Tetracyclic Chromane Derivatives from *Rhododendron anthopogonoides*

Naoki Iwata and Susumu Kitanaka*

College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan

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Four new chromane derivatives, anthopogochromane (**1**), anthopogochromene A (**2**), anthopogochromene B (**3**), and anthopogochromene C (**4**), and two known compounds, daurichromenic acid (**5**) and ilicicolinic acid B (**6**), have been isolated from the Chinese medicinal plant *Rhododendron anthopogonoides*. The *S* absolute configuration of the stereogenic carbons in the chromane and chromene rings of **1**−**4** was determined from their circular dichroism spectra. Compounds **1** and **2** inhibited compound 48/80-induced histamine release from rat peritoneal mast cells.

Rhododendron anthopogonoides belongs to the family Ericaceae and is distributed throughout southern China. This plant is used in traditional medicine as an expectorant and for chronic bronchitis. The crude drug contains several flavonoids, essential oils, triterpenes, and tannins.[1] In our screening program for antiallergic agents from natural sources, we found that a 60% EtOH extract of *R. anthopogonoides* inhibited compound 48/80-induced histamine release from rat mast cells. As part of our ongoing phytochemical and antiallergic agent studies on *Rhododendron* plants,[2] we report on the isolation and structure elucidation of four new chromane derivatives (**1**−**4**) and the known compounds daurichromenic acid (**5**)[3] and ilicicolinic acid B (**6**),[4] as well as their inhibitory effects on histamine release.

The 60% EtOH extract of the leaves and twigs of *R. anthopogonoides* was fractionated into *n*-hexane, EtOAc, *n*-BuOH, and aqueous layers. The hexane layer showed the most potent inhibitory activity on histamine release; thus this fraction was separated using normal- and reversed-phase chromatography and HPLC employing a normal-phase column to give compounds **1**−**6**.

Anthopogochromane (**1**) was isolated as a light yellow oil, $[\alpha]_D^{23}$ −166.3 (*c* 1.0, CH_3OH), and HRFABMS showed a quasimolecular ion peak at *m*/*z* 385.2014 $[M - H]^-$ corresponding to the molecular formula $C_{23}H_{30}O_5$, indicating nine degrees of unsaturation. The UV spectrum showed absorption maxima at 275 and 227 nm, and the IR spectrum showed absorption bands at 3409, 1700, 1620, and 1455 cm^{-1}, suggesting the presence of hydroxy and carbonyl groups and an aromatic ring. The ^1H NMR spectrum indicated the presence of two secondary methyl groups, two tertiary methyl groups, an aromatic methyl group, and a pentasubstituted aromatic ring (Table 1). The 23 carbon signals observed in the ^{13}C NMR spectrum and DEPT experiment revealed the presence of a carbonyl carbon at δ_C 210.2, a carboxylic carbon at δ_C 174.8, a benzene ring, four methylene carbons, four aliphatic methine carbons, a quaternary carbon, an oxygen-bearing quaternary carbon, and five methyl carbons (Table 2). The HMBC correlations of H_3-21 with C-6, C-7, and C-8; H-8 with C-4a, C-6, C-7, C-8a, and C-21; and H-4 with C-2, C-4a, C-5, and C-8a indicated that a hydrogen-bonded hydroxy group, a carboxylic group, and a methyl group were located at C-5, C-6, and C-7, respectively. The partial structural units [CH_2 (H-9)−CH_2 (H-10), CH (H-3)−CH (H-4)−CH (H-13), and a 3-methylbutyryl group (O═C−C-15−C-16−CH_3-17−CH_3-18)] were deduced from the $^1H-^1H$ COSY and HMBC spectra (Figure 1). In the HMBC spectrum, H-13 showed correlations with C-14 and C-15; the methyl protons (H_3-19) showed correlations with C-3, C-11, C-12, and C-13; and H_3-20 showed correlations with C-2, C-3, and C-9. These results together with the partial structural units indicated the presence of the 5,6,7-trisubstituted chromane ring, a cyclobutane ring (C-3−C-4−C-13−C-12), a cyclohexane ring (C-2−C-3−C-12−C-11−C-10−C-9), and a 3-methylbutyryl group located at C-13. The presence of an alicyclic system was supported by the degree of unsaturation of **1**. The tetracyclic compound **1** was similar to rhododaurichromanic acids A and B.[3] It is known

Table 1. ^1H NMR Data for Compounds **1**−**4** [300 MHz, $CDCl_3$, TMS, δ (ppm) J = Hz]

position	1	2	3	4
3	1.88 d (9.1)	5.46 d (10.0)	5.45 d (10.0)	5.46 d (10.0)
4	4.24 dd (9.1, 8.5)	6.72 d (10.0)	6.62 d (10.0)	6.72 d (10.0)
6			6.12 br s	
8	6.33 s	6.21 s	6.20 br s	6.21 s
9	2.15 m	1.79, 1.68 m	1.73, 1.65 m	1.81, 1.67 m
10	1.38 m	2.12 m	2.12 m	2.19 m
11	1.61 m	5.23 t (6.6)	5.23 t (6.6)	5.23 t (6.6)
13	3.00 d (8.5)	3.00 s	2.99 s	3.01 s
15	2.25 m	2.28 d (7.0)	2.28 d (7.1)	6.08 s
16	2.13 m	2.15 m	2.15 m	
17	0.84 d (6.2)	0.89 d (6.4)	0.89 d (6.4)	1.87 s
18	0.84 d (6.2)	0.89 d (6.4)	0.89 d (6.4)	2.14 s
19	1.13 s	1.58 s	1.58 s	1.59 s
20	1.05 s	1.37 s	1.37 s	1.41 s
21	2.52 s	2.53 s	2.19 s	2.53 s
5-OH	11.75 s	11.74 s		11.76 s

Table 2. ^{13}C NMR Data for Compounds **1**−**4** (75 MHz, $CDCl_3$, TMS)[a]

position	1	2	3	4
2	77.2 s	79.9 s	78.0 s	79.9 s
3	46.3 d	125.8 d	126.5 d	125.9 d
4	23.8 d	116.7 d	116.9 d	116.6 d
4a	113.3 s	106.8 s	106.6 s	106.8 s
5	160.3 s	160.3 s	151.0 s	160.3 s
6	104.7 d	103.6 s	108.3 d	103.5 s
7	142.3 s	144.2 s	139.3 s	144.1 s
8	114.6 d	111.9 d	109.4 d	111.9 d
8a	160.9 s	158.4 s	153.7 s	158.4 s
9	36.2 t	41.4 t	40.8 t	41.5 t
10	17.2 t	23.0 t	23.1 t	23.0 t
11	34.0 t	129.1 d	129.3 d	128.5 d
12	38.8 s	128.9 s	128.8 s	129.9 s
13	56.4 d	54.4 t	54.5 t	55.3 t
14	210.2 s	209.6 s	209.6 s	199.0 s
15	51.9 t	50.7 t	50.7 t	122.6 d
16	24.1 d	24.6 d	24.6 d	155.7 s
17	22.8 q	22.7 q	22.7 q	20.9 q
18	22.8 q	22.7 q	22.7 q	27.9 q
19	25.4 q	16.6 q	16.6 q	16.6 q
20	29.0 q	27.3 q	29.5 q	27.4 q
21	24.4 q	24.7 q	21.7 q	24.7 q
COOH	174.8 s	175.5 s		175.2 s

[a] The multiplicities of carbon signals were determined using the DEPT method and are indicated as s, d, t, and q.

* To whom correspondence should be addressed. Tel: 0081-47-465-5356. Fax: 0081-47-465-5440. E-mail: kitanaka.susumu@nihon-u.ac.jp.

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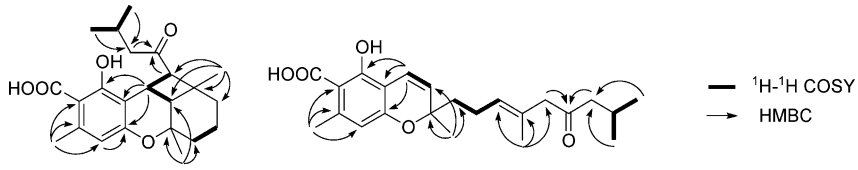


1 **2**

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations of compounds **1** and **2**.

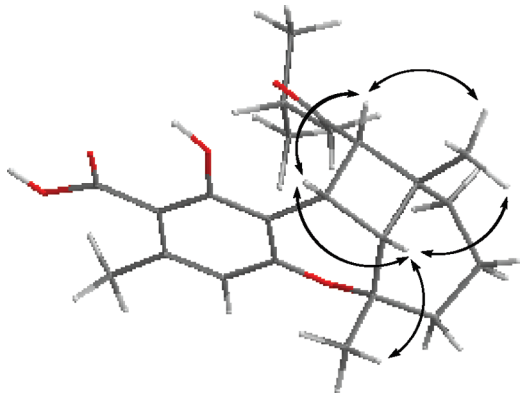


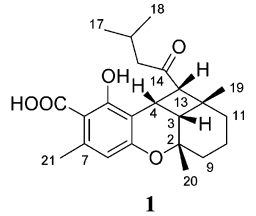
Figure 2. NOE correlations of compound **1**.

that these compounds originated by cyclization of C-3−C-4 and C-11−C-12 in **5**. The presence of the tetracyclic chromane derivatives has been identified in several natural products.[5−7] However, the tetracyclic-type chromane derivative designated as **1**, originated by cyclization of C-3−C-4 and C-12−C-13, has not yet been reported. The relative configuration of **1** was assigned on the basis of the difference NOE experiments (Figure 2). Irradiation of H-3 enhanced the signal intensity of H-4, H_3-19, and H_3-20; irradiation of H-4 enhanced the signal intensity of H-3 and H-13; and irradiation of H-13 enhanced the signal intensity of H-4 and H_3-19. These results suggest that H-3, H-4, H-13, H_3-19, and H_3-20 have the same orientation. On the basis of the above findings, the structure of **1** was established as shown in Chart 1.

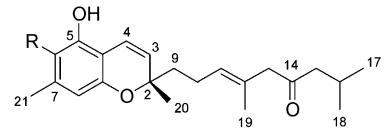
Anthopogochromene A (**2**) was obtained as a colorless oil, $[\alpha]_D^{23}$ +83.0 (*c* 1.0, CH_3OH). The molecular formula was deduced to be $C_{23}H_{30}O_5$ by HRFABMS. The IR spectrum exhibited absorption bands at 3225, 1702, 1619, and 1455 cm^{-1}, and the UV spectrum displayed maxima at 325, 285, and 247 nm. The ^{1}H NMR spectrum indicated one aromatic methyl group, the presence of a pentasubstituted aromatic ring, a pair of *cis*-coupled olefinic protons at δ_H 5.46 (d, J = 10.0 Hz, H-3) and 6.72 (d, J = 10.0 Hz, H-4), and a hydrogen-bonded hydroxy proton (Table 1). The presence of a pentasubstituted aromatic ring as seen in **1** and a 2,6-dimethyl-6-nonaen-4-one unit[8] were deduced from the ^{1}H−^{1}H COSY and HMBC spectra. In the HMBC spectrum, H_3-20 showed long-range correlations with C-2, C-3, and C-9; H-4 showed cross-peaks with C-4a, C-5, and C-8a. These findings indicate the presence of a 5,6,7-substituted chromene ring and that a 2,6-dimethylnona-6-en-4-one side chain was located at C-2 in the chromene ring. This arrangement was supported by the observation of a fragment at *m*/*z* 168 $[M - C_{12}H_{11}O_4]^+$ on FABMS of **2**. The NOESY spectrum established that the C-11/12 double bond of **2** has *E* geometry due to the correlations of H-9 and H-11, and H-10 and H_3-19. Thus, the structure of **2** was established as 2-(12,16-dimethyl-14-oxo-11*E*-nonenyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene-6-carboxylic acid.

Anthopogochromene B (**3**) showed a molecular formula of $C_{22}H_{30}O_3$ and was obtained as a light yellow oil, $[\alpha]_D^{23}$ +261.3 (*c* 1.0, CH_3OH). The UV spectrum displayed maxima at 301, 261, and 224 nm, and the IR spectrum showed absorption bands at 3384, 1699, 1623, and 1452 cm^{-1}. The ^{1}H and ^{13}C NMR spectral data of **3** were similar to those of **2**. However, a carboxyl group (δ_C 175.5) and a hydrogen-bonded hydroxy proton (δ_H 11.74) in **2** were absent in **3** and were replaced by a *meta*-coupled aromatic proton at δ_H 6.12 (H-6). In the HMBC spectrum, the aromatic methyl (H_3-21) showed long-range correlations with C-6, C-7, and C-8; H-6 showed cross-peaks with C-4a, C-5, C-8, and CH_3-21. Thus, the structure

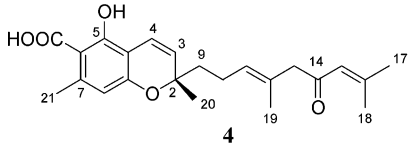
Chart 1. Structures of Compounds **1**−**6**



1



2 R = COOH
3 R = H



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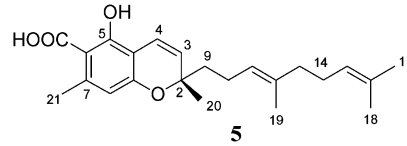

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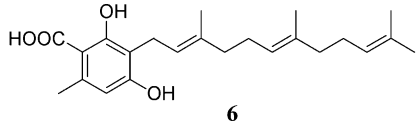

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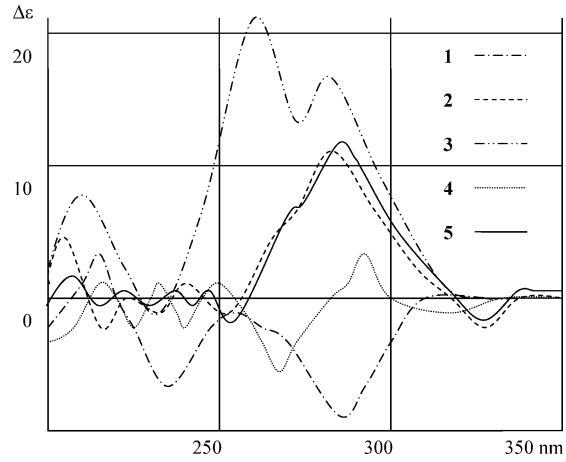
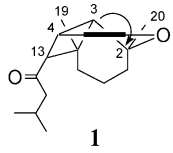


Figure 3. CD spectra of compounds **1**−**5**.



1

P-helicity ⇒ negative ^1L_b band

Figure 4. Helicity of the chromane ring for compound **1**.

of **3** was established as 2-(12,16-dimethyl-14-oxo-11*E*-nonenyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene.

Anthopogochromene C (**4**), a light yellow oil, $[\alpha]_D^{23}$ −69.7 (*c* 1.0, CH_3OH), showed a molecular formula of $C_{23}H_{28}O_5$. The ^1H and ^{13}C NMR spectral data of **4** were similar to those of **2**, except for a 3-methylbutyryl-2-ene unit (C-14−C-18) in **4** instead of the 3-methylbutyryl unit in **2**. In the HMBC spectrum, the aromatic methine proton (H-15) showed long-range correlations with C-13, C-14, C-16, CH_3-17, and CH_3-18; H-13 showed cross-peaks with C-11, C-12, C-14, C-15, and CH_3-19. Thus, the structure of **4** was established as 2-(12,16-dimethyl-14-oxo-11*E*,15-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene-6-carboxylic acid.

Compounds **5** and **6** were determined to be daurichromenic acid (**5**) and ilicicolinic acid B (**6**) by comparison of their physical data with those reported. This is the first time that compounds **5** and **6** have been isolated from *R. anthopogonoides*.

The CD spectra of **1**−**5** are shown in Figure 3. The 2*S* absolute configuration in **1** was established on the basis of the negative Cotton effects for the ^1L_b band at 270−290 nm due to the *P*-helicity of the chromane ring system (Figure 4).[9−13] The 2*S* absolute configuration in **2**−**4** was determined by comparison of their CD spectra with those of **5**.

We have examined the inhibitory activity of **1**−**6** on compound 48/80-induced histamine release from peritoneal mast cells in rats. The inhibitory effects of **1** (IC_{50} = 114 μM) and **2** (IC_{50} = 63 μM) were comparable to that of the potent anti-inflammatory drug indomethacin (IC_{50} = 250 μM); however, the other compounds did not possess any inhibitory activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-36 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-300. UV spectra were obtained on a UV-160 (Shimadzu) and MS on a Hitachi M-200 spectrometer. CD spectra were obtained with a JASCO J-600 spectrophotometer. NMR spectra were taken on a Mercury-300BB Varian FT NMR spectrometer, using TMS as an internal standard. Column chromatography was performed on silica gel (Wako gel C-300, Wako Pure Chemical Industry Ltd.). HPLC was carried out with silica gel (YMC-Pack SIL-06, YMC-Guardpack SIL-06) and reversed-phase columns (CAPCELL PAK C-18).

Plant Material. The leaves and twigs of *R. anthopogonoides* were collected in Kangding County of Sichuan Province in China, in July 2003. A voucher specimen has been deposited at the College of Pharmacy, Nihon University.

Extraction and Isolation. The leaves and twigs of *R. anthopogonoides* (860 g) were crushed and extracted twice with 60% EtOH. Evaporation of the solvent under reduced pressure gave the 60% EtOH extract [193 g, inhibitory effect IC_{50} = 22.9 μg/mL]. The extract was dissolved and suspended in water and partitioned with *n*-hexane, EtOAc, and *n*-BuOH, successively. Evaporation of solvent yielded the *n*-hexane (13.0 g, 18.6 μg/mL), EtOAc (40.4 g, 53.7 μg/mL), *n*-BuOH (38.8 g, >100 μg/mL), and aqueous fractions (55.1 g, >100 μg/mL). The hexane fraction was subjected to silica gel column chromatography with *n*-hexane−EtOAc (98:2 to 0:100) to give fractions 1 (0.33 g), 2 (4.19 g), 3 (2.00 g), 4 (0.84 g), and 5 (1.18 g). Fraction 4 was chromatographed on a silica gel column using *n*-hexane−EtOAc (90:10) to give four fractions (4-1−4-4), and fraction 4-3 (0.20 g) was purified by HPLC (column: CAPCELL PAK C-18, 70% CH_3CN) to give **1** (7.8 mg) and **6** (11.3 mg). Fraction 3 was chromatographed on an ODS column using 80% CH_3OH to give four fractions (3-1−3-4), and fraction 3-4 (0.10 g) was purified by HPLC (column: silica gel YMC-Pack SIL-06, *n*-hexane−EtOAc, 93:7) to give **2** (21.7 mg). Fraction 3-2 (0.18 g) was purified by HPLC (column: silica gel YMC-Pack SIL-06, *n*-hexane−EtOAc, 92:8) to give **3** (18.8 mg). Fraction 5 was purified by HPLC (column: silica gel YMC-Pack SIL-06, *n*-hexane−EtOAc, 90:10) to give **4** (12.8 mg). Fraction 2 was purified by HPLC (column: silica gel YMC-Pack SIL-06, *n*-hexane−EtOAc, 95:5) to give **5** (76.2 mg).

Anthopogochromane (1): colorless, amorphous powder; $[\alpha]_D^{23}$ −166.3 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3409, 2957, 2931, 1700, 1620, 1579, 1455, 1374, 1178, 1127, 1059 cm^{-1}; UV (MeOH) λ_{max} (log ε) 275 (3.08), 227 (4.08), 208 (4.51) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 385 $[M - H]^-$, 306, 199, 168, 151, 138, 122, 92; HRFABMS *m/z* 385.2014 (calcd for $C_{23}H_{29}O_5$ 385.2015); CD (MeOH) λ_{max} $\Delta\varepsilon_{291}$ −9.6, $\Delta\varepsilon_{237}$−8.0.

Anthopogochromene A (2): colorless oil; $[\alpha]_D^{23}$ +83.0 (*c* 1.0, MeOH); IR (liquid film) ν_{max} 3225, 2960, 2929, 1702, 1647, 1619, 1565, 1455, 1121, 1061 cm^{-1}; UV (MeOH) λ_{max} (log ε) 325 (3.50), 285 (3.60), 247 (4.46) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 385 $[M - H]^-$, 306, 199, 168, 153, 122, 91; HRFABMS *m/z* 385.2019 (calcd for $C_{23}H_{29}O_5$ 385.2015); CD (MeOH) λ_{max} $\Delta\varepsilon_{288}$ +10.3, $\Delta\varepsilon_{268}$ +5.3.

Anthopogochromene B (3): light yellow oil; $[\alpha]_D^{23}$ +261.3 (*c* 1.0, MeOH); IR (liquid film) ν_{max} 3384, 2958, 2927, 1699, 1623, 1578, 1452, 1366, 1142, 1066 cm^{-1}; UV (MeOH) λ_{max} (log ε) 301 (3.64), 261 (3.99), 224 (4.51) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 342 $[M]^+$, 327, 304, 257, 242, 227, 215, 201, 187, 175, 161, 149, 145, 137, 131, 121, 115, 107; HREIMS *m/z* 342.2186 (calcd for $C_{22}H_{30}O_3$ 342.2195); CD (MeOH) λ_{max} $\Delta\varepsilon_{290}$ +16.6, $\Delta\varepsilon_{265}$ +20.6.

Anthopogochromene C (4): light yellow oil; $[\alpha]_D^{23}$ −69.7 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3428, 2969, 2934, 1619, 1453, 1380, 1265, 1174, 1122, 1061 cm^{-1}; UV (MeOH) λ_{max} (log ε) 299 (3.57), 257 (3.96), 220 (4.51) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 385 $[M - H]^-$, 305, 199, 168, 152, 138, 122, 92; HRFABMS *m/z* 383.1854 (calcd for $C_{23}H_{27}O_5$ 383.1858); CD (MeOH) λ_{max} $\Delta\varepsilon_{297}$ +3.7, $\Delta\varepsilon_{269}$ −4.7.

Inhibitory Activity on Histamine Release. The preparation of mast cells and the assay of histamine release from mast cells were performed by the modified method of Hirai et al.[14] Male Wister rats (Japan SLC, Shizuoka) weighing 180−200 g were exsanguinated and injected intraperitoneally with 10 mL of Tyrode solution. The abdominal region was massaged for 3 min, and then the peritoneal exudates were collected. The peritoneal cavity fluid containing mast cells was suspended in phosphate-buffered saline (PBS), then layered on bovine serum albumin (*d* = 1.068) in a test tube on ice for 20 min. After centrifugation at 300 rpm at 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 3 mL of PBS (pH 7.0) and suspended in the same medium. Cell viability was determined using trypan blue (10 μL) at 37 °C for 10 min, followed by the addition of histamine releasers (compound 48/80, 5 μg/mL). The mixture was incubated again for 10 min, the quantity of histamine released was expressed in peak height, and percent inhibition was then calculated. Indomethacin was used as a standard drug.

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References and Notes

(1) *Chinese Materia Medica*; Jiangsu New Medical College, Ed.; Shanghai People's Pub. House: Shanghai, 1985; pp 1275−1273.
(2) Iwata, N.; Wang, N. L.; Yao, X. S.; Kitanaka, S. *J. Nat. Prod.* **2004**, *67*, 1106–1109.
(3) Kashiwada, Y.; Yamazaki, K.; Ikeshiro, Y.; Yamagishi, T.; Fujioka, T.; Mihashi, K.; Mizuki, K.; Cosentino, L. M.; Fowke, K.; Morris-Natschke, S. L.; Lee, K. H. *Tetrahedron* **2001**, *57*, 1559–1563.
(4) Jpn. Kokai Tokkyo Koho, 05255184, 1993.
(5) Ganoi, Y.; Mechoulan, R. *J. Am. Chem. Soc.* **1971**, *93*, 217–224.
(6) Jefferies, P. R.; Worth, G. K. *Tetrahedron* **1973**, *29*, 903–908.
(7) Gonzalez, J. G.; Olivares, E. M.; Delle Monache, F. *Phytochemistry* **1995**, *38*, 485–489.
(8) McPhail, K.; Davies-Coleman, M. T.; Starmer, J. *J. Nat. Prod.* **2001**, *64*, 1183–1190.
(9) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177–2215.
(10) Snatzke, G.; Kajtar, M.; Snatzke, F. In *Fundamental Aspects and Development in Optical Rotatory Dispersion and Circular Dichroism*; Ciardelli, F.; Salvadori, P., Eds.; Heyden & Son Ltd: London, 1973; pp 148−172.
(11) Ferreira, D.; Marais, J. P. J.; Slade, D.; Walker, L. A. *J. Nat. Prod.* **2004**, *67*, 174–178.
(12) Nozoe, S.; Hirai, K. *Tetrahedron* **1974**, *30*, 2773–2776.
(13) Crabbe, P. *Chem. Ind.* **1969**, 917–918.
(14) Hirai, Y.; Takase, H.; Yasuhara, T.; Nakajima, T. *Shoyakugaku Zasshi* **1983**, *37*, 374–380.

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