Dammarane-Type Glycosides from *Gynostemma pentaphyllum* and Their Effects on IL-4-Induced Eotaxin Expression in Human Bronchial Epithelial Cells

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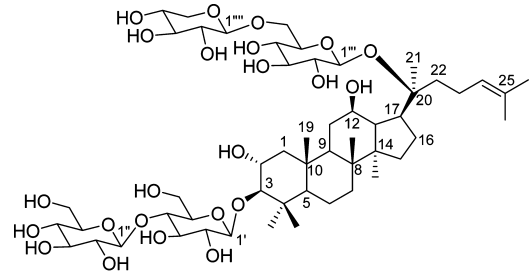
Two new dammarane-type glycosides, 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (**1**) and 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene-3-*O*-β-D-glucopyranosyl-20-*O*-[β-D-6-*O*-acetylglucopyranosyl-(1→2)-β-D-glucopyranoside] (**2**), were isolated from a MeOH extract of the leaves of *Gynostemma pentaphyllum*. Their structures were elucidated by 1D and 2D NMR spectroscopic interpretation as well as by chemical studies. The isolated compounds showed potential inhibitory effects on eotaxin expression in BEAS-2B bronchial epithelial cells.

Asthma is defined as an obstructive disease of the pulmonary airways resulting from spasms of airway muscle, increased mucus secretion, and inflammation. The interaction between bronchial epithelial cells and eosinophils is an important feature of an asthma attack. Most evidence suggests that eosinophilic infiltration and activation may account for the spasmodic and cyclic nature of hyper-reactive airways.[1] The mechanisms underlying the selective recruitment of eosinophils are complex and include multistep processes, probably mediated by the cooperative action between cytokines that cause eosinophil priming and increase survival, interleukin [IL]-3 and IL-5 and granulocyte macrophage colony stimulating factor, and those that activate the endothelium IL-1, tumor necrosis factor (TNF)-α, IL-4, IL-13, and eosinophil-selective chemoattractant molecules, especially C−C chemokines.[2] Eotaxin is a C−C chemokine implicated in the recruitment of eosinophils in a variety of inflammatory disorders and, unlike all other eosinophil chemoattractants, is eosinophil-specific.[3] This characterizes eotaxin as a key mediator in allergic diseases of which eosinophilic infiltration is characteristic.[3,4] Cultured bronchial epithelial cells, including the normal bronchial epithelium, have been observed to produce eotaxin after stimulation with TNF-α and T helper (Th) type 2 cytokines.[5]

Gynostemma belongs to the family Cucurbitaceae, and plants in this genus are known in mainland China as "xiancao", or "herb of immortality".[6] They have been used in folk medicine to lower cholesterol levels, regulate blood pressure, strengthen the immune system, and reduce inflammation.[7] *Gynostemma* species taste sweet and aromatic and can be taken either as a tea or with alcohol. Previous investigations of this genus have shown the occurrence of dammarane-type glycosides called the gypenosides, which are structurally related to ginseng saponins[8] and exhibit various biological activities.[9] Certain gypenosides have been reported to inhibit the proliferation of Hep-3B and HA22T cells, by affecting calcium and sodium currents in a dose-dependent manner.[10] In Vietnam, *Gynostemma pentaphyllum* is used as a traditional tea due to its sweetness, has efficacy in the treatment of elevated cholesterol, and exhibited antitumor, antioxidant, antidiabetes, and hypoglycemic effects,[11] as reported in a number of studies.[9,12] Recently, the effect exerted by *G. pentaphyllum* extract and its dammarane constituents on PTP1B has been studied.[13] However, no data are available on the influence of the components on eotaxin expression in BEAS-2B bronchial epithelial cells in vitro. In the present study, we report the isolation and structure elucidation of two new dammarane-type glycosides (**1** and **2**). In addition, the ability of these gypenosides to suppress eotaxin expression stimulated by the Th2 cytokine IL-4 alone and/or in combination with TNF-α in BEAS-2B cells was determined.



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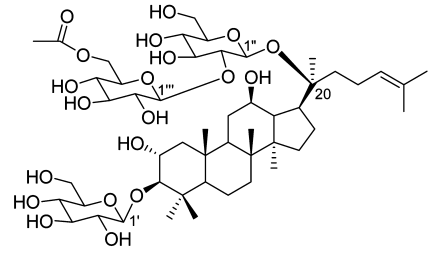

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Results and Discussion

Repeated column chromatography of the *n*-BuOH-soluble fraction of the 70% EtOH extract of *G. pentaphyllum* leaves resulted in the purification of **1** and **2**. Compound **1** was obtained as an amorphous powder. The molecular weight was determined from

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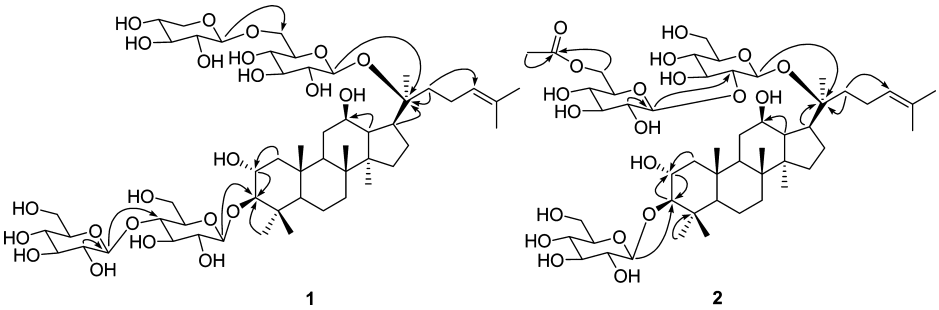

Figure 1. Key HMBC correlations of compounds **1** and **2**.

the positive HRFABMS at *m*/*z* 1117.5775 for the $[M + Na]^+$ ion (calcd for $C_{53}H_{90}O_{23}Na$, 1117.5770 $[M + Na]^+$). The ^{13}C and DEPT NMR spectra gave 53 signals, of which 23 were assigned to the sugar moieties and 30 to a triterpene moiety. The ^1H NMR spectrum of **1** showed eight singlets assignable to the aglycon methyls at δ 0.95−1.72, two of which were diagnostic for methyls linked to a sp^2 carbon at δ 1.72 (C-26) and 1.66 (C-27). The ^1H NMR spectrum of **1** also showed an olefinic proton signal at δ 5.13 (1H, t, $J = 6.0$ Hz, H-24). Two β-hydroxy substituents were evident from the chemical shifts and the J values of protons ascribable to H-3α at δ 3.04 (1H, d, $J = 9.6$ Hz) and H-12α at δ 4.04 (1H, br d, $J = 10.0$ Hz). The coupling behavior of H-3 (9.6 Hz) and a NOESY correlation between H-2 at δ 3.88 (1H, m) and H-19 (3H, s) suggested the presence of 2α-hydroxy substitution. On the basis of the ^1H and ^{13}C NMR data, the aglycon of **1** was identified as 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene.[14] Additionally, signals for four anomeric protons at δ 4.79 (1H, d, $J = 8.0$ Hz), 4.60 (1H, d, $J = 8.0$ Hz), 4.49 (1H, d, $J = 8.0$ Hz), and 4.33 (1H, d, $J = 8.0$ Hz) were observed in the ^1H NMR spectrum, suggesting the presence of four sugar units. In comparison with the corresponding signals in a model compound reported in the literature,[14] glycosidations at C-3 and C-20 were indicated by the significant downfield shift observed for these carbon signals in **1**. Acid hydrolysis of **1** yielded D-glucose and D-xylose (3:1), which were confirmed by GC analysis. The chemical shifts, the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^1H NMR spectrum, as well as the ^{13}C NMR data, indicated that both sugar units have a β-configuration, and these were confirmed from the $^1H-^1H$ COSY and HMQC spectra (Figure S3, Supporting Information). The linkage sites and sequences of the four saccharides and the aglycon were deduced from a HMBC experiment. Correlations were observed between H-1′ of Glc-1 and C-3 of the aglycon and between H-1″ of the Glc-2 and C-4′ of Glc-1. In addition, the correlations between H-1‴ of Glc-3 and C-20 of the aglycon and between H-1⁗ of the Xyl and C-6‴ of Glc-3 were also observed (Figure S4, Supporting Information). Thus, the structure of **1** was elucidated as 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside].

Compound **2** was isolated as an amorphous powder. Its molecular formula was established as $C_{50}H_{84}O_{20}$ from the HRFABMS quasi-molecular ion at *m*/*z* 1027.5454 (calcd for $C_{50}H_{84}O_{20}Na$, 1027.5453 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** indicated that they have the same aglycon moiety, 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene. Signals for three anomeric protons at δ 4.42 (1H, d, $J = 7.5$ Hz), 4.60 (1H, d, $J = 7.5$ Hz), and 4.71 (1H, d, $J = 7.5$ Hz) were observed in the ^1H NMR spectrum, suggesting the presence of three sugar units. In comparison with the corresponding signals in a model compound[14] and compound **1**, glycosidation at C-3 and C-20 was indicated by the significant downfield shifts observed for these carbon signals in **2**. By GC analysis of the acetate derivatives of the component monosaccharides, it was clear after acid hydrolysis that **2** contains three units of β-D-glucose. The structures of these sugar moieties were

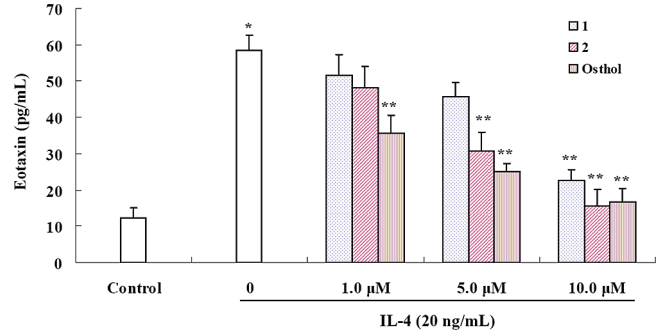

Figure 2. BEAS-2B cells were pretreated with **1** and **2** and the positive control osthol 2 h before IL-4 stimulation (20 ng/mL). Cell supernatants were collected for measurement of eotaxin concentration after 48 h incubation. The control value was obtained in the absence of IL-4 and the test compounds. These data represent the average values of three repeated experiments. *$p < 0.05$ vs control, **$p < 0.001$ vs stimulated groups.

determined from the $^1H-^1H$ COSY, TOCSY, and HMQC data. The linkage sites and sequences of the three saccharides and of the aglycon were also determined from a HMBC experiment (Figure S7, Supporting Information). A HMBC correlation of H-3 to C-1′ (δ 105.6) indicated that Glc-1 is connected to the aglycon at C-3 (δ 96.6). Also, other HMBC correlations were observed between H-1″ of Glc-2 and C-20 (δ 85.0) of the aglycon and between H-1‴ of the Glc-3 and C-2″ (δ 75.3) of Glc-2. In addition, the ^1H and ^{13}C NMR spectra of **2** showed the signals of an acetyl group at δ_H 2.04 (3H, s) and δ_C 172.9 and 20.7. A correlation was observed between H-6‴ of Glc-3 and the carbonyl carbon of this acetyl group (Figure 1). Thus, the structure of **2** was elucidated as 2α,3β,12β,20*S*-tetrahydroxydammar-24-ene-3-*O*-β-D-glucopyranosyl-20-*O*-[β-D-6-*O*-acetylglucopyranosyl-(1→2)-β-D-glucopyranoside].

The ability of the isolated compounds, **1** and **2**, to regulate cytokine-induced eotaxin expression was investigated in the BEAS-2B human bronchial epithelial cell line. In a preliminary study, the cytotoxic effects of gypenosides **1** and **2** were evaluated using the MTT assay,[15] and these compounds did not affect the cell viability of BEAS-2B cells in either the presence or absence of IL-4, even at a dose of 10 μM, after a period of 48 h. There was no significant difference between the control and treated cells. Then, gypenosides **1** and **2** were tested in the suppression of IL-4-induced eotaxin production in BEAS-2B cells. During an incubation time of 48 h, BEAS-2B cells produced 12.4 ± 2.5 pg/mL eotaxin in the resting stage (Figure 2). However, after IL-4 stimulation (20 ng/mL), eotaxin production increased dramatically to 58.5 ± 4.7 pg/mL. Gypenosides **1** and **2** (1.0, 5.0, and 10.0 μM) significantly downregulated IL-4-induced eotaxin production in a dose-dependent manner. Osthol, a positive control,[16,17] significantly inhibited IL-4-induced eotaxin production (16.8 ± 3.5 pg/mL) at a concentration of 10.0 μM. To examine whether the tested compounds could inhibit eotaxin production in the combination of IL-4 and TNF-α stimulation, the cells were preincubated with compounds for 2 h and then

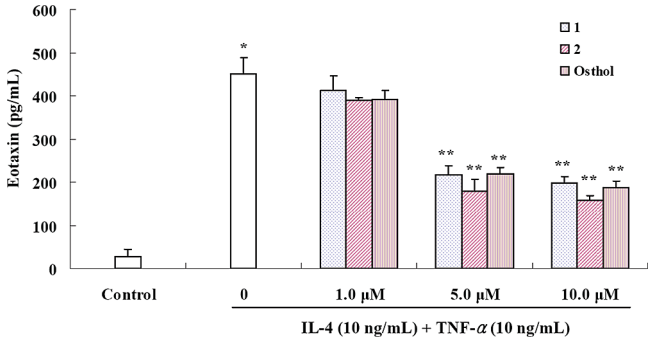

Figure 3. BEAS-2B cells were pretreated with **1** and **2** and the positive control osthol 2 h before IL-4 (10 ng/mL) plus TNF-α (10 ng/mL) stimulation. Cell supernatants were collected for measurement of eotaxin concentration after 48 h incubation. The control value was obtained in the absence of IL-4 and TNF-α and the test compounds. These data represent the average values of three repeated experiments. *$p < 0.05$ vs control, **$p < 0.001$ vs stimulated groups.

activated with IL-4 (10 ng/mL) and TNF-α (10 ng/mL). The BEAS-2B cells produced 28.1 ± 3.5 pg/mL eotaxin in the resting stage, but, after stimulation, eotaxin production increased to 450.7 ± 38.4 pg/mL. Gypenoside **1** (1.0, 5.0, and 10.0 μM) suppressed IL-4- and TNF-α-induced eotaxin expression of BEAS-2B cells with the production decreasing from 411.6 ± 35.2 to 197.2 ± 15.5 pg/mL. In the same manipulation, gypenoside **2** reduced eotaxin production to 157.2 ± 11.8 pg/mL at a concentration of 10 μM (Figure 3). In this experiment, osthol, at a concentration of 10.0 μM, decreased eotaxin production to 187.3 ± 15.2 pg/mL. Eosinophils including eotaxin are important inflammatory effector cells that accumulate at the site of allergic inflammation, e.g., the airway submucosa.[18,19] The activated eosinophils release cytotoxic molecules such as major basic proteins, eosinophil peroxidase, eosinophilic cationic protein, lipid mediators, and cytokines that cause tissue damage and consequently result in the manifestations of allergic diseases, such as allergic asthma. The interaction of bronchial epithelium with eosinophils represents a crucial mechanism in the initiation of local inflammation in allergic asthma.[5] In this study, we examined the release of the inflammatory chemokine eotaxin, stimulated by IL-4 and the combination of IL-4 and TNF-α in BEAS-2B cells, which mimics the in vivo conditions in bronchial allergic asthma. The suppressive effect of these two gypenosides, **1** and **2**, on IL-4-induced eotaxin expression demonstrated herein suggests that *G. pentaphyllum* and its components may be of benefit in allergic diseases.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1020 polarimeter using a 100 mm glass microcell. IR spectra (KBr) were recorded on a Bruker Equinox 55 FT-IR spectrometer. NMR spectra were obtained on a Varian Inova 400 MHz spectrometer with TMS as the internal standard. FABMS and HR-FABMS data were obtained on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. For column chromatography, silica gel (63−200 μm particle size), RP-18 (75 μm particle size, Merck, Germany), and YMC gel (ODS-A, S-150 μm, YMC Co., Ltd., Japan) were used. TLC was carried out with Merck silica gel 60 F254 and RP-18 F254 plates. HPLC was carried out using a Gilson system with a UV detector and an Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech, Korea). GC system: GC-14BPF column, 5% OV-225/AW-DMCS-Chromosorb W (80−100 mesh), 3 mm i.d. × 2.5 m; column temperature, 210 °C; injection temperature, 250 °C; carrier gas, N_2 at a flow rate of 25 mL/min; detector, FID (Shimadzu, Japan).

Chemicals and Reagents. Solvents were purchased from Samchun Chemicals Company. RPMI 1640, FBS, PBS buffer, penicillin−streptomycin, and 10% trypsin-EDTA were purchased from Gibco (Carlsbad, CA). MTT reagent and DMSO were obtained from Sigma Aldrich. Osthol was isolated and identified from the root of *Angelica dahurica* by K.H.B.[20]

Plant Material. The leaves of *Gynostemma pentaphyllum* were collected from Da Bac, Hoa Binh Province, Vietnam, in July 2008 and identified by Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi University of Pharmacy. A voucher specimen (0152-b) was deposited in the herbarium of the Hanoi University of Pharmacy.

Extraction and Isolation. The dried leaves (0.5 kg) of *G. pentaphyllum* were extracted with 2 L of 70% ethanol, three times. The 70% EtOH extract was combined and concentrated to yield a residue that was suspended in water and then successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH to afford $CHCl_3$-, EtOAc-, and *n*-BuOH-soluble fractions. The *n*-BuOH-soluble fraction (5.3 g) was separated by silica gel (63−200 μm particle size) column chromatography using a gradient of $CHCl_3$−MeOH−H_2O (from 60:1:0.1 to 5:1:0.1) to yield five subfractions (B.1−B.5) according to their TLC profiles. Subfraction B.3 was chromatographed over a YMC column (150 μm particle size) using a gradient of MeOH−H_2O (from 2:1 to 10:1) to yield seven further subfractions, B.3.1−B.3.7. The B.3.2 fraction was purified by semipreparative HPLC [RS Tech Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeOH−H_2O (63:37) with 0.1% formic acid; flow rate 2 mL/min; UV detection at 205 nm], resulting in the isolation of compound **2** (15.1 mg; t_R = 39.5 min). The B.3.5 subfraction was also purified by semipreparative HPLC using the above conditions, resulting in the isolation of compound **1** (14.1 mg; t_R = 81.7 min).

2α,3β,12β,20*S*-Tetrahydroxydammar-24-ene-3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-20-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (1): white, amorphous powder, $[\alpha]_D^{25}$ +6.5 (*c* 0.10, MeOH), IR (KBr) ν_{max} 3438, 2930, 1665, 1636, 1270, 1175, 1087, 1042, 810 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 1117.5775 $[M + Na]^+$ (calcd for $C_{53}H_{90}O_{23}Na$, 1117.5770).

2α,3β,12β,20*S*-Tetrahydroxydammar-24-ene-3-*O*-β-D-glucopyranosyl-20-*O*-[β-D-acetylglucopyranosyl-(1→2)-β-D-glucopyranoside] (2): white, amorphous powder; $[\alpha]_D^{25}$ −1.8 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3435, 2941, 2855, 1758, 1645, 1450, 1327, 1309, 1281, 1075, 1030 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 1027.5454 $[M + Na]^+$ (calcd for $C_{50}H_{84}O_{20}Na$, 1027.5453).

Determination of Sugar Components. The monosaccharide subunits of **1** and **2** were obtained by acid hydrolysis. Compounds **1** and **2** (4 mg each) in 10% HCl−dioxane (1:1, 1 mL) were each heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 mL × 3). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with $CHCl_3$−MeOH−H_2O (55:45:10) and compared with authentic samples. Each sugar residue was then dissolved in 2 mL of H_2O, 15 mg of $NaBH_4$ was added, and the mixture was left to stand for 2 h at ambient temperature. Several drops of 25% HOAc were added until the pH value was 4−5. After co-distillation with CH_3OH to remove the extra boracic acid and water, the resulting products were put into a vacuum-desiccator overnight and then heated at 110 °C for 15 min to further remove the water. Next, 3 mL of acetic anhydride was added and the solution was kept at 100 °C for 1 h. Then the solution was cooled and co-distillated with toluene several times. The acetate derivatives were dissolved in $CHCl_3$, washed with distilled water and then anhydrous sodium sulfate, filtered, and concentrated to 0.1 mL. The acetate derivatives were subjected to GC analysis to identify the sugars. Column temperature 210 °C; injection temperature 250 °C; carrier gas N_2 at a flow rate of 25 mL/min; D-glucose and D-xylose, 17.40 and 7.54 min, respectively.

Cell Culture. Human BEAS-2B bronchial epithelial cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI-1640 medium (Invitrogen, Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Gibco) at 37 °C in 5% CO_2 in a humidified incubator. Cells were centrifuged and resuspended in fresh media in 12-well plates at a concentration of 5 × 10^5/mL for 12 h before experimental use. When BEAS-2B cells reached 80% confluence in the 12-well plates, the culture medium was replaced with RPMI-1640 without FBS. The recom-

Table 1. ^{1}H and ^{13}C NMR (CD_3OD) Spectroscopic Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1	2.03 (dd, 4.4, 12.0), 1.10 (m)	48.0	2.32 (m), 1.10 (m)	47.8
2	3.88 (m)	68.2	3.87 (m)	68.2
3	3.04 (d, 9.6)	96.8	2.96 (d, 9.5)	96.6
4		41.9		41.8
5	0.75 (m)	57.3	0.80 (m)	57.2
6	1.45 (m), 1.37 (m)	19.4	1.53 (m), 1.31 (m)	19.3
7	1.42 (m), 1.28 (m)	35.8	1.25 (m), 1.56 (m)	35.7
8		40.0		41.0
9	1.51 (m)	51.1	1.56 (m)	51.0
10		38.9		38.8
11	1.93 (m), 1.04 (m),	31.0	2.11 (m), 1.12 (m)	31.1
12	4.04 (br d, 10.0)	70.1	3.87 (m)	71.0
13	1.82 (m)	49.5	1.93 (m)	49.7
14		52.5		52.5
15	1.90 (m), 1.15 (m)	31.6	1.59 (m), 0.95 (m)	31.6
16	2.24 (m),	27.4	1.78 (m)	27.2
17	2.35 (m)	52.9	2.29 (m)	53.1
18	1.16 (s)	16.4	0.93 (s)	16.3
19	1.03 (s)	17.9	0.87 (s)	17.9
20		85.0		85.0
21	1.39 (s)	22.4	1.34 (s)	22.8
22	1.52 (m), 1.83 (m)	36.8	2.09 (m), 1.87 (m)	36.6
23	2.01 (m)	23.9	2.06 (m)	24.2
24	5.13 (t, 6.0)	126.1	5.11 (t, 6.0)	125.9
25		132.3		132.4
26	1.72 (s)	26.1	1.68 (s)	25.7
27	1.66 (s)	18.1	1.62 (s)	17.9
28	0.95 (s)	17.9	0.98 (s)	16.2
29	1.01 (s)	28.8	1.10 (s)	28.6
30	0.96 (s)	17.5	1.02 (s)	18.0
$OCOCH_3$				172.9
$OCOCH_3$			2.04	20.7
	C-3-Glc-1		C-3-Glc-1	
1′	4.79 (d, 8.0)	104.9	4.42 (d, 7.5)	105.6
2′	3.30 (m)	75.3	3.36 (m)	76.2
3′	3.41 (m)	78.0	3.45 (m)	78.1
4′	3.27 (m)	77.6	3.28 (m)	71.3
5′	3.79 (m)	78.6	3.75 (m)	78.0
6′	3.66 (m), 3.92 (br d, 10.4)	62.4	4.32 (dd, 1.6, 10.0), 3.75 (m)	62.6
	Glc-2		C-20-Glc-1	
1″	4.49 (d, 8.0)	104.3	4.60 (d, 7.5)	98.3
2″	3.37 (m)	76.7	3.32 (m)	82.4
3″	3.65 (m)	78.1	3.60 (m)	78.3
4″	3.39 (m)	72.1	3.71 (m)	71.5
5″	3.64 (m)	78.3	3.43 (m)	75.5
6″	3.79 (dd, 5.4, 11.2), 3.90 (m)	63.3	3.87 (dd, 2.0, 10.5), 3.71 (m)	62.4
	C-20-Glc-1		Glc-2	
1‴	4.33 (d, 8.0)	105.6	4.71 (d, 7.5)	104.7
2‴	3.88 (m)	75.2	3.45 (m)	75.3
3‴	3.52 (m)	78.5	3.63 (m)	78.0
4‴	3.35 (m)	71.7	3.42 (m)	71.9
5‴	3.65 (m)	77.6	3.69 (m)	78.8
6‴	4.04 (br d, 10), 3.85 (m)	68.2	4.16 (dd, 4.4, 11.0), 3.75 (m)	65.1
	Xyl-1			
1⁗	4.60 (d, 8.0)	98.1		
2⁗	3.75 (m)	76.7		
3⁗	3.67 (m)	77.4		
4⁗	3.65 (m)	74.9		
5⁗	3.87 (dd, 5.2, 11.6), 3.21 (m)	66.8		

[a] Data were assigned on the basis of H−H COSY, HMQC, and HMBC experiments. The chemical shifts are in ppm, and coupling constants (*J* in Hz) are in parentheses.

binant human IL-4 and TNF-α were purchased from R&D Systems (Minneapolis, MN). The cells were then pretreated with different concentrations of isolated compounds (1, 5, and 10 μM) for 2 h before stimulation with IL-4 (20 ng/mL) alone or IL-4 (10 ng/mL) combined with TNF-α (10 ng/mL). Cell supernatants were collected after 48 h for ELISA of eotaxin. Culture supernatants were used to detect the production of eotaxin using sandwich ELISA from R&D Systems, performed according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc., Waltham, MA).

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Supporting Information Available: Spectroscopic data, consisting of ^{1}H, ^{13}C NMR, COSY, and HMBC spectra of compounds **1** and **2**, are available free of charge via the Internet at http://pubs.acs.org.

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