Bioactive Constituents from Chinese Natural Medicines. XX.[1] Inhibitors of Antigen-Induced Degranulation in RBL-2H3 Cells from the Seeds of *Psoralea corylifolia*

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The ethyl acetate-soluble fraction of the methanolic extract from the seeds of *Psoralea corylifolia* was found to inhibit the release of β-hexosaminidase, as a marker of antigen-IgE-induced degranulation in RBL-2H3 cells. Sixteen constituents were isolated from the ethyl acetate-soluble fraction and several constituents, Δ^1,3-hydroxybakuchiol (IC_{50}=49 μM), Δ^3,2-hydroxybakuchiol (69 μM, bavachin (58 μM), and psoralidin (*ca.* 100 μM), showed inhibitory activities against the antigen-induced degranulation.

Key words *Psoralea corylifolia*; degranulation inhibitor; Δ^1,3-hydroxybakuchiol; Δ^3,2-hydroxybakuchiol; bavachin

The Legminosae plant, *Psoralea corylifolia* L., is widely distributed from India to Southeast Asian countries. The seeds of this plant have been used as a tonic, to treat uterine hemorrhage, and as a coronary vasodilatory agent in Chinese traditional medicine.[2—5] Previously, several meroterpenoid[6—11] and flavonoid[12—19] constituents were isolated from this natural medicine. In addition, biological studies to determine its antioxidative,[20] antiplatelet,[21] and DNA polymerase and topoisomerase II inhibitory activities[22] were also reported. In the course of our characterization studies on the bioactive constituents in Chinese natural medicines,[1,23—34] the ethyl acetate (EtOAc)-soluble fraction of the methanolic extract from this natural medicine was found to inhibit antigen-IgE-induced degranulation in RBL-2H3 cells. From this active fraction, two meroterpene constituents (**1**, **2**)[35] were newly isolated together with 14 known constituents. This paper deals with the isolation and structural confirmation of the two compounds (**1**, **2**) as well as the inhibitory effects of the constituents from *P. corylifolia* on antigen-induced degranulation in RBL-2H3 cells.

The seeds of *P. corylifolia* were extracted with methanol under reflux. The methanolic extract was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish the EtOAc- and H_2O-soluble fractions. The EtOAc-acetate-soluble fraction inhibited antigen-induced degranulation in RBL-2H3 cells [inhibition (%): 39.3±2.5 ($p<0.05$) at 30 μg/ml], but the H_2O-soluble fraction did not [inhibition (%): 6.3±4.4 at 30 μg/ml]. The EtOAc-soluble fraction was subjected to ordinary and reverse-phase silica gel column chromatography and finally HPLC to furnish **1** (0.011% from the natural medicine), **2** (0.009%), bakuchiol[6–10] (**3**, 8.19%), Δ^1,3-hydroxybakuchiol[11] (**4**, 0.016%), Δ^3,2-hydroxybakuchiol[11] (**5**, 0.007%), bavachin[36,37] (**6**, 0.59%), bavachinin[12] (**7**, 1.87%), 6-prenylnaringenin[38] (**8**, 0.019%), isovabachalcone[39] (**9**, 0.83%), psoralidin[40] (**10**, 0.084%), neobavaisoflavone[41] (**11**, 0.75%), corylifol A[42] (**12**, 0.20%), angelicin[43] (**13**, 0.099%), *p*-hydroxybenzaldehyde[44] (**14**, 0.004%), linoleic acid[44] (0.055%), and linolenic acid[44] (0.020%).

Structureal Confirmation of 1 and 2 Compound **1** was obtained as a colorless oil and exhibited a positive optical rotation ($[\alpha]_D^{25}$ +16.6° in $CHCl_3$). In the UV spectrum of **1**, absorption maxima were observed at 227 (log ε 4.19) and 278 (3.43) nm. The IR spectrum of **1** showed absorption bands at 3389, 1615, 1599, 1520, 1456, and 1050 cm^{-1} assignable to hydroxyl and ether functional groups and an aromatic ring. In the EI-MS of **1**, a molecular ion peak was observed at *m/z* 288 (M^+) and high-resolution EI-MS analysis revealed the molecular formula of **1** to be $C_{18}H_{24}O_3$. The ^{1}H- ($CDCl_3$, Table 1) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[45] showed signals assignable to three methyls [δ 1.02, 1.03 (3H each, both d, *J*=7.0 Hz, 1, 18-H_3), 1.05 (3H, s, 15-H_3)], two methylenes and a methine [δ 1.64 (1H, br dd, *J*=*ca.* 12, 14 Hz, 4β-H), 1.69 (1H, m, 5β-H), 1.78 (1H, br dd, *J*=*ca.* 5, 14 Hz, 4α-H), 1.83 (1H, ddd, *J*=5.2, 12.2, 14.3 Hz, 5α-H), 2.08 (1H, m, 2-H)], two methines bearing an oxygen function [δ 3.97, 5.10 (1H each, both br s, 7, 8-H)], an *exo*-methylene [δ 5.08 (1H, dd, *J*=1.2, 11.0 Hz), 5.14 (1H, dd, *J*=1.2, 17.8 Hz), 17-H_2], an olefin [δ 6.11 (1H, dd, *J*=11.0, 17.8 Hz, 16-H)], and *ortho*-coupled A_2B_2-type aromatic protons [δ 6.75, 7.21 (2H each, both d, *J*=8.5 Hz, 11,13, 10,14-H)]. As shown in Fig. 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of partial structures in bold lines. In the heteronuclear multiple-bond correlations (HMBC) experiment on **1**, long-range correlations were observed between the following protons and carbons (1-H_3, 18-H_3 and 2-C; 1-H_3, 2-H, 4-H_2, 7-H, 8-H, 18-H_3 and 3-C; 2-H and 4-C; 15-H_3 and 5-C; 5-H_2, 7-H_2, 15-H_3, 16-H, 17-H_2 and 6-C; 15-H_3 and 7-C; 10,14-H and 8-C; 8-H, 11,13-H and 9-C; 8-H and 10,14-C; 10,14-H and 12-C; 16-H and 15-C; 15-H_3 and 16-C), as shown in Fig. 1. Thus the connectivities of quaternary carbons (3, 6, 9, 12-C) in **1** were clarified and the planar structure of **1** was elucidated. Next, the stereostructure of **1** was characterized in a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs (4β-H, 5β-H and 15-H_3; 7α-H and 10,14-H, 15-H_3, 16-H; 8-H and 10,14-H, 15-H_3), as shown in Fig. 1. On the basis of those findings and comparison of the NMR (CD_3OD) data of **1** with those of psoracorylifol B,[46] the structure of **1** was confirmed to be the same as that of psoracorylifol B, except for the $[\alpha]_D$ value.[47]

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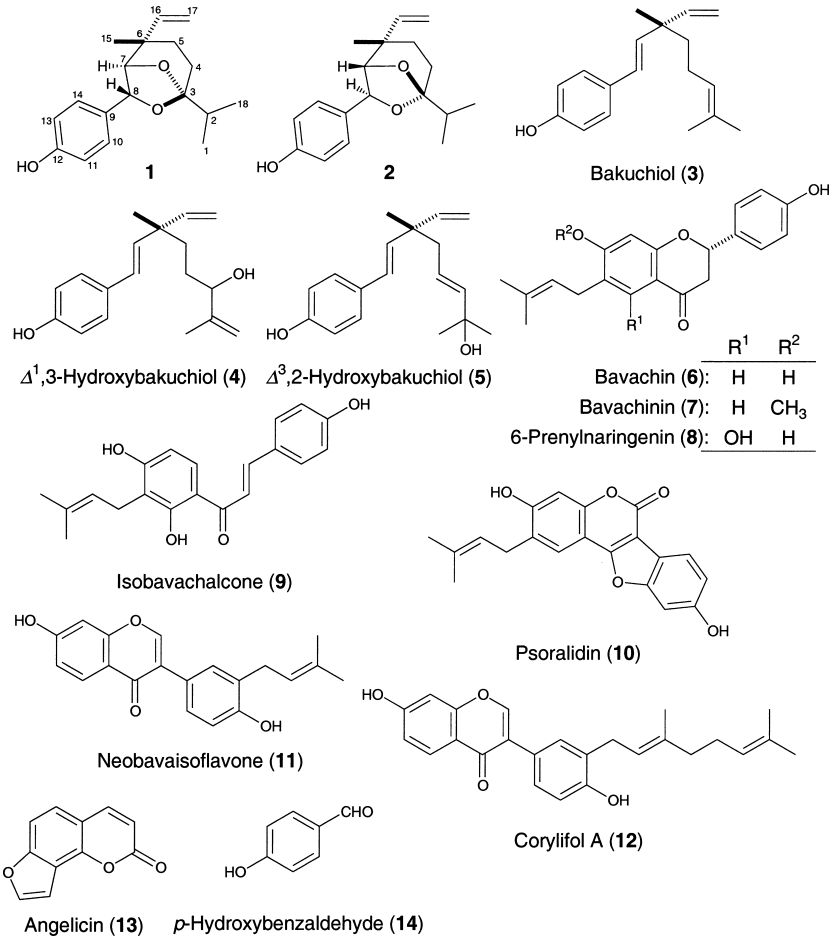

Chart 1

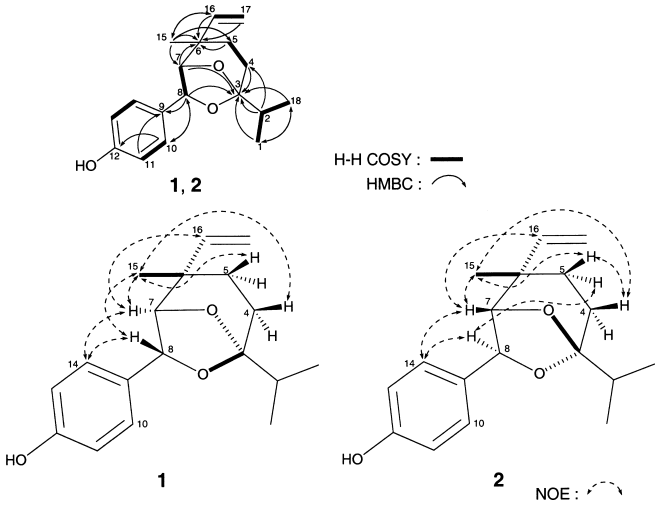

Fig. 1

Compound **2** was also isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{25}$ −23.5° in $CHCl_3$). The UV spectrum of **2** showed absorption maxima at 227 (log ε 4.03) and 277 (3.35) nm. The IR spectrum of **2** showed absorption bands at 3389, 1615, 1599, 1516, 1456, and 1038 cm^{-1} ascribable to hydroxyl and ether functions and an aromatic ring. The molecular formula $C_{18}H_{24}O_3$ of **2**, which was the same as that of **1**, was determined based on the molecular ion peak in EI-MS and by high-resolution MS measurement. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were superimposable on those of **1**. That is, the proton and carbon signals in the ^{1}H- ($CDCl_3$, Table 1) and ^{13}C-NMR (Table 2) spectra[45] of **2** showed signals assignable to three methyls [δ 1.05, 1.06 (3H each, both d, J=7.0 Hz, 1, 18-H_3), 1.19 (3H, s, 15-H_3)], two methylenes and a methine [δ 1.46 (1H, br dd, J=*ca.* 6, 14 Hz, 5β-H), 1.70 (1H, br dd, J=*ca.* 6, 14 Hz, 4α-H), 1.82 (1H, ddd, J=5.2, 12.3, 13.8 Hz, 4β-H), 2.04 (1H, ddd, J=6.1, 12.3, 14.4 Hz, 5α-H), 2.10 (1H, m, 2-H)], two methines bearing an oxygen function [δ 3.91, 4.97 (1H each, both br s, 7, 8-H)], an *exo*-methylene [δ 5.06 (1H, dd, J=1.2, 17.7 Hz), 5.14 (1H, dd, J=1.2, 11.0 Hz), 17-H_2], an olefin [δ 5.78 (1H, dd, J=11.0, 17.7 Hz, 16-H)], and *ortho*-coupled A_2B_2-type aromatic protons [δ 6.72, 7.18 (2H each, both d, J=8.6 Hz, 11,13, 10,14-H)]. The planar structure of **2** was confirmed by ^{1}H–^{1}H COSY and HMBC experiments to be the same as that of **1** (Fig. 1). The stereostructure of **2** was determined in a NOESY experiment, in which correlations were observed between the following proton pairs (4β-H and 5β-H, 15-H_3; 5α-H and 8-H; 5β-H and 15-H_3; 7-H and 10,14-H, 15-H_3, 16-H; 8-H and 10,14-H). Finally, by comparison of the NMR data (CD_3OD) of **2** with those of psoracorylifol C, **2** was identified as psoracorylifol C.[47]

Inhibitory Effects on the Release of β-Hexosaminidase in RBL-2H3 Cells Histamine, which is released from mast cells when stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in *in vitro* experiments on immediate allergic reactions. β-Hex-

Table 1. ^{1}H-NMR (500 MHz, $CDCl_3$) Data of **1** and **2**

H-	**1**	**2**
	δ (J Hz)	
1	1.02 (3H, d, 7.0)[a]	1.05 (3H, d, 7.0)[a]
2	2.08 (1H, m)	2.10 (1H, m)
4α	1.78 (1H, br dd, *ca.* 5, 14)	1.70 (1H, br dd, *ca.* 6, 14)
4β	1.64 (1H, br dd, *ca.* 12, 14)	1.82 (1H, ddd, 5.2, 12.3, 13.8)
5α	1.83 (1H, ddd, 5.2, 12.2, 14.3)	2.04 (1H, ddd, 6.1, 12.3, 14.4)
5β	1.69 (1H, m)	1.46 (1H, br dd, *ca.* 6, 14)
7	3.97 (1H, br s)	3.91 (1H, br s)
8	5.10 (1H, br s)	4.97 (1H, br s)
10, 14	7.21 (2H, d, 8.5)	7.18 (2H, d, 8.6)
11, 13	6.75 (2H, d, 8.5)	6.72 (2H, d, 8.6)
15	1.05 (3H, s)	1.19 (3H, s)
16	6.11 (1H, dd, 11.0, 17.8)	5.78 (1H, dd, 11.0, 17.7)
17	5.08 (1H, dd, 1.2, 11.0)	5.14 (1H, dd, 1.2, 11.0)
	5.14 (1H, dd, 1.2, 17.8)	5.06 (1H, dd, 1.2, 17.7)
18	1.03 (3H, d, 7.0)[a]	1.06 (3H, d, 7.0)[a]

a) May be interchangeable within the same column.

Table 2. ^{13}C-NMR (125 MHz, $CDCl_3$) Data of **1** and **2**

C-	**1**	**2**
1	18.1 (q)[a]	18.1 (q)[a]
2	35.4 (d)	35.5 (d)
3	112.3 (s)	112.6 (s)
4	26.4 (t)	25.8 (t)
5	28.8 (t)	27.0 (t)
6	38.6 (s)	38.9 (s)
7	88.8 (d)	88.1 (d)
8	77.9 (d)	78.4 (d)
9	134.4 (s)	134.7 (s)
10, 14	127.8 (d)	127.8 (d)
11, 13	115.3 (d)	115.2 (d)
12	155.4 (s)	155.2 (s)
15	23.4 (q)	22.6 (q)
16	143.6 (d)	144.4 (d)
17	112.5 (t)	114.0 (t)
18	17.5 (q)[a]	17.6 (q)[a]

a) May be interchangeable within the same column.

Table 3. Inhibitory Effects of Constituents from *P. corylifolia* on the Release of β-Hexosaminidase from RBL-2H3 Cells

	Inhibition (%)				IC_{50} (μM)
	0 μM	30 μM	60 μM	100 μM	
1	0.0±1.0	—	—	1.9±7.9	>100
2	0.0±5.3	—	—	41.6±2.0**	>100
Bakuchiol (**3**)	0.0±2.0	−9.7±2.0	−6.1±1.3	−9.9±1.8	>100
Δ^1,3-Hydroxybakuchiol (**4**)	0.0±4.2	17.4±4.8*	65.7±2.6**	94.3±1.0**	49
Δ^3,2-Hydroxybakuchiol (**5**)	0.0±1.1	7.9±2.3	37.1±3.0**	83.6±1.0**	69
Bavachin (**6**)	0.0±1.7	−8.4±1.5**	51.7±1.9**	99.5±0.8**	58
Bavachinin (**7**)	0.0±1.3	—	—	13.3±2.6	>100
6-Prenylnaringenin (**8**)	0.0±7.1	—	—	2.5±7.5	>100
Isobavachalcone (**9**)	0.0±5.6	—	—	21.5±6.7	>100
Psoralidine (**10**)	0.0±3.9	−7.8±1.9	12.7±2.3*	49.1±3.0**	*ca.* 100
Neobavaisoflavone (**11**)	0.0±5.6	—	—	−4.3±3.4	>100
Corylifol A (**12**)	0.0±1.3	—	—	23.0±3.6*	>100
Angelicin (**13**)	0.0±2.0	—	—	15.0±2.7**	>100
p-Hydroxybenzaldehyde (**14**)	0.0±2.0	—	—	–1.4±1.3	>100

	Inhibition (%)					IC_{50} (μM)
	0 μM	30 μM	100 μM	300 μM	1000 μM	
Tranilast[52]	0.0±1.7	8.2±1.8	22.4±2.5*	56.9±3.4**	75.0±0.6**	282
Ketorifen fumarate[52]	0.0±1.8	7.7±1.5	27.6±2.2*	80.7±1.8**	100.7±1.1**	158

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, * p<0.05, ** p<0.01. *a*) Cytotoxic effect was observed.

osaminidase is also stored in the secretory granules of mast cells and is released concomitantly with histamine when mast cells are immunologically activated.[48,49] Therefore it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells.

As a part of our characterization studies on the bioactive components of natural medicines, we previously reported several inhibitors of the release of β-hexosaminidase such as diarylheptanoids,[23,50,51] sesquiterpenes,[52] diterpenes,[53] flavonoids,[54] anthraquinones,[27] stilbenes,[28] phenanthrenes,[28] phenylpropanoids,[55] and alkaloids.[29,30] In our continuous search for antiallergic principles from natural sources, we examined the effects of isolated constituents from this natural medicine on the inhibition of the release of β-hexosaminidase (Table 3). As a result, the principal meroterpene bakuchiol (**3**) did not show inhibitory effects, but the related compounds Δ^1,3-hydroxybakuchiol (**4**, 49 μM) and Δ^3,2-hydroxybakuchiol (**5**, 64 μM), the prenyl flavanone bavachin (**6**, 58 μM), and the coumestan derivative psoralidin (**10**, *ca.* 100 μM) showed inhibitory activity, and their activities were stronger than those of two antiallergic compounds, tranilast (282 μM) and ketotifen fumarate (158 μM).[51] Furthermore, the effects of the active compounds **4**—**6** and **10** did not inhibit the enzyme activity of β-hexosaminidase at 100 μM (data not shown). These results indicate that **4**—**6** and **10** inhibited the antigen-induced degranulation but not the enzyme activity of β-hexosaminidase.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shi-

madzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; 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, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

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); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The seeds of *P. corylifolia* L. were purchased from Kunming, Yunnan province, China, in September 2001, and identified by one of authors (M. Y.). A voucher specimen (2001.09. Yunnan-24) of this plant is on file in our laboratory.

Extraction and Isolation The seeds of *P. corylifolia* (958 g) were powdered and extracted with methanol three times under reflux. Evaporation of the solvent under reduced pressure provided the methanolic extract (293 g, 30.6%). The methanolic extract (259 g) was partitioned in an EtOAc–H_2O (1 : 1, v/v) mixture. Removal of the solvents *in vacuo* from the EtOAc- and H_2O-soluble fraction yielded 165 g (19.5%) and 94 g (11.1%) of the residues, respectively.

Normal-phase silica gel column chromatography [3.0 kg, *n*-hexane–EtOAc (15 : 1→10 : 1→5 : 1→1 : 1, v/v)→$CHCl_3$: MeOH : H_2O (6 : 4 : 1, v/v/v)→MeOH] of the EtOAc-soluble fraction (140 g) gave nine fractions [fr. 1 (2.4 g), fr. 2 (15.5 g), fr. 3 (57.8 g), fr. 4 (5.6 g), fr. 5 (4.5 g), fr. 6 (9.7 g), fr. 7 (14.8 g), fr. 8 (6.5 g), and fr. 9 (22.7 g)]. Fraction 3 (50.0 g) was separated by reverse-phase silica gel column chromatography [1.5 kg, MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] to furnish five fractions {fr. 3-1 (0.06 g), fr. 3-2 (0.58 g), fr. 3-3 [=bakuchiol (**3**, 47.10 g, 8.18%)], fr. 3-4 (0.24 g), and fr. 3-5 (2.00 g)}. Fraction 3-2 (580 mg) was separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., Kyoto, Japan, 250×20 mm i.d.), MeOH–H_2O (75 : 25, v/v)] to give *p*-hydroxybenzaldehyde (**14**, 22 mg, 0.0038%). Fraction 4 (5.10 g) was subjected to reverse-phase silica gel column chromatography [160 g, MeOH–H_2O (30 : 70→50 : 50→65 : 35→80 : 20, v/v)→MeOH] to furnish 13 fractions {fr. 4-1 (20 mg), fr. 4-2 (128 mg), fr. 4-3 [=angelicin (**13**, 600 mg, 0.099%)], fr. 4-4 (200 mg), fr. 4-5 (47 mg), fr. 4-6 (297 mg), fr. 4-7 (106 mg), fr. 4-8 (214 mg), fr. 4-9 [=linolenic acid (121 mg, 0.020%)], fr. 4-10 [=linoleic acid (335 mg, 0.055%)], fr. 4-11 (226 mg), fr. 4-12 (1787 mg), and fr. 4-13 (982 mg)}. Fraction 4-6 (267 mg) was separated by HPLC [MeOH–H_2O (75 : 25, v/v)] to give **1** (60 mg, 0.011%) and **2** (50 mg, 0.0091%). Fraction 4-8 (212 mg) was purified by HPLC [MeOH–H_2O (80 : 20, v/v)] to give **3** (54 mg, 0.0096%). Fraction 6 (9.3 g) was separated by reverse-phase silica gel column chromatography [300 g, MeOH–H_2O (60 : 40→70 : 30→80 : 20, v/v)→MeOH] to furnish six fractions {fr. 6-1 (142 mg), fr. 6-2 (307 mg), fr. 6-3 (1340 mg), fr. 6-4 (800 mg), Fr. 6-5 [=bavachinin (**7**, 5500 mg, 1.78%)], and Fr. 6-6 (1152 mg)}. Fraction 6-3 (1340 mg) was separated by HPLC [isopropanol–H_2O (40 : 60, v/v)] to give Δ^1,3-hydroxybakuchiol (**4**, 49 mg, 0.016%) and Δ^3,2-hydroxybakuchiol (**5**, 21 mg, 0.007%). Fraction 6-4 (800 mg) was purified by HPLC [MeOH–H_2O (80 : 20, v/v)] to give **7** (507 mg, 0.094%). Fraction 7 (12.4 g) was subjected to reverse-phase silica gel column chromatography [400 g, MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] to furnish 11 fractions {fr. 7-1 (75 mg), fr. 7-2 (60 mg), fr. 7-3 (104 mg), fr. 7-4 (170 mg), fr. 7-5 [=bavachin (**6**, 1581 mg, 0.28%)], fr. 7-6 (877 mg), fr. 7-7 (390 mg), fr. 7-8 (235 mg), fr. 7-9 [=isobavachalcone (**9**, 4300 mg, 0.77%)], fr. 7-10 (1800 mg), and fr. 7-11 (2808 mg)}. Fraction 7-6 (220 mg) was separated by HPLC [MeOH–1% aqueous AcOH (70 : 30, v/v)] to give **6** (117 mg, 0.30%). Fraction 7-7 (390 mg) was separated by HPLC [MeOH–1% aqueous AcOH (70 : 30, v/v)] to give **6** (26 mg, 0.005%), neobavaisoflavone (**11**, 187 mg, 0.041%), and 6-prenylnaringenin (**8**, 65 mg, 0.012%). Fraction 7-8 (203 mg) was separated by HPLC [MeOH–1% aqueous AcOH (75 : 25, v/v)] to give **8** (32 mg, 0.007%), **9** (27 mg, 0.006%), and **11** (84 mg, 0.017%). Fraction 7-5 (1581 mg) was separated by HPLC [MeOH–H_2O (80 : 20, v/v)] to give **9** (82 mg, 0.052%), psoralidin (**10**, 133 mg, 0.084%), and corylifol A (**12**, 88 mg, 0.056%). Fraction 8 (6.3 g) was subjected to reverse-phase silica gel column chromatography [200 g, MeOH–H_2O (60 : 40→70 : 30→75 : 25→80 : 20, v/v)→MeOH] to furnish nine fractions {fr. 8-1 (36 mg), fr. 8-2 (70 mg), fr. 8-3 (294 mg), fr. 8-4 (156 mg), fr. 8-5 [=**11** (3701 mg, 0.69%)], fr. 8-6 [=**12** (904 mg, 0.14%)], fr. 8-7 (96 mg), fr. 8-8 (203 mg), and fr. 8-9 (840 mg)}.

The known compounds were identified by comparison of their physical data ([α]$_D$, UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS] with reported values[6—12,36—43] or commercial samples.[44]

1: Colorless oil, $[\alpha]_D^{25}$ +16.6° (*c*=0.63, $CHCl_3$), $[\alpha]_D^{26}$ +1.5° (*c*=1.27, CH_3OH). High-resolution EI-MS: Calcd for $C_{18}H_{24}O_3$ (M^+): 288.1725; Found: 288.1718. UV [MeOH, nm (log ε)]: 227 (4.19), 278 (3.43). IR (film): 3389, 1615, 1599, 1520, 1456, 1050 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: see Table 1. ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: see Table 2. EI-MS (%): *m*/*z* 288 (M^+, 1), 270 (M^+-H_2O, 3), 185 (100).

2: Colorless oil, $[\alpha]_D^{25}$ −23.5° (*c*=0.74, $CHCl_3$), $[\alpha]_D^{27}$ −7.9° (*c*=2.00, CH_3OH). High-resolution EI-MS: Calcd for $C_{18}H_{24}O_3$ (M^+): 288.1725; Found: 288.1731. UV [MeOH, nm (log ε)]: 227 (4.03), 277 (3.35). IR (film): 3389, 1615, 1599, 1516, 1456, 1038 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: see Table 1. ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: see Table 2. EI-MS (%): *m*/*z* 288 (M^+, 1), 270 (M^+-H_2O, 4), 185 (100).

Bioassay. Effects on the Release of β-Hexosaminidase from RBL-2H3 Cells Inhibitory effects on the release of β-hexosaminidase in RBL-2H3 (cell no. JCRB0023, obtained from Health Science Research Resources Bank, Osaka, Japan) were evaluated by a method reported previously.[51] Briefly, RBL-2H3 cells in 24-well plates (2×10^5 cells/well in MEM containing 10% FCS, penicillin 100 units/ml, streptomycin 100 μg/ml) were sensitized with anti-DNP IgE (0.45 μg/ml). The cells were washed with Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), and 40 mM NaOH, pH 7.2] supplemented with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% bovine serum albumin (BSA) (incubation buffer) and then incubated in 160 μl of the incubation buffer for 10 min at 37 °C. After that, 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. 10 μg/ml) at 37 °C for 10 min to stimulate the cells to degranulate. The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μl) was transferred into 96-well plates and incubated with 50 μl of substrate (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, 1 mM) in citrate buffer 0.1 M (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μl of stop solution (Na_2CO_3/$NaHCO_3$ 0.1 M, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to incubation buffer (final DMSO concentration, was 0.1%). The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated using the following equation, and IC_{50} values were determined graphically:

$$\text{inhibition (\%)} = [1-(T-B-N)/(C-N)]\times 100$$

where the control (C) was DNP-BSA (+), test sample (−); test (T), DNP-BSA (+), test sample (+); blank (B), DNP-BSA (−), test sample (+); and normal (N), DNP-BSA (−), test sample (−).

To clarify that the anti allergic effects of samples we are due to the inhibition of β-hexosaminidase release, and not a false positive from the inhibition of β-hexosaminidase activity, the cell suspension in PBS was sonicated. The solution was then centrifuged, and the supernatant was diluted with the incubation buffer and adjusted to equal the enzyme activity of the degranulation tested above. The enzyme solution (45 μl) and test sample solution (5 μl) were transferred into a 96-well microplate and enzyme activity was examined as described above.

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47) We presented the isolation and structural elucidation of psoraleanols A (**1**) and B (**2**) from the seeds of *P. corylifolia* at the 52nd Annual Meeting of the Japanese Society of Pharmacognosy on September 16, 2005.[35] Recently, Yin *et al.* have reported the isolation and structural elucidation of psoracorylifols B and C from the seeds of this plant. Since the physical data of psoraleanols A (**1**) and B (**2**) were very similar to those of psoracorylifols B and C, respectively, except for the $[\alpha]_D$ values [psoracorylifol B: $[\alpha]_D^{20}$ 0 (c=0.61, MeOH), psoracorylifol C: $[\alpha]_D^{20}$ −30.7° (c=0.39, MeOH)],[47] the relative stereostructures of **1** and **2** seem to be the same as those of psoracorylifols B and C, respectively.
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