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ISOFLAVANONES IN ROOTS OF *SOPHORA SECUNDIFLORA**

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Key Word Index—*Sophora secundiflora*; Leguminosae; roots; isoflavanones; secundiflorols D–F.

Abstract—Three new isoflavanones, secundiflorols D–F, were isolated from the roots of *Sophora secundiflora* in addition to the nine known flavonoids, geraldol, pseudobaptigenin, pratensein, formononetin, calycosin, secundifloran and secundiflorols A–C. The structures of secundiflorols D–F were established as 5′-α,α-dimethylallyl-7,2′,3′-trihydroxy-4′-methoxyisoflavanone, 5′-α,α-dimethylallyl-5,7,3′-trihydroxy-2′,4′-dimethoxyisoflavanone and (3*R*)-2′-γ,γ-dimethylallyl-7,3′-dihydroxy-4′-methoxyisoflavanone, respectively, by means of spectral evidence.

INTRODUCTION

In the previous paper we reported the isolation and structural elucidation of 13 phenolic compounds, including the three new isoflavonoids, secundiflorols A–C, from the roots of *Sophora secundiflora* [2]. In our further study on the chemical constituents of these roots, three other new isoflavanones, named secundiflorols D–F (**1**–**3**), were isolated from an ether-soluble fraction. The present paper deals with the structural elucidation of these compounds.

RESULTS AND DISCUSSION

A MeOH extract of the roots of *S. secundiflora* was partitioned between Et_2O and H_2O. After concentration, the Et_2O extract was subjected to column chromatography on silica gel eluting with benzene–ethyl acetate. Respective fractions were further purified by low-pressure liquid chromatography on ODS using MeOH–H_2O and recrystallization to give **1**–**3**.

Compound **1**, obtained as needles, mp 248–250°, gave a $[M]^+$ at *m/z* 370 in the EI-mass spectrum. Absorption bands (3400 and 1660 cm^{-1}) in the IR spectrum showed the presence of hydroxyl groups and a carbonyl group. The ^1H NMR spectrum (Table 1) also exhibited the presence of an ABX-system based on two protons at C-2 (δ 4.42 and 4.56) and a proton at C-3 (δ-4.08) due to an isoflavanone skeleton. The spectrum also showed the presence of an α,α-dimethylallyl group [δ 1.30 (Me × 2), 4.88 (*dd*, *J* = 17.7, 1.1 Hz), 4.90 (*dd*, *J* = 10.6, 1.1 Hz) and 6.06 (*dd*, *J* = 17.7, 10.6 Hz], a methoxyl group (δ 3.67) and three hydroxyl groups (δ 8.49, 8.58 and 10.54 both of which were exchangeable with D_2O). In the aromatic region of the spectrum, signals of the remaining four protons were observed as a one-proton singlet (δ 6.42) due to the B-ring proton; an ABC system [δ 6.33 (1H, *d*, *J* = 2.5 Hz), 6.51 (1H, *dd*, *J* = 8.6, 2.5 Hz) and 7.66 (1H, *d*, *J* = 8.6 Hz)] could be assigned to the protons at C-8, C-6 and C-5 in the A-ring of the skeleton.

The EI-mass spectrum of **1** (Scheme 1) showed major ion peaks at *m/z* 234, 219 and 137, amongst which *m/z* 234 and 137 were caused by a retro-Diels-Alder rearrangement. In view of the ^1H NMR spectral data, the ion peak at *m/z* 137 was attributable to the A-ring, indicating that the ring had a hydroxyl group. Another significant ion at *m/z* 234 showed that two hydroxyls, the methoxyl group and the α,α-dimethylallyl group were attached to the B-ring. In the ^{13}C NMR spectrum (Table 2), three quaternary carbons bearing an oxygen function appeared at δ 138.6, 144.1 and 147.5 which were assignable to the carbons of a 1,2,3-trioxygenated benzene ring. As the methoxyl carbon was observed at δ 59.2, both *ortho*-positions were occupied by some substituents. The substitution was additionally supported by NOESY, HSQC and HMBC spectra. These chemical shift values were good

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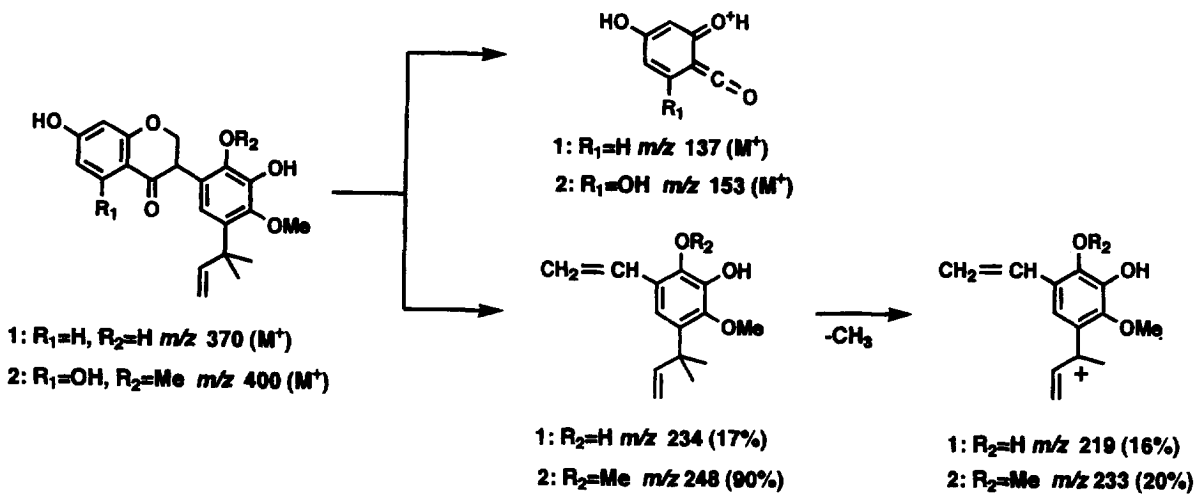
Table 1. ^{1}H NMR spectral data of compounds **1–3**

H	1*	2†	3*
2a	4.42 *dd* (11.0, 5.5)	4.46 *dd* (11.0, 5.5)	4.44 *d* (7.9)
2b	4.56 *t* (11.0)	4.55 *t* (11.0)	4.44 *d* (7.9)
3	4.08 *dd* (11.0, 5.5)	4.29 *dd* (11.0, 5.5)	4.05 *t* (7.9)
5	7.66 *d* (8.6)	—	7.68 *d* (8.6)
6	6.51 *dd* (8.6, 2.5)	5.96 *d* (2.1)	6.52 *dd* (8.6, 2.4)
8	6.33 *d* (2.5)	5.97 *d* (2.1)	6.36 *d* (2.4)
5′	—	—	6.74 *d* (8.6)
6′	6.42 *s*	6.68 *s*	6.47 *d* (8.6)
1″	—	—	3.34 *br d* (6.4)
2″,3″	1.30 *s*	1.37 *s*	5.03 *t* (6.4)‡
4″	6.06 *dd* (17.7, 10.6)	6.13 *dd* (17.7, 10.7)	1.61 *s*
5″	4.88 *dd* (17.7, 1.1)	4.92 *dd* (10.7, 1.2)	1.65 *s*
	4.90 *dd* (10.6, 1.1)	4.94 *dd* (17.7, 1.2)	—
OMe	3.67 *s*	3.77 *s*, 3.79 *s*	3.75 *s*
OH	8.49 *br s*	7.80, 9.50 *br s*	8.44 *s*
OH	8.58 *br s*	12.28 *s* (C-5)	10.58 *s*
OH	10.54 *br s*	—	—

Coupling constants (in parentheses) in Hz.
* In DMSO-d_6.
† In Me_2CO-d_6.
‡ 2″-H.

agreement with those of secundiflorol and secundiflorol C [2]. Consequently, the structure of **1** is 5′-α, α-dimethylallyl-7,2′,3′-trihydroxy-4′-methoxyisoflavanone, named secundiflorol D.

Compound **2**, obtained as solid, gave a $[M]^+$ at *m/z* 400 in the EI-mass spectrum. IR absorption bands (3400 and 1640 cm^{-1}) showed the presence of hydroxyls and a chelated carbonyl group. The ^{1}H NMR spectrum (Table 1) showed the presence of an ABX-system based on protons at C-2 (δ 4.46 and 4.55) and at C-3 (δ 4.29) in an isoflavanone, the same as in **1**. The spectrum also exhibited the presence of an α,α-dimethylallyl group [δ 1.37 (Me × 2), 4.92 (*dd*, *J* = 10.7, 1.2 Hz), 4.94 (*dd*, *J* = 17.7, 1.2 Hz) and 6.13 (*dd*, *J* = 17.7, 10.7 Hz)], two methoxyl groups (δ 3.77, 3.79) and three hydroxyl groups (δ 7.80, 9.50 and 12.28; exchangeable with D_2O). In the aromatic region of the spectrum, signals of the remaining three protons appeared in a one proton singlet (δ 6.68) due to the B-ring and two *meta*-coupled protons at δ 5.96 (1H, *d*, *J* = 2.1 Hz) and 5.97 (1H *d*, *J* = 2.1 Hz). A base peak at *m/z* 153 in the EI-mass spectrum supported that **2** had a 5,7-dihydroxyl substitution on the A-ring. The chemical shift value of the B-ring carbons were good agreement with those of secundiflorols A and B [2]. In the NOESY spectrum, significant NOEs were observed between the methoxyl methyl protons and methyl protons in an α,α-dimethylallyl group and between the proton of C-3 and the methoxyl methyl protons (Fig. 1). The B-ring moiety thus had a 5′-α,



Scheme 1. Mass spectral fragmentation of compounds **1** and **2**.

Table 2. ^{13}C NMR spectral data of compounds 1–3

C	1*	2†	3*
2	70.1	71.4	71.4
3	47.7	47.7	47.7
4	190.4	198.1	190.9
5	128.9	165.6	129.0
6	110.5	96.9	110.6
7	164.2	167.2	164.5
8	102.3	95.6	102.4
9	163.3	164.5	163.2
10	114.1	103.5	114.1
1′	117.1	123.4	127.3
2′	144.1	147.1	127.5
3′	138.6	144.4	143.9
4′	147.5	148.7	146.4
5′	131.0	137.4	109.3
6′	117.9	118.8	119.0
1″	39.7	41.1	25.0
2″	27.9	28.4	123.3
3″	27.9	28.4	130.3
4″	148.7	149.5	25.4
5″	109.4	110.1	17.8
OMe	59.2	60.3, 60.9	55.7

All carbons assigned with the aid of HSQC and HMBC.
* In DMSO-d_6.
† In Me_2CO-d_6.

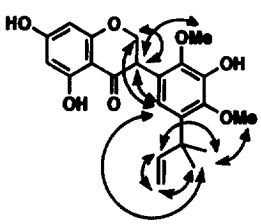
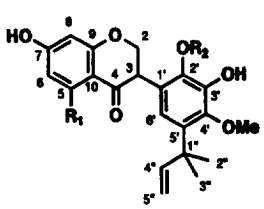
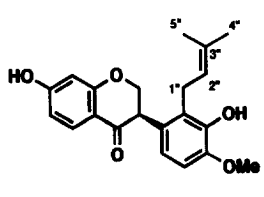


Fig. 1. NOEs in NOESY spectrum of compound **2**.

α-dimethylallyl-3′-hydroxy-2′,4′-dimethoxyl substitution. Therefore, the total structure of **2** was 5′-α, α-dimethylallyl-5,7,3′-trihydroxy-2′,4′-dimethoxyisoflavanone, named secundiflorol E.



1: R_1=H, R_2=H
2: R_1=OH, R_2=Me



3

Compound **3**, obtained as pale yellow needles, mp 188–190°, gave a $[M]^+$ at *m/z* 354 in the EI-mass spectrum. The ^1H NMR spectrum (Table 1) showed the presence of an A_2B-system due to two protons at C-2 (δ 4.44, *d*, *J* = 7.9 Hz) and a proton at C-3 (δ 4.05, *t*, *J* = 7.9 Hz) in an isoflavanone skeleton, as well as a methoxyl (δ 3.75) and two hydroxyl groups (δ 8.44 and 10.58; both exchangeable with D_2O). The difference from the other two isoflavanones is that **3** has a γ,γ-dimethylallyl group [δ 1.61, 1.65 (Me × 2), 3.34 (2H *br d*, *J* = 6.4 Hz), 5.03 (1H, *t*, *J* = 6.4 Hz)] instead of the α,α-dimethylallyl groups in **1** and **2**. In addition to the above data, a significant fragment ion at *m/z* 137 in the EI-mass spectrum demonstrated that the A-ring had a 7-hydroxyl substitution. Because two important cross-peaks were observed in the HSQC spectrum of **3**, the carbon signals at δ 109.3 and 119.0 were assigned to C-5′ and C-6′, and the proton signals at δ 6.74 and 6.47 were then ascribed to H-5′ and H-6′, respectively. The positions of the γ,γ-dimethylallyl, hydroxyl and methoxyl group on the B-ring were deduced from the HSQC and NOESY spectra, leading to precise assignment of the carbon signals [δ 127.3 (C-1′), 127.5 (C-2′), 143.9 (C-3′), 146.4 (C-4′), and 25.0 (C-1″)]. The signal of the methylene protons (δ 3.34) was correlated with the signals of C-1′, C-2′ and C-3′ ($^2J_{C\text{-}H}$ and $^3J_{C\text{-}H}$) in the HMBC spectrum. In the NOESY spectrum, cross-peaks were observed at δ 6.74 (H-5′) and 3.75 (OMe), 4.44 (H-2) and 6.47 (H-6′), and 4.05 (H-3) and 3.34 (H-1″), respectively. The chemical shift value of C-1″ (δ25.0) was in good agreement with the situation that one of the *ortho*-positions to a prenyl group was replaced with an alkyl or alkenyl group, and another was with an oxygenated substituent (type 3) [3]. Therefore, the B-ring was concluded to have 2′-γ,γ-dimethylallyl-3′-hydroxy-4′-methoxyl substitution. The configuration at C-3 was determined as *R* from the observation that the CD spectrum indicated a positive Cotton effect at 330 nm [4]. The structure of **3** was thus (3*R*)-2′-γ,γ-dimethylallyl-7,3′-dihydroxy-4′-methoxyisoflavanone, named secundiflorol F.

Compounds **4** and **5** were characterized as geraldol [5, 6] and pseudobaptigenin [7] by means of spectral analysis. As the ^{13}C NMR spectrum of geraldol has not been assigned yet, the assignment is given in the Experimental.

EXPERIMENTAL

General. Mps: uncorr. MS: direct inlet, 70 eV. ^1H and ^{13}C NMR: 500 and 125 MHz, respectively.

Plant material. Roots of *S. secundiflora* (Ort.) Lag. ex DC. were collected in the Amistad Recreation Area near Del Rio, Texas, U.S.A., in April 1983. A voucher specimen is deposited in the Garden of Medicinal Plants in Josai University.

Extraction and isolation. Air-dried and cut roots (550 g) were extracted 3 × with MeOH under reflux. The MeOH extract (102 g) after conc was shaken with Et_2O and H_2O. The Et_2O extract was concd and the residue (61.2 g) chromatographed on silica gel CC with benzene–EtOAc (10:0–1:1) (5 were monitored by TLC) to elute a mixt. of **5** and pratensein, a mixt. of **2**, formononetin and secundiflorols A and C, **1** and secundifloran, a mixt. of secundiflorol B, **3** and **4** (131 mg) and calycosin (234 mg), successively. The mixt. of **5** and pratensein was rechromatographed on silica gel CC with *n*-hexane–Me_2CO (4:1) to give **5** (4.2

mg) and pratensein (9 mg). The other mixed frs were subjected to low-pressure LC on octadecyl-bonded silica gel (ODS) (CPO-HS-221-20, Kusano Sci., Japan) eluting with MeOH–H_2O (4 : 1) to yield **2** (30 mg), formononetin (142 mg), secundiflorol A (8 mg), secundiflorol C (764 mg), **1** (240 mg) and secundifloran (16 mg). A mixt. of secundiflorol B and **3** was also applied to LC on ODS eluting with MeOH–H_2O (3 : 1) to give secundiflorol B (191 mg) and **3** (67 mg).

Compound 1 (*secundiflorol D*). Needles (*n*-hexane–Me_2CO), mp 248–250°. $[\alpha]_D$ 0°. HR-EIMS: *m/z* 370.1415 $[M]^+$, (Calcd for $C_{21}H_{22}O_6$, 370.1416). EIMS *m/z* (rel. int.): 370 $[M]^+$, 47), 234 (17), 219 (16), 137 (100). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950 (CH), 1660 (C=O), 1600, 1580 (arom. C=C). UV λ_{max}^{MeOH} nm: 292, 336sh, $+AlCl_3$: 293, 342sh, +NaOAc: 290sh, 330. ^1H and ^{13}C NMR: Tables 1 and 2.

Compound 2 (*secundiflorol E*). Solid. $[\alpha]_D$ 0°. HR-EIMS: *m/z*: 400.1520 $[M]^+$, (Calcd for $C_{22}H_{24}O_7$ 400.1522). EIMS *m/z* (rel. int.): 400 $[M]^+$, 72), 385 (12), 248 (90), 233 (20), 221 (15), 201 (42), 173 (21), 153 (100). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950 (CH), 1640 (C=O), 1600, 1540 (arom. C=C). UV λ_{max}^{MeOH} nm): 292, 336sh, $+AlCl_3$: 308, 362sh, +NaOAc: 290sh, 330. ^1H and ^{13}C NMR: Tables 1 and 2.

Compound 3 (*secundiflorol F*). Pale yellow needles (*n*-hexane–Me_2CO), mp 188–190°. $[\alpha]_D^{22}$ −16° (*c* 0.5, MeOH). HR-EIMS: *m/z*: 354.1467 $[M]^+$, Calcd for $C_{21}H_{22}O_5$, 354.1468). EIMS *m/z* (rel. int.): 354 $[M]^+$, 47), 298 (17), 218 (16), 137 (100). IR ν_{max}^{KBr} cm^{-1}): 3400 (OH), 2950 (CH), 1670 (C=O), 1600, 1540 (arom. C=C). UV λ_{max}^{MeOH} nm): 292, 336sh, $+AlCl_3$: 295, 341sh, +NaOAc: 290sh, 330. CD (*c* 5.0×10^{-3}, MeOH) $[\theta]$ (nm): 0, (250), −4248 (300), 0 (315), +7080 (330), 0(350). ^1H and ^{13}C NMR: Tables 1 and 2.

Compound 4 (*geraldol*). Yellow needles (MeOH), mp > 300°. ^1H NMR (DMSO-d_6) δ: 3.90 (3H, *s*, OMe), 6.96 (1H, *dd*, *J* = 8.9, 2.4 Hz, H-6), 6.99 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.02 (1H, *d*, *J* = 2.4 Hz, H-8), 7.75 (1H, *dd*, *J* = 8.5, 2.2 Hz, H-6′), 7.83 (1H, *d*, *J* = 2.2 Hz, H-2′), 7.98 (1H, *d*, *J* = 8.9 Hz, H-5), 9.14, 9.71, 10.78 (each 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 55.8 (OMe), 102.1 (C-8), 111.7 (C-2′), 114.3 (C-10), 114.8 (C-6), 115.6 (C-5′), 121.5 (C-6′), 122.6 (C-1′), 126.5 (C-5), 137.3 (C-3), 145.0 (C-2), 147.4 (C-3′), 148.4 (C-4′), 156.4 (C-9), 162.3 (C-7), 172.1 (C-4).

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