

In modern medicine, the pre-eminent position of steroids is well recognised with the result that steroids rank next only to life-saving drugs in importance. Solasodine – a steroid present in *Solanum viarum* Dunal, (syn. *S. khasianum* var. *chatterjeeanum*) is a major source of steroidal raw material. The mucilaginous coating around the seed (exotesta) in this species has been reported to be very rich in solasodine (1, 2). Even though there are several reports regarding the distribution and accumulation of solasodine, its exact site of synthesis remains unknown. The study of ontogeny of the single-layered exotesta through histochemical and ultrastructural methods supported the implication of exotesta in alkaloid synthesis and storage (3). This study revealed rapid expansions of this layer during development and its transformation into a mucilaginous layer. Corroboratory evidence for the site of solasodine synthesis was sought through reciprocal grafts involving diploid and induced autotetraploid steroid-bearing *S. viarum* and steroid-free, edible *S. melongena* L. (brinjal) var. "Arka Shirish". The results are presented in this communication.

In fruits from brinjal scions grafted upon *S. viarum* (diploid), the presence of solasodine could not be detected (at 0.1 % level of sensitivity). But the fruits harvested from diploid and autotetraploid scions of *S. viarum* grafted upon brinjal stocks recorded  $2.23 \pm 0.39$  and  $2.14 \pm 0.13$  percent (dry weight basis) solasodine, respectively. These results indicate that the synthesis of solasodine is scion-specific. In this species, scion specificity of solasodine content was reported by Nanaiah (4) from a study of self- and reciprocal-grafts involving diploids and induced autotetraploids. Nanaiah found that the higher solasodine content (upto 50 %) in berries of induced autotetraploids over diploids of *S. viarum* was not affected in reciprocal grafts involving either diploidy or autotetraploidy as stock. This is understandable considering the fact that cholesterol, which is involved in the biosynthesis of solasodine, is translocated from leaves into berries in *S. viarum* as reported by Nes et al. (5). On the other hand, in tobacco, another solanaceous taxon, the roots are established to be the sites of alkaloid synthesis (6).

## Materials and Methods

Seedlings of both brinjal (*S. melongena* var. "Arka Shirish") and *S. viarum* (diploid and autotetraploid) were raised in a nursery. Both the species are cultivated. Scions of brinjal were grafted on diploid and autotetraploid *S. viarum* rootstocks and vice versa on two-months-old seedlings. After graft union and establishment of the scion on the rootstock, they were transplanted and grown in the field. The plants with brinjal scions upon autotetraploid *S. viarum* rootstock failed to establish.

Care was taken to remove flowers which were already present on the plant at the time of planting. Newly formed flowers were retained on the plant for the fruits to set. These fruits from the individual grafts were harvested separately and analysed for solasodine using the colorimetric method suggested by Birner (7).

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## A Novel Flavonoid from *Sophora flavescens*

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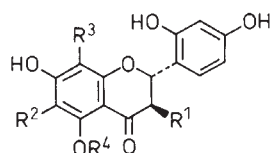
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**Abstract:** A new dihydroflavonol named kosamol A (**1**) has been isolated from the roots of *Sophora flavescens* (Leguminosae) along with twelve related flavonoids. The structure of **1** was determined to be (2*R*,3*R*)-5,7,2',4'-tetrahydroxy-6-(3-hydroxy-3-methylbutyl)-8-lavandulylflavanonol on the basis of spectral analyses.

The shrub, *Sophora flavescens* Aiton (Leguminosae) is spread widely in northeast Asian countries; it is currently rare in the wild, but commonly cultivated and available commercially in Korea. It has been applied frequently in folk medicine as an antipyretic, analgesic, anthelmintic, and as a stomachic. As constituents of this species, alkaloids (1, 2), pterocarpanes (3, 4), and flavonoids (4–12) have so far been reported. As a part of a search for potent antitumor substances from natural resources, we recently found that the methanolic extracts of the roots of *S. flavescens* showed a moderate cytotoxicity against several cultured human tumor cell lines (13).

In the course of investigation of cytotoxic principles from the MeOH extracts, we isolated a novel dihydroflavonol (flavanonol), tentatively named as kosamol A (**1**), as well as twelve other related flavonoids **2–13**, i.e., kushenol B, E, L, M, N, H, K, sophoraflavanone G, kurarinone, kuraridin, nor-kuraridin and kurarinol (Fig. 1). Flavonoids **2–13** had been isolated earlier from this species or from other species in the *Sophora* genus (4–12). In this report, we describe the isolation and structure elucidation of **1** on the basis of spectral analyses.



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<b>1</b>	—OH	—X	—Y	—H
<b>2</b>	—OH	—W	—Y	—H
<b>3</b>	—H	—W	—W	—H
<b>4</b>	—H	—W	—Y	—H
<b>5</b>	—H	—H	—Y	—H
<b>6</b>	—OH	—W	—W	—H
<b>7</b>	—H	—H	—Y	—CH <sub>3</sub>
<b>8</b>	—OH(α)	—H	—Y	—CH <sub>3</sub>
<b>9</b>	—H	—H	—Z	—H
<b>10</b>	—H	—H	—Z	—CH <sub>3</sub>
<b>11</b>	—OH	—H	—Z	—CH <sub>3</sub>
<b>12</b>	—OH(α)	—H	—Z	—CH <sub>3</sub>

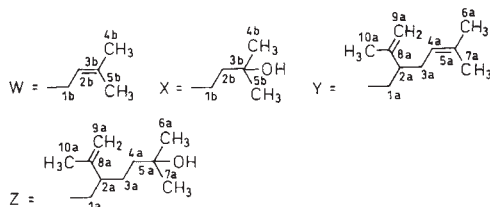


Fig. 1 Flavonoids from *Sophora flavescens*.

Purification of the EtOAc-soluble part of the extract of *S. flavescens* by repeated chromatography with silica gel and RP-18 resulted in the isolation of **1** as pale yellow amorphous powder. In the high resolution mass spectrum ( $[M]^+ m/z = 526.2563$ ), the empirical formula of **1** was confirmed as  $C_{30}H_{38}O_8$  (calculated 526.2567). The UV absorption pattern of **1** was identical with that of **2** (kushenol M,  $M_r$ : 508), which suggested the dihydroflavonol (flavanonol) skeleton of **1**. Compound **2** is a typical dihydroflavonol isolated from this species and has been identified to bear two side chains, an isoprenyl and a lavandulyl (5-methyl-2-isopropenyl-hex-4-enyl) at the C-6 and at C-8 positions of the A ring respectively, as illustrated in Fig. 1 (6, 10).

All proton and carbon signals of **1** were completely assigned by the aid of  $^1H$ - $^1H$  COSY,  $^{13}C$ - $^1H$  COSY, and COLOC (correlated spectroscopy for long range coupling) experiments. Most of proton and carbon signals of **1** were almost superimposable with those of **2**, except for slight differences of chemical shifts ( $\delta$ ). However, some proton signals due to the isoprenyl moiety had completely disappeared, whereas other new signals were observed which were assignable to two pairs of methylene protons at  $\delta = 1.52$  (2H, m),  $\delta = 2.64$  (2H, m) and a six-proton singlet at  $\delta = 1.15$ . These signals were established as the protons attached to C-2(2b), C-1(1b), C-4(4b), and C-5(5b) of the 3-hydroxy-3-methylbutyl group, respectively, by the aid of  $^{13}C$ - $^1H$  COSY and COLOC spectra of **1** (Table 1). It suggested that **1** does not bear the isoprenyl group but the 3-hydroxy-3-methylbutyl group, which was verified by the difference of mass ( $\Delta M = 18 [H_2O]$ ) between **1** and **2**. The linkage positions of the 3-hydroxy-3-methylbutyl group and of lavandulyl group

of **1** was unequivocally established to be at C-6 and at C-8 of the A ring by the COLOC experiment (Table 1), i.e., the methylene protons ( $\delta = 2.55$ ) at C-1(1a) of the lavandulyl group showed correlations with C-7, 8, 9, and other methylene protons ( $\delta = 2.64$ ) at C-1(1b) of the 3-hydroxy-3-methylbutyl group were correlated with C-5 of the A ring, respectively. The configuration of **1** was determined as *2R,3R*, by the CD analysis, which showed a positive maximum at 320 nm and a negative maximum at 295 nm (14, 15). Therefore, the structure of kosamol A (**1**), a novel dihydroflavonol from *S. flavescens* was established as (2*R,3R*)-5,7,2',4'-tetrahydroxy-6-(3-hydroxy-3-methylbutyl)-8-lavandulylflavanonol on the basis of spectral analyses. It could be postulated that **2** was biogenetically hydrated by some enzymatic reaction to yield **1**.

Table 1 Chemical shifts and correlations of **1** in DMSO- $d_6$ .

Position	$^1H$ shift	$^{13}C$ shift	COLOC
C-2	5.26 (d, 11.2)	77.6(d*)	C3; C4; C1'; C2'; C6'
C-3	4.62 (d, 11.2)	70.7 (d)	C2; C4
C-4		199.0(s)	
C-5		158.4(s)	
C-6		108.9(s)	
C-7		162.6(s)	
C-8		106.7(s)	
C-9		158.3(s)	
C-10		100.4(s)	
C-1'		114.1(s)	
C-2'		157.2(s)	
C-3'	6.32 (d, 2.2)	102.4(d)	C1'; C2'; C4'; C5'
C-4'		158.5(s)	
C-5'	6.32 (dd, 2.2, 8.5)	106.2(d)	C1'
C-6'	7.13 (d, 8.5)	129.6(d)	C2; C2'; C4'
OH-5	12.17 (brs)		C5; C6; C10
C-1a	2.55 (2H, m)	27.0(t)	C7; C8; C9; C8a
C-2a	2.36 (m)	46.5(d)	
C-3a	1.92 (2H, m)	30.7(t)	
C-4a	4.86 (t, 6.6)	123.5(d)	
C-5a		130.6(s)	
C-6a	1.52 (3H, s)	25.6(q)	C4a; C5a
C-7a	1.45 (3H, s)	17.7(q)	C4a; C5a
C-8a		147.8(s)	
C-9a	4.52, 4.44 (each 1H, brs)	110.9(t)	C2a
C-10a	1.52 (3H, s)	18.7(q)	C2a; C8a; C9a
C-1b	2.64 (2H, m)	17.0(t)	C5
C-2b	1.52 (2H, m)	42.3(t)	
C-3b		69.4(s)	
C-4b	1.15 (3H, s)	29.3(q)	C2b; C3b
C-5b	1.15 (3H, s)	29.3(q)	C2b; C3b

\* Multiplicities were established by DEPT pulse sequence.

## Materials and Methods

The roots of *S. flavescens* were collected from Cheongju, Korea, in October 1993. A sample was identified by the Herbarium of Natural Products Research Institute of Seoul National University, Korea, where a voucher specimen (SNU-9-357) is deposited.

The dried and chopped roots (3 kg) were extracted with MeOH at room temperature. Concentration of the solvents afforded an extract of about 240 g, which was suspended in  $H_2O$  and partitioned with  $CH_2Cl_2$  (35 g) and EtOAc (50 g), successively. The EtOAc soluble fraction was subjected to column chromatography.

graphy over silica gel 60 (70–230 mesh, 2 kg; column  $\varnothing$  = 15 cm) and eluted under gradient conditions with  $\text{CH}_2\text{Cl}_2$  in MeOH to afford six fractions:  $F_1$  MeOH 1% (41),  $F_2$  MeOH 2% (41),  $F_3$  MeOH 5% (41),  $F_4$  MeOH 10% (41),  $F_5$  MeOH 20% (41), and  $F_6$  MeOH 30% (41).  $F_3$  was purified again by CC over RP-18 (100 g; column  $\varnothing$  = 1.5 cm) using 60% MeOH as eluent, and finally purified by repeated preparative TLC on a silica gel plate (Merck) using the following system [hexane-EtOAc-AcOH (20:10:1)] ( $R_f$  = 0.2) to afford 85 mg of **1** as a pale yellow amorphous powder.  $F_1$ ,  $F_2$ ,  $F_4$ ,  $F_5$ ,  $F_6$ , and the  $\text{CH}_2\text{Cl}_2$  soluble fractions were also subjected to CC in a similar manner to yield twelve known flavonoid constituents, i.e., **3** (kushenol E, 55 mg), **4** (kushenol B, 250 mg), **5** (sophoraflavanone G, 480 mg), **6** (kushenol L, 30 mg), **2** (kushenol M, 500 mg), **13** (kuraridin, 110 mg), **7** (kurarinone, 120 mg), **8** (kushenol N, 20 mg), **1** (kosamol A, 85 mg), **9** (norkurarinol, 28 mg), **10** (kurarinol, 500 mg), **11** (kushenol H, 110 mg), and **12** (kushenol K, 100 mg) in decreasing  $R_f$  order. Compounds **2**–**13** were identified by comparison of spectroscopic data with those of authentic samples (4–12). Copies of the original spectra are obtainable from the author of correspondence.

All NMR spectra were obtained on Bruker AM-300 and Bruker AMX-500 spectrometers. High and low resolution MS were taken with a direct inlet and recorded with a JMS-DX303 mass spectrometer (JEOL). The optical rotations were determined on Autopol III automatic polarimeter. CD spectra were recorded on JASCO J-720 spectropolarimeter.

Kosamol A (**1**):  $\text{C}_{30}\text{H}_{38}\text{O}_8$  ( $M_r$ : 526) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : +36° (c 1.0, MeOH); IR (KBr):  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) = 3300, 1630, 1600, 1450; UV  $\lambda_{\text{max}}$  nm (MeOH): 298, 350; CD (c = 2.2  $\times$  10<sup>-4</sup>, MeOH)  $\theta$  (nm): +830 (320) (positive maximum), –1995 (295) (negative maximum); HR-MS: 526.2563 (calculated 526.2567); LR-MS (rel. int.): 526 ( $M^+$ , 10), 508 ( $M^+$  –  $\text{H}_2\text{O}$ , 40), 490 (20), 453 (20), 385 (100), 367 (80), 357 (60), 311 (70), 233 (95), 117 (90);  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and COLOC: Table 1. Kushenol M (**2**):  $\text{C}_{30}\text{H}_{36}\text{O}_7$  ( $M_r$ : 508) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : +18° (c 0.1, MeOH). Kushenol E (**3**):  $\text{C}_{25}\text{H}_{28}\text{O}_6$  ( $M_r$ : 424) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : –42° (c 0.1, MeOH). Kushenol B (**4**):  $\text{C}_{30}\text{H}_{36}\text{O}_6$  ( $M_r$ : 492) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : –38° (c 0.1, MeOH). Sophoraflavanone G (**5**):  $\text{C}_{25}\text{H}_{28}\text{O}_6$  ( $M_r$ : 424) pale yellow needles in  $\text{CHCl}_3$ /hexane; m.p. 175 °C. Kushenol L (**6**):  $\text{C}_{25}\text{H}_{28}\text{O}_7$  ( $M_r$ : 440) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : +12° (c 0.1, MeOH). Kurarinone (**7**):  $\text{C}_{26}\text{H}_{30}\text{O}_6$  ( $M_r$ : 438) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : +12° (c 0.1, MeOH); **7** was converted to the corresponding chalcone, kuraridin (**13**) by the treatment of  $\text{NH}_3$ . Kushenol N (**8**):  $\text{C}_{26}\text{H}_{30}\text{O}_7$  ( $M_r$ : 454) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : –52° (c 0.1, MeOH). Norkurarinol (**9**):  $\text{C}_{25}\text{H}_{30}\text{O}_7$  ( $M_r$ : 442) colorless amorphous powder;  $[\alpha]_D^{25}$ : –25° (c 0.1, MeOH). Kurarinol (**10**):  $\text{C}_{26}\text{H}_{32}\text{O}_7$  ( $M_r$ : 456) colorless needles in Hexane/EtOAc;  $[\alpha]_D^{25}$ : –48° (c 1.0, MeOH). Kushenol H (**11**):  $\text{C}_{26}\text{H}_{32}\text{O}_8$  ( $M_r$ : 472) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : +15° (c 1.0, MeOH). Kushenol K (**12**):  $\text{C}_{26}\text{H}_{32}\text{O}_8$  ( $M_r$ : 472) pale yellow amorphous powder;  $[\alpha]_D^{25}$ : –81° (c 1.0, MeOH). Kuraridin (**13**):  $\text{C}_{21}\text{H}_{30}\text{O}_6$  ( $M_r$ : 438) yellow amorphous powder, a chalcone of **7**;  $[\alpha]_D^{25}$ : +6° (c 0.1, MeOH).

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## Nealoetin A: A New C-Glucofuranosyl Chromone from *Aloe barbadensis*

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**Abstract:** The first C-glucofuranosyl compound, named as nealoetin A, was isolated from the leaves of *Aloe barbadensis*. Its structure was determined to be 8- $\alpha$ -D-glucofuranosyl-7-hydroxy-5-methyl-2-(2-oxopropyl)-4H-1-benzopyran-4-one on the basis of chemical and spectral evidence.

*Aloe* has long been used in folk medicine to treat constipation, burns and dermatitis (1). Anthraquinones, anthrones, chromones, and their C-glycosyl derivatives (2–9) were reported to be present in various species of *Aloe*. In particular, aloetin (3) was reported as the first natural C-glucopyranosyl chromone. In the course of searching for bioactive components from the leaves of *Aloe barbadensis*, we isolated a C-glucofuranosyl analogue of aloetin.