Notes

A New Prenylated Flavonol from the Roots of *Sophora flavescens*

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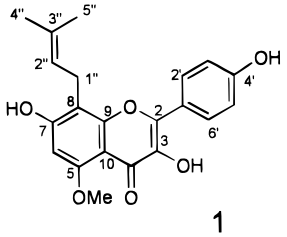
A new prenylated flavonol, sophoflavescenol (**1**), together with five known flavonoids, kurarinol, kushenol K, kushenol H, trifolirhizin, and kuraidin, were isolated from the roots of *Sophora flavescens*. The structure of **1** was determined by spectroscopic analysis. Among the five known flavonoids, kurarinol, kushenol K, and kushenol H showed weak antiviral activity against Herpes simplex virus types I and II.

Sophorae Radix, the dried roots of *Sophora flavescens*, a well-known Chinese traditional medicine, has been used as a diuretic, stomachic, antipyretic, and anthelmintic.[1,2] The plant, *S. flavescens* Ait. (Leguminosae) grows as a perennial herb and is widely distributed in Korea.[3] Phytochemical studies of this plant have so far yielded such alkaloids as flavonoids, chromones, and saponins.[4−8] During our search for antiviral compounds from natural products, we carried out antiherpetic assays on about 300 species of plants. Among them, the crude ethyl-acetate extract of the roots of *S. flavescens* showed antiherpetic activity.[17]

The MeOH extract of the roots of *S. flavescens* was suspended in water and then consecutively partitioned with dichloromethane, ethyl acetate, and butanol. The ethyl acetate extract showed weak antiviral activity against Herpes simplex virus types I and II (HSV-1 and 2) (EC_{50} values of >125 μg/mL and 93.3 ± 12.5 μg/mL, respectively). The extract was separated by means of bioassay-directed chromatographic fractionation on a Si gel column using a stepwise solvent gradient method (EtOAc−MeOH−H_2O). The active fractions were further purified by chromatography on Sephadex LH-20 and Si gel to afford the new sophoflavescenol (**1**). In addition to compound **1**, five known flavonols—kurarinol,[5,10] kushenol K,[11] kushenol H,[12,14] trifolirhizin,[5,15] and kuraidin[13]—were also isolated.



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Compound **1** was obtained as yellow needles. Its molecular formula $C_{21}H_{19}O_6$ was deduced from HREIMS (found *m/z* 368.1261, calcd 368.1260). The UV spectrum exhibited absorption maxima 267 and 366 nm, characteristic for a flavonol skeleton.[9] The ^{1}H NMR spectrum of **1** revealed the presence of H-3′, -5′ and H-2′, -6′ protons of the B ring as an AB quartet at δ 6.92 and 7.99 (each 2H, J = 8.8 Hz). In the aromatic region of the ^{1}H NMR spectrum of **1**, a singlet appearing at δ 6.45 was assigned to the proton of a trisubstituted ring A. Three aromatic hydroxyl groups appeared at δ 8.57, 9.94, and 10.53 (broad singlets), and a signal at δ 3.80 showed the presence of a methoxy group of **1**. Additionally, signals at δ 5.18, 3.46 were assigned to the olefinic and methylene protons; signals at δ 1.63, 1.76 were also assigned to the methyl protons.

Table 1. Chemical Shifts, ^{1}H−^{1}H COSY, and HMBC NMR Correlations of **1** in DMSO-d_6

position	δ ^{13}C	δ ^{1}H (multiplicity, J_{H-H})	^{1}H−^{1}H COSY[a]	HMBC
2	141.6 (s[b])			
3	136.8 (s)			
4	171.2 (s)			
5	157.9 (s)			
6	95.4 (d)	6.45 (s)	5-OCH_3	C-5, 7, 8, 10
7	159.5 (s)			
8	106.8 (s)			
9	155.4 (s)			
10	105.2 (s)			
1′	122.3 (s)			
2′	128.6 (d)	7.99 (d, 8.8)	3′, 5′	C-2, 4′
3′	115.4 (d)	6.92 (d, 8.8)	2′, 6′	C-1′, 4′
4′	158.5 (s)			
5′	115.4 (d)	6.92 (d, 8.8)	2′, 6′	C-1′, 4′
6′	128.6 (d)	7.99 (d, 8.8)	3′, 5′	C-2, 4′
1″	21.4 (t)	3.46 (d, 6.3)	2″, 4″, 5″	C-7, 8, 9, 2″, 3″
2″	122.7 (d)	5.18 (t-like, 6.0)	1″, 4″, 5″	C-4″, 5″
3″	130.9 (s)			
4″	25.4 (q)	1.63 (s)	1″, 2″	C-1″, 2″, 5″
5″	17.8 (q)	1.76 (s)	1″, 2″	C-1″, 2″, 4″
5-OCH_3	55.7 (q)	3.80 (s)	6	C-5
3-OH		8.57 (br.s)		C-2
7-OH		10.53 (br.s)		
4′-OH		9.94 (br.s)		C-3′, 5′

[a] Major ^{1}H−^{1}H correlations observed in COSY-45 and long-range COSY-45 experiments. [b] Multiplicities were established by DEPT experiment.

The ^{13}C NMR data (Table 1) of **1** also supported the above assignments. The spectrum of **1** showed characteristic peaks of the kampferol moiety and signals at δ 21.4, 122.7, 130.9, 25.4, 17.8 and 55.7, assignable to the prenyl and methoxy groups. In the ^{1}H−^{1}H homonuclear COSY spectrum, the olefinic proton at δ 5.18 showed coupling to methylene protons (δ 3.46) and two methyl groups (δ 1.63 and 1.76). Also, the methoxy protons at δ 3.80 were

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coupled to a proton (H-6) at δ 6.45. These couplings required the presence of three substituents, a 3,3-dimethylallyl, a hydroxyl, and a methoxy group in A ring of the flavonol skeleton. The UV spectral data with shift reagents indicated that free hydroxyl groups were located at C-3, -7, and -4′ of the flavonoid skeleton.[9] After addition of NaOMe and $AlCl_3$ + HCl, absorption maxima at 267 and 366 nm shifted to 268, 322, and 407 and to 265, 307, and 423 nm, respectively. Also, after addition of NaOAc, absorption maxima at 267 and 366 nm shifted to 277, 313, and 385 nm. From the UV shift phenomenon and $^1H-^1H$ homonuclear correlations, the attachments of the methoxy, hydroxyl, and 3,3-dimethylallyl group in **1** were found to be at C-5, C-7, and C-8 of flavonol, respectively. These results were further supported by HMBC experiments. The proton signal at δ 3.80 of methoxy group showed long-range correlations with the C-5 at δ 157.9 and proton signals of H-1″ at δ 3.46 were correlated with C-7, -8, -9, -2″ and -3″. In the HMQC spectrum for **1**, all direct ^1J connectivities between carbons and protons were determined. The assignments of the ^1H and ^{13}C NMR chemical shift values of **1** were based on the HMQC and HMBC correlations. On the basis of the foregoing findings, compound **1** was determined to be a 5-methoxy-7,4′-dihydroxy-8-(3,3-dimethylallyl)flavonol, named sophoflavescenol. A structurally related compound, noranhydroicaritin, was previously isolated from *Sophora angustifolia* by Komatsu et al.[16] Compound **1** differs by the presence of a methoxy substituent in C-5 on A ring instead of a hydroxy group.

The antiviral activities of the six isolated compounds were tested against HSV-1, 2 according to established methods.[17] Kurarinol was weakly active against HSV-1 (EC_{50} 60 ± 3.4 μg/mL), whereas the remaining five isolates, including **1**, were inactive at EC_{50} > 300 μg/mL. The EC_{50} values for kurarinol, kushenol K, and kushenol H against HSV-2 were 44 ± 2.9 μg/mL, 147 ± 8.8 μg/mL, and 162 ± 6.5 μg/mL, respectively. Trifolirhizin, kuraidin, and **1** were inactive.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas−Hoover capillary melting apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV 240 UV−vis recording spectrophotometer. IR spectra were recorded on a Perkin−Elmer 16F−PC FT-IR spectrophotometer using KBr pellets. ^1H NMR spectra were recorded on a Bruker AMX-500 (500 MHz) spectrometer, using TMS as internal standard. ^{13}C NMR spectra were recorded on a Bruker AMX-500 (125 MHz) spectrometer. $^1H-^1H$ COSY, HMQC, and HMBC NMR spectra were obtained with the usual pulse sequences, and data processing was performed with the Bruker software. HRMS and LRMS were obtained on a VG70-VSEQ mass spectrometer (VG Analytical, UK). TLC and column chromatography were carried out on precoated Si gel F_{254} plates (Merck, art. 5715), RP-18 F_{254s} plates (Merck, art. 15423), Si gel 60 (Merck, 230−400 mesh), Lichroprep RP-18 (Merck, 40−63 μm), Sephadex LH-20 (Sigma), and MCI gel CHP20P (Mitsubishi Kasei Corporation, 150−300 μm).

Plant Material. The roots of *Sophora flavescens* Ait. were collected at Chang-young, Kyungsangnamdo, Korea, in February 1994. The voucher specimen (518−18) has been deposited in the laboratory of the Korea Institute of Science and Technology (KIST).

Extraction and Isolation. Fresh roots (9 kg) of *S. flavescens* were extracted with MeOH twice at room temperature. The MeOH extract was evaporated to dryness (257 g) under reduced pressure, suspended in distilled H_2O, and partitioned with CH_2Cl_2, EtOAc, and BuOH consecutively. The EtOAc extract, which showed antiviral activity, was subjected to column chromatography over Si gel (700 g) eluting sequentially with EtOAc−MeOH−H_2O (70:5:2), EtOAc−MeOH−H_2O (100:10:7), and EtOAc−MeOH−H_2O (100:13.5:10). Fractions were combined based on their TLC pattern to yield fractions designated as E1−E8. Fraction E2 was found to be active in an antiviral assay. Fraction E2 (5.2 g) was further chromatographed on a Sephadex LH-20 column (MeOH, 4.5 × 40 cm) and a Si gel column (300 g) eluting sequentially with hexane−CH_2Cl_2−MeOH (10:10:2), hexane−CH_2Cl_2−MeOH (5:10:3), and CH_2Cl_2−MeOH−H_2O (50:10:1) to afford six fractions (E2A−E2F). Fraction E2C (0.52 g) was chromatographed on a Sephadex LH-20 (MeOH, 2.5 × 36 cm) and was recrystallized from MeOH to afford sophoflavescenol (**1**) (27 mg). Fraction E2D (1.8 g) was purified by column chromatography on RP-18 (70% MeOH, 3 × 30 cm) to give kurarinol (**2**) (530 mg). Fraction E2E (1.1 g) was subjected to a series of purification steps, including Sephadex LH-20, RP-18, and MCI gel column chromatography, to obtain kushenol K (**3**) (180 mg), kushenol H (**4**) (167 mg), and trifolirhizin (**5**) (38 mg). Also fraction E2B (0.47 g) was chromatographed on a Si gel (CH_2Cl_2−MeOH−H_2O, 100:13:1) and RP-18 (70% MeOH) to give kuraridin (**6**) (24 mg).

Sophoflavescenol (1): yellow needles; mp 273−275 °C; UV (MeOH) λ_{max} (log ϵ) 262 (sh) (4.52), 267 (4.53), 306 (sh) (4.25), 366 (4.53) nm; (MeOH + NaOMe) λ_{max} 268, 322, 407 nm; (MeOH + $AlCl_3$) λ_{max} 260 (sh), 267, 307 (sh), 343 (sh), 426 nm; (MeOH + $AlCl_3$/HCl) λ_{max} 258 (sh), 265, 307 (sh), 423 nm; (MeOH + NaOAc) λ_{max} 270 (sh), 277, 313 (sh), 385 nm; (MeOH + NaOAc/H_3BO_3) λ_{max} 268, 282 (sh), 364, 430 nm; IR (KBr) ν_{max} 3424, 1608, 1564, 1514 cm^{-1}; ^1H and ^{13}C NMR data; see Table 1; EIMS *m/z* 368 [M^+] (100), 351 (6.4), 339 (6.3), 322 (21.5), 313 (23.3), 294 (6.8), 283 (12.5), 267 (8.5), 255 (7.5), 179 (9.4), 159 (10.1), 121 (49.8); HREIMS *m/z* [M]$^+$ 368.1261, calcd for $C_{21}H_{19}O_6$ 368.1260.

Anti-HSV Activity. The anti-HSV activity assay was carried out according to a CPE (cytopathic effect)−MTT assay.[17] Vero cells (3×10^3 cells/well) were seeded into a 96-well plate. After 3−4 days of incubation, a confluent monolayer was generally obtained. After washing the cells, 100 μL of the virus solution, diluted with DME medium supplemented with 2% FBS, which was equivalent to 50% cell culture inhibitory dose ($CCID_{50}$), was added to each well and incubated for 60 min at 37 °C. After absorption of the virus, the culture medium was removed, and 100 μL of culture medium, including various concentrations of sample, was added to each well in duplicate, then incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 3 days. After removing the culture medium, MTT assay was carried out as described previously.[18] The antiviral effective concentration was expressed as an EC_{50}, the concentration of the sample required to inhibit virus-induced CPE by 50%. To make clear the cytotoxicity of sample, mock-infected cells were also prepared simultaneously. After addition of various concentrations of sample to each confluent monolayer in duplicate, further incubation followed for 3 days. After removing the culture medium, MTT assay was carried out. CC_{50} (50% cytotoxic concentration) was determined by comparing the relative cell number of the sample treated well with the cell number of the nontreated well. Compounds were tested at concentrations below 300 μg/mL.

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