

## An Efficient Preparation of Acetyl Isoflavone Glucoside

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Received April 16, 1997; accepted June 13, 1997

Minor components of isoflavones in soybeans, 7-(6-*O*-acetyl- $\beta$ -D-glucopyranosyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (6''-*O*-acetyl daidzin, **5**) and 7-(6-*O*-acetyl- $\beta$ -D-glucopyranosyl)-5-hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (6''-*O*-acetyl genistin, **6**), were efficiently prepared from 6''-*O*-malonyl daidzin (**3**) or 6''-*O*-malonyl genistin (**4**) by decarboxylation in *N,N*-dimethylformamide at 60 °C in 46% or 57% yield, respectively. This reaction may explain the increase in the amount of the acetate during toasting of soybeans.

**Key words** isoflavone; decarboxylation; daidzin; genistin; acetyl glucoside; malonyl glucoside

Soybeans are an essential part of the Japanese diet, being used in soy sauce, miso, tofu, natto, and soymilk. Soybeans are also eaten as a health food in the United States.<sup>1)</sup> They contain a variety of biologically active compounds,<sup>2)</sup> including isoflavones. It has been reported that isoflavones in soybeans, daidzein (**1**) and genistein (**2**) (Fig. 1), have estrogenic,<sup>3)</sup> antioxidative,<sup>4)</sup> antifungal,<sup>5)</sup> and aromatase-inhibitory activities.<sup>6)</sup> Genistein (**2**) is a particularly potent inhibitor of tyrosine protein kinase<sup>7)</sup> and DNA topoisomerase.<sup>8)</sup>

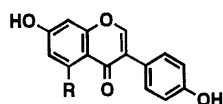
Compounds **1** and **2** mainly exist in 6''-*O*-malonyl glucoside form in soybeans, that is, 6''-*O*-malonyl daidzin (**3**) and 6''-*O*-malonyl genistin (**4**) (Fig. 2).<sup>9)</sup> We have reported the extraction of these malonates **3** and **4** from soybeans.<sup>10)</sup> 6''-*O*-Acetyl daidzin (**5**) and 6''-*O*-acetyl genistin (**6**) are known to be trace ingredients in soybeans.<sup>1,11)</sup> There have been few studies on the biological activities of the acetyl derivatives **5** and **6** because of the difficulty in obtaining them in large amounts.<sup>12)</sup> These two acetates, however, are expected to possess more potent activities than the isoflavones **1** and **2**. In this paper we report an efficient method of preparing these acetyl glucosides.

or **6** is the decarboxylation of the malonyl glucosides **3** or **4**, which are readily available in large quantities.<sup>10)</sup> There have been many reports on decarboxylation of various esters or carboxylic acids.<sup>13)</sup> According to Westheimer and Jones,<sup>14)</sup> the polarity of solvents does not affect the rate of decarboxylation. We initially used H<sub>2</sub>O, MeOH, or EtOH, in which malonyl glucosides **3** and **4** are highly soluble. When the malonate **3** was treated at 40 °C in each solvent, daidzin (**7**) was formed in low yield, instead of the desired acetate **5** because of the direct hydrolysis or alcoholysis of the malonate (entries 1–3 in Table 1). In less polar solvents, *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, or 1,4-dioxane, the desired product was not obtained because of poor solubility of **3** (entries 4–6 in Table 1).

Next, aprotic polar solvents were tested: CH<sub>3</sub>CN, *N,N*-dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), and *N,N*-dimethylformamide (DMF). As we expected, decarboxylation in these solvents gave the desired acetate **5**; although the yield was poor in CH<sub>3</sub>CN and DMA, good results were obtained in DMSO and DMF. We selected DMF for use as a solvent because of

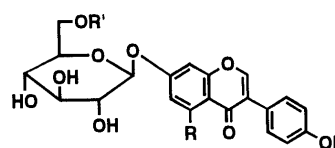
### Results and Discussion

An obvious approach to preparing acetyl glucosides **5**



1: R=H, daidzein  
2: R=OH, genistein

Fig. 1



3: R=H, R'=COCH<sub>2</sub>COOH, 6''-*O*-malonyl daidzin  
4: R=OH, R'=COCH<sub>2</sub>COOH, 6''-*O*-malonyl genistin  
5: R=H, R'=COCH<sub>3</sub>, 6''-*O*-acetyl daidzin  
6: R=OH, R'=COCH<sub>3</sub>, 6''-*O*-acetyl genistin  
7: R=H, R'=H, daidzin  
8: R=OH, R'=H, genistin

Fig. 2

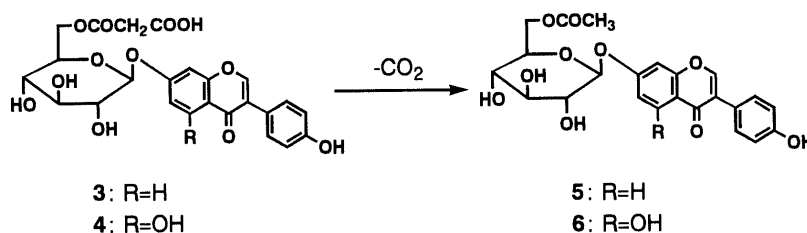


Fig. 3

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Table 1. Decarboxylation of **3** in Various Solvents at 40 °C

Entry	Solvent	<b>5</b> (yield, <sup>a</sup> %)	<b>7</b> (yield, %)
1	H <sub>2</sub> O	ND <sup>b)</sup>	15
2	MeOH	ND	10
3	EtOH	ND	9
4	<i>n</i> -Hexane	ND	ND
5	CH <sub>2</sub> Cl <sub>2</sub>	ND	ND
6	1,4-Dioxane	ND	ND
7	CH <sub>3</sub> CN	4	ND
8	DMA	15	ND
9	DMSO	75	ND
10	DMF	79	ND

a) Determined by HPLC with CH<sub>3</sub>CN: 0.1% aqueous AcOH (3:7, v/v).  
b) Not detected.

Table 2. Decarboxylation of **4** by Various Methods

Entry	Solvent	Additive	Temp. (°C)	<b>6</b> (yield, <sup>a</sup> %)
1	H <sub>2</sub> O	H <sub>2</sub> SO <sub>4</sub>	20	ND <sup>b)</sup>
2	Cyclohexanol	Cyclohexenone	100	ND
3	Toluene	DMAP	100	ND
4	DMSO	NaCl	40	31
5	DMSO	Na <sub>3</sub> PO <sub>4</sub>	40	64
6	DMSO	NaOAc	40	46
7	DMF	— <sup>c)</sup>	40	76

a) Determined by HPLC with CH<sub>3</sub>CN: 0.1% aqueous AcOH (3:7, v/v).  
b) Not detected. c) No additive.

the high yield and convenient work-up (entries 7–10 in Table 1).

Other methods were tested to obtain higher yields of acetyl glucosides using 6''-*O*-malonylgenistin (**4**) as a starting material. When the malonate **4** was dissolved in 30% H<sub>2</sub>SO<sub>4</sub> at 20 °C,<sup>15)</sup> decomposition occurred, affording a complex mixture without the desired acetate **6** (entry 1 in Table 2). When **4** was added to cyclohexanol in the presence of cyclohexenone and the reaction mixture was heated to 100 °C,<sup>16)</sup> no reaction occurred (entry 2 in Table 2). Reflux of **4** in toluene with 4-dimethylaminopyridine (DMAP)<sup>17)</sup> did not give the acetate **6** (entry 3 in Table 2). Reaction of **4** in DMSO at 40 °C with NaCl, Na<sub>3</sub>PO<sub>4</sub>, or NaOAc<sup>18)</sup> afforded the desired acetate **6** in 31, 64, or 46% yield, respectively (entries 4–6 in Table 2). Thus, no reported method was superior to our simple technique of heating **4** in DMF (entry 7 in Table 2).

On the basis of these results, we turned our attention to the relationship between yield and reaction time in this decarboxylation of **3** at various temperatures. As shown in Fig. 4, the reaction in DMF was completed within 30 min at above 80 °C, followed by gradual decomposition. At 60 °C, the reaction was completed within 1 h without decomposition. At 20 and 40 °C, the reaction rates were so slow that large-scale preparation was rather impractical under these conditions. At 0 °C, the reaction did not occur at all. Similar results were found in the case of another malonate **4**, as indicated in Fig. 5. The optimal conditions for preparation of the acetates were thus heating at 60 °C in DMF. This method was used to prepare the acetates **5** and **6** in 46% and 57% isolated yields, respectively.

In conclusion, we have developed a very efficient and convenient method for preparation of 6''-*O*-acetyldaidzin

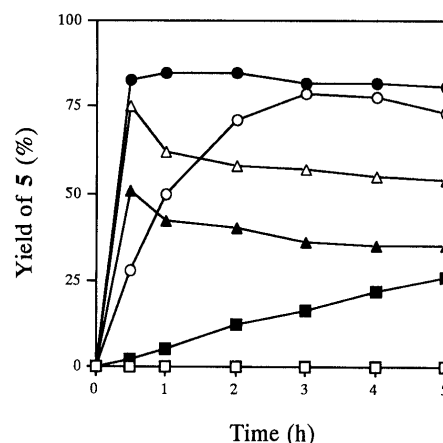


Fig. 4. Time Courses of Decarboxylation of **3** at Various Temperatures  
Temperatures: □, 0 °C; ■, 20 °C; ○, 40 °C; ●, 60 °C; △, 80 °C; ▲, 100 °C.

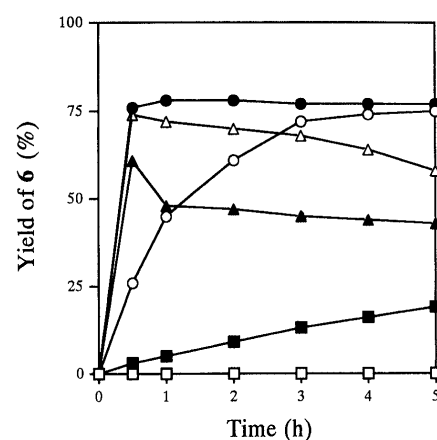


Fig. 5. Time Courses of Decarboxylation of **4** at Various Temperatures  
Temperatures: □, 0 °C; ■, 20 °C; ○, 40 °C; ●, 60 °C; △, 80 °C; ▲, 100 °C.

(**5**) and 6''-*O*-acetylgenistin (**6**) from the malonyl derivatives **3** and **4**. This reaction is considered to be the reason why the amount of the acetate increases during toasting of soybeans.<sup>1)</sup> This also demonstrates that the interior of toasted soybeans is a hydrophobic environment. This method makes it possible to obtain these acetates on a large scale for further studies.

#### Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. <sup>1</sup>H-NMR spectra were taken at 199.50 MHz and <sup>13</sup>C-NMR spectra were taken at 50.10 MHz with a JEOL JNM-FX200 spectrometer and tetramethylsilane as an internal standard. IR spectra were taken with a JASCO FT/IR-7300 spectrometer. MS were determined with a Hitachi M-80 spectrometer. HPLC was performed on a YMC-Pack ODS-AQ312 column (6.0 mm i.d. × 150 mm) with a flow rate of 1.0 ml/min using a JASCO pump (PU-980) and an ultraviolet detector (JASCO UV-970) at room temperature. Column chromatography was performed on YMC-GEL ODS AQ 120-S50 (250–350 mesh, from Yamamura Chemical Laboratories Co., Ltd.) and HP-20 gel (from Mitsubishi Chemical Ind.).

**Preparation of 6''-*O*-Malonyldaidzin (**3**) and 6''-*O*-Malonylgenistin (**4**)**  
Dehulled soybeans (3.0 kg) were extracted in H<sub>2</sub>O (30 l) at 50 °C for 2 h with the pH adjusted to 8.0, then the pH of the extract (25 l) was adjusted to 4.0 by adding concentrated HCl. The mixture was allowed to stand for 2 h, then the supernatant (20 l) was obtained by decantation. The solution was chromatographed on an HP-20 gel column using an EtOH–H<sub>2</sub>O gradient of 5–50% to give solutions of crude **3** and crude **4**, respectively. The solution of crude **3** was adjusted to pH 8.0, then concentrated, followed by chromatography of the residue on an octadecyl

silica (ODS) gel column with EtOH-H<sub>2</sub>O (1:9, v/v) to afford pure **3** (1.15 g) as the sodium salt. The solution of crude **4** was similarly treated to afford pure **4** (1.36 g) as the sodium salt. <sup>1</sup>H- or <sup>13</sup>C-NMR data for **3** and **4** thus prepared were in accord with those previously reported.

**Decarboxylation of 6''-O-Malonyldaidzin (3) (Entries 1–10 in Table 1)** 6''-O-Malonyldaidzin (**3**, 10 mg, 0.02 mmol) was added to each solvent (10 ml), and the solution was stirred at 40 °C for 5 h. The yield was determined by HPLC analysis.

**Decarboxylation of 6''-O-Malonylgenistin (4) (Entry 1 in Table 2)** 6''-O-Malonylgenistin (**4**, 10 mg, 0.02 mmol) was added to concentrated H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O (3:7, v/v), and the mixture was stirred at 20 °C for 5 h. The yield was determined by HPLC analysis.

**Entry 2 in Table 2** 6''-O-Malonylgenistin (**4**, 0.5 g, 1.0 mmol) and cyclohexenone (5 mg, 0.05 mmol) were added to cyclohexanol (2.5 ml), and the mixture was refluxed for 5 h. The yield was determined by HPLC analysis.

**Entry 3 in Table 2** 6''-O-Malonylgenistin (**4**, 0.5 g, 1.0 mmol) and DMAP (62 mg, 0.5 mmol) were added to 1.0 M phosphate buffer (pH 7.0, 2.5 ml) and toluene (2.5 ml), and the mixture was refluxed for 5 h. The yield was determined by HPLC analysis.

**Entries 4–6 in Table 2** 6''-O-Malonylgenistin (**4**, 10 mg, 0.02 mmol) and a salt (100 mg) were added to DMSO (10 ml), and the mixture was heated at 40 °C for 5 h. The yield was determined by HPLC analysis.

**Preparation of 6''-O-Acetyldaidzin (5)** 6''-O-Malonyldaidzin (**3**, 15.00 g, 0.03 mol) was added to DMF (300 ml), and the solution was kept at 60 °C for 3 h until HPLC indicated that the reaction was complete. The solvent was evaporated off and the residue was chromatographed on an ODS gel column with EtOH-H<sub>2</sub>O (1:4, v/v) to give crude 6''-O-acetyldaidzin (**5**). Recrystallization from MeOH afforded analytically pure **5** (5.24 g, 46%). mp 184–186 °C. IR (KBr) cm<sup>-1</sup>: 1738 (C=O), 1625, 1605. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.02 (3H, s), 3.65–3.80 (1H, m), 4.12 (1H, dd, *J*=11.8, 7.0 Hz), 4.35 (1H, d, *J*=11.8 Hz), 5.05–5.20 (1H, m), 5.14 (1H, d, *J*=7.0 Hz), 5.20–5.25 (1H, m), 5.35–5.45 (1H, m), 6.81 (2H, d, *J*=8.5 Hz), 7.14 (1H, dd, *J*=8.8, 2.1 Hz), 7.23 (1H, d, *J*=2.1 Hz), 7.41 (2H, d, *J*=8.5 Hz), 8.06 (1H, d, *J*=8.8 Hz), 8.35 (1H, s), 9.44 (1H, s). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ: 20.8 (C-2''), 63.5 (C-6''), 69.9 (C-4''), 73.1 (C-2'), 74.1 (C-5'), 76.3 (C-3'), 100.1 (C-1'), 103.7 (C-8), 115.2 (C-3',5'), 115.7 (C-6), 118.8 (C-10), 122.6 (C-1'), 124.0 (C-3), 127.2 (C-5), 130.2 (C-2',6'), 153.4 (C-2), 157.2 (C-9), 157.3 (C-4'), 161.4 (C-7), 170.4 (C-1''), 175.2 (C-4). MS *m/z*: 458 (M<sup>+</sup>). Anal. Calcd for C<sub>23</sub>H<sub>22</sub>O<sub>10</sub>: C, 60.26; H, 4.84; O, 34.90. Found: C, 60.22; H, 4.87; O, 34.91.

**Preparation of 6''-O-Acetylgenistin (6)** 6''-O-Malonylgenistin (**4**, 6.00 g, 0.01 mol) was added to DMF (150 ml), and the solution was kept at 60 °C for 3 h until HPLC indicated that the reaction was complete. Work-up was as described for 6''-O-acetyldaidzin (**5**). Recrystallization from EtOH-H<sub>2</sub>O afforded analytically pure **6** (2.62 g, 57%), mp 195–197 °C. IR (KBr) cm<sup>-1</sup>: 1734 (C=O), 1655, 1615. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.02 (3H, s), 3.65–3.80 (1H, m), 4.08 (1H, dd, *J*=11.8, 7.2 Hz), 4.33 (1H, d, *J*=11.8 Hz), 5.05–5.20 (1H, m), 5.14 (1H, d,

*J*=7.2 Hz), 5.20–5.25 (1H, m), 5.35–5.45 (1H, m), 6.81 (2H, d, *J*=8.5 Hz), 7.14 (1H, d, *J*=2.1 Hz), 7.41 (2H, d, *J*=8.5 Hz), 7.79 (1H, d, *J*=2.1 Hz), 8.35 (1H, s), 9.44 (1H, s). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ: 20.7 (C-2''), 61.7 (C-6''), 70.1 (C-4''), 73.2 (C-5'), 73.8 (C-2'), 76.1 (C-3'), 94.9 (C-8), 99.7 (C-6), 99.8 (C-1''), 106.2 (C-10), 115.2 (C-3',5'), 121.2 (C-1'), 122.9 (C-3), 130.3 (C-2',6'), 154.6 (C-2), 157.2 (C-9), 157.3 (C-4'), 161.4 (C-5), 162.8 (C-7), 170.4 (C-1''), 175.2 (C-4). MS *m/z*: 474 (M<sup>+</sup>). Anal. Calcd for C<sub>23</sub>H<sub>22</sub>O<sub>11</sub>: C, 58.23; H, 4.67; O, 37.10. Found: C, 58.24; H, 4.71; O, 37.05.

## References and Notes

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