Studies on Nepalese Medicinal Resources: Chemical Analysis and Biological Activities of *Diplomorpha canescens* and their Comparison with *Diplomorpha ganpi* and *Diplomorpha sikokiana* from Japan

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2013

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A thesis submitted to the Graduate School of Pharmaceutical Sciences, Kumamoto University for the partial fulfillment of a degree of

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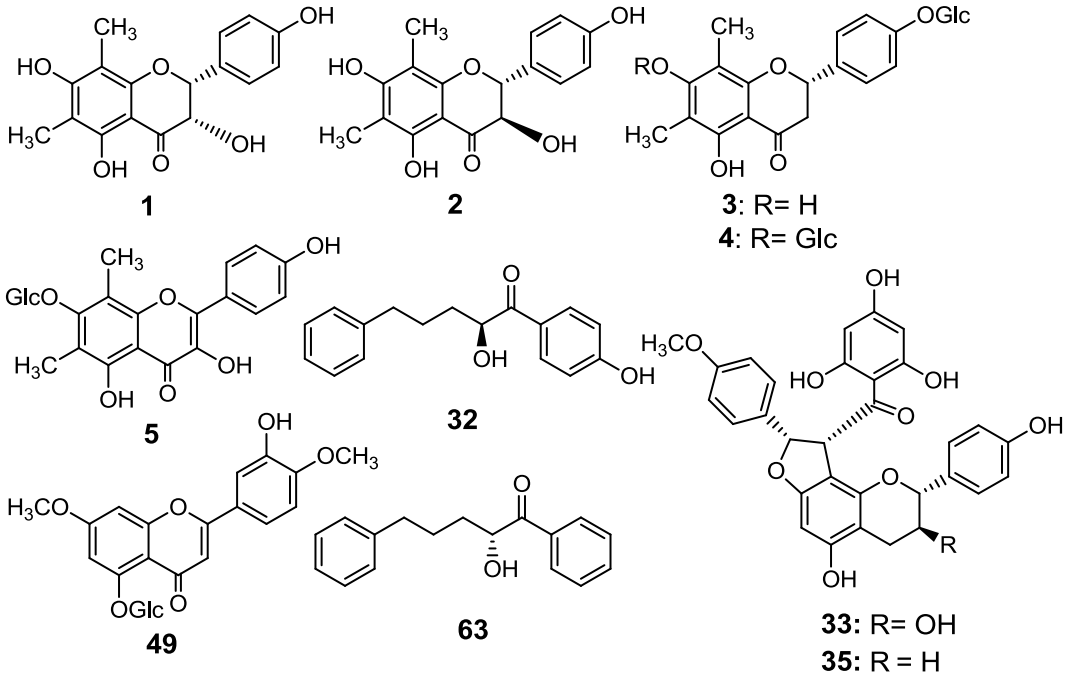
Diplomorpha canescens is a widely abundant plant in the hilly region of Nepal and traditionally used as the remedy for toothache. Roots and flowers of *D. canescens* are used for the treatment of various disorders in traditional Chinese medicine. In addition, it has commercial importance as the main ingredient for the preparation of handmade paper and current paper currency. Therefore this study is aimed to explore the medicinal and commercial importance of *D. canescens* from Nepal. For this purpose, detailed chemical analysis was carried out on one species *D. canescens* from Nepal and two species *D. ganpi* and *D. sikokiana* from Japan. In total, 8 new compounds together with 40 known compounds were isolated from *D. canescens* and their structures were elucidated based on the mainly spectroscopic and some chemical methods. In addition, 2 new and 33 known compounds from *D. ganpi* and 23 known compounds from *D. sikokiana* were isolated and identified. All these compounds from *D. canescens* and *D. ganpi* were reported for the first time. Moreover, antioxidative activity and tyrosinase inhibitory activity on some of the isolated compounds were carried out.

Six new compounds such as (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**), (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**), farrerol 4'-*O*-*β*-D-glucopyranoside (**3**), diplomorphanin A (**4**) diplomorphanin B (**5**) and diplomorphanone A (**32**) together with 26 known compounds from the aerial parts and 2 new compounds such as 14''-*O*-methyldihydrodaphnodorin B (**33**) and 14''-*O*-methyldaphnodorin J (**35**) along with 16 known compounds were isolated from the roots of *D. canescens*.

One new compound, pilloin 5-*O*-*β*-D-glucopyranoside (**49**) along with 22 known compounds from the stems and 1 new compound, diplomorphanone B (**63**) along with 12 known compounds from the roots of *D. ganpi* were isolated and their structure were identified.

Fourteen known compounds from the stems, 13 known compounds from the roots and 5 known compounds from the leaves of *D. sikokiana* were isolated.



Structures of new compounds isolated from *Diplomorpha* plants

Among these isolated compounds, 19 compounds including flavonoids, lignans and chlorogenic acid were evaluated for their antioxidant activities. Quercetin (**11**), luteolin 7-methyl ether (**51**), hypolaetin 8-*O*-*β*-D–glucuronopyranoside (**53**), kaempferol (**8**), luteolin 7-methyl ether-5-*O*-*β*-D–glucopyranoside (**19**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), quercetin 3-*O*-*β*-D–rhamnopyranoside (**52**), chlorogenic acid (**58**), (-)-pinoresinol (**22**) and (-)-syringaresinol 4-*O*-*β*-D–glucopyranoside (**44**) showed potent antioxidant activity with Trolox equivalent (mmol TE/mol) being 2117, 1962, 1888, 1581, 1312, 1215, 1133, 842, 841 and 650, respectively.

Similarly, 30 of the isolated compounds including flavonoids, biflavonoids and lignans were evaluated for their mushroom tyrosinase inhibitory activity. (-)-Syringaresinol (**43**) was the most potent compound with 96.3±2.1% inhibition followed by quercetin (**11**), kaempferol (**8**), farrerol 7-*O*-*β*-D–glucopyranoside (**6**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), genkwanin 5-*O*-*β*-D–glucopyranoside (**16**), rhamnocitrin 3-*O*-*β*-D–glucopyranoside (**10**), apigenin (**14**), syringin (**43**), 3(*S*)-hydroxy-1,5-diphenylpentanone (**65**) and rhamnetin 3-*O*-*β*–D–glucopyranoside (**13**).

Acknowledgement

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1. Introduction

1.1. Medicinal plants, drug discovery and traditional medicines

Medicinal plants have been used as therapeutic agents from ancient times and they also serve as important source of modern drugs.[1-5)] Plant derived natural products have played an important role in modern drug discovery and development.[4)] About 25% of the drugs prescribed worldwide come from plants.[2)] Taxol from *Taxus brevifolia* and *Taxus baccata,* vinblastine and vincristine from *Catharanthus roseus*, digoxin from *Digitalis* spp., morphine from *Papaver somniferum* are some examples of drugs of natural product origin in market.[2-4)] More than 60% of anti-tumor and anti-infective drugs already on market or under clinical trial are estimated to be of plant origin.[2,5)] Specially for oncological and antihypertensive area, natural products are the main sources of new drugs.[4)] Many new molecules based on the natural products of plant origin are under clinical trial.[4,5)]

Medicinal plant based traditional medicine is the dominant or perhaps only form of primary healthcare for at least 4.5 billion people.[1)] More than 60% of the world population and 60–90% of the population of developing countries (80% in Nepal , 70% in India, 80% in Pakistan, 65% in Sri Lanka, 90% in Bangladesh, 85% in Burma, and 60% in Indonesia) rely on traditional medicine, and about 85% of these traditional remedies are derived from plants.[6)] All the traditional medicine systems in the world including Ayurveda, traditional Chinese medicine (TCM), Kampo medicines, Homeopathy, Unani, Tibetan traditional medicine, etc. use plants as the primary source of medicine. Parallel to the allopathic system, traditional medicine is encouraged in all spheres because of its efficacy, availability, safety, and affordability as compared to allopathic drugs.[7-10)] Thus, it is very important to focus on isolation and identification the chemical constituents form the medicinal plants and their bioactivity evaluations to provide the scientific evidences for traditional medicines and also to discover lead molecules for new therapeutic agents.

1.2. Nepalese medicinal resources

Nepal is located between the Tibetan Autonomous Region of China in the North and India in the South, East and West. Nepal covers small area of 147,181 Km^2 (26°22′ N - 30° 27′ N and 80°4′ E - 88°22′ E) but due to wide range of altitude variation the climate ranges from tropical to alpine region. Nepal is rich in biodiversity and accommodates all types of world

agro-climate for cultivation and conservation of wide varieties of biological resources.[11] Naturally due to wide variation in climate, good level of diversity in the flora and fauna, including aromatic and medicinal plants are present in Nepal. Nepal contains about 7000 plant species and among them about 700 (10%) are supposed to be of known medicinal value. Many of such plants never came on screening for biological activities and chemical analysis. Therefore, it is very important to carry out researches on identification, chemical analysis and biological activities on Nepalese medicinal resources for the resource and product developments. [11,12]

There have been many attempts on the documentation of medicinal plants of Nepal including "Plants and People of Nepal" by Manandhar NP (2002),[11] "Ethnobotany of Nepal" by Rajbhandari KR (2001),[13] "A Handbook of Medicinal Plants of Nepal" by Watanabe T, Rajbhandari KR, Malla KJ, Yahara S (2005)[14] and others.[6,9,15] Many medicinal plants reported in these text as traditional medicines have not been studied for ther chemical constituents. During the preparation of "A Handbook of Medicinal Plants of Nepal Supplement I" by Watanabe T, Rajbhandari KR, Malla KJ, Devkota HP, Yahara S (2013),[16] we encountered many valuable medicinal plants whose chemical analysis and biological activity evaluation would be essential for the development of evidence based medicines and standardization of the traditional remedies. One of such plant species was *Diplomorpha canescens* (Meisn.) C. A. Meyer (Syn: *Wikstroemia canescens* Meisn.) belonging to family Thymelaeaceae, which is widely used in Nepal as a remedy for toothache. *Diplomorpha canescens* is also used in traditional Chinese medicine for the treatment various disorders including cancer.[17-19] As *Diplomorpha canescens* is also used to prepare handmade paper in Nepal, local users have also experienced its usefulness to prevent dryness of skin. We collected the aerial parts and roots of *Diplomorpha canescens* from Nepal and performed chemical analysis, antioxidant and tyrosinase inhibitory activity evaluation of isolated compounds. On the basis of interesting results from these experiments, we then decided to perform the chemical analysis on *Diplomorpha ganpi,* a rare plant from Aso, Kumamoto and *Diplomorpha sikokiana* from Kochi, Japan.

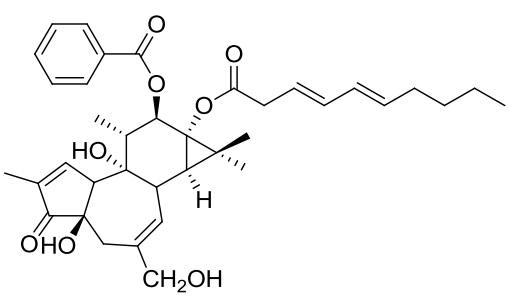
1.3. *Diplomorpha* plants

Various plants of Thymelaeaceae family such as *Diplomorpha canescens* (Meisn.) C. A. Meyer (Syn: *Wikstroemia canescens* Meisn.),[11,16-19] *Wikstroemia indica* (L.) C. A.

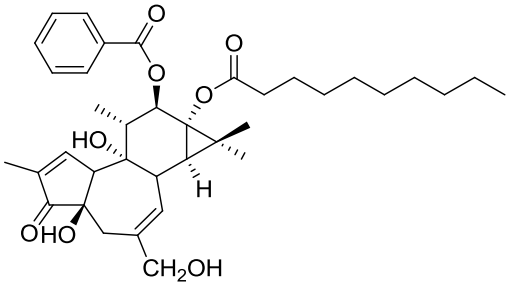
Meyer,[20,21] *Stellera chamaejasme* Linn. (Syn. *Wikstroemia chamaejasme* (Linn.) Domke.[14,22] are widely used as traditional medicines in many Asian countries including China, Nepal, India and Japan. Many bioactive constituents including diterpene ester[17,22] flavonoids,[23-25] biflavonoids,[26-28] lignans,[29] coumarins[30,31] etc. have been isolated from these species. Although some species of *Diplomorpha* genus are used in traditional medicines but detailed chemical and bioactivity analysis have not been performed. The present study is focused on the isolation, identification and anti-oxidative and tyrosinase inhibitory activities of chemical constituents from *Diplomorpha canescens* collected in Nepal and *Diplomorpha ganpi* (Sieb. et Zucc.) Nakai and *Diplomorpha sikokiana* (Franchet & Savatier) Honda collected in Japan.

Figure 1. *Diplomorpha canescens*

Diplomorpha canescens, locally called as "Phurkepaat" in Nepali is widely distributed throughout Nepal, Afghanistan, northern India, Sri Lanka and southwest China.[11,14] Traditionally in Nepal, fiber from bark of stem is used to prepare handmade Nepalese paper.[11] Stems are used in toothache in Nepal.[14] Roots are called as "Sanhijyou" in traditional Chinese medicine (TCM) and used for the treatment of many disorders[18] and in antitumour therapy.[17] Flowers of this plant are the source of "Jouka", a TCM used for the treatment of cough and balancing water in body.[19] Methanol extract of the aerial parts showed potent tyrosinase inhibitory activity.[32] Previous phytochemical studies have reported two tigliane type diterpene esters, wikstroemia factors C_1 and C_2 from the root of this plant. Among them, wikstroemia factor C_1 showed potent irritant activity on mouse ear.[17]



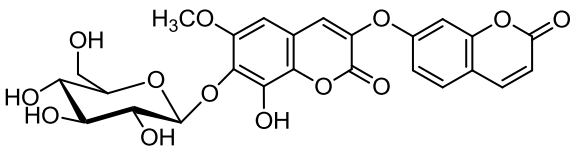
wikstroemia factor C_1



wikstroemia factor C_2

Diplomorpha ganpi (Syn: *Wikstroemia ganpi* (Sieb. et Zucc.) Maxim) known as “Koganpi” in Japanese is distributed mainly in Honshu, Shikoku and Kyushu (including Mt. Aso), Japan.[33] Although the flowers of *Diplomorpha ganpi* are also used as source of “Jouka,” [19] no chemical constituent analysis has been carried out, apart from a report on daphnin from stem bark. [34]

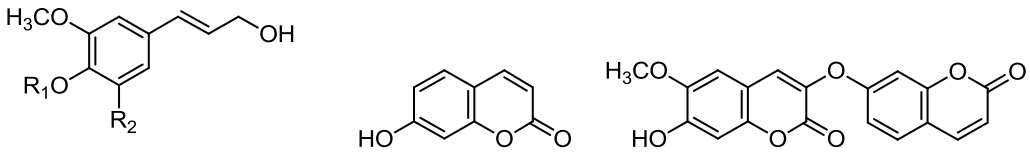
Figure 2. *Diplomorpha ganpi*



daphnin

Diplomorpha sikokiana (Syn: *Wikstroemia sikokiana* Franchet & Savatier) known as "Ganpi" in Japanese is distributed mainly in Honshu, Shikoku and Kyushu islands of Japan.[33] Flowers of *Diplomorpha sikokiana* are also used as source of "Jouka." Previous studies have reported three phenylpropanoid glucosides, syringin, syringinoside, coniferinoside;[35] two coumarins, daphnoretin and umbelliferone;[36] thirteen biflavonoids, neochamaejasmins A and B, sikokianins A, B, and C, wikstrols A and B, [36,37] chamaejasmenins A and B,[38] isochamaejasmenin, genkwanol B, daphnodorin B, dihydrodaphnodorin B; two flavonoids, apigenin 4',7-dimethylether 5-*O*-primeveroside and yuenkanin; a diarylpentanoid, (-)-*erythro*-1,5-diphenylpentane-1,3-diol;[38] from the roots of *Diplomorpha sikokiana.* Seven lignans, (-)-pinoresinol, (+)-matairesinol, (+)-wikstromol, (-)-lariciresinol, (-)-secolariciresinol, (+)-kusunokinin, (+)-methyltrachelogenin[39-42] were isolated from the stems of *Diplomorpha sikokiana.*

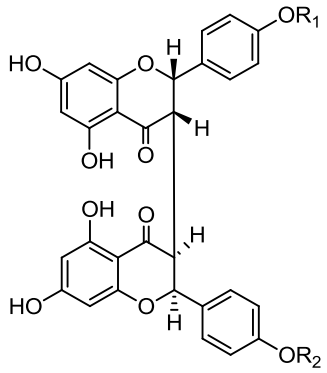
Figure 3. *Diplomorpha sikokiana*



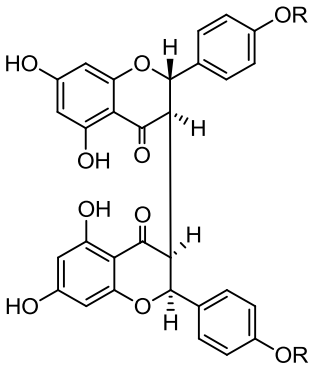
syringin: R_1 = Glc, R_2 = OCH_3
syringinoside: R_1 = Glc-(6)-Glc, , R_2 = OCH_3
coniferinoside: R_1 = Glc-(6)-Glc, R_2 = H

umbelliferone

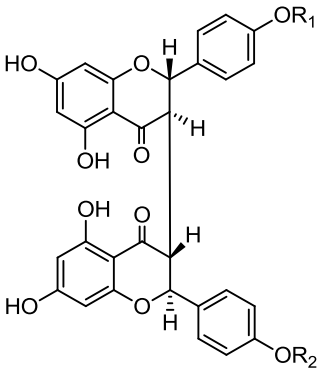
daphnoretin



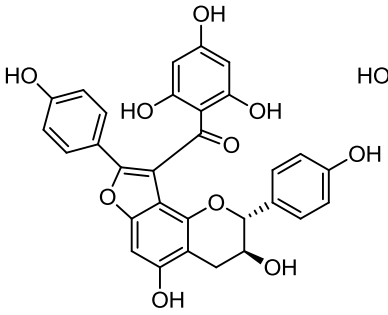
neochamaejasmin A: R_1 = R_2 = H
sikokianin A: R_1 = CH_3, R_2 = H
chamaejasmenin B: R_1 = R_2 = CH_3



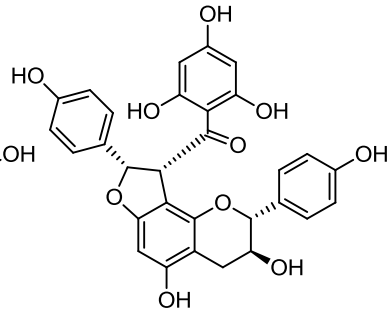
neochamaejasmin B: R H
sikokianin B: R_1 = CH_3



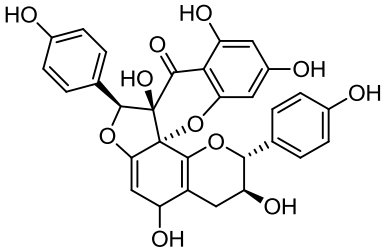
isochamaejasmin: R_1 = R_2 = H
sikokianin C: R_1 = CH_3, R_2 = H
chamaejasmenin A: R_1 = R_2 = CH_3



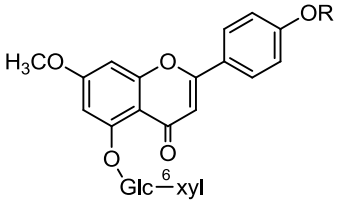
daphnodorin B



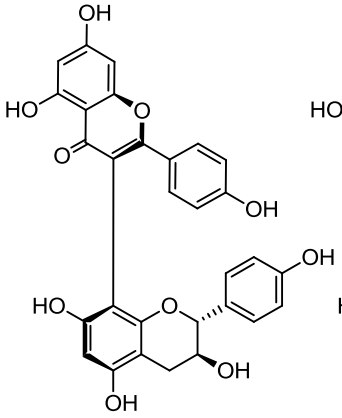
dihydrodaphnodorin B



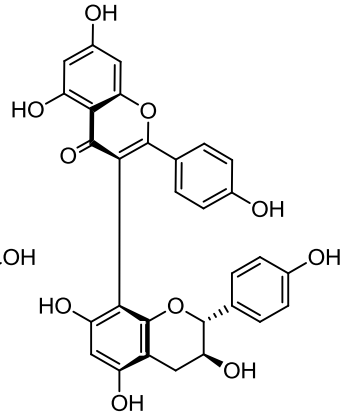
genkwanol B



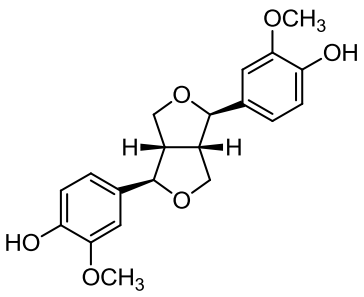
apigenin 4', 7-dimethylether
5-*O*-primeveroside: R = CH_3
yuenkanin: R = H



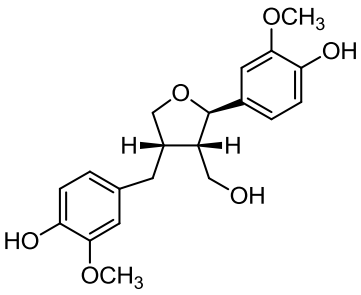
wikstrol A



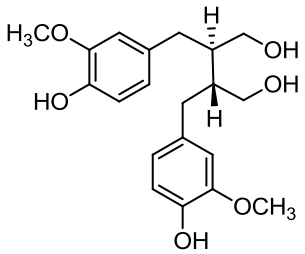
wikstrol B



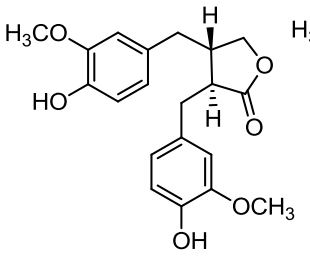
(-)-pinoresinol



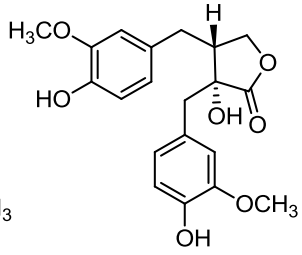
(-)-lariciresinol



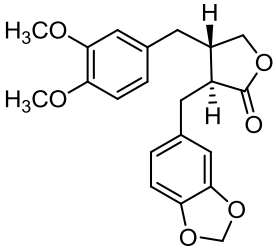
(-)-secoisolariciresinol



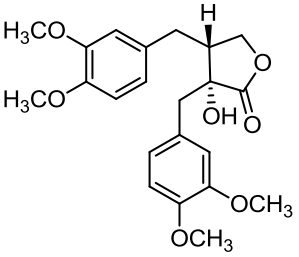
(+)-matairesinol



(+)-wikstromol



(+)-kusunokinin



(+)-methyltrachelogenin

1.4. Antioxidative activity

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the various forms of activated oxygen and nitrogen. These include free radicals such as superoxide ions (O_2^{-}), hydroxyl (HO˙) and nitric oxide radicals (NO˙) along with non-free radical species such as hydrogen peroxide (H_2O_2) and nitrous acid (HNO_2). Reactive oxygen and nitrogen species are together involved in normal cell regulation process.[43] Overproduction of these free radicals weakens the natural antioxidant system, first resulting in oxidative stress, and then leading to oxidative injury and finally to numerous disease states including cardiovascular diseases,[44-45] diabetes,[46,47] retinal ischemia, cancer, neurodegenerative disorders such as Parkinson's disease,[48] Alzheimer's disease[49] and aging processes.[50]

Cellular radical scavenging systems include the enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). External sources of antioxidative protection include antioxidants like vitamin C, vitamin E, flavonoids, polyphenols, carotenoids as well as minerals such as selenium and zinc.[51,52]

Various antioxidants are contained in most foods and medicinal plants. They are polyphenols, vitamins, carotenoids and flavonoids in vegetables and fruits. The recent study on

antioxidative substances in foods and medicinal plants is a comparatively new province. The digestion, absorption, biological activity and metabolic pathway in each food and medicinal plant are still complicated because a number of substances are included in various proportions in one plant. Meanwhile, their safety has been established to a certain extent for empirical and traditional use from ancient times. Great efforts have been made in an attempt to find the safe and potent natural antioxidants from plant resources for natural antioxidants which seems to be safer causing fewer adverse effects.[53-55)] Flavonoids, tannins and polyphenolic compounds play important role in biological system. Therefore, the study of antioxidative substances in foods and medicinal plants would be important, and such antioxidant substances might be applied for treatment and prevention of human disease.

1.5. Tyrosinase inhibitory activity

Tyrosinase (EC 1.14.18.1) is the key enzyme in the melanogenesis (melanin biosynthesis) and participates in the oxidation of tyrosine to dopaquinone via L-3, 4–dihydroxyphenylalanine (L-DOPA). Melanogenesis is the process by which melanin is produced and distributed in the skin and hair follicles by the melanocytes. The synthesis of melanin starts with the conversion of L-tyrosine to L-DOPA (Figure 4). The subsequent oxidation of L–DOPA yields dopaquinone, which is the initial step in melanin synthesis.[56-58)]

Although melanin in human skin acts as a major defense mechanism against ultraviolet light from the sun, the production of abnormal pigmentation such as melasma, freckles, age spots, liver spots and other forms of melanin hyperpigmentation can be serious aesthetic problem.[59)]

Melanin biosynthesis can be inhibited by avoiding ultraviolet (UV) light exposure, by inhibiting melanocytes metabolism and proliferation, by inhibiting tyrosinase activity, or by removing melanin by corneal ablation.[60)] It has been recently shown that the other factors such as metal ions and the tyrosinase related proteins, TRP-1 and TRP-2 also contribute to the melanin biosynthesis.[61)] However, tyrosinase plays the crucial role in melanogenesis. Therefore, many tyrosinase inhibitors that suppress melanogenesis have been actively studies with the aim of developing preparations for the treatment of hyperpigmentation.[56,61,62)] Studies on mushroom tyrosinase inhibition are preferred as mushroom tyrosinase is commercially available and inexpensive.[56)]

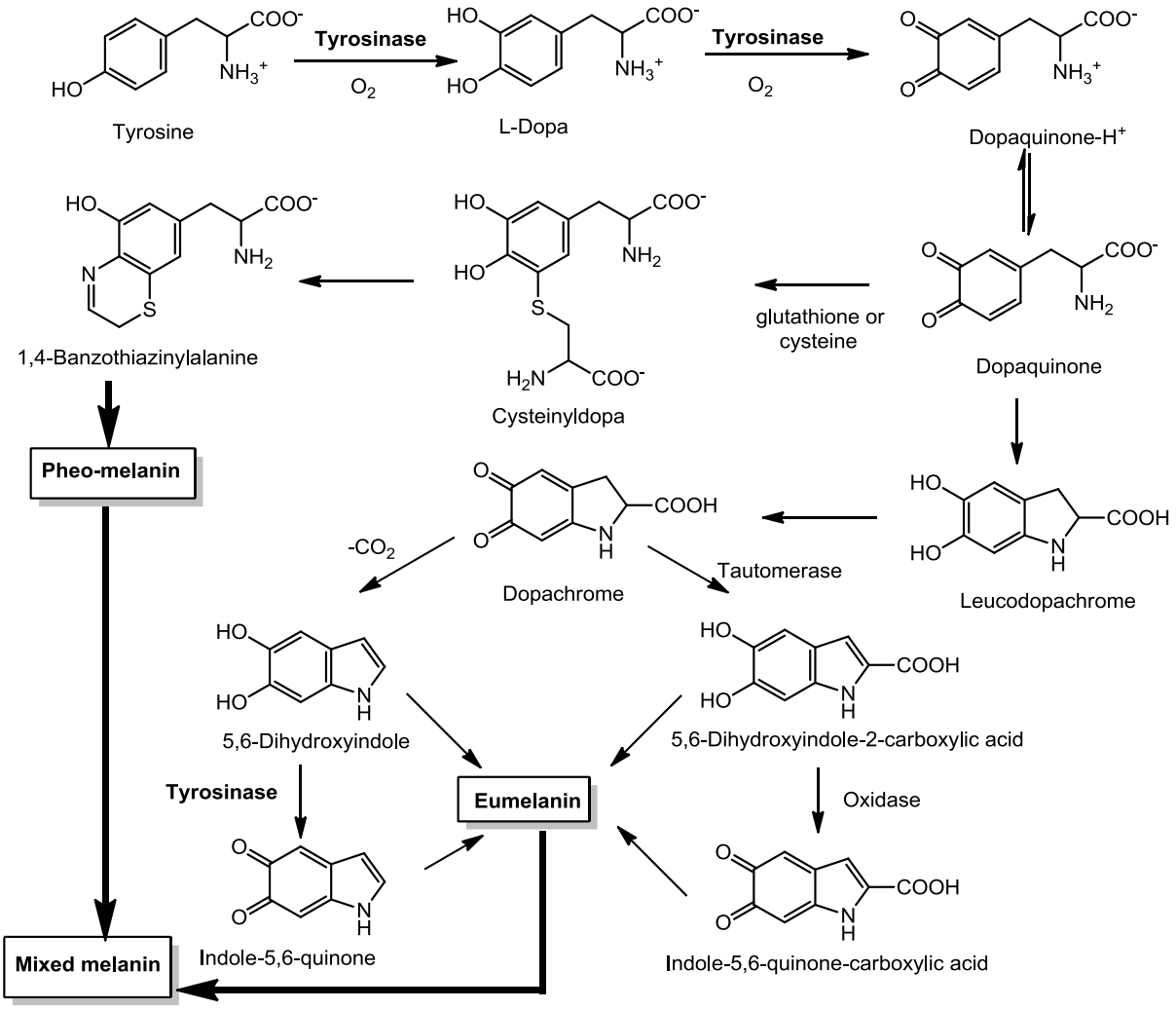


Figure 4. The pathway of melanogenesis[56)]

Disorders of hyperpigmentation are difficult to treat, particularly in darker-skinned individuals. The goal is to reduce the hyperpigmentation without causing undesirable hypopigmentation or irritation in the surrounding normally pigmented skin. The psychosocial impact caused by these disorders must be considered. Various tyrosinase inhibitors as hypopigmenting agents have been reported from both natural and synthetic sources with diverse mechanisms of actions but only a few of them are marketed as skin whitening agents, primarily due to various safety concerns.[63-65)] The most commonly used hypopigmenting agents are phenolic agents such as hydroquinone, arbutin, licorice extract, aloe extract etc.[65,66)] There are other phenolic agents, such as N-acetyl-4 cystaminyl phenol (NCAP) that are currently being studied and developed.[64)] The non-phenolic agents which include tretinoin, adapalene, topical corticosteroids, azelaic acid, kojic acid are also used for the treatment of

hyperpigmentation.[63] Phenolic compounds specially flavonoids and chalcones have shown potent tyrosinase inhibitory activities.[56,59,67-69] Thus, evaluation of compounds isolated from *Diplomorpha* plants as tyrosinase inhibitors may be beneficial for the development of new and more efficient remedies.

The main objectives of present study were the extraction, isolation and identification (structure elucidation) of the chemical constituents from the aerial parts and roots of a Nepalese medicinal plant, *Diplomorpha canescens*, evaluation of antioxidant and tyrosinase inhibitory activity of these isolated compounds and comparison with the *Diplomorpha ganpi* and *Diplomorpha sikokiana* from Japan.

2. Extraction, isolation and structure elucidation

The aerial parts and roots of *Diplomorpha canescens* were collected from Daman, Nepal; stems and roots of *Diplomorpha ganpi* were collected from Kumamoto, Japan and stems, leaves and roots of *Diplomorpha sikokiana* were collected from Kochi, Japan. Each of these plant parts were extracted with 70% MeOH or MeOH and the extracts were then subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH-20, octadecyl silica (ODS) and silica gel column to isolate pure compounds. Thin layer chromatography (TLC) profiles ($CHCl_3$:MeOH:H_2O=8:2:0.1) of plant parts are shown in Figure 5.

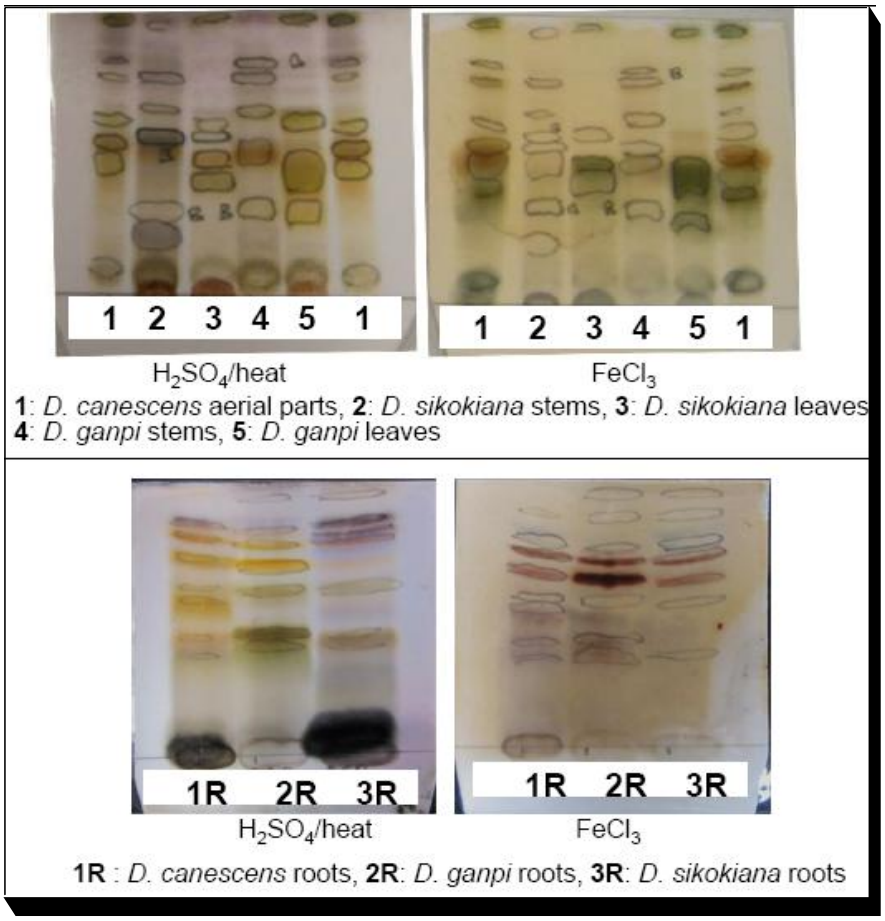


Figure 5. TLC profile of aerial parts of *Diplomorpha* plants

The structures of new compounds were elucidated on the basis of spectroscopic techniques especially mass and NMR spectra including one (1D) and two-dimensional (2D) NMR such as ^1H-^1H correlation spectroscopy (^1H-^1H COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC). All of the known compounds were identified by using physical and spectroscopic data including melting points, optical rotation, NMR data and with comparison to literature data.

2.1. Compounds from the aerial parts of *Diplomorpha canescens*

The fresh aerial parts of *D. canescens* were collected from Daman, Nepal in August 2007 and then shade dried for one month. The dried aerial parts were extracted with 70% MeOH. Then the extract was subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH-20, octadecyl silica (ODS) and silica gel to afford 32 compounds as shown in Chart 1.

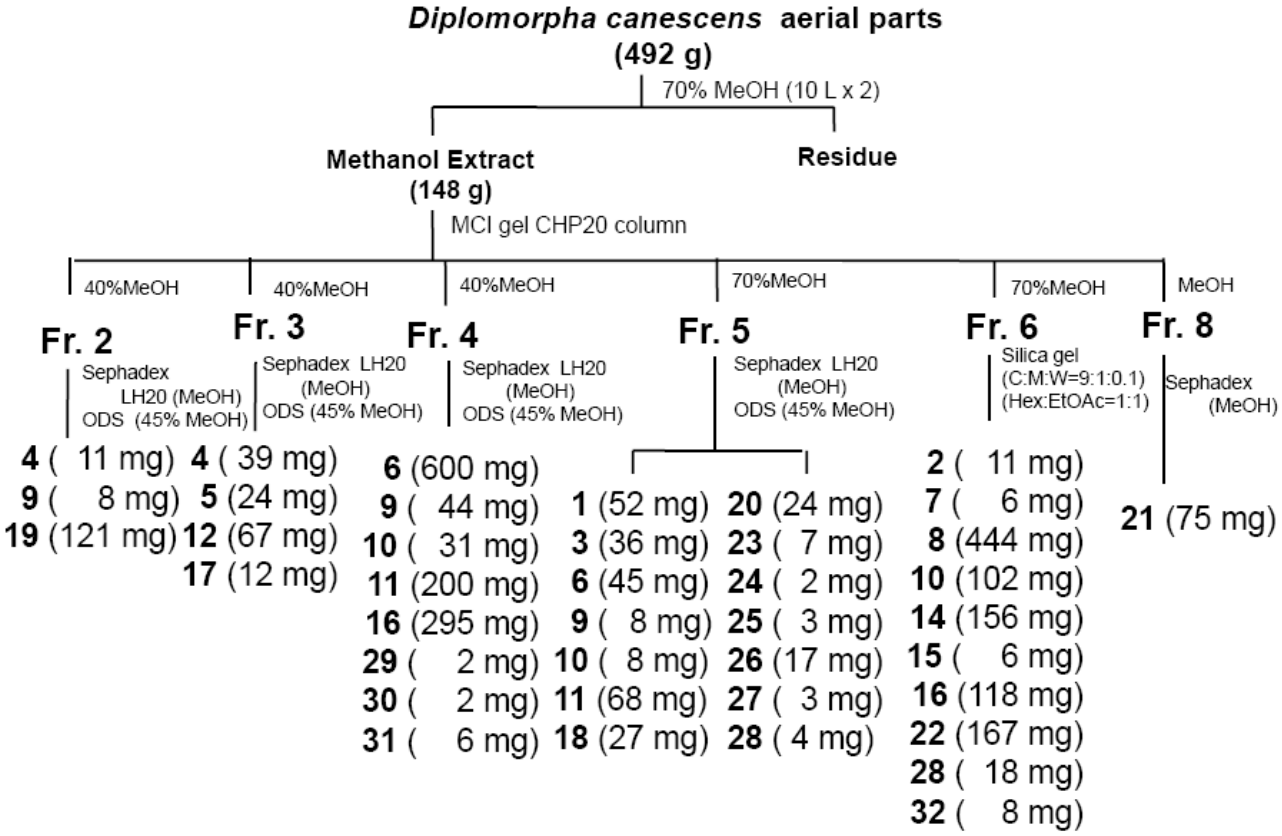


Chart 1. Extraction and isolation of compounds from aerial parts of *Diplomorpha canescens*

From the detailed spectral analysis, these compounds were identified to be five new *C*-methyl flavonoids, (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**), (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**), farrerol 4'-*O*-*β*-D-glucopyranoside (**3**), farrerol 4',7-di-*O*-*β*-D-glucopyranoside (**4**), 6,8-di-*C*-methylkaempferol 7-*O*-*β*-D-glucopyranoside (**5**); one new diarylpentanoid, diplomorphanone A (**32**) (Figure 6) along with 14 known flavonoids, farrerol 7-*O*-*β*-glucopyranoside (**6**),[70] farrerol (**7**),[70,71] kaempferol (**8**),[72] kaempferol-3-*O*-*β*-D-glucopyranoside (**9**),[72] rhamnocitrin 3-*O*-*β*-D-glucopyranoside (**10**),[72] quercetin (**11**),[72] quercetin 3-*O*-*β*-D-glucopyranoside (**12**),[73] rhamnetin 3-*O*-*β*-D-glucopyranoside (**13**),[72] apigenin (**14**),[72] genkwanin (**15**),[74] genkwanin 5-*O*-*β*-D-glucopyranoside (**16**),[75,76] genkwanin 5-*O*-primeveroside (**17**),[76] luteolin (**18**),[77]

luteolin 7-methyl ether-5-*O*-*β*-D-glucopyranoside (**19**),[78] four lignans, (-)-lariciresinol (**20**),[79,80] (-)-dihydrosesamin (**21**),[81] (-)-pinoresinol (**22**),[79,82] (±)-dehydrodiconiferyl alcohol (**23**);[83,84] three phenylpropanoid derivatives, coniferyl aldehyde (**24**),[85] sinapyl aldehyde (**25**),[85,86] *p*-coumaric acid methyl ester (**28**);[87] two coumarin derivatives, rutarensin (**26**)[88], umbelliferone (**31**)[89] and three related phenolic compounds, syringaldehyde (**27**),[85] *p*-hydroxybenzaldehyde (**29**)[90] and *p*-hydroxyacetophenone (**30**)[91] (Figure 7). Although Chen *et al.*[92] have reported the chemical structure similar to that of **1** and **2,** there has been no report regarding the isolation, synthesis, physical and spectral data and absolute configuration of these compounds in literature. Hence, we report these compound **1** and **2** as new natural compounds. All of these known compounds were identified by using physical data and spectroscopic data including melting point, optical rotation, NMR data and with comparison to literature data. All of these compounds were isolated for the first time from this plant.

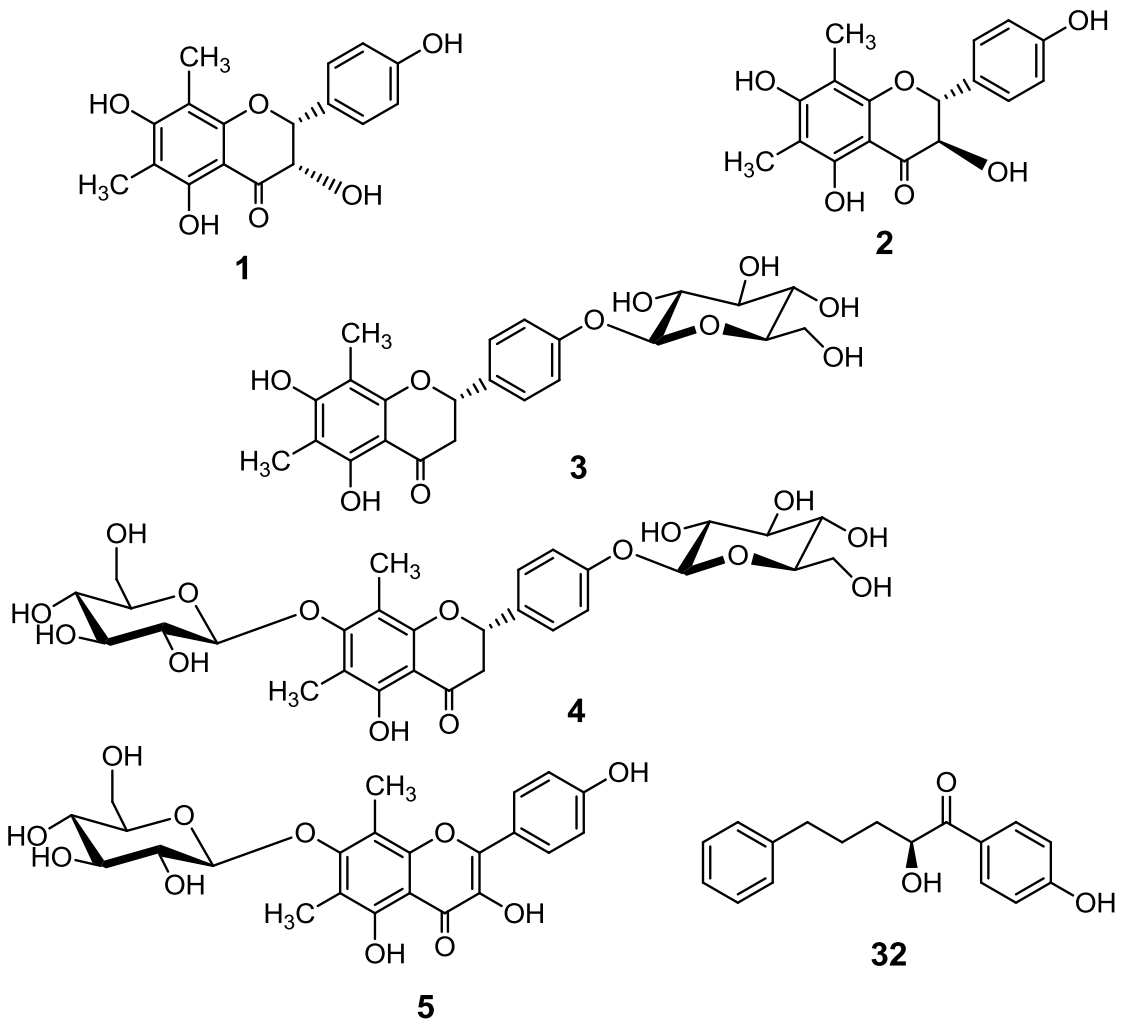


Figure 6. Structures of new compounds isolated from the aerial parts of *D. canescens*

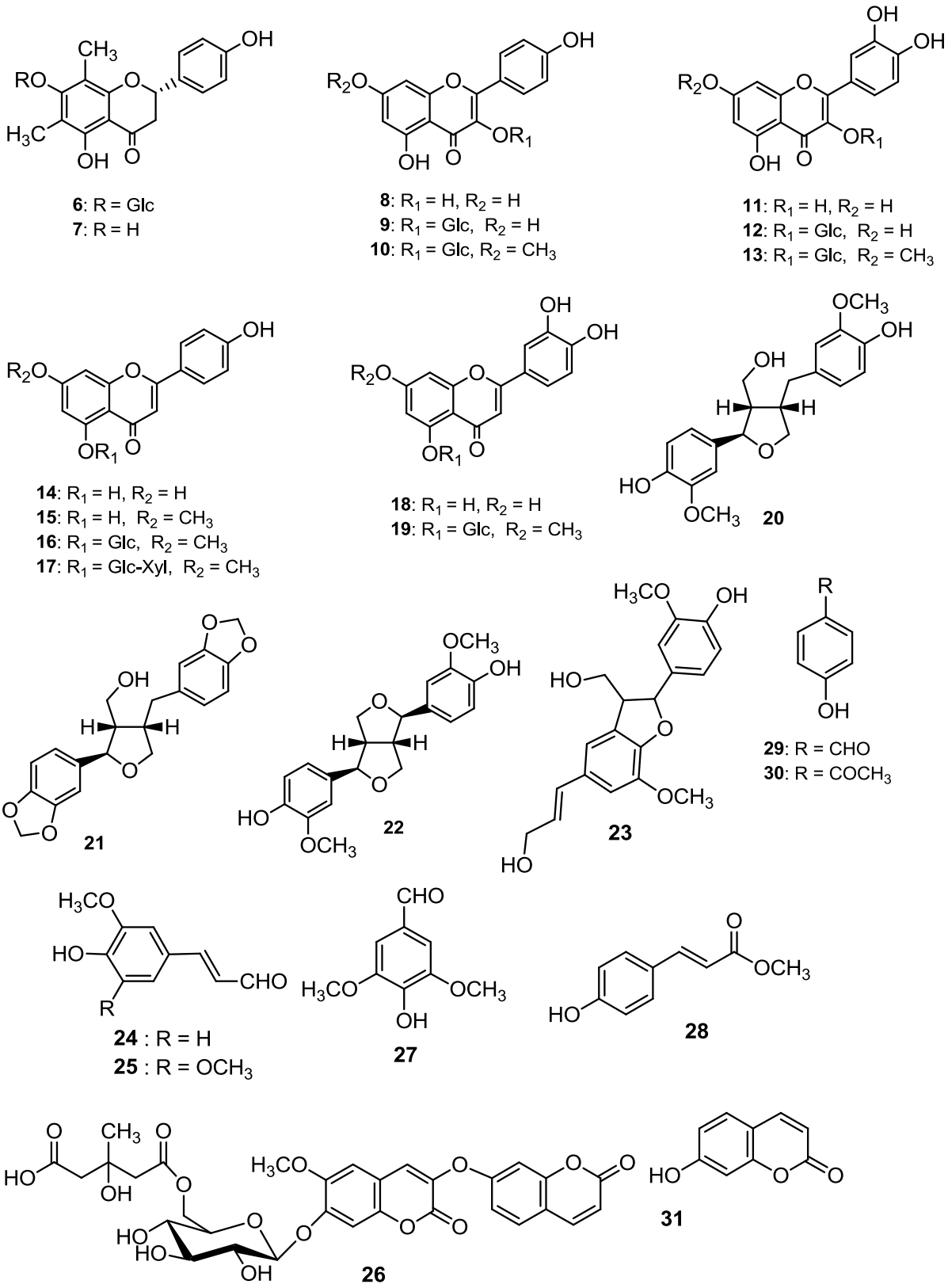


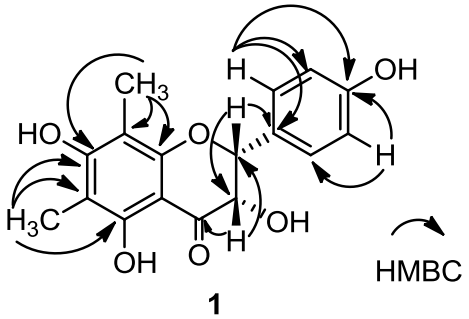
Figure 7. Structures of known compounds isolated from the aerial parts of *D. canescens*

2.1.1. New compounds

2.1.1.1. (2*R*,3*S*)-6,8-Di-*C*-methyldihydrokaempferol (1)

Compound **1** was obtained as pale yellow amorphous powder, $[\alpha]_D^{20}$ -80.5º. The HRFABMS of **1** showed the quasi-molecular ion $[M+H]^+$ peak at *m*/*z* 317.0997 (calcd. for $C_{17}H_{17}O_6$, 317.1025) supporting the formula $C_{17}H_{16}O_6$. The ^{1}H-NMR spectrum of **1** (Table 1, Figure 10) showed signals due to two aromatic methyl groups at δ 2.04 (3H, s) and 2.00 (3H, s), and the proton resonances at 6.79 (2H, d, *J* = 8.2 Hz, $C_{3'}$-H, $C_{5'}$-H) and 7.35 (2H, d, *J* = 8.2 Hz, $C_{2'}$-H, $C_{6'}$-H). The two resonances at δ 5.32 (1H, d, *J* = 2.7 Hz, C_3-H) and 4.20 (1H, d, *J* = 2.7 Hz, C_2-H) ppm were the characteristics of dihydroflavonol skeleton with *cis* stereochemistry.

The ^{13}C-NMR (Figure 10) also supported the presence of a dihydroflavonol moiety and two aromatic methyl groups at δ 7.4 and 8.1 ppm. In the HMBC spectrum, proton resonances for $C_{3'}$-H, $C_{5'}$-H and $C_{2'}$-H, $C_{6'}$-H had long range correlation with carbon resonance at δ 158.4 (C-4'). Similarly, the proton signal for C_2-H (δ 4.20) showed correlations with carbons at δ 128.6 (C-1'), 129.6 (C-2',6') and with the carbons of the dihydroflavonol skeleton at δ 128.6 (C-3), 197.0 (C-4) and 158.7 (C-9). The C_6-methyl signal (δ 2.00) had correlation with carbons at δ 160.7 (C-5), 105.0 (C-6) and 164.4 (C-7). Similarly, C_8-methyl signal (δ 2.04) had correlation with carbons at δ 158.7 (C-9), 104.2 (C-8) and 164.4 (C-7). The key HMBC correlations have been given in Figure 8.



1

Figure 8. Key HMBC correlations observed in the spectrum of **1**.

The CD spectrum of **1 (**Figure 12) showed the positive Cotton effect at 349 nm suggesting the absolute configuration at the C-2 position is *R*.[93)] Depending upon the CD data and the coupling constant (*J* = 2.7 Hz) of C_2-H and C_3-H, the configuration of dihydroflavonol was found to be 2*R*,3*S* having 2α equatorial aryl group and 3α axial hydroxyl group (Figure 9).

The structure of **1** was finally concluded as (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol.

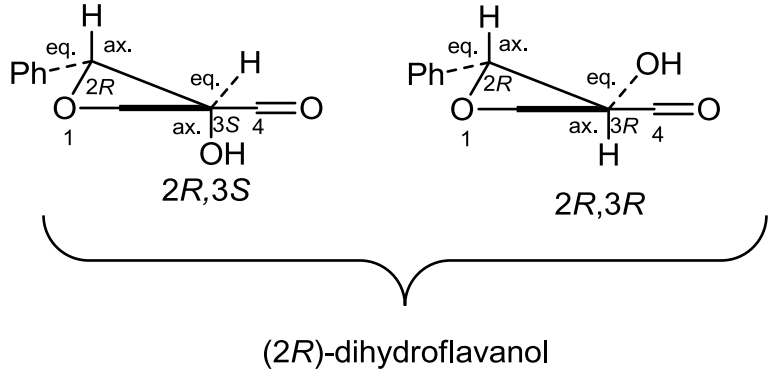


Figure 9. Hetero-ring conformations of the 2(*R*)-dihydroflavanols with equatorial C2-aryl groups.

Table 1. NMR spectroscopic data for **1** and **2** in CD_3OD

	1		**2**	
position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	73.0	4.20, d (2.7)	73.7	4.49, d (11.7)
3	82.5	5.32, d (2.7)	84.8	4.92, d (11.7)
4	197.0		199.0	
5	160.7		160.1	
6	105.0		105.2	
7	164.4		164.5	
8	104.2		104.3	
9	158.7		159.1	
10	101.7		101.7	
1'	128.6		129.7	
2'	129.6	7.35, d (8.2)	130.3	7.37, d (8.3)
3'	115.9	6.79, d (8.2)	116.2	6.83, d (8.3)
4'	158.4		159.0	
5'	115.9	6.79, d (8.2)	116.2	6.83, d (8.3)
6'	129.6	7.35, d (8.2)	130.3	7.37, d (8.3)
6-CH_3	7.4	2.00, s	7.4	1.94, s
8-CH_3	8.1	2.04, s	8.0	2.01, s

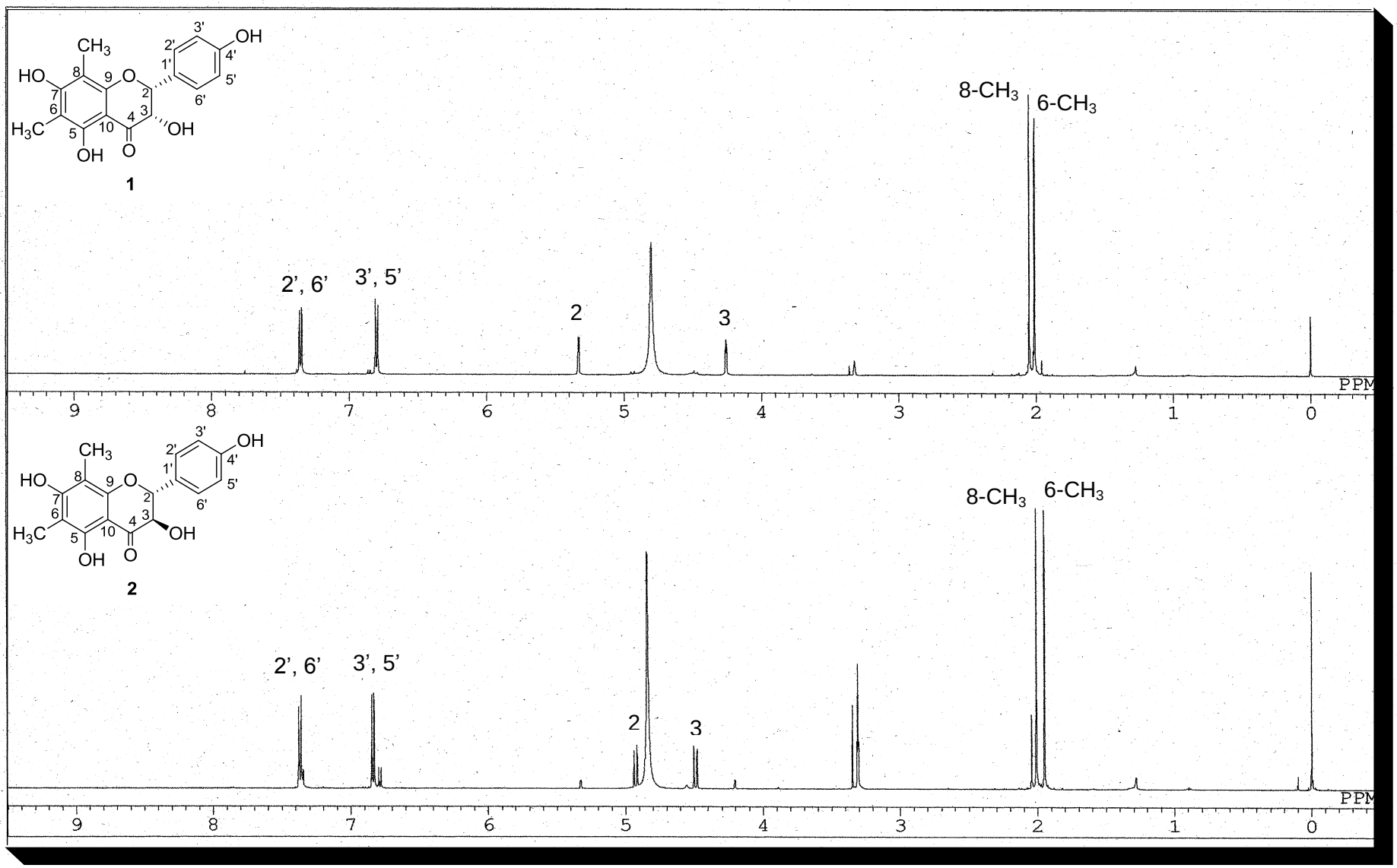


Figure 10. ^{1}H-NMR spectra of (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**) and (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**) in CD_3OD

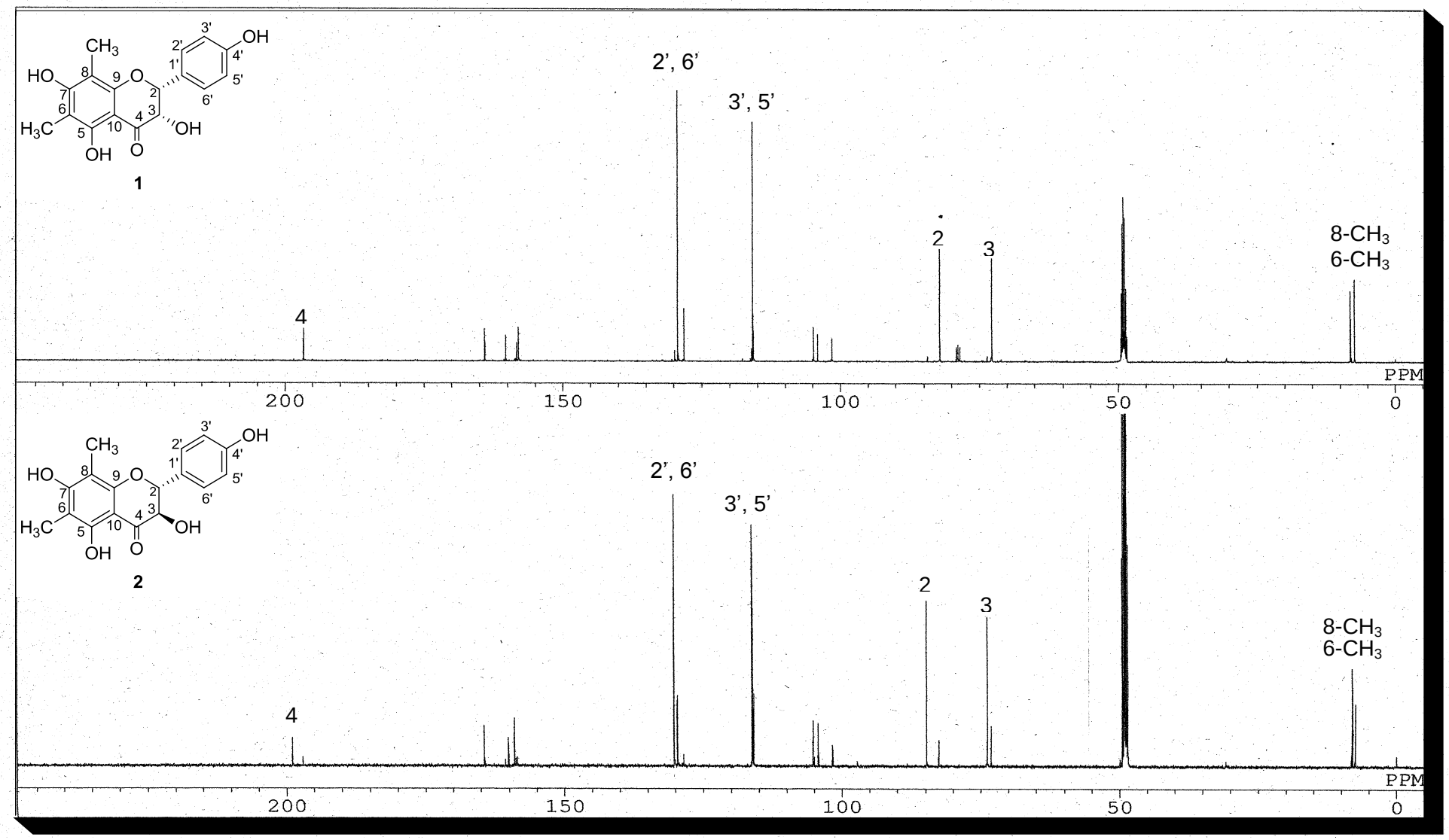


Figure 11. ^{13}C-NMR spectra of (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**) and (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**) in CD_3OD

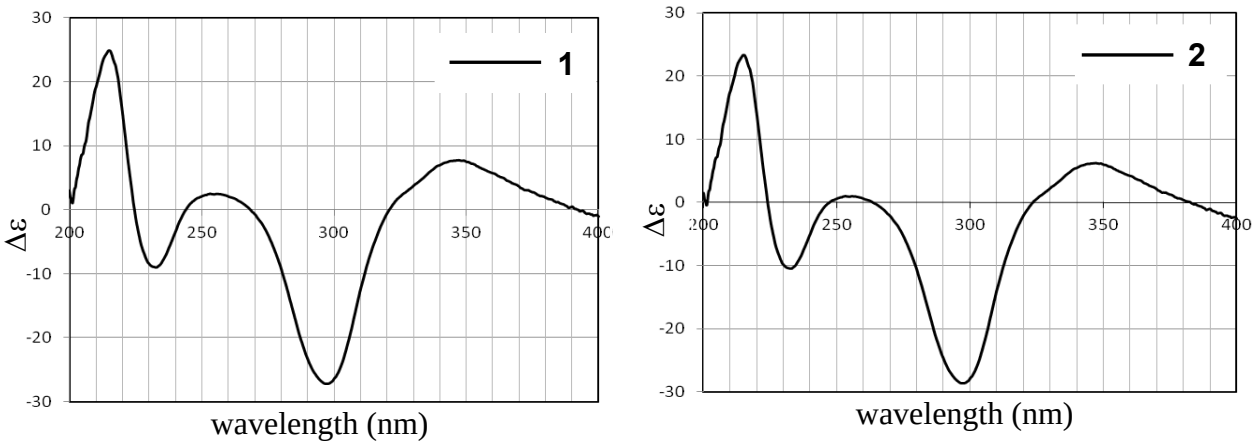


Figure 12. Circular dichroism (CD) spectra of **1** and **2**

2.1.1.2. (2*R*,3*R*)-6,8-Di-*C*-methyldihydrokaempferol (2)

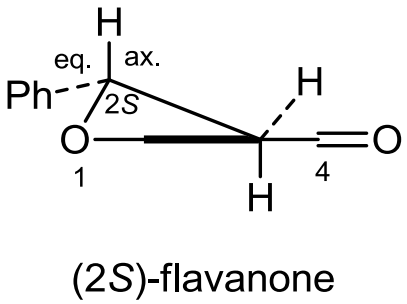
Compound **2** was obtained as pale yellow amorphous powder, $[\alpha]_D^{21}$ +4.8º. The HRFABMS of **2** showed the quasi-molecular ion $[M+H]^+$ peak at *m*/*z* 317.1065 (calcd. for $C_{17}H_{17}O_6$, 317.1025) supporting the formula $C_{17}H_{16}O_6$. The ^{1}H- and ^{13}C- NMR (Table 1, Figure 10, 11) data of **2** were similar to that of **1** except that the resonance at δ 4.92 (1H, d, *J* = 11.7 Hz, H-3) and 4.49 (1H, d, *J* = 11.7 Hz, H-2) revealed the *trans* stereochemistry of the dihydroflavonol moiety between C_2-H and C_3-H. All other signals were assigned on the basis of those of **1**. The CD spectra of **2** (Figure 12) also showed the positive Cotton effect at 347 nm suggesting 2*R* configuration.[93)] Depending upon the CD data and the coupling constant (*J* = 11.7 Hz) of C_2-H and C_3-H, the configuration of dihydroflavonol was found to be 2*R*,3*R* having 2α equatorial aryl group and 3β equatorial hydroxyl group. On the basis of these data, the structure of **2** was concluded as (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol.

2.1.1.3. Farrerol 4'-*O*-β-D-glucopyranoside (3)

Compound **3** was obtained as pale yellow amorphous powder, $[\alpha]_D^{22}$ –36.5º. The HR-FABMS of **3** showed the quasi-molecular ion $[M+H]^+$ at *m*/*z:* 463.1654 supporting the formula $C_{23}H_{26}O_{10}$. The ^{1}H-NMR spectrum of **3** (Table 14) showed signals due to two aromatic methyl groups at δ_H 1.96 (3H, s) and 1.95 (3H, s), and the proton resonances at δ_H 7.44 (2H, d, *J* = 8.8 Hz, $C_{2'}$-H, $C_{6'}$-H) and 7.08 (2H, d, *J* = 8.8 Hz, $C_{3'}$-H, $C_{5'}$-H) were characteristic of *p*-substituted phenyl ring. An anomeric proton of a sugar in β-configuration was observed at δ 4.89 (1H, d, *J* = 7.6 Hz). Similarly, three resonances at δ 5.48 (1H, dd, *J* = 3.0, 13.0 Hz,

C_2-H), 3.20 (1H, dd, *J* = 13.0, 16.9 Hz, C_3-Ha) and 2.80 (1H, dd, *J* = 3.0, 16.9 Hz, C_3-Hb) were characteristic of C ring of a flavanone moiety.

The ^{13}C-NMR (Table 15) and distortionless enhancement by polarization transfer (DEPT) spectrum showed signals equivalent to 23 carbons. Among them, seventeen carbon signals were assignable to 6,8-di-*C*-methyl-5,7,4'-trihydroxyflavanone or farrerol (**7**)[70,71] and six carbons signals (δ 60.7, 69.8, 73.2, 76.6, 77.0, 100.4) were assignable to β-glucopyranosyl moiety. All of these assignments were made on the basis of HMQC and HMBC correlations. In the HMBC spectrum, proton resonances at δ7.44 ($C_{2'}$-H, $C_{6'}$-H) and 7.08 ($C_{3'}$-H, $C_{5'}$-H) had long range correlation with carbon resonance at δ 157.3 (C-4'). Similarly, the signal for anomeric proton at δ4.89 had correlations with C-4', which revealed that the glucose molecule was attached to the 4' position in the B ring. The signal for C_5-OH (δ 12.36) had correlations with carbons at δ158.4 (C-5), 103.4 (C-6) and 101.7 (C-10). The key HMBC correlations have been given in Figure 14.



(2*S*)-flavanone

Figure 13. Hetero-ring conformations of the 2(*S*)- flavanone with equatorial C2-aryl groups.

The circular dichroism (CD) spectrum of **3** showed the positive Cotton effect at 345 nm suggesting the absolute configuration at the C-2 position is *S*,[93] which was also similar to that of farrerol (**7**). Compound **3** on acid hydrolysis gave farrerol (**7**) and glucose, which were identified by co-TLC with authentic samples. The absolute configuration of D-glucopyranoside moiety in compound **3** was confirmed by the application of Klyne's rule[94] as the molecular rotation difference (-104.7°) between compound **3** (-168.6°) and farrerol (**7**) (-63.9°) was similar to the molecular rotation ($[M]_D$) of methyl-β-D-glucopyranoside (-66.3°).[95-97] On the basis of these data, the structure of **3** was concluded as farrerol 4'-*O*-β-D-glucopyranoside.

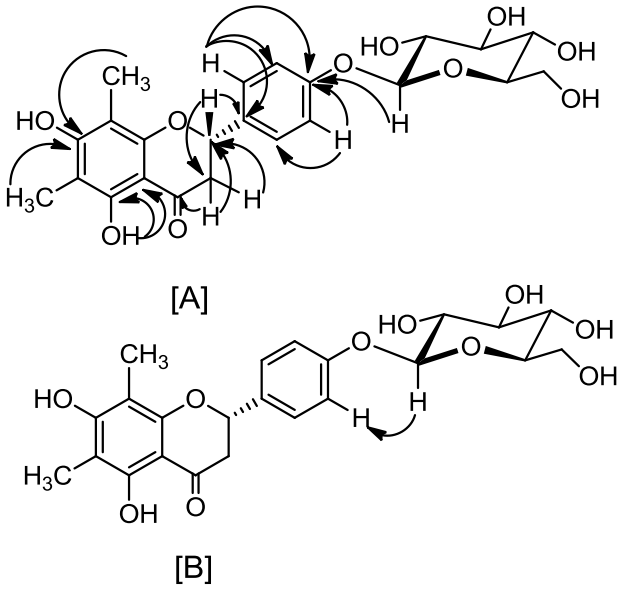


Figure 14. [A] Key HMBC correlations observed in the spectrum of **3**. [B] NOEs observed in the difference NOE experiments of **3**.

2.1.1.4. Diplomorphanin A (4)

Diplomorphanin A (**4**) was obtained as pale yellow amorphous powder, $[\alpha]_D^{21}$ -22.2. The HR-FAB-MS of **4** showed the quasi-molecular ion $[M+Na]^+$ peak at *m/z:* 647.1979 supporting the formula $C_{29}H_{36}O_{15}$. The ^{1}H-NMR spectrum of **4** (Table 2) showed signals due to two aromatic methyl groups at δ_H 2.07 (3H, s) and 2.09 (3H, s), and the proton resonances at δ_H 7.45 (2H, d, J = 8.5 Hz, $C_{2'}$-H, $C_{6'}$-H) and 7.09 (2H, d, J = 8.5 Hz, $C_{3'}$-H, $C_{5'}$-H) were characteristic of *p*-substituted phenyl ring. Signals for two anomeric protons were observed at δ 4.59 (1H, d, J = 7.6 Hz) and 4.89 (1H, d, J = 7.6 Hz). Similarly, three resonances at δ 5.58 (1H, dd, J = 2.7, 12.3 Hz, C_2-H), 3.32 (1H, dd, J = 12.3, 16.0 Hz, C_3-Ha) and 2.87 (1H, dd, J = 2.7, 16.0 Hz, C_3-Hb) were characteristic of C ring of a flavanone moiety.

The ^{13}C-NMR (Table 2) and distortionless enhancement by polarization transfer (DEPT) spectra showed signals equivalent to 29 carbons. Among them, seventeen carbon signals were assignable to 6,8-di-*C*-methyl-5,7,4'-trihydroxyflavanone or farrerol (**7**)[70,71] and twelve carbons signals were assignable to two units of β-glucopyranosyl moiety which was also supported by the acid hydrolysis of **4** affording **7** and D-glucose.

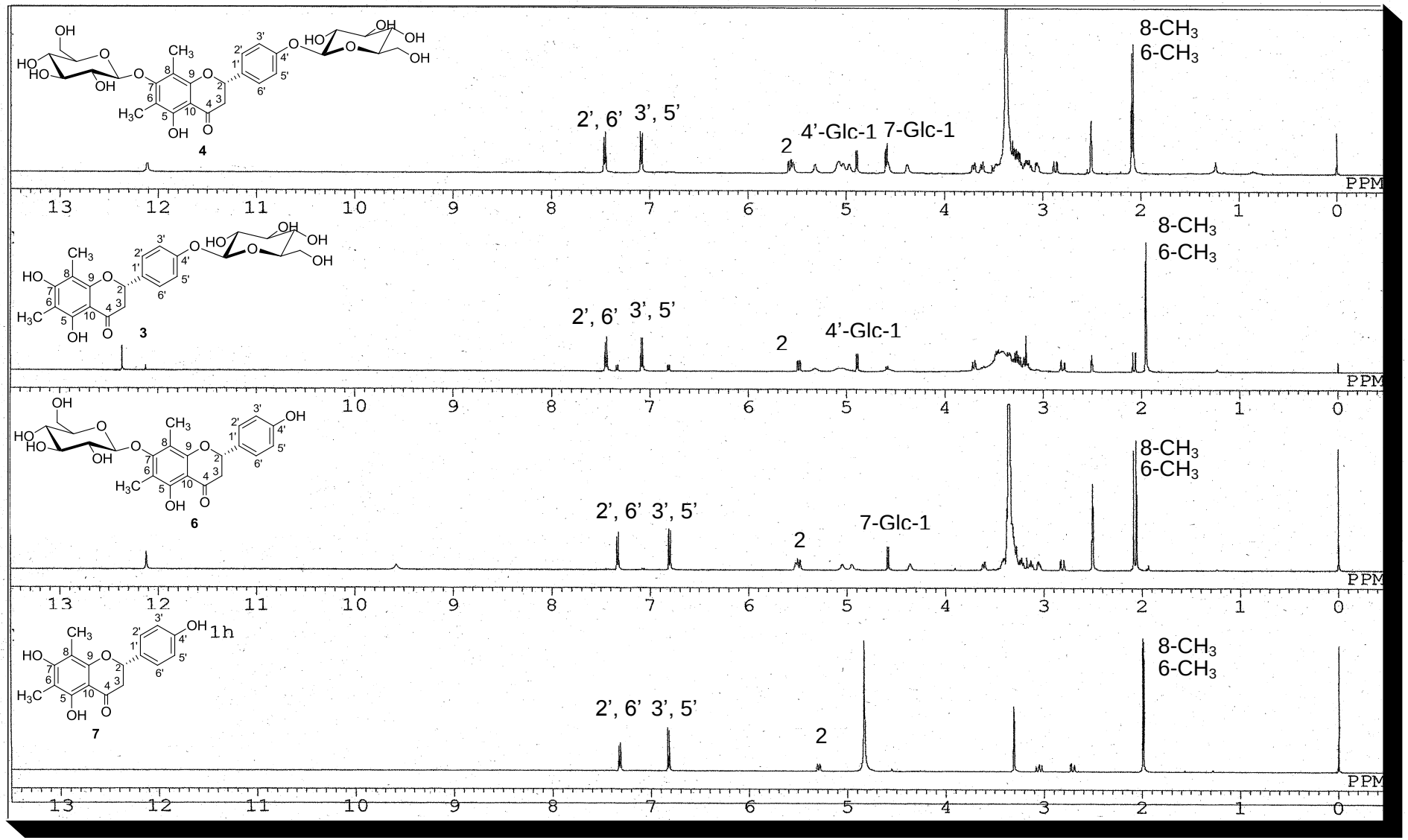


Figure 15. ^{1}H-NMR spectra of farrerol 4'-*O*-*β*-D-glucopyranoside (**3**), diplomorphanin A (**4**), farrerol 7-*O*-*β*-D-glucopyranoside (**6**) in DMSO-d_6 and farerol (**7**) in CD_3OD.

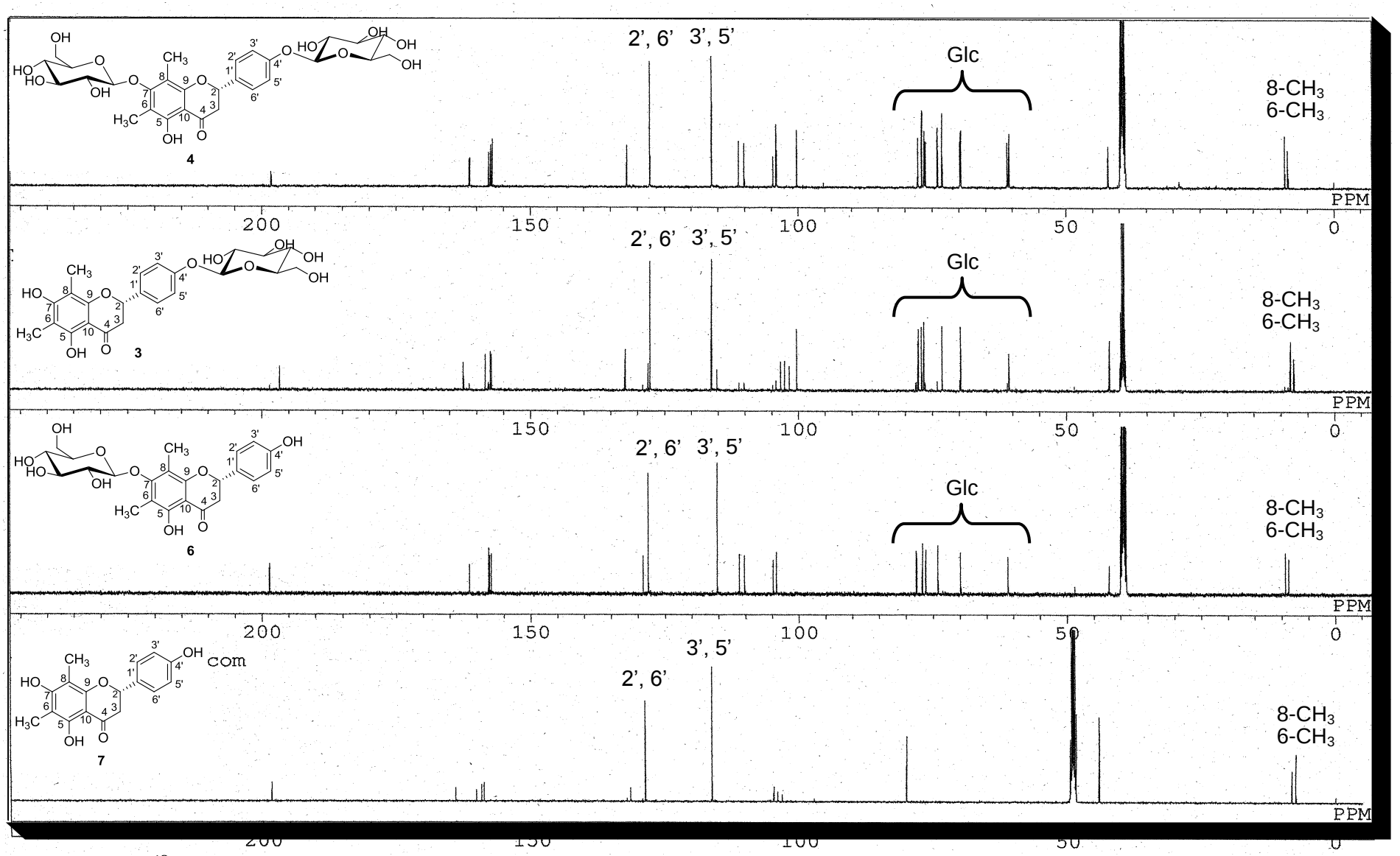


Figure 16. ^{13}C-NMR spectra of farrerol 4'-*O*-*β*-D-glucopyranoside (**3**), diplomorphanin A (**4**), farrerol 7-*O*-*β*-D-glucopyranoside (**6**) in DMSO-d_6 and farerol (**7**) in CD_3OD.

Table 2. NMR spectroscopic data for **3**, **4** and **6** in DMSO-d_6

	3		**4**		**6**	
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	77.7	5.48, dd (3.0, 13.0)	77.8	5.58, dd (2.7, 12.3)	78.0	5.49, m
3	42.0	2.80, dd (3.0, 16.9) 3.20, dd (13.0, 16.9)	42.2	2.87, dd (2.7, 16.0) 3.32, dd (12.3, 16.0)	42.2	2.78, dd (2.9, 7.2) 3.30, m
4	196.7		198.4		198.6	
5	158.4		157.9		157.9	
6	103.4		111.2		111.1	
7	162.5		164.1		164.4	
8	102.6		110.2		110.1	
9	157.4		157.2		157.4	
10	101.7		104.8		104.8	
1'	132.4		132.1		129.0	
2'	127.7	7.44, d (8.8)	127.8	7.45, d (8.5)	128.0	7.32, d (8.4)
3'	116.3	7.08, d (8.8)	116.2	7.09, d (8.5)	115.2	6.80, d (8.4)
4'	157.3		157.3		157.6	
5'	116.3	7.08, d (8.8)	116.2	7.09, d (8.5)	115.2	6.80, d (8.4)
6'	127.7	7.44, d (8.8)	127.7	7.45, d (8.5)	128.0	7.32, d (8.4)
6-CH_3	7.6[c]	1.95, s[c]	8.7	2.09, s	8.7	2.08, s
8-CH_3	8.3 [c]	1.96, s[c]	9.3	2.07, s	9.3	2.05, s
C_5-OH		12.10, s		12.10, s		12.12, s
4'-*O*-Glc-1	100.4	4.89, d (7.6)	100.3	4.89, d (7.6)		
4'-*O*-Glc-2	73.2	3.26, m	73.2	3.22—3.41		
4'-*O*-Glc-3	76.6	3.30, m	77.0	3.22—3.41		
4'-*O*-Glc-4	69.8	3.17, m	69.7	3.22—3.41		
4'-*O*-Glc-5	77.0	3.30, m	76.3	3.22—3.41		
4'-*O*-Glc-6	60.7	3.70, brd (10.0) 3.45, m	60.7	3.70, brd (10.0) 3.22—3.41		
7-*O*-Glc-1			104.2	4.59, d (7.6)	104.1	4.57, d (7.3)
7-*O*-Glc-2			74.1	3.22—3.41	74.0	3.22, m
7-*O*-Glc-3			77.0	3.22—3.41	77.0	3.31, m
7-*O*-Glc-4			69.8	3.22—3.41	69.8	3.17, m
7-*O*-Glc-5			76.6	3.22—3.41	76.3	3.31, m
7-*O*-Glc-6			61.0	3.61, brd (10.9) 3.22—3.41	61.0	3.61, brd (11.3) 3.43, m

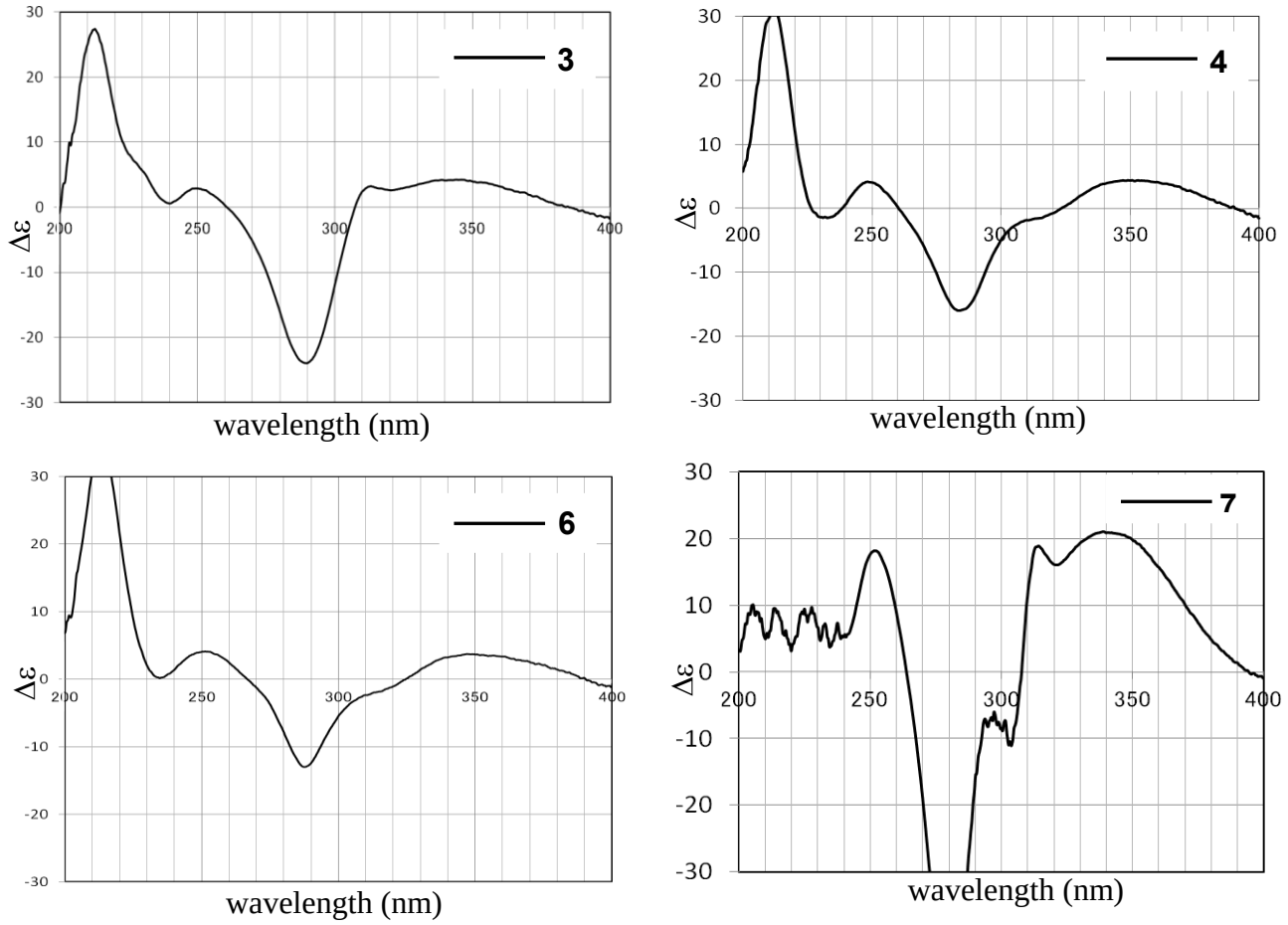


Figure 17. Circular dichroism (CD) spectra of **3**, **4**, **6** and **7**

The attachments of *O*-*β*-D-glucopyranosyl moieties in C-4' and C-7 position were confirmed on the basis of heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) correlations and differential NOE spectra. In the HMBC spectrum, proton resonances at δ 7.45 ($C_{2'}$-H, $C_{6'}$-H) and 7.09 ($C_{3'}$-H, $C_{5'}$-H) had long range correlation with carbon resonance at δ 157.3 (C-4'). Similarly, the signal for an anomeric proton at δ 4.89 had correlations with C-4', which revealed that the one glucose moeity was attached to the 4' position in the B ring. Correlation of anomeric proton at δ 4.59 with carbon at 164.1 (C-7) suggested the attachment of second glucopyranosyl moiety in C-7 which was also supported by the downfield shift of A ring carbons. The signal for C_5-OH (δ 12.10) had correlations with carbons at δ 157.9 (C-5), 111.2 (C-6) and 104.8 (C-10). Key HMBC correlations are given in Figure 18. In the differential NOE spectra, irradiation of C_6-CH_3 proton (δ 2.09 ppm) enhanced the proton signal at δ 4.59 ($C_{7\text{-}Glc}$-C_1-H) and 12.10 (C_5-OH). Also the irradiation of C_8-CH_3 proton (δ 2.07 ppm) enhanced the proton signal at δ 4.59 ($C_{7\text{-}Glc}$-C_1-H).

The circular dichroism (CD) spectrum of **4** showed the positive Cotton effect at 347 nm suggesting the absolute configuration at the C-2 position is *S*.[93] Hence, on the basis of these data the structure of **4** was assigned to be farrerol 4',7-di-*O*-*β*-D-glucopyranoside.

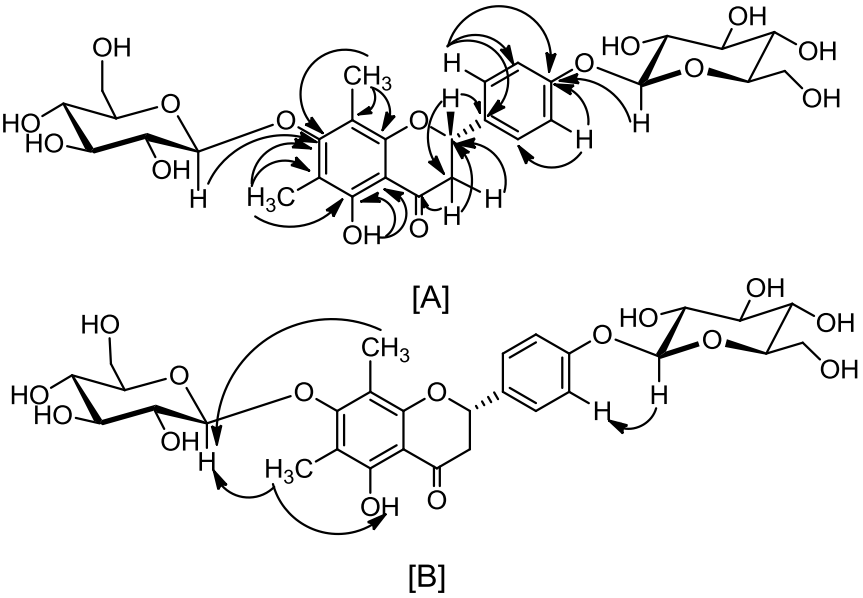


Figure 18. [A] Key HMBC correlations observed in the spectrum of **4**. [B] NOEs observed in the difference NOE experiments of **4**.

The ^{1}H and ^{13}C-NMR data for two known compounds farrerol 7-*O*-*β*-D glucopyranoside (**6**) and farrerol (**7**) are given in Table 6 and 7 are given in Table 3.

2.1.1.5. Diplomorphanin B (5)

Diplomorphanin B (**5)** was obtained as pale yellow amorphous powder, $[\alpha]_D^{21}$ +2.6. The HR-FAB-MS of **5** showed the quasi-molecular ion $[M-H]^-$ peak at *m*/*z:* 475.1280 supporting the formula $C_{23}H_{24}O_{11}$. The ^{1}H-NMR of **5** (Table 4) showed two signals due to two aromatic methyl groups at δ_H 2.21 (3H, s) and 2.42 (3H, s), and the proton resonances at δ_H 8.11 (2H, d, J = 8.7 Hz, $C_{2'}$-H, $C_{6'}$-H) and 6.97 (2H, d, J = 8.7 Hz, $C_{3'}$-H, $C_{5'}$-H) were characteristic of *p*-substituted phenyl ring. Signal for an anomeric proton was observed at δ 4.66 (1H, d, J = 7.7 Hz).

Table 3. NMR spectroscopic data for **6** and **7** in CD_3OD

	6		**7**	
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	80.0	5.27, brs	80.1	5.29, brd (12.8)
3	44.2	2.74, d (17.1) 3.31—3.78	44.1	2.71, brd (17.1) 3.05, dd (12.8, 17.1)
4	199.8		198.4	
5	159.2		159.4	
6	113.0		104.8	
7	162.8		164.2	
8	111.9		104.1	
9	159.8		160.3	
10	106.3		103.3	
1'	131.2		131.6	
2'	128.9	7.29, d (8.5)	128.8	7.31, d (8.8)
3'	116.4	6.81, d (8.5)	116.4	6.82, d (8.8)
4'	158.8		158.8	
5'	116.4	6.81, d (8.5)	116.4	6.82, d (8.8)
6'	128.9	7.29, d (8.5)	128.8	7.31, d (8.8)
6-CH_3	9.2[a]	2.11, s[a]	7.4[a]	1.98, s[a]
8-CH_3	9.8[a]	2.13, s[a]	8.2[a]	1.99, s[a]
Glc-1	105.3	4.73, d (7.3)		
Glc-2	75.7	3.31—3.78		
Glc-3	77.9	3.31—3.78		
Glc-4	71.5	3.31—3.78		
Glc-5	78.0	3.31—3.78		
Glc-6	62.7	3.31—3.78		

[a]assignments may be reversed in same column.

The ^{13}C-NMR (Table 4) and DEPT spectra showed signals equivalent to 23 carbons. Among them, 17 carbon signals were assignable to 6,8-di-*C*-methylkaempferol (**5a**)[98] and the six carbon signals at δ 61.1, 71.5, 74.0, 76.3, 77.0 and 104.0 were assignable to a β-glucopyranosyl moiety which was also supported by the acid hydrolysis of **5** affording **5a** and D-glucose. In the HMBC spectrum, proton resonances for $C_{3'}$-H, $C_{5'}$-H and $C_{2'}$-H, $C_{6'}$-H had long range correlation with carbon resonance at δ 159.4 (C-4'). The C_6-methyl signal (δ2.21) had correlation with carbons at δ 154.9 (C-5), 113.2 (C-6) and 158.5 (C-7). Similarly, C_8-methyl signal (δ2.42) had correlation with carbons at δ 150.0 (C-9), 109.8 (C-8) and 158.5 (C-7). The signal for C_5-OH had correlations with carbons at δ154.9 (C-5), 113.2 (C-6) and 106.0 (C-10). The anomeric proton signal at δ4.66 had correlation with carbon at 158.5 (C-7) which suggested that the glucopyranosyl moiety was attached at C-7 position. Key HMBC correlations have been given in Figure 19.

In the differential NOE spectra, irradiation of C_6-CH_3 proton (δ2.21 ppm) enhanced the proton signal at δ 4.66 (Glc-C_1-H) and 12.58 (C_5-OH) and the irradiation of C_8-CH_3 proton (δ2.42 ppm) enhanced the proton signal at δ 4.66 (Glc-C_1-H) which also supported the above assignments. Hence, on the basis of these data the structure of **5** was assigned to be 6,8-di-*C*-methylkaempferol 7-*O*-β-D-glucopyranoside.

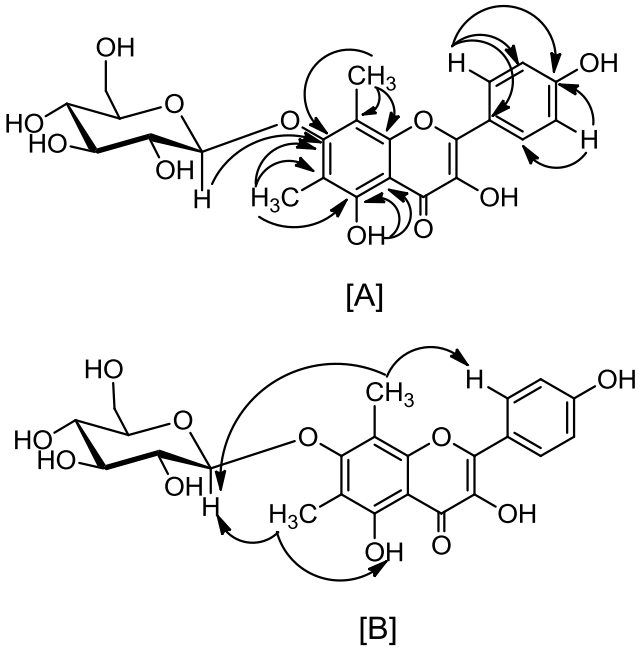


Figure 19. [A] Key HMBC correlations observed in the spectrum of **5**. [B] NOEs observed in the difference NOE experiments of **5**.

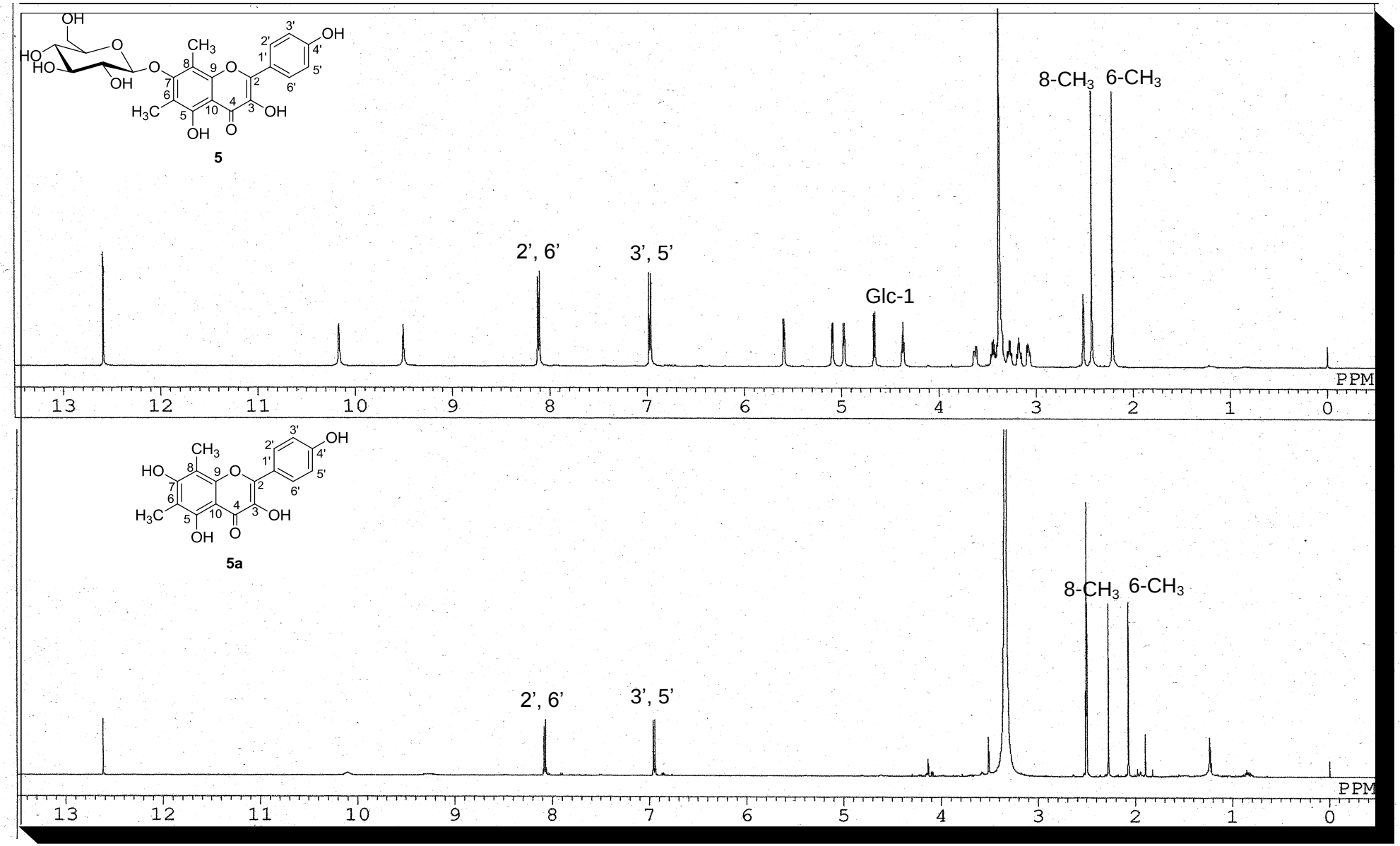


Figure 20. [1]H-NMR spectra of diplomorphanin B (**5**) and 6,8-di-*C*-methylkaempferol (**5a**) in DMSO-d_6

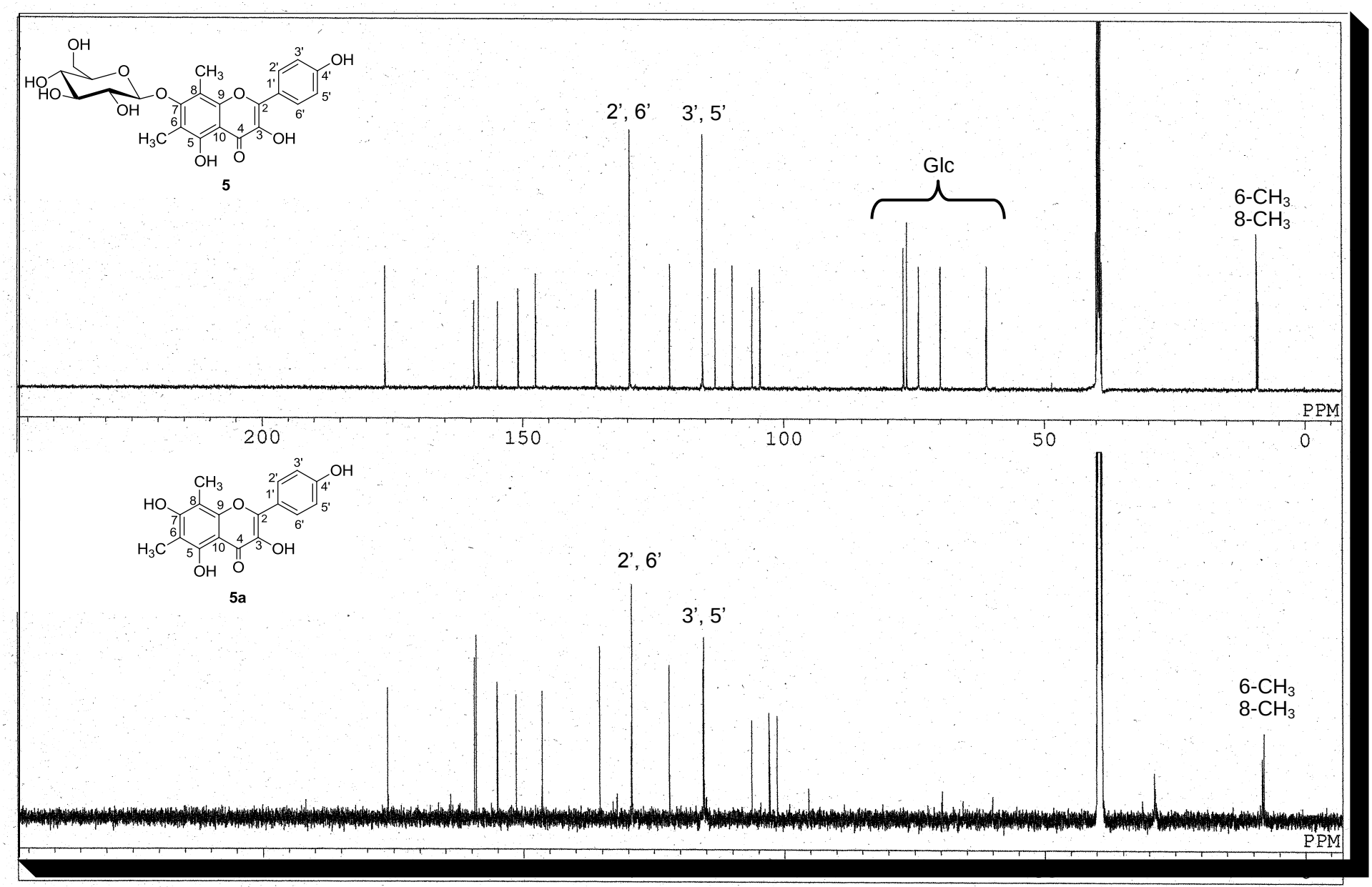


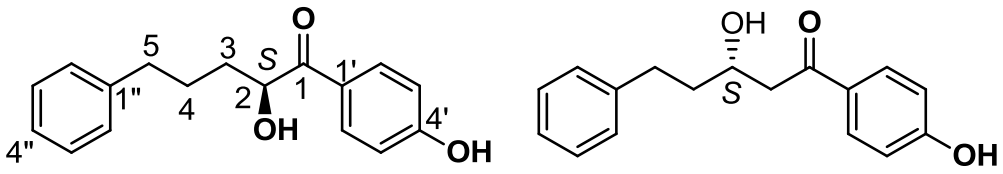
Figure 21. ^{13}C-NMR spectra of diplomorphanin B (**5**) and 6,8-di-*C*-methylkaempferol (**5a**) in DMSO-d_6

Table 4. NMR spectroscopic data for **5** and **5a** in DMSO-d_6

	5		**5a**	
Position	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	147.5		146.5	
3	136.0		135.5	
4	176.5		176.1	
5	154.9		155.0	
6	113.2		106.3	
7	158.5		159.1	
8	109.8		101.4	
9	150.0		151.4	
10	106.0		102.9	
1'	121.8		122.0	
2'	129.5	8.11, d (8.7)	129.4	8.07, d (8.8)
3'	115.5	6.97, d (8.7)	115.5	6.95, d (8.8)
4'	159.4		159.4	
5'	115.5	6.97, d (8.7)	115.5	6.95, d (8.8)
6'	129.5	8.11, d (8.7)	129.4	8.07, d (8.8)
6-CH_3	8.9	2.21, s	8.0	2.07, s
8-CH_3	9.2	2.42, s	8.2	2.27, s
C_5-OH		12.58, s		12.61, s
7-*O*-Glc-1	104.0	4.66, d (7.7)		
7-*O*-Glc-2	74.0	3.27, m		
7-*O*-Glc-3	77.0	3.37, m		
7-*O*-Glc-4	71.5	3.17, m		
7-*O*-Glc-5	76.3	3.37, m		
7-*O*-Glc-6	61.1	3.62, brd (11.4) 3.42, m		

2.1.1.6. Diplomorphanone A (32)

Diplomorphanone A (**32**) was isolated as a pale yellowish oil; $[\alpha]_D^{20}$ -31.4 (*c* 0.74, $CHCl_3$). The HR-FABMS of **32** showed the quasi-molecular ion $[M+Na]^+$ at *m/z*: 293.1151, supporting the molecular formula $C_{17}H_{18}O_3$ (calcd. for $C_{17}H_{18}O_3Na$, 293.1154). The ^{1}H-NMR spectroscopic data of compound **32** (Table 1) showed the proton resonances at δ_H 7.78 (2H, d, *J* = 8.8 Hz, $C_{2'}$-H, $C_{6'}$-H) and 6.87 (2H, d, *J* = 8.8 Hz, $C_{3'}$-H, $C_{5'}$-H) characteristic of 1,4-di-substituted benzene ring. Similarly, the proton resonances at δ_H 7.13 (2H, brd, *J* = 7.7 Hz, $C_{2''}$-H, $C_{6''}$-H), 7.24 (2H, t, *J* = 7.7 Hz, $C_{3''}$-H, $C_{5''}$-H) and 7.16 (1H, d, *J* = 7.7 Hz, $C_{4''}$-H) were characteristic of mono-substituted benzene ring. Signal for methine proton of a secondary hydroxyl group was observed at δ_H 5.02 (1H, m, C_2-H) along with six protons in the aliphatic region (δ_H 2.63 (2H, m, C_5-H), 1.89 (2H, m, C_3-Ha, C_4-Ha), 1.76 (1H, m, C_4-Hb) and 1.58 (1H, m, C_3-Hb). The ^{13}C-NMR (Table 5) showed signals equivalent to 17 carbons including one di-substituted benzene ring, one mono-substituted benzene ring, one ketone (δ_c 200.1, C-1,) one hydroxyl methine group (δ_c 72.5, C-2) and three methylene groups (δ_c 35.5, 35.3, 26.3). These observations suggested that compound **32** may be a diarylpentanoid derivative. By comparing the literature data, **32** was found to be an isomer of daphneolone, (*S*)-3-hydroxy-1-(4-hydroxyphenyl)-5-phenyl-1-pentanone, isolated from *Daphne odora* Thunb.[99] which has characteristically different NMR pattern for the pentan-1-one moiety.



diplomorphanone A **(32)** daphneolone

Figure 22. Structures of diplomorphanone A (**32**) and daphneolone

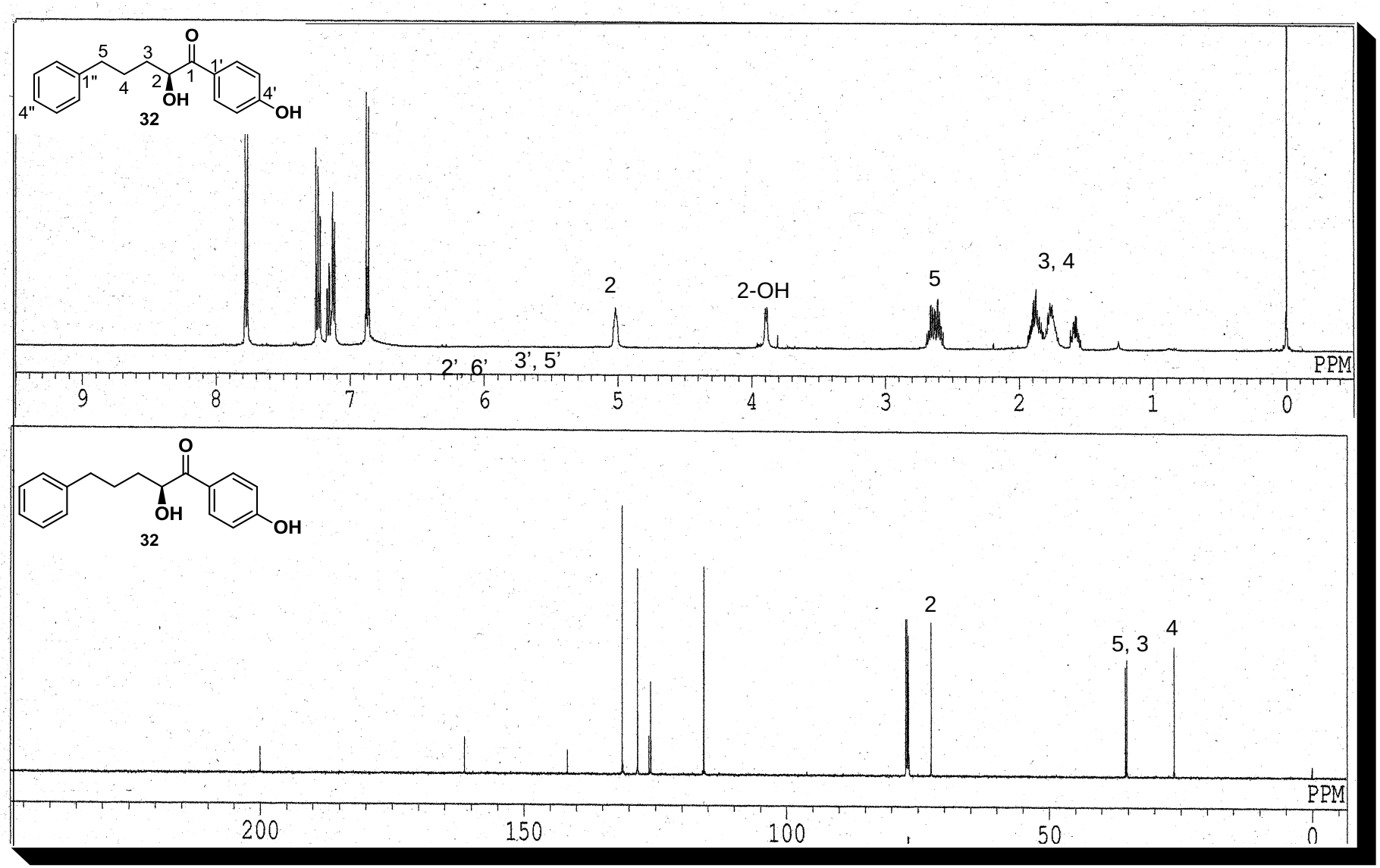


Figure 23. ^{1}H- and ^{13}C- NMR spectra of diplomorphanone A (**32**) in $CDCl_3$

Table 5. ^{1}H- and ^{13}C- NMR spectroscopic data of **32** and daphneolone in $CDCl_3$

position	**32**		daphneolone[99)]	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	200.1		200.0	
2	72.5	5.02, m	46.6	3.08, m
3	35.5	1.89, m	68.9	4.17, m
		1.58, m		
4	26.3	1.89, m	40.3	1.83, m
		1.76, m		
5	35.3	2.63, m	32.9	2.71, m
1'	126.1		130.4	
2', 6'	131.3	7.78, d (8.8)	132.0	7.86, d (7.0)
3', 5'	115.9	6.87, d (8.8)	112.4	6.82, d (7.0)
4'	161.3		163.9	
1"	141.7		143.4	
2", 6"	128.4	7.13, brd (7.7)	129.4	7.18, m
3", 5"	128.4	7.24, t (7.7)	129.4	7.18, m
4"	125.9	7.16, d (7.7)	126.7	7.14, m
2-OH		3.89, d (6.4)		

All assignments were made on the basis of ^{1}H-^{1}H COSY, HMQC and HMBC correlations. From the ^{1}H-^{1}H COSY spectrum, partial structure in pentanone moiety was found to be (—CH(OH) —CH_2—CH_2— CH_2—) as correlations were observed between C_2-OH (δ_H 3.89) and C_2-H (δ_H 5.02); C_2-H (δ_H 5.02) and C_3-Hb (δ_H 1.58); C_3-Hb (δ_H 1.58) and C_4-Hb (δ_H 1.76); C_4-Hb (δ_H 1.76) and C_5-H (δ_H 2.63). In the HMBC spectrum, proton resonances at δ_H 5.02 (C_2-H), 3.89 (C_2-OH), 1.89 (C_3-Ha), 7.78 ($C_{2'}$-H, $C_{6'}$-H) and 6.87 ($C_{3'}$-H, $C_{5'}$-H), had correlations with carbon at δ_c 200.1 (C-1). Proton resonances at δ_H 7.78 ($C_{2'}$-H, $C_{6'}$-H) and 6.87 ($C_{3'}$-H, $C_{5'}$-H), also had correlation with carbon at δ_c 161.3 (C-4') and δ_c 126.1 (C-1'). Similarly, proton at δ_H 2.63

(C_5-H), 7.13 ($C_{2''}$-H, $C_{6''}$-H) and 7.24 ($C_{3''}$-H, $C_{5''}$-H) had correlations with carbon at δ_c 141.7 (C-1''). Key HMBC correlations are also given in Figure 24. The 2*S* configuration was assigned on the basis of similar optical rotation to that of (*S*)-2-hydroxy-1-phenyl-1-pentanone, $[\alpha]_D^{20}$ -19.9 (*c* 2.4, $CHCl_3$);[100] (*S*)-α-hydroxy butanones[101] and related ketones.[102,103] Hence, the structure of **32** was assigned to be 2(*S*)-hydroxy-1-(4-hydroxyphenyl)-5-phenyl-1-pentanone.

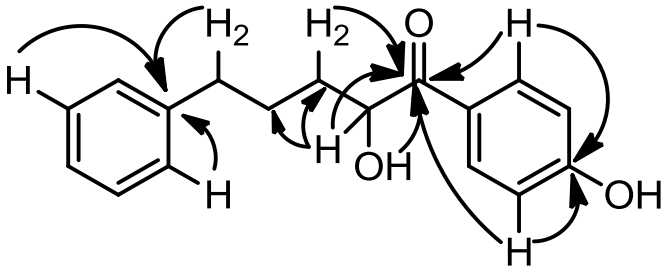


Figure 24. Key HMBC correlations observed in the spectrum of **32**.

2.1.2. Known compounds

The ^{1}H-NMR data for known flavonoids kaempferol (**8**), kaempferol 3-*O*-*β*-D-glucopyranoside (**9**), rhamnocitrin 3-*O*-*β*-D-glucopyranoside (**10**), quercetin (**11**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**) and rhamnetin 3-*O*-*β*-D-glucopyranoside (**13**) are given in Table 6.

Similarly, ^{1}H-NMR data for known flavonoids, apigenin (**14**), genkwanin (**15**), genkwanin 5-*O*-*β*-D-glucopyranoside (**16**), genkwanin 5-*O*-primeveroside (**17**), luteolin (**18**) and luteolin 7-methyl ether-5-*O*-*β*-D-glucopyranoside (**19**) are given in Table 7.

The ^{13}C-NMR data compounds **8—13** are given in Table 8.

Table 6. ^{1}H NMR data of compounds **8—13** [$\delta_{H,}$ mult. (J in Hz)]

Position	**8**[a]	**9**[b]	**10**[c]	**11**[a]	**12**[a]	**13**[a]
6	6.23, d (1.8)	6.26, s	6.32, d (1.8)	6.21, d (1.8)	6.21, d (1.8)	6.37, d (2.4)
8	6.47, d (1.8)	6.40, s	6.57, d (1.8)	6.44, d (1.8)	6.42, d (1.8)	6.37, d (2.4)
2'	8.07, d (8.5)	7.97, d (8.5)	8.06, d (9.1)	7.70, d (1.8)	7.59, s	7.62, d (2.4)
3'	6.96, d (8.5)	6.96, d (8.5)	6.89, d (9.1)			
5'	6.96, d (8.5)	6.96, d (8.5)	6.89, d (9.1)	6.92, d (8.5)	6.85, d (9.1)	6.86, d (8.5)
6'	8.07, d (8.5)	7.97, d (8.5)	8.06, d (9.1)	7.57, dd (1.8, 8.5)	7.58, d (9.1)	7.61, dd (2.4, 8.5)
OCH_3			3.87, s			3.86, s
Glc-1		5.02, d (6.7)	5.23, d (7.3)		5.46, d (7.3)	5.48, d (7.3)
Glc-2		3.17—3.90	3.19—3.70		3.10—3.59	3.10—3.59
Glc-3		3.17—3.90	3.19—3.70		3.10—3.59	3.10—3.59
Glc-4		3.17—3.90	3.19—3.70		3.10—3.59	3.10—3.59
Glc-5		3.17—3.90	3.19—3.70		3.10—3.59	3.10—3.59
Glc-6		3.17—3.90	3.19—3.70		3.10—3.59	3.10—3.59
5-OH	12.64, s			12.5, s	12.63, s	12.63, s

[a]in DMSO-d_6, [b]in CD_3OD+D_2O, [c]in $CD_3OD+CDCl_3$

Table 7. ^{1}H NMR data of compounds **14—19** [$\delta_{H,}$ mult. (*J* in Hz)]

C No.	**14**[a]	**15**[a]	**16**[a]	**17**[a]	**18**[a]	**19**[a]
3	6.76, s	6.83, s	6.71, s	6.70, s	6.66, s	6.60, s
6	6.21, d (1.8)	6.37, d (2.1)	6.91, d (2.4)	6.87, d (2.4)	6.19, d (2.1)	6.90, d (2.4)
8	6.49, d (1.8)	6.96, d (2.1)	7.05, d (2.4)	7.03, d (2.4)	6.45, d (2.1)	7.01, d (2.4)
2'	7.92, d (8.5)	7.95, d (8.8)	7.93, d (9.1)	7.93, d (8.5)	7.40, d (2.1)	7.43, d (2.4)
3'	6.94, d (8.5)	6.93, d (8.8)	6.93, d (9.1)	6.93, d (8.5)		
5'	6.94, d (8.5)	6.93, d (8.8)	6.93, d (9.1)	6.93, d (8.5)	6.90, d (8.2)	6.89, d (7.9)
6'	7.92, d (8.5)	7.95, d (8.8)	7.93, d (9.1)	7.93, d (8.5)	7.42, dd (2.1, 8.2)	7.42, dd (2.4, 7.9)
OCH_3		3.87, s	3.91, s	3.90, s		3.90, s
Glc-1			4.77, d (7.3)	4.77, d (7.3)		4.77, d (7.9)
Glc-2			3.10—3.78	2.98—3.99		3.10—3.78
Glc-3			3.10—3.78	2.98—3.99		3.10—3.78
Glc-4			3.10—3.78	2.98—3.99		3.10—3.78
Glc-5			3.10—3.78	2.98—3.99		3.10—3.78
Glc-6			3.10—3.78	2.98—3.99		3.10—3.78
Xyl-1				4.20, d (7.3)		
Xyl-2				2.98—3.99		
Xyl-3				2.98—3.99		
Xyl-4				2.98—3.99		
Xyl-5				2.98—3.99		
5-OH	12.97, s	12.95, s			12.97, s	

[a]in DMSO-d_6

Table 8. ^{13}C NMR data of compounds **8—19**

C No.	**8**[a]	**9**[b]	**10**[c]	**11**[a]	**12**[a]	**13**[a]	**14**[a]	**15**[a]	**16**[a]	**17**[a]	**18**[a]	**19**[a]
2	146.8	157.0	158.1	146.8	156.2	156.2	163.8	164.0	161.4	161.3	163.8	163.6
3	135.6	135.1	135.7	135.7	133.4	133.5	102.9	102.9	103.6	105.8	102.8	105.9
4	175.9	179.0	179.4	175.8	177.5	177.5	181.7	181.8	177.0	176.8	181.7	176.9
5	160.7	161.6	162.5	160.7	161.2	160.8	161.5	161.2	158.4	158.1	161.4	158.5
6	98.2	100.0	99.0	98.1	98.7	97.8	98.8	97.9	103.0	102.9	98.8	103.4
7	163.9	164.6	167.1	163.8	164.1	165.0	164.2	165.0	163.5	163.6	164.1	161.6
8	93.5	95.3	93.2	93.3	93.5	95.3	94.0	92.6	96.4	96.2	93.8	96.5
9	156.2	159.2	159.3	156.1	156.3	156.5	157.4	157.1	158.0	158.4	157.3	158.2
10	103.0	105.6	106.4	102.9	104.0	104.9	103.7	104.5	109.0	109.1	103.7	109.3
1'	121.7	122.6	124.0	121.9	121.2	121.0	121.2	120.9	121.0	121.1	121.5	121.5
2'	129.5	132.0	132.2	115.4	115.2	115.1	128.4	128.5	128.1	128.2	113.3	113.3
3'	115.9	116.2	116.0	145.0	144.8	144.7	115.9	115.9	115.9	115.9	145.7	145.8
4'	159.1	160.2	161.4	147.6	148.5	148.5	161.2	161.2	160.8	160.8	149.7	149.4
5'	115.9	116.2	116.0	115.5	116.2	116.3	115.9	115.9	115.9	115.9	116.0	116.0
6'	129.5	132.0	132.2	119.9	121.6	121.6	128.4	128.5	128.1	128.2	118.9	118.6
OCH_3			56.5			56.0		55.9	56.0	56.1		56.1
Glc-1		103.7	104.6		100.9	100.8			105.7	103.7		104.1
Glc-2		74.9	75.6		74.1	74.0			73.4	73.3		73.6
Glc-3		77.4	78.0		77.5	77.5			77.4	75.9		75.8
Glc-4		70.5	71.3		69.9	69.8			69.9	69.5		70.0
Glc-5		77.0	77.9		76.5	76.4			75.6	75.6		77.6
Glc-6		61.8	62.7		61.0	60.9			60.8	68.6		61.0
Xyl-1										104.9		
Xyl-2										73.4		
Xyl-3										76.5		
Xyl-4										69.5		
Xyl-5										65.6		

[a]in DMSO-d_6, [b]in CD_3OD+D_2O, [c]in $CD_3OD+CDCl_3$

2.2. Compounds from the roots of *Diplomorpha canescens*

The dried roots of *D. canescens* (500 g) were extracted twice with 70% MeOH (4.5 l) and extracts were evaporated under reduced pressure to give 70% MeOH extract (104 g). The extract was then separated into water soluble part (45 g) and water insoluble part (59 g). Water insoluble part was subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH-20, octadecyl silica (ODS) and silica gel to afford two new biflavonoids 14”-*O*-methyldihydrodaphnodorin B (**33**) and 14”-*O*-methyldaphnodorin J (**35**) along with 16 known compounds.

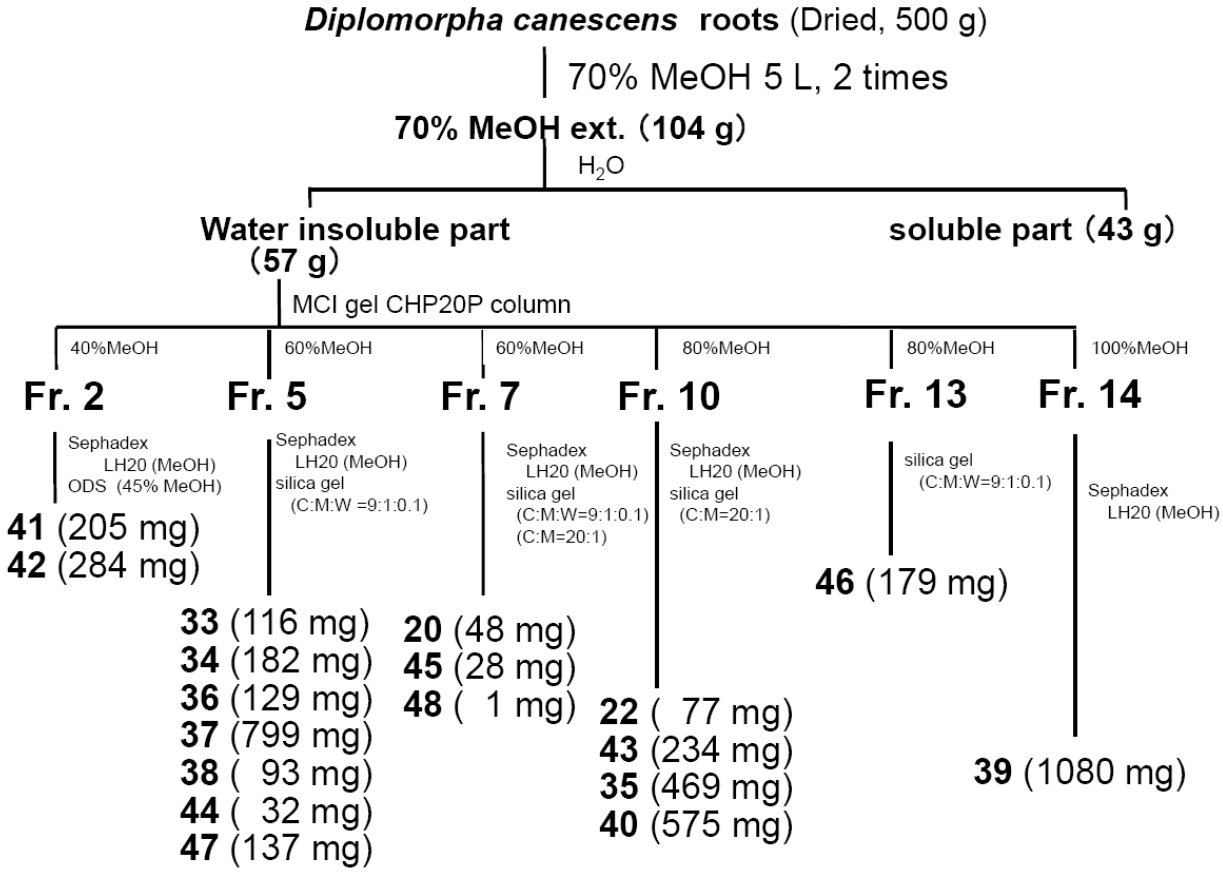


Chart 2. Extraction and isolation of compounds from roots of *Diplomorpha canescens*

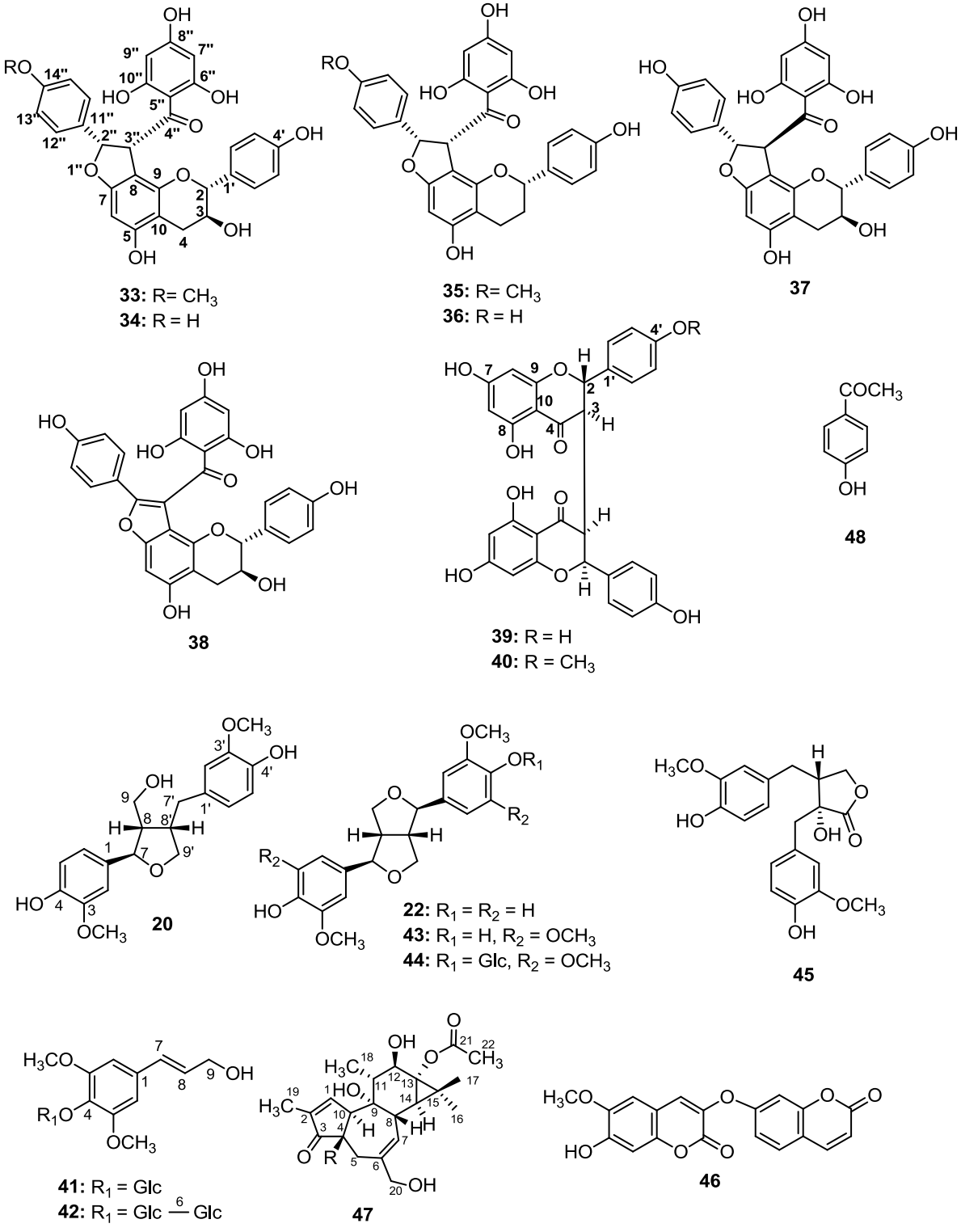


Figure 25. Structures of the compounds isolated from roots of *Diplomorpha canescens*

From the detailed spectral analysis, structures of known compounds were identified to be six biflavonoids, dihydrodaphnodorin B (**34**),[104,105] daphnodorin J (**36**),[104,105] 3''-epi-dihydrodaphnodorin B (**37**),[106] daphnodorin B (**38**),[107-109] neochamaejasmin B(**39**),[36,110] sikokianin B (**40**);[36] five lignans, (-)-syringaresinol (**43**),[112] (-)-syringaresinol 4-*O*-β-D-glucopyranoside (**44**),[86] (+)-nortrachelogenin (**45**),[113] (-)-lariciresinol (**20**), (-)-pinoresinol (**22**); two phenylpropanoids, syringin (**41**),[35] syringinoside (**42**);[35,111] daphnoretin (**46**),[114] phorbol 13-acetate (**47**)[115] and methyl paraben (**48**)[116] (Figure 25). All of these compounds were isolated for the first time from this plant.

2.2.1. New compounds

2.2.1.1. 14''-*O*-Methyldihydrodaphnodorin B (33)

Compound **33** was obtained as pale yellow amorphous powder, $[\alpha]_D^{20}$ +25.5º (*c* 0.84, MeOH). The HRFABMS of **33** showed the quasi-molecular ion $[M-H]^-$ at *m/z:* 557.1475 supporting the formula $C_{31}H_{26}O_{10}$. The ^{1}H-NMR spectrum of **33** (Table 9) showed signals due to two pair of oxyphenyl groups at 7.19, 6.68 (each 2H, d, *J* = 8.5 Hz) and 7.09, 6.67 (each 2H, d, 8.5 Hz); a 2,4,6-trioxyphenyl group at 5.65 (2H, s), a pair of coupled benzylmethine protons at 6.07, 5.96 (each 1H, d, *J* = 10.3 Hz); and a set of protons resembling 5,7,8,4'-substituted flavanol moiety at 4.71 (1H, d, *J* = 7.3 Hz), 3.92 (1H, brd, *J* = 7.3, 12.1 Hz), 2.83 (1H, dd, *J* = 4.8, 16.1) and 2.57 (1H, dd, *J* = 7.3, 16.1) and a methoxyl proton at 3.67 (3H, s). All these ^{1}H- and ^{13}C-NMR data were similar to that of dihydrodaphnodorin B (**34**)[104] except for the methoxyl group which suggested that **33** was a methyl ether derivative of **34**. The attachment of methyl group at 14''-*O*-position was confirmed on the basis of 2D-NMR data including ^{1}H-^{1}H COSY, HMBC and HMQC. In HMBC spectra, proton signal for methoxyl group at δ 3.67 had correlation with carbon at δ 166.0 (C-14'') which also correlated to protons at δ 7.19 (H-12'',H-16'') and 6.68 (H-13'',H-15''). Similarly, protons at δ 7.19 (H-12'',H-16'') also had correlation with carbon at δ 88.8 (C-2''). Key HMBC correlations are given in Figure 28.

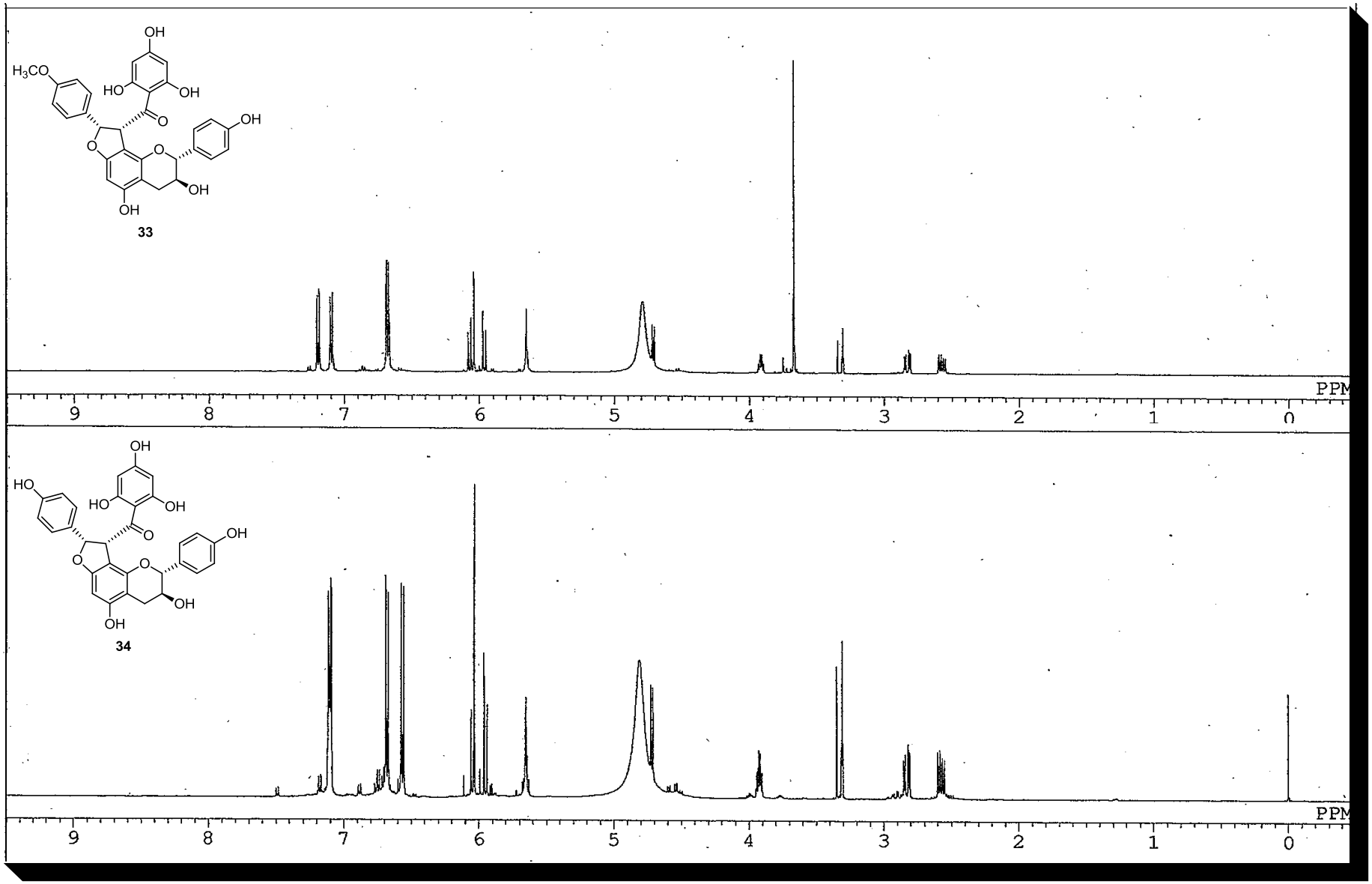


Figure 26. ^{1}H-NMR spectra of 14”-*O*-methyldihydrodaphnodorin B (**33**) and dihydrodaphnodorin B (**34**) in CD_3OD

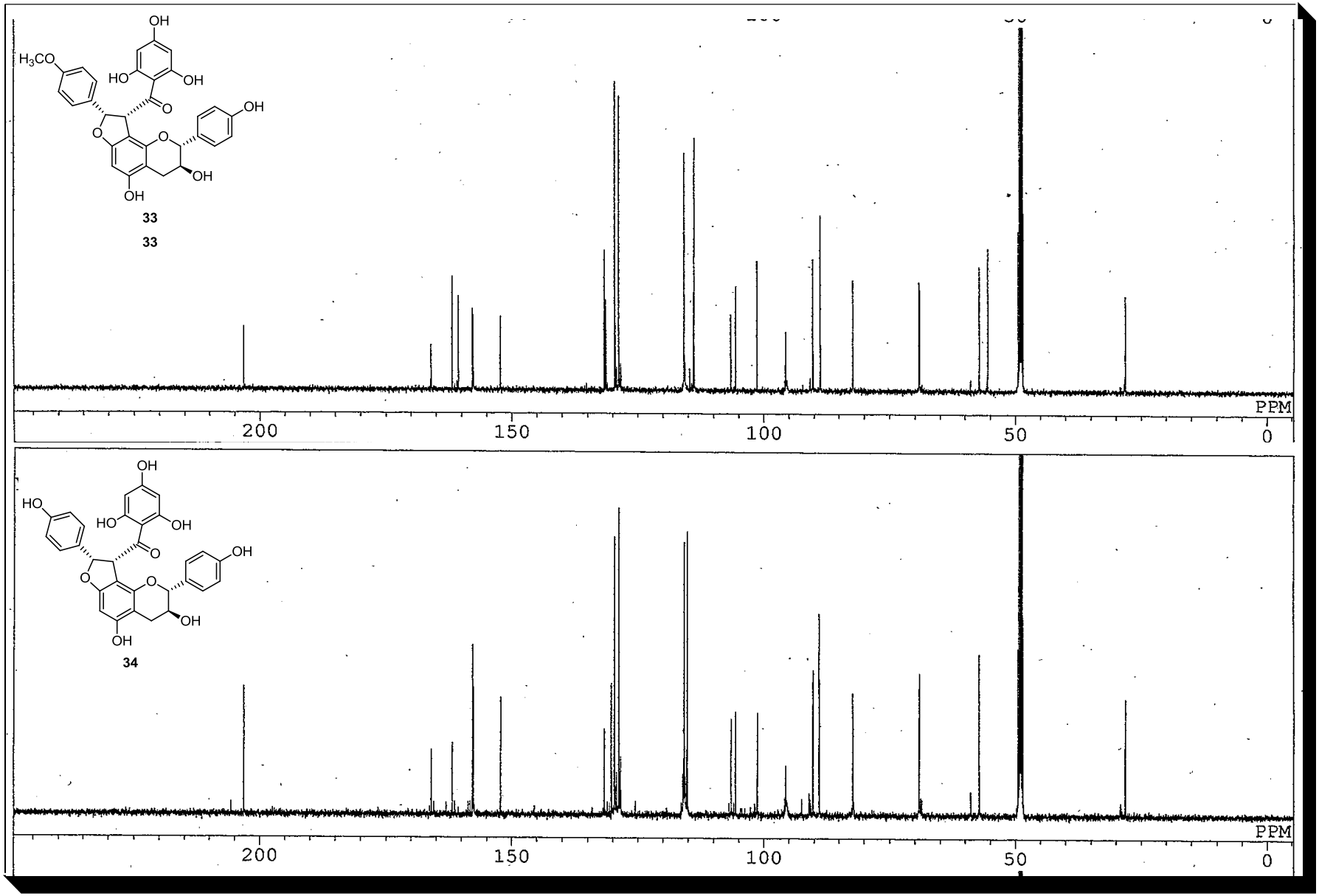


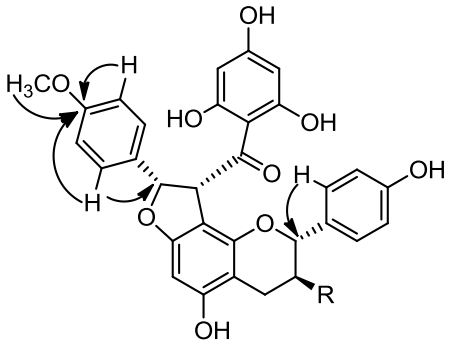
Figure 27. ^{13}C-NMR spectra of 14''-*O*-methyldihydrodaphnodorin B (**33**) and dihydrodaphnodorin B (**34**) in CD_3OD

Table 9. ^{1}H NMR data of compounds **33—38** in CD_3OD

C No.	**33**	**34**	**35**	**36**	**37**	**38**
2	4.71,d (7.3)	4.72,d (7.3)	4.87,br d (8.5)	4.89,dd (1.8, 10.5)	4.54,d (7.6)	4.51,d (8.2)
3	3.92, brd (7.3, 12.1)	3.93, brd (7.3, 12.1)	2.10, m	2.10, m	3.99, brd (7.6, 12.3)	3.87, m
			1.78, m	1.78, m		
4	2.83, dd (4.8, 16.1)	2.83, dd (4.8, 16.1)	2.70, m	2.70, m	2.83, dd (5.4, 16.1)	3.01, dd (5.5, 16.0)
	2.57, dd (7.3, 16.1)	2.57, dd (7.3, 16.1)	2.60, m	2.60, m	2.57, dd (7.3, 16.1)	2.62, dd (8.8, 16.0)
6	6.04, s	6.03, s	6.02, s	6.01, s	5.99, s	6.55, s
2',6'	7.09, d (8.5)	7.09, d (8.5)	7.02, d (8.5)	7.02, d (8.5)	7.11, d (8.5)	7.49, d (8.8)
3',5'	6.67, d (8.5)	6.67, d (8.5)	6.63, d (8.5)	6.63, d (8.5)	6.70, d (8.5)	6.75, d (8.8)
2"	6.07, d (10.3)	6.04, d (10.3)	6.08, d (10.3)	6.06, d (10.6)	5.67, d (5.4)	
3"	5.96, d (10.3)	5.95, d (10.3)	5.95, d (10.3)	5.95, d (10.6)	5.89, d (5.4)	
7",9"	5.65, s	5.65, s	5.69, s	5.69, s	5.64, s	5.68, s
12",16"	7.19, d (8.5)	7.11, d (8.5)	7.19, d (8.5)	7.11, d (8.5)	7.17, d (8.5)	6.96, d (8.2)
13",15"	6.68, d (8.5)	6.56, d (8.5)	6.68, d (8.5)	6.56, d (8.5)	6.74, d (8.5)	6.67, d (8.2)
OCH_3	3.67, s		3.67, s	3.67, s		

Table 10. ^{13}C NMR data of compounds **33—38** in CD_3OD

C No.	**33**	**34**	**35**	**36**	**37**	**38**
2	82.2	82.2	78.4	78.4	82.5	82.7
3	69.1	69.1	31.7	31.7	68.6	69.0
4	28.2	28.1	20.4	20.4	29.1	29.9
5	166.0	166.0	166.0	166.0	165.6	154.9
6	90.2	90.2	89.9	89.8	91.0	90.8
7	157.6	157.6	157.3	157.3	166.6	149.8
8	106.5	106.5	106.5	106.5	106.8	111.6
9	161.8	161.8	161.5	161.5	161.6	154.6
10	101.3	101.2	103.3	103.3	101.7	104.5
1'	131.6	131.6	134.6	134.6	131.1	130.8
2', 6'	128.8	128.8	127.5	127.5	129.2	128.4
3', 5'	115.8	115.8	115.8	115.8	116.1	115.8
4'	152.2	152.2	153.2	153.2	158.3	157.7
2”	88.8	89.0	88.7	89.9	90.7	118.7
3”	57.2	57.1	57.2	57.2	58.9	149.2
4”	203.1	203.2	203.3	203.2	205.8	196.7
5”	105.6	105.6	106.1	106.0	105.9	107.9
6”,10”	157.7	157.7	157.3	157.7	158.4	167.4
7”,9”	95.6	95.6	95.6	95.6	97.0	95.7
8”	160.6	157.8	160.5	157.8	157.9	157.8
11”	131.3	130.2	131.5	130.2	134.1	123.5
12”,16”	129.5	129.6	129.5	129.6	128.5	129.0
13”,15”	113.9	115.2	113.9	115.2	115.8	116.4
14”	166.0	157.8	166.0	157.8	152.4	158.7
OCH_3	55.5		55.5			



33: R= OH
35: R = H

Figure 28. Key HMBC correlations observed in the spectrum of **33** and **35**.

The CD spectral data (Figure 29) was also similar to that of daphnodorin J (**36**)[104)] which suggested the *R* configuration at C-2 carbon and *trans* configuration in between C-2 and C-3 proton was concluded on the basis of large coupling constant (7.3 Hz) between these two protons. Similarly, the relative configuration between C-2" and C-3" was decided to be *cis* on the basis of the coupling constant (10.3 Hz) in ^{1}H- NMR spectra of **33** but the absolute configuration remains to be determined. Finally, the relative structure for **33** was decided to be 14"-*O*-methyldihydrodaphnodorin B as shown in Figure 25.

2.2.1.2. 14"-*O*-Methyldaphnodorin J (35)

Compound **35** was obtained as pale yellow amorphous powder, $[\alpha]_D^{20}$ +37.7º (*c* 0.71, MeOH). The HRFABMS of **35** showed the quasi-molecular ion $[M-H]^-$ at *m/z:* 541.1524 supporting the formula $C_{31}H_{26}O_9$. The ^{1}H-NMR spectrum of **35** (Figure 30, Table 9) showed signals due to two pair of oxyphenyl groups at 7.19, 6.68 (each 2H, d, *J* = 8.5 Hz) and 7.09, 6.67 (each 2H, d, 8.5 Hz); a 2,4,6-trioxyphenyl group at 5.65 (2H, s), a pair of coupled benzylmethine protons at 6.08, 5.95 (each 1H, d, *J* = 10.3 Hz); and a set of protons resembling 5,7,8,4'-substituted flavan moiety at 4.87 (1H, brd, *J* = 8.7 Hz), 2.70, 2.60, 2.10, 1.78 (each 1H, m) and a methoxyl proton at 3.67(3H, s). All these ^{1}H- and ^{13}C-NMR data except for methyl group were similar to that of dihydrodaphnodorin A or daphnodorin J (**36**) which suggested that **35** was a methyl ether derivative of **36**. Comparing the spectral data of **35** with **33**, the presence of a methylene carbon (δc 31.7) in **35** instead of methine carbon (δc 69.1) in **33** also suggested the above statement. The

attachment of methyl group at 14”-*O*-position was confirmed on the basis of 2D-NMR data including ^{1}H-^{1}H COSY, HMBC and HMQC as for **33**. Key HMBC correlations are given in Figure 28.

The CD spectral data (Figure 29) was also similar to that of daphnodorin J (**36**)[104] which suggested the *S* configuration at C-2 carbon. Similarly, the relative configuration between C-2” and C-3” was decided to be *cis* on the basis of the coupling constant (10.6 Hz) in ^{1}H- NMR spectra of **35** but the absolute configuration remains to be determined. Finally, the relative structure for **35** was decided to be 14”-*O*-methyldihydrodaphnodorin A or 14”-*O*-methyldaphnodorin J as shown in Figure 25.

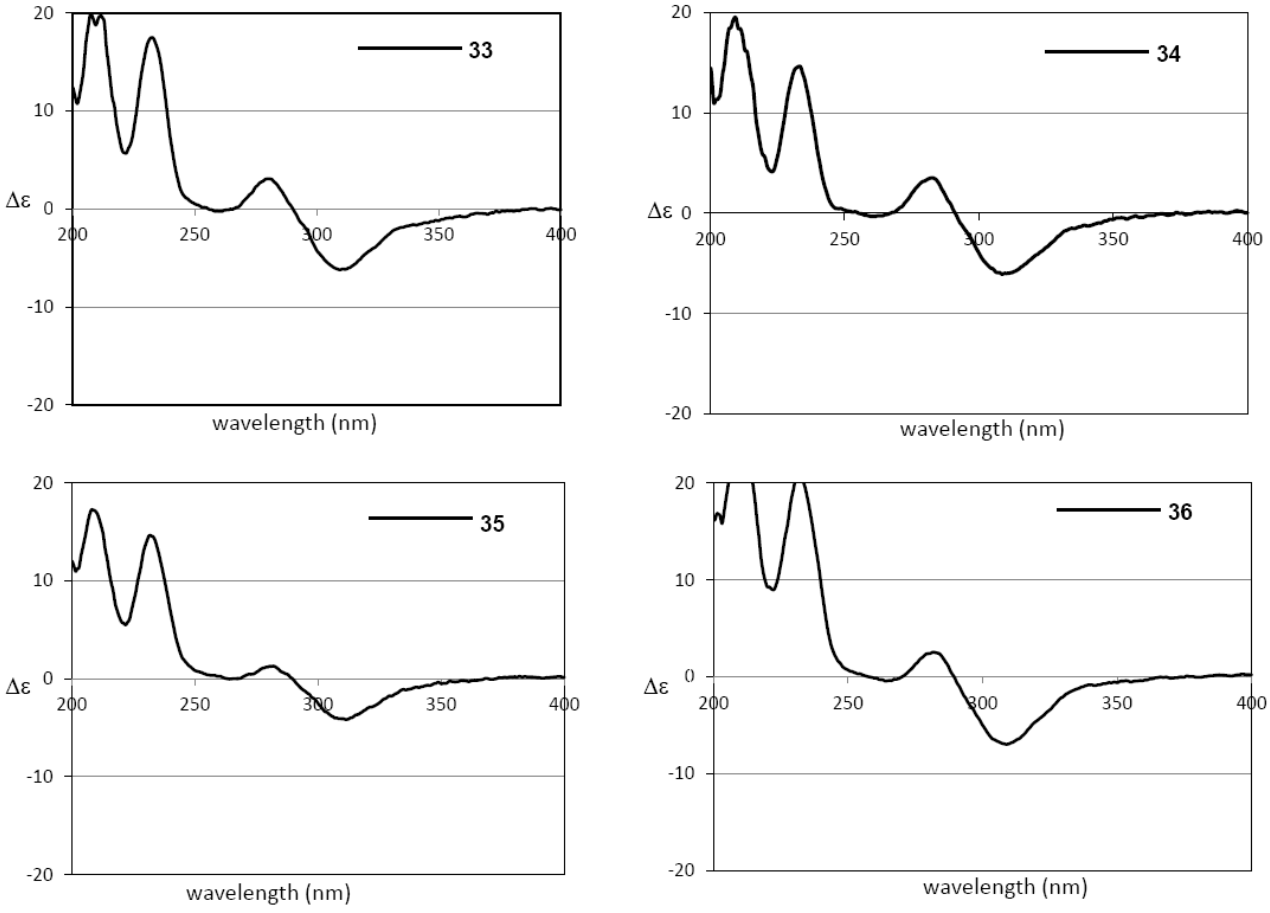


Figure 29. CD spectral data for compounds **33**-**36**.

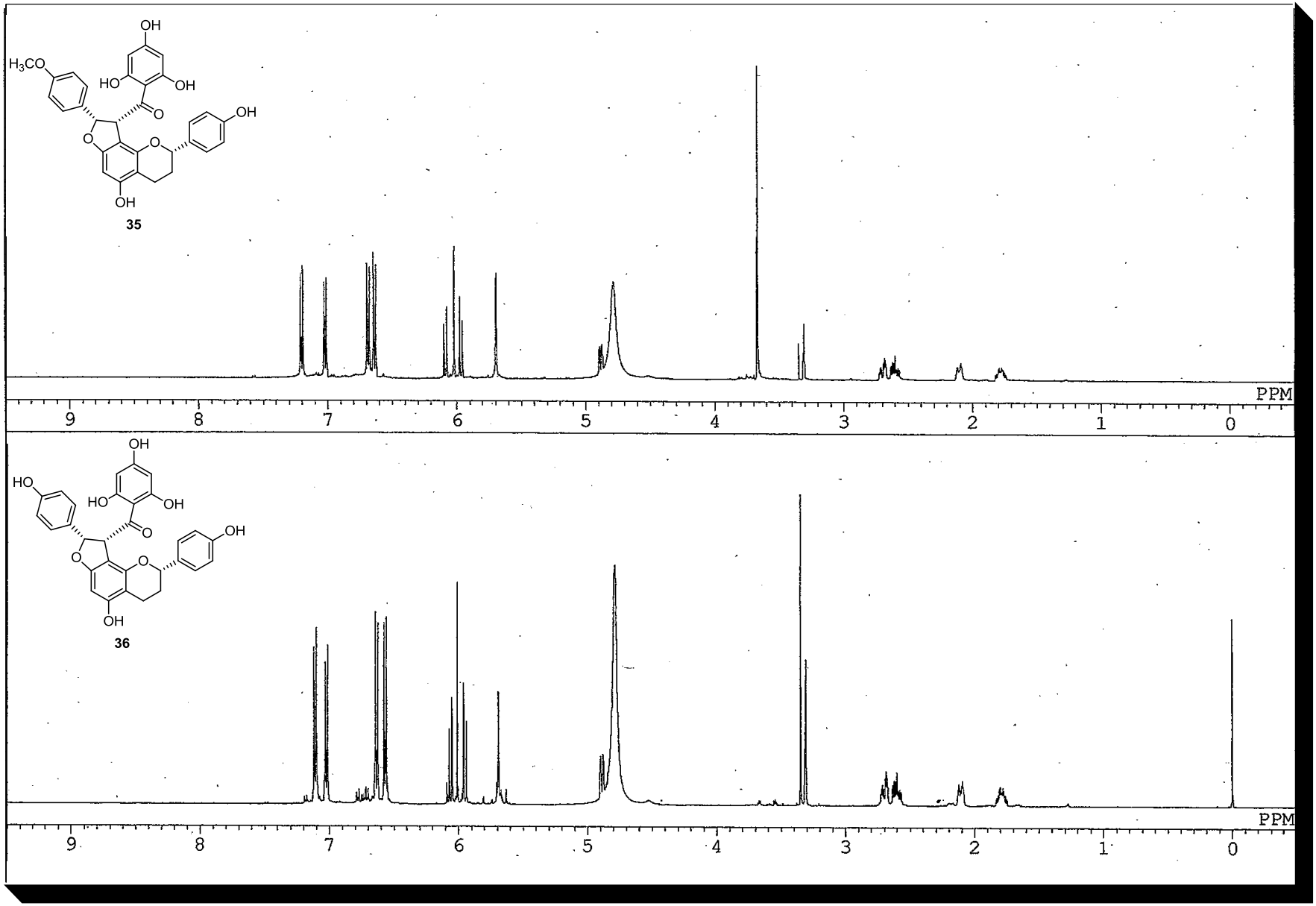


Figure 30. ^{1}H-NMR spectra of 14”-*O*-methyldaphnodorin J (**35**) and daphnodorin J (**36**) in CD_3OD

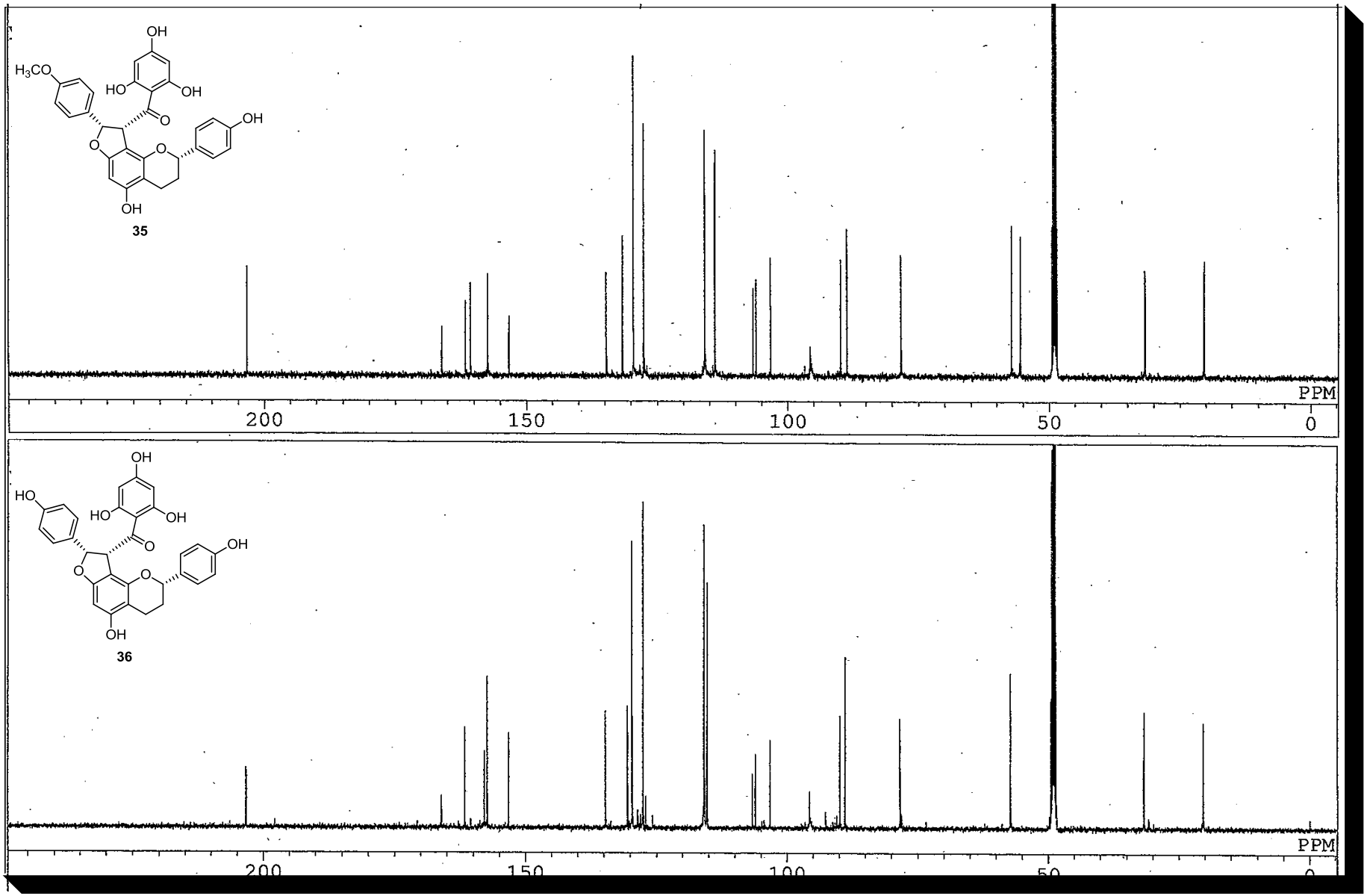


Figure 31. ^{13}C-NMR spectra of 14”-*O*-methyldaphnodorin J (**35**) and daphnodorin J (**36**) in CD_3OD

2.2.2. Known compounds

The NMR spectral data for known compounds neochamaejasmin B (**39**) and sikokianin B (**40**) are given in Table 11.

Table 11. NMR data of compounds **39—40** in CD_3OD

	39		**40**	
Position	δ_C	$\delta_{H,}$ mult. (*J* in Hz)	δ_C	$\delta_{H,}$ mult. (*J* in Hz)
2	83.1	5.15, d (8.8)	82.8	5.19, d (8.8)
3	50.7	3.27, dd (3.6, 8.8)	50.7	3.32, dd (3.6, 8.8)
4	198.5		196.0	
5	165.1		165.1	
6	97.2	5.79, d (2.1)	97.0	5.78, d (2.1)
7	168.2		168.0	
8	96.3	5.98, d (2.1)	96.0	5.97, d (2.1)
9	163.3		163.2	
10	105.0		105.0	
1’	129.0		130.1	
2’,6’	130.1	6.93, d (8.5)	128.4	7.03, d (8.5)
3’, 5’	116.4	6.65, d (8.5)	114.7	6.74, d (8.5)
4’	158.8		161.3	
2’’	81.4	5.53, brs	81.4	5.51, brs
3’’	49.5	3.15, brs	49.5	3.23, brs
4’’	196.2		198.5	
5’’	165.4		165.4	
6’’	97.0	5.76, d (2.1)	97.2	5.76, d (2.1)
7’’	168.0		168.2	
8’’	95.9	5.88, d (2.1)	96.4	5.85, d (2.1)
9’’	165.0		165.0	
10’’	105.8		103.7	
1’’’	128.7		128.6	
2’’’, 6’’’	128.4	7.15, d (8.5)	128.4	7.15, d (8.5)
3’’’, 5’’’	116.1	6.76, d (8.5)	116.3	6.78, d (8.5)
4’’’	158.5		158.5	
OCH_3			55.7	3.74, s

The ^{13}C-NMR data for compounds syringin (**41**), syringinoside (**42**), (-)-syringaresinol (**43**), (-)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**44**) and (+)-nortrachelogenin (**45**) are given in Table 12.

Table 12. ^{13}C NMR data of compounds **41-45**

C No.	**41**[a]	**42**[a]	**43**[b]	**44**[b]	**45**[b]
1	135.0	134.4	132.0	138.7	129.4
2	105.3	105.2	102.7	103.9	114.3[d]
3	153.8	153.7	147.2	153.5	146.7[e]
4	135.2	135.1	134.3	135.1	144.3[f]
5	153.8	153.7	147.2	153.5	111.2[g]
6	105.3	105.2	102.7	103.9	122.4
7	131.1	131.2	86.0	86.4	31.1
8	129.9	129.7	54.3	54.4[e]	48.4
9	63.2[d]	63.1[d]	71.7	72.3[d]	69.0
1’			132.0	131.9	125.2
2’			102.7	103.7	114.6 [d]
3’			147.2	148.4	147.2 [e]
4’			134.3	135.4	144.8 [f]
5’			147.2	148.4	113.1[g]
6’			102.7	103.7	120.3
7’			86.0	86.8	37.9
8’			54.3	55.6[e]	76.2
9’			71.7	72.4 [d]	178.7
OCH_3	57.2	57.1	56.3	56.8, 56.6	55.0
Glc-1	104.7	104.7, 103.5		105.5	
Glc-2	75.1	74.3, 74.8		74.8	
Glc-3	77.1	76.9, 77.0		76.8	
Glc-4	70.5	70.4, 70.8		70.4	
Glc-5	77.6	77.1, 76.9		77.2	
Glc-6	61.8[d]	68.7, 61.8[d]		62.1	

[a]in $CD_3OD + D_2O$, [b]in $CDCl_3$, [c]in $CDCl_3$+CD_3OD, [d-f]assignments may be reversed in the same column.

2.3. Compounds from stems of *Diplomorpha ganpi*

The fresh stems of *D. ganpi* (3 kg) were extracted twice with MeOH (20 L) and extracts were evaporated under reduced pressure to give MeOH extract (122 g) which was then separated into water soluble part (92 g) and water insoluble part (30 g). Water soluble fraction of the MeOH extract of stems of *D. ganpi* was subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH-20, octadecyl silica (ODS) and silica gel to afford a new compound, pilloin 5-*O*-*β*-D-glucopyranoside (**49**) along with 22 known compounds.

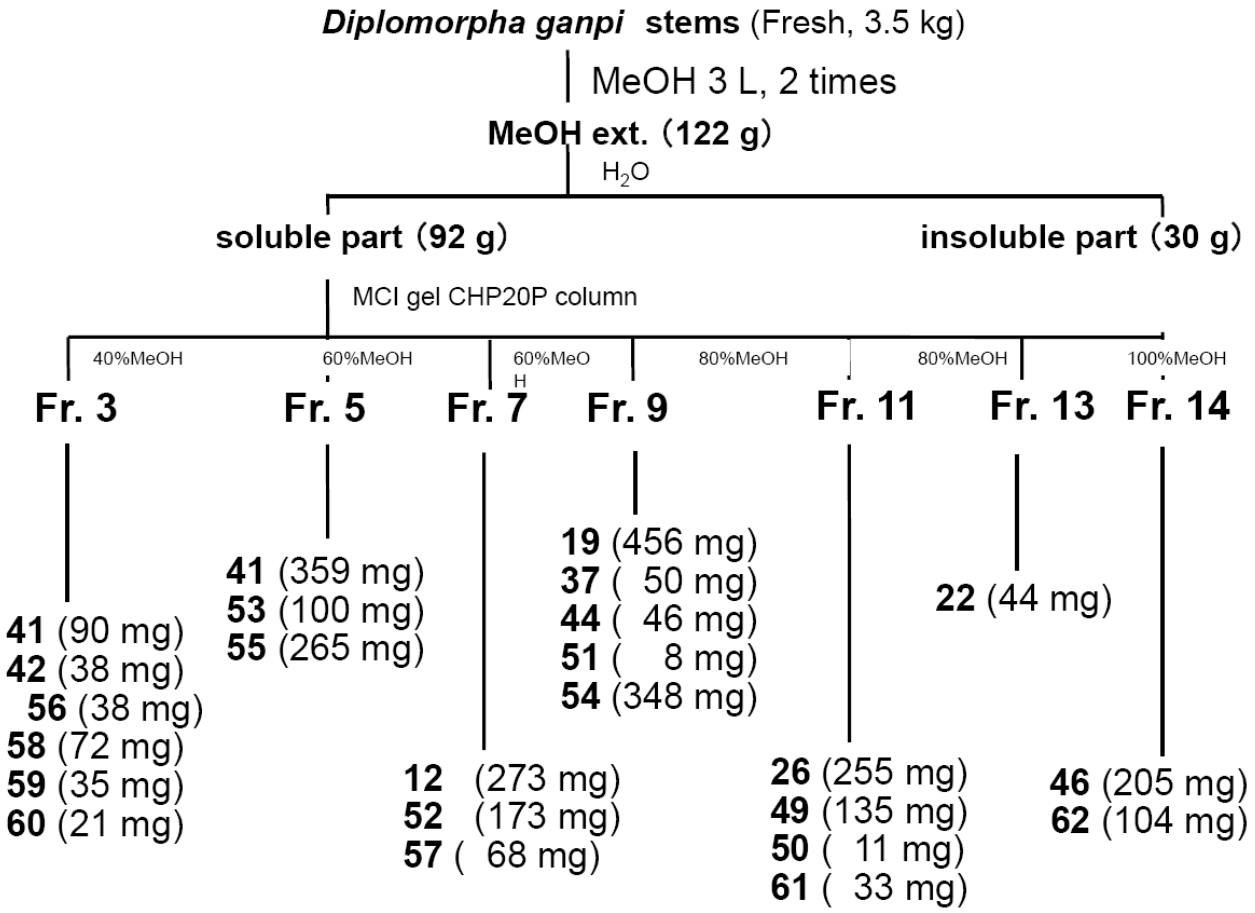


Chart 3. Extraction and isolation of compounds from *Diplomorpha ganpi* stems

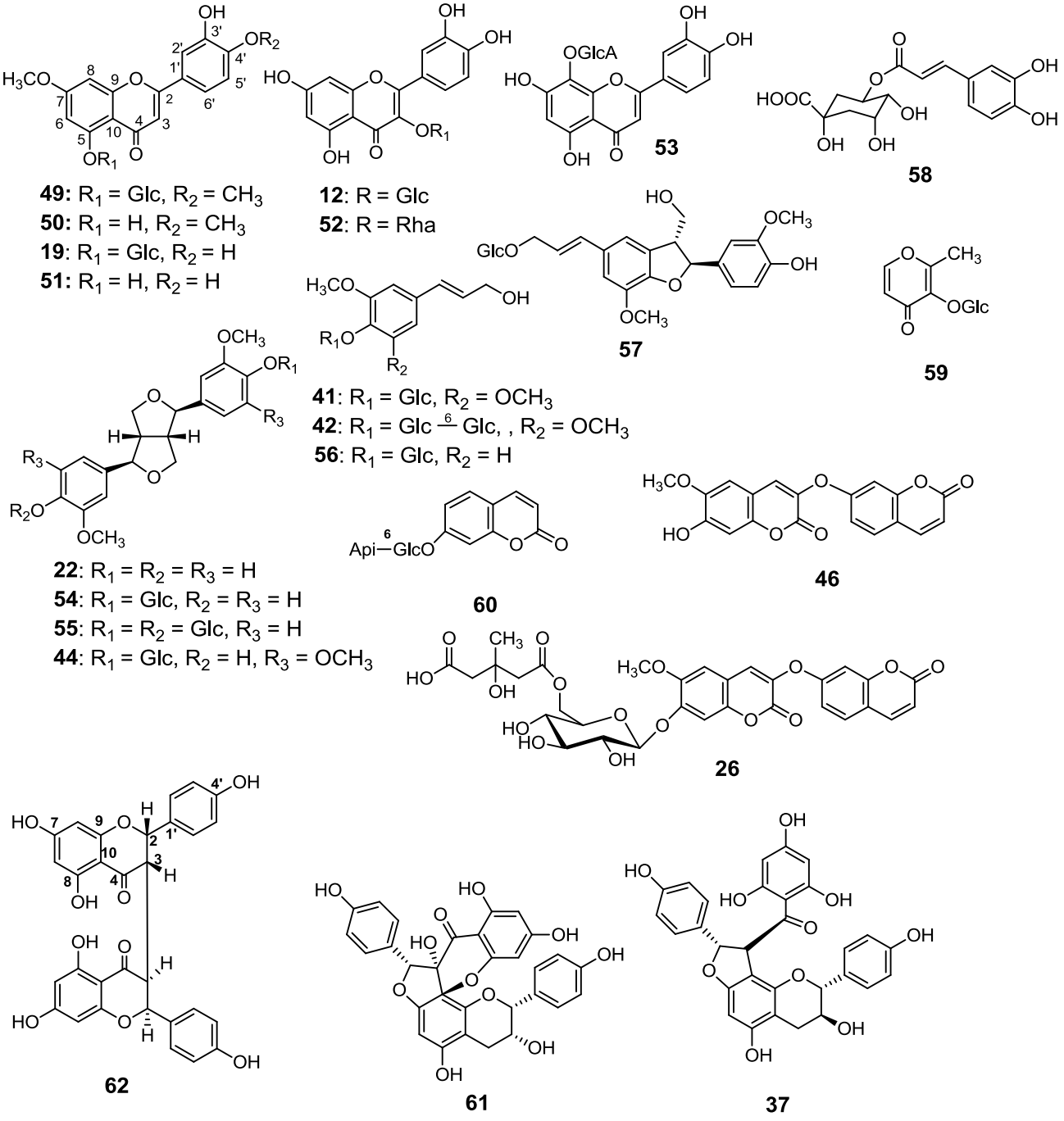


Figure 32. Compounds isolated from the stems of *Diplomorpha ganpi*

Structures of known compounds including six flavonoids, pilloin (**50**),[117,118] luteolin 7-methyl ether 5-*O*-β-D-glucopyranoside (**19**),[78] luteolin 7-methyl ether (**51**),[119] quercetin 3-*O*- β–D–glucopyranoside (**12**), quercetin 3-*O*-α–L-rhamnopyranoside (**52**), hypolaetin 8-*O*-β–D–glucuronopyranoside (**53**);[120] five lignans, (-)-pinoresinol (**22**), (-)-pinoresinol 4-*O*-β-D-glucopyranoside (**54**),[121] (-)-pinoresinol 4,4'-di-*O*-β-D-glucopyranoside (**55**),[82] syringaresinol 4-*O*-β-D-glucopyranoside (**44**),[86] (7*S*,8*R*) dehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside (**57**);[122] three phenylpropanoids, syringin (**41**),[35] syringinoside (**42**),[35,111] coniferin (**56**);[35,110] three biflavonoids, stelleranol (**61**),[25] neochamaejasmin A (**62**),[36,110] and 3''-epi-dihydrodaphnodorin B (**37**);[106] chlorogenic acid (5**8**),[123,124] maltol 3-*O*-β-D-glucopyranoside (5**9**);[123] three coumarins, apiosylskimmin (**60**),[125] daphnoretin (**46**)[114] and rutarensin (**26**) (Figure 32) were elucidated on the basis of spectral data and comparison with literature. All of these compounds were isolated for the first time from this plant.

2.3.1. New compound

2.3.1.1. Pilloin 5-*O*-β-D-glucopyranoside (49)

Compound **49** was obtained as white amorphous powder, mp 165-168ºC showing levorotatory optical activity, $[\alpha]_D^{21}$ -45º (*c* 0.66, pyridine). The HR-FABMS of **49** showed the quasi-molecular ion $[M+H]^+$ at *m/z:* 477.1401 supporting the formula $C_{23}H_{24}O_{11}$ (calcd. for $C_{23}H_{25}O_{11}$, 477.1397).. On magnesium-hydrochloric acid (Mg-HCl) reaction, it gave yellow color suggesting a flavone derivative. The ^{1}H-NMR spectrum (Table 13) displayed signals for two methoxy groups at δ_H 3.91 and 3.87; six aromatic protons: one singlet at δ_H 6.67; two doublets at δ_H 6.91 and 7.03 (*J* = 2.4 Hz each) characteristic of *meta*-protons on a tetrasubstituted benzene ring; three protons at δ_H 7.09 (d, *J* = 8.8 Hz), 7.44 (d, *J* = 2.4 Hz) and 7.54 (dd, *J* = 2.4, 8.8 Hz) in an ABX system of a benzene ring; and an anomeric proton at δ_H 4.76 (d, *J* = 7.6 Hz). The ^{13}C-NMR (Table 13) and DEPT spectrum showed signals equivalent to 23 carbons. Among them, 17 carbon signals were assigned by analysis of HMQC and HMBC spectra, to those of luteolin 7,4'-dimethyl ether (pilloin) (**50**) and six carbons signals (δ 60.9, 69.9, 73.5, 75.7, 77.6, 104.1) were assignable to β-glucopyranosyl moiety.

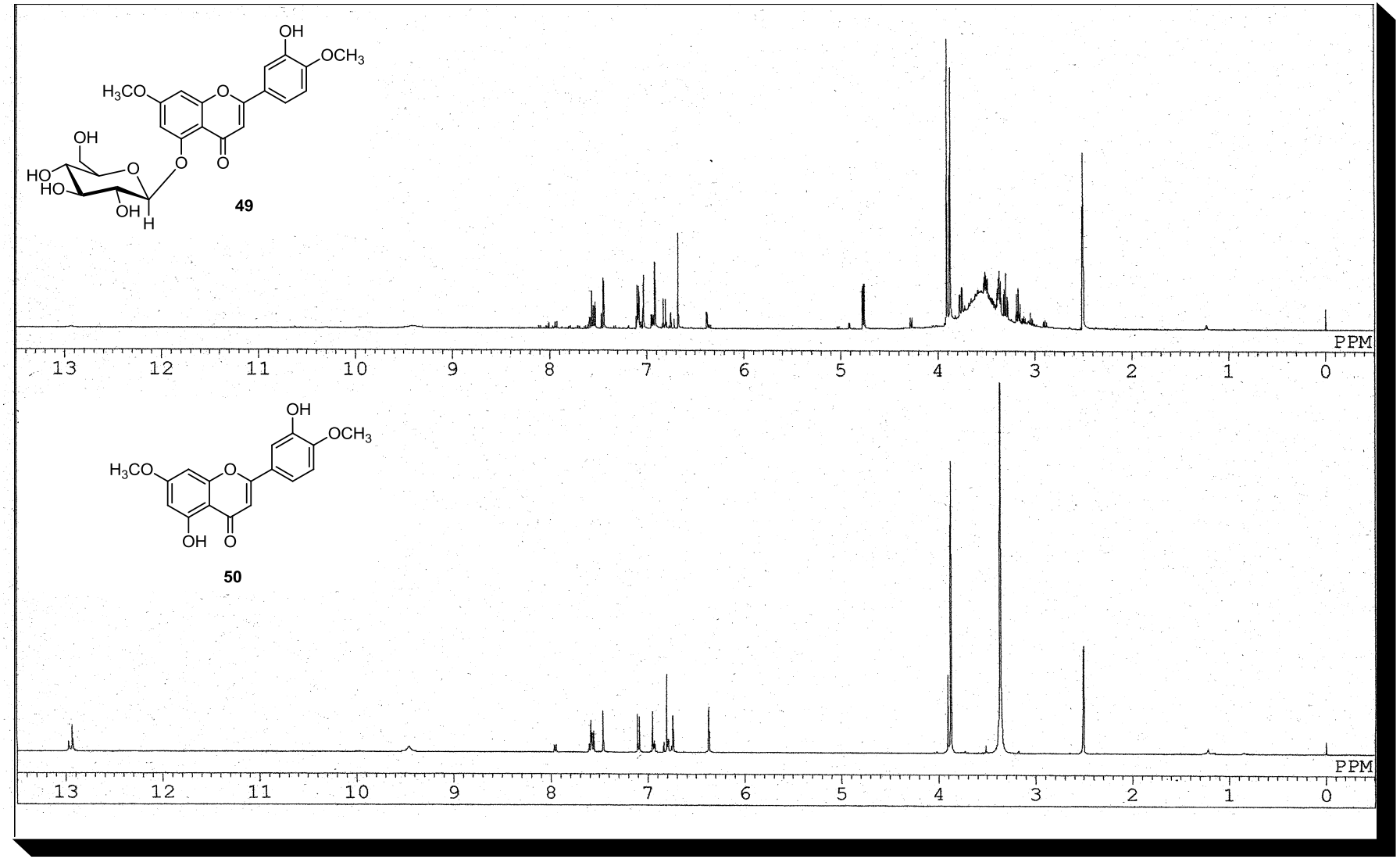


Figure 33. ^{1}H-NMR spectra of pilloin 5-*O*-*β*-D-glucopyranoside (**49**) and pilloin (**50**) in DMSO-d_6

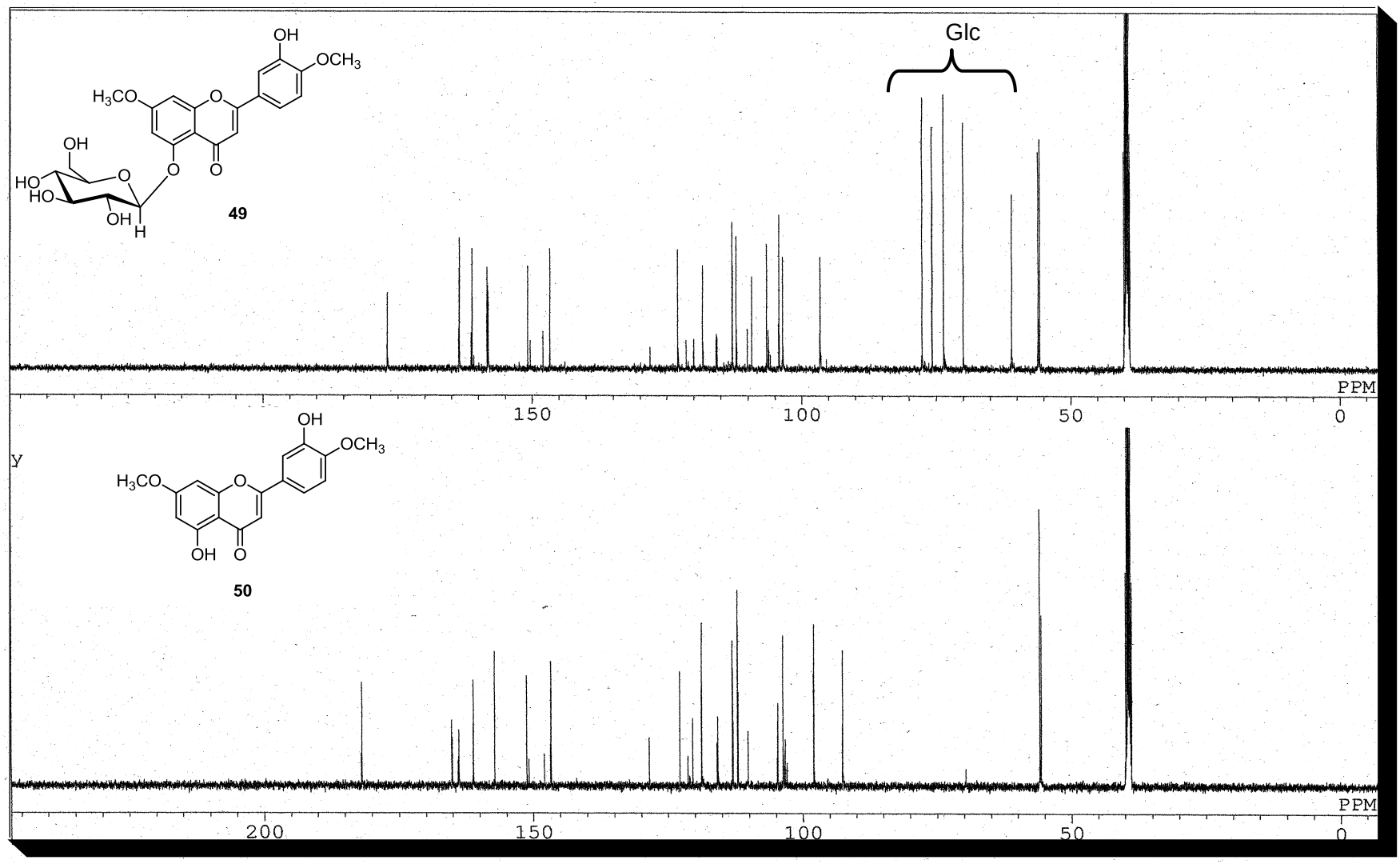


Figure 34. ^{13}C-NMR spectra of pilloin 5-*O*-*β*-D-glucopyranoside (**49**) and pilloin (**50**) in DMSO-d_6

Table 13. NMR spectroscopic data for **49—51 and 19** in DMSO-d_6

Carbon	**49**		**50**		**19**		**51**	
no.	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	161.1		165.1		161.5		165.0	
3	106.4	6.67, s	103.6	6.80, s	105.8	6.59, s	103.0	6.37, s
4	176.8		181.8		176.8		181.8	
5	158.2		161.6		158.3		161.1	
6	103.5	6.91, d (2.4)	97.9	6.37, d (2.1)	103.4	6.92, d (2.1)	97.9	6.37, d (2.1)
7	163.5		163.8		163.5		164.2	
8	96.4	7.03, d (2.4)	92.6	6.76, d (2.1)	96.4	6.99, d (2.1)	92.5	6.71, d (2.1)
9	158.8		157.2		158.1		157.1	
10	109.2		104.6		109.2		104.6	
1’	122.9		122.8		121.4		121.3	
2'	112.8	7.44, d (2.4)	113.0	7.46, d (2.1)	113.2	7.43, d (2.4)	113.4	7.43, d (2.1)
3’	146.7		146.7		145.7		145.7	
4’	150.8		151.2		149.3		149.8	
5'	112.1	7.09, d (8.8)	112.0	7.10, d (8.5)	115.9	6.89, d (7.9)	115.9	6.90, d (8.2)
6'	118.3	7.54, dd (2.4, 8.8)	118.7	7.56, dd (2.1, 8.5)	118.5	7.42, dd (2.4,7.9)	119.6	7.45, d (2.1, 8.2)
C_7-OCH_3	56.0	3.91, s	56.0	3.87, s	56.0	3.91,s	56.0	3.87, s
$C_{4'}$-OCH_3	55.7	3.87, s	55.7	3.87, s				
C_5-OH				12.95, s				12.97, s
Glc-1	104.1	4.76, d (7.6)			104.2	4.77, d (7.6)		
Glc-2	73.5	3.34, m			73.6	3.36, m		
Glc-3	75.7	3.32, m			75.7	3.32, m		
Glc-4	69.9	3.16, t (9.1)			69.9	3.19, m		
Glc-5	77.6	3.34, m			77.6	3.34, m		
Glc-6	60.9	3.76, brd (10) 3.50, m			60.9	3.78, brd (10) 3.52, m		

For acid hydrolysis, a solution of compound **49** (10.8 mg) in 2M HCl (4 mL) in a sealed tube was heated at 70º C for 3 hr. The aglycone (**50**, 6.6 mg) was extracted with EtOAc and confirmed by ^{1}H-NMR data and co-TLC with authentic sample. D-Glucose (3.7 mg) was obtained from aq. layer and confirmed by optical rotation, $[\alpha]_D^{23}$ +58º (*c* 0.37, H_2O) and co-TLC with authentic sample. In the HMBC spectrum, the signal for anomeric proton at δ_H 4.76 and C_6-H at δ_H 6.91 had long range correlation with carbon resonance at δ 158.2 (C-5), which suggested that the glucosyl residue was located at the 5-*O*-position of the flavone skeleton. This was also supported by the differential NOE spectrum in which irradiation of the anomeric proton signal (δ_H 4.76) caused the enhancement of the C_6-H (δ_H 6.91). Key HMBC and NOE correlations are given in Figure 35.

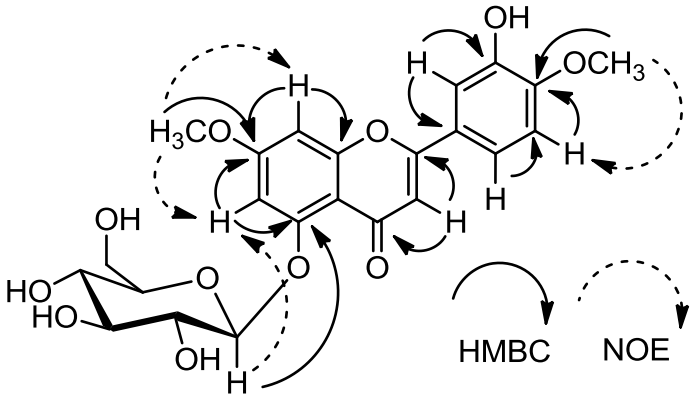


Figure 35. Key HMBC and NOE correlations of **49**

The absolute configuration of D-glucopyranosyl moiety in compound **49** was further supported by Klyne's rule as the the sign of molecular rotation ($[M]_D$) of **49** (-213.2°) was similar with that of methyl β-D-glucopyranoside (-64°, 90% EtOH).[95-97] Thus, structure of compound **49** was confirmed as pilloin 5-*O*-β-D-glucopyranoside. Although similar structures as 5-*O*-xylosylglucoside and 3'-*O*-glucoside of luteolin 7,4'-dimethyl ether (pilloin) have been reported from *Ovidia pillo-pillo* (Thymelaeaceae)[126] and *Gelonium multiflorum* (Euphorbiaceae)[127] respectively, this is the first report on isolation and structure elucidation of compound **49**.

2.3.2. Known compounds

The NMR spectral data for compounds **50** and **19** are given in Table 13. Similarly, the NMR data of quercetin 3-*O*-α–L-rhamnopyranoside (**52**) and hypolaetin 8-*O*-β–D–glucuronopyranoside (**53**) are given in Table 14.

Table 14. NMR spectroscopic data for **52 and 53**

Carbon	**52**[a]		**53**[b]	
no.	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
2	158.4*		164.6	
3	136.2		102.6	6.64, s
4	176.5		181.6	
5	163.0		157.3*	
6	99.7	6.19, d (2.1)	99.0	6.27, s
7	165.7		157.2*	
8	94.7	6.34, d (2.1)	125.4	
9	159.2*		149.4	
10	105.8		103.3	
1’	122.9		121.4	
2'	116.3	7.34, d (2.1)	113.8	7.68, brs
3’	146.3		146.0	
4’	149.6		149.8	
5'	116.9	6.91, d (8.2)	115.9	6.87, d (8.4)
6'	122.8	7.32, d (2.1,8.2)	119.2	7.51, d (2.1,8.4)
Gly-1	103.4	5.36, d (1.5)	106.5	4.73, d (7.6)
Gly-2	71.9*		73.9	
Gly-3	71.8*		76.3	
Gly-4	72.1*		75.5	
Gly-5	73.2		71.6	
Gly-6	17.6	0.96, d (6.1)	170.9	

[a]in CD_3OD, [b]in DMSO-d_6

The ^{13}C-NMR spectral data for compounds (-)-pinoresinol (**22**), (-)-pinoresinol 4-*O*-*β*-D-glucopyranoside (**54**), (-)-pinoresinol 4,4'-di-*O*-*β*-D-glucopyranoside (**55**), coniferin (**56**) and (7*S*,8*R*) dehydrodiconiferyl alcohol 9'-*O*-*β*-D-glucopyranoside (**57**) are given in Table 15.

Table 15. ^{13}C NMR data of compounds **22**, **54—57**

C No.	**22**[a]	**54**[b]	**55**[c]	**56**[d]	**57**[e]
1	132.7	137.3	135.2	133.6	137.9
2	108.6	111.6	110.4	117.5	111.2
3	146.6	147.2	145.8	150.4	150.8
4	145.1	149.0	148.9	147.1	147.6
5	114.2	117.9	115.2	111.4	117.9
6	118.8	119.7	118.2	120.8	119.4
7	85.7	86.9	84.8	131.1	88.8
8	54.0	55.2	53.7	128.8	57.9
9	71.5	72.66	71.0	63.4*	74.2
1'	132.7	137.1	135.2		130.0
2'	108.6	110.9	110.4		112.2
3'	146.6	147.4	145.8		145.5
4'	145.1	150.8	148.9		149.3
5'	114.2	116.0	115.2		132.3
6'	118.8	120.0	118.2		116.6
7'	85.7	87.4	84.8		134.2
8'	54.0	55.4	53.7		124.3
9'	71.5	72.6	71.0		64.8
OCH_3	55.8	56.7, 56.4	55.7	56.8	56.7, 55.2
Glc-1		102.7	100.1	102.3	102.6
Glc-2		74.8	73.2	74.5	74.8
Glc-3		77.7	76.8	77.7	77.7
Glc-4		71.2	69.6	70.9	71.2
Glc-5		78.1	77.0	77.2	78.1
Glc-6		62.4	60.7	62.1*	62.4

[a]in $CDCl_3$, [b]in CD_3OD, [c]in DMSO-d_6, [d]in CD_3OD +D_2O [e]in $CDCl_3$+CD_3OD
*assignments may be reversed in the same column.

2.4. Compounds from the roots of *Diplomorpha ganpi*

The fresh roots of *Diplomorpha ganpi* (750 g) were extracted twice with MeOH (3 L) and extracts were evaporated under reduced pressure to give MeOH extract (98 g) which was separated into water soluble (19 g) and water insoluble (79 g) fractions. The water insoluble fraction was subjected to Sephadex LH20 and silica gel column to afford one new diarylpentanoid, diplomorphanone B (**63**) and four known diarylpentanoids, 1,5-diphenyl-1-pentanone (**64**),[128] (*S*)-3-hydroxy-1,5-diphenyl-1-pentanone (**65**),[129] 3-methoxy-1,5-diphenyl-1-pentanone (**66**),[130,131] 1,5-diphenyl-2-penten-1-one (**67**).[128] Eight known compounds dihydrodaphnodorin B (**34**), daphnodorin B (**38**), syringin (**41**), syringinoside (**42**), apiosylskimmin (**60**), (+)-afzelechin (**68**),[132] sinapyl alcohol (**69**),[133] and sikokianin A (**70**) (Figure 36) were isolated from water soluble fraction by MCI gel CHP20P, Sephadex LH20 and ODS column chromatography (Chart 4). All of these compounds were isolated for the first time from this plant.

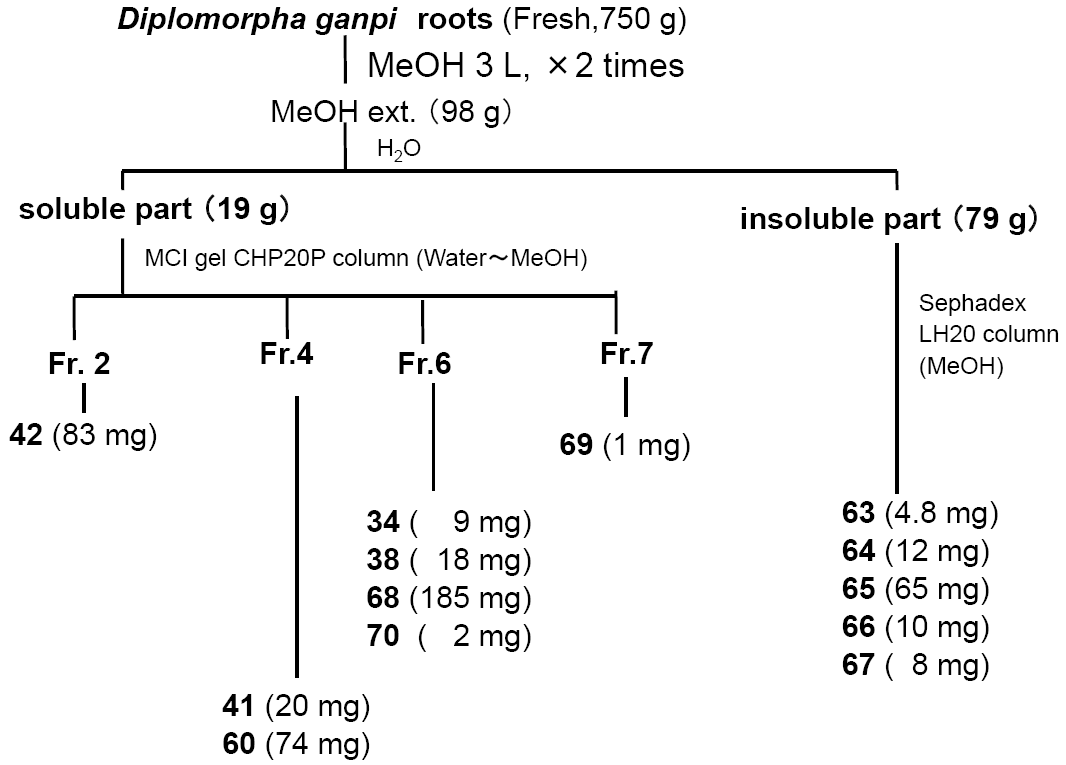


Chart 4. Extraction and isolation of compounds from roots of *Diplomorpha ganpi*

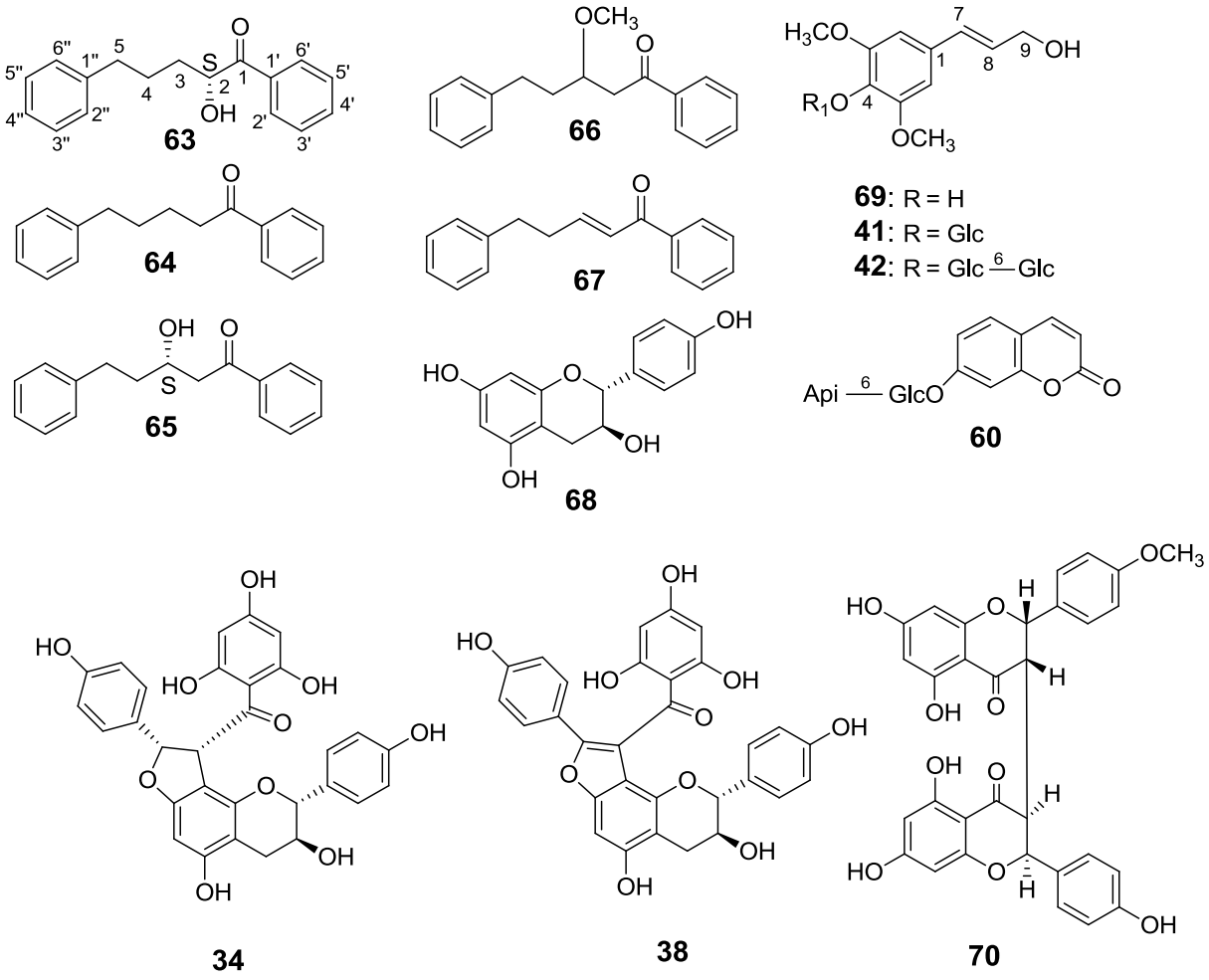


Figure 36. Compounds isolated from the roots of *Diplomorpha ganpi*

2.4.1. New compound

2.4.1.1. Diplomorphanone B (63)

Diplomorphanone B (**63**) was isolated as a pale yellowish oil; $[\alpha]_D^{20}$ +30.1 (*c* 0.41, $CHCl_3$). The HR-FABMS of **63** showed the quasi-molecular ion $[M+H]^+$ at *m/z*: 255.1378, supporting the molecular formula $C_{17}H_{18}O_2$ (calcd. for $C_{17}H_{19}O_2$, 255.1385). The ^{1}H-NMR spectra of **63** was almost similar to that of **32** except the fact that **63** was found to be 4'-dehydroxy derivative of **32**, which was characterized by the presence of five aromatic protons at δ_H 7.85 (2H, dd, *J* = 1.2, 8.1 Hz, $C_{2'}$-H, $C_{6'}$-H), 7.47 (2H, t, *J* = 8.1 Hz, $C_{3'}$-H, $C_{5'}$-H) and 7.60 (1H, dd, *J* = 1.2, 8.1 Hz, $C_{4'}$-H).

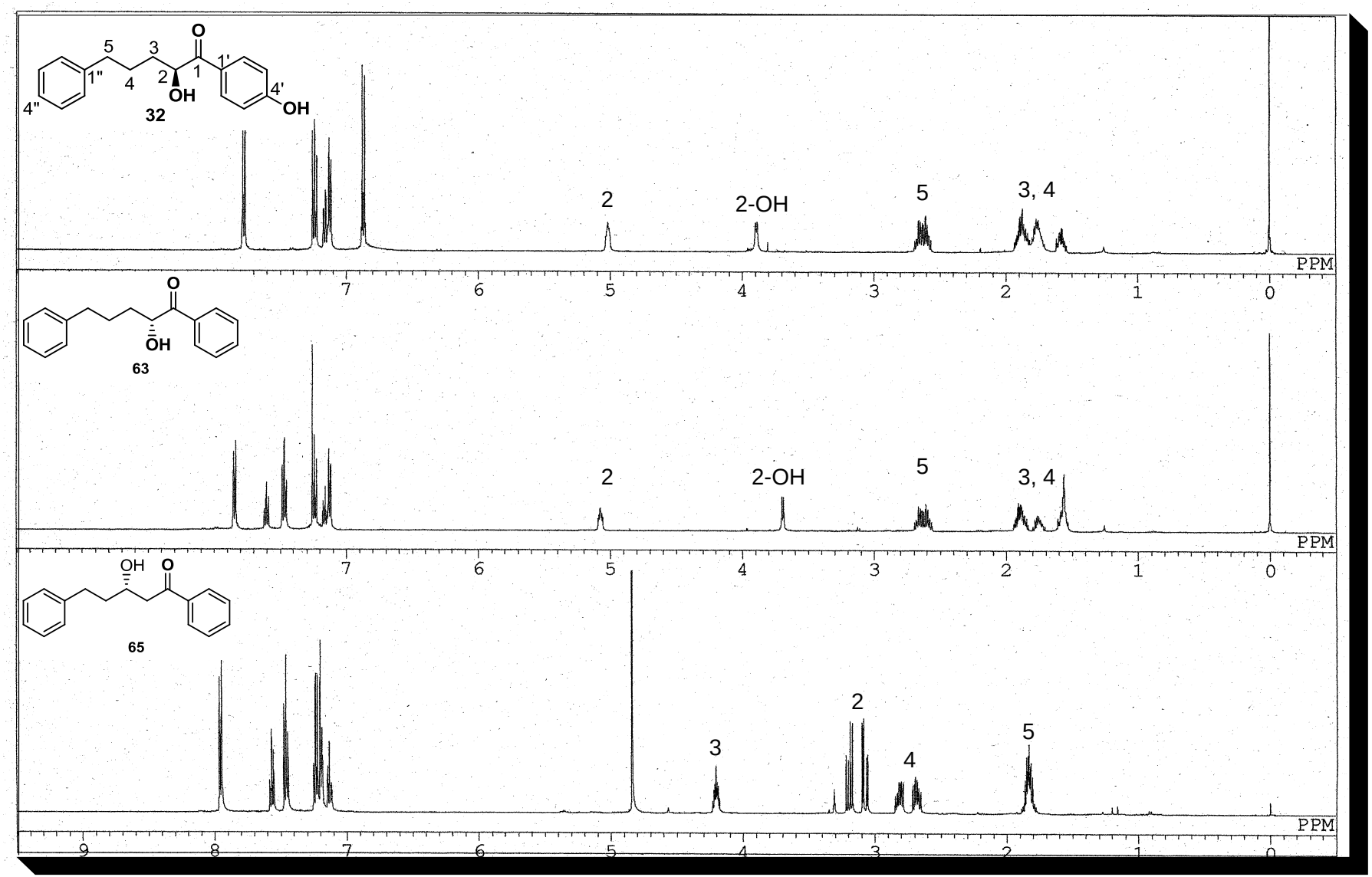


Figure 37. ^{1}H-NMR spectra of diplomorphanone A (**32**), diplomorphanone B (**63**) and (*S*)-3-hydroxy-1,5-diphenyl-1-pentanone (**65**) in $CDCl_3$

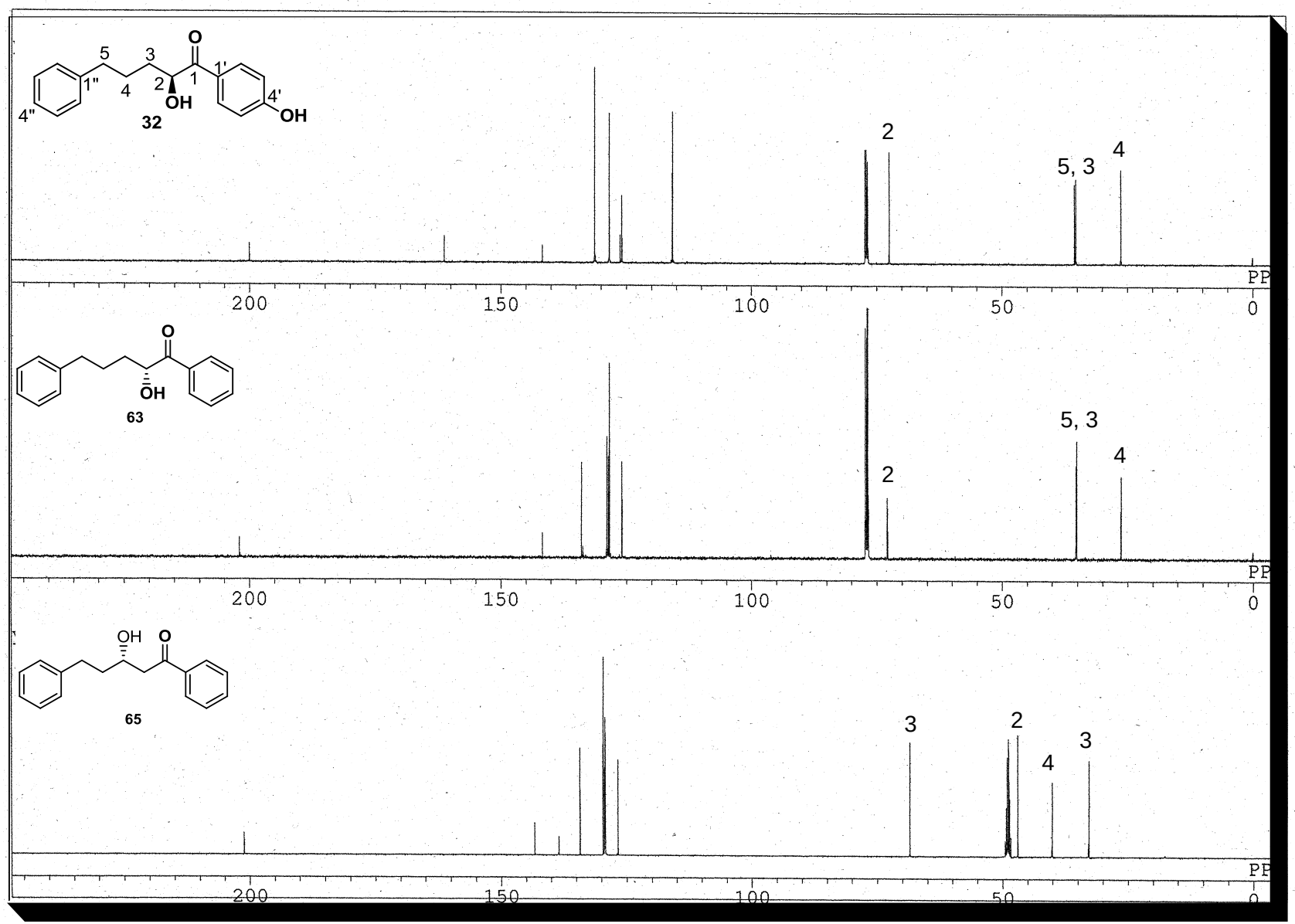


Figure 38. ^{13}C-NMR spectra of diplomorphanone A (**32**), diplomorphanone B (**63**) and (*S*)-3-hydroxy-1,5-diphenyl-1-pentanone (**65**) in $CDCl_3$.

Table 16. ^{1}H- and ^{13}C- NMR spectroscopic data of **32** and **63** and **65** in $CDCl_3$

position	**32**		**63**		**65**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
C-1	200.1		202.0		201.2	
C-2	72.5	5.02, m	72.9	5.07, m	47.0	3.07, dd (4.4, 16.3) 3.19, dd (8.0, 16.3)
C-3	35.5	1.89, m	35.2	1.89, m	68.5	4.21, m
		1.58, m		1.58, m		
C-4	26.3	1.89, m	26.3	1.89, m	40.2	1.84, m
		1.76, m		1.76, m		
C-5	35.3	2.63, m	35.3	2.61, m	32.9	2.83, m
C-1'	126.1		133.6		138.6	
C-2', 6'	131.3	7.78, d (8.8)	128.5	7.85, dd (1.2, 8.1)	129.1[c]	7.94, d (7.3)
C-3', 5'	115.9	6.87, d (8.8)	128.9	7.47, t (8.1)	129.4[c]	7.46, t (7.3)
C-4'	161.3		133.9	7.60, dd (1.2, 8.1)	134.3	7.57, d (7.3)
C-1"	141.7		141.7		143.3	
C-2", 6"	128.4	7.13, brd (7.7)	128.3	7.13, brd (7.6)	129.2[c]	7.19, d (7.3)
C-3", 5"	128.4	7.24, t (7.7)	128.3	7.24, t (7.6)	129.7[c]	7.23, t (7.3)
C-4"	125.9	7.16, d (7.7)	125.8	7.17, d (7.6)	126.7	7.13, d (7.3)
2-OH		3.89, d (6.4)		3.96, d (1.8)		

The ^{13}C-NMR spectra also showed signals for two mono-substituted benzene rings in **63** (Table 17). All other spectral data including ^{1}H-^{1}H COSY, HMQC and HMBC were similar to that of **32** (Fig. 35). The optical rotation of **63** was found to be opposite to that of **32** thus the configuration at C-2 was assigned to be *R* which was also supported by the similar optical rotation to that of (*R*)-2-hydroxy-1-phenyl-1-pentanone, $[\alpha]_D^{20}$ +17.3 (*c* 1.3, $CHCl_3$); (*R*)-α-hydroxy butanones and related ketones. Hence, the structure of **63** was assigned to be 2(*R*)-hydroxy-1,5-diphenyl-1-pentanone. Although synthesis of **63** has been reported by Vitale *et al.*[134] but absolute configuration, physiochemical and spectral data were not reported. Thus we report compound **63** as a new natural product.

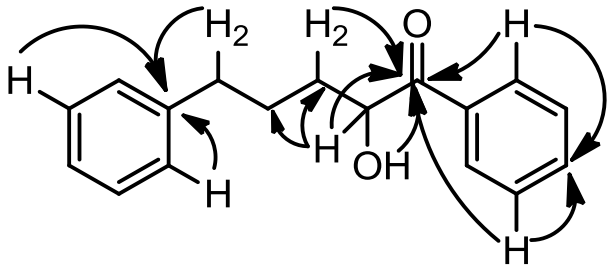


Figure 39. Key HMBC correlations observed in the spectrum of **32**.

2.4.2. Known compounds

NMR spectroscopic data for 1,5-diphenyl-1-pentanone (**64**), 3-methoxy-1,5-diphenyl-1-pentanone (**66**) and 1,5-diphenyl-2-penten-1-one (**67**) are given in Table 17.

Compounds **3**, **4** and **6** have been previously isolated from the roots of *Stellera chamaejasme* Linn. (Thymelaeaceae) as potent botanical aphicides. Compound **5,** $[\alpha]_D^{20}$ ~0 (*c* 0.86, $CHCl_3$), was isolated as a racemic mixture in the present study which may be a artifact during isolation procedures.

Table 17. ^{1}H- and ^{13}C- NMR spectroscopic data of **64**, **66** and **67** in CD_3OD

position	**64**		**66**		**67**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
C-1	202.8		198.9		192.9	
C-2	39.3	3.01, d (7.3)	43.2	2.97, dd (5.8, 16.3) 3.34, dd (6.6, 16.3)	127.8	6.94, d (15.4)
C-3	25.1	1.70, m	77.1	3.93, m	150.4	7.03, dd (6.5, 15.4)
C-4	32.2	1.70, m	31.5	2.70, m 2.79, m	35.4[c]	2.85, m
C-5	36.7	2.64, d (7.3)	36.3	1.90, m	35.5[c]	2.65, m
C-1'	138.3		137.3		139.0	
C-2', 6'	129.1[a]	7.95, dd (1.2, 7.9)	128.4[c]	7.94, dd (1.2,7.6)	129.6[d]	7.86, dd (1.4,8.0)
C-3', 5'	129.3[c]	7.46, t (7.3)	128.6[c]	7.46, t (7.6)	129.7[d]	7.47, t (8.0)
C-4'	134.2	7.57, dd (1.2, 7.9)	133.1	7.57, d (7.6)	134.1	7.58, d (1.4,8.0)
C-1"	143.5		142.0		142.3	
C-2", 6"	129.4[c]	7.17, dd (1.2, 7.3)	128.4[c]	7.19, d (7.6)	129.5[d]	7.23, d (1.8,8.0)
C-3", 5"	129.7[c]	7.23, t (7.3)	128.2[c]	7.27, t (7.6)	129.4[d]	7.27, t (8.0)
C-4"	126.7	7.13, d (1.2, 7.3)	125.8	7.17, d (7.6)	127.2	7.17, brd (8.0)
OCH_3			57.1	3.37, s	192.9	

[c,d] assignments may be reversed in the same column with same alphabets.

2.5. Known compounds from stems of *Diplomorpha sikokiana*

Fresh stems of *Diplomorpha sikokiana* (417 g) were extracted twice with MeOH (3 l) and extracts were evaporated under reduced pressure to give MeOH extract (26 g). The extract was then dissolved in water and subjected to MCI gel CHP20P column followed by Sephadex LH-20, ODS or silica to obtain 14 known compounds (Chart 5).

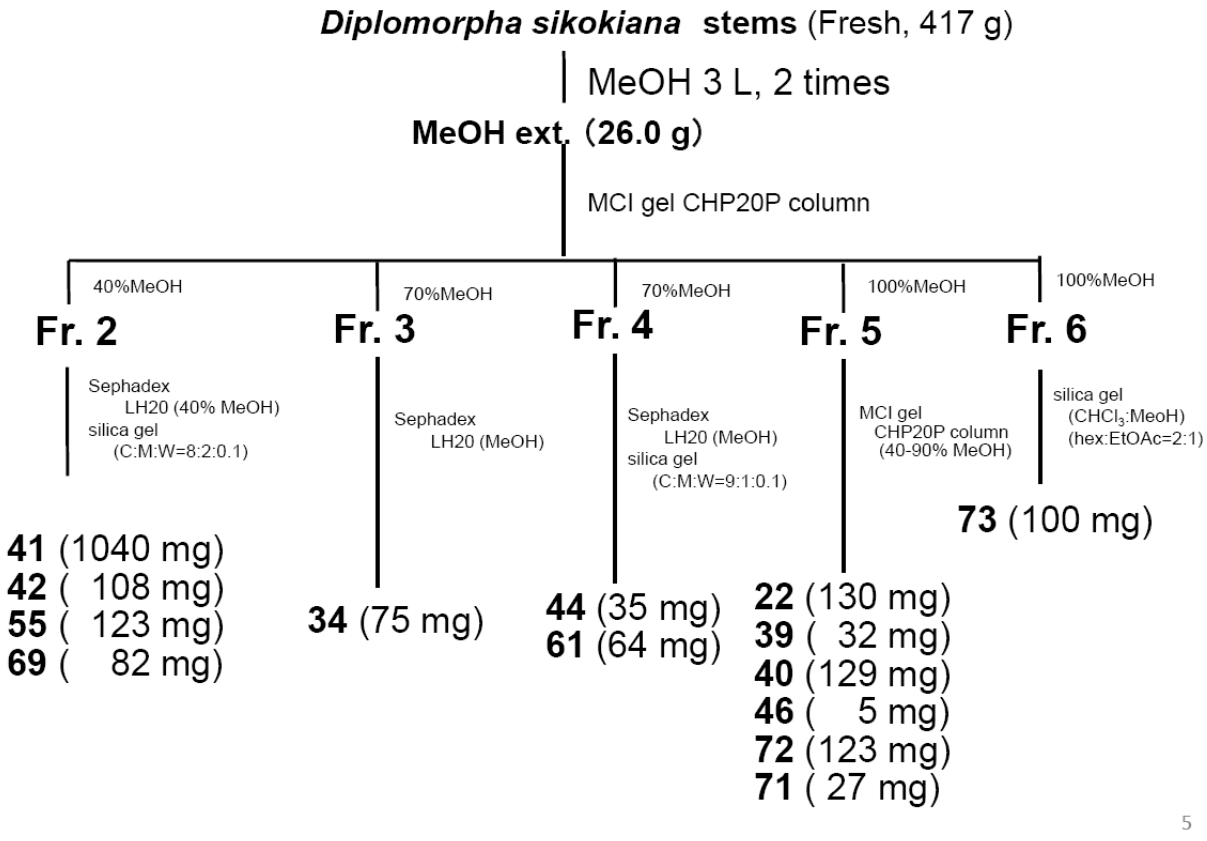


Chart 5. Extraction and isolation of compounds from stems of *Diplomorpha sikokiana*

Structures of these compounds were elucidated to be two phenylpropanoids, syringin (**41**), syringinoside (**42**); five biflavonoids, neochamaejesmin B (**39**), sikokianin B (**40**), chamaejasmenin B (**71**),[26] stelleranol (**61**) and dihydrodaphnodorin B (**34**); three lignans, pinoresinol (**22**), syringaresinol 4-*O*-β-D-glucopyranoside (**44**), pinoresinol 4,4'di-*O*-β-D-glucopyranoside (**55**); one flavonoid, apigenin 4',7-dimethylether 5-*O*-primeveroside (**72**);[135] two coumarins, apiosylskimmin (**60**), daphnoretin (**45**) and β-sitosterol (**72**),[136] on the basis of spectral data and comparison with literature values. Compounds **44**, **55**, **61**, **72** and **73** were isolated for the first time from this plant.

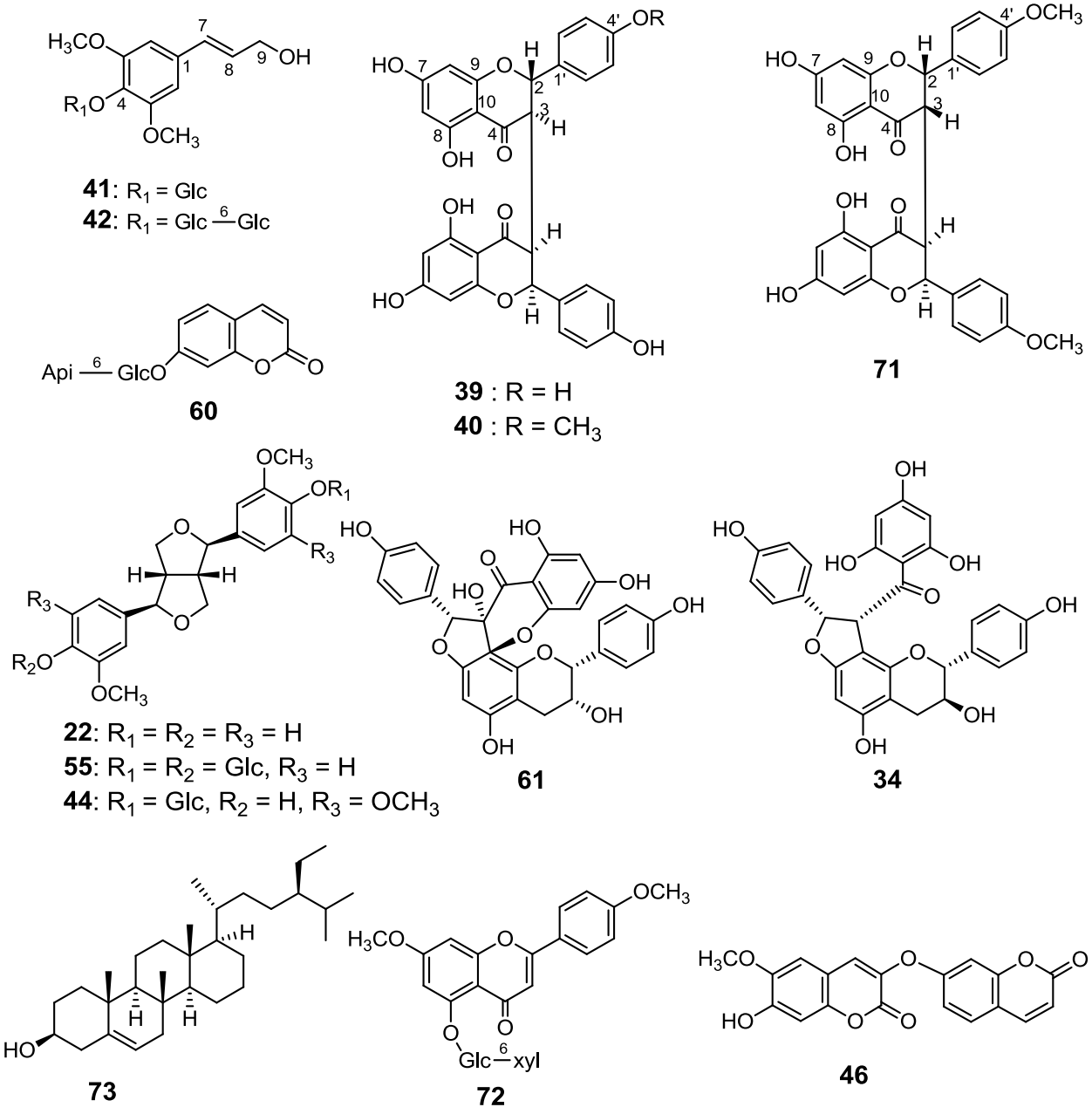


Figure 40. Structures of the compounds isolated from the stems of *Diplomorpha sikokiana*

The NMR spectroscopic data for neochamaejasmin A (**62**), sikokianin A (**70**) and chamaejasmenin B (**71)** are given in Table 18.

Table 18. NMR data of compounds **62**, **70** and **71** in CD_3OD

	62		**70**		**71**	
Position	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
2	82.4	4.85, brs[a]	81.7	5.35, brs	81.7	5.35, brs
3	49.5	3.32, dd (1.5, 3.3)	48.1	3.35, dd (1.5, 3.3)	48.1	3.35, dd (1.5, 3.3)
4	196.8		197.5		197.5	
5	165.3		165.1		165.1	
6	97.2	5.76, d (2.1)	97.8	5.74, d (2.1)	97.8	5.77, d (2.1)
7	168.2		168.2		168.2	
8	96.0	5.90, d (2.1)	95.8	5.88, d (2.1)	95.8	5.90, d (2.1)
9	164.3		164.5		164.5	
10	102.7		103.7		103.7	
1’	128.9		128.9		128.9	
2’,6’	130.7	7.03, d (8.5)	129.4	7.12, d (8.5)	129.4	7.02, d (8.5)
3’, 5’	116.5	6.80, d (8.5)	114.8	6.94, d (8.5)	114.8	6.75, d (8.5)
4’	159.6		160.6		160.6	
2’’	82.4	4.85, brs	82.4	4.85, brs	81.7	5.35, brs
3’’	49.5	3.32, dd (1.5, 3.3)	49.5	3.32, dd (1.5, 3.3)	48.1	3.35, dd (1.5, 3.3)
4’’	196.8		196.8		197.5	
5’’	165.3		165.3		165.1	
6’’	97.2	5.76, d (2.1)	97.2	5.74, d (2.1)	97.8	5.77, d (2.1)
7’’	168.2		168.2		168.2	
8’’	96.0	5.90, d (2.1)	96.0	5.88, d (2.1)	95.8	5.90, d (2.1)
9’’	164.3		164.3		164.5	
10’’	102.7		102.7		103.7	
1’’’	128.9		128.9		128.9	
2’’’, 6’’’	130.7	7.03, d (8.5)	130.7	7.01, d (8.5)	129.4	7.02, d (8.5)
3’’’, 5’’’	116.5	6.80, d (8.5)	116.5	6.80, d (8.5)	114.8	6.75, d (8.5)
4’’’	159.6		159.6		160.6	
OCH_3			55.7	3.81, s	55.7	3.83, s

2.6. Known compounds from roots of *Diplomorpha sikokiana*

Fresh roots of *Diplomorpha sikokiana* (100 g) were extracted twice with MeOH (700 ml) and extracts were evaporated under reduced pressure to give MeOH extract (7.7 g). The extract was then dissolved in water and subjected to MCI gel CHP20P column followed by Sephadex LH-20, ODS or silica to obtain 13 known compounds.

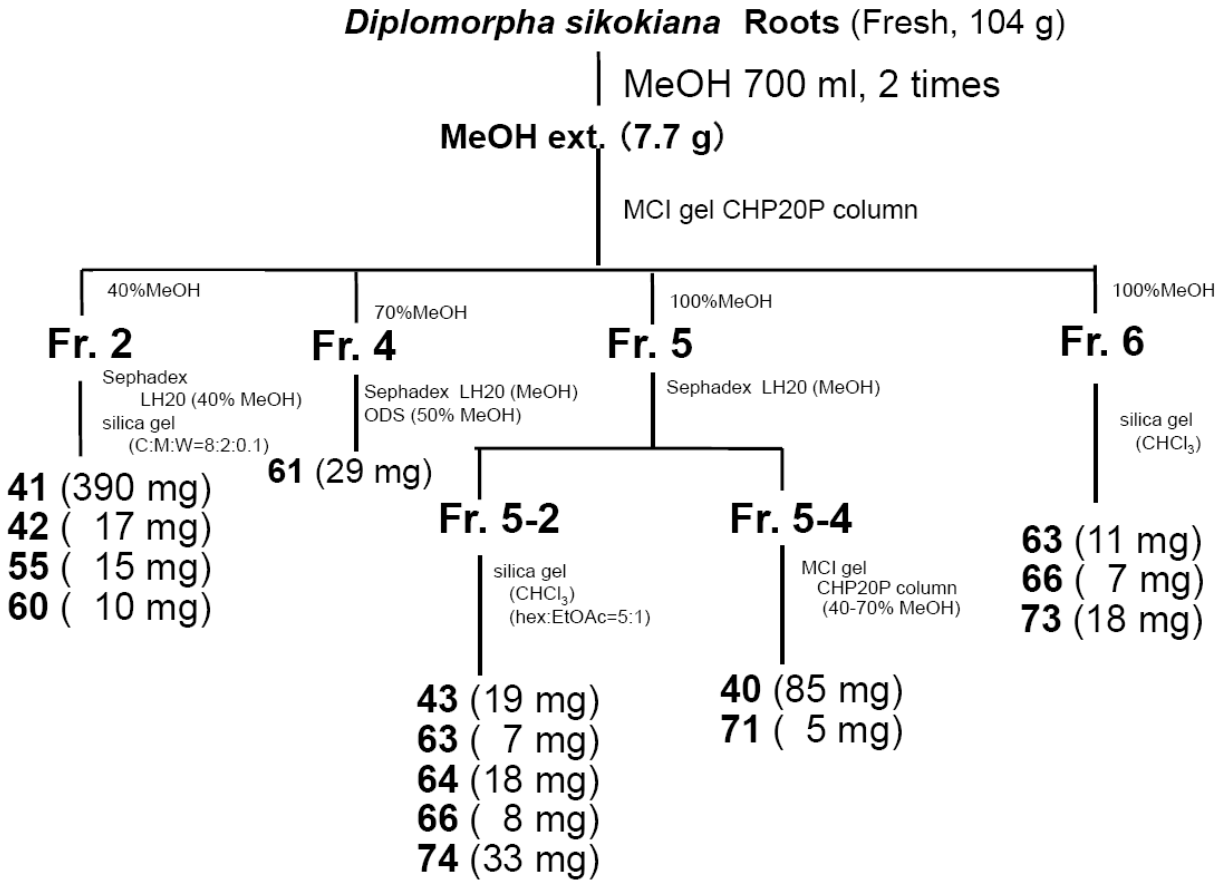


Chart 6. Extraction and isolation of compounds from rootss of *Diplomorpha sikokiana*

Structures of these compounds were elucidated to be four diarylpentanoids, 1,5-diphenyl-1-pentanone (**63**), 1,5-diphenyl-2-penten-1-one (**66**), 1,3-dihydroxy-1,5-diphenylpentane (**74**),[38] (-)-*erythro*-1,5-diphenylpentane-1,3-diol (**64**), two phenylpropanoids, syringin (**41**), syringinoside (**42**); three biflavonoids, sikokianin B (**37**), stelleranol (**61**), chamaejasmenin B (**71**); three lignans, pinoresinol (**22**), (-)-syringaresinol (**43**), (-)-pinoresinol 4,4' di-*O*-β-D-glucopyranoside (**55**), a coumarin,

apiosylskimmin (**60**) and *β*-sitosterol (**73**). Compounds **43**, **55**, **60**, **63**, **64**, **66** and **73** were isolated for the first time from this plant.

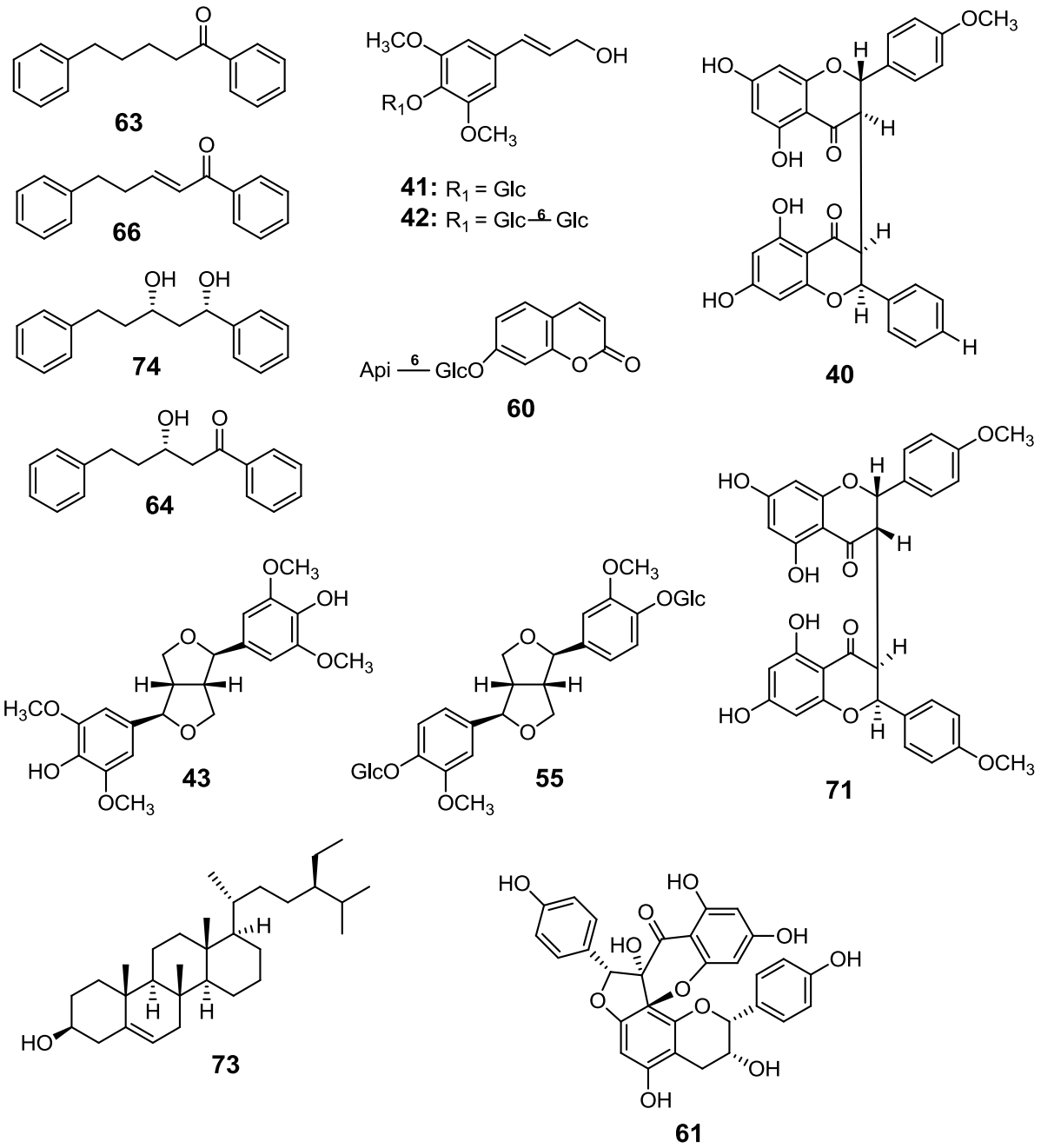


Figure 41. Structures of the compounds isolated from the roots of *Diplomorpha sikokiana*

2.7. Known compounds from leaves of *Diplomorpha sikokiana*

Fresh leaves of *Diplomorpha sikokiana* (95 g) were extracted twice with MeOH (700 ml) and extracts were evaporated under reduced pressure to give MeOH extract (18 g) which was then separated into water soluble (14 g) and water insoluble (4 g) fractions. The water soluble fraction then dissolved in water and subjected to MCI gel CHP20P column followed by Sephadex LH-20, ODS or silica to obtain 5 known compounds such as genkwanin 5-*O*-primeveroside (**17**), quercetin 3-*O*-α-L-rhamnopyranoside (**52**), apigenin 4', 7-dimethylether 5-*O*-primeveroside (**72**), kaempferol 3-*O*-α-L-rhamnopyranoside (**75**) and tiliroside (**76**)[72] (Figure 42). Compounds **52**, **75** and **76** were isolated for the first time from this plant.

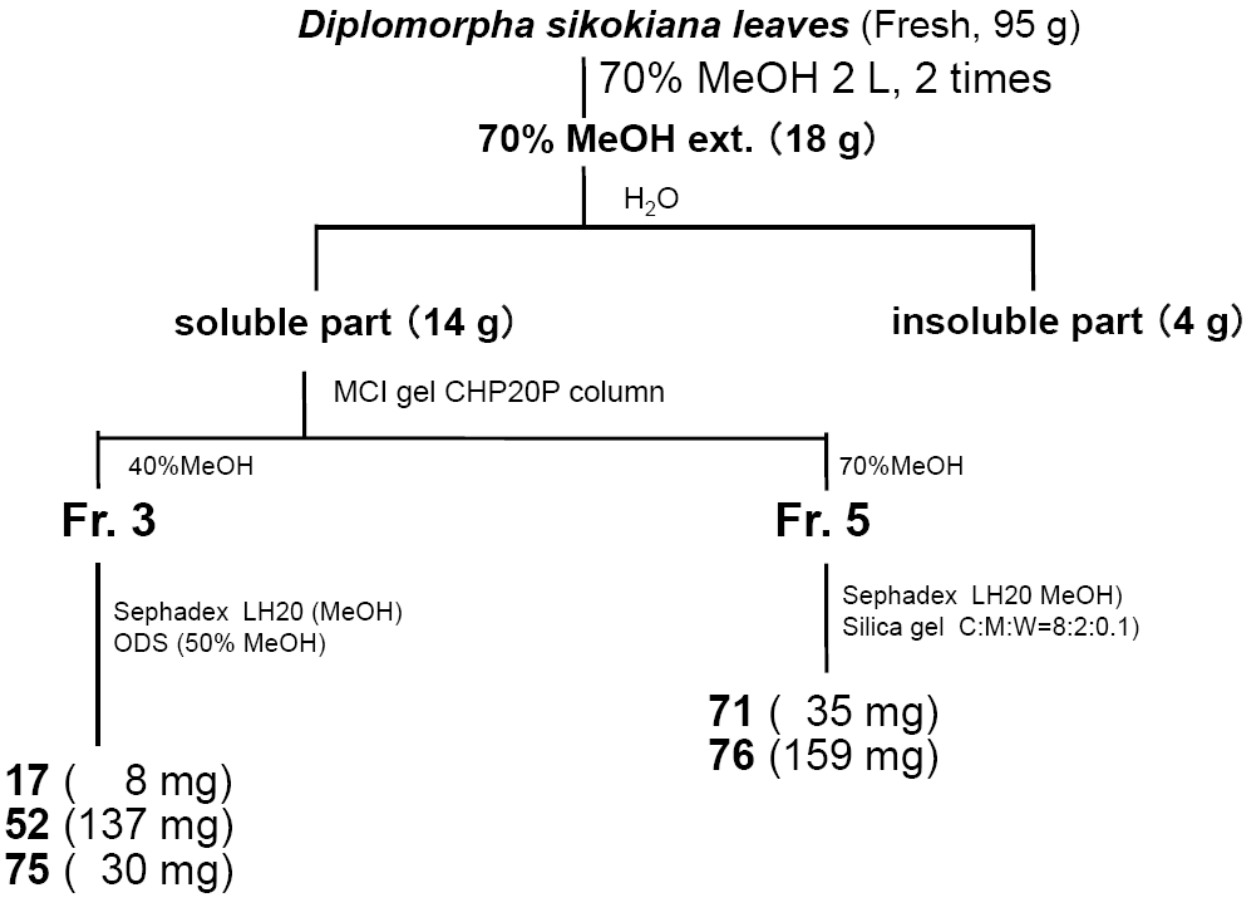


Chart 7. Structures of the compounds isolated from the leaves of *Diplomorpha sikokiana*

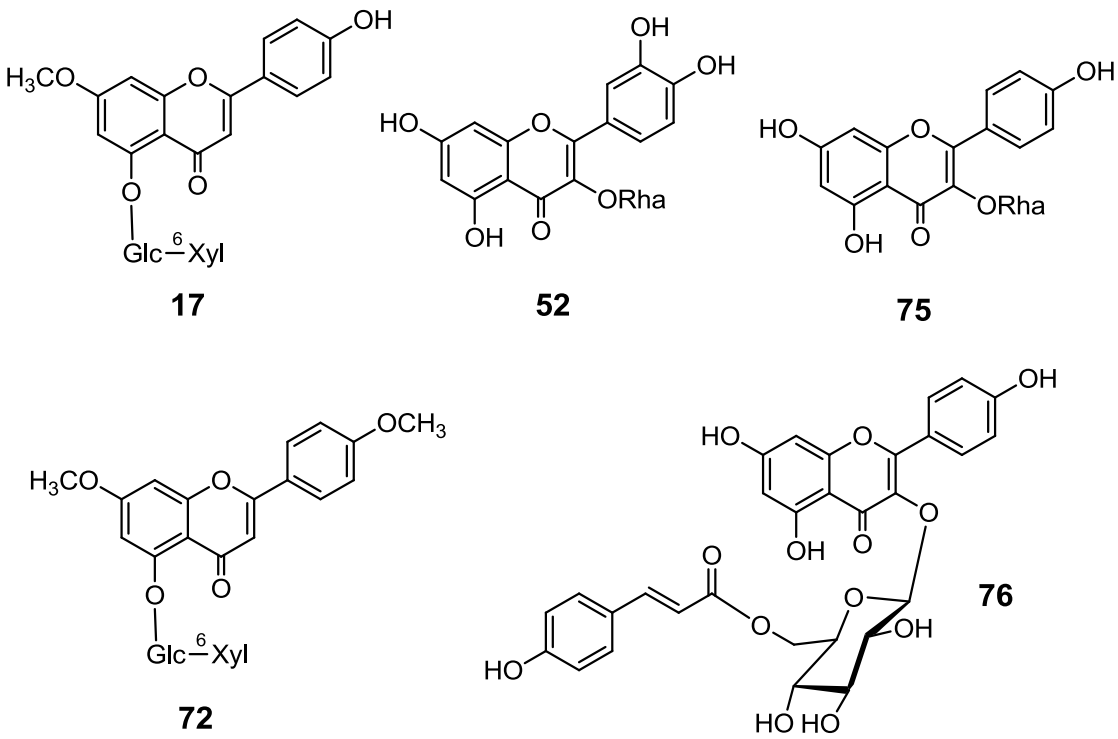


Figure 42. Structures of the compounds isolated from the leaves of *Diplomorpha sikokiana*

Table 19. NMR spectroscopic data for compounds **72**, **75** and **76** in DMSO-d_6

Carbon	**72**		**75**		**76**	
no.	δ_C	δ_H, mult.	δ_C	δ_H, mult.	δ_C	δ_H, mult.
2	161.0		158.4		156.4[b]	
3	106.4	6.77, s	136.2		133.0	
4	176.8		179.5		177.4	
5	158.0		163.1		159.9	
6	102.8	6.88, d (2.1)	99.8	6.19, d (2.1)	98.7	6.16, d (2.1)
7	163.6		165.7		164.1	
8	96.5	7.05, d (2.1)	94.7	6.36, d (2.1)	93.6	6.39, d (2.1)
9	158.4		159.2		156.3[b]	
10	109.1		105.9		103.8	
1’	122.7		122.6		120.7	
2'	128.0	8.03, d (8.8)	131.8	7.75, d (8.8)	130.1	7.99, d (8.8)
3’	114.5	7.11, d (8.8)	116.5	6.93, d (8.8)	115.0	6.79, d (8.8)
4’	162.0		161.4		159.9[c]	
5'	114.5	7.11, d (8.8)	116.5	6.93, d (8.8)	115.0	6.79, d (8.8)
6'	128.0	8.03, d (8.8)	131.8	7.75, d (8.8)	130.1	7.99, d (8.8)
7-OCH_3	56.1	3.90, s				
4’-OCH_3	55.5	3.80, s				
1”	103.7	4.79, d (7.3)	103.4	5.37, d (1.5)	100.9	5.45, d (7.3)
2”	73.3	2.98—3.99	71.9[a]		74.2[d]	3.20—4.30
3”	75.9	2.98—3.99	71.8[a]		76.2	3.20—4.30
4”	69.5	2.98—3.99	72.1[a]		74.1	3.20—4.30
5”	75.6	2.98—3.99	73.2		74.1[d]	3.20—4.30
6”	68.6	2.98—3.99	17.6	0.93, d (5.7)	62.9	3.20—4.30
1’’’	104.9	4.20, d (7.3)			124.9	
2’’’	73.4	2.98—3.99			133.0	7.37, d (8.8)
3’’’	76.5	2.98—3.99			115.7	6.86, d (8.5)
4’’’	69.5	2.98—3.99			159.7[c]	
5’’’	65.6	2.98—3.99			115.7	6.86, d (8.5)
6’’’					133.0	7.37, d (8.8)
7’’’					144.5	7.35, d (14.0)
8’’’					113.6	
9’’’					166.2	

[a,b,c,d]Assignments may be reversed in same column.

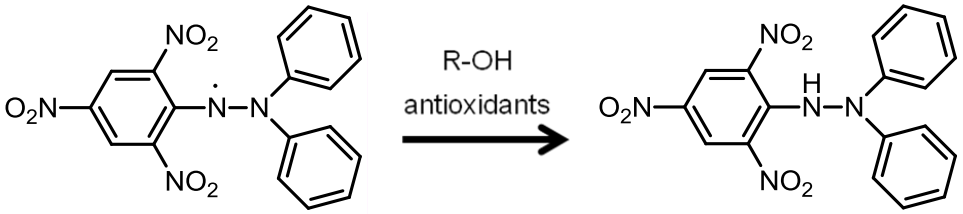
3. Biological activities

Most of the compounds isolated from Diplomorpha plants were phenolic compounds including flavonoids, lignans, coumarins and phenylpropanoids, etc. Thus, some of the isolated compounds were evaluated for their antioxidative and tyrosinase inhibitory activities.

3.1. Antioxidative activity

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effect of free radicals in the human body, and to prevent the deterioration of fats and others constituents of food stuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources.[137] There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants.

One such method that is currently popular is based upon the use of the stable free radical 1,1-diphenyl-2-picryl hydrazyl (*α*,*α*-diphenyl-*β*-picrylhydrazyl: DPPH). The molecule of DPPH is characterized as a stable free radical by virtue of the dislocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise, as would be the case with most other free radicals. The dislocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives reduced form with the loss this violet colour.[138]

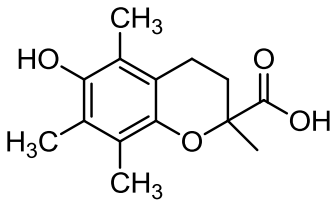


DPPH (free radical)
UV max. 517 nm
(violet colour)

DPPH (non radical)
(pale yellow colour)

Various phenolic compounds including flavonoids and lignans have been reported as potent antioxidants. In the present study, antioxidant activity was evaluated using according to method of Suda *et al* (2006)[139] with slight modification. Using the standard calibration curve of Trolox with correlation coefficient (R^2) more than 0.99, the free radical scavenging activity

of each compound was expressed as mmol of Trolox equivalent per mol of compound (mmol TE/mol). Trolox is a synthetic water soluble derivative of vitamin E.



Trolox

Total 19 compounds including flavonids, lignans and chlorogenic acid were evaluated for their antioxidant activity (Table 20). Among them, quercetin (**11**), luteolin 7-methyl ether (**51**), hypolaetin 8-*O*-*β*-D–glucuronopyranoside (**53**), kaempferol (**8**), luteolin 7-methyl ether 5-*O*-*β*-D–glucopyranoside (**19**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), quercetin 3-*O*-*α*-L–rhamnopyranoside (**52**), chlorogenic acid (**58**), (-)-pinoresinol (**22**), (-)-syringaresinol 4-*O*-*β*-D–glucopyranoside (**44**) showed potent antioxidant activity with trolox equivalent (mmol TE/mol) being 2117, 1962, 1888, 1581, 1312, 1215, 1133, 842, 841 and 650, respectively (Figure 43).

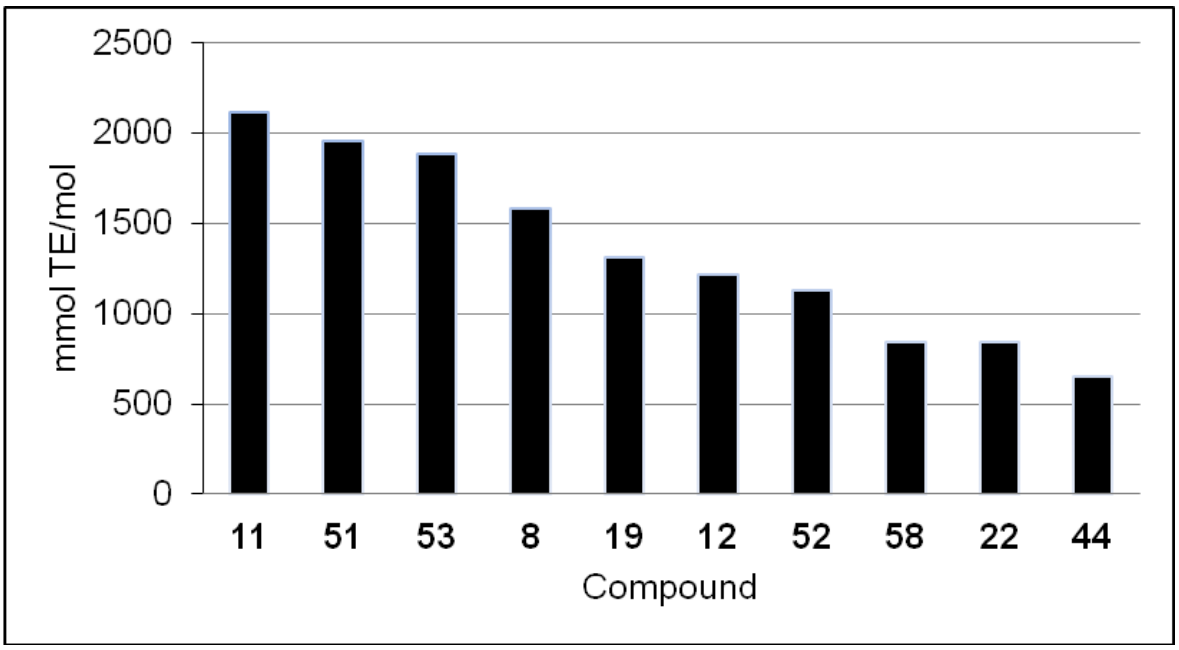


Figure 43. Graphical representation of potent antioxidant compounds with their Trolox equivalents (TE).

Table 20. Antioxidant activity of isolated compounds expressed as mmol TE/mol.

S. No.	Compound	Mol. formula	Mol. weight	mmol TE/g	mmol TE/mol
1	quercetin (**11**)	$C_{15}H_{10}O_7$	302	7.01	2117
2	luteolin 7-methyl ether (**51**)	$C_{16}H_{12}O_6$	300	6.54	1962
3	hypolaetin 8- *O*-*β*-D – glucuronopyranoside (**53**)	$C_{21}H_{18}O_{13}$	478	3.95	1888
4	kaempferol (**8**)	$C_{15}H_{10}O6$	286	5.53	1581
5	luteolin 7-methyl ether- 5-*O*-*β*-D–glucopyranoside (**19**)	$C_{22}H_{22}O_{11}$	462	2.84	1312
6	quercetin -3-*O*-*β*-D-glucopyranoside (**12**)	$C_{21}H_{20}O_{12}$	464	2.62	1215
7	quercetin 3-*O*-*α*-L – rhamnopyranoside (**52**)	$C_{21}H_{20}O_{11}$	448	2.53	1133
8	chlorogenic acid (**58**)	$C_{16}H_{18}O_9$	354	2.38	842
9	(-)-pinoresinol (**22**)	$C_{20}H_{22}O_6$	358	2.35	841
10	(-)-syringaresinol 4-*O*-*β*-D – glucopyranoside (**44**)	$C_{28}H_{36}O_{13}$	580	1.12	650
11	(-)-pinoresinol 4-*O*-*β*-D – glucopyranoside (**54**)	$C_{26}H_{32}O_{11}$	520	0.55	286
12	kaempferol-3-*O*-*β*-D – glucopyranoside (**9**)	$C_{21}H_{20}O_{11}$	448	0.50	224
13	apigenin (**14**)	$C_{15}H_{10}O_5$	270	0.32	86
14	genkwanin 5-*O*-*β*-D – glucopyranoside (**16**)	$C_{22}H_{22}O_{10}$	446	0.16	71
15	rhamnocitrin 3-*O*-*β*-D – glucopyranoside (**10**)	$C_{22}H_{22}O_{11}$	462	0.12	<50
16	(2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**)	$C_{17}H_{16}O_6$	316	0.14	<50
17	pillion 5-*O*-*β*-D – glucopyranoside (**49**)	$C_{23}H_{24}O_{11}$	476	0.03	<50
18	(-)-pinoresinol 4,4’-di- *O*-*β*-D – glucopyranoside (**55**)	$C_{26}H_{32}O_{11}$	682	0.01	<50
19	farrerol 7- *O*-*β*-D – glucopyranoside (**6**)	$C_{23}H_{26}O_{10}$	462	0.01	<50

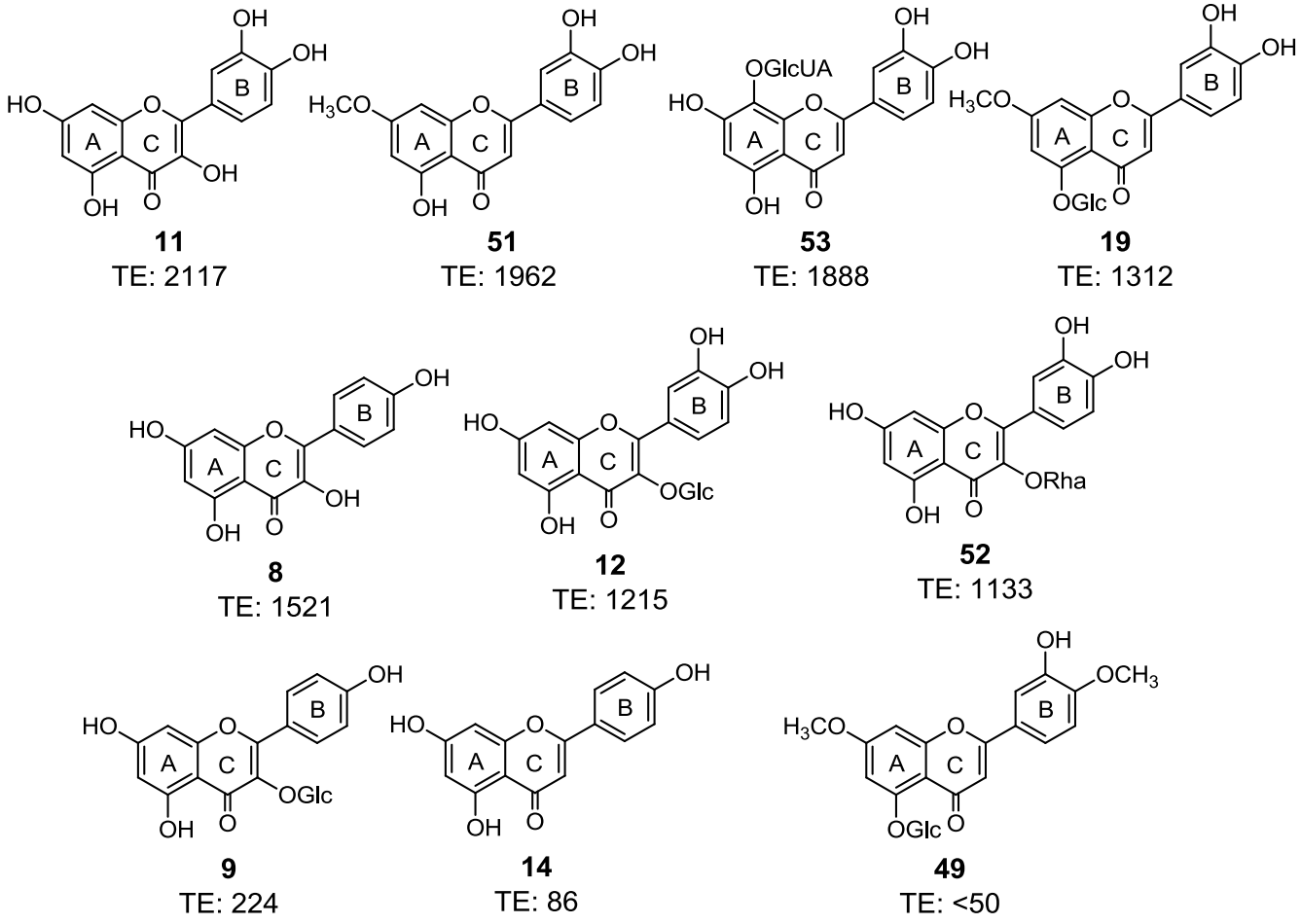


Figure 44. Structure activity relationships (SAR) of antioxidative activity of flavonoids

We have also analysed the structure activity relationships (SAR) of isolated flavonoids for antioxidant activity. Among the tested compounds, quercetin (11) having 3',4'-dihydroxy group in the B- ring and free 3-hydroxy group, showed most potent free radical scavenging activity (Figure 44). Other three flavonoids with free 3',4'-dihydroxy group in the B-ring, luteolin 7-methyl ether (51), hypolaetin 8-*O*-*β*-D–glucuronopyranoside (53) and luteolin 7-methyl ether 5-*O*-*β*-D–glucopyranoside (19) also showed potent but slightly weaker activity than that of quercetin (11). Kaempferol (8) which has one less hydroxyl group in the B-ring showed weaker activity than quercetin (11), which suggests that free 3',4'-dihydroxy group in the B- ring is essential for the antioxidant activity. Subsitution of 3-hydroxyl group by sugar moieties in 11 reduced the activity as in quercetin 3-*O*-*β*-D-glucopyranoside (12), quercetin 3-*O*-*α*-L–rhamnopyranoside (52) and lack of 3-hydroxy group in apigenin (14) had

very weak activity which suggests that the 3-hydroxyl group may also play important role in antioxidant activity. Substitution of any hydroxyl groups in B-ring also weakens the activity as observed in pillion 5-*O*-*β*-D–glucopyranoside (19) as compared to luteolin 7-methyl ether 5-*O*-*β*-D–glucopyranoside (19) (Figure 44). These structure activity relationships are similar to previous reports.[55,140-141)]

3.2. Tyrosinase inhibitory activity

Tyrosinase is the key enzyme in the melanogenesis (melanin biosynthesis) and participates in the oxidation of tyrosine to dopaquinone via L-3, 4–dihydroxyphenylalanine (L-DOPA). Tyrosinase inhibitory activity assays are one of the widely used assays to evaluate the inhibition of melanin biosynthesis. Studies on mushroom tyrosinase inhibition are preferred as mushroom tyrosinase is commercially available and inexpensive. In the present study 30 of the isolated compounds including flavonoids, biflavonoids and lignans were evaluated for their mushroom tyrosinase inhibitory activities according to the method by Jiang *et al* (2012)[142)] with slight modifications using L-DOPA as a substrate. Present result (Table 21) shows their primary screening data for tyrsinase inhibition at the concentration of 1 mg/mL.

Many of these compounds showed potent inhibitory activity on tyrosinase**.** (-)-Syringaresinol (**43**) was the most potent compounds with 96.3±2.1 % inhibition followed by quercetin (**11**), kaempferol (**8**), farrerol 7-*O*-*β*-D–glucopyranoside (**6**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), genkwanin 5-*O*-*β*-D–glucopyranoside (**16**), rhamnocitrin 3-*O*-*β*-D–glucopyranoside (**10**), apigenin (**14**), syringin (**43**), 3(*S*)-hydroxy-1,5-diphenylpentanone (**65**) and rhamnetin 3-*O*-*β*–D–glucopyranoside (**13**).

The present study shows the tyrosinase inhibitory activity of these compounds only in one concentration level. Further dose-dependent studies and calculation of EC_{50} values should be carried out for the potent compounds.

Table 21. Tyrosinase inhibitory activity of isolated compounds

S. No.	Compound [a)]	% Tyrosinase inhibitory activity[b)]
1	(-)-syringaresinol (**43**)	96.3±2.1
2	quercetin (**11**)	81.3±3.2
3	kaempferol (**8**)	78.5±1.9
4	farrerol 7- *O*-*β*-D–glucopyranoside (**6**)	76.8±2.1
5	quercetin 3-*O*-*β*-D-glucopyranoside (**12**)	75.7±3.3
6	genkwanin 5-*O*-*β*-D–glucopyranoside (**16**)	69.4±2.1
7	rhamnocitrin 3-*O*-*β*-D–glucopyranoside (**10**)	69.0±4.3
8	apigenin (**14**)	67.3±4.1
9	syringin (**43**)	66.9±3.4
10	3(*S*)-hydroxy-1,5-diphenylpentanone (**65**)	66.7±3.2
11	rhamnetin 3-*O*-*β*-D–glucopyranoside (**13**)	66.2±1.9
12	neochamaejasmin B (**39**)	64.4±4.3
13	pillion 5-*O*-*β*-D –glucopyranoside (**49**)	64.1±3.5
14	luteolin (**18**)	63.4±2.1
15	daphnoretin (**46**)	62.4±1.8
16	kaempferol 3-*O*-*β*-D –glucopyranoside (**9**)	60.6±2.2
17	neochamaejasmin A (**62**)	59.2±5.1
18	chlorogenic acid (**58**)	57.4±4.7
19	(-)-pinoresinol 4,4'-di-*O*-*β*-D –glucopyranoside (**55**)	55.6±3.8
20	afzelechin (**68**)	55.2±4.3
21	sikokianin B (**40**)	54.6±2.7
22	(-)-dihydrosesamin (**21**)	53.8±3.9
23	(2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**)	53.5±4.7
24	luteolin 7-methyl ether-5-*O*-*β*-D–glucopyranoside (**19**)	51.4±2.8
25	hypolaetin 8-*β*-D –glucuronopyranoside (**53**)	50.6± 4.1
26	syringinoside (**42**)	49.8±3.8
27	(-)-pinoresinol (**20**)	48.2±4.3
28	rutarensin (**26**)	48.2±3.9
29	quercetin 3-*O*-*β*-D –rhamnopyranoside (**52**)	39.8±2.7
30	(-)-lariciresinol (**20**)	38.4±4.6

[a)]sample concentration was 1mg/mL. [b)] mean±s.d., n=3.

4. Conclusion

The present study was focused on the chemical analysis and biological activity evaluation of *Diplomorpha* plants from Nepal and Japan which are used in traditional medicines. We have carried out the chemical analysis on aerial parts and roots of *Diplomorpha canescens* from Nepal and stems and roots of *Diplomorpha ganpi* and stems, roots and leaves of *Diplomorpha sikokiana* from Japan.

From the 70% MeOH extract of aerial parts of *Diplomorpha canescens,* five new C-methyl flavonoids such as (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**), (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**),[143] farrerol 4'-*O*-*β*-D-glucopyranoside (**3**),[144] farrerol 4',7-di-*O*-*β*-D-glucopyranoside (**4**) and 6,8-di-*C*-methylkaempferol 7-*O*-*β*-D-glucopyranoside (**5**)[145] and one new diarylpentanoid, diplomorphanone A (**32**)[146] were isolated from along with 26 known phenolic compounds including 14 known flavonoids, farrerol 7-*O*-*β*-glucopyranoside (**6**), farrerol (**7**), kaempferol (**8**), kaempferol 3-*O*-*β*-D-glucopyranoside (**9**), rhamnocitrin 3-*O*-*β*-D-glucopyranoside (**10**), quercetin (**11**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), rhamnetin 3-*O*-*β*-D-glucopyranoside (**13**), apigenin (**14**), genkwanin (**15**), genkwanin 5-*O*-*β*-D-glucopyranoside (**16**), genkwanin 5-*O*-primeveroside (**17**), luteolin (**18**), luteolin 7-methyl ether-5-*O*-*β*-D-glucopyranoside (**19**), four lignans, (-)-lariciresinol (**20**), (-)-dihydrosesamin (**21**), (-)-pinoresinol (**22**) (±)-dehydrodiconiferyl alcohol (**23**); three phenylpropanoid derivatives, coniferyl aldehyde (**24**), sinapyl aldehyde (**25**), *p*-coumaric acid methyl ester (**28**); two coumarin derivatives, rutarensin (**26**), umbelliferone (**31**) and three related phenolic compounds, syringaldehyde (**27**), *p*-hydroxy benzaldehyde (**29**) and *p*-hydroxy acetophenone (**30**).

From the 70% MeOH extract of *Diplomorpha canescens,* two new biflavonoids, 14''-*O*-methyldihydrodaphnodorin B (**33**) and 14''-*O*-methyldaphnodorin J (**35**) were isolated along with 16 known compounds including be six biflavonoids, dihydrodaphnodorin B (**34**), daphnodorin J (**36**), 3''-epi-dihydrodaphnodorin B (**37**), daphnodorin B (**38**), neochamaejesmin B (**39**), sikokianin B (**40**); five lignans, (-)-syringaresinol (**43**), (-)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**44**), (+)-nortrachelogenin (**45**), (-)-lariciresinol (**20**), (-)-pinoresinol (**22**); two phenylpropanoids, syringin (**41**), syringinoside (**42**);

daphnoretin (**46**), phorbol 13-acetate (**47**) and methyl paraben (**48**). All these compounds were isolated for the first time from this plant.

From the MeOH extract of stems of *Diplomorpha ganpi*, one new flavones glucoside, pilloin 5-*O*-*β*-D-glucopyranoside (**49**)[146] along with 22 known compounds including six flavonoids, pilloin (**50**), luteolin 7-methyl ether-5-*O*-*β*-D-glucopyranoside (**19**), luteolin 7-methyl ether (**51**), quercetin 3-*O*- *β*–D–glucopyranoside (**12**), quercetin 3-*O*-*α*–L-rhamnopyranoside (**52**), hypolaetin 8-*O*-*β*–D–glucuronopyranoside (**53**); five lignans, (-)-pinoresinol (**22**), (-)-pinoresinol 4-*O*-*β*-D-glucopyranoside (**54**), (-)-pinoresinol 4,4'-di-*O*-*β*-D-glucopyranoside (**55**), syringaresinol 4-*O*-*β*-D-glucopyranoside (**44**), (7*S*,8*R*) dehydrodiconiferyl alcohol 9'-*O*-*β*-D-glucopyranoside (**57**); three phenylpropanoids, syringin (**41**), syringinoside (**42**), coniferin (**56**); four biflavonoids, stelleranol (**61**), neochamaejasmin A (**62**), dihydrodaphnodorin B (**34**), and 3''-epi-dihydrodaphnodorin B (**37**); chlorogenic acid (5**8**), maltol 3-*O*-*β*-D-glucopyranoside (**59**); and three coumarins, apiosylskimmin (**60**), daphnoretin (**46**) and rutarensin (**26**).

From the MeOH extract of roots of *Diplomorpha ganpi*, one new diarylpentanoid, diplomorphanone B (**63**)[146] was isolated along with 12 known compounds including four diarylpentanoids, 1,5-diphenyl-1-pentanone (**64**), (*S*)-3-hydroxy-1,5-diphenyl-1-pentanone (**65**), 3-methoxy-1,5-diphenyl-1-pentanone (**66**), 1,5-diphenyl-2-penten-1-one (**67**); three biflavonoids, dihydrodaphnodorin B (**34**), daphnodorin B (**38**), sikokianin A (**70**); three phenylpropanoid derivatives, syringin (**41**), syringinoside (**42**), sinapyl alcohol (**69**); a coumarin, apiosylskimmin (**60**); and a flavanol, (+)-afzelechin (**68**). All these compounds were isolated for the first time from this plant.

From the MeOH extract of stems of *Diplomorpha sikokiana*, fourteen known compounds including two phenylpropanoids, syringin (**41**), syringinoside (**42**); five biflavonoids, neochamaejesmin B (**39**), sikokianin B (**40**), chamaejasmenin B (**71**), stelleranol (**61**) and dihydrodaphnodorin B (**34**); two lignans, (-)-syringaresinol 4-*O*-*β*-D-glucoside (**44**), (-)-pinoresinol 4,4' di-*O*-*β*-D-glucoside (**55**); one flavonoid, apigenin 4',7-dimethylether 5-*O*-primeveroside (**72**); two coumarins, apiosylskimmin (**60**), daphnoretin (**45**) and *β*-sitosterol (**72**) were isolated and identified.

From the MeOH extract of roots of *Diplomorpha sikokiana*, 13 known compounds including four diarylpentanoids, 1,5-diphenyl-1-pentanone (**63**), 1,5-diphenyl-2-penten-1-one (**66**), (-)-*erythro*-1,5-diphenylpentane-1,3-diol (**74**), 3(*S*)-hydroxy-1,5-diphenyl-1-pentanone (**64**), two phenylpropanoids, syringin (**41**), syringinoside (**42**); three biflavonoids, sikokianin B (**40**), stelleranol (**61**), chamaejasmenin B (**71**); three lignans, (-)-pinoresinol (**22**), (-)-syringaresinol (**43**), (-)-pinoresinol 4,4' -di-*O*-β-D-glucopyranoside (**55**), a coumarin, apiosylskimmin (**60**) and β-sitosterol (**73**) were isolated and identified.

From the MeOH extract of leaves of *Diplomorpha sikokiana*, five known flavonoids, genkwanin 5-*O*-primeveroside (**17**), quercetin 3-*O*-α-L-rhamnopyranoside (**52**), apigenin 4', 7-dimethylether 5-*O*-primeveroside (**72**), kaempferol 3-*O*-α-L-rhamnopyranoside (**75**) and tiliroside (**76**) were isolated and identified. All these compounds except **17**, **22**, **34**, **39**, **40**, **41**, **42**, **45**, **71** and **72** were isolated for the first time from *Diplomorpha sikokiana*.

Altogether 76 different compounds were isolated and identified and among them 10 compounds were new ones. All these three species of *Diplomorpha* were found to contain many similar chemical constituents including flavonoids, biflavonoids, lignans, phenylpropanoids and coumarins. Chemical analysis was performed on the whole aerial parts of *Diplomorpha canescens* (including both stems and leaves) and leaves of *Diplomorpha ganpi* were not studies in present study, so direct comparison of chemical constituents would be difficult. But, on the basis of isolated compounds, flavonoids and lignans were the major compounds present in aerial parts (leaves and stems) and biflavonoids were main constituents in roots of these three species. Diarylpentanoids were isolated from the roots of *Diplomorpha ganpi* and *Diplomorpha sikokiana* but not from the roots of *Diplomorpha canescens*, whereas one diarylpentanoid was isolated from the aerial parts of *Diplomorpha canescens* but not from the aerial parts of other two species.

The general distribution of isolated compounds in different plants parts of species included in study is presented in Table 22.

Table 22. Distribution of compounds in studied *Diplomorpha* plants parts

	CA	CR	GS	GR	SS	SR	SL
Flavonoids							
1	O						
2	O						
3	O						
4	O						
5	O						
6	O						
7	O						
8	O						
9	O						
10	O						
11	O						
12	O		O				
13	O						
14	O						
15	O						
16	O						
17	O						O
18	O						
19	O		O				
49			O				
50			O				
51			O				
52			O				O
53			O				
68				O			
72					O		O
75							O
76							O
Lignans							
20	O	O					
21	O						
22	O	O	O		O	O	
23	O						
43		O				O	
44		O	O		O		
45		O					
54			O				
55			O		O	O	
57			O				
Coumarins							
26	O		O				
31	O						
46		O	O		O		
60			O	O	O	O	

	CA	CR	GS	GR	SS	SR	SL
Phenylpropanoids							
24	O						
25	O						
28	O						
41		O	O	O	O	O	
42		O	O	O	O	O	
56			O				
69				O			
Biflavonoids							
33		O					
34		O		O	O		
35		O					
36		O					
37		O	O		O	O	
38		O		O	O		
39		O					
40		O					
61			O		O	O	
62			O				
70				O			
71					O	O	
Diarylpentanoids							
32	O						
63				O		O	
64				O		O	
65				O			
66				O		O	
67				O			
74						O	
Others							
27	O						
29	O						
30	O						
47		O					
48		O					
58			O				
59			O				
73					O	O	

CA: *D. canescens* aerial parts, CR: *D. canescens* roots, GS: *D. ganpi* stems, GR: *D. ganpi* roots, SS: *D. sikokiana* stems, SR: *D. sikokiana* roots, SL: *D. sikokaina* leaves

Among these isolated compounds, 19 compounds including flavonoids, lignans and chlorogenic acid were evaluated for their antioxidant activity. Among them, quercetin (**11**), luteolin 7-methyl ether (**51**), hypolaetin 8-*O*-*β*-D–glucuronopyranoside (**53**), kaempferol (**8**), luteolin 7-methyl ether- 5-*O*-*β*-D–glucopyranoside (**19**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), quercetin 3-*O*-*α*-L–rhamnopyranoside (**52**), chlorogenic acid (**58**), (-)-pinoresinol (**22**), (-)-syringaresinol 4-*O*-*β*-D–glucopyranoside (**44**) showed potent antioxidant activity with Trolox equivalent (mmol TE/mol) being 2117, 1962, 1888, 1581, 1312, 1215, 1133, 842, 841 and 650, respectively.

Similarly, 30 of the isolated compounds including flavonoids, biflavonoids and lignans were evaluated for their mushroom tyrosinase inhibitory activity. (-)-Syringaresinol (**43**) was the most potent compounds with 96.3±2.1% inhibition followed by quercetin (**11**), kaempferol (**8**), farrerol 7-*O*-*β*-D–glucopyranoside (**6**), quercetin 3-*O*-*β*-D-glucopyranoside (**12**), genkwanin 5-*O*-*β*-D–glucopyranoside (**16**), rhamnocitrin 3-*O*-*β*-D–glucopyranoside (**10**), apigenin (**14**), syringin (**43**), 3(*S*)-hydroxy-1,5-diphenylpentanone (**65**) and rhamnetin 3-*O*-*β*–D–glucopyranoside (**13**). Further dose-dependent studies and calculation of EC_{50} values should be carried out for the potent compounds.

In conclusion, this study provided the idea about the chemical constituents of three *Diplomorpha* species and 10 new compounds were isolated and identified along with 66 known compounds. Some of the isolated compounds showed potent antioxidant and tyrosinase inhibitory activities. This study provides the evidences for the traditional use of these medicinal plant species. Isolated compounds may also help in the discovery and development of new chemical moiety as pharmaceutical agents.

5. Experimental

General experimental procedures

Melting points were measured on Yanaco Micromelting point apparatus (MP-J3, MP-S3) and were uncorrected.Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C- and 2D-NMR spectra were measured on a JEOL α-500 spectrometer (^{1}H: 500 MHz and ^{13}C: 125 MHz). Chemical shifts are given in ppm with reference to TMS. Mass spectra were recorded on JEOL JMS 700 MStation mass spectrometer. CD spectra were recorded on JASCO J-810 spectropolarimeter. Column chromatography was carried out with silica gel 60 (0.040-0.063 mm, Merck), MCI gel CHP20P (75-150 μm, Mitsubishi Chemical Industries Co., Ltd.), Saphadex LH-20 (Amersham Pharmacia Biotech) and Chromatorex ODS (30-50 μm, Fuji Silysia Chemical Co., Ltd.). TLC was performed on a precoated silica gel 60 F_{254} (0.2 mm, aluminum sheet, Merck).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was from Wako Pure Chemicals, Osaka, Japan. Trolox was from Calbiochem (Denmark). 2-Morpholinoethanesulfonic acid, monohydrate (MES) buffer was from Dojindo Chemical Research, Kumamoto, Japan. Mushroom tyrosinase and L-DOPA were from Sigma-Adrich (St. Louis, MO, USA). Absorbance was recorded on Immuno-Mini NJ-2300 Microtiter Plate Reader, Biotech Pvt. Ltd. (Tokyo, Japan) for DPPH and on xMark Microplate Spectrophotometer, Bio-Rad Laboratories, Inc. (Tokyo, Japan) for tyrosinase.

Plant materials

Aerial parts and roots of *Diplomorpha canescens* were collected in August, 2007 from Daman, Nepal. The specimen was identified by Mr. Kuber Jung Malla, Scientic Officer, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The voucher specimens have been deposited at Kochi Prefectural Makino Botanical Garden, Kochi, Japan.

Fresh stems and roots of *Diplomorpha ganpi* were collected from Aso, Kumamoto, Japan in September, 2008 and voucher specimens have been deposited at Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Fresh stems, leaves and roots of *Diplomorpha sikokiana* were collected from Kochi, Japan in July, 2012 and voucher specimens have been deposited at Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Extraction and isolation of compounds from aerial parts of *Diplomorpha canescens*

The shade dried aerial parts of *D. canescens* (492 g) were extracted twice with 70% MeOH (3 l) and extracts were evaporated under reduced pressure to give 70% MeOH extract (148.7 g) which was then dissolved in water and subjected to MCI gel CHP20P column eluting with water, 40% MeOH, 70% MeOH and 100% MeOH to give 8 fractions.

Fraction 2 (16.3 g) was subjected to Sephadex LH 20 column (MeOH) and then ODS (30% MeOH) to afford **19** (121 mg), **9** (41 mg) and **4** (11 mg).

Fraction 3 (10.9 g) was separated into MeOH soluble (3-1) and MeOH insoluble (3-2) parts. MeOH soluble part (3-1, 8.5 g) was then applied on Saphadex LH-20 column (MeOH) to give seven subfraction. Subfraction 3-1-4 was again applied on ODS column (30-50% MeOH) to give further 12 subfractions. Among them, subfraction 3-1-4-5 was applied on silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to obtain **12** (67 mg) and **4** (39 mg). Subfraction 3-1-5 and 3-1-4-10 were obtained as **5** (24 mg) and **17** (12 mg).

Fraction 4 (10.0 g) was subjected to Sephadex LH-20 column (MeOH) to give 8 subfractions. Among them, subfraction 4-4 (3.0 g) was applied on ODS column (30-50% MeOH) to obtain **9** (44 mg), **10** (31 mg), **16** (13 mg) and **6** (39 mg). Subfraction 4-3 (1.9 g) was subjected to Saphadex LH-20 column (MeOH) and then ODS column (30% MeOH) to obtain **16** (282 mg), **29** (2 mg), **30** (2 mg), **31** (6 mg), **6** (569 mg). Subfraction 4-7 was obtained as **11** (200 mg).

Fraction 5 (2.5 g) was subjected Saphadex LH-20 column (MeOH) to obtain 9 subfractions. The subfraction 5-5 was then subjected to ODS column (45% MeOH) to give 11 subfractions. Subfractions 5-5-3, 5-5-5, 5-5-7 and 5-5-10 afforded pure compounds **9** (8 mg), **6** (45 mg), **13** (34 mg) and **10** (8 mg), respectively. Subfraction 5-5-6 (151 mg) was subjected to MCI gel column (30% MeOH), Saphadex LH-20 column ($CHCl_3$:MeOH=3:1) and then silica gel column ($CHCl_3$:MeOH:H_2O=9:2:0.1) to obtain compound **1** (52 mg) and **3** (36 mg).

Compound **18** (27 mg) and **11** (68 mg) were obtained from the recrysatllization (MeOH:H_2O) of subfraction 5-7 (135.7 mg) and 5-8 (80 mg), respectively. Subfraction 5-3 was applied on silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) to obtain 10 subfractions. Subfraction 5-3-1 was then applied on silica gel column elution with hexane:EtOAc (3:1) to obtain **28** (4 mg), **27** (3 mg), **24** (2 mg) and **25** (3 mg). Subfraction 5-3-2 (73 mg) was applied on silica gel column eluting with hexane:EtOAc (1:2) to obtain **20** (24 mg) and **23** (7 mg). Subfraction 5-3-10 (63 mg) was applied on silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) to obtain **26** (17 mg).

Fraction 6 (6.4 g) was then subjected Saphadex LH-20 column (MeOH) to afford 8 subfractions. Subfraction 6-3 (1.5 g) was then applied on silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) to afford 8 subfractions (6-3-1—6-3-8). Subfraction 6-3-1 (462 mg) was applied on silica gel column eluting with hexane:EtOAc (1:1) to give further 12 subfractions (6-3-1-1—6-3-1-12). Subfraction 6-3-1-2 was again applied on silica gel column eluting with hexane:EtOAc (1:1) and then $CHCl_3$:MeOH (20:1) to obtain **7** (6 mg), and **28** (18 mg) and **32** (8 mg). Subfraction 6-3-1-3 and 6-3-1-7 were obtained as pure compounds **15** (6 mg) and **22** (167 mg) respectively. Subfraction 6-3-2 was again applied on silica gel column eluting with hexane:EtOAc (1:1) to give **2** (11 mg). Subfraction 6-3-4 and 6-3-6 were obtained as pure compounds **10** (102 mg) and **16** (79 mg) respectively. Compound **14** (156.5 mg) and **8** (444 mg) were obtained from the recrysatllization (MeOH:H_2O) of subfraction 6-4 (689.9 mg) and 6-5 (684 mg), respectively. Subfraction 6-8 (253 mg) was applied on silica gel column ($CHCl_3$:MeOH:H_2O=9:2:0.1) to obtain compound **16** (39 mg).

Fraction 8 (3.8 g) was dissolved in MeOH and filtered to give MeOH soluble and insoluble parts. MeOH soluble part was then applied on Saphadex LH-20 column (MeOH) and silica gel column (hexane:EtOAc=2:1) to obtain **21** (75 mg).

Extraction and isolation of compounds from roots of *Diplomorpha canescens*

The dried roots of *D. canescens* (500 g) were extracted twice with 70% MeOH (4.5 l) and extracts were evaporated under reduced pressure to give 70% MeOH extract (104 g). The extract was then separated into water soluble part (45 g) and water insoluble part (59 g). Water insoluble part was dissolved in 40% MeOH and subjected to MCI gel CHP20P column eluting with 40%, 60%, 80% and 100% MeOH to give 16 fractions.

Fraction 2 (2.7 g) was subjected to MCI gel CHP20P column (10—20% MeOH), Saphadex LH-20 column (MeOH) and ODS column (20—40% MeOH) to **41** (205 mg) and **42** (284 mg).

Fraction 5 (7.0 g) was subjected to Saphadex LH-20 column (MeOH) to afford 7 subfractions (5-1—7). Subfraction 5-2 (1035 mg) was applied on silica gel column ($CHCl_3$:MeOH:H_2O=9:1:0.1) to obtain compounds **44** (32 mg) and **47** (47 mg). Subfraction 5-6 (2656 mg) was applied on ODS column (30—60% MeOH) to obtain compounds **33** (116 mg) and **37** (799 mg). Subfraction 5-7 (802 mg) was applied on ODS column (40—70% MeOH) to obtain compounds **34** (182 mg), **36** (129 mg) and **38** (93 mg).

Fraction 7 (4.4 g) was subjected to Saphadex LH-20 (MeOH) and silica column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) and $CHCl_3$:MeOH (20:1) to obtain **20** (48 mg), **45** (28 mg) and **48** (1 mg).

Fraction 10 (3.1 g) was subjected to Saphadex LH-20 column (MeOH) to afford 10 subfractions (10-1—10). Subfraction 10-2 (371 mg) and 10-4 (129 mg) were subjected to silica column ($CHCl_3$:MeOH= 20:1) to obtain **43** (243 mg) and **22** (77 mg), respectively. Subfractions 10-8 and 10-10 were obtained as **35** (469 mg) and **40** (465 mg), respectively.

Fractions 13 (787 mg) and 14 (2.6 g) were subjected to Sephadex column (MeOH) to obtain **46** (179 mg) and **39** (1080 mg), respectively.

Extraction and isolation of compounds from stems of *Diplomorpha ganpi*

Fresh stems of *D. ganpi* (3 kg) were extracted twice with MeOH (20 L) and extracts were evaporated under reduced pressure to give MeOH extract (122 g). The extract was then separated into water soluble part (92 g) and water insoluble part (30 g). Water soluble part was dissolved in water and subjected to MCI gel CHP20P column eluting with water, 40%, 60%, 80% and 100% MeOH to give 17 fractions and each fraction was monitored with TLC.

Fraction 3 (1.8 g) was subjected to Saphadex LH-20 column eluting with MeOH to afford 9 subfractions (3-1—9). Subfraction 3-2 (891 mg) was subjected to ODS column eluting with 30% MeOH to give 6 subfractions (3-2-1—6). Among them, subfraction 3-2-1 (230 mg) was

further subjected to ODS column (20% MeOH) to obtain compounds **58** (64 mg) and **59** (35 mg). Subfraction 3-2-3 (196 mg) was further subjected silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to obtain **42** (38 mg), **56** (90 mg) and apiosylskimmin **60** (21 mg). Similarly, subfraction 3-2-5 (99 mg) was subjected silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to obtain **41** (58 mg).

Fraction 5 (7.7 g) was subjected to Saphadex LH-20 column (MeOH) to afford 5 subfractions (5-1—5). Subfraction 5-1 (5.3 g) was applied on ODS column (30—50% MeOH) and then silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to afford **41** (359 mg) and **55** (265 mg). Subfraction 5-4 was obtained as **53** (100 mg).

Fraction 7 (3.8 g) was subjected to Saphadex LH-20 column (MeOH) to afford 7 subfractions (7-1—7). Subfraction 7-2 (1.5 g) was applied on ODS column (30% MeOH) to obtain **57** (68 mg). Subfraction 7-4 (1.2 g) was applied on ODS column (35—40% MeOH) to obtain **12** (273 mg) and **52** (173 mg).

Fraction 9 (5.2 g) was subjected to Saphadex LH-20 column (MeOH) to afford 9 subfractions (9-1—9). Subfraction 9-2 (1.7 g) was applied on ODS column (40% MeOH) and then silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:2:0.1) to afford **54** (348 mg) and **44** (46 mg). Subfraction 9-3 (1.3 g) was recrystallized on MeOH:H_2O (1:1) to afford **19** (456 mg). Subfraction 9-5 (348 mg) was subjected to silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:2:0.1) to afford **51** (8 mg) and mixture of **34** and **37** (33 mg)..

Fraction 11 (3.3 g) was subjected to Saphadex LH-20 column (MeOH) to afford 5 subfractions (11-1—5). Among them, subfraction 11-2 (1.8 g) was subjected to ODS column (50% MeOH) and then silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to afford compounds **49** (135 mg) and **26** (255 mg). Subfraction 11-3 (616 mg) was subjected to ODS column (50% MeOH) and then Saphadex LH-20 column (MeOH) to afford compound **61** (33 mg) and **51** (11 mg).

Fraction 13 (759 mg) was subjected to Saphadex LH-20 column (MeOH) and then silica gel column eluting with $CHCl_3$:MeOH (10:1, 30:1) to afford **22** (44 mg).

Fraction 14 (1.0 g) was subjected to silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) to afford **46** (205 mg) and **62** (86 mg).

Extraction and isolation of compounds from roots of *Diplomorpha ganpi*

The fresh roots of *D. ganpi* (750 g) were extracted twice with MeOH (3 L) and extracts were evaporated under reduced pressure to give MeOH extract (98 g) which was separated into water soluble (19 g) and water insoluble (79 g) fractions. The water insoluble fraction was then subjected to Saphadex LH-20 column (MeOH) to give 8 fractions. Fraction 3 (3.2 g) was re-chromatographed on ODS (70% MeOH) and silica gel (hexane:EtOAc = 10:1, hexane:EtOAc = 20:1 and hexane:acetone = 10:1) to afford **63** (4.8 mg), **64** (12 mg), **65** (65 mg), **66** (10 mg) and **67** (8 mg).

Similarly, water soluble fraction was dissolved in water and subjected to MCI gel CHP20P column and eluted with water, 40% MeOH, 70% MeOH and 100% MeOH to give 9 fractions. Fraction 2 (653 mg) was then subjected to ODS (30% MeOH) and Saphadex LH-20 column (MeOH) to obtain **42** (83 mg). Fraction 4 (346 mg) was applied on ODS (30% MeOH) and Saphadex LH-20 column (MeOH) to afford **41** (20 mg) and **60** (74 mg). Fraction 6 (561 mg) was applied on ODS (50% MeOH) and silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to obtain **68** (185 mg), **34** (9.9 mg) and **38** (18.1 mg). Similarly, fraction 7 (3.1 g) was applied on silica gel column eluting with $CHCl_3$:MeOH:H_2O (8:2:0.1) to obtain **69** (1 mg).

Extraction and isolation of compounds from stems of *Diplomorpha sikokiana*

Fresh stems of *Diplomorpha sikokiana* (417 g) were extracted twice with MeOH (3 l) and extracts were evaporated under reduced pressure to give MeOH extract (26 g). The extract was then dissolved in water and subjected to MCI gel CHP20P column eluting with water, 40%, 70% and 100% MeOH to give 7 fractions.

Fraction 2 (4.3 g) was subjected to MCI gel CHP20P column (10—20% MeOH), Saphadex LH-20 column (MeOH) and ODS column (20—40% MeOH) to obtain **41** (1044 mg), **42** (108 mg), **55** (123 mg) and **60** (82 mg).

Fraction 3 (1.0 g) was subjected to Saphadex LH-20 column (MeOH) and ODS column (40% MeOH) to obtain **34** (75 mg).

Fraction 4 (3.1 g) was subjected to Saphadex LH-20 column (MeOH) to afford 3 subfractions (4-1—3). Subfraction 4-1 (1.4 g) was subjected to silica gel column ($CHCl_3$:MeOH:H_2O=9:1:0.1) to obtain **44** (35 mg). Subfraction 4-2 (1.6 g) was subjected to ODS column (40% MeOH) to obtain **61** (64 mg).

Fraction 5 (2.2 g) was subjected to Saphadex LH-20 column (MeOH) to afford 5 subfractions (5-1—5). Subfraction 5-2 (945 mg) was subjected to silica gel column ($CHCl_3$:MeOH:H_2O=9:1:0.1) to obtain **22** (130 mg) and **72** (123 mg). Subfraction 5-3 (62 mg) was subjected to silica gel column ($CHCl_3$:MeOH:H_2O=9:1:0.1) to obtain **46** (5 mg). Subfraction 5-4 (707 mg) was subjected to MCI gel column CHP20P (60—90% MeOH) to obtain **37** (32 mg), **38** (129 mg) and **71** (27 mg).

Fraction 6 (790 mg) was subjected to silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) and then hexane:EtOAc (2:1) o obtain **73** (100 mg).

Extraction and isolation of roots of *Diplomorpha sikokiana*

Fresh roots of *Diplomorpha sikokiana* (100 g) were extracted twice with MeOH (700 ml) and extracts were evaporated under reduced pressure to give MeOH extract (7.7 g). The extract was then dissolved in water and subjected to MCI gel CHP20P column eluting with water, 40%, 70% and 100% MeOH to give 6 fractions.

Fraction 2 (420 mg) was subjected to MCI gel CHP20P column (10—20% MeOH), Saphadex LH-20 column (MeOH) and ODS column (20—40% MeOH) to obtain **41** (390 mg), **42** (17 mg), **55** (15 mg) and **60** (10 mg).

Fraction 4 (325 mg) was subjected to Saphadex LH-20 column (MeOH) and then ODS column (5% MeOH) to obtain **61** (29 mg).

Fraction 5 (1.4 g) was subjected to Saphadex LH-20 column (MeOH) to afford 5 subfractions (5-1—5). Subfraction 5-2 (665 mg) was subjected to silica gel column ($CHCl_3$:MeOH:H_2O=9:1:0.1) to obtain **43** (19 mg), **63** (7 mg), **64** (7 mg), **66** (8 mg) and **74** (33 mg). Subfraction 5-4 (441 mg) was subjected to MCI gel column CHP20P (40—90% MeOH) to obtain **37** (85 mg), and **70** (4 mg).

Fraction 6 (191 mg) was subjected to silica gel column eluting with $CHCl_3$:MeOH:H_2O (9:1:0.1) and then hexane:EtOAc (2:1) o obtain **63** (11 mg), **66** (7 mg) and **73** (18 mg).

Extraction and isolation of leaves of *Diplomorpha sikokiana*

Fresh leaves of *Diplomorpha sikokiana* (95 g) were extracted twice with MeOH (700 ml) and extracts were evaporated under reduced pressure to give MeOH extract (18 g) which was then separated into water soluble (14 g) and water insoluble (4 g) fractions. The water soluble fraction was dissolved in water and subjected to MCI gel CHP20P column eluting with water, 40%, 70% and 100% MeOH to give 6 fractions.

Fraction 3 (1.4 g) was subjected to Saphadex LH-20 column (MeOH) and ODS column (5% MeOH) to obtain **17** (8 mg), **52** (137 mg) and **75** (30 mg).

Fraction 4 (853 mg) was subjected to Saphadex LH-20 column (MeOH) to obtain 3 fractions (4-1—3). Fraction 4-1 (506 mg) and 4-2 (331 mg) were subjected separately to silica gel column ($CHCl_3$:MeOH:H_2O=8:2:0.1) to obtain **72** (35 mg) and **76** (159 mg).

(2*R*,3*S*)-6,8-Di-*C*-methyldihydrokaempferol (1)

A pale yellow amorphous powder; $[\alpha]_D^{20}$- 80.5º (*c* 0.52, MeOH); ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 1; HRFABMS *m*/*z* 317.0997 $[M+H]^+$ (calcd. for $C_{17}H_{17}O_6$, 317.1025); CD (MeOH, *c* 0.022): △ε (nm) –27.1 (297), +7.7 (349).

(2*R*,3*R*)-6,8-Di-*C*-methyldihydrokaempferol (2)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ + 4.8º (*c* 0.48, MeOH); ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 1; HRFABMS *m*/*z* 317.1065 $[M+H]^+$ (calcd. for $C_{17}H_{17}O_6$, 317.1025); CD (MeOH, *c* 0.020): △ε (nm) – 28.6 (297), +6.2 (347).

Farrerol 4'-*O*-*β*-D-glucopyranoside (3)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ - 36.5º (*c* 0.66, MeOH).; ^{1}H-NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), Table 2; HRFABMS *m*/*z*$[M+H]^+$ at *m*/*z* 463.1654 (calcd. for $C_{23}H_{27}O_{10}$, 463.1604); CD (MeOH, *c* 0.024): △ε (nm) – 23.9 (290), +4.2 (345).

Acid hydrolysis of farrerol 4'-*O*-β-D-glucopyranoside (3)

A solution of compound **3** (1 mg) in 2N HCl (0.2 ml) in a sealed microtube was heated at 70º C for 4 hours and then the solution was subjected to silica gel TLC along with authentic samples using 10% sulphuric acid as a detection reagent. Glucose was detected using developing solvents *n*-BuOH:AcOH:H_2O (5:1:4, upper layer) and $CHCl_3$:MeOH:H_2O (6:4:1). Similarly, the aglycone, farrerol (**7**) was detected using developing solvent $CHCl_3$:MeOH (9:1).

Diplomorphanin A (4)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ – 22.2º (*c* 0.66, MeOH).; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 2; HRFABMS *m/z:* 647.1979 $[M+Na]^+$ (calcd. for $C_{29}H_{36}O_{15}Na$, 647.1952); CD (MeOH, *c* 0.024): $\triangle\varepsilon$ (nm) –15.9 (283), +4.4 (347).

Acid hydrolysis of diplomorphanin A (4)

Acid hydrolysis was performed by heating a solution of compound **4** (8.0 mg) in 2M HCl (2 mL) in a sealed tube at 70ºC for 3 hr. The aglycone (**7**, 4.0 mg) was extracted with EtOAc and the structure was confirmed as farrerol[4)] by comparing its NMR data and co-TLC with an authentic sample. D-Glucose (3.3 mg) was obtained from the aq. layer and confirmed by comparing its optical rotation, $[\alpha]_D^{21}$ +75.0 (*c* 0.33, H_2O), and co-TLC with an authentic sample.

Dipomorphanin B (5)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +2.6º (*c* 0.50, pyridine); ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 4; HRFABMS *m/z:* 475.1280 $[M-H]^-$ (calcd. for $C_{23}H_{23}O_{11}$, 475.1240).

Acid hydrolysis of diplomorphanin B (5)

Acid hydrolysis was performed by heating a solution of compound **5** (6.0 mg) in 2M HCl:dioxane (1:1, 1 mL) in a sealed tube at 80ºC for 3 hr. The mixture was evaporated and applied to silica gel column ($CHCl_3$:MeOH:H_2O = 7:3:0.5) to obtain aglycone (**5a**, 2.5 mg) and D-glucose (1.3 mg). The aglycone **5a** was confirmed as 6,8-di-C-methyl kaempferol by comparing its NMR data and D-glucose was confirmed by comparing its optical rotation, $[\alpha]_D^{21}$ +73.0 (*c* 0.13, H_2O), and co-TLC with an authentic sample.

6,8-Di-C-methyl kaempferol (5a)

A pale yellow amorphous powder; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 4.

Farrerol 7-*O*-β-D-glucopyranoside (6)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +7.2º (*c* 0.57, MeOH);); ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 2; ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 3; CD (MeOH, *c* 0.024): $\triangle\varepsilon$ (nm) – 12.9 (288), +3.7 (349).

Acid hydrolysis of farrerol 7-*O*-β-D -glucopyranoside (6)

Acid hydrolysis was performed by heating a solution of compound **6** (12.0 mg) in 2M HCl (2 mL) in a sealed tube at 70ºC for 3 hr. The aglycone (**7**, 7.9 mg) was extracted with EtOAc and the structure was confirmed as farrerol by comparing its NMR data and co-TLC with an authentic sample. D-Glucose (3.6 mg) was obtained from the aq. layer and confirmed by comparing its optical rotation, $[\alpha]_D^{21}$ +85.0 (*c* 0.36, H_2O), and co-TLC with an authentic sample.

Farrerol (7)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ -21.3º (*c* 0.62, MeOH); ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 3. CD (MeOH, *c* 0.090): $\triangle\varepsilon$ (nm) – 53.8 (281), +20.8 (341).

Kaempferol (8)

A pale yellow crystal; mp 272ºC (decomp.); ^{1}H-NMR (DMSO-d_6), Table 6 and ^{13}C NMR (DMSO-d_6), Table 8.

Kaempferol 3-*O*-β-D-glucopyranoside (9)A pale yellow amorphous powder; $[\alpha]_D^{19}$ -35.8º (*c* 0.75, pyridine); ^{1}H-NMR (CD_3OD), Table 6 and ^{13}C NMR (CD_3OD), Table 8.

Rhamnocitrin 3-*O*-β-D-glucopyranoside (10)

A pale yellow amorphous powder; $[\alpha]_D^{19}$ -54.7º (*c* 0.82, pyridine); ^{1}H-NMR ($CDCl_3$:CD_3OD=1:1), Table 6 and ^{13}C NMR ($CDCl_3$:CD_3OD=1:1), Table 8.

Quercetin (11)

A pale yellow crystal; ^{1}H-NMR (DMSO-d_6), Table 6 and ^{13}C NMR (DMSO-d_6), Table 8.

Quercetin 3-*O*-*β*-D-glucopyranoside (12)

A pale yellow amorphous powder; $[\alpha]_D^{19}$ -30.1º (*c* 0.57, pyridine); ^{1}H-NMR (DMSO-d_6), Table 6 and ^{13}C NMR (DMSO-d_6), Table 8.

Rhamnetin -*O*-*β*-D-glucopyranoside (13)

A pale yellow amorphous powder; $[\alpha]_D^{19}$ -38.5º (*c* 0.47, pyridine); ^{1}H-NMR (DMSO-d_6), Table 6 and ^{13}C NMR (DMSO-d_6), Table 8.

Apigenin (**14**)

A pale yellow crystal; ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

Genkwanin (**15**)

A pale yellow amorphous powder; ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

Genkwanin 5-*O*-*β*-D-glucopyranoside (**16**)

A pale yellow crystal; $[\alpha]_D^{19}$ -58.3º (*c* 0.59, pyridine); mp 192ºC (decomp.); ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

Genkwanin 5-*O*-primeveroside (17)

A pale yellow crystal; $[\alpha]_D^{19}$ -50.7º (*c* 1.12, pyridine); ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

Luteolin (18)

A pale yellow crystal; ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

Luteolin 7-methyl ether-5-*O*-*β*-D-glucopyranoside (19)

A pale yellow amorphous powder; $[a]_D^{19}$ -55.3º (*c* 1.09, pyridine); ^{1}H-NMR (DMSO-d_6) Table 7 and ^{13}C NMR (DMSO-d_6), Table 8.

(-)-Lariciresinol (20)

A pale yellow substance; $[\alpha]_D^{19}$ -11.8º (*c* 0.48, $CHCl_3$); ^{1}H-NMR (CD_3OD) δ 6.88 (1H, s), 6.63-6.73 (4H, m, H-2, 5,5', 6), 6.64 (1H, d, *J* = 8.0 Hz, H-6'), 3.99 (1H, dd, *J* = 7.6, 7.3 Hz, Hb-9'), 3.85, 3.84 (3H each, s, OCH_3), 3.83 (1H, m, Hb-9), 3.73 (1H, dd, *J* = 6.9, 7.3 Hz,

Ha-9'), 3.65 (1H, dd, *J* = 6.5, 10.8 Hz, Ha-9), 2.93(1H, dd, *J* = 4.3, 13.4 Hz, Ha-7'), 3.73 (1H, m, Ha-8'), 2.48 (1H, dd, *J* = 11.7, 12.8 Hz, Ha-7'), 2.39 (1H, 3.73 (1H, d, *J* = 6.9 Hz, Ha-8); ^{13}C-NMR (CD_3OD) δ: 148.6, 148.5 (C-3,3'), 146.6 (C-4), 145.3 (C-4'), 135.3 (C-1), 133.1 (C-1'), 121.9 (C-6'), 119.5 (C-6), 115.9 (C-5'), 115.7 (C-5), 113.1 (C-2'), 110.3 (C-2), 83.7 (C-7), 73.3 (C-9'), 60.3 (C-9), 56.3 (OCH3x2), 53.5 (C-8), 43.4 (C-8'), 33.8 (C-7').

(-)-Dihydrosesamin (21)

A pale yellow substance; $[\alpha]_D^{19}$ -5.5º (*c* 0.71, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 6.60-6.81 (6H, m, H-2, 2', 5,5', 6, 6'), 5.91, 5.91 (2H each, -OCH_2O-), 4.77 (1H, d, *J* = 6.1 Hz, Ha-7), 4.02 (1H, dd, *J* = 6.7, 8.4 Hz, Hb-9'), 3.83 (1H, dd, *J* = 6.7, 10.7 Hz, Ha-9'), 3.69 (2H, m, H-9), 2.84 (1H, dd, *J* = 5.1, 13.8 Hz, Ha-7'), 2.66 (1H, m, H-8'), 2.50 (1H, dd, *J* = 10.7, 13.8 Hz, Hb-7'), 2.32 (1H, m, H-8); ^{13}C-NMR ($CDCl_3$) δ: 147.8 (C-4), 147.7 (C-3'), 146.8 (C-3), 145.9 (C-4'), 137.0 (C-1), 134.2 (C-1'), 121.4 (C-6'), 119.0 (C-6), 108.9 (C-5'), 108.3 (C-5), 108.0 (C-2'), 106.3 (C-2), 100.9, 100.8 (C- OCH_2O-), 82.8 (C-7), 72.8 (C-9'), 60.7 (C-9), 52.6 (C-8), 42.3 (C-8'), 33.2 (C-7').

(-)-Pinoresinol (22)

A pale yellow substance; $[\alpha]_D^{19}$ -51.8º (*c* 0.79, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 6.89 (2H, d, *J* = 1.8 Hz, H-2,2'), 6.87 (2H, d, *J* = 8.2 Hz, H-5,5'), 6.81 (2H, dd, *J* = 1.8, 8.2 Hz, H-6, 6'), 4.73 (2H, d, *J* = 4.3 Hz, H-7,7'), 4.22 (2H, dd, *J* = 7.0, 9.6 Hz, H-9,9'), 3.874 (2H,dd, *J* = 3.9, 8.7 Hz, Ha-9,9'), 3.871 (6H, s, OCH_3), 3.08 (2H, m, H-8,8'); ^{13}C-NMR ($CDCl_3$) δ: 146.7 (C-3,3'), 145.2 (C-4,4'), 132.8 (C-1,1'), 118.9 (C-6,6'), 114.3 (C-5,5'), 108.7 (C-2,2'), 85.8 (C-7,7'), 71.6 (C-9,9'), 55.9 (OCH_3), 54.1 (C-8,8').

Dehydrodiconiferyl alcohol (23)

A pale yellow substance; $[\alpha]_D^{19}$ —0.0º (*c* 0.79, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 6.95 (1H, s, H-4 or H-6), 6.94 (1H, d, *J*=1.8 Hz, H-2'), 6.91 (1H, s, H-4 or H-6), 6.85 (1H, dd, *J*= 1.8, 8.5 Hz, H-6'), 6.79 (1H, d, *J*= 8.5 Hz, H-5'), 6.54 (1H, d, *J*= 15.8 Hz, H-10), 6.22 (1H, dt, *J*= 15.8,6.1 Hz, H-11), 5.55 (1H, d, *J*= 6.4 Hz, H-2), 4.20 (2H, d, *J*= 6.1Hz, H-12), 3.90, 3.84 (3H each, OCH_3), 3.81 (2H, m, H-13), 3.55 (1H, m, H-3). ^{13}C-NMR ($CDCl_3$) δ: 148.7 (C-3' or C-9), 148.5 (C-3' or C-9), 146.9 (C-4'), 144.9 (C-8), 133.9 (C-1'), 131.9 (C-6), 131.6 (C-10), 129.7 (C-4), 127.2 (C-11), 119.5 (C-6'), 116.1 (C-5), 115.7 (C-5'), 111.4 (C-7), 110.2 (C-2'), 89.0 (C-2), 64.5 (C-13), 63.5 (C-12), 56.5 (OCH_3), 56.3 (C-8'), 54.6 (C-.3).

Coniferyl aldehyde (24)

A pale brown substance; ^{1}H-NMR ($CDCl_3$) δ 9.65 (1H, d, J = 7.9 Hz, H-9), 7.40 (1H, d, J = 15.6 Hz, H-7), 7.13 (1H, dd, J = 1.8, 8.1 Hz, H-6), 7.07 (1H, d, J = 1.8 Hz, H-2), 6.96 (1H, d, J = 8.1 Hz, H-5), 6.60 (1H,dd, J = 7.9, 15.5 Hz, H-8), 3.95 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$) δ: 193.6 (C-9), 153.0 (C-7), 148.9 (C-3), 146.9 (C-4), 128.3 (C-1), 126.5 (C-8), 124.0 (C-6), 114.9 (C-5), 109.5 (C-2), 56.0 (OCH_3).

Sinapyl aldehyde (25)

A pale brown substance; ^{1}H-NMR ($CDCl_3$) δ 9.66 (1H, d, J = 7.6 Hz, H-9), 7.38 (1H, d, J = 15.9 Hz, H-7), 6.81 (2H, s, H-2,6), 6.61 (1H, dd, J = 7.6, 15.9 Hz, H-8), 3.94 (6H, s, OCH_3); ^{13}C-NMR ($CDCl_3$) δ: 193.3 (C-9), 153.0 (C-7), 147.8 (C-3,5), 138.1 (C-4), 126.8 (C-8), 125.6 (C-1), 105.6 (C-2,6), 56.4 (OCH_3).

Rutarensin (26)

A white amorphous powder; $[\alpha]_D^{29}$ -116.8º (c 0.50, MeOH:H_20=1:1); ^{1}H-NMR (DMSO-d_6) δ 8.04 (1H, d, J = 9.5 Hz, H-4'), 7.86 (1H, s, H-4), 7.72 (1H, d, J = 8.4 Hz, H-5'), 7.27 (1H, s, H-5), 7.23 (1H, s, H-8), 7.22 (1H, s, H-8'), 7.14 (1H, d, J = 8.4 Hz, H-6'), 6.38 (1H, d, J = 9.5 Hz, H-3'), 5.15 (1H, d, J = 7.3 Hz, H-1''), 4.26 (1H, brd, J = 10.6 Hz, H-6a''), 4.05 (1H, dd, J = 12.1, 6.2 Hz, H-6b''), 3.80 (3H, s, OCH_3), 3.71-3.74 (1H, m), 2.49 (1H, d. J = 13.9 Hz, H-4a'''), 2.42 (1H, d. J = 3.9 Hz, H-4b'''), 2.32 (1H, d. J = 15.0 Hz, H-2a'''), 2.15 (1H, d. J = 15.0 Hz, H-2b'''), 1.12 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6) δ 173.4 (5'''), 170.4 (C-1'''), 159.9 (C-2'), 159.3 (C-2), 156.7 (C-7'), 154.9 (C-9'), 148.6 (C-9), 146.6 (C-6), 146.2 (C-7), 143.9 (C-4'), 137.0 (C-3), 129.8 (C-4), 129.7 (C-5'), 114.5 (C-10), 113.9 (C-6'), 113.5 (C-3'), 112.2 (C-10'), 109.5 (C-5), 104.3 (C-8'), 102.9 (C-8), 99.3 (C-1''), 76.4 (C-3''), 73.7 (C-5''), 72.9 (C-2''), 69.6 (C-4''), 68.8 (C-3'''), 62.9 (C-6''), 46.1 (C-4'''), 45.9 (C-2'''), 27.7 (CH_3).

Syringaldehyde (27)

A pale brown substance; ^{1}H-NMR ($CDCl_3$) δ 9.82 (1H, s , H–7), 7.15 (2H, s, H-2,6), 3.97 (6H, s, OCH_3). ^{13}C-NMR ($CDCl_3$) δ 190.7(C-7), 147.4 (C-3,5), 140.8 (C-4), 128.4 (C-1), 106.7 (C-2,6), 56.5 (OCH_3).

***p*-Coumaric acid methyl ester (28)**

A white amorphous powder; ^{1}H-NMR ($CDCl_3$) δ 7.61 (1H, d, J = 15.9 Hz, H-7), 7.42 (2H, d, J = 8.5 Hz, H-2.6), 6.82 (2H, d, J = 8.5 Hz, H-3,5), 6.28 (1H, d, d, J = 15.9 Hz, H-8), 3.77

(3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$) δ 169.5 (C-9), 160.7 (C-4), 146.3 (C-7), 130.8 (C-2, 6), 126.8 (C-1), 116.6 (C-3,5), 114.6 (C-8).

_p_-Hydroxy benzaldehyde (29)

A white amorphous powder; ^{1}H-NMR (DMSO-d_6) δ 6.90 (2H, d, J=8.5 Hz, C_3 and C_5-H), 7.74 (2H, d, J=8.5 Hz, C_2 and C_6-H), 9.76 (1H, s, C_7-H); ^{13}C-NMR (DMSO-d_6): δ 190.1 (C-7), 163.3 (C-1), 132.0 (C-3,5), 128.4 (C-4), 115.8 (C-2,6).

_p_-Hydroxy-acetophenone (30)

A white amorphous powder; ^{1}H-NMR (DMSO-d_6) δ 2.47 (3H, s, C_8-H), 6.84 (2H, d, J=8.5 Hz, $C_{2'}$ and $C_{6'}$-H), 7.82 (2H, d, J=8.9 Hz, $C_{3'}$ and $C_{5'}$-H), 10.38 (1H, s, C_4-OH); ^{13}C-NMR (DMSO-d_6) δ 196.0 (C-7), 161.9 (C-1), 130.6 (C-3,5), 128.5 (C-4), 115.0 (C-2,6), 26.0 (C-8).

Umbelliferone (31)

A white amorphous powder; ^{1}H-NMR (DMSO-d_6) δ 6.14 (1H, d, J=9.5 Hz, C_4-H), 6.72 (1H, d, J=2.2 Hz, C_8-H), 6.79 (1H, dd, J=2.4, 8.6 Hz, C_6-H), 7.48 (1H, d, J=8.2 Hz, C_5-H), 7.90 (1H, d, J=9.5 Hz, C_3-H); ^{13}C-NMR (DMSO-d_6): ^{13}C-NMR (DMSO-d_6): 161.4 (C-2), 160.5 (C-7), 155.5 (C-9), 144.5 (C-4), 129.7 (C-5), 111.4 (C-6), 111.3 (C-3, 10), 102.3 (C-8).

Diplomorphanone A (32)

Pale yellowish oil; $[\alpha]_D^{20}$ -31.4 (c 0.74, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$), Table 5; HR-FABMS m/z 293.1151 $[M+Na]^+$, calcd. for $C_{17}H_{18}O_3Na$, 293.1154.

14”-_O_-Methyldihydrodaphnodorin B (33)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ + 25.5º (c 0.84, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10; HRFABMS m/z 557.1475 $[M-H]^-$ (calcd. for $C_{31}H_{25}O_{10}$, 557.1448); CD (MeOH, c 0.14): $\triangle\varepsilon$ (nm) –0.26 (260), +3.07 (280), -6.02 (309).

Dihydrodaphnodorin B (34)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +10.8 º (c 0.50, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10; CD (MeOH, c 0.14): $\triangle\varepsilon$ (nm) –0.34 (261), +3.50 (282), -6.10 (309).

14”-*O*-Methyldaphnodorin J (35)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +37.7 º (*c* 0.71, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10; HRFABMS *m/z* 541.1524 $[M-H]^-$ (calcd. for $C_{31}H_{25}O_9$, 541.1499); CD (MeOH, *c* 0.10): $\triangle\varepsilon$ (nm) –0.03 (264), +1.28 (282), -4.09 (309).

Daphnodorin J (36)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +37.3 º (*c* 0.74, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10; CD (MeOH, *c* 0.10): $\triangle\varepsilon$ (nm) –0.44 (264), +2.53 (281), -6.90 (308).

3”-epi-Dihydrodaphnodorin B (37)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ -6.0 º (*c* 0.92, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10.

Daphnodorin B (38)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +68.7º (*c* 0.58, MeOH); ^{1}H-NMR (CD_3OD), Table 9 and ^{13}C-NMR (CD_3OD), Table 10.

Sikokianin B (39)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +195.9º (*c* 1.19, MeOH); ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 11.

Neochamaejasmin B (40)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +213.3º (*c* 0.89, MeOH); ^{1}H-NMR (CD_3OD) and ^{13}C NMR (CD_3OD), Table 12.

Syringin (41)

A white amorphous powder; $[\alpha]_D^{29}$ -24.2º (*c* 0.93, MeOH); ^{1}H-NMR (CD_3OD) δ 6.80 (2H, s, H-2,6), 6.57 (1H, d, *J* = 15.8 Hz, H-7), 6.36 (1H, dt, *J* = 15.8, 5.7 Hz, H-8), 4.92 (1H, d, *J* = 7.3 Hz, glc-1), 4.27 (2H, d, *J* = 5.7 Hz, H-9), 3.88 (6H, s, OCH_3x2); ^{13}C-NMR (CD_3OD), Table 12.

Syringinoside (42)

A white amorphous powder; $[\alpha]_D^{29}$ -41.3º (*c* 0.89, MeOH: H_2O=1:1); ^{1}H-NMR

(CD_3OD+D_2O) δ 6.81 (2H, s, H-2,6), 6.57 (1H, d, *J* = 15.8 Hz, H-7), 6.37 (1H, dt, *J* = 15.8, 5.7 Hz, H-8), 4.99 (1H, d, *J* = 7.3 Hz, glc-1), 4.33 (1H, d, *J* = 7.3 Hz, glc-1), 4.27 (2H, d, *J* = 5.7 Hz, H-9), 3.88 (6H, s, OCH_3x2); ^{13}C-NMR (CD_3OD+D_2O), Table 12.

(-)-Syringaresinol (43)

A white amorphous powder; $[\alpha]_D^{21}$ -47.2º (*c* 0.44, $CHCl_3$); ^{1}H-NMR ($CDCl_3+CD_3OD$) δ 6.66 (4H, s, H-2,6, H-2',6'), , 4.72 (2H, d, *J* = 3.7 Hz, H-7'), 4.30 (2H, m, H-9β,9β'), 3.92 (2H, m, H-9α,9α'), 3.889 (3H, s, OCH_3), 3.14 (2H, m, H-8,8'); ^{13}C-NMR ($CDCl_3+CD_3OD$), Table 12.

(-)-Syringaresinol 4-*O*-*β*-D-glucopyranoside (44)

A white amorphous powder; $[\alpha]_D^{21}$ -25.8º (*c* 0.33, $CHCl_3$); ^{1}H-NMR ($CDCl_3+CD_3OD$) δ 6.66 (2H, s, H-2,6), 6.61 (2H, s, H-2',6'), 4.76 (1H, d, *J* = 3.6 Hz, H-7), 4.72 (1H, d, *J* = 3.7 Hz, H-7'), 4.72 (1H, d, *J* = 7.6 Hz, glc-1), 4.30 (2H, m, H-9β,9β'), 3.92 (2H, m, H-9α,9α'), 3.889, 3.881 (3H each, s, OCH_3), 3.14 (2H, m, H-8,8'); ^{13}C-NMR ($CDCl_3+CD_3OD$), Table 12.

(+)-Nortrachelogenin (45)

A white amorphous powder; $[\alpha]_D^{21}$ +3.5 º (*c* 0.24, MeOH); ^{1}H-NMR ($CDCl_3+CD_3OD$), 6.57-6.86 (6H, m, H-2,2',5,5',6,6'), 4.08 (2H, dd, *J* = 7.3, 8.8 Hz, H9b), 3.86, 3.85 (3H each, s, OCH_3), 3.68 (1H, m, Hb-9a), 3.07 (2H, m H-7'), 2.79-2.64 (3H, m, H-7, H-8); ^{13}C-NMR ($CDCl_3+CD_3OD$), Table 12.

Daphnoretin (46)

A white amorphous powder; ^{1}H-NMR (DMSO-d_6) δ 8.03 (1H, d, *J* = 9.4 Hz, H-4'), 7.86 (1H, s, H-4), 7.71 (1H, d, *J* = 8.8 Hz, H-5'), 7.21 (1H, s, H-5), 7.17 (1H, d, *J* = 2.4 Hz, H-8'), 7.11 (1H, d, J = 8.8 Hz, H-6'), 6.87 (1H,s, H-8), 6.37 (1H, d, *J* = 9.4 Hz, H-3'), 3.82 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6) δ 59.9 (C-2'), 159.6 (C-2), 156.9 (C-7'), 154.9 (C-9'), 150.3 (C-7), 147.4 (C-9), 145.6 (C-6), 144.0 (C-4'), 135.6 (C-3), 130.8 (C-4), 129.8 (C-5'), 114.3 (C-10'), 113.8 (C-3'), 113.4 (C-6'), 110.1 (C-10), 109.3 (C-5), 103.9 (C-8'), 102.7 (C-8), 56.0 (OCH_3).

Phorbol-13-acetate (47)

A white amorphous powder; $[\alpha]_D^{21}$ +55.9 º (*c* 0.91, MeOH); ^{1}H-NMR ($CDCl_3+CD_3OD$) δ 7.57 (1H, s, H-1), 5.60 (1H, d, *J* = 4.2 Hz, H-7), 3.96 (3H, m, H-12, H-20), 3.24 (1H, m,

H-8), 3.12 (1H, m, H-10), 2.53 (1H, d, *J* = 18.1 Hz, H-5b), 2.43 (1H, d, *J* = 18.1 Hz, H-5a), 2.12 (3H, s, H-22), 2.20 (1H, m, H-11), 1.76 (3H, s, H-19), 1.23 (3H, s, H-17), 1.22 (3H, s, H-16), 1.07 (3H, d, *J* = 6.7 Hz, H-18), 1.03 (3H, d, *J* = 5.7 Hz, H-14); ^{13}C-NMR (DMSO-d_6) δ 210.0 (C-3), 175.0 (C-21), 160.7 (C-1), 141.4(C-2), 133.6 (C-6), 132.1(C-7), 78.8 (C-4), 77.0 (C-12, 68.6 (C-13), 67.5 (C-20), 56.9 (C-10), 45.1 (C-11), 39.1 (C-8), 37.9 (C-5), 35.8 (C-14), 26.5 (C-15), 23.8 (C-16), 21.0 (C-22), 16.9 (C-18), 15.1 (C-17), 10.1(C-19).

Methyl paraben (48)

A pale white amorphous powder; ^{1}H-NMR ($CDCl_3$), δ: 7.95 (2H, d, *J* = 8.8 Hz, H-2,6), 6.85 (2H, d, *J* = 8.8 Hz, H-3,5), 3.88 (3H, s, OCH_3).

Pilloin 5-*O*-β-D-glucopyranoside (49)

A white amorphous powder; $[a]_D^{21}$ - 44.8º (*c* 0.66, pyridine); ^{1}H-NMR (DMSO-*d*) and ^{13}C-NMR (DMSO-d_6), Table 13; HR-FAB-MS *m*/*z*: 477.1401 $[M+H]^+$ (calcd. for $C_{23}H_{25}O_{11}$, 477.1397).

Acid hydrolysis of pilloin 5-*O*-β-D-glucopyranoside (49)

A solution of compound **49** (10.8 mg) in 2M HCl (4 mL) in a sealed tube was heated at 70º C for 3 hr. The aglycone (**50**, 6.6 mg) was extracted with EtOAc and confirmed by ^{1}H-NMR data and co-TLC with authentic sample. D-Glucose (3.7 mg) was obtained from aq. layer and confirmed by optical rotation, $[\alpha]_D^{23}$ +58º (*c* 0.37, H_2O) and co-TLC with authentic sample.

Pilloin (50)

A pale yellow amorphous powder; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 13.

Luteolin 7-methyl ether (51)

A pale yellow amorphous powder; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), Table 13.

Quercetin 3-*O*-α-L-rhamnoside (52)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ – 105.3º (*c* 0.82, pyridine); ^{1}H-NMR (CD_3OD), Table 7 and ^{13}C NMR (CD_3OD), Table 14.

Hypolaetin 8-*O*-*β*-D-glucuronopyranoside (53)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ -25.6º (*c* 0.46, pyridine); ^{1}H-NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), Table 14.

(-)-Pinoresinol 4-*O*-*β*-D-glucopyranoside (54)

A white amorphous powder; $[\alpha]_D^{21}$ - 80.6º (*c* 0.62, MeOH); ^{1}H-NMR (CD_3OD) δ 7.15 (1H, d, *J* = 8.5 Hz, H-5), 7.00 (1H, s, H-2), 6.92 (1H, s, H-2'), 6.88 (1H, dd, *J* = 1.4, 8.5 Hz, H-6'), 6.78 (1H, dd, *J* = 1.5, 8.5 Hz, H-6), 6.77 (1H, d, *J* = 8.5 Hz, H-5'), 4.88 (1H, d, *J* = 7.3 Hz, glc-1), 4.73 (1H, d, *J* = 3.6 Hz, H-7), 4.68 (1H, d, *J* = 3.9 Hz, H-7'), 4.21 (2H, m, H-9β,9β'), 3.84, 3.83 (3H each, s, OCH_3), 3.82 (2H, m, H-9α,9α'), 3.09 (2H, m, H-8,8'); ^{13}C-NMR (CD_3OD), Table 15.

(-)-Pinoresinol 4, 4'-di-*O*-*β*-D-glucopyranoside (55)

A white amorphous powder; $[\alpha]_D^{21}$ -70.9º (*c* 0.38, pyridine); ^{1}H-NMR (DMSO-d_6)) δ 7.04 (2H, d, *J* = 8.2 Hz, H-5,5'), 6.95 (2H, s, H-2,2'), 6.85 (2H, d, *J* = 8.2 Hz, H-6, 6'), 5.07 (2H, d, *J* = 4.3 Hz, H-7,7'), 4.88 (2H, d, *J* = 7.3 Hz, glc-1), 4.14 (2H, dd, *J* = 6.7,8.5 Hz, H-9,9'), 3.76 (6H, s, OCH_3), 3.14 (2H, m, H-8,8'); ^{13}C-NMR (DMSO-d_6), Table 15.

Coniferin (56)

A white amorphous powder; $[a]_D^{21}$ -25.2º (*c* 0.66, MeOH:H_2O=1;1); ^{1}H-NMR (CD_3OD+D_2O) δ 7.12 (1H, d, *J* = 8.2 Hz, H-5), 7.09 (1H, d, *J* = 1.8 Hz, H-2), 6.99 (1H, dd, *J* = 1.8, 8.2 Hz, H-6), 6.56 (1H, d, *J* = 15.8 Hz, H-7), 6.28 (1H, dt, *J* = 15.8, 5.7 Hz, H-8), 4.95 (1H, d, *J* = 7.6 Hz, glc-1), 4.23 (2H, d, *J* = 5.7 Hz, H-9), 3.88 (3H, s, $OCH_{3)}$; ^{13}C-NMR (CD_3OD+D_2O), Table 15.

Dehydrodiconiferyl alcohol 9'-O-*β*–D-glucopyranoside (57)

A white amorphous powder; $[a]_D^{21}$ - 45.08º (*c* 0.35, MeOH:H_2O=1:1); ^{1}H-NMR (CD_3OD) δ: 7.12 (1H, s, H-6'), 7.10 (1H, s, H-6'), 7.01 (1H, s, H-2), 6.92 (1H, d, *J* = 8.5 Hz, H-5), 6.90 (1H, dd, *J* = 2.1, 8.5 Hz, H-6), 6.54 (1H, d, *J* = 15.8 Hz, H-7'), 6.15 (1H, dt, *J* =, 15.8, 5.7 Hz, H-8'), 5.56 (1H, d, *J* = 5.7 Hz, H-7), 4.88 (1H, d, *J* = 7.0 Hz, glc-1), 4.03 (2H, d, *J* = 5.7 Hz, H-9'), 3.85, 3.82 (3H each, s, OCH_3); ^{13}C-NMR (CD_3OD), Table 15.

Chlorogenic acid (58)

A white amorphous powder; $[a]_D^{21}$ - 21.0º (*c* 0.46, H_2O); ^{1}H-NMR (CD_3OD+D_2O) δ 7.57 (1H, d, *J* = 15.8 Hz, H-7'), 7.09 (1H, s, H-2'), 6.98 (1H, d, *J* = 7.9 Hz, H-6'), 6.85 (1H, d, *J* = 7.9 Hz, H-5'), 6.32 (1H, d, *J* = 15.8 Hz, H-8'), 5.36 (1H, m, H-5), 4.30 (1H, brs, H-3), 3.85 (1H, brd, *J* = 9.7 Hz, H-4), 2.1-1.8 (4H, m, H-2,6); ^{13}C-NMR (CD_3OD+D_2O) δ 180.7 (C-7), 169.6 (C-9'), 148.5 (C-4'), 147.1 (C-7'), 145.7 (C-3'), 127.7 (C-1'), 123.3 (C-6'), 116.8 (C-5'), 115.6 (C-8'), 115.2 (C-2'), 78.9 (C-1), 73.9 (C-4), 72.3 (C-3), 71.9 (C-5), 39.6 (C-6), 37.9 (C-2).

Maltol 3-*O*-β-D-glucopyranoside (59)

A white amorphous powder; ^{1}H-NMR (CD_3OD+D_2O) δ 8.05 (1H, d, *J* = 5.4 Hz, H-6), 6.52 (1H, d, *J* = 5.4 Hz, H-5), 4.84 (1H, d, *J* = 7.3 Hz, glc-1), 2.48 (3H, s, H-7); ^{13}C-NMR (CD_3OD+D_2O) δ: 177.3 (C-4), 165.0 (C-2), 157.3 (C-6), 143.2 (C-3), 117.1 (C-5), 104.8 (glc-1), 78.1 (glc-3), 77.5 (glc-5), 75.1 (glc-2), 70.8 (glc-4), 62.2 (glc-6), 15.9 (C-7).

Apiosylskimmin (60)

A white amorphous powder; $[\alpha]_D^{21}$ - 59.8º (*c* 1.00, pyridine); ^{1}H-NMR (CD_3OD+D_2O) δ 7.95 (1H, d, *J* = 9.4 Hz, H-4), 7.61 (1H, d, *J* = 9.1 Hz, H-5), 7.11 (1H, d, *J* = 9.1 Hz, H-6), 7.10 (1H, *d*, *J* = 2.4 Hz, H-8), 6.34 (1H, d, *J* = 9.4 Hz, H-3), 5.05 (1H, d, *J* = 7.6 Hz, glc-1), 5.02 (1H, d, *J* = 3.0 Hz, api-1). ^{13}C-NMR (CD_3OD+D_2O) δ: 164.0 (C-2), 161.7 (C-7), 156.3 (C-9), 146.1 (C-4), 130.5 (C-5), 115.5 (C-3 or C-6), 115.3 (C-10), 114.5 (C-3 or C-6), 110.7 (api-1), 104.9 9C-8), 101.6 (glc-1), 80.3 (api-3), 78.0 (glc-5), 77.4 (api-2), 76.8 (glc-3), 74.8 (api-4), 74.4 (glc-2), 71.2 (glc-4), 68.8 (glc-6), 65.2 (api-5).

Stelleranol (61)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ -90.5 º (*c* 0.28, MeOH); ^{1}H-NMR (CD_3OD), δ 7.22 (2H, d, *J* = 8.5 Hz, H-2''', H-6'''), 6.83 (2H, d, *J* = 8.5 Hz, H-3''', H-5'''), 6.71 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.62 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.16 (1H, d, *J* = 2.0 Hz, H-8''), 6.14 (1H, d, *J* = 2.0 Hz, H-6''), 6.09 (1H, s, H-2''), 5.68 (1H, s, H-6), 4.96 (1H, s, H-2), 4.18 (1H, brs, H-3), 2.65 (1H, d, *J* = 17.5 Hz, H-4), 2.49 (1H, dd, *J* = 3.8, 17.3 Hz, H-4). ^{13}C-NMR (CD_3OD) δ 193.1 (C-4''), 188.7 (C-5), 170.4 (C-7), 169.6 (C-7''), 166.1 (C-9''), 163.1 (C-5''), 160.3 (C-9), 159.9 (C-4'''), 158.6 (C-4'), 131.7 (C-2''', 6'''), 130.4 (C-1'), 129.2 (C-2', 6'), 124.7 (C-1'''), 116.7 (C-3''', 5'''), 116.5 (C-3', 5'), 111.1 (C-10) ,102.9 (C-6), 102.0 (C-10''), 99.0 (C-6''), 98.4 (C-8''), 92.2 (C-2''), 87.5 (C-8), 82.3 (C-2), 82.1 (C-3''), 66.5 (C-3), 28.6 (C-4).

Neochamaejasmin A (62)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +37.7º (*c* 0.89, MeOH); ^{1}H-NMR (CD_3OD and ^{13}C-NMR (CD_3OD), Table 18.

Diplomorphanone B (63)

Pale yellowish oil; $[\alpha]_D^{20}$ +30.1 (*c* 0.41, $CHCl_3$), HR-FABMS m/z 255.1378 $[M+H]^+$, calcd. for $C_{17}H_{19}O_2$, 255.1385; ^{1}H-NMR ($CDCl_3$) and Table 9 and ^{13}C-NMR ($CDCl_3$), Table 16.

1,5-Diaryl-pentan-1-one (64)

Pale yellowish oil; ^{1}H-NMR ($CDCl_3$) and Table 9 and ^{13}C-NMR ($CDCl_3$), Table 17.

1,5-Diphenyl- 3-hydroxy-pentan-1-one (65)

Pale yellowish oil; $[\alpha]_D^{20}$ +36.5 (*c* 1.51, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) and Table 9 and ^{13}C-NMR ($CDCl_3$), Table 16.

1,5-Diphenyl-3-methoxy-pentan-1-one (66)

Pale yellowish oil; $[\alpha]_D^{20}$ 0.0 (*c* 0.86, $CHCl_3$), ^{1}H-NMR ($CDCl_3$) and Table 9 and ^{13}C-NMR ($CDCl_3$), Table 17.

1,5-Diphenyl-2-penten-1-one (67)

Pale yellowish oil; ^{1}H-NMR ($CDCl_3$) and Table 9 and ^{13}C-NMR ($CDCl_3$), Table 17.

(+)-Afzelechin (68)

A white amorphous powder, $[\alpha]_D^{27} = +9.54^\circ$ (*c* 1.00, MeOH); ^{1}H-NMR (DMSO-d_6) δ 2.31 (1H, dd, *J*=8.3, 16.1 Hz, C_{4ax}-H), 2.71 (1H, dd, *J*=5.4, 16.1 Hz, C_{4eq}-H), 3.87 (1H, m, C_3-H), 4.53 (1H, d, *J*=7.9 Hz, C_2-H), 5.70 (1H, d, *J*=2.1 Hz, C_6-H), 5.90 (1H, d, *J*=2.4 Hz, C_8-H), 6.74 (2H, d, *J*=8.5 Hz, $C_{5'}$ and $C_{3'}$-H), 7.15 (2H, d, *J*=8.5 Hz, $C_{2'}$ and $C_{6'}$-H); ^{13}C-NMR (DMSO-d_6) δ 157.4 (C-4'), 155.9 (C-9), 156.9 (C-7), 156.6 (C-5), 130.4 (C-1'), 129.0 (C-2', 6'), 115.3 (C-3', 5'), 99.6 (C-10), 94.4 (C-6), 95.7 (C-8), 81.5 (C-2), 66.8 (C-3), 28.7 (C-4).

Sinapyl alcohol (69)

A white amorphous powder; ^{1}H-NMR (DMSO-d_6) δ 6.68 (2H, s, C_2 and C_6-H), 6.41 (1H, d, *J*=15.9 Hz, C_7-H), 6.22 (1H, dt, *J*=5.4, 15.9 Hz, C_8-H), 4.77 (1H, t, *J*=5.5 Hz, C_9-OH), 4.08

(2H, t, *J*=4.5 Hz, C_9-H), 3.76 (6H, s, OCH_3), ^{13}C-NMR (DMSO-d_6) 148.5 (C-2, C-5), 135.7 (C-4), 129.7 (C-7), 128.4 (C-8), 127.9 (C-1), 104.3 (C-3, C-5), 62.1 (C-9), 56.4 (OCH_3).

Sikokianin A (70)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +63.4 (*c* 0.22, MeOH); ^{1}H-NMR (CD_3ODand ^{13}C-NMR (CD_3OD), Table 18.

Chamaejasmenin B (71)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ +98.7º (*c* 0.46, MeOH); ^{1}H-NMR (DMSO-d_6), Table 7 and ^{13}C NMR (DMSO-d_6), Table 9. ^{1}H-NMR (CD_3OD and ^{13}C-NMR (CD_3OD), Table 18.

Apigenin 4,7'-dimethylether 5-*O*-primeversoide (72)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ -37.4º (*c* 0.68, pyridine); ^{1}H-NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), Table 19.

β-Sitosterol (73)

A white amorphous powder; ; $[\alpha]_D^{21}$ -26.3º (*c* 0.69, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.34 (1H, d, *J*=5.5, H-6), 3.53 (1H, m, H-3), 1.00 (3H, s, H-29), 0.91 (3H, d, *J*=6.4 Hz, H-19), 0.85 (3H, d, *J*=7.3 Hz, H-24), 0.83 (3H, d, *J*=6.6 Hz, H-26), 0.81 (3H, d, *J*=6.4 Hz, H-27), 0.68 (3H, s, H-28); ^{13}C-NMR ($CDCl_3$) δ 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.7 (C-17), 50.2 (C-9), 45.8 (C-22), 42.3 (C-4,13), 39.8 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-18), 33.9 (C-20), 31.9 (C-7,8), 31.7 (C-2), 29.2 (C-24), 28.2 (C-16), 26.2 (C-15), 24.3 (C-21), 23.1 (C-23), 21.1 (C-11), 19.8 (C-26), 19.4 (C-27), 19.1 (C-19), 18.7 (C-28), 11.9 (C-24), 11.8 (C-29).

(-)-*Erythro*-1,5-diphenylpentane-1,3-diol (74)

Pale yellowish oil; $[\alpha]_D^{19}$ -22.2º (*c* 0.47, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.09-7.36 (10H, complex, H-2-6, H-2'-6'), 4.60 (1H, d, *J*=4.6 Hz, H-1), 3.78 (1H, m, H-3), 2.57 (1H, m, H-5), 1.80, 1.61, 1.42, 1.30 (1H each, m, H-2, H-4); ^{13}C NMR ($CDCl_3$) δ 142.2, 140.3 (C-1,C-1'), 128.3, 128.2, 127.8, 126.7, 126.0, 125.6 (C-2-6,C-2'-6'), 77.0 (C-1), 74.9 (C-3), 35.7 (C-5), 31.1, 27.6 (C-2, C-4).

Kaempferol 3-O-α-L-rhamnopyranoside (75)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ - 120.2º (*c* 0.73, pyridine); ^{1}H-NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), Table 19.

Tiliroside (76)

A pale yellow amorphous powder; $[\alpha]_D^{21}$ - 74.2º (*c* 0.70, pyridine); ^{1}H-NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6), Table 19.

Measurement of DPPH free radical scavenging activity

DPPH free radical scavenging activity was measured according to method of Suda *et al* (2006)[139] with slight modification. Briefly, 40 µl of MES buffer (pH 6.0), 80 µl of sample solution at different concentration (in DMSO:Ethanol=1:1) and 40 µl of DPPH solution (800 mM in EtOH) were mixed in a 96-well plate and kept in dark at room temp. for 20 minutes. Then the absorbance was measured at 510 nm. Using the standard calibration curve of Trolox at concentrations 40, 60, 80 and 120 µM, the free radical scavenging activity of each compound was expressed as mmol of Trolox equivalent per mol of compound (mmol TE/mol).

Measurement of tyrosinase inhibitory activity

Mushroom tyrosinase inhibitory activity was measured according to method of Jiang *et al* (2012)[142] with slight modification using 96 well microplate reader. Briefly, 70 µL of phosphate buffer (pH 6.8, 0.067 M), 30 µL of mushroom tyrosinase (71.5 Units/mL) and 20 µl of sample solution (1 mg/mL in DMSO) were mixed in each well and preincubated for 5 minutes at 25°C in incubator. Then, 30 µL of L-DOPA (10 mM) was added to the reaction mixture and absorbance was measure at 475 nm (A1). The reaction mixture was then incubated for 2 minutes at room temperature and absorbance was measured again at 475 nm (A2). The difference between these two absorbance (A2—A1) was represented as $\Delta A_{sample.}$. Blank absorbance (ΔA_{blank}) and control absorbances ($\Delta A_{control}$) were recorded in the same way without L-DOPA and sample solution, respectively. Then the percentage tyrosinase inbibition was calculated by the formula as below.

$$\text{Tyrosinase inhibition } (\%) = [1- (\Delta A_{sample} - \Delta A_{blank})/\Delta A_{control}] \times 100$$

Each sample was analyzed as triplicate.

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