Antipruritic Dinaphthofuran-7,12-dione Derivatives from the Pericarp of *Impatiens balsamina*

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Dinaphthofuran-7,12-dione derivatives named balsaminones A (**1**) and B (**2**) were isolated from the pericarp of *Impatiens balsamina* L. together with the known compound 2-methoxy-1,4-naphthoquinone (**3**). Their structures were elucidated by spectral techniques. These compounds have significant antipruritic activity.

The aerial parts of *Impatiens balsamina* L. (Balsaminaceae) serve as a Chinese herbal medicine for the treatment of articular rheumatism, bruises, and beriberi.[1] The flowers of *I. balsamina* have been reported to contain flavonols,[2] flavonoid pigments,[3,4] and anthocyanin pigments.[5]

In some areas of Japan, in folk medicine, juice squeezed from the white flower petals of *I. balsamina* is painted topically on the skin to alleviate lesions of several types of dermatitis, including urticaria. In previous studies, we confirmed that this folkloric use of the juice could be verified by pharmacological evidence using newly developed antianaphylactic[6−8] and antipruritic assays.[9] From the white petals, we have isolated antianaphylactic,[10] antihistamic,[11] and antipruritic[9] compounds.

In our continuing search for biologically active substances from *I. balsamina*, we report herein the isolation and structure elucidation of two novel dinaphthofurans, balsaminone A (**1**) and balsaminone B (**2**), together with the known 2-methoxy 1,4-naphthoquinone (**3**)[10] from the pericarp of the fruit.

Balsaminone A (**1**) was obtained as red needles. The EI-MS spectrum showed a molecular ion peak at *m/z* 344. It decomposes gradually and more readily in $CHCl_3$ solution. The IR spectrum suggested the presence of hydroxyl groups at 3450 cm^{-1} and conjugated ketone at 1665 and 1640 cm^{-1}. The absorption bands at 205, 222, 267, 330(sh), and 475 nm suggested a 5-hydroxy-dinaphthofurandione structure.[12]

The ^1H NMR spectrum of **1** showed a singlet at δ 4.09 due to methoxyl protons, a singlet at δ 6.33 due to a hydroxyl proton, a set of complex symmetrical multiplets characteristic of an A_2X_2 system at δ 8.30 and 7.80 (each 2H), and three multiplets corresponding to an ABX_2 system at δ 8.48, 8.34, and 7.67 (2H) (Table 1). The UV and ^1H NMR data suggested the presence of two naphthalene moieties with four adjacent protons. The ^{13}C NMR spectrum of **1** displayed signals for all 21 carbons in the molecule: one methoxyl, two carbonyl, and 18 aromatic carbons, of which eight were protonated, six were quaternary, and four bore oxygen (Table 1). All signal assignments of ^1H NMR and ^{13}C NMR spectra were confirmed by $^1H-^1H$ COSY, C−H COSY, and HMQC.

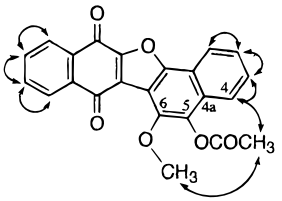

Figure 1. NOESY interactions of balsaminone A monoacetate (**4**).

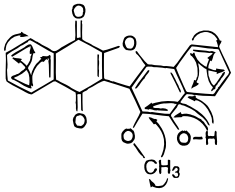

Figure 2. Long-range correlation in the HMBC spectrum of balsaminone A (**1**).

The above data suggested three possible dinaphthofurandione structures (1A−C) for **1**. Of these, structure 1A could explain the downfield shift of H-1 (δ 8.47) in **1**, which indicates the peripheral location of the proton with respect to the furan oxygen.[13] UV absorption of **1** at λ_{max} 475 nm implied the location of the hydroxyl group at C-5 when compared with those of 2,2-dinaphthofuran-1,4-quinone derivatives.[12] Additional support was provided by NMR data (Table 1) of the monoacetate (**4**) obtained by acetylation of **1**. The upfield shift (0.43 ppm) of H-4 in ^1H NMR indicated the location peripheral to the acetyl group, and downfield shifts at C-5 (0.9 ppm), C-4a (3.1 ppm), C-6 (2.6 ppm), and C-13a (3.4 ppm) were observed in the ^{13}C NMR.[14] In the NOESY spectrum of **4** (Figure 1), a correlation was observed between H-4 and acetyl-CH_3, also indicating their peripheral location. Further, the bonding pattern was confirmed by the HMBC correlation as shown in Figure 2. Thus, balsaminone A was established as 5-hydroxy-6-methoxy-dinaphtho[1,2-b:2′,3′-d]furan-7,12-dione (**1**).

Balsaminone B(**2**) was obtained as orange needles. The positive FABMS showed a molecular ion peak at *m/z* 507$[M + 1]^+$, and the EIMS showed an ion peak at *m/z* 344, equivalent to the molecular ion of **1**. The IR spectrum of **2** suggested the presence of hydrogen-bonded hydroxyl groups and conjugated ketone showing

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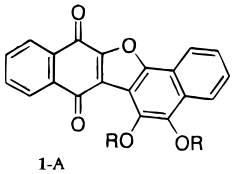
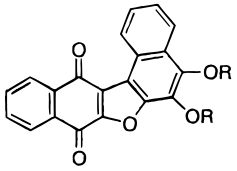
Table 1. ^{1}H and ^{13}C NMR Spectral Data[a] for Compounds **1**, **4**, and **2**

	balsaminone A (**1**)[b]		balsaminone A acetate (**4**)[b]		balsaminone B (**2**)[c]	
carbon no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	121.2	8.48 m	121.7	8.52 m	121.0	9.00 br d (8.1)[d]
2	127.8	7.67 m	128.8	7.68 m	128.2	7.58 m
3	127.1	7.67 m	126.9	7.68 m	126.9	7.58 m
4	123.1	8.34 m[e]	122.0	7.89 m[e]	125.1	8.30 br d (8.1)
4a	125.6		128.7		126.5	
5	142.8		143.7		144.1	
6	134.7		137.3		134.3	
6a	114.6		115.5		116.7	
6b	118.9		118.8		119.0	
7	174.6		174.7		174.8	
7a	133.7		133.7		132.5	
8	127.4	8.30 m	127.5	8.30 m	127.5	8.34 m
9	134.2	7.80 m	134.3	7.82 m	134.4	7.72 m
10	133.8	7.80 m	133.8	7.82 m	133.9	7.72 m
11	126.7	8.30 m	126.7	8.30 m	126.5	8.34 m
11a	132.3		132.0		131.3	
12	180.2		179.4		179.4	
12a	153.1		153.4		153.8	
13a	148.8		152.2		150.9	
13b	125.0		125.7		126.0	
OMe	63.4	4.09 s	63.0		63.2	4.47 s
*C*OMe			169.0			
CO*C*H$_3$			20.6			
OH		6.33 s		2.54 s		
glucose moiety						
1′					106.0	6.05 d (7.7)
2′					76.0	4.51 m
3′					78.9	4.39 m
4′					71.8	4.39 m
5′					78.6	3.93 m
6′					62.6	4.27/4.39 m

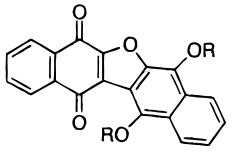
[a] Assignments based on HMQC and HMBC. [b,c]Data recorded in $CDCl_3$[b] or in pyridine-d_5, respectively, at 125 MHz (^{13}C) and 500 MHz (^{1}H). [d] Values in parentheses are coupling constants in Hertz (500 MHz). [e] Partially overlapped with δ 8.30.



1-A



1-B

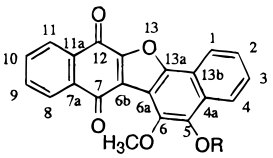


1-C

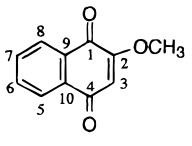
absorbance at 3500−3000 cm^{-1} (broad) and at 1668 cm^{-1}, respectively, while the UV spectrum had λ_{max} values at 205, 217, 264, 325, 369, and 427 nm. The ^{1}H and ^{13}C NMR data of **2** indicated that it may be a monoglucoside of balsaminone A (**1**). Acid hydrolysis of **2** yielded an aglycon and a sugar. The aglycon was shown to be **1** by comparison of its IR, MS, and ^{1}H NMR data with those obtained for **1** (Table 1). The sugar unit was determined to be D-glucose as evidenced by comparative TLC and NMR analysis (Table 1). Downfield shifts at C-4a (0.9 ppm) and C-13a (2.1 ppm) in the ^{13}C NMR of **2** suggested the location of a glucose moiety at C-5-OH. The hypsochromic shift of wavelength of **2** (λ_{max} 427 nm) from **1** (475 nm) also supports the location of the glucose at the phenolic OH at C-5.[12] Thus, balsaminone B was established as being 5-β-D-glucosyloxy-6-methoxy-dinaphtho[1,2-b:2′,3′-d]furan-7,12-dione (**2**).

Although the dinaphthofurandione structure has been derived synthetically from dinaphthoquinone by photorearrangement,[12] it is rare to isolate dinaphthofurandione derivatives from natural sources. Methyl 5-hydroxy-7,12-dioxo-dinaphtho[1,2-b:2′,3′-d]furan-6-carboxylate is a natural isolate from the stem of *Paulownia tomentosa* Steudel,[15] the structure of which was determined by crystallography.

As the antipruritic activity was reported previously for the naphthoquinone derivative (**3**)[9] isolated from the white petals, we considered it worth investigating the same biological effect for balsaminones A (**1**) and B (**2**). As shown in Figure 3, 10 mg/kg of **2** and **3** given to mice significantly inhibited ($p<0.05$) scratching of the nose,



1: R= H
2: R= β-D-glc
4: R= $COCH_3$



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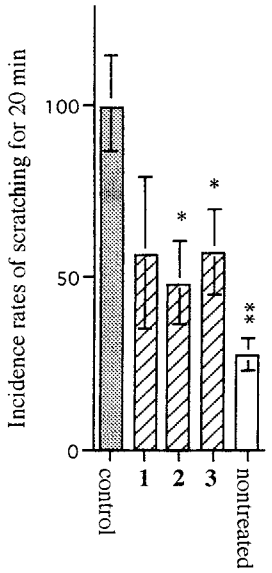

Figure 3. Antipruritic effects of compounds **1**−**3** from the pericarps of *Impatiens balsamina* L. The bar labeled as "control" refers to nontreated mice injected subcutaneously with compound 48/80 at 3 mg/kg. Compounds **1**, **2**, and **3** were administered orally at a dose of 10 mg/kg 24 h before injection of compound 48/80. All values are mean ± SEM for 5 mice. * $p<0.05$ and ** $p<0.001$ (compared with control by Student's *t*-test).

which had been induced by compound 48/80, an agent causing histamine release. Compound **1** showed slightly different results compared with the control group, although the difference was not significant. The mechanisms of action are currently under investigation.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM-GSX 500 spectrometers (TMS as internal reference). IR spectra were recorded on a Shimadzu 435 spectrometer and the UV absorption spectra with a Hitachi 323. MS spectra (70 eV) were performed on a JEOL JMS-DX 303 spectrometer.

Plant Material. *I. balsamina* was planted in our university medicinal plant garden and verified by Dr. G. Yoneda (Faculty of Pharmaceutical Sciences,Osaka University). The pericarp of the fruit was collected in August 1997. A voucher specimen is kept in our laboratory.

Extraction and Isolation. Fresh pericarp (404 g) of *I. balsamina* was repeatedly extracted with MeOH at room temperature. The precipitate (3.6 g) from the MeOH extract was filtered, dried, and then subjected to Si gel column chromatography using $CHCl_3$ to afford six fractions: fraction 1 (157 mg), fraction 2 (989 mg), fraction 3 (94 mg), fraction 4 (63 mg), fraction 5 (51 mg), and fraction 6 (1.2 g). Fraction 2 was recrystallized from MeOH to give 2-methoxy-1,4-naphthoquinone (**3**) (933 mg). Fraction 5 was rechromatographed on Si gel using a $CHCl_3$−MeOH gradient system and was recrystallized from pyridine−EtOH to give compound **1** (19.2 mg).

The filtrate of MeOH extract was evaporated under vacuum to yield a residue (2.58 g) that was chromatographed on a Si gel column by use of a $CHCl_3$−MeOH gradient system. The eluate by $CHCl_3$−MeOH (9:1) was recrystallized from pyridine−EtOH to afford compound **2** (4.5 mg).

Balsaminone A (1): red needles (pyridine−EtOH); mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 205 (4.45), 222 (4.39), 267 (4.69), 330 (sh) (3.72), 475 (3.64) nm; IR (KBr) ν_{max} : 3450 (br, OH), 1665, 1640, 1583, (quinone C═O and C═C), 1540, 1509, 1210 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m/z* 344 $[M]^+$ (65), 329 $[M - CH_3]^+$ (100), 301 $[329 - CO]^+$ (7), 273 $[301 - CO]^+$ (8), 245 $[273 - CO]$(5), 189 $[C_{11}H_8O_3 + H]^+$ (17), 188 $[C_{11}H_8O_3]^+$ (6), 172 $[188 - O]^+$ (3).

Balsaminone B (2): orange needles (pyridine−EtOH); mp >300 °C; IR (KBr) ν_{max} 3500−3000 (br, OH), 1670, 1650, 1630 (quinone C═O and C═C), 1540 cm^{-1}; UV (MeOH) λ_{max} (log ϵ): 205(4.40), 264(4.67), 325(sh) (3.67), 427 (3.57) nm; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m/z* 344 $[M - GLC]^+$ (76), 329 $[344 - CH_3]^+$ (100), 301 $[329 - CO]^+$ (6), 273 $[301 - CO]^+$ (6), 245 $[273 - CO]$(4), 189 $[C_{11}H_9O_3 + H]^+$ (13); FABMS *m/z* 507 $[M + 1]^+$.

2-Methoxy-1,4-naphthoquinone (3): yellow needles (MeOH); mp 186−189 °C; IR (KBr) ν_{max} 1680 (quinone C═O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.91 (3H, s, OCH_3), 6.18 (1H, s, H-3), 7.71 (1H, td, J = 1.5, 7.5 Hz, H-7), 7.75 (1H, td, J = 1.5, 7.5 Hz, H-6), 8.08 (1H, dd, J = 1.5, 7.5 Hz, H-8), 8.13 (1H, dd, J = 1.5, 7.5 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.4 (11), 109.9, (3), 126.2 (8), 126.7 (5), 131.1 (9), 132.1 (10), 133.3 (7) 134.3 (6), 160.4 (2), 180.0 (1), 184.7 (4); EIMS *m/z* 188 $[M]^+$ (100), 158 $[M - CH_2O]^+$ (3), 130 $[158 - CO]^+$ (1).

Animals. Male ddY mice (SPF grade), 7 weeks old, were obtained from Japan SLC (Shizuoka, Japan) and housed at 24 °C. Food and water were available ad libitum.

Assay for Antipruritic Activities on Compound 48/80-evoked Scratching Behavior. The antipruritic activities of compounds **1**−**3** were measured as previously reported.[9] A 3-mg/mL saline solution of compound 48/80, a degranulation agent, and 1-mg/mL saline solutions of compounds **1**, **2**, and **3** were prepared. The solution of compound 48/80 was administered at 3 mg/kg sc into the base of the neck on the back of mice to provoke scratching behavior. Next, the solution of compound **1**, **2**, or **3** was administered at 10 mg/kg po to nontreated mice 24 h before injection of compound 48/80 (n = 5 per group). As a control, nontreated mice were only injected with compound 48/80. Incidences of nose scratching were counted for 20 min.

Statistical Analysis. All values are means ± S. E. M. The data were evaluated by Student's *t*-test.

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