v/v) to afford nine subfractions. Further separation of

subfraction F3-3 (0.6 g) over ODS-A CC (40 × 3 cm i.d.; Acetone/H_2O, 1:1 → 1:0, v/v), followed by preparative HPLC using CH_3CN/H_2O (55:45, v/v) yielded compound **25** (19.4 mg). Subfractions F3-5 (0.4 g), F3-8 (2.2 g) and F3-9 (0.1g) were separated and purified in a similar manner to afford compounds **26** (2.9 mg), **17** (13.1 mg) and **20** (9.8 mg), respectively. F6 (3.9 g) was subjected to ODS-A CC (50 × 5 cm i.d.) eluting with acetone/H_2O (1:9 → 9:1, v/v) to afford compound **14** (56.2 mg) and nine other subfractions. Subfraction F6-3 (158 mg) was purified by preparative HPLC (CH_3CN/H_2O, 23:77, v/v) to yield compounds **2** (9.4 mg), **5** (3.6 mg) and **21** (8.9 mg). Preparative HPLC allowed for the purification of subfractions F6-4 (48 mg) and F6-9 (167 mg) yielded compounds **1** (6.9 mg) and **16** (122.5 mg), respectively. Moreover, purification of subfraction F6-5 (108 mg) yielded compounds **13** (25.9 mg) and **27** (2.1 mg), while purification of subfraction F6-7 (67 mg) yielded compounds **10** (6.1 mg) and **15** (23.5 mg). F7 (7 g) was subjected to the ODS-A CC and eluted with MeOH/H_2O (15:85 → 95:5, v/v) to afford 16 subfractions. Subfraction F7-4 (904 mg) was directly purified by repeatedly preparative HPLC with CH_3CN/H_2O (14:76, v/v) to yield compound **18** (4.9 mg). Subfraction F7-5 (2.1 g) was purified by normal-phase PTLC using $CHCl_3$/MeOH (5:1, v/v), followed by preparative HPLC using CH_3CN/H_2O (15:85, v/v) to yield compounds **7** (18.9 mg), **8** (8.2 mg) and **9** (245.2 mg). With a similar process, subfraction F7-8 (55 mg) was purified to yield compounds **4** (5.1 mg) and **6** (3.7 mg); subfractions F7-10 (11 mg), F7-13 (39 mg) and F7-14 (16 mg) were purified to yield compounds **3** (2.1 mg), **11** (36.3 mg) and **12** (9.2 mg), respectively; and F7-15 (92 mg) was purified to yield compounds **22** (9.7 mg), **23** (22.1 mg) and **24** (15.7 mg). F8 (0.3 g) was subjected to the ODS-A CC and eluted with MeOH/H_2O (20:80 → 60:40, v/v) to afford four subfractions. Subfractions F8-3 (26.2 mg) and 8-4 (19.6 mg) were purified by preparative HPLC (CH_3CN/H_2O, 24:76, v/v) to yield compounds **19** (2.1 mg) and **28** (2.4 mg), respectively.

4.3 Extraction and isolation of compounds from GMR

The chopped GMR (1.5 kg) was successively extracted with $CHCl_3$ (9 L × 4, each 1.5 h) and MeOH (9 L × 4, each 1.5 h) under sonication at room temperature. The combined MeOH extract was evaporated to dryness *in vacuo* to yield crude extract (562.1 g). Part of the crude extract (500 g) was suspended in H_2O (5 L) and passed through a Diaion HP-21 resin column successively eluted with water, 30%, 60% and 9% aqueous MeOH. The 30% MeOH elute (107.6 g) was then separated over a silica gel column eluting with a gradient solvent condition of $CHCl_3$/MeOH (95:5 → 60:40, v/v) to give nine fractions F1-F9. F2 (2.9 g) was subjected to ODS-A CC eluting with MeOH/H_2O (20:80 → 100:0, v/v) to give seven subfractions F2-1 to F2-7. Subfraction F2-2 was purified by normal-phase PTLC using $CHCl_3$/MeOH (2:98, v/v) and followed by preparative HPLC using CH_3CN/H_2O (15:85, v/v) to afford **43** (17.7 mg). With a similar process, subfraction F2-3 was purified to afford **42** (3.6 mg) and F2-6 was purified to afford **39** (3.1 mg) and **40** (5.1 mg). F3 (66.9 g) was re-dissolved in EtOH in an open conical flask and allowed to stand at room temperature for 3 days. The resulting precipitate was collected by filtration to afford **9** (9.1 g). The filtrate was evaporated to dryness and subsequently subjected to ODS-A CC eluting with an isocratic system of MeOH/H_2O (50:50, v/v) to give five subfractions F3-1 to F3-5. Subfractions F3-2 and F3-4 were purified by preparative HPLC using CH_3CN/H_2O (16:84 v/v) to afford **29** (22.5 mg) and **30** (11.5 mg), respectively. Subfraction F3-5 was separated over ODS-A CC (MeOH/H_2O, 5:95 → 60:40, v/v) and then purified by preparative HPLC using CH_3CN/H_2O (10:90 v/v) to afford **7** (28.3 mg), **8** (85.9 mg), **41** (12.8 mg) and **50** (3.4 mg). F4 (4.8 g) was subjected to ODS-A CC eluting with an isocratic system of CH_3CN/H_2O (20:80, v/v) to give three subfractions F4-1 to F4-3. Purification of subfraction F4-1 by repeated preparative HPLC using CH_3CN/H_2O

(11:89 v/v) afforded **36** (32.8 mg) and **48** (8.2 mg); purification of F4-2 afforded **37** (12.8 mg) and **44** (1.4 mg). F7 (13.2 g) was subjected to Sephadex LH-20 CC eluting with an isocratic system of MeOH/H_2O (60:40, v/v) to give four subfractions F7-1 to F7-4. Subfraction F7-1 was purified by preparative HPLC using CH_3CN/H_2O (5:95 v/v) to afford **38** (3.9 mg) and **45** (2.9 mg). Subfraction F7-2 was further separated over ODS-A CC (MeOH/H_2O, 8:92 → 15:85, v/v) and then purified by normal-phase PTLC using $CHCl_3$/MeOH (84:16, v/v) to afford **31** (11.8 mg), **32** (20.9 mg), **53** (4.7 mg) and **55** (1.0 mg). Subfraction F7-3 was re-subjected to Sephadex LH-20 CC eluting with an isocratic system of MeOH/H_2O (60:40, v/v) and then purified by preparative HPLC using CH_3CN/H_2O (15:85 v/v) to afford **51** (77.1 mg) and **52** (42.8 mg). F8 (6.1 g) was subjected to ODS-A CC, eluting with an isocratic system of CH_3CN/H_2O (10:90, v/v) to give four subfractions F8-1 to F8-4. Subfraction F8-1 was subjected to Sephadex LH-20 CC eluting with an isocratic system of MeOH/H_2O (60:40, v/v), then purified by preparative HPLC using CH_3CN/H_2O (10:90 v/v) to afford **34** (5.8 mg), **35** (9.0 mg) and **56** (1.4 mg). Subfraction F8-2 was purified by preparative HPLC using CH_3CN/H_2O (15:85 v/v) to afford **49** (8.5 mg); F8-3 was purified to afforded **33** (17.6 mg) and **46** (28.3 mg).

The 60% MeOH elute (36.2 g) was priorly analyzed by HPLC methods and found a dominant peak together with several minor peaks. The main peak was identified as 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**) on the basis of its retention time, UV absorption and co-HPLC injection with the reference compound isolated from the 30% MeOH portion. In order to identify the residue peaks, part of the 60% MeOH portion (5.0 g) was subjected to ODS-A CC eluting with CH_3CN/H_2O (20:80 → 100:0, v/v) to give four fractions F10-F13. F11 was then subjected to Sephadex LH-20 CC eluting with an isocratic system of MeOH/H_2O (60:40, v/v) to give four subfractions F11-1 to

F11-4. Subfraction F11-2 was purified by preparative HPLC using CH_3CN/H_2O (20:80 v/v) to afford **21** (28.6 mg) and **47** (9.2 mg); F11-3 was purified by preparative HPLC using CH_3CN/H_2O (25:75 v/v) to afford **54** (160.8 mg).

The water soluble portion was found to contain loganic acid (**LA**), one of the main constitutes in *Gentiana* drugs, by HPLC analysis. Subsequently, HPLC-guided isolation of this compound was conducted. The water soluble portion (1.0 g used) was re-dissolved in 80% MeOH and subjected to Sephadex LH-20 CC eluting with an isocratic system of $MeOH/H_2O$ (60:40, v/v), then purified by preparative HPLC using CH_3CN/H_2O (12:88 v/v) to afford **LA** (210.0 mg).

4.4 Extraction and isolation of compounds from GR

The chopped GR (100 g) was extracted with MeOH (0.5 L × 4, each 0.5 h) under sonication at room temperature. The crude extract was concentrated *in vacuo* and subjected to solvent-solvent partitioning with 0.1 L × 4 CH_3Cl, and then with 0.1 L × 4 *n*-BuOH. The combined CH_3Cl phase (CF) was concentrated *in vacuo*, re-dissolved with 5 mL MeOH, and then transferred into an opened vial. After allowing the mixture to stand for 24 h, yellow flocculent and needle crystals were formed. The crystal mass was transferred to a dry paper towel, then carefully separated using a small lab spatula to afford **64** (needle crystal, 2.1 mg) and **65** (flocculent crystal, 4.7 mg). The supernatant was concentrated *in vacuo* and separated over a silica gel column eluting with a gradient solvent condition of $CHCl_3$/MeOH (9:1 → 3:7, v/v) to give three fractions CF-1 to CF-3. CF-1 (202 mg) was subjected to ODS-A CC eluting with $MeOH/H_2O$ (10:90 → 25:75, v/v) to afford two subfractions CF-1-1 and CF-1-2.

Purification of CF-1-1 by preparative HPLC using CH_3CN/H_2O (8:92 v/v) afforded **60** (33.2 mg). With a similar process, purification of CF-1-2 afforded **44** (4.0 mg) and **59** (3.7 mg). CF-2 (99 mg) was subjected to Sephadex LH-20 CC eluting with an isocratic system of $MeOH/H_2O$ (80:20, v/v) to afford three subfractions CF-2-1 to CF-2-3. CF-2-2 was purified by preparative HPLC using CH_3CN/H_2O (28:72 v/v) to afford **27** (7.9 mg), **57** (5.0 mg) and a mixture, which was further purified by preparative HPLC using CH_3CN/H_2O (12:78 v/v) to afford **43** (2.0 mg) and **58** (4.9 mg). CF-2-3 was purified by repeated preparative HPLC using CH_3CN/H_2O (25:75 v/v) to afford to afford **66** (4.9 mg), **67** (3.1 mg) and **68** (3.8 mg).

The combined *n*-BuOH phase (BF) was separated over ODS-A CC ($MeOH/H_2O$, 12:88 → 50:50, v/v) to afford three fractions BF-1 to BF-3. BF-2 was purified by normal-phase PTLC using $CHCl_3$/MeOH (4:1, v/v) and followed by preparative HPLC using CH_3CN/H_2O (11:89, v/v) to afford **36** (5.4 mg) and **37** (6.2 mg). BF-3 was further separated over a silica gel column eluting with a gradient solvent condition of $CHCl_3$/ MeOH (9:1 → 3:7, v/v) to afford five subfractions BF-3-1 to BF-3-5. BF-3-2 was purified by preparative HPLC using CH_3CN/H_2O (24:76 v/v) to afford **21** (4.9 mg) and **61** (23.1 mg); BF-3-3 was purified by preparative HPLC using CH_3CN/H_2O (20:80 v/v) to afford **9** (3.6 mg); BF-3-4 was purified by normal-phase PTLC using $CHCl_3$/MeOH (5:1, v/v) to afford **62** (5.7 mg); BF-3-5 was purified by preparative HPLC using CH_3CN/H_2O (18:82 v/v) to afford **63** (6.8 mg) and **69** (4.3 mg).

4.5 Acidic hydrolysis and sugar analyses

Acidic hydrolysis was carried out using a similar procedure as reported [Tanaka *et al.*, 2007]. Briefly, each compound (0.5 mg) was dissolved in 1.0 N HCl (1.0 mL) and heated in a water bath (90 °C) for 3 h. The filtrate from the hydrolysate was

neutralized with Amberlite IRA-400 resin and concentrated to dryness *in vacuo*. The residue was then dissolved in pyridine (0.1 mL) and successively reacted with L-cysteine methyl ester hydrochloride and phenyl isothiocyanate at 60 °C for 1 h. Finally, the reaction mixture was subjected to HPLC system to determine the D/L configuration of sugar moieties by comparing their retention times (t_R) with those of authentic D/L-glucose and D/L-xylose derivatives (t_R: D-glucose, 21.5 min; L-glucose, 20.4 min; D-xylose, 25.3 min; L-xylose, 23.4 min).

4.6 Cell culture and treatment

The RAW264 cells (Riken Cell Bank, Tsukuba, Japan) were cultured in MEM supplemented with 10% FBS in a humidified 5% CO_2 at 37 °C. For all experiments, cells were grown to 80–90% confluence, then harvested with trypsin-EDTA and diluted to a suspension in fresh media. The suspended cells were seeded into 96-well plates at a density of 1×10^5 cells/well and allowed to adhere for 6 h, followed by treatment with LPS (0.1 μg/mL) for 24 h in the presence or absence of test samples. For measurement of NO, IL-6 and TNF-α productions, cells treated with 0.1% DMSO in medium were served as blank, while treated with 0.1% DMSO in the presence of LPS (0.1 μg/mL) were served as vehicle control. Parthenolide was used as a positive control. Each sample was tested in triplicate of three independent experiments.

4.7 Cell viability assay

Cell viability was assessed by CCK-8 assay, according to the manufacturer's instructions. Briefly, after treatment with crude extract (max. conc. 250.00 μg/mL), test compound (max. conc. 100.00 μM), parthenolide (max. conc. 10.00 μM) or vehicle

control, 10 μL of WST-8 solution was added to each well and incubated for 2 h at 37 °C. The absorbance at 405 nm was measured on a microplate reader. The percentage of cell viability was calculated according to the following formula: Cell viability (%) = (mean absorbance of test samples / mean absorbance of vehicle control) × 100%.

4.8 Measurement of NO production

NO production was determined by measuring the accumulation of nitrite in the cell culture supernatant using Griess reagent [Wang *et al.*, 2013]. The absorbance at 560 nm was measured on a microplate reader. The nitrite concentration was calculated from the calibration curve prepared using $NaNO_2$ standards. Inhibition activity was calculated according to the following formula: Inhibition activity (%) = $[(A_c - A_s) / (A_c - A_b)] \times 100\%$, where A_c, A_s and A_b represent the nitrite concentration (μM) of vehicle control, test sample and blank, respectively.

4.9 Measurement of IL-6 and TNF-α productions

IL-6 and TNF-α levels in the cell-free supernatant were measured using commercially available ELISA kits, according to the manufacturer's instructions. Absorbance at 405 nm was measured on a microplate reader. Inhibition activity of the test compounds was calculated according to the following formula: Inhibition activity (%) = $[(A_c - A_s) / (A_c - A_b)] \times 100\%$, where A_c, A_s and A_b are absorbance of vehicle control, test sample and blank, respectively.

4.10 Quantitative analysis of chemical composition in GSR, GMR and GR

4.10.1 Sample preparation

Each of the fine powered samples (0.5 g) was accurately weighted and extracted four times with 5 mL of methanol by ultrasonication for 40 min at room temperature. After centrifugations at 3500 rpm for 5 min, the supernatant was combined and concentrated *in vacuo*. The residue was dissolved in methanol and transferred into a 5.0 mL volumetric flask, then diluted to the indicated volume with methanol. Prior to injection all extracted solutions were filtered with 0.20 μm polytetrafluoroethylene (PTFE) syringe filter (ADVANTEC, Toyo Roshi Kaisha, Ltd., Japan).

4.10.2 HPLC analysis

HPLC analysis was performed on a Shimadzu HPLC system equipped with YMC-pack ODS-A column (250×20 mm i.d.). The mobile phase was a binary eluent of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B) with the following gradient program: 0 min, 10% B; 2 min, 10% B; 15 min, 15% B; 40 min, 40% B; 50 min, 95% B; 58min, 95% B. The detection wavelength range of the DAD was 190-400 nm with a monitoring wavelength set at 254 nm. The flow rate was 1.0 ml/min and the column temperature was set to 35 °C. The injection volume was set to 10 μL.

4.10.3 Peak assignment

All the reference compounds (**1**-**69** and **LA**) were accurately weighted and dissolved in methanol to produce stock solutions. Gentiopicroside (**9**) was used as the internal reference (IS). Each stock solution was mixed with IS solution and then diluted with methanol to yield a mixture solution (0.1mg/mL containing 0.1mg/mL IS).

The retention time (t_R) of each assigned peak was recorded in the chromatograms at 254 nm, then *RRT* was calculated. Finally peak assignments were made by comparing their *RRT*s and characteristic UV/vis absorption spectra from the DAD with those of the reference compounds.

4.10.4 Calibration

Linear gradient analysis for each of the 17 compounds (**LA**, **9**, **11**, **12**, **13**, **14**, **16**, **17**, **33**, **36**, **37**, **54**, **61**, **64**, **66**, **67** and **69**) was performed at six concentration levels by the external method. Calibration curves were constructed by plotting the peak area (y) versus the concentration of each injected reference compound (x). The calibration curves of the reference compounds were summarized: loganic acid (**LA**), y = 1993676.6275x + 100358.6517 (R^2 = 0.9991) within the linear range from 0.069 to 2.214 mg/mL; gentiopicroside (**9**), y = 3155404.6602x − 55952.9304 (R^2 = 0.9992) within the linear range from 0.117 to 3.741 mg/mL; 4'''-*O*-*β*-D-glucopyranosyl-trifloroside (**11**), y = 5896860.7141x + 30714.7702 (R^2 = 0.9995) within the linear range from 2.536 × 10^{-3} to 0.616 mg/mL; 4'''-*O*-*β*-D-glucopyranosylscabraside (**12**), y = 7759377.4452x + 4373.1542 (R^2 = 0.9990) within the linear range from 2.583 × 10^{-3} to 0.083 mg/mL; gelidosidce (**13**), y = 9000106.5922x + 6307.06292 (R^2 = 0.9999) within the linear range from 1.754 × 10^{-3} to 0.426 mg/mL; trifloroside (**14**), y = 9821829.8354x − 51675.0157 (R^2 = 0.9991) within the linear range from 3.103 × 10^{-3} to 0.754 mg/mL; scabraside (**16**), y = 9799539.4069x + 4933.2537 (R^2 = 0.9994) within the linear range from 4.183 × 10^{-3} to 0.134 mg/mL; deglucosyltrifloroside (**17**), y = 8848678.0984x − 7155.7512 (R^2 = 0.9998) within the linear range from 4.809 × 10^{-3} to 0.154 mg/mL; 6'-*O*-*β*-D-glucopyranosylgentiopicroside (**33**), y = 1752315.9681x + 51571.8344 (R^2 = 0.9989) within the linear range from 0.015 to 3.553 mg/mL;

swertiamarin (**36**), y = 3228799.4453x + 50979.7015 (R^2 = 0.9990) within the linear range from 0.030 to 0.963 mg/mL; sweroside (**37**), y = 4567847.8413x + 74387.7202 (R^2 = 0.9994) within the linear range from 0.009 to 2.068 mg/mL; macrophylloside D (**54**), y = 9515526.5212x + 5166.6667 (R^2 = 0.9994) within the linear range from 2.898 $\times$ 10^{-3} to 0.464 mg/mL; amarogentin (**61**), y = 10906561.8551x + 4061.0846 (R^2 = 0.9995) within the linear range from 3.424 $\times$ 10^{-3} to 0.110 mg/mL; gentisin (**64**), y = 16298646.0792x + 25507.5000 (R^2 = 0.9996) within the linear range from 4.920 $\times$ 10^{-3} to 0.079 mg/mL; gentioside (**66**), y = 7009496.2704x – 2716.9700 (R^2 = 0.9991) within the linear range from 3.881 $\times$ 10^{-3} to 0.124 mg/mL; 1-hydroxy-3-methoxy-7-*O*-primeverosylxanthone (**67**), y = 5774843.0874x – 2237.6818 (R^2 = 0.9991) within the linear range from 3.880 $\times$ 10^{-3} to 0.124 mg/mL; and isovetexin (**69**), y = 15336838.2107x – 4793.7503 (R^2 = 0.9999) within the linear range from 1.157 $\times$ 10^{-3} to 0.281 mg/mL.

4.11 Statistical analysis

Statistical analysis of the data was performed with the statistical package SPSS 19.0 and Excel 2007. To work with the *in vitro* anti-inflammatory activity test, the IC_{50} values were calculated using SigmaPlot (Version 11.5) with a four parameter logistic nonlinear regression model with the sample absorbencies over the log concentrations of the test samples. The results are expressed as the mean ± SD of three independent experiments. One-way analysis of variance (ANOVA) following Dunnett's test was used for statistical analysis. Results with p value < 0.05 were considered statistically significant.

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