An Efficient Preparation of Acetyl Isoflavone Glucoside

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Minor components of isoflavones in soybeans, 7-(6-*O*-acetyl-β-D-glucopyranosyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (6″-*O*-acetyldaidzin, 5) and 7-(6-*O*-acetyl-β-D-glucopyranosyl)-5-hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (6″-*O*-acetylgenistin, 6), were efficiently prepared from 6″-*O*-malonyldaidzin (3) or 6″-*O*-malonylgenistin (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

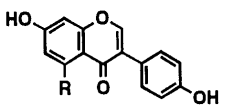
Soybeans are an essential part of the Japanese diet, being used in soy sauce, miso, tofu, natto, and soymilk. Soybeans are also eaten as a health food in the United States.[1] They contain a variety of biologically active compounds,[2] including isoflavones. It has been reported that isoflavones in soybeans,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

Compounds **1** and **2** mainly exist in 6″-*O*-malonyl glucoside form in soybeans, that is, 6″-*O*-malonyldaidzin (**3**) and 6″-*O*-malonylgenistin (**4**) (Fig. 2).[9] We have reported the extraction of these malonates **3** and **4** from soybeans.[10] 6″-*O*-Acetyldaidzin (**5**) and 6″-*O*-acetylgenistin (**6**) are known to be trace ingredients in soybeans.[1,11] 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.[12] 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.

Results and Discussion

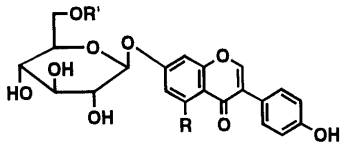
An obvious approach to preparing acetyl glucosides **5** or **6** is the decarboxylation of the malonyl glucosides **3** or **4**, which are readily available in large quantities.[10] There have been many reports on decarboxylation of various esters or carboxylic acids.[13] According to Westheimer and Jones,[14] the polarity of solvents does not affect the rate of decarboxylation. We initially used H_2O, 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_2Cl_2, 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_3CN, *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_3CN and DMA, good results were obtained in DMSO and DMF. We selected DMF for use as a solvent because of



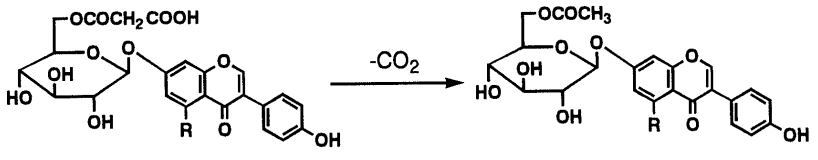
1: R=H, daidzein
2: R=OH, genistein

Fig. 1



3: R=H, R'=$COCH_2COOH$, 6"-*O*-malonyldaidzin
4: R=OH, R'=$COCH_2COOH$, 6"-*O*-malonylgenistin
5: R=H, R'=$COCH_3$, 6"-*O*-acetyldaidzin
6: R=OH, R'=$COCH_3$, 6"-*O*-acetylgenistin
7: R=H, R'=H, daidzin
8: R=OH, R'=H, genistin

Fig. 2



3: R=H
4: R=OH

5: R=H
6: R=OH

Fig. 3

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

Entry	Solvent	**5** (yield,[a] %)	**7** (yield, %)
1	H_2O	ND[b]	15
2	MeOH	ND	10
3	EtOH	ND	9
4	*n*-Hexane	ND	ND
5	CH_2Cl_2	ND	ND
6	1,4-Dioxane	ND	ND
7	CH_3CN	4	ND
8	DMA	15	ND
9	DMSO	75	ND
10	DMF	79	ND

a) Determined by HPLC with CH_3CN: 0.1% aqueous AcOH (3:7, v/v). *b*) Not detected.

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

Enty	Solvent	Additive	Temp. (°C)	**6** (yield,[a] %)
1	H_2O	H_2SO_4	20	ND[b]
2	Cyclohexanol	Cyclohexenone	100	ND
3	Toluene	DMAP	100	ND
4	DMSO	NaCl	40	31
5	DMSO	Na_3PO_4	40	64
6	DMSO	NaOAc	40	46
7	DMF	—[c]	40	76

a) Determined by HPLC with CH_3CN: 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_2SO_4 at 20 °C,[15] 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,[16] no reaction occurred (entry 2 in Table 2). Reflux of **4** in toluene with 4-dimethylaminopyridine (DMAP)[17] did not give the acetate **6** (entry 3 in Table 2). Reaction of **4** in DMSO at 40 °C with NaCl, Na_3PO_4, or NaOAc[18] 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

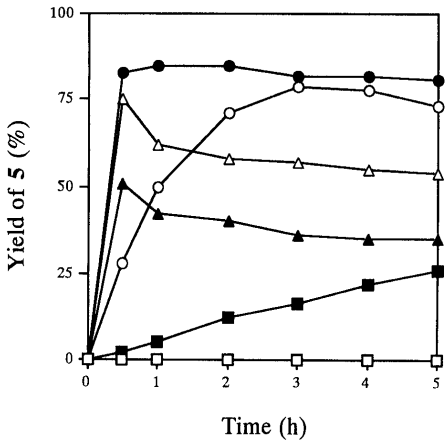

Fig. 4. Time Courses of Decarboxylation of **3** at Various Temperatures
Temperatures: □, 0 °C; ■, 20 °C; ○, 40 °C; ●, 60 °C; △, 80 °C; ▲, 100 °C.

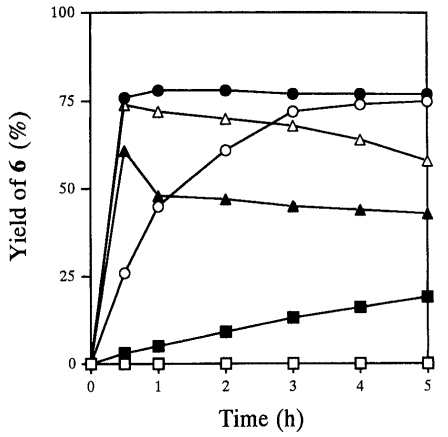

Fig. 5. Time Courses of Decarboxylation of **4** at Various Temperatures
Temperatures: □, 0 °C; ■, 20 °C; ○, 40 °C; ●, 60 °C; △, 80 °C; ▲, 100 °C.

(**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.[1] 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. ^{1}H-NMR spectra were taken at 199.50 MHz and ^{13}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_2O (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_2O 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_2O (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. ^{1}H- or ^{13}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_2SO_4–H_2O (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_2O (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^{-1}: 1738 (C=O), 1625, 1605. ^{1}H-NMR (DMSO-d_6) 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). ^{13}C-NMR (DMSO-d_6) 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^+). *Anal.* Calcd for $C_{23}H_{22}O_{10}$: 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_2O afforded analytically pure **6** (2.62 g, 57%), mp 195—197 °C. IR (KBr) cm^{-1}: 1734 (C=O), 1655, 1615. ^{1}H-NMR (DMSO-d_6) 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). ^{13}C-NMR (DMSO-d_6) 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^+). *Anal.* Calcd for $C_{23}H_{22}O_{11}$: C, 58.23; H, 4.67; O, 37.10. Found: C, 58.24; H, 4.71; O, 37.05.

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