PERGAMON

Phytochemistry 52 (1999) 311–319

PHYTOCHEMISTRY

Antioxidants from *Lespedeza homoloba* (II)

Toshio Miyase[a,*], Mitsuaki Sano[a], Kyoji Yoshino[b], Kazuhiko Nonaka[b]

[a]*School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka, 422-8526, Japan*
[b]*Department of Chemistry and Biochemistry, Numazu College of Technology, 3600 Ooka, Numazu, Shizuoka, 410-8501, Japan*

Received 30 June 1998; received in revised form 29 September 1998

Abstract

The stems of *Lespedeza homoloba* yielded fifteen new isoflavonoids and a new stilbenoid having antioxidative activity. Their structures were determined by analysis of spectroscopic evidence.

Keywords: *Lespedeza homoloba*; Leguminosae; Antioxidant; Isoflavonoid

1. Introduction

Lespedeza species (Leguminosae) grow in North America and Eastern Asia and hybridize with each other easily. Woody *Lespedeza* have many unique isoflavonoids, like pterocarp-6a-en and isoflav-3-en (Miyase et al., 1999; Ueno, Ichikawa, Miyase et al., 1973; Ueno, Ichikawa & Fukushima, 1973; Miyase, Ueno, Noro & Fukushima, 1980; Ueno, Ichikawa, Fukushima et al., 1973; Miyase, Ueno, Noro & Fukushima, 1981). In the preceding paper (Miyase et al., 1999), we reported the isolation of seven isoflavonoids and four stilbenoids and their antioxidative and antiallergic activity. As a continuation of the investigation of antioxidants from *Lespedeza homoloba* Nakai, the ether soluble fraction afforded fifteen new isoflavonoids and a new stilbenoid.

2. Results and discussion

Fractions C–K [see Experimental and preceding paper (Miyase et al., 1999)] were separated by preparative HPLC on a reversed phase column (ODS, PhA) to afford fifteen new isoflavonoids and a new stilbenoid.

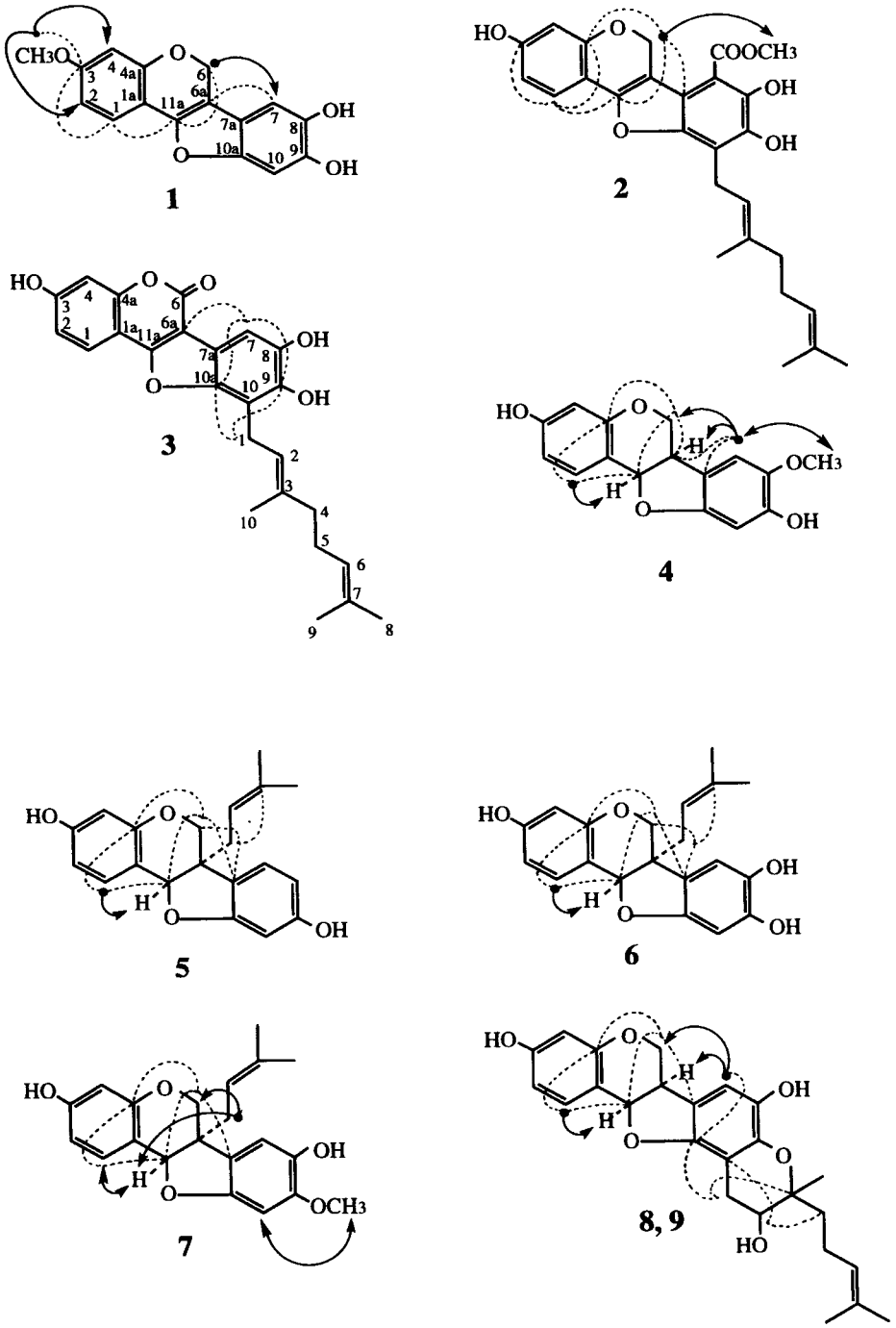
Lespedezol A_4 (**1**) showed a molecular ion peak at *m/z*: 284 in the FAB-MS. The UV and the ^{1}H NMR spectra suggested that **1** had a pterocarp-6a-en skeleton showing absorption maxima at 243 sh and 338 nm and a characteristic proton signal at δ 5.53 (2H, *s*) due to H_2-2 (Miyase et al., 1999). The ^{1}H NMR spectrum revealed a methoxyl proton signal at δ 3.79 (3H, *s*), two singlet aromatic proton signals at δ 6.91 and 7.05 and ABX-type aromatic proton signals at δ 6.48 (1H, *s*, J = 2 Hz); 6.56 (1H, *dd*, J = 8, 2 Hz); 7.33 (1H, *d*, J = 8.5 Hz). In the NOE experiment, an irradiation of a methylene proton signal at δ 5.53 enhanced the proton signal at δ 6.91 and an irradiation of a methoxyl proton signal at δ 3.79 enhanced the proton signals at δ 6.48 and 6.56. From these data, the structure of lespedezol A_4 was determined to be **1**.

The NMR data of lespedezol A_5 (**2**) were similar to those of lespedezol A_2 (Miyase et al., 1999) except for the presence of a carbomethoxyl signal [δ 3.99 (3H, *s*); 52.5 171.5] in the place of a singlet aromatic proton signal. ABX-type proton signals at δ 6.38 (1H, *d*, J = 2 Hz); 6.49 (1H, *dd*, J = 8.5, 2 Hz); 7.30 (1H, *d*, J = 8.5 Hz) were assigned to H-4, H-2 and H-1, respectively, by comparing with those of lespedezol A_2. Irradiation of a methylene proton signal at δ 5.60 due to H_2-6 enhanced the carbomethoxyl proton signal.

* Corresponding author. Tel.: +81-54-264-5661; fax: +81-54-264-5661.

E-mail address: miyase@ys7.u-shizuoka-ken.ac.jp (T. Miyase)

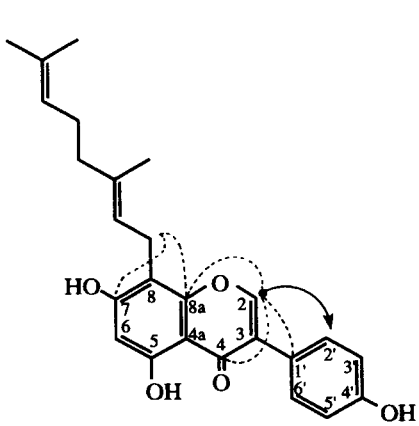
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00194-6

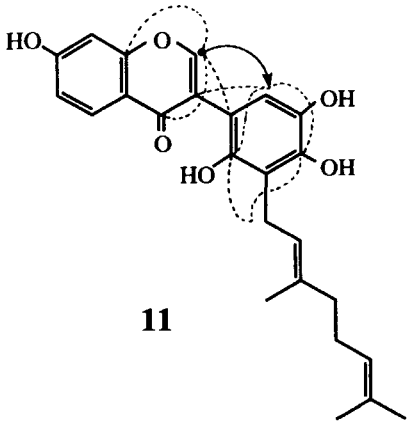


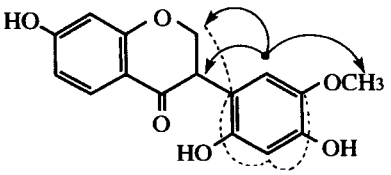
These results led us to conclude the structure of lespedezol A_4 to be **2**.

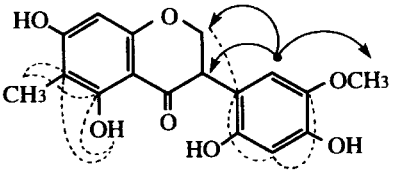
Lespedezol A_6 (**3**) showed a pseudo-molecular ion peak at *m/z*: 421 $[M+H]^+$ in the FAB-MS and a geranyl side chain like lespedezols A_2 and A_5 (**2**) in the NMR spectra. An unsaturated ester carbonyl carbon was observed in the ^{13}C NMR spectrum at δ 158.6 which was assigned to C-2 of a coumestan skeleton (Shiozawa, Urata, Kinoshita & Saitoh, 1989). The ^1H NMR spectrum revealed a singlet proton signal at δ 7.31 (1H, *s*) and ABX-type proton signals at δ 6.93 (1H, *d*, J = 2 Hz); 7.00 (1H, *dd*, J = 8.5, 2 Hz); 7.88 (1H, *d*, J = 8.5 Hz) in the aromatic proton region. HMBC correlations were observed between the singlet proton (δ 7.31) and the carbons at δ 104.1, 144.0, and 149.9 due to C-6a, C-9 and C-10a, respectively, and between the methylene proton signal at δ 3.73 (2H, *br d*, J = 7 Hz) due to H_2-1 of the geranyl side chain, and the carbons at δ 144.0 and 149.9 due to C-9 and C-10a, respectively. Therefore, the structure of lespedezol A_6 deduced to be **3**.

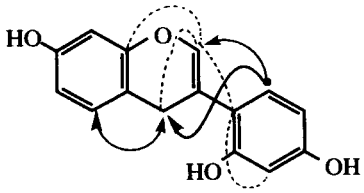
The ^1H NMR spectrum of lespedezol D_1 (**4**) showed

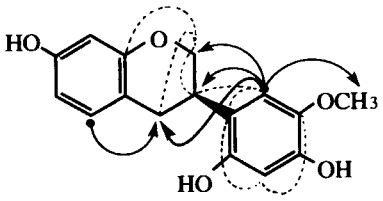


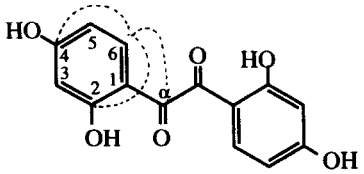


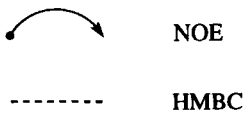












characteristic proton signals of a pterocarpan skeleton at δ 3.54 (1H, *m*), 3.60 (1H, *dd, J* = 10, 10 Hz); 4.26 (1H, *dd, J* = 10, 4 Hz); 5.43 (1H, *br d, J* = 6.5 Hz) due to H-6a, H_2-6 and H-11a), respectively, and a methoxyl proton signal at δ 3.80 (3H, *s*), two singlet aromatic proton signals at δ 6.33 and 7.01 and ABX-type proton signals at δ 6.36 (1H, *d, J* = 2.5 Hz); 6.55 (1H, *dd, J* = 8.5, 2.5 Hz); 7.30 (1H, *d, J* = 8.5 Hz) due to the A ring protons. In NOE experiments, irradiation at the singlet aromatic proton signal at δ 7.01 enhanced the methoxyl proton signal at δ 3.80 and the proton signals at δ 3.54, 3.60 and 4.26 due to H-6a, H-6β and H-6α, respectively. Compound **4** showed a positive Cotton effect at 292 nm and a negative Cotton effect at 284 nm. These results led to the structure of lespedezol D_1 as **4** (Tanaka H, Tanaka T & Etoh, 1997).

The FAB-MS of lespedezol D_2 (**5**) showed a pseudo molecular ion peak at *m/z*: 325 $[M+H]^+$. The ^1H NMR spectrum was similar to that of lespein (Ueno et al., 1973) except for the absence of a set of isopentenyl proton signals, and showed two sets of ABX-type pro-

ton signals [δ 6.36 (1H, *d*, J = 2.5 Hz); 6.54 (1H, *dd*, J = 8.5, 2.5 Hz); 7.28 (1H, *d*, J = 8.5 Hz) and δ 6.26 (1H, *d*, J = 2 Hz); 6.38 (1H, *dd*, J = 8, 2 Hz); 7.07 (1H, *d*, J = 8 Hz)] in the aromatic region. Irradiation of a proton signal at δ 7.28 enhanced the proton signal at δ 5.12 (1H, *br s*) due to H-11a. The CD spectrum showed a positive Cotton effect at 288 nm and negative Cotton effects at 274 and 235 nm. Therefore, the structure of lespedezol D_2 was deduced to be **5** (Tanaka et al., 1997).

Lespedezols D_3 (**6**) and D_4 (**7**) showed similar NMR data to those of **5**. The ^{1}H NMR spectrum of **6** showed two singlet aromatic proton signals at δ 6.32 (1H, *s*) and 6.78 (1H, *s*) due to H-10 and H-7, respectively, by comparing the chemical shifts with those of **5**. The ^{1}H NMR spectrum of **7** showed a methoxyl proton signal at δ 3.80 (3H, *s*). NOEs were observed at the singlet aromatic proton signal at δ 6.47 (1H, *s*) on irradiation at the methoxyl proton signal. These results led us to conclude the structures of lespedezols D_3 and D_4 to be **6**, and **7**, respectively.

Lespedezols D_5 (**8**) and D_6 (**9**) had the same molecular ion peak at m/z: 424 $[M]^+$ in the FAB-MS and showed similar NMR data except for the signals due to a C10-side chain. Lespedezol D_5 (**8**) afforded triacetate (**8a**) on acetylation with acetic anhydride and pyridine. Compound **8a** revealed an aliphatic acetoxyl signal at δ 2.05 (3H, *s*) and two aromatic acetoxyl signals at δ 2.27 and 2.29 (each 3H, *s*) in the ^{1}H NMR spectrum. By comparing the NMR data with those of lespedezols A_1 (Miyase et al., 1999) and D_1 (**4**), the structure of lespedezols D_5 and D_6 were elucidated to be **8** and **9**, respectively. These two compounds were assumed to be steroisomers at C-2 and C-3 in the side chain.

The ^{1}H NMR spectra of lespedezols E_1 (**10**) and E_2 (**11**) revealed a characteristic olefinic proton signal at δ 8.24 and 8.21, respectively, due to H-2 of an isoflavone and signals due to a geranyl side chain. Lespedezol E_1 (**10**) showed A_2B_2-type proton signals at δ 6.90 (2H, *d*, J = 8.5 Hz); 7.47 (2H, *d*, J = 8.5 Hz) due to the B-ring protons and a singlet aromatic proton at δ 6.37 (1H, *s*). In the ^{13}C NMR spectrum of lespedezol E_1 (**10**), a protonated aromatic carbon was observed at δ 99.5, which was assigned to C-6 by comparing the chemical shifts of C-6 and C-8 of 5,7-dihydroxy isoflavones (Agrawal & Rastogi, 1981). The ^{1}H NMR spectrum of lespedezol E_2 (**11**) showed ABX-type aromatic proton signals at δ 6.99 (1H, *d*, J = 2 Hz); 7.09 (1H, *dd*, J = 9, 2 Hz); 8.15 (1H, *d*, J = 9 Hz) due to H-8, H-6 and H-5, respectively, and a singlet aromatic proton signal at δ 6.65 (1H, *s*). The singlet signal at δ 6.65 was enhanced on irradiation of the signal at δ 8.21 and was assigned to H-6′. Therefore, the structures of these isoflavones were concluded to be **10** and **11**.

The ^{1}H NMR spectrum of lespedol D (**12**) showed ABX-type proton signals at δ 4.16 (1H, *dd*, J = 10, 5 Hz); 4.54 (1H, *dd*, J = 11, 5 Hz); 4.68 (1H, *dd*, J = 11, 10 Hz) which were characteristic of an isoflavanone (Miyase et al., 1981). In an aromatic proton region, ABX-type proton signals [δ 6.41 (1H, *d*, J = 2 Hz); 6.58 (1H, *dd*, J = 8.5, 2 Hz); 7.77 (1H, *d*, J = 8.5 Hz)] and two singlet proton signals at δ 6.46 (1H, *s*) and 6.78 (1H, *s*) were observed. On irradiation of the latter singlet signal, NOEs were observed at the methoxyl signal at δ 3.71 and at the ABX-type proton signals in the aliphatic proton region.

The ^{1}H NMR spectrum of lespedol E (**13**) suggested that **13** had the same substitution pattern in the B-ring as **12** and a singlet aromatic and methyl proton signals at δ 6.03 (1H, *br s*) and 1.97 (3H, *br s*). The methyl proton signal had correlation peaks with carbon signals at δ 162.8 and 103.9 in the HMBC spectrum. The two carbon signals were also correlated with a hydrogen-bonded hydroxyl proton signal at δ 12.62 (1H, *s*). So the position of the vinyl methyl group was deduced to be C-6.

The ^{1}H NMR spectrum of lespedezol F_1 (**14**) revealed the presence of an olefinic proton signal at δ 6.87 (1H, *dd*, J = 1.5, 1.5 Hz) and an equivalent methylene proton signal at δ 3.61 (2H, *br s*), which were coupled to each other, and two sets of ABX-type proton signals [δ 6.38 (1H, *d*, J = 2.5 Hz); 6.53 (1H, *dd*, J = 8, 2.5 Hz); 6.94 (1H, *br d*, J = 8 Hz), δ 6.37 (1H, *dd*, J = 8, 2.5 Hz); 6.45 (1H, *d*, J = 2.5 Hz); 7.01 (1H, *d*, J = 8 Hz)]. Irradiation of the aromatic proton signal at δ 7.01 enhanced the olefinic proton signal (δ 6.87) and the methylene proton signal (δ 3.61). Irradiation of the methylene proton signal (δ 3.61) enhanced the aromatic proton signal at δ 6.94. In the HMQC spectrum, the olefinic proton signal was correlated to the carbon signal at δ 139.2 and the methylene proton signal was correlated to the carbon signal at δ 27.5. These results led the structure of lespedezol F_1 to be **14**. This is the first report on an isoflav-2-en as a natural product to our knowledge.

Lespedezol G_1 (**15**) was assumed to be an isoflavan from the ^{1}H NMR spectrum. In the aromatic proton region, ABX-type [δ 6.28 (1H, *d*, J = 2.5 Hz); 6.36 (1H, *dd*, J = 8, 2.5 Hz); 6.89 (1H, *br d*, J = 8 Hz)] and two singlet proton signals [δ 6.48 (1H, *s*); 6.78 (1H, *s*)] were observed. On irradiation of the singlet proton signal at δ 6.78, NOEs were observed at δ 3.74 (3H, *s*) due to a methoxyl proton, a methine proton signal at δ 3.48 (1H, *m*) and two sets of methylene proton signals at δ 4.00 (1H, *dd*, J = 10, 10 Hz); 4.22 (1H, *ddd*, J = 10, 4, 2 Hz); 2.79 (1H, *ddd*, J = 15.5, 5, 2 Hz); 2.98 (1H, *ddd*, J = 15.5, 11, 1 Hz). The CD spectrum showed a positive Cotton effect at 288 nm and a negative one at 233 nm, suggesting that lespede-

Table 1
^{1}H NMR spectral data of compounds **1**–**9**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
6	5.53 *s*	5.60 *s*		3.60 *dd* (10, 10)	3.68 *d* (11)	3.66 *d* (11)	3.70 *d* (11)	3.57 *dd* (10.5, 10.5)	3.58 *dd* (10.5, 10.5)
6				4.26 *dd* (10, 4)	4.02 *dd* (11, 1)	4.01 *dd* (11, 1)	4.03 *dd* (11, 1)	4.24 *dd* (10.5, 4.5)	4.24 *dd* (10.5, 4.5)
6a				3.54 *m*				3.50 *m*	3.51 *ddd* (10.5, 7, 4.5)
11a				5.43 *br d* (6.5)	5.12 *br s*	5.03 *br s*	5.08 *br s*	5.40 *d* (7)	5.42 *d* (7)
1	7.33 *d* (8.5)	7.30 *d* (8.5)	7.88 *d* (8.5)	7.30 *d* (8.5)	7.28 *d* (8.5)	7.26 *d* (8.5)	7.27 *br d* (8)	7.31 *d* (8.5)	7.31 *d* (8.5)
2	6.56 *dd* (8.5, 2)	6.49 *dd* (8.5, 2)	7.00 *dd* (8.5, 2)	6.55 *dd* (8.5, 2.5)	6.54 *dd* (8.5, 2.5)	6.54 *dd* (8.5, 2.5)	6.54 *dd* (8, 2.5)	6.55 *dd* (8.5, 2.5)	6.54 *dd* (8.5, 2.5)
4	6.48 *d* (2)	6.38 *d* (2)	6.93 *d* (2)	6.36 *d* (2.5)	6.36 *d* (2.5)	6.36 *d* (2.5)	6.35 *d* (2.5)	6.36 *d* (2.5)	6.35 *d* (2.5)
7	6.91 *s*		7.31 *s*	7.01 *s*	7.07 *d* (8)	6.78 *s*	6.78 *s*	6.68 *s*	6.68 *s*
8					6.38 *dd* (8, 2)				
10	7.05 *s*			6.33 *s*	6.26 *d* (2)	6.32 *s*	6.47 *s*		
OMe	3.79 *s*			3.80 *s*			3.80 *s*		
COOMe		3.99 *s*							
Me									
Side chain									
1		3.65 *br d* (7.5)	3.73 *br d* (7)		2.46 *br d* (7.5)	2.43 *br d* (7.5)	2.45 *br d* (7)	2.52 *dd* (16.5, 7.5)	2.61 *dd* (17, 8)
1								2.94 *dd* (16.5, 5.5)	2.84 *dd* (17, 5.5)
2		5.38 *m*	5.46 *m*		5.24 *m*	5.25 *m*	5.24 *m*	3.91 *dd* (7.5, 5.5)	3.88 *m*
4		1.99 *m*	2.01 *m*		1.66 *d* (10)	1.67 *br s*	1.67 *d* (1)	1.72 *m*	1.71 *m*
5		2.06 *m*	2.09 *m*		1.49 *br s*	1.50 *br s*	1.50 *d* (1)	2.21 *m*	2.19 *m*
6		5.03 *m*	5.04 *m*					5.13 *m*	5.11 *m*
8		1.55 *br s*	1.52 *d* (1)					1.66 *d* (1)	1.64 *d* (1)
9		1.50 *br s*	1.50 *br s*					1.60 *br s*	1.57 *br s*
10		1.89 *br s*	1.93 *d* (1)					1.23 *s*	1.26 *s*

zol G_1 had a 3*S*-isoflavan skeleton (Zeng, Li, Xu & Zhu, 1996).

Lespedezol H_1 (**16**) showed a pseudo-molecular ion peak at *m/z*: 275 $[M+H]^+$ in the FAB-MS. The ^{1}H NMR spectrum showed three aromatic protons as an ABX-type at δ 6.47 (1H, *d*, $J = 2$ Hz); 6.50 (1H, *dd*, $J = 9$, 2 Hz); 7.47 (1H, *d*, $J = 9$ Hz). The ^{13}C NMR spectrum revealed a carbonyl carbon signal at δ 195.6 which was correlated to the proton signal at δ 7.47 in the HMBC spectrum. These results suggested a symmetrical structure as shown.

The antioxidative activities against lipid peroxidation in rat brain homogenate, chelating and O_2^- radical scavenging of these compounds are listed in Table 5. In general, catechol-type compounds showed a strong antioxidative activity against lipid peroxidation in the rat brain homogenate and a superoxide anion radical scavenging activity. Most compounds had a weak or no ability to form a Fe^{2+}-complex and the effect of geranyl and isoprenyl side chain in these activities was unclear.

3. Experimental

General instrumentation and plant material [see preceding paper (Miyase et al., 1999)] with the following exception a: JASCO J-20A automatic recording spectropolarimeter for CD spectra.

3.1. Extraction and isolation: see preceding paper (Miyase et al., 1999)

Fr. C (1.770 g) afforded compounds **2** (28 mg) and **7** (16 mg) after repeated preparative HPLC on a reverse phase (ODS, PhA) column using acetonitrile–water

Table 2
^{1}H NMR spectral data of compounds **10**–**15**

	10	**11**	**12**	**13**	**14**	**15**
2	8.24 *s*	8.21 *s*	4.54 *dd* (11, 5)	4.43 *dd* (10.5, 5.5)	6.87 *dd* (1.5, 1.5)	4.00 *dd* (10, 10)
2			4.68 *dd* (11,10)	4.58 *dd* (10.5, 10.5)		4.22 *ddd* (10, 4, 2)
3			4.16 *dd* (10, 5)	4.25 *dd* (10.5, 5.5)		3.48 *m*
4					3.61 *br s*	2.79 *ddd* (15.5, 5, 2)
4						2.98 *ddd* (15.5, 11, 1)
5		8.15 *d* (9)	7.77 *d* (8.5)		6.94 *br d* (8)	6.89 *br d* (8)
6	6.37 *s*	7.09 *dd* (9, 2)	6.58 *dd* (8.5, 2)		6.53 *dd* (8, 2.5)	6.36 *dd* (8, 2.5)
8		6.99 *d* (2)	6.41 *d* (2)	6.03 *br s*	6.38 *d* (2.5)	6.28 *d* (2.5)
2′	7.47 *d* (8.5)					
3′	6.90 *d* (8.5)		6.46 *s*	6.47 *s*	6.45 *d* (2.5)	6.48 *s*
5′	7.47 *d* (8.5)				6.37 *dd* (8, 2.5)	
6′	6.90 *d* (8.5)	6.65 *s*	6.78 *s*	6.79 *br s*	7.01 *d* (8)	6.78 *s*
OMe			3.71 *s*	3.72 *s*		3.74 *s*
Me				1.97 *br s*		
C_5–OH				12.62 *s*		
Side chain						
1	3.46 *br d* (7)	3.48 *br d* (7)				
2	5.27 *m*	5.37 *m*				
4	1.98 *m*	1.97 *m*				
5	2.03 *m*	2.08 *m*				
6	5.05 *m*	5.11 *m*				
8	1.58 *br s*	1.62 *d* (1)				
9	1.54 *br s*	1.57 *br s*				
10	1.82 *br s*	1.81 *d* (1)				

system as a solvent and UV detection (280 nm). Fr. E (3.992 g) afforded compounds **1** (18 mg), **4** (9 mg), **8** (38 mg) and **10** (17 mg), fr. G (3.549 g) afforded compounds **3** (51 mg), **5** (133 mg), **9** (21 mg) and **11** (8 mg), fr. H (1.961 g) afforded compound **16** (138 mg), fr. I (2.690 g) afforded compounds **6** (35 mg) and **13** (14 mg), fr. J (717 mg) afforded compound **15** (20 mg), fr. K (3.817 g) afforded compounds **12** (8 mg) and **14** (34 mg), following treatment similar to that for fr. C. The R*f* values in TLC [Silicagel GF_{254}, $CHCl_3$–MeOH–AcOH (94:5:1), coloring agent: 50% H_2SO_4] were as follows: **1** (0.57), **2** (0.60), **3** (0.43), **4** (0.48), **5** (0.43), **6** (0.33), **7** (0.53), **8** (0.47), **9** (0.49), **10** (0.48), **11** (0.43), **12** (0.27), **13** (0.26), **14** (0.23), **15** (0.35), **16** (0.53).

3.2. Lespedezol A_4 (*1*)

Amorphous powder, UV λ_{max} nm (log ϵ): 243 sh (4.09), 338 (4.36). FAB-MS *m/z*: 284 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.3. Lespedezol A_5 (*2*)

Amorphous powder UV λ_{max} nm (log ϵ): 275 (4.14), 389 (4.16). FAB-MS *m/z*: 464 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.4. Lespedezol A_6 (*3*)

Amorphous powder, UV λ_{max} nm (log ϵ): 254.5 (4.30), 355 (4.36). FAB-MS *m/z*: 421 $[M+H]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.5. Lespedezol D_1 (*4*)

Amorphous powder, UV λ_{max} nm (log ϵ): 227 sh (4.16), 287.5 (3.82), 2.99 (3.80), 329.5 sh (3.49). $[\alpha]_D$ -97.1° (MeOH; *c* 0.46). CD $[\theta]_{284}$ −24,280, $[\theta]_{292}$ +6480 (MeOH; *c* 0.0106). FAB-MS *m/z*: 286 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.6. Lespedezol D_2 (*5*)

Amorphous powder, UV λ_{max} nm (log ϵ): 228 sh (4.17), 282 sh (3.92), 287 (3.96), 305 sh (3.37). $[\alpha]_D$ -136.9° (MeOH; *c* 0.16). CD $[\theta]_{235}$ −76,400, $[\theta]_{274}$ −7360, $[\theta]_{288}$ +12,450 (MeOH; *c* 0.0229). FAB-MS *m/z*: 324 $[M]^+$, 325 $[M+H]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.7. Lespedezol D_3 (*6*)

Amorphous powder, UV λ_{max} nm (log ϵ): 225 sh (4.06), 281 (3.69), 301 (3.65). $[\alpha]_D$ -113.2° (MeOH; *c* 0.70). CD $[\theta]_{233}$ −48,500, $[\theta]_{291}$ +3630 (MeOH; *c* 0.0150). FAB-MS *m/z*: 340 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

Table 3
^{13}C NMR spectral data of compounds **1**–**9**[a]

	1	2	3	4	5	6	7	8	9
6	66.1	68.5	158.5	67.2	70.8	70.7	70.7	67.3	67.2
6a	107.3	107.1	104.1	41.2	47.2	47.6	47.9	41.4	41.4
11a	147.2	149.4	160.2	78.9	83.0	82.4	82.5	78.7	78.6
1a	110.9	109.2	106.4	113.1	112.4	112.5	112.6	113.3	113.3
1	121.4	122.1	123.3	133.0	133.5	133.5	133.5	133.0	133.0
2	107.9	109.4	114.3	110.4	110.5	110.4	110.5	110.4	110.4
3	161.8	160.3	161.6	159.6	159.8	159.7	159.8	159.5	159.5
4	103.3	104.1	104.2	103.9	103.8	103.7	103.8	103.9	103.9
4a	155.9	156.4	156.0	157.7	157.1	157.0	157.1	157.7	157.7
7a	118.6	116.8	115.4	117.9	122.3	121.4	122.3	117.4	117.4
7	104.4	101.3	103.5	110.5	125.0	111.7	111.1	109.4	109.4
8	143.6	141.4	144.3	148.6	108.3	139.7	141.6	140.7	140.7
9	144.7	149.3	144.0	142.8	161.8	146.3	148.6	141.5	141.5
10	99.2	119.8	113.6	98.7	98.7	98.8	96.1	105.3	105.2
10a	150.6	148.6	149.9	155.1	159.8	153.9	153.3	151.1	151.2
OMe	55.7			57.6					
CO		171.5							
COOMe		52.5							
Side chain									
1		24.0	23.7		31.5	31.3	31.5	27.2	27.2
2		121.6	122.4		120.0	120.0	120.0	67.6	67.4
3		136.8	136.4		135.3	135.2	135.3	80.1	80.0
4		40.4	40.4		26.0	26.0	26.0	38.3	38.4
5		27.3	27.3		18.0	18.0	18.0	22.3	22.3
6		125.0	125.0					125.6	125.6
7		131.7	131.7					131.7	131.7
8		25.7	25.7					25.8	25.8
9		17.7	17.6					17.7	17.7
10		16.4	16.4					18.6	18.5

[a] Assigned by HMQC and HMBC spectra.

3.8. Lespedezol D_4 (*7*)

Amorphous powder, UV λ_{max} nm (log ϵ): 231 sh (4.05), 302.5 (3.86), 330.5 sh (3.12). $[\alpha]_D$ −156.9° (MeOH; *c* 0.88). CD $[\theta]_{233}$ −89,000, $[\theta]_{277}$ −11,100, $[\theta]_{290}$ +6670 (MeOH; *c* 0.0191). FAB-MS *m/z*: 354 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.9. Lespedezol D_5 (*8*)

Amorphous powder, UV λ_{max} nm (log ϵ): 226.5 sh (4.24), 281.5 (3.87), 330.5 (3.92), 335 (3.79), 350.5 sh (3.66). $[\alpha]_D$ −49.9° (MeOH; *c* 1.03). CD $[\theta]_{239}$ −41,370, $[\theta]_{290}$ +12,180 (MeOH; *c* 0.0369). FAB-MS *m/z*: 424 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.10. Lespedezol D_6 (*9*)

Amorphous powder, UV λ_{max} nm (log ϵ): 228 sh (4.14), 282.5 (3.75), 300 (3.86), 335.5 sh (3.30). $[\alpha]_D$ −120.9° (MeOH; *c* 1.23). CD $[\theta]_{235}$ −42,400, $[\theta]_{289}$ +10,100 (MeOH; *c* 0.0126). FAB-MS *m/z*: 424 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 1 and 3.

3.11. Lespedezol E_1 (*10*)

Amorphous powder, UV λ_{max} nm (log ϵ): 264.5 (4.30), 341 (4.01). FAB-MS *m/z*: 407 $[M+H]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2, 3 and 4.

3.12. Lespedezol E_2 (*11*)

Amorphous powder, UV λ_{max} nm (log ϵ): 260 (4.29), 288 (4.26). FAB-MS *m/z*: 422 $[M]^+$, 423 $[M+H]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2 and 4.

3.13. Lespedol D (*12*)

Amorphous powder, UV λ_{max} nm (log ϵ): 226 sh (4.23), 278 (4.13), 315 sh (3.92). $[\alpha]_D$ 0° (MeOH; *c* 0.71). CD no Cotton effect (MeOH; *c* 0.016). FAB-MS *m/z*: 302 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2 and 4.

3.14. Lespedol E (*13*)

Amorphous powder, UV λ_{max} nm (log ϵ): 226.5 sh (4.27), 295 (4.26), 330 sh (3.85). $[\alpha]_D$ 0° (MeOH; *c*

Table 4
^{13}C NMR spectral data of compounds **10**–**15**

	10	11	12	13	14	15
2	154.3	156.3	71.7	71.0	139.2	70.6
3	123.2	111.7	47.8	47.4	112.4	32.9
4	182.0	179.0	191.6	198.1	27.5	31.2
4a	106.3	117.3	115.5	103.4	111.9	114.4
5	156.4	128.7	130.1	162.8	130.6	131.0
6	99.5	116.7	111.3	103.9	111.6	108.8
7	162.2	164.1	165.2	164.8	157.6	157.5
8	107.3	103.0	103.5	95.2	103.4	103.7
8a	161.5	158.8	164.7	162.0	152.2	156.2
1	123.7	125.6	113.5	113.1	117.6	118.8
2	131.1	149.6	150.8	150.8	156.9	150.2
3	116.0	119.1	104.6	104.5	104.0	104.3
4	158.4	146.2	147.8	148.0	158.4	146.9
5	116.0	138.9	141.9	142.0	107.8	142.0
6	131.1	114.3	115.2	115.0	130.2	113.1
OMe			57.4	57.4		57.4
Me				7.1		
Side Chain						
1	22.0	23.9				
2	123.1	124.2				
3	135.7	134.7				
4	40.4	40.6				
5	27.3	27.5				
6	125.1	125.6				
7	131.6	131.6				
8	25.8	25.8				
9	17.7	17.7				
10	16.2	16.3				

1.21). CD no Cotton effect (MeOH; *c* 0.0103). FAB-MS *m/z*: 333 $[M+H]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2 and 4.

3.15. Lespedezol F_1 (**14**)

Amorphous powder, UV λ_{max} nm (log ϵ): 272 (3.85). FAB-MS *m/z*: 256 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2 and 4.

3.16. Lespedezol G_1 (**15**)

Amorphous powder, UV λ_{max} nm (log ϵ): 221 sh (4.13), 287 (3.89). $[\alpha]_D$ −16.7° (MeOH; *c* 0.98). CD $[\theta]_{233}$ −10,030, $[\theta]_{288}$ +1000 (MeOH; *c* 0.0402). FAB-MS *m/z*: 288 $[M]^+$. ^{1}H and ^{13}C NMR spectra: Tables 2 and 4.

3.17. Lespedezol H_1 (**16**)

Amorphous powder, UV λ_{max} nm (log ϵ): 232 (4.25), 287 (4.24), 332.5 (4.31). FAB-MS *m/z*: 275 $[M+H]^+$. ^{1}H NMR δ: 6.47 (2H, *d, J* = 2 Hz, H-3), 6.50 (2H, *dd, J* = 9, 2 Hz, H-5), 7.47 (2H, *d, J* = 9 Hz, H-6). ^{13}C NMR δ: 104.0 (C-3), 110.2 (C-5), 111.5 (C-1), 135.7 (C-6), 167.2 (C-4), 167.4 (C-2), 195.6 (C-α).

3.18. Acetylation of lespedezol D_5 (**8**)

Compound **8** (2 mg) was acetylated with pyridine and acetic anhydride (each 0.2 ml) at room tempera-

Table 5
Antioxidative activities of isoflavonoids and stilbenoid from *Lespedeza homoloba*[c]

Compound	Anti-oxidative activity IC_{50} (μM)[a]	Fe^{2+}-complex % vs EGCg[b]	O_2^- radical scavenging activity (%)
1	0.2	22.9	85.1
2	0.4	−26.0	66.8
3	0.4	−5.4	24.2
4	–[d]	0.1	−15.5
5	–[d]	5.3	2.5
6	0.5	0.7	98.6
7	0.1	3.4	14.7
8	0.2	3.9	−5.9
9	0.1	−9.3	−12.7
10	–[d]	6.5	89.1
11	0.4	12.6	54.7
12	–[d]	−0.4	−14.7
13	–[d]	2.3	11.7
14	0.4	1.1	54.9
15	–[d]	6.1	9.2
16	–[d]	5.2	61.4
EGCg[e]	0.07	100.0	83.7

[a] Suppression of autooxidation of rat brain homogenates.
[b] Ferrous tartrate-method, the final concentration of all fractions was 0.033 mg/ml.
[c] Phenazine methosulfate (PMS)-nitro blue tetrazolium (NBT)-method, the final concentration of all fractions was 0.020 mg/ml.
[d] –: Not determined.
[e] EGCg: epigallocatechin gallate.

ture overnight. The reagents were evaporated and the acetate **8a** (2 mg) was obtained. ^{1}H NMR δ: 2.05 (3H, *s*, OAc), 2.27 (3H, *s*, OAc), 2.29 (3H, *s*, OAc).

3.19. Antioxidative activities

Antioxidative activity in rat brain homogenate, formation and determination of the Fe^{2+} complex and determination of superoxide anion radical scavenging activity were by the methods in the previous paper (Miyase et al., 1999) Table 5.

Acknowledgements

This work was supported by a grant from the Program for Promotion of Basic Research Activities for Innovative Biosciences.

References

Agrawal, P. K., & Rastogi, R. P. (1981). *Heterocycles*, *16*, 2181.
Miyase, T., Ueno, A., Noro, T., & Fukushima, S. (1980). *Chemical and Pharmaceutical Bulletin*, *28*, 1172.
Miyase, T., Ueno, A., Noro, T., & Fukushima, S. (1981). *Chemical and Pharmaceutical Bulletin*, *29*, 2205.
Miyase T., Sano M., Nakai H., Muraoka M., Nakazawa M., Suzuki M., Yoshino K., Nishihara Y., Tanai J. 1999 Phytochemistry, submitted.
Shiozawa, T., Urata, S., Kinoshita, T., & Saitoh, T. (1989). *Chemical and Pharmaceutical Bulletin*, *37*, 2239.
Tanaka, H., Tanaka, T., & Etoh, H. (1997). *Phytochemistry*, *45*, 835.
Ueno, A., Ichikawa, M., & Fukushima, S. (1973). *Chemical and Pharmaceutical Bulletin*, *21*, 2712.
Ueno, A., Ichikawa, M., Miyase, T., Fukushima, S., Saiki, Y., & Morinaga, K. (1973). *Chemical and Pharmaceutical Bulletin*, *21*, 1734.
Ueno, A., Ichikawa, M., Fukushima, S., Saiki, Y., Noro, T., Morinaga, K., & Kuwano, H. (1973). *Chemical and Pharmaceutical Bulletin*, *21*, 2715.
Zeng, J-F., Li, G-L., Xu, X., & Zhu, D-Y. (1996). *Phytochemistry*, *43*, 893.