HETEROCYCLES, Vol. 57, No. 11, 2002, pp. 1983 - 1988, Received, 12th August, 2002

STRUCTURES AND RADICAL SCAVENGING ACTIVITIES OF NOVEL NOR-STILBENE DIMER, LONGUSONE A, AND NEW STILBENE DIMERS, LONGUSOLS A, B, AND C, FROM EGYPTIAN HERBAL MEDICINE *CYPERUS LONGUS*

Toshio Morikawa, Fengming Xu, Hisashi Matsuda, and Masayuki Yoshikawa*

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan

Abstract — The methanolic extract of the whole plant of *Cyperus longus* originating in Egypt was found to show scavenging activity for DPPH radical. By bioassay–guided separation, a nor-stilbene dimer, longusone A, with a tropilene structure and three stilbene dimers, longusols A, B, and C, were isolated as the active constituents together with 10 phenolic compounds. The structures of new compounds were elucidated on the basis of chemical and physicochemical evidence.

The Cyperaceae plant, *Cyperus longus* L., is widely distributed in the Middle Eastern areas and 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] However, the pharmaceutical activity and bioactive constituents of this natural medicine are left uncharacterized. In the course of our characterization studies on the bioactive constituents from natural medicines, the methanolic extract from the dried whole plant of *C. longus* was found to show scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. By bioassay–guided separation, a novel nor-stilbene dimer termed longusone A (**1**) with a tropilene structure and three new stilbene dimers named longusols A (**2**), B (**3**), and C (**4**) were isolated from the methanolic extract together with 10 phenolic constituents. This paper deals with the isolation and structure elucidation of four new constituents and the radical scavenging activities of 14 phenolic constituents from this natural medicine.

The methanolic extract from the dried whole plant of *C. longus* originating in Egypt showed scavenging activity for DPPH radical as shown in Table 1. The methanolic extract was partitioned into a mixture of ethyl acetate (EtOAc) and water to provide the EtOAc–soluble portion and H_2O–soluble portion. The EtOAc–soluble portion, which exhibited potent scavenging activity, was subjected to silica gel and ODS column chromatographies and finally HPLC to give longusone A (**1**, 0.0010% from natural medicine), longusols A (**2**, 0.0005%), B (**3**, 0.0010%), and C (**4**, 0.0004%) together with luteolin (**5**,[2] 0.0033%), (+)-catechin (**6**,[3] 0.0018%), (–)-epicatechin (**7**,[3] 0.0048%), resveratrol (**8**,[4] 0.0006%), piceatannol (**9**,[4] 0.0018%), *trans*-scirpusins A (**10**,[5] 0.028%) and B (**11**,[5] 0.0079%), cassigarols E (**12**,[6] 0.0013%) and G (**13**,[6] 0.0002%), and pallidol (**14**,[7] 0.0005%).

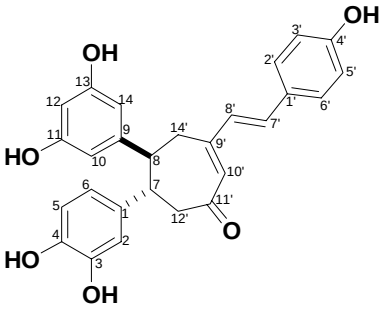
Longusone A (**1**),[8] a pale yellow powder, $[\alpha]_D^{24}$ +11.4°(*c*=1.20, MeOH), $C_{27}H_{24}O_6$, EI-MS *m/z*: 444 (M^+, 8) and 94 (base peak), showed absorption maxima at 283 nm (log ε 4.0) and 357 nm (4.4) suggestive of a strong conjugated chromophore in the UV spectrum. The IR spectrum of **1** showed absorption bands at 3346, 1601, 1570, 1458, 1169, and 961 cm^{-1} ascribable to hydroxyl, conjugated

Table 1. DPPH Radical Scavenging Activities of MeOH Extract and EtOAc- and H_2O-Soluble Portions from *C. longus*

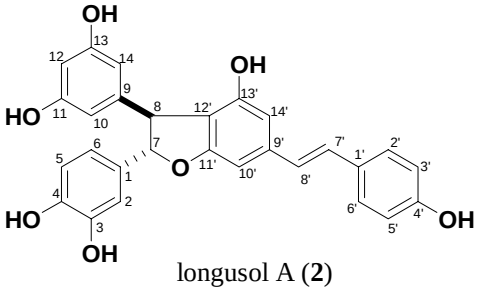
	DPPH radical SC_{50}[a]
MeOH extract	22
EtOAc-soluble portion	16
H_2O-soluble portion	26

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

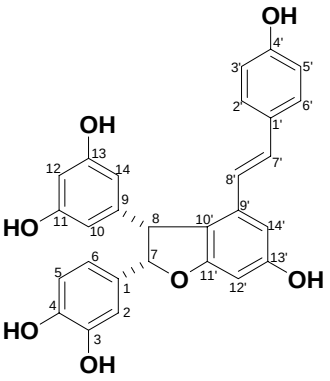
carbonyl, aromatic ring, and olefin functions. The ^{1}H- and ^{13}C-NMR (C_5D_5N, Table 2) spectra[9] of **1** showed signals assignable to two methylenes [δ 3.26 (dd, *J*=7.0, 14.6 Hz, 14'β-H), 3.35 (dd, *J*=4.3, 14.6 Hz, 14'α-H), 3.29 (dd, *J*=7.1, 14.3



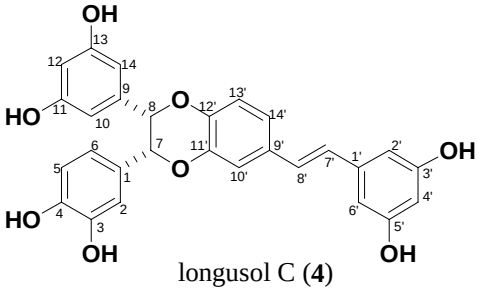
longusone A (**1**)



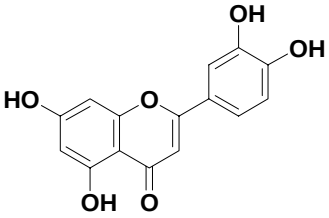
longusol A (**2**)



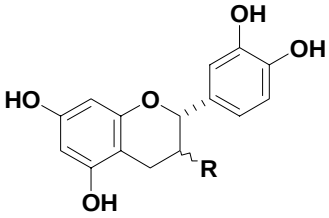
longusol B (**3**)



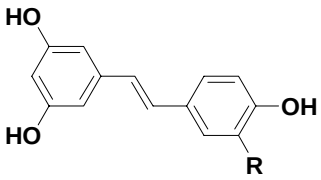
longusol C (**4**)



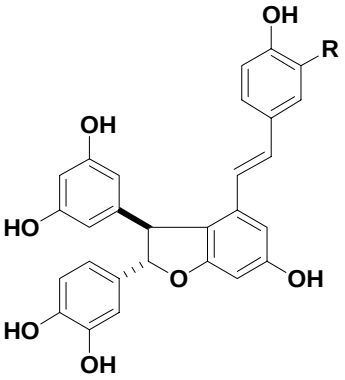
luteolin (**5**)



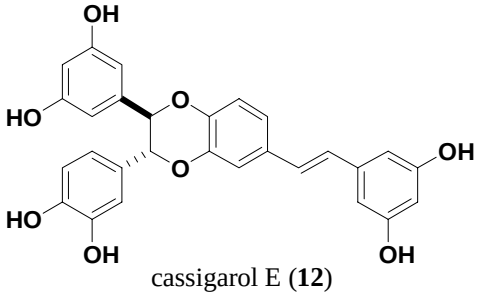
(+)-catechin (**6**): R=β-OH
(–)-epicatechin (**7**): R=α-OH



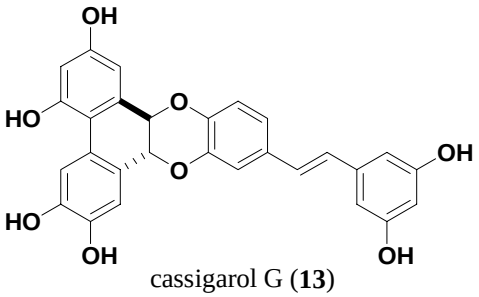
resveratrol (**8**): R=H
piceatannol (**9**): R=OH



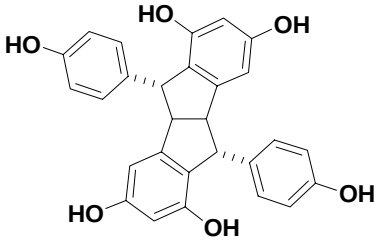
trans-scirpusin A (**10**): R=H
trans-scirpusin B (**11**): R=OH



cassigarol E (**12**)



cassigarol G (**13**)



pallidol (**14**)

Chart 1

Hz, 12'α-H), 3.38 (dd, *J*=4.2, 14.3 Hz, 12'β-H)], two benzyl 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 and 14-H), 6.84 [δ (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 H–H COSY and HMBC experiments. Thus, the H–H COSY experiments on **1** indicated the presence of a partial structure shown in bold line in Figure 1 (C-12'—C-7—C-8—C-14'). In the HMBC experiment, long-range correlations were observed between the 8, 8', 14'-protons and 9'-carbon and between the 7, 10', 12'-protons and 11'-carbon. The substitution pattern of the tropilene ring was also clarified by HMBC experiment, which showed long-range correlations between the 7-proton and 1-carbon, between the 8-proton and 9-carbon, between the 8'-proton and 9'-carbon, and between the 7'-proton and 2', 6'-carbons. The relative stereostructure of the 7 and 8-positions in the tropilene moiety was characterized by nuclear Overhauser 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.

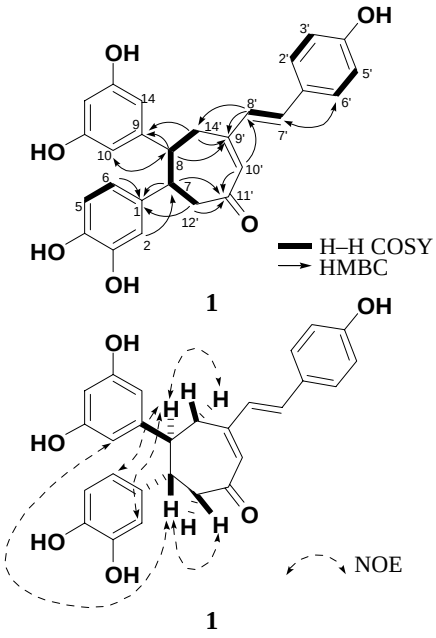


Figure 1

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

	1[a]	**1**[b]	**2**[b]	**3**[b]	**4**[b]		**1**[a]	**1**[b]	**2**[b]	**3**[b]	**4**[b]
C-1	137.2	137.5	135.0	130.2	129.2	C-1'	128.3	129.1	130.3	130.2	141.1
C-2	116.2	115.6	113.4	115.2	115.7	C-2'	129.6	129.9	128.6	128.6	106.0
C-3	147.0	145.7	146.1	145.1	145.9	C-3'	116.7	116.5	11.3	116.2	159.8
C-4	145.6	144.4	146.3	145.2	146.2	C-4'	160.1	159.5	158.1	158.1	103.0
C-5	116.3	116.1	116.1	115.3	115.7	C-5'	116.7	116.5	116.3	116.2	159.8
C-6	119.5	119.8	118.2	119.6	120.4	C-6'	.6	129.9	128.6	128.6	106.0
C-7	46.9	47.2	94.5	90.7	80.0	C-7'	135.4	137.4	129.2	130.6	128.6
C-8	52.4	52.9	56.6	53.6	80.4	C-8'	129.0	128.9	126.8	123.8	129.1
C-9	149.0	148.6	146.6	143.5	140.0	C-9'	155.2	158.3	141.5	136.7	133.0
C-10	107.1	107.3	106.9	109.1	107.1	C-10'	131.5	130.7	99.3	121.6	115.8
C-11	160.2	159.0	159.4	158.5	159.1	C-11'	201.6	205.3	163.2	162.4	145.6
C-12	102.4	101.6	101.8	101.6	103.1	C-12'	50.5	50.6	115.2	97.0	145.1
C-13	160.2	159.0	159.4	158.5	159.1	C-13'			155.5	159.1	118.2
C-14	107.1	107.3	106.9	109.1	107.1	C-14'	34.5	35.0	107.7	105.0	121.3

Measured in [a]pyridine-d_5 and [b]CD_3OD at 125 MHz.

Longusol A (**2**), a pale yellow powder, $[\alpha]_D^{24}$ +93.0° (*c*=0.60, MeOH), $C_{28}H_{22}O_7$,[10] showed absorption bands at 3389, 1605, 1512, 1431, 1156, and 961 cm^{-1} due to hydroxyl, aromatic ring, and olefin functions in the IR spectrum, while its UV spectrum showed absorption maxima at 285 (sh, log ε 4.2), 308 (sh, 4.4), and 326 (4.4) nm due to a stilbene chromophore. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 2) spectra[9] of **2** showed signals assignable to a dihydrofuran moiety [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: *meta*-coupled A_2B-type protons [δ 6.12 (2H, d, *J*=2.0 Hz, 10 and 14-H), 6.14 [δ (1H, t, *J*=2.0 Hz, 12-H)], *meta*-coupled protons [δ 6.50, 6.62 (1H each, both br s, 14' and 10'-H)], *ortho*- and *meta*-coupled 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', 2', and 6'-H)]. The proton and carbon signals of **2** were similar to those of *trans*-scirpusin A (**10**), except for the signals due to the benzene ring in the benzofuran moiety. The planar structure of **2** was constructed on the basis of H–H COSY and HMBC experiments, which showed long-range correlations between the 7-proton and 1-carbon, between the 8-proton and 9-carbon, between the 8'-proton and 9'-carbon, and between the 7'-proton and 1'-carbon (Figure 2). Furthermore, the relative stereostructure of **2** was characterized on the basis of the NOESY experiment, in which the NOE correlations were observed between the following proton pairs of **2** (2-, 6-H and 8-H; 10-, 14-H and 7-H). Consequently, the structure of **2** was determined as shown.

Longusol B (**3**),[11] a pale yellow powder, $[\alpha]_D^{26}$ +64.9° (*c*=0.40, MeOH), $C_{28}H_{22}O_7$, showed absorption maxima at 288 nm (sh,

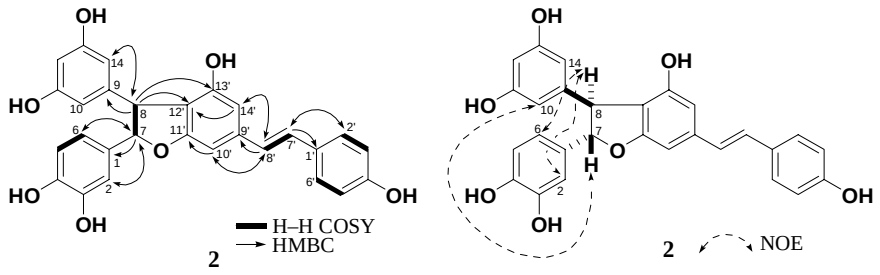


Figure 2

log ε 4.4), 310 nm (sh, 4.3), and 322 nm (4.3) assignable to a stilbene chromophore in the UV spectrum. The IR spectrum of **3** showed absorption bands at 3346, 1605, 1509, 1458, 1237, and 1159 cm^{-1} ascribable to hydroxyl, aromatic ring, and olefin functions. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 2) spectra[9] of **3** were superimporsable on those of **10**, except for the signals due to the dihydrofuran moiety. The planar structure of **3** was confirmed to be the same as that of **10** using H–H COSY and HMBC experiments (Figure 3). In the NOESY experiment of **3**, NOE correlation was observed between the 7-proton and the 8-proton, so that the structure of **3** was confirmed to be as the *cis*-type isomer of **10**.

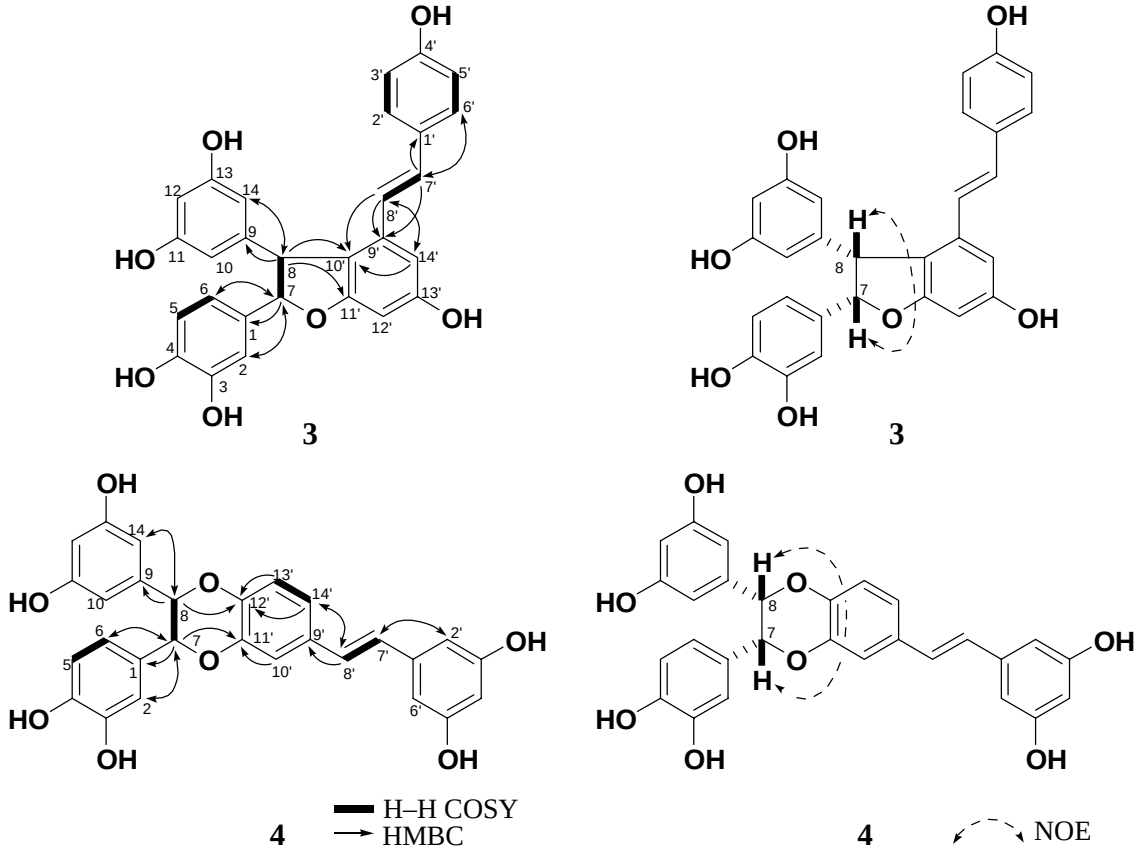


Figure 3

Longusol C (**4**),[12] a pale yellow powder, $[\alpha]_D^{24}$ +9.4°(*c*=0.30, MeOH), $C_{28}H_{22}O_8$, the positive-ion fast atom bombardment (FAB)-MS *m/z*: 487 $(M+H)^+$, showed absorption bands at 3410, 1649, 1619, 1509, and 1157 cm^{-1} ascribable to hydroxyl, aromatic ring, and olefin functions in its IR spectrum. The UV spectrum of **4** showed absorption maxima at 307 nm (sh, log ε 3.9) and 323 nm (4.0) ascribable to a stilbene chromophore. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 2) spectra[9] of **4** showed signals due to a 1,4-dioxoran moiety [δ 5.24, 5.29 (1H each, both d, *J*=3.0 Hz, 8 and 7-H)], *trans*-olefinic protons [δ 6.87, 6.96 (1H each, both d, *J*=16.4 Hz, 8' and 7'-H)], and 12 aromatic protons including two A_2B-type protons [δ 6.06 (2H, d, *J*=2.1 Hz, 10 and 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' and 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 superimposable on those of cassigarol E (**12**),[6] except for signals due to the 1,4-dioxane part. As shown in Figure 3, the planar structure of **4** was elucidated on the basis of H–H COSY and HMBC experiments and the relative stereostructure of **4** was elucidated by NOESY experiment, in which the NOE correlations were observed between the 7-proton and the 8-proton. Consequently, the structure of **4** was elucidated as the *cis*-type isomer of **12**.

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.[13] When this compound accepts an electron or hydrogen radical to become a more stable compound, the absorption vanishes. Previously, we already reported the DPPH radical and $^{\bullet}O_2^-$ scavenging activities of several natural medicines, such as the rhizomes of *Rheum undulatum*[4] and the fruit hulls of *Garcinia mangostana*.[14] In our continuing studies on antioxidative principles from natural medicines, isolated constituents from the methanolic extract of *C. longus* were examined. As shown in Table 3, *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**, **10**—**13**) were stronger than those of monomers (**8**, **9**), except for pallidol (**14**).

Table 3. DPPH Radical Scavenging Activities 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 (**5**)	4.8
(+)-Catechin (**6**)	6.0
(–)-Epicatechin (**7**)	7.0
Resveratrol (**8**)	24
Piceatannol (**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.

REFERENCES AND NOTES

1 a) J. B. Harborne, *Phytochemistry*, 1971, **10**, 1569; b) Y. D. Sadykov and Y. M. Begovatov, *Izv. Akad. Nauk Tadzh. SSR, Otd. Fiz.-Mat. Khim. Geol. Nauk*, 1990, **4**, 31.

2 a) M. Yoshikawa, T. Morikawa, T. Murakami, I. Toguchida, S. Harima and H. Matsuda, *Chem. Pharm. Bull.*, 1999, **47**, 340. b) M. Yoshikawa, T. Morikawa, I. Toguchida, S. Harima and H. Matsuda, *Chem. Pharm. Bull.*, 2000, **48**, 651. c) H. Matsuda, T. Morikawa, I. Toguchida, S. Harima and M. Yoshikawa, *Chem. Pharm. Bull.*, 2002, **50**, 972.

3 M. Yoshikawa, T. Morikawa, E. Fujiwara, T. Ohgushi, Y. Asao and H. Matsuda, *Heterocycles*, 2001, **55**, 1653.

4 a) H. Matsuda, T. Kageura, T. Morikawa, I. Toguchida, S. Harima and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 323. b) H. Matsuda, T. Morikawa, I. Toguchida, J.-Y. Park, S. Harima and M. Yoshikawa, *Bioorg. Med. Chem.*, 2001, **9**, 41. c) T. Kageura, H. Matsuda, T. Morikawa, I. Toguchida, S. Harima, M. Oda and M. Yoshikawa, *Bioorg. Med. Chem.*, 2001, **9**, 1887.

5 K. Nakajima, H. Taguchi, T. Endo and I. Yosioka, *Chem. Pharm. Bull.*, 1978, **26**, 3050.

6 K. Baba, T. Kido, M. Taniguchi and M. Kozawa, *Phytochemistry*, 1994, **36**, 1509.

7 M. A. Khan, S. G. Nabi, S. Prakash and A. Zaman, *Phytochemistry*, 1986, **25**, 1945.

8 **1**: High-resolution EI-MS: Calcd for $C_{27}H_{24}O_6$ (M^+): 444.1573. Found: 444.1569. ^{1}H-NMR (CD_3OD) δ 2.98, 3.10 (2H each, both m, 12' and 14'-H_2), 3.05, 3.16 (1H each, both m, 8 and 7-H), 6.05 (1H, t, *J*=2.1 Hz, 12-H), 6.08 (2H, d, *J*=2.1 Hz, 10 and 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' and 8'-H), 6.73, 7.27 (2H each, both d, *J*=8.6 Hz, 3', 5'-H and 2', 6'-H).

9 The ^{1}H- and ^{13}C-NMR spectra of **1**—**4** were assigned with the aid of homo- and hetero-correlation spectroscopies (^{1}H–^{1}H, ^{13}C–^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.

10 **2**: High-resolution EI-MS: Calcd for $C_{28}H_{22}O_7$ (M^+): 470.1365. Found: 470.1352. EI-MS *m/z*: 470 (M^+, 2), 94 (100).

11 **3**: High-resolution EI-MS: Calcd for $C_{28}H_{22}O_7$ (M^+): 470.1365. Found: 470.1366. ^{1}H-NMR (CD_3OD) δ 4.55, 5.76 (1H each, both d, *J*=8.4 Hz, 8 and 7-H), 5.78 (2H, d, *J*=2.2 Hz, 10 and 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' and 7'-H), 6.67, 7.11 (2H each, both d, *J*=8.6 Hz, 3', 5'-H and 2', 6'-H). EI-MS *m/z*: 470 (M^+, 6), 94 (100).

12 **4**: High-resolution positive-ion FAB-MS: Calcd for $C_{28}H_{23}O_8$ $(M+H)^+$: 487.1393. Found: 487.1407. Positive-ion FAB-MS *m/z*: 487 $(M+H)^+$.

13 a) M. S. Blois, *Nature*, 1958, **181**, 1199. b) M. Uchiyama, Y. Suzuki and K. Fukuzawa, *Yakugaku Zasshi*, 1968, **88**, 678.

14 M. Yoshikawa, E. Harada, A. Miki, K. Tsukamoto, S. Q. Liang, J. Yamahara and N. Murakami, *Yakugaku Zasshi*, 1994, **114**, 129.