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氏名（本籍）	ｴﾙﾃﾞﾈﾁﾒｸﾞ　ｾﾚﾝｹﾞ Erdenechimeg　Selenge（モンゴル）
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論文審査委員	主査　　教　授　　浪　越　通　夫 副査　　教　授　　山　下　幸　和 副査　　教　授　　柴　田　信　之

Studies on phytochemical constituents and their biological activities from Mongolian medicinal plants, *Dracocephalum* and *Chamaerhodos* species

A dissertation presented

by

Erdenechimeg Selenge Scholar

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List of Abbreviations

$[\alpha]_D$	specific rotation
CD	circular dichroism
p-DAB	*p*-dimethylaminobenzaldehyde
DMSO	dimethyl sulfoxide
EI	electron ionization
EtOH	ethanol
FAB	fast atom bombardment
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
IC_{50}	50% inhibitory concentration
IR	infrared
MeOH	methanol
MTPA	α-methoxy-α-(trifluoromethyl) phenylacetic acid
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PGME	phenylglycine methyl ester
ROE	nuclear Overhauser effect in the rotating frame
TFA	trifluoroacetic acid
TMS	tetramethylsilane
UV	ultraviolet

Abstract

Traditional Mongolian Medicine (TMM) has been revived and continues to be practiced widely, playing vital role in the health care needs of a large portion of the population of Mongolia. It includes the use of crude drugs, acupuncture, moxibustion, cupping, and massage. Most of the crude drugs used in medicine are derived from plant sources, while the others from animal and mineral sources.

In Mongolia, over 800 plant species are recognized as medicinal plants. Since ancient times, these plants are used for remedy and to prevent various infectious and non-infectious diseases, as well as improving the fertility of livestock. Some of typical medicinal plants are easily accessible within the country, and are widely used by Mongolian nomads as not only preventing and treating illnesses, but also a tonic to improve the health, because they cannot obtain modern drugs easily in nomadic life. Even though city dwellers have access to modern medication use of traditional medicine is quite popular, as they have a few side effects.

TMM is being used for since several generations, and the traditional knowledge is extremely valuable. Hence a policy of state of Mongolia, making National herbal pharmacopoeia is needed and discussed. Thus it is the demand of the hour to conduct study of TMM using scientific approaches, so that traditional knowledge can be backed up by scientific data. In case of the medicinal plants, vegetation surveys and ecological researches have been done more than phytochemical study.

On the other hand, studies of Mongolian medicinal plants are still at a nascent stage and even phytochemical constituents and the basic biological activities have not yet been investigated sufficiently. So, there is a real need for scientific studies and knowledge about TMM to provide scientific rationality. The knowledge of the basic

scientific data of phytochemical constituents will contribute to the pharmacopoeia, which specifies effective and safe use of each medicinal plant for patients.

Mongolian extreme climate damages skin and induces many other skin problems during the whole year, and increase especially in winter. Hence much attention has been paid to skin inflammation and its related diseases including allergies, severe rashes, dryness, and aging of skin by Mongolians. There are a lot of medicinal plants which have been handed down through the history, for skin care and protection from inflammation and its related diseases. A major focus of this study was identification of active components and action mechanisms of the plants in skin-care.

At first 51 extractions of Mongolian medicinal plants were tested for their hyaluronidase inhibitory and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. Then, out of these extracts, 2 genus and 4 species were picked up, which showed significant activity and are commonly used as herbal medicine in TMM.

Dracocephalum L. is one of the important members of Lamiaceae family for TMM. The genus consists of 17 species distributed in Mongolia and traditionally used for the treatment of inflammatory diseases, rheumatism, and external injury. Especially, *D. foetidum* has been widely used as traditional medicine among Mongolian nomads. In this research, two *Dracocephalum* plants, *D. ruyschiana* and *D. foetidum* were revealed to have hyaluronidase inhibitory activity, which is known to be related with anti-inflammatory mechanism. The phytochemical constituents were isolated from the two plants by chromatography and chemical structures were determined by using instrumental analyses.

Ten new and 19 known compounds were identified from *D. ruyschiana*, and 13 new and 13 known compounds were identified from *D. foetidum*. Plants were found to

contain polyphenolic compounds such as phenylpropanoids and flavonoids. Rosmarinic acid was obtained as one of the main constituents of *D. foetidum*, but it was not found in *D. ruyschiana*, even when these are from same genus. The structure determination process is presented in **Chapter 2** and **3**.

Chapter 2 presents isolation and structure elucidation of five new flavone tetraglycosides, five new benzyl alcohol glycosides, and 19 known compounds from *D. ruyschiana*.

In **Chapter 3**, three new limonene glycosides, a new caffeic acid trimer, four new rosmarinic acid derivatives, five new acacetin acyl glycosides, as well as 13 known compounds from *D. foetidum* were characterized.

Chamaerhodos plants, *C. erecta* and *C. altaica* were revealed having potent antioxidant activity by screening of 23 Mongolian medicinal plants, and the plants are known to be used for skin-care, traditionally. Phytochemical investigations of *C. erecta* and *C. altaica* were followed the same processes as *Dracocephalum* plants and identified 4,5-dihydroxybenzaldehyde-3-*O*-β-D-glucopyranoside from *C. erecta* and quercetin-3-*O*-β-D-glucuronopyranosyl-4′-*O*-β-D-glucopyranoside from *C. altaica* as new compounds with 37 known compounds. A number of hydrolyzable tannins were isolated as typical constituents of *Chamaerhodos* plants. These results are explained in **Chapter 4**.

To elucidate skin-care effects and biological activities of the obtained 91 compounds (**1** - **91**), four basic tests hyaluronidase inhibitory, DPPH radical scavenging, Advanced glycation endproducts (AGEs) inhibitory, and tyrosinase inhibitory activities were evaluated. The tests were related with anti-inflammatory, antioxidant, antipigmentation activities, and their results are discussed in **Chapter 5**.

Highlight of the four assays is detailed in here. Rosmarinic acid derivative (**34**), and acacetin glycosides (**43** and **46**) showed stronger hyaluronidase inhibitory activity than positive control disodium cromoglicate. Some of flavone glycosides, catechin, and some of hydrolyzable tannins showed moderate activity. Hyaluronidase inhibitory activity is expected to be involved anti-inflammatory and anti-allergic reactions, and this activity can be used as primary screen of anti-allergic effects.

Antioxidant activities of rosmarinic acid and hydrolyzable tannins were more than the positive control trolox, while some flavonoid glycosides and rosmarinic acid derivatives were similar to that of trolox.

A series of flavonols and their glycosides, catechins, and hydrolyzable tannins showed AGEs inhibitory activities. It is thought that the prevention of AGEs formation is promoted by antioxidant compounds, and almost of these active compounds also had DPPH radical scavenging activity. Antioxidant activity of natural products protects cells against the damaging effects of free radicals and is expected to be useful for the prevention and treatment of many diseases including skin inflammations, allergies, and aging-related diseases.

Although tyrosinase inhibitory effects of all compounds of *D. foetidum* were examined, they did not show any significant activity.

Dracocephalum and *Chamaerhodos* plants which contain rosmarinic acid and its derivatives, some flavonoid glycosides, and hydrolyzable tannins as potent hyaluronidase inhibitors and antioxidants may be useful in cosmetic for anti-inflammation, anti-allergies, and antioxidation.

It is rational that nomadic Mongolians used *Dracocephalum* and *Chamaerhodos* plants for their ailments because the present study showed that constituents from those

have beneficial biological effects. These four medicinal plants have been important parts for TMM. The scientific data are expected to be useful and important information for the crude drugs which are being used by Mongolian people and generate data for the Mongolian National herbal pharmacopoeia.

Basic studies like above would increase understanding of the value of medicinal plants in Mongolia and increase the evidence for the efficacious use of herbs in health care.
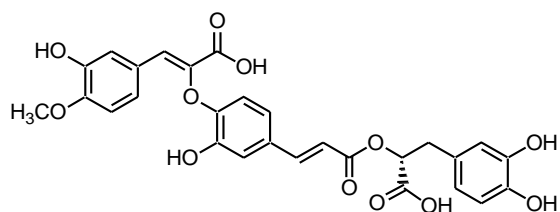
34
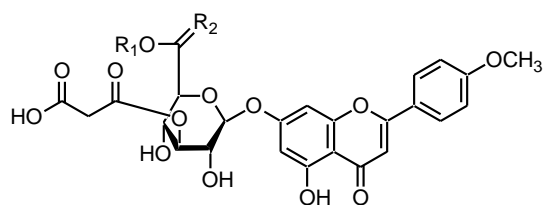
43 R_1 = malonyl, R_2 = H_2

46 R_1 = H, R_2 = O

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1. Flavone Tetraglycosides and Benzyl Alcohol Glycosides from the Mongolian Medicinal Plant *Dracocephalum ruyschiana*

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2. Phytochemical constituents of Mongolian traditional medicinal plants, *Chamaerhodos erecta* and *C. altaica*, and its constituents prevent the extracellular matrix degradation factors

   Erdenechimeg Selenge, Gendaram Odontuya, Toshihiro Murata, Kenroh Sasaki, Kyoko Kobayashi, Javzan Batkhuu, Fumihiko Yoshizaki. *Journal of Natural Medicines*, 2013, 67, 867-875.

3. Monoterpene glycosides, phenylpropanoids, and acacetin glycosides from *Dracocephalum foetidum*

   Erdenechimeg Selenge, Toshihiro Murata, Shiho Tanaka, Kenroh Sasaki, Javzan Batkhuu, Fumihiko Yoshizaki. *Phytochemistry*, 2014, In Press.

Chapter 1. Introduction

Nowadays people from around the world are having great interest in natural herbal medicines and are seeking more herbal remedies, products, and supplements. At the same time Traditional Mongolian Medicine (TMM) has been revived and continues to be practiced widely, playing vital role in the health-care needs of a large portion of the population of Mongolia.

TMM has a known history of more than 2500 years and has been passed from one generation to the next via oral traditions (Zhang, 2001). TMM based on the experiences of nomadic people, has its own unique medical theory, techniques, and medications in Mongolia. Some aspects of TMM along with elements from other Asian systems, such as Tibetan medicine, Ayurveda, and traditional Chinese medicine have been integrated into the Mongolian medical system (WHO, 2013).

From the 1930's until the end of the 1980's, traditional medicine was unrecognized. Socio-economic changes in Mongolia during the 1990's led to the development of the national culture, including revival of TMM (Pitschmann et al., 2013; Zhang, 2001). Nowadays, traditional medicine is officially recognized as part of Mongolian medical heritage (Zhang, 2001).

In TMM, the physicians diagnose the diseases by reading the pulses, examining the tongue, checking the urine by smell, color, and taste, as well as questioning the patients (Pitschmann et al., 2013). TMM includes the use of crude drugs, acupuncture, moxibustion, cupping, and massage. Most of the crude drugs used in medicine are derived from plant sources, while the others from animal and mineral sources. The physicians substitute plants, exchange plant parts or alter the formula of the recipe, depending on the patients (Gerke, 2004).

In Mongolia, over 800 plant species are recognized as medicinal plants. Since ancient times, these plants are used for remedy and prevent various infectious and non-infectious diseases as well as improving the fertility of livestock. Some of typical medicinal plants are easily accessible within the country, and are widely used by Mongolian nomads as not only preventing and treating illnesses, but also a tonic to improve the health, because they cannot obtain modern drugs easily in nomadic life. Even though city dwellers have access to modern medication use of traditional medicine is quite popular, as they have a few side effects.

There are several research institutions, universities, government agencies, and private companies involved in the research, protection, and commercial utilization of medicinal plants in Mongolia. Also many laboratories have formed research collaborations with other countries (Pitschmann et al., 2013). Since the last decade, Department of Pharmacognosy of Tohoku Pharmaceutical University has been collaborating with Laboratory of Bioorganic chemistry and Pharmacognosy of National University of Mongolia, to find and develop new drug candidates from medicinal plants used in traditional medicine, for various inflammations, cancer, Alzheimer, diabetes, and so on.

Mongolia currently has no pharmacopoeia for traditional medicine. In place of a national herbal pharmacopoeia, many reliable resources are used, including the Chinese pharmacopoeia and State pharmacopoeia of the USSR, and these are legally binding (WHO, 2012). Herbal pharmacopoeia specifies botany, chemistry, harvesting, growing, drying, storage, purity standards, dosage, side effects, contraindications, and drug interactions of each medicinal plant, as well as herbal pharmacopoeia is to promote the responsible use of herbal medicines and ensure they are used with the highest possible

degree of efficacy and safety. Quality, safety, and efficacy are the main requirements for the application of medicinal plants and herbal medicinal products (Ajazuddin and Saraf, 2012).

TMM is being used for since several generations, and traditional knowledge is extremely valuable. Hence a policy of state of Mongolia, making National herbal pharmacopoeia is needed and discussed. Thus it is the demand of the hour to conduct study of TMM using scientific approach, so that traditional knowledge can be backed up by scientific data. In case of the medicinal plants, vegetation surveys and ecological researches have been done more than phytochemical study.

On the other hand studies of Mongolian medicinal plants are still at a nascent stage and even phytochemical constituents and the basic biological activities have not yet been investigated sufficiently. So, there is a real need for scientific studies and knowledge about TMM to provide scientific rationality. The knowledge of the basic scientific data of phytochemical constituents will contribute to the pharmacopoeia, which specifies effective and safe use of each medicinal plant for patients.

In Mongolia, much attention has been paid to skin inflammation and its related diseases including allergies, severe rashes, dryness, and aging. Because Mongolian extreme climate damages skin and induces many other skin problems during the whole year, and increase especially in winter. There are a lot of medicinal plants which have been handed down through the history, for skin care and protection from inflammation and its related diseases.

Searching new skin-care ingredients from Mongolian plants becomes a great interest and could also be used as skin-care products best suited to the harsh climate of Mongolia to relieve the skin problems. Most widely used form is herbal extract for

skin-care and primarily added to the preparations due to several associated properties such as antioxidants. Also they have been used for the topical anti-inflammatory properties (Kole et al., 2005). Anti-inflammatory agents may be used in many different types of skin care products for sun protection, acne treatment, anti-aging skin-care products, and so on.

There are various methods to evaluate the potential of natural products for use in skin-care. Four (1-4) of the most common, simple, and related tests on natural products can be evaluating their hyaluronidase inhibitory, antioxidant, advanced glycation end products (AGEs) production inhibitory, and tyrosinase inhibitory activities (Fig. 1).

1. Hyaluronidase (EC 3.2.1.35) is an enzyme that decomposes hyaluronic acid resulting in reduced dermal hydration, disorganization of collagen and elastin fibers, and increased skin wrinkling and folding (Fig. 1). Hyaluronidase inhibitors are known to have potential benefits in preventing and treating wrinkling and inflammations (Mitra and Babu, 2010). In addition, hyaluronidases have been recognized in a number of physiological and pathological processes such as embryogenesis, angiogenesis, disease progression, wound healing, bacterial pathogenesis, and the diffusion of systemic toxins/venoms (Girish and Kemparaju, 2007). The modulation of hyaluronidase by suitable inhibitors will be useful for not only inflammations, but also normal homeostasis in the body (Gonzalez-Pena et al., 2013).

2. Skin produces free radicals or reactive oxygen species due to environmental pollutants, food contaminants, cosmetic products, drugs, etc, which lead to oxidative stresses and inflammatory responses in the dermal or epidermal layer of the connective tissues resulting aging and damage to cell membranes, lipids, proteins, and DNA (Athar, 2002; Yamamoto, 2001).
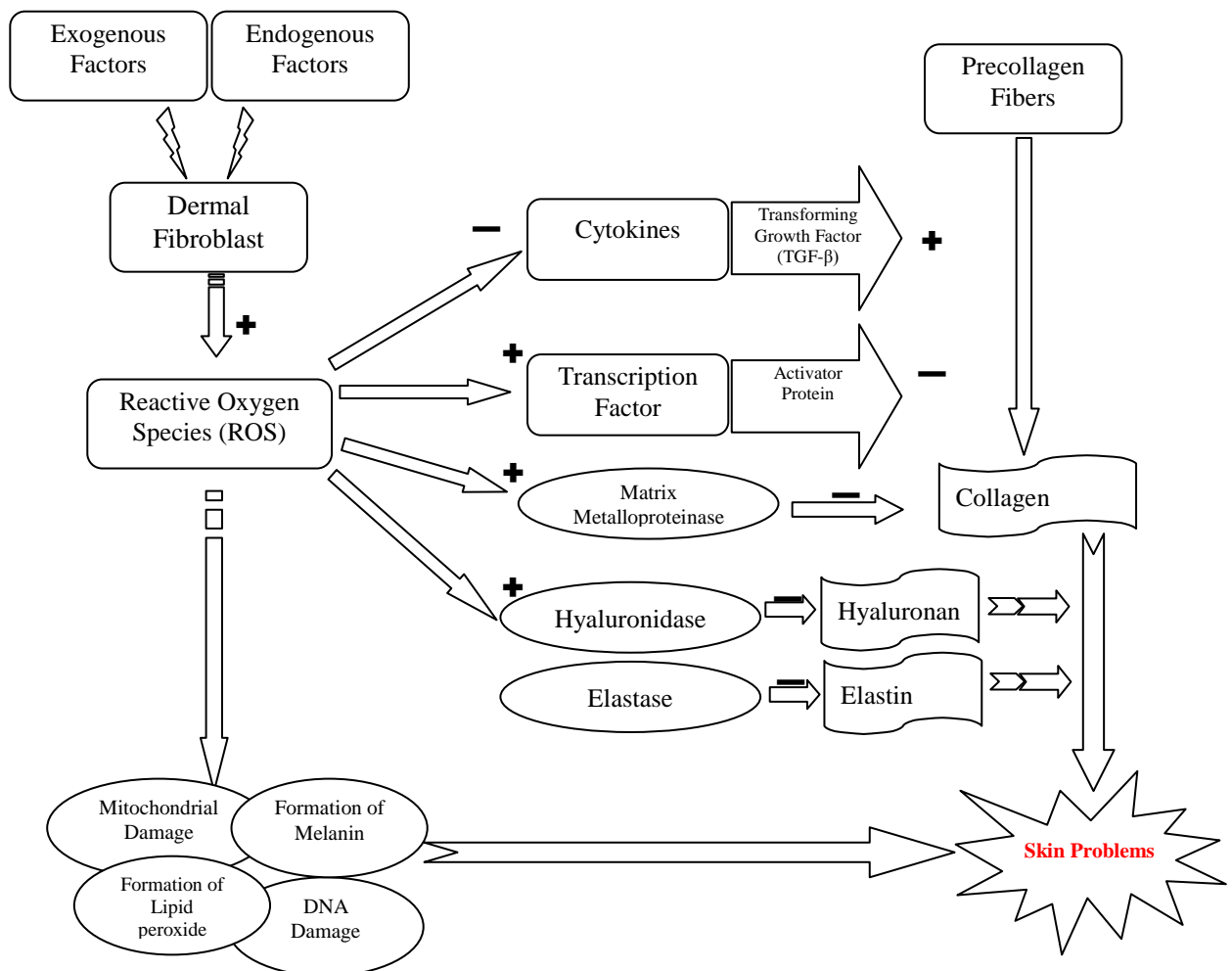
Fig. 1 Pathway of skin problems (+, induction; -, inhibition)

3.   AGEs production in organs is induced by hyperglycemia and is one of the causes of diabetic complications (Sourris et al., 2009). Moreover, AGEs production accumulate in the skin are correlated with aging and modifies elastin and collagen (Dyer et al., 1993; Mizutari et al., 1997). When AGEs production accumulate, they induce cross-linking of collagen and reduce skin degradability and dermal regeneration (Wondrak et al., 2002). Thus, the discovery and investigation of AGEs production inhibitors would offer a potential therapeutic approach for the prevention of skin complications.

4. Tyrosinase (EC 1.14.18.1) is responsible for biosynthesis of melanin in melanocytes of human skin, and epidermal hyper-pigmentation might cause various dermatological disorders, such as melasma, freckles, and age spots (Li et al., 2010). It catalyzes the key step of the formation of melanin, the oxidation of diphenol to quinines (Pan et al., 2011). Tyrosinase inhibitors such as kojic acid, arbutin, and ascorbic acid have been used for prevention and treatment of hyperpigmentation. Some commercially available chemical and fungal derived skin-lightening agents have been proven to have chronic, cytotoxic, and mutagenic effects on humans (Nerya et al., 2003; Wang et al., 2006). Therefore, there is a need for alternative herbal derived and pharmaceutical agents for the treatment of hyperpigmentation of human skin (Yesilada, 2005).

At first 51 extractions of Mongolian medicinal plants were tested for their hyaluronidase inhibitory and DPPH radical scavenging activities. Then, out of these extracts, 2 genus and 4 species were picked up, which showed significant activity and are commonly used as herbal medicine in TMM. Their phytochemical and biological knowledge were revealed in this research.

Dracocephalum L. is one of the important members of Lamiaceae family for TMM and consists of around 60 species distributed in the temperate regions of the Northern hemisphere (Sonboli et al., 2011; Zeng et al., 2010). In the flora of Mongolia, the genus is represented by 17 species, which are mainly distributed in the northern and eastern parts of the country (Ligaa, 2005).

Dracocephalum plants, which are used in the traditional medicine, for a long period, for the treatment of various inflammatory diseases, rheumatism, and external injury (Ligaa, 2005), also showed a potent hyaluronidase inhibitory activity in our screening (Murata et al., 2012). Especially, *D. foetidum* has been widely used as traditional

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medicine among Mongolian nomads. Recently, much attention has been paid to *Dracocephalum* species and their chemical constituents, because of their diverse effects, such as antioxidant, anti-inflammatory, antihypoxic, and immunomodulatory activities. Plants in this genus typically contain terpenoids and flavonoids (Zeng et al., 2010). In this research, two *Dracocephalum* plants, *D. ruyschiana* and *D. foetidum* were revealed to have hyaluronidase inhibitory activity, which is known to concern anti-inflammatory mechanism. The phytochemical constituents were isolated from the two plants by chromatography and chemical structures were determined by using instrumental analyses.

Ten new and 19 known compounds were identified from *D. ruyschiana*, and 13 new and 13 known compounds were identified from *D. foetidum*. The structure determination process is presented in **Chapter 2** and **3**.

Chapter 2 presents isolation and structure elucidation of five new flavone tetraglycosides (**1-5**), five new benzyl alcohol glycosides (**7-9**, **12**, and **13**), and 19 known compounds (**6**, **10**, **11**, and **14-29**) from *D. ruyschiana*.

In **Chapter 3**, three new limonene glycosides (**30-32**), a new caffeic acid trimer (**34**), four new rosmarinic acid derivatives (**37-39** and **41**), five new acacetin acyl glycosides (**42-46**), as well as 13 known compounds (**33**, **35**, **36**, **40**, and **47-55**) from *D. foetidum* were characterized.

Chamaerhodos plants, *C. erecta* and *C. altaica* were revealed having potent antioxidant activity by screening of 23 Mongolian medicinal plants, and the plants are known to be used for skin-care, traditionally. Plants in the genus *Chamaerhodos* Bge, which belongs to the family Rosaceae, are herbs or subshrubs, glandular pilose and distributed in Asia and North America. There are 6 species of *Chamaerhodos* in

Mongolia (Gubanov, 1996; Ligaa, 2005). The plants belonging to Rosaceae family were very active against DPPH free radical in our screening (Selenge, 2010).

Phytochemical investigations of *C. erecta* and *C. altaica* were followed the same processes as *Dracocephalum* plants and identified 4,5-dihydroxybenzaldehyde-3-*O*-β-D-glucopyranoside (**56**) from *C. erecta* and quercetin-3-*O*-β-D-glucurono pyranosyl-4′-*O*-β-D-glucopyranoside (**57**) from *C. altaica* as new compounds with 37 known compounds (**14-16** and **56-91**). These results are explained in **Chapter 4**.

To elucidate skin-care effects and biological activities of the obtained 91 compounds, four basic tests hyaluronidase inhibitory, DPPH radical scavenging, AGEs production inhibitory, and tyrosinase inhibitory activities were evaluated. The tests were related with anti-inflammatory, antioxidant, antipigmentation activities and their results are discussed in **Chapter 5**.

Phytochemical constituents of these plants are expected to contribute to biological effects and usage of them in skin-care. This study will serve as a part of primary reference for Mongolians, especially health care providers, manufacturers, and regulators as well as it can contribute to confirm scientific rationality of traditional medicinal plants. These conclusions are summarized in **Chapter 6**.

Chapter 2. Phytochemical constituents of *Dracocephalum ruyschiana* L.

2.1 Introduction

Dracocephalum ruyschiana L. (fam. Lamiaceae, Mongolian name: Ruishiin shimeldeg), is a traditional medicinal plant widely distributed in Mongolia around Khentei, Khangai, Mongol-Daurian, Great Khingan, Khobdo, and Middle Khalkha. It is typical habitat includes larch and mixed forests, their fringes and meadow slopes (Grubov, 1982). The aerial parts are widely used for the treatment of gastric ulcers, laryngitis, headache, acute respiratory infection, diarrhea, and rheumatoid arthritis (Ligaa, 2005). It has also been shown to have hepatoprotective effects, antimicrobial activity against Gram-positive bacteria, and antispasmodic effects (Ligaa, 2005). Moreover, certain flavone glycosides, benzyl alcohol glycosides, and phenylpropanoids isolated from *D. ruyschiana* have been confirmed to have some pharmacological effects, such as anti-inflammatory (Zeng et al., 2010), hepatoprotective (Perez-Alvarez et al., 2001), antimicrobial activities (Rigano et al., 2007). Caffeic acid and α-hydroxydihydrocaffeic acid were reported as the chemical constituents of *D. ruyschiana* (Zeng et al., 2010).

2.2 Results and Discussion

From the extract of aerial parts of *D. ruyschiana* L., five new flavone tetraglycosides (**1-5**) (Fig. 2-1), five new benzyl alcohol glycosides (**7-9**, **12**, and **13**) (Fig. 2-2, 2-3), and 19 known compounds (**6, 10-11, 14-29**) were isolated (Fig. 2-4). The tetraglycosides contained a 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[β-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl moiety. The benzyl alcohol glycosides had acyl groups on their glycosyl or aglycone moieties.

Similar tetraglycosides comprising a rhamnopyranosyl and three glucopyranosyl

units were identified in *Peganum harmala* (Ahmed and Saleh, 1987), *P. nigellastrum* (Yang et al., 2010), and *Coptis japonica var. dissecta* (Yoshikawa et al., 1997). Similar triglycosides were found in species such as *Calamintha glandulosa*, *Micromeria* spp (Marin et al., 2001)., *Valeriana jatamansi* (Tang et al., 2003), *Sclerochiton vogelii* (Lamidi et al., 2006), and *Robinia pseudoacacia* (Veitch et al., 2010). Some of the flavone tri- and tetraglycosides have an *O*-acetyl group on a glycosidic moiety. Flavone monodesmosides containing four glycosidic moieties seemed to be rare. On the other hand, benzyl alcohol, the aglycone moiety of **7-9**, is known as an important aroma substance with a variety of biological activity (Scognamiglio et al., 2012). Two of the new benzyl alcohol glycosides (**12** and **13**) are esters of gastrodin, one of the major bioactive components from *Gastrodia elata* Bl., exhibiting cardiac hypertrophy protective, anticonvulsant, and neuroprotective effects (Shu et al., 2012).

2.2.1 Isolation of Known Compounds

Known compounds were identified from spectroscopic data as diosmetin-7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (**6**) (Yang et al., 2010), benzyl-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**10**) (Kawahara et al., 2005), benzyl-*O*-β-D-glucopyranoside (**11**) (Seigler et al., 2002), kaempferol-3-*O*-β-D-glucopyranoside (**14**) (Han et al., 2004), quercetin-3-*O*-β-D-glucopyranoside (**15**) (Han et al., 2004), quercetin-3-*O*-β-D-glucuronopyranoside (**16**) (Seto et al., 1992), chlorogenic acid (**17**) (Pauli et al., 1999), 3,5-dicaffeoylquinic acid (**18**) (Kim et al., 2011), 3,4-dicaffeoylquinic acid (**19**) (Kim et al., 2011), 3-p-(*E*)-coumaroyl-5-(*E*)-caffeoylquinic acid (**20**) (Kim et al., 2011; Pauli et al., 1999), (7S,8R)-dihydrodehydro diconiferyl alcohol-9′-*O*-β-D-glucopyranoside (**21**) (Kuang et al., 2009; Matsuda et al.,

1996; Otsuka et al., 2000), (7*S*, 8*R*)-urolignoside (**22**) (Kuang et al., 2009; Matsuda et al., 1996; Otsuka et al., 2000), citrusin C (**23**) (Teng et al., 2005), *trans-p*-coumaric acid (**24**) (Salum et al., 2010), methyl *trans-p*-coumaric acid (**25**) (Kwon and Kim, 2003), *trans*-ferulic acid (**26**) (Salum et al., 2010), *cis-p*-coumaric acid (**27**) (Salum et al., 2010), *p*-hydroxybenzaldehyde (**28**) (Kim et al., 2003), and 4,4′-dihydroxydiphenyl methane (**29**) (Hejaz et al., 2004).

2.2.2   Isolation and structure elucidation of new compounds

Five new flavone tetraglycosides (**1**-**5**) and five new benzyl alcohol glycosides (**7**-**9**, **12**, and **13**) were isolated.

2.2.2.1 Flavone tetraglycosides

Compounds **1**-**5** were isolated as amorphous solids. Compound **1** was demonstrated to have the molecular formula $C_{40}H_{52}O_{24}$ on the basis of HRFABMS (m/z 939.2752, [M +Na]$^+$). In the ^{13}C-NMR spectrum, 40 carbons (Table. 2-1) were observed; 14 aromatic/ olefinic carbons (δ_C 94.9, C-8; 99.8, C-6; 103.8, C-3; 105.5, C-10; 114.8, C-3′ and -5′; 122.7, C-1′; 128.5, C-2′ and -6′; 157.0, C-9; 161.1, C-5; 162.5, C-4′; 162.7, C-7; 164.0, C-2) and a carbonyl carbon (δ_C 182.1) suggested a flavone skeleton. An *O*-methyl signal at δ_H 3.85 (3H, H-4′-OMe) was long-range coupled with C-4′ (δ_C 162.5) in the HMBC spectrum. These results demonstrated the presence of an acacetin skeleton. Four anomeric protons in the ^{1}H-NMR spectrum and a methyl (δ_C 17.8) and 23 oxygenated carbons in the ^{13}C-NMR spectrum suggested the presence of four glycosidic units. The glycosidic components and their absolute configurations were determined as one L-rhamnose and three D-glucose units from the NMR data and HPLC sugar analysis, as described in the Experimental Section (Tanaka et al., 2007). The coupling constants of the anomeric protons of the three D-glucopyranosyl moieties were 7.5 Hz, indicating

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anomeric β-configurations (Veitch et al., 2010). Similarly, the anomeric proton (δ$_H$ 4.54, br s, H-Rha-1) of the L-rhamnopyranosyl moiety indicated an α-anomeric configuration (Veitch et al., 2010). An anomeric proton at δ$_H$ 5.20 (d, J =7.5 Hz, H-Glc-I-1) was correlated with a proton at δ$_H$ 3.48 (dd, J = 9.5, 7.5 Hz, H-Glc-I-2) and with the corresponding carbon at δ$_C$ 83.0 (C-Glc-I-2) according to COSY and HMQC data. In addition, an anomeric proton at δ$_H$ 4.62 (1H, d, J = 7.5 Hz, HGlc-II-1) was correlated with a proton at δ$_H$ 3.25 (dd, J = 9.0, 7.5 Hz, H-Glc-II-2) and with the corresponding carbon at δ$_C$ 83.1 (C-Glc-II-2). The signals of the ^{13}C-NMR spectrum were similar to those of acacetin-7-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (Tang et al., 2003), except for the presence of the third β-D-glucopyranosyl unit of **1**. The C-Glc-II-2 signal was shifted downfield relative to that of the acacetin triglycoside, which suggested that the third glucopyranosyl unit is coupled to the carbon. In the HMBC spectrum, an anomeric proton at δ$_H$ 4.51 (d, J = 7.5 Hz, H-Glc-III-1) was long-range coupled with C-Glc-II-2. The anomeric proton of the second glucopyranosyl unit (H-Glc-II-1) was long-range coupled with C-Glc-I-2. A lower shifted C-6 signal at δ$_C$ 65.9 (C-Glc-I-6) was long-range coupled with H-Rha-1. The signal of the acacetin moiety (C-7) was long-range coupled with H-Glc-I-1. From these results, the structure of **1** was determined as acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Fig. 2-1 New compounds (**1-5**)

1 R_1= H, R_2= Me, R_3= H, R_4= H

2 R_1= H, R_2= Me, R_3= Ac, R_4= H

3 R_1= H, R_2= Me, R_3= H, R_4= Ac

4 R_1= OH, R_2= Me, R_3= H, R_4= Ac

5 R_1= H, R_2= H, R_3= H, R_4= Ac
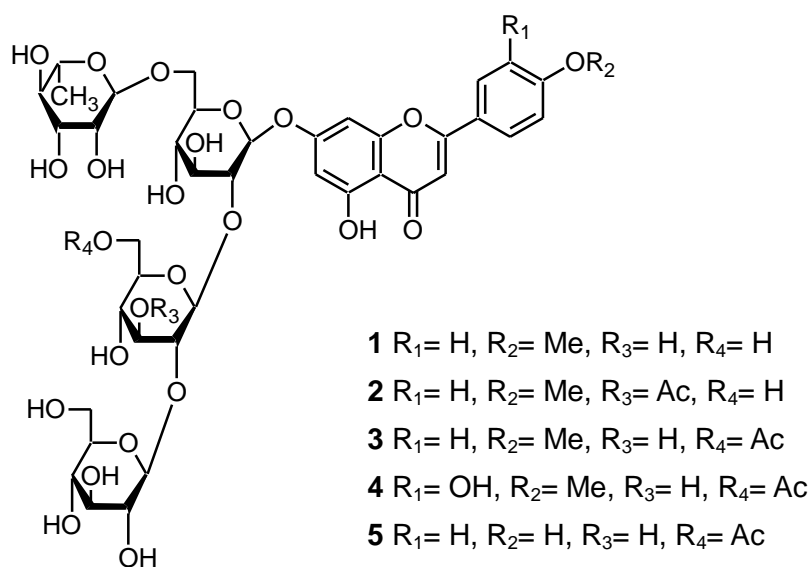
The molecular formula of **2** was established as $C_{42}H_{54}O_{25}$ on the basis of the HRFABMS (m/z 959.3031 [M+H]$^+$), which was C_2H_2O more than that of **1**. In the ^{1}H- and ^{13}C-NMR spectra, signals of an *O*-acetyl group (δ_H 2.03, 3H, s; δ_C 21.1 and 169.5) were observed. Other signals were similar to those of **1**. The differential HOHAHA spectrum showed the correlations of the signals of the second glucosyl moiety [δ_H 4.73 (d, *J* = 7.5 Hz, H-Glc-II-1), 3.49 (dd, *J* = 9.0, 7.5 Hz, H- Glc-II-2), 5.00 (dd, *J* = 9.5, 9.0 Hz, H-Glc-II-3), 3.44 (t, *J* = 9.5 Hz, H-Glc-II-4), 3.30 (overlapped, H-Glc-II-5), 3.30 (overlapped, H-Glc-II-6), and 3.47 (dd, *J* = 11.5, 2.0 Hz, HGlc-II-6)]. The H-Glc-II-3 signal was long-range coupled with the carbonyl carbon signal of the *O*-acetyl group (δ_C 169.5). Thus, the structure of **2** was identified as acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-3-*O*-acetyl-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **3** had an [M+H]$^+$ ion peak in HRFABMS at m/z 959.3026, corresponding to the molecular formula $C_{42}H_{54}O_{25}$, the same as that of **2**. Its ^{1}H- and ^{13}C-NMR spectra (in DMSO-d_6, Table. 2-1) were similar to those of **2**. The ^{1}H- and

^{13}C-NMR spectra recorded in pyridine-d_5 (see Experimental Section) were almost superimposable onto those of peganetin (Ahmed and Saleh, 1987). The HMBC spectrum showed that an anomeric proton at δ_H 5.63 (d, J = 7.5 Hz, H-Glc-I-1) was long-range coupled with C-7 (δ_C 164.0) of an acacetin moiety. Similarly, H-Glc-II-1 (δ_H 5.27, d, J = 8.0 Hz) was long-range coupled with C-Glc-I-2 (δ_C 84.7), HGlc-III-1 (δ_H 5.31, d, J = 7.5 Hz) with C-Glc-II-2 (δ_C 85.2), and H-Rha-I-1 (δ_H 5.41, br s) with C-Glc-I-6 (δ_C 67.2). In the differential HOHAHA spectrum (in pyridine-d_5), the anomeric proton of Glc-II correlated with H-Glc-II-6 (δ_H 4.80, dd, J = 12.0, 3.0 Hz and 4.86, dd, J = 12.0, 1.0 Hz). The H-Glc-II-6 protons were long-range coupled with an *O*-acetyl carbonyl carbon at δ_C 170.9 in the HMBC spectrum. Hence, the structure of **3** was determined as acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-6-*O*-acetyl-β-D-gluco pyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside. Assignments of NMR signals were as shown in Table. 2-1 (in DMSO-d_6) and the Experimental Section (in pyridine-d_5).

The molecular formula of **4** was suggested as $C_{42}H_{54}O_{26}$ on the basis of HRFABMS (m/z 975.2988 [M+H]$^+$), which was one oxygen atom more than that of **3**. Signals of glycosyl and an *O*-acetyl moiety in the ^{1}H- and ^{13}C-NMR spectra (in DMSO-d_6, Table. 2-1) were similar to those of **3**. The aromatic protons (δ_H 7.44, d, J = 2.0 Hz, H-2′; 7.13, d, J = 8.5 Hz, H-5′; 7.57, dd, J = 8.5, 2.0 Hz, H-6′), and an *O*-methyl singlet at δ_H 3.88 suggested that the aglycone moiety of **4** is diosmetin. In the differential HOHAHA spectra, correlations of glycosidic signals such as from H-Glc-II-1 (δ_H 4.68, d, J = 8.0 Hz) to H-Glc-II-6 (δ_H 4.00, 1H, m; 4.01, 1H, m) were observed. Consequently, compound **4** was identified as diosmetin-7-*O*-β-D-glucopyranosyl-(1→2)-6-*O*-acetyl-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

In the ^{1}H-NMR spectrum of **5** (Table. 2-1), an AA′BB′ spin system (δ_H 6.95, 2H, d, $J = 9.0$ Hz, H-3′ and -5′; 7.94, 2H, d, $J = 9.0$ Hz, H2′ and -6′), two *m*-coupled protons (δ_H 6.46, 1H, $J = 2.0$ Hz, H-6; 6.71, 1H, $J = 2.0$ Hz, H-8), and a singlet (δ 6.84, 1H, H-3) were observed in the aromatic/olefinic region. These showed the presence of apigenin as the aglycone moiety. The molecular formula of **5** was suggested to be $C_{41}H_{52}O_{25}$ on the basis of the HRFABMS (m/z 967.2713 $[M+Na]^+$), which was CH_2O less than that of **4**. These results suggested that **5** was apigenin-7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

2.2.2.2 Benzyl alcohol glycosides

Compounds **7−9**, **12**, and **13** were isolated as colorless powders. Compound **7** was established to have the molecular formula, $C_{28}H_{34}O_{12}$, based on the HRFABMS (m/z 563.2117, $[M+H]^+$). Five aromatic protons (δ_H 7.23, 1H, m, H-4; 7.29, 2H, m, H-3 and -5; 7.41, 2H, d, $J = 7.0$ Hz, H-2 and -6) and two oxygenated methylene protons in the ^{1}H-NMR spectrum (Table. 2-2) at δ_H 4.64 (d, $J = 11.5$ Hz, H-7) and 4.87 (d, $J = 11.5$ Hz, H-7) suggested the presence of a benzyl group. Spin systems at δ_H 6.80 (2H, d, $J = 8.5$ Hz, H-3′ and -5′), 7.43 (2H, d, $J = 8.5$ Hz, H-2′ and -6′), 6.36 (1H, d, $J = 16.0$ Hz, H-8′), and 7.59 (1H, d, $J = 16.0$ Hz, H-7′), and a carbonyl carbon at δ_C 168.6 (C-9′) indicated a *trans*-*p*-coumaroyl moiety. Two anomeric, a methyl, and nine oxygenated carbons suggested the presence of two glycosidic moieties. After acid hydrolysis of **7**, the sugars were identified as D-glucose and L-rhamnose by the same method as for **1** (Tanaka et al., 2007). The coupling constant of H-Glc-1 (δ_H 4.36, d, $J = 8.0$) indicated a β-D-gluco pyranosyl moiety (Kawahara et al., 2005; Veitch et al., 2010). The H-Glc-1 signal was long-range coupled with a benzylic oxygenated carbon at δ_C 71.8 (C-7) in the HMBC

spectrum. The anomeric proton at δ_H 4.95 (d, J = 1.5 Hz, H-Rha-1) and the methyl doublet (δ_H 1.32, d, J = 6.5 Hz, H-Rha-6) indicated the presence of an α-L-rhamnopyranosyl moiety (Kawahara et al., 2005; Veitch et al., 2010). The H-Rha-1 signal correlated with H-Rha-2 (δ_H 5.20, dd, J = 3.5, 1.5 Hz) in the COSY spectrum. In the HMBC spectrum, the H-Rha-1 signal was long-range coupled with C-Glc-6 (δ_C 67.8), and H-Rha-2 was long-range coupled with the acyl carbonyl carbon (C-9′). From these results, the structure of **7** was determined as benzyl-2-*O-trans-p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

The molecular formula of **8** was determined as $C_{28}H_{34}O_{12}$ on the basis of the HRFABMS (m/z 563.2129 [M+H]$^+$), which was the same as that of **7**. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **7**, except for signals of the acyl group. The coupling constant of H-7′ (δ_H 6.88, d, J = 13.0 Hz) and H-8′ (δ_H 5.82, d, J = 13.0 Hz) suggested that the acyl group was a *cis-p*-coumaroyl moiety. Therefore, the structure of **8** was determined as benzyl-2-*O-cis-p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **9** also had the molecular formula $C_{28}H_{34}O_{12}$ on the basis of the HRFABMS (m/z 563.2143, [M+H]$^+$). Although its NMR spectra were similar to those of **7**, the H-Rha-4 signal (δ_H 5.08, t, J = 9.0 Hz) in **9** was shifted downfield, instead of the H-Rha-2 proton. In the HMBC spectrum, the H-Rha-4 signal correlated with the carbonyl carbon at δ_C 168.9 (C-9′). Hence, the structure of **9** was determined as benzyl-4-*O-trans-p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.
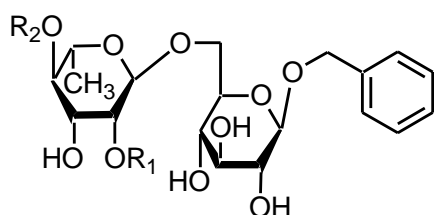
7	R_1= *trans-p*-coumaroyl, R_2= H
8	R_1= *cis-p*-coumaroyl, R_2= H
9	R_1= H, R_2= *trans-p*-coumaroyl

Fig. 2-2 New compounds (**7-9**)

The molecular formula of **12** was established as $C_{26}H_{32}O_{12}$ on the basis of the HRFABMS (m/z 559.1797 [M+Na]$^+$). Aromatic ring protons (δ_H 7.96, 2H, br d, J = 7.5 Hz, H-2′ and -6′; 7.65, 1H, m, H-4′; 7.40, 2H, m, H-3′ and -5′), an AA′BB′ spin system (δ_H 7.40, d, J = 9.0 Hz, H-2 and -6; 7.00, d, J = 9.0 Hz, H-3 and -5), an oxymethylene singlet (δ_H 5.27, 2H, H-7), an oxygenated carbon at δ_C 157.1 (C-4), and an ester carbonyl carbon at δ_C 165.6 (C-7′) were observed in the ^{1}H- and ^{13}C-NMR spectra of **12**, indicating the presence of a benzyl and a benzoyl moiety. The H-7, -2′, and -6′ signals were long-range coupled with C-7′ (Fig. 2-3), demonstrating the presence of a 4-hydroxybenzyl benzoate aglycone moiety. Signals of two glycosidic units were also observed in the ^{1}H- and ^{13}C-NMR spectra. HPLC sugar analysis, as described in the Experimental Section, and coupling patterns of anomeric protons at δ_H 5.00 (d, J = 7.5 Hz, H-Glc-1) and 5.12 (d, J = 1.5 Hz, H-Rha-1) confirmed the β-D-glucopyranosyl and α-L-rhamnopyranosyl moieties (Tanaka et al., 2007; Veitch et al., 2010). In the COSY spectrum, the H-Glc-1 signal correlated with H-Glc-2 (δ_H 3.47, overlapped). The corresponding carbon (δ_C 76.3, C-Glc-2) was determined using the HMQC data. In the HMBC spectrum (Fig. 2-3), the H-Rha-1 signal correlated with C-Glc-2, and H-Glc-1 with C-4 (δ_C 157.1). From these results, the structure of **12** was formulated as [(benzoxy)methyl]phenyl-4-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **13** had an [M+H]$^+$ ion peak in the HRFABMS at m/z 431.1558, corresponding to a molecular formula of $C_{19}H_{26}O_{11}$. The oxymethylene protons at δ_H

5.07 (2H, s, H-7) and AA′BB′ systems (δ_H 7.31, 2H, d, J = 9.0 Hz, H-2 and -6; 7.08, 2H, d, J = 9.0 Hz, H-3 and -5) indicated that **13** also contains a 4-hydroxybenzyl moiety. Two carbonyl carbons [δ_C 174.8 (C-5′) and 172.5 (C-1′)] and an oxygenated carbon [δ_C 70.7 (C-3′)] were observed in the ^{13}C-NMR spectrum. In the HMBC spectrum (Fig. 2-3), a methyl singlet at δ_H 1.35 (3H, H-6′) was long-range coupled with C-3′ and two methylene signals [δ_C 46.3 (C-2′) and 45.8 (C-4′)]. The methylene protons at δ_H 2.70 (2H, br s, H-2′) and the H-7 signal correlated with C-1′ in the HMBC spectrum. These data suggested the presence of the ester of 3-hydroxy-3-methyl pentanedioic acid. A glycosidic unit was determined as the β-D-glucopyranosyl by sugar analysis using HPLC (see Experimental Section) and a coupling constant of the anomeric proton at δ_H 4.90 (d, J = 7.5 Hz, H-Glc-1). The H-Glc-1 signal correlated with C-4 (δ_C 159.1) in the HMBC spectrum. These results suggested that compound **13** was [(3-hydroxy-3-methylglutaryl)methyl]phenyl-4-*O*-β-D-glucopyranoside. The absolute configuration of C-3′ could not be defined.

H ⤻ C: key HMBC correlations
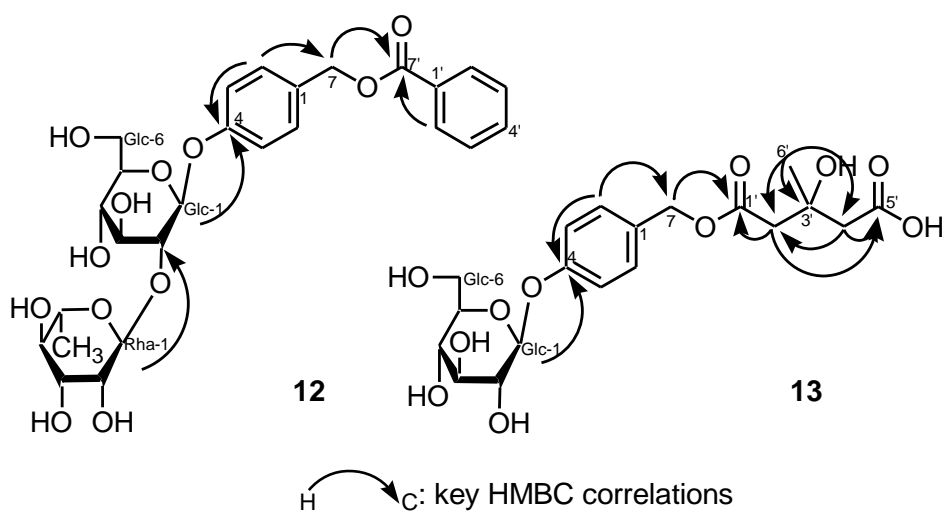
Fig. 2-3 New compounds (**12-13**)

14 R$_1$= H, R$_2$= β-D-glucopyranosyl
15 R$_1$= OH, R$_2$= β-D-glucopyranosyl
16 R$_1$= OH, R$_2$= β-D-glucuronide

24 R$_1$= H, R$_2$= H,
25 R$_1$= H, R$_2$= Me
26 R$_1$= OMe, R$_2$= H

21 R$_1$= H, R$_2$= β-D-glucopyranosyl
22 R$_1$= β-D-glucopyranosyl, R$_2$= H

17 R$_1$= E-caffeoyl, R$_2$= H, R$_3$= H
18 R$_1$= E-caffeoyl, R$_2$= H, R$_3$= E-caffeoyl
19 R$_1$= E-caffeoyl, R$_2$= E-caffeoyl, R$_3$= H
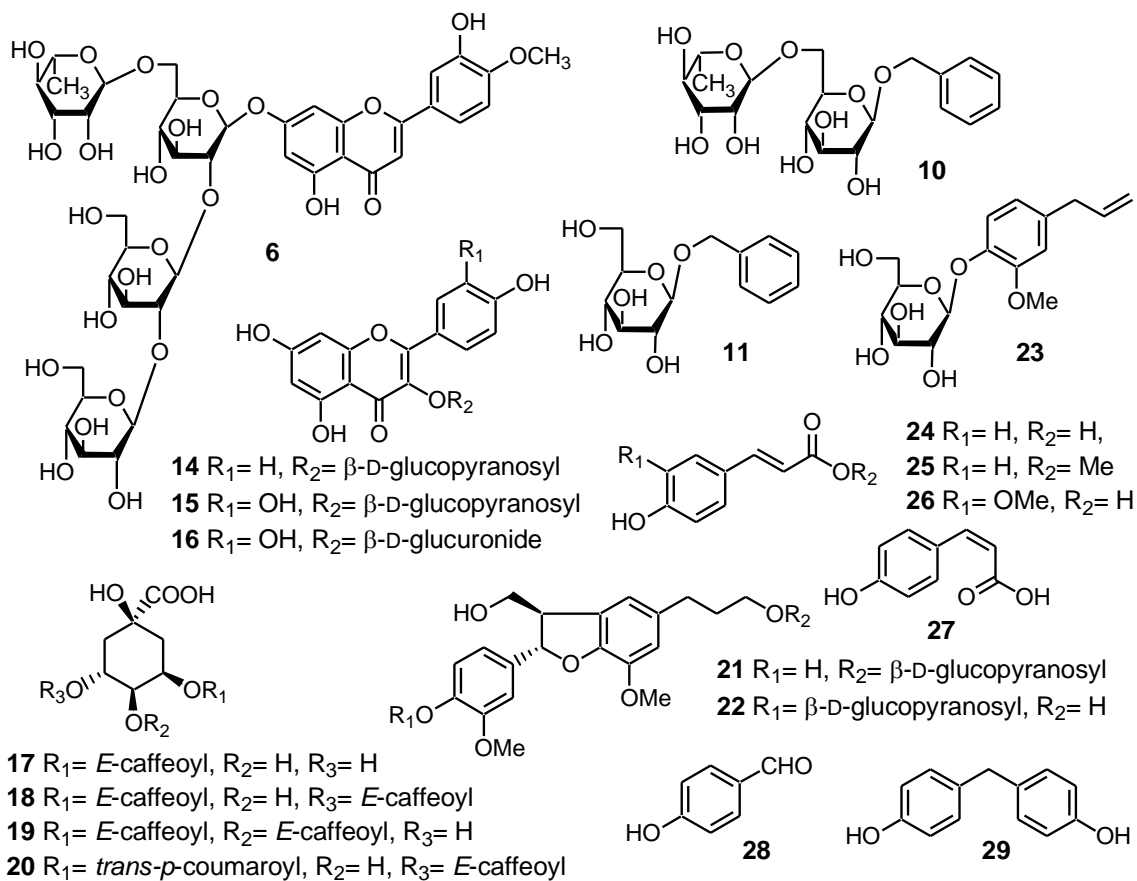**20** R$_1$= trans-p-coumaroyl, R$_2$= H, R$_3$= E-caffeoyl

Fig. 2-4 Known compounds (**6, 10-11, 14-29**)

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Table 2-1. [^1]H and [^13]C NMR Spectroscopic Data (in DMSO-d_6) of Compounds 1-5

position	1 δH (J in Hz)	1 δC	1 HMBC (H to C)	2 δH (J in Hz)	2 δC	2 HMBC (H to C)	3 δH (J in Hz)	3 δC	3 HMBC	4 δH (J in Hz)	4 δC	4 HMBC	5 δH (J in Hz)	5 δC	5 HMBC (H to C)
aglycone															
2		164.0			163.9			163.9			164.2			164.3	
3	6.91, s	103.8	2, 4, 10, 1'	6.94, s	103.8	2, 4, 10, 1'	6.91, s	103.8	2, 4, 10, 1'	6.80, s	103.8	4, 10, 1'	6.84, s	103.1	4, 10, 1'
4		182.1			182.0			182.0			181.9			181.9	
5		161.1			161.1			161.2			161.1			161.1	
6	6.49, d (1.5)	99.8	5, 7, 8, 10	6.51, d (2.0)	99.6	5, 7	6.46, d (2.0)	99.6	7, 8, 10	6.47, d (2.0)	99.5	5, 7, 8, 10	6.46, d (2.0)	99.4	7, 8, 10
7		162.7			162.4			162.7			162.6			162.6	
8	6.83, d (1.5)	94.9	6, 7, 9, 10	6.84, d (2.0)	94.7	7, 9	6.72, d (2.0)	94.6	4, 6, 7, 9, 10	6.73, d (2.0)	94.5	4, 6, 7, 9, 10	6.71, d (2.0)	94.6	9, 10
9		157.0			156.9			156.9			156.9			156.8	
10		105.5			105.4			105.4			105.4			105.3	
1'		122.7			122.7			122.7			122.9			121.0	
2'	8.02, d (8.5)	128.5	2, 4', 6'	8.05, d (9.0)	128.4	4', 6', 2	8.04, d (8.5)	128.4	3', 4', 6'	7.44, d (2.0)	113.1	2, 3', 4', 6'	7.94, d (9.0)	128.6	2, 4', 6'
3'	7.14, d (8.5)	114.8	1', 4', 5'	7.16, d (9.0)	114.7	1', 4', 5'	7.13, d (8.5)	114.7	1', 4', 5'		146.8		6.95, d (9.0)	116.0	1', 5'
4'		162.5			162.5			162.4			151.3			161.3	
5'	7.14, d (8.5)	114.8	1', 4', 3'	7.16, d (9.0)	114.7	1', 3', 4'	7.13, d (8.5)	114.7	1', 3', 4'	7.13, d (8.5)	112.2	1', 3'	6.95, d (9.0)	116.0	1', 3'
6'	8.02, d (8.5)	128.5	2, 2', 4'	8.05, d (9.0)	128.4	2', 4', 2	8.04, d (8.5)	128.4	2', 3', 4'	7.57, dd (8.5, 2.0)	118.9	2, 2', 4'	7.94, d (9.0)	128.6	2, 2', 4'
4'-OCH$_3$	3.85, s	55.6	4'	3.87, s	55.5	4'	3.85, s	55.6	4'	3.88, s	55.8	4'			
sugar-1															
Glc-I-1	5.20, d (7.5)	98.3	7	5.25, d (7.5)	98.2	7	5.23, d (7.5)	98.0	7, Glc-I-2	5.26, d (7.5)	98.0	7	5.24, d (7.5)	98.0	7
Glc-I-2	3.48, dd (9.5, 7.5)	83.0		3.47, dd (9.0, 7.5)	82.8		3.47, dd (9.5, 7.5)	83.0	Glc-I-1	3.47, dd (9.5, 7.5)	83.2		3.48, dd (9.0, 7.5)	83.2	Glc-I-1
Glc-I-3	3.57, m	75.3		3.58, dd (9.5, 9.0)	75.0		3.61, dd (9.5, 9.0)	75.3		3.61, t (9.0)	75.2		3.61, t (9.0)	75.4	
Glc-I-4	3.24^a	68.7		3.29, t (9.5)	68.7		3.24, t (9.0)	68.6		3.25^a	68.5		3.21, t (9.0)	68.5	
Glc-I-5	3.66, m	75.4		3.70, m	75.3		3.45, m	75.5		3.47^a	75.4		3.44, m	75.4	
Glc-I-6	3.45^a	65.9	Rha-1	3.48^a	65.9		3.46, dd (11.5, 4.5)	65.9		3.46^a	65.8	Rha-1	3.48^a	65.8	Rha-1
	3.85, br d (11.5)			3.88, br d (11.5)			3.86, br d (11.5)			3.85, br d (11.5)			3.85, dd (11.5, 1.5)		
sugar-2															
Glc-II-1	4.62, d (7.5)	102.4	Glc-I-2	4.73, d (7.5)	102.1	Glc-I-2	4.67, d (7.5)	102.5	Glc-I-2	4.68, d (8.0)	102.4	Glc-I-2	4.68, d (7.5)	102.4	Glc-I-2
Glc-II-2	3.25, dd (9.0, 7.5)	83.1	Glc-II-1	3.49, dd (9.0, 7.5)	78.3	Glc-II-1	3.30, dd (9.0, 7.5)	83.0	Glc-II-1	3.31^a	82.9	Glc-II-1	3.30^a	83.0	Glc-II-1
Glc-II-3	3.45^a	76.1		5.00, dd (9.5, 9.0)	76.4	Ac C=O, Glc-II-2	3.48^a	75.7		3.48, t (9.0)	75.7		3.49^a	75.7	
Glc-II-4	3.18, t (9.0)	69.2		3.44, t (9.5)	67.1		3.19, t (9.5)	69.2		3.20, t (9.0)	69.2		3.19, t (9.0)	69.2	
Glc-II-5	3.45, m	76.5		3.30^a	76.4		3.46, m	73.2		3.47, m	73.2		3.45, m	73.2	
Glc-II-6	3.40, dd (11.5, 5.0)	60.3		3.30^a	59.6		4.01, dd (12.0, 4.5)	63.1	Ac C=O, Glc-II-5	4.00, m	63.0	Ac C=O	4.00, dd (12.0, 4.5)	63.1	
	3.45^a			3.47, dd (11.5, 2.0)			4.04, dd (12.0, 2.0)			4.01, m			4.03, dd (12.0, 3.0)		
Ac C=O					169.5			170.2			170.1			170.2	
Ac CH3				2.03, s	21.1	Ac C=O	1.89, s	20.4	Ac C=O	1.89, s	20.4	Ac C=O	1.89, s	20.4	
sugar-3															
Glc-III-1	4.51, d (7.5)	104.2	Glc-II-2	4.35, d (7.5)	103.2	Glc-II-2	4.52, d (7.5)	104.2	Glc-II-2	4.54, d (8.0)	104.1	Glc-II-2	4.52, d (8.0)	104.2	Glc-II-2
Glc-III-2	3.03, dd (8.5, 7.5)	74.6	Glc-III-1	2.92, dd (8.5, 7.5)	73.4		3.04, dd (8.5, 7.5)	74.6	Glc-III-1	3.05, dd (9.0, 8.0)	74.6	Glc-III-1	3.05, dd (9.0, 8.0)	74.6	
Glc-III-3	3.16^a	76.2		3.13, dd (9.0, 8.5)	76.5		3.21, dd (9.0, 8.5)	76.0		3.22, t (9.0)	76.0		3.20^a	76.0	
Glc-III-4	3.08, t (9.0)	69.7		3.04, t (9.0)	69.8		3.09, t (9.0)	69.7		3.09, t (9.0)	69.6		3.09, t (9.0)	69.6	
Glc-III-5	3.20, m	77.4		3.20, m	77.2		3.21, m	77.4		3.22, m	77.4		3.19^a	77.4	
Glc-III-6	3.50^a	60.9		3.48, dd (11.5, 5.0)	61.0		3.50, dd (11.5, 5.5)	60.9		3.52, dd (12.0, 6.0)	60.8		3.52, dd (12.0, 6.0)	60.8	
	3.73, brd (11)			3.74, dd (11.5, 2.0)			3.75, br d (11.5)			3.77, dd (12.0, 2.0)			3.76, dd (12.0, 2.0)		
sugar-4															
Rha-1	4.54, brs	100.5	Glc-I-6, Rha-5	4.56, br s	100.5	Glc-I-6, Rha-5	4.55, brs	100.1	Glc-I-6, Rha-5	4.55, brs	100.5	Glc-I-6, Rha-5	4.55, d (1.0)	100.5	Glc-I-6, Rha-5
Rha-2	3.66, dd (4.0, 1.5)	70.4		3.68, dd (3.5, 1.0)	70.3		3.67, dd (3.5, 1.5)	70.3		3.67, br d (3.5)	70.3		3.67, dd (3.0, 1.0)	70.3	
Rha-3	3.44, m	70.8		3.45, m	70.7		3.45, m	70.8		3.47, m	70.7		3.45^a	70.7	
Rha-4	3.15, t (9.5)	72.1		3.16, t (9.5)	72.0		3.18^a	72.1		3.17, t (9.0)	72.1		3.17, t (9.0)	72.0	
Rha-5	3.41, dd (9.5, 6.5)	68.4		3.49, dd (9.5, 6.5)	68.3		3.42, dd (9.0, 6.0)	68.3		3.42, dd (9.0, 6.5)	68.3		3.41, dd (9.5, 6.5)	68.3	
Rha-6	1.06, d (6.5)	17.8		1.08, d (6.5)	17.8	Rha-4, Rha-5	1.07, d (6.0)	17.8	Rha-4, Rha-5	1.07, d (6.5)	17.7	Rha-4, Rha-5	1.07, d (6.5)	17.7	Rha-4, Rha-5

^{a} Unclear signal pattern due to overlapping signals.

Table 2-2. ^{1}H and ^{13}C NMR Spectroscopic Data (in methanol-d_4) of Compounds 7-9

position	7 δ_H (J in Hz)	δ_C	HMBC (H to C)	8 δ_H (J in Hz)	δ_C	HMBC (H to C)	9 δ_H (J in Hz)	δ_C	HMBC (H to C)
aglycone									
1		138.9			139.0			138.8	
2	7.41, d (7.0)	129.3	4	7.39, br d (8.0)	129.4	4, 6, 7	7.42, d (8.5)	129.2	4, 7
3	7.29, m	129.3	1, 4	7.28, m	129.3	1, 5	7.30, br t (8.5)	129.3	1
4	7.23, m	128.7	2, 3	7.25, m	128.7	2	7.23, m	128.7	2, 3
5	7.29, m	129.3	1, 4	7.28, m	129.3	1, 3	7.30, br t (8.5)	129.3	1
6	7.41, d (7.0)	129.3	4	7.39, br d (8.0)	129.4	2, 4, 7	7.42, d (8.5)	129.2	4, 7
7	4.64, d (11.5)	71.8	1	4.63, d (12.0)	71.8	Glc-1	4.65, d (12.0)	71.9	1, Glc-1
	4.87, d (11.5)			4.84, d (12.0)			4.90, d (12.0)		
p-coumaroyl									
1'		127.2			127.6			127.1	
2'	7.43, d (8.5)	131.3	4', 6', 7'	7.66, d (8.5)	134.0	4', 7'	7.39, d (8.5)	131.2	3', 6', 7'
3'	6.80, d (8.5)	116.8	1', 4', 5'	6.71, d (8.5)	115.9	1', 5'	6.81, d (8.5)	116.8	
4'		161.3			160.2			161.1	
5'	6.80, d (8.5)	116.8	1', 3', 4'	6.71, d (8.5)	115.9	1', 3'	6.81, d (8.5)	116.8	
6'	7.43, d (8.5)	131.3	2', 4', 7'	7.66, d (8.5)	134.0	4', 7'	7.39, d (8.5)	131.2	
7'	7.59, d (16.0)	147.1	2', 6', 8', 9'	6.88, d (13.0)	145.9	9'	7.63, d (16.0)	146.8	1', 2', 8', 9'
8'	6.36, d (16.0)	114.9	1', 9'	5.82, d (13.0)	116.3	1'	6.31, d (16.0)	115.1	1', 9'
9'		168.6			167.6			168.9	
sugar									
Glc-1	4.36, d (8.0)	103.3	7	4.33, d (8.0)	103.3	7	4.39, d (8.0)	103.3	7, Glc-5
Glc-2	3.26, dd (9.0, 8.0)	75.1		3.24^a	75.2		3.31^a	75.0	
Glc-3	3.36, t, (9.0)	78.0		3.34, t, (9.0)	78.1		3.39^a	78.0	
Glc-4	3.29^a	71.8		3.28^a	71.9		3.36^a	71.6	
Glc-5	3.43, m	77.4		3.42, m	77.4		3.44, m	76.6	Glc-4
Glc-6	3.72, dd (11.5, 6.5)	67.8	Rha-1	3.70, dd (12.0, 6.0)	68.0	Rha-1	3.73, dd (11.5, 6.0)	68.2	Rha-1
	4.00, dd (11.5, 2.0)			4.00, dd (12.0, 2.0)			4.03, dd (11.5, 2.0)		
Rha-1	4.95, d (1.5)	99.4	Glc-6,Rha-2,Rha-5	4.91, d (1.5)	99.6	Glc-6	4.87, br s	102.1	Glc-6,Rha-2,Rha-5
Rha-2	5.20, dd (3.5, 1.5)	74.0	9'	5.17, dd (3.5, 1.5)	73.9	9'	3.97^a	72.3	
Rha-3	3.94, dd (9.5, 3.5)	70.7		3.91, dd (9.5, 3.5)	70.7		3.98^a	70.4	
Rha-4	3.48, t (9.5, 9.5)	74.4		3.36, t (9.5)	74.3		5.08, t (9.0)	75.5	
Rha-5	3.77, dd (9.5, 6.5)	69.9		3.73, dd (9.5, 6.0)	70.0		3.97^a	67.7	Rha-4
Rha-6	1.32, d (6.5)	18.1	Rha-4,Rha-5	1.28, d (6.5)	18.1		1.19, d (6.5)	18.0	Rha-4,Rha-5

^{a} Unclear signal pattern due to overlapping signals.

Chapter 3. Phytochemical constituents of *Dracocephalum foetidum* Bunge.

3.1 Introduction

Dracocephalum foetidum Bunge. (fam. Lamiaceae, Mongolian name: Umkhii shimeldeg), is widely distributed in Mongolia around Khubsugul, Khentei, Khangai, Mongol-Daurian, Khobdo, Mongolian Altai, Middle Khalkha, Eastern Mongolia, Depression of Great Lakes, Valley of Lakes, Eastern Gobi, and Gobi-Altai. It is typical habitat includes river banks, pebbles, bottoms of cornices of dry riverbeds, and small river valleys, foot rocks, screes, sandy steppes, rubbly, and stony steppe slopes (Grubov, 2001; Ligaa, 2005). It has been used for treatment of various inflammatory conditions, such as oral cavity diseases, rheumatic edema, and wounds. In addition, leaves and flowers of *D. foetidum* are used as a traditional medicine among Mongolian nomads to wash their faces and hands to prevent bacterial and fungal infections (Shatar and Altantsetseg, 2000). Flowers of the plant are also used to treat fever and suppurative diseases (Batkhuu et al., 2005). The herb contains flavonoids, triterpenoids and essential oils (Ligaa, 2005). There have been few studies on *D. foetidum*, and the only report on the chemical constituents of *D. foetidum* focused on the essential oil components that are effective against bacteria (Lee et al., 2007).

3.2 Results and Discussion

Aerial parts of *D. foetidum*, including flowers, were extracted with acetone-H_2O (8:2), and the extract was partitioned between H_2O and diethyl ether. The H_2O fraction was then subjected to various chromatographic purification procedures to obtain four limonene glycosides (**30-33**), eight rosmarinic acid derivatives (**34-41**), and 14 flavones (**42-55**). (Fig. 3-1).

3.2.1 Isolation of known compounds

The present study identified the following known compounds by comparison of their spectroscopic data with those reported in the literature limonene-10-ol 10-O-β-D-glucopyranoside (**33**) (Saeidnia et al., 2004), rosmarinic acid (**35**) (Dapkevicius et al., 2002), 3′-O-methyl-rosmarinic acid (**36**) (Murata et al., 2012), rosmarinic acid-3-O-β-D-glucopyranoside (**40**) (Tezuka et al., 1998), acacetin-7-O-β-D--glucopyranoside (**47**) (Li et al., 2008), acacetin-7-O-(6″-malonyl)-β-D--glucopyranoside (**48**) (Sugawara and Igarashi, 2009), acacetin-7-O-α-L-rhamno-pyranosyl-(1-6)-β-D-glucopyranoside (**49**) (Piao et al., 2003), acacetin-7-O-β-D-glucuronopyranoside (**50**) (Kartnig et al., 1993; Lee et al., 2002), apigenin-7-O-(6″-malonyl)-β-D-glucopyranoside (**51**) (Svehlikova et al., 2004), apigenin-7-O-β-D-glucuronopyranoside (**52**) (Flamini et al., 2001; Vanhoenacker et al., 2002), luteolin-7-O-β-D-glucuronopyranoside (**53**) (Vanhoenacker et al., 2002), diosmetin-7-O-β-D-glucuronopyranoside (**54**) (Murata et al., 2010a), and apigenin (**55**) (Ha et al., 2012), respectively. Limonene glycosides, a number of flavones and their glycosides, and rosmarinic acid have already been isolated from various species of *Dracocephalum* (Zeng et al., 2010).

3.2.2 Isolation and structure elucidation of new compounds

3.2.2.1 Monoterpene glycosides

Limonene glycosides **30-32** were isolated as colorless gums; ^{1}H- and ^{13}C-NMR spectroscopic data (measured in CD$_3$OD at 30 °C) are shown in Table. 3-1. The NMR signals of **30-32** were similar to compound **33** and limonene-10-ol 10-O-β-D-glucopyranosyl- (1→2)-β-D-glucopyranoside (Saeidnia et al., 2004).

Compound **30** was deduced to have the molecular formula C$_{28}$H$_{40}$O$_{17}$ based on

HRFABMS (m/z 671.2162, calcd for $C_{28}H_{40}O_{17}Na$, 671.2162). Its molecular formula had an additional $C_6H_4O_6$ compared to limonene-10-ol 10-O-β-D-glucopyranosyl -(1→2)-β-D-glucopyranoside. It was considered that this $C_6H_4O_6$ corresponded to two malonyl moieties, and carbonyl carbons ($δ_C$ 168.7, 170.1) of the malonyl moieties were observed in the ^{13}C-NMR spectrum. The proton ($δ_H$ 3.39, br s) and carbon ($δ_C$ 41.5) signals of the malonyl methylene were confirmed by analysis of the HMQC and HMBC spectra. After hydrolysis of **30**, sugar analysis showed the presence of a D-glucopyranosyl moiety (Tanaka et al., 2007). Two anomeric protons and their coupling constants suggested that the two sugars were β-D-glucopyranosyl (Saeidnia et al., 2004). The oxymethylene proton and carbon signals of the glucopyranosyl moiety ($δ_H$ 4.27, 1H, dd, J = 12.0, 6.0 Hz, H-6′; 4.47, 1H, dd, J = 12.0, 2.0 Hz, H-6′; 4.23, 1H, dd, J = 11.5, 5.5 Hz, H-6″; 4.43, 1H, dd, J = 11.5, 2.0 Hz, H-6″) were shifted to a lower field than those of limonene-10-ol 10-O-β-D-glucopyranosyl-(1→2)-β-D-gluco-pyranoside (Saeidnia et al., 2004). In the HMBC spectrum (Fig. 3-2), the H-6′ and H-6″ protons were long-range coupled with the carbonyl carbon of the malonyl moieties ($δ_C$ 168.7). These data and the other HMBC correlations established that **30** was limonene-10-ol 10-O-6-malonyl-β-D-glucopyranosyl-(1→2)-6-malonyl-β-D-gluco-pyranoside as shown in Fig. 3-1.

30 R$_1$ = 6-malonyl-β-D-glucopyranosyl, R$_2$ = malonyl
31 R$_1$ = β-D-glucopyranosyl, R$_2$ = malonyl
32 R$_1$ = 6-malonyl-β-D-glucopyranosyl, R$_2$ = H
33 R$_1$ = H, R$_2$ = H

35 R$_1$ = H
36 R$_1$ = Me
37 R$_1$ = 6-malonyl-β-D-glucopyranosyl
38 R$_1$ = 6-feruloyl-β-D-glucopyranosyl
39 R$_1$ = 6-synapoyl-β-D-glucopyranosyl
40 R$_1$ = β-D-glucopyranosyl

42 R$_1$ = H, R$_2$ = malonyl, R$_3$ = H, R$_4$ = H$_2$
43 R$_1$ = H, R$_2$ = malonyl, R$_3$ = malonyl, R$_4$ = H$_2$
44 R$_1$ = acetyl, R$_2$ = H, R$_3$ = H, R$_4$ = O
45 R$_1$ = malonyl, R$_2$ = H, R$_3$ = H, R$_4$ = O
46 R$_1$ = H, R$_2$ = malonyl, R$_3$ = H, R$_4$ = O
47 R$_1$ = H, R$_2$ = H, R$_3$ = H, R$_4$ = H$_2$
48 R$_1$ = H, R$_2$ = H, R$_3$ = malonyl, R$_4$ = H$_2$
49 R$_1$ = H, R$_2$ = H, R$_3$ = α-L-rhamnopyranosyl, R$_4$ = H$_2$
50 R$_1$ = H, R$_2$ = H, R$_3$ = H, R$_4$ = O

feruloyl:

synapoyl:

malonyl:

51 R$_1$ = H, R$_2$ = H, R$_3$ = 6-malonyl-β-D-glucopyranosyl
52 R$_1$ = H, R$_2$ = H, R$_3$ = β-D-glucuronopyranosyl
53 R$_1$ = OH, R$_2$ = H, R$_3$ = β-D-glucuronopyranosyl
54 R$_1$ = OH, R$_2$ = Me, R$_3$ = β-D-glucuronopyranosyl
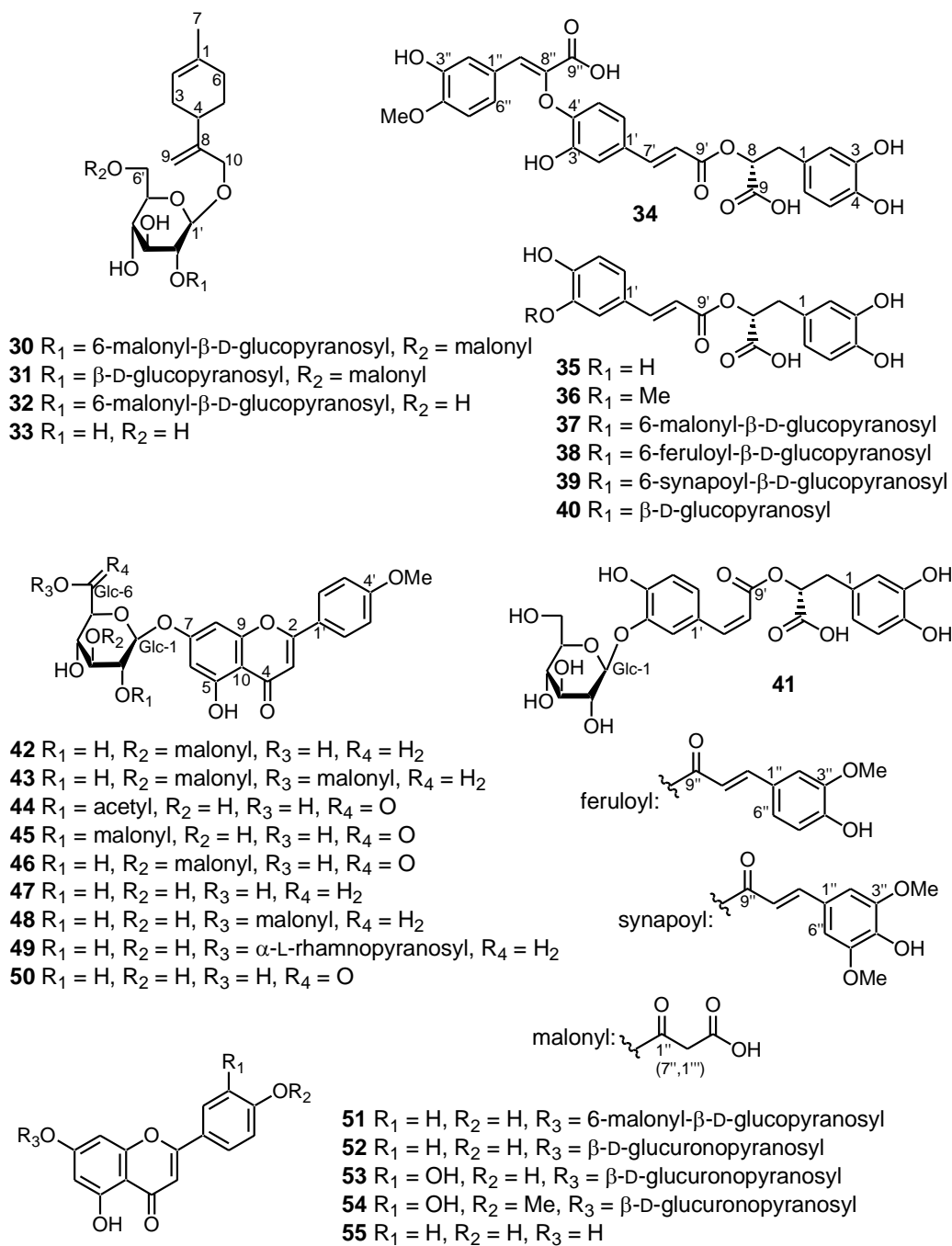**55** R$_1$ = H, R$_2$ = H, R$_3$ = H

Fig. 3-1 Compounds from *D. foetidum* (**30-55**)

25

Compound **31** had an molecular formula of $C_{25}H_{38}O_{14}$, which included the absence of a

$C_3H_2O_3$ group as compared to **30** [HRFABMS, **31**: *m/z* 585.2156, calcd for

$C_{25}H_{38}O_{14}Na$, 585.2159]. This showed that **31** has only one malonyl moiety. The proton

signals of oxymethylene in the glucose of **31** (δ_H 3.63, 1H, dd, *J* = 12.0, 6.0 Hz, H-6″;

3.81, 1H, dd, *J* = 12.0, 2.0 Hz, H-6″) were shifted to a higher field than those of **30**. In

the differential HOHAHA spectra of **31**, the anomeric proton of the second glucose

moiety (δ_H 4.63, 1H, d, *J* = 7.5 Hz, H-1″) was correlated with the oxymethylene protons

(H-6″). On the other hand, H-6′ protons (δ_H 4.28, 1H, dd, *J* = 12.0, 6.0 Hz; 4.48, 1H, dd,

J = 12.0, 2.0 Hz) were long-range coupled with the malonyl carbonyl carbon (δ_C 168.6,

C-7′) in the HMBC spectrum. These data suggested that **31** has a malonyl moiety at the

C-6 position of the first glucose moiety. Thus **31** was identified as limonene-10-ol

10-*O*-β-D-glucopyranosyl-(1→2)-6-malonyl-β-D-glucopyranoside   (Fig.   3-1).   The

molecular formula of compound **32** was $C_{25}H_{38}O_{14}$, which was the same as **31** and thus

also suggested the absence of a $C_3H_2O_3$ as compared to **30** [HRFABMS, **32:** *m/z*

585.2167, calcd for $C_{25}H_{38}O_{14}Na$, 585.2159], and thus **32** also had only one malonyl

moiety. The proton signals of oxymethylene in the glucose of **32** (δ_H 3.67, 1H, dd, *J* =

12.0, 5.5 Hz, H-6′; 3.86, 1H, dd, *J* = 12.0, 2.0 Hz, H-6′) were shifted to a higher field

than those of **30**. In the differential HOHAHA spectra of **32**, the anomeric proton of the

first glucose moiety (δ_H 4.43, 1H, d, *J* = 7.0 Hz, H-1′) was correlated with the

oxymethylene protons (H-6′). In the HMBC spectrum, the H-6″ protons (δ_H 4.23, 1H, dd,

J = 12.0, 5.5 Hz; 4.41, 1H, dd, *J* = 12.0 Hz) were long-range coupled with the malonyl

carbon (δ_C 168.7, C-7″). These data suggested that **32** has a malonyl moiety at the C-6

position of the second glucose moiety, not at the first glucose moiety. Thus, **32** was

identified   as   limonene-10-ol   10-*O*-6-malonyl-β-D-glucopyranosyl-(1→2)-β-D-gluco-

pyranoside, as shown in Fig. 3-1.

The absolute configuration at C-4 of **30-32** was not determined. Compound **33** was isolated from *D. kotschyi* (Saeidnia et al., 2004), and *d*-limonene derivatives were also determined. The expected aglycones of **30-33** from *D. foetidum* are likely to be *d*-limonene derivatives.
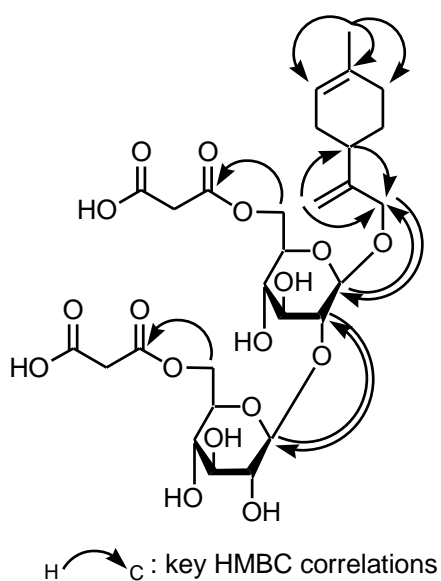

H ⤻ C : key HMBC correlations

Fig. 3-2 Compound **30**

3.2.2.2 Rosmarinic acid derivatives

The molecular formula of **34** ($C_{28}H_{25}O_{12}$, *m/z* 553.1358 $[M+H]^+$, calcd for $C_{28}H_{25}O_{12}$, 553.1346) was established using HRFABMS. The ^1H- and ^{13}C-NMR spectroscopic data (measured in acetone-d_6 at 30 °C) are shown in Table. 3-2. Three sets of coupling system protons (δ_H 6.85, 1H, d, *J* = 2.0 Hz, H-2, 6.75, 1H, d, *J* = 8.0 Hz, H-5, 6.67, 1H, dd, *J* = 8.0, 2.0 Hz, H-6; 7.29, 1H, d, *J* = 2.0 Hz, H-2′, 6.82, 1H, d, *J* = 8.0 Hz, H-5′, 7.07, 1H, dd, *J* = 8.0, 2.0 Hz, H-6′; 7.38, 1H, d, *J* = 2.0 Hz, H-2″, 6.97, 1H, d, *J* = 8.0 Hz, H-5″, 7.26, 1H, dd, *J* = 8.0, 2.0 Hz, H-6″) and 27 carbon signals, except for the presence of a methoxy signal at δ_C 56.2, suggested that **34** was a

phenylpropanoid trimer. These signals were very similar to those of melitric acid A (Agata et al., 1993). The methoxy proton signal at δ_H 3.87 (3H, s) was long-range coupled with the carbon signal at δ_C 150.2 (C-4″) in the HMBC spectrum and was correlated with the H-5″ in the differential NOE spectrum (Fig. 3-3). From these data, **34** was identified as (αR)-α-[[(2E)-3-[4-[[(1Z)-1-carboxy-2-(3-hydroxy-4-methoxy phenyl)ethenyl]oxy]-3-hydroxyphenyl]-1-oxo-2-propen-1-yl]oxy]-3,4-dihydroxybenzen epropanoic acid, which is the 4″-methoxy derivative of melitric acid A, as shown in Fig. 3-1.
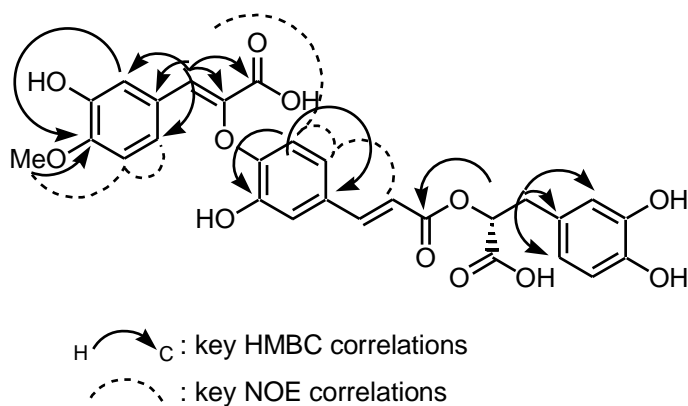

H ⌒▸ C : key HMBC correlations

⌒ ⌒ : key NOE correlations

Fig. 3-3 Compound **34**

The ^{1}H- and ^{13}C-NMR spectroscopic data (measured in CD$_3$OD at 30 °C) of compounds **37-39** are shown in Table. 3-2. The signals corresponding to rosmarinic acid and glucopyranosyl moieties were observed in the NMR spectra, which were similar to those of **40**. In addition, the signals of the acyl moieties of **37-39** were observed in each respective spectrum. Their Glc-6 protons and carbons were downfield shifted relative to that of **40**, suggesting an acyl moiety bond to C-6 of each glucopyranosyl of **37-39**.

For **37**, typical malonyl carbonyl carbons (δ_C 168.9 and 170.3), such as **30-32**, were observed in the ^{13}C-NMR spectrum, which suggested that the acyl moiety of **37** was malonyl. The molecular formula C$_{27}$H$_{28}$O$_{16}$ established using HRFABMS (*m/z* 609.1428

[M+H]$^+$, calcd for $C_{27}H_{29}O_{16}$, 609.1455), which, when compared with **40**, corresponded to an additional malonyl unit, $C_3H_2O_3$, and supported this conclusion. The sugar analysis using HPLC (Tanaka et al., 2007) and the coupling constant of the anomeric proton (δ_H 4.85, d, J = 7.5 Hz) suggested that the glucosyl moiety was β-D-glucopyranosyl. The Glc-6 protons (δ_H 4.32, dd, J = 12.0, 7.0 Hz; 4.56, dd, J = 12.0, 1.5 Hz) and carbon (δ_C 65.6) of **37** were downfield shifted relative to those of **40**. These shifts suggested that the malonyl group connected to C-6 of the β-D-glucopyranosyl unit. Consequently, compound **37** was identified as 3-(6-malonyl-β-D-glucopyranosyl) -rosmarinic acid.

The molecular formula $C_{34}H_{34}O_{16}$ for **38** was determined by HRFABMS (*m/z* 721.1727, calcd for $C_{34}H_{34}O_{16}Na$, 721.1743). In the ^{1}H-NMR spectrum, the ABX system proton signals (δ_H 7.00, 1H, d, J = 2.0 Hz, H-2″, 6.76, 1H, d, J = 8.0 Hz, H-5″, 6.90, 1H, dd, J = 8.0, 2.0 Hz, H-6″) and *trans* olefinic proton signals (δ_H 7.53, 1H, d, J = 16.0 Hz, H-7″, 6.27, 1H, d, J = 16.0 Hz, H-8″) were observed. The singlet methoxy proton (δ_H 3.83, 3H) was correlated with the H-2″ in the NOE spectrum. These data suggested that **38** posseses a feruloyl moiety instead of a malonyl moiety for **37**. Hence, **38** was identified as 3-(6-feruloyl-β-D-glucopyranosyl)-rosmarinic acid.

The molecular formula $C_{35}H_{36}O_{17}$ for **39** was determined by HRFABMS (*m/z* 751.1843, calcd for $C_{35}H_{36}O_{17}Na$, 751.1849). In the NOE spectrum, the H-2″ and 6″ singlet proton signals (δ_H 6.73, 2H) correlated with the methoxy proton signals (δ_H 3.85, 6H) and the *trans* olefinic proton signals (δ_H 7.53, 1H, d, J = 16.0 Hz, H-7″, 6.30, 1H, d, J = 16.0 Hz, H-8″). These data suggested that **39** possesses a synapoyl moiety, instead of the malonyl moiety of **37**. Hence, **39** was identified as 3-(6-synapoyl-β-D-glucopyranosyl)-rosmarinic acid.

The molecular formula of **41** (HRFABMS, $C_{24}H_{27}O_{13}$, m/z 523.1459 [M+H]$^+$, calcd for $C_{24}H_{27}O_{13}$, 523.1451) was the same as that of **40**. The ^{1}H- and ^{13}C-NMR signals of **41** were similar to **40**, except for a caffeoyl moiety on the rosmarinic acid moiety (Table. 3-2). The coupling constant of the olefinic protons (δ_H 6.88, 1H, d, J = 13.0 Hz, H-7′, 5.82, 1H, d, J = 13.0 Hz, H-8′) suggested that **41** has a *cis*-oriented caffeoyl moiety (Wei et al., 2004). The NOE correlation between H-7′ and H-8′ supported this conclusion. Therefore, **41** was identified as the *cis*-isomer of 3-*O*-β-D-glucopyranosyl- rosmarinic acid, as shown in Fig. 3-1.

The absolute stereochemistries of the C-8′ of **37-39** and **41** were determined to be *R* from the retention time of the amide derivative of the (*S*)-2-phenylglycine methyl ester and 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid after acidic hydrolysis of the compounds (Murata et al., 2010b).

3.2.2.3 Acacetin acyl glycosides

Compounds **42-46** were obtained as colorless powders with UV (measured in MeOH) and NMR (measured in DMSO-d_6 at 30 °C, Table. 3-3) data. Their UV spectra were very similar to that of acacetin (Greenham et al., 2003). A carbonyl, a methoxy, and 14 phenolic or olefinic carbons were observed in the ^{13}C-NMR spectrum of each compound, which suggested the presence of acacetin as the aglycone of **42-46** (Selenge et al., 2013). The fragment ion peak (m/z 285, $[C_{16}H_{12}O_5+H]^+$) in the FABMS spectra of **42-46** supported this conclusion.

The ^{1}H- and ^{13}C-NMR signals of **42** and **43** showed that they had glycosyl and malonyl moieties. Sugar analysis of **42** and **43** using HPLC suggested the presence of D-glucopyranosyl moieties. The coupling constants of the anomeric proton signals (**42**: J = 8.0 Hz; **43**: J = 7.5 Hz) showed the β-configuration for the D-glucopyranosyl

component.

The molecular formula of **42** was determined as $C_{25}H_{24}O_{13}$ on the basis of HRFABMS (*m/z* 533.1290, calcd for $C_{25}H_{25}O_{13}$, 533.1295), which, when compared with **47**, corresponded to an additional malonyl unit of the formula $C_3H_2O_3$. The methylene proton and carbon signals (δ_H 3.41, 2H, s; δ_C 41.6) and two carbonyl carbon signals (δ_C 166.4, 167.8) indicated a malonyl moiety. The Glc-3 proton and carbon signals (δ_H 4.96, 1H, t, *J* = 9.0 Hz; δ_C 78.4) were downfield shifted relative to those of **47**. In addition, in the HMBC spectrum of **42**, the H-Glc-3 proton was long-range coupled with the malonyl carbonyl carbon (δ_C 166.4). From these data, **42** was determined as acacetin-7-*O*-(3-*O*-malonyl)-β-D-glucopyranoside.

The molecular formula of **43** was determined as $C_{28}H_{26}O_{16}$ on the basis of HRFABMS (*m/z* 619.1284 calcd for $C_{28}H_{27}O_{16}$, 619.1298), which contained an additional malonyl group, $C_3H_2O_3$, as compared to **42**. The Glc-6 proton and carbon signals (δ_H 4.20, 1H, dd, *J* = 12.0, 6.5 Hz; 4.38, 1H, dd, *J* = 12.0, 2.0 Hz; δ_C 63.5) were downfield shifted relative to those of **42**. The H-Glc-6 proton was long-range coupled with the malonyl carbonyl carbon (δ_C 167.7) in the HMBC spectrum of **43**. From these data, **43** was determined as acacetin-7-*O*-(3,6-*O*-dimalonyl)-β-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR signals of **44** were similar to those of **50**, except for presence of signals of an acetyl moiety (δ_H 2.04, 3H, s; δ_C 20.7, 169.1). The sugar moiety of **44** was determined as β-D-glucuronic acid on the basis of sugar analysis using HPLC and the coupling constant of the anomeric proton signal (δ_H 5.58, 1H, d, *J* = 8.0 Hz, H-Glc A-1). The anomeric proton coupled with δ_H 4.84 (1H, dd, *J* = 9.0, 8.0 Hz, H-Glc A-2). The H-Glc A-2 was long-range coupled with the acetyl carbonyl carbon. Thus, **44** was identified as acacetin-7-*O*-(2-*O*-acetyl)-β-D-glucuronopyranoside.

The ^{1}H- and ^{13}C-NMR signals of **45** were similar to those of **44**. The malonyl proton and carbon signals (δ_H 3.38, 2H, s; δ_C 165.9, 167.5) were observed in the NMR spectra of **45** instead of the acetyl signals of **44**. These data showed that **45** was acacetin-7-*O*-(2-*O*-malonyl)-β-D-glucuronopyranoside.

The ^{1}H- and ^{13}C-NMR signals of **46** were similar to those of **45**. The molecular formula of **46** (HRFABMS *m/z* 547.1093, calcd for $C_{25}H_{23}O_{14}$, 547.1087) was the same as that of **45**. The assignments of proton signals of the glycosyl unit were determined by ^{1}H-^{1}H COSY correlations. The H-Glc A-3 proton signals (δ_H 5.00, 1H, t, J = 9.5 Hz) were long-range coupled with the malonyl carbonyl carbon (δ_C 166.4). Thus, **46** was identified as acacetin-7-*O*-(3-*O*-malonyl)-β-D-glucuronopyranoside.

Table 3-1. ^{1}H and ^{13}C NMR Spectroscopic Data (in methanol-d_4) of Compounds 30-32

position	30 δ$_H$ (*J* in Hz)	30 δ$_C$	30 HMBC (H to C)	31 δ$_H$ (*J* in Hz)	31 δ$_C$	31 HMBC (H to C)	32 δ$_H$ (*J* in Hz)	32 δ$_C$	32 HMBC (H to C)
1		134.7			134.7			134.7	
2	5.40, br s	122.0		5.40, m	121.8	6, 7	5.40, br s	121.9	3, 4, 6, 7
3	1.80 - 2.00^a / 2.19, m	32.3		1.84 - 1.95^a / 2.19, m	32.2		1.91, m / 2.18, m	32.3	
4	2.36, m	37.6		2.33, m	37.5		2.30, m	37.7	8
5	1.49^a / 1.85^a	29.2		1.50, m / 1.85^a	29.1		1.49, m / 1.85, m	29.2	
6	1.80 - 2.00^a / 2.06, m	31.6		1.84 - 1.95^a / 2.08, m	31.6		1.97, m / 2.06, m	31.6	
7	1.64, s	23.7	1, 2, 6	1.64, s	23.7	1, 2, 6	1.64, s	23.7	1, 2, 6
8		151.7			151.5			151.8	
9	4.91, br s / 5.12, br s	110.9	4, 8, 10	4.93, br s / 5.13, br s	111.4	4, 8, 10	4.91, br s / 5.13, m	110.5	4, 8, 10
10	4.11, br d (12.5) / 4.34, br d (12.5)	72.2	4, 8, 9, 1'	4.14, br d (12.5) / 4.36, br d (12.5)	72.6	4, 8, 9, 1'	4.12, br d (12.5) / 4.42, br d (12.5)	72.0	4, 8, 9, 1'
1'	4.42, d (7.0)	101.7	10	4.43, d (7.5)	101.8		4.43, d (7.0)	101.8	10
2'	3.45^a	83.3	1"	3.56, dd (9.0, 7.5)	82.0	1', 1"	3.45, dd (9.0, 7.0)	83.4	1', 1"
3'	3.50, t (9.0)	78.0	2', 4'	3.37, dd (9.5, 9.0)	78.1		3.55, t (9.0)	78.1	2'
4'	3.33^a	71.5		3.49, t (9.5)	71.4		3.36, dd (9.5, 9.0)	71.5	
5'	3.45^a	75.1		3.47, m	75.2		3.24, m	75.9	
6'	4.27, dd (12.0, 6.0) / 4.47, dd (12.0, 2.0)	65.5	7'	4.28, dd (12.0, 6.0) / 4.48, dd (12.0, 2.0)	65.4	7'	3.67, dd (12.0, 5.5) / 3.86, dd (12.0, 2.0)	62.7	
7'		168.7			168.6				
8'	3.39, brs	41.5			missing				
9'		170.1			170.1				
1"	4.59, d (7.5)	105.3	2'	4.63, d (7.5)	104.8	2'	4.60, d (8.0)	105.3	2'
2"	3.23, dd (9.0, 7.5)	75.9	1", 4"	3.20, dd (9.0, 7.5)	75.9	1"	3.24^a	75.9	
3"	3.36, t (9.0)	77.7		3.37, dd (9.5, 9.0)	77.9		3.37, dd (9.5, 9.0)	77.7	
4"	3.35, dd (9.5, 9.0)	71.4		3.25^a	71.8		3.31, dd (9.5, 9.0)	71.4	
5"	3.45^a	75.4		3.25^a	75.9		3.44, m	75.4	
6"	4.23, dd (11.5, 5.5) / 4.43, dd (11.5, 2.0)	65.6	7"	3.63, dd (12.0, 6.0)	63.0		4.23, dd (12.0, 5.5) / 4.41, br d (12.0)	65.6	7"
7"		168.7						168.7	
8"	3.39, brs	41.5					3.40, s	42.0	7", 9"
9"		170.1						170.1	

^{a} Unclear signal pattern due to overlapping signals.

Table 3-2. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 34, 37-39, and 41

position	34[b] δH (J in Hz)	δC	HMBC (H to C)	NOE (H to H)	37[c] δH (J in Hz)	δC	HMBC (H to C)	NOE (H to H)	38[c] δH (J in Hz)	δC	HMBC (H to C)	NOE (H to H)	39[c] δH (J in Hz)	δC	HMBC (H to C)	NOE (H to H)	41[c] δH (J in Hz)	δC	HMBC (H to C)	NOE (H to H)
1		129.1				129.4				129.2				129.2				129.4		
2	6.85, d (2.0)	117.3	4		6.75, d (2.0)	117.7	4, 6		6.72, d (2.0)	117.6	4, 6, 7		6.71, d (2.0)	117.8	4		6.70, d (2.0)	117.6	4	
3		145.6				146.2				146.2				146.2				146.1		
4		144.8				145.3				145.3				145.4				145.3		
5	6.75, d (8.0)	116.0	1, 3	6, 7"	6.69, d (8.0)	116.4	1, 3		6.68, d (8.0)	116.4	1, 3, 4		6.68, d (8.0)	116.4	3		6.67, d (8.5)	116.2	1, 3	
6	6.67, dd (8.0, 2.0)	121.7	4	5, 7, 8	6.62, dd (8.0, 2.0)	121.9			6.58, dd (8.0, 2.0)	122.0	2, 4, 7		6.58, dd (8.0, 2.0)	122.1	1, 4		6.54, dd (8.5, 2.0)	121.9	4	
7	3.04, dd (14.0, 8.5); 3.13, dd (14.0, 4.0)	37.5	1, 2, 6, 8, 9		3.01, dd (14.5, 8.5); 3.09, dd (14.5, 3.0)	38.0	8		2.94, dd (14.0, 8.0); 3.00, dd (14.0, 4.5)	37.9	1		2.93, dd (14.5, 8.0); 2.99, dd (14.5, 8.0)	37.9			2.93, dd (14.5, 8.5); 3.06, dd (14.5, 4.5)	37.9	1	
8	5.23, dd (8.5, 4.0)	73.8	1, 9, 9'		5.19, dd (8.5, 3.0)	74.9			5.05, m	74.8	1		5.03, dd (8.0, 4.5)	74.8			5.12, dd (8.5, 4.0)	74.8	1	
9		171.0				173.7				173.4				173.3				173.6		
1'		130.7				127.9				127.8				127.8				128.2		
2'	7.29, d (2.0)	116.5	4'		7.42, d (2.0)	118.4	4', 7'		7.40, d (2.0)	117.6	4', 6'		7.42, d (2.0)	117.6	1', 4'		8.02, d (2.0)	120.4	3', 4'	
3'		148.2				146.9				146.8				146.8				146.2		
4'		147.6				151.4				151.3				151.4				150.1		
5'	6.82, d (8.0)	115.6	1', 3', 4'		6.87, d (8.0)	117.6	1', 4'		6.85, d (8.0)	117.7	3'		6.85, d (8.0)	117.6	3'		6.79, d (8.5)	116.5	3', 4'	
6'	7.07, dd (8.0, 2.0)	121.8	4'		7.20, dd (8.0, 2.0)	126.3	4', 7'		7.12, dd (8.0, 2.0)	126.6	4'		7.11, d (8.0, 2.0)	126.7	4'		7.15, dd (8.5, 2.0)	129.0	4'	
7'	7.59, d (16.0)	145.9	1', 9'		7.61, d (16.0)	147.2	9'		7.53, d (16.0)	147.1	9'		7.52, d (16.0)	147.0	1', 9'		6.88, d (13.0)	146.5	1', 6', 9'	
8'	6.41, d (16.0)	116.8	1', 9'		6.35, d (16.0)	115.6	1', 9'		6.34, d (16.0)	115.6	1'		6.34, d (16.0)	115.5	1'		5.82, d (13.0)	116.4	1'	
9'		166.6				168.3				168.3				168.2				167.3		
Glc-1					4.85, d (7.5)	104.0		2'	4.90, d (7.0)	103.4		2'	4.90, d (7.5)	103.6		2'	4.75[a]	103.7	3'	2'
Glc-2					3.52[a]	74.9			3.55[a]	74.8			3.55[a]	74.8			3.49[a]	74.9		
Glc-3					3.52[a]	77.4			3.55[a]	77.5			3.55[a]	77.6			3.49[a]	77.7		
Glc-4					3.40[a]	71.6			3.44, m	72.2			3.42, m	72.2			3.49a	71.1		
Glc-5					3.73, m	75.7			3.83[a]	75.7			3.83[a]	75.7			3.49a	78.0		
Glc-6					4.32, dd (12.0, 7.0); 4.56, dd (12.0, 1.5)	65.6			4.39, dd (11.5, 7.0); 4.62, dd (11.5, 2.0)	64.9	9"		4.37, dd (12.0, 7.0); 4.65, dd (12.0, 2.0)	64.9			3.76, dd (12.5, 4.5); 3.91, dd (12.5, 2.0)	62.2		
1"		126.5				168.9				127.7				126.7						
2"	7.38, d (2.0)	117.4	3", 4", 7"		missing				7.00, d (2.0)	112.2	6"		6.73, s	107.1	4", 6", 7"					
3"		147.4				170.3				149.3				149.4						
4"		150.2								150.6				139.7						
5"	6.97, d (8.0)	112.3	1", 3", 4"	6", 4"-OMe					6.76, d (8.0)	116.6	3"			149.4						
6"	7.26, dd (8.0, 2.0)	124.3	7"	3", 7"					6.90, dd (8.0, 2.0)	124.0	2", 4"		6.73, s	107.1	2", 4", 7"					
7"	7.38, s	128.5	8", 9"	5					7.53, d (16.0)	147.3	6"		7.53, d (16.0)	147.6	9"					
8"		139.1							6.27, d (16.0)	115.2	1", 9"		6.30, d (16.0)	115.7	1"					
9"		164.4								169.2				169.1						
3"-OCH$_3$									3.83, s	56.6	3"		3.85, s	57.0	3"					
4"-OCH$_3$	3.87, s	56.2	4"	5"																
5"-OCH$_3$													3.85, s	57.0	5"					

[a] Unclear signal pattern due to overlapping signals.

[b] in acetone-d_6

[c] in CD$_3$OD

Table 3-3. ^{1}H and ^{13}C NMR Spectroscopic Data (in DMSO-d_6) of Compounds 42-46

position	42 δ_H (J in Hz)	42 δ_C	42 HMBC (H to C)	43 δ_H (J in Hz)	43 δ_C	43 HMBC (H to C)	44 δ_H (J in Hz)	44 δ_C	44 HMBC (H to C)	45 δ_H (J in Hz)	45 δ_C	45 HMBC (H to C)	46 δ_H (J in Hz)	46 δ_C	46 HMBC (H to C)
2		163.8			163.9			163.9			163.9			163.9	
3	6.96, s	103.8	2, 10, 1'	6.96, s	103.8	2, 4, 1'	6.96, s	103.8	2, 10, 1'	6.96, s	103.8	2, 10, 1'	6.95, s	103.8	2, 4, 1'
4		182.0			182.0			182.0			182.0			182.0	
5	12.93, s	161.1	6, 10		161.0		12.95, s	161.2	6, 10	12.93, s	161.1		12.95, s	161.2	6, 10
6	6.48, d (2.0)	99.5	5, 8	6.47, d (2.0)	99.6	5, 7, 8, 10	6.44, d (2.0)	99.1	7, 8, 10	6.43, d (2.0)	99.3	8, 10	6.51, d (2.0)	99.4	7, 10
7		162.6			162.4			161.7			161.9			162.2	
8	6.88, d (2.0)	94.9	6, 9, 10	6.86, d (2.0)	94.8	6, 7, 9, 10	6.83, d (2.0)	94.9	6, 7, 9, 10	6.80, d (2.0)	95.0	6, 7, 9, 10	6.90, d (2.0)	94.8	7, 9, 10
9		156.9			156.9			156.8			156.8			156.9	
10		105.4			105.6			105.7			105.7			105.6	
1'		122.6			122.6			122.5			122.6			122.6	
2'	8.07, d (9.0)	128.4	2, 4', 6'	8.07, d (8.5)	128.4	2, 3', 4', 6'	8.06, d (9.0)	128.4	2, 4', 6'	8.06, d (9.0)	128.4	2, 4', 6'	8.06, d (9.0)	128.4	2, 4', 6'
3'	7.14, d (9.0)	114.6	1', 4', 5'	7.13, d (8.5)	114.5	1', 5'	7.13, d (9.0)	114.6	1', 4', 5'	7.13, d (9.0)	114.6	1', 4', 5'	7.13, d (9.0)	114.6	1', 4', 5'
4'		162.4			162.4			162.4			162.4			162.5	
5'	7.14, d (9.0)	114.6	1', 3', 4'	7.13, d (8.5)	114.5	1', 3'	7.13, d (9.0)	114.6	1', 3', 4'	7.13, d (9.0)	114.6	1', 4', 3'	7.13, d (9.0)	114.6	1', 3', 4'
6'	8.07, d (9.0)	128.4	2, 2', 4'	8.07, d (8.5)	128.4	2, 2', 3', 4'	8.06, d (9.0)	128.4	2, 2', 4'	8.06, d (9.0)	128.4	2, 2', 4'	8.06, d (9.0)	128.4	2, 2', 4'
4'-OCH$_3$	3.87, s	55.5	4'	3.87, s	55.5	4'	3.87, s	55.5	4'	3.87, s	55.5	4'	3.87, s	55.6	4'
Glc or Glc A -1	5.27, d (8.0)	99.3	7	5.31, d (7.5)	99.1	7	5.58, d (8.0)	96.7	7	5.54, d (8.0)	96.7	7	5.48, d (8.0)	98.8	7
Glc or Glc A -2	3.45^a	70.9		3.50, dd (9.5, 7.5)	70.8	Glc-1, Glc-3	4.84, dd (9.0, 8.0)	72.9	Glc A-1, Glc A-3, 1''	4.85, dd (9.0, 8.0)	73.6	1''	3.53, dd (9.5, 8.0)	70.5	
Glc or Glc A -3	4.96, t (9.0)	78.4	Glc-2, 1''	4.98, t (9.5)	77.9	Glc-2, Glc-4, 1''	3.54^a	73.0		3.57, dd (9.5, 9.0)	73.0	Glc-2, Glc A-4	5.00, t (9.5)	77.4	1''
Glc or Glc A -4	3.42^a	67.2		3.46, dd (9.5, 9.0)	67.3	Glc-5	3.54^a	71.2		3.51, dd (9.5, 9.0)	71.2	Glc A-5	3.64, t (9.5)	69.0	Glc A-6
Glc or Glc A -5	3.61, m	76.6		3.94, m	73.3		4.16, d (9.0)	75.3	Glc A-4, Glc A-6	4.12, d (9.0)	75.1	Glc A-4, Glc A-6	4.23, d (9.5)	74.9	Glc A-6
Glc or Glc A -6	3.53, dd (12.0, 6.0); 3.72, dd (12.0, 2.0)	60.1		4.20, dd (12.0, 6.5); 4.38, dd (12.0, 2.0)	63.5	1'''		169.5			169.6			169.3	
1''		166.4			166.4			169.1			165.9			166.4	
2''	3.41, s	41.6	1'', 3''	3.42, s	41.6	1'', 3''	2.04, s	20.7	2''	3.38, s	41.6	1'', 3''	3.43, s	41.5	1'', 3''
3''		167.8			167.7						167.5			167.8	
1'''					166.7										
2'''				3.42, s	41.2	1''', 3'''									
3'''					167.7										

^{a} Unclear signal pattern due to overlapping signals.

35

Chapter 4. Phytochemical Constituents of *Chamaerhodos erecta* (L.) and *Chamaerhodos altaica* (Laxm.)

4.1 Introduction

Chamaerhodos erecta (L.) (fam. Rosaceae, Mongolian name: Tseh tumen tana), a biennial plant found throughout Mongolia except in the Gobi desert (Batkhuu et al., 2005; Grubov, 1982), around Khubsugul, Khentei, Khangai, Mongol-Daurian, Great Khingan, Khobdo, Mongolian Altai, Middle Khalkha, East Mongolia, Depression of Great Lakes, Valley of Lakes, East Gobi, and Gobi-Altai (Batkhuu et al., 2005; Grubov, 1982; Gubanov, 1996). It is typical habitat includes sandy steppes, steppe debris, stony slopes, dry larch forests, rocks, and waterside pebbles (Grubov, 2001). *C. erecta* is used in Mongolian traditional medicine to treat high temperature, tachycardia, face, and foot swelling, arthritis, scorbutus, itching allergies, and ulcers. Moreover, it has laxative activity with other plant drugs (Khaidav et al., 1985). *Chamaerhodos altaica* (Laxm.) (fam. Rosaceae, Mongolian name: Altain tumen tana) is typical habitat includes debris, stony steppe slopes of mountains, sandy steppes, montane steppes, and rocks (Grubov, 2001). It is also used in Mongolian traditional medicine to treat hepatic disorders, meal poisoning, hemorrhage, scurvy, and rheumatism (Batkhuu et al., 2005; Khaidav et al., 1985). Currently, very little research related to *C. erecta* and *C. altaica* has been reported. The presence of tannins, flavonoids, and triterpenes in the aerial parts of *C. erecta* was noted by Russian researchers (Sokolova, 1987).

4.2 Results and Discussion

Air-dried aerial parts of *C. erecta* were separately extracted with methanol (MeOH) and water then concentrated *in vacuo*. The MeOH extract was subsequently fractionated into chloroform ($CHCl_3$) and *n*-BuOH fractions. The methanol extract, fractions, and

water residue were all screened for DPPH-scavenging activity. The results showed that the *n*-BuOH fraction had the strongest scavenging activity. The CHCl$_3$ fraction, water residue and water extract exhibited less activity, which indicated fewer antioxidative compounds in these parts.

The *n*-BuOH fraction was then subjected to repeated liquid column chromatography using Sephadex LH-20, MCI-gel CHP20P, Sepra RP-18, and HPLC, as well as preparative chromatography to afford 18 compounds (**58**, **14-16**, **64-67**, **73**, **74**, **83-86**, **88**, **90**, and **91**) including a new simple phenol derivative (**56**) (Fig. 4-1, 4-2). The major compounds in the *n*-BuOH extract of aerial parts of *C. erecta* were quercetin and kaempferol derivatives (**14-16**, **58**, and **64-67**), as well as hydrolyzable tannins (**83-86**, **88**, and **90**), tannin-related ellagic acid (**91**), and a new compound: 4,5-dihydroxybenzaldehyde-3-*O*-β-D-glucopyranoside (**56**).

In a similar way, air-dried aerial parts of *C. altaica* were separately extracted with acetone-water (4:1), then concentrated *in vacuo*. The acetone extract was subsequently fractionated into water and diethyl-ether fractions. The water extract was separated using column chromatography to yield compounds **57-63**, **14-16**, **68-72**, **75-83**, and **87-90**. Compound **57** was a new flavonol diglycoside (Fig. 4-1, 4-2). *C. altaica* also contained quercetin and kaempferol derivatives (**14-16**, **57-63**, and **68**) and hydrolyzable tannins (**83**, **85**, and **87-90**). In addition, an isoflavone glucoside (**69**), catechins (**70** and **71**), a lignan glucoside (**75**), and aromatic glycosides (**76-82**) were isolated from this plant.

56

14 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-glucopyranosyl, R$_4$ = H
15 R$_1$ = OH, R$_2$ = H, R$_3$ = β–D-glucopyranosyl, R$_4$ = H
16 R$_1$ = OH, R$_2$ = H, R$_3$ = β–D-glucuronopyranosyl, R$_4$ = H
57 R$_1$ = OH, R$_2$ = β–D-glucopyranosyl, R$_3$ = β–D-glucuronopyranosyl, R$_4$ = H
58 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-(6''-*O-E-p*-coumaroyl) glucopyranosyl, R$_4$ = H
59 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-(6''-*O-Z-p*-coumaroyl) glucopyranosyl, R$_4$ = H
60 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-xylopyranosyl-(1-2)-β–D-glucopyranosyl, R$_4$ = H
61 R$_1$ = OH, R$_2$ = H, R$_3$ = β–D-xylopyranosyl-(1-2)-β–D-glucopyranosyl, R$_4$ = H
62 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-glucopyranosyl, R$_4$ = β–D-glucuronopyranosyl
63 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-(6''-*O-E-p*-coumaroyl) glucopyranosyl, R$_4$ = β–D-glucopyranosyl
64 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-glucuronopyranosyl, R$_4$ = H
65 R$_1$ = H, R$_2$ = H, R$_3$ = β–D-glucuronopyranosyl methyl ester, R$_4$ = H
66 R$_1$ = OH, R$_2$ = H, R$_3$ = H, R$_4$ = H
67 R$_1$ = H, R$_2$ = H, R$_3$ = H, R$_4$ = H

69

72

70 R = H
71 R = β–D-glucopyranosyl

68

76

75

73 R = α-OH
74 R = β-OH

77

78 R$_1$ = acetyl, R$_2$ = H, R$_3$ = β–D-glucopyranosyl
79 R$_1$ = H, R$_2$ = acetyl, R$_3$ = β–D-glucopyranosyl
80 R$_1$ = H, R$_2$ = CH$_2$CH$_2$COOH, R$_3$ = β–D-glucopyranosyl
81 R$_1$ = β–D-glucopyranosyl, R$_2$ = OMe, R$_3$ = OH
82 R$_1$ = OH, R$_2$ = COOH, R$_3$ = 6-galloyl-β–D-glucopyranosyl

Fig. 4-1 Structures of compounds **(14-16, 56-82)**

4.2.1 Isolation of known compounds

Compounds **14-16** and **58-91** were determined by comparing their physico-chemical characteristics and spectral data with those in the literature as well as by direct comparison with reference samples or those of the authentic samples. Thus, **58** was identified as kaempferol-3-*O*-β-D-(6″-*O*-*trans*-*p*-coumaroyl)-glucopyranoside (Tsukamoto et al., 2004), **59** as kaempferol-3-*O*-β-D-(6″-*O*-*cis*-*p*-coumaroyl)-glucopyranoside (Tsukamoto et al., 2004), **60** as kaempferol-3-*O*-β-D-xylopyranosyl (1→2)-*O*-β-D-glucopyranoside (Cui et al., 1993), **61** as quercetin-3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (Webby, 1991), **62** as kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-β-D-glucuronopyranoside (Budzianowski, 1991), **63** as kaempferol-3-*O*-β-D-(6″-*O*-*trans*-*p*-coumaroyl)-glucopyranosyl-7-*O*-β-D-glucopyranoside (Mousallami et al., 2002), **16** as quercetin-3-*O*-β-D-glucuronopyranoside (Seto et al., 1992), **15** as quercetin-3-*O*-β-D-glucopyranoside (Kazuma et al., 2003), **14** as kaempferol-3-*O*-β-D-glucopyranoside (Kazuma et al., 2003), **64** as kaempferol-3-*O*-β-D-glucuronopyranoside (Harborne and Saleh, 1971), **65** as quercetin-3-*O*-β-D-glucuronopyranoside methyl ether (Nawwar et al., 1984), **66** as quercetin (Harborne, 1994; Harborne and Mabry, 1982), **67** as kaempferol (Harborne, 1994; Harborne and Mabry, 1982), **68** as potentilin A (Xu et al., 2010), **69** as sissotrin (Vitor et al., 2004), **70** as (+)-catechin (Foo and Karchesy, 1989), **71** as (+)-catechin-7-*O*-β-D-glucopyranoside (Raab et al., 2010), **72** as tryptophan, **73** as tormentic acid (Taniguchi et al., 2002), **74** as euscaphic acid (Chen et al., 2008), **75** as lariciresinol-4′-β-D-glucopyranoside (Sugiyama and Kikuchi, 1993), **76** as *trans*-cinnamoyl-1-*O*-β-L-arabinopyranosyl-(1→6)-*O*-β-D-glucopyranoside (Latza et al., 1996), **77** as benzyl-β-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside (Kawahara et

al., 2005), **78** as picein (Perry et al., 1996), **79** as *m*-acetyl-β-D-glucopyranoside (Otani et al., 2008), **80** as *m*-hydrocinnamoyl-β-D-glucopyranoside, **81** as tachioside (Zhong et al., 1999), **82** as shomaside F (Iwanaga et al., 2010), **83** as strictinin (Yagi et al., 2009), **84** as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranoside (Kiss et al., 2008), **85** as eugeniin (Hatano et al., 1988; Nonaka et al., 1980), **86** as casuarictin (Okuda et al., 1983), **87** as 1,2,6-tri-*O*-galloyl-β-D-glucopyranoside (Yoshida et al., 1984), **88** as potentillin (Gupta et al., 1982; Okuda et al., 1982), **89** as pedunculagin (Hatano et al., 1988; Okuda et al., 1983), **90** as agrimoniin (Okuda et al., 1984), and **91** as ellagic acid (Li et al., 1999) (Fig. 4-2).

83 R$_1$ = gallyol, R$_2$ = H, R$_3$ = H, R$_4$-R$_5$ = (*S*)-HHDP
84 R$_1$ = gallyol, R$_2$ = gallyol, R$_3$ = gallyol, R$_4$ = gallyol, R$_5$ = gallyol
85 R$_1$ = gallyol, R$_2$ = gallyol, R$_3$ = gallyol, R$_4$-R$_5$ = (*S*)-HHDP
86 R$_1$ = gallyol, R$_2$-R$_3$ = (*S*)-HHDP, R$_4$-R$_5$ = (*S*)-HHDP
87 R$_1$ = gallyol, R$_2$ = gallyol, R$_3$ = H, R$_4$ = H, R$_5$ = gallyol

88 R$_1$ = gallyol, R$_2$-R$_3$ = (*S*)-HHDP, R$_4$-R$_5$ = (*S*)-HHDP
89 R$_1$ = H, R$_2$-R$_3$ = (*S*)-HHDP, R$_4$-R$_5$ = (*S*)-HHDP

90 R$_2$-R$_3$ = (*S*)-HHDP, R$_4$-R$_5$ = (*S*)-HHDP

(*S*)-HHDP

91
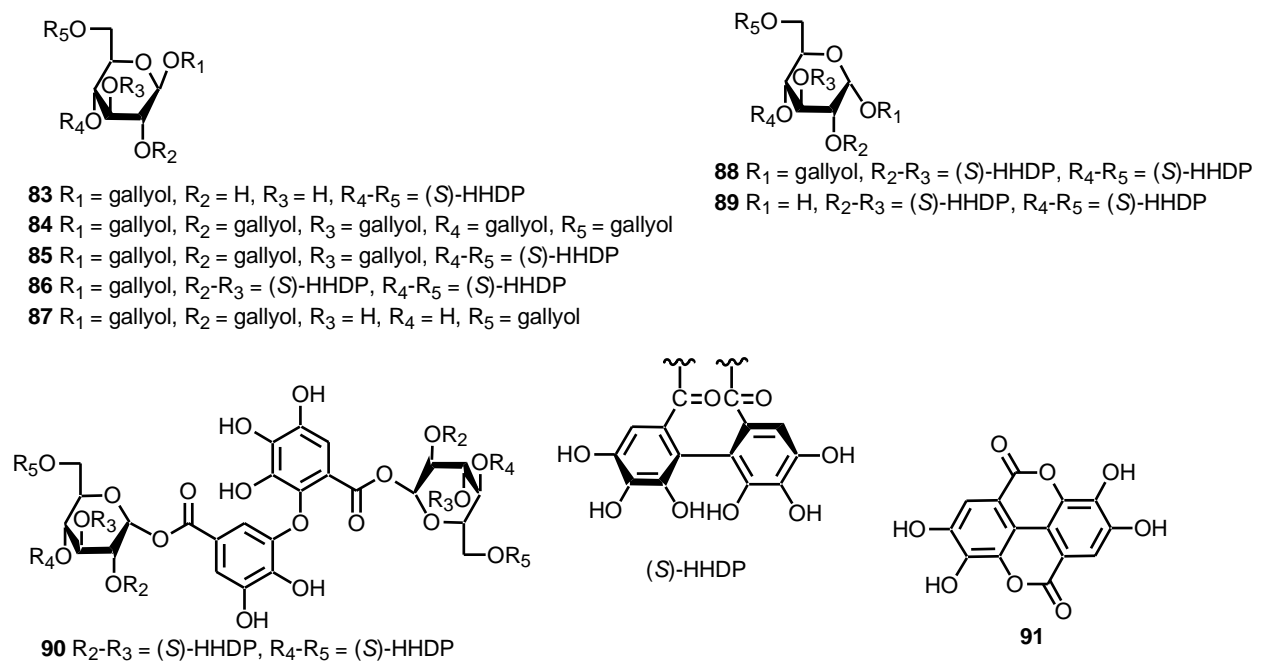
Fig. 4-2 Structures of tannins **(83-91)**

4.2.2 Isolation and structure elucidation of new compounds

Compound **56**, a colorless amorphous powder, was determined using TLC purple at 254 nm and blue fluorescence at 356 nm. A high-resolution (HR)-FAB-MS analysis showed a [M+Na]$^+$ at *m/z* 339.0701 corresponding to the molecular formula $C_{13}H_{16}O_9$. The ^{1}H-NMR spectrum of **56** showed two meta-coupled doublets at the aromatic proton field δ_H 7.20 (1H, d, *J* = 2.0 Hz) and 7.37 (1H, d, *J* = 2.0 Hz), which were attributed to H-2 and H-6. This spectrum also indicated a signal at δ_H 9.72 (1H, s, H-7), which was assigned to the aldehyde proton and the anomer proton doublet of the sugar at δ_H 5.01 (1H, d, *J* = 8.0 Hz), respectively. Whereas the other protons of the sugar appeared at δ_H 3.95-3.55 ppm field. A sugar analysis showed the presence of a D-glucose unit (Tanaka et al., 2007). The anomer proton coupling constant indicated that the sugar was in a β-configuration (Feng et al., 1988). The ^{13}C-NMR spectrum of **56** indicated the presence of an aldehyde carbon at δ_C 193.1, and one six member aromatic carbon group at δ_C 112.0, 112.1, 128.5, 143.3, 146.8 and 147.1 ppm, respectively. This spectrum also indicated six carbons of the sugar at δ_C 61.8, 70.5, 74.0, 76.6, 77.5 and 103.0, which corresponded to β-D-glucopyranose (Agrawal, 1992; Feng et al., 1988). Proton and carbon signals were assigned with the help of HMQC, HMBC, and COSY. The HMBC spectrum of **56** indicated correlations between an aldehyde proton and C-1 (δ_C 128.5), C-2 (δ_C 112.0) and C-6 (δ_C 112.1); H-2 and C-3 (δ_C 147.1), C-4 (δ_C 143.3) and C-6 (δ_C 112.1); H-6 and C-2 (δ_C 112.0), C-4 (δ_C 143.3) and C-5 (δ_C 146.8); and the anomer proton of the sugar and C-3 (δ_C 147.1). Based on the physico-chemical properties as well as one and two dimensional NMR spectroscopic analyses, **56** was identified as a new compound, 4,5-dihydroxy benzaldehyde-3-*O*-β-D-glucopyranoside (Fig. 4-1), which is closely related to a castamollissin isolated from *Castanea mollissima* (Feng et

al., 1988).

Compound **57** had a molecular formula $C_{27}H_{28}O_{18}$, as determined by HR-FAB-MS (*m/z* 641.1354 [M+H]$^+$). ^{1}H- and ^{13}C-NMR spectra recorded in DMSO-d_6 are shown in the experimental section. In the aromatic region of the ^{1}H-NMR spectrum, two sets of coupling proton signals [δ_H 6.22 (1H, d, *J* = 2.0 Hz, H-6), 6.44 (1H, d, *J* = 2.0 Hz, H-8)] and [δ_H 7.67 (1H, d, *J* = 2.0 Hz, H-2′), 7.20 (1H, d, *J* = 8.5 Hz, H-5′), 7.58 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′)] suggested the presence of a quercetin moiety as the aglycone of **57**. In the ^{13}C-NMR spectrum, signals for two anomeric carbons, nine oxygenated carbons, and a carboxy carbon were observed, which were assigned to two sugar units. Sugar analysis after acid hydrolyses revealed that the two sugars were D-glucose and D-glucuronic acid. The coupling constants of anomeric proton signals at δ_H 5.50 (1H, d, *J* = 7.5, H-Glc A-1) and 4.88 (1H, d, *J* = 7.5, H-Glc-1) suggested that both corresponding carbons were in a β-configuration. The ^{13}C-NMR spectrum was almost superimposable onto those of quercetin-3-*O*-β-D-glucopyranosyl-4′-*O*-β-D-gluco pyranoside (Jaramillo et al., 2011), except that **57** had a β-D-glucuronic acid moiety instead of a 3-*O*-β-D-glucopyranosyl moiety. The H-Glc-1 anomeric proton signal was long-range coupled with the C-4′ carbon signal at δ_C 147.6 in the HMBC spectrum. A higher-shifted C-3 carbon signal at δ_C 133.6 suggested the presence of 3-*O*-β-D-glucuronopyranoside (Jaramillo et al., 2011; Kajjout and Rolando, 2011). The anomeric proton signal of β-D-glucuronopyranoside (H-Glc A-1) correlated with the H-Glc A-5 (δ_H 3.56, d, *J* = 9.5 Hz) proton signal in the NOE spectrum. Consequently, the structure of **57** was determined as quercetin-3-*O*-β-D-glucuronopyranosyl-4′-*O*-β-D-glucopyranoside (Fig. 4-1).

Chapter 5. Biological evaluation

In the present study, the isolated compounds (**14-16**, **30-35**, **38**, **40**, **43**, **46-58**, **60**, **62**, **64**, **66-68**, **70-72**, **75**, **77**, **78**, **81**, and **83-91**) from *D. foetidum*, *C. erecta*, and *C. altaica* were investigated for hyaluronidase inhibitory activity. Compounds **1-30**, **34**, **35**, **38**, **40**, **41**, **43**, **46-63**, and **66-91** from *D. ruyschiana*, *D. foetidum*, *C. erecta*, and *C. altaica* were screened for antioxidant activity. Compounds **1-29**, **56-60**, **62**, **66-68**, **70-72**, **75**, **76**, **78-81**, and **83-91** from *D. ruyschiana*, *C. erecta*, and *C. altaica* were screened for AGEs production inhibitory activity. In addition, compounds **30**, **34**, **35**, **38**, **40**, **41**, **43**, and **46-55** from *D. foetidum* were screened for tyrosinase inhibitory activity.

5.1 Hyaluronidase inhibitory activity

The inhibitory effects of compounds **14-16**, **30-35**, **38**, **40**, **43**, **46-58**, **60**, **62**, **64**, **66-68**, **70-72**, **75**, **77-78**, **81**, and **83-91** hyaluronidase were examined (Table. 5-1). Among them, compounds rosmarinic acid derivative (**34**), acacetin glycosides (**43**), and (**46**) showed stronger hyaluronidase inhibitory activity (IC_{50} value of 0.22, 0.25, and 0.19 mM) compared to disodium cromoglicate (DSCG, IC_{50} value of 0.65 mM). Some of flavone glycosides (**50-53**), catechin (**70**), and some of tannins (**84**, **85**, **87**, **88**, and **90**) showed moderate activity.

Phenylpropanoid oligomers, some flavonoid glucuronopyranosides, catechins, and hydrolyzable tannins were reported as hyaluronidase inhibitors (Kakegawa et al., 1985; Murata et al., 2010a, 2012; Murata et al., 2010b; Terauchi et al., 2007). Also the results suggested that the galloyl group contributes the higher activity than the hexahydroxydiphenoyl (HHDP) group in tannins.

Rosmarinic acid (**35**) is potent hyaluronidase inhibitor (Ippoushi et al., 2000). On the other hand, the glycosides of rosmarinic acid did not show significant values (**38** and

40). Compound **34** was a trimer of phenylpropanoid, which showed lower IC_{50} value (0.22 mM) than those of rosmarinic acid (IC_{50} value of 0.75 mM) and DSCG as a positive control.

Likewise, a new acacetin malonyl glucoside (**43**) and a new acacetin malonyl glucuronopyranoside (**46**) had potent activity. The flavone glycosides with inhibitory activity isolated in the present study (**43**, **46**, and **50-53**) have glucuronic acid and/or malonyl moieties. Also some of flavonoid glucuronopyranosides, including **16** and **64** are known as hyaluronidase inhibitors, although their IC_{50} value could not be determined (maximal concentration: 1.0 mM), which were obtained from *Fragaria ananassa* Duch. (Rosaceae) (Terauchi et al., 2007) and *Meehania fargesii* (H.LE´ V.) C. Y. Wu (Lamiaceae) (Murata et al., 2010a). Compound **57** is also a flavonoid glucuronopyranoside and its inhibition level was similar to **16**. Compounds **15** and **16** have inhibitory effects on compound 48/80-induced histamine release from peritoneal mast cell of rats (Terauchi et al., 2007).

5.2 DPPH radical scavenging activity

DPPH radical scavenging assay of crude extracts, fractions and compounds was carried out according to known methods (Mensor et al., 2001). Briefly, solutions with 25, 50, 100 and 200 μg/mL of MeOH were prepared from each extract and fractions. Moreover, concentrations of isolated compounds were 5, 10, 20, 40, 100 and 200 μg/mL in MeOH.

The antioxidant activity of **1-29**, **30**, **34**, **35**, **38**, **40**, **41**, **43**, **46-55**, **56-63**, and **66-91** on the stable free radical DPPH was examined (Table. 5-2).

Among these compounds, **35** and **83-91** showed antioxidative activity (IC_{50} value of 13.6, 8.4, 5.5, 6.0, 6.4, 11.5, 5.9, 9.2, 4.8, and 22.0 μM, respectively) than the positive

control trolox (IC$_{50}$ value of 25.9 µM), while compounds, **16**, **18-19**, **38**, **41**, **53**, and **82** were similar (IC$_{50}$ value of 26.4 – 39.6 µM) to that of trolox. The antioxidant activity of tannins, rosmarinic acid, some of flavone glycosides, and their derivatives have already been reported by others (Tagashira and Ohtake, 1998). The antioxidant activity of the flavonoids was variable, and those with a catechol B-ring (luteolin glycosides) were more active than those without (apigenin glycosides) (Mosquera et al., 2007; Okawa et al., 2001). A number of them are known as typical antioxidants, and their DPPH radical scavenging activity have been reported previously (Fukuda et al., 2003; Gao et al., 2010). The compounds were tested for antioxidant activity using DPPH. Although the new compounds were not active, phenylpropanoylquinic acid derivatives were revealed as radical scavengers in *D. ruyschiana*.

In the case of these plants, rosmarinic acid, its derivatives, some flavone glycosides, and tannins seem to be one of the important constituents for its antioxidative activity.

5.3 AGEs production inhibitory activity

Incubation of D-glucose and BSA induced the production of fluorescent AGEs. The results of AGEs production inhibitory activity are shown in Table. 5-3. AGEs production inhibitory of **1-29**, **56-60**, **62**, **66-68**, **70-72**, **75**, **76**, **78-81**, and **83-91** on the AGEs formation was examined. A series of flavonols and their glycosides, catechins, and tannins showed over 29.4 % inhibitory activity in 300µM. In particular, compounds **14**, **16**, **58**, **59**, **68**, **84**, **85**, **86**, **88**, **90**, and **91** demonstrated potent activity (IC$_{50}$ 91.1–294.6 µM) like that reported for active flavonols: quercetin (**66**, IC$_{50}$ 424.0 µM) and kaempferol (**67**, IC$_{50}$ 381.2 µM) (Shimoda et al., 2011). It is thought that the prevention of AGEs production formation is promoted by antioxidant compounds (Peng et al., 2011), and almost of these active compounds also had DPPH radical scavenging

activity. On the other hand, although catechins and the galloyl group were indicated to have preventative activities, there are few reports about the activity of hydrolyzable tannins as inhibitors (Lee et al., 2008; Peng et al., 2011; Wu and Yen, 2005). In this study, ellagic acid, and typical hydrolyzable tannins were identified as inhibitors. These compounds are also expected to be useful to treat diseases by preventing excess AGEs production.

5.4 Tyrosinase inhibitory activity

Inhibitory effects of compounds **30, 34-35**, **38**, **40-41**, **43**, and **46-55** tyrosinase were examined (Table. 5-4). Among them, some of rosmarinic acid derivatives and some acacetin glycosides showed 8.7-16.2% in 100 μM. Kojic acid as a positive control exhibited 65.5% inhibitory activity in 100μM.

Table. 5-1 Hyaluronidase Inhibitory Activity of Compounds

compound	IC$_{50}$ (mM)	compound	IC$_{50}$ (mM)
14	ND[a]	60	ND[a]
15	ND[a]	62	ND[a]
16	ND[a]	64	ND[a]
30	ND[a]	66	ND[a]
31	ND[a]	67	ND[a]
32	ND[a]	68	ND[a]
33	ND[a]	70	0.84
34	0.22	71	ND[a]
35	0.75	72	ND[a]
38	ND[a]	75	ND[a]
40	ND[a]	77	ND[a]
43	0.25	78	ND[a]
46	0.19	81	ND[a]
47	ND[a]	83	ND[a]
48	ND[a]	84	0.60
49	ND[a]	85	0.51
50	0.55	86	ND[a]
51	0.99	87	0.79
52	0.56	88	0.89
53	0.79	89	ND[a]
54	ND[a]	90	0.58
55	ND[a]	91	ND[a]
56	ND[a]	DSCG	0.65
57	ND[a]	Rosmarinic acid	1.36
58	ND[a]		

[a] Not determined : Inhibitory activity at 1.0 mM was under 35%

47

Table. 5-2 DPPH Radical-Scavenging Activity of Compounds

compound	IC$_{50}$ (μM)	compound	IC$_{50}$ (μM)
1	ND^b	51	312.0
2	ND^b	52	126.4
3	ND^b	53	39.6
4	ND^b	54	446.0
5	ND^b	55	ND^b
6	ND^b	56	400.0
7	ND^b	57	ND^b
8	ND^b	58	ND^b
9	ND^b	59	266.0
10	ND^b	60	ND^b
11	223.0	61	69.0
12	ND^b	62	ND^b
13	ND^b	63	ND^b
14	ND^b	66	65.2
15	60.6	67	86.7
16	37.2	68	ND^b
17	45.5	69	ND^b
18	32.4	70	130.0
19	28.9	71	95.8
20	57.4	72	656.0
21	284.0	73	ND^b
22	ND^b	74	ND^b
23	328.0	75	332.0
24	ND^b	76	ND^b
25	ND^b	77	ND^b
26	62.4	78	ND^b
27	ND^b	79	ND^b
28	ND^b	80	ND^b
29	ND^b	81	198.0
30	ND^b	82	34.6
34	61.0	83	8.4
35	13.6	84	5.5
38	26.4	85	6.0
40	63.2	86	6.4
41	32.8	87	11.5
43	ND^b	88	5.9
46	ND^b	89	9.2
47	408.0	90	4.8
48	ND^b	91	22.0
49	ND^b	rutin	38.7
50	ND^b	trolox	25.9

^{b} Not determined : IC$_{50}$ value > 200 μg/ml

Table. 5-3 AGEs production Inhibitory Activity of Compounds

compound	Inhibition % (in 300 μM)	compound	Inhibition % (in 300 μM)
1	18.1	58	54.8
2	22.2	59	53.6
3	6.4	60	34.8
7	4.3	62	31.2
8	23.2	66	47.4
9	6.2	67	44.9
10	3.8	68	67.7
11	15.1	70	29.4
12	17.6	71	38.5
13	13.6	72	-5.7
14	50.1	75	-1.4
15	48.4	76	7.0
16	53.1	78	12.9
17	8.7	79	6.5
18	26.5	80	-0.2
19	52.0	81	0.0
20	40.6	83	52.9
21	5.8	84	59.6
22	6.0	85	61.6
23	25.6	86	61.0
24	9.6	87	47.6
25	4.5	88	64.8
26	38.0	89	48.8
27	7.0	90	67.2
29	11.0	91	55.3
56	44.8	quercetin	50.1
57	40.4	kaempferol	48.4

Table. 5-4 Results of Tyrosinase Inhibitory Activity

compound	Inhibition % (in 100 μM)	compound	Inhibition % (in 100 μM)
30	4.1	48	6.8
34	13.8	49	2.7
38	2.1	50	16.2
40	12.2	53	0.0
41	8.7	54	6.1
43	4.1	55	0.7
46	2.9	kojic acid	65.50

Chapter 6. Conclusions

Mongolians have ancient practices utilizing medicinal plants for their daily life to prevent and cure various diseases. Although studies of Mongolian medicinal plants are still at a nascent stage and even phytochemical constituents and the basic biological activities have not yet been investigated sufficiently. So, there is a real need for scientific studies and knowledge about TMM to provide scientific rationality.

In Mongolia, much attention has been paid to skin inflammation and its related diseases including allergies, severe rashes, dryness, and aging. There are a lot of medicinal plants which have been handed down through the history, for skin care and protection from inflammation and diseases.

In this research, 51 extractions of Mongolian medicinal plants were tested for their hyaluronidase inhibitory and DPPH radical scavenging activities. Out of them, two *Dracocephalum* and two *Chamaerhodos* plants were picked up, which showed significant activity and are commonly used as herbal medicines in TMM. The phytochemical constituents from the four plants were isolated by chromatography and chemical structures were determined by using instrumental analyses. Then the biological activities concerning skin-care were estimated.

Five new flavone tetraglycosides (**1-5**) (Fig. 2-1) and five new benzyl alcohol glycosides (**7-9**, **12**, and **13**) (Fig. 2-2, 2-3) were isolated from *D. ruyschiana* together with 19 known compounds (**6**, **10**, **11**, and **14-29**) (Fig. 2-4). The 7-O-β-D-gluco pyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-gluco pyranosyl moiety in flavone, which is a monodesmoside, were yielded high amount (0.73%) from the plant.

From the aerial parts of *D. foetidum*, three new limonene glycosides (**30-32**), a new

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caffeic acid trimer (**34**), four new rosmarinic acid derivatives (**37-39** and **41**), five new acacetin acyl glycosides (**42-46**) as well as 13 known compounds (**33**, **35**, **36**, **40**, and **47-55**) were characterized (Fig. 3-1).

Plants were found to contain polyphenolic compounds such as phenylpropanoids and flavonoids. Rosmarinic acid was obtained as one of the main constituents of *D. foetidum*, but it was not found in *D. ruyschiana*, even when these are from same genus.

C. erecta and *C. altaica* were revealed having potent antioxidant activity by screening of 23 Mongolian medicinal plants, and the plants are known to be used for skin-care, traditionally. Chemical investigation of the *C. erecta* and *C. altaica* has resulted in the isolation and structure elucidation of 4,5-dihydroxybenzaldehyde-3-*O*-β-D-glucopyranoside (**56**) and quercetin-3-*O*-β-D-glucuronopyranosyl-4′-*O*-β-D-glucopyranoside (**57**) as well as 37 known compounds (**14-16** and **56-91**) (Fig. 4-1). Flavonol glycosides and hydrolyzable tannins were typical and main constituents of *Chamaerhodos* two plants. These results are explained in **Chapter 4**.

The isolated compounds have a basic phenolic skeleton, although the flavones and the phenylpropanoids also have attached sugar units. These are very common metabolites in the plant kingdom, and they show various biological activities.

In order to identify skin-care effects and biological activities of the obtained total 91 compounds from *Dracocephalum* and *Chamaerhodos* plants, four basic tests hyaluronidase inhibitory, DPPH radical scavenging, AGEs production inhibitory, and tyrosinase inhibitory activities were evaluated. The tests were related with anti-inflammatory, antioxidant, antipigmentation activities and their results were discussed. Compounds **14-16**, **30-35**, **38**, **40**, **43**, **46-58**, **60**, **62**, **64**, **66-68**, **70-72**, **75**, **77**, **78**, **81**, and **83-91** from *D. foetidum*, *C. erecta*, and *C. altaica* were investigated for

hyaluronidase inhibitory activity. Compounds **1-30**, **34**, **35**, **38**, **40**, **41**, **43**, **46-63**, and **66-91** from *D. ruyschiana*, *D. foetidum*, *C. erecta*, and *C. altaica* were screened for antioxidant activity. Compounds **1-29**, **56-60**, **62**, **66-68**, **70-72**, **75**, **76**, **78-81**, and **83-91** from *D. ruyschiana*, *C. erecta*, and *C. altaica* were screened for AGEs production inhibitory activity. In addition, compounds **30**, **34**, **35**, **38**, **40**, **41**, **43**, and **46-55** from *D. foetidum* were screened for tyrosinase inhibitory activity.

Rosmarinic acid derivative (**34**) and acacetin glycosides (**43** and **46**) showed stronger hyaluronidase inhibitory activity than positive control disodium cromoglicate. Some of flavone glycosides (**50-53**), catechin (**70**), and some of tannins (**84**, **85**, **87**, **88**, and **90**) showed moderate activity (Table. 5-1).

Antioxidant activities of rosmarinic acid (**35**) and tannins (**83-91**) were more than the positive control trolox, while some flavone glycosides and rosmarinic acid derivatives (**16**, **18-19**, **38**, **41**, **53**, and **82**) were similar to that of trolox (Table. 5-2). The antioxidant activities of rosmarinic acid, tannins, some of flavone glycosides, and their derivatives have already been reported by others.

A series of flavonols and their glycosides, catechins, and tannins showed AGEs production inhibitory activities. It is thought that the prevention of AGEs production formation is promoted by antioxidant compounds, and almost of these active compounds also had DPPH radical scavenging activity. On the other hand, although catechins and the galloyl group were indicated to have preventative activities, there are few reports about the activity of hydrolyzable tannins as inhibitors (Lee et al., 2008; Peng et al., 2011; Wu and Yen, 2005). In this study, ellagic acid, and typical hydrolyzable tannins were identified as inhibitors (Table. 5-3).

Although tyrosinase inhibitory effects of all compounds of *D. foetidum* were

examined, they did not show any significant activity (Table. 5-4).

From consideration of the above results, in the case of these plants, flavone glycosides, rosmarinic acid and its derivatives, and hydrolyzable tannins seem to be important constituents for their biological activities.

Some flavonoids, including flavones, flavonol, their glycosides, and methylated and acetylated derivatives were revealed from four plants in this study. A number of isolated flavones is conjugated to sugar residues and is abound as β-glycoside. To these glycosides malonyl or acetyl residues attached. The evidence in the literature suggests that biological activity of flavonoids does not depend upon the type of glycoside form. However some results show that there is a certain difference in absorption rate between aglycone and conjugated forms in favor of aglycones (Kren and Martinkova, 2001).

Flavonoids are present in a variety of plants and are used as important components of our diets. Actually, their biological and pharmacological effects, including antioxidant, anti-mutagenic, anti-carcinogenic, anti-viral, and anti-inflammatory properties, have been demonstrated in numerous studies (Nakagawa et al., 1999; Takano et al., 2004). The occurrence of flavonoids at such high concentrations in *Dracocephalum* and *Chamaerhodos* plants is related to their anti-inflammatory properties. Plants with anti-inflammatory properties often have a high level of flavonoids (Aburjai and Natsheh, 2003). These properties are potentially beneficial in preventing diseases and utilizing the skin-care.

Rosmarinic acid and its derivatives were revealed as the antioxidative and hyaluronidase inhibitory components from *D. foetidum*. Rosmarinic acid is the most abundant constituent in Lamiaceae family and was obtained as one of the main constituents of *D. foetidum*, but it was not found in *D. ruyschiana*. Rosmarinic acid and

its derivatives have interesting properties which have led to broad range of applications from food preservatives to cosmetics. The presence of them has beneficial and health promoting effects as well as they contribute to the antioxidant activity of plants used in skin-care products, such as *Rosmarinus officinalis* and *Sanicula europaea* (D'Amelio, 1999; Petersen and Simmonds, 2003). Rosmarinic acid and its derivatives were seemed to be potent medicinal properties of *D. foetidum*.

From *Chamaerhodos* plants, a number of hydrolyzable tannins were isolated, which exhibit a wide variety of beneficial biological activities in mammals. They have been considered health promoting components in plant derived foods and beverages and also reported to possess anticarcinogenic and anti-mutagenic potentials as well as antimicrobial properties (Amarowicz, 2007). Likewise, tannins are potent antioxidants that may protect against UV light and, in turn, reduce the risk of skin cancer and premature aging (Gali-Muhtasib et al., 1999). Isolated hydrolyzable tannins were revealed as potent active constituents that may contribute to the pharmaceutical effects of two *Chamaerhodos* species.

This study suggests that phytochemical constituents of these four plants, which contain hyaluronidase inhibitors and antioxidants, may be useful in skin-care for anti-inflammation, anti-allergies, and antioxidation.

It is rational that nomadic Mongolians used *D. ruyschiana*, *D. foetidum*, *C. erecta*, and *C. altaica* for their ailments because the present study showed that constituents from those have beneficial biological effects. These four medicinal plants have been important parts for TMM. The scientific data are expected to be useful and important information for the crude drugs which are being used by Mongolian people and generate data for the Mongolian National herbal pharmacopoeia.

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Chapter 7. Experimental Section

7.1 General

Optical rotations were recorded on a P-2300 polarimeter (Jasco Co., Tokyo, Japan). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz), ^{1}H-^{1}H COSY, HMQC (optimized for $^1J_{C-H}$ = 145 Hz), and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) spectra were recorded on a JNM-AL400 FT-NMR spectrometer (Jeol Ltd., Tokyo, Japan), and chemical shifts are given as δ values with TMS as an internal standard. HRFABMS data were obtained on a JMS700 mass spectrometer (Jeol Ltd.), using either an *m*-nitrobenzyl alcohol or glycerol matrix. A porous polymer gel (Diaion HP-20, 60 × 300 mm, Mitsubishi Chemical Co., Tokyo, Japan) and octadecyl silica (ODS) (Cosmosil 140 C_{18}-OPN, 150 g, Nacalai Tesque, Kyoto, Japan) were used for column chromatography (CC). Preparative HPLC was performed on a Jasco 2089 and detected with UV at 210 nm (columns, Cosmosil AR-II, 20 × 250 mm, Nacalai Tesque; Cosmosil 5PE-MS, 20 × 250 mm, Nacalai Tesque; Develosil C_{30}-UG-5, 20 × 250 mm, Nomura Chemical Co. Ltd., Aichi, Japan; Mightysil RP-18 GP, 10 × 250 mm, Kanto Chemical Co. Inc., Tokyo, Japan).

Sephadex LH-20 (Pharmacia, Uppsala, Sweden), MCI gel-CHP-20P (75-150 μm, Mitsubishi Chemical Co., Tokyo, Japan), and Sepra C18-E (50 μm, 65 A°) were used for column chromatography. Preparative and analytical TLC was carried out on pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) and spots were detected under UV radiation (254 nm) and by spraying with 1% methanolic diphenylboric acid-β-ethylamino ester (NP), 5% ethanolic polyethylene glycol (PEG).

7.2 *Dracocephalum ruyschiana*

Aerial parts of *D. ruyschiana* were collected in July 2011 in Mongolia (N

48°04.550′; E 106°22.611′). Prof. Ch. Sanchir from the Institute of Botany, Mongolian Academy of Sciences, identified the plant species. A voucher specimen (№ 90.7.7.11A) is deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia.

7.2.1 Extraction and Isolation

Dried aerial parts of *D. ruyschiana* (95 g) were extracted with acetone-H_2O (8:2) at room temperature for 2 weeks (3 L). The extract was concentrated at reduced pressure, and the solid (60.6 g) residue was suspended in H_2O (1.5 L) and subjected to extraction with Et_2O (3 × 1 L). The aqueous layer extract (25.1 g) was dissolved in H_2O, passed through a porous polymer gel (Diaion HP-20, 70 × 180 mm), and eluted with H_2O (fraction 1A, 13.29 g), MeOH-H_2O (50:50) (fr. 1B, 2.55 g), and MeOH (fr. 1C, 9.52 g). Fr. 1B was applied to a reversed-phase column using an ODS-packed column (ODS-SM-50C-M, Yamazen Co., Osaka, Japan) and eluted with MeOH-H_2O (10:90, V/V) (frs. 2A-2E) and MeOH-H_2O (30:70, V/V) (frs. 2F-2I). Compounds **10** (18.6 mg, from fr. 2H), **11** (1.9 mg, from fr. 2G), **13** (3.0 mg, from fr. 2D), **29** (1.9 mg, from fr. 2D), and **17** (9.2 mg, from fr. 2B) were purified by HPLC [columns: AR-II, mobile phases CH_3CN-H_2O containing 0.2% TFA (5:95, 10:90, and 15:85, V/V); 5PE-MS, mobile phases CH_3CN-H_2O containing 0.2% TFA (10:90, V/V); Mightysil RP-18, mobile phases CH_3CN-H_2O containing 0.2% TFA (5:95 and 10:90, V/V)]. Fr. 1C was applied to a reversed-phase column using an ODS-packed column (ODS-SM-50C-M) and eluted with MeOH-H_2O (30:70, V/V) (frs. 3A-3I) and MeOH-H_2O (50:50, V/V) (frs. 3J-3M). Each fraction was subjected to HPLC [columns: AR-II, mobile phases CH_3CN-H_2O containing 0.2% TFA (15:85, 20:80, and 25:75, V/V); 5PE-MS, mobile phases CH_3CN-H_2O containing 0.2% TFA (15:85 and 20:80, V/V); Mightysil RP-18,

mobile phases CH$_3$CN-H$_2$O containing 0.2% TFA (15:75, 20:80, and 25:75, V/V) and MeOH-H$_2$O containing 0.2% TFA (30:70, V/V)] to yield the compounds described below. Compound **9** (1.7 mg) was purified from fr. 3M (228 mg). Compounds **3** (47.6 mg), **7** (28.3 mg), **8** (6.0 mg), and **9** (12.6 mg) were purified from fr. 3L (522 mg); **3** (267 mg) and **12** (123 mg) were purified from fr. 3K (2.04 g); **1** (31.9 mg) and **2** (13.3 mg) were purified from fr. 3J; **1** (55.7 mg) and **25** (0.5 mg) were purified from fr. 3I (309 mg); **1** (2.9 mg) was purified from fr. 3H (273.0 mg); **1** (1.5 mg), **4** (14.5 mg), **6** (3.5 mg), **14** (7.0 mg), **20** (2.3 mg), and **26** (0.6 mg) were purified from fr. 3G (224 mg); **5** (4.3 mg), **14** (2.0 mg), **15** (12.8 mg), **18** (2.9 mg), **19** (1.7 mg), **23** (3.5 mg), and **26** (6.7 mg) were purified from fr. 3F (215 mg); **16** (58.2 mg), **18** (48.4 mg), **21** (5.4 mg), and **26** (3.1 mg) were purified from fr. 3E (466.7 mg); **22** (5.4 mg), **24** (18.6 mg), and **27** (2.1 mg) were purified from fr. 3D (163 mg); **10** (18.6 mg), **26** (12.8 mg), and **28** (6.9 mg) were purified from frs. 3B and 3C (215 mg).

Data of new compounds

Acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyra nosyl-(1→6)]-β-D-glucopyranoside (**1**): colorless, amorphous solid; $[\alpha]^{23}_{D}$ −61.1 (*c* 0.37, DMSO); ^{1}H-NMR (DMSO-*d$_6$*, 400 MHz) and ^{13}C-NMR (DMSO-*d$_6$*, 100 MHz), see Table. 2-1; HRFABMS (positive) m/z 939.2752 [M+Na]$^+$ (calcd for C$_{40}$H$_{52}$O$_{24}$Na, 939.2745).

Acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-3-*O*-acetyl-β-D-glucopyranosyl-(1→2)-[α-L-r hamnopyranosyl-(1→6)]-β-D-glucopyranoside (**2**): colorless, amorphous solid; $[\alpha]^{21}_{D}$ −51.5 (*c* 0.54, DMSO); ^{1}H-NMR (DMSO-*d$_6$*, 400 MHz) and ^{13}C-NMR (DMSO-*d$_6$*, 100 MHz), see Table. 2-1; HRFABMS (positive) m/z 959.3031 [M+H]$^+$ (calcd for C$_{42}$H$_{55}$O$_{25}$, 959.3032).

Acacetin-7-*O*-β-D-glucopyranosyl-(1→2)-6-*O*-acetyl-β-D-glucopyranosyl-(1→2)-[α-L-r

hamnopyranosyl-(1→6)]-β-D-glucopyranoside (**3**): colorless, amorphous solid; [α]$^{21}_D$

−59.8 (c 4.28, DMSO); ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100

MHz), see Table. 2-1; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 6.87 (1H, s, H-3), 7.00 (1H, d,

J = 2.0 Hz, H-6), 7.07 (1H, d, J = 2.0 Hz, H-8), 8.02 (2H, d, J = 9.0 Hz, H-2′, 6′), 7.18

(2H, d, J = 9.0 Hz, H-3′, 5′), 3.71 (3H, s, H-OMe of C-4′), 5.63 (1H, d, J = 7.5 Hz,

H-Glc-I-1), 4.10 (1H, dd, J = 9.0, 7.5 Hz, H-Glc-I-2), 4.49 (1H, t, J = 9.0 Hz, H-Glc-I-3),

4.19 (1H, t, J = 9.0 Hz, H-Glc-I-4), 4.22 (1H, m, H-Glc-I-5), 4.07 (1H, m, HGlc-I-6),

4.60 (1H, br d, J = 11.5 Hz, H-Glc-6), 5.27 (1H, d, J = 8.0 Hz, H-Glc-II-1), 4.08 (1H, dd,

J = 9.0, 8.0 Hz, H-Glc-II-2), 4.27 (1H, t, J = 9.0 Hz, H-Glc-II-3), 4.09 (1H, t, J = 9.0 Hz,

H-Glc-II-4), 4.05 (1H, m, H-Glc-II-5), 4.80 (1H, dd, J = 12.0, 3.0 Hz, H-Glc-II-6), 4.86

(1H, dd, J = 12.0, 1.0 Hz, H-Glc-II-6), 2.00 (3H, s, H-Ac), 5.31 (1H, d, J = 7.5 Hz,

H-Glc-III-1), 4.09 (1H, dd, J = 9.0, 7.0 Hz, H-Glc-III-2), 4.18 (1H, dd, J = 9.5, 9.0 Hz,

H-Glc-III-3), 4.11 (1H, t, J = 9.5 Hz, H-III-4), 4.03 (1H, m, H-Glc-III-5), 4.32 (1H, dd,

J = 12.0, 5.0 Hz, H-Glc-III-6), 4.58 (1H, br d, J = 12.0 Hz, H-Glc-III-6), 5.41 (1H, br s,

H-Rha-1), 4.64 (1H, dd, J = 3.5, 1.5 Hz, H-Rha-2), 4.52 (1H, dd, J = 9.0, 3.5 Hz,

H-Rha-3), 4.18 (1H, m, H-Rha-4), 4.25 (1H, m, H-Rha-5), 1.54 (1H, d, J = 1.5 Hz,

H-Rha-6); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 164.6 (C-2), 104.6 (C-3), 182.8 (C-4),

162.6 (C-5), 100.9 (C-6), 164.0 (C-7), 95.5 (C-8), 157.8 (C-9), 106.6 (C-10), 123.6

(C-1′), 128.8 (C-2′, -6′), 115.0 (C-3′, -5′), 163.1 (C-4′), 55.5 (C-OMe of 4′), 100.0

(CGlc-I-1), 84.7 (C-Glc-I-2), 77.3 (C-Glc-I-3), 69.9 (C-Glc-I-4), 77.2 (C-Glc-I-5), 67.2

(C-Glc-I-6), 104.4 (C-Glc-II-1), 85.2 (C-Glc-II-2), 77.5 (C-Glc-II-3), 70.6 (C-Glc-II-4),

74.9 (C-Glc-II-5), 64.2 (C-Glc-II-6), 179.0 (C-Ac-C=O), 20.7 (C-Ac-Me), 106.7

(C-Glc-III-1), 76.4 (C-Glc-III-2), 77.7 (C-Glc-III-3), 71.1 (C-Glc-III-4), 79.3

(C-Glc-III-5), 62.4 (C-Glc-III-6), 102.3 (C-Rha-1), 72.0 (C-Rha-2), 72.7 (C-Rha-3), 74.0 (C-Rha-4), 69.7 (C-Rha-5), 18.5 (C-Rha-6); HRFABMS (positive) m/z 959.3026 $[M+H]^+$ (calcd for $C_{42}H_{55}O_{25}$, 959.3032).

Diosmetin-7-*O*-β-D-glucopyranosyl-(1→2)-6-*O*-acetyl-β-D-glucopyranosyl-(1→2)-[α-L -rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (**4**): colorless, amorphous solid; $[α]^{21}_D$ −49.2 (*c* 1.30, DMSO); ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz), see Table. 2-1; HRFABMS (positive) m/z 975.2988 $[M+H]^+$ (calcd for $C_{42}H_{55}O_{26}$, 975.2981).

Apigenin-7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyra nosyl-(1→6)]-β-D-glucopyranoside (**5**): colorless, amorphous solid; $[α]^{21}_D$ −71.0 (*c* 0.31, DMSO); ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz), see Table. 2-1; HRFABMS (positive) m/z 967.2713 $[M+Na]^+$ (calcd for $C_{41}H_{52}O_{25}Na$, 967.2694).

Benzyl-2-*O*-*trans*-*p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**7**): colorless powder; $[α]^{21}_D$ −32.7 (*c* 2.81, MeOH); ^{1}H-NMR (methanol-d_4, 400 MHz) and ^{13}C-NMR (methanol-d_4, 100 MHz), see Table. 2-2; HRFABMS (positive) m/z 563.2117 $[M+H]^+$ (calcd for $C_{28}H_{35}O_{12}$, 563.2129).

Benzyl-2-*O*-*cis*-*p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**8**): colorless powder; $[α]^{23}_D$ −26.7 (*c* 0.39, MeOH); ^{1}H-NMR (methanol-d_4, 400 MHz) and ^{13}C-NMR (methanol-d_4, 100 MHz), see Table. 2-2; HRFABMS (positive) m/z 563.2129 $[M+H]^+$ (calcd for $C_{28}H_{35}O_{12}$, 563.2129).

Benzyl-4-*O*-*trans*-*p*-coumaroyl-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**9**): colorless powder; $[α]^{21}_D$ −20.2 (*c* 9.68, MeOH); ^{1}H-NMR (methanol-d_4, 400 MHz) and ^{13}C-NMR (methanol-d_4, 100 MHz), see Table. 2-2; HRFABMS (positive) m/z 563.2143

[M + H]$^+$ (calcd for C$_{28}$H$_{35}$O$_{12}$, 563.2129).

[(Benzoxy)methyl]phenyl-4-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**12**): colorless powder; [α]^{21}$_D$ −56.3 (*c* 1.44, MeOH); ^{1}H-NMR (DMSO-*d*$_6$, 400 MHz) δ 7.40 (2H, d, J = 9.0 Hz, H-2, -6), 7.00 (2H, d, J = 9.0 Hz, H-3, -5), 5.27 (2H, s, H-7), 7.96 (2H, d, J = 7.5 Hz, H-2′, -6′), 7.51 (2H, br t, J = 7.5 Hz, H-3′, -5′), 7.65 (1H, br t, J = 7.5 Hz, H-4′), 5.00 (1H, d, J = 7.5 Hz, H-Glc-1), 3.47 (overlapped, H-Glc-2), 3.45 (overlapped, H-Glc-3), 3.18 (overlapped, H-Glc-4), 3.34 (overlapped, H-Glc-5), 3.44 (1H, dd, J = 12.5, 5.0 Hz, H-Glc-6), 3.68 (overlapped, H-Glc-6), 5.12 (1H, d, J = 1.5 Hz, H-Rha-1), 3.67 (overlapped, H-Rha-2), 3.33 (dd, J = 9.5, 2.5 Hz, H-Rha-3), 3.18 (overlapped, H-Rha-4), 3.84 (m, H-Rha-5), 1.17 (3H, d, J = 6.5 Hz, H-Rha-6); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 129.3 (C-1), 129.1 (C-2, -6), 115.8 (C-3, -5), 157.1 (C-4), 65.9 (C-7), 129.6 (C-1′), 129.8 (2′, 6′), 128.7 (C-3′, -5′), 133.3 (C-4′), 165.6 (C-7′), 98.2 (C-Glc-1), 76.3 (C-Glc-2), 77.4 (C-Glc-3), 71.9 (C-Glc-4), 76.8 (C-Glc-5), 60.5 (C-Glc-6), 100.3 (C-Rha-1), 70.4 (C-Rha-2), 70.5 (C-Rha-3), 69.8 (C-Rha-4), 68.2 (C-Rha-5), 18.0 (C-Rha-6); HRFABMS (positive) m/z 559.1797 [M+H]+ (calcd for C$_{26}$H$_{32}$O$_{12}$Na, 559.1791).

[(3-Hydroxy-3-methylglutaryl)methyl]phenyl-4-*O*-β-D-glucopyranoside (**13**): colorless powder; [α]^{21}$_D$ −35.7 (c 0.28, MeOH); ^{1}H-NMR (methanol-*d*$_4$, 400 MHz) δ 7.31 (2H, d, J = 9.0 Hz, H-2, -6), 7.08 (2H, d, J = 9.0 Hz, H-3, -5), 5.07 (2H, s, H-7), 2.70 (2H, br s, H- 2′), 2.63 (2H, s, H-4′), 1.35 (3H, s, H-6′), 4.90 (1H, d, J = 7.5 Hz, HGlc-1), 3.35−4.00 (overlapped, H-Glc-2, -3, -4, -5), 3.70 (dd, J = 12.0, 5.0 Hz, H-Glc-6), 3.89 (dd, J = 12.0, 2.0 Hz, H-Glc-6); ^{13}C-NMR (methanol-*d*$_4$, 100 MHz) δ 131.4 (C-1), 130.9 (C-2, 6), 117.7 (C-3,5), 159.1 (C-4), 66.9 (C-7), 172.5 (C-1′), 46.3 (C-2′), 70.7 (C-3′), 45.8 (C-4′), 174.8 (C-5′), 27.7 (C-6′), 102.3 (C-Glc-1), 74.9 (C-Glc-2), 78.2 (C-Glc-3),

71.4 (C-Glc-4), 78.0 (C-Glc-5), 62.5 (C-Glc-6); HRFABMS (positive) m/z 431.1558 [M+H]$^+$ (calcd for $C_{19}H_{27}O_{11}$, 431.1553).

7.2.2 Acid hydrolysis and identification of sugar components

Compounds **1**, **7**, **12** (each 5.0 mg), and **13** (1.0 mg) were separately hydrolyzed with 7% HCl (1 mL) at 60°C for 2 h. Each reaction mixture was neutralized using an Amberlite IRA400 column, (Sigma-Ardrich Co. LLC., St. Louis, MO, USA), and each eluate was concentrated. Residues were individually stirred with L-cysteine methyl ester (5 mg) and *O*-tolyl isothiocyanate (10 μL) in pyridine (0.5 mL), using the procedure reported by Tanaka and colleagues (Tanaka et al., 2007).

Each of the mixtures was analyzed by HPLC (column, Cosmosil 5C$_{18}$-AR II column, 4.6 × 250 mm, Nacalai tesque; mobile phase, CH$_3$CN-0.2% TFA in H$_2$O (25:75), 1.0 mL/min; detector, UV at 210 nm) at 20°C. D-Glucose (t_R 22.3 min) was identified as the glycosidic moieties of **1**, **7**, **12**, and **13** by comparison with authentic samples of D-glucose derivatives (t_R 22.3 min) and L-glucose (t_R 20.3 min) derivatives. L-Rhamnose (t_R 37.6 min) was identified as the glycosidic moiety of **1**, **7**, and **12** by comparison with authentic samples of L-rhamnose (t_R 37.6 min) and D-rhamnose (using D-cysteine methyl ester and L-rhamnose, t_R 21.0 min) derivatives.

7.3 *Dracocephalum foetidum*

Aerial parts of flowering *D. foetidum* were collected in July 2012 in Mongolia (N 47°47.188′; E 106°40.335′). Prof. Ch. Sanchir from the Institute of Botany, Mongolian Academy of Sciences, identified the plant species. A voucher specimen (№ 90.7.1.12A) is deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia.

7.3.1 Extraction and isolation

Dried aerial parts of *D. foetidum* (315 g) were extracted with acetone-H_2O (8:2) at room temperature for 2 weeks (3.0 L). The extract was concentrated at reduced pressure, and the solid (97.2 g) residue was suspended in H_2O (1.0 L) and subjected to extraction with Et_2O (2 × 1 L). The aqueous layer extract (77.97 g) was dissolved in H_2O, passed through a porous polymer gel (Diaion HP-20, 70 × 180 mm), and eluted with H_2O (fraction 1A, 59.64 g), MeOH-H_2O (1:4) (fr. 1B, 3.65 g), MeOH-H_2O (2:3) (fr. 1C, 3.70 g), MeOH-H_2O (3:2) (fr. 1D, 4.86 g), MeOH-H_2O (4:1) (fr. 1E, 4.98 g), and MeOH (fr. 1F, 2.62 g). Fr. 1C was applied to a reversed-phase column using an ODS-packed column (ODS-SM-50C-M, Yamazen Co., Osaka, Japan) and was eluted with MeOH/H_2O (20:80, V/V) (frs. 2B-2E), MeOH/H_2O (30:70, V/V) (frs. 2F-2I), and MeOH/H_2O (40:60, V/V) (frs. 2J-2K). Compound **53** (29.1 mg) was purified from fr. 2B (1.03 g); **37** (1.8 mg), **40** (34.7 mg), **41** (4.8 mg), **52** (0.6 mg) were from fr. 2C (661.1 mg); **35** (73.6 mg), **40** (8.4 mg), **52** (1.5 mg) were from fr. 2D, E (543.1 mg); **35** (7.5 mg) was from fr. 2F, G (78.6 mg); **53** (4.1 mg) was from fr. 2H (35.9 mg); **35** (33.7 mg) was from fr. 2K (93.2 mg), by HPLC [columns: AR-II, mobile phases CH_3CN/H_2O containing 0.2% TFA (15:85→20:80, V/V); 5PE-MS, mobile phases CH_3CN/H_2O containing 0.2% TFA (20:80, V/V); Mightysil RP-18, mobile phases CH_3CN/H_2O containing 0.2% TFA (15:85→20:80→30:70, V/V)]. Fr. 1D was applied to a reversed-phase column using an ODS-packed column (ODS-SM-50C-M) and eluted with MeOH/H_2O (30:70, V/V) (frs. 3A-3F), MeOH/H_2O (40:60, V/V) (frs. 3G-3L), and MeOH/H_2O (50:50, V/V) (frs. 3M-3N). Compound **35** (42.1 mg) was purified from fr. 3H (61.1 mg); **52** (46.9 mg) was from fr. 3K (117.4 mg), by HPLC [columns: AR-II, mobile phase CH_3CN/H_2O containing 0.2% TFA (20:80, V/V); C_{30}-UG-5, mobile phase CH_3CN/H_2O containing 0.2% TFA (25:75, V/V)]. Fr. 1E was subjected to

reversed-phase CC using an ODS-packed column (ODS-SM-50C-M) and was eluted with MeOH/H_2O (40:60, V/V) (frs. 4C-4D), MeOH/H_2O (50:50, V/V) (frs. 4E-4G), and MeOH/H_2O (60:40, V/V) (frs. 4H-4I). Fr. 4C (401.8 mg) was subjected to HPLC [columns: AR-II, mobile phases CH_3CN/H_2O containing 0.2% TFA (25:75, 30:70, 40:60, V/V); C_{30}-UG-5, mobile phases CH_3CN/H_2O containing 0.2% TFA (25:75, 30:70, 35:65, V/V); Mightysil RP-18, mobile phases CH_3CN/H_2O containing 0.2% TFA (25:75, 30:70, 35:65, V/V)] to yield compounds **36** (1.8 mg), **38** (13.2 mg), **39** (3.5 mg), **50** (12.6 mg), **51** (31.3 mg), and **54** (6.7 mg). Compounds **32** (27.3 mg), **34** (7.1 mg), and **50** (18.9 mg) were purified from fr. 4D (297.0 mg); **30** (8.4 mg), **31** (13.8 mg), **46** (6.8 mg), **47** (4.2 mg), and **50** (17.9 mg) were from fr. 4E (474.5 mg); **30** (11.9 mg), **33** (3.7 mg), **47** (4.1 mg), **48** (5.4 mg), and **49** (1.5 mg) were from fr. 4F, G (470.0 mg), by HPLC with same condition mentioned above. Fr. 1F was applied to a reversed-phase column using an ODS-packed column (ODS-SM-50C-M) and eluted with MeOH/H_2O (50:50, V/V) (frs. 5A-5H), MeOH/H_2O (60:40, V/V) (frs. 5I-5J) and MeOH/H_2O (80:20, V/V) (frs. 5K-5M). Each fraction was subjected to HPLC [columns: AR-II, C_{30}-UG-5, mobile phases CH_3CN/H_2O containing 0.2% TFA (30:70 and 35:65, V/V); Mightysil RP-18, mobile phases CH_3CN/H_2O containing 0.2% TFA (25:75, 30:70, and 35:65, V/V) and MeOH/H_2O containing 0.2% TFA (30:70, V/V)] to yield the compounds described below. Compound **50** (64.1 mg) was from fr. 5D (216.2 mg); **44** (5.7 mg) and **46** (20.2 mg) were from fr. 5E (363.1 mg); **42** (2.5 mg), **43** (21.0 mg), **45** (1.2 mg), **46** (1.4 mg), **47** (7.2 mg), **49** (2.2 mg), and **50** (26.5 mg) were from fr. 5F (339.8 mg); **42** (0.8 mg), **46** (4.2 mg), **48** (93.8 mg), and **55** (5.7 mg) were from fr. 5G (216.7 mg); **43** (10.9 mg), **48** (33.5 mg), and **55** (1.6 mg) were from fr. 5H (211.6 mg); **44** (9.1 mg) and **50** (5.8 mg) were from fr. 5I, J (284.6 mg).

Data

2-[4-Methyl-1-cyclohex-3-enyl]prop-2-en-1-O-6-malonyl-β-D-glucopyranosyl-(1→2)-6-malonyl-β-D-glucopyranoside (**30**): Colorless amorphous solid; $[\alpha]^{23}_{D}$ -32.2 (c 1.03) MeOH; HRFABMS (positive) m/z 671.2162 [M+Na]$^+$ (calcd for $C_{28}H_{40}O_{17}Na$, 671.2162)

2-[4-Methyl-1-cyclohex-3-enyl]prop-2-en-1-O-β-D-glucopyranosyl-(1→2)-6-malonyl-β-D-glucopyranoside (**31**): Colorless amorphous solid; $[\alpha]^{23}_{D}$ -42.1 (c 1.34) MeOH; HRFABMS (positive) m/z 585.2156 [M+Na]$^+$ (calcd for $C_{25}H_{38}O_{14}Na$, 585.2159)

2-[4-Methyl-1-cyclohex-3-enyl]prop-2-en-1-O-6-malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**32**): Colorless amorphous solid; $[\alpha]^{24}_{D}$ -47.0 (c 2.6) MeOH; HRFABMS (positive) m/z 585.2167 [M+Na]$^+$ (calcd for $C_{25}H_{38}O_{14}Na$, 585.2159)

4-O-(8-Z-Isoferuloyl)-rosmarinic acid (**34**): Colorless amorphous solid; $[\alpha]^{21}_{D}$ +36.56 (c 0.64) MeOH; HRFABMS (positive) m/z 553.1358 [M+H]$^+$ (calcd for $C_{28}H_{25}O_{12}$, 553.1346)

3-(6-Malonyl-β-D-glucopyranosyl)-rosmarinic acid (**37**): Colorless amorphous solid; $[\alpha]^{23}_{D}$ -24.3 (c 0.14) MeOH; HRFABMS (positive) m/z 609.1428 [M+H]$^+$ (calcd for $C_{27}H_{29}O_{16}$, 609.1455); CD (c = 0.2, MeOH) nm ([θ]): 293 (2300), 259 (2800), 220 (-3300), 207 (-5500); UV (MeOH) λ_{max} (log ε): 323 (4.06), 287 (4.09).

3-(6-Feruloyl-β-D-glucopyranosyl)-rosmarinic acid (**38**): Colorless amorphous solid; $[\alpha]^{22}_{D}$ -37.1 (c 1.1) MeOH; HRFABMS (positive) m/z 721.1727 [M+Na]$^+$ (calcd for $C_{34}H_{34}O_{16}Na$, 721.1743); CD (c = 0.2, MeOH) nm ([θ]): 337 (2700), 294 (8200), 262 (3600), 226 (-2400), 205 (-6400); UV (MeOH) λ_{max} (log ε): 321 (4.16).

3-(6-Synapoyl-β-D-glucopyranosyl)-rosmarinic acid (**39**): Colorless amorphous solid; $[\alpha]^{22}_{D}$ -37.9 (c 0.28) MeOH; HRFABMS (positive) m/z 751.1843 [M+Na]$^+$ (calcd for

C$_{35}$H$_{36}$O$_{17}$Na, 751.1849); CD (c = 0.2, MeOH) nm ([θ]): 295 (7500), 259 (3400), 238 (-1500), 222 (-1300), 206 (-9800); UV (MeOH) λ_{max} (log ε): 321 (4.16).

3-O-β-D-Glucopyranosyl-7,8-*cis*-rosmarinic acid (**41**): Colorless amorphous solid; [α]^{22}$_D$ -59.4 (c 0.64) MeOH; HRFABMS (positive) m/z 523.1459 [M+H]$^+$ (calcd for C$_{24}$H$_{27}$O$_{13}$, 523.1451); CD (c = 0.2, MeOH) nm ([θ]): 312 (1600), 275 (-1400), 224 (-4200), 209 (-5100); UV (MeOH) λ_{max} (log ε): 316 (4.07), 287 (4.11).

Acacetin-7-O-(3-O-malonyl)-β-D-glucopyranoside (**42**): Colorless amorphous powder; [α]^{25}$_D$ -19.8 (c 0.33) DMSO; HRFABMS (positive) m/z 533.1290 [M+H]$^+$ (calcd for C$_{25}$H$_{25}$O$_{13}$, 533.1295); UV (MeOH) λ_{max} (log ε): 325 (4.10), 270 (4.07).

Acacetin-7-O-(3,6-O-dimalonyl)-β-D-glucopyranoside (**43**): Colorless amorphous powder; [α]^{23}$_D$ -2.9 (c 3.19) DMSO; HRFABMS (positive) m/z 619.1284 [M+H]$^+$ (calcd for C$_{28}$H$_{27}$O$_{16}$, 619.1298); UV (MeOH) λ_{max} (log ε): 326 (4.13), 270 (4.08).

Acacetin-7-O-(2-O-acetyl)-β-D-glucuronopyranoside (**44**): Colorless amorphous powder; [α]^{25}$_D$ -17.7 (c 1.48) DMSO; HRFABMS (positive) m/z 503.1176 [M+H]$^+$ (calcd for C$_{24}$H$_{23}$O$_{12}$, 503.1189); UV (MeOH) λ_{max} (log ε): 326 (4.11), 270 (3.85).

Acacetin-7-O-(2-O-malonyl)-β-D-glucuronopyranoside (**45**): Colorless amorphous powder; [α]^{24}$_D$ -57.5 (c 0.12) DMSO; HRFABMS (positive) m/z 547.1093 [M+H]$^+$ (calcd for C$_{25}$H$_{23}$O$_{14}$, 547.1087); UV (MeOH) λ_{max} (log ε): 326 (4.25), 270 (4.20).

Acacetin-7-O-(3-O-malonyl)-β-D-glucuronopyranoside (**46**): Colorless amorphous powder; [α]^{22}$_D$ -13.5 (c 3.38) DMSO; HRFABMS (positive) m/z 547.1093 [M+H]$^+$ (calcd for C$_{25}$H$_{23}$O$_{14}$, 547.1087); UV (MeOH) λ_{max} (log ε): 325 (4.04), 270 (4.00).

7.3.2 Acid hydrolysis and identification of sugar components

Compounds **30** (1 mg), **31** (1.5 mg), **38** (1.9 mg), **43** (2.5 mg), **44** (2.0 mg), and **46** (7.6 mg), were separately hydrolyzed with 2N HCl (0.5 ml) at 60°C for 3 h. Each

reaction mixture was neutralized using an Amberlite IRA400 column, (Sigma-Ardrich Co. LLC., St. Louis, MO, USA), and each eluate was concentrated. Residues were individually stirred with L-cystein methyl ester (5 mg) and O-tolyl isothiocyanate (20 μL) in pyridine (0.5 ml), using the procedure reported by Tanaka and colleagues (Tanaka et al., 2007).

Each of the mixtures was analyzed by HPLC (column, Cosmosil $5C_{18}$-AR II column, 4.6×250 mm, Nacalai tesque; mobile phase, CH_3CN-0.2% TFA in H_2O (25:75), 1.0 ml/min; detector, UV at 210 nm) at 20°C. D-Glucose (t_R 16.4 min) was identified as the glycosidic moiety of **30**, **31**, **38**, and **43** in comparison with the authentic samples of D-glucose derivatives (t_R 16.4 min) and L-glucose derivatives (t_R 15.3 min). D-Glucuronic acid (t_R 16.2 min) was identified as the glycosidic moiety of **44** and **46** by comparison with authentic samples of D-glucuronic acid derivatives (t_R 16.2 min) and L-glucuronic acid derivatives (using D-cysteine methyl ester and D-glucuronopyranoside, t_R 15.1 min).

7.3.3 Alkaline hydrolysis of compounds 37-39 and 41 and condensation with (*S*)-phenylglycine methyl ester

Each compound (**37-39** and **41**: each 1.0 mg) was dissolved in 10% NaOH (0.5 mL) and stirred for 2 h at room temperature. Each of the reaction mixtures was passed through a Dowex 50W×2 column (5×70 mm, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and eluted with H_2O (30 mL). Each residue was dissolved in DMF and (*S*)-phenylglycine methyl ester (PGME), benzotriazol-1-yl-oxy-tris-pyrrolidinophos-phonium hexafluorophosphate, 1-hydroxybenzotriazole, and N-methylmorpholine were added as reported previously (Murata et al., 2012). Each mixture was then stirred for 10 h at room temperature to give (*S*)-PGME amide; t_R = 17.1 min in the HPLC analysis

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[column, Shiseido Capcell Pak C18 column (4.6 × 250 mm, Shiseido, Tokyo, Japan); solvent, CH_3CN-H_2O containing 0.2% TFA (22.5:77.5); flow rate, 1.0 mL/min; detector, UV 210 nm]. The retention time of (*S*)-PGME amide of (2*R*)-3-(3,4-dihydroxy-phenyl)-2-hydroxypropanoic acid was 17.1 min and that of (*R*)-PGME amide of (2*R*)-3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid was 17.8 min, which corresponded with authentic samples.

7.4 *Chamaerhodos erecta* and *Chamaerhodos altaica*

Aerial parts of *C*. *erecta* were collected in Tuv province (N 47˚46.016′; E 107˚34.430′), Mongolia in July, 2009. Voucher specimens (№ 48.16.5.09A) are deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia. The aerial parts of *C*. *altaica* were collected in Tuv province (N 48˚04.459′; E 106˚22.098′), Mongolia in June, 2011. Voucher specimens (№ 48.16.2.11A) are deposited at the same herbarium. Prof. Ch. Sanchir from the Institute of Botany, Mongolian Academy of Sciences identified both plant species.

7.4.1 Extraction and Isolation

Dried aerial parts of *C*. *erecta* (450 g) were chopped into small pieces and fully extracted with 4.5 L MeOH at ambient temperature to yield 67 g of a thick extract after evaporation *in vacuo* at 40°C. The thick extract was suspended in H_2O and successively fractionated with $CHCl_3$ and *n*-BuOH to yield 18 g and 19 g, respectively. Each fraction including the MeOH extract and H_2O residue was analyzed by TLC in the solvent systems $CHCl_3$-MeOH (9:1) and $CHCl_3$-MeOH-H_2O (7:3:0.4), and detected by spraying with NP/PEG and 5% H_2SO_4 following heating at 110°C. The *n*-BuOH fraction (19 g) was dissolved in $CHCl_3$-MeOH (1:2) and subjected to column chromatography over

Sephadex LH-20 (100 g) eluted with 1900 ml of CHCl$_3$-MeOH (1:2) and 1900 ml of MeOH. Sixty eluents (50 ml each) were collected. According to the TLC analysis, the eluents were reduced to 16 fractions – Fr. 1 (96.3 mg), Fr. 2 (1.65 g), Fr. 3 (2.23 g), Fr. 4 (3.62 g), Fr. 5 (1.17 g), Fr. 6 (435.7 mg), Fr. 7 (253.5 mg), Fr. 8 (183.7 mg), Fr. 9 (194.5 mg), Fr. 10 (1.07 g), Fr. 11 (269.3 mg), Fr. 12 (235.7 mg), Fr. 13 (723.8 mg), Fr. 14 (487.7 mg), Fr. 15 (478.5 mg), and Fr. 16 (2.56 g). Fr. 4-9 were subjected to column chromatography (3 x 54 cm, 2 x 57 cm and 1.5 x 34 cm) with MCI-gel CHP 20P and reversed-phase sepra C18-E (2 x 60 cm, 1 x 40 cm and 0.5 x 35 cm) and eluted with MeOH-H$_2$O (60:40, 70:30, and 80:20, V/V). The final purification of isolated compounds was carried out by column chromatography (0.5 x 30 cm and 1 x 35 cm) with Sephadex LH-20 eluting with MeOH (60:40, 70:30, and 80:20, V/V) as well as by preparative chromatography on pre-coated silica gel 60 F$_{254}$ plates. Compound **1** (267 mg) from Fr. 4-7; **3** (90 mg) from Fr. 4 and 5; **9** (50 mg) from Fr. 5 and 6; **10** (7.0 mg) from Fr. 4-6; **11** (21 mg) from Fr. 4 and 5; **12** (7.0 mg) from Fr. 4 and 5; **13** (1.8 mg) from Fr. 8 and 9; **14** (0.8 mg) from Fr. 6 and 7; **15** (0.3 mg) from Fr. 6 and 7; **21** (3.0 mg) from Fr. 4; **22** (3.0 mg) from Fr. 4; and **39** (282 mg) from Fr. 5 and 6 were isolated. Whereas, compound **31** (6.7 mg) from Fr. 10 and 11; **32** (2.6 mg) from Fr. 16; **33** (14.1 mg) from Fr. 12-15; **34** (3.3 mg) from Fr. 15; **36** (10.6 mg) from Fr. 12-15; and **38** (20.0 mg) from Fr. 16 were isolated by using preparative HPLCs [columns, AR-II, mobile phase, CH$_3$CN/H$_2$O containing 0.2 % TFA (15:85, V/V) or (20:80); Mightysil RP-18 GP, mobile phase, CH$_3$CN/H$_2$O containing 0.2 % TFA (15:85 and 20:80, V/V)].

Aerial parts of *C. altaica* (457 g) were chopped into small pieces and fully extracted with 5.0 L acetone-H$_2$O (8:2) at 60°C to yield 39.3 g of an extract after evaporation *in vacuo* at 40°C. The extract was suspended in H$_2$O (2.0 L) and subjected

to extraction with diethyl ether (1.0 L) three times. The aqueous layer extract (23.8 g) was dissolved in H_2O and passed through a porous polymer gel (Diaion HP-20, 70 x 270 mm) eluted with H_2O (fr. 1A, 14.47 g), EtOH-H_2O (30:70) (fr. 1B, 3.10 g), and EtOH (fr. 1C, 6.56 g). The fr.1B was chromatographed on a reversed-phase column using an ODS packed column (ODS-SM-50C-M) and eluted with 10% (fr.s 2A-2G) and 30% MeOH (fr.s 2H-2M). Compounds were isolated by using preparative HPLCs [columns, AR-II, mobile phases CH_3CN/H_2O containing 0.2% TFA (15:85 and 20:80, V/V); 5PE-MS, mobile phases CH_3CN/H_2O containing 0.2% TFA (12.5:87.5, 15:85, and 20:80, V/V); C_{30}UG-5, mobile phases CH_3CN/H_2O containing 0.2% TFA (10:90, 15:85, and 20:80, V/V); Mightysil RP-18 GP, mobile phases CH_3CN/H_2O containing 0.2 % TFA (15:85 and 20:80, V/V)] from each fraction as below, **19** (4.4 mg), **29** (2.6 mg), and **37** (3.3 mg) from Fr. 2B and 2C; **19** (5.3 mg), **20** (3.8 mg), **26** (6.9 mg), and **28** (1.1 mg) from Fr. 2D; **18** (3.3 mg), **20** (1.9 mg), and **26** (6.8 mg) from Fr. 2E; **7** (0.7 mg), **18** (8.3 mg), **27** (1.1 mg), **30** (0.7 mg) and **31** (2.1 mg) from Fr. 2F; **35** (10.1 mg) from Fr. 2H; **7** (8.6 mg), **33** (1.3 mg), **35** (1.1 mg), and **38** (40.3 mg) from Fr. 2I; **10** (8.1 mg), and **25** (5.1 mg) from Fr. 2J; **6** (3.1 mg), **7** (4.5 mg), and **9** (41.8 mg) from Fr. 2K, 2L and 2M. Secondly, the aerial parts of *C. altaica* (380 g) were chopped into small pieces and fully extracted with 5.0 L acetone-H_2O (4:1) at 60°C to yield 54.6 g of an extract after evaporation *in vacuo* at 40°C. The extract was suspended in H_2O (1.5 L) and subjected to extraction with Et_2O (1.0 L) three times. The aqueous layer extract (32.7 g) was chromatographed on a reversed-phase column using ODS (Cosmosil 140C18-OPN, 150 g) and eluted with H_2O (Fr. 3A, 27.48 g), EtOH-H_2O (20:80) (Fr. 3B, 2.14 g), EtOH-H_2O (40:60) (Fr. 3C, 1.96 g), and EtOH-H_2O (80:20) (Fr. 3D, 0.81 g). Compounds **2** (18.8 mg), **5** (0.7 mg), **10** (2.8 mg), **11** (23.7 mg), **24** (1.2 mg), and **33**

(8.2 mg) from Fr. 3B; **3** (225.6 mg), **5** (2.9 mg), **8** (4.2 mg), **10** (0.7 mg), **11** (25.4 mg), **16** (17.9 mg), and **17** (3.9 mg) from Fr. 3C; **3** (0.9 mg), **4** (1.5 mg), and **17** (19.4 mg) from Fr. 3D were isolated.

Data

4,5-dihydroxybenzaldehyde-3-*O*-β-D-glucopyranoside (**56**): Colorless amorphous powder; $[\alpha]^{23}_D$ -111.1 (*c* 0.18, MeOH), HR-FAB-MS *m/z*: 339.0701 [M+Na]$_+$ (Calcd for $C_{13}H_{16}O_9Na$: 339.0691). ^{1}H-NMR (acetone-d_6 with 10% D_2O, 400 MHz) δ 9.72 (1H, s, C-7), 7.37 (1H, d, *J* = 2.0 Hz, H-2), 7.20 (1H, d, *J* = 2.0 Hz, H-6), 5.01 (1H, d, *J* = 8.0 Hz, H-Glc-1), 3.95 (1H, dd, *J* = 12.0, 2.0 Hz, H-Glc-6a), 3.79 (1H, dd, *J* = 12.0, 5.0, H-Glc-6b), 3.6-3.7 (3H, m, H-Glc-2, 3, and 5), 3.55 (1H, m, H-Glc-4). ^{13}C-NMR (acetone-d_6 with 10% D_2O, 100 MHz) δ 193.1 (C-7), 147.1 (C-3), 146.8 (C-5), 143.3 (C-4), 128.5 (C-1), 112.1 (C-6), 112.0 (C-2), 103.0 (C-Glc-1), 77.5 (CGlc-3), 76.6 (C-Glc-5), 74.0 (C-Glc-2), 70.5 (C-Glc-4), 61.8 (C-Glc-6). HMBC correlations: H-2/C-3, C-4, C-6; H-6/C-2, C-4, C-5; H-7/C-1, C-2, C-6; H-Glc-1/C-3.

Quercetin-3-*O*-β-D-glucuronopyranosyl-4`-*O*-β-D-glucopyranoside (**57**): $[\alpha]^{21}_D$ -58.3 (*c* 0.23, MeOH), HR-FAB-MS *m/z*: 641.1354 [M+H]$^+$ (Calcd for $C_{27}H_{28}O_{18}$: 641.1353). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 6.22 (1H, d, *J* = 2.0 Hz, C-6), 6.44 (1H, d, *J* = 2.0 Hz, H-8), 12.50 (1H, s, H-5-OH), 10.91 (1H, br s, H-7-OH), 7.67 (1H, d, *J* = 2.0 Hz, H-2′), 7.20 (1H, d, *J* = 8.5 Hz, H-5′), 7.58 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′), 8.94 (1H, br s, H-3′-OH), 5.50 (1H, d, *J* = 7.5, H-Glc A-1), 3.25 (overlapped, H-Glc A-2), 3.30 (overlapped, H-Glc A-3), 3.36 (overlapped, H-Glc A-4), 3.56 (1H, d, *J* = 9.5 Hz, H-Glc A-5), 4.88 (1H, d, *J* = 7.5, H-Glc-1), 3.20-3.40 (overlapped, H-Glc-2, 3, 4, and 5), 3.50 (1H, dd, *J* = 11.5, 5.0 Hz, H-Glc-6a), 3.73 (1H, br d, *J* = 11.5 Hz, H-Glc-6b). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 155.5 (C-2), 133.6 (C-3), 177.2 (C-4), 161.2 (C-5), 98.8 (C-6),

164.3 (C-7), 93.7 (C-8), 156.3 (C-9), 104.0 (C-10), 124.2 (C-1′), 116.4 (C-2′), 146.2 (C-3′), 147.6 (C-4′), 115.4 (C-5′), 121.0 (C-6′), 100.9 (C-Glc A-1), 73.8 (C-Glc A-2), 76.0 (C-Glc A-3), 71.3 (C-Glc A-4), 75.8 (C-Glc A-5), 169.6 (C- Glc A-6), 101.4 (C-Glc A-1), 73.3 (C-Glc A-2), 75.8 (C-Glc A-3), 69.7 (C-Glc A-4), 77.2 (C-Glc A-5), 60.6 (C-Glc A-6). HMBC correlations: H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-10; H-5-OH/C-5, C-6, C-10; H-2′/C-2, C-3′, C-4′; H-5′/C-1′, C-3′, C-4′; H-6′, C-2′, C-4′; H-Glc-1/C-4′. NOE correlations: H-5′/H-Glc-1; H-Glc A-1/H-Glc A-5.

7.4.2 Identification of sugar components

Compounds **56** (1.0 mg) and **57** (2.0 mg) were hydrolyzed with 7% HCl (0.5 mL) at 60°C for 1 h. The reaction mixture was concentrated. Residues were individually stirred with L-cysteine methyl ester (5 mg) and *O*-tolyl isothiocyanate (10 μL) in pyridine (0.4 mL), as reported by Tanaka and colleagues (Tanaka et al., 2007). The reaction mixture of **56** was analyzed by HPLC (column, Cosmosil 5C$_{18}$–AR II, 4.6 x 250 mm; mobile phase, CH$_3$CN-0.2 % TFA in H$_2$O (25:75), 1.0 mL/min; detector, UV at 210 nm) at 20°C. D-Glucose (t_R 15.7 min) was identified as the sugar moiety of **56** based on comparisons with authentic samples of D-glucose derivative (t_R 15.7 min) and L-glucose derivative (t_R 14.3 min). The reaction mixture of **57** was analyzed by HPLC (column, Develosil C$_{30}$-UG-5, 4.6 x 250 mm; mobile phase, CH$_3$CN-0.2 % TFA in H$_2$O (25:75), 0.8 mL/min; detector, UV at 210 nm) at 20°C. D-Glucose (t_R 33.1 min) was identified as the sugar moiety of **56** based on comparisons with authentic samples of D-glucose derivative (t_R 33.1 min) and L-glucose derivative (t_R 30.6 min). D-Glucuronic acid (t_R 34.5 min) was identified as the sugar moiety of **57** based on comparisons with authentic samples of D-glucuronic acid derivative (t_R 34.5 min) and L-glucuronic acid derivative (using D-cystein methyl ester and D-glucuronic acid, t_R 34.1 min).

7.5 Hyaluronidase inhibitory assay

Assays were conducted in accordance with the Morgan-Elson method, which was modified by Davidson and Aronson. Hyaluronidase activity was measured as described previously (Murata et al., 2010). Disodium cromoglicate (Wako Pure Chemical Industries, Ltd.) was used as a positive control. The final concentration of hyaluronidase (Type IV-S from Bovine Testes, Sigma-Aldrich Co. LLC.) was 400 units/mL. Sodium hyaluronate from Cockscomb was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan).

7.6 Measurement of DPPH radical scavenging activity

A modified version of the method of Mensor (Mensor et al., 2001) was used to measure the free radical scavenging activity of the pure compounds with DPPH. In a 96-well microplate, of sample (100 μL) at different concentrations (in absolute MeOH) was added into wells containing of 0.06 mM DPPH in MeOH (100 μL) and was mixed well. The absorbance was measured at 510 nm exactly at 30 min by a microplate reader ImmunoMini NJ-2300 (Cosmo Bio Co., Ltd., Tokyo, Japan), and the percent inhibition was calculated. IC_{50} values expressed the concentration of the sample required to scavenge 50% of the DPPH free radicals. All samples were run in triplicate. Rutin (Wako Pure Chemical Industries, Ltd. Japan) and Trolox (Tokyo Chemical Industry Co., Ltd. Japan) were used as positive controls.

7.7 AGEs production inhibitory assay

The assay was carried out in accordance with the previous report (Shimoda et al., 2011). D-Glucose (10%) and BSA (1%) were dissolved in PBS (pH 7.2). Samples were dissolved in PBS including 10% DMSO (final concentration 300 μM). The glucose-BSA solution (900 μl) and sample solution (100 μl) were mixed, and the

mixture was incubated for 48 h at 60°C. Fluorescence (F) was measured at 440 nm excited at 375 nm, after the mixture was diluted (1:10). The inhibitory activity was calculated as follows: Inhibitory activity (%) = [1 − (Fsample − Fsample$_{Blank}$)/(F control − F normal)] x 100. PBS buffer was added in place of the sample solution in PBS as a control (F control). F sample: sample solution and glucose-BSA solution, F sample$_{Blank}$: sample solution without incubation, F normal: glucose-BSA solution without incubation. Quercetin (**14**) and kaempferol (**15**) were used as positive controls (Shimoda et al., 2011; Wu and Yen, 2005). All data are expressed as the mean ± standard error (S.E.).

7.8 Tyrosinase inhibitory assay

Tyrosinase activity inhibition was assayed using spectrometric method (Sasaki and Yoshizaki, 2002) with l-dopa as the substrate. The assay medium, consisting of 0.1 mL of mushroom tyrosinase solution (625 units/mL), 0.9 mL of 1/15 M phosphate buffered saline (PBS) buffer solution (2.0mM), 1.0 mL of sample solution containing 5mM dimethyl sulfoxide (DMSO), was mixed and pre-incubated at 25 °C for 10 min. Then, a reaction was carried out adding 0.03% l-dopa solution. A control reaction (A) was conducted without the test sample, and a blank reaction (B) was used for non-active heated mushroom tyroshinase. The absorbance was measured at 475 nm after incubation, giving the sample value as C. The percentage of inhibition of tyrosinase was calculated as follows: tyrosinase inhibition (%)=(A-C)/(A-B)x100, where the absorbance value for 5 min incubation was determined. Kojic acid was used as the positive control.

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I hope that I can become a bridge between Japan and Mongolia. I also hope that someday I will be able to set up an excellent research institute in Mongolia, so that our future generation can produce high quality research in Mongolia.

<div align="right">

I HAVE A DREAM *(Martin Luther King)*
Erdenechimeg SELENGE
2014 年 2 月 20 日

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