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NAPHTHOQUINONES AND COUMARINS FROM *IMPATIENS BALSAMINA* ROOT CULTURES

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Key Word Index—*Impatiens balsamina*; Balsaminaceae; root cultures; naphthoquinones; coumarins; methylene-3,3′-bilawsone; lawsone; 2-methoxy-1,4-naphthoquinone; scopoletin; isofraxidin.

Abstract—A novel natural bisnaphthoquinone, methylene-3,3′-bilawsone, was isolated from root cultures of *Impatiens balsamina*, along with two naphthoquinones (lawsone and 2-methoxy-1,4-naphthoquinone), two coumarin derivatives (scopoletin and isofraxidin) and a sterol (spinasterol).

INTRODUCTION

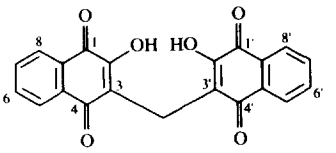
Root cultures of *Impatiens balsamina* have been established in our laboratory in order to study their ability to biosynthesize secondary metabolites. The cultures were initiated from young leaf explants using B5 medium supplemented with 0.1 mg l^{-1} NAA, 0.1 mg l^{-1} kinetin and 1.0 mg l^{-1} BA. Analysis of the ethyl acetate and methanolic extracts of the cultured roots by TLC revealed the presence of naphthoquinone and coumarin derivatives. We report here on the isolation and structural elucidation of these compounds and of a new natural naphthoquinone derivative.

RESULTS AND DISCUSSION

The dried roots of *I. balsamina* root cultures afforded six compounds (**1**–**6**). Compounds **1**, **2**, **3** and **5** were identified as the known compounds 2-methoxy-1,4-naphthoquinone, lawsone (2-hydroxynaphthoquinone), spinasterol and scopoletin, respectively. The naphthoquinones **1** and **2** [1–4] and the coumarin **5** [4, 5] have been reported to be present in *I. balsamina* leaves, and the sterol **3** [6, 7] has been found in the seeds.

The NMR spectra of compound **1** have been reported previously [3], here, we show the complete and unambiguous assignments using 2D NMR techniques (Fig. 1).

Compound **4** exhibited a UV band with λ_{max} 271.5 nm, suggesting the presence of the same chromophore as the other naphthoquinones. It was identified as diphthiocol by the following spectroscopic data. The proton noise decoupled ^{13}C NMR spectrum (Table 1) suggested that the molecule contained 11 carbons, yet the results of the high resolution mass spectrum gave a molecular formula



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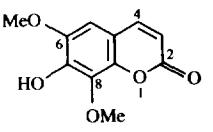

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Table 1. ^{13}C NMR data for compounds **1**, **2** and **4** (100 MHz)

C	**1** ($CDCl_3$)	**2** (CD_3OD)	**4** (DMSO-*d*)*
1	180.0	182.9	183.5
2	160.5	158.9	121.9
3	110.0	111.3	155.0
4	184.9	185.0	180.7
4a	132.1	133.2	129.8
5	126.3	126.3	125.8
6	134.4	135.2	133.0
7	133.4	133.6	134.4
8	126.8	126.5	125.5
8a	131.1	131.0	131.9
1′		—	183.5
2′		—	121.9
3′		—	155.0
4′			180.7
4a′		—	129.8
5′		—	125.9
6′		—	133.0
7′		—	134.4
8′		—	125.5
8a′		—	131.9
2-CH_2		—	17.9
OMe	56.5	—	

*At 125 MHz.

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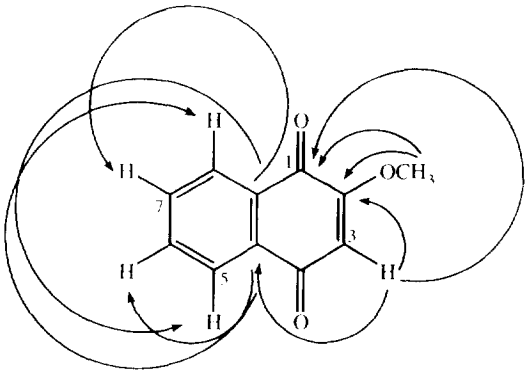

Fig. 1. Important ^{13}C–^{1}H long-range correlations observed by heteronuclear COSY of compound **1**.

of $C_{21}H_{12}O_6$ (m/z 360.0631 $[M]^+$; calc. for $C_{21}H_{12}O_6$: 360.0628). This inconsistency suggested that 10 signals were doubled. The pattern was similar to that of **2**, except for the absence of the signal at δ111.3 (C-3 of **2**), and the appearance of signals at δ121.9 (C-3 of diphthiocol) and 17.9 (C-9 of diphthiocol). A DEPT pulse sequence and single frequency off resonance spectra showed that those two signals were those of quaternary and methylene carbons, respectively.

The ^{1}H NMR of **4** also showed close similarity to that of **2** (Table 2). The fine splitting in the aromatic region was also the same as **2**, indicating that this molecule was composed of the same naphthoquinone moiety. The results of 2D NMR of **4** are shown by correlation arrows in Fig. 2. Following the additive rule in chemical shift assignment, bis-benzylmethylene gives the reasonable chemical shift values of 3.9–4.0 ppm. It is plausible that the methylene carbon bonds to two aromatic rings or similar systems can afford the same electronic atmosphere as a benzene ring. This consideration was supported by the result of the mass measurement. Therefore, the structure of **4** was clearly identified as methylene-3,3′-bilawsone (diphthiocol).

Compound **6** was identified by ^{1}H and ^{13}C NMR comparisons (Table 3). Irradiation of the methoxyl protons at δ3.94 enhanced the H-5 signal at δ6.66 by 12.83%. This compound is, therefore, isofraxidin (8-methoxyscopoletin), which has been isolated from the bark of German ash wood [8].

EXPERIMENTAL

General. TLC: silica gel precoated Al sheets (Merck); CC: silica gel (40–63 μm, Merck) and Sephadex LH-20 (Pharmacia); MPLC: prepacked Lobar column (Li Chroprep, Diol, 40–63 μm, Merck) at a flow rate of 2 ml min^{-1}; MS: Finnigan MAT TSQ 70 triple stage quadrupole instrument and JEOL HX-110; NMR: 500 MHz (^{1}H) and 100 MHz (^{13}C).

Root cultures. Root cultures of *I. balsamina* L. were initiated from young leaf explants on B5 solid medium containing 0.1 mg l^{-1} NAA, 0.1 mg l^{-1} kinetin, 1.0 mg l^{-1} BA and 20 g l^{-1} sucrose. The roots were formed after 2 weeks of incubation (25°, continuous light 2000 lux). After 3 weeks, the root culture was transferred into B5 liquid medium containing the same supplements as the solid medium (55 ml medium in 250 ml Erlenmeyer flask). The culture was maintained by transfer of 2 g fr. wt tissue into 55 ml fresh medium every 3 weeks and maintained at 25°, 80 rpm, in continuous light.

Extraction and isolation. Three kg fr. wt of *I. balsamina* root cultures were harvested (from 300 flasks) and dried at 60° for 12 hr to yield 150 g dried root. The dried ground root (100 g) was successively extracted under reflux with EtOAc and MeOH. Both extracts were concd separately *in vacuo* to yield 2.0 g EtOAc extract and 22 g MeOH extract. The EtOAc extract was sepd into 9 frs (200 ml each) on a silica gel (acidified by 0.5 N oxalic acid) column (3 × 20 cm) using step gradient elution ($CHCl_3$–MeOH, 100:0 → 1:19). Further purification of fr. 1 on an RP-18 Lobar column using step gradient

Table 2. ^{1}H NMR data for compounds **1**, **2** and **4** (500 MHz)

H	**1** ($CDCl_3$)	**2** (CD_3OD)	**4** (DMSO-*d*)
3	6.18 *s*	6.25 *s*	
5	8.09 *dd* (7.32, 1.23)	8.04 *d* (7.95)	7.98 *dd* (7.01, 1.5)
6	7.76 *ddd* (7.93, 7.33, 1.22)	7.88 *ddd* (7.96, 7.29, 1.32)	7.78 *ddd* (7.5, 7.5, 1.5)
7	7.72 *ddd* (7.32, 7.33, 1.83)	7.83 *dd* (7.95, 7.29)	7.83 *ddd* (7.5, 7.5, 1.5)
8	8.14 *dd* (7.33, 1.83)	8.09 *d* (7.96)	7.97 *dd* (7.5, 1.5)
5′			
6′			
7′			
8′			
2-CH_2			3.765
OMe	3.91 *s*		

Coupling constants (J in Hz) are given in parentheses.

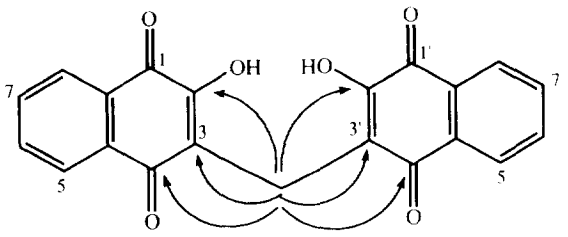

Fig. 2. Important ^{13}C–^{1}H long-range correlations observed by heteronuclear COSY of compound **4**.

Table 3. ^{13}C NMR (125 MHz, $CDCl_3$) and ^{1}H NMR (500 MHz, $CDCl_3$) data for compound **6**

C	^{13}C NMR	^{1}H NMR
2	160.62	
3	103.21	6.28 *d* (10.0)
4	143.81	7.60 *d* (10.0)
5	113.49	6.66 *s*
5a	111.20	
6	144.60	
7	134.48	
8	143.05	
8a	142.46	
6-OMe	56.49	3.94 *s*
8-OMe	61.60	4.09 *s*

Coupling constants (*J* in Hz) are given in parentheses.

elution (MeOH–H_2O, 7:3 → 100:0) yielded **1** (3 mg) as yellow needles, whereas further purification of frs 2 and 3 using the same column and conditions yielded **2** (2 mg). Compound **3** was also obtained from the crude EtOAc extract by CC on a silica gel (oxalic acid) column (5 × 40 cm) using step gradient elution (C_6H_6–Me_2CO, 100:0 → 4:1) to yield 16 frs, 10 ml each. Crystallization of fr. 10 gave **3** (5 mg) as needles.

The methanolic extract was first partitioned between EtOAc and H_2O. Further sepn of the EtOAc fr. by gel filtration on a Sephadex LH-20 column (5 × 20 cm) using MeOH as eluent yielded 4 frs (I–IV). Purification of fr. III by repeated gel filtration on a Sephadex LH-20 column (MeOH) afforded compound **4** (15.1 mg). Purification of fr. II by Sephadex LH-20 CC (EtOH) yielded 3 frs (A–C). Fr. B was subjected to CC on silica gel column using step gradient elution ($CHCl_3$–Me_2CO, 100:0 → 3:2) and 4 frs were collected (1–4). Crystallization of frs 2 and 3 in $CHCl_3$–hexane yielded **5** (2 mg) and **6** (9 mg).

2-Methoxy-1,4-naphthoquinone (**1**). Yellow needles, mp 182–183°. IR ν_{max}^{KBr} cm^{-1}: 1680 (C=O), 1645 (C=O), 1605 (C=C, Ar), 1240 (C–O); EIMS *m/z*: 188 $[M]^+$, 173 $[M - Me]^+$, 157 $[M - OMe]^+$; ^{13}C and ^{1}H NMR: Tables 1 and 2 respectively.

Lawsone (*2-hydroxy-1,4-naphthoquinone*) (**2**). Yellow crystals, mp 192°. IR ν_{max}^{KBr} cm^{-1}: 3160 (OH), 1674 (C=O), 1630 (C=C, Ar), 1220 (C–O); EIMS *m/z*: 174 $[M]^+$, 146 $[M - CO]^+$, 118 $[M - 2 \times CO]^+$, 105; ^{13}C and ^{1}H NMR: Tables 1 and 2, respectively.

Spinasterol (**3**). White needles, mp 169–170°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1625 (C=C), 1450 (C–H), 1380 (C–H); EIMS *m/z*: 412 $[M]^+$, 397 $[M - Me]^+$, 369 $[M - Me - H_2O]^+$, 300 $[C_{22}H_{46}]^+$.

Diphthiocol (**4**). Yellow crystals, mp 229°. UV λ_{max}^{MeOH} nm: 271.5; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1674 (C=O), 1605, 1570 (C=C, Ar), 1455 (C=C), 1260, 1210 (C–O); EIMS *m/z*: 360 $[M]^+$, 342 $[M - H_2O]^+$; 314 $[M - H_2O - CO]^+$, 188 $[C_{11}H_8O_3]^+$, 173 $[C_{10}H_5O_3]^+$; HRFAB-MS *m/z*: 360.0631 $[M]^+$ (calc. 360.0628 for $C_{21}H_{12}O_6$), 342.0497 $[M - H_2O]^+$ (calc. 342.0494 for $C_{21}H_{10}O_5$), 314.0584 $[M - H_2O - CO]^+$ (calc. 314.0583 for $C_{20}H_{10}O_4$); ^{13}C and ^{1}H NMR: Tables 1 and 2, respectively.

Scopoletin (*7-hydroxy-6-methoxycoumarin*) (**5**). Crystals, mp 204°. IR ν_{max}^{KBr} cm^{-1}: 3325 (OH), 1720 (C=O), 1610, 1560, 1510 (C=C, Ar) 1300, 1130 (C(O) – O).

Isofraxidin (**6**). Red rosette aggregates, mp 148° [9]. EIMS *m/z*: 222 $[M]^+$, 207 $[M - Me]^+$, 194 $[M - CO]^+$, 179 $[C_9H_7O_4]^+$, ^{13}C and ^{1}H NMR: Table 3.

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