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ISOFLAVONOIDS IN ROOTS OF *SOPHORA SECUNDIFLORA*

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Key Word Index—*Sophora secundiflora*; Leguminosae; roots; 3-hydroxyisoflavanone; isoflavone; secundiflorol A–C.

Abstract—From the roots of *Sophora secundiflora*, three new isoflavonoids, secundiflorols A–C, were isolated in addition to 10 known flavonoids. The structures were determined by spectral analysis including 2D-NMR techniques.

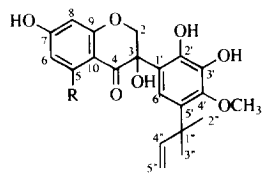
INTRODUCTION

In continuous studies on chemosystematics of the genus *Sophora* (Leguminosae), we have previously determined the structures of flavonoids and stilbenoids in several *Sophora* species; *S. leachiana* [1–4] in the U.S.A., *S. koreensis* (= *Echinosophora koreensis*) [5] in Korea, *S. exigua* in Thailand [6], *S. fraseri* [7] in Australia and *S. prostrata* [8] and *S. teraptera* [9, 10] in New Zealand. In the present paper, we describe the isolation and structural determination of 13 phenolic compounds including three new isoflavonoids in the roots of *S. secundiflora* (Ort.) DC. This evergreen shrub or tree, native to Mexico and the southwestern U.S.A., is classified in the subgenus *Styphnolobium* according to the treatment of the genus *Sophora* by Tsoong and Ma [11, 12]. Previous studies have reported isoflavonoids [13, 14], flavones, flavonols and their glycosides [15, 16] as chemical constituents of the species.

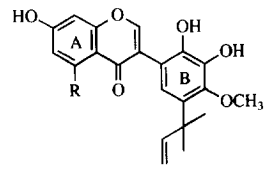
RESULTS AND DISCUSSION

An acetone extract of the roots of *S. secundiflora* was subjected to silica gel column chromatography eluted with *n*-hexane–acetone. Respective *n*-hexane–acetone (5:1) and (1:1) fractions were further separated with vacuum liquid chromatography and purified by preparative TLC and recrystallization to give **1**–**13**.

Compound **1**, obtained as needles gave $[M]^+$ at *m/z* 402 in the mass spectrum. The IR absorption bands (3400 and 1640 cm^{-1}) showed the presence of hydroxyls and a chelated α,β-unsaturated carbonyl group. The ^1H



1: R = OH 2: R = H



3: R = OH 4: R = H

(Table 1) and the ^{13}C NMR (Table 2) spectrum also showed the presence of an α,α-dimethylallyl group [δ 1.38 (Me × 2), 4.91 (*dd*, J = 11, 1 Hz), 4.93 (*dd*, J = 18, 1 Hz) 6.12 (*dd*, J = 18, 11 Hz)]. The ^1H NMR spectrum further exhibited a set of *meta*-coupled protons [δ 5.96 and 5.98 (each J = 2 Hz)], two doublets at δ 4.28 and 4.84 (each J = 11 Hz) near an oxygen function and an aromatic singlet proton (δ 7.04) in addition to methoxyl (δ 3.77) and chelated hydroxyl group (δ 11.98). The above doublets in a large coupling constant (δ 4.28 and 4.84) were assignable to either H-3 and H-2 in a flavanonol (3-hydroxyflavanone) skeleton or to H-2 in a 3-hydroxyisoflavanone [17]. Since the two protons were correlated with a carbon signal at δ74.7 tentatively assigned to C-2 in the ^{13}C–^1H COSY spectrum, they could be allotted to hydrogens at C-2, which indicated that **1** was a 3-hydroxyisoflavanone. The *meta*-coupled protons at δ 5.96 and 5.98 (each J = 2 Hz) and the base peak at *m/z* 153 in EI-mass spectrum supported **1** having a 5,7-dihydroxyl substitution on the A ring. A significant fragment ion at *m/z* 250 showed that two hydroxyls, the methoxy and the α,α-dimethylallyl group were located on the B ring. Three quaternary carbons bearing an oxygen function appeared at δ139.5, 143.8 and 149.8

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Table 1. ^{1}H NMR spectral data of compounds **1**–**4** (in Me_2CO-d_6)

H	1	2	3	4
2	4.28 *d* (11)	4.37 *d* (11)	8.31 *s*	8.45 *s*
3	4.84 *d* (11)	4.90 *m*[a]		
2		7.78 *d* (9)		8.17 *d* (9)
6	5.96 *d* (2)	6.61 *dd* (9, 2)	6.35 *d* (2)	7.12 *dd* (9, 2)
8	5.98 *d* (2)	6.40 *d* (2)	6.50 *d* (2)	7.03 *d* (2)
6′	7.04 *s*	6.89 *s*	6.73 *s*	6.73 *s*
2″,3″	1.38 *s*	1.38 *s*	1.44 *s*	1.45 *s*
4″	6.12 *dd* (18, 11)	6.08 *dd* (18, 11)	6.20 *dd* (18, 11)	6.20 *dd* (18, 11)
5′	4.91 *dd* (11, 1)	4.90 *m*[a]	4.93 *br d* (18)	4.92 *br d* (18)
	4.93 *dd* (18,1)		4.95 *br d* (11)	4.95 *br d* (11)
OMe	3.77 *s*	3.77 *s*	3.86 *s*	3.88 *s*
OH	7.90 *br s*	7.35 *s* ($C_{4'}$)	7.40 *br s* ($C_{4'}$)	7.28 *br s* ($C_{4'}$)
	11.98 *s*	8.24 *br s* ($C_{2'}$)	8.14 *s* ($C_{2'}$)	9.50 *br s* (C_7)
		9.50 *br s* (C_7)	9.83 *br s* (C_7)	
			12.54 *s* (C_5)	

Multiplicity and *J* values are given in parentheses. Assignment of hydroxyl groups is based on that of **2**.

[a]Overlapped.

Table 2. ^{13}C NMR spectral data of compounds **1**–**4** (in Me_2CO-d_6)

C	1	2	3	4
2	74.7	74.7	157.1	156.8
3	75.1	75.3	123.0	124.9
4	195.6	190.8	182.4	179.2
5	166.1	130.5	163.6	128.6
6	97.1	111.9	100.4	116.9
7	166.7	165.7	165.6	164.3
8	95.7	103.3	94.7	103.0
9	164.1	164.1	159.1	159.0
10	101.8	115.1	105.9	115.2
1′	119.1	119.1	114.1	117.1
2′	143.8	144.4	144.1	144.4
3′	139.5	139.9	141.0	141.6
4′	149.8	148.5	148.6	148.6
5′	132.9	132.5	134.1	134.3
6′	116.1	116.1	118.8	117.9
1″	41.0	41.0	41.0	41.0
2″,3″	28.1	28.4	28.5	28.4
4″	149.8	149.7	149.8	149.9
5′	109.9	109.9	110.0	110.0
OMe	60.0	59.8	59.9	59.7

All carbons of **1** were assigned by the aid of ^{13}C–^{1}H COSY and COLOC spectrum.

were assignable to the carbons of a 1,2,3-trioxygenated benzene ring. As the methoxyl carbon was observed at δ60.0, both *ortho*-positions were occupied by a substituent. In the ^{1}H NMR spectrum, NOEs were observed when both the aromatic singlet (δ7.04) assignable to H-6′ and the methoxyl group when a methyl of the α, α-dimethylallyl group was irradiated and vice versa, the methyl of the group was enhanced in addition to a hydroxyl group when the methoxyl was irradiated (Fig.1), which indicated that the B ring moiety was a 5′-α,α dimethylallyl-2′, 3′-dihydroxy-4′-methoxyl substitution. This substitution was confirmed by the COLOC spectrum in Fig. 2. Consequently, **1** is 5′-α, α-dimethylallyl-3,5,7,2′,3′-pentahydroxyl 4′-methoxy-isoflavanone, and named secundiflorol A.

Compound **2**, obtained as needles, gave $[M]^+$ at *m/z* 386 in the EI-mass spectrum. In the ^{1}H NMR spectrum, two one-proton doublets at δ4.37 and 4.90 (each J = 11 Hz) were assigned to H-2 in a 3-hydroxyisoflavanone the same as **1**. The ^{1}H NMR spectrum also exhibited three protons at δ6.40 (*d*, J = 2 Hz), 6.61 (*dd*, J = 9, 2 Hz) and 7.78 (*d*, J = 9 Hz) in an ABX spin system. In addition to the above data, a significant fragment ion at *m/z* 137 in the EI-mass spectrum (Fig. 3)

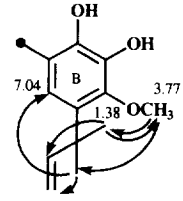

Fig. 1. NOEs in DIFNOE of **1**.

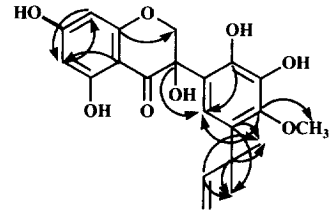

Fig. 2. COLOC spectrum of **1** (J = 10 Hz).

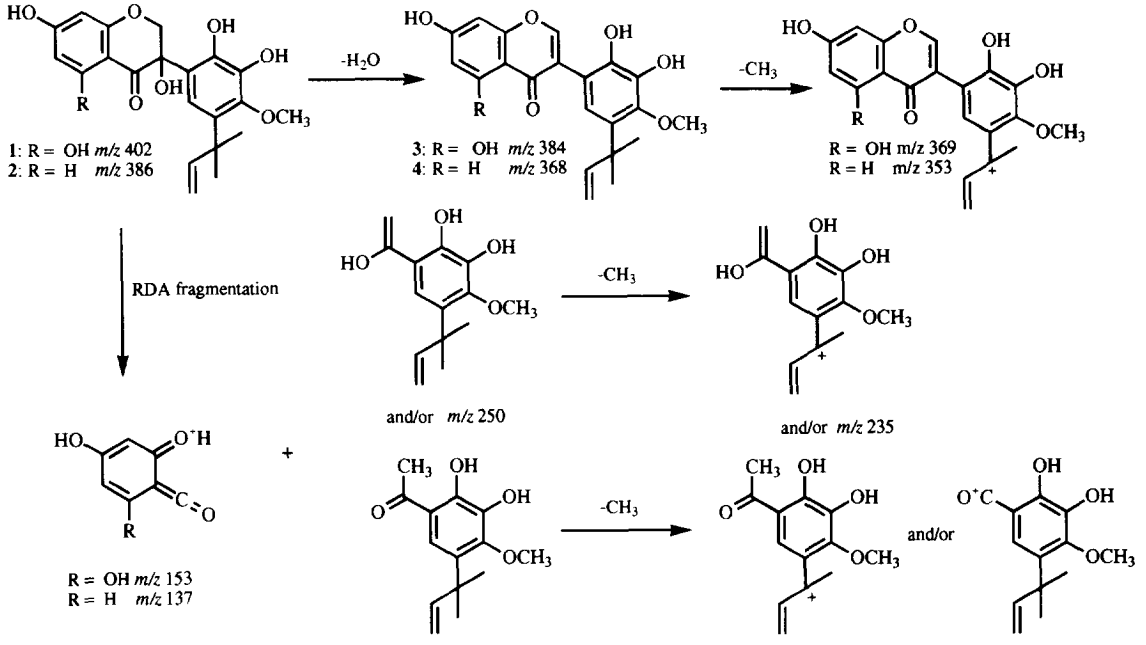

Fig. 3. EI-mass spectral fragmentation of **1**–**4**.

supported the A ring moiety being a 7-hydroxyl substitution. The ^{1}HNMR spectrum further showed an aromatic singlet (δ6.89) as well as the presence of an α,α-dimethylallyl [δ1.38 (Me × 2), 4.90 (*m*, $=CH_2$ and 6.08 (*dd*, J = 18, 11 Hz)], a methoxyl (δ3.77) and three hydroxyl groups (δ7.35, 8.24 and 9.50). In the difference NOE spectrum, NOEs were observed at a methyl in the α, α-dimethylallyl group and one of hydroxyl groups when the methoxyl was irradiated. The B ring moiety was then a 5′-α,α-dimethylallyl-2′, 3′-dihydroxy-4′-methoxyl substitution. Therefore, **2** is 5′-α,α-dimethylallyl-3,5,7,2′,3′-tetrahydroxy-4′-methoxyisoflavanone, which has been already isolated from the same plant and named secundifloran [14], but the ^{13}C NMR spectrum has not been assigned before.

Compounds **3** and **4**, obtained as a pale yellow oil, gave $[M]^+$ at *m/z* 384 and 368 in the EI-mass spectrum. The UV data and singlet proton observed in a lower field (**3**:δ8.31, **4**:8.41) in the ^{1}H NMR spectrum indicated that **3** and **4** were both isoflavones. In addition, the EI-mass spectral fragment ions caused by RDA cleavage (Fig. 3) and the ^{1}HNMR spectral data (Table 1) supported that the A rings in **3** and **4** were a 5,7-dihydroxyl and a 7-hydroxyl substitution, respectively. The B ring moiety of **3** and **4** was commonly a 5′-α,α-dimethylallyl-2′,3′-dihydroxy-4′-methoxyl substitution, which was supported by NOE experiments. Therefore, **3** and **4** are 5′-α,α-dimethylallyl-5,7,2′,3′-tetrahydroxy-4′-methoxyisoflavone and 5′-α,α-dimethylallyl-7,2′,3′-trihydroxy-4′-methoxyisoflavone, and are named secundiflorols B and C, respectively.

Compounds **5**–**12** were determined to be gancaonin B (**5**) [18], formononetin (**6**), calycosin (**7**), cladrin (**8**) [19], genistein (**9**), pratensein (**10**), medicapin (**11**) and 6-γ,γ-dimethylallyl-5,7,3′,4′,-tetrahydroxyflavanone (**12**) [20], respectively, by means of spectral analysis.

Flavonoid compounds with a 2′,3′,4′,-trioxygenated substitution on the B ring are very rare in the genus *Sophora*, but have been found in other leguminous plants such as *Dalbergia* [21] except for **9**.

EXPERIMENTAL

Plant material. The roots of *S. ophora secundiflora* were collected at Kingsville, Kleberg Co., Texas, U.S.A. in August 1993. A voucher specimen, Burandt no.2535, is deposited in the private herbarium of the collector.

Extraction and isolation. The air-dried and pulverized roots of *S. secundiflora* (700 g) were extracted with Me_2CO (3 l × 3) at room temp. and the solutions concd *in vacuo* to give brownish syrup (55 g). A part of the extract (50 g) was chromatographed on silica gel (1 kg) eluted with varying solutions of *n*-hexane–Me_2CO, 4:1 (frs 1–27), 5:3 (frs 28–50) and 1:1 (frs 50–63). Each fraction was 300 ml. frs 28–30 were recombined (800 mg) and further purified by VLC (Kiesel gel H) to give **11** (350 mg), frs 61–63 (1.7 g) were subjected to VLC eluted with cyclohexane–Me_2CO (3:1) (subfrs 1–10). Upon recombination, subfrs 5–7 were purified by prep. TLC (C_6H_6– EtOAc, 10:1) to give **1** (50 mg) and **2** (35 mg). The subfrs 6–9 were repeatedly purified by VLC and prep. TLC developed with C_6H_6–EtOH (10:1), $CHCl_3$–Me_2CO (10:1) and $CHCl_3$–EtOAc (10:1) to give **3** (8 mg), **4** (10 mg), **5** (22 mg), **6** (6 mg), **7** (120 mg), **8** (10 mg), **9** (4 mg), **10** (9 mg) and **12** (15 mg) with respect.

Compound **1** (*secundiflorol A*). Needles; mp 213 –214° (*n*-hexane–Me_2CO); $[\alpha]_D$ 0° EI-MS *m/z* (rel. int.): 402

([M]$^+$, 63), 384 (36), 369 (35), 351 (8), 256 (10), 250 (10), 235 (17), 199 (50), 193 (17), 153 (100), 137 (28), 105 (35), 91 (52); IR ν^{KBr} cm^{-1}: 3400, 2950, 1640, 1583; UV λ^{MeOH} nm: 281, 310sh, + $AlCl_3$: 310, 355, + $AlCl_3$/HCl: 309, 362, + NaOMe: 329, + NaOAc: 330 + NaOAc/H_3BO_3: 286, 330sh. ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

Compound **2** (*secundifloran*). Needles; mp 218–220° (*n*-hexane–Me_2CO); $[\alpha]_D$ 0°; UV λ^{MeOH} nm: 280, 310sh; EI-MS *m/z* (rel. int.): 386([M]$^+$, 27), 368 (46), 353 (31), 250 (8), 235 (13), 193 (15), 178 (13), 137 (100). ^{1}H and ^{13}C NMR spectral data and their assignments are listed in Tables 1 and 2.

Compound **3** (*secundiflorol B*). A pale yellow oil; EI-MS *m/z* (rel. int.) 384 ([M]$^+$, 100), 369 (60), 354 (38), 339 (15), 153 (23); UV λ^{MeOH} nm: 261, 295sh, 325sh, + $AlCl_3$: 269, 304, 371, + $AlCl_3$/HCl: 269, 306, 365, + NaOMe: 267, 321, + NaOAc: 271, 327, + NaOAc/H_3BO_3: 261, 300sh, 340sh. ^{1}H and ^{13}C NMR spectral data are presented in Tables 1 and 2.

Compound **4** (*secundiflorol C*). A pale yellow oil; EI-MS *m/z* (rel. int.): 368 ([M]$^+$, 63), 353 (20), 325 (100), 313 (79), 149 (18); 137 (8); UV λ^{MeOH} nm: 249, 261sh, 295sh, + $AlCl_3$: 267, 308sh, 355, + $AlCl_3$/HCl: 266, 307, 361, + NaOMe: 327, + NaOAc: 276, 333, + NaOAc/H_3BO_3: 250, 267, 300sh. ^{1}H and ^{13}C NMR spectral data are shown in Tables 1 and 2.

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