Structures of Novel Norstilbene Dimer, Longusone A, and Three New Stilbene Dimers, Longusols A, B, and C, with Antiallergic and Radical Scavenging Activities from Egyptian Natural Medicine *Cyperus longus*

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The methanolic extract of the whole plant of *Cyperus longus* originating in Egypt was found to show antiallergic effect on ear passive cutaneous anaphylaxis reactions in mice. By bioassay-guided separation, 11 stilbenes and stilbene dimers including a novel norstilbene dimer, longusone A, and three new stilbene dimers, longusols A, B, and C, were isolated. Their structures were elucidated on the basis of chemical and physicochemical evidence. Among the isolates, longusol B (IC_{50}=96 μM), luteolin (3.0 μM), resveratrol (17 μM), piceatannol (24 μM), and cassigarols E (84 μM) and G (84 μM) were found to inhibit the release of β-hexosaminidase, as a marker of antigen-induced degranulations, in rat basophilic leukemia (RBL-2H3) cells. In addition, the methanolic extract and the constituents showed 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (SC_{50}=22 μg/ml and 2.8—29 μM, respectively).

Key words *Cyperus longus*; longusone; longusol; stilbene dimer; antiallergic activity; radical scavenging activity

Egyptian traditional herbal medicine, *Cyperus longus* L. (Cyperaceae), is widely distributed in the Middle Eastern areas. The whole plant of *C. longus* has been used as a diuretic and tonic in Egyptian traditional medicine. As chemical constituents of this natural medicine, several flavonoids and alkaloids have been reported.[1,2] However, the pharmacological effects and bioactive constituents of this natural medicine are left uncharacterized. Previously, we have reported on bioactive constituents from several Egyptian and Yemeni natural medicines such as *Anastatica hierochuntica*,[3,4] *Nigella sativa*,[5,6] *Crinum yemense*,[7] *Dichrocephala integrifolia*,[8] *Citrullus colocynthis*,[9] and *Bryonia cretica*.[10,11] We previously reported that the methanolic extract of the whole plant of *C. longus* exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging[12] and hepatoprotective activities.[13] By bioassay-guided separation, a novel norstilbene dimer, longusone A (**1**) with a tropilene skeleton, and three new stilbene dimers, longusols A (**2**), B (**3**), and C (**4**), and six sesquiterpenes, cyperusols A_1, A_2, B_1, B_2, C, and D, were isolated from the whole plant of *C. longus*.[12,13] As a continuing study, we also found that the methanolic extract from this herbal medicine showed antiallergic effects on ear passive cutaneous anaphylaxis (PCA) reactions in mice, and several constituents also inhibited the release of β-hexosaminidase, as a marker of antigen-induced degranulations, in rat basophilic leukemia (RBL-2H3) cells. This paper deals with the full account of isolation and structure elucidation of four new constituents (**1**—**4**) and antiallergic and radical scavenging activities of several constituents from this herbal medicine.

The methanolic extract from the dried whole plant of *C. longus* originating in Egypt, which showed an antiallergic effect on ear PCA reactions in mice at a dose of 500 mg/kg, *per os* (*p.o.*) (Table 1) and DPPH radical scavenging activity (the concentration required for a 50% reduction of 40 μM DPPH radical, SC_{50}=22 μg/ml, as shown in Table 2). The methanolic extract was partitioned into a mixture of EtOAc and water to furnish the EtOAc- and H_2O-soluble fractions. The EtOAc-soluble fraction, which was obtained as the active fraction, was subjected to silica gel and octadecyl silica (ODS) column chromatography and finally HPLC to furnish a new norstiblene dimer, longusone A (**1**, 0.0010%, from natural medicine) and three new stilbene dimers, longusols A (**2**, 0.00050%), B (**3**, 0.0010%), and C (**4**, 0.00036%), together with a flavone, luteolin[14] (**5**, 0.0033%), two flavan-3-ols, (+)-catechin[15] (**6**, 0.0018%) and (−)-epicatechin[16] (**7**, 0.0048%), and seven stilbenes, resveratrol[17] (**8**, 0.00056%), piceatannol[17] (**9**, 0.0018%), *trans*-scirpusins A[18] (**10**,

Table 1. Effects of the MeOH Extract and Its EtOAc- and H_2O-Soluble Fractions from *C. longus* on Ear PCA Reactions in Mice

Treatment	Dose (mg/kg, *p.o.*)	*n*	Leakage of dye (O.D. at 620 nm)	Inhibition (%)
Normal (PBS)	—	8	0.099±0.013**	—
Control	—	8	0.324±0.035	—
MeOH ext.	500	7	0.154±0.019**	75.6
EtOAc-Soluble fraction	100	5	0.257±0.024	29.9
	200	8	0.165±0.029**	71.0
H_2O-Soluble fraction	1000	6	0.245±0.048	32.4
Normal (PBS)	—	5	0.050±0.009**	—
Control	—	8	0.309±0.054	—
Tranilast	100	8	0.171±0.016**	53.0
	200	8	0.125±0.017**	71.2

Values represent the means±S.E.M. Significantly different from the control group, ** $p<0.01$.

Table 2. DPPH Radical Scavenging Activity of MeOH Extract and Its EtOAc- and H_2O-Soluble Fractions from *C. longus*

Treatment	DPPH radical SC_{50} (μg/ml)[a)]
MeOH extract	22
EtOAc-Soluble fraction	16
H_2O-Soluble fraction	26

a) Concentration required for 50% reduction of 40 μM DPPH radical.

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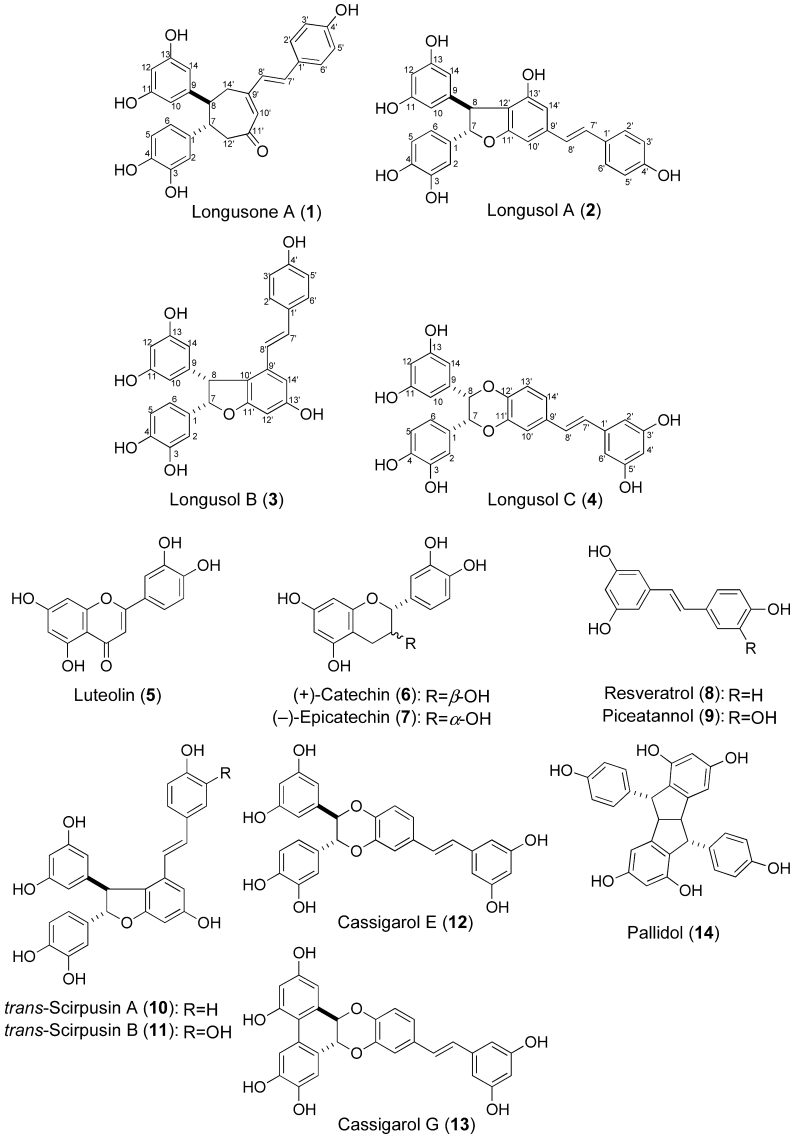

Chart 1

0.028%) and B[18] (**11**, 0.0079%), and cassigarols E[19] (**12**, 0.0013%) and G[19] (**13**, 0.00021%), and pallidol[20] (**14**, 0.00053%).

Structure of Longusone A (1) Longusone A (**1**) was isolated as a pale yellow powder with positive optical rotation ($[\alpha]_D^{24}$ +11.4 in MeOH). The electron ionization (EI)-MS of **1** showed a molecular ion peak at *m*/*z* 444 (M^+) and the molecular formula $C_{27}H_{24}O_6$ of **1** was determined from the molecular ion peaks and by high-resolution EI-MS measurement. The IR spectrum of **1** showed absorption bands at 3346, 1601, 1570, 1458, 1169, and 961 cm^{-1} due to hydroxyl, olefin functions, and benzene ring, while absorption maxima due to strong conjugated chromophore were observed at 283 (log ε 3.97) and 357 (4.39) nm in the UV spectrum. The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 3)[21] spectra of **1** showed signals assignable to two methylenes [δ 3.26 (dd, J=7.0, 14.6 Hz, 14′β-H), 3.35 (dd, J=4.3, 14.6 Hz, 14′α-H), 3.29 (dd, J=7.1, 14.3 Hz, 12′α-H), 3.38 (dd, J=4.2, 14.3 Hz, 12′β-H)], two methine protons [δ 3.47, 3.62 (1H each, both m, H-8 and 7)], an olefin proton [δ 6.45 (1H, s, 10′-H)], *trans*-olefinic protons [δ 6.91, 6.99 (1H each, both d, J=15.8 Hz, 8′ and 7′-H)], and 10 aromatic protons including resveratrol-type dihydroxybenzene (*meta*-coupled A_2B-type protons) [δ 6.76 (2H, d, J=2.1 Hz, 10,14-H), 6.84 (1H, t, J=2.1 Hz, 12-H)], pyrocatechol-type dihydroxybenzene (*ortho*- and *meta*-coupled ABC-type protons) [δ 6.74 (1H, dd, J=2.2, 8.2 Hz, 6-H), 7.06 (1H, d, J=8.2 Hz, 5-H), 7.22 (1H, d, J=2.2 Hz, 2-H)], and *p*-phenol (*ortho*-coupled A_2B_2-type protons) [δ 7.15, 7.46 (2H each, both d, J=8.6 Hz, 3′,5′-H and 2′,6′-H)]. The tropilene structure in **1** was constructed on the basis of ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple bond correlation (HMBC) experiments. Thus, the ^{1}H–^{1}H COSY experiments on **1** indicated the presence of a partial structure shown in bold line in Fig. 1 (C-12′–C-7–C-8–C-14′). In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs (2-H and 7-C; 6-H and 1-C; 7-H and 1, 11′-C; 8-H and 9, 10,14, 9′-C; 10,14-H and 8-C; 2′,6′-H and 7′-C; 7′-H and 2′,6′-C; 8′-H and 9′, 14′-C; 10′-H and 8′, 11′-C; 12′-H_2 and 1, 11′-C; 14′-H_2 and 9, 9′-C). The relative stereostructure of the 7 and 8-positions in the tropilene moiety was characterized by nuclear Over-

Table 3. ^{13}C-NMR Data for Longusone A (**1**) and Longusols A—C (**2**—**4**)

Position	**1**[a)]	**1**[b)]	**2**[b)]	**3**[b)]	**4**[b)]
1	137.2	137.5	135.0	130.2	129.2
2	116.2	115.6	113.4	115.2	115.7
3	147.0	145.7	146.1	145.1	145.9
4	145.6	144.4	146.3	145.2	146.2
5	116.3	116.1	116.1	115.3	115.7
6	119.5	119.8	118.2	119.6	120.4
7	46.9	47.2	94.5	90.7	80.0
8	52.4	52.9	56.6	53.6	80.4
9	149.0	148.6	146.6	143.5	140.0
10	107.1	107.3	106.9	109.1	107.1
11	160.2	159.0	159.4	158.5	159.1
12	102.4	101.6	101.8	101.6	103.1
13	160.2	159.0	159.4	158.5	159.1
14	107.1	107.3	106.9	109.1	107.1
1′	128.3	129.1	130.3	130.2	141.1
2′	129.6	129.9	128.6	128.6	106.0
3′	116.7	116.5	116.3	116.2	159.8
4′	160.1	159.5	158.1	158.1	103.0
5′	116.7	116.5	116.3	116.2	159.8
6′	129.6	129.9	128.6	128.6	106.0
7′	135.4	137.4	129.2	130.6	128.6
8′	129.0	128.9	126.8	123.8	129.1
9′	155.2	158.3	141.5	136.7	133.0
10′	131.5	130.7	99.3	121.6	115.8
11′	201.6	205.3	163.2	162.4	145.6
12′	50.5	50.6	115.2	97.0	145.1
13′			155.5	159.1	118.2
14′	34.5	35.0	107.7	105.0	121.3

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

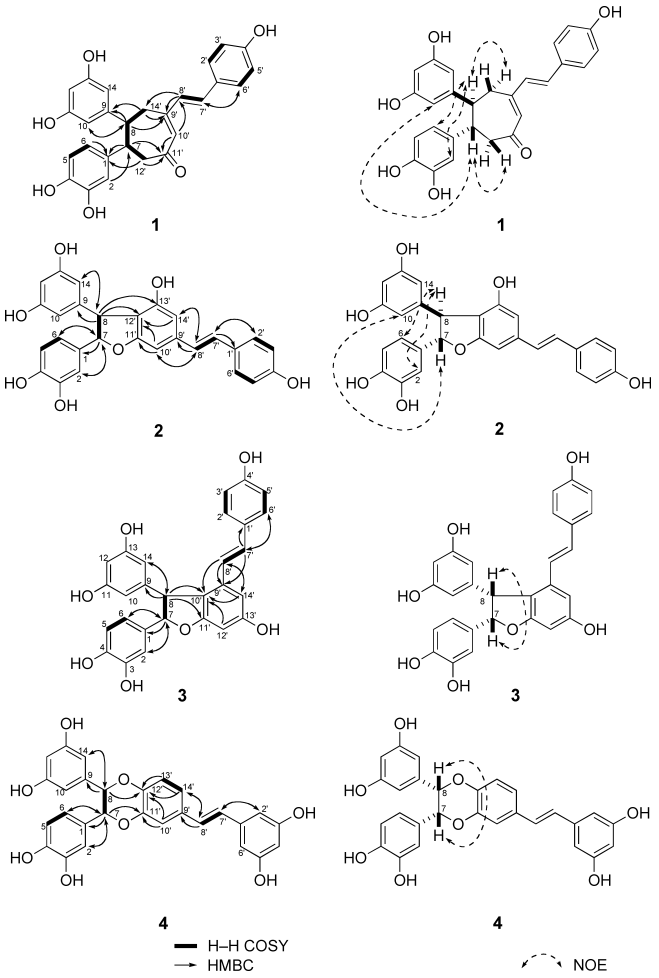

Fig. 1. ^{1}H–^{1}H COSY, HMBC, and NOE Correlations of **1**—**4**

hauser effect spectroscopy (NOESY) experiment on **1**, in which the NOE correlations were observed between the following proton pairs (7-H and 10,14-H, 12′β-H; 8-H and 2-H, 6-H, 14′α-H). On the basis of this evidence, the structure of **1** was determined as shown.

Structures of Longusols A (2), B (3), and C (4) Longusol A (**2**) was isolated as a pale yellow powder with positive optical rotation ($[\alpha]_D^{24}$ +93.0 in MeOH). The EI-MS of **2** showed a molecular ion peak at *m/z* 470 (M^+) and the molecular formula $C_{28}H_{22}O_7$ of **2** was determined from the molecular ion peaks and by high-resolution EI-MS measurement. The IR spectrum of **2** showed absorption bands at 3389, 1605, 1512, 1431, 1156, and 961 cm^{-1} due to hydroxyl, olefin functions, and benzene ring, while its UV spectrum showed absorption maxima at 285 (sh, log ε 4.20), 3.08 (sh, 4.36), and 326 (4.39) nm due to a stilbene chromophore. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 3)[21] spectra of **2** showed signals assignable to dihydrobenzofuran moieties [δ 4.33, 5.25 (1H each, both d, J=5.0 Hz, 8 and 7-H)], *trans*-olefinic protons [δ 6.87, 7.00 (1H each, both d, J=16.3 Hz, 8′ and 7′-H)], and 12 aromatic protons including A_2B-type protons {[δ 6.12 (2H, d, J=2.0 Hz, 10,14-H), 6.14 (1H, t, J=2.0 Hz, 12-H)], *meta*-coupled proton pairs [δ 6.50, 6.62 (1H each, both br s, 14′ and 10′-H)], ABC-type protons [δ 6.65 (1H, dd, J=2.0, 8.2 Hz, 6-H), 6.72 (1H, d, J=8.2 Hz, 5-H), 6.76 (1H, d, J=2.0 Hz, 2-H)], and A_2B_2-type protons [δ 6.76, 7.36 (2H each, both d, J=8.4 Hz, 3′,5′-H and 2′,6′-H)]}. The proton and carbon signals of **2** were similar to those of **10**,[18] except for the signal due to the benzene ring in the benzofuran moiety. The ^{1}H–^{1}H COSY experiment on **2** indicated the presence of four partial structures written in bold lines, as shown in Fig. 1. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **2** (2,6-H and 7-C; 7-H and 1, 2,6-C; 8-H and 9, 10,14, 12′-C; 10,14-H and 8-C; 2′,6′-H and 7′-C; 7′-H and 1′, 2′,6′-C; 8′-H and 9′, 10′,14′-C; 10′,14′-H and 8′, 11′, 12′-C). Furthermore, the relative stereostructure of **2** was characterized on the basis of the NOESY experiment, in which correlations were observed between the following proton pairs (2,6-H and 8-H; 10,14-H and 7-H). Consequently, the stereostructure of **2** was determined as shown.

Longusol B (**3**) was also isolated as a pale yellow powder with positive optical rotation ($[\alpha]_D^{26}$ +64.9 in MeOH). The IR spectrum of **3** showed absorption bands at 3346, 1605, 1509, 1458, 1237, and 1159 cm^{-1} ascribable to hydroxyl and olefin functions and aromatic ring. The UV spectrum of **3** showed absorption maxima at 288 nm (sh, log ε 4.38), 310 nm (sh, 4.33), and 322 nm (4.31) assignable to a stilbene chromophore. The molecular formula $C_{28}H_{22}O_7$ of **3** was determined by high-resolution EI-MS measurement. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 3) spectra[21] of **3** were superimposable on those of **10**,[18] except for the signals due to the dihydrofuran moiety [δ 4.55, 5.76 (1H each, both d, J=8.4 Hz, 8, 7-H)]. The planar structure of **3** was determined by ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1) to be the same as that of **10**. In the NOESY experiment of **3**, the NOE correlation was observed between the 7-proton and the 8-

proton, so that the relative stereostructure of **3** was elucidated to be the *cis*-type isomer in dihydrofuran part of **10**.

Longusol C (**4**) was isolated as a pale yellow powder with positive optical rotation ($[\alpha]_D^{24}$ +9.4 in MeOH). In the positive-ion fast atom bombardment (FAB)-MS of **4**, a quasimolecular ion peak was observed at *m/z* 487 $(M+H)^+$. The molecular formula of **4** was determined to be $C_{28}H_{22}O_8$ by high resolution FAB-MS measurement. In IR spectrum of **4**, absorption bands at 3410, 1649, 1619, 1509, and 1157 cm^{-1} due to hydroxyl and olefin functions and aromatic ring were observed, while its UV spectrum showed absorption maxima at 307 nm (sh, log ε 3.89) and 323 nm (3.95) ascribable to a stilbene chromophore. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 3) spectra[21] of **4** showed signals assignable to a 1,4-dioxane moiety [δ 5.24, 5.29 (1H each, both d, J=3.0 Hz, 8, 7-H)], *trans*-olefinic protons [δ 6.87, 6.96 (1H each, both d, J=16.4 Hz, 8′, 7′-H)], and 12 aromatic protons including two A_2B-type protons [δ 6.06 (2H, d, J=2.1 Hz, 10,14-H), 6.11 (1H, t, J=2.1 Hz, 12-H), 6.17 (1H, t, J=2.1 Hz, 4′-H), 6.47 (2H, d, J=2.1 Hz, 2′,6′-H)], and two ABC-type protons [δ 6.39 (1H, dd, J=2.2, 8.1 Hz, 6-H), 6.56 (1H, d, J=2.2 Hz, 2-H), 6.58 (1H, d, J=8.1 Hz, 5-H), 6.95 (1H, d, J=8.4 Hz, 13′-H), 7.09 (1H, dd, J=2.1, 8.4 Hz, 14′-H), 7.14 (1H, d, J=2.1 Hz, 10′-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **4** were found to be very similar to those of **12**,[19] except for the signals due to the 1,4-dioxane part. The planar structure of **4** was constructed on the basis of the ^{1}H–^{1}H COSY and HMBC experiments as shown in Fig. 1. Finally, the relative stereostrucure of **4** was elucidated by a NOESY experiment, in which the NOE correlation was observed between the 7-proton and 8-proton. Therefore, the stereostructure of **4** was determined to be *cis*-type isomer in 1,4-dioxane part of **12**.

Effect on Ear Passive Cutaneous Anaphylaxis (PCA) Reactions in Mice Type I allergy is induced by certain types of antigens such as foods, dust mites, medicines, cosmetics, mold spores and pollen. This class of antigens induces the production of antigen-specific immunoglobulin E (IgE) antibodies that bind to receptors on mast cells or basophils. The early phase of type I allergy, which is degranulation of mast cells or basophils, occurs within minutes and then the mediators such as histamine and serotonin stored in granulates are released from the cells. These mediators induce vasodilation, mucous secretion and bronchoconstriction. Passive cutaneous anaphylaxis (PCA) reaction is a useful and popular experimental model for the early phase of type I allergy and the intensity of the PCA reaction is usually evaluated by the amount of Evans blue dye leaked at the IgE injection site.[22] As shown in Table 1, the methanolic extract of *C. longus* and its EtOAc-soluble fraction were found to inhibit PCA reactions in mice as doses of 500 and 200 mg/kg. *p.o.*, respectively.

Inhibitory Effects of the Constituents from *C. longus* on the Release of β-Hexosaminidase in RBL-2H3 Cells Histamine, which is released from mast cells on stimulation by an antigen or a degranulation inducer, is usually determined as a degranulation marker *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 immunologi-

Table 4. Inhibitory Effects of Constituents from *C. longus* on the Release of β-Hexosaminidase from RBL-2H3 Cells

Treatment	Inhibition (%)				IC_{50} (μM)
	0	10	30	100 (μM)	
Longusone A (**1**)	0.0±1.3	3.4±0.6	2.9±0.5	18.6±0.7**	>100
Longusol A (**2**)	0.0±2.2	4.2±0.4	11.3±2.5**	12.7±1.0**	>100
Longusol B (**3**)	0.0±2.8	10.2±2.8*	22.0±2.0**	28.2±1.5**	>100
Longusol C (**4**)	0.0±1.3	14.4±1.4**	22.1±1.3**	51.6±2.4**	96
(+)-Catechin[26] (**6**)					>100
(−)-Epicatechin[26] (**7**)					>100
Resveratrol[27] (**8**)	0.0±5.7	26.7±4.3**	71.0±2.6**	102.1±0.3**	17
Piceatannol[27] (**9**)	0.0±3.6	6.6±7.3	78.7±4.0**	105.1±0.8**	24
trans-Scirpusin A (**10**)	0.0±1.2	9.4±2.2	10.5±2.0*	17.3±1.9**	>100
trans-Scirpusin B (**11**)	0.0±2.1	7.0±1.6	9.2±2.2	13.4±1.7**	>100
Cassigarol E (**12**)	0.0±3.3	20.7±1.8**	17.5±1.6**	60.0±0.8**	84
Cassigarol G (**13**)	0.0±2.8	11.6±1.7**	12.0±2.4**	61.1±1.5**	84
Pallidol (**14**)	0.0±2.3	5.3±1.2	4.8±1.2	5.2±1.6	>100

Treatment	Inhibition (%)				IC_{50} (μM)
	0	1	3	10 (μM)	
Luteolin[26] (**5**)	0.0±2.4	14.1±2.7**	49.2±2.3**	93.4±1.3**	3.0

Treatment	Inhibition (%)				IC_{50} (μM)
	0	30	100	300 (μM)	
Ketotifen fumarate	0.0±5.2	10.8±5.4	30.6±4.8**	68.9±2.4**	176
Tranilast	0.0±3.5	25.4±4.3**	44.5±4.4**	75.3±0.2**	112

Values represent the means±S.E.M. (n=4). Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

Table 5. DPPH Radical Scavenging Activity of Constituents from *C. longus*

Treatment	DPPH radical SC_{50} (μM)[a)]
Longusone A (**1**)	4.6
Longusol A (**2**)	9.3
Longusol B (**3**)	4.3
Longusol C (**4**)	5.0
Luteolin[29)] (**5**)	4.8
(+)-Catechin (**6**)	6.0
(−)-Epicatechin (**7**)	7.0
Resveratrol[35)] (**8**)	24
Piceatannol[35)] (**9**)	11
trans-Scirpusin A (**10**)	8.2
trans-Scirpusin B (**11**)	2.8
Cassigarol E (**12**)	3.2
Cassigarol G (**13**)	4.5
Pallidol (**14**)	29
α-Tocopherol	11
Gallic acid	3.9

a) Concentration required for 50% reduction of 40 μM DPPH radical. Values in parentheses represent the inhibition (%) at 100 μM.

cally activated.[23,24)] Therefore it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells. In our characterization studies on antiallergic constituents from the natural medicines,[9,16,25)] effects of the EtOAc-soluble fraction of the methanolic extract from *C. longus* was found to inhibit the release of β-hexosaminidase in RBL-2H3 cells (inhibition: 75.6±4.8% at 300 μg/ml). Among the isolates, longusol C (**4**, IC_{50}=96 μM) and cassigarols E (**12**, 84 μM) and G (**13**, 84 μM) inhibited the release of β-hexosaminidase, whose activities were stronger than those of antiallergic compounds, tranilast (IC_{50}=112 μM) and ketotifen fumarate (176 μM). However, a flavone constituent, luteolin (**5**, 3.0 μM),[26)] and stilbene monomer constituents, resveratrol (**8**, 17 μM)[27)] and piceatannol (**9**, 24 μM),[27)] were more stronger than those of stilbene dimmers as shown in Table 4.

DPPH Radical Scavenging Activity of the Constituents from *C. longus* Active oxygen species and free radicals react with biomolecular constituents (*e.g.* lipid, protein, and DNA) to cause certain clinical diseases, such as cerebral ischemia, atherosclerosis, inflammation, diabetes, and cancer.[28)] The DPPH radical, which is stable and shows an absorption at 517 nm, has been used as a convenient tool for the radical scavenge assay, and this assay is independent of any enzyme activity.[29,30)] When this compound accepts an electron or hydrogen radical to become a more stable compound, the absorption vanishes. In our studies on antioxidative principles from natural medicines,[31)] DPPH radical scavenging activities of the methanolic extract of *C. longus* and isolated constituents were examined. As shown in Table 5, *trans*-scirpusin B (**11**) was found to show the most potent DPPH radical scavenging activity (SC_{50}=2.8 μM) and the scavenging activities of stilbene dimers (**1**—**4** and **10**—**13**, 2.8—9.3 μM) were stronger than those of monomers (**8**, 24 μM and **9**, 11 μM), except for pallidol (**14**, 29 μM).

Experimental

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel $60F_{254}$ (Merck, Ltd., Japan, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, Ltd., Japan, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, Ltd., Japan, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material This item was described in a previous report.[12)]

Extraction and Isolation Dried whole plants of *C. longus* L. (3.2 kg) were finely cut and extracted three times with methanol (MeOH) under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the methanolic extract (589 g, 18.4%). The MeOH extract (570 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture, and removal of the solvent *in vacuo* from the EtOAc and H_2O-soluble fractions yielded 104 g (3.4%) and 466 g (15.0%) of residues, respectively. The EtOAc-soluble fraction (94 g) was subjected to ordinary-phase silica gel column chromatography [2.0 kg, *n*-hexane–EtOAc (10 : 1→5 : 1→2 : 1→1 : 1, v/v)→$CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give seven fractions [Fr. 1 (37.54 g), Fr. 2 (6.93 g), Fr. 3 (14.92 g), Fr. 4 (19.45 g), Fr. 5 (6.25 g), Fr. 6 (2.30 g), and Fr. 7 (5.61 g)]. Fraction 3 (12.98 g) was subjected to ordinary-phase silica gel column chromatography [400 g, *n*-hexane–EtOAc (2 : 1, v/v)→$CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give nine fractions [Fr. 3-1 (0.33 g), Fr. 3-2 (1.57 g), Fr. 3-3 (7.86 g), Fr. 3-4 (0.35 g), Fr. 3-5 (0.21 g), Fr. 3-6 (0.12 g), Fr. 3-7 (0.95 g), Fr. 3-8 (0.87 g), and Fr. 3-9 (0.22 g)]. Fr. 3-7 (0.95 g) was subjected to reversed-phase silica gel column chromatography [30 g, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] to afford four fractions [Fr. 3-7-1 (194 mg), Fr. 3-7-2 (24 mg), Fr. 3-7-3 (622 mg), and Fr. 3-7-4 (55 mg)]. Fr. 3-7-1 (194 mg) was purified by HPLC [YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–H_2O (30 : 70, v/v)] to give (+)-catechin (**6**, 43.3 mg, 0.0018%) and (−)-epicatechin (**7**, 118.0 mg, 0.0048%). Fr. 3-7-3 (622 mg) was also purified by HPLC [MeOH–H_2O (50 : 50, v/v)] to give longusone A (**1**, 23.1 mg, 0.00095%), longusol A (**2**, 12.2 mg, 0.00050%), and *trans*-scirpusin A (**10**, 520.0 mg, 0.022%). Fr. 3-8 (0.87 g) was subjected to reversed-phase silica gel column chromatography [25 g, MeOH–H_2O (30 : 70→45 : 55→60 : 40, v/v)→MeOH] to afford seven fractions [Fr. 3-8-1 (28 mg), Fr. 3-8-2 (265 mg), Fr. 3-8-3 (270 mg), Fr. 3-8-4 (138 mg), Fr. 3-8-5 (69 mg), Fr. 3-8-6 (25 mg), and Fr. 3-8-7 (45 mg)]. Fr. 3-8-2 (265 mg) was further separated by HPLC [MeOH–H_2O (45 : 55, v/v)] to give *trans*-scirpusin B (**11**, 191.6 mg, 0.0079%) and pallidol (**14**, 13.0 mg, 0.00053%). Fr. 3-8-3 (270 mg) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give *trans*-scirpusin A (**10**, 150.9 mg, 0.0064%), resveratrol (**8**, 13.7 mg, 0.00056%), and piceatannol (**9**, 43.1 mg, 0.0018%). Fr. 3-8-4 (138 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give longusol B (**3**, 24.3 mg, 0.0010%) and luteolin (**5**, 80.8 mg, 0.0033%). Fr. 3-8-5 (69 mg) was separated by HPLC [MeOH–H_2O (55 : 45, v/v)] to give longusol C (**4**, 8.7 mg, 0.00036%) and cassigarol E (**12**, 32.4 mg, 0.0013%). Fr. 3-8-6 (25 mg) was purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to afford cassigarol G (**13**, 5.3 mg, 0.00021%). The known compounds (**5**—**14**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values[12,14)] or those of commercial samples.[13)]

Longusone A (**1**): A pale yellow powder, $[\alpha]_D^{24}$ +11.4 (c=1.20, MeOH). High-resolution EI-MS: Calcd for $C_{27}H_{24}O_6$ (M^+) 444.1573; Found 444.1569. UV [MeOH, nm, (log ε)]: 283 (3.97), 357 (4.39). IR (KBr, cm^{-1}): 3346, 1601, 1570, 1458, 1169, 961. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 3.26 (1H, dd, J=7.0, 14.6 Hz, 14′β-H), 3.29 (1H, dd, J=7.1, 14.3 Hz, 12′α-H), 3.35 (1H, dd, J=4.3, 14.6 Hz, 14′α-H), 3.38 (1H, dd, J=4.2, 14.3 Hz, 12′β-H), 3.47, 3.62 (1H each, both m, 8, 7-H), 6.45 (1H, s, 10′-H), 6.74 (1H, dd, J=2.2, 8.2 Hz, 6-H), 6.76 (2H, d, J=2.1 Hz, 10, 14-H), 6.84 (1H, t, J=2.1 Hz, 12-H), 6.91, 6.99 (1H each, both d, J=15.8 Hz, 8′, 7′-H), 7.06 (1H, d, J=8.2 Hz, 5-H), 7.22 (1H, d, J=2.2 Hz, 2-H), 7.15, 7.46 (2H each, both d, J=8.6 Hz, 3′, 5′-H and 2′, 6′-H). ^{1}H-NMR (500 MHz, CD_3OD) δ: 2.98, 3.10 (2H each, both m, 12′, 14′-H_2), 3.05, 3.16 (1H each, both m, 8, 7-H), 6.05 (1H, t, J=2.1 Hz, 12-H), 6.08 (2H, d, J=2.1 Hz, 10, 14-H), 6.21

(1H, s, 10′-H), 6.37 (1H, dd, J=2.0, 8.1 Hz, 6-H), 6.51 (1H, d, J=2.0 Hz, 2-H), 6.58 (1H, d, J=8.1 Hz, 5-H), 6.66, 6.83 (1H each, both d, J=16.1 Hz, 7′, 8′-H), 6.73, 7.27 (2H each, both d, J=8.6 Hz, 3′,5′-H and 2′,6′-H). ^{13}C-NMR (125 MHz, pyridine-d_5, CD_3OD) δ_C: given in Table 3. EI-MS (*m/z*, %): 444 (M^+, 18), 429 (10), 324 (19), 309 (6), 120 (58), 94 (100), 66 (15).

Longusol A (**2**): A pale yellow powder, $[\alpha]_D^{24}$ +93.0 (c=0.60, MeOH). High-resolution EI-MS: Calcd for $C_{28}H_{22}O_7$ (M^+) 470.1365; Found 470.1352. UV [MeOH, nm, (log ε)]: 285 (4.20), 308 (sh, 4.36), 326 (4.39). IR (KBr, cm^{-1}): 3389, 1605, 1512, 1431, 1156, 961. ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.33, 5.25 (1H each, both d, J=5.0 Hz, 8, 7-H), 6.12 (2H, d, J=2.0 Hz, 10, 14-H), 6.14 (1H, t, J=2.0 Hz, 12-H), 6.50, 6.62 (1H each, both br s, 14′, 10′-H), 6.65(1H, dd, J=2.0, 8.2 Hz, 6-H), 6.72 (1H, d, J=8.2 Hz, 5-H), 6.76 (1H, d, J=2.0 Hz, 2-H), 6.76, 7.36 (2H each, both d, J=8.4 Hz, 3′, 5′-H and 2′, 6′-H), 6.87, 7.00 (1H each, both d, J=16.3 Hz, 8′, 7′-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 3. EI-MS (*m/z*, %): 470 (M^+, 2), 228 (2), 124 (6), 110 (20), 94 (100), 66 (23).

Longusol B (**3**): A pale yellow powder, $[\alpha]_D^{26}$ +64.9 (c=0.40, MeOH). High-resolution EI-MS: Calcd for $C_{28}H_{22}O_7$ (M^+) 470.1365; Found 470.1366. UV [MeOH, nm, (log ε)]: 288 (4.38), 310 (sh, 4.33), 322 (4.31). IR (KBr, cm^{-1}): 3346, 1605, 1509, 1458, 1237, 1159. ^{1}H-NMR (500 MHz, CD_3OD) δ: 4.55, 5.76 (1H each, both d, J=8.4 Hz, 8, 7-H), 5.78 (2H, d, J=2.2 Hz, 10, 14-H), 6.31 (1H, d, J=1.9 Hz, 12′-H), 6.50 (1H, t, J=2.2 Hz, 12-H), 6.50 (1H, dd, J=1.9, 8.4 Hz, 6-H), 6.56 (1H, d, J=8.4 Hz, 5-H), 6.61 (1H, d, J=1.9 Hz, 2-H), 6.63 (1H, d, J=1.9 Hz, 14′-H), 6.64, 6.85 (1H each, both d, J=16.2 Hz, 8′, 7′-H), 6.67, 7.11 (2H each, both d, J=8.6 Hz, 3′, 5′-H and 2′, 6′-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 3. EI-MS (*m/z*, %): 470 (M^+, 6), 376 (2), 347 (1), 244 (6), 110 (14), 94 (100), 66 (15).

Longusol C (**4**): A pale yellow powder, $[\alpha]_D^{24}$ +9.4 (c=0.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{28}H_{23}O_8$ $(M+H)^+$ 487.1393; Found 487.1407. UV [MeOH, nm, (log ε)]: 307 (sh, 3.89), 323 (3.95). IR (KBr, cm^{-1}): 3410, 1649, 1619, 1509, 1430, 1157. ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.24, 5.29 (1H each, both d, J=3.0 Hz, 8, 7-H), 6.06 (2H, d, J=2.1 Hz, 10, 14-H), 6.11 (1H, t, J=2.1 Hz, 12-H), 6.17 (1H, t, J=2.1 Hz, 4′-H), 6.39 (1H, dd, J=2.2, 8.1 Hz, 6-H), 6.47 (2H, d, J=2.1 Hz, 2′, 6′-H), 6.56 (1H, d, J=2.2 Hz, 2-H), 6.58 (1H, d, J=8.1 Hz, 5-H), 6.87, 6.96 (1H each, both d, J=16.4 Hz, 8′, 7′-H), 6.95 (1H, d, J=8.4 Hz, 13′-H), 7.09 (1H, dd, J=2.1, 8.4 Hz, 14′-H), 7.14 (1H, d, J=2.1 Hz, 10′-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 3. Positive-ion FAB-MS: *m/z* 487 $(M+H)^+$.

Animals Male ddY mice weighing about 25—30 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 24—26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effects on PCA Reactions in Mice Experiments on the effects of the methanolic extract and its fractions from the whole plants of *C. longus* on ear PCA reactions were performed according to the method reported previously[32] with a slight modification. Briefly, 10 μl of anti-dinitrophenyl (DNP) IgE diluted in phosphate buffered saline (PBS) (20 μg/ml) or PBS alone (normal group) was injected intradermally into both ears of male ddY mice. Forty-seven hours later, test compounds suspended in 5% acacia solution were administrated orally. After 1 h, 0.25 ml of PBS which contained 2% Evans blue and 0.25 mg of DNP-bovine serum albumin (BSA) was injected into the vein. Thirty minutes later, mice were killed by cervical dislocation and the both ears were removed and incubated with 1 M KOH solution overnight at 37 °C to dissolve them. The solution was then mixed with 4.5 ml of a mixture of acetone–0.2 M H_3PO_4 (13 : 5, v/v). After centrifugation at 4000 rpm for 10 min, absorbance of the supernatant was measured at 620 nm using a spectrophotometer (Beckmann DU 530). An antiallergic agent, tranilast,[33] was used as a reference compound.

Inhibitory Effect on the Release of β-Hexosaminidase in RBL-2H3 Cells The 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 a method reported previously.[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-Aldrich, Japan) 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] supplemented with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA, and incubated in 160 μl of the buffer for an additional 10 min at 37 °C. Aliquots (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:

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

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

DPPH Radical Scavenging Activity The free radical scavenging activity of test samples were assessed using the DPPH radical.[29,30] An ethanol solution of DPPH (200 μM, 0.5 ml) radical was mixed with different concentrations of each test compound (0—100 μM, 1.0 ml) in ethanol and 0.1 M acetate buffer (pH 5.5, 1.0 ml), and the absorbance change at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, SC_{50}) of 40 μM DPPH radical solution was determined graphically.

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis.

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