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Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia pandurata*

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Abstract

The cyclohexenyl chalcone derivative [(−)-hydroxypanduratin A], together with the previously known panduratin A, sakuranetin, pinostrobin, pinocembrin, and dihydro-5,6-dehydrokawain were isolated from the chloroform extract of the red rhizome variety of *Boesenbergia pandurata* (Robx.) Schltr. [currently known as *Boesenbergia rotunda* (L.) Mansf., Kulturpfl.]. Their structures were assigned on the basis of their spectroscopic data. (−)-Hydroxypanduratin A and (−)-panduratin A showed significant topical anti-inflammatory activity in the assay of TPA-induced ear edema in rats.

Keywords: *Boesenbergia pandurata*; *Boesenbergia rotunda*; Zingiberaceae; Red rhizome variety; Cyclohexenyl chalcone derivatives; Flavanones; 2-Pyrone; Anti-inflammatory activity

1. Introduction

Boesenbergia pandurata (Robx.) Schltr. (local name: krachai-dang), currently known as *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. (Larsen, 1996), is a perennial herb of the family Zingiberaceae. The fresh rhizome is used in cooking, also in Thai folk medicine as an aphrodisiac, and for the treatment of colic disorder. Previous investigations of the yellow rhizomes of *B. pandurata* dealt with the constituents of the essential oil (Lawrence et al., 1971); isolation of (±)-pinostrobin and (±)-alpinetin (Mongkolsuk and Dean, 1964); (±)-boesenbergin A, (±)-boesenbergin B, (±)-panduratin A, 2′,6′-dihydroxy-4′-methoxychalcone, cardamonin, pinostrobin and pinocembrin (Jaipetch et al., 1982; Mahidol et al., 1984). Apart from the yellow rhizomes, other varieties of *B. pandurata* have also been studied in our laboratory. Eleven flavonoids were reported to be the constituents of the black rhizome variety (Jaipetch et al., 1983). The white rhizome variety was found to contain croteepoxide, (+)-zeylenol, boesenboxide as well as isopimaric acid and 2′-hydroxy-4,4′,6′-trimethoxychalcone (Pancharoen et al., 1984; Tantiwachwuttikul et al., 1987) Chemical investigation of the hexane extract of the red rhizomes of *B. pandurata* resulted in the isolation of (±)-panduratin A, pinostrobin, together with boesenbergin A and rubranine (Combes et al., 1970; Tantiwachwuttikul et al., 1984).

In continuation of our previous investigations on the constituents of *B. pandurata*, we now report the phytochemical studies of the chloroform extract of the red rhizome variety of *B. pandurata*. Six compounds were isolated and the structures were established as (−)-(2,4,6-trihydroxyphenyl)[3′-methyl-2′-(3″-methylbut-2″-enyl)-6′-phenylcyclohex-3′-enyl]methanone [(−)-hydroxypanduratin A, **1**], along with (−)-(2,6-dihydroxy-4-methoxyphenyl)[3′-methyl-2′-(3″-methylbut-2″-enyl)-6′-phenylcyclohex-3′-enyl]methanone [(−)-panduratin A, **2**], 5,4′-dihydroxy-7-methoxyflavanone (sakuranetin, **3**), 5-hydroxy-7-methoxyflavanone (pinostrobin, **4**), 5,7-dihydroxyflavanone (pino-

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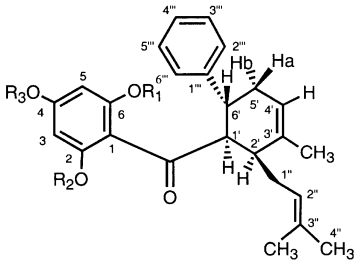
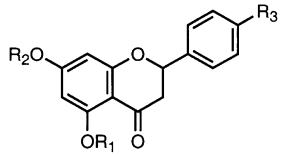
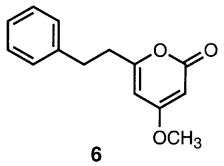
cembrin, **5**), and dihydro-5,6-dehydrokawain (**6**). It should be noted that compounds **1**, **3** and **6** have not been isolated from any variety of *B. pandurata* and **1** is a new compound.



1 $R_1 = R_2 = R_3 = H$
2 $R_1 = R_2 = H$, $R_3 = CH_3$
7 $R_1 = R_2 = R_3 = CH_3$





3 R_1= H, $R_2 = CH_3$, R_3 = OH
4 R_1 = H, $R_2 = CH_3$, R_3 = H
5 $R_1 = R_2 = R_3 = H$

6

2. Results and discussion

The structure of compound **1** was established on the basis of its spectral data, making use of DEPT, COSY, HMQC and HMBC experiments in combination with ^1H and ^{13}C NMR spectroscopic data.

Compound **1**, $[\alpha]_{589}^{28}$ −10.44° (EtOH, *c* 0.35), was obtained as a pale yellow solid and analyzed as $C_{25}H_{28}O_4$. The IR spectrum displayed a broad hydrogen-bonded O–H stretching band at 3500–3100 cm^{-1} and a C–O stretching band at 1235 cm^{-1} which suggested that compound **1** was phenolic. A strong absorption band at 1630 cm^{-1} was characterized for a conjugated ketone with intramolecular hydrogen bonding. The ultraviolet spectrum [λ_{max}^{EtOH}at 293 (log ε 4.32) and 223 (log ε 4.23) nm] supported the presence of a conjugated ketone in the structure. In addition, the EI mass spectrum of compound **1** showed an $[M]^+$ at *m*/*z* 392 which was 14 mass units less than that of panduratin A. Moreover, treatment of compound **1** with dimethyl sulphate and potassium carbonate in acetone at reflux temperature for 24 h gave a fully methylated product **7** which exhibited identical ^1H NMR spectral data with those of the fully methylated product of panduratin A. This evidence suggested that compound **1** was a hydroxy analogue of panduratin A.

The ^1H NMR spectral data of **1** in $CDCl_3$ were quite similar to those of panduratin A (**2**) (Mahidol et al., 1984; Tantiwachwuttikul et al., 1984), except that no signal of *O*-methoxyl protons was observed in **1**. However, weak signals and poor resolution were obtained, due to its poor solubility. For detailed analysis, the spectra were recorded in CD_3COCD_3 (Table 1). The ^1H NMR spectra of this compound showed a broad singlet at δ 11.74 (2H) which was assigned to the two phenolic hydroxyl groups at C-2 and C-6, while another broad singlet at δ 9.17 (1H) was referred to the resonance of the phenolic hydroxyl group at C-4. These observations confirmed that compound **1** was a trihydroxy derivative. The signals centered at δ 7.04 (*m*, 1H), δ 7.17 (*m*, 2H) and δ 7.19 (*m*, 2H) were recognized as the resonances of the five protons of a monosubstituted benzene, while the two magnetically equivalent aromatic protons resonating as a broad singlet at δ 5.88, were assigned to H-3 and H-5. The presence of a γ,γ-dimethylallyl moiety was indicated by the signals at δ 1.51 (*br s*, 6H, 2×CH_3), δ 2.10 (*m*, 1H, allylic H), δ 2.26 (*m*, 1H, allylic H) and δ 4.92 (*m*, 1H, olefinic H). Since the signal of the methylene protons (δ 2.10 and 2.26) of the γ,γ-dimethylallyl unit appeared to be magnetically non-equivalent, it is suggested that this group is located at an asymmetric centre. The correlations observed in the COSY spectrum (H-1″a/H-1″b, H-1″a/H-2″, H1″b/H-2″, H-1″a/3″-Me, H-1″b/3″-Me, H-1″a/H-4″, H-1″b/H4″, H1″a/H2′ and H-1″b/H-2′) confirmed that the γ,γ-dimethylallyl group was connected to C-2′. The resonances at δ 5.41(*m*, H-4′), δ 4.82 (*dd*, H-1′), δ 3.45 (*br ddd*, H-6′), δ 2.69 (*m*, H-2′), δ 2.35 (*m*, H-5′a), δ 1.95 (*m*, H-5′b) and δ 1.76 (*br ddd*, 3′-Me) agreed well with the data of the substituted cyclohexenyl unit found in panduratin A. Other correlations found in the COSY and HMBC spectra (See Table 1) linked all the connections in the structure. Establishment of the relative stereochemistry at C-1′, C-2′ and C-6′ was done by comparing the coupling constants of $J_{1',6'}$ and $J_{1',2'}$ with those observed in panduratin A (Tantiwachwuttikul et al., 1984), saggenon D and saggenon C (Nomura et al., 1982). The data lead to the conclusion that H-1′ and H-2′ have a *cis* relationship ($J_{1',2'}$ = 4.6 Hz), and H-1′ and H-6′ are *trans*-oriented ($J_{1',6'}$ = 11.6 Hz).

The optically active (−)-panduratin A (**2**) ($[\alpha]_{589}^{29}$ −24.62°, EtOH, *c* 0.07), sakuranetin (**3**) pinostrobin (**4**) and pinocembrin (**5**) were identified by comparison of their spectral data with those reported in the literature (Mongkolsuk and Dean, 1964; Jaipetch et al., 1982; Tantiwachwuttikul et al., 1984; Mahidol et al., 1984; Liu et al., 1992). Since the values for the optical rotations of the isolated compounds **3**, **4** and **5** are rather low, so they are almost racemic. The known 2-pyrone, dihydro-5,6-dehydrokawain (**6**), has been previously found in the rhizomes of *Alpinia speciosa* and *Alpinia kumatahe* (Zingiberaceae) (Kimura et al., 1966; Itokawa et al., 1981).

Compounds **1** and **2** were assayed for topical anti-inflammatory activity in the experimental model of

Table 1
NMR spectral data for compound **1** [(−)-hydroxypanduratin A] in CD_3COCD_3

Position	δ_H[a]	δ_C[a]	DEPT	COSY[b]	HMQC[c]	HMBC[c]
1	–	106.17	C	–	–	H-5 and H-3
2	–	164.82	C	–	–	H-3
3	5.88 (*br s*)	95.88	CH	–	H-3	H-5
4	–	164.82	C	–	–	H-5 and H-3
5	5.88 (*br s*)	95.88	CH	–	H-5	H-3
6	–	164.82	C	–	–	H-5
1′	4.82 (*dd*)[d]	54.48	CH	H-6′, H-2′ (w)	H-1′	H-6′, H-2′, H-1″a
2′	2.69 (*m*)	43.36	CH	H-1′ (w), H-1″a, H-1″b	H-2′	H-6′, 3′-Me, H-1′, H-1″a, H-1″b
3′	–	137.87	C	–	–	H-5′a, H-5′b, 3′-Me, H-2′, H-1′, H-1″a, H-1″b
3′-Me	1.76 (app *ddd*)[e]	23.02	CH_3	H-5′a, H-5′b, H-4′	3′-Me	H-2′
4′	5.41 (*m*)	121.72	CH	H-5′a, 3′-Me	H-4′	H-5′a, H-5′b (w), 3′-Me, H-2′
5′	(a) 2.35 (*m*)	36.81	CH_2	H-6′, H-5′b, H-4′, 3′-Me	H-5′a	H-6′
	(b) 1.95 (*m*)			H-6′, H-5′a, 3′-Me	H-5′b	
6′	3.45 (*br ddd*)[f]	37.84	CH	H-5′a, H-5′b, H-1′	H-6′	H-5′a, H-5′b (w), H-2′, H-1′, H-6‴ and H-2‴
1″	(a) 2.26 (*m*)	29.54	CH_2	H-2′, H-4″ and 3″-Me (w), H-2″, H-1″b	H-1″a	H-2′, H-1′, H-2″ (w)
	(b) 2.10 (*m*)			H-2′, H-4″and 3″-Me (w), H-2″, H-1″a	H-1″b	
2″	4.92 (*m*)	125.42	CH	H-4″ and 3″-Me, H-1″a, H-1″b	H-2″	H-2′, H-4″ and 3″-Me, H-1″a, H-1″b
3″	–	131.67	C	–	–	H-4″ and 3″-Me, H-1″a, H-1″b
3″-Me	1.51 (*br s*)	17.97	CH_3	H-2″, H-1″a (w), H-1″b (w)	3″-Me	H-4″, H-2″
4″	1.51 (*br s*)	25.86	CH_3	H-2″, H-1″a (w), H-1″b (w)	H-4″	3″-Me, H-2″
1‴	–	148.30	C	–	–	H-6′, H-5‴, H-3‴
2‴	7.19 (*m*)	127.99	CH	H-3‴	H-2‴	H-6′, H-6‴, H-4‴
3‴	7.17 (*m*)	128.91	CH	H-4‴, H-2‴	H-3‴	H-5‴
4‴	7.04 (*m*)	126.16	CH	H-5‴, H-3‴	H-4‴	H-6‴, H-2‴
5‴	7.17 (*m*)	128.91	CH	H-6‴, H-4‴	H-5‴	H-3‴
6‴	7.19 (*m*)	127.99	CH	H-5‴	H-6‴	H-6′, H-4‴, H-2‴
4-OH	9.17 (*br s*)	–	–	–	–	–
2,6-OH	11.74 (*br s*)	–	–	–	–	–
C=O	–	207.00	C=O	–	–	H-5 (w) and H-3 (w), H-6′, H-1′

[a] CD_3COCD_3 signals at δ_H 2.04 and δ_C 29.80 as references.
[b] Protons correlating with proton resonance.
[c] Protons correlating with carbon resonance.
[d] $J_{1',6'} = 11.6$ Hz, $J_{1',2'} = 4.6$ Hz.
[e] $J_{3'\text{-Me},4'}$, $J_{3'\text{-Me},5'a}$, $J_{3'\text{-Me},5'b} \sim 1.8$ Hz.
[f] $J_{6',1'} = 11.6$ Hz, $J_{6',5'b} = 10.7$ Hz, $J_{6',5'a} = 6.3$ Hz, *J* values were determined from decoupling experiments; (w) = weak correlation.

TPA-induced ear edema in rats. Challenge of the rat ear with the inflammogen TPA (12-*O*-tetradecanoylphorbol-13-acetate; 4 μg/ear) provoked maximum edematous response 8 h after application. Pretreatment of the rat ear by topical application of compound **1** or **2** (20–2000 μg/ear) significantly ($P < 0.01$) inhibited TPA-induced ear edema formation in a dose-dependent manner (Table 2). The ID_{50}-values of **1** and **2** were determined as 84 and 12 μg/ear, respectively. The presence of these anti-inflammatory compounds in *B. pandurata* may very well be related to the use of this plant in traditional medicine.

3. Experimental

3.1. General

Mps: uncorr (electrothermal apparatus); IR: Nujol; UV: EtOH. NMR spectra were recorded at the Central Instrumental Unit of Mahidol University on a Brüker DPX 300 in CD_3COCD_3 or $CDCl_3$. MS was measured at 70 eV. CC was carried out on silica gel 60 (70–230 mesh).

3.2. Plant material

Red rhizomes of *B. pandurata* were collected from Kanchanaburi Province of Thailand. The plant was identified by one of us (TS) and a voucher specimen (BKF 68909) has been deposited at the Forest Herbarium, Royal Forestry Department, Bangkok.

3.3. Extraction

Oven dried (40–60 °C) finely powdered rhizomes of *B. pandurata* (red rhizomes) (9.2 kg) were extracted in a Soxhlet with boiling hexane (3×8 l) for 107 h, then with boiling $CHCl_3$ (3×8 l) for 129 h and finally with boiling MeOH (3×8 l) for 129 h. Evaporation of solvents yielded 740, 835 and 604 g of the extracts, respectively.

Table 2
Effect of topical application of compound **1** and panduratin A (**2**) on TPA induced ear edema in rats

Treatment	*n*	Edema thickness (μm)					% Inhibition				
		2 h	4 h	6 h	8 h	10 h	2 h	4 h	6 h	8 h	10 h
Vehicle	26	77±6	187±9	208±8	217±9	179±8					
*Compound **1***											
20 μg/ear	14	36±8*	98±7*	156±10*	127±9*	94±6*	53	48	25	41	47
200	14	41±5*	75±6*	100±6*	98±5*	82±5*	47	60	52	55	54
2000	14	41±5*	47±5*	61±5*	64±6*	48±6*	47	75	71	71	73
*Compound **2***											
20 μg/ear	12	29±5*	85±10*	99±8*	100±8*	81±8*	62	55	52	54	58
200	12	23±5*	43±6*	51±6*	57±6*	44±6*	70	77	75	74	75
2000	12	11±3*	24±5*	23±5*	18±4*	11±4*	86	87	89	92	94
Diclofenac											
600 μg/ear	14	32±4*	36±5*	54±5*	94±4*	49±6*	58	81	74	57	73

* $p \leqslant 0.01$.

3.4. Isolation

The $CHCl_3$ extract (343 g) was subjected to coarse separation by flash CC, gradient eluting with various proportions of EtOAc-*n*-hexane, followed by 2%, 10% of MeOH–EtOAc and finally with MeOH. Various fractions were obtained and then submitted to flash chromatography (EtOAc–hexane gradient) to afford pinostrobin (**4**) (119.8 g), (−)-panduratin A (**2**) (9.5g), (−)-hydroxypanduratin A (**1**) (0.51 g), pinocembrin (**5**) (50.5 g), dihydro-5,6-dehydrokawain (**6**) (0.91 g) and sakuranetin (**3**) (0.67 g).

3.5. (−)-(2,4,6-Trihydroxyphenyl)[3′-methyl-2′-(3″-methylbut-2″-enyl)-6′-phenylcyclohex-3′-enyl]methanone (*1*)

Amorphous from EtOAc–*n*-hexane, mp 213.0–214.2 °C (Found: C, 76.5; H, 7.1. $C_{25}H_{28}O_4$ requires: C, 76.5; H, 7.2); $[\alpha]_{589}^{28}$ −10.44° (EtOH, *c* 0.35); IR $\nu_{max}^{Nujol}cm^{-1}$; 3500–3100 (OH), 1630 (C=O), 1600, 1565, 1520, 1450, 1380, 1235, 1175, 1145, 1075, 1020, 830, 810, 755, 700; UV λ_{max}^{EtOH}nm (log ε); 293 (4.32), 223 (4.23) (*sh*); EIMS *m*/*z* (rel. int.): 392 [M^+] (3), 323 (6), 257 (57), 219 (6), 171 (4), 153 (100), 111 (4), 91 (10), 69 (12), 55 (3), 41 (12), 32 (9), 28 (41); ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data: see Table 1.

3.6. Anti-inflammatory assays

Topical anti-inflammatory activity of compounds **1** and **2** were assessed by the method as described previously (Pongprayoon et al., 1996).

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