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The Structure of Kushenol M from *Sophora flavescens*

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The linkage pattern of two side chains *i.e.*, a isopentenyl and a lavandulyl group in kushenol M(**I**), a flavonoid from *Sophora flavescens* was established by the aid of 2-D NMR techniques, especially DEPT, ^{13}C-^{1}H COSY and COLOC experiments. Thus, **I** was unequivocally determined as (2*R*,3*R*)-5,7,2′,4′-tetrahydroxy-6-isopentenyl-8-lavandulylflavanonol.

Key words: Kushenol M, Flavonoid, *Sophora flavescens*, NMR, COLOC

INTRODUCTION

Kushenols are members of the flavanone or flavonol (dihydoflavonol) classes of compounds and are abundant constituents of *Sophora* species. During our search for bioactive components from the extract of the root of *Sophora flavences*, we recently isolated several known flavonoids including kushenol B, E, L and M (I). These substances show moderate cyctotoxicity against cultured human tumor cells *in vitro*.

During the course of our investigation, we realized that the structures of some of the kushenols remain partially unknown. In particular, a complete structural assignment for Kushenol M (I) had not been proven despite extensive characterization by Wu *et al.* (ref.). Specifically, the points of attachment of the isopentyl and lavanduyl side chains were unknown and two isomeric structures were postulated for Kushenol M (I) as shown in Chart 1.

Herein we report the structure elucidation of kushenol M (I). The exact placement of the side chains was unequivocally established by high field NMR spectroscopy studies using correlated spectroscopy for long range coupling (COLOC) techniques. Thus, the structure of (I) was determined to be (2*R*,3*R*)-5,7,2′,4′-tetrahydroxy-6-isopentyl-8-lavanduylflavanol.

MATERIALS AND METHOD

All NMR spectra were obtained on a Bruker AM-300 or Bruker AMX-500 spectrometer. Low resolution MS (70 eV) were taken with a direct inlet and recorded with a JMS-DX303 mass spectrometer (JEOL).

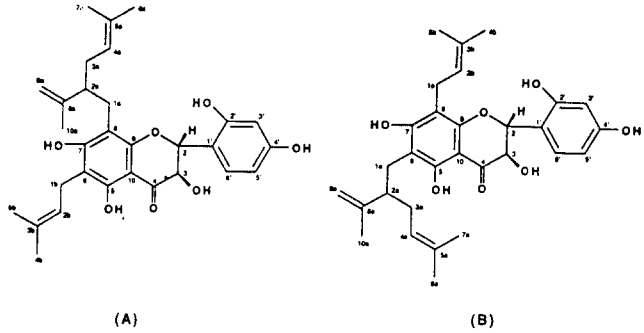

Chart 1. Tentative structures of kushenol M(**I**), postulated by Wu *et al.* (1985c).

Extraction and Isolation of kushenol M (I)

The dried roots (1.2 kg) of *Sophora flavescens* were purchased from the local Korean market and refluxed twice with MeOH for 3 hours. Concentration of the extracts afforded 90 g of crude MeOH extract, which was suspended in H_2O and partitioned with CH_2Cl_2 and EtOAc, successively. The CH_2Cl_2 soluble fraction (16 g) was subjected to column chromatography on SiO_2, using CH_2Cl_2 with increasing amounts of MeOH as the eluent. Six subfractions were obtained. Subfraction No. 4 (3.2 g) was purified with an RP-18 column, which finally afforded kushenol M (**I**, 450 mg) as a pale yellow amorphous powder, as well as four other flavonoids which were indentified as kushenol E (30 mg), kushenol B (130 mg), sophoraflavanone G (240 mg) and kushenol L (15 mg), respectively.

kushenol M(**I**) pale yellow amorphous powder. $[\alpha]_D$

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Table I. Chemical shifts and correlations of kushenol M in DMSO-d_6

Position	^{1}H shift	^{13}C shift	COLOC
C-2	5.28 (d, 11.3)	77.8(d*)	C3; C4; C1'; C2'
C-3	4.62 (d, 11.3)	70.9(d)	C2; C4
C-4		199.2(s)	
C-5		158.4(s)	
C-6		108.0(s)	
C-7		162.3(s)	
C-8		106.8(s)	
C-9		158.7(s)	
C-10		100.7(s)	
C-1'		114.2(s)	
C-2'		157.3(s)	
C-3'	6.35 (d, 2.0)	102.6(d)	C1'; C2'
C-4'		158.5(s)	
C-5'	6.29 (dd, 2.0, 8.5)	106.4(d)	C1'
C-6'	7.16 (d, 8.5)	129.6(d)	C2'; C4'
OH-5	12.16 (brs)		C5; C6; C10
C-1a	2.49 (2H, m)	27.1(t)	C8; C9
C-2a	2.35 (m)	46.6(d)	
C-3a	1.91 (2H, m)	30.7(t)	
C-4a	4.84 (t-like m)	123.6(d)	
C-5a		130.7(s)	
C-6a	1.48 (3H, s)	25.7(q)	C4a; C5a
C-7a	1.41 (3H, s)	17.7(q)	C4a; C5a
C-8a		147.9(s)	
C-9a	4.52, 4.44 (each 1H, brs)	111.1(t)	
C-10a	1.50 (3H, s)	18.9(q)	C8a; C9a
C-1b	3.19 (2H, m)	21.2(t)	C5
C-2b	5.05 (*t-like* m)	123.0(d)	
C-3b		130.5(s)	
C-4b	1.56 (3H, s)	25.7(q)	C3b
C-5b	1.66 (3H, s)	17.9(q)	C3b

*Multiplicities were established by DEPT.

+15 (c=0.1, MeOH), UV (λ_{max} : MeOH); nm (logε): 297 (4.5), 347 (4.0). MS *m/z* (rel. int. %); 508 (M^+, 15), 490 (M^+-H_2O, 5), 385 (82), 367 (33), 311 (35), 233 (100), 177 (67), 152 (12), 135 (10), 123 (25), 109 (15). ^{1}H-NMR, ^{13}C-NMR, DEPT and ^{13}C-^{1}H COSY: Table I, COLOC: Fig. 1.

RESULTS AND DISCUSSION

The repeated chromatographic separation of the CH_2Cl_2 soluble fraction of the MeOH extract from *Sophora flavescens*, actually by the activity-guided fractionation led to the isolation of a known constituent, kushenol M(**I**), which had been reported by Wu *et al.* (1985c).

The proton NMR spectrum of **I** showed signals due to a hydrogen-bonded hydroxy proton (δ; 12.16, C_5-OH), H-2 (δ; 5.28, d, J=11.3 Hz) and H-3 (δ; 4.62, d, J=11.3 Hz) of the flavanonol, a lavandulyl(5-methyl-2-isopropenyl-hex-4-enyl) group [δ; 1.41 (3H, s, -CH_3), 1.48 (3H, s, -CH_3), 1.50 (3H, s, -CH_3), 1.91 (2H, m), 2.35 (1H, m), 2.49 (2H, m), 4.44 (1H, brs), 4.52 (1H, brs) and 4.84 (1H, *t-like* m)], an isopentenyl group

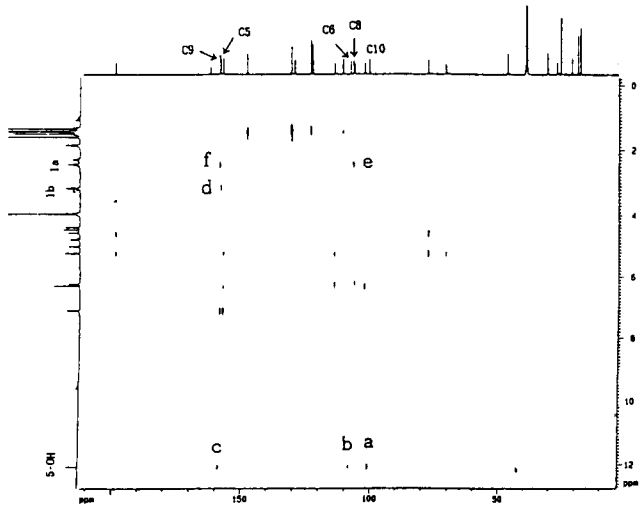

Fig. 1. COLOC spectrum of kushenol M(**I**).
Each cross peaks was due to the correlation between 5-OH and C10 (a), 5-OH and C6 (b), 5-OH and C5 (c), 1b-H and C5 (d), 1a-H and C8 (e) and between 1a-H and C9 (f).

[δ; 1.56 (3H, s, -CH_3), 1.66 (3H, s, -CH_3), 3.19 (2H, m) and 5.05 (1H, t-like m)] and three aromatic protons [δ; 6.29 (1H, dd, J=2.0, 8.5 Hz), 6.35 (1H, d, J=2.0 Hz) and 7.16 (1H, d, J=8.5 Hz)]. The chemical shifts and the splitting patterns of the three aromatic protons are consistent with a 2,4-dihydroxyphenyl moiety as the B ring of **I**. In the mass spectrum(MS) of **I**, the fragment, *m/z* 385 [M^+-123(C_9H_{15})], corresponds to a lavandulyl group. Other fragments, *m/z* 233 [M^+-123(C_9H_{15})-152($C_8H_8O_3$)], *m/z* 177 [M^+-123(C_9H_{15})-152($C_8H_8O_3$)-56(C_4H_8)] and *m/z* 152 ($C_8H_8O_3$), due to the retro Diels-Alder cleavage of the flavanonol, indicate that the B ring of **I** has just two hydroxy groups and does not contain either the lavandulyl or isopentenyl group.

All proton and carbon signals including thirteen quarternary carbons were completely assigned by the aid of 2-D NMR experiments, *i.e.*, ^{13}C-^{1}H COSY, DEPT and COLOC. The position of the isopentenyl and lavandulyl moieties, were unequivocally established by the COLOC experiment. The distinctive proton signal at δ12.16, which is assigned to a chelated hydroxyl proton at C-5, caused three cross peaks (*a*, *b* and *c*) with carbon signals at δ 100.7, δ 108.0 and δ 158.4 (Fig. 1). Hence it could be deduced that these carbon signals must correspond to C-5, C-6 and C-10, respectively. Furthermore, the carbon signal at δ 158.4 showed a cross peak(*d*) with the proton signal at δ 3.19, which was assigned to the two protons of the isopentenyl group(H-1b) and had been established by the ^{13}C-^{1}H COSY experiment. Therefore, the carbon signal at δ 158.4 should correspond to C-5, and the isopentenyl residue must be attached to the C-6 position. Additional spectroscopic evidence, *i.e.*, two cross peaks, e

and *f* (Fig. 1), due to the correlation between the two α protons of the lavandulyl group, H-1a (δ 2.49) and C-8 (δ 106.8), and also between H-1a (δ 2.49) and C-9 (δ 158.7), verified that the linkage point of the lavandulyl residue was at the C-8 position. Concerning the stereochemistry of **I** at C2 and C3, Wu *et al.* (1985c) had already confirmed the configuration of **I** as 2*R*,3*R* according to the CD(circular dichroism) experiment (Gaffield, 1970).

In conclusion, our assignment for kushenol M (I) is in agreement with the structure proposed by Wu *et al.* (1985c) *i.e.*, (2*R*,3*R*)-5,7,2′,4′-tetrahydroxy-6-isopentenyl-8-lavandulylflavanonol, by the aid of 2-D NMR techniques.

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REFERENCES CITED

Gaffield, W., Circular dichroism, optical rotatory dispersion and absolute configuration of flavanones, 3-hydroxyflavanones and their glycosides. determination of aglycone chirality in flavanone glycosides. *Tetrahedron,* 26, 4093-4108 (1970).

Ryu, S. Y., Choi, S. U., Lee, S. H., No, Z. and Ahn, J. W., Antitumor activity of *Sophora flavescens*, Abstract for the 43th annual convention of Korean Pharmaceutical Society. p.218 (1994).

Wu, L., Miyase, T., Ueno, A., Kuroyanagi, M., Noro, T. and Fukushima, S., Studies on the constituents of Sophora flavescens Ait. II. *Chem. Pharm. Bull.,* 33 (8), 3231-3236 (1985a).

Wu, L., Miyase, T., Ueno, A., Kuroyanagi, M., Noro, T. and Fukushima, S., Studies on the constituents of *Sophora flavescens* Ait. III. *Yakugaku Zasshi,* 105 (8), 736-741 (1985b).

Wu, L., Miyase, T., Ueno, A., Kuroyanagi, M., Noro, T., Fukushima, S. and Sasaki, S., Studies on the constituents of *Sophora flavescens* Ait. IV. *Yakugaku Zasshi,* 105(11), 1034-1039 (1985c).