Kyoto Pharmaceutical University

Doctoral Dissertation

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Bioactive constituents from the flowers of *Hydrangea macrophylla* var. *thunbergii* and *Osmanthus fragrans* var. *aurantiacus*

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Index

Abstract

Flowers have been used as not only ornamental purposes but also medicines and foods. However, the chemical constituents and pharmacological properties of medicinal flowers have not yet been clarified. As a part of the characterization studies on the bioactive constituents of medicinal flowers, I focused on the bioactive constituents from the flowers of *Hydrangea macrophylla* var. *thunbergii* and *Osmanthus fragrans* var. *aurantiacus*.

1. Hydrangeamines A and B, Novel Polyketide-Type Pseudoalkaloid-Coupled Secoiridoid Glycosides from the Flowers of *H. macrophylla* var. *thunbergii*

The Saxifragaceae plant, *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO, is native to Japan. The processed leaves of this plant (Hydrangea Dulcis Folium) are currently used as a natural medicine for an oral refrigerant and as a sweetener for diabetic patients. Moreover, these preparations are listed in the Japanese Pharmacopoeia. Several chemical constituents of the processed leaves and the dried leaves of this plant have been isolated including dihydroisocoumarins and their glycosides, benzylidenephthalides, phthalides and secoiridoid glycosides. However, a full chemical analysis of the flowers from this plant has not yet been performed. I have examined the chemical constituents from the flowers of *H. macrophylla* var. *thunbergii*. Namely, a methanolic extract of the flowers of *H. macrophylla* var. *thunbergii* collected in Nagano province was partitioned between EtOAc-H_2O to furnish an EtOAc-soluble fraction and an aqueous layer. The aqueous layer was further extracted with 1-butanol (1-BuOH) to give a 1-BuOH and a H_2O soluble fraction. The 1-BuOH-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatography (CC) and repeated HPLC to give hydrangeamines A (**1**) and B (**2**) together with hydrangenoside A (**3**) and hydramacrosides A (**4**) and B (**5**). The absolute stereostructures of the two hydrangeamines were characterized on the basis of chemical and physiochemical analyses, which included the application of CD for the 4,5-dihydro-[6*H*]-pyrano chromophore. The side chain (C-12~C-25) of **1** and **2** were deduced to be biosynthesized through intermediates by the shikimate-malonate pathway, followed by the formation of the polyketide amine part. This is the first isolation of the unique polyketide-type pseudoalkaloid-coupled secoiridoid glycosides.

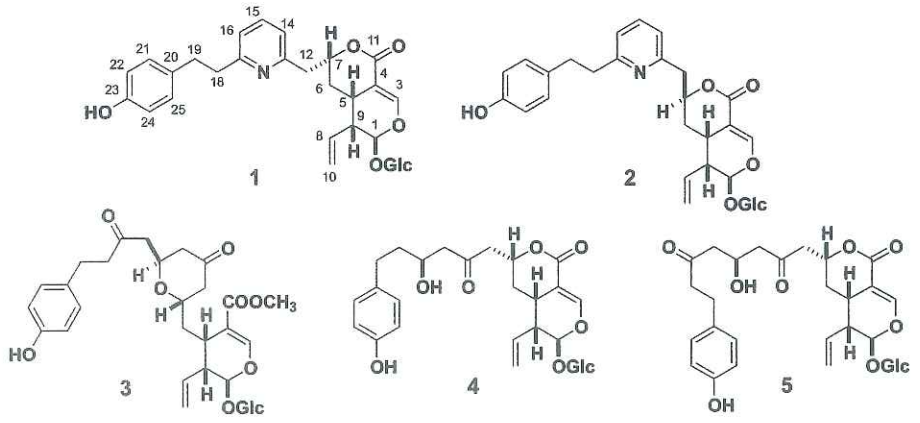


Figure 1 Structures of secoiridoid glycosides from the flowers of *H. macrophylla* var. *thunbergii*

2. Structures of Dihydroisocoumarin Glycosides from the Flowers of *H. macrophylla* var. *thunbergii*

Six dihydroisocoumarin glucosides, florahydrosides I (**6**) and II (**7**), thunberginol G

8-*O*-*β*-D-glucopyranoside (**8**), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**), 4-hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**), and thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**), were isolated from the flowers of *H. macrophylla* var. *thunbergii* together with 20 known compounds including dihydroisocoumarin derivatives and acylated quinic acid analogs. The chemical structures of the new compounds were elucidated on the basis of chemical and physicochemical evidence.

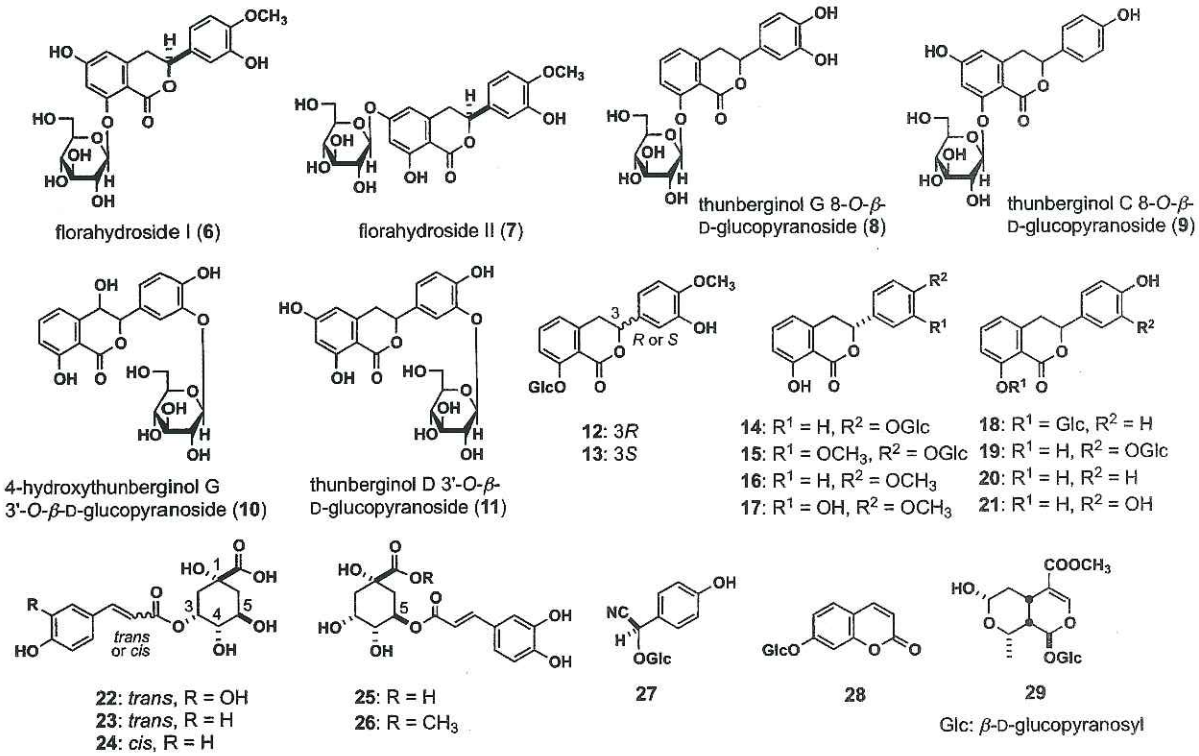


Figure 2 Structures of dihydroisocoumarins, quinic acid analogs, and glucosides from the flowers of *H. macrophylla* var. *thunbergii*

3. Aldose Reducatase inhibitors from *H. macrophylla* var. *thunbergii*

The inhibitory effects on aldose reductase of the isolated constituents from the flowers of *H. macrophylla* var. *thunbergii* were examined. Among the constituents, neochlorogenic acid (**22**) inhibited aldose reductase [IC_{50} = 5.6 μM]. In addition, chlorogenic acid methyl ester (**26**) showed the inhibitory effect [IC_{50} = 2.9 μM]. On the other hand, dihydroisocoumarin glucosides (**6–15**, **18**, and **19**) and dihydroisocoumarins (**16** and **17**) lacked the inhibitory effects [IC_{50} > 100 μM]. Hydrangenol (**20**), thunberginol G (**21**), taxiphyllin (**27**), and umbelliferone glucoside (**28**) exhibited moderate inhibitory effects [IC_{50} = 48–69 μM]. Next, the inhibitory effects on aldose reductase of caffeoylquinic acid analogs were examined for the structure-activity relationship study. The inhibitory effect of D-quinic acid with *trans-p*-caffeoyl group at the 5-position was stronger than those of D-quinic acids with *trans-p*-caffeoyl group at the 3 or 4-positions.

4. Inhibitory Effects on Nitric Oxide Production from the Flowers of *O. fragrans* var. *aurantiacus*

Seventy compounds including several new megastigmane glycosides, sesquiterpenes, and triterpenes were isolated from the flowers of *Osmanthus fragrans* var. *aurantiacus* (sweet osmanthus; kinmokusei in Japanese). Among them, several triterpenes showed inhibitory effects on nitric oxide production.

Chapter 1 Hydrangeamines A and B, Novel Polyketide-Type Pseudoalkaloid-Coupled Secoiridoid Glycosides from the Flowers of *H. macrophylla* var. *thunbergii*

1.1 Introduction

The Saxifragaceae plant, *Hydrangea* (*H.*) *macrophylla* SERINGE var. *thunbergii* MAKINO, is native to Japan. The processed leaves of this plant (Hydrangea Dulcis Folium) are currently used as a natural medicine for an oral refrigerant and as a sweetener for diabetic patients. In addition, these preparations are listed in the Japanese Pharmacopoeia. Previously, we reported the isolation and structure determination of many chemical constituents with anti-diabetic, anti-allergic, and anti-bacterial activities from the processed leaves and the dried leaves of this plant.[1-6] However, a full chemical analysis of the flowers from this plant has not yet been performed. In the course of our chemical and pharmacological studies on Hydrangea plants[1-8] and medicinal flowers,[8-14] we have examined the chemical constituents from the flowers of *H. macrophylla* var. *thunbergii*. Two novel secoiridoid glycosides with a pyridine ring named hydrangeamines A (**1**) and B (**2**) along with three known secoiridoid glycosides, hydrangenoside A (**3**), hydramacrosides A (**4**) and B (**5**) were isolated from the flowers of *H. macrophylla* var. *thunbergii* cultivated in Nagano prefecture, Japan together with 40 known compounds including five acylated quinic acid analogs.

1.2 Extraction and Isolation

A methanol (MeOH) extract (32.2% from the flowers of *H. macrophylla* var. *thunbergii* cultivated in Nagano province) was partitioned between EtOAc-H_2O (1:1) to furnish an EtOAc-soluble fraction (8.8%) and an aqueous layer. The aqueous layer was further extracted with 1-butanol (1-BuOH) to give a 1-BuOH (12.5%) and a H_2O (11.0%) soluble fraction. The 1-BuOH-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatography (CC) and repeated HPLC to give hydrangeamines A (**1**, 0.0009%) and B (**2**, 0.0004%) together with hydrangenoside A (**3**, 0.0077%)[9-14] hydramacrosides A (**4**, 0.0091%) and B (**5**, 0.0083%).[12, 15] (Figure 1)

1.3 Absolute Stereostructures of Novel Polyketide-Type Pseudoalkaloid-Coupled Secoiridoid Glycosides from the Flowers of *H. macrophylla* var. *thunbergii*

Hydrangeamine A (**1**) was isolated as a white amorphous powder with negative optical rotation ($[\alpha]_D^{18}$ −130.8° in MeOH). Its IR spectrum showed absorption bands at 3420, 1697, 1618, 1508 and 1071 cm^{-1} ascribable to hydroxy, lactone, aromatic ring, and ether functions. Its UV spectrum showed absorption maxima ascribable to enone and aromatic ring components at 227 and 275 nm, respectively. In the positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 570 $(M+H)^+$ and *m/z* 592 $(M+Na)^+$ and the molecular formula $C_{30}H_{35}NO_{10}$ of **1** was determined by HRMS measurement of the

quasimolecular ion peak $(M+Na)^+$. Acid hydrolysis of **1** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[16] The ^1H (methanol-d_4) and ^{13}C NMR spectra of **1** (Table **1**), which were assigned by various NMR experiments, showed signals due to the secoiridoid lactone moiety (C-1~C-11) [δ5.54 (br d, J = 2.1 Hz, H-1), 7.58 (d, J = 2.8 Hz, H-3), 3.14 (m, H-5), 1.47 (ddd, J = 13.0, 13.0, 11.7 Hz, H-6α), 1.81 (ddd, J = 13.0, 4.1, 1.4 Hz, H-6β), 4.76 (m, H-7), 5.50 (ddd, J = 17.2, 10.3, 10.3 Hz, H-8), 2.67 (1H, m, H-9), 5.24 (dd, J = 10.3, 1.7 Hz, H-10α), 5.28 (dd like, J = 17.2, 1.7 Hz, H-10β)] and the side chain including pyridine and *p*-hydroxyphenyl moieties [δ7.16 (d, J = 7.6 Hz, H-14), 7.61 (dd, J = 7.6, 7.6 Hz, H-15), 7.05 (d, J = 7.6 Hz, H-16), 6.92 (d, J = 8.2 Hz, H-21, 25), 6.63 (d, J = 8.2 Hz, H-22, 24)] together with a β-D-glucopyranoside moiety [4.67 (d, J = 7.6 Hz, H-1')]. In the ^{13}C NMR spectrum of **1**, the carbon signals due to the secoiridoid lactone β-D-glucopyranoside part of **1** were superimposable on those of **4** and **5** except for the signals around the 7-position. As shown in Figure 3, the double quantum filter correlation spectroscopy (DQF-COSY) experiment of **1** indicated the presence of certain structures (highlighted by bold lines). Heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments revealed long-range correlations between the following protons and carbons: H-1 and C-3, 5; H-3 and C-11; H-7 and C-5; H-9 and C-4; H-10 and C-9; H-12 and C-6, 13, 14; H-14 and C-12, 16; H-15 and C-13, 17; H-16 and C-14, 18; H-19 and C-20; H-21 and C-19, 23; H-22 and C-20; H-1' and C-1.

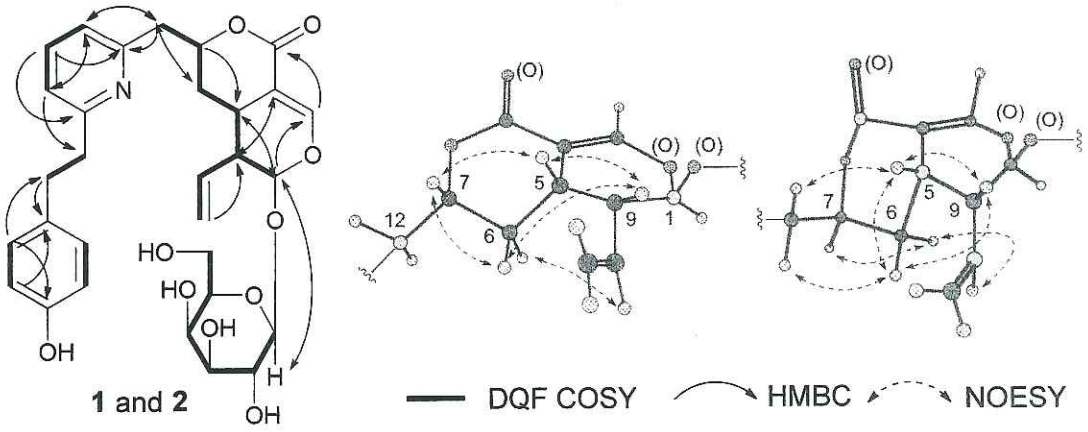


Figure 3 DQF-COSY, HMBC, and NOE correlations of hydrangeamines A (**1**) and B (**2**)

The relative stereostructure of **1** was deduced by comparing the ^1H and ^{13}C NMR data with those of known secoiridoid glucosides such as (7*R*)-caffeoyloxysweroside[17] and (7*S*)-caffeoyloxysweroside.[17] Nuclear Overhauser enhancement spectroscopy (NOESY) showed NOE correlations between the following proton pairs: H-5β and H-7β, 9β; H-6β and H-7β, 9β; H-6α and H-8. Finally, the absolute configuration of **1** was characterized by circular dichroism (CD).

It was previously reported that the absolute stereostructures of secoiridoids with a 4,5-dihydro-[6*H*]-pyrano skeleton were determined based on CD spectra.[18, 19] For example, Yu et al. reported the CD spectrum of a secoiridoid, loniphenyruviridoside B with a 4,5-dihydro-[6*H*]-pyrano skeleton, which displayed a negative Cotton effect with a peak at 232 nm ($\Delta\varepsilon$ −8.26 in MeOH) resulting from the $\pi\rightarrow\pi^*$ transition of the 4,5-dihydro-[6*H*]-pyrano chromophore. The Cotton effect indicated a 1*S*,

5*S*, 9*R* configuration by application of the helicity rule to the 4,5-dihydro-[6H]-pyrano chromophore as described elsewhere.[18, 19] The CD data of **1** with a 4,5-dihydro-[6*H*]-pyrano moiety showed a negative Cotton effect with a peak at 245 nm ($\Delta\varepsilon$ −10.78 in MeOH) similar to that of loniphenyruviridoside B. Hence, the absolute configurations at the 1, 5 and 9 positions were determined to be *S*, *S*, *R*, respectively. Consequently, the structure of **1** was determined as shown in Figure **1** and the absolute configuration at the 1, 5, 7, and 9 positions of **1** were indicated to be *S*, *S*, *R*, and *R*, respectively.

Hydrangeamine B (**2**), a colorless amorphous powder, exhibited a negative optical rotation ($[\alpha]_D^{16}$ −79.2° in MeOH). In the FABMS of **2**, two quasimolecular ion peaks were observed at *m/z* 570 $(M+H)^+$ and *m/z* 592 $(M+Na)^+$, and the molecular formula $C_{30}H_{35}NO_{10}$ was determined by HRMS. The IR and UV spectra of **2** were similar to those of **1**. Acid hydrolysis of **2** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[16] The proton and carbon signals of **2** in the ^1H and ^{13}C NMR spectra were superimposable on those of **1**, except for the signals due to the 5, 6, and 7-positions. The planar structure of **2** was characterized by means of DQF-COSY and HMBC experiments. In addition, the relative stereostructure of **2** was characterized by NOESY experiments, which showed NOE correlations between the following proton pairs: H-5β and H-6β, 9β, H_2-12; H-6β and 9β, H_2-12; H-6α and H-7, 8. In addition, the signals at C-5, C-6 and C-7 of **2** were observed at higher fields (4.5, 3.1 and 0.9 ppm, respectively) than those of **1**. These results suggested that **2** is a C-7 epimer of **1**. The CD data of **2** showed a negative Cotton effect with a peak at 246 nm ($\Delta\varepsilon$ −13.14 in MeOH). Thus, the absolute configurations at the 1, 5 and 9 positions were determined to be *S*, *S*, *R*, respectively. The structure of **2** is shown in Figure **1**. The absolute configuration at the 1, 5, 7 and 9 positions of **2** are *S*, *S*, *S*, *R*, respectively.

In conclusion, two novel secoiridoid glycosides with a pyridine ring, hydrangeamines A (**1**) and B (**2**), were isolated from the flowers of *H. macrophylla* var. *thunbergii* cultivated in Japan. Secoiridoid glucosides, hydrangenoside A (**3**) and hydramacroside B (**5**), were deduced to be biosynthesized through an aldol-type condensation of secologanin (intermediate B) with the polyketide unit (intermediate A) formed by the shikimate-malonate pathway.[9-11]

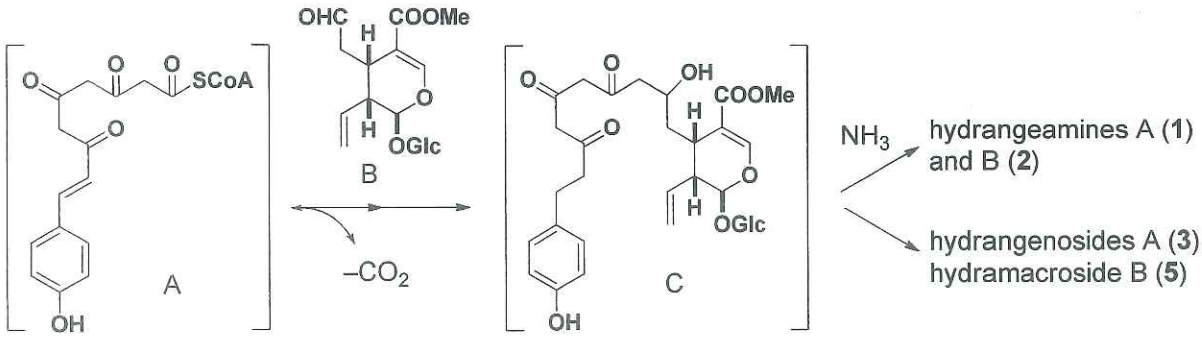


Figure 4 Plausible biosynthetic route of hydrangeamines A (**1**) and B (**2**)

Our results suggest the side chain (C-12—C-25) of **1** and **2** are also biosynthesized through intermediates (C) by the shikimate-malonate pathway, followed by the formation of the polyketide amine part of the molecule as shown in Figure **4**. To the best of our knowledge, this is the first isolation of the unique

polyketide-type pseudoalkaloid-coupled secoiridoid glycosides. The biosynthesis of the novel secoiridoid with a pyridine ring will be the focus of further studies.

Table 1 ^{1}H and ^{13}C NMR (methanol–d_4, 600/150 MHz) data for **1** and **2**

No.	**1**[a]		**2**[a]	
	δ_H	δ_C	δ_H	δ_C
1	5.53 br d (2.1)	98.0	5.55 br d (1.4)	98.0
3	7.58 d (2.8)	154.2	7.58 d (2.8)	154.2
4		105.5		105.2
5	3.14 m	28.0	3.37 m	23.5
6α	1.48 ddd (13.0, 13.0, 11.7)	31.2	1.80 ddd (13.6, 12.4, 4.8)	28.1
6β	1.79 ddd (1.4, 4.1, 13.0)		1.62 ddd (13.6, 5.5, 2.8)	
7	4.75 m	80.4	4.87 m	79.5
8	5.49 ddd (17.2, 10.3, 10.3)	133.3	5.51 ddd (17.2, 9.6, 9.6)	133.2
9	2.67 m	43.8	2.56 m	43.8
10	5.23 dd (10.3, 1.7)	120.9	5.22 dd like (9.6, 1.4)	121.1
	5.28 dd like (17.2, 1.7)		5.23 dd like (17.2, 1.4)	
11		168.4		168.5
12	3.05 dd (13.7, 5.5)	44.7	3.07 dd (13.7, 6.8)	43.1
	3.12 dd (13.7, 7.6)		3.32 m	
13		157.7		158.0
14	7.15 d (7.6)	123.4	7.13 d (7.6)	122.9
15	7.60 dd (7.6, 7.6)	138.7	7.61 dd (7.6)	138.9
16	7.04 d (7.6)	122.8	7.05 d (7.6)	122.9
17		162.4		162.6
18	2.99 m	41.0	2.99 m	41.0
19	2.89 m	36.6	2.88 m	36.5
20		133.3		133.3
21	6.91 d (8.2)	130.5	6.92 d (8.2)	130.5
22	6.63 d (8.2)	116.1	6.62 d (8.2)	116.1
23		156.5		156.6
24	6.63 d (8.2)	116.1	6.62 d (8.2)	116.1
25	6.91 d (8.2)	130.5	6.92 d (8.2)	130.5
1'	4.66 d (7.6)	99.7	4.66 d (7.6)	99.7
2'	3.19 dd (8.9, 7.6)	74.7	3.23 dd (8.9, 7.6)	74.7
3'	3.32 m	78.4	3.32 m	78.5
4'	3.26 dd like (8.9, 8.9)	71.5	3.26 m	71.6
5'	3.35 m	77.8	3.37 m	77.8
6'	3.64 dd (11.6, 6.1)	62.7	3.64 dd (11.6, 6.1)	62.7
	3.87 dd (11.6, 2.1)		3.87 dd (11.6, 2.1)	

[a]Coupling constants (J in Hz) are given in parentheses.

Chapter 2 Structures of New Dihydroisocoumarin Glycosides from the Flowers of *H. macrophylla* var. *thunbergii* and Qualitative Analysis of Cyanogenic Glycoside [(2*R*)-Taxiphyllin and Hydracyanoside A] from Several Parts of *H. macrophylla* var. *thunbergii*

2.1 Introduction

Six dihydroisocoumarin glucosides, florahydrosides I (**6**) and II (**7**), thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**), 4-hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**), and thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**), were isolated from the flowers of *H. macrophylla* var. *thunbergii* together with 20 known compounds including dihydroisocoumarin derivatives and acylated quinic acid analogs. The chemical structures of the new compounds were elucidated on the basis of chemical and physicochemical evidence.

2.2 Extraction and Isolation

The EtOAc- and 1-BuOH-soluble fractions were subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give florahydrosides I (**6**, 0.068%) and II (**7**, 0.014%), thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**, 0.035%), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**, 0.045%), 4-hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**, 0.020%), and thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**, 0.027%) (Figure 1) together with 3*R*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**12**, 3.81%),[13, 14] 3*S*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**13**, 0.043%),[13, 14] (+)-hydrangenol 4'-*O*-*β*-D-glucopyranoside (**14**, 0.0040%),[20, 21] 3*R*-thunberginol I 4'-*O*-*β*-D-glucopyranoside (**15**, 0.056%),[22, 23] (+)-3-(4-methoxyphenyl)-8-hydroxy-3,4-dihydroisocoumarin (**16**, hydrangenol monomethyl ether, 0.011%),[14, 24] phyllodulcin (**17**, 0.19%),[21, 23-26] hydrangenol 8-*O*-*β*-D-glucopyranoside (**18**, 2.87%),[13, 14, 22, 23] thunberginol G 3'-*O*-*β*-D-glucopyranoside (**19**, 0.16%),[13, 14, 24, 27] hydrangenol (**20**, 4.79%),[25, 26, 28] thunberginol G (**21**, 0.067%),[4, 5, 27, 29] neochlorogenic acid (**22**, 0.075%)[28-32] 3-*O*-*trans*-*p*-coumaroyl-D-quinic acid (**23**, 0.27%),[30-33] 3-*O*-*cis*-*p*-coumaroyl-D-quinic acid (**24**, 0.029%),[31, 33] chlorogenic acid (**25**, 0.054%),[32, 34] chlorogenic acid methyl ester (**26**, 0.049%),[8, 33-35] taxiphyllin (**27**, 0.076%),[7, 8, 34, 36, 37] umbelliferone glucoside (**28**, 0.11%),[37-39] α-morroniside (**29**, 0.021%),[39, 40] shikimic acid (**30**, 0.36%),[41] 3-(*β*-D-glucopyranosyloxy)-4-hydroxy-benzaldehyde, (**31**, 0.031%),[42] 7-methoxy-8-*O*-*β*-D-glucopyranosyl coumarin (**32**, 0.015%),[43] loganoside (**33**, 0.025%),[44] *trans*-*p*-coumaric acid (**34**, 0.013%),[32, 40, 45] vogeloside (**35**, 0.12%),[46] 2-hydroxy-4-(*β*-D-glucopyranosyloxy)-6-[2-(4-hydroxyphenyl)ethyl]-benzoic acid, (**36**, 1.00%),[47] deoxyloganic acid (**37**, 0.48%),[48] naringenin 7-glucoside (**38**, 0.0038%),[49] quercetol 3-glucoside (**39**, 0.0013%),[50] thunberginol E 3'-*O*-*β*-D-glucopyranoside (**40**, 0.0041%),[14, 51] gingerglycolipid A (**42**, 0.0026%),[48] 1-*O*-palmitoyl-3-*O*-[α-D-galactopyranosyl(1→6)-*β*-D-galactopyranosyl]-sn-glycerol (**43**,

0.0012%),[52] *p*-hydroxybenzaldehyde (**44**, 0.1520%),[53, 54] *trans*-cinnamic acid (**45**, 0.0190%),[55-57] skimmetine (**46**, 0.0455%),[58] naringenin (**47**, 0.0065%),[59] hydrangeic acid (**48**, 0.0455%).[60] (Figures 2 and 5)

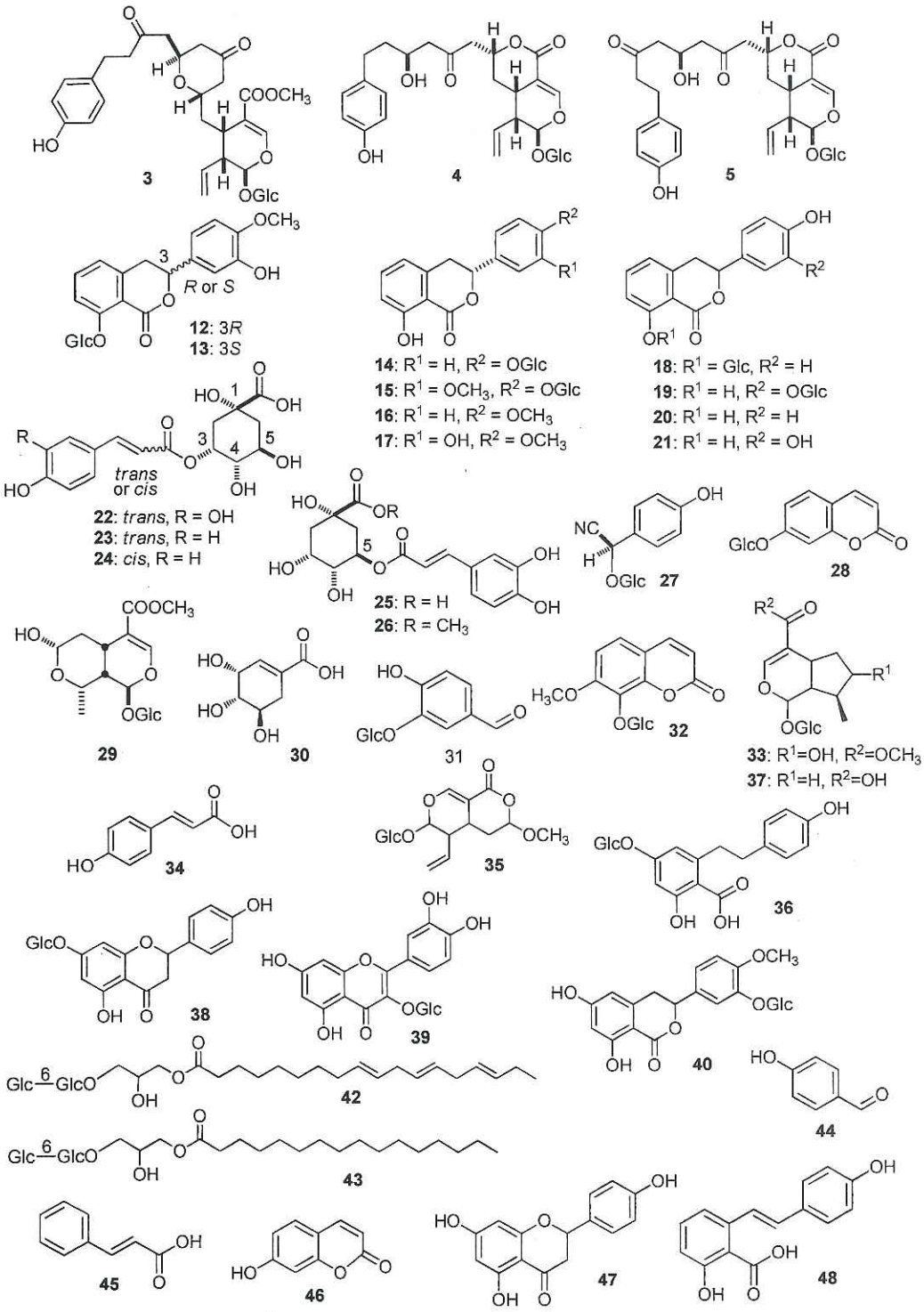


Figure 5 Structures of known compounds from the flowers of *H. macrophylla* var. *thunbergii*

2.3 Structure Elucidation of New Dihydroisocoumarin Glycosides from the Flowers of *H. macrophylla* var. *thunbergii*

Florahydrosides I (**6**) and II (**7**) were isolated as a white amorphous powder with negative optical rotation (**6**: $[\alpha]_D^{20}$ –8.0°, **7**: $[\alpha]_D^{25}$ –21.0°, in MeOH). Their IR spectra showed absorption bands due to hydroxy (**6** and **7**: 3400 cm^{-1}), lactone (**6**: 1684 cm^{-1}, **7**: 1686 cm^{-1}), aromatic ring (**6**: 1610, 1508 cm^{-1}, **7**: 1618, 1510 cm^{-1}), and ether functions (**6**: 1071 cm^{-1}, **7**: 1073 cm^{-1}). In the FAB-MS spectra of **6** and **7**, the common quasimolecular ion peak was observed at *m/z* 487 ($[M+Na]^+$) and the molecular formula $C_{22}H_{24}O_{11}$ was determined by high-resolution (HR) MS measurement. Acid hydrolysis of **6** and **7** with 5% aqueous H_2SO_4 liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector. The ^1H NMR (methanol-d_4) and ^{13}C NMR (Table **2**) spectra of **6**, which were assigned by various NMR experiments, showed signals assignable to a dihydroisocoumarin moiety [δ 2.93 (dd, J = 16.5, 2.1 Hz, H-4a), 3.14 (dd, J = 16.5, 12.4 Hz, H-4b), 5.29 (dd, J = 12.4, 2.1 Hz, H-3), 6.45 (br s, H-5), 6.78 (br s, H-7)], ABX-type aromatic ring [δ 6.90 (1H, dd, J = 8.2, 1.4 Hz, H-6'), 6.92 (1H, d, J = 8.2 Hz, H-5'), 6.94 (1H, d, J = 1.4 Hz, H-2')], a methoxyl group [δ 3.85 (s, OCH_3)], and a β-D-glucopyranosyl moiety [δ 4.81 (1H, d, J = 7.6 Hz, H-1")]. The proton and carbon signals of the 3-phenyl-dihydroisocoumarin (aglycon) part in the ^1H- and ^{13}C NMR spectra of **6** were superimposable on those of 3*R*-thunberginol E,[27] except for the signals around the 8-positon, while the proton and carbon signals due to the dihydroisocoumarin part including glycoside moiety were very similar to those of 3*S*-scorzocreticoside I [3,4-dihydro-6,8-dihydroxy-3*S*-(*p*-methoxyphenethyl)-isocoumarin][61, 68] As shown in Figure **6**, the double quantum filter correlation spectroscopy (DQF-COSY) experiment of **6** indicated the presence of certain structures (highlighted by bold lines). Heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments revealed long-range correlations between the following protons and carbons: H-3 and C-4a, 1', 6'; H-4 and C-5, 8a; H-5 and C-4, 8a; H-7 and C-6, 8, 8a; H-2' and C-4'; H-5' and C-1'; H-6' and C-3, 1', 2', 4'; H-1" and C-8; OCH_3 and C-4'. In addition, the position of the glucoside linkage was confirmed by a nuclear Overhauser effect spectroscopy (NOESY) experiment, which was showed NOE correlations between H-7 and H-1". Next, the absolute configuration at the 3-position of **1** was identified by circular dichroism (CD) spectrum. Namely, **1** showed a positive Cotton effect at 236 nm ($\Delta\varepsilon$ +8.62) and a negative Cotton effect at 255 nm ($\Delta\varepsilon$ –0.79) for 3*S*-dihydroisocoumarin.[23] On the basis of all this evidence, the chemical structure of florahydrosides I (**6**) was determined to be 3*S*-thunberginol E 8-*O*-β-D-glucopyranoside.

On the other hand, the ^1H NMR (methanol-d_4) and ^{13}C NMR (Table 2) spectra of **7**, showed signals assignable to thunberinol E with a β-D-glucopyranosyl moiety as well as **6**. In addition, on the basis of the comparison of the NMR data for **7** with those of **6** and the DQF-COSY and HMBC experiments on **7** (Figure **6**), the position of the glucoside linkage on **7** was determined to be the 6-position. Next, the absolute configuration at the 3-position of **7** was found to be *S* by the CD spectrum as well as **6**. On the basis of all this evidence, the chemical structure of florahydrosides II (**7**) was determined to be 3*S*-thunberginol E 6-*O*-β-D-glucopyranoside.

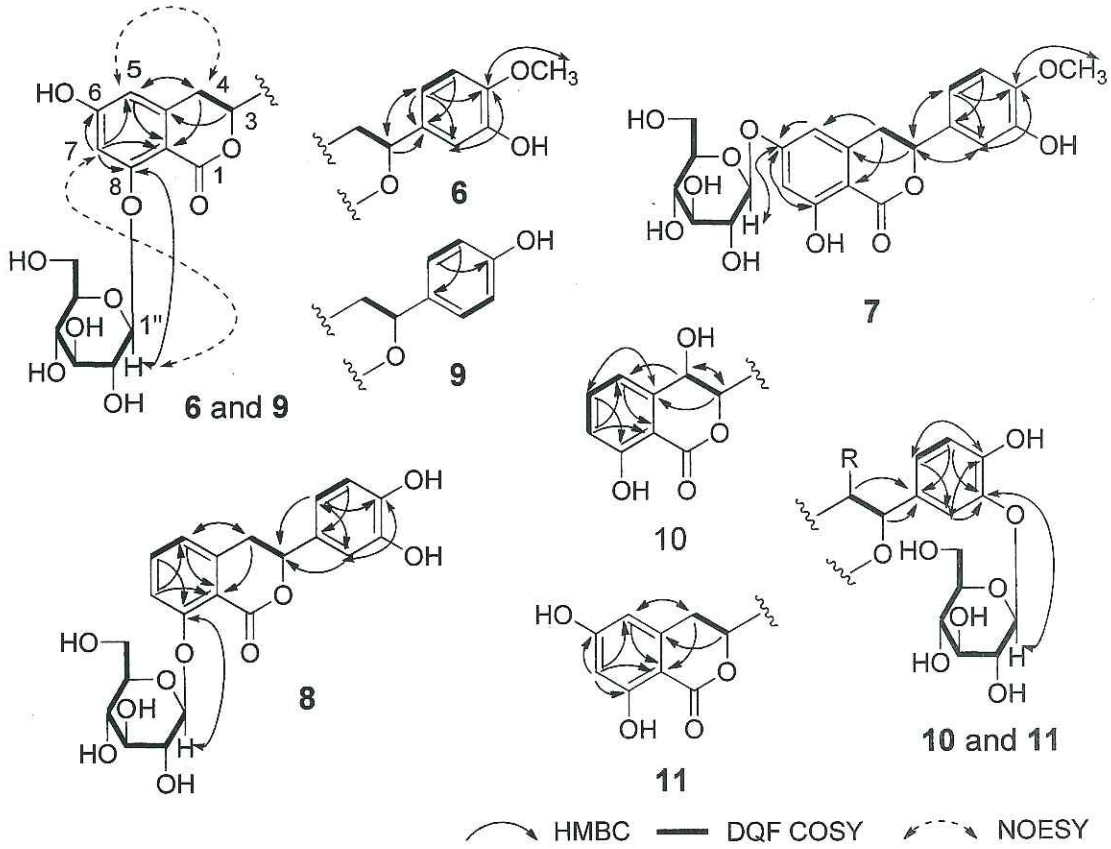


Figure 6 Important 2D NMR correlations of **6-11.**

Table 2 ^{13}C NMR (methanol-d_4, 150 MHz) data for compounds **6–11**

Position	**6**	**7**	**8**	**9**	**10**	**11**
1	166.4	171.4	166.0/165.4	166.7/166.7	171.8/171.8	171.7/171.7
3	80.9	82.0	81.8/81.0	81.1/80.5	85.1/84.7	81.8/81.9
4	37.2	35.9	37.1/36.7	37.3/36.9	75.7/75.3	35.7/35.7
4a	145.5	143.4	143.7/143.2	145.5/145.2	150.2/150.3	143.6/143.7
5	110.3	108.3	123.2/122.7	110.7/110.2	116.8/116.8	108.0/108.0
6	165.4	165.2	136.6/136.3	166.2/166.2	137.0/137.1	166.4/166.4
7	105.3	103.6	118.2/117.0	105.6/104.6	115.6/115.7	102.2/102.2
8	163.2	165.2	160.8/160.3	163.3/162.8	158.0/158.0	165.6/165.7
8a	107.2	104.1	116.0/115.8	106.5/106.5	113.5/113.6	101.7/101.7
1'	132.7	132.6	131.1/131.3	130.8/130.9	132.1/131.9	131.5/131.6
2'	114.6	114.5	114.8/114.6	129.1/128.9	118.3/117.4	117.2/117.4
3'	147.7	147.7	146.6/146.5	116.3/116.3	146.5/146.4	146.7/146.7
4'	149.4	149.5	147.1/146.8	159.1/158.9	148.5/148.1	148.8/148.9
5'	112.5	112.5	116.3/116.3	116.3/116.3	116.6/116.4	117.0/117.1
6'	119.1	119.0	119.4/119.1	129.1/128.9	124.0/123.9	123.0/123.2
OCH_3	56.4	56.4	–	–	–	–
Glc-1"	105.1	101.4	105.1/103.1	105.2/103.3	104.5/104.1	104.0/104.3
2"	74.9	74.7	74.7/ 75.0	74.9/74.5	74.9/74.8	74.9/74.9
3"	77.1	77.9	77.2/77.8	77.2/77.7	77.7/77.6	77.6/77.6
4"	71.1	71.2	71.3/71.2	71.2/71.1	71.3/71.3	71.4/71.5
5"	78.7	78.4	78.8/ 78.5	78.7/78.4	78.2/78.4	78.3/78.4
6"	62.5	62.4	62.6/62.5	62.5/62.5	62.4/62.5	62.5/62.5

Compounds **8–11** were obtained as 3-epimeric mixtures.

Thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**) and thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**), which were obtained as a white amorphous powder and a mixture of diastereoisomers, showed absorption bands due to hydroxy, lactone, aromatic ring, and ether functions in their IR spectra. In the FAB-MS spectra of **8** and **9**, the common quasimolecular ion peak was observed at *m/z* 457 ($[M+Na]^+$) and the molecular formula $C_{21}H_{22}O_{10}$ was determined by HRMS measurement. Acid hydrolysis of **8** and **9** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector. The ^1H NMR (methanol-d_4) and ^{13}C NMR (Table 2) spectra of **8**, which were assigned by various NMR experiments, revealed the products to be a *ca.* 2:1 mixture of two diastereoisomers. Namely, the ^1H NMR showed double signals assignable to a dehydroisocoumarin moiety [δ 3.09 (0.67H, dd, J = 16.5, 2.8 Hz, H-4a), 3.16 (0.33H, dd, J = 16.5, 2.8 Hz, H-4a), 3.28 (1H, m, H-4b), 5.36 (0.67H, dd, J = 11.7, 2.8 Hz, H-3), 5.41 (0.33H, dd, J = 11.7, 2.8 Hz, H-3), 7.04 (0.33H, d like, J = 7.6 Hz, H-5), 7.10 (0.67H, d like, J = 8.2 Hz, H-5), 7.29 (0.33H, d like, J = 7.6 Hz, H-7), 7.40 (0.67H, d like, J = 8.2 Hz, H-7), 7.40 (0.67H, dd, J = 8.2, 8.2 Hz, H-6), 7.53 (0.33H, dd, J = 7.6, 7.6 Hz, H-6)], an ABX-type aromatic ring [δ 6.79 (0.67H, d, J = 8.5 Hz, H-5'), 6.79 (0.33H, m, H-5'), 6.82 (0.67H, dd, J = 8.5, 2.0 Hz, H-6'), 6.82 (0.33H, m, H-6'), 6.88 (0.33H, br s, H-2'), 6.93 (0.67H, d, J = 2.0 Hz, H-2')], and a *β*-D-glucopyranosyl moiety [δ 4.90 (0.67H, d, J = 7.6 Hz, H-1"), 4.94 (0.33H, d, J = 8.3 Hz, H-1")]. The proton and carbon signals of the aglycon part in the ^1H and ^{13}C NMR spectra of **8** were superimposable on those of thunberginol G (**21**),[4, 29] except for the signals around the 8-positon,[5, 25] while the proton and carbon signals due to the isocoumarin part including glycoside moiety were very similar to those of **12**, **13**, and **18**.[14, 23]

As shown in Figure **6**, the HMBC experiments revealed long-range correlations between the following protons and carbons: H-4 and C-5, 8a; H-5 and C-4, 8a; H-6 and C-8; H-7 and C-5, 8a; H-2' and C-3, 4'; H-5' and C-1'; H-6' and C-3, 2', 4'; H-1" and C-8. Furthermore, the 3-position of dihydroisocoumarins with the 4'-hydroxyphenyl group such as hydrangenol, thunberginols B, C, and E, were reported to display tautomer-like behavior.[23] Therefore, **8** was displayed to be a 3-epimeric mixture of thunberiginol G glycoside. On the basis of all this evidence, the chemical structure of **8** was determined to be thunberginol G 8-*O*-*β*-D-glucopyranoside.

On the other hand, the ^1H NMR (methanol-d_4) and ^{13}C NMR (Table **2**) spectra of **9** revealed the products to be a *ca.* 7:3 mixture of two diastereoisomers. The proton and carbon signals of the dihydroisocoumarin part in the ^1H and ^{13}C NMR spectra of **9** were superimposable on those of **6**, while the proton and carbon signals due to the 3-phenyl part were very similar to those of hydrangenol 8-*O*-*β*-D-glucopyranoside (**18**).[14, 23] On the basis of the DQF-COSY and HMBC experiments (Figure **6**), the structure of **9** was determined to be thunberginol C[21, 25, 27] with glucoside. Next, the position of the glucoside linkage in **9** was confirmed based on HMBC and NOESY experiments, which was showed long-range correlations between H-1" and C-8 and NOE correlations between H-7 and H-1", respectively. Consequently, the chemical structure of **9** was determined to be thunberginol C 8-*O*-*β*-D-glucopyranoside.

4-Hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**) and thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**), which were obtained as a mixture of diastereoisomers, showed absorption

bands due to hydroxy, lactone, aromatic ring, and ether functions in their IR spectra. The common molecular formula ($C_{21}H_{22}O_{11}$) of **10** and **11** was determined from the quasimolecular ion peak (*m/z* 473 $[M+Na]^+$) in the positive-ion FABMS and by HRMS measurement. Acid hydrolysis of **10** and **11** liberated D-glucose. The ^1H NMR (methanol-d_4) and ^{13}C NMR (Table **2**) spectra of **10**, showed signals assignable to a dihydroisocoumarin moiety [*ca.* 11:9 mixture of two diastereoisomers (3-epimeric mixture), δ 4.81 (0.55H, d, J = 5.5 Hz, H-4), 4.91 (0.45H, d, J = 5.5 Hz, H-4), 5.60 (0.55H, d, J = 5.5 Hz, H-3), 5.61 (0.45H, d, J = 5.5 Hz, H-3), 6.80 (0.55H, d like, J = 7.9 Hz, H-5), 6.82 (0.45H, d like, J = 7.9 Hz, H-5), 7.40 (0.55H, dd, J = 7.9, 7.9 Hz, H-6), 7.45 (0.45H, dd, J = 7.9, 7.9 Hz, H-6), 6.53 (0.55H, d like, J = 7.9 Hz, H-7), 6.74 (0.45H, overlap, H-7)], ABX-type aromatic ring, and a β-D-glucopyranosyl moiety. The proton and carbon signals in the ^1H and ^{13}C NMR spectra of **10** were superimposable on those of thunberginol G 3'-*O*-β-D-glucopyranoside (**19**),[14, 27] except for the signals around the 4-positon, The structure of dihydroisocoumarin moiety including the two hydroxy groups on **10** was confirmed based on DQF-COSY and HMBC experiments, which was showed long-range correlations between the following protons and carbons: H-3 and C-4; H-4 and C-3, 5; H-5 and C-8a; H-6 and C-8; H-7 and C-8a. On the basis of all this evidence, the chemical structure of **10** was determined to be 4-hydroxythunberginol G 3'-*O*-β-D-glucopyranoside.

On the other hand, the proton and carbon signals of the aglycon part in the ^1H and ^{13}C NMR spectra of **11** were superimposable on those of thunberginol B,[25] except for the signals around the 3'-positon, while the proton and carbon signals due to the 3-benzene ring including a glucoside moiety were very similar to those of **10**. In addition, on the basis of the DQF-COSY and HMBC experiments (Figure 6), the chemical structure of **11** were determined to be thunberginol B 3'-*O*-β-D-glucopyranoside.

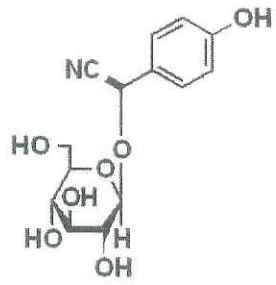
2.4 Qualitative Analysis of Cyanogenic Glycoside [(2*R*)-taxiphyllin and Hydracyanoside A] from Several Parts of *H. macrophylla* var. *thunbergii*

Hydrangea macrophylla (Thunb.) Ser. is another Saxifragaceae plant widely cultivated in many countries including Japan and China. The blossoms are mainly used for ornamental purposes. In 2008, the cases of food poisoning that present symptoms of vomiting etc. after eating the leaves of *H. macrophylla* were generated at Osaka and Ibaraki prefectures in Japan. At first, the Ministry of Health, Labor, and Welfare of Japan advised the possibility that the food poisoning was caused by cyanogenic glycosides in the leaves of *H. macrophylla*. Previously, the chemical constituents from the leaves and stems of *H. macrophylla* and isolated several of cyanogenic glycosides named hydracyanosides A-C and taxiphyllin were examined, so that the cause of the food poisoning remained to be clarified.[7] In the course of our chemical and pharmacological studies on Hydrangea plants[1-8] and medicinal flowers,[8, 35, 62-66] i have examined the qualitative analysis of cyanogenic glycoside [(2*R*)-taxiphyllin and Hydracyanoside A] (Figure **7**) from Several Parts of *Hydrangea macrophylla* var. *thunbergii*.

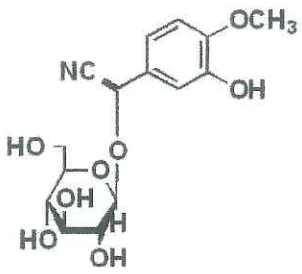
From the results of HPLC Chromatomaps, (2*R*)-taxiphyllin and hydracyanoside A were detected from the leaves of *H. Macrophylla* (Figure 8-A. 八仙花, Sichuan, China), (2*R*)-taxiphyllin was detected from the flowers, stems, and fresh leave of *H. macrophylla* var. *thunbergii* (Figure 8-B—D. アマチャ, Nagano,

Japan), it was suggested that hydracyanoside A has the possibility to be utilized as a chemotaxonomic marker for the identification of the species, *H. macrophylla* (Thunb.)Ser. and *H. macrophylla* var. *thunbergii*. The discovery of cyanogenic glycoside may provide useful insights into the taxonomy of this genus.

In addition, as shown in Figure 8-E, (2*R*)-taxiphyllin was not detected from the processed leaves; comparison of HPLC chromatograms between the fresh and processed leaves, I suppose that (2*R*)-taxiphyllin can be degraded in the process. The process of deactivation by enzymes is necessary for *H. macrophylla* var. *thunbergii* as the resource of food and drink.



(2*R*)-Taxiphilin (**27**)



Hydracyanoside A

Figure 7 Structures of (2*R*)-taxiphyllin and hydracyanoside A

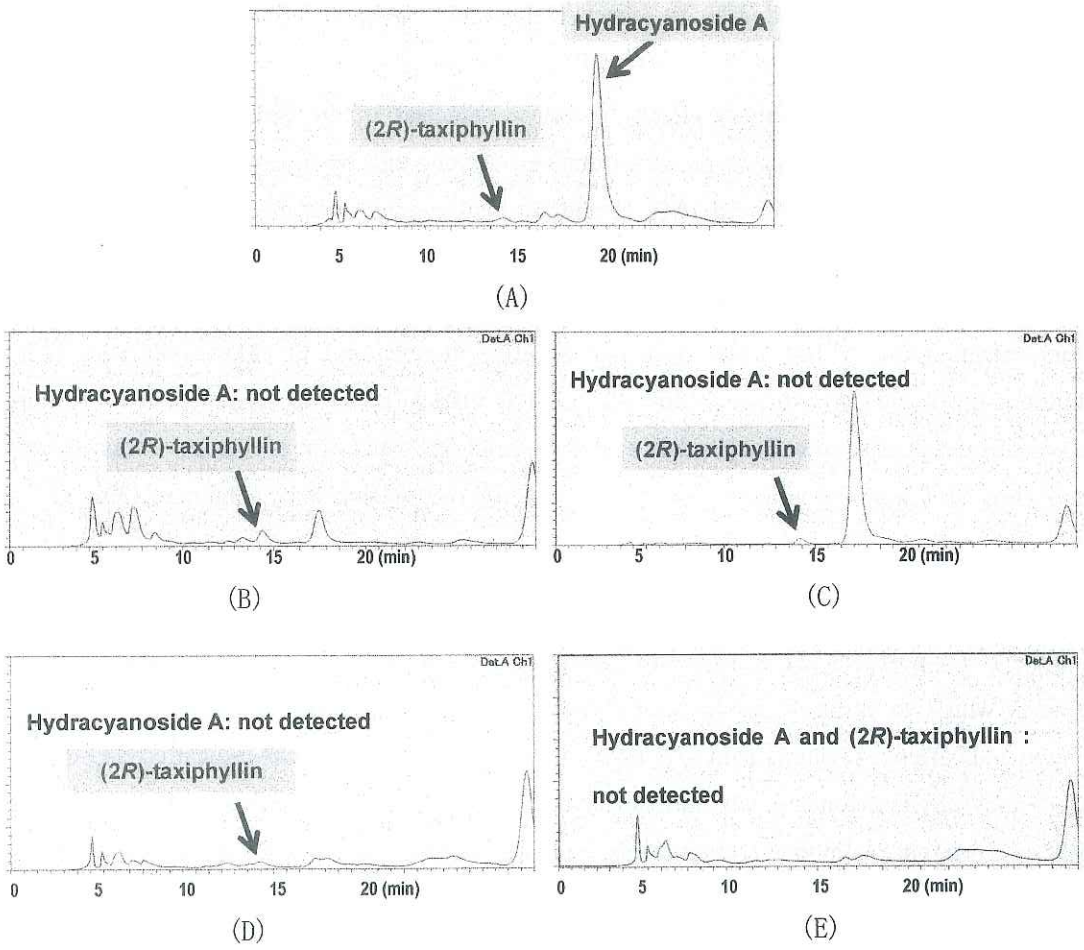


Figure 8 HPLC chromatomap of several parts of *H. macrophylla*

(A) *H. macrophylla* (八仙花, leaves, Sichuan, China); (B) *H. macrophylla* var. *thunbergii* (アマチャ, flowers, Nagano, Japan); (C) *H. macrophylla* var. *thunbergii* (アマチャ, stems, Nagano, Japan); (D) *H. macrophylla* var. *thunbergii* (アマチャ, fresh leaves, Nagano, Japan); (E) *H. macrophylla* var. *thunbergii* (アマチャ, processed leaves , Nagano, Japan).

Chapter 3 Aldose Reducatase Inhibitors from the Flowers of *H. macrophylla* var. *thunbergii*

As a key enzyme in the polyol pathway, aldose reductase has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in chronic complications of diabetes such as cataracts.[1, 35, 41-46] Previously, we have reported that various constituents such as acylated quinic acids, flavonoids, and terpenoids from natural medicines and medicinal foods inhibited aldose reductase inhibitory effect.[8, 13, 20, 22, 66-68] Furthermore, the inhibitory effects of the isolated compounds and its related compounds on aldose reductase were investigated. This chapter deals with the structure elucidation of new compounds and the inhibitory effects of constituents and its related compounds, acylated quinic acid analogs, on aldose reductase.

In the present study, the inhibitory effects on aldose reductase of the isolated constituents from the flowers of *H. macrophylla* var. *thunbergii* were examined. Among the constituents, neochlorogenic acid (**22**) inhibited aldose reductase [IC_{50} = 5.6 μM]. In addition, chlorogenic acid methyl ester (**26**) showed the inhibitory effect [IC_{50}=2.9 μM] in agreement with the previous study.[8] On the other hand, dihydroisocoumarin glycosides (**6-15**, **18**, and **19**) and dihydroisocoumarins (**16** and **17**) lacked the inhibitory effects [IC_{50} > 100 μM]. Hydrangenol (**20**), thunberginol G (**21**), taxiphyllin (**27**), and umbelliferone glucoside (**28**) exhibited moderate inhibitory effects [IC_{50} = 48−69 μM]. Next, the inhibitory effects on aldose reductase of caffeoylquinic acid analogs **49-52**, which was previously obtained from *Ilex paraguariensis*,[69, 70] were examined for the structure-activity relationship study (Figure 9, Table 3).

The inhibitory effect of D-quinic acid with *trans-p*-caffeoyl group at the 5-position [chlorogenic acid (**25**, reference compound,[71] IC_{50} = 0.41 μM]] was stronger than those of D-quinic acids with *trans-p*-caffeoyl group at the 3 or 4-positions [**22** or 4-*O*-*trans-p*-caffeoyl-D-quinic acid (**49**, IC_{50} = 11.8 μM)]. In addition, quinic acids with two cafferoyl groups [3,4-*O*-*trans-p*-dicaffeoyl-D-quinic acid (**50**, IC_{50} = 0.34 μM), 3,5-*O*-*trans-p*-dicaffeoyl-D-quinic acid (**51**, IC_{50} = 0.31 μM), and 4,5-*O*-*trans-p*-dicaffeoyl-D-quinic acid (**52**, IC_{50} = 0.29 μM)] exhibited potent inhibitory effects. Those biological effects were equal to or stronger than that of a reference compound, chlorogenic acid (**25**). Further study for the development of acylated quinic acid analogs as potent anti-cataract agents are expected.

49: $R^1 = R^3 = H$, R^2 = caffeoyl **51**: $R^1 = R^3$ = caffeoyl, $R^2 = H$
50: $R^1 = R^2$ = caffeoyl, $R^3 = H$ **52**: $R^2 = R^3$ = caffeoyl, $R^1 = H$

Figure 9 Structures of caffeoylquinic acid analogs (**49-52**) from *Ilex paraguariensis*

Table 3 Inhibitory effects on aldose reductase of compounds from the flowers of *H. macrophylla* var. *thunbergii*

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
Hydrangeamine A (**1**)	>100 (49.2)	Chlorogenic acid (**25**)	0.4
Hydrangeamine B (**2**)	92.8	Chlorogenic acid methyl ester (**26**)	2.9
Hydrangenoside A (**3**)	>100 (48.7)	Taxiphyllin (**27**)	54.1
Hydramacroside A (**4**)	68.8	Umbelliferone glucoside (**28**)	68.6
Hydramacroside B (**5**)	>100 (30.6)	*α*-Morroniside (**29**)	>100 (21.0)
Florahydroside I (**6**)	>100 (28.7)	Shikimic acid (**30**)	>100 (0.0)
Florahydroside II (**7**)	>100 (26.8)	**31**	>100 (48.3)
Thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**)	>100 (34.2)	**32**	>100 (30.2)
Thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**)	>100 (39.0)	Loganoside (**33**)	>100 (9.6)
4-Hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**)	>100 (30.1)	*trans*-*p*-Coumaric acid (**34**)	29.9
Thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**)	>100 (4.4)	Vogeloside (**35**)	>100 (-0.4)
3*R*-Phyllodulcin 8-*O*-*β*-D-glucopyranoside (**12**)	>100 (47.3)	**36**	>100 (25.7)
3*S*-Phyllodulcin 8-*O*-*β*-D-glucopyranoside (**13**)	>100 (17.8)	Deoxyloganic acid (**37**)	>100 (25.9)
(+)-Hydrangenol 4'-*O*-*β*-D-glucopyranoside (**14**)	>100 (35.4)	Gingerglycolipid A (**42**)	88.3
3*R*-Thunberginol I 4'-*O*-*β*-D-glucopyranoside (**15**)	>100 (15.7)	**43**	>100 (11.5)
(+)-3-(4-methoxyphenyl)-8-hydroxy-3,4-dihydroisocoumarin (**16**)	>100 (41.0)	*p*-Hydroxybenzaldehyde (**44**)	78.5
Phyllodulcin (**17**)	>100 (20.1)	*trans*-Cinnamic acid (**45**)	>100 (15.1)
Hydrangenol 8-*O*-*β*-D-glucopyranoside (**18**)	>100 (25.5)	Skimmetine (**46**)	>100 (38.7)
Thunberginol G 3'-*O*-*β*-D-glucopyranoside (**19**)	>100 (38.2)	Hydrangeic acid (**48**)	10.6
Hydrangenol (**20**)	47.8		
Thunberginol G (**21**)	58.3	4-*O*-*trans*-*p*-caffeoyl-D-quinic acid (**49**)	11.8
Neochlorogenic acid (**22**)	5.6	3,4-*O*-*trans*-*p*-dicaffeoyl-D-quinic acid (**50**)	0.34
3-*O*-*trans*-*p*-Coumaroyl-D-quinic acid (**23**)	11.5	3,5-*O*-*trans*-*p*-dicaffeoyl-D-quinic acid (**51**)	0.31
3-*O*-*cis*-*p*-Coumaroyl-D-quinic acid (**24**)	55.2	4,5-*O*-*trans*-*p*-dicaffeoyl-D-quinic acid (**52**)	0.29

( ): Inhibition% at 100μM

Chapter 4 Bioactive Constituents from the Flowers of *O. fragrans* var. *aurantiacus*

4.1 Introduction

Osmanthus fragrans var. *aurantiacus* (kinmokusei in Japanese) is a species of *Osmanthus* native to Asia, it is valued for its delicate fruity-floral apricot aroma. The flower is used in perfumery and foods, such as sweet osmanthus wine, tea, sugar, juices, cakes and sauces.[72, 73] There are many medical products made out of sweet osmanthus buds, leaves, bark and flowers: a decoction of the stem bark is used in the treatment of boils, carbuncles etc. A decoction of the lateral roots is used in the treatment of dysmenorrhoea, rheumatism, bruises etc. An essential oil obtained from the flowers is used as an insect repellent for clothes. They are also added to herbal medicines in order to disguise obnoxious flavors.[73, 74]

In the present study, 4 new megastigmane glycosides were isolated from the flowers of *Osmanthus fragrans* var. *aurantiacus* together with 121 known compounds. The chemical structures of the new compounds were elucidated on the basis of chemical and physicochemical evidence. Furthermore, the inhibitory effects of several isolated triterpenes on nitric oxide production were examined. This chapter deals with the structure elucidation of the new compounds and the inhibitory effects of triterpene compounds on nitric oxide production.

4.2 Extraction and Isolation

The flowers (2.0 kg) of *Osmanthus fragrans* var. *aurantiacus* cultivated in China was extracted with MeOH. Evaporation of the solvent under reduced pressure provided the MeOH extract (874.9 g, 43.8%). A part of the MeOH extract (824.7 g) was partitioned into 1-hexane, $CHCl_3$, EtOAc, 1-BuOH, and H_2O respectively mixture to yield 1-Hexane-soluble fraction (85.7 g, 4.6%), $CHCl_3$-soluble fraction (53.3 g, 2.8%), EtOAc-soluble fraction (149.8 g, 7.9%), 1-BuOH-soluble fraction (256.3 g, 13.6%) and H_2O-fraction (303.4 g, 16.1%).

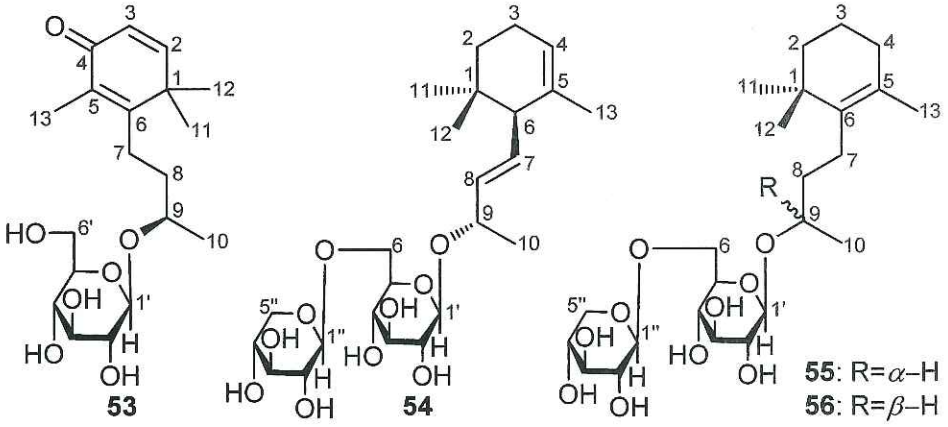


Figure 10 Structures of the new compounds from the flowers of *O. fragrans* var. *aurantiacus*

The 1-BuOH-soluble fraction and $CHCl_3$-soluble fraction were subjected to normal-phase and reversed-phase silica gel column chromatography (CC) and repeated HPLC to give florafragrosides A (**53**, 0.0016%), B (**54**, 0.0010%), C (**55**, 0.0069%), and D (**56**, 0.0574%) (Figure 10) together with 2α,3β,29-trihydroxy-olean-12-en-28-oic acid, (**57**, 0.0085%),[75] cleroindicin F (**58**, 0.0081%),[76] hexahydro-3α-hydroxy-4-methoxy-6(2*H*)-benzofuranone (**59**, 0.0039%),[77] linaloolpyran oxides C (**60**, 0.0316%),[78] and D (**61**, 0.0046%),[79] (*S*)-menthiafolic acid (**62**, 0.0055%),[80] (6*E*,9*S*)-9-hydroxy-4,6-megastigmadien-3-one (**63**, 0.0082%),[81] (6*Z*,9*S*)-9-hydroxy-4,6-megastigmadien-3-one (**64**, 0.0072%),[81] 4-(3-hydroxy-1-butenyl)-isophorone (**65**, 0.0061%),[82] blumenol A (**66**, 0.0019%),[83] 4-oxo-7,8-dihydro-β-ionol (**67**, 0.0373%),[84] (3*E*)-4-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, (**68**, 0.0033%),[85] 4-oxo-7,8-dihydro-β-ionol (**69**, 0.0051%),[86] 2,6-dimethyl-7-octene-2,3,6-triol (**70**, 0.0334%),[87] 7-octene-1,3,6-triol, 6-methyl-2-methylene- (**71**, 0.0023%),[88] 2*E*,6*R*-betulalbuside A (**72**, 0.0106%),[89] 2*E*,6*S*-betulalbuside A (**73**, 0.0020%),[90] larixic acid (**74**, 0.0015%),[91], (4*E*)-3,5,5-trimethyl-4-[3-(β-D-glucopyranosyloxy)butylidene]-2-cyclohexen-1-one (**75**, 0.0033%),[92-95] [*R*-[*R**,*S**-(*E*)]]-3,5,5-trimethyl-4-[3-(β-D-glucopyranosyloxy)-1-butenyl]-2-cyclohexen-1-one (**76**, 0.0013%),[96] 9ξ-*O*-β-D-glucopyranosyloxy-5-megastigmen-4-one (**77**, 0.0116%),[97] geranyl β-primeveroside- (**78**, 0.0032%),[98, 99] jasminoside O (**79**, 0.0169%),[100] salidroside (**80**, 1.4242%),[101] benzeneethanol, 4-hydroxy- (**81**, 0.1893%),[102] *trans*-*p*-coumarinic acid (**34**, 0.0008%),[45] *trans*-ferulic acid (**82**, 0.0127%),[103] 4',5,7-trihydroxy-flavanone (**83**, 0.0045%),[104] vanillic acid (**84**, 0.0189%),[105] cyclolariciresinol (**85**, 0.0129%),[106] (3α*S*,4*S*,6α*S*)-4-(3,4-dimethoxyphenyl)tetrahydro-1*H*,3*H*-furo[3,4-c]furan-1-one (**86**, 0.0028%),[107] dihydro-5-(3-hydroxyhexyl)-2(3*H*)-furanone, (**87**, 0.0029%),[108] linalool oxide β-D-glucoside (**88**, 0.0111%),[109] foliamenthic acid (**89**, 0.0019%),[110] 10-hydroxygeraniol (**90**, 0.0062%),[111] 8-hydroxypinoresinol (**91**, 0.0040%),[112] phillyroside (**92**, 0.0669%),[113] (+)-epipinoresinol (**93**, 0.0028%),[113] (-)-phillygenin (**94**, 0.0293%),[114] (+)-pinoresinol (**95**, 0.0140%),[113] 10-acetoxyligustroside (**96**, 0.0315%),[115] 3'-*O*-D-glucopyranosyl ligustroside (**97**, 0.0045%),[116] ligustroside (**98**, 0.0962%),[116] isoacteoside (**99**, 0.0033%),[117] verbascoside (**100**, 12.1658%),[118] oleoacteoside (**101**, 0.0082%),[119] messagenic acid I (**102**, 0.0011%),[120] platanic acid (**103**, 0.0099%),[121] 19α-hydroxyasiatic acid (**104**, 0.0082%),[122] arjunolic acid (**105**, 0.0180%),[123] asiatic acid (**106**, 0.0080%),[123] 4-epi-spathodic acid (**107**, 0.0085%),[124] rotundic acid (**108**, 0.0125%),[125] benthamic acid (**109**, 0.0019%),[126] myrianthic acid (**110**, 0.0114%),[122] isoarjunolic acid (**111**, 0.0040%),[127] esculentic acid (**112**, 0.0102%),[128] queretaroic acid (**113**, 0.0038%),[129] 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid (**114**, 0.0037%),[130] euscophic acid (**115**, 0.0062%),[131] tormentolic acid (**116**, 0.0080%),[130] siaresinolic acid (**117**, 0.0207%),[132] maslinin acid (**118**, 0.0170%),[133] oleanolic acid (**119**, 0.0219%),[134] 3-epi-corosolic acid (**120**, 0.0157%),[135] β-ursolic acid (**121**, 0.0316%),[136] 2α-hydroxy ursolic acid (**122**, 0.0074%),[137] α-ilexanolic acid (**123**, 0.0013%),[138] (2α,3β)-2,19-dihydroxy-3-[[(2*E*)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-urs-12-en-28-oic acid (**124**, 0.0055%),[139] (2α,3β)-3-[[(2*E*)-3-(4-hydroxyphenyl)-1-oxo-2-propenyl]oxy]-2,19-dihydroxy-urs-12-en-28-oic acid (**125**, 0.0010%).[140] (Figures 11-13)

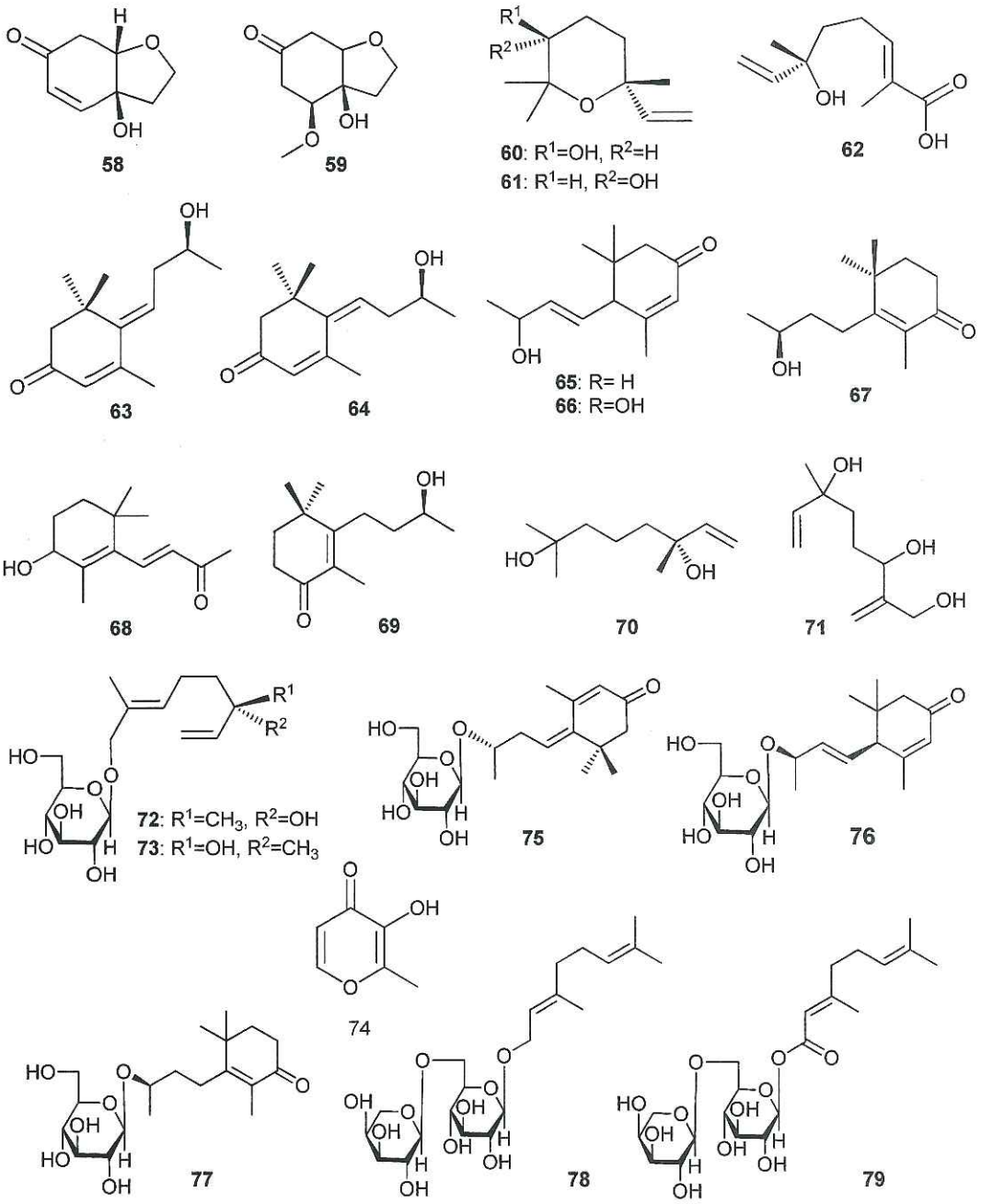


Figure 11 Structures of the known compounds from the flowers of *O. fragrans* var. *aurantiacus* (1)

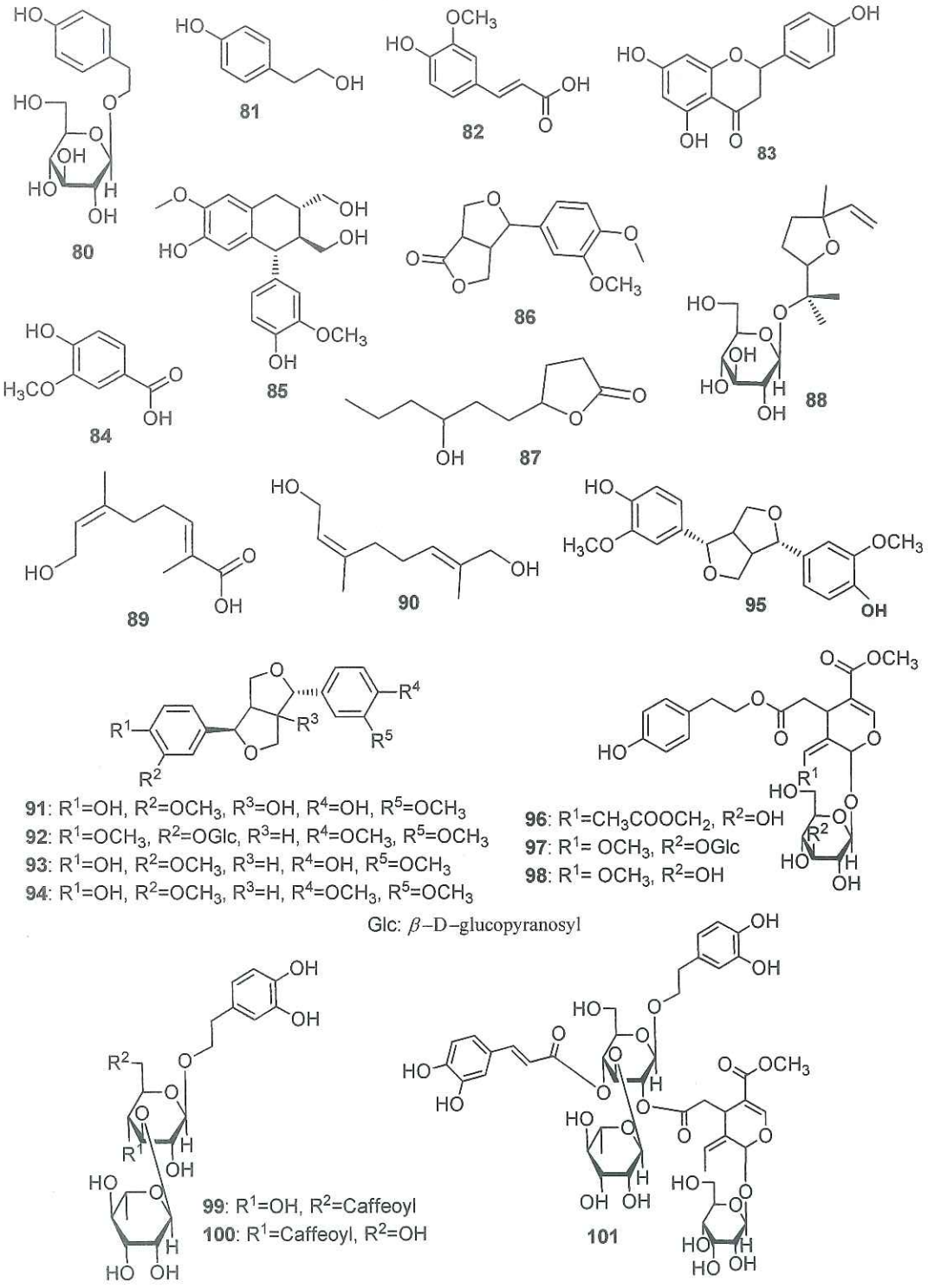


Figure 12 Structures of the known compounds from the flowers of *O. fragrans* var. *aurantiacus* (2)

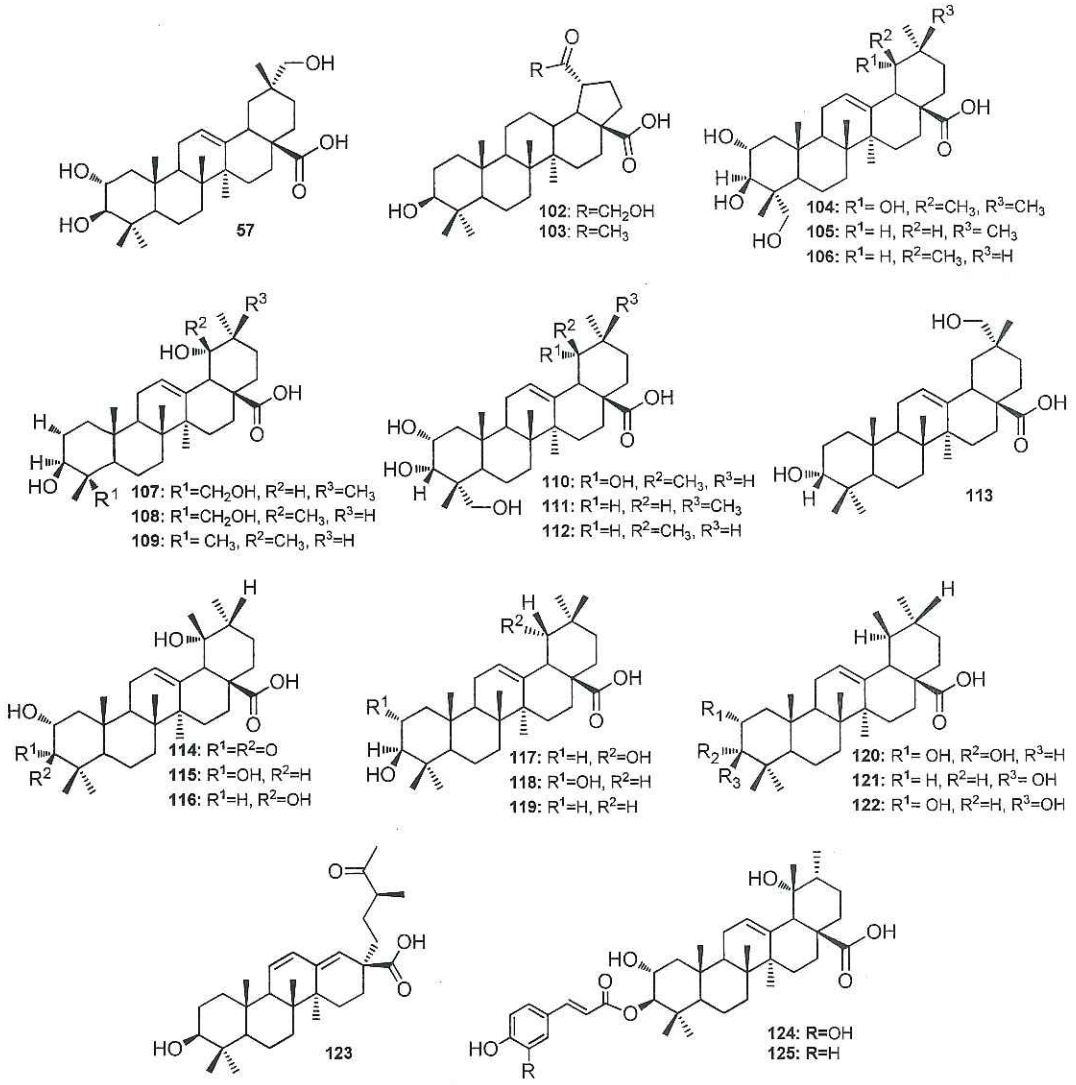


Figure 13 Structures of the known compounds from the flowers of *O. fragrans* var. *aurantiacus* (3)

4.3 Structure Elucidation of New Megastigmane Glycosides from the Flowers of *O. fragrans* var. *aurantiacus*

Florafragroside A (**53**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{27}$ –9.0°, in MeOH). Its IR spectra showed absorption bands at 3339, 1653, and 1074 cm^{-1} due to hydroxy, α, β-unsaturated carbonyl, and ether functions. In the FAB-MS spectra of **53**, the common quasimolecular ion peak was observed at *m/z* 393 ($[M+Na]^+$) and the molecular formula $C_{19}H_{30}O_7$ was determined by HR MS measurement. The ^1H NMR (methanol-d_4) and ^{13}C NMR (Table 4) spectra of **53**, which were assigned by various NMR experiments revealed signals for two methylenes (δ_H 1.81, 1.90, and 1.40, 1.81), four methyls including a gem-dimethyl group (δ_H 1.06, 1.08, 1.85, and 2.30), an oxymethine (δ_H 4.16, brs,H-9), an *trans*-olefin pair [δ_H 6.12 (d, J=16.50 Hz, H-2), δ_C 134.0 and δ_H 7.30 (d, J=16.50 Hz, H-3), δ_C 144.9], and an α, β-unsaturated carbonyl [δ_C 134.4 (C-5), δ_C 141.4 (C-6), and δ_C 201.1 (C-4)], all of which constituted the aglycon part of the molecule together with a β-D-glucopyranoside moiety [δ_H 4.35 (d, J = 7.6 Hz, H-1'), δ_C 101.9]. Acid hydrolysis of **53** with 5% aqueous H_2SO_4 liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.

In the ^{13}C NMR spectrum of **53**, aglycon part of **53** were superimposable on those of 9ξ-*O*-*β*-D-glucopyranosyloxy-5-megastigmen-4-one[97] and foliasalacioside D[141] except for the signals around the 7-9 positions. As shown in Figure 14, the structure of **53** was characterized by means of DQF-COSY and HMBC experiment (Figure 14). The positions of glycoside linkages were determined by a HMBC experiment, which showed long-range correlations between H-1' and C-9. Furthermore, the configuration of the 9-position in **53** was characterized by comparison of the ^{13}C NMR data as described in the references [9*R*: δ_C-9(75.7-76.8), δ_C-10(19.7-20.4), δ_C-1'(102.0-102.9); 9*S*: δ_C-9(77.7-78.1), δ_C-10(21.8-22.0), δ_C-1'(103.7-103.9)].[141-143] Namely, the signals of C-9, -10, and -1' in **53** were observed at [δ_C 76.2(C-9), 19.1(C-10), 101.9(C-1')], respectively, so that the configuration of the 9-position was determined to be *R*, and the total structure of **53** was characterized as shown in Figure 10.

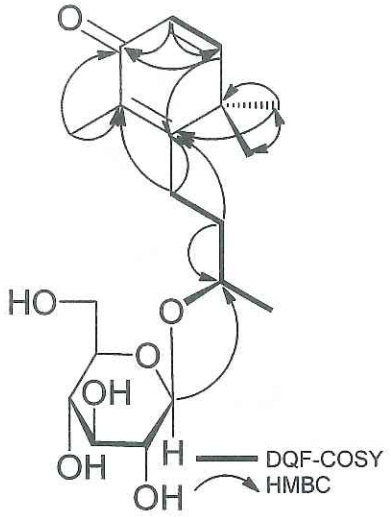


Figure 14 DQF-COSY and HMBC correlations of **53**

Florafragroside B (**54**) was isolated as an amorphous powder. Its molecular formula was derived as $C_{24}H_{40}O_{10}$ by HR-FAB-MS (*m/z* 511.2514 $[M+Na]^+$). The ^1H NMR spectrum of **54** showed signals assignable to a vinyl proton at δ_H5.41(brs) and two mutually coupled vinyl protons at δ_H 5.52 (dd, *J*=8.94, 15.12 Hz) and δ_H 5.34 (dd, *J*=8.22, 15.12 Hz). Proton signals due to two methines [δ_H4.40 (t, *J*=8.22, H-9), and δ_H2.14 (d, *J*=8.94, H-6)], two isolated methylenes [δ_H1.18, 1.49(m, m, H_2-2), and δ_H2.02(brs, H_2-3)], a vinyl methyl (δ_H1.63, brs, H_3-13), a secondary methyl [δ_H1.26 (d, *J*=6.90 Hz, H_3-10)], and two tertiary methyl groups (δ_H0.83, 0.89, H_3-11 and 12, each s) were observed in the aliphatic region. The presence of two sugar residues was suggested by two doublet signals [δ_H4.33 (*J* =8.28 Hz), δ_H4.30 (*J* =7.56 Hz)] due to anomeric protons.

The ^{13}C NMR spectrum of **54** showed 24 carbon signals, among which 13 resonances were similar to those corresponding to a *α*-ionol moiety of (6*R*, 9*R*)-*α*-ionol 9-*O*-*α*-L-arabinofuranosyl (1→6)-*β*-D-glucopyranoside. The remaining 11 carbon resonances (Table 4) could be superimposed on signals due to *β*-glucopyranosyl and *β*-xylopyranosyl units in eriojaposide A.[144] Acid hydrolysis of **54** with 5% aqueous H_2SO_4 liberated D-glucose and D-xylose, which were identified by HPLC analysis using an optical rotation detector.

The locations of the sugar residues in **54** were established by the HMBC experiment (Figure 15). Hence,

the anomeric proton signal (δ_H4.33, H-1') of glucose, which was assigned from the DQF-COSY NMR spectrum, was correlated through a threebond coupling with C-9 (δ_C 75.4) of the aglycon in the HMBC experiment. The other anomeric proton (xylose) at δ_H4.30 was also correlated with C-6' of glucose at δ_C 69.9. A xylopyranosyl-(1"→6')-glucopyranosyl moiety as the disaccharide sugar chain was thus allocated to C-9 of a α-ionol moiety. The absolute configuration at C-6 of the aglycon was determined by CD spectra.[145] The CD spectra of **54** showed Cotton effect at [243 nm ($\Delta\varepsilon$ +3.15 in MeOH)], so that the stereostructure at the 6-position in **54** was confirmed to be *R* orientation. The absolute configuration at C-9 was assigned as *S* on the basis of a diagnostic chemical shift of the C-9 signal (δ_C 75.4) in the ^{13}C NMR spectrum [9*R*: δ_C-9(77.3-79.1), δ_C-10(21.2-21.8), δ_C-1'(102.2-103.0); 9*S*: δ_C-9(74.7-76.3), δ_C-10(22.3-22.6), δ_C-1'(100.5-101.7)].[141-143] Consequently, the structure of **54** was determined as shown in Figure 10.

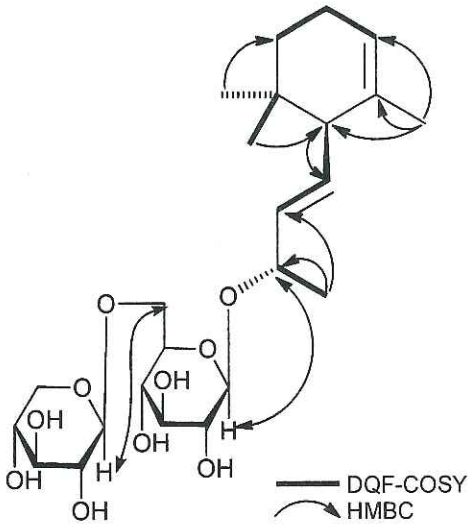


Figure 15 DQF-COSY and HMBC correlations of **54**

Florafragroside C (**55**) and D (**56**) were isolated as a white amorphous powder with negative optical rotation (**55**: $[\alpha]_D^{24}$ –8.4°, **56**: $[\alpha]_D^{26}$ –48.9°, in MeOH). Their IR spectra showed absorption bands due to hydroxy and double bonds functions. In the FAB-MS spectra of **55** and **56**, the common quasimolecular ion peak was observed at *m/z* 513 ($[M+Na]^+$) and the molecular formula $C_{24}H_{42}O_{10}$ was determined by high-resolution (HR) FAB-MS measurement. Acid hydrolysis of **55** and **56** with 5% aqueous H_2SO_4 liberated D-glucose and D-xylose, which were identified by HPLC analysis using an optical rotation detector.

The ^{1}H NMR (methanol-d_4) and ^{13}C NMR (Table 4) spectra of **55** and **56**, which were assigned by various NMR experiments, showed signals assignable to four methyls[**55**: δ_H0.99, 0.99,1.60 (all s, H_3-11,12,13), 1.18(d, *J*=6.18, H_3-10); **56**: δ_H1.00, 1.00,1.60 (all s, H_3-11,12,13), 1.25(d, *J*=6.18, H_3-10)], a methane bearing an oxygen function [**55**: δ_H3.84 (m, H-9); **56**: δ_H3.82 (dd, *J*=6.18, 12.36, H-9)], a tetra-substituted double bond [**55**: δ_C127.9 (C-5), 138.5 (C-6); **56**: δ_C127.9 (C-5), 138.3 (C-6)], five methylenes [**55**: δ_C41.1 (C-2), 20.6 (C-3), 33.8 (C-4) , 25.7 (C-7), 39.1 (C-8); **56**: δ_C41.1 (C-2), 20.6 (C-3), 33.7 (C-4) , 25.3 (C-7), 38.1 (C-8)], and a quaternary carbon [**55**: δ_C36.0 (C-1); **56**: δ_C35.9 (C-1)] together with a β-D-glucopyranosyl part [**55**: δ_H4.32 (d, *J*=6.90, H-1'); **56**: δ_H4.33 (d, *J*=7.56, H-1')] and a β-D-xylopyranosyl part [**55**: δ_H4.33 (d, *J*=6.84, H-1"); **56**: δ_H4.33 (d, *J*=7.56, H-1")].

As shown in Figure 16, the DQF-COSY experiments on **55** and **56** indicated the presence of partial structure written in bold lines, and in the HMBC experiments, long-range correlations were observed between the follow protons and carbons: H_2-2 and C-1, 4; H_2-3 and C-1; H_2-7 and C-5, 6; H_3-10 and C-8, 9; H_3-11 and C-2; H_3-12 and C-6; H_3-13 and C-4, 5, 6; H-1' and C-9; H-1" and C-6', so that the planar structure of the aglycon part and the positions of the glycoside linkages in **55** and **56** were characterized.

The correlations of DQF-COSY and HMBC experiments between **55** and **56** were the same, but in the ^{1}H NMR and ^{13}C NMR spectra, all the signals of **55** were superimposable on those of **56** respectively except for the signals around the positions of C-8, 9, 10 and 1' [**55**: δ_C39.1, 76.3, 19.8 and 102.3; **56**:38.1, 78.0, 21.9 and 103.9]. So that **55** and **56** were identified as a pair of diastereoisomers, the absolute configurations at C-9 of **55** and **56** were assigned as *R* and *S* respectively on the basis of a diagnostic chemical shift of these signals in the ^{13}C NMR spectrum.[141-143] Consequently, the structure of **55** and **56** were determined as shown in Figure 10.

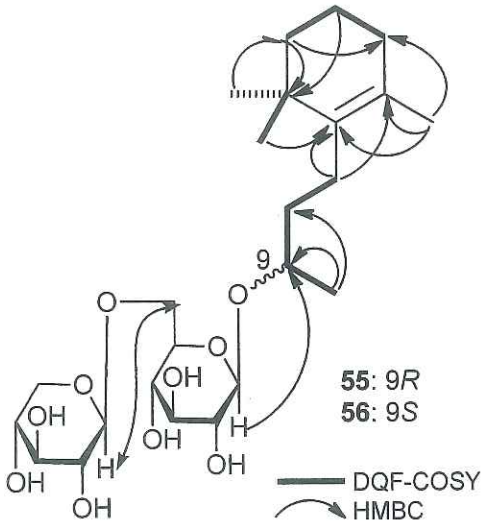


Figure 16 DQF-COSY and HMBC correlations of **55** and **56**

Table 4 ^{1}H and ^{13}C NMR (methanol–d_4, 600/150 MHz) data for the new megastigmane glycosides **53-56**

No.	**53**		**54**		**55**		**56**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		35.5		32.7		36.0		35.9
2	6.12, d, *J*=16.50	134.0	1.18, m; 1.49, m	32.9	1.42, d, *J*=6.18	41.1	1.42, m	41.1
3	7.30, d, *J*=16.50	144.9	2.02, brs	24	1.58, t, *J*=6.18	20.6	1.57, m	20.6
4		201.1	5.41, brs	122.1	1.89, t, *J*=6.18	33.8	1.90, t, *J*=6.18	33.7
5		134.4		135.2		127.9		127.9
6		141.4	2.14, d, *J*=8.94	55.5		138.5		138.3
7	1.81, d, *J*=9.60 1.90, m	24.9	5.52, dd, *J*=8.94,15.12	136.1	1.99, m 2.19, m	25.7	2.00, td, *J*=4.80, 13.74 2.19, td, *J*=4.80, 13.74	25.3
8	1.40, m 1.81, d, *J*=9.60	35.5	5.34, dd, *J*=8.22,15.12	134.1	1.53, m 1.64, m	39.1	1.53, m 1.65, m	38.1
9	4.16, brs	76.2	4.40, t. *J*=8.22	75.4	3.84, m	76.3	3.82, dd, *J*=6.18, 12.36	78.0
10	1.85, s	19.1	1.26, d, *J*=6.90	22.4	1.18, d, *J*=6.18	19.8	1.25, d, *J*=6.18	21.9
11	1.08, s	29.3	0.83, s	32.9	0.99, s	29.2	1.00, s	29.1
12	1.06, s	27.6	0.89, s	32.7	0.99, s	29.2	1.00, s	29.1
13	2.30, s	27.2	1.63, brs	23.5	1.60, s	20.2	1.60, s	20.2
1'	4.35, d, *J*=7.56	101.9	4.33, d, *J*=8.28	100.8	4.32, d, *J*=6.90	102.3	4.33, d, *J*=7.56	103.9
2'	3.16, dd, *J*=8.28,9.66	75.0	3.20, overlapped	74.9	3.12-3.22, overlapped	74.9	3.20, m	74.8
3'	3.35, t, *J*=8.94	78.3	3.20-3.30, overlapped	78.2	3.34, overlapped	78.1	3.35, m	78.0
4'	3.24-3.28, overlapped	71.9	3.20-3.30, overlapped	71.6	3.34, overlapped	71.5	3.35, m	71.4
5'	3.24-3.28, overlapped	78.0	3.20-3.30, overlapped	76.9	3.40, m	76.9	3.42, m	76.8
6'	3.67, dd, *J*=5.46,11.64 3.87, dd, *J*=2.10,12.36	63.0	3.72, m 4.04, d, *J*=10.98	69.9	3.73, dd, *J*=5.46, 11.64 4.05, d, *J*=11.04	69.8	3.75, dd, *J*=5.46,11.70 4.05, dd, *J*=2.04,11.70	69.7
1"			4.30, d, *J*=7.56	105.6	4.33, d, *J*=6.84	105.5	4.33, d, *J*=7.56	105.4
2"			3.20, overlapped	74.9	3.12-3.22, overlapped	75.1	3.18, m	75.1
3"			3.20-3.30, overlapped	77.8	3.34, overlapped	77.6	3.31, m	77.6
4"			3.47, m	71.2	3.47, m	71.2	3.48, m	71.1
5"			3.85, dd, *J*=5.46, 11.64 3.20,overlapped	66.9	3.18, m 3.84, m	66.8	3.19, m 3.85, dd, *J*=5.46, 11.7	66.8

4.4 Inhibitory Effects on Nitric Oxide Production from the Flowers of *O. fragrans* var. *aurantiacus*

NO is a highly reactive free radical implicated in macrophage-mediated cytotoxicity and the inhibition of cellular proliferation. It is produced from L-arginine by NO synthase (NOS), one form of which (Type II, or iNOS) is induces by various cytokines and inflammatory mediator.[145-148] Various infectious diseases and physical, chemical, and immunological factors participate in inflammation-related carcinogenesis. Under inflammatory conditions, reactive oxygen and nitrogen species are generated from inflammatory and epithelial cells, and so the suppression of signaling molecules for iNOS expression may have great potential for the prevention and treatment of inflammation associated carcinogenesis.[149-152] In murine macrophage RAW264.7 cells, lipopolysaccharide (LPS) alone induces the transcription and protein synthesis of iNOS, and increased NO production. Using the Griess reaction, a spectrophotometric determination of nitrite (NO_2^-) was carried out to quantify levels in the conditioned medium of RAW264.7 cells treated with LPS. This cell-based assay system has been used for drug screening and the evaluation of potential inhibitors of the pathways leading to the induction of iNOS and NO production[153, 154].

In the present study, we examined the inhibitory effects of isolated constituents from the flowers of *Osmanthus fragrans* var. *aurantiacus* on NO production induced by LPS. Our results showed that several triterpenes showed moderate inhibitory effects on nitric oxide production in LPS-activated RAW264.7. cells **124** and **125** showed the moderate inhibitory effects with IC_{50} values of 12.6 and 19.1 μM. Other triterpenes showed weaker inhibitory effects. These findings indicate that acyl group, such as *trans-p*-caffeoyl proup or *trans-p*-coumaroyl group, is an important functional group for the inhibition. The structure-activity relationships of this type of triterpene should be studied further.

Conclusion

1. Aldose Reducatase inhibitors from *H. macrophylla* var. *thunbergii*

Hydrangea (*H.*) *macrophylla* SERINGE var. *thunbergii* MAKINO, is native to Japan. The processed leaves of this plant (Hydrangea Dulcis Folium) are currently used as a natural medicine for an oral refrigerant and as a sweetener for diabetic patients, and these preparations are also listed in the Japanese Pharmacopoeia. Chemical investigation of the MeOH-eluted fraction let to isolation of two novel polyketide-type pseudoalkaloid-coupled secoiridoid glycosides, hydrangeamines A(**1**) and B(**2**), and six new dihydroisocoumarin glucosides, florahydrosides I (**6**) and II (**7**), thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**), 4-hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (**10**), and thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**) from the flowers of *H. macrophylla* var. *thunbergii* together with 40 known compounds including dihydroisocoumarin derivatives and acylated quinic acid analogs. Among the constituents, neochlorogenic acid (**22**) inhibited aldose reductase [IC_{50} = 5.6 μM]. In addition, chlorogenic acid methyl ester (**26**) showed the inhibitory effect [IC_{50} = 2.9 μM]. On the other hand, dihydroisocoumarin glucosides (**6**–**15**, **18**, and **19**) and dihydroisocoumarins (**16** and **17**) lacked the inhibitory effects [IC_{50} > 100 μM]. Hydrangenol (**20**), thunberginol G (**21**), taxiphyllin (**27**), and umbelliferone glucoside (**28**) exhibited moderate inhibitory effects [IC_{50} = 48–69 μM]. Next, the inhibitory effects on aldose reductase of caffeoylquinic acid analogs were examined for the structure-activity relationship study. The inhibitory effect of D-quinic acid with *trans-p*-caffeoyl group at the 5-position was stronger than those of D-quinic acids with *trans-p*-caffeoyl group at the 3 or 4-positions.

2. Inhibitory Effects on Nitric Oxide Production from the Flowers of *Osmanthus fragrans* var. *aurantiacus*

Osmanthus fragrans var. *aurantiacus* (sweet osmanthus; kinmokusei in Japanese) is a species of *Osmanthus* native to Asia, it is valued for its delicate fruity-floral apricot aroma. The flower is used in perfumery and foods and there are many medical products made out of sweet osmanthus buds, leaves, bark and flowers. 73 compounds including several new megastigmane glycosides (**53**—**56**) were isolated from the flowers of *Osmanthus fragrans* var. *aurantiacus*. The chemical structures of these compounds were elucidated on the basis of chemical and physicochemical evidence. Among them, the triterpene with acyl group at the 3-position showed inhibitory effects on nitric oxide production.

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Experimental

The following instruments were used to obtain physical data: specific rotations, a Horiba SEPA-300 digital polarimeter (l = 5 cm); IR spectra, a Shimadzu FTIR-8100 spectrometer; CD spectra, a JASCO J-720WI spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, a JEOL JMS-SX 102A mass spectrometer; ^{1}H NMR spectra, JEOL JNM-ECA600 (600 MHz) spectrometers; ^{13}C NMR spectra, JEOL JNM-ECA600 (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC, a Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors. YMC-Pack ODS-A (YMC), COSMOSIL-5C_{18}-PAQ (Nacalai Tesque) and COSMOSIL-Cholester (Nacalai Tesque){[250 x 4.6 mm i.d. (5 μm) for analytical purposes] and [250 x 20 mm i.d. (5 μm) for preparative purposes]} columns were used. The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography (CC), Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reverse-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm). Detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

1. Experimental for *H. macrophylla* var. *thunbergii*

1.1 Material

Flowers of *Hydrangea macrophylla* SERINGE var. *thunbergi*i MAKINO (Saxifragaceae), which were cultivated in Nagano prefecture, Japan, in 2010, were purchased from Kurohime Medical herb Tea Co., Ltd. (Nagano, Japan). A voucher of the plant is on file in our laboratory (KPU Medicinal Flower-2010-HM).

1.2 Extraction and Isolation

The flowers (647.4 g) of *Hydrangea macrophylla* var. *thunbergi*i was extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (208.5 g, 32.20%). Part of the MeOH extract (198.4 g) was partitioned into an EtOAc-H_2O (1: 1, v/v) mixture to yield an EtOAc-soluble fraction (53.9 g, 8.75%) and an aqueous fraction; The aqueous fraction was partitioned into an H_2O-1-BuOH (1: 1, v/v) mixture to yield an 1-BuOH-soluble fraction (76.9 g, 12.49%) and H_2O fraction (67.5 g, 10.96%).

The BuOH–soluble fraction (72.5 g) was subjected to ordinary–phase silica gel column chromatography {500 g, $CHCl_3$–MeOH–H_2O [(20: 3: 1, v/v/v, lower layer) →(10: 3: 1, v/v/v, lower layer) →(7: 3: 1, v/v/v, lower layer) →(6: 4: 1, v/v/v, lower layer)]→MeOH} to give 2 fractions {fr. X (44.16 g), fr. Y (16.42 g) }. Fr. X (42.60 g) was subjected to reversed–phase silica gel column chromatography [740 g, MeOH–H_2O (0: 100→10: 90→20: 80→30: 70→40: 60→50: 50, v/v)→MeOH] to give 11 fractions {fr. X–1 (0.38 g), fr. X–2 (1.85 g), fr. X–3 (0.39 g), fr. X–4 (4.73 g), fr. X–5 (4.69 g), fr. X–6 (3.54 g), fr. X–7 (6.23 g) , fr. X–8 (14.54 g) , fr. X–9 (6.11 g) , fr. X–10 (0.92 g), and fr. X–11(2.75 g)}. Fr. X–1 and fr. X–2 was purified by HPLC [MeOH: H_2O (20: 70), HPLC column: PAQ(250×20 mm i.d., S–5 μm, 12 nm)] to give Shikimic acid (**30**, 413.2 mg, 0.3600%). Fr. X–4 was purified by HPLC [MeOH: H_2O (30: 70), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (20: 80), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give taxiphyllin (**2**, 23.6 mg, 0.0764%), neochlorogenic acid (**25**, 16.6 mg, 0.0537%), 3-(*β*-D-glucopyranosyloxy)-4-hydroxy-benzaldehyde, (**31**, 9.6 mg, 0.0311%), umbelliferone glucoside (**28**, 13.8 mg, 0.11%), morroniside (**29**, 6.4 mg, 0.0207%), 5-p-*cis*-coumaroylquinic acid (**24**, 8.8 mg, 0.0285%), 5-p-*trans*-coumaroylquinic acid (**23**, 70.3 mg, 0.27%), chlorogenic acid (**22**, 23.1 mg, 0.0747%). Fr. X–5 was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (30: 70), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give umbelliferone glucoside (**28**, 10.5 mg, 0.1016%), 3-*O*-*trans*-*p*-coumaroyl-D-quinic acid (**23**, 6.6 mg, 0.0639%), chlorogenic acid (**22**, 1.1 mg, 0.0106%), methyl chlorogenate (**26**, 5.1 mg, 0.0494%), hydrangenol 8-*O*-glucoside (**18**, 197.4 mg, 2.78%). Fr. X–6 was purified by HPLC [MeOH: H_2O (45: 55), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (40: 60), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give hydrangenol 8-*O*-glucoside (**18**, 42.0 mg, 0.0916%), 8-(*β*-D-glucopyranosyloxy)-7-methoxy-2*H*-1-benzopyran-2-one (**32**, 4.2 mg, 0.0146%),

3,4-dihydro-8-hydroxy-3- [3-(*β*-D-glucopyranosyloxy)-4-hydroxyphenyl]-1*H*-2-benzopyran-1-one, (**10**, 5.8 mg, 0.0202%), loganoside (**33**, 6.9 mg, 0.0245%), thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**, 1.8 mg, 0.1197%), *trans*-p-coumaric acid (**34**, 3.7 mg, 0.0131%), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**, 24.6 mg, 0.4585%), vogeloside (**35**, 39.6 mg, 0.1200%), benzoic acid, 2-hydroxy-4-(*β*-D-glucopyranosyloxy)-6-[2-(4-hydroxyphenyl)ethyl]- (**36**, 117.2 mg, 1.0000%), 3*R*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**12**, 535.8 mg, 2.0857%), 3*S*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**13**, 2.09%), deoxyloganic acid (**37**, 11.1 mg, 0.4300%). Fr. X–7 was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (40: 60), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give hydrangenol 8-*O*-glucoside (**18**, 27.7 mg, 0.2364%), thunberginol G 8-*O*-*β*-D-glucopyranoside (**8**, 4.1 mg, 0.0350%), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**, 5.3 mg, 0.0452%), deoxyloganic acid (**37**, 5.8 mg, 0.0495%). Fr. X–8 was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (40: 60), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give hydrangenol 8-*O*-glucoside (**18**, 86.4 mg, 0.7411%), thunberginol C 8-*O*-*β*-D-glucopyranoside (**9**, 17.5 mg, 0.0996%), 3*R*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**12**, 175.9 mg, 1.0010%), 3*S*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**13**, 3.70 mg, 0.032%), naringenin 7-glucoside (**38**, 1.0 mg, 0.0019%). Fr. X–9 was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm) & MeOH: H_2O (35: 65), HPLC column: Cholester water (250×20 mm i.d., S–5 μm, 12 nm)] to give 3*R*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**12**, 339.3 mg, 0.073%), 3*S*-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**13**, 5.60 mg, 0.011%), naringenin 7-glucoside (**38**, 1.0 mg, 0.0019%), florahydroside I (**6**, 35.9 mg, 0.0677%), florahydroside II (**7**, 6.3 mg, 0.0137%), thunberginol D 3'-*O*-*β*-D-glucopyranoside (**11**, 14.3 mg, 0.0270%), quercetol 3-glucoside (**39**, 0.7 mg, 0.0013%), 3,4-dihydro-6,8-dihydroxy-3-(*β*-D-glucopyranosyloxy)-3-(3-hydroxy-4-methoxyphenyl)-1*H*-2-benzopyran -1-one (**40**, 2.2 mg, 0.0041%), hydramacroside A (**4**, 4.9 mg, 0.0091%), hydramacroside B (**5**, 4.5 mg, 0.0083%), 3-[4-(*β*-D-glucopyranosyloxy)phenyl]-3,4-dihydro-8-hydroxy-1*H*-2-benzopyran-1-one (**14**, 2.1 mg, 0.0040%), 1*H*-2-benzopyran-1-one, 3-[3-(*β*-D-glucopyranosyloxy)-4-hydroxyphenyl]-3,4-dihydro-8-hydroxy- (**19**, 102.0, 0.1600%). Fr. X–10 was purified by HPLC [MeOH: H_2O (45: 55), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm)] to give hydramacroside B (**5**, 3.4 mg, 0.0055%%), 3-[3-(*β*-D-glucopyranosyloxy)-4-hydroxyphenyl]-3,4-dihydro-8-hydroxy-1*H*-2-benzopyran-1-one (**19**, 52.4, 0.0700%), 3-[4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]-3,4-dihydro-8-hydroxy-1*H*-2-benzopyran-1-one (**15**, 34.2 mg, 0.0558%), hydrangenoside A (**3**, 5.8 mg, 0.0077%). Fr. X-11 (2.75 g) was subjected to ordinary–phase silica gel column chromatography {300 g, $CHCl_3$→$CHCl_3$–MeOH (10: 1) →$CHCl_3$–MeOH–H_2O [(10: 3: 1, v/v/v, lower layer) →(7: 3: 1, v/v/v, lower layer) →(6: 4: 1, v/v/v, lower layer)]→MeOH} to give 7 fractions {fr. X-11–1 (151.8 mg), fr. X-11–2 (56.2 mg), fr. X-11–3 (382.5 mg), fr. X-11–4 (45.6 mg), fr. X-11–5 (67.3 mg), fr. X-11–6 (733.7 mg), fr. X-11–7 (220.4 mg) and fr. X-11–8 (251.9 mg)}. Fr. X-11–6 & fr. X-11–7 were purified by HPLC [MeOH: H_2O (40: 60), HPLC column:

YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm)] to give hydrangenol 8-*O*-glucoside (**18**, 14.6 mg, 0.0023%), hydrangeamine A (**1**, 5.8 mg, 0.0009%), hydrangeamine B (**2**, 2.4 mg, 0.0004%); Fr. X-11–8 was purified by HPLC [MeOH: H_2O (85: 15), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm)] to give gingerglycolipid A (**42**, 16.0 mg, 0.0026%), hexadecanoic acid, 3-[(6-*O*-*α*-D-galactopyranosyl-*β*-D-galactopyranosyl)oxy]-2-hydroxypropyl ester, (*S*)- (**43**, 7.4 mg, 0.0012%).

The EtOAc–soluble fraction (20.0 g) was subjected to ordinary–phase silica gel column chromatography {300 g, EtOAc: Hex [(1: 10) →(1: 5) →(1: 2)]→$CHCl_3$–MeOH [(20: 1) →(10: 1)]→MeOH} to give 12 fractions {fr. E1 (26.3 mg), fr. E2 (1510 mg) , fr. E3 (330 mg) , fr. E4 (910 mg), fr. E5 (447 mg), fr. E6 (1310 mg), fr. E7 (1320 mg), fr. E8 (750 mg), fr. E9 (870 mg), fr. E10 (1440 mg), fr. E11 (360 mg), fr. E12 (10.72 g)}. Fr. E3 was purified by HPLC [MeOH: H_2O (95: 5), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm)] to give hydrangenol monomethyl ether (**16**, 2.1 mg, 0.0105%); Fr. E4, E5, E6 and E8 were purified by HPLC [MeOH: H_2O (60: 40), HPLC column: YMC–Pack ODS–A(250×20 mm i.d., S–5 μm, 12 nm)] to give *P*-hydroxybenzaldehyde (**44**, 30.4 mg, 0.1520%), *trans*-cinnamic acid (**45**, 3.8 mg, 0.0190%), hydrangenol (**20**, 957.6 mg, 4.7900%), skimmetine (**46**, 9.1 mg, 0.0455%), thunberginol G (**21**, 13.3 mg, 0.0665%), naringenin (**47**, 1.3 mg, 0.0065%), phyllodulcin (**17**, 38.6 mg, 0.1930%), hydrangeic acid (**48**, 9.1 mg, 0.0455%).

1.3 Isolates

Hydrangeamine A (1): white amorphous powder

$[\alpha]_D^{18}$= –130.83° (C= 0.29, MeOH)

UV (MeOH) λmax (log ε) 204(4.28), 228 (4.17), 275(3.82) nm

CD (MeOH) 245 ($\Delta\varepsilon$ -10.78)

IR (film, MeOH) ν max 3421, 2364, 1697, 1618, 1508, 1071 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table **1**

Positive-ion FAB-MS: *m/z* 592 $[M+Na]^+$; HR-FAB-MS: *m/z* 592.2154

Calcd for $C_{30}H_{35}NO_{10}Na$ $[M+Na]^+$: *m/z* 592.2159

Hydrangeamine B (2): white, amorphous powder

$[\alpha]_D^{16}$= –79.21° (C= 0.12, MeOH)

UV (MeOH) λmax (log ε) 202(4.29), 227 (4.16), 275(3.72) nm

CD (MeOH) 246 ($\Delta\varepsilon$ -13.14)

IR (film, MeOH) ν max 3421, 2364, 1697, 1618, 1508, 1071 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz), see Table **1**

Positive-ion FAB-MS: *m/z* 592 $[M+Na]^+$; HR-FAB-MS: *m/z* 592.2153

Calcd for $C_{30}H_{35}NO_{10}Na$ $[M+Na]^+$: *m/z* 592.2159

Florahydroside I (6): white amorphous powder

$[\alpha]_D^{20}$ –8.0° (*c* = 0.50, MeOH)

IR (KBr): ν_{max} 3400, 1684, 1610, 1508, 1071 cm^{-1}

CD (MeOH) λ_{max} ($\Delta\varepsilon$) 236 (+8.62), 255 (–0.79)

^1H NMR (methanol-d_4, 600 MHz) δ 2.93 (dd, *J* = 16.5, 2.1 Hz, H-4a), 3.14 (dd, *J* = 16.5, 12.4 Hz, H-4b), 3.85 (s, OCH_3), 4.81 (1H, d, *J* = 7.6 Hz, H-1''), 5.29 (dd, *J* = 12.4, 2.1 Hz, H-3), 6.45 (br s, H-5), 6.78 (br s, H-7), 6.90 (1H, dd, *J* = 8.2, 1.4 Hz, H-6'), 6.92 (1H, d, *J* = 8.2 Hz, H-5'), 6.94 (1H, d, *J* = 1.4 Hz, H-2');

^{13}C NMR: given in Table **2**;

Positive-ion FAB-MS: *m/z* 487 $[M+Na]^+$; HR-FAB-MS: *m/z* 487.1214

Calcd for $C_{22}H_{24}O_{11}Na$ $[M+Na]^+$: *m/z* 487.1216

Florahydroside II (7): white amorphous powder

$[\alpha]_D^{25}$ –21.0° (*c* = 0.50, MeOH)

IR (KBr): ν_{max} 3400, 1686, 1618, 1510, 1073 cm^{-1}

CD (MeOH) λ_{max} ($\Delta\varepsilon$) 231 (+3.88), 249 (–3.14)

^1H NMR (methanol-d_4, 600 MHz) δ 3.01 (dd, *J* = 16.5, 2.0 Hz, H-4a), 3.15 (dd, *J* = 16.5, 12.4 Hz, H-4b), 3.77 (s, OCH_3), 4.92 (1H, d, *J* = 7.6 Hz, H-1''), 5.40 (dd, *J* = 12.4, 2.0 Hz, H-3), 6.46 (br s, H-7), 6.47 (br s, H-5), 6.83 (1H, d, *J* = 8.2 Hz, H-5'), 6.85 (1H, br d, *J* = 8.2 Hz, H-6'), 6.85 (1H, br s, H-2')

^{13}C NMR: given in Table **2**

Positive-ion FAB-MS: *m/z* 487 $[M+Na]^+$; HR-FAB-MS: *m/z* 487.1220

Calcd for $C_{22}H_{24}O_{11}Na$ $[M+Na]^+$: *m/z* 487.1216

Thunberginol G 8-*O*-*β*-D-glucopyranoside (8): white amorphous powder

IR (KBr): ν_{max} 3400, 1684, 1618, 1509, 1070 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz, *ca.* 2:1 mixture of two diastereoisomers) δ 3.09 (0.67H, dd, J = 16.5, 2.8 Hz, H-4a), 3.16 (0.33H, dd, J = 16.5, 2.8 Hz, H-4a), 3.28 (1H, m, H-4b), 4.90 (0.67H, d, J = 7.6 Hz, H-1"), 4.94 (0.33H, d, J = 8.3 Hz, H-1"), 5.36 (0.67H, dd, J = 12.4, 2.8 Hz, H-3), 5.41 (0.33H, dd, J = 10.4, 2.8 Hz, H-3), 6.79 (0.67H, d, J = 8.5 Hz, H-5'), 6.79 (0.33H, m, H-5'), 6.82 (0.67H, dd, J = 8.5, 2.0 Hz, H-6'), 6.82 (0.33H, m, H-6'), 6.88 (0.33H, br s, H-2'), 6.93 (0.67H, d, J = 2.0 Hz, H-2'), 7.04 (0.33H, d like, J = 7.6 Hz, H-5), 7.10 (0.67H, d like, J = 8.2 Hz, H-5), 7.29 (0.33H, d like, J = 7.6 Hz, H-7), 7.40 (0.67H, d like, J = 8.2 Hz, H-7), 7.40 (0.67H, dd, J = 8.2, 8.2 Hz, H-6), 7.53 (0.33H, dd, J = 7.6, 7.6 Hz, H-6)

^{13}C NMR: given in Table **2**

Positive-ion FAB-MS: *m/z* 457 $[M+Na]^+$; HR-FAB-MS: *m/z* 457.1105

Calcd for $C_{21}H_{22}O_{10}Na$ $[M+Na]^+$: *m/z* 457.1111

Thunberginol C 8-*O*-*β*-D-glucopyranoside (9): white amorphous powder

IR (KBr): ν_{max} 3400, 1684, 1617, 1509, 1071 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz, *ca.* 7:3 mixture of two diastereoisomers) δ 2.95 (0.70H, dd, J = 16.5, 2.0 Hz, H-4a), 3.03 (0.30H, dd, J = 16.5, 2.0 Hz, H-4a), 3.22 (0.70H, dd, J = 16.5, 13.1 Hz, H-4b), 3.24 (0.30H, m, H-4b), 4.82 (1H, d, J = 6.8 Hz, H-1"), 5.34 (0.70H, dd, J = 13.1, 2.0 Hz, H-3), 5.41 (0.30H, dd, J = 11.0, 2.8 Hz, H-3), 6.80 (0.70H, d, J = 8.9 Hz, H-3',5'), 6.78 (0.30H, d, J = 8.9 Hz, H-3',5'), 7.27 (0.30H, d, J = 8.9 Hz, H-2',6'), 7.31 (0.70H, d, J = 8.9 Hz, H-2',6'), 6.40 (0.30H, br s, H-5), 6.43 (0.70H, br s, H-5), 6.66 (0.30H, br s, H-7), 6.75 (0.70H, br s, H-7)

^{13}C NMR: given in Table **2**

Positive-ion FAB-MS: *m/z* 457 $[M+Na]^+$; HR-FAB-MS: *m/z* 457.1107

Calcd for $C_{21}H_{22}O_{10}Na$ $[M+Na]^+$: *m/z* 457.1111

4-Hydroxythunberginol G 3'-*O*-*β*-D-glucopyranoside (10): white amorphous powder

IR (KBr): ν_{max} 3400, 1682, 1619, 1508, 1073 cm^{-1}

^1H NMR [methanol-d_4, 600 MHz, *ca.* 11:9 mixture of two diastereoisomers (3-epimeric mixture)] δ 4.58 (0.45H, d, J = 7.6 Hz, H-1"), 4.69 (0.45H, d, J = 7.6 Hz, H-1"), 4.81 (0.55H, d, J = 5.5 Hz, H-4), 4.91 (0.45H, d, J = 5.5 Hz, H-4), 5.60 (0.55H, d, J = 5.5 Hz, H-3), 5.61 (0.45H, d, J = 5.5 Hz, H-3), 6.53 (0.55H, d like, J = 7.9 Hz, H-7), 6.74 (0.45H, overlap, H-7), 6.74 (0.45H, d, J = 8.2 Hz, H-5'), 6.76 (0.55H, d, J = 8.2 Hz, H-5'), 6.86 (0.45H, dd, J = 8.2, 2.0 Hz, H-6'), 6.80 (0.55H, d like, J = 7.9 Hz, H-5), 6.82 (0.45H, d

like, J = 7.9 Hz, H-5), 6.88 (0.55H, dd, J = 8.2, 2.0 Hz, H-6'), 7.10 (0.45H, d, J = 2.0 Hz, H-2'), 7.22 (0.55H, d, J = 2.0 Hz, H-2'), 7.40 (0.55H, dd, J = 7.9, 7.9 Hz, H-6), 7.45 (0.45H, dd, J = 7.9, 7.9 Hz, H-6)

^{13}C NMR: given in Table **2**

Positive-ion FAB-MS: *m/z* 473 $[M+Na]^+$; HR-FAB-MS: *m/z* 473.1056

Calcd for $C_{21}H_{22}O_{11}Na$ $[M+Na]^+$: *m/z* 473.1060

Thunberginol D 3'-*O*-*β*-D-glucopyranoside (11): white amorphous powder

IR (KBr): ν_{max} 3400, 1680, 1610, 1508, 1073 cm^{-1}

^{1}H NMR (methanol-d_4, 600 MHz, *ca.* 1:1 mixture of two diastereoisomers) δ2.93 (0.50H, dd, J = 15.8, 3.4 Hz, H-4a), 2.95 (0.50H, dd, J = 15.8, 3.4 Hz, H-4a), 3.17 (1H, m, H-4b), 4.78 (0.50H, d, J = 7.6 Hz, H-1"), 4.80 (0.50H, d, J = 8.3 Hz, H-1"), 5.47 (1H, dd like, J = 11.7, 3.4 Hz, H-3), 6.87 (0.50H, d, J = 8.2 Hz, H-5'), 6.88 (0.50H, d, J = 8.2 Hz, H-5'), 7.05 (0.50H, dd, J = 8.2, 2.0 Hz, H-6'), 7.07 (0.50H, dd, J = 8.2, 2.0 Hz, H-6'), 7.33 (0.50H, d, J = 2.0 Hz, H-2'), 7.35 (0.50H, d, J = 2.0 Hz, H-2'), 6.22 (1H, br s, H-7), 6.26 (1H, br s, H-5)

^{13}C NMR: given in Table **2**

Positive-ion FAB-MS: *m/z* 473 $[M+Na]^+$; HR-FAB-MS: *m/z* 473.1056

Calcd for $C_{21}H_{22}O_{11}Na$ $[M+Na]^+$: *m/z* 473.1060

1.4 Acid Hydrolyses of 1-2 and 6-11

Compounds **1-2** and **6-11** (1.0 mg each) were dissolved in 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v, 1.0 mL), and each solution was heated at 80 °C for 1 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and filtrated, and the solution was partitioned with EtOAc to give two layers. The aqueous layer was evaporated and then subjected to HPLC analysis to identify the D-glucose under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm; detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, MeCN–H_2O (85:15, v/v); flow rate, 0.50 ml/min; column temperature, room temperature. Identification of D-glucose was carried out by comparison of its retention time and optical rotation with that of an authentic sample [t_R: 18.9 min (positive optical rotation)].

1.5 Qualitative Analysis of Cyanogenic Glycoside

Column: COSMOSIL 5C18-PAQ [4.6 x 250 mm, 5 *m*m (Nakarai Tesque)]

Detection: UV [240 nm and 280 nm, SPD-M10Avp, Diode Array Detector (Shimadzu)]

Eluent: CH_3CN-H_2O [15:85 (v/v)] ; Flow rate: 1.0 mL/min; Injection volume: 10 mL

Column temperature: 40 °C [CTO-10ACvp Column Oven (Shimadzu)]

Sample preparaion: Each plant (1.00 g) was extracted with MeOH under reflux. Evaporation of the solvent under reduced pressure provided a MeOH extract. Next, MeOH (10 mL) was added to the extract, and then the solution was filtrated.

1.6 Bioassay

The experiments were performed as described in the previous reports.[66] The supernatant fluid of rat lens homogenate was used as a crude enzyme. The enzyme suspension was diluted to produce *ca.* 10 nmoL/tube of NADP in the following reaction. The incubation mixture contained phosphate buffer 135 mM (pH 7.0), Li_2SO_4 100 mM, NADPH 0.03 mM, DL-glyceraldehyde 1 mM as a substrate, and 100 μL of enzyme fraction, with 25 μL of sample solution, in a total volume of 0.5 mL. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μL of HCl 0.5 M. Then, 0.5 mL of NaOH 6 M containing imidazole 10 mM was added, and the solution was heated at 60 °C for 20 min to convert NADP into a fluorescent product. Fluorescence was measured using a fluorescence microplate reader (FLUOstar OPTIMA, BMG Labtechnologies) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Each test sample was dissolved in DMSO. Measurements were performed in duplicate, and IC_{50} values were determined graphically.

2. Experimental for *O. fragrans* var. *aurantiacus*

2.1 Material

Flowers of *Osmanthus fragrans* var. *aurantiacus*, which were cultivated in China, in 2011, were purchased from Kurohime medical herb tea Co., Ltd. (Nagano, Japan). A voucher of the plant is on file in our laboratory (KPU Medicinal Flower-2011-OF).

2.2 Extraction and Isolation

The flowers (2.0 kg) of *Osmanthus fragrans* var. *aurantiacus* cultivated in China was extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (874.92 g, 43.75%). Part of the MeOH extract (824.74 g) was partitioned into Hex–H_2O (1: 1, v/v) mixture to yield Hex–soluble fraction (85.68 g, 4.55%) and an aqueous fraction; The aqueous fraction was partitioned into $CHCl_3$–H_2O (1: 1, v/v) mixture to yield $CHCl_3$–soluble fraction (53.25 g, 2.82%) and an aqueous fraction; The aqueous fraction was partitioned into EtOAc–H_2O (1: 1, v/v) mixture to yield EtOAc–soluble fraction (149.76 g, 7.94%) and an aqueous fraction; The aqueous fraction was partitioned into H_2O–BuOH (1: 1, v/v) mixture to yield an BuOH–soluble fraction (256.25 g, 13.59%) and H_2O fraction (303.44 g, 16.10%). Part of the BuOH–soluble fraction (150.0 mg) was purified by HPLC [MeOH: H_2O (35: 65), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give salidroside (**80**, 15.3 mg, 1.4242%), verbascoside (**100**, 123.6 mg, 12.1658%); The $CHCl_3$–soluble fraction (48.25 g) was subjected to normal–phase silica gel column chromatography [SiO_2, 700 g, Hex→Hex: EtOAc (2: 1, v/v)→$CHCl_3$→$CHCl_3$: MeOH(10: 1, v/v) →$CHCl_3$: MeOH: H_2O(10: 3: 1→7: 3: 1→6: 4: 1, v/v/v, lower layer)] to give 6 fractions [fr. GC–1 (47.2 mg), fr. GC–2 (649.3 mg), fr. GC–3 (3.96 g), fr. GC–4 (26.04 g), fr. GC–5 (5.77 g), fr. GC–6 (8.90 g)].

The MeOH soluble part of fr. GC–4 (20.15 g) was subjected to reversed–phase silica gel column chromatography [ODS, 450 g, MeOH: H_2O (10: 90→20: 80→40: 60→50: 50→60: 40→80: 20, v/v)→MeOH] to give 24 fractions [fr. GC-4A–1 (170 mg), fr. GC-4A–2 (280 mg), fr. GC-4A–3 (570 mg), fr. GC-4A–4 (690 mg), fr. GC-4A–5 (380 mg), fr. GC-4A–6 (380 mg), fr. GC-4A–7 (350 mg) , fr. GC-4A–8 (640 mg) , fr. GC-4A–9 (400 mg) , fr. GC-4A–10 (1.25 g) , fr. GC-4A–11 (280 mg) , fr. GC-4A–12 (630 mg) , fr. GC-4A–13 (340 mg) , fr. GC-4A–14 (220 mg) , fr. GC-4A–15 (340 mg) , fr. GC-4A–16 (350 mg) , fr. GC-4A–17 (260 mg) , fr. GC-4A–18 (400 mg) , fr. GC-4A–19 (1000 mg) , fr. GC-4A–20 (2.42 g) , fr. GC-4A–21 (5.88 g) , fr. GC-4A–22 (3.67 g) , fr. GC-4A–23 (930 mg) and fr. GC-4A–24 (1.21 g)]. Fr. GC-4A–2 (280 mg) was purified by HPLC [MeOH: H_2O (30: 70), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give cleroindicin F (**58**, 15.3 mg, 0.0081%) and (*3αR,4R,7αR*)-hexahydro-3α-hydroxy-4-methoxy-6(2H)-benzofuranone (**59**, 7.4 mg, 0.0039%); Fr. GC-4A–3 (570 mg) was purified by HPLC [MeOH: H_2O (30: 70), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give larixic acid (**74**, 2.9 mg, 0.0015%), benzeneethanol, 4-hydroxy-

(**81**, 320.9 mg, 0.1702%), 7-octene-1,3,6-triol, 6-methyl-2-methylene- (**71**, 4.4 mg, 0.0023%), linaloolpyran oxide C (**60**, 59.6 mg, 0.0316%), and (linaloolpyran oxide D, **61**, 8.6 mg, 0.0046%); Fr. GC-4A–4 (690 mg) was purified by HPLC [H_2O, HPLC column: PAQ (250×20 mm i.d., S–5 μm, 12 nm)] to give benzeneethanol, 4-hydroxy- (**81**, 36.1 mg, 0.0191%); Fr. GC-4A–5 (380 mg) was purified by HPLC [MeOH: H_2O (30: 70), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give vanillic acid (**84**, 35.7 mg, 0.0189%), 2,6-dimethyl-7-octene-2,3,6-triol (**70**, 63.0 mg, 0.0334%), and blumenol A (**66**, 3.6 mg, 0.0019%); Fr. GC-4A–8 (640 mg) was purified by HPLC [MeOH: H_2O (30: 70), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give cyclolariciresinol (**85**, 16.4 mg, 0.0087%), (*3αS,4S,6αS*)-4-(3,4-dimethoxyphenyl)tetrahydro-1H,3H-furo[3,4-c]furan-1-one (**86**, 5.3 mg, 0.0028%), *trans*-ferulic acid (**82**, 24.0 mg, 0.0127%), 2(3H)-furanone, dihydro-5-(3-hydroxyhexyl)- (**87**, 5.4 mg, 0.0029%), linalool oxide *β*-D-glucoside (**88**, 20.9 mg, 0.0111%), and (*S*)-menthiafolic acid (**62**, 10.3 mg, 0.0055%); Fr. GC-4A–9 (400 mg) was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give *trans-p*-coumaric acid (**34**, 1.6 mg, 0.0008%), cyclolariciresinol (**85**, 8.0 mg, 0.0042%), 10-hydroxygeraniol (**90**, 11.7 mg, 0.0062%), foliamenthic acid (**89**, 3.5 mg, 0.0019%), and 8-hydroxypinoresinol (**91**, 7.4 mg, 0.0040%); Fr. GC-4A–10 (1.25 g) was subjected to Sephadex LH-20 column chromatography (200 g, MeOH) and finally HPLC [MeOH: H_2O (50: 50), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give (*6E,9S*)-9-hydroxy-4,6-megastigmadien-3-one (**63**, 15.4 mg, 0.0082%), (*6Z,9S*)-9-hydroxy-4,6-megastigmadien-3-one (**64**, 13.5 mg, 0.0072%), 4-(3-hydroxy-1-butenyl)-isophorone (**65**, 11.5 mg, 0.0061%), 4-Oxo-7,8-dihydro-*β*-ionol (**67**, 70.4 mg, 0.0373%), (*3E*)-4-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one (**68**, 6.3 mg, 0.0033%), (+)-pinoresinol (**95**, 26.4 mg, 0.0140%), and (+)-epipinoresinol (**93**, 5.3 mg, 0.0028%); Fr. GC-4A–11 (280 mg) was purified by HPLC [MeOH: H_2O (50: 50), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give 4-oxo-7,8-dihydro-*β*-ionol (**69**, 9.6 mg, 0.0051%) and flavanone, 4',5,7-trihydroxy- (**83**, 8.5 mg, 0.0045%); Fr. GC-4A–12 (630 mg) was purified by HPLC [MeOH: H_2O (55: 45), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give (-)-phillygenin (**94**, 55.3 mg, 0.0293%); Fr. GC-4A–16 (350 mg) was purified by HPLC [MeOH: H_2O (70: 30), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give 19*α*-hydroxyasiatic acid (**104**, 15.5 mg, 0.0082%), myrianthic acid (**110**, 21.5 mg, 0.0114%), 2*α*,3*β*,29-trihydroxy-olean-12-en-28-oic acid (**57**, 16.0 mg, 0.0085%), 4-epi-spathodic acid (**107**, 16.0 mg, 0.0085%), and rotundic acid (**108**, 10.7 mg, 0.0057%); The mixture of fr. GC-4A–17 (260 mg) , fr. GC-4A–18 (400 mg) and fr. GC-4A–19 (1000 mg) was subjected to Sephadex LH-20 column chromatography (200 g, MeOH) to give 8 fractions [fr. GC–4AS-1 (101.6 mg), fr. GC–4AS-2 (105.5 mg), fr. GC–4AS-3 (35.7 mg), fr. GC–4AS-4 (140.9 mg), fr. GC–4AS-5 (59.8 mg), fr. GC–4AS-6 (770.2 mg) , fr. GC–4AS-7 (338.7 mg) , fr. GC–4AS-8 (38.1 mg)]. Fr. GC–4AS-6 (770.2 mg) was purified by HPLC [MeOH: H_2O (80: 20), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give arjunolic acid (**105**, 34.0 mg, 0.0180%), asiatic acid (**106**, 15.0 mg, 0.0080%), messagenic acid I (**102**, 2.0 mg, 0.0011%), siaresinolic acid (**117**, 39.0 mg, 0.0207%),

3-epi-corosolic acid (**120**, 29.6 mg, 0.0157%), maslinin acid (**118**, 32.1 mg, 0.0170%), rotundic acid (**108**, 12.8 mg, 0.0068%), queretaroic acid (**113**, 7.1 mg, 0.0038%), 2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid (**114**, 6.9 mg, 0.0037%), isoarjunolic acid (**111**, 7.5 mg, 0.0040%), esculentic acid (**112**, 19.2 mg, 0.0102%), platanic acid (**103**, 18.6 mg, 0.0099%), α-ilexanolic acid (**123**, 2.4 mg, 0.0013%), euscophic acid (**115**, 11.7 mg, 0.0062%), and tormentolic acid (**116**, 15.1 mg, 0.0080%); Fr. GC–4AS-8 (38.1 mg) was purified by HPLC [MeOH: H_2O (80: 20), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give *2α,3β*-3-[[(2*E*)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-2,19-dihydroxy-urs-12-en-28-oic acid, (**124**, 10.3 mg, 0.0055%) and 2α,3β-2,19-dihydroxy-3-[[(2*E*)-3-(4-hydroxyphenyl)-1-oxo-2-propenyl]oxy]-urs- 12-en-28-oic acid (**125**, 1.9 mg, 0.0010%). Fr. GC-4A–22 (3.67 g) was purified by HPLC [MeOH: H_2O (90: 10), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give benthamic acid (**109**, 3.6 mg, 0.0019%), 2α-hydroxy ursolic acid (**122**, 13.9 mg, 0.0074%), oleanolic acid (**119**, 43.1 mg, 0.0219%), and β-ursolic acid (**121**, 59.6 mg, 0.0316%);

Fr. GC–5 (5.77 g) was subjected to reversed–phase silica gel column chromatography [ODS, 700 g, MeOH: H_2O (40: 60→60: 40→70: 30→80: 20→90: 10, v/v)→MeOH] to give 10 fractions[fr. GC-5–1 (100 mg), fr. GC-5–2 (402 mg), fr. GC-5–3 (551 mg), fr. GC-5–4 (302 mg), fr. GC-5–5 (338 mg), fr. GC-5–6 (147 mg), fr. GC-5–7 (426 mg) , fr. GC-5–8 (143 mg) , fr. GC-5–9 (313 mg) , fr. GC-5–10 (1.56 g)]. Fr. GC-5–2 (402 mg) was purified by HPLC [MeOH: H_2O (45: 55), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give *2E,6R*-betulalbuside A (**72**, 19.9 mg, 0.0106%), *2E,6S*-betulalbuside A (**73**, 3.8 mg, 0.0020%), 2-cyclohexen-1-one, 4-[3-(*β*-D-glucopyranosyloxy)butylidene]-3,5,5-trimethyl-, (*4E*)- (**75**, 6.3 mg, 0.0033%), and 2-cyclohexen-1-one, 4-[3-(*β*-D-glucopyranosyloxy)-1-butenyl]-3,5,5-trimethyl-, [*R*-[*R**,*S**-(*E*)]]- (**76**, 2.4 mg, 0.0013%); Fr. GC-5–3 (551 mg) was purified by HPLC [MeOH: H_2O (45: 55), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give phillyroside (**92**, 126.1 mg, 0.0669%), 9*ξ*-*O*-*β*-D-glucopyranosyloxy-5-megastigmen-4-one (**77**, 21.8 mg, 0.0116%), 10-acetoxyligustroside (**96**, 59.3 mg, 0.0315%), 3'-*O*-D-glucopyranosyl ligustroside (**97**, 8.5 mg, 0.0045%), ligustroside (**98**, 181.3 mg, 0.0962%), and Florafragroside A (**53**, 3.0 mg, 0.0016%); The mixture of fr. GC-5–5 (338 mg) and fr. GC-5–6 (147 mg) was subjected to Sephadex LH-20 column chromatography (200 g, MeOH) and finally HPLC [MeOH: H_2O (60: 40), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give geranyl *β*-primeveroside-(**78**, 6.0 mg, 0.0032%); Fr. GC-5–7 (426 mg) was purified by HPLC [MeOH: H_2O (70: 30), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give Florafragrosides B (**54**, 1.8 mg, 0.0010%), C (**55**, 13.1 mg, 0.0069%), and D (**56**, 108.3 mg, 0.0574%);

Fr. GC–6 (8.90 g) was subjected to reversed–phase silica gel column chromatography [ODS, 700 g, MeOH: H_2O (20: 80→80: 20, v/v)→MeOH] to give 11 fractions [fr. GC-6–1 (100 mg), fr. GC-6–2 (720.6 mg), fr. GC-6–3 (89.9 mg), fr. GC-6–4 (100.2 mg), fr. GC-6–5 (309.4 mg), fr. GC-6–6 (1.72 g), fr. GC-6–7 (125.2 mg) , fr. GC-6–8 (69.2 mg) , fr. GC-6–9 (133.5 mg) , fr. GC-6–10 (188.8 g) , fr. GC-6–11 (165.7 mg)]. Fr. GC-6–9 (133.5 mg) was purified by HPLC [MeOH: H_2O (40: 60), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give Isoacteoside (**99**, 6.3 mg, 0.0033%); Fr.

GC-6–10 (188.8 g) was purified by HPLC [MeOH: H_2O (50: 50), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give oleoacteoside (**101**, 15.5 mg, 0.0082%); Fr. GC-6–11 (165.7 mg) was purified by HPLC [MeOH: H_2O (60: 40), HPLC column: Cosmosil-5C_{18}-MS-II (250×20 mm i.d., S–5 μm, 12 nm)] to give jasminoside O (**79**, 31.8 mg, 0.0169%).

2.3 Isolates

Florafragroside A (53)

white amorphous powder

$[\alpha]_D^{27}$ –9.0° (c = 0.50, MeOH)

IR (KBr): ν_{max} 3339, 1653, and 1074 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR: given in Table **4**

Positive-ion FAB-MS: *m/z* 393 $[M+Na]^+$; HR-FAB-MS: *m/z* 393.1892

Calcd for $C_{19}H_{30}O_7Na$ $[M+Na]^+$: *m/z* 393.1889

Florafragroside B (54)

white amorphous powder

$[\alpha]_D^{27}$ +58.1° (c = 0.50, MeOH)

IR (KBr): ν_{max} 3389, 2361, 1684, and 1170 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR: given in Table **4**

Positive-ion FAB-MS: *m/z* 511 $[M+Na]^+$; HR-FAB-MS: *m/z* 511.2514

Calcd for $C_{24}H_{40}O_{10}Na$ $[M+Na]^+$: *m/z* 511.2519

Florafragroside C (55)

white amorphous powder

$[\alpha]_D^{24}$ –8.4°(c = 0.50, MeOH)

IR (KBr): ν_{max} 3740, 2866, 2368, 1684, and 1076 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR: given in Table **4**

Positive-ion FAB-MS: *m/z* 513 $[M+Na]^+$; HR-FAB-MS: *m/z* 513.2682

Calcd for $C_{24}H_{42}O_{10}Na$ $[M+Na]^+$: *m/z* 513.2678

Florafragroside D (56)

white amorphous powder

$[\alpha]_D^{26}$ –48.9°(c = 0.50, MeOH)

IR (KBr): ν_{max} 3737, 2928, 2325, 1647, and 1078 cm^{-1}

^1H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR: given in Table **4**

Positive-ion FAB-MS: *m/z* 513 $[M+Na]^+$; HR-FAB-MS: *m/z* 513.2681

Calcd for $C_{24}H_{42}O_{10}Na$ $[M+Na]^+$: *m/z* 513.2676

2.4 Acid hydrolyses of 53-56

Compounds **53-56** (1.0 mg each) were dissolved in 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v, 1.0 mL), and each solution was heated at 80 °C for 1 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and filtrated, and the solution was partitioned with EtOAc to give two layers. The aqueous layer was evaporated and then subjected to HPLC analysis to identify the D-glucose and D-xylose under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm; detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, MeCN–H_2O (75:25, v/v); flow rate, 0.80 ml/min; column temperature, room temperature. Identification of D-glucose and D-xylose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with that of an authentic sample [t_R: 9.3 min (D-xylose, positive optical rotation), 12.3 min (D-glucose, positive optical rotation)], respectively.

2.5 Bioassay

2.5.1 Cell Culture

The murine macrophage cells (RAW264.7, ATCC No. TIB-71) were obtained from Dainippon Pharmaceutical, Osaka, Japan and cultured in Dulbecco's modified Eagle's medium (DMEM, 4500 mg/L glucose) supplemented with 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100μg/mL) (Sigma Chemical Co., St. Louis, MO, U.S.A.). The cells were incubated at 37°C in 5% CO_2/air.

2.5.2 Effects on Production of NO in LPS-Stimulated RAW264.7 Macrophages

The total amount of nitrite in a medium is used as an indicator of NO synthesis. The screening test for NO production using RAW264.7 cells was described previously.[155] Briefly, RAW264.7 cells were cultured in DMEM, and the suspension seeded into a 96-well microplate at 2.5×10^5 cells/100 μL/well. After 6 h, nonadherent cells were removed by washing with phosphate buffered saline (PBS), the adherent cells were cultured in 100 μL of fresh medium containing the test compounds for 10 min, and then 100 μL of the medium containing LPS (from *E. coli*, 055: B5, Sigma) was added to stimulate the cells for 18 h (final

concentration of LPS, 10μg/mL). The nitrite concentration was measured from the supernatant by a Griess reaction. NO production in each well was assessed by measuring the accumulation of nitrite in the culture medium using Griess reagent. Inhibition (%) was calculated using the following formula and the IC_{50} was determined graphically (n=4).

$$\textbf{Inhibition (\%)} = [(A\text{-}B) / (A\text{-}C)] \times 100$$

A–C: nitrite concentration (μM). A: LPS (+), sample (–); B: LPS (+), sample (+); C: LPS (−), sample (−)

Cytotoxicity was evaluated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*tetrazolium bromide (MTT) colorimetric assay according to a previous report with a slight modification.[155)]

2.5.3 Statistical Analysis

All data are expressed as the mean±S.E.M. The data analysis was performed with a oneway analysis of variance (1-ANOVA), followed by Dunnett's test. A p value of less than 0.05 was considered to be significant.

References

1) Zhang H., Matsuda H., Yamashita C., Nakamura S., Yoshikawa M., *Eur J Pharmacol,* **606**, 255-261 (2009).

2) Kurume A., Kamata Y., Yamashita M., Wang Q., Matsuda H., Yoshikawa M., Kawasaki I., Ohta S., *Chem Pharm Bull (Tokyo),* **56**, 1264-1269 (2008).

3) Matsuda H., Wang Q., Matsuhira K., Nakamura S., Yuan D., Yoshikawa M., *Phytomedicine,* **15**, 177-184 (2008).

4) Wang Q., Matsuda H., Matsuhira K., Nakamura S., Yuan D., Yoshikawa M., *Biol Pharm Bull,* **30**, 388-392 (2007).

5) Zhang H., Matsuda H., Kumahara A., Ito Y., Nakamura S., Yoshikawa M., *Bioorg Med Chem Lett,* **17**, 4972-4976 (2007).

6) Wang Z. N., Seikou; Matsuda, Hisashi; Wu, Lijun; Yoshikawa, Masayuki., *Heterocycles,* **81**, 909-916 (2010).

7) Nakamura S., Wang Z., Xu F., Matsuda H., Wu L., Yoshikawa M., *Tetrahedron Letters,* **50**, 4639-4642 (2009).

8) Fujimoto K., Nakamura S., Matsumoto T., Ohta T., Ogawa K., Tamura H., Matsuda H., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **61**, 445-451 (2013).

9) Uesato S., Takeda Y., Hashimoto T., Uobe K., Inouye H., Taguchi H., Endo T., *Helv Chim Acta,* **67**, 2111-2127 (1984).

10) Uesato S., Hashimoto T., Takeda Y., Uobe K., Inouye H., *Chemical & pharmaceutical bulletin* **29**, 3421-3424 (1981).

11) Inouye H., Takeda Y., Uesato S., Uobe K., Hashimoto T., Shingu T., *Tetrahedron Letters,* **21**, 1059-1062 (1980).

12) Yoshikawa M., Ueda T., Matsuda H., Yamahara J., Murakami N., *Chem Pharm Bull (Tokyo),* **42**, 1691-1693 (1994).

13) Xie H., Wang T., Matsuda H., Morikawa T., Yoshikawa M., Tani T., *Chem Pharm Bull (Tokyo),* **53**, 1416-1422 (2005).

14) Masayuki Yoshikawa T. M., Tomohiko Ueda, Hiroshi Shimoda, Johji Yamahara, and Hisashi Matsuda, *Heterocycles,* **50**, 411-418 (1999).

15) Matsuda H., Shimoda H., Uemura T., Ueda T., Yamahara J., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **47**, 1753-1758 (1999).

16) Matsuda H., Nakamura S., Fujimoto K., Moriuchi R., Kimura Y., Ikoma N., Hata Y., Muraoka O., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **58**, 1617-1621 (2010).

17) Ohashi K., Tanikawa T., Okumura Y., Kawazoe K., Tatara N., Minato M., Shibuya H., Kitagawa I., *Chem Pharm Bull (Tokyo),* **42**, 1791-1797 (1994).

18) Ferreira D., Marais J. P. J., Slade D., Walker L. A., *Journal of Natural Products,* **67**, 174-178 (2004).

19) Yu Y., Song W., Zhu C., Lin S., Zhao F., Wu X., Yue Z., Liu B., Wang S., Yuan S., Hou Q., Shi J., *Journal of Natural Products,* **74**, 2151-2160 (2011).

20) Morikawa T., Kishi A., Pongpiriyadacha Y., Matsuda H., Yoshikawa M., *J Nat Prod,* **66**, 1191-1196 (2003).

21) Yoshikawa M., Matsuda H., Shimoda H., Shimada H., Harada E., Naitoh Y., Miki A., Yamahara J.,

Murakami N., *Chem Pharm Bull (Tokyo),* **44**, 1440-1447 (1996).

22) Matsuda H., Morikawa T., Toguchida I., Harima S., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **50**, 972-975 (2002).

23) Yoshikawa M. U., Tomohiko; Shimoda, Hiroshi; Murakami, Toshiyuki; Yamahara, Johji; Matsuda, Hisashi, *Chemical & Pharmaceutical Bulletin* **47**, 383-387 (1999).

24) Valcic S., Zapp J., Becker H., *Phytochemistry,* **44**, 89-99 (1997).

25) Yoshikawa M., Uchida E., Chatani N., Murakami N., Yamahara J., *Chem Pharm Bull (Tokyo),* **40**, 3121-3123 (1992).

26) Uchida K., Fukuda T., Iwao M., *Tetrahedron,* **63**, 7178-7186 (2007).

27) Yoshikawa M., Uchida E., Chatani N., Kobayashi H., Naitoh Y., Okuno Y., Matsuda H., Yamahara J., Murakami N., *Chem Pharm Bull (Tokyo),* **40**, 3352-3354 (1992).

28) Mandal S. K., Roy S. C., *Tetrahedron Letters,* **48**, 4131-4134 (2007).

29) Mandal S. K., Roy S. C., *Tetrahedron,* **64**, 11050-11057 (2008).

30) Yoshida T., Chen L., Shingu T., Okuda T., *Chem. Pharm. Bull.,* **36**, 2940-2949 (1988).

31) dos Santos A. R. P. d. B., Michely; Santin, Silvana Maria de Oliveira; Sarragiotto, Maria Helena; Conceicao de Souza, Maria; Eberlin, Marcos Nogueira; Meurer, Eduardo Cesar. , *Quimica Nova* **27**, 525-527 (2004).

32) Jin H., Tanaka T., Kouno I., Ishimaru K., *Journal of Natural Medicines,* **61**, 351-354 (2007).

33) Ossipov V., Nurmi K., Loponen J., Haukioja E., Pihlaja K., *Journal of Chromatography A,* **721**, 59-68 (1996).

34) Zhu X., Dong X., Wang Y., Ju P., Luo S., *Helv Chim Acta,* **88**, 339-342 (2005).

35) Nakamura S., Nakashima S., Tanabe G., Oda Y., Yokota N., Fujimoto K., Matsumoto T., Sakuma R., Ohta T., Ogawa K., Nishida S., Miki H., Matsuda H., Muraoka O., Yoshikawa M., *Bioorg Med Chem,* **21**, 779-787 (2013).

36) Towers G. H. N., McInnes A. G., Neish A. C., *Tetrahedron,* **20**, 71-77 (1964).

37) Seigler D. S., *Phytochemistry,* **66**, 1567-1580 (2005).

38) Yang W., Ye M., Liu M., Kong D., Shi R., Shi X., Zhang K., Wang Q., Lantong Z., *Journal of Chromatography A,* **1217**, 4587-4600 (2010).

39) Lin H., Feng B., Shi L., Tang L., Wang Y., *Zhongguo Yaoxue Zazhi (Beijing, China),* **44**, 1851-1853 (2009).

40) Gross G.-A., Sticher O., *Helv Chim Acta,* **69**, 1113-1119 (1986).

41) Humphreys J. L., Lowes D. J., Wesson K. A., Whitehead R. C., *Tetrahedron,* **62**, 5099-5108 (2006).

42) Helferich B., Papalambrou P., *Justus Liebigs Annalen der Chemie,* **551**, 242-248 (1942).

43) Wang Z. B., Gao H. Y., Xu F. M., Wu L. J., *Chinese Chemical Letters,* **21**, 702-705 (2010).

44) Garcia J., Chulia A. J., *Planta Med*, 327-329 (1986).

45) Salum M. L., Robles C. J., Erra-Balsells R., *Org Lett,* **12**, 4808-4811 (2010).

46) Recio-Iglesias M.-C., Marston A., Hostettmann K., *Phytochemistry,* **31**, 1387-1389 (1992).

47) Kunz S., Becker H., *Phytochemistry,* **31**, 3981-3983 (1992).

48) Yoshikawa M., Hatakeyama S., Taniguchi K., Matuda H., Yamahara J., *Chem Pharm Bull (Tokyo),* **40**, 2239-2241 (1992).

49) Rayyan S., Fossen T., Solheim Nateland H., Andersen O. M., *Phytochem Anal,* **16**, 334-341 (2005).

50) Manguro L. O., Ugi I., Lemmen P., Hermann R., *Phytochemistry,* **64**, 891-896 (2003).

51) Umehara K., Matsumoto M., Nakamura M., Miyase T., Kuroyanagi M., Noguchi H., *Chem Pharm Bull (Tokyo),* **48**, 566-567 (2000).

52) Kim J. S., Shim S. H., Lee S., Chae S., Han S. J., Kang S. S., Lee Y. S., Jung S. H., Shin K. H., *Nat.*

Prod. Sci., **10**, 341-343 (2004).

53) Kim H., Ralph J., Lu F., Ralph S. A., Boudet A.-M., MacKay J. J., Sederoff R. R., Ito T., Kawai S., Ohashi H., Higuchi T., *Org. Biomol. Chem.,* **1**, 268-281 (2003).

54) Peng Y.-Y., Zeng Y., Qiu G., Cai L., Pike V. W., *J. Heterocycl. Chem.,* **47**, 1240-1245 (2010).

55) Oyamada J., Hashimoto T., Kitamura T., *Journal of Organometallic Chemistry,* **694**, 3626-3632 (2009).

56) Salum M. a. L., Robles C. J., Erra-Balsells R., *Org Lett,* **12**, 4808-4811 (2010).

57) Cheng K., Lee Y. S., Rothman R. B., Dersch C. M., Bittman R. W., Jacobson A. E., Rice K. C., *J Med Chem,* **54**, 957-969 (2011).

58) Zolek T., Paradowska K., Wawer I., *Solid State Nucl Magn Reson,* **23**, 77-87 (2003).

59) Jeon S., Chun W., Choi Y., Kwon Y., *Arch Pharm Res,* **31**, 978-982 (2008).

60) Yoshikawa M., Harada E., Naitoh Y., Inoue K., Matsuda H., Shimoda H., Yamahara J., Murakami N., *Chem Pharm Bull (Tokyo),* **42**, 2225-2230 (1994).

61) Paraschos S., Magiatis P., Kalpoutzakis E., Harvala C., Skaltsounis A. L., *J Nat Prod,* **64**, 1585-1587 (2001).

62) Nakamura S., Fujimoto K., Nakashima S., Matsumoto T., Miura T., Uno K., Matsuda H., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **60**, 752-758 (2012).

63) Fujimoto K., Nakamura S., Nakashima S., Matsumoto T., Uno K., Ohta T., Miura T., Matsuda H., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **60**, 1188-1194 (2012).

64) Nakamura S., Moriura T., Park S., Fujimoto K., Matsumoto T., Ohta T., Matsuda H., Yoshikawa M., *J Nat Prod,* **75**, 1425-1430 (2012).

65) Matsuda H., Hamao M., Nakamura S., Kon'i H., Murata M., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **60**, 674-680 (2012).

66) Matsuda H., Asao Y., Nakamura S., Hamao M., Sugimoto S., Hongo M., Pongpiriyadacha Y., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **57**, 487-494 (2009).

67) Morikawa T., Xie H., Wang T., Matsuda H., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **56**, 1438-1444 (2008).

68) Matsuda H., Morikawa T., Toguchida I., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **50**, 788-795 (2002).

69) Hussein G. M., Matsuda H., Nakamura S., Akiyama T., Tamura K., Yoshikawa M., *Phytomedicine,* **19**, 88-97 (2011).

70) Hussein G. M., Matsuda H., Nakamura S., Hamao M., Akiyama T., Tamura K., Yoshikawa M., *Biol Pharm Bull,* **34**, 1849-1855 (2011).

71) Kim C.-S., Kim J., Lee Y., Sohn E., Jo K., Kim J., *Arch Pharm Res,* **34**, 847-852 (2011).

72) Kaiser R., *ACS Symp. Ser.,* **802**, 160-182 (2002).

73) Baldermann S., Kato M., Kurosawa M., Kurobayashi Y., Fujita A., Fleischmann P., Watanabe N., *J. Exp. Bot.,* **61**, 2967-2977 (2010).

74) Watanabe N., Watanabe S., Nakajima R., Moon J. H., Shimokihara K., Inagaki J., Etoh H., Asai T., Sakata K., Ina K., *Biosci., Biotechnol., Biochem.,* **57**, 1101-1106 (1993).

75) Ina H., Ohta Y., Iida H., *Phytochemistry,* **24**, 2655-2657 (1985).

76) Chen Y. Q., Shen Y. H., Su Y. Q., Kong L. Y., Zhang W. D., *Chem Biodivers,* **6**, 779-783 (2009).

77) Hase T., Kawamoto Y., Ohtani K., Kasai R., Yamasaki K., Picheansoonthon C., *Phytochemistry,* **39**, 235-241 (1995).

78) Winterhalter P., Katzenberger D., Schreier P., *Phytochemistry,* **25**, 1347-1350 (1986).

79) Bartschat D., Beck T., Mosandl A., *J. Agric. Food Chem.,* **45**, 4554-4557 (1997).

80) Dawson G. W., Pickett J. A., Smiley D. W., *Bioorg Med Chem,* **4**, 351-361 (1996).

81) D'Abrosca B., DellaGreca M., Fiorentino A., Monaco P., Oriano P., Temussi F., *Phytochemistry,* **65**, 497-505 (2004).
82) Habib Ur R., Arfan M., Atta Ur R., Choudhary M. I., Khan A. M., *J. Chem. Soc. Pak.,* **25**, 337-340 (2003).
83) Siddiqui B. S., Kardar M. N., Ali S. T., Khan S., *Helvetica Chimica Acta,* **86**, 2164-2169 (2003).
84) Macias F. A., Lacret R., Varela R. M., Nogueiras C., Molinillo J. M., *Phytochemistry,* **69**, 2708-2715 (2008).
85) Libsu S., Singh A. K., *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.,* **46B**, 1297-1302 (2007).
86) Lin T.-C., Fang J.-M., Cheng Y.-S., *Phytochemistry,* **51**, 793-801 (1999).
87) Ishikawa T., Kondo K., Kitajima J., *Chem Pharm Bull (Tokyo),* **51**, 32-39 (2003).
88) Tan R. X., Jakupovic J., Bohlmann F., Jia Z. J., Huneck S., *Phytochemistry,* **30**, 583-587 (1991).
89) Morikawa H., Kasai R., Otsuka H., Hirata E., Shinzato T., Aramoto M., Takeda Y., *Chem Pharm Bull (Tokyo),* **52**, 1086-1090 (2004).
90) Park S. H., Nhiem N. X., Kiem P. V., Choi E. M., Kim J. A., Kim Y. H., *Arch Pharm Res,* **33**, 75-80 (2010).
91) Sukhov B. G., Mukha S. A., Antipova I. A., Medvedeva S. A., Larina L. I., Chipanina N. N., Kazheva O. N., Shilov G. V., Dyachenko O. A., Trofimov B. A., *ARKIVOC (Gainesville, FL, U. S.)*, 139-149 (2008).
92) Sakho M., Chassagne D., Crouzet J., *J. Agric. Food Chem.,* **45**, 883-888 (1997).
93) Kodama H., Fujimori T., Kato K., *Phytochemistry,* **23**, 583-585 (1984).
94) Khan S. H., Mosihuzzaman M., Nahar N., Rashid M. A., Rokeya B., Ali L., Khan A. K. A., *Pharm. Biol. (Lisse, Neth.),* **41**, 512-515 (2003).
95) Kaisar M. A., Rahman M. S., Rahman M. Z., Hasan C. M., Rashid M. A., *J. Pharm. Nutr. Sci.,* **1**, 87-95 (2011).
96) Pabst A., Barron D., Sémon E., Schreier P., *Phytochemistry,* **31**, 1649-1652 (1992).
97) Sarker S. D., Dinan L., Šik V., Rees H. H., *Phytochemistry,* **45**, 1431-1433 (1997).
98) Sekiwa Y., Kobayashi A., Kubota K., Takenaka M., *Natural Product Letters,* **15**, 267-274 (2001).
99) Yoshikawa K., Nagai M., Wakabayashi M., Arihara S., *Phytochemistry,* **34**, 1431-1433 (1993).
100) Yu Y., Gao H., Dai Y., Wang Y., Chen H.-R., Yao X.-S., *Helvetica Chimica Acta,* **93**, 763-771 (2010).
101) Shi T., Chen H., Jing L., Liu X., Sun X., Jiang R., *Synthetic Communications,* **41**, 2594-2600 (2011).
102) Takaya Y., Furukawa T., Miura S., Akutagawa T., Hotta Y., Ishikawa N., Niwa M., *J Agric Food Chem,* **55**, 75-79 (2007).
103) Prachayasittikul S., Suphapong S., Worachartcheewan A., Lawung R., Ruchirawat S., Prachayasittikul V., *Molecules,* **14**, 850-867 (2009).
104) Jeon S. H., Chun W., Choi Y. J., Kwon Y. S., *Arch Pharm Res,* **31**, 978-982 (2008).
105) Lee C.-k., Lu C.-k., Kuo Y.-H., Chen J.-Z., Sun G.-Z., *J. Chin. Chem. Soc. (Taipei, Taiwan),* **51**, 437-441 (2004).
106) Jiang Z. H., Tanaka T., Sakamoto M., Jiang T., Kouno I., *Chem Pharm Bull (Tokyo),* **49**, 1036-1038 (2001).
107) Ming D. S., Yu D. Q., Yu S. S., Liu J., He C. H., *J Asian Nat Prod Res,* **1**, 221-226 (1999).
108) Abeysekera A. M., Mahatantila C., Sajeevani J. R. D. M., *J. Natl. Sci. Found. Sri Lanka,* **36**, 185-190 (2008).
109) Jiang L., Kojima H., Yamada K., Kobayashi A., Kubota K., *J Agric Food Chem,* **49**, 5888-5894 (2001).
110) Morimoto H., Kurimoto K., Imada I., *Chem. Pharm. Bull.,* **33**, 3182-3186 (1985).
111) Nakai T., Yajima A., Akasaka K., Kaihoku T., Ohtaki M., Nukada T., Ohrui H., Yabuta G., *Biosci*

Biotechnol Biochem, **69**, 2401-2408 (2005).
112) Tsukamoto H., Hisada S., Nishide S., *Chem Pharm Bull (Tokyo),* **32**, 4482-4489 (1984).
113) Kwak J. H., Kang M. W., Roh J. H., Choi S. U., Zee O. P., *Arch Pharm Res,* **32**, 1681-1687 (2009).
114) Iida T., Nakano M., Ito K., *Phytochemistry,* **21**, 673-675 (1982).
115) Inouye H., Inoue K., Nishioka T., Kaniwa M., *Phytochemistry,* **14**, 2029-2032 (1975).
116) Sakamoto S., Machida K., Kikuchi M., *Heterocycles,* **77**, 557-563 (2009).
117) Kim K. H., Kim S., Jung M. Y., Ham I. H., Whang W. K., *Arch Pharm Res,* **32**, 7-13 (2009).
118) Kobayashi H., Oguchi H., Takizawa N., Miyase T., Ueno A., Usmanghani K., Ahmad M., *Chem Pharm Bull (Tokyo),* **35**, 3309-3314 (1987).
119) Kikuchi M., Yamauchi Y., Takahashi Y., Sugiyama M., *Journal of the Pharmaceutical Society of Japan,* **109**, 366-371 (1989).
120) Macías F. A., Simonet A. M., Galindo J. C. G., Pacheco P. C., Sánchez J. A., *Phytochemistry,* **49**, 709-717 (1998).
121) Denisenko M. V., Samoshina N. F., Denisenko V. A., Dmitrenok P. S., *Chemistry of Natural Compounds,* **47**, 741-748 (2011).
122) Seto T., Tanaka T., Tanaka O., Naruhashi N., *Phytochemistry,* **23**, 2829-2834 (1984).
123) Bisoli E., Garcez W. S., Hamerski L., Tieppo C., Garcez F. R., *Molecules,* **13**, 2717-2728 (2008).
124) Amimoto K., Yoshikawa K., Arihara S., *Chem Pharm Bull (Tokyo),* **41**, 77-80 (1993).
125) Fang S.-y., He Z.-s., Fan G.-j., Wu H.-m., Xu J.-f., *Journal of Natural Products,* **59**, 304-307 (1996).
126) Kakuno T., Yoshikawa K., Arihara S., *Phytochemistry,* **31**, 2809-2812 (1992).
127) Mahato S. B., Kundu A. P., *Phytochemistry,* **37**, 1517-1575 (1994).
128) Wang F., Ding L., Wang H., *Zhongguo Zhongyao Zazhi,* **30**, 1929-1932 (2005).
129) Shao C.-J., Kasai R., Xu J.-D., Tanaka O., *Chem Pharm Bull (Tokyo),* **37**, 42-45 (1989).
130) Taniguchi S., Imayoshi Y., Kobayashi E., Takamatsu Y., Ito H., Hatano T., Sakagami H., Tokuda H., Nishino H., Sugita D., Shimura S., Yoshida T., *Phytochemistry,* **59**, 315-323 (2002).
131) Cheng J.-J., Zhang L.-J., Cheng H.-L., Chiou C.-T., Lee I. J., Kuo Y.-H., *Journal of Natural Products,* **73**, 1655-1658 (2010).
132) Mimaki Y., Fukushima M., Yokosuka A., Sashida Y., Furuya S., Sakagami H., *Phytochemistry,* **57**, 773-779 (2001).
133) Huang Y., Aisa H. A., Isaev M. I., *Chemistry of Natural Compounds,* **45**, 921-924 (2009).
134) Salem M. M., Werbovetz K. A., *J Nat Prod,* **69**, 43-49 (2006).
135) Wen X., Xia J., Cheng K., Zhang L., Zhang P., Liu J., Zhang L., Ni P., Sun H., *Bioorganic & Medicinal Chemistry Letters,* **17**, 5777-5782 (2007).
136) Wang M. Y., Yang L., Tu Y. Y., *Zhongguo Zhong Yao Za Zhi,* **31**, 307-308 (2006).
137) Yao Z., Li J., Zhou S., Zhang Y., Tu P., *Zhongguo Zhong Yao Za Zhi,* **34**, 999-1001 (2009).
138) Kakuno T., Yoshikawa K., Arihara S., Takei M., Endo K., *Tetrahedron,* **47**, 7219-7226 (1991).
139) De Tommasi N., De Simone F., Pizza C., Mahmood N., Moore P. S., Conti C., Orsi N., Stein M. L., *Journal of Natural Products,* **55**, 1067-1073 (1992).
140) Numata A., Yang P., Takahashi C., Fujiki R., Nabae M., Fujita E., *Chem Pharm Bull (Tokyo),* **37**, 648-651 (1989).
141) Zhang Y., Nakamura S., Pongpiriyadacha Y., Matsuda H., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **56**, 547-553 (2008).
142) Matsunami K., Otsuka H., Takeda Y., *Chemical and Pharmaceutical Bulletin,* **58**, 438-441 (2010).
143) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *Journal of the American Chemical Society,* **113**, 4092-4096 (1991).

144) Ito H., Kobayashi E., Li S. H., Hatano T., Sugita D., Kubo N., Shimura S., Itoh Y., Yoshida T., *J Nat Prod,* **64**, 737-740 (2001).
145) Punjabi C. J., Laskin D. L., Heck D. E., Laskin J. D., *J Immunol,* **149**, 2179-2184 (1992).
146) Mills C. D., Shearer J., Evans R., Caldwell M. D., *J Immunol,* **149**, 2709-2714 (1992).
147) Lorsbach R. B., Murphy W. J., Lowenstein C. J., Snyder S. H., Russell S. W., *J Biol Chem,* **268**, 1908-1913 (1993).
148) Lavnikova N., Drapier J. C., Laskin D. L., *J Leukoc Biol,* **54**, 322-328 (1993).
149) Rahat M. A., Hemmerlein B., *Front Physiol,* **4**, 144 (2013).
150) Nairz M., Schleicher U., Schroll A., Sonnweber T., Theurl I., Ludwiczek S., Talasz H., Brandacher G., Moser P. L., Muckenthaler M. U., Fang F. C., Bogdan C., Weiss G., *J Exp Med,* **210**, 855-873 (2013).
151) Bosca L., Zeini M., Traves P. G., Hortelano S., *Toxicology,* **208**, 249-258 (2005).
152) MacMicking J., Xie Q. W., Nathan C., *Annu Rev Immunol,* **15**, 323-350 (1997).
153) Lala P. K., Chakraborty C., *Lancet Oncol,* **2**, 149-156 (2001).
154) Hiraku Y., Kawanishi S., Ichinose T., Murata M., *Ann N Y Acad Sci,* **1203**, 15-22 (2010).
155) Hegazy M. E., Matsuda H., Nakamura S., Yabe M., Matsumoto T., Yoshikawa M., *Chem Pharm Bull (Tokyo),* **60**, 363-370 (2012).