Absolute Stereostructures of Three New Sesquiterpenes from the Fruit of *Alpinia oxyphylla* with Inhibitory Effects on Nitric Oxide Production and Degranulation in RBL-2H3 Cells[1]

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The 80% aqueous acetone extract and the ethyl acetate-soluble portion from the dried fruit of *Alpinia oxyphylla* MIQUEL were found to show inhibitory effects on nitric oxide production in lipopolysaccharide-activated macrophages and antigen-induced degranulation in RBL-2H3 cells. A new eudesmane-type sesquiterpene, oxyphyllol A, and two eremophilane-type sesquiterpenes, oxyphyllols B and C, were isolated from the ethyl acetate-soluble portion, together with 16 known constituents. The absolute stereostructures of oxyphyllols A, B, and C were determined on the basis of chemical and physicochemical evidence. The effects of isolated components on nitric oxide production were examined, and nine constituents including oxyphyllol A and nootkatone were found to show inhibitory activity. On the other hand, five constituents inhibited the release of β-hexosaminidase from RBL-2H3 cells.

The Zingiberaceae plant *Alpinia* (*A.*) *oxyphylla* MIQUEL has been widely cultivated in South China. The fruit of this plant has been used as a medicine for intestinal disorders and urosis in Chinese traditional medicine and is listed in Japanese Pharmacopeia XIV as an aromatic stomachic. Recently, we reported isolation and elucidation of the structures of four norsesquiterpenes named oxyphyllenodiols A and B and oxyphyllenones A and B from the kernels of *A. oxyphylla*.[2] As part of our continuing studies of this herbal medicine, we found that the 80% aqueous acetone extract and the ethyl acetate (AcOEt)-soluble portion from the fruit of *A. oxyphylla* inhibited nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages and the release of β-hexosaminidase from RBL-2H3 cells. From the AcOEt-soluble portion, we isolated three new sesquiterpenes called oxyphyllols A (**1**), B (**2**), and C (**3**) together with 16 known compounds.

This paper deals with the elucidation of the structures of three new sesquiterpenes (**1**−**3**) and the inhibitory effects of the isolated components from the AcOEt-soluble portion on NO production in LPS-activated macrophages and the release of β-hexosaminidase from RBL-2H3 cells.

Results and Discussion

The 80% aqueous acetone extract of the fruit of *A. oxyphylla*, which was cultivated in Hainan island (China), showed inhibitory effects on NO production and release of β-hexsosaminidase as shown in Tables 1 and 2. The aqueous acetone extract was partitioned into a mixture of AcOEt and water to furnish the AcOEt-soluble portion and an aqueous phase. The aqueous phase was further extracted with 1-butanol (1-BuOH) to give the 1-BuOH-soluble portion and H_2O-soluble portion. The AcOEt-soluble portion was found to show inhibitory effects on NO production and release of β-hexosaminidase, while the 1-BuOH-soluble and H_2O-soluble portions lacked the activity (Tables 1 and 2). The AcOEt-soluble portion was subjected to normal- and reversed-phase silica gel column chromatography and finally HPLC to furnish oxyphyllols A (**1**, 0.0038% from the natural medicine), B (**2**, 0.030%), and C (**3**, 0.027%), nootkatone (**4**,[3] 0.17%), **5**[4] (0.0020%), selin-11-en-4α-ol (**6**,[5] 0.0009%), isocyperol (**7**,[6] 0.0020%), oxyphyllenodiols A (**8**,[2] 0.020%) and B (**9**,[2] 0.0031%), oxyphyllenones A (**10**,[2] 0.0063%) and B (**11**,[2] 0.0021%), (*E*)-labda-8(17),12-diene-15,16-dial (**12**,[7] 0.0011%), tectochrysin (**13**,[8,9] 0.0013%), and izalpinin (**14**,[9] 0.0006%) together with five free fatty acids, palmitoleic acid[10] (0.0095%), oleic acid[10] (0.082%), linoleic acid[10] (0.020%), linolenic acid[10] (0.0015%), and lignoceric acid[10] (0.040%).Oxyphyllol A (**1**) was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{26}$ +17.7°). The EIMS of **1** showed a molecular ion peak at *m/z* 220 [M^+] in addition to fragment ion peaks at *m/z* 202 [$M^+ - H_2O$] and 121 [base peak]. The molecular formula of **1**, $C_{15}H_{24}O$, was determined from the molecular ion peak observed in the EIMS and by HREIMS measurement. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl, olefin, and *exo*-methylene functions at 3360, 1653, and 1456 cm^{-1}, respectively. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 3) spectra of **1** showed signals assignable to three methyls [δ 0.88 (both, H_3-14), 1.63, 1.82 (both br s, H_3-15 and 13)], an *exo*-methylene [δ 5.03 (dd, J = 1.2, 1.2 Hz), 5.07 (br s), H_2-12], and an olefin [δ 5.33 (br s, H-6)] together with five methylenes (H_2-1, 2, 3, 8, 9), a methine (H-7), and four quaternary carbons (C-4, 5, 10, 11).

The planar structure of **1** was constructed on the basis of $^1H-^1H$ COSY and HMBC experiments. Thus, the $^1H-^1H$ COSY experiment on **1** indicated the presence of two partial structures shown as bold lines in Figure 1. In the HMBC experiment, long-range correlations were observed between the following protons and carbons (H_3-15 and C-3−5; H_3-14 and C-1, 5, 10; H-7 and C-11; H_2-12 and C-7, 13; H_3-13 and C-7, 11, 12), so that the connectivities of the quaternary carbons (C-4, 5, 10, 11) in **1** were clarified.[11] The above evidence indicated that the skeleton of oxyphyllol A (**1**) was 5,11-eudesmadien-4-ol.

The relative stereostructure of **1** was characterized by NOESY experiment, which showed NOE correlations between the following proton pairs (H_3-14 and H-1β, 2β, 9β, H_3-15; H-8α, H-9α and H-7) as shown in Figure 1. To elucidate the absolute stereostructure, **1** was chemically related to **6**, the absolute configuration of which has been reported.[5] Thus, hydrogenation of **1** in the presence of 10% Pd−C in MeOH selectively furnished **1a**,[12] whereas **1a** was

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Table 1. Inhibitory Effects of 80% Aqueous Acetone Extract and AcOEt-, 1-BuOH-, and H_2O-Soluble Portions from *Alpinia oxyphylla* on NO Production in LPS-Activated Macrophages[a]

	inhibition (%)					
	0 μg/mL	3 μg/mL	10 μg/mL	30 μg/mL	100 μg/mL	IC_{50} (μg/mL)
80% aq acetone extr	0.0 ± 4.1	18.4 ± 1.2**[b]	46.7 ± 2.8**[b]	97.7 ± 1.3**[b]	104.2 ± 1.3**[b,c]	10
AcOEt-soluble portion	0.0 ± 2.1	30.0 ± 4.6**[b]	77.6 ± 2.4**[b]	99.9 ± 0.6**	108.8 ± 0.2**[b,c]	5.5
1-BuOH-soluble portion	0.0 ± 6.3	7.0 ± 4.5	−4.4 ± 4.7	1.9 ± 7.0	22.4 ± 6.1*[b]	>100
H_2O-soluble portion	0.0 ± 9.4	−9.4 ± 8.7	−12.6 ± 6.5	22.2 ± 4.4	35.9 ± 4.4**[b]	>100

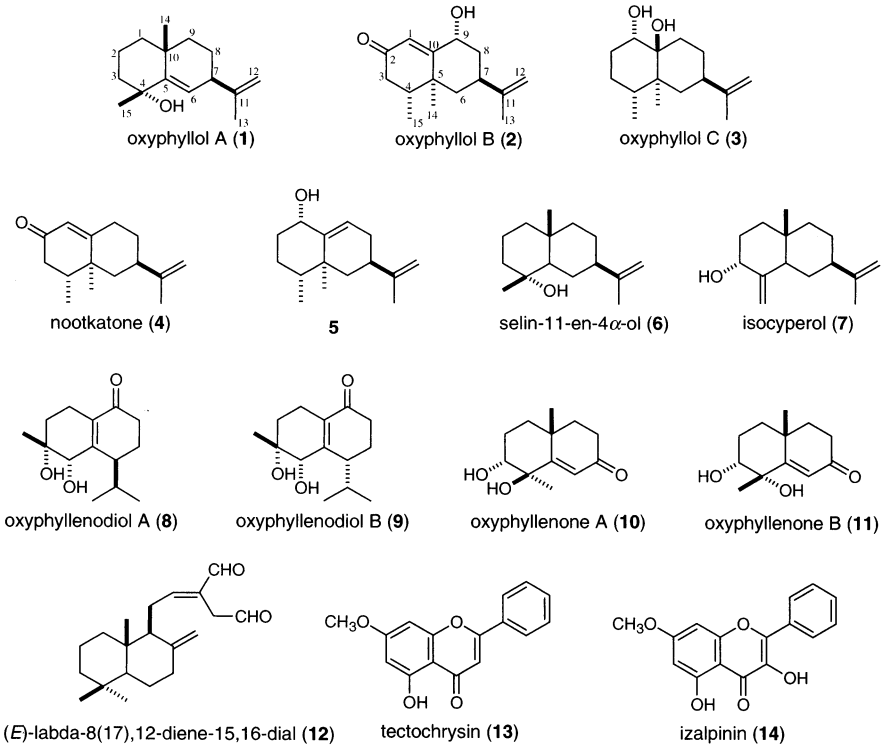
[a] Each value represents the mean± SEM ($N = 4$). [b]Significantly different from the control: $*p < 0.05$, $**p < 0.01$. [c] Cytotoxic effect was observed.

Table 2. Inhibitory Effects of 80% Aqueous Acetone Extract and AcOEt-, 1-BuOH-, and H_2O-Soluble Portions from *Alpinia oxyphylla* on the Release of β-Hexosaminidase from RBL-2H3 Cells

	inhibition (%)				
	0 μg/mL	30 μg/mL	100 μg/mL	300 μg/mL	IC_{50} (μg/mL)
80% aq acetone extr.	0.0 ± 1.3	13.0 ± 1.2**[b]	43.8 ± 1.1**[b]	74.0 ± 0.7**[b]	141
AcOEt-soluble portion	0.0 ± 1.5	7.7 ± 1.4**[b]	36.2 ± 0.9**[b]	84.0 ± 1.0**[b]	158
1-BuOH-soluble portion	0.0 ± 5.2	−9.8 ± 2.7	−14.5 ± 2.4*[b]	−14.8 ± 1.6*[b]	–
H_2O-soluble portion	0.0 ± 3.5	−0.7 ± 1.6	−6.0 ± 1.9	−2.9 ± 2.6	–

[a] Each value represents the mean ± SEM ($N = 4$). [b] Significantly different from the control: $*p < 0.05$, $**p < 0.01$.

Chart 1



also obtained by hydrogenation of **6** from the less hindered α-side. Consequently, the absolute stereostructure of oxyphyllol A was determined to be the 4*R*,7*R*,10*R* configuration. Oxyphyllol B (**2**)[13] was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{28}$ +10.4°). The molecular formula of **2**, $C_{15}H_{22}O_2$, was determined from the molecular ion peak at *m*/*z* 234 $[M]^+$ in EIMS and by HREIMS measurement. In the UV spectrum of **2**, an absorption maximum was observed at 232 nm (log ϵ 3.76), suggestive of an α,β-unsaturated carbonyl function, while the IR spectrum of **2** showed absorption bands at 3436, 1667, and 1456 cm^{-1} ascribable to hydroxyl, α,β-unsaturated carbonyl, and *exo*-methylene functions. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 3) spectra of **2** showed signals assignable to three methyls [δ 0.95 (d, $J = 6.7$ Hz, H_3-15), 1.32, 1.77 (both s, H_3-14, 13)], a methine bearing the oxygen function [δ 4.45 (dd, $J = 2.8$, 3.0 Hz, H-9)], an *exo*-methylene [δ 4.75 (br s), 4.77 (dd, $J = 1.2$, 1.5 Hz), H_2-12], and an α,β-unsaturated carbonyl function [δ 5.88 (s, H-1); δ_C 126.9 (d, C-1), 201.0 (s, C-2), 168.8 (s, C-10)] together with three methylenes (H_2-3, 6, 8), two methines (H-4, 7), and two quaternary carbons (C-5, 11). The planar structure of **2** was constructed on the basis of $^1H-^1H$ COSY and HMBC experiments,[11] in addition to the relative stereostructure of **2** elucidated by the NOESY experiment as shown in Figure 2.

Next, the absolute stereostructure of **2** was determined by application of the CD excitation chirality method for an allylic benzoate (**2b**).[14] Thus, **2** was treated with $NaBH_4$ in the presence of $CeCl_3$ to give the reductant **2a**. By treatment of **2a** with *p*-bromobenzoyl chloride, the 2-*O*-*p*-bromobenzoate (**2b**) was selectively obtained, because of steric hindrance of the axial 9α-hydroxyl group. The stereostructure of **2b** was determined by NOESY experiment (Figure 2), in which the NOE correlations were observed between the 2β-proton and 4-proton. The 2-*O*-*p*-

Table 3. ^{13}C NMR Data for Oxyphyllols A (**1**), B (**2**), and C (**3**) and Its Derivative (**2a**)

	1[a]	**1**[b]	**2**[a]	**2**[b]	**2a**[a]	**3**[a]	**3**[b]
C-1	37.6	38.7	126.9	126.9	129.6	75.7	75.9
C-2	32.4	33.4	201.0	199.5	67.9	29.1	30.4
C-3	33.4	34.4	42.2	42.7	38.6	25.3	26.8
C-4	75.3	74.3	41.1	41.6	40.0	34.9	35.2
C-5	134.3	134.9	38.7	39.3	37.7	39.2	40.0
C-6	121.2	121.4	43.6	44.4	44.3	38.6	39.2
C-7	43.4	43.6	33.9	34.8	34.6	39.3	40.4
C-8	23.0	23.3	37.8	39.2	37.0	25.6	26.6
C-9	38.1	38.0	73.0	72.7	74.5	31.4	32.7
C-10	32.4	32.8	168.8	169.1	146.8	73.9	73.6
C-11	146.8	148.6	148.8	149.6	149.8	150.5	151.5
C-12	113.4	112.4	109.3	109.4	108.9	108.4	108.6
C-13	18.7	19.2	20.9	21.0	21.0	20.8	21.0
C-14	15.9	16.1	18.0	18.2	19.9	15.7	16.0
C-15	21.2	21.3	14.4	14.6	15.1	15.1	15.7

[a] Measured in $CDCl_3$ at 125 MHz. [b] Measured in pyridine-d_5 at 125 MHz.

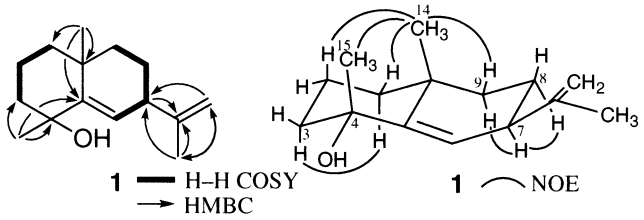


Figure 1.

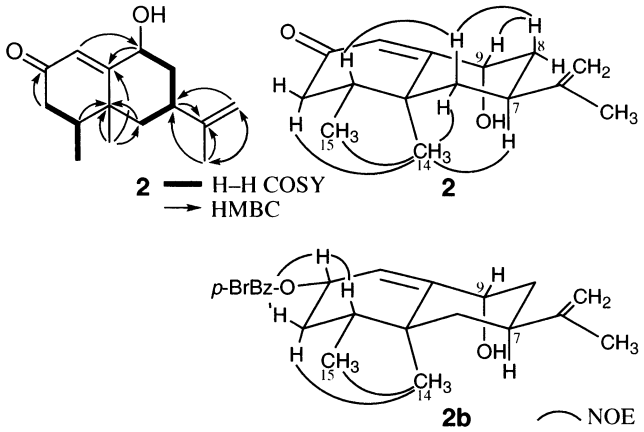


Figure 2.

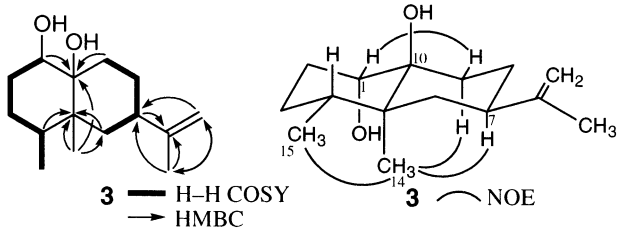
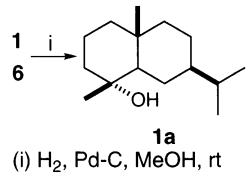


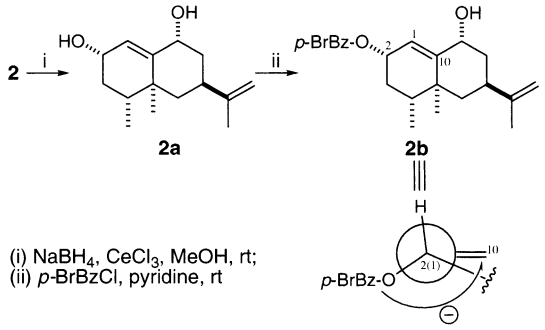
Figure 3.

bromobenzoate (**2b**) showed a negative Cotton effect [245 nm ($\Delta\epsilon$ −3.75)], which indicated the 2-position of **2b** to be *S*. On the basis of this evidence, the absolute stereostructure of **2** was determined. Oxyphyllol C (**3**) was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{29}$ +6.5°). The molecular formula $C_{15}H_{26}O_2$ of **3** was also determined from the molecular ion peak at *m*/*z* 238 [M^+] in the EIMS of **3** and by HREIMS measurement. The IR spectrum of **3** showed absorption bands ascribable to hydroxyl and *exo*-methylene groups at 3475 and 1456 cm^{-1}. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 3) spectra of **3** showed signals

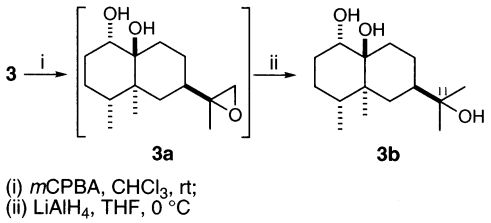
Scheme 1



Scheme 2



Scheme 3



assignable to three methyls [δ 0.76 (d, J = 7.0 Hz, H_3-15), 1.08, 1.73 (both s, H_3-14, 13)], a methine bearing the oxygen function [δ 3.52 (dd, J = 2.4, 3.1 Hz, H-1)], and an *exo*-methylene [δ 4.69, 4.72 (both s, H_2-12)] together with five methylenes (H_2-2, 3, 6, 8, 9), two methines (H-4, 7), and three quaternary carbons (C-5, 10, 11). The partial structures of **3**, indicated by bold lines in Figure 3, were clarified by H−H COSY, and long-range correlations were observed in the HMBC experiment.[11] In addition, the relative structure of **3** was suggested on the basis of ^1H NMR (*vide ante*) and the NOESY experiment as shown in Figure 3. Finally, the absolute stereostructure of **3** was determined following chemical modification. Thus, **3** was oxidized with *m*CPBA to give the 11,12-epoxide derivative (**3a**), which was subjected to $LiAlH_4$ reduction to give 1α,10*β*,11-trihydroxy-4α,5α,7*β*-eremophilane (**3b**), the absolute configuration of which has been reported.[15]

The inorganic free radical NO has been implicated in physiological and pathological processes such as vasodilation, nonspecific host defense, ischemia reperfusion injury, and chronic or acute inflammation.[16] NO is produced by the oxidation of L-arginine catalyzed by NO synthase (NOS). In the NOS family, inducible NOS in particular is involved in pathological overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1*β*, tumor necrosis factor-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells.

As a part of our studies to characterize the bioactive components of natural medicines, we have reported several NO production inhibitors, i.e., higher unsaturated fatty acids,[17] polyacetylenes,[18,19] coumarins,[18] flavonoids,[19] stilbenes,[20] lignans,[21] sesquiterpenes,[2,19,22] diterpenes,[23] and triterpenes.[24] As part of our continuing screening, the effects of isolated constituents from *A. oxyphylla* on NO production from LPS-activated macrophages were exam-

Table 4. Inhibitory Effects of Constituents from *Alpinia oxyphylla* on NO Production in LPS-Activated Macrophages[a]

compound	inhibition (%)					
	0 μM	3 μM	10 μM	30 μM	100 μM	IC_{50} (μM)
oxyphyllol A (**1**)	0.0 ± 2.4	12.5 ± 2.4**[b]	19.3 ± 2.3**[b]	40.4 ± 2.8**[b]	74.2 ± 1.3**[b]	42
nootkatone (**4**)	0.0 ± 3.5	−6.2 ± 2.9	10.7 ± 3.2*[b]	44.8 ± 2.1**[b]	86.9 ± 1.9**[b]	34
5	0.0 ± 0.9	5.8 ± 2.3	1.8 ± 3.0	31.0 ± 3.0**[b]	65.3 ± 1.1**[b]	48
selin-11-en-4α-ol (**6**)	0.0 ± 5.6	2.5 ± 4.4	19.2 ± 2.5*[b]	42.2 ± 4.9**[b]	73.3 ± 1.0**[b]	39
isocyperol (**7**)	0.0 ± 1.8	10.3 ± 0.9**[b]	23.9 ± 2.3**[b]	64.4 ± 1.4**[b]	96.8 ± 0.3**[b]	21
oxyphyllenodiol A (**8**)[2]	0.0 ± 4.7	11.8 ± 3.3	16.9 ± 3.8*[b]	52.5 ± 5.9**[b]	101.7 ± 0.4**[b]	28
oxyphyllenodiol B (**9**)[2]	0.0 ± 2.6	−4.2 ± 1.9	−0.4 ± 6.2	19.6 ± 3.2**[b]	40.1 ± 3.6**[b]	>100
oxyphyllenone A (**10**)[2]	0.0 ± 3.1	1.6 ± 3.9	−2.9 ± 3.5	43.0 ± 2.2**[b]	85.7 ± 3.5**[b]	35
oxyphyllenone B (**11**)[2]	0.0 ± 4.5	−8.1 ± 3.9	0.4 ± 3.4	19.2 ± 2.3*[b]	41.2 ± 4.9**[b]	>100
12	0.0 ± 3.3	−4.7 ± 1.8	9.3 ± 3.8	68.1 ± 0.7**[b]	95.6 ± 1.5**[b,c]	22
tectochrysin (**13**)	0.0 ± 3.3	−0.1 ± 3.8	19.2 ± 3.0**[b]	60.6 ± 2.3**[b]	87.6 ± 0.8**[b,c]	23
izalpinin (**14**)	0.0 ± 1.8	6.3 ± 2.2	14.6 ± 2.7	13.4 ± 2.1	41.5 ± 9.0**[b,c]	>30
L-NMMA	0.0 ± 1.1	2.0 ± 1.6	17.7 ± 2.8**[b]	52.3 ± 1.5**[b]	79.2 ± 0.9**[b]	28

[a] Each value represents the mean± SEM ($N = 4$). [b] Significantly different from the control: *$p < 0.05$, **$p < 0.01$. [c] Cytotoxic effect was observed.

Table 5. Inhibitory Effects of Constituents from *Alpinia oxyphylla* on the Release of β-Hexosaminidase from RBL-2H3 Cells[a]

compound	inhibition (%)			
	0 μM	10 μM	30 μM	100 μM
oxyphyllol A (**1**)	0.0 ± 1.8	−8.3 ± 2.0*[b]	−15.1 ± 2.1**[b]	−24.0 ± 2.5**[b]
oxyphyllol C (**3**)	0.0 ± 1.8	−1.1 ± 2.0	2.4 ± 1.2	8.2 ± 1.7*[b]
nootkatone (**4**)	0.0 ± 1.8	2.0 ± 1.7	8.2 ± 1.3*[b]	25.8 ± 1.8**[b]
5	0.0 ± 2.6	−5.0 ± 2.2	−9.2 ± 1.4*[b]	−8.0 ± 0.6*[b]
selin-11-en-4α-ol (**6**)	0.0 ± 1.7	2.9 ± 1.6	4.4 ± 1.7	11.9 ± 1.4**[b]
isocyperol (**7**)	0.0 ± 2.3	−5.3 ± 2.3	−11.9 ± 2.9**[b]	−28.9 ± 1.1**[b]
oxyphyllenodiol A (**8**)	0.0 ± 1.5	1.1 ± 2.1	0.4 ± 1.8	1.7 ± 3.0
oxyphyllenodiol B (**9**)	0.0 ± 0.9	−4.3 ± 1.4	−3.3 ± 1.5	−3.1 ± 1.2
oxyphyllenone A (**10**)	0.0 ± 0.5	−3.3 ± 1.2	−4.5 ± 1.4	−4.7 ± 1.0
oxyphyllenone B (**11**)	0.0 ± 1.0	−0.8 ± 0.8	−1.3 ± 1.1	−1.8 ± 0.5
12	0.0 ± 3.0	−0.9 ± 1.6	13.1 ± 1.8**[b]	42.0 ± 2.9**[b]
tectochrysin (**13**)	0.0 ± 1.1	11.9 ± 2.4**[b]	40.8 ± 1.9**[b]	75.1 ± 0.8**[b]
izalpinin (**14**)	0.0 ± 0.9	1.6 ± 2.0	8.7 ± 2.1	27.5 ± 1.6**[b]
curcumin	0.0 ± 1.8	2.2 ± 2.0	14.2 ± 1.6**[b]	62.6 ± 1.0**[b]

[a] Each value represents the mean ± SEM ($N = 4$). [b] Significantly different from the control: *$p < 0.05$, **$p < 0.01$.

ined,[2] and the results are summarized in Table 4. Seven sesquiterpenes, **1** (IC_{50} = 42 μM), **4** (34 μM), **5** (48 μM), **6** (39 μM), **7** (21 μM), **8** (28 μM), and **10** (35 μM), a diterpene, **12** (22 μM), and a flavone, **13** (23 μM), were found to inhibit NO production.

Furthermore, we also examined the inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells. Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in in vitro experiments on immediate allergic reactions. β-Hexosaminidase is also stored in secretory granules of mast cells and is also released concomitantly with histamine when mast cells are immunologically activated.[25] Therefore, it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. Previously, we reported inhibitors of the release of β-hexosaminidase isolated from *Myrica rubra*.[26] In addition, the aqueous ethanolic extract of *A. oxyphylla* was reported to show the above inhibitory activity, while the active components were left uncharacterized. In our continuous search for antiallergic principles from natural sources, we examined the effects of constituents from the fruit of *A. oxyphylla* on the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP IgE. Antiallergic compounds, tranilast and ketotifen fumarate, showed weak inhibition, with IC_{50} values of 0.49 and 0.22 mM, respectively. On the other hand, luteolin and curcumin, which were reported to inhibit the degranulation in this experimental model, showed relatively strong inhibition, with IC_{50} values of 3.0 and 82 μM, respectively.[26] As shown in Table 5, two sesquiterpenes (**4**, **6**), a diterpene (**12**), and two flavonoids (**13**, IC_{50} = 49 μM; **14**) were found to show significant inhibitory activity against the release of β-hexosaminidase from RBL-2H3 cells.

Experimental Section

General Experimental Procedures. 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-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; GC−MS, EIMS, and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H NMR spectra, JEOL LNM-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL LNM-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector.

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

Plant Material. The fruit of *A. oxyphylla* was cultivated in Hainan Island, China, and purchased in 1998. It was identified by one of the authors, M. Yoshikawa, professor of Kyoto Pharmaceutical University. A voucher of the plant is on file in our laboratory.

Extraction and Isolation. The dried fruit of *A. oxyphylla* (7.5 kg) was cut and extracted three times with 80% aqueous

acetone at room temperature. Evaporation of the solvent under reduced pressure provided the aqueous acetone extract (800 g, 10.7%). The aqueous acetone extract (420 g) was partitioned in an AcOEt−H_2O (1:1, v/v) mixture. The aqueous layer was extracted with 1-BuOH, and removal of the solvent *in vacuo* from the AcOEt-, 1-BuOH-, and H_2O-soluble portions yielded 208 g (5.3%), 43 g (1.1%), and 169 g (4.3%) of the residue, respectively.

Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 3.0 kg), *n*-hexane−AcOEt (15:1−5:1−1:1, v/v)−AcOEt−MeOH] of the AcOEt-soluble portion (125 g) gave eight fractions [1 (2.9 g), 2 (6.9 g), 3 (11.4 g), 4 (14.1 g), 5 (36.3 g), 6 (12.3 g), 7 (21.0 g), 8 (20.1 g)]. Fraction 3 (9.2 g) was separated by reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Co., Ltd., 300 g), MeOH−H_2O (70:30−90:10, v/v)−MeOH] to furnish seven fractions [3-1 (718 mg), 3-2 (3.32 g), 3-3 (1.93 g), 3-4 (280 mg), 3-5 (632 mg), 3-6 (1.56 g), 3-7 (760 mg)]. Among them, fractions 3-2, 3-6, and 3-7 were identified as nootkatone (**4**, 0.17%), oleic acid (0.082%), and lignoceric acid (0.040%), respectively. Fraction 3-3 (680 mg) was further separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d.), (1) MeOH−H_2O (75:25, v/v), (2) CH_3CN−H_2O (65:35, v/v)] to give oxyphyllol A (**1**, 26 mg, 0.0038%), **5** (14 mg, 0.0020%), selin-11-en-4α-ol (**6**, 6 mg, 0.0009%), isocyperol (**7**, 14 mg, 0.0020%), tectochrysin (**13**, 9 mg, 0.0013%), and izalpinin (**14**, 4 mg, 0.0006%). Fraction 3-4 (130 mg) was purified by HPLC [CH_3CN−H_2O (80:20, v/v)] to furnish (*E*)-labda-8(17),12-diene-15,16-dial (**12**, 10 mg, 0.0011%) and linolenic acid (14 mg, 0.0015%). Fraction 3-5 (150 mg) was further separated by HPLC [MeOH−H_2O (90:10, v/v)] to give palmitoleic acid (4 mg, 0.0095%) and linoleic acid (90 mg, 0.020%). Fraction 5 (21.1 g) was subjected to reversed-phase silica gel column chromatography [600 g, MeOH−H_2O (80:20−90:10, v/v)−MeOH] to furnish eight fractions [5-1 (2.03 g), 5-2 (4.77 g), 5-3 (3.23 g), 5-4 (3.86 g), 5-5 (560 mg), 5-6 (1.40 g), 5-7 (520 mg), 5-8 (4.73 g)]. Fraction 5-3 (610 mg) was further purified by HPLC [MeOH−H_2O (75:25, v/v)] to give oxyphyllols B (**2**, 78 mg, 0.030%) and C (**3**, 71 mg, 0.027%). Fraction 6 (10.9 g) was separated by reversed-phase silica gel column chromatography [300 g, MeOH−H_2O (50:50−70:30, v/v)−MeOH] to furnish six fractions [6-1 (1.42 g), 6-2 (3.24 g), 6-3 (900 mg), 6-4 (2.46 g), 6-5 (1.14 g), 6-6 (1.74 g)]. Fraction 6-2 (580 mg) was further purified by HPLC [MeOH−H_2O (60:40, v/v)] to give oxyphyllenodiols A (**8**, 75 mg, 0.020%) and B (**9**, 12 mg, 0.0031%). Fraction 7 (15.5 g) was purified by reversed-phase silica gel column chromatography [500 g, MeOH−H_2O (50:50−70:30, v/v)−MeOH] to give six fractions [7-1 (1.82 g), 7-2 (2.90 g), 7-3 (810 mg), 7-4 (4.40 g), 7-5 (5.17 g), 7-6 (400 mg)]. Fraction 7-1 (440 mg) was further purified by HPLC [MeOH−1% aqueous AcOH (35:65, v/v)] to give oxyphyllenones A (**10**, 26 mg, 0.0063%) and B (**11**, 9 mg, 0.0021%).

The known compounds (**4**−**14**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR, MS) with reported values.[2−9] Free fatty acids such as palmitoleic acid, oleic acid, linoleic acid, linolenic acid, and lignoceric acid were identified by GM−MS with reference to commercial samples purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan).[10]

Oxyphyllol A (1): colorless oil; $[\alpha]_D^{26}$ +17.7° (*c* 0.30, $CHCl_3$); IR (film) ν_{max} 3360, 2923, 1653, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, s, H_3-14), 1.23 (1H, ddd, *J* = 4.0, 6.4, 17.4 Hz, H-9α), 1.25 (1H, ddd, *J* = 6.4, 12.2, 17.7 Hz, H-1α), 1.36 (1H, m, H-1*β*), 1.37 (1H, m, H-3α), 1.38 (1H, m, H-9*β*), 1.63 (3H, br s, H_3-15), 1.80 (1H, m, H-7), 1.82 (3H, br s, H_3-13), 1.84 (1H, m, H-2*β*), 1.96 (1H, d-like, H-8*β*), 2.05 (1H, ddd-like, H-2α), 2.10 (1H, m, H-8α), 2.18 (1H, ddd, *J* = 2.7, 2.8, 13.1 Hz, H-3*β*), 5.03 (1H, dd, *J* = 1.2, 1.2 Hz, H-12a), 5.07 (1H, br s, H-12b), 5.33 (1H, br s, H-6); ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.93 (3H, s, H_3-14), 1.26 (1H, ddd, *J* = 6.5, 12.6, 18.1 Hz, H-9α), 1.34 (1H, m, H-9*β*), 1.35 (2H, m, H_2-1), 1.63 (1H, m, H-3α), 1.64 (3H, d, *J* = 1.9 Hz, H_3-15), 1.92 (1H, d-like, H-7), 1.99 (3H, s, H_3-13), 1.96 (2H, m, H_2-8), 2.09 (1H, m, H-2*β*), 2.28 (1H, m, H-2α), 2.42 (1H, ddd, *J* = 2.4, 2.7, 13.1 Hz, H-3*β*), 5.08, 5.15 (1H each, both s, H_2-12), 5.33 (1H, br s, H-6); ^{13}C NMR data, see Table 3; EIMS *m*/*z* 220 [M]$^+$ (48), 202 (32), 121 (100); HREIMS *m*/*z* 220.1820 (calcd for $C_{15}H_{24}O$, 220.1827).

Oxyphyllol B (2): colorless oil; $[\alpha]_D^{28}$ +10.4° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (3.76); IR (film) ν_{max} 3436, 2938, 1667, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.95 (3H, d, *J* = 6.7 Hz, H_3-15), 1.10 (1H, m, H-6*β*), 1.32 (3H, s, H_3-14), 1.53 (1H,ddd, *J* = 3.0, 12.5, 15.8 Hz, H-8*β*), 1.77 (3H, s, H_3-13), 1.97 (1H, ddd-like, H-6α), 2.00 (1H, ddq, *J* = 4.0, 14.0, 6.7 Hz, H-4), 2.08 (1H, ddd, *J* = 2.7, 2.8, 15.8 Hz, H-8α), 2.25 (1H, dd, *J* = 4.0, 17.4 Hz, H-3*β*), 2.35 (1H, dd, *J* = 14.0, 17.4 Hz, H-3α), 2.79 (1H, dddd, *J* = 2.7, 3.1, 12.5, 12.8 Hz, H-7), 4.45 (1H, dd, *J* = 2.8, 3.0 Hz, H-9), 4.75 (1H, br s, H-12a), 4.77 (1H, dd, *J* = 1.2, 1.5 Hz, H-12b), 5.88 (1H, s, H-1); ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.84 (3H, d, *J* = 6.8 Hz, H_3-15), 1.12 (1H, m, H-6*β*), 1.44 (3H, s, H_3-14), 1.54 (1H,ddd, *J* = 3.1, 13.1, 16.5 Hz, H-8*β*), 1.77 (3H, s, H_3-13), 1.93 (1H, ddq, *J* = 4.9, 13.8, 6.5 Hz, H-4), 2.00 (1H, br d, *J* = ca. 13 Hz, H-6α), 2.24 (1H, ddd, *J* = 2.7, 3.2, 16.5 Hz, H-8α), 2.30 (1H, dd, *J* = 4.9, 17.1 Hz, H-3*β*), 2.39 (1H, dd, *J* = 13.8, 17.1 Hz, H-3α), 3.09 (1H, br dd, *J* = ca.13, 13 Hz, H-7), 4.60 (1H, dd, *J* = 2.7, 3.1 Hz, H-9), 4.85 (1H, dd, *J* = 1.5, 1.6 Hz, H-12a), 4.87 (1H, d, *J* = 0.6 Hz, H-12b), 6.05 (1H, s, H-1); ^{13}C NMR data, see Table 3; EIMS *m*/*z* 234 [M]$^+$ (36), 216 (82), 191 (100); HREIMS *m*/*z* 234.1621 (calcd for $C_{15}H_{22}O_2$, 234.1620).

Oxyphyllol C (3): colorless oil; $[\alpha]_D^{29}$ +6.5° (*c* 1.60, $CHCl_3$); IR (film) ν_{max} 3475, 2932, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.76 (3H, d, *J* = 7.0 Hz, H_3-15), 1.08 (3H, s, H_3-14), 1.25 (1H, m, H-9*β*), 1.30 (1H, dd-like, H-3*β*), 1.40 (2H, m, H_2-6), 1.57 (1H, m, H-2α), 1.58 (1H, m, H-3α), 1.64 (2H, m, H_2-8), 1.73 (3H, s, H_3-13), 1.80 (1H, m, H-4), 2.14 (1H, ddd-like, H-2*β*), 2.24 (1H, m, H-9α), 2.27 (1H, m, H-7), 3.52 (1H, dd, *J* = 2.4, 3.1 Hz, H-1), 4.69, 4.72 (1H each, both br s, H-12); ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.87 (3H, d, *J* = 6.7 Hz, H_3-15), 1.40 (1H, dd-like, H-3*β*), 1.42 (3H, s, H_3-14), 1.52 (1H, dd, *J* = 4.3, 12.8 Hz, H-6α), 1.60 (1H, br d, *J* = ca. 14 Hz, H-9*β*), 1.70 (1H, ddd-like, H-8α), 1.79 (3H, s, H_3-13), 1.86 (1H, br d, *J* = ca. 13 Hz, H-2α), 2.00 (1H, dddd, *J* = 4.3, 12.5, 13.1, 17.7 Hz, H-3α), 2.02 (1H, dd, *J* = 12.5, 12.8 Hz, H-6*β*), 2.16 (1H, dddd, *J* = 4.6, 12.8, 13.2, 17.7 Hz, H-8*β*), 2.38 (1H, m, H-4), 2.45 (1H, m, H-7), 2.62 (1H, dddd, *J* = 3.9, 5.5, 12.7, 13.4 Hz, H-2*β*), 2.70 (1H, ddd, *J* = 4.6, 13.7, 13.8 Hz, H-9α), 3.99 (1H, br s, H-1), 4.80, 4.91 (1H each, both s, H_2-12); ^{13}C NMR data, see Table 3; EIMS *m*/*z* 238 [M]$^+$ (5), 220 (49), 109 (100); HREIMS *m*/*z* 238.1945 (calcd for $C_{15}H_{26}O_2$, 238.1933).

Hydrogenation of Oxyphyllol A (1) and 6. A solution of **1** (3.5 mg, 0.016 mmol) in MeOH (2.0 mL) was treated with H_2 in the presence of 10% Pd−C (5 mg) at room temperature (25 °C) for 2 h. The catalyst was removed by filtration, and the solvent of the filtrate was evaporated under reduced pressure to give a product. The product was purified by HPLC [CH_3CN−H_2O (70:30, v/v)] to give **1a** (2.8 mg, 80%). By a similar procedure, **6** (2.1 mg, 0.010 mmol) in MeOH (2.0 mL) was hydrogenated in the presence of 10% Pd−C (5 mg), and the mixture was stirred at room temperature for 2 h. The reaction mixture was treated as described above to furnish **1a** (2.2 mg, quant.). Compound **1a** was identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H NMR, MS) with reported values.[12]

$NaBH_4$−$CeCl_3$ Reduction of Oxyphyllol B (2). A solution of **2** (8.6 mg, 0.037 mmol) in MeOH (2.0 mL) was treated with $NaBH_4$ (1.0 mg) in the presence of $CeCl_3$·7H_2O (5.0 mg), and the mixture was stirred at 0 °C for 40 min. The reaction mixture was quenched in acetone, then poured into ice−water, and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and then dried over $MgSO_4$ powder. Removal of the solvent under reduced pressure yielded a residue, which was purified by normal-phase silica gel column chromatography [200 mg, *n*-hexane−AcOEt (2:1, v/v)] to give **2a** (8.4 mg, 97%).

2a: colorless oil; $[\alpha]_D^{26}$ +31.2° (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3446, 2926, 1653, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, d, *J* = 6.5 Hz, H_3-15), 1.23 (3H, s, H_3-14), 1.74 (3H, s, H_3-13), 2.72 (1H, dddd, *J* = 2.3, 3.4, 12.4, 13.1 Hz, H-7), 4.27 (1H, ddd, *J* = 2.1, 6.4, 6.7 Hz, H-2), 4.35 (1H, dd, *J* = 3.0, 3.1 Hz, H-9), 4.72, 4.73 (1H each, both s, H_2-12), 5.61 (1H, br

s, H-1); ^{13}C NMR data, see Table 3; EIMS *m/z* 236 $[M]^+$ (27), 218 (30), 135 (100); HREIMS *m/z* 236.1772 (calcd for $C_{15}H_{24}O_2$, 236.1776).

Treatment of 2a with *p*-Bromobenzoyl Chloride. A solution of **2a** (4.0 mg, 0.017 mmol) in dry pyridine (1.0 mL) was treated with *p*-bromobenzoyl chloride (7.5 mg, 0.034 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into ice−water, and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by normal-phase silica gel column chromatography [200 mg, *n*-hexane−AcOEt (7:1, v/v)] to furnish **2b** (6.0 mg, 85%).

2b: colorless oil; $[\alpha]_D^{27}$ −34.1° (*c* 0.20, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\epsilon$) 245 (−3.75); UV (MeOH) λ_{max} (log ϵ) 245 (4.31); IR (film) ν_{max} 2939, 1717, 1269 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.92 (3H, d, J = 6.7 Hz, H_3-15), 1.10 (1H, dd-like, H-6β), 1.26 (3H, s, H_3-14), 1.48 (1H, m, H-8β), 1.72 (1H, dd-like, H-3α), 1.76 (3H, s, H_3-13), 1.92 (1H, m, H-6α), 1.94 (1H, m, H-3β), 2.02 (1H, d-like, H-8α), 2.74 (1H, br dd, J = ca. 12, 13 Hz, H-7), 4.38 (1H, br s, H-9), 4.74, 4.75 (1H each, both br s, H_2-12), 5.57 (1H, br dd, J = ca. 8, 9 Hz, H-2), 5.66 (1H, br s, H-1), 7.58, 7.82 (2H each, both d, J = 8.5 Hz, H−Ph); EIMS *m/z* 418, 420 $[M]^+$ (2), 400, 402 (12), 135 (100); HREIMS *m/z* 418.1145 (calcd for $C_{22}H_{27}O_3Br$, 418.1143).

Conversion from 3 to 3b. A solution of **3** (10.8 mg,0.045 mmol) in dry $CHCl_3$ (1.5 mL) was treated with *m*-chloroperbenzoic acid (*m*-CPBA, 15.5 mg, 0.090 mmol), and the whole mixture was stirred at room temperature for 45 min. The reaction mixture was poured into ice−water, and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by normal-phase silica gel column chromatography [600 mg, *n*-hexane−AcOEt (2:1, v/v)] to furnish the epoxide mixture (**3a**, 8.6 mg, 75%). A solution of the epoxide mixture (**3a**, 2.0 mg, 0.008 mmol) in dry THF (0.5 mL) was treated with $LiAlH_4$ (1.0 mg), and the whole mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with saturated aqueous ether, and the whole mixture was poured into ice−water. The whole was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$ powder. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [100 mg, *n*-hexane−AcOEt (2:1, v/v)] to furnish **3b** (2.0 mg, 99%). Compound **3b** was identified by comparison of the physical data ($[\alpha]_D$, IR, ^{1}H NMR, MS) with reported values.[15]

NO Production from Macrophages Stimulated by LPS. Peritoneal exudate cells were collected from the peritoneal cavities of male ddY mice by washing with 6−7 mL of ice-cold phosphate-buffered saline (PBS), and cells (5×10^5 cells/well) were suspended in 200 μL of RPMI 1640 supplemented with 5% fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) and precultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing the cells with PBS, and the adherent cells (more than 95% macrophages as determined by Giemsa staining) were cultured in fresh medium containing 10 μg/mL LPS and test compound (3−100 μM) for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite in the culture medium using Griess reagent. Cytotoxicity was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay. Briefly, after 20 h incubation with test compounds, MTT (10 μL, 5 mg/mL in PBS) solution was added to the wells. After 4 h culture, the medium was removed, and 2-propanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm (reference, 655 nm). N^G-Monomethyl-L-arginine (L-NMMA) was used as a reference compound. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated by the following formula, and the IC_{50} was determined graphically (N = 4):

$$\text{inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

$A - C$: NO_2^- concentration (μM) [*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)].

Inhibitory Effects on the Release of β-Hexosaminidase from RBL-2H3 Cells. Inhibitory effects of test samples on the release of β-hexosaminidase from RBL-2H3 cells were evaluated by the following procedure.[27] RBL-2H3 cells were grown in Minimum Essential Medium Eagle (MEM) containing fetal calf serum (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL). Before the experiment, cells were dispensed into 24-well plates at a concentration of 2×10^5 cells/well using a medium containing 0.45 μg/mL of anti-DNP IgE and incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then, cells were washed twice with 500 μL of siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 40 mM NaOH, pH 7.2] and incubated in 160 μL of siraganian buffer (supplemented with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA) for an additional 10 min at 37 °C. Then, 20 μL of test sample solution was added to each well and incubated for 10 min, followed by addition of 20 μL of antigen (DNP−BSA, final concentration was 10 μg/mL) at 37 °C for 10 min to stimulate the cells to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μL) was transferred into a 96-well microplate and incubated with 50 μL of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μL of stop solution (0.1 M Na_2CO_3−$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to siraganian buffer (final DMSO concentration was 0.1%).To estimate the spontaneous release of β-hexosaminidase from cells, the same procedure was followed (normal), but without adding antigen and IgE. Blank absorbance of the test material was measured to eliminate interference caused by the color of the test material itself. For this, only test material and substrate were added without adding cell extract (blank). Thus, the inhibition % of the release of β-hexosaminidase by the test material was calculated by the following equation.

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

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

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.

Tranilast, ketotifen fumarate, luteolin, and curcumin were used as reference compounds.

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

References and Notes

(1) This paper is "Bioactive Constituents of Chinese Natural Medicines. VIII." in our serial studies.
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(10) GC−MS conditions were as follows. Column: Hewlett-Packard, HP-INNOWax (Cross-Linked PEG), 30 m × 0.25 mm i.d., film thickness 0.25 mm; column temperature: 200 °C (1 min), 200−250 °C (10 °C/min), 250 °C (30 min); inlet temperature: 250 °C; injection: splitless (purge off time, 1 min); carrier gas: He, 1.2 mL/min (79 kPa); interface temperature: 250 °C; ion source temperature: 200 °C; ionization energy: 70 eV (EI); t_R: palmitoleic acid (9.30 min), oleic acid (12.44 min), linoleic acid (13.59 min), linolenic acid (15.40 min), and lignoceric acid (36.52 min).
(11) The ^{1}H and ^{13}C NMR spectra of **1**−**3** were assigned with the aid of homo- and heterocorrelation spectroscopy (^{1}H−^{1}H, ^{13}C−^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteonuclear multiple bond connectivity (HMBC) experiments.
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