Pergamon

PII: S0031-9422(97)00371-3

Phytochemistry, Vol. 46, No. 5, pp. 921–928, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

PHENOLIC GLUCOSIDES FROM THE ROOT OF *PUERARIA LOBATA*

KAZUHIRO HIRAKURA,* MAKOTO MORITA, KAORU NAKAJIMA, KOH SUGAMA, KOJI TAKAGI, KAZUAKI NIITSU, YUKINOBU IKEYA, MASAO MARUNO, and MINORU OKADA

Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki, 300-11, Japan

(*Received in revised form* 24 *March* 1997)

Key Word Index—*Pueraria lobata*; Leguminosae; root; isolfavone; butenolide; 6″-*O*-malonyldaidzin; kuzubutenolide A; 3′-hydroxy-4′-*O*-β-D-glucosylpuerarin; 3′-methoxydaidzin.

Abstract—Six phenolic glucosides were isolated from the methanolic extract of the root of *Pueraria lobata*. Three of them were new compounds and their structures were proved to be kuzubutenolide A, 3′-hydroxy-4′-*O*-β-D-glucosylpuerarin and 3′-methoxydaidzin. © 1997 Elsevier Science Ltd

INTRODUCTION

The root of *Pueraria lobata* is one of the oriental medicinal plants has been used as an antipyretic, antispasmodic and migraine agent. Many isoflavones such as daidzin (**1**) and puerarin (**2**) [1–4], and sapogenols [5, 6] have been reported from this plant. In this paper, we report the isolation of new phenolic compounds from *Pueraria* root along with **1** and **2**. These structures were elucidated by spectral and chemical methods.

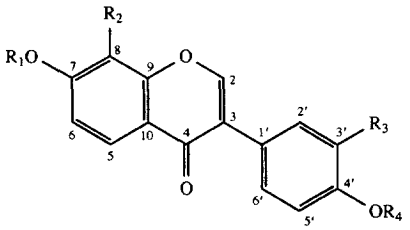
RESULTS AND DISCUSSION

Two different samples of the dried root of *Pueraria lobata* were extracted with methanol. The methanol extract of the first sample was chromatographed on DIAION HP-20, Sephadex LH-20 and preparative HPLC successively to afford 6″-*O*-malonyldaidzin (**3**). In contrast, the second methanol extract was chromatographed on DIAION HP-20, silica gel and MCI CHP-20P successively to afford kuzubutenolide A (**4**), crude daidzin and 3′-hydroxy-4′-*O*-β-D-glucosylpuerarin (**5**). The crude daidzin sample was acetylated, and after purification by preparative HPLC and hydrolysis afforded 3′-methoxydaidzin (**6**) and daidzin (**1**). 6″-*O*-malonyldaidzin (**3**) [7–9] was obtained as an unstable colourless powder. The UV spectrum of **3** exhibited λ_{max} at 202, 211 (*infl.*), 221, 233 (*infl.*), 250, 260 and 294 nm, which was similar to that of **1**. The IR spectrum of **3** showed the presence of hydroxyl (*br* 3376 cm^{-1}), carbonyl ester (*br* 1720 cm^{-1}) and conjugated carbonyl (1620 cm^{-1}) groups. The molecular formula of **3** was determined to be $C_{24}H_{22}O_{12}$ by HR-FAB-MS. The ^1H NMR spectrum of **3** was similar to that of **1**, except that the H-6″ proton signals of **3** appeared about 1.0 ppm downfield from those of **1** (Table 1). The ^{13}C NMR spectrum of **3** was also similar to that of **1**, except that the C-5″ and C-6″ carbon signals of **3** showed acylation shifts (Table 2). These data suggested that the hydroxyl group at C-6″ in **3** was acylated. Methylation of **3** with CH_2N_2 at −78° gave a monomethyl ether (**3a**). The appearance of the peak at *m/z* 417($[M+H-100]^+$) in the FD-mass spectrum of **3a** showed the presence of a methyl malonyl group in **3a**. Methanolysis of **3** gave a dimethyl malonate and **1**. Thus **3** is daidzein 7-*O*-β-D-(6″-*O*-malonyl)glucoside.

Compound **6** was obtained as colourless needles (MeOH), mp 226–228°. The UV spectrum of **6** gave absorption bands similar to those of **1** [λ_{max} at 202, 219, 249 (*infl.*), 265 and 291 (*infl.*) nm]. The IR spectrum of **6** showed the presence of hydroxyl groups (*br* 3448 cm^{-1}), carbonyl group (1638 cm^{-1}), and aromatic ring (1608 and 850 cm^{-1}). The molecular formula of **6** was determined to be $C_{22}H_{22}O_{10}$ by HR-FAB mass spectroscopy, corresponding to a methoxydaidzin such as calycosin 7-*O*-β-D-glucoside (**8**) [10–12]. Acidic hydrolysis of **6** gave glucose and **7**. The ^{13}C NMR spectrum of **6** was similar to that of **8** [10]. However, a comparison of the ^1H NMR spectra of **6** and **8**, indicated that the chemical shifts of the B-ring protons were different (Tables 1 and 2) [10, 11]. In an NOE experiment on the peracetate (**6a**) of **6**, the NOE was observed only at H-2′ (δ_H 7.30), when the methoxy protons (δ_H 3.89) were irradiated. The Gibbs test on **6** was negative, whilst on **8** it was positive [10]. Therefore, the location of the methoxy group was confirmed to be at C-3′. From these results, **6** is 3-methoxydaidzin.

Compound **4** was obtained as colourless needles

* Author to whom correspondence should be addressed.



1: R_1 = β-D-Glc, R_2 = R_3 = R_4 = H

1a: R_1 = β-D-Glc(Ac)$_4$, R_2 = R_3 = H, R_4 = Ac

2: R_1 = R_3 = R_4 = H, R_2 = β-D-Glc

5: R_1 = H, R_2 = β-D-Glc, R_3 = OH, R_4 = β-D-Glc

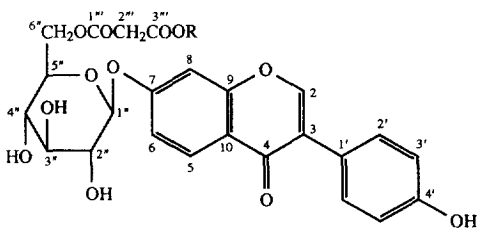
6: R_1 = β-D-Glc, R_2 = R_4 = H, R_3 = OMe

6a: R_1 = β-D-Glc(Ac)$_4$, R_2 = H, R_3 = OMe, R_4 = Ac

7: R_1 = R_2 = R_4 = H, R_3 = OMe

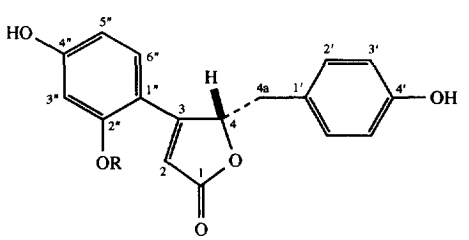
8: R_1 = β-D-Glc, R_2 = H, R_3 = OH, R_4 = Me

10: R_1 = R_4 = H, R_2 = β-D-Glc, R_3 = OH



3: R = H

3a: R = Me



4: R = β-D-Glc

9: R = H

(MeOH–H_2O), mp 169–171°. The UV spectrum of **4** exhibited λ_{max} at 200, 219, 227 (*infl.*), 243 (*infl.*), 289 and 316 nm. The IR spectrum of **4** showed the presence of hydroxyl (*br* 3300 cm^{-1}), carbonyl (1692 cm^{-1}), and double band and aromatic ring (1604 and 1516 cm^{-1}). The molecular formula of **4** was determined to be $C_{23}H_{24}O_{10}$ by HR-FAB mass spectroscopy. The ^1H NMR and ^{13}C NMR spectra of **4** were similar to those of 3-(2,4-dihydroxyphenyl)-4-(4-hydroxybenzyl)but-2-en-4-olide (**9**) [13, 14], except for the signals due to a sugar moiety (Tables 3 and 4). Acidic hydrolysis of **4** gave glucose, whilst enzymatic hydrolysis gave **9** and glucose, suggesting that **4** was glucosylated **9**. The location of the glucose was confirmed to be at C-2″-OH from a cross peak between the anomeric proton and C-2″ carbon in the HMBC experiment. This was further supported by the fact that the Gibbs test on **4** was negative, whilst on **9** it was positive. Finally, the structure of kuzubutenolide A (**4**), including the absolute configuration at C-4, was determined as (4*R*)-3-(2-β-D-glucopyranoxyloxy-4-hydroxyphenyl)-4-(4-hydroxybenzyl) but-2-en-4-olide by X-ray analysis (Fig. 1).

Compound **5** was obtained as powder. The UV spectrum of **5** exhibited λ_{max} at 202, 220, 244 (*infl.*), 250, 289 and 310 (*infl.*), and was similar to that of PG-1 (**10**) [3, 4]. The IR spectrum of **5** showed the presence of hydroxyl (*br* 3400 cm^{-1}) and carbonyl (1628 cm^{-1}) groups, double bond and aromatic ring (1588 and 880 cm^{-1}). The molecular formula of **5** was determined to be $C_{27}H_{30}O_{15}$ by HR-FAB mass spectroscopy. The ^1H and ^{13}C NMR spectra of **5** were similar to those of **10** except for the presence of a sugar moiety in the B-ring (Tables 1 and 2). Acidic hydrolysis of **5** gave glucose and **10**. The location of the glucose in the B-ring was confirmed to be at C-4′-OH by an HMBC experiment and the fact that the Gibbs test on **5** was positive. From these results, **5** is 3′-hydroxy-4′-*O*-β-D-glucosyl-puerarin.

EXPERIMENTAL

General. ^1H NMR spectra were measured at 200, 400 and 500 MHz, and ^{13}C NMR spectra were measured at 50, 100 and 125 MHz. Chemical shifts are expressed in δ ppm from TMS as int. standard (except for spectra measured in D_2O) and coupling constants (J) are given in Hz. FAB MS were taken in the positive ion mode, using *m*-nitrobenzyl alcohol as matrix. Silica gel (Merck, 70–230 mesh) was used for CC. Silica gel $60F_{254}$ (0.25 mm) and cellulose F(0.1 mm) were used for TLC and prep. TLC. Prep. HPLC employed at CIG 22 $\phi \times 100$ column system (Kusano Scientific Co., Tokyo) and the stationary phase used was silica gel (50 μm).

Plant material. The root of *Pueraria lobata* used to isolate **3** was purchased from Uchida wakanyaku Co. Ltd. (Japan). Compounds **4**, **5** and **6** were isolated from the root of *Pueraria lobata* purchased from Shibata Co. Ltd. (Japan).

Isolation of compound **3**. Dried and crushed root (3 kg) was extracted × 5 with MeOH (each 18 *l*) at room temp. to give an MeOH extract (595 g). The extract was chromatographed on a DIAION HP-20 (1.5 *l*) column, eluting with H_2O (8 *l*), 50% MeOH (8 *l*) and MeOH (8 *l*) successively. These eluates were concd. under red. pres. to give: H_2O fr. (34.3 g), 50% MeOH fr. (259 g) and MeOH fr. (260 g). A portion of 50% MeOH fr. (45 g) was divided into 15 parts and each part was rechromatographed on a Sephadex LH-20 column (2 $\phi \times 45$ cm), eluting with H_2O to give crude **3** (2.9 g). Crude **3** was purified by prep. HPLC (μ-

Table 1. ^{1}H NMR spectral data for compounds **1–3**, **5**, **6**, **8** (δ in DMSO-d_6)

H	**1**	**2**	**3**	**5**	**6**	**8** [10]
2	8.35 *s*	8.26 *s*	8.36 *s*	8.06 *s*	8.42 *s*	8.39 *s*
5	8.05 *d*	7.96 *d*	8.07 *d*	7.77 *d*	8.06 *d*	8.06 *d*
	(8.8)	(8.8)	(8.9)	(8.9)	(8.8)	(8.8)
6	7.14 *dd*	6.99 *d*	7.17 *dd*	6.68 *d*	7.15 *dd*	7.15 *dd*
	(8.8, 2.4)	(8.8)	(8.9, 2.2)	(8.9)	(1.9, 8.8)	(2.2, 8.8)
8	7.22 *d*	—	7.22 *d*	—	7.23 *d*	7.24 *d*
	(2.4)	—	(2.3)	—	(1.9)	(2.2)
2′	7.41 *d*	7.42 *d*	7.41 *d*	7.13 *d*	7.19 *d*	6.97 *br s*
	(8.7)	(8.8)	(8.6)	(2.2)	(1.9)	—
3′	6.82 *d*	6.82 *d*	6.83 *d*	—	—	—
	(8.7)	(8.8)	(8.6)	—	—	—
5′	—	—	—	7.15 *d*	6.83 *d*	6.97 *br s*
	—	—	—	(8.3)	(8.3)	
6′	—	—	—	6.96 *dd*	7.02 *dd*	7.08 *br s*
	—	—	—	(2.2, 8.3)	(1.9, 8.3)	
7-*O*-Glc-1(1″)	5.09 *d*	—	5.13 *d*	—	5.11 *d*	5.11 *d*
	(7.5)	—	(7.5)	—	(7.3)	(7.3)
2(2″)	3.20 *m*	—	3.45 *m*	—	3.19 *m*	
3(3″)	3.30–3.36 *m*	—	3.26 *m*	—	3.27–3.34 *m*	
4(4″)	3.44–3.52 *m*	—	3.35 *m*	—	3.45–3.51 *m*	
5(5″)		—		—	3.67–3.74 *m*	
6(6″)	3.74 *m*	—	4.27 *dd*	—		
		—	(2, 12)	—		
	3.46 *m*	—	4.13 *dd*	—		
		—	(6.6, 12)	—		
8-*C*-Glc-1	—	4.90 *d*	—	4.87 *d*	—	—
	—	(10.4)	—	(9.8)	—	—
2	—	4.00 *m*	—	4.09 *br t* (9)	—	—
3	—	3.75 *m*	—	3.46 *br t* (9)	—	—
4	—	3.54 *m*	—	3.32–4.41 *m*	—	—
5	—	3.34 *m*	—		—	—
6	—	3.55 *br d*	—	3.73 *dd*	—	—
	—	(12)	—	(2.8, 11)	—	—
	—	3.74 *br d*	—	3.63 *dd*	—	—
		(12)	—	(4.4, 11)	—	—
4′-*O*-Glc-1	—	—	—	4.71 *d*	—	—
	—	—	—	(7.2)	—	—
2	—	—	—		—	—
3	—	—	—		—	—
4	—	—	—	3.21–4.41 *m*	—	—
5	—	—	—		—	—
6	—	—	—	3.78 *dd*	—	—
	—	—	—	(2.3, 11.7)	—	—
	—	—	—	3.59 *dd*	—	—
	—	—	—	(5.7, 11.7)	—	—
OMe	—	—	—	—	3.80(3′-OMe)	3.80(4′-OMe)
OH	9.48, 5.39, 5.10, 5.04, 4.58	4.68			9.08(4′-OH), 5.41, 5.05, 4.59	
malonyl group	—	—	3.50–3.60 *m*	—	—	—

Coupling constants (J) in Hz are given in parentheses.

Bondapack C_{18}), eluting with THF–H_2O (7:93) to give **3** (283 mg).

Isolation of compounds **4** *and* **6**. Dried and crushed root (99 kg) was divided into 13 parts and each part was extracted ×3 by refluxing with MeOH (each 36 *l*) to give an MeOH extract (11.8 kg). The MeOH extract was divided into 12 parts and each part was chromatographed on a DIAION HP-20 column (12

Table 2. ^{13}C NMR Spectra data of **1–3**, **5**, **6**, **8** (δ in DMSO-d_6)

	1	**2**	**3**	**5**	**6**	**8** [10]
C-2	153.1	152.0	153.2	151.1	153.4	153.4
3	123.7[a]	122.9[a]	123.6	127.9	123.6[a]	124.3
4	174.7	174.6	174.7	173.7	174.6	174.5
5	126.9	125.9	127.0	124.8	126.9	126.9
6	115.4	114.8	115.2	118.7	115.5	115.5
7	161.3	160.6	161.1	N.D.(163.7)*	161.3	161.3
8	103.3	113.2	103.5	N.D.(113.2)*	103.3	103.3
9	157.2	155.7	156.9[a]	156.9	156.9	156.9
10	118.5	116.8	118.5	N.D.(119.2)*	118.4	118.4
1′	122.2[a]	122.4[a]	122.4	121.9	122.7[a]	123.5
2′	129.9	129.6	129.9	116.5	113.2	111.8
3′	114.9	114.7	114.9	146.4[a]	147.1[b]	145.9
4′	157.0	156.9	157.2[a]	144.4[a]	146.5[b]	147.5
5′	115.5	114.7	114.9	117.3	115.1	116.3
6′	129.9	129.6	129.9	119.2	121.5	119.6
7-*O*-Glc-1(1″)	100.1	—	99.7	—	100.0	99.9
2(2″)	73.1	—	73.1	—	73.1	73.0
3(3″)	76.4	—	76.2	—	76.5	76.4
4(4″)	69.6	—	69.9	—	69.6	69.5
5(5″)	77.2	—	73.9	—	77.1	77.1
6(6″)	60.6	—	63.4	—	60.6	60.6
8-*C*-Glc-1	—	73.4	—	74.5	—	—
2	—	70.9	—	70.0	—	—
3	—	78.5	—	78.7	—	—
4	—	70.2	—	70.8	—	—
5	—	81.2	—	80.6	—	—
6	—	61.1	—	60.6	—	—
4′-*O*-Glc-1	—	—	—	102.8	—	—
2	—	—	—	73.0	—	—
3	—	—	—	75.9	—	—
4	—	—	—	69.9	—	—
5	—	—	—	76.8	—	—
6	—	—	—	60.7	—	—
OMe	—	—	—	—	55.6(3′-OMe)	55.6(4′-OMe)
	—	—	168.0(*br*)	—	—	—
malonyl group	—	—	48.5(*br*)	—	—	—
	—	—	170.4(*br*)	—	—	—

Signal assignments were based on ^{13}C–^{1}H COSY and DEPT experiments.
[a,b] Assignments may be interchanged in each column.
* Measured in D_2O (TSP as int. standard).

$\phi \times 76$ cm), eluting with H_2O (36 *l*), 50% MeOH (36 *l*) and MeOH (36 *l*), successively. The eluates were concd. under red. pres. to give a 50% MeOH fr. (2.4 kg). This fr. was divided into 7 parts and each part was chromatographed on a silica gel (3.2 kg) column, eluting with $CHCl_3$–MeOH–H_2O (14:2.5:0.1) and MeOH, to give frs A, B, C and D (MeOH eluate). The frs B and C were acetylated in the usual way to give mainly crude acetyl diadzin (117 g), and then were purified by prep. HPLC (silica gel) eluting with C_6H_6–$CHCl_3$–EtOAc (1:4:1) to give **6a** (15 g) and **1a** (87 g). Compound **6a** was hydrolysed with 1 M KOH (75% MeOH) and recrystallized from MeOH–H_2O to give **6** (2.5 g). Compound **1a** was also hydrolysed by the above method and recrystallized from THF to give **1** (53 g). On the other hand, fr. A was rechromatographed on an MCI GEL CHP-20P column eluting with MeOH–H_2O to give a fr. containing **4**. Compound **1** was removed from this fr. by crystallization with MeOH–H_2O. The mother liquor was evapd. to give crude **4**, which was recrystallized from MeOH–H_2O to give pure **4** (0.14 g). The fr. D (1.4 kg) was divided into 4 parts and one of the parts was rechromatographed on a silica gel column, eluting with $CHCl_3$–MeOH–H_2O (7:1.8:0.2) and MeOH to give frs E (7 g), F (66.3 g), G (67.4 g) and H (45.4 g, MeOH eluate). Frs F and G were rechromatographed on an MCI GEL CHP-20P column eluting with MeOH–H_2O, then the frs containing **2** were combined and recrystallized from H_2O to give **2** (35 g). Fr. H

Table 3. ^{1}H NMR spectral data for compounds **4** and **9** (δ in CD_3OD)

	4	**9** [13]
2	6.12 *d*	6.17 *d*
	(1.4)	(1.5)
4	6.11 *ddd*	5.85 *ddd*
	(1.4, 3.4, 6.4)	(1.5, 3.4, 6.7)
4a	3.20 *dd*	3.23 *dd*
	(3.4, 14.4)	(3.4, 15.5)
	2.78 *dd*	2.74 *dd*
	(6.4, 14.4)	(6.7, 15.5)
2′	6.91 *d*	6.92 *d*
	(8.6)	(8.5)
3′	6.63 *d*	6.67 *d*
	(8.6)	(8.5)
3″	6.80 *d*	6.59 *d*
	(2.3)	(2.2)
5″	6.58 *dd*	6.52 *dd*
	(2.3, 8.6)	(2.2, 8.4)
6″	7.35 *d*	7.40 *d*
	(8.6)	(8.4)
Glc-1	5.01 *d*	—
	(7.1)	—
2		—
3	3.40–3.52 *m*	—
4		—
5		—
6	3.96 *dd*	—
	(2.3, 12.1)	—
	3.76 *dd*	—
	(5.7, 12.1)	—

Coupling constants (J) in Hz are given in parentheses. Signal assignments were based on ^{1}H–^{1}H COSY spectrum.

Table 4. ^{13}C NMR spectral data for compounds **4** and **9**

	4[1]	**9**[2] [14]
1	176.4	172.9
2	113.7	111.1
3	168.9	165.0
4	86.2	82.6
4a	39.4	38.4
1′	128.0	126.0
2′, 6′	131.9	130.1
3′, 5′	115.8	114.6
4′	157.2	155.7
1″	112.8	108.6
2″	158.3	158.0
3″	103.8	102.7
4″	163.5	161.2
5″	111.4	107.7
6″	132.6	130.8
Glc-1	102.0	—
2	74.7	—
3	78.3	—
4	71.3	—
5	78.5	—
6	62.6	—

Signal assignments were based on ^{13}C-^{1}H COSY and DEPT experiments.
[1] Measured in CD_3OD.
[2] Measured in DMSO-d_6.

was rechromatographed on an MCI GEL CHP-20P column, eluting with MeOH–H_2O, to give **5** (0.27 g).

6″-O-*malonyl daidzin* (**3**). Colourless powder. $[\alpha]_D$ −45.2° (DMSO; *c* 0.16). IR ν_{max}^{KBr} cm^{-1}: 3376 (*br* OH), 1720 (C=O), 1620 (C=O), 1250, 1198, 1072, 1019, 956, 924. UV λ_{max}^{MeOH} nm (log ε): 202 (4.44), 211 (*infl.* 4.33), 221 (4.21), 233 (*infl.* 4.26), 250 (*infl.* 4.35), 260 (4.38), 294 (*infl.* 3.93). EIMS (70 eV) *m/z* (rel. int.): 284 (10), 254 (100), 137 (82). HR-FABMS *m/z*: 503.1164 (Calcd for $C_{24}H_{23}O_{12}$ $[M+H]^+$: 503.1184). ^{1}H NMR see Table 1. ^{13}C NMR see Table 2.

Methylation of **3** *with* CH_2N_2. An MeOH soln of **3** (323 mg) was added to CH_2N_2–Et_2O (excess amount) at −78° and stirred for 48 hr. The reaction mixt. was concd under red. pres., and purified by prep. TLC [$CHCl_3$–MeOH–H_2O (65:35:10) lower phase] to give a powder **3a** (28 mg). IR ν_{max}^{KBr} cm^{-1}: 3324 (*br* OH), 2924, 1748 (C=O), 1720 (C=O), 1628 (C=O), 1450, 1256, 1072, 1024. UV λ_{max}^{MeOH} nm (log ε): 201 (4.47), 212 (*infl.* 4.34), 231 (4.27), 250 (*infl.* 4.38), 261 (4.41), 303 (*infl.* 3.89). FDMS *m/z*: 517 $[M+H]^+$, 417 [M+H-methyl malonate]$^+$. ^{1}H NMR (acetone-d_6, 200 MHz): δ 3.50 (2H, *s*, H-2‴), 3.55–3.65 (3H, *m*, H-2″, 3″ and 4″), 3.90 (1H, *ddd*, J = 2, 7 and 9 Hz, H-5″), 4.29 (1H, *dd*, J = 7 and 12 Hz, H-6″), 4.59 (1H, *dd*, J = 2 and 12 Hz, H-6″), 4.87 (*br* OH), 5.22 (1H, *d*, J = 8 Hz, H-1″), 6.89 (2H, *d*, J = 9 Hz, H-3′), 7.48 (2H, *d*, J = 9 Hz, H-2′), 7.22 (1H, *d*, J = 2 Hz, H-8), 7.16 (1H, *dd*, J = 2 and 8 Hz, H-6), 8.14 (1H, *d*, J = 8 Hz, H-5), 8.21 (1H, *s*, H-2), 8.50 (1H, *br*, OH). ^{13}C NMR (acetone-d_6, 125 MHz): δ 41.6 (*t*, C-2‴), 52.5 (*q*, OMe), 65.2 (*t*, C-6″), 71.1 (*d*, C-4″), 74.5 (*d*, C-2″), 75.1 (*d*, C-5″), 77.8 (*d*, C-3″), 101.4 (*d*, C-1″), 104.6 (*d*, C-8), 115.9 (*d* × 2, C-3′), 116.3 (*d*, C-6), 120.3 (*s*, C-10), 124.2 (*s*, C-1′ or C-3), 125.4 (*s*, C-3 or C-1′), 128.2 (*d*, C-5), 131.1 (*d* × 2, C-2′), 153.6 (*d*, C-2), 158.3 (*s*, C-4′ or C-9), 158.4 (*s*, C-9 or C-4′), 162.5 (*s*, C-7), 167.2 (*s*, C-3‴ or C-1‴), 167.6 (*s*, C-1‴ or C-3‴), 175.8 (*s*, C-4).

Methanolysis of **3**. An MeOH soln (10 ml) of **3** (27 mg) was refluxed for 40 min. After cooling, a part of the reaction mixt. was added to CH_2N_2–Et_2O to give dimethyl malonate which was identified by GC-MS [column: 3% silicone OV-225, 2 m × 3 mm; carrier

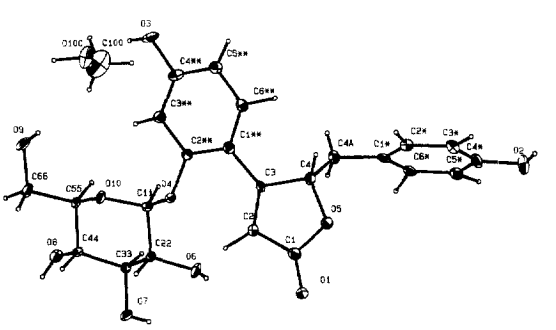


Fig. 1. ORTEP drawing of compound **4**.

gas: He at 35 ml min^{-1}; temp.: 80°; injection temp. 120°; Sep. temp.: 150°] comparison with an authentic sample. The residual reaction mixt. was recrystallized from MeOH to give colourless prisms (4 mg) which were identified as daidzin (**1**) by mixed melting point.

3′-Methoxydaidzin (**6**). Colourless needles (MeOH–H_2O). mp 226–228°. $[\alpha]_D$ −24.3° (DMSO; *c* 0.15). IR ν_{max}^{KBr} cm^{-1}: 3448 (*br* OH), 3308 (OH), 2920 (C-H), 1638 (C=O), 1620, 1608, 1172, 986, 850. UV λ_{max}^{EtOH} nm (log ε): 202 (4.50), 219 (4.46), 249 (*infl.* 4.31), 265 (4.40), 291 (*infl.* 4.18). HR-FABMS *m/z*: 447.1295 (Calcd for $C_{22}H_{23}O_{10}$ $[M+H]^+$: 447.1291). ^1H NMR see Table 1. ^{13}C NMR see Table 2.

Acid hydrolysis of **6**. Compound **6** (0.10 g) was refluxed with 3% aq. HCl (10 ml) for 1 hr. After cooling, the reaction mixt. was extracted with EtOAc (10 ml × 3). The aq. layer was concd under red. pres. to give a sugar residue. The sugar was identified as glucose by comparison with a standard sample, using silica gel TLC [R_f 0.13, $CHCl_3$–MeOH–H_2O (65:35:10) lower phase, R_f 0.20 MeCN–H_2O (17:3)], detected with 10% H_2SO_4, and cellulose TLC [R_f 0.13, 1-BuOH–pyridine–H_2O (6:4:3)], detected with aniline-phthalate reagent [15]. The EtOAc layer was washed with H_2O (10 ml × 2), dried over $MgSO_4$ and concd under red. pres. The residue was purified by prep. HPLC, eluting with *n*-hexane–Me_2CO (3:2), then recrystallized from MeOH to give **7** (colourless needles, 23 mg), mp 257–260°. IR ν_{max}^{KBr} cm^{-1}: 3516 (OH), 3100 (*br* OH), 1620 (C=O), 1588, 1512, 1452, 1288, 1194, 956, 912. UV λ_{max}^{EtOH} nm (log ε): 203 (4.50), 216 (4.42), 239 (*infl.* 4.33), 249 (4.35), 261 (4.35), 289 (*infl.* 4.17), 309 (*infl.* 4.00). EIMS 70 eV, *m/z* (rel. int.): 284 $[M]^+$ (100), 269 $[M\text{-}Me]^+$ (9), 148 (12), 137 (25). ^1H NMR (DMSO-d_6, 400 MHz): δ 3.80 (3H, *s*, OMe), 6.81 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.85 (1H, *d*, *J* = 2.4 Hz, H-8), 6.93 (1H, *dd*, *J* = 2.4 and 8.3 Hz, H-6), 6.99 (1H, *dd*, *J* = 1.9 and 8.3 Hz, H-6′), 7.16 (1H, *d*, *J* = 1.9 Hz, H-2′), 7.97 (1H, *d*, *J* = 8.3 Hz, H-5), 8.31 (1H, *s*, H-2), 9.00 (*br* OH), 10.5 (*br* OH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 55.6 (*q*, OMe), 102.0 (*d*, C-8), 113.3 (*d*, C-2′), 115.0 (*d*, C-6 or C-5′), 115.1 (*d*, C-5′ or C-6), 116.6 (*s*, C-10), 121.5 (*d*, C-6′), 122.9 (*s*, C-3 or C-1′), 123.4 (*s*, C-1′ or C-3), 127.2 (*d*, C-5), 146.4 (*s*, C-3′ or C-4′), 147.1 (*s*, C-4′ or C-3′), 152.9 (*d*, C-2), 157.3 (*s*, C-9), 162.5 (*s*, C-7), 174.6 (*s*, C-4).

Pentaacetyl 3′-methoxydaidzin (**6a**). Colourless prisms (MeOH). mp 186–187°. $[\alpha]_D$ −26.3° ($CHCl_3$; *c* 0.19). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3024, 1758 (C=O), 1646 (C=O), 1626, 1444, 1036. UV $\lambda_{max}^{dioxane}$ nm (log ε): 252 (4.46), 282 (*infl.* 4.08), 304 (3.93). EIMS (70 eV) *m/z* (rel. int.): 656 $[M]^+$ (3.5), 614 $[M\text{-}42]^+$ (16), 331 (21), 169 (96), 109 (49), 43 (100). ^1H NMR ($CDCl_3$, 500 MHz): δ 2.05, 2.07, 2.08, 2.10 and 2.33 (each, 3H, *s*, Ac), 3.89 (3H, *s*, 3′-OMe), 3.96 (1H, *ddd*, *J* = 2.6, 5.7 and 8.3 Hz, Glc-5), 4.22 (1H, *dd*, *J* = 2.6 and 12.4 Hz, Glc-6), 4.29 (1H, *dd*, *J* = 5.7 and 12.4 Hz, Glc-6), 5.19 (1H, *t-like*, *J* = 10.0 Hz, Glc-4), 5.24 (1H, *d-like*, *J* = 7.7 Hz, Glc-1), 5.30–5.37 (2H, *m*, Glc-2 and Glc-3), 7.04 (1H, *dd*, *J* = 1.9 and 8.1 Hz, H-6′), 7.05 (1H, *dd*, *J* = 0.4 and 2.3 Hz, H-8), 7.06 (1H, *dd*, *J* = 2.3 and 8.8 Hz, H-6), 7.09 (1H, *d*, *J* = 8.1 Hz, H-5′), 7.30 (1H, *d*, *J* = 1.9 Hz, H-2′), 7.99 (1H, *s*, H-2), 8.25 (1H, *dd*, *J* = 0.4 and 8.8 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.5 (× 2), 20.6 (× 2) and 20.7 (each, *q*, Ac), 56.0 (*q*, 3′-OMe), 61.9 (*t*, Glc-6), 68.2 (*d*, Glc-4), 71.1 (*d*, Glc-2), 72.5 (*d*, Glc-3 or Glc-5), 72.6 (*d*, Glc-5 or Glc-3), 98.4 (*d*, Glc-1), 104.4 (*d*, C-8), 113.7 (*d*, C-2′), 115.5 (*d*, C-6), 120.3 (*s*, C-10), 120.9 (*d*, C-6′), 122.8 (*d*, C-5′), 124.9 (*s*, C-3), 128.2 (*d*, C-5), 130.5 (*s*, C-1′), 140.0 (*s*, C-4′), 151.0 (*s*, C-3′), 153.0 (*d*, C-2), 157.3 (*s*, C-9), 160.7 (*s*, C-7), 169.0, 169.2, 169.4, 170.1 and 170.4 (each *s*, Ac), 175.4 (*s*, C-4).

Kuzubutenolide A (**4**). Pale yellow prisms (MeOH–H_2O) mp 169–171°. $[\alpha]_D$ +66.2° (DMSO; *c* 0.10). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 3300 (*br* OH), 2916 (C—H), 1692 (C=O), 1604, 1516, 1244, 1110, 1074, 1032, 990, 962. UV λ_{max}^{EtOH} nm (log ε): 200 (4.39), 219 (4.29), 227 (*infl.* 4.25), 243 (*infl.* 4.02), 289 (4.11), 316 (4.22). HR-FABMS *m/z*: 461.1420 (Calcd for $C_{23}H_{25}O_{10}$ $[M+H]^+$: 461.1447). CD (MeOH; $c\ 3.14 \times 10^{-3}$): $[\theta]_{229}$ −44, $[\theta]_{244}$ −39000 (negative maximum), $[\theta]_{285}$ +37000 (positive maximum), $[\theta]_{297}$ +31000, $[\theta]_{308}$ +33000 (positive maximum). ^1H NMR see Table 3. ^{13}C NMR: see Table 4.

X-Ray analysis of **4**. The crystal size of **4** was 0.05 × 0.02 × 0.12 mm. The unit cell dimension was obtained by least-squares refinement using 15 centred reflections for which $20° < \theta < 30°$ (graphite monochromatized CuKα, λ = 1.54184 Å). Data were collected with an Enraf-Nonius CAD-4 System and using the ω–2θ mode with CuKα radiation. Lorenz and polarization, absorption and extinction corrections were applied. The structure was solved by direct methods using an Enraf-Nonius SDP Program and was refined by full matrix least squares. All non-H atoms were refined anisotropically. The parameters were refined by full matrix least squares. Crystal data: $C_{23}H_{24}O_{10}$+MeOH, Orthorhombic, space group $P2_12_12_1$, *a* = 9.060(1), *b* = 28.177(2), *c* = 8.644(1) Å, α = 90.02(1), β = 89.99(1), γ = 90.01(1) deg., *V* = 2206.6(4) $Å^3$, D_{calcd} = 1.386 g cm^{-3}, μ(CuKα) = 8.8 cm^{-1}. The model was refined to *R* = 0.047 for 1277 reflections with $I > 3\sigma$. The other detail parameters as well as bond lengths and angles will be deposited at the Cambridge Crystallographic Data Centre, U.K.

Acid hydrolysis of **4**. Compound **4** (1 mg) was refluxed with 3% HCl for 1 hr. After cooling, the reaction mixt. was extracted with EtOAc (1 ml × 2). The aq. layer was concd under red. pres. to give a sugar residue. The sugar was identified as glucose by comparison with a standard sample.

Enzymatic hydrolysis of **4** *with β-glucosidase*. A mixt. of **4** (21 mg), β-glucosidase (excess amount) and H_2O (3 ml) was incubated at 37° for 20 hr. The reaction mixt. was added to H_2O (8 ml) and extracted with EtOAc (10 ml × 3). The EtOAc layer was dried over $MgSO_4$ and concd under red. pres. The residue was recrystallized from MeOH to give **9** (10 mg), mp 215–217° (decomp.). $[\alpha]_D$ +133.4° (MeOH; *c* 1.01). IR

ν_{max}^{KBr} cm^{-1}: 3600 (*br* OH), 1688 (C=O), 1612, 1572, 1266, 1024, 836, 818. UV λ_{max}^{MeOH} nm (log ε): 201 (4.36), 220 (4.27), 241 (*infl.* 3.92), 286 (4.05), 326 (4.19). EIMS 70 eV, *m/z* (rel. int.): 298 $[M]^+$ (8.7), 254 (98), 239 (100), 161 (39), 107 (97). CD (MeOH; *c* 3.14×10^{-3}): $[\theta]_{230}$ +7000 (positive maximum), $[\theta]_{246}$ −20 000 (negative maximum), $[\theta]_{284}$ +31 000 (positive maximum), $[\theta]_{301}$ +16 000 and $[\theta]_{318}$ +21 000 (positive maximum). ^{1}H NMR (acetone-d_6, 500 MHz): δ 2.76 (1H, *dd*, *J* = 6.7 and 14.6 Hz, H-4a), 3.24 (1H, *dd*, *J* = 3.3 and 14.6 Hz, H-4a), 5.86 (1H, *ddd*, *J* = 1.4, 3.3 and 6.7 Hz, H-4), 6.17 (1H, *d*, *J* = 1.4 Hz, H-2), 6.53 (1H, *dd*, *J* = 2.4 and 8.6 Hz, H-5″), 6.59 (1H, *d*, *J* = 2.4 Hz, H-3″), 6.69 (1H, *d*, *J* = 8.6 Hz, H-3′), 6.94 (2H, *d*, *J* = 8.6 Hz, H-2′), 7.39 (1H, *d*, *J* = 8.6 Hz, H-6″). ^{13}C NMR (acetone-d_6, 125 MHz); δ 40.2 (*t*, C-4a), 84.5 (*d*, C-4), 104.6 (*d*, C-3″), 109.6 (*d*, C-5″), 11.3 (*s*, C-1″), 114.1 (*d*, C-2), 116.1 (*d*×2, C-3′), 128.4 (*s*, C-1′), 131.9 (*d*×2, C-2′), 132.3 (*d*, C-6″), 157.4 (*s*, C-4′), 159.1 (*s*, C-2″ or C-4″), 162.6 (*s*, C-4″ or C-2″), 166.2 (*s*, C-3), 174.1 (*s*, C-1).

3′-hydroxy-4′-O-β-D-glucosyl puerarin (**5**). Colourless powder. $[\alpha]_D$ −17.7° (MeOH; *c* 0.14). IR ν_{max}^{KBr} cm^{-1}: 3400 (*br* OH), 2880 (C—H), 1628 (C=O), 1588, 1508, 1190, 880. UV λ_{max}^{MeOH} nm (log ε): 202 (4.41), 220 (4.34), 244 (*infl.* 4.33), 250 (4.34), 289 (4.02), 310 (*infl.* 3.90). HR-FABMS *m/z*: 595.1664 (Calcd for $C_{27}H_{31}O_{15}$ $[M+H]^+$: 595.1663). ^{1}H NMR see Table 1. ^{13}C NMR see Table 2.

Acid hydrolysis of **5**. Compound **5** (20 mg) was refluxed with dil. HCl for 5 hr. After cooling, the reaction mixt. was chromatographed on an MCI GEL CHP-20P column, eluting with H_2O and 50% MeOH, successively. The H_2O eluate was concd under red. pres. to give a sugar residue. The sugar was identified as glucose by comparison with a standard sample. The 50% MeOH eluate was concd under red. pres. and recrystallized from H_2O to give colourless needles (7 mg) which were identified as PG-1 (**10**) by mixed melting point.

Colourless needles (H_2O). mp 209–211°. $[\alpha]_D$ +13.7° (DMSO; *c* 0.11). IR ν_{max}^{KBr} cm^{-1}: 3400 (*br* OH), 2924 (C—H), 1628 (C=O), 1586, 1290, 1080. UV λ_{max}^{MeOH} nm (log ε): 202 (4.49), 220 (4.39), 241 (*infl.* 4.34), 250 (4.35), 264 (*infl.* 4.28), 291 (4.11), 310 (*infl.* 3.95). FABMS *m/z*: 433 $[M+H]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 3.48 (1H, *ddd*, *J* = 2.2, 5.2 and 9.9 Hz, Glc-5), 3.51–3.54 (2H, *m*, Glc-3 and Glc-4), 3.76 (1H, *br dd*, *J* = 5 and 12 Hz, Glc-6), 3.89 (1H, *dd*, *J* = 2.3 and 12.1 Hz, Glc-6), 4.10 (1H, *br*, Glc-2), 5.10 (1H, *d*, *J* = 9.9 Hz, Glc-1), 6.82 (1H, *d*, *J* = 8.1 Hz, H-5′), 6.86 (1H, *dd*, *J* = 2.0 and 8.1 Hz, H-6′), 6.98 (1H, *d*, *J* = 8.9 Hz, H-6), 7.02 (1H, *d*, *J* = 2.0 Hz, H-2′), 8.05 (1H, *d*, *J* = 8.9 Hz, H-5), 8.15 (1H, *s*, H-2). ^{13}C NMR (125 MHz, CD_3OD): δ 62.8 (*t*, Glc-6), 71.8 (*d*, Glc-4), 73.1 (*d*, Glc-2), 75.8 (*d*, Glc-1), 80.1 (*d*, Glc-3), 82.8 (*d*, Glc-5), 113.2 (*s*, C-8), 116.4 (*d*, C-2′), 116.7 (*d*, C-6), 117.5 (*d*, C-5′), 118.6 (*s*, C-10), 121.8 (*d*, C-6′), 124.8 (*s*, C-3 or C-1′), 125.8 (*s*, C-1′ or C-3), 128.1 (*d*, C-5), 146.3 (*s*, C-3′ or C-4′), 146.8 (*s*, C-4′ or C-3′), 154.5 (*d*, C-2), 158.0 (*br s*, C-9), 163.0 (*s*, C-7), 178.3 (*s*, C-4).

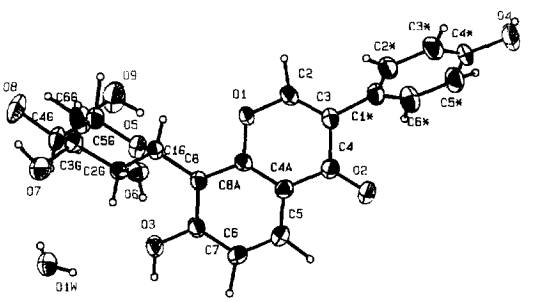


Fig. 2. ORTEP drawing of compound **2**.

X-Ray analysis of **2**. The crystal size of **2** was $0.02 \times 0.17 \times 0.42$ mm. The unit cell dimension was obtained by least-squares refinement using 15 centred reflections for which $20° < \theta < 30°$ (graphite monochromatized CuKα, λ = 1.54184 Å). Data were collected with an Enraf-Nonius CAD-4 System and using the ω–2θ mode with CuKα radiation. Lorenz and polarization, absorption and extinction corrections were applied. The structure was solved by direct methods using an Enraf-Nonius SDP Program and was refined by full matrix least squares. All non-H atoms were refined anisotropically (Fig. 2). Crystal data: $C_{21}H_{20}O_9 + H_2O$, Triclinic, space group P1, *Z* = 2, *a* = 6.3523(3), *b* = 11.4794(4), *c* = 14.1346(3) Å, α = 73.971(2), β = 88.145(3), γ = 88.466(4) deg., *V* = 989.47(7) $Å^3$, D_{calcd} = 1.396 g cm^{-3}. μ(CuKα) = 8.9 cm^{-1}. The model refined to *R* = 0.053 for 3778 reflections with $I > 3\sigma$. The other parameters as well as bond lengths and angles will be deposited at the Cambridge Crystallographic Data Centre, U.K.

Acknowledgements—The authors are grateful to Prof. T. Nohara, Faculty of Pharmaceutical Sciences, Kumamoto University, for providing an authentic sample of PG-1.

REFERENCES

1. Shibata, S., Murakami, T. and Nishikawa, Y., *Yakugaku Zasshi*, 1959, **79**, 757.
2. Murakami, T., Nishikawa, Y. and Ando, T., *Chemical and Pharmaceutical Bulletin*, 1960, **8**, 688.
3. Ohshima, Y., Okuyama, T., Takahashi, K., Takizawa, T. and Shibata, S., *Planta Medica*, 1988, **54**, 250.
4. Kinjo, J., Furusawa, J., Baba, J., Takeshita, T., Yamasaki, M. and Nohara, T., *Chemical and Pharmaceutical Bulletin*, 1987, **35**, 4846.
5. Kinjo, J., Miyamoto, I., Murakami, K., Kida, K., Tomimatsu, T., Yamasaki, M. and Nohara, T., *Chemical and Pharmaceutical Bulletin*, 1985, **33**, 1293.

6. Arao, T., Kinjo, J., Nohara, T. and Isobe, R., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 1176.
7. Hirakura, K., Nakajima, K. Morita, M., Taguchi, H. and Mitsuhashi, H., *Abstracts of Papers, The 108th Annual Meeting of Pharmaceutical Society of Japan*. Hiroshima, April 1988.
8. Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K. and Okubo, K., *Agriculture and Biological Chemistry*, 1991, **55**, 2227.
9. Prak, H., Hakamatsuka, T., Noguchi, H., Sankawa, U. and Ebizuka, Y., *Chemical and Pharmaceutical Bulletin*, 1992, **40**, 1978.
10. Cui, B., Nakamura, M., Kinjio, J. and Nohara, T., *Chemical and Pharmaceutical Bulletin*, 1993, **41**, 178.
11. Markham, K. R., Mabry, T. J. and Swift, T. W., *Phytochemistry*, 1968, **7**, 803.
12. Lü, G., Lü, S., Zhang, G., Xu, S., Li, D. and Huarg, Q., *Zhong Cao Yao*, 1984, **15**, 452.
13. Bezuidenhoudt, B. C. B., Swanepoel, A., Brandt, E. V. and Ferreira, D., *Journal of the Chemical Society, Perkin Transactions I*, 1990, 2599.
14. Nohara, T., Kinjo, J., Furusawa, J., Sakai, Y., Inoue, M., Shirataki, Y., Ishibashi, Y., Yokoe, I. and Komatsu, M., *Phytochemistry*, 1993, **33**, 1207.
15. Partridge, M., *Nature*, 1949, **164**, 443.