New Farnesane-Type Sesquiterpenes, Hedychiols A and B 8,9-Diacetate, and Inhibitors of Degranulation in RBL-2H3 Cells from the Rhizome of *Hedychium coronarium*

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Two new farnesane-type sesquiterpenes, hedychiols A and B 8,9-diacetate, were isolated from the methanolic extract of the fresh rhizome of *Hedychium coronarium* KOEN. cultivated in Japan. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence. The inhibitory effects of isolated constituents on the release of β-hexosaminidase from RBL-2H3 cells were examined, and hedychilactone A and coronarin D were found to show the inhibitory activity.

Key words hedychiol; *Hedychium coronarium*; degranulation inhibitor; hedychilactone A; coronarin D; Zingiberaceae

Hedychium coronarium KOEN. (Zingiberaceae), which has many common names such as butterfly ginger, butterfly lily, cinnamon jasmine, garland flower, and ginger lily, is widely cultivated in India, Southeast Asian countries, South China, Japan, and Brazil. The rhizomes of *H. coronarium* ("土羌活" in Chinese, Hanashukusha in Japanese) have been used for the treatment of headache, lancinating pain, contusion inflammation and sharp pain due to rheumatism in Chinese traditional medicine. In the course of characterization studies on the bioactive constituents of Zingiberaceae natural medicines,[1—12] we have already reported that three new labdane-type diterpene lactones, hedychilactones A (**3**), B (**4**), and C (**5**), were isolated from the methanolic extract of the fresh rhizome of Japanese *H. coronarium*.[1,2] In addition, the methanolic extract, coronarin D (**6**), which was obtained as a principal labdane-type diterpene, and its methyl ether (**7**) were found to show an inhibitory effect on the increase in vascular permeability induced by acetic acid in mice.[1,2] Furthermore, we also examined the inhibitory effects of the isolated constituents on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.[1,2] Among them, labdane-type diterpenes, including **3** and **6**, showed potent inhibitory activity, and one of the mechanisms of action of NO production inhibitory activity was suggested to be attributed to inhibitory activity against the induction of inducible nitric oxide synthase (iNOS) in LPS-activated macrophages.[2]

As a continuing study of this natural medicine, we additionally isolated two new farnesane-type sesquiterpenes, hedychiols A (**1**) and B 8,9-diacetate (**2**), from the methanolic extract of the fresh rhizome of *H. coronarium*. This paper deals with the structural elucidation of farnesane-type sesquiterpenes (**1**, **2**). In addition, we have described the inhibitory effect of isolated constituents from the fresh rhizome of *H. coronarium* on an immediate allergic reaction by monitoring the release of β-hexosaminidase from rat basophilic

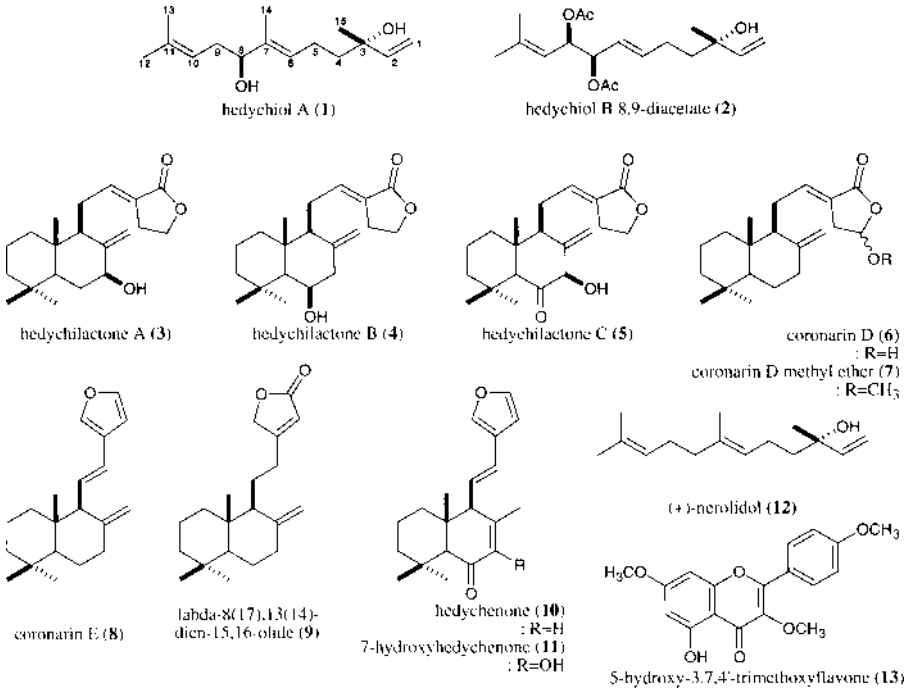

Chart 1

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leukemia (RBL-2H3) cells.

Isolation of Chemical Constituents from the Fresh Rhizome of *H. coronarium* The methanolic extract from the fresh rhizome of *H. coronarium* was partitioned into a mixture of ethyl acetate (AcOEt) and water to furnish the AcOEt-soluble portion and H_2O-soluble portion, as described.[2] The AcOEt-soluble fraction was subjected to ordinary- and reversed-phase silica gel column chromatography, and finally HPLC, to furnish two farnesane-type sesquiterpenes, hedychiols A (**1**, 0.00084% from the fresh rhizome) and B 8,9-diacetate (**2**, 0.000086%), together with 11 constituents including hedychilactones A—C (**3**—**5**).

Stereostructures of Hedychiols A (1) and B 8,9-Diacetate (2) Hedychiol A (**1**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{25}$ −2.4°). The IR spectrum of **1** showed absorption bands due to hydroxyl and *exo*-methylene functions at 3372 and 1375 cm^{-1}. The molecular formula $C_{15}H_{26}O_2$ of **1** was determined from the quasimolecular ion peak at *m/z* 261 $(M+Na)^+$ in the positive-ion fast atom bombardment (FAB)-MS and by high-resolution MS measurement. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR spectra (Table 1) of **1** showed signals assignable to four tertiary methyls [δ 1.29, 1.62, 1.63, 1.71 (all s, 15, 14, 13, 12-H_3)], a methine bearing a hydroxyl group [δ 3.97 (dd, *J*=7.3, 7.3 Hz, 8-H)], an *exo*-methylene [δ 5.06 (dd, *J*=0.9, 10.7 Hz), 5.22 (dd, *J*=0.9, 17.4 Hz), 1-H_2], and three olefins [δ 5.08 (dd-like, 10-H), 5.40 (dd, *J*=7.0, 7.3 Hz, 6-H), 5.91 (dd, *J*=10.7, 17.4 Hz, 2-H)], together with three methylenes (4, 5, 9-H_2) and three quaternary carbons (3, 7, 11-C), which were superimporsable on those of (+)-nerolidol (**12**), expect for a signal due to the secondary hydroxyl group. In addition, the connectivity of the ^{1}H–^{1}H and the quaternary carbons in **1** was clarified by ^{1}H–^{1}H correlation spectroscopy (H–H COSY) and heteronuclear multiple bond correlation (HMBC) experiments. Thus, the H–H COSY experiment on **1** indicated the presence of the partial structures shown as bold lines in Fig. 1 (1-C–2-C, 4-C–6-C, 8-C–10-C). In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **1** (1-H_2 and 2-C; 6-H and 8-C; 8-H and 6-C, 10-C; 12-H_3 and 10-C, 11-C, 13-C; 13-H_3 and 10-C, 11-C, 12-C; 14-H_3 and 6-C, 7-C, 8-C; 15-H_3 and 1-C, 3-C, 4-C), so that the connectivities of the quaternary carbons (3, 7, 11-C) in **1** were clarified. The above-mentioned evidence led us to confirm the planar structure of **1**.

Next, the absolute stereostructure of the 8-position of **1** was determined by application of modified Mosher's method[13] to the 8-mono-(*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetate (MTPA esters, **1a**, **1b**), which were prepared by esterification of the 8-hydroxyl group in **1** with (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP). As shown in Fig. 1, signals due to protons at C-4, 5, 6, and 14 of the (*R*)-MTPA ester (**1a**) appeared at higher fields than those of the (*S*)-MTPA ester (**1b**) [$\Delta\delta$: positive], while signals due to protons attached to C-9, 10, 12, and 13 of **1a** were observed at a lower field as compared to those of **1b** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 8-position in **1** has been determined to be *S*.

To elucidate the absolute stereostructure of the 3-position, **1** was chemically related to (+)-nerolidol (**12**), whose absolute configuration has been reported to be an *S*-configuration.[14] As shown in Fig. 2, ozone degradation of **12** in MeOH followed by treatment with $NaBH_4$ furnished 1,4-pentandiol[15] and the triol (**12a**).[16] On the other hand, **12a** was also obtained from **1** by the same procedure. Consequently, the absolute stereostructure of **1** was determined to be as shown.

Hedychiol B 8,9-diacetate (**2**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{21}$ −18.8°). The molecular formula $C_{19}H_{30}O_5$ of **2** was determined from the quasimolecular ion peak at *m/z* 361 $(M+Na)^+$ in the positive-ion FAB-MS and by high-resolution MS measurement. The IR spectrum of **2** showed absorption bands of hydroxyl, olefin and *exo*-methylene functions at 3459, 1740, and 1374 cm^{-1}. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2** showed signals assignable to four tertiary methyls [δ 1.27,

Table 1. ^{13}C-NMR Data for Hedychiols A (**1**) and B 8,9-Diacetate (**2**)

	1	**2**
C-1	111.8	111.9
C-2	144.9	144.8
C-3	73.3	73.2
C-4	41.8	41.4
C-5	22.3	22.4
C-6	126.0	130.9
C-7	137.1	130.4
C-8	77.1	79.9
C-9	34.2	70.7
C-10	120.2	119.5
C-11	134.6	139.8
C-12	25.9	25.9
C-13	18.0	18.6
C-14	11.7	12.7
C-15	27.8	27.9
$\underline{C}H_3CO$–		21.1
		21.1
$CH_3\underline{C}O$–		170.1
		170.1

Measured in $CDCl_3$, at 125 MHz.

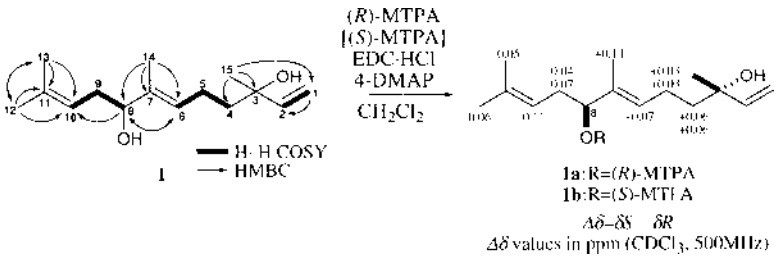

Fig. 1

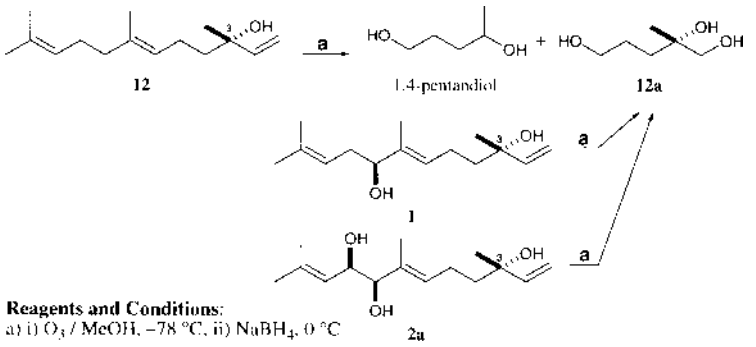

Fig. 2

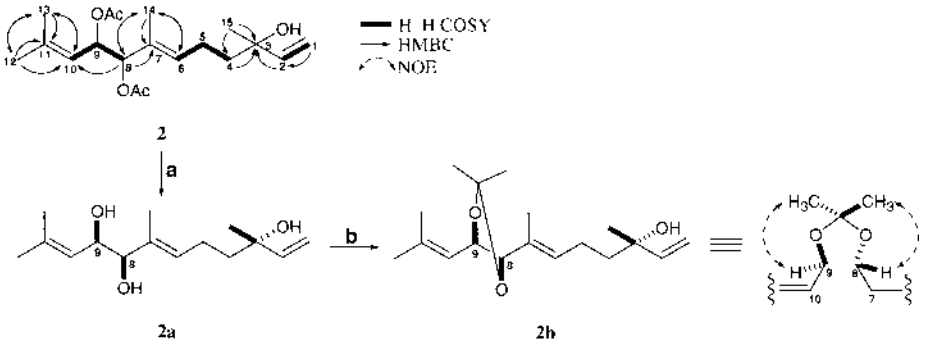

Fig. 3

1.59, 1.70, 1.74 (all s, 15, 14, 13, 12-H_3)], two acetyl methyls [δ 2.01, 2.04 (both s)], three olefins [δ 5.00 (br d, J=*ca.* 10 Hz, 10-H), 5.49 (dd, J=7.3, 7.3 Hz, 6-H), 5.89 (dd, J=10.7, 17.4 Hz, 2-H)], an *exo*-methylene [δ 5.06 (dd, J=1.2, 10.7 Hz), 5.20 (dd, J=1.2, 17.4 Hz), 1-H_2)], and two methines bearing an acetyl group [δ 5.15 (d, J=7.9 Hz, 8-H), 5.72 (dd, J=7.9, 9.5 Hz, 9-H)], together with two methylenes (4, 5-H_2) and three quaternary carbons (3, 7, 11-C). Next, the connectivities of the ^{1}H–^{1}H and the quaternary carbons in **2** were clarified by H–H COSY and HMBC experiments. As shown in Fig. 3, the H–H COSY experiment on **2** indicated the presence of three partial structures shown as bold lines (1-C–2-C, 4-C–6-C, 8-C–10-C), whereas, in the HMBC experiment, long-range correlations were observed between the following protons and carbons (1-H_2 and 2-C; 2-H_2 and 3-C; 4-H_2 and 3-C; 8-H and 7-C, 10-C, 14-C; 12-H_3 and 10-C, 11-C, 13-C; 13-H_3 and 10-C, 11-C, 12-C; 14-H_3 and 6-C, 7-C, 8-C; 15-H_3 and 3-C, 4-C). Thus, the connectivities of the quaternary carbons (3, 7, 11-C) in **2** were clarified.

Next, the relative stereostructure of the 8 and 9-hydroxyl groups in **2** was clarified by nuclear Overhauser effect spectroscopy (NOESY) experiment on the 8,9-acetonide (**2b**). Namely, treatment of **2** with 0.1% sodium methoxide (NaOMe) furnished the desacetyl derivative, hedychiol B (**2a**), which was subsequently treated with 2,2-dimethoxypropane to give the 8,9-acetonide (**2b**). The NOESY experiment on **2b** showed NOE correlations between the 8,9-protons and acetonide methyls, as shown in Fig. 3, so that the stereostructure of the 8 and 9-positions of **2** was determined.[17]

The absolute stereostructure of the 3-position of **2** was determined by a similar method used for **1**. As shown in Fig. 2, **12a** was also obtained by ozone oxidation of **2a**, followed by $NaBH_4$ reduction. Consequently, the absolute stereostructure of the 3-position of **2** was determined to be an *S*-configuration.

Inhibitory Effect of Constituents from *H. coronarium* on the Release of β-Hexosaminidase from RBL-2H3 Cells

Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in *in vitro* experiments on immediate allergic reactions. β-Hexosaminidase is also stored in the secretory granules of mast cells, and is released concomitantly with histamine when mast cells are immunologically activated.[18,19] Therefore, it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. Previously, we reported inhibitors of the release of β-hexosaminidase isolated from *Myrica rubra*.[20] In our continuous search for antiallergic principles from natural sources, we examined the effects of constituents from the fresh rhizome of *H. coronarium* on the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP IgE. Two antiallergic compounds, tranilast and ketotifen fumarate, showed weak inhibition with IC_{50} values of 0.49 and 0.22 mM, respectively. On the other hand, luteolin and curcumin, which were reported to inhibit degranulation in this experimental model,[20,21] showed relatively strong inhibition, with IC_{50} values of 3.0 and 82 μM, respectively. As shown in Table 2, hedychilactone A (**3**) and coronarin D (**6**) showed inhibitory activity. Especially, **6** was found to show strong activity (IC_{50}=57 μM). In addition, hedychenone (**10**) enhanced the release of β-hexosaminidase from RBL-2H3 cells.

Table 2. Inhibitory Effects of Constituents from *H. coronarium* on the Release of β-Hexosaminidase from RBL-2H3 Cells

	Inhibition (%)			
	0 μM	10 μM	30 μM	100 μM
Hedychiol A (**1**)	0.0±3.2	−1.0±1.5	−6.4±2.9	−3.5±2.8
Hedychiol B 8,9-diacetate (**2**)	0.0±1.7	0.1±1.6	1.5±1.1	11.4±1.2**
Hedychilactone A (**3**)	0.0±1.9	−0.1±1.7	6.0±2.3	39.1±2.7**
Hedychilactone B (**4**)	0.0±5.8	14.1±2.2	27.2±4.2**	−3.5±2.2
Hedychilactone C (**5**)	0.0±2.3	−1.8±1.1	−2.3±2.7	−0.5±2.1
Coronarin D (**6**)	0.0±1.9	4.6±1.8	23.0±2.7**	93.5±0.4**
Coronarin D methyl ether (**7**)	0.0±2.9	−3.0±3.8	−14.5±3.3*	−18.2±2.7**
Coronarin E (**8**)	0.0±2.6	−6.7±1.9	−9.8±1.4*	−13.8±2.1**
Labda-8(17),13(14)-dien-15,16-olide (**9**)	0.0±7.3	−6.4±4.1	−25.2±3.9*	−13.3±4.5
Hedychenone (**10**)	0.0±2.1	−37.2±3.1**	−51.7±0.2**	−45.7±1.8**
7-Hydroxyhedychenone (**11**)	0.0±3.4	−4.7±3.5	−11.2±3.4	−17.7±3.1**
(+)-Nerolidol (**12**)	0.0±3.5	−2.1±3.5	2.2±2.6	11.8±1.3*

Each value represents the mean±S.E.M. ($n=4$). Significantly different from the control: * $p<0.05$, ** $p<0.01$.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; electron impact (EI)-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector; ozone generator, Nihon Ozone O-3-2 ozone generator.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Isolation of Hedychiols A (1) and B 8,9-Diacetate (2) Fraction 7 (2.8 g), obtained from the AcOEt-soluble portion of the fresh rhizome of *H. coronarium* (cultivated in Kagawa Prefecture, Japan), and isolated hedychilactones A—C (**3**—**5**), and coronarin D (**6**), as reported previously,[2] were further separated by reversed-phase silica gel chromatography [84 g, MeOH–H_2O (70 : 30→80 : 20→90 : 10)→MeOH] and finally HPLC [YMC-pack ODS-A 250×20 mm i.d., MeOH–H_2O (65 : 35 or 80 : 20)] to furnish hedychiols A (**1**, 54 mg) and B 8,9-diacetate (**2**, 6 mg).

Hedychiol A (**1**): Colorless oil, $[\alpha]_D^{26}$ −2.4° ($c=0.800$, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{26}O_2Na$ $(M+Na)^+$: 261.1839. Found: 261.1830. IR (film): 3372, 2971, 2859, 1375 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, s, 15-H_3), 1.59 (2H, m, 4-H_2), 1.62, 1.63, 1.71 (3H each, all s, 14, 13, 12-H_3), 2.07 (2H, m, 5-H_2), 2.23 (2H, m, 9-H_2), 3.97 (1H, dd, $J=7.3$, 7.3 Hz, 8-H), 5.06 (1H, dd, $J=0.9$, 10.7 Hz, 1-H), 5.08 (1H, dd-like, 10-H), 5.22 (1H, dd, $J=0.9$, 17.4 Hz, 1-H), 5.40 (1H, dd, $J=7.0$, 7.3 Hz, 6-H), 5.91 (1H, dd, $J=10.7$, 17.4 Hz, 2-H). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 261 $(M+Na)^+$.

Hedychiol B 8,9-Diacetate (**2**): Colorless oil, $[\alpha]_D^{21}$ −18.8° ($c=0.300$, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{19}H_{30}O_5Na$ $(M+Na)^+$: 361.1991. Found : 361.1996. IR (film) 3459, 2971, 2929, 1740, 1374 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, s, 15-H_3), 1.54 (2H, m, 4-H_2), 1.59, 1.70, 1.74 (3H each, all s, 14, 13, 12-H_3), 2.01, 2.04 (3H each, both s, C$\underline{H_3}$CO–), 2.03 (2H, m, 5-H_2), 5.00 (1H, br d, *J=ca.* 10 Hz, 10-H), 5.06 (1H, dd, $J=1.2$, 10.7 Hz, 1-H), 5.15 (1H, d, $J=7.9$ Hz, 8-H), 5.20 (1H, dd, $J=1.2$, 17.4 Hz, 1-H), 5.49 (1H, dd, $J=7.3$, 7.3 Hz, 6-H), 5.72 (1H, dd, $J=7.9$, 9.5 Hz, 9-H), 5.89 (1H, dd, $J=10.7$, 17.4 Hz, 2-H). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 361 $(M+Na)^+$.

Preparation of the (*R*)-MTPA Ester (1a) and the (*S*)-MTPA Ester (1b) from Hedychiol A (1) A solution of **1** (2.2 mg, 9.7 μmol) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-MTPA (6.8 mg, 29 μmol) in the presence of EDC·HCl (6.0 mg, 29 μmol) and 4-DMAP (2.4 mg, 19 μmol), and the mixture was stirred under reflux for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was treated in the usual manner to give a residue which was purified by silica gel column chromatography (0.2 g, *n*-hexane–AcOEt=10 : 1) to give **1a** (3.0 mg, 70%). Through a similar procedure, **1b** (3.1 mg, 53%) was prepared from **1** (3.0 mg, 13.2 μmol) using (*S*)-MTPA (9.2 mg, 39.5 μmol), EDC·HCl (8.1 mg, 39.5 μmol) and 4-DMAP (3.2 mg, 26.4 μmol).

1a: Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.29, 1.48 (3H each, both s, 15, 14-H_3), 1.52 (2H, m, 4-H_2), 1.59, 1.69 (3H each, both s, 13, 12-H_3), 2.04 (2H, m, 5-H_2), 2.27 (1H, ddd, $J=5.9$, 8.0, 14.7 Hz, 9-H), 2.47 (1H, ddd, $J=8.0$, 8.2, 14.7 Hz, 9-H), 3.54 (3H, s, –OCH_3), 5.03 (1H, dd, $J=8.0$, 8.0 Hz, 10-H), 5.08 (1H, dd, $J=1.2$, 10.6 Hz, 1-H), 5.22 (1H, dd, $J=1.2$, 17.4 Hz, 1-H), 5.32 (1H, dd, $J=5.9$, 8.2 Hz, 8-H), 5.48 (1H, dd, $J=7.9$, 7.9 Hz, 6-H), 5.90 (1H, dd, $J=10.6$, 17.4 Hz, 2-H), 7.36—7.50 (5H, m, Ph).

1b: Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, s, 15-H_3), 1.58 (2H, m, 4-H_2), 1.54, 1.62, 1.63 (3H each, all s, 13, 14, 12-H_3), 2.07 (2H, m, 5-H_2), 2.23, 2.40 (1H each, both m, 9-H_2), 3.51 (3H, s, –OCH_3), 4.92 (1H, dd, $J=7.0$, 7.3 Hz, 10-H), 5.07 (1H, dd, $J=1.2$, 10.7 Hz, 1-H), 5.21 (1H, dd, $J=1.2$, 17.4 Hz, 1-H), 5.34 (1H, dd, $J=5.8$, 8.0 Hz, 8-H), 5.55 (1H, dd, $J=7.3$, 7.3 Hz, 6-H), 5.91 (1H, dd, $J=10.7$, 17.4 Hz, 2-H), 7.37—7.50 (5H, m, Ph).

Deacetylation of Hedychiol B 8,9-Diacetate (2) A solution of **2** (2.9 mg, 8.6 μmol) in 0.1% NaOMe–MeOH (0.8 ml) was stirred at room temperature for 4 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue which was purified by silica gel column chromatography (0.2 g, *n*-hexane–AcOEt=5 : 1) to furnish hedychiol B (**2a**, 2.1 mg, 96%).

Hedychiol B (**2a**): Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, s, 15-H_3), 1.56 (2H, m, 4-H_2), 1.60, 1.68, 1.72 (3H each, all s, 14, 13, 12-H_3), 2.07 (2H, m, 5-H_2), 3.82 (1H, d, $J=7.2$ Hz, 8-H), 4.30 (1H, dd, $J=7.2$, 8.7 Hz, 9-H), 5.07 (1H, dd, $J=1.2$, 10.8 Hz, 1-H), 5.14 (1H, d, $J=8.7$ Hz, 10-H), 5.21 (1H, dd, $J=1.2$, 17.2 Hz, 1-H), 5.46 (1H, dd, $J=7.2$, 7.2 Hz, 6-H), 5.90 (1H, dd, $J=10.8$, 17.2 Hz, 2-H). Positive-ion FAB-MS *m/z*: 277 $(M+Na)^+$.

Preparation of the Acetonide (2b) from Hedychiol B (2a) A solution of **2a** (0.8 mg, 3.1 μmol) in 2,2-dimethoxypropane (0.5 ml) was treated with Dowex HCR-W2 (H^+ form, 10 mg), and the mixture was stirred at room temperature for 2 h. The resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure yielded **2b** (1.1 mg, quant.).

2b: Colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, s, 15-H_3), 1.43, 1.45 (3H each, both s, ($C\underline{H_3}$)$_2$–C–), 1.52 (2H, m, 4-H_2), 1.64, 1.65, 1.75 (3H each, all s, 14, 13, 12-H_3), 2.05 (2H, m, 5-H_2), 3.97 (1H, d, $J=8.5$ Hz, 8-H), 4.44 (1H, dd, $J=8.5$, 8.9 Hz, 9-H), 5.07 (1H, dd, $J=1.2$, 10.7 Hz, 1-H), 5.14 (1H, d, $J=8.9$ Hz, 10-H), 5.22 (1H, dd, $J=1.2$, 17.4 Hz, 1-H), 5.50 (1H, dd, $J=7.0$, 7.0 Hz, 6-H), 5.91 (1H, dd, $J=10.7$, 17.4 Hz, 2-H). Positive-ion FAB-MS *m/z*: 317 $(M+Na)^+$.

Conversion from (+)-Nerolidol (12), and Hedychiols A (1) and B (2a) to 12a A solution of **12** (25.0 mg, 0.11 mmol) in MeOH (2.0 ml) was oxidized with O_3 gas (3.0 g/h) at −78 °C for 2 h to give ozonide. Successively, the reaction mixture was treated with $NaBH_4$ (5.0 mg) and the mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched with acetone (2.0 ml). Removal of the solvent under reduced pressure furnished a residue which was purified by silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O=10 : 3 : 1 (lower layer)] to give 1,4-pentandiol (6.4 mg, 55%)

and triol (**12a**, 2.8 mg, 19%). Compound **12a** was identified by comparison of its physical data ($[\alpha]_D$, MS, ^{1}H-NMR) with reported values.[16] Through a similar procedure, **1** (5.0 mg, 0.021 mmol) and **2a** (1.2 mg, 0.005 mmol) in MeOH (1.0 ml) were treated with O_3 gas (3.0 g/h) at −78 °C for 2 h, and successively treated with $NaBH_4$ (2.0 mg), then the mixtures were stirred at 0 °C for 30 min, respectively. Work-up of the reaction mixtures, as described above, gave products which were purified by silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O=10 : 3 : 1 (lower layer)] to furnish **12a** (2.2 mg from **1**, 43%; 0.3 mg from **2a**, 47%).

Bioassay. Inhibitory Effect on the Release of β-Hexosaminidase from RBL-2H3 Cells Inhibitory effects of test samples on the release of β-hexosaminidase from RBL-2H3 cells were evaluated by the following procedure.[21] RBL-2H3 cells were grown in Minimum Essential Medium Eagle (MEM, Sigma Co., Ltd.) containing fetal calf serum (10%), penicillin (100 units/ml), and streptomycin (100 μg/ml). Before the experiment, cells were dispensed into 24-well plates at the concentration of 2×10^5 cells/well using a medium containing 0.45 μg/ml of anti-DNP IgE, and these were incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then, cells were washed twice with 500 μl of siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 40 mM NaOH, pH 7.2], then incubated in 160 μl of siraganian buffer (5.6 mM glucose, 1 mM $CaCl_2$, 0.1% BSA were added) for an additional 10 min at 37 °C. Next, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by the addition of 20 μl of antigen (DNP-BSA, final concentration was 10 μg/ml) at 37 °C for 10 min to stimulate the cells to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μl) was transferred into a 96-well microplate and incubated with 50 μl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured by a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to siraganian buffer (final DMSO concentration was 0.1%).

For estimating the spontaneous release of β-hexosaminidase from cells, exactly the same procedure was followed (Normal), but without adding antigen. Blank absorbance of the test material was measured to eliminate interference caused by the color of the test material itself. For this, only test material and substrate were added without adding the cell extract (Blank). Thus, the inhibition % of the release of β-hexosaminidase by the test material was calculated by the following equation.

$$\text{inhibition (\%)}=\left[1-\frac{T-B-N}{C-N}\right]\times100$$

Control (*C*): antigen-IgE response was evoked without test sample; Test (*T*): antigen-IgE response was evoked in the presence of test sample; Blank (*B*): only test sample and substrate were added; Normal (*N*): antigen-IgE response was not evoked, test sample was not added.

In this condition, it was calculated that 40—50% of β-hexosaminidase was released from the cells in the control groups by determination of the total β-hexosaminidase activity after sonication of the cell suspension.

Statistics Values are expressed as means±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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