Isoflavanones from the Heartwood of *Swartzia polyphylla* and Their Antibacterial Activity against Cariogenic Bacteria

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The methanolic extract of *Swartzia polyphylla* Dc. heartwood had antibacterial activity against cariogenic bacteria, the mutans Streptococci. The chromatographic purification of the extract afforded seven flavonoids. Among them, three known isoflavanones, dihydrobiochanin A, ferreirin and darbergioidin, and one new isoflavanone, 5,2′,4′-trihydroxy-7-methoxyisoflavanone (dihydrocajanin) had potent antibacterial activity against cariogenic bacteria. This effect was not detected on isoflavone derivatives. A comparative antibacterial study of various flavonoids was further performed, and their structure–activity relationship was discussed.

Keywords *Swartzia polyphylla*: Leguminosae: isoflavanone; antibacterial activity; cariogenic bacteria; 5,2′,4-trihydroxy-7-methoxyisoflavanone

Introduction

Dental caries is oral infectious disease. Current evidence supports the concept that gram-positive bacteria involving mutans Streptococci play a key role in the pathogenesis of dental caries in humans.[1] These bacteria synthesize extracellular adhesive, insoluble glucan from sucrose and adhere to tooth surfaces, forming dental plaque which obstructs the diffusion of organic acids produced by oral microorganisms. The decline of pH value on the tooth surface leads to decay of enamel lesions. To prevent dental caries, it is important to control the growth of these plaque forming bacteria in the oral cavity by using an antibacterial substance.[2]

Swartzia polyphylla Dc. is a tree which grows in South America. In the present study, we observed that the methanolic extract prepared from the heartwood of *S. polyphylla* exhibited potent antibacterial activity against cariogenic bacteria. *Swartzia* is tropical genus of the Leguminosae. Recently, a chemical and biological investigation carried out on *Swartzia* sp. (*S. madagascariensis*, *S. laevicarpa*, *S. simplex*, *S. leiocalycina*) reported the antifungal activity of various pterocarpanoids[3] and the molluscicidal effect of trieterpenoid saponins.[4] However, the constituents of *S. polyphylla* have not been reported. The present paper is concerned with antibacterial isoflavanones against cariogenic bacteria isolated from *S. polyphylla* and their structure–activity relationships.

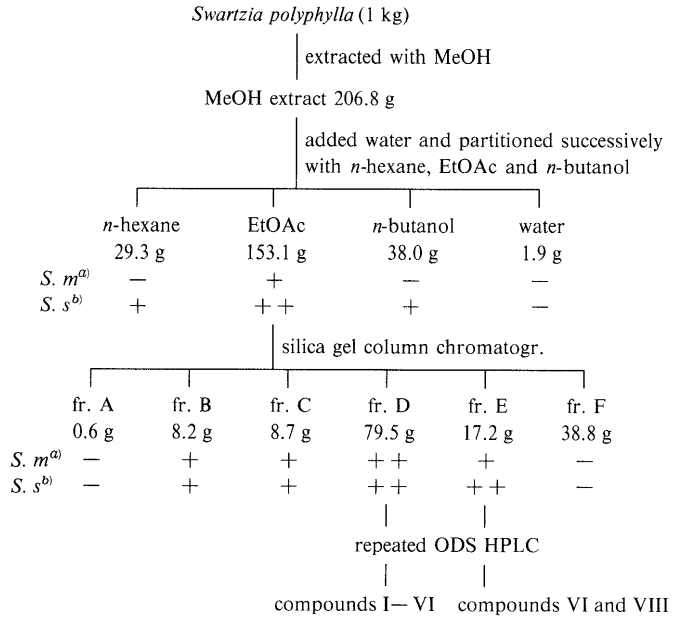

Chart 1. Antibacterial Activity of Fractions Separated from *S. polyphylla*

Antibacterial activity was determined by paper disk assay. ++: inhibitory zone was formed at 50 μg/disk, +: at 500 μg/disk, —: no inhibitory zone was formed at 500 μg/disk. *a*) *S. mutans* Ingbritt, *b*) *S. sobrinus* 6715.

Results and Discussion

The heartwood of *Swartzia polyphylla* Dc. (Leguminosae) was extracted with methanol and the extract showed appreciable antibacterial activity against *Streptococcus mutans* Ingbritt and *S. sobrinus* 6715. In a partition using several solvents, the antibacterial activity was concentrated in ethyl acetate soluble fraction as shown in Chart 1. This fraction was subjected to silica gel column chromatography to afford six fractions. Fraction D and fr. E possessing antibacterial activity were further separated to give six flavonoids (I—VI) and two flavonoids (VI, VII) respectively. Among the seven flavonoids isolated from *S. polyphylla*, five of the compounds were identified as (*S*)-naringenin (I),[5] formononetin (II),[6] biochanin A (III),[7] ferreirin (V)[8] and darbergioidin (VII)[9] by comparison of physical and spectroscopic data with those in a previous report.

Compound IV, $C_{16}H_{14}O_5$, was obtained as colorless crystals, mp 175—177 °C and showed negative in the

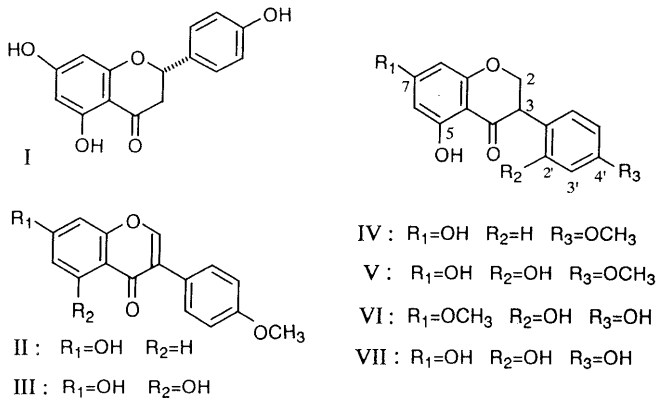

Fig. 1

magnesium–hydrochloric acid (Mg–HCl) test and a dark purple color to ferric chloride ($FeCl_3$) reaction. The infrared (IR) spectrum suggested the presence of hydroxyl (3370 cm^{-1}) and carbonyl (1640 cm^{-1}) groups. ^{1}H-nuclear magnetic resonance (NMR) spectrum of compound IV showed the presence of an isoflavanone skeleton with signals at 4.58 (2-H), 3.97 (3-H) and one methoxyl group at δ 3.77. The structural feature was also corroborated by clearly different ^{13}C signals at δ 72.0 (C-2) and 50.9 (C-3) from those of the flavanone derivatives. In the mass spectrum (MS) of compound IV the fragment ions of *m/z* 286 [M^+], 152, 134, derived from the retro Diels–Alder fragmentation, suggested that ring A of the isoflavanone skeleton had two hydroxyl groups and ring B had one methoxyl group. The methoxyl group of ring B was located at the C-4′ position, because the A_2B_2-type proton signals were observed. Thus, compound IV was established to be 5,7-dihydroxy-4′-methoxyisoflavanone (dihydrobiochanin A). The physical and spectroscopic data of hydrogenated biochanin A with 10% Pd–C were identical with those of compound IV. Schleper *et al.* reported that dihydrobiochanin A could be converted from biochanin A by NADPH-dependent oxidoreductase obtained from fungus *Fusarium javanicum*.[10] This is the first isolation from a natural source.

Compound VI was obtained as colorless crystals, mp 214—216 °C and showed negative in Mg–HCl test and a dark purple color to $FeCl_3$ reaction. The high resolution (HR) MS data of compound VI supported the molecular formula of $C_{16}H_{14}O_6$ (M^+ 302.0783, requires 302.0790). The IR spectrum showed the absorption bands at 3430 and 1650 cm^{-1} which were characteristic of hydroxyl and carbonyl groups. The ^{1}H-NMR spectrum of compound VI also suggested an isoflavanone skeleton with signals at δ 4.47 (dd, J=5.5, 10.6 Hz, 2-H_α), 4.63 (t, J=10.8 Hz, 2-H_β) and 4.27 (dd, J=5.5, 10.6 Hz, 3-H), and one methoxyl group with signal at δ 3.84. The MS of compound VI showed a typical retro Diels–Alder fragmentation giving rise to *m/z* 302 [M^+], 167 and 136, with the result that ring A of the isoflavanone skeleton had one hydroxyl group and one methoxyl group, and ring B had two hydroxyl groups. The ^{1}H-NMR signal at δ 12.34 (s, exchangeable with D_2O) was characteristic of C-5 hydroxyl proton with hydrogen bond and 6-H and 8-H were characterized by the signals at δ 6.02 (d, J=2.2 Hz) and 6.04 (d, J=2.2 Hz). Therefore, this compound was found to have the hydroxyl group at C-5 and the methoxyl group at C-7 in ring A. Then, ABX-type proton signals in an aromatic ring were observed at δ 6.45 (d, J=2.2 Hz), 6.34 (dd, J=2.2, 8.3 Hz) and 6.93 (d, J=8.3 Hz) in ring B. The two hydroxyl groups of ring B were determined to be at C-2′ and C-4′ positions since the ^{13}C-NMR signals of C-3 and C-1′ appeared at δ 47.4 and 113.6. If the two hydroxyl groups were at C-3′ and C-4′ positions in ring B, the signals of C-3 and C-1′ would shift downfield to 50—52 and 120—130, respectively.[11] The position of the hydroxyl groups was also shown to be the same as those in darbergioidin (VII) by comparison of the chemical shifts of the aromatic proton signals. Based on these data, compound VI was established as 5,2′,4′-trihydroxy-7-methoxyisoflavanone (dihydrocajanin). The ^{1}H and ^{13}C-NMR assignments of the four isoflavanones (IV, V, VI, VII) which exhibited antibacterial activity are listed in Tables I and II.

Table I. ^{1}H-NMR Chemical Shifts of Isoflavanones IV, V, VI, VII, δ (ppm) from TMS in Acetone-d_6 (*J*/Hz in Parentheses, 400 MHz)

H	IV	V	VI	VII
2	4.58 m	4.46 dd (5.4, 11.0) 4.61 t (10.7)	4.47 dd (5.5, 10.6) 4.63 t (10.8)	4.44 dd (5.4, 10.9) 4.59 t (10.7)
3	3.97 t (6.7)	4.27 dd (5.4, 11.0)	4.27 dd (5.5, 10.6)	4.23 dd (5.4, 10.9)
6	5.98 s	5.96 s	6.02 d (2.2)	5.95 d (2.0)
8	5.98 s	5.97 s	6.04 d (2.2)	5.96 d (2.0)
2′	7.25 d (8.7)			
3′	6.90 d (8.7)	6.50 d (2.3)	6.45 d (2.2)	6.45 d (2.2)
5′	6.90 d (8.7)	6.42 dd (2.3, 8.4)	6.34 dd (2.2, 8.3)	6.33 dd (2.2, 8.3)
6′	7.25 d (8.7)	7.03 d (8.4)	6.93 d (8.3)	6.91 s (8.3)
OH	12.22 s	12.34 s	12.34 s 8.61 br s 8.31 br s	12.35 s
OMe	3.77 s	3.72 s	3.84 s	

Table II. ^{13}C-NMR Chemical Shifts of Isoflavanones IV, V, VI, VII, δ (ppm) from TMS in Acetone-d_6 (100 MHz)

C	IV	V	VI	VII
2	72.0 (t)	71.0 (t)	71.2 (t)	71.0 (t)
3	50.9 (d)	47.3 (d)	47.4 (d)	47.2 (d)
4	187.5 (s)	198.2 (s)	198.8 (s)	198.4 (s)
4a	103.1 (s)	103.6 (s)	104.2 (s)	103.5 (s)
5	165.7 (s)	165.6 (s)	165.4 (s)	165.5 (s)
6	97.0 (d)	96.9 (d)	95.5 (d)	96.9 (d)
7	167.7 (s)	167.2 (s)	168.6 (s)	167.2 (s)
8	95.7 (d)	95.7 (d)	94.3 (d)	95.6 (d)
8a	164.2 (s)	164.5 (s)	164.4 (s)	164.4 (s)
1′	128.6 (s)	115.1 (s)	113.6 (s)	113.7 (s)
2′	130.5 (d)	157.0 (s)	157.0 (s)	157.0 (s)
3′	114.9 (d)	102.7 (d)	103.8 (d)	103.8 (d)
4′	160.1 (s)	161.3 (s)	158.9 (s)	158.8 (s)
5′	114.9 (d)	105.9 (d)	107.9 (d)	107.8 (d)
6′	130.5 (d)	131.7 (d)	131.8 (d)	131.6 (d)
OMe	55.5 (q)	55.4 (q)	56.2 (q)	

The multiplications of carbon signals were determined by distortionless enhancement by polarization transfer (DEPT) method.

The minimum inhibitory concentrations (MIC) of compounds I—VII against cariogenic bacteria were estimated as shown in Table III. It was found that isoflavanone derivatives, dihydrobiochanin A (IV), ferreirin (V), dihydrocajanin (VI), darbergioidin (VII) possessed relatively strong antibacterial activity, dihydrobiochanin A (IV) was the most active component. These isoflavanones were principal constituents of the heartwood of *S. polyphylla*, and it was thought that the antibacterial activity of *S. polyphylla* against cariogenic bacteria was due to these isoflavanone derivatives. Naringenin (I), one of the flavanone derivatives also had antibacterial activity, however, it was not as strong as that of isoflavanones. In contrast, isoflavone derivatives such as formononetin (II) and biochanin A (III) showed no inhibitory activity at 400 μg/ml against any of the organisms. Isoflavanone and flavanone structures have no double bond at the C-2 position in ring C, only isoflavone structure contains a double bond at the C-2 position in ring C. It is suggested that this structural feature could be related to the antibacterial activity. We therefore further examined the antibacterial assay using various natural and standard flavonoids in an attempt to explain the structural–activity relationship. Antibacterial potencies of these flavonoids were estimated by the paper disk method, and the results

TABLE III. Minimal Inhibitory Concentration (MIC) of Compounds I—VII Obtained from *S. polyphylla* against Cariogenic Bacteria as Determined by the Agar Dilution Method

Strain	Serotype	MIC (μg/ml)							
		I	II	III	IV	V	VI	VII	C.G.[a]
S. cricetus HS6	a	>400	>400	>400	200	400	400	400	6.25
S. rattus FAI	b	200	>400	>400	25	100	100	100	1.56
S. mutans Ingbritt	c	200	>400	>400	50	100	100	200	1.56
S. sobrinus B13	d	400	>400	>400	50	50	100	100	1.56
S. mutans LA7	e	400	>400	>400	50	50	100	100	0.75
S. mutans OMZ-175	f	400	>400	>400	50	100	100	200	1.56
S. sobrinus 6715	g	400	>400	>400	100	100	100	200	1.56

a) Chlorhexidine gluconate.

TABLE IV. Antibacterial Activity of Flavonoids from *S. polyphylla* and Related Compounds against *S. mutans* Ingbritt and *S. sobrinus* 6715

Compound	Structure	Growth inhibition	
		S. m[a] Ingbritt	*S. s*[b] 6715
Flavone			
1 Flavone		−	−
2 Apigenin	5,7,4′-OH	−	−
3 Diosmin	5,7,3′-OH, 4′-OCH_3	−	−
Flavanone			
4 Flavanone		−	−
5 (*S*)-Naringenin (I)	5,7,4′-OH	++	+
6 (*RS*)-Naringenin		++	+
7 Isosakuranetin	5,7,-OH, 4′-OCH_3	−	−
8 Eriodictyol	5,7,3′,4′-OH	−	−
9 Hesperetin	5,7,3′-OH, 4′-OCH_3	−	−
Isoflavone			
10 Formononetin (II)	7-OH, 4′-OCH_3	−	−
11 Genistein	5,7,4′-OH	−	−
12 Biochanin A (III)	5,7-OH, 4′-OCH_3	−	−
13 Pseudobaptigenin	7-OH, 3′,4′-OCH_2O–	−	−
Isoflavanone			
14 Dihydrogenistein	5,7,4′-OH	++++	+++
15 Dihydrobiochanin A (IV)	5,7-OH, 4′-OCH_3	++++	++++
16 (*R*)-Ferreirin	5,7,2′-OH, 4′-OCH_3	+++	+++
17 (*S*)-Ferreirin		+++	+++
18 (*RS*)-Ferreirin (V)		++++	++++
19 (*R*)-Dihydrocajanin	5,2′,4′-OH, 7-OCH_3	+++	++++
20 (*S*)-Dihydrocajanin		+++	++++
21 (*RS*)-Dihydrocajanin (VI)		++++	++++
22 Darbergioidin (VII)	5,7,2′,4′-OH	+++	+++
23 Dihydrocajanin methylate	5,7,2′,4′-OCH_3	−	−
24 Dihydrocajanin acetylate	5,2′,4′-OAc, 7-OCH_3	−	−

Antibacterial activity was determined by paper disk assay ++++: inhibitory zone was formed at 62.5 μg/disk, +++: at 125 μg/disk, ++: at 250 μg/disk, +: at 500 μg/disk, −: no inhibitory zone was formed at 500 μg/disk. *a*) *S. mutans*, *b*) *S. sobrinus*.

are summarized in Table IV. These flavonoids may be grouped into four categories: flavones (**1**—**3**), flavanones (**4**—**9**), isoflavones (**10**—**13**) and isoflavanones (**14**—**24**). Compounds **14**—**22** belonging to isoflavanones showed strong inhibitory activity, with an inhibitory zone at 125 μg/disk or less against both organisms, but flavones, flavanones and isoflavones did not show any inhibitory effect except for compounds **5** and **6**. It is interesting that only isoflavanones have significant antibacterial action against cariogenic bacteria. Perrin and Cruickshank[12] reported a similar effect of various naturally occurring pterocarpans and related compounds against the fungus *Monilinia fructicola*, and proposed that the angle of the B/C ring junction is very important in high fungitoxicity. With regard to substitutions, no significant difference in inhibitory potency was observed among isoflavanones **14**—**22**. Methylation and acetylation of the phenolic hydroxyl groups of dihydrocajanin resulted in the loss of antibacterial activity, which showed that free hydroxyl groups are very important for the inhibitory effect. The antibacterial examination of the *rectus*-, *sinister*- and racemic forms of ferreirin (**16**, **17**, **18**) and dihydrocajanin (**19**, **20**, **21**) suggested that there were no appreciable differences in inhibitory activity among the stereoisomers, and that the inhibitory potency of respective stereoisomers was a little weaker than that of the racemic compound. Except for isoflavanones, only naringenin had antibacterial activity, but its potency was lower than that of isoflavanones. The structurally similar flavanones, isosakuranetin, eriodictyol and hesperetin showed no inhibitory activity at 500 μg/disk against either organism.

There have been many reports relative to the antifungal and antibacterial activities of flavonoid,[13] but very few on those of isoflavanone.[14] In the present study, we confirmed that the isoflavanones isolated from *S. polyphylla* had a potent antibacterial activity. Our findings suggest that these compounds may be effective agents for preventing the growth of cariogenic bacteria.

Experimental

All melting points were recorded on a Yanagimoto MP-3 micro melting point apparatus and were uncorrected. Spectral data were obtained on the following instruments: optical rotation on a JASCO DIP-4, circular dichroism (CD) on a JASCO J-500C, IR on a JASCO A-300, UV on a Shimadzu UV-160, NMR on a Bruker AM400 and MS on a Hitachi M-80. High-performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-8A, LC-9A system with Senshu Pak ODS-5251-SH column with 5 μm octadecyl silica (ODS) as the reversed phase. Optical resolution was carried out using a Daicel chiralcel OD column.

Chemicals The standard samples apigenin, diosmin, flavanone, hesperetin, eriodictyol, isosakuranetin, genistein, and pseudobaptigenin were purchased from Extrasynthese, France, flavone, and (*RS*)-naringenin were purchased from Sigma Chemical Co., U.S.A.

Test Bacterial Strains and Culture Media The seven bacterial strains tested were: *Streptococcus cricetus* HS6 (serotype a), *S. rattus* FAI (serotype b), *S. mutans* Ingbritt (serotype c), *S. sobrinus* B13 (serotype d), *S. mutans* LA7 (serotype e), *S. mutans* OMZ175 (serotype f), and *S. sobrinus* 6715 (serotype g). These organisms were cultured on brain heart infusion (BHI) agar (Difco) at 37 °C and used for antibacterial assay.

Assay of Antibacterial Activity Paper Disk Method: One loopful of the organism was precultured in 70 ml of BHI broth (Difco) overnight to prepare the seeded solution. The turbidity of the seeded solution was adjusted to optical density (OD) 1.0 (550 nm). Then, 1.0 ml of the cell suspension was diluted with 10 ml of BHI agar in a petri dish. After cooling, the paper disk (8 mm in diameter, Advantec) which contained the test substance was placed on the seeded medium and incubated at 37 °C for

24 h. Diameter of the inhibitory zone was measured.

Measurement of Minimum Inhibitory Concentration (MIC): Two-fold serial dilutions of the tested substances were prepared with methanol and sterilized by passing through a filter (0.22 μm, Millipore). Five-tenths ml of a given dilution of test substance was added to 19.5 ml of BHI agar. As a control, a plate of culture medium was prepared by adding 0.5 ml of methanol. One loopful of 3 d cultured cells were suspended in 2 ml of BHI broth. One loopful of each cell suspension was then inoculated onto the BHI agar containing the test substance. The plates were incubated at 37 °C for 24 h, and the bacterial growth on each medium was examined.

Extraction and Isolation The heartwood of *S. polyphylla* purchased at a Peruvian market (1 kg) was extracted with hot methanol and concentrated *in vacuo* to give methanolic extract (206.8 g). The extract was separated into *n*-hexane, ethyl acetate, *n*-butanol and water soluble fractions, respectively. The active fraction (ethyl acetate soluble fraction) was applied to silica gel column chromatography. The column was eluted with dichloromethane–methanol (100 : 0—50 : 50, v/v) and separated into six fractions. Chromatographic purification of fractions D and E by ODS HPLC with methanol–water (70 : 30—55 : 45, v/v) led to the isolation of compound I—VII.

Optical Resolution of Isoflavanones All isoflavanones separated in this study were racemic compounds at C3 chiral center. The optical isomers of ferreirin (V) and dihydrocajanin (VI) were separated by chiralcel OD HPLC as the solvents *n*-hexane–isopropanol–trifluoroacetic acid (TFA) (90 : 10 : 0.5, v/v) for ferreirin and (80 : 20 : 0.5, v/v) for dihydrocajanin by monitoring UV absorption at 300 nm. All these separated compounds had optical activity and the absolute configuration was determined by circular dichroism (CD) spectral analysis in comparison with previous reports.[15]

5,7,4′-Trihydroxyflavanone (I) ((*S*)-Naringenin) Colorless crystals mp 248—250 °C. $[\alpha]_D$ −11.4° (*c* = 0.55, MeOH). IR (KBr): 3100, 1640, 1600, 1520, 1460, 1310, 1250 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 213 (4.43), 291 (4.23). MS *m/z* (%): 272 [M$^+$] (100), 179 (22), 153 (70), 120 (42). CD (EtOH) [θ] (nm): +2730 (326), −24520 (290). ^{1}H-NMR (acetone-d_6) δ: 12.15 (1H, s, 5-OH), 7.38 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.90 (2H, d, *J* = 8.5 Hz, H-3′, 5′), 5.96 (2H, s, H-6, 8), 5.42 (1H, dd, *J* = 2.9, 12.8 Hz, H-2), 3.15 (1H, dd, *J* = 12.8, 17.1 Hz, H-3$_\alpha$), 2.72 (1H, dd, *J* = 2.9, 17.1 Hz, H-3$_\beta$).

5-Hydroxy-4′-methoxyisoflavone (II) (Formononetin) Colorless needles mp 259—261 °C. IR (KBr): 3150, 1640, 1620, 1520, 1460, 1280, 1250 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 211 (4.24), 249 (4.39). MS *m/z*(%): 268 [M$^+$] (53), 132 (100), 117 (35), 89 (84). ^{1}H-NMR (DMSO-d_6) δ: 8.32 (1H, s, H-2), 7.97 (1H, d, *J* = 8.8 Hz, H-5), 7.51 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 6.99 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 6.94 (1H, dd, *J* = 2.2, 8.8 Hz, H-6), 6.87 (1H, d, *J* = 2.2 Hz, H-8), 3.79 (3H, s, 4′-OCH$_3$).

5,7-Dihydroxy-4′-methoxyisoflavone (III) (Biochanin A) White amorphous powder mp 216—218 °C. IR (KBr): 3400, 1660, 1630, 1520, 1440, 1360, 1250, 1190 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 211 (4.44), 262 (4.59). MS *m/z* (%):284 [M$^+$] (100), 152 (18), 132 (50), 89 (17). ^{1}H-NMR (DMSO-d_6) δ: 12,91 (1H, s, 5-OH), 8.34 (1H, s, H-2), 7.49 (2H, d, *J* = 8.7 Hz, H-2′, 6′), 6.99 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.23 (1H, d, *J* = 2.0 Hz, H-6), 3.79 (3H, s, 4′-OCH$_3$).

5,7-Dihydroxy-4′-methoxyisoflavanone (IV) (Dihydrobiochanin A) Colorless crystals mp 175—177 °C. $[\alpha]_D$ 0° (*c* = 0.56, MeOH). IR (KBr): 3370, 1640, 1620, 1580, 1520, 1480, 1450, 1280, 1240, 1160 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 214 (4.42), 224 (4.41), 292 (4.28). MS *m/z* (%): 286 [M$^+$] (15), 152 (10), 134 (100), 119 (14).

5,7,2′-Trihydroxy-4′-methoxyisoflavanone (V) (Ferreirin) Colorless crystals mp 208—210 °C. $[\alpha]_D$ 0° (*c* = 0.55, MeOH). IR (KBr): 3400, 1640, 1610, 1580, 1520, 1470, 1440, 1380, 1280, 1230, 1200, 1160 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 211 (4.42), 288 (4.25). MS *m/z* (%): 302 [M$^+$] (84), 153 (84), 150 (100). NOE: irradiation at 3.72 (4′-OCH$_3$) produced enhancement at 6.50 (H-3′) and 6.42 (H-5′). (*R*)-Ferreirin: $[\alpha]_D$ +23.8° (*c* = 0.37, MeOH). CD (EtOH) [θ] (nm): +1212 (350), −8788 (307), +5151 (280). (*S*)-Ferreirin: $[\alpha]_D$ −24.8° (*c* = 0.36, MeOH). CD (EtOH) [θ] (nm): −2273 (342), +7273 (307), −8333 (280).

5,2′,4′-Trihydroxy-7-methoxyisoflavanone (VI) (Dihydrocajanin) Colorless crystals mp 214—216 °C. $[\alpha]_D$ 0° (*c* = 0.55, MeOH). IR (KBr): 3430, 1650, 1610, 1590, 1530, 1510, 1460, 1385, 1300, 1275, 1220, 1210, 1190, 1160 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 211 (4.50), 287 (4.32). MS *m/z* (%); 302 [M$^+$] (25), 167 (100), 136 (18), 28 (56). (*R*)-Dihydrocajanin: $[\alpha]_D$ +27.6° (*c* = 0.19, MeOH). CD (EtOH) [θ] (nm): +1212 (350), −5303 (310), +3939 (280). (*S*)-Dihydrocajanin: $[\alpha]_D$ −22.5° (*c* = 0.21, MeOH). CD (EtOH) [θ] (nm): −1970 (343), +4091 (310), −4545 (280).

5,7,2′,4′-Tetrahydroxyisoflavanone (VII) (Darbergioidin) Colorless crystals mp 228—230 °C. $[\alpha]_D$ 0° (*c* = 1.12, MeOH). IR (KBr): 3350, 1640, 1520, 1480, 1450, 1390, 1280, 1160, 1100 cm^{-1}. UV λ_{max}^{EtOH} nm (log ε): 210 (4.45), 288 (4.28). MS *m/z* (%): 288 [M$^+$] (30), 153 (100), 136 (28), 43 (55).

Methylation of Dihydrocajanin A solution of dihydrocajanin (60 mg) in methanol (4 ml) was treated with diazomethane. After standing at room temperature for 3 h, the solvent was removed. Chromatographic purification of the residue by silica gel HPLC with *n*-hexane–ethyl acetate (80 : 20, v/v) gave the methyl ether of dihydrocajanin (37.0 mg) as white amorphous solid. mp 102—104 °C. IR (KBr): 1640, 1570, 1510, 1460, 1430, 1390, 1260, 1200, 1160, 1100, 1030, 830 cm^{-1}. MS *m/z* (%): 344 [M$^+$] (10), 330 (18), 164 (100), 148 (28), 121 (20). ^{1}H-NMR (acetone-d_6) δ: 7.09 (1H, d, *J* = 8.3 Hz, H-6′), 6.62 (1H, d, *J* = 2.2 Hz, H-3′), 6.52 (1H, dd, *J* = 2.2, 8.3 Hz, H-5′), 6.07 (1H, d, *J* = 2.0 Hz, H-8), 6.04 (1H, d, *J* = 2.0 Hz, H-6), 4.58 (1H, t, *J* = 11.0 Hz, H-2$_\alpha$), 4.45 (1H, dd, *J* = 5.5, 11.0 Hz, H-2$_\beta$), 4.32 (1H, dd, *J* = 5.5, 11.0 Hz, H-3), 3.87, 3.86, 3.81 (3H, 3H, 3H × 2, s, s, s, 5, 7, 2′, 4′-OCH$_3$).

Acetylation of Dihydrocajanin A solution of dihydrocajanin (30 mg) and a small amount of 4-dimethyl amino pyridine in pyridine (5 ml) was treated with acetic anhydride (5 ml). After standing at room temperature for 2 h, the solvent was removed. The acetate of dihydrocajanin (13.2 mg) gave colorless prisms. mp 168—170 °C. IR (KBr): 1770, 1680, 1620, 1570, 1440, 1370, 1190, 1150 cm^{-1}. MS *m/z* (%): 428 (5), 368 (10), 326 (30), 284 (8), 209 (24), 167 (100), 136 (56). ^{1}H-NMR ($CDCl_3$) δ: 7.19 (1H, d, *J* = 8.3 Hz, 6′-H), 7.03 (1H, d, *J* = 2.3 Hz, 3′-H), 6.98 (1H, dd, *J* = 2.3, 8.3 Hz, 5′-H), 6.39 (1H, d, *J* = 2.5 Hz, 8-H), 6.29 (1H, d, *J* = 2.5 Hz, 6-H), 4.53 (2H, m, 2-H$_{\alpha\beta}$), 4.11 (1H, m, 3-H), 3.84 (3H, s, 7-OCH$_3$), 2.32, 2.28, 2.24 (3H, 3H, 3H, s, s, s, 5, 2′, 4′-OAc).

Hydrogenation of Biochanin A and Genistein Hydrogenation was performed according to the method of Farkas *et al.*[16] Biochanin A (100 mg) and genistein (25 mg) were hydrogenated with 10% Pd–C in acetic acid until the uptake of 1 eq of H_2. The crude products were recrystallized from 50% aqueous MeOH to afford dihydrobiochanin A (74.0 mg) and dihydrogenistein (13.2 mg). Dihydrobiochanin A: Colorless crystals. The spectroscopic and physical properties were identical to compound IV. Dihydrogenistein: Colorless crystals. mp 200—202 °C. IR (KBr): 3370, 1640, 1600, 1520, 1480, 1450, 1230, 1160, 1100, 1030, 837 cm^{-1}. ^{1}H-NMR (acetone-d_6) δ: 7.18 (2H, dd, *J* = 2.0, 8.5 Hz, H-2′, 6′), 6.83 (2H, dd, *J* = 2.0, 8.5 Hz, H-3′, 5′), 5.97 (2H, s, H-6, 8), 4.61 (2H, m, 2-H$_{\alpha\beta}$), 3.97 (1H, t, *J* = 5.8 Hz, H-3).

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