Structures of New Flavonoids and Benzofuran-Type Stilbene and Degranulation Inhibitors of Rat Basophilic Leukemia Cells from the Brazilian Herbal Medicine *Cissus sicyoides*

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Three new flavonoid glycosides (cissosides I, II, and III) and a new benzofuran-type stilbene (cissusin) were isolated from the methanolic extract of the aerial parts of *Cissus sicyoides* cultivated in Brazil. Their structures were elucidated on the basis of chemical and physicochemical evidence. The inhibitory effects of the isolated constituents on the release of β-hexosaminidase as a marker of degranulation in rat basophilic leukemia (RBL-2H3) cells were examined. Cissusin, flavonols (kaempferol, quercetin), flavones (7,3′,4′-trihydroxyflavone, lanceolatin B), pterocarpanes (homopterocarpin), chalcones (isoliquiritigenin, *E*-7-*O*-methylpongamol), and tryptanthrin markedly inhibited the release of β-hexosaminidase.

Key words *Cissus sicyoides*; flavonoid; benzofuran-type stilbene; cissoside; cissusin; degranulation inhibitor

Cissus sicyoides (Vitaceae) (Brazilian name: *Insulina*) is a herbaceous plant widely distributed in Brazil. Plant infusions are used in Brazilian folk medicine to treat diabetes mellitus, while the leaves are used externally against rheumatism and wounds.[1] Bioactive studies indicated that the water extract of *C. sicyoides* (500 mg/kg body weight) improved hyperglycemia after the oral administration of maltose to KK-A^y mice.[2] Previous phytochemical investigations of this plant yielded coumarins, flavonoids, and anthocyanins.[1,3] In the course of our characterization studies of traditional Brazilian medicines,[4—7] we systematically investigated the chemical constituents of the methanolic extract from the aerial parts of *C. sicyoides*, which resulted in the isolation of three new flavonoid glycosides, cissosides I (**1**), II (**2**), and III (**3**), and the new benzofuran-type stilbene cissusin (**4**), together with 39 known constituents. This paper deals with the isolation and structural elucidation of four new constituents (**1**—**4**), and the inhibitory effects of both the extracts and isolated compounds on the release of β-hexosaminidase from RBL-2H3 cells.

The methanolic extract from the dried aerial parts of *C. sicyoides* (cultivated in Brazil) was partitioned into a mixture of ethyl acetate (EtOAc) and water to furnish the EtOAc-soluble fraction and an aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH- and H_2O-soluble fractions. The EtOAc- and *n*-BuOH-soluble fractions were subjected to silica gel and octadecyl silica (ODS) column chromatography and finally to HPLC to furnish three new flavonoid glycosides, cissoside I (**1**, 0.0018% from natural medicine), cissoside II (**2**, 0.00029%), and cissoside III (**3**, 0.00038%); a new benzofuran-type stilbene, cissusin (**4**, 0.00054%); 18 flavonols, kaempferol (**5**, 0.0011%),[8] kaempferol-3-*O*-α-L-rhamnopyranoside (**6**, 0.00023%),[8] kaempferol-3-*O*-β-D-glucopyranoside (**7**, 0.00029%),[9] kaempferol-3-*O*-α-L-arabinofuranoside (**8**, 0.00024%),[8] kaempferol-7-*O*-α-L-rhamnopyranoside (**9**, 0.00065%),[10] kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**10**, 0.010%),[8] kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**11**, 0.00039%),[10] kaempferol-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside (**12**, 0.0010%),[11] kaempferol-3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside (**13**, 0.0019%),[12] kaempferol-3-*O*-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**14**, 0.0041%),[8] quercetin (**15**, 0.00077%),[9] quercetin-3-*O*-α-L-rhamnopyranoside (**16**, 0.00045%),[9] quercetin-3-*O*-β-D-glucopyranoside (**17**, 0.00017%),[9] quercetin-3-*O*-β-D-galactopyranoside (**18**, 0.000092%),[9] quercetin-3-*O*-α-L-arabinopyranoside (**19**, 0.00051%),[9] quercetin-3-*O*-α-L-arabinofuranoside (**20**, 0.00057%),[9] rutin (**21**, 0.017%),[13] quercetin-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**22**, 0.00043%),[11] two flavones 7,3′,4′-trihydroxyflavone (**23**, 0.00039%),[14] and lanceolatin B (**24**, 0.00094%)[15]; three pterocarpanes, medicarpin (**25**, 0.00011%),[16] homopterocarpin (**26**, 0.00018%),[16] and melilotocarpan D (**27**, 0.00043%)[17]; three chalcones, isoliquiritigenin (**28**, 0.00075%),[18] pongamol (**29**, 0.0018%),[19] and *E*-7-*O*-methylpongamol (**30**, 0.0017%)[19]; a benzofuran-type stilbene, 2-(2′,4′-dimethoxyphenyl)-5,6-methylenedioxybenzofuran (**31**, 0.00016%)[20]; a lignan, (+)-pinoresinol (**32**, 0.00068%)[21]; an indole quinazoline alkaloid, tryptanthrin (**33**, 0.0024%)[22]; a chromone, 7-hydroxychromone (0.000085%)[23]; seven phenolics, syringaldehyde (0.00011%),[24] 4-hydroxybenzoic acid (0.00028%),[25] vanillic acid (0.00078%),[25] 4-hydroxycinnamic acid (0.00017%),[26] gallic acid (0.0069%),[27] methyl gallate (0.00034%),[28] and glucosyl salicylate (0.00084%)[29]; an indole, indican (0.0037%)[30]; and a catechin, epicatechin 3-*O*-gallate (0.0030%).[31]

Cissoside I (**1**) was obtained as a yellow powder with negative optical rotation ($[\alpha]_D^{27}$ −176.8°). In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m*/*z* 629 $(M+Na)^+$, and high-resolution (HR) positive-ion FAB-MS analysis revealed the formula of **1** to be $C_{28}H_{30}O_{15}$. The IR spectrum of **1** suggested the presence of hydroxyl (3415 cm^{-1}), acetyl carbonyl (1720 cm^{-1}), and γ-pyrone (1655 cm^{-1}) groups, and an aromatic ring (1601, 1493 cm^{-1}). Its UV spectrum in methanol showed absorption maxima at 266 nm (log ε 4.32) (band II) and 347 nm (4.21) (band I), suggesting the presence of a flavonol skeleton. The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra of **1**, which were as-

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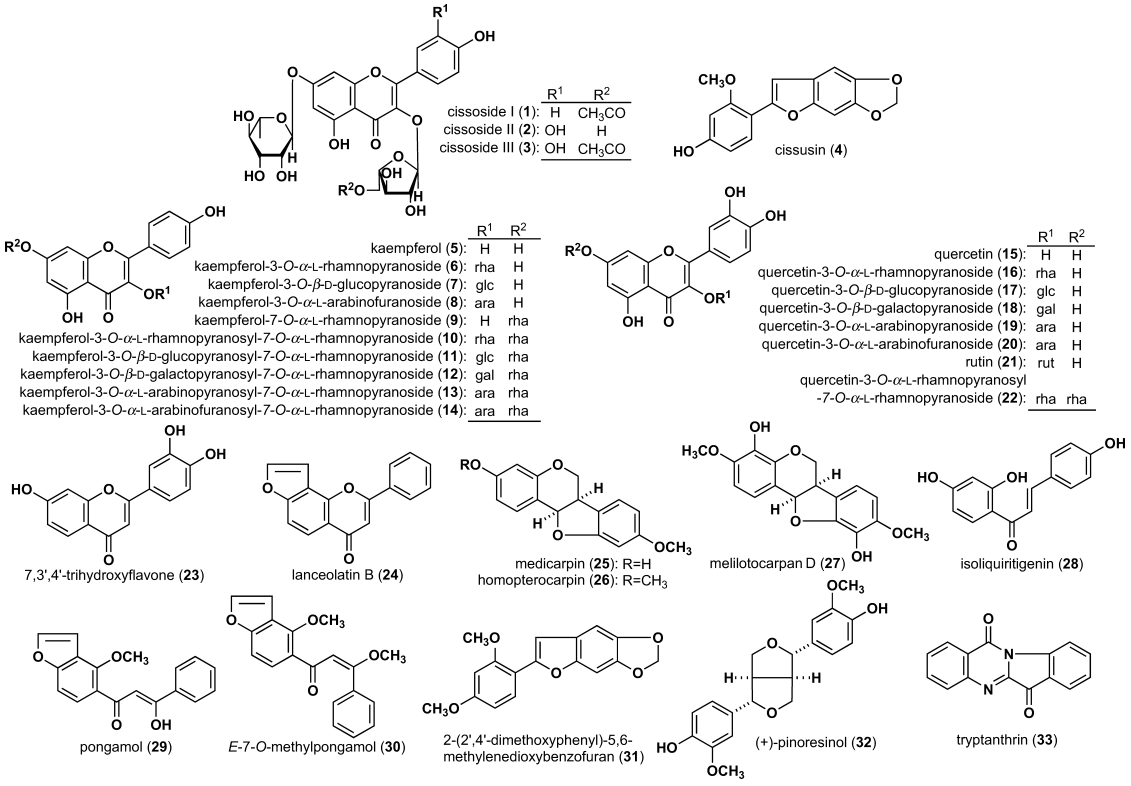

Chart 1. Structures of Principal Constituents Isolated from the Aerial Parts of *C. sicyoides*

Table 1. ^{13}C-NMR Data for **1**—**4**, **14** and **31**

	1[a]	**14**[a]	**2**[a]	**3**[a]		**4**[b]	**31**[b]
C-2	158.6	158.3	158.5	158.8	C-2	152.1	152.1
C-3	135.0	135.1	135.1	135.1	C-3	104.7	104.8
C-4	179.1	179.3	179.3	179.2	C-3a	99.3	99.3
C-5	162.3	162.3	162.3	162.3	C-4	145.6	145.6
C-6	100.4	100.4	100.3	100.3	C-5	144.2	144.2
C-7	162.8	162.8	162.8	162.8	C-6	93.2	93.2
C-8	94.9	94.9	94.8	94.8	C-7	123.2	123.2
C-9	157.0	157.0	157.0	157.0	C-7a	148.8	148.8
C-10	106.9	106.9	106.9	106.9			
C-1′	121.8	121.8	122.2	122.2	C-1′	112.9	113.0
C-2′	131.8	131.8	116.6	116.6	C-2′	157.4	157.2
C-3′	116.5	116.5	147.5	147.5	C-3′	99.3	98.8
C-4′	161.9	161.9	150.9	150.9	C-4′	156.6	160.5
C-5′	116.5	116.5	117.1	117.1	C-5′	107.5	104.8
C-6′	131.8	131.8	122.7	122.7	C-6′	127.5	127.3
C-1″	110.0	110.0	109.9	109.9	O$\underline{C}$H$_2$O	101.1	101.1
C-2″	83.7	83.7	83.6	83.6	O$\underline{C}$H$_3$	55.5	55.5×2
C-3″	79.4	78.9	79.0	79.5			
C-4″	84.2	88.6	88.6	84.2			
C-5″	64.9	62.5	62.4	64.8			
C-1‴	100.0	100.0	100.0	100.0			
C-2‴	71.6	71.6	71.6	71.6			
C-3‴	72.4	72.4	72.4	72.4			
C-4‴	73.6	73.6	73.6	73.6			
C-5‴	71.5	71.5	71.5	71.5			
C-6‴	18.7	18.7	18.7	18.7			
$\underline{C}$H$_3\underline{C}$O	20.6			20.6			
	170.5			170.5			

a) Measured in pyridine-d_5 at 150 MHz. *b*) Measured in $CDCl_3$ at 150 MHz.

signed by various NMR experiments (distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond connectivtiy (HMBC)), showed signals assignable to a kaempferol part {two *meta*-coupled aromatic protons

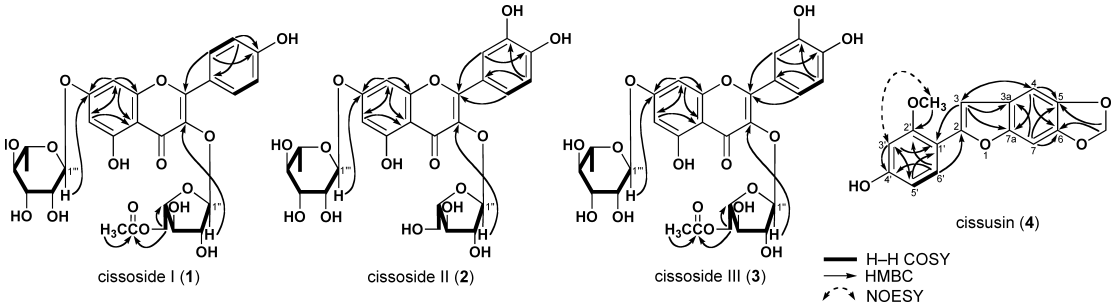

Fig. 1. Significant H–H COSY, HMBC, and NOE Correlations for **1**—**4**

[δ 6.78, 6.96 (1H each, both d, J=2.1 Hz, H-6, -8)], four *ortho*-coupled aromatic protons [δ 7.33, 8.32 (2H each, both d, J=8.6 Hz, H-3′, -5′, -2′, -6′)]}, an arabinofuranosyl moiety {δ [4.42 (1H, dd, J=6.8, 12.4 Hz), 4.62 (1H, dd, J=3.4, 12.4 Hz) H_2-5′], 4.64 (1H, m, H-3″), 4.66 (1H, m, H-4″), 5.25 (1H, br d, J=2.8 Hz, H-2″), 6.49 (1H, br s, H-1″)}, a rhamnopyranosyl moiety [1.68 (3H, d, J=6.1 Hz, H_3-6‴), 4.31 (1H m, H-5‴), 4.41 (1H dd, J=8.9, 9.6 Hz, H-4‴), 4.67 (1H, dd, J=3.4, 9.6 Hz, H-3‴), 4.73 (1H, br s, H-2‴), 6.26 (1H, br s, H-1‴)], and an acetyl group [δ 1.88 (3H, s, $-CH_3$)]. The position of the acetyl group in **1** was confirmed in an HMBC experiment (Fig. 1), in which a long-range correlation was observed between the proton-5″ [4.42 (1H, dd, J=6.8, 12.4 Hz), 4.62 (1H, dd, J=3.4, 12.4 Hz) H_2-5′] and the acetyl carbonyl carbon (δ_C 170.5). In addition, the alkaline hydrolysis of **1** with 0.5% sodium methoxide (NaOMe)–MeOH at room temperature furnished kaempferol 3-*O*-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**14**).[8] These findings led us to formulate the structure of cissoside I to be kaempferol 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**1**).

Cissoside II (**2**) was obtained as a yellow powder with negative optical rotation ($[\alpha]_D^{26}$ −181.5°). In the positive-ion FAB-MS of **2**, a quasimolecular ion peak was observed at *m*/*z* 603 $(M+Na)^+$, and HR-FAB-MS analysis established its formula to be $C_{26}H_{28}O_{15}$. Its IR spectrum showed absorption bands at 3400, 1655, 1609, and 1509 cm^{-1} ascribable to hydroxyl and carbonyl functions and aromatic rings. The UV spectrum of **2** in methanol showed absorption maxima at 258 nm (log ε 4.54) (band II) and 349 nm (3.93) (band I), suggesting the presence of a flavonol skeleton. The acid hydrolysis of **2** with 1 M of hydrochloric acid (HCl)–1,4-dioxane (1 : 1, v/v) liberated quercetin, L-arabinose, and L-rhamnose, which were identified with HPLC analysis using a refractive index or optical rotation detector.[32,33] The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra of **2** showed signals assignable to a quercetin part {two *meta*-coupled aromatic protons [δ 6.75, 6.84 (1H each, both d, J=2.0 Hz, H-6, -8)], three *ortho*- and *meta*-coupled aromatic protons [δ 7.37 (1H, d, J=8.2 Hz, H-5′), 7.99 (1H, dd, J=2.0, 8.2 Hz, H-6′), 8.19 (1H, d, J=2.0 Hz, H-2′)]}, an α-L-arabinofuranosyl moiety {δ [4.10 (1H, dd, J=4.1, 11.7 Hz), 4.17 (1H, dd, J=2.7, 11.7 Hz), H_2-5″], 4.75 (1H, m, H-4″), 4.91 (1H, m, H-3″), 5.20 (1H, br d, J=2.8 Hz, H-2″), 6.53 (1H, br s, H-1″)}, and an α-L-rhamnopyranosyl moiety [1.68 (3H, d, J=6.2 Hz, H_3-6‴), 4.30 (1H m, H-5‴), 4.42 (1H dd, J=8.9, 9.6 Hz, H-4‴), 4.66 (1H, dd, J=2.8, 9.6 Hz, H-3‴), 4.71 (1H, br s, H-2‴), 6.22 (1H, br s, H-1‴)]. The linkages of the sugar moieties in **2** were clarified in HMBC experiments (Fig. 1). Thus long-range correlations were observed between H-1″ [6.53 (1H, br s)] and C-3 (δ_C 135.1) as well as between H-1‴ [6.22 (1H, br s)] and the carbon-7 (δ_C 162.8). On the basis of this evidence, the structure of cissoside II was determined to be quercetin 3-*O*-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**2**).

Cissoside III (**3**) was also obtained as a yellow powder. Its positive-ion FAB-MS and HR-FAB-MS analysis revealed the formula of **3** to be $C_{28}H_{30}O_{16}$. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra of **3** were very similar to those of **2**, except for the signals due to an additional acetyl group [(δ_C 20.6, 170.5), (δ_H 1.89, s)]. Comparison of the ^{13}C-NMR spectra (Table 1) of **3** with those of **2** revealed an acylation shift around the C-5″ position of the α-L-arabinofuranosyl moiety. This was also confirmed in an HMBC experiment, as shown in Fig. 1. Furthermore, the alkaline hydrolysis of **3** with 0.5% NaOMe–MeOH at room temperature furnished cissoside II (**2**). On the basis of the above-mentioned evidence, the structure of cissoside III was established to be quercetin 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**3**).

Cissusin (**4**) was isolated as a colorless powder. The molecular formula of **4** was established to be $C_{16}H_{12}O_5$ by HR-EI-MS measurement. The IR spectrum of **4** suggested the presence of a hydroxyl group (3433 cm^{-1}), an aromatic ring (1623, 1614, 1592, 1501 cm^{-1}), and a methylenedioxy group (1036, 945 cm^{-1}). Its UV spectrum showed absorption maxima at 330 nm (log ε 4.38) and 345 nm (4.39), suggesting the presence of a 2-arylbenzofuran structure.[20] The ^{1}H- and ^{13}C-NMR ($CDCl_3$, Table 1) spectra of **4** showed signals assignable to one methoxyl group [δ 3.93 (3H, s, $OC\underline{H}_3$-2′)], one methylenedioxy group [δ 5.97 (2H, s, $OC\underline{H}_2O$-5, 6)], three *ortho*- and *meta*-coupled aromatic protons [δ 6.52 (1H, d, J=2.1 Hz, H-3′), 6.54 (1H, dd, J=2.1, 8.9 Hz, H-5′), 7.81 (1H, d, J=8.9 Hz, H-6′)], three single aromatic protons [δ 6.92, 6.98, 7.06 (1H each, all s, H-4, 7, 3). The structure of **4** was confirmed in ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC experiments. As shown in Fig. 1, the former indicated the presence of one partial structure shown by the bold line, while long-range correlations in the HMBC experiment on **4** were observed between the following proton and carbon pairs: H-3 and C-3a, -4, -7a, 1′; H-4 and C-3, -5, -6, -7a; $OC\underline{H}_2O$-5, -6 and C-5, 6; H-7 and C-3a, -5, -6; $OC\underline{H}_3$-2′ and C-2′; H-3′ and C-1′, -4′, -5′; H-5′ and C-1′, -3′; H-6′ and C-2, -2′, -4′. The position of a methoxyl group was also supported by the observation of a nuclear Overhauser effect (NOE) at H-3′ upon irradiation of $OC\underline{H}_3$-2′. Furthermore,

Table 2. Inhibitory Effects of Constituents (**4**, **5**, **15**, **23**—**33**) from *C. sicyoides* on the Release of β-Hexosaminidase by RBL-2H3 Cells

	Inhibition (%)						Inhibition (%) of β-hexosaminidase at 100 μM
	0 μM	3 μM	10 μM	30 μM	100 μM	IC_{50} (μM)	
Cissusin (**4**)	0.0±3.5	—	12.0±4.5	24.1±6.1**	51.6±2.7**	95	2.0
Kaempferol (**5**)	0.0±2.9	—	30.8±5.1**	79.6±2.5**	93.2±0.6**	15	3.6
Quercetin (**15**)[a]	0.0±8.6	46.0±3.4**	78.8±1.2**	87.3±0.7**	90.1±0.4**	3.3	4.8
7,3′,4′-Trihydroxyflavone (**23**)	0.0±6.1	1.6±5.2	30.4±3.5**	71.8±2.1**	90.8±2.0**	17	5.2
Lanceolatin B (**24**)	0.0±6.3	20.7±4.7*	52.7±4.5**	55.8±3.9**	69.0±3.6**	*ca.* 11	4.3
Medicarpin (**25**)	0.0±4.1	—	—	—	34.4±4.9**	>100	—
Homopterocarpin (**26**)	0.0±3.5	—	16.4±4.5**	28.1±1.8**	74.7±0.1**	54	1.5
Melilotocarpan D (**27**)	0.0±6.0	—	—	—	16.9±5.8	—	—
Isoliquiritigenin (**28**)	0.0±1.4	15.4±2.2**	28.1±2.8**	54.5±3.2**	91.3±0.3**	24	5.3
Pongamol (**29**)	0.0±1.6	—	—	—	36.7±2.6**	>100	—
E-7-*O*-methylpongamol (**30**)	0.0±3.7	—	8.6±3.0	51.5±1.4**	81.1±2.4**	29	−5.5
31	0.0±4.1	—	—	—	25.7±3.6**	>100	—
(+)-Pinoresinol (**32**)	0.0±2.6	—	—	—	−11.3±3.5	—	—
Tryptanthrin (**33**)	0.0±3.5	—	19.4±4.0**	85.6±1.7**	89.4±0.7**	*ca.* 19	−1.1

	Inhibition (%)					Inhibition (%) of β-hexosaminidase at 300 μM
	0 μM	30 μM	100 μM	300 μM	IC_{50} (μM)	
Ketotifen fumarate	0.0±5.2	10.8±5.4	30.6±4.8**	68.9±2.4	176	7.3
Tranilast	0.0±6.3	25.4±4.3**	44.5±4.4**	75.3±0.2**	112	7.1

Each value represents mean±S.E.M. (n=4). Significantly different from the control, * $p<0.05$, ** $p<0.01$. *a*) Inhibition at 1 μM was 6.7±7.6%.

treatment of **4** with trimethylsilyldiazomethane ($TMSCHN_2$) yielded **31**.[20] Consequently, the structure of **4** was determined to be as shown.

Inhibitory Effects of the Constituents of *C. sicyoides* on the Release of β-Hexosaminidase by RBL-2H3 Cells Histamine, which is released from mast cells upon stimulation with an antigen or a degranulation inducer, is usually used 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.[34,35] Therefore it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells.

As a part of our characterization studies of bioactive components from natural medicines, we previously reported various inhibitors of the release of β-hexosaminidase including flavonoids and lignans.[36—38] The glycosides (**6**—**14**, **16**—**22**) were not subjected to the examination, since the glycoside moiety of flavonoids markedly reduces their activity in this *in vitro* bioassay method.[37] As a continuing study of the antiallergic constituents of natural medicines,[39—48] the effects of the principal constituents of *C. sicyoides* lacking a glycoside moiety on the release of β-hexosaminidase in RBL-2H3 cells were examined. As shown in Table 2, cissusin (**4**, IC_{50}=95 μM), kaempferol (**5**, 15 μM), quercetin (**15**, 3.3 μM), 7,3′,4′-trihydroxyflavone (**23**, 17 μM), tryptanthrin (**33**, *ca.* 19 μM), lanceolatin B (**24**, *ca.* 11 μM), homopterocarpin (**26**, 54 μM), isoliquiritigenin (**28**, 20 μM), and *E*-7-*O*-methylpongamol (**30**, 29 μM) inhibited the release of β-hexosaminidase without inhibiting its enzyme activity [inhibition (%): *ca.* 5.3% at 100 μM] and their activities were more potent than those of the antiallergic compounds tranilast (112 μM) and ketotifen fumarate (176 μM).

With regard to structural requirements for the activity, **5** and **23** showed less activity than **15** and luteolin (IC_{50}=2.9 μM, data not shown) indicating that the 3′,4′-dihydroxyl group at the B-ring and the 5-hydroxyl group are important for the greater activity, in agreement with our previous results.[37] The 4′-hydroxyl group of the benzofuran-type stilbene **4** is suggested to be important for the activity, since the methylated compound **31** showed less activity than **4**. Among the pterocarpans, **26** showed stronger activity than **25** and **27**, suggesting that the 7-methoxy group is important, but the 8- and/or 3′-hydroxyl groups reduced the activity. The detailed structural requirements for the activity should be studied further.

In conclusion, three new flavonoid glycosides, cissosides I (**1**), II (**2**), and III (**3**), and a new benzofuran-type stilbene, cissusin (**4**), were isolated from the methanolic extract of the aerial parts of *C. sicyoides* cultivated in Brazil. Their structures were elucidated on the basis of chemical and physicochemical evidence. The inhibitory effects of the isolated constituents on the release of β-hexosaminidase as a marker of degranulation in RBL-2H3 cells were examined. We found that cissusin (**4**), flavonols [kaempferol (**5**), quercetin (**15**)], flavones [7,3′,4′-trihydroxyflavone (**23**), lanceolatin B (**24**)], a pterocarpane [homopterocarpin (**26**)], chalcones [isoliquiritigenin (**28**), *E*-7-*O*-methylpongamol (**30**)], and tryptanthrin (**33**) markedly inhibited the release of β-hexosaminidase, and several structural requirements of the active compounds for the inhibition were suggested.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS, CI-MS, and high-resolution CI-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) and JNM-ECA600 (600 MHz) spectrometers; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) and JNM-ECA600 (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. For HPLC, COSMOSIL 5C18-PAQ 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., Aichi, Japan, 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, 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) (reversed phase); reversed-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 aerial parts of *C. sicyoides* cultivated in Brazil were obtained in January 2005 *via* Tamura Pharmaceutical Co., Ltd. (Wakayama, Japan). A voucher specimen (2005.01, Brazil-02) is on file in our laboratory.

Extraction and Isolation The aerial parts of *C. sicyoides* (4.5 kg) were powdered and extracted three times with methanol (MeOH) under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the methanolic extract (199.7 g, 4.4%). The MeOH extract (174.7 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture, and removal of the solvent *in vacuo* yielded an EtOAc-soluble fraction (59.3 g, 1.5%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (39.4 g, 1.0%) and a water-soluble fraction (76.0 g, 1.9%).

The EtOAc-soluble fraction (44.3 g) was subjected to ordinary-phase silica gel column chromatography [1.4 kg, *n*-hexane–EtOAc (10 : 1→5 : 1→2 : 1→1 : 1→1 : 2, v/v)→$CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give six fractions [Fr. 1 (9.27 g), Fr. 2 (7.48 g), Fr. 3 (11.14 g), Fr. 4 (5.16 g), Fr. 5 (4.12 g), and Fr. 6 (6.28 g)]. Fr. 1 (9.27 g) was subjected to reversed-phase silica gel column chromatography [270 g, MeOH–H_2O (30 : 70→45 : 55→60 : 40→75 : 25, v/v)→MeOH] to give seven fractions [Fr. 1-1 (110 mg), Fr. 1-2 (151 mg), Fr. 1-3 (658 mg), Fr. 1-4 (1922 mg), Fr. 1-5 (751 mg), Fr. 1-6 (814 mg), and Fr. 1-7 (3884 mg)]. Fr. 1-2 (151 mg) and Fr. 1-3 (658 mg) were purified using HPLC [CH_3CN–H_2O (60 : 40, v/v)] to give pongamol (**29**, 52.1 mg, 0.0018%). Fr. 2 (7.48 g) was subjected to reversed-phase silica gel column chromatography [220 g, MeOH–H_2O (20 : 80→30 : 70→45 : 55→60 : 40→75 : 25, v/v)→MeOH] to give 11 fractions [Fr. 2-1 (330 mg), Fr. 2-2 (473 mg), Fr. 2-3 (318 mg), Fr. 2-4 (270 mg), Fr. 2-5 (310 mg), Fr. 2-6 (240 mg), Fr. 2-7 (200 mg), Fr. 2-8 (256 mg), Fr. 2-9 (335 mg), Fr. 2-10 (658 mg), and Fr. 2-11 (2860 mg)]. Fr. 2-1 (330 mg) was separated using HPLC [CH_3CN–H_2O (10 : 90, v/v)] to give 4-hydroxybenzoic acid (8.2 mg, 0.00028%), vanillic acid (23.0 mg, 0.00078%), and methyl gallate (10.0 mg, 0.00034%). Fr. 2-2 (473 mg) was purified using HPLC [CH_3CN–H_2O (15 : 85, v/v)] to give 7-hydroxychromone (2.5 mg, 0.000085%), syringaldehyde (3.2 mg, 0.00011%), and 4-hydroxycinnamic acid (5.0 mg, 0.00017%). Fr. 2-4 (270 mg) was separated using HPLC [CH_3CN–H_2O (25 : 75, v/v)] to give melilotocarpan D (**27**, 12.7 mg, 0.00043%) and (+)-pinoresinol (**32**, 20.0 mg, 0.00068%). Fr. 2-5 (310 mg) was purified using HPLC [MeOH–H_2O (50 : 50, v/v)] to give medicarpin (**25**, 3.1 mg, 0.00011%) and homopterocarpin (**26**, 5.2 mg, 0.00018%). Fr. 2-6 (240 mg) was separated using HPLC [MeOH–H_2O (55 : 45, v/v)] to give isoliquiritigenin (**28**, 11.5 mg, 0.00039%) and *E*-7-*O*-methylpongamol (**30**, 10.0 mg, 0.00034%). Fr. 2-8 (256 mg) was separated using HPLC [MeOH–H_2O (65 : 35, v/v)] to give tryptanthrin (**33**, 27.1 mg, 0.00091%), isoliquiritigenin (**28**, 10.7 mg, 0.00036%), and *E*-7-*O*-methylpongamol (**30**, 23.1 mg, 0.00078%). Fr. 2-9 (335 mg) was separated using HPLC [CH_3CN–H_2O (40 : 60, v/v)] to give cissusin (**4**, 15.8 mg, 0.00054%), lanceolatin B (**24**, 27.7 mg, 0.00094%), *E*-7-*O*-methylpongamol (**30**, 17.5 mg, 0.00059%), and 2-(2′,4′-dimethoxyphenyl)-5,6-methylenedioxybenzofuran (**31**, 4.6 mg, 0.00016%). Fr. 3 (11.14 g) was subjected to reversed-phase silica gel column chromatography [330 g, MeOH–H_2O (20 : 80→30 : 70→45 : 55→70 : 30, v/v)→MeOH] to give nine fractions [Fr. 3-1 (448 mg), Fr. 3-2 (603 mg), Fr. 3-3 (574 mg), Fr. 3-4 (492 mg), Fr. 3-5 (1064 mg), Fr. 3-6 (1430 mg), Fr. 3-7 (772 mg), Fr. 3-8 (1335 mg), and Fr. 3-9 (2645 mg)]. Fr. 3-5 (1064 mg) was separated using HPLC [MeOH–H_2O (40 : 60, v/v)] to give kaempferol-3-*O*-α-L-arabinofuranoside (**8**, 7.0 mg, 0.00024%), quercetin-3-*O*-α-L-arabinopyranoside (**19**, 15.0 mg, 0.00051%), and quercetin-3-*O*-α-L-arabinofuranoside (**20**, 16.7 mg, 0.00057%). Fr. 3-6 (1430 mg) was separated using HPLC [MeOH–H_2O (50 : 50, v/v)] to give kaempferol (**5**, 32.4 mg, 0.0011%), quercetin (**15**, 22.5 mg, 0.00077%), kaempferol-3-*O*-β-D-glucopyranoside (**7**, 8.6 mg, 0.00029%), kaempferol-3-*O*-α-L-rhamnopyranoside (**6**, 6.6 mg, 0.00023%), kaempferol-7-*O*-α-L-rhamnopyranoside (**9**, 19.0 mg, 0.00065%), and 7,3′,4′-trihydroxyflavone (**23**, 11.3 mg, 0.00039%). Fr. 4 (5.16 g) was subjected to reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (10 : 90→30 : 70→50 : 50→75 : 25, v/v)→MeOH] to give 12 fractions [Fr. 4-1 (170 mg), Fr. 4-2 (287 mg), Fr. 4-3 (318 mg), Fr. 4-4 (107 mg), Fr. 4-5 (556 mg), Fr. 4-6 (254 mg), Fr. 4-7 (906 mg), Fr. 4-8 (540 mg), Fr. 4-9 (606 mg), Fr. 4-10 (140 mg), Fr. 4-11 (110 mg), and Fr. 4-12 (824 mg)]. Fr. 4-2 (287 mg) was separated using HPLC [MeOH–H_2O (10 : 90, v/v)] to give gallic acid (202.8 mg, 0.0069%). Fr. 4-3 (318 mg) was separated using HPLC [MeOH–H_2O (15 : 85, v/v)] to give indican (34.7 mg, 0.0012%). Fr. 4-5 (556 mg) was separated using HPLC [MeOH–H_2O (30 : 70, v/v)] to give epicatechin 3-*O*-gallate (60.4 mg, 0.0021%). Fr. 4-7 (906 mg) and Fr. 4-8 (540 mg) were separated using HPLC [MeOH–H_2O (40 : 60, v/v)] to give cissoside I (**1**, 51.8 mg, 0.0018%), cissoside II (**2**, 8.5 mg, 0.00029%), cissoside III (**3**, 11.2 mg, 0.00038%), kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**10**, 51.7 mg, 0.0018%), and kaempferol-3-*O*-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**14**, 120.5 mg, 0.0041%). Fr. 4-9 (606 mg) was separated using HPLC [MeOH–H_2O (60 : 40, v/v)] to give tryptanthrin (**33**, 42.1 mg, 0.0015%). Fr. 5 (4.12 g) was subjected to reversed-phase silica gel column chromatography [120 g, MeOH–H_2O (15 : 85→30 : 70→45 : 55→70 : 30, v/v)→MeOH] to give 10 fractions [Fr. 5-1 (384 mg), Fr. 5-2 (270 mg), Fr. 5-3 (170 mg), Fr. 5-4 (233 mg), Fr. 5-5 (278 mg), Fr. 5-6 (960 mg), Fr. 5-7 (280 mg), Fr. 5-8 (326 mg), Fr. 5-9 (335 mg), and Fr. 5-10 (540 mg)]. Fr. 5-2 (270 mg) was separated using HPLC [MeOH–H_2O (30 : 70, v/v)] to give epicatechin 3-*O*-gallate (26.4 mg, 0.00090%). Fr. 5-5 (278 mg) was separated using HPLC [CH_3CN–H_2O (15 : 85, v/v)] to give quercetin-3-*O*-α-L-rhamnopyranoside (**16**, 13.2 mg, 0.00045%), quercetin-3-*O*-β-D-glucopyranoside (**17**, 5.0 mg, 0.00017%), quercetin-3-*O*-β-D-galactopyranoside (**18**, 2.7 mg, 0.000092%), and rutin (**21**, 8.6 mg, 0.00029%). Fr. 5-6 (960 mg) was separated using HPLC [MeOH–H_2O (45 : 55, v/v)] to give kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**10**, 129.9 mg, 0.0044%).

The *n*-BuOH-soluble fraction (27.7 g) was subjected to ordinary-phase silica gel column chromatography [800 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give seven fractions [Fr. 1 (0.35 g), Fr. 2 (1.69 g), Fr. 3 (4.33 g), Fr. 4 (3.46 g), Fr. 5 (6.40 g), Fr. 6 (4.84 g), and Fr. 7 (2.45 g)]. Fr. 3 (4.33 g) was subjected to reversed-phase silica gel column chromatography [120 g, MeOH–H_2O (10 : 90→30 : 70→45 : 55→75 : 25, v/v)→MeOH]] to give eight fractions [Fr. 3-1 (165 mg), Fr. 3-2 (603 mg), Fr. 3-3 (407 mg), Fr. 3-4 (445 mg), Fr. 3-5 (359 mg), Fr. 3-6 (575 mg), Fr. 3-7 (406 mg), and Fr. 3-8 (740 mg)]. Fr. 3-2 (603 mg) was separated by HPLC [MeOH–H_2O (10 : 90, v/v)] to give glucosyl salicylate (23.3 mg, 0.00084%) and indican (70 mg, 0.0025%). Fr. 3-6 (575 mg) was separated using HPLC [CH_3CN–H_2O (20 : 80, v/v)] to give kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**10**, 88.6 mg, 0.0032%) and kaempferol-3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside (**13**, 52.0 mg, 0.0019%). Fr. 4 (3.46 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (20 : 80→30 : 70→45 : 55→60 : 40, v/v)→MeOH]] to give six fractions [Fr. 4-1 (430 mg), Fr. 4-2 (646 mg), Fr. 4-3 (737 mg), Fr. 4-4 (264 mg), Fr. 4-5 (103 mg), and Fr. 4-6 (730 mg)]. Fr. 4-3 (737 mg) was separated using HPLC [CH_3CN–H_2O (15 : 85, v/v)] to give kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**11**, 10.7 mg, 0.00039%), kaempferol-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside (**12**, 28.0 mg, 0.0010%), rutin (**21**, 91.2 mg, 0.0033%), and quercetin-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**22**, 12.0 mg, 0.00043%). Fr. 4-4 (264 mg) was separated using HPLC [CH_3CN–H_2O (20 : 80, v/v)] to give kaempferol-3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**10**, 22.0 mg, 0.00079%). Fr. 5 (6.40 g) was subjected to reversed-phase silica gel column chromatography [180 g, MeOH–H_2O (30 : 70→50 : 50→75 : 25, v/v)→MeOH]] to give seven fractions [Fr. 5-1 (1250 mg), Fr. 5-2 (508 mg), Fr. 5-3 (422 mg), Fr. 5-4 (700 mg), Fr. 5-5 (859 mg), Fr. 5-6 (623 mg), and Fr. 5-7 (1377 mg)]. Fr. 5-3 (422 mg) and Fr. 5-4 (700 mg) were separated using HPLC [CH_3CN–H_2O (15 : 85, v/v)] to give rutin (**21**, 357.8 mg, 0.013%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-, ^{13}C-NMR, MS) with reported values[8—15,17—29,31] or those of authentic samples.[16,30]

Cissoside I (**1**): A yellow powder, $[\alpha]_D^{27}$ −176.8° (*c*=0.70, MeOH). UV λ_{max} (MeOH) nm (log ε): 266 (4.32), 347 (4.21). IR (KBr) cm^{-1}: 3415, 1720, 1655, 1601, 1493, 1375, 1208, 1179, 1120, 1063. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 6.78, 6.96 (1H each, both d, *J*=2.1 Hz, H-6, 8), 7.33, 8.32

(2H each, both d, J=8.6 Hz, H-3′, -5′, -2′, -6′), [H-arabinose part: 1.88 (3H, s, C$\underline{H}_3$CO–), 4.42 (1H dd, J=6.8, 12.4 Hz, H_a-5″), 4.62 (1H dd, J=3.4, 12.4 Hz, H_b-5″), 4.64 (1H, m, H-3″), 4.66 (1H, m, H-4″), 5.25 (1H, br d, J=2.8 Hz, H-2″), 6.49 (1H, br s, H-1″)], [H-rhamnose part: 1.68 (3H, d, J=6.1 Hz, H_3-6‴), 4.31 (1H m, H-5‴), 4.41 (1H dd, J=8.9, 9.6 Hz, H-4‴), 4.67 (1H, dd, J=3.4, 9.6 Hz, H-3‴), 4.73 (1H, br s, H-2‴), 6.26 (1H, br s, H-1‴)]. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: see Table 1. Positive-ion FAB-MS m/z: 629 $(M+Na)^+$. HR-FAB-MS m/z: 629.1476 [Calcd for $C_{28}H_{30}O_{15}Na$ $(M+Na)^+$: 629.1482].

Cissoside II (**2**): A yellow powder, $[\alpha]_D^{26}$ −181.5° (c=0.50, MeOH). UV λ_{max} (MeOH) nm (log ε): 258 (4.54), 349 (3.93). IR (KBr) cm^{-1}: 3400, 1655, 1609, 1509, 1460, 1364, 1200, 1169, 1115, 1065. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 6.75, 6.84 (1H each, both d, J=2.0 Hz, H-6, -8), 7.37 (1H, d, J=8.2 Hz, H-5′), 7.99 (1H, dd, J=2.0, 8.2 Hz, H-6′), 8.19 (1H, d, J=2.0 Hz, H-2′), [H-arabinose part: 4.10 (1H dd, J=4.1, 11.7 Hz, H_a-5″), 4.17 (1H dd, J=2.7, 11.7 Hz, H_b-5″), 4.75 (1H, m, H-4″), 4.91 (1H, m, H-3″), 5.20 (1H br d, J=2.8 Hz, H-2″), 6.53 (1H, br s, H-1″)], [H-rhamnose part: 1.68 (3H, d, J=6.2 Hz, H_3-6‴), 4.30 (1H m, H-5‴), 4.42 (1H dd, J=8.9, 9.6 Hz, H-4‴), 4.66 (1H, dd, J=2.8, 9.6 Hz, H-3‴), 4.71 (1H, br s, H-2‴), 6.22 (1H, br s, H-1‴)]. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: see Table 1. Positive-ion FAB-MS m/z: 603 $(M+Na)^+$. HR-FAB-MS m/z: 603.1322 [Calcd for $C_{26}H_{28}O_{15}Na$ $(M+Na)^+$: 603.1326].

Cissoside III (**3**): A yellow powder, $[\alpha]_D^{26}$ −187.9° (c=0.70, MeOH). UV λ_{max} (MeOH) nm (log ε): 257 (4.25), 350 (3.93). IR (KBr) cm^{-1}: 3397, 1717, 1655, 1603, 1509, 1370, 1206, 1177, 1115, 1061. ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 6.75, 6.84 (1H each, both d, J=2.0 Hz, H-6, -8), 7.37 (1H, d, J=8.2 Hz, H-5′), 7.99 (1H, dd, J=2.0, 8.2 Hz, H-6′), 8.19 (1H, d, J=2.0 Hz, H-2′), [H-arabinose part: 1.89 (3H, s, C$\underline{H}_3$CO–), 4.42 (1H dd, J=6.9, 12.4 Hz, H_a-5″), 4.62 (1H dd, J=3.4, 12.4 Hz, H_b-5″), 4.65 (1H, m, H-3″), 4.75 (1H, m, H-4″), 5.22 (1H, br d, J=3.5 Hz, H-2″), 6.47 (1H, br s, H-1″)], [H-rhamnose part: 1.68 (3H, d, J=6.2 Hz, H_3-6‴), 4.30 (1H m, H-5‴), 4.41 (1H, dd, J=8.9, 9.6 Hz, H-4‴), 4.66 (1H, dd, J=3.4, 9.6 Hz, H-3‴), 4.71 (1H, br s, H-2‴), 6.22 (1H, br s, H-1‴)]. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: see Table 1. Positive-ion FAB-MS m/z: 645 $(M+Na)^+$. HR-FAB-MS m/z: 645.1429 [Calcd for $C_{28}H_{30}O_{16}Na$ $(M+Na)^+$: 645.1432].

Cissusin (**4**): A colorless powder. UV λ_{max} (MeOH) nm (log ε): 212 (4.37), 282 (4.06), 330 (4.38), 345 (4.39). IR (KBr) cm^{-1}: 3433, 1623, 1614, 1592, 1501, 1458, 1321, 1202, 1173, 1036, 945, 845. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.93 (3H s, OC$\underline{H}_3$-2′), 5.97 (2H, s, OC$\underline{H}_2$O-5, -6), 6.52 (1H, d, J=2.1 Hz, H-3′), 6.54 (1H, dd, J=2.1, 8.9 Hz, H-5′), 6.92 (1H, s, H-4), 6.98 (1H, s, H-7), 7.06 (1H, s, H-3), 7.81 (1H, d, J=8.9 Hz, H-6′). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: see Table 1. EI-MS m/z: 284 (M^+) (100). HR-EI-MS m/z: 284.0687 (Calcd for $C_{16}H_{12}O_5$: 284.0685).

Deacylation of 1 and 3 A solution of **1** (5.2 mg) in 0.5% NaOMe–MeOH (1.0 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form), and the resin was removed by filtration. Evaporation of the solvent under reduced pressure furnished a residue, which was purified by silica gel chromatography [0.8 g, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower layer)] to give **14** (4.1 mg, 84.7%). Using a similar procedure, **2** (3.3 mg, 78.6%) was obtained from **3** (4.5 mg).

Kaempferol-3-*O*-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**14**): A yellow powder, $[\alpha]_D^{28}$ −170.6° (c=0.80, MeOH). ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 6.80, 6.98 (1H each, both d, J=2.1 Hz, H-6, -8), 7.33, 8.40 (2H each, both d, J=8.2 Hz, H-3′, -5′, -2′, -6′), [H-arabinose part: 4.09 (1H dd, J=4.1, 11.7 Hz, H_a-5″), 4.15 (1H dd, J=2.7, 11.7 Hz, H_b-5″), 4.68 (1H, m, H-4″), 4.92 (1H, m, H-3″), 5.26 (1H, br d, J=2.8 Hz, H-2″), 6.57 (1H, br s, H-1″)], [H-rhamnose part: 1.67 (3H, d, J=6.2 Hz, H_3-6‴), 4.30 (1H m, H-5‴), 4.40 (1H dd, J=8.9, 9.6 Hz, H-4‴), 4.67 (1H, dd-like, H-3‴), 4.73 (1H, br s, H-2‴), 6.27 (1H, br s, H-1‴)]. ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: see Table 1. Positive-ion FAB-MS m/z: 587 $(M+Na)^+$. HR-FAB-MS m/z: 587.1374 [Calcd for $C_{26}H_{28}O_{14}Na$ $(M+Na)^+$: 587.1377]. The physical data agreed with published data.[8]

Acid Hydrolysis of 2 A solution of **2** (2.0 mg) in HCl–1,4-dioxane 1 M (1 : 1, v/v, 1.0 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The EtOAc layer was subjected to HPLC analysis under the following conditions: HPLC column, YMC-Pack ODS-A, (YMC, 4.6 mm i.d.×250 mm); detection, RID-6A refractive index; mobile phase, CH_3OH–H_2O (50 : 50, v/v); and flow rate, 0.8 ml/min. Identification of quercetin from **2** present in the EtOAc layer was carried out by comparison of its retention time with that of an authentic sample. t_R: 23.8 min. In addition, the aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN–H_2O (85 : 15, v/v); flow rate, 0.7 ml/min; and column temperature, room temperature. Identification of L-rhamnose (i) and L-arabinose (ii) from **2** present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R: (i) 8.7 min (negative optical rotation); (ii) 11.4 min (positive optical rotation).

Methylation of 4 with $TMSCHN_2$ A solution of **4** (3.4 mg) in MeOH (1 ml) was treated with trimethylsilyldiazomethane ($TMSCHN_2$) (10% in hexane, 0.2 ml), and the mixture was stirred at room temperature for 3 h. Removal of the solvent under reduced pressure gave a residue, which was purified using silica gel column chromatography [0.8 g, hexane–EtOAc (30 : 1, v/v)] to give **31** (2.8 mg, 78.5%).

2-(2′,4′-Dimethoxyphenyl)-5,6-methylenedioxybenzofuran (**31**): A colorless powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.86, 3.96 (3H each, both s, OC$\underline{H}_3$-4′, -2′), 5.98 (2H, s, OC$\underline{H}_2$O-5, 6), 6.55 (1H, d, J=2.1 Hz, H-3′), 6.59 (1H, dd, J=2.1, 8.6 Hz, H-5′), 6.93 (1H, s, H-4), 7.00 (1H, s, H-7), 7.09 (1H, s, H-3), 7.88 (1H, d, J=8.6 Hz, H-6′). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: see Table 1. EI-MS m/z: 298 (M^+) (100). HR-EI-MS m/z: 298.0836 (Calcd for $C_{17}H_{14}O_5$: 298.0841). The physical data agreed with published data.[20]

Inhibitory Effects on the Release of β-Hexosaminidase by RBL-2H3 Cells The inhibitory effects of the test samples on the release of β-hexosaminidase from RBL-2H3 cells (Cell No. JCRB0023, obtained from Health Science Research Resources Bank, Osaka, Japan) were evaluated using the method reported previously[37—48] with some modifications. Briefly, RBL-2H3 cells were dispensed into 48-well plates at a concentration of 4×10^4 cells/well using Eagle's minimum essential medium (MEM, Sigma) containing fetal calf serum (10%), penicillin (100 units/ml), streptomycin (100 μg/ml), and 0.45 μg/ml of anti-dinitrophenyl (DNP) IgE. The mixture was incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then, the cells were washed twice with 200 μl of Siraganian buffer [NaCl 119 mM, KCl 5 mM, $MgCl_2$ 0.4 mM, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) 25 mM, and NaOH 40 mM, pH 7.2], and incubated in 80 μl of Siraganian buffer [glucose 5.6 mM, $CaCl_2$ 1 mM, and 0.1% bovine serum albumin (BSA) were added] for an additional 10 min at 37 °C. Aliquots (10 μl) of test sample solution were added to each well and incubated for 10 min, followed by the addition of 10 μl of antigen (dinitrophenylated bovine serum albumin (DNP-BSA), final concentration 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 (40 μl) was transferred into a 96-well microplate and incubated with 40 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 2 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 using 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 0.1%).

The percentage of inhibition of the release of β-hexosaminidase by the test material was calculated using the following equation, and IC_{50} values were determined graphically:

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

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

Under these conditions, it was calculated that 10—15% of β-hexosaminidase was released from the cells in the control groups by determination of the total β-hexosaminidase activity after treatment with 0.05% Triton X-100.

To clarify whether the antiallergic effects of samples were due to the inhibition of hexosaminidase release, but not a false positive due to the inhibition of β-hexosaminidase activity, the following assay was carried out. The supernatant (36 μl) of the control group as an enzyme solution, substrate solution (40 μl), and test sample solution (4 μl) were transferred into a 96-well microplate and enzyme activity was examined as described above.

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

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