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Stilbene oligomers in roots of *Sophora davidii*

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Abstract

Three stilbene oligomers, davidiols A–C were isolated from the roots of *Sophora davidii* in addition to the seven known phenols, leachianone A, sophoraflavanones G, H and I, miyabenol C, α-viniferin and ε-viniferin. Their structures and relative configurations were established by means of 2D-NMR spectroscopy including COLOC and PSNOESY. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Sophora davidii; Leguminosae; Stilbene Oligomer; Davidiol A; Davidiol B; Davidiol C

1. Introduction

In a previous paper, we reported the structural elucidation of the first naturally occurring resveratrol pentamer, davidiol D (Ohyama, Ichise, Tanaka, Iinuma & Burandt, 1996), from the roots of *Sophora davidii* (Franchet) Skeels (syn. *S. viciifolia*) (Isely, 1981). Further examination of the acetone extract of the roots resulted in isolation of three new stilbene oligomers named davidiols A–C, as well as seven known phenolic compounds (leachianone A, sophoraflavanones G–I, miyabenol C, α-viniferin and ε-viniferin). The structural determinations are described below.

2. Results and discussion

Davidiol A (**1**), obtained as a colorless powder, $[\alpha]_D^{29}$ − 272°, gave a $[M-H]^-$ at *m/z* 679 in the negative ion FABMS attributable to the empirical formula $C_{42}H_{31}O_9$. Absorption bands were also observed at 285 and 219 nm in the UV spectrum. The ^{1}H-NMR spectrum (Table 1) showed three sets of *ortho*-coupled aromatic protons in A_2X_2 spin system [δ 6.78, 7.21 (2H each, *d*, J = 8.8 Hz, H-3(5)a and H-2(6)a; 6.60, 7.04 (2H, *d*, J = 8.8 Hz, H-3(5)b and H-2(6)b; 6.61, 6.76 (2H each, *d*, J = 8.3 Hz, H-3(5)c and H-2(6)c], a set of three aromatic protons in an A_2X spin system [δ 6.19 (1H, *t*, J = 2.9 Hz, H-12c), 6.42 (2H, *d*, J = 2.9 Hz, H-14c)], a set of *meta*-coupled aromatic protons [δ 6.44, 6.55 (1H each, *br s*, H-12a and H-14a)], an aromatic proton in singlet (δ 6.02, H-12b) as well as eight hydroxyl protons [δ 6.56, 7.93, 7.96 (×2), 7.99, 8.07, 8.30, 8.31]. The ^{1}H-NMR and ^{1}H–^{1}H long range COSY spectra indicated the presence of a set of mutually coupled benzyl methine protons [δ 4.40 (*d* like *m*, H-8a), 6.08 (*d*, J = 2.9 Hz, H-7a)] and a sequence of successively coupled benzyl methine protons [δ 4.38 (*d*, J = 9.8 Hz, H-7c), 2.97 (*dd*, J = 11.7 and 9.8 Hz, H-8c), 4.23 (*d*, J = 11.7 Hz, H-8b), 5.27 (*br s*, H-7b)]. The CH COSY spectrum supplied the complete assignment of all protonated carbons as shown in Table 2. In the COLOC spectrum of **1** (Fig. 1), C-3(5)a, C-3(5)b and C-3(5)c were correlated with the hydroxyl protons at δ 8.31, 7.93 and 7.99, respectively, which indicated the presence of three 4-

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Table 1
^{1}H-NMR spectral data for **1**–**3** in acetone-d_6[a]

	1	**2**	**3**
2(6)a	7.21 (*d*, 8.8)	6.98 (*d*, 8.8)	6.96 (*d*, 8.3)
3(5)a	6.78 (*d*, 8.8)	6.86 (*d*, 8.8)	6.70 (*d*, 8.3)
7a	6.08 (*d*, 2.9)	5.05 (*br s*)	5.19 (*d*, 2.9)
8a	4.40 (*m*)	3.13 (*br s*)	4.64 (*d*, 2.9)
10a		5.90 (*d* like *m*)	
12a	6.44 (*br s*)	6.33 (*t* like *m*)	6.23 (*d* like *m*)
14a	6.55 (*br s*)	5.90 (*d* like *m*)	6.36 (*d* like *m*)
2(6)b	7.04 (*d*, 8.8)	6.12 (*d*, 8.3)	7.08 (*d*, 8.3)
3(5)b	6.60 (*d*, 8.8)	6.54 (*d*, 8.3)	6.68 (*d*, 8.3)
7b	5.27 (*br s*)	4.10 (*br s*)	6.00 (*br s*)
8b	4.23 (*d*, 11.7)	2.82 (*br s*)	3.69 (*br s*)
12b	6.02 (*s*)	6.17 (*d* like *m*)	6.22 (*d*, 2.0)
14b		6.00 (*d* like *m*)	6.69 (*m*)
2(6)c	6.76 (*d*, 8.3)	6.44 (*m*)[b]	7.35 (*d*, 8.8)
3(5)c	6.61 (*d*, 8.3)	6.44 (*m*)[b]	6.79 (*d*, 8.8)
7c	4.38 (*d*, 9.8)	3.98 (*br d*, 9.8)	6.01 (*d*, 11.2)
8c	2.97 (*dd*, 11.7, 9.8)	2.95 (*d*, 9.8)	4.80 (*d*, 11.2)
10c	6.42 (*d*, 2.9)		
12c	6.19 (*t*, 2.9)	6.38 (*br s*)	
14c	6.42 (*d*, 2.9)	6.68 (*br s*)	6.73 (*s*)
2(6)d			7.07 (*d*, 8.8)
3(5)d			6.76 (*d*, 8.8)
7d			5.48 (*d*, 6.3)
8d			4.37 (*d*, 6.3)
10(14)d			6.23 (*d*, 2.0)
12d			6.27 (*t*, 2.0)
OHs (C No.)	6.56 (13b), 7.93 (4b), 7.96 (11c, 13c), 7.99 (4c), 8.07 (13a), 8.30 (11a), 8.31 (4a) (each *br s*)	3.78 (7c), 7.82 (11c), 7.96 (4b), 8.03 (4c), 8.10 (13c), 8.19 (11a, 13a), 8.28 (13b), 8.32 (4a) (each *br s*)	8.15 (11*d*, 13*d*), 8.31 (4a, 13b), 8.36 (4b), 8.40 (13a), 8.45 (4c), 8.47 (4d) (each *br s*)

[a] All spectra were measured at 400 MHz.
[b] Overlapping signals.

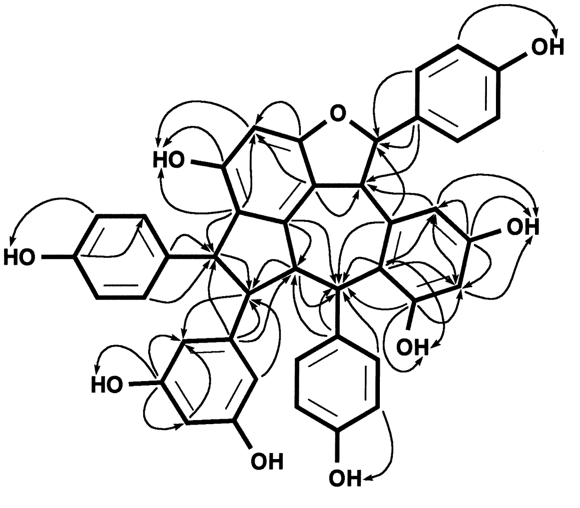


Fig. 1. CH long range correlations in the COLOC spectrum (J = 8 Hz) of **1**.

hydroxyphenyl groups (rings A_1, B_1 and C_1). The presence of a 3,5-dihydroxyphenyl group (ring C_2) was also established by the CH long range correlations between the hydroxyl groups at δ 7.96 (overlapping) and the carbon signals at δ 158.9 (C-13c) correlated with H-12c and 14c in the COLOC spectrum. H-14c was coupled with quaternary carbons at δ 156.8 (C-13a) and 157.9 (C-11a) which were additionally coupled with the hydroxyl groups at δ 8.07 and 8.30, indicating the occurrence of a 3,5-dihydroxy-1,2-disubstituted benzene ring (ring A_2). A hydroxyl group (δ 6.56) was correlated with an aromatic carbon (δ 96.0, C-12b) possessing a proton in singlet (H-12b) which had three long range correlations to the carbons at C-10b, 11b and 14b, substantiating the presence of a *penta*-substituted benzene ring such as ring B_2. These results showed that **1** was a resveratrol (3,5,4′-trihydroxystilbene) trimer bearing a dihydrobenzofuran ring in the molecule. The carbons at δ 129.9 [C-2(6)c], δ 108.3 (C-10c, 14c) and δ 129.6 [C-2(6)b] were coupled with the methine protons at H-7c, H-8c and

H-7b via ^{3}J in the COLOC spectrum. C-14b was further coupled with the benzyl methine proton at δ 4.38 (H-7c), indicating that C-14b was linked towards C-7c. The mutually coupled methine protons (H-7a and H-8a) were coupled with C-2(6)a and C-14a via ^{3}J correlations. As the carbon signal at δ 118.1 (C-10a), which was correlated to H-14a, had a cross peak with H-7b, the linkage between C-10a and C-7b was revealed. The linkage between C-8a and C-10b was deduced by the correlations such as C-10b/H-8a. These correlations observed at C-2(6)b/H-7b, C-2(6)c/H-7c and C-10c, 14c/H-8c indicated that rings B_1, C_1 and C_2 were substituted at C-7b, 7c and 8c, respectively. The ^{3}J long range correlations observed at C-1b, C-9c/H-8b indicated that H-8b existed between H-7b and H-8c. Finally, the planar structure of **1** was determined as Fig. 1 in consideration of the additional long range correlations observed at C-14a/H-8a, C-9a/H-7b, C-11a/H-7b, C-9b/H-7b, C-9b/H-8c and C-14b/H-7c. The relative sterostrucure of **1** was confirmed by the analysis of phase sensitive NOESY (PSNOESY) spectrum as shown in Fig. 2.

Table 2
^{13}C-NMR spectral data for **1**–**3** in acetone-d_6[a]

Number	1	2	3
1a	134.4	134.3	133.1
2(6)a	128.1	127.5	127.2
3(5)a	116.0	116.0	116.0
4a	155.8	157.8	157.7
7a	85.8	93.1	95.9
8a	50.4	54.3	55.8
9a	147.1	148.9	141.7
10a	118.1	106.9	119.9
11a	157.9	159.8	160.9
12a	101.3	101.7	97.9
13a	156.8	159.8	159.4
14a	104.0	106.9	108.5
1b	137.5	136.5	132.0
2(6)b	129.6	129.6	128.3
3(5)b	115.4	115.2	115.7
4b	158.1	156.9[b]	158.0
7b	36.5	53.2	86.4
8b	51.4	54.4	45.6
9b	143.1	147.8	140.1
10b	119.2	118.5	120.8
11b	159.4	161.6	160.4
12b	96.0	95.4	96.4
13b	154.7	159.9	159.4
14b	122.2	104.5	105.9
1c	134.1	136.5	131.2
2(6)c	129.9	128.7	129.2
3(5)c	115.6	116.1	116.1
4c	156.6	155.8[b]	158.4
7c	56.2	77.0	90.1
8c	67.5	63.8	52.8
9c	143.9	149.8	138.4
10c	108.3	122.8	121.3
11c	158.9	155.0	157.2
12c	102.0	102.3	111.2
13c	158.9	158.7	164.1
14c	108.3	107.4	99.7
2(6)d			128.2
3(5)d			116.2
4d			158.2
7d			94.5
8d			55.8
9d			145.1
10(14)d			107.1
11(13)d			159.6
12d			102.2

[a] All spectra were measured at 100 MHz. All carbons were assigned by means of CH COSY and COLOC spectra.

[b] Interchangeable.

Davidiol B (**2**), a brown solid, $\{\alpha\}_{D}^{29} - 82°$, gave an $[M\text{-}H]^-$ ion at m/z 697 corresponding to the empirical formula, $C_{42}H_{31}O_{10}$. The ^{1}H-NMR and HH long range COSY spectra exhibited the presence of three 4-hydroxyphenyl groups (rings A_1, B_1 and C_1), a 3,5-dihydroxyphenyl group (ring A_2), two 3,5-dioxygenated-2-substituted phenyl groups (rings B_2 and C_2) and three pair of mutually coupled methine protons [δ 3.98 (1H, *br d*, J = 9.8 Hz, H-7c), 2.95 (1H, *d*, J = 9.8 Hz, H-8c); 5.05, 3.13 (1H each, *br s*, H-7a and 8a); 4.10, 2.82 (1H each, *br s*, H-7b and 8b] as well as nine hydroxyl groups [δ 3.78 (*br s*); 7.82, 7.96, 8.03, 8.10, 8.19 (×2), 8.28, 8.32 (each *br s*, phenolic)]. The hydroxyl group caused a cross peak between H-7c in the HH long range COSY spectrum. The CH COSY spectrum supplied the complete assignment of all protonated carbons as shown in Table 2. In the COLOC spectrum (Fig. 3), H-7a and H-8a were correlated to C-2(6a) and C-10(14a), respectively. Moreover, those protons were coupled respectively with C-10b and C-11b, indicating that ring B_2 was fused to the dihydroxybenzofuran ring. The long range correlation via ^{3}J observed between C-10b/H-8b indicated that C-8b was

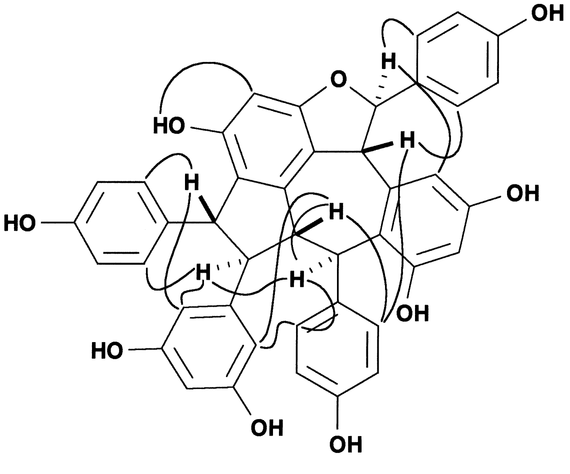


Fig. 2. NOE interactions in the PSNOESY spectrum of **1**.

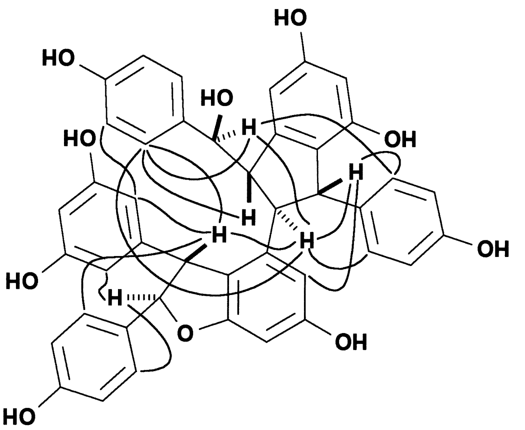


Fig. 3. CH long range correlations in the COLOC spectrum (J = 8 Hz) of **2**.

linked to ring B_2. Furthermore, H-7b was coupled with C-2(6)c on ring B_1. The linkage between C-7b and C-10c was established by correlation of C-11c/H-7b and C-10c/H-7b. On the other hand, the ^3J correlation between C-2(6)c and H-7c suggested that ring C_1 was attached at C-7c. Finally, the linkages between C-8b/C-8c and C-8c/C-9c, established by the long range correlations between C-9c/H-8c and C-9c/H-8b, substantiated the planar structure of **2** as shown in Fig. 3. The relative stereochemistry of **2** could be determined by the observation of PSNOESY spectrum as shown in Fig. 4.

Davidiol C (**3**), obtained as a brown solid, $\{\alpha\}_D^{29} - 124°$, gave an $[M-H]^-$ ion at m/z 903 corresponding to the empirical formula, $C_{56}H_{39}O_{12}$. The ^{1}H-NMR and HH long range COSY spectra exhibited the presence of four 4-hydroxyphenyl groups (ring A_1, B_1, C_1 and D_1)], a 3,5-dihydroxyphenyl group (ring D_2), two 3,5-dioxygenated-2-substituted phenyl groups (ring A_2 and B_2)], a penta-substituted benzene ring derived from a 3,5-dihydroxyphenyl group (ring C_2) and four mutually coupled methine protons [δ 5.19, 4.64 (each d, J = 2.9 Hz, H-7a and H-8a); 6.00, 3.69 (each $br\ s$, H-7b and H-8b); 6.01, 4.80 (each d, J = 11.2 Hz, H-7c and H-8c); 5.48, 4.37 (each d, J = 6.3 Hz, H-7d and H-8d)] as well as eight hydroxyl groups [δ 8.15, 8.31 (×2), 8.36, 8.40, 8.45, 8.47 (each $br\ s$)]. The following correlations revealed the presence of four resveratrol units in the molecule; C-2(6)a-/H-7a, C-14a/H-8a, C-2(6)b-/H-7b, C-14b/H-8b, C-2(6)c/H-7c), C-14c/H-8c, C-2(6)d/H-7d), C-11d(14)d/H-8d. The quaternary carbon resonanced at δ 119.9 was correlated to H-14a in the COLOC spectrum, indicating that the carbon was assigned to C-10a, whereas a carbon at δ 160.9 was assigned to C-11a by correlation with H-12a. Those two carbons showed correlations between H-7b and H-8b, so that the linkages between C-10a/C-8b and C-11a/C-7b through an oxygen at C-11a were established. Linkages between C-10b/C-8c, C-11b/c-7c, C-10c/C-8a and C-11c/C-7a were deduced in the same manner. The CH long range correlations between C-12c/H-8d and C-13c/H-7d indicated that C-8d and C-7d were fused at C-12c and the oxygen was attached to C-13c, respectively. Consequently, the planar structure of **3** was determined as shown in Fig. 5. The relative stereochemistry could also be determined as shown in Fig. 6 by observation of PSNOESY spectrum. However, the absolute stereochemical configuration for **1**, **2** and **3** have not yet been determined.

Seven known phenolic compounds were identified as

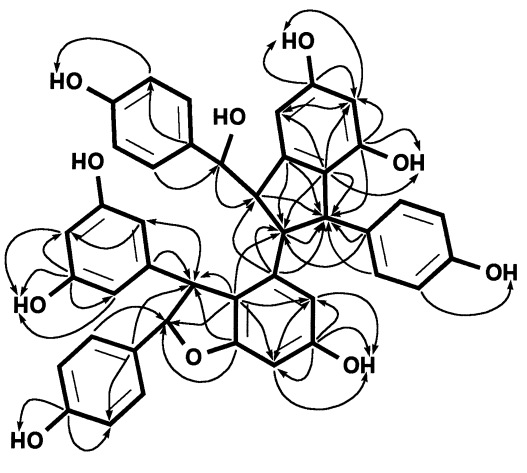


Fig. 4. NOE interactions in the PSNOESY spectrum of **2**.

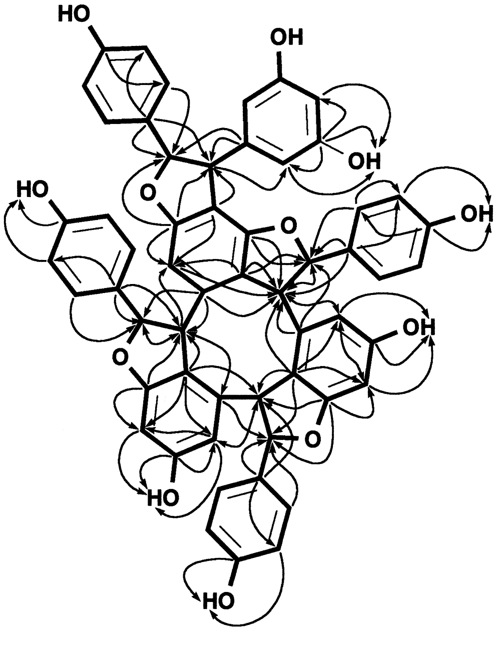
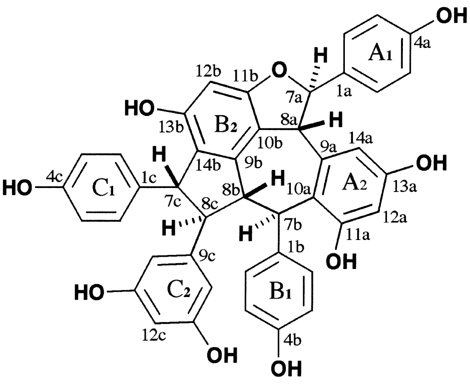
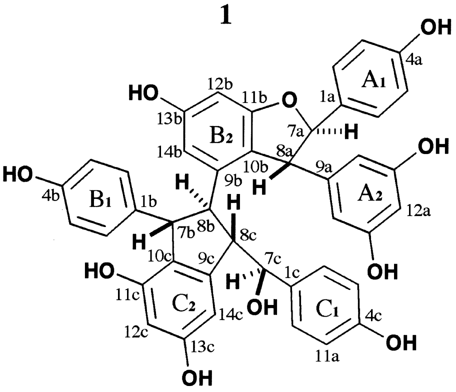


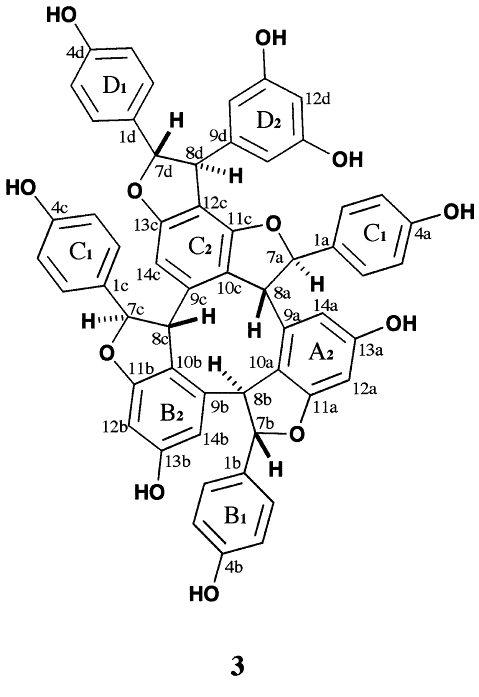
Fig. 5. CH long range correlations in the COLOC spectrum (J = 8 Hz) of **3**.



1



2



3

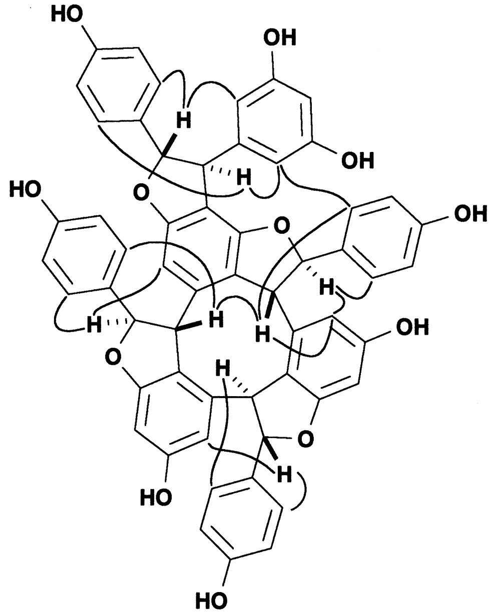


Fig. 6. NOE interactions in the PSNOESY spectrum of **3**.

four prenylated flavanones [leacihianone A (Iinuma, Tanaka, Mizuno, Shirataki, Yokoe, Komatsu & Lang, 1990), sophoraflavanone G (Shirataki, Yokoe, Noguchi, Tomimori & Komatsu, 1988), sophoraflavanones H and I (Shirataki, Noguchi, Yokoe, Tomimori & Komatsu, 1991)] and three resveratrol oligomers [miyabenol C (Kurihara, Kawabata, Ichikawa, Mishima & Mizutani, 1991), α-viniferin and ε-viniferin (Langcake & Pryce, 1977)] following analysis of their spectral data and direct comparison with authentic samples.

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR spectra were recorded in acetone-d_6 solution on JNM-EX-400 spectrometers (JEOL). Negative ion FABMS was measured on a JMS-DX-300 spectrometer equipped with JMA-3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrophotometer (Shimadzu), whereas optical rotations were obtained using a DIP-370 instrument (JASCO). Silica gel 60 (70–230 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography; Silica gel 60H (Merck) was used for vacuum liquid chromatography.

3.2. Plant material

Roots of *S. davidii* were collected in Texas, USA in May 1994. A voucher specimen is deposited in the herbarium of Gifu Pharmaceutical University.

3.3. Extraction and isolation

An acetone extract (50 g) of the dried and pulverized roots (900 g) of *S. davidii* was subjected to column chromatography on silica gel [eluted with benzene–acetone (15:1) and subsequent gradients) with eight fractions being collected. The fraction eluted with benzene–acetone (10:1) was further subjected to chromatography on silica gel eluted with *n*-hexane–acetone (3:1) as eluant to give leachianone A (43 mg), sophoraflavanone G (120 mg), sophoraflavanone H (45 mg), sophoraflavanone I (22 mg) and ε-viniferin (45 mg), respectively. The benzene–acetone (5:1) to (2:1) eluted fractions were combined and applied to a silica gel column eluted with a gradient of $CHCl_3$–MeOH. The $CHCl_3$–MeOH (10:1) eluted fraction was next loaded onto a Sephadex LH 20 column, this being eluted with MeOH, and further purified by silica gel vacuum liquid chromatography [benzene–acetone (2:1) and $CHCl_3$–MeOH (5:1)] and preparative TLC [$CHCl_3$–MeOH–H_2O (45:15:2)], respectively, to give **1** (40 mg), **2** (52 mg), **3** (32 mg), α-viniferin (25 mg) and miyabenol C (13 mg).

*3.4. Compound **1** (davidiol A)*

Colorless powder; $\{\alpha\}_D^{29} - 272°$ (c = 0.18, MeOH); Negative ion FABMS $[MH]^-$: m/z 679; UV λ_{max} (nm, MeOH): 285 (4.08), 219 (4.83).

*3.5. Compound **2** (davidiol B)*

Brown solid; $\{\alpha\}_D^{29} - 82°$ (c = 0.04, MeOH); Negative ion FABMS $[MH]^-$: m/z 697; UV λ_{max} (nm, MeOH): 284 (4.15), 223sh, 214 (4.96).

*3.6. Compound **3** (davidiol C)*

Brown powder; $\{\alpha\}_D^{29} - 124°$ (c = 0.11, MeOH); Negative ion FABMS $[MH]^-$: m/z 903; UV λ_{max} (nm, MeOH): 290sh, 284 (4.15), 225 (4.94).

The ^1H- and ^{13}C-NMR spectral data are summarized in Tables 1 and 2.

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