Structures and Histamine Release Inhibitory Effects of Prenylated Orcinol Derivatives from *Rhododendron dauricum*[1]

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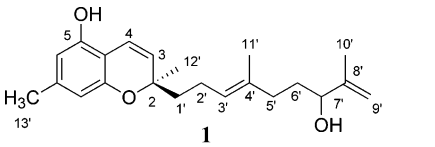
Four new prenylated orcinol derivatives, daurichromenes A−D (**1**−**4**), along with three known compounds, confluentin (**5**), grifolin (**6**), and orcinol (**7**), have been isolated from the Chinese medicinal plant *Rhododendron dauricum*. Their structures were established as 2*R*-(7′-hydroxy-4′,8′-dimethyl-3′*E*,8′-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene (**1**), 2*R*-(3′-hydroxy-8′-methyl-4′-methyliden-7′-nonaenyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene (**2**), 2*R*-(8′-hydroxy-4′,8′-dimethyl-3′*E*,6′*Z*-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene (**3**), and 2*R*-(9′-hydroxy-4′,8′-dimethyl-3′*E*,7′*E*-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene (**4**) by analysis of spectral data. The absolute configuration of the asymmetric carbons at the chromene ring in **1**−**5** was determined as *R* from their circular dichroism spectra. Compounds **1**−**6** significantly inhibited compound 48/80-induced histamine release from rat peritoneal mast cells.

Rhododendron dauricum belongs to the family Ericaceae and is distributed throughout northern China, inner Mongolia, eastern Siberia, and Hokkaido. The dried leaves of this plant are called "Manshanhong" in China and are used in traditional medicine as an expectorant and for acute and chronic bronchitis. The crude drug contains several flavonoids, essential oils, grayanotoxin I, coumarins, and tannins.[2−4] Recently, the isolation of active anti-HIV compounds, rhododaurichromanic acid and daurichromenic acid, from the leaves and twigs of *R. dauricum* has been reported.[5,6] During our program of screening for antiallergic agents from natural sources, we found a *R. dauricum* extract that inhibits histamine release from rat mast cells induced by compound 48/80. Therefore, we studied the chemical constituents of this plant in an effort to identify the antiallergic agents. The 60% EtOH extract of the leaves and twigs of *R. dauricum* was dissolved and suspended in water and partitioned with *n*-hexane, EtOAc, and *n*-BuOH, respectively. Bioassay-guided fractionation led to the isolation of four new prenylated orcinol derivatives, daurichromenes A−D (**1**−**4**), and three known compounds, confluentin (**5**),[7,8] grifolin (**6**),[9,10] and orcinol (**7**). In this paper, we describe the structure elucidation and the inhibition of histamine release by these compounds.



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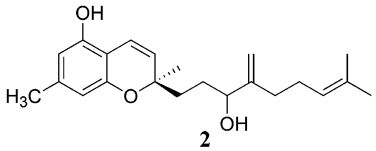

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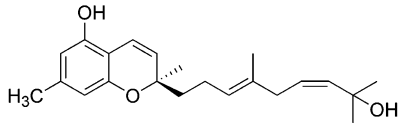

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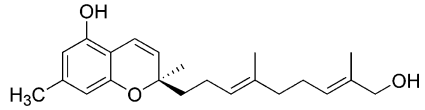

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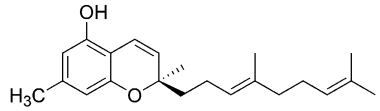

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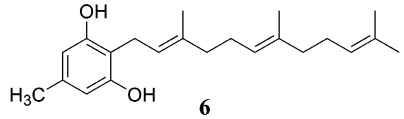

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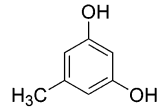

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Results and Discussion

Daurichromene A (**1**) was isolated as a light yellow oil, $[\alpha]^{26}_{D}$ −30.4° (*c* 0.20, CH_3OH), and the molecular formula of **1** was established as $C_{22}H_{30}O_3$ by HREIMS, which showed a molecular ion peak at *m*/*z* 342.2258. The UV spectrum showed maxima at 280 and 230 nm, and the IR spectrum showed an absorption band at 3363 cm^{-1} for at least one hydroxyl group. The ^1H NMR spectrum indicated the presence of one aromatic methyl proton at δ_H 2.20 (CH_3-13′), *meta*-coupled aromatic protons at δ_H 6.11 (H-6) and 6.23 (H-8), and a pair of *cis*-coupled olefinic protons at δ_H 5.48 (d, J = 10.1 Hz, H-3) and 6.61 (d, J = 10.1 Hz, H-4) (Table 1). The ^{13}C NMR and DEPT spectra of **1** showed 22 carbon signals, including aromatic carbons, three double bonds, four methylenes, one oxygen-bearing methine, one oxygen-bearing quaternary carbon, and four methyl carbons (Table 2). In the HMBC spectrum, the aromatic methyl proton at δ_H 2.20 (CH_3-13′) showed long-range

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**4** [500 MHz, $CDCl_3$, TMS, δ (ppm) J = Hz]

proton	1	2	3	4
3	5.48 d (10.1)	5.47 d (9.4)	5.47 d (10.1)	5.49 d (10.0)
4	6.61 d (10.1)	6.61 d (9.4)	6.61 d (10.1)	6.61 d (10.0)
6	6.11 br s	6.11 br s	6.12 br s	6.12 br s
8	6.23 br s	6.22 br s	6.23 br s	6.24 br s
1′	1.76 m	1.78 m	1.78 m	1.74 m
2′	2.12 m	1.65, 1.76 m	2.12 m	2.12 m
3′	5.16 t (7.0)	4.08 t (6.1)	5.13 t (7.1)	5.12 t (7.2)
5′	2.00 m	2.13 m	2.64 d (6.1)	1.99 m
6′	1.63 m	2.15 m	5.56 m	2.09 m
7′	4.04 t (6.1)	5.11 t (7.0)	5.61 br t (7.0)	5.34 t (7.0)
9′	4.84, 4.93 m	1.68 s	1.31 s	3.99 s
10′	1.72 s	1.61 s	1.31 s	1.65 s
11′	1.59 s	4.87, 5.03 m	1.55 s	1.58 s
12′	1.37 s	1.37 s	1.37 s	1.37 s
13′	2.20 s	2.20 s	2.20 s	2.20 s

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**5** (125 MHz, $CDCl_3$, TMS)[a]

carbon	1	2	3	4	5
2	78.2 s	78.2 s	78.2 s	78.2 s	78.2 s
3	127.0 d	126.8 d	127.1 d	127.1 d	127.2 d
4	116.8 d	117.0 d	116.8 d	116.8 d	116.7 d
4a	106.8 s	106.6 s	106.7 s	106.8 s	106.8 s
5	151.1 s	151.1 s	151.1 s	151.1 s	151.0 s
6	108.3 d	108.4 d	108.3 d	108.3 d	108.3 d
7	139.5 s	139.6 s	139.6 s	139.6 s	139.5 s
8	109.7 d	109.8 d	109.8 d	109.8 d	109.3 d
8a	154.1 s	153.9 s	154.1 s	154.1 s	154.1 s
1′	41.0 t	37.1 t	41.0 t	41.0 t	41.0 t
2′	22.7 t	30.0 t	22.7 t	22.6 t	22.6 t
3′	124.6 d	75.5 d	125.1 d	124.4 d	124.0 d
4′	134.9 s	151.1 s	133.9 s	134.8 s	135.3 s
5′	35.6 t	31.5 t	42.3 t	39.2 t	39.7 t
6′	33.1 t	26.6 t	125.3 d	26.1 t	26.7 t
7′	75.8 d	124.0 d	139.2 d	126.1 d	124.4 d
8′	147.8 s	131.9 s	70.8 s	134.7 s	131.3 s
9′	111.1 t	25.7 q	29.8 q	69.1 t	25.7 q
10′	17.6 q	17.7 q	29.8 q	13.7 q	17.7 q
11′	15.9 q	109.6 t	16.0 q	15.9 q	16.0 q
12′	26.3 q	26.5 q	26.4 q	26.3 q	26.3 q
13′	21.5 q	21.5 q	21.5 q	21.5 q	21.5 q

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

correlations with the carbon signals at δ_C 108.3 (C-6), 139.5 (C-7), and 109.7 (C-8). The proton signal of the methyl at δ_H 1.37 (CH_3-12′) showed long-range correlations with the carbon signals at δ_C 78.2 (C-2), 127.0 (C-3), and 41.0 (C-1′); δ_H 5.48 (H-3) showed cross-peaks with the carbon signals at δ_C 78.2 (C-2), 116.8 (C-4), 41.0 (C-1′), and 26.3 (CH_3-12′); and δ_H 6.61 (H-4) showed cross-peaks with the carbon signals at δ_C 78.2 (C-2), 127.0 (C-3), 106.8 (C-4a), 151.1 (C-5), and 154.1 (C-8a). From this evidence, the presence of a 5,7-disubstituted chromene ring was postulated. This arrangement was supported by the observation of a fragment at m/z 175 $[M - C_{11}H_{11}O_2]^+$ in the EIMS of **1**. The ^{1}H and ^{13}C NMR spectral data of **1** were very similar to those of **5**, except for one *exo*-methylene (δ_H 4.84, 4.93; δ_C 111.1) and one oxygen-bearing methine carbon (δ_C 75.8, C-7′) in **1** instead of the one methyl (C-9′) and one aromatic methine (C-7′) in **5**. In the HMBC spectrum, an oxygen-bearing methine proton at δ_H 4.04 (H-7′) showed long-range correlations with carbon signals at δ_C 35.6 (C-5′), 33.1 (C-6′), 147.8 (C-8′), 111.1 (C-9′), and 17.6 (CH_3-10′); δ_H 4.84, 4.93 (H-9′) showed correlations with δ_C 75.8 (C-7′), 147.8 (C-8′), and 17.6 (CH_3-10′); and δ_H 2.00 (H-5′) showed cross-peaks with C-3′, 4′, 5′, 6′, and 11′. Furthermore, comparison of the H-6′ signal of **1** (δ_H 1.63) with that of H-6′ of **5** (δ_H 2.04) showed that it was shifted upfield by 0.41. Therefore, the hydroxyl group was located at C-7′ and the *exo*-methylene at C-9′. The NOESY spectrum established that the C-3′/4′ double bond of **1** is *E*, due to the correlations of H-2′ and CH_3-11′, H-3′, and H-5′. Thus, the structure of **1** was established as 2-(7′-hydroxy-4′,8′-dimethyl-3′*E*,8′-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene.

Daurichromene B (**2**) was obtained as a light yellow oil, $[\alpha]^{26}_D$ −27.7° (*c* 0.13, CH_3OH). The molecular formula, $C_{22}H_{30}O_3$, was the same as that of **1**. The IR spectrum exhibited an absorption band at 3349 cm^{-1}, and the UV spectrum displayed maxima at 280 and 230 nm. The ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of the isomeric **1**, which differs from **2** only in the positions of the hydroxyl and *exo*-methylene groups. In the HMBC spectrum of **2**, δ_H 4.08 (H-3′) showed cross-peaks with δ_C 37.1 (C-1′), 30.0 (C-2′), 151.1 (C-4′), and 109.6 (C-11′), placing the hydroxyl group at C-3′. In addition, δ_H 4.87, 5.03 (H-11′) showed long-range correlations with the carbon signals at δ_C 75.5 (C-3′), 151.1 (C-4′), and 31.5 (C-5′), indicating that the *exo*-methylene was located at C-4′. Thus, the structure of **2** was established as 2-(3′-hydroxy-8′-methyl-4′-methyliden-7′-nonaenyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene.

Daurichromene C (**3**) possessed the same molecular formula as **1**, $C_{22}H_{30}O_3$, and was obtained as a light yellow oil, $[\alpha]^{26}_D$ −32.0° (*c* 0.10, CH_3OH). The UV spectrum showed maxima at 280 and 230 nm, and the IR spectrum showed an absorption band at 3363 cm^{-1} for at least one hydroxyl group. The ^{1}H and ^{13}C NMR spectral data of **3** indicated two equivalent methyl carbons (δ_C 29.8, CH_3-9′, 10′), one methine carbon (δ_C 125.3, C-6′), and one oxygen-bearing quaternary carbon (δ_C 70.8, C-8′) in place of the two methyl carbons [δ_C 25.7 (CH_3-9′), δ_C 17.7 (CH_3-10′)], one methylene (δ_C 26.7, C-6′), and one quaternary carbon (δ_C 131.3, C-8′) in **5**. In the HMBC spectrum of **3**, δ_H 5.56 (H-6′) showed cross-peaks with C-4′ (δ_C 133.9), C-5′ (δ_C 42.3), and C-8′ (δ_C 70.8), and δ_H 1.31 (CH_3-9′,10′) showed cross-peaks with C-7′ (δ_C 139.2) and C-8′ (δ_C 70.8). Thus, the hydroxyl group was located at C-8′. The position of the double bond was determined by comparison of the ^{1}H NMR spectra of **5**; a downfield shift of H-6′ (3.5 ppm) demonstrated that the C-7′ to C-8′ double bond of **5** was translocated at C-6′ to C-7′ in **3**. The configuration of C-3′/4′ was 3′*E* as indicated by the ^{13}C NMR chemical shift of CH_3-11′ at δ_C 16.0 (less than 20 ppm). The coupling constant of the olefinic H-6′, H-7′ (J = 7.0 Hz) bond proved the geometry to be 6′*Z*, which was confirmed by the H-5′ methylene chemical shift at δ_H 2.64 (this value in the geometrical isomers was moved upfield to δ_H 2.0−2.1).[11,12] Thus, the structure of **3** was established as 2-(8′-hydroxy-4′,8′-dimethyl-3′*E*,6′*Z*-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene.

Daurichromene D (**4**), light yellow oil, $[\alpha]^{26}_D$ −26.0° (*c* 0.10, CH_3OH), showed a molecular formula of $C_{22}H_{30}O_3$. The ^{1}H and ^{13}C NMR spectral data of **4** were almost the same as those of **5**, except for one oxymethylene (δ_C 69.1, C-9′). In the HMBC spectrum of **4**, the proton signal of the methyl at δ_H 1.65 (CH_3-10′) showed long-range correlations with the carbon signals at δ_C 126.1 (C-7′), 134.7 (C-8′), and 69.1 (C-9′); δ_H 3.99 (H-9′) showed cross-peaks with the carbon signals at δ_C 126.1 (C-7′), 134.7 (C-8′), and 13.7 (CH_3-10′); and δ_H 5.34 (H-7′) showed cross-peaks with the carbon signals at δ_C 69.1 (C-9′) and 13.7 (CH_3-10′) and was considered to be located on the oxymethylene at C-9′. Thus, the C-9′ methyl group in **5** was hydroxylated [δ_H 3.99 (2H), δ_C 69.1] in **4**. The NOESY spectrum established that the C-3′/4′ and C-7′/8′ double bonds of **5** were *E*, due to the

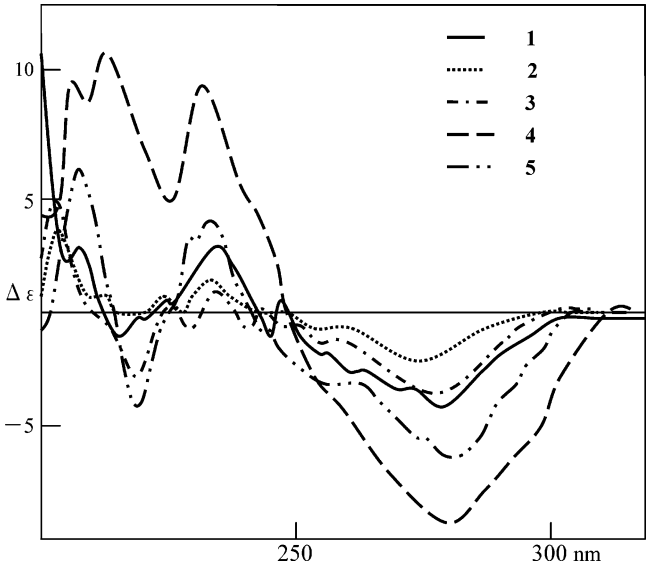

Figure 1. CD spectra of compounds **1**−**5** (in MeOH).

correlations of H-2′ and CH_3-11′, H-3′ and H-5′, H-6′ and CH_3-10′, and H-7′ and H-9′. Thus, the structure of **4** was established as 2*R*-(9′-hydroxy-4′,8′-dimethyl-3′*E*,7′*E*-nonadienyl)-5-hydroxy-2,7-dimethyl-2*H*-chromene.

The circular dichroism (CD) spectra of **1**−**5** are shown in Figure 1. The absolute C-2 configurations were established as *R* by Crabbe's rule.[13,14] The *P*-helicity of the α-chromene ring culminated in negative Cotton effects for the band at 270−280 nm.

We have examined the inhibitory activity of compounds **1**−**7** on compound 48/80-induced histamine release from peritoneal mast cells in rats (**1**: $IC_{50} = 1.6\ \mu L$, **2**: $IC_{50} = 4.4\ \mu L$, **3**: $IC_{50} = 87.4\ \mu L$, **4**: $IC_{50} = 1.3\ \mu L$, **5**: $IC_{50} = 205.0\ \mu L$, **6**: $IC_{50} = 81.6\ \mu L$). The inhibitory effects of compounds **1**−**6** were much higher than that of the potent antiinflammatory drug indomethacin ($IC_{50} = 250\ \mu L$); however, **7** did not possess any inhibitory activity. These compounds are likely to be responsible for the histamine release inhibitory effect of *R. dauricum*, and the results suggest that the presence of an orcinol-connected isoprenyl side chain is important to the histamine inhibitory potency. Prenylated orcinol derivatives with an inhibitory effect on histamine release were previously unknown. Thus, this study presents natural products as a potential source of a new generation of drugs for the treatment of various inflammatory diseases.

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 mass spectra (MS) on a Hitachi M-200 spectrometer. CD spectra were obtained with a JASCO J-600 spectrophotometer. NMR spectra were taken on a JNM-GSK 500 FT NMR spectrometer, using tetramethylsilane (TMS) as an internal standard. Column chromatography was performed on silica gel (Wako gel C-200 and C-300, Wako Pure Chemical Industry Ltd.). Thin-layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 10% (v/v) H_2SO_4 in ethanol solution and then heating on a hot plate. High-performance liquid chromatography (HPLC) was carried out with silica gel YMC-Pack SIL-06 and YMC-Guardpack SIL-06, gel permeation chromatography (GPC) with Asahi pak GS-310, and reverse phase with Fluofix 1NW-125, Capcell Pak C-18.

Plant Material. The leaves and twigs of *R. dauricum* L. were collected in Da-Hi-Shan County of LiaoNing Province in China in July 1999 and identified by Prof. Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, China). A voucher specimen is deposited in the Department of Natural Products Chemistry of Shenyang Pharmaceutical University.

Extraction and Isolation. The leaves and twigs of *R. dauricum* (3.0 kg) were crushed and extracted twice with 60% EtOH. Evaporation of the solvent under reduced pressure gave the 60% EtOH extract [426.0 g, inhibitory effect 97.1% (100 μg/mL)]. The extract was dissolved and suspended in water and partitioned with *n*-hexane, EtOAc, and *n*-BuOH, respectively. Evaporation of solvent yielded a hexane fraction (18.8 g, 99.9%), an EtOAc fraction (65.5 g, 94.2%), an *n*-BuOH fraction (148.5 g, 81.1%), and an aqueous fraction (44.3 g, 80.6%). The hexane fraction was subjected to a silica gel column chromatography with hexane−EtOAc (20:1 to 0:1) to give fractions 1 (0.05 g, 99.6%), 2 (0.36 g, 67.1%), 3 (0.28 g, 71.9%), 4 (2.9 g, 97.8%), 5 (3.5 g, 92.9%), 6 (1.5 g, 101.7%), 7 (3.6 g, 94.1%), and 8 (3.2 g, 100.3%). Fraction 4 was chromatographed on a silica gel column using hexane−EtOAc (15:1) to give two fractions (4-1 and 4-2), and fraction 4-1 (1.2 g) was purified by HPLC (column: Capcell Pak C-18, 90% CH_3CN) to give **5** (1.2 g). Fraction 5 was purified by HPLC (column: silica gel YMC-Pack SIL-06, hexane−EtOAc, 20:1) to give three fractions (5-1−5-3), with fraction 5-2 (0.83 g) purified by HPLC (column: Asahi Pak GS-310, 85% MeOH) to give **6** (92.6 mg). Fraction 7 was chromatographed on a silica gel column using hexane−EtOAc (9:1) to give four fractions (7-1−7-4), and fraction 7-2 (0.25 g) was purified by HPLC (column: Capcell Pak C-18, 70% MeOH) to give **1** (27.2 mg) and **2** (3.0 mg). Fraction 7-3 (0.22 g) was purified by HPLC (column: Capcell Pak C-18, 70% MeOH) to give **3** (6.0 mg) and **4** (5.0 mg). The EtOAc fraction was subjected to silica gel column chromatography with $CHCl_3$−MeOH (98:2 to 0:1) to give 10 fractions (1−10). Fraction 4 (2.3 g) was chromatographed on a Sephadex LH-20 column using MeOH−$CHCl_3$ (80:20) to give seven fractions (4-1−4-7), and fraction 4-3 (0.19 g) was purified by HPLC (column: Capcell Pak C-18, 30% MeOH) to give **7** (13.8 mg).

Daurichromene A (1): light yellow oil; $[\alpha]^{26}_D$ −30.4 (*c* 0.20, CH_3OH); IR (liquid film) ν_{max} 3363, 1624, 1063 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 280 (3.90), 230 (4.37) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 342 $[M]^+$, 327, 272, 215, 203, 193, 175, 161, 137, 123, 107, 91, 81, 55; HREIMS *m*/*z* 342.2258 (calcd for $C_{22}H_{30}O_3$ 342.2195); CD (CH_3OH) λ_{max} $\Delta\epsilon_{274}$ −2.29, $\Delta\epsilon_{239}$ +1.14.

Daurichromene B (2): light yellow oil; $[\alpha]^{26}_D$ −27.7 (*c* 0.13, CH_3OH); IR (liquid film) ν_{max} 3349, 1621, 1051 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 280 (4.06), 230 (4.54) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 342 $[M]^+$, 324, 273, 255, 187, 175, 161, 137, 121, 107, 91, 81, 69, 55; HREIMS *m*/*z* 342.2258 (calcd for $C_{22}H_{30}O_3$ 342.2195); CD (CH_3OH) λ_{max} $\Delta\epsilon_{283}$ −0.58, $\Delta\epsilon_{241}$ +0.50.

Daurichromene C (3): light yellow oil; $[\alpha]^{26}_D$ −32.0 (*c* 0.10, CH_3OH); IR (liquid film) ν_{max} 3363, 1624, 1064 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 280 (3.81), 230 (4.27) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 342 $[M]^+$, 327, 272, 215, 175, 161, 137, 125, 106, 91, 81, 67, 55; HREIMS *m*/*z* 342.2194 (calcd for $C_{22}H_{30}O_3$ 342.2195); CD (CH_3OH) λ_{max} $\Delta\epsilon_{280}$ −2.00, $\Delta\epsilon_{237}$ +0.38.

Daurichromene D (4): light yellow oil; $[\alpha]^{26}_D$ −26.0 (*c* 0.10, CH_3OH); IR (liquid film) ν_{max} 3333, 1625, 1075 cm^{-1}; UV (CH_3OH) λ_{max} (log ϵ) 280 (3.70), 230 (4.17) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 342 $[M]^+$, 324, 268, 215, 175, 161, 137, 121, 107, 91, 81, 67, 55; HREIMS *m*/*z* 342.2195 (calcd for $C_{22}H_{30}O_3$ 342.2195); CD (CH_3OH) λ_{max} $\Delta\epsilon_{276}$ −12.0, $\Delta\epsilon_{237}$ +9.07.

Assay of 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.[15] 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.

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