Medicinal Foodstuffs. XXXI.[1] Structures of New Aromatic Constituents and Inhibitors of Degranulation in RBL-2H3 Cells from a Japanese Folk Medicine, the Stem Bark of *Acer nikoense*

Toshio MORIKAWA, Jing TAO, Kazuho UEDA, Hisashi MATSUDA, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan.
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Four new aromatic constituents, rhododendroketoside, (−)-sakuraresinoside, acernikol, and nikoenoside, were isolated from a Japanese folk medicine, the stem bark of *Acer nikoense* MAXIM. The structures of the new constituents were determined on the basis of chemical and physicochemical evidence. The principle cyclic diarylheptanoids were found to show inhibitory effects on the release of β-hexosaminidase in RBL-2H3 cells.

Key words *Acer nikoense*; degranulation inhibitor; cyclic diarylheptanoid; rhododendroketoside; (−)-sakuraresinoside; nikoenol

The Aceraceae plant *Acer nikoense* MAXIM is indigenous to Japan (Japanese name, *megusurinoki*) and its stem bark has been used as a folk medicine for the treatment of hepatic disorders and eye disease. Recently, the stem bark of this plant has been consumed as a health food in Japan. Among the chemical constituents of this folk medicine, diarylheptanoids and three phenolic compounds were characterized.[2—5] In addition, the methanolic extract was reported to have hepatoprotective effects and (+)-rhododendrol (**15**) was isolated as the active principle.[2,6]

During the course of our studies on bioactive constituents of natural medicines[7—13] and medicinal foodstuffs,[14—19] we have reported three new cyclic diarylheptanoids called aceroside B_1 (**5**) and B_2 (**6**) and aceroketoside (**7**) from the stem bark of *A. nikoense* as well as the inhibitory effects of the principal diarylheptanoids on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophages.[1]

As a continuation of the characterization studies on bioactive constituents of the stem bark of *A. nikoense*, we have isolated four new aromatic compounds called rhododendroketoside (**1**), (−)-sakuraresinoside (**2**), acernikol (**3**), and nikoenoside (**4**) together with six known compounds (**9**, **14**, **17**, **27**—**29**). In this paper, we describe the isolation and structure elucidation of new aromatic constituents (**1**—**4**) and the inhibitory effects of the principal constituents (**8**, **10**—**13**, **15**, **20**—**25**, **31**) on the release of β-hexosaminidase in RBL-2H3 cells.

The methanolic extract from the stem bark of *A. nikoense* collected in Miyagi prefecture, Japan, was partitioned into an ethyl acetate (EtOAc)–water mixture to furnish the EtOAc-soluble portion and aqueous phase. The aqueous phase was extracted with *n*-butanol (*n*-BuOH) to give the *n*-BuOH-soluble and H_2O-soluble potions as previously described.[1] The *n*-BuOH-soluble portion was subjected to normal-phase silica gel column chromatography to provide six fractions. Previously, three then new cyclic diarylheptanoids (**5**—**7**) and 20 known compounds (**8**, **10**—**13**, **15**, **16**, **18**—**26**, **30**—**33**) were isolated from the EtOAc-soluble portion and fractions 2 and 3 of the *n*-BuOH-soluble portion.[1] In the present experiments, the fractions of the *n*-BuOH-soluble portion were further separated by normal-phase and reverse-phase silica gel column chromatography and repeated HPLC to give rhododendroketoside (**1**, 0.0009%), (−)-sakuraresinoside (**2**, 0.0002%), acernikol (**3**, 0.0020%), and nikoenoside (**4**, 0.0015%) together with acerosides III (**9**,[20] 0.0075%) and VIII (**14**,[21] 0.0038%), apiosylepirhododendrin (**17**,[20] 0.027%), kelampayoside A (**27**,[22] 0.0011%), 1-[α-L-rhamnosyl(1→6)-β-D-glucopyranosyloxy]-3,4,5-trimethoxybenzene (**28**,[23] 0.0010%), and benzyl-*O*-α-L-rhamnosyl(1→6)-β-D-glucopyranoside (**29**,[24] 0.0003%). Among them, compounds **18**, **19**, **21**, and **25**—**33** were first isolated from this plant.

Stereostructures of Rhododendroketoside (1), (−)-Sakuraresinoside (2), Acernikol (3), and Nikoenoside (4) Rhododendroketoside (**1**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{22}$ −84.6°). The IR spectrum of **1** showed absorption bands at 1736, 1597, 1516, and 1460 cm^{-1} ascribable to carbonyl and aromatic functions and strong absorption bands at 3380 and 1043 cm^{-1} suggestive of its oligoglycosidic structure. The UV spectrum of **1** showed absorption maxima at 223 (log ε 3.90) and 279 (3.26) nm. In the negative-ion fast atom bombardment (FAB)-MS of **1**, a quasimolecular ion peak was observed at *m/z* 457 $(M-H)^-$ in addition to a fragment ion peak at *m/z* 165 $(M-C_{11}H_{17}O_9)^-$, which was derived by cleavage of the glycoside linkage at the 1′-position, while the positive-ion FAB-MS showed a quasimolecular ion peak at *m/z* 481 $(M+Na)^+$.

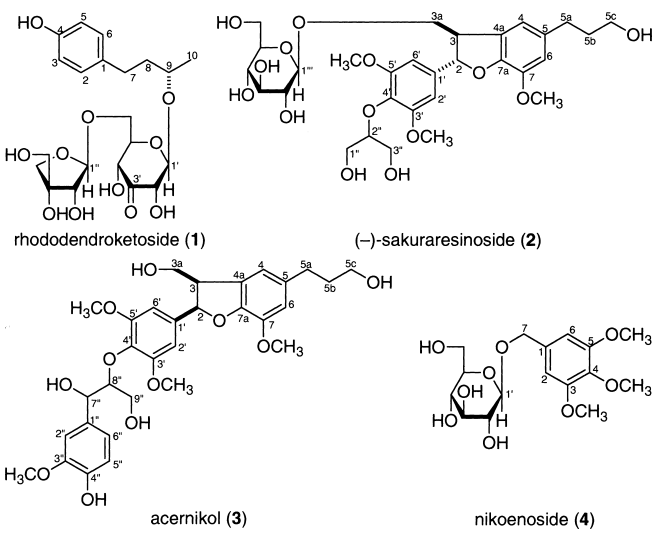

Chart 1

* To whom correspondence should be addressed. e-mail: shoyaku@mb.kyoto-phu.ac.jp

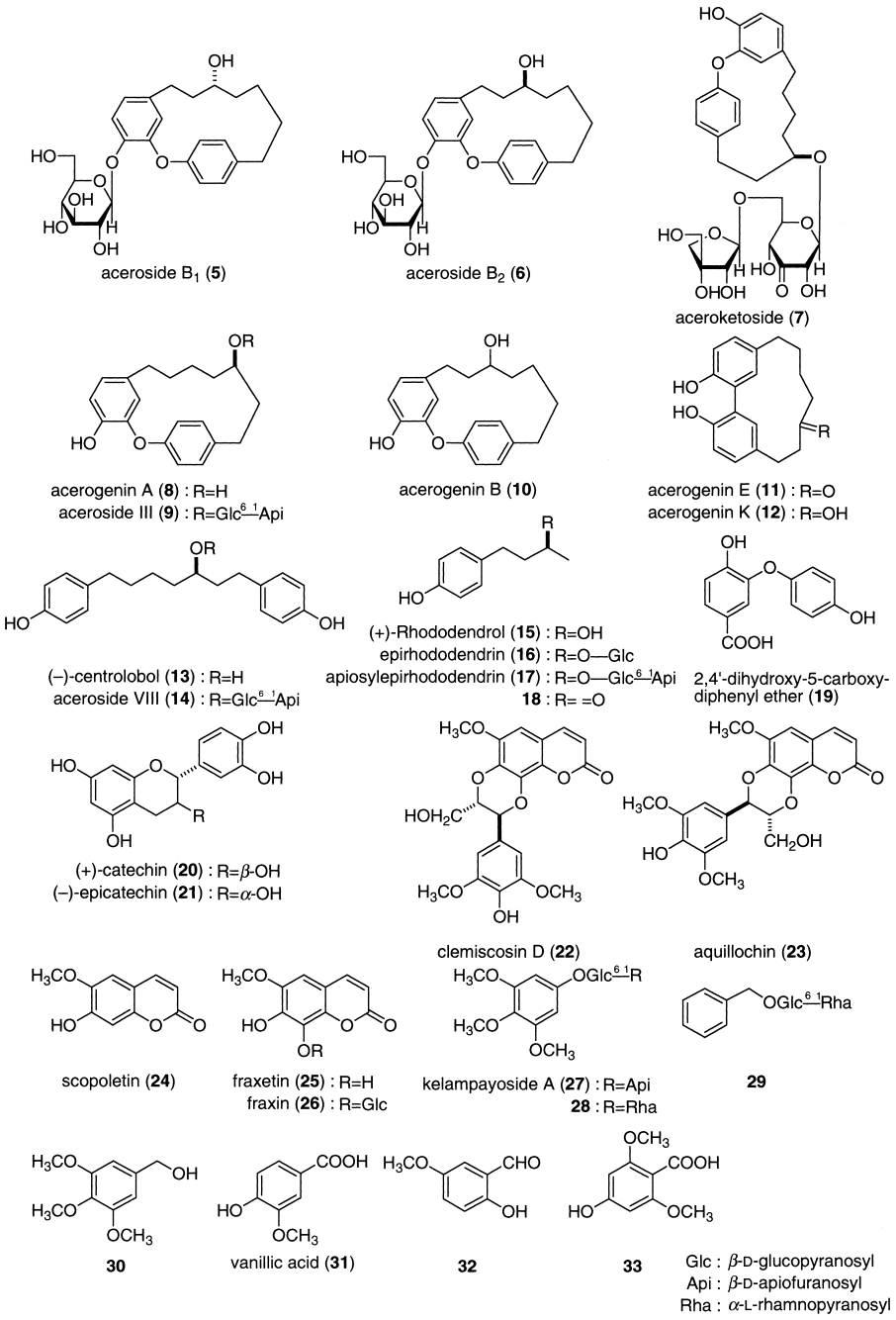

Chart 2

The molecular formula $C_{21}H_{30}O_{11}$ of **1** was determined from the quasimolecular ion peaks observed in the FAB-MS and by high-resolution FAB-MS measurement.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[25] of **1** showed signals assignable to a methyl [δ 1.45 (3H, d, J=6.1 Hz, 10-H_3)], two methylenes [δ 1.82, 2.04 (1H each, both m, 8-H_2), 2.86 (2H, m, 7-H_2)], a methine bearing an oxygen function [δ 4.06 (1H, m, 9-H)], an apiofuranosyl part [δ 5.80 (1H, d, J=2.2 Hz, 1″-H)], a ketohexopyranosyl part [δ 3.97 (1H, m, 5′-H), 4.25, 4.65 (1H each, both dd, J=5.8, 9.5 Hz, 6′-H_2), 4.69 (1H, d, J=7.5 Hz, 2′-H), 4.70 (1H, d-like, 4′-H), 4.91 (1H, d, J=7.5 Hz, 1′-H)], and a *p*-substituted benzene ring [δ 7.12 (2H, d, J=8.2 Hz, 3, 5-H), 7.22 (1H, d, J=8.2 Hz, 2, 6-H)]. The proton and carbon signals due to the aglycon moiety in the ^{1}H- and ^{13}C-NMR spectra of **1** were superimposable on those of (+)-rhododendrol glycosides, epirhododendrin (**16**),[1,26] and apiosylepirhododendrin (**17**),[20] whereas the proton and carbon signals due to the diglycoside part were very similar to those of aceroketoside (**7**).[1] Enzymatic hydrolysis of **1** with naringinase furnished (+)-rhododendrol (**15**). The position of the ketone function in **1** was clarified by heteronuclear multiple-bond correlation (HMBC) experiments on **1**, which showed long-range correlations between the 1′, 2′, 4′, and 5′-protons and the 3′-carbon (Fig. 1). Furthermore, reduction of **1** with sodium borohydride ($NaBH_4$) in MeOH yielded a mixture of **17** and its 3′-epimer, which was subjected to acid hydrolysis with 1 M HCl to liberate D-glucose, D-allose, and D-apiose. These monosaccharides were identified by HPLC analysis using an optical rotation detector.[1,9,27] On the basis of this

Table 1. ^{13}C-NMR Data for Rhododendroketoside (**1**), (−)-Sakuraresinoside (**2**), Acernikol (**3**), Nikoenoside (**4**), and (−)-Sakuraresinol (**2a**)

	1[a]	**4**[b]		**2**[b]	**2a**[b]	**3**[b]
C-1	133.2	134.9	C-2	89.0	88.6	88.6
C-2	130.0	106.1	C-3	53.2	55.8	55.8
C-3	116.2	154.1	C-3a	72.3	65.2	65.1
C-4	157.0	138.1	C-4	118.2	117.9	118.0
C-5	116.2	154.1	C-4a	129.2	129.6	129.6
C-6	130.0	106.1	C-5	137.3	137.3	137.2
C-7	30.9	71.3	C-5a	32.9	32.9	32.9
C-8	39.6		C-5b	35.8	35.8	35.8
C-9	76.8		C-5c	62.2	62.2	62.3
C-10	22.3		C-6	114.3	114.3	114.3
			C-7	145.3	145.3	145.3
C-1′	105.7	102.8	C-7a	147.5	147.5	147.5
C-2′	78.7	75.0				
C-3′	207.4	77.9	C-1′	139.8	139.9	139.8
C-4′	74.2	71.3	C-2′	104.0	103.9	104.0
C-5′	76.9	77.9	C-3′	154.7	154.7	154.7
C-6′	68.7	62.7	C-4′	136.3	136.5	136.3
			C-5′	154.7	154.7	154.7
C-1″	111.3		C-6′	104.0	103.9	104.0
C-2″	77.8					
C-3″	80.5		C-1″	62.2	62.2	133.8
C-4″	75.2		C-2″	84.8	84.9	111.5
C-5″	65.5		C-3″	62.2	62.2	148.7
			C-4″			146.7
3-OCH$_3$		56.5	C-5″			115.7
4-OCH$_3$		60.1	C-6″			120.7
5-OCH$_3$		56.5	C-7″			74.1
			C-8″			87.4
			C-9″			61.7
			C-1‴	104.1		
			C-2‴	75.2		
			C-3‴	78.3		
			C-4‴	71.1		
			C-5‴	78.1		
			C-6‴	62.6		
			7-OCH$_3$	56.9	56.9	56.9
			3′-OCH$_3$	56.8	56.7	56.7
			5′-OCH$_3$	56.8	56.7	56.7
			3″-OCH$_3$			56.4

Measured in *a*) pyridine-d_5 and *b*) CD_3OD at 125 MHz.

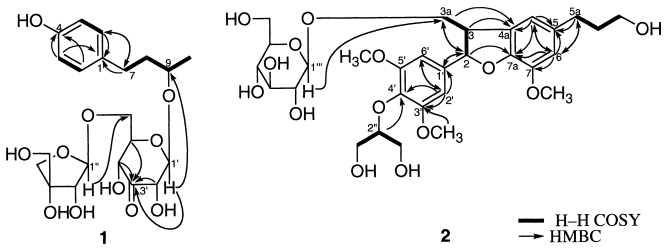

Fig. 1

evidence, the structure of rhododendroketoside was determined to be the 3′-keto derivative (**1**) of **17**.

(−)-Sakuraresinoside (**2**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −8.8°). The IR spectrum of **2** showed absorption bands at 3453, 1595, 1508, and 1032 cm^{-1} ascribable to glycosidic and aromatic functions, while its UV spectrum showed absorption maxima at 235 (sh, log ε 4.17), and 281 (3.58) nm. The molecular formula $C_{30}H_{42}O_{14}$ of **2** was characterized from the negative- and positive-ion FAB-MS and by high-resolution MS measurement. The negative-ion FAB-MS of **2** showed a quasimolecular ion peak at *m/z* 625 $(M-H)^-$ and a fragment ion peak at *m/z* 463 $(M-C_6H_{11}O_5)^-$, while a quasimolecular ion peak was observed at *m/z* 649 $(M+Na)^+$ in the positive-ion FAB-MS. Acid hydrolysis of **2** liberated D-glucose,[1,9,27] whereas (−)-sakuraresinol (**2a**)[28,29] was obtained by enzymatic hydrolysis of **2** with β-glucosidase. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[25] of **2** indicated the presence of a (−)-sakuraresinol moiety [δ 1.81 (2H, m, 5b-H$_2$), 2.62 (2H, t, J=6.4 Hz, 5a-H$_2$), 3.55 (2H, t, J=6.4 Hz, 5c-H$_2$), 3.67 (1H, m, 3-H), 3.74 (4H, dd, J=1.2, 4.9 Hz, 1″, 3″-H$_2$), 3.82 (6H, s, 3′, 5′-OCH$_3$), 3.87 (3H, s, 7-OCH$_3$), 3.92, 4.09 (1H each, both m, 3a-H$_2$), 3.99 (1H, t, J=4.9 Hz, 2″-H), 5.68 (1H, d, J=5.8 Hz, 2-H), 6.74 (1H, br s, 6-H), 6.76 (1H, br s, 4-H), 6.78 (2H, br s, 2′, 6′-H)], and a β-D-glucopyranosyl part [δ 4.39 (1H, d, J=7.9 Hz, 1‴-H)]. By comparison of the carbon signals in the ^{13}C-NMR spectrum of **2** with those of **2a**, a glycosylation shift was observed around the 3a-position of **2**. The position of the glucoside linkage was confirmed by HMBC experiments, which showed long-range correlations between the 1‴-proton and 3a-carbon, and between the 3a-proton and 1‴-carbon. Consequently, the structure of (−)-sakuraresinoside was determined to be sakuraresinol 3a-*O*-β-D-glucopyranoside (**2**). In addition, the absolute stereostructures of **2** and **2a** were determined by circular dichronic (CD) spectroscopic analysis. Thus, the CD spectra of **2** and **2a** showed a negative Cotton effect [**2**: $\Delta\varepsilon$ −0.89 (223 nm), **2a**: $\Delta\varepsilon$ −0.71 (225 nm)], which indicated the absolute configuration of the 2-position to be in the *S* orientation, respectively.[30] On the basis of this evidence, the absolute stereostructures of **2** and **2a** were elucidated as shown.

Acernikol (**3**) was also obtained as a white powder with positive optical rotation ($[\alpha]_D^{24}$ +4.7°). The IR spectrum of **3** showed absorption bands at 3431, 1610, 1508, and 1038 cm^{-1} ascribable to hydroxyl and aromatic functions. In the UV spectrum of **3**, absorption maxima were observed at 235 (sh, log ε 4.33), and 281 (3.77) nm. The IR and UV spectra of **3** resembled those of **2** and **2a**. The electron impact (EI)-MS of **3** showed a molecular ion peak at *m/z* 586 (M^+) and high-resolution MS analysis revealed the molecular formula of **3** to be $C_{31}H_{38}O_{11}$. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[25] of **3** showed signals assignable to two methylenes [δ 1.81 (2H, m, 5b-H$_2$), 2.62 (2H, t, J=7.3 Hz, 5a-H$_2$)], a methine [δ 3.45 (1H, m, 3-H)], three methylenes and three methines bearing an oxygen function [δ 3.56 (2H, m, 5c-H$_2$), 3.58, 3.90 (1H each, both m, 9″-H$_2$), 3.75, 3.81 (1H each, both m, 3a-H$_2$), 4.23 (1H, m, 8″-H), 4.89 (1H, d, J=4.9 Hz, 7″-H), 5.54 (1H, d, J=6.1 Hz, 2-H)], four methoxyls [δ 3.77 (6H, s, 3′, 5′-OCH$_3$), 3.80 (3H, s, 3″-OCH$_3$), 3.87 (3H, 7-OCH$_3$)], and seven aromatic protons [δ 6.70 (2H, d, J=2.0 Hz, 2′, 6′-H), 6.73 (1H, d, J=2.0 Hz, 4-H), 6.74 (1H, d, J=8.0 Hz, 5″-H), 6.75 (1H, d, J=2.0 Hz, 6-H), 6.77 (1H, d, J=2.0, 8.0 Hz, 6″-H), 6.96 (1H, d, J=2.0 Hz, 2″-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were superimposable on those of dihydrobuddlenol B (**3a**),[28] except for the signals due to the dihydrofuran ring. The planar structure of **3** was confirmed by ^{1}H–^{1}H correlation spectroscopy (H–H COSY) and HMBC experiments, as shown in Fig. 2. The relative stereostructure of the dihydrofuran ring in **3** was clarified by difference nuclear Overhauser effect spectroscopy experiments, which

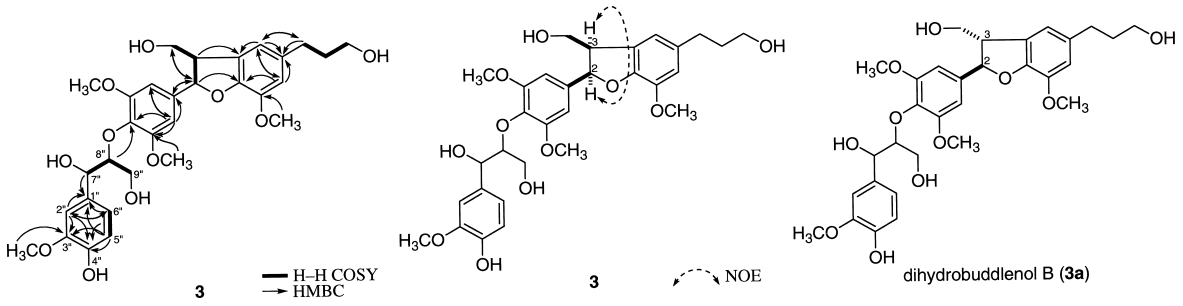

Fig. 2

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

	Inhibition (%)			
	0 μM	10 μM	30 μM	100 μM
Acerogenin A (**8**)	0.0±1.5	2.3±1.7	11.2±3.1**	40.0±1.1**
Acerogenin B (**10**)	0.0±2.4	5.6±2.5	34.9±2.2**	86.5±1.2**
Acerogenin E (**11**)	0.0±2.0	9.9±2.5	23.6±1.0**	47.9±1.0**
Acerogenin K (**12**)	0.0±1.0	19.8±1.6**	48.1±2.0**	87.5±0.8**
(−)-Centrolobol (**13**)	0.0±5.0	−9.8±2.8	−21.1±4.8	−17.2±3.8
(+)-Rhododendrol (**15**)	0.0±1.0	8.9±1.4	0.9±3.1	2.2±0.5
(+)-Catechin (**20**)	0.0±1.6	5.3±3.3	0.1±2.0	2.1±2.5
(−)-Epicatechin (**21**)	0.0±1.5	3.1±1.3	4.5±1.9	−3.9±1.2
Clemiscosin D (**22**)	0.0±1.0	5.7±3.6	17.8±2.3	1.1±3.5
Aquillochin (**23**)	0.0±2.5	0.3±1.7	2.3±2.0	−1.7±3.3
Scopoletin (**24**)	0.0±3.0	0.5±3.4	−4.7±5.0	1.9±3.4
Fraxin (**25**)	0.0±2.8	1.7±4.1	−4.9±3.9	−7.7±4.4
Vanillic acid (**31**)	0.0±1.5	3.7±3.9	12.7±2.6	12.1±1.5

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

showed a nuclear Overhauser effect correlation between the 2- and 3-proton pair. This evidence led us to clarify the structure of acernikol to be the *cis*-type isomer (**3**) of **3a** at the dihydrofuran ring. Moreover, the CD spectrum of **3** showed a positive Cotton effect at 220 nm ($\Delta\varepsilon$ +0.41), which indicated the absolute configuration of the 2-position of **3** to be in the *R* orientation.[30] On the basis of this evidence, the absolute stereostructure of the 2- and 3-positions of **3** was elucidated as shown.

Nikoenoside (**4**), obtained as a white powder with negative optical rotation ($[\alpha]_D^{24}$ −57.7°), showed an absorption maximum at 271 (log ε 3.06) nm in the UV spectrum. The IR spectrum of **4** showed absorption bands at 3453, 1595, 1508, and 1032 cm^{-1}, suggestive of glycosidic and aromatic functions. The molecular formula $C_{16}H_{24}O_9$ of **4** was determined by a quasimolecular ion peak [*m/z* 359 $(M-H)^-$ and *m/z* 383 $(M+Na)^+$] in the negative- and positive-ion FAB-MS and by high-resolution MS analysis. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[25] of **4** indicated the presence of a 3,4,5-trimethoxylbenzyl moiety [δ 3.74 (3H, 4-OCH_3), 3.83 (6H, s, 3, 5-OCH_3), 4.64, 4.84 (1H each, both d, J=13.2 Hz, 7-H_2), 6.76 (2H, br s, 2, 6-H)] and a β-D-glucopyranosyl part [δ 4.33 (1H, d, J=7.4 Hz, 1′-H)]. Acid hydrolysis of **4** liberated D-glucose,[1,9,27] while 3,4,5-trimethoxybenzyl alcohol (**30**)[1] was obtained by enzymatic hydrolysis of **4** with β-glucosidase. On the basis of these findings, the structure of nikoenoside (**4**) was elucidated to be 3,4,5-trimethoxybenzyl β-D-glucopyranoside.

Inhibitory Effect of Constituents from *A. nikoense* on the Release of β-Hexosaminidase in 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.[31,32] 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,[8] diterpenes,[33] and flavonoids.[34] In our continuous search for antiallergic principles from natural sources, we examined the effects of constituents from the stem bark of *A. nikonense* on the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP IgE (Table 2). As a result, the biphenyl-type diarylheptanoids acerogenins B (**10**, IC_{50}=50 μM) and K (**12**, 33 μM) showed inhibitory activity, and their activities were stronger than those of two antiallergic compounds, tranilast (0.49 mM) and ketotifen fumarate (0.22 mM).[33]

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu 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, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index detector.

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.

Extraction and Isolation Fraction 2-2 (566 mg) obtained from the *n*-BuOH-soluble portion of the dried stem bark of *A. nikoense* (8.5 kg) and isolated (+)-rhododendrol (**15**), 4-(4-hydroxyphenyl)-2-butanone (**18**), and scopoletin (**24**) as reported previously,[1] was further separated by HPLC [MeOH–H_2O (45 : 55, v/v)] to furnish acernikol (**3**, 12 mg, 0.0020%). Fraction 3-3 (720 mg), from which epirhododendrin (**16**), (+)-catechin (**20**), (−)-epicatechin (**21**), and fraxin (**26**) were previously isolated,[1] was subjected to HPLC [MeOH–H_2O (30 : 70, v/v)] to give nikoenoside (**4**, 77 mg, 0.0015%). Fraction 4 (28.0 g) was subjected to reverse-phase silica gel column chromatography [540 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give 9 fractions [fr. 4-1 (2.39 g), 4-2 (0.46 g), 4-3 (0.28 g), 4-4 (1.83 g), 4-5 (0.87 g), 4-6 (0.79 g), 4-7 (0.41 g), 4-8 (1.13 g), 4-9 (19.84 g)]. Fraction 4-2 (460 mg) was further purified by HPLC [MeOH–H_2O (25 : 75, v/v)] to give kelampayoside A (**27**, 56 mg, 0.0011%), 1-[α-L-rhamnosyl-(1→6)-β-D-glucopyranosyl)-3,4,5-trimethoxybenzene (**28**, 54 mg, 0.0010%), and benzyl-*O*-α-L-rhamnosyl(1→6)-β-D-glucopyranoside (**29**, 13 mg, 0.0003%). Fraction 4-3 (280 mg) was subjected by HPLC [MeOH–H_2O (30 : 70, v/v)] to furnish rhododendroketoside (**1**, 22 mg, 0.0009%) and apiosylepirhododendrin (**17**, 762 mg, 0.027%). Fraction 4-4 (1.00 g) was separated by HPLC [MeOH–H_2O (30 : 70, v/v)] to give (−)-sakuraresinoside (**2**, 13 mg, 0.0002%). Fraction 4-8 (560 mg) was further purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to give acerosides III (**9**, 192 mg, 0.0075%) and VIII (**14**, 97 mg, 0.0038%).

The known constituents were identified by comparison of their physical data (IR, UV, and ^{1}H- and ^{13}C-NMR) with reported values.[20—24]

Rhododendroketoside (**1**): A white powder, $[\alpha]_D^{22}$ −84.6° (c=0.20, EtOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{30}O_{11}Na$ $(M+Na)^+$ 481.1686; Found 481.1692. UV [EtOH, nm (log ε)]: 223 (3.90), 279 (3.26). IR (KBr): 3380, 2934, 1736, 1597, 1516, 1460, 1043 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.45 (3H, d, J=6.1 Hz, 10-H_3), 1.82, 2.04 (1H each, both m, 8-H_2), 2.86 (2H, m, 7-H_2), 3.97 (1H, m, 5′-H), 4.06 (1H, m, 9-H), 4.25, 4.65 (1H each, both dd, J=5.8, 9.5 Hz, 6′-H_2), 4.69 (1H, d, J=7.5 Hz, 2′-H), 4.70 (1H, d-like, 4′-H), 4.91 (1H, d, J=7.5 Hz, 1′-H), 5.80 (1H, d, J=2.2 Hz, 1″-H), 7.12 (2H, d, J=8.2 Hz, 3,5-H), 7.22 (1H, d, J=8.2 Hz, 2,6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 481 $(M+Na)^+$. Negative-ion FAB-MS: m/z 457 $(M-H)^-$, 165 $(M-C_{11}H_{17}O_9)^-$.

(−)-Sakuraresinoside (**2**): A white powder, $[\alpha]_D^{22}$ −8.8° (c=0.20, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{42}O_{14}Na$ $(M+Na)^+$ 649.2472; Found 649.2481. CD [MeOH, nm ($\Delta\varepsilon$)]: 223 (−0.89), 246 (+0.85). UV [EtOH, nm (log ε)]: 235 (sh, 4.17), 281 (3.58). IR (KBr): 3453, 2936, 1595, 1508, 1466, 1327, 1032 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.81 (2H, m, 5b-H_2), 2.62 (2H, t, J=6.4 Hz, 5a-H_2), 3.55 (2H, t, J=6.4 Hz, 5c-H_2), 3.67 (1H, m, 3-H), 3.74 (4H, dd, J=1.2, 4.9 Hz, 1″, 3″-H_2), 3.82 (6H, s, 3′,5′-OCH_3), 3.87 (3H, s, 7-OCH_3), 3.92, 4.09 (1H each, both m, 3a-H_2), 3.99 (1H, t, J=4.9 Hz, 2″-H), 4.39 (1H, d, J=7.9 Hz, 1‴-H), 5.68 (1H, d, J=5.8 Hz, 2-H), 6.74 (1H, br s, 6-H), 6.76 (1H, br s, 4-H), 6.78 (2H, br s, 2′,6′-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 649 $(M+Na)^+$. Negative-ion FAB-MS: m/z 625 $(M-H)^-$, 463 $(M-C_6H_{11}O_5)^-$.

Acernikol (**3**): A white powder, $[\alpha]_D^{22}$ +4.7° (c=0.20, MeOH). High-resolution EI-MS: Calcd for $C_{31}H_{38}O_{11}$ $(M)^+$ 586.2414; Found 586.2411. CD [MeOH, nm ($\Delta\varepsilon$)]: 220 (+0.41), 244 (−0.46). UV [EtOH, nm (log ε)]: 235 (sh, 4.33), 281 (3.77). IR (KBr): 3431, 2961, 1610, 1508, 1465, 1337, 1038 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.81 (2H, m, 5b-H_2), 2.62 (2H, t, J=7.3 Hz, 5a-H_2), 3.45 (1H, m, 3-H), 3.56 (2H, m, 5c-H_2), 3.58, 3.90 (1H each, both m, 9″-H_2), 3.75, 3.81 (1H each, both m, 3a-H_2), 3.77 (6H, s, 3′,5′-OCH_3), 3.80 (3H, s, 3″-OCH_3), 3.87 (3H, 7-OCH_3), 4.23 (1H, m, 8″-H), 4.89 (1H, d, J=4.9 Hz, 7″-H), 5.54 (1H, d, J=6.1 Hz, 2-H), 6.70 (2H, d, J=2.0 Hz, 2′,6′-H), 6.73 (1H, d, J=2.0 Hz, 4-H), 6.74 (1H, d, J=8.0 Hz, 5″-H), 6.75 (1H, d, J=2.0 Hz, 6-H), 6.77 (1H, d, J=2.0, 8.0 Hz, 6″-H), 6.96 (1H, d, J=2.0 Hz, 2″-H). ^{13}C-NMR (125 MHz CD_3OD) δ_C: given in Table 1. EI-MS: m/z 586 (M^+, 13), 137 (100).

Nikoenoside (**4**): A white powder, $[\alpha]_D^{22}$ −57.7° (c=0.20, EtOH). High-resolution positive-ion FAB-MS: Calcd for $C_{16}H_{24}O_9Na$ $(M+Na)^+$ 383.1318; Found 383.1327. UV [EtOH, nm (log ε)]: 271 (3.06). IR (KBr): 3453, 2936, 1595, 1508, 1466, 1327, 1032 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.74 (3H, 4-OCH_3), 3.83 (6H, s, 3,5-OCH_3), 4.33 (1H, d, J=7.4 Hz, 1′-H), 4.64, 4.84 (1H each, both d, J=13.2 Hz, 7-H_2), 6.76 (2H, br s, 2,6-H). ^{13}C-NMR (125 MHz CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 383 $(M+Na)^+$. Negative-ion FAB-MS: m/z 359 $(M-H)^-$.

$NaBH_4$ Reduction and Acid Hydrolysis of Rhododendroside (1) A solution of **1** (10 mg) in MeOH (1.0 ml) was treated with $NaBH_4$ (2.0 mg) and the mixture was stirred at room temperature for 1 h. The reaction mixture was quenched in acetone, and then removal of the solvent under reduced pressure yielded a reduction mixture. Apiosylepirhododendrin (**17**)[20] was detected in the reduction mixture by TLC analysis [solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer)]. Then a solution of the reduction mixture in 1 M HCl (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into icewater and neutralized with Amberlite IRA-400 (OH^- form), and the residue was removed by filtration. Then the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Shodex Asahipak NH-2P-50-4E, 4.6 mm i.d.×250 mm (Showa Denko Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN–H_2O (75 : 25, v/v); flow rate 0.8 ml/min; and column temperature, room temperature. Identification of D-apiose,[1] D-allose, and D-glucose present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R: 6.4 min (D-apiose, positive optical rotation), 9.7 min (D-allose, positive optical rotation), and 11.1 min (D-glucose, positive optical rotation).

Enzymatic Hydrolysis of Rhododendroside (1) A solution of **1** (4.9 mg) in 0.2 M acetate buffer (pH 4.0, 2.0 ml) was treated with naringinase (10 mg from *Penicillium decumbens*, Sigma Chemical Co., Ltd., St. Louis, MO, U.S.A.) and the mixture was stirred at 40 °C for 4 d. After the addition of EtOH to the reaction mixture, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography [0.5 g, $CHCl_3$–MeOH–H_2O (20 : 3 : 1, lower layer)] to give (+)-rhododendrol (**15**,[1] 1.3 mg, 73%).

Acid Hydrolysis of (−)-Sakuraresinoside (2) and Nikoenoside (4) A solution of **2** or **4** (5 mg each) in 1 M HCl (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into icewater and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Shodex Asahipak NH-2P-50-4E, 4.6 mm i.d.×250 mm; detection, optical rotation; mobile phase, CH_3CN–H_2O (75 : 25, v/v); flow rate 0.8 ml/min; and column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of authentic sample. t_R: 11.1 min (D-glucose, positive optical rotation).

Enzymatic Hydrolysis of (−)-Sakuraresinoside (2) and Nikoenoside (4) A solution of **2** (5.6 mg) or **4** (15.0 mg) in 0.2 M acetate buffer (pH 4.4, 2.0 ml) was treated with β-glucosidase (10 mg from almond, Oriental Yeast Co., Ltd., Tokyo, Japan), and the mixture was stirred at 38 °C for 4 h. After the addition of EtOH to the reaction mixture, the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography [0.5 g, *n*-hexane–EtOAc (1 : 1, v/v)] to give (−)-sakuraresinol (**2a**,[28] 3.4 mg, 82%) or 3,4,5-trimethoxybenzyl alcohol (**30**,[1] 8.0 mg, 97%).

(−)-Sakuraresinol (**2a**): A white powder, $[\alpha]_D^{26}$ −14.1° (c=0.20, MeOH). High-resolution positive-ion EI-MS: Calcd for $C_{24}H_{32}O_9$ (M^+) 464.2047; Found 464.2046. CD [MeOH, nm ($\Delta\varepsilon$)]: 225 (−0.71), 247 (+0.53). UV [EtOH, nm (log ε)]: 240 (sh, 3.90), 281 (3.20). IR (KBr): 3389, 2961, 1602, 1508, 1465, 1422, 1333, 1217 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.81 (2H, m, 5b-H_2), 2.63 (2H, m, 5a-H_2), 3.47 (1H, m, 3-H), 3.56 (2H, m, 5c-H_2), 3.74 (4H, d, J=5.2 Hz, 1″,3″-H_2), 3.76, 3.83 (1H each, both m, 3a-H_2), 3.82 (6H, s, 3′,5′-OCH_3), 3.88 (3H, s, 7-OCH_3), 3.99 (1H, t, J=5.2 Hz, 2″-H), 5.56 (1H, d, J=5.8 Hz, 2-H), 6.72, 6.76 (1H each, both br s, 4,6-H), 6.74 (2H, br s, 2′,6′-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 1. EI-MS: m/z 464 (M^+, 51), 446 (M^+-H_2O, 19), 372 ($M^+-C_3H_8O_3$, 100).

Bioassay

Inhibitory Effect on the Release of β-Hexosaminidase in RBL-2H3 Cells Inhibitory effects of 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 by the method reported previously.[8,33,34] Briefly, RBL-2H3 cells were dispensed into 24-well plates at a concentration of 2×10^5 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-DNP IgE, and these were incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then the 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), and 40 mM NaOH, pH 7.2], then incubated in 160 μl of siraganian buffer [5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% bovine serum albmin (BSA) were added] for an additional 10 min at 37 °C. Then aliquots of 20 μl of test sample solution were 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 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 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 percent inhibition of the release of β-hexosaminidase by the test material was calculated using the following equation, and IC_{50} values were determined graphically:

$$\text{inhibition (\%)} = \left(1 - \frac{T - B - N}{C - N}\right) \times 100$$

Control (*C*): DNP-BSA (+), test sample (−); Test (*T*): DNP-BSA (+), test sample (+); Blank (*B*): DNP-BSA (−), test sample (+); Normal (*N*): DNP-BSA (−), test sample (−).

Under these conditions, 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 mean±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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