Isolation of 6″-*O*-Acetylgenistin and 6″-*O*-Acetyldaidzin from Toasted Defatted Soyflakes

Efi Farmakalidis and Patricia A. Murphy*

The isoflavones, 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin, were isolated and purified from toasted defatted soyflakes. Their structure was confirmed with nuclear magnetic resonance spectroscopy, mass spectroscopy, ultraviolet and infrared spectroscopy, and acidic and enzymatic hydrolysis. Defatted soyflakes contained 52 ppm of 6″-*O*-acetyldaidzin and 88 ppm of 6″-*O*-acetylgenistin. Acetone with 0.1 N HCl was found superior to 80% methanol for extraction of these compounds. Four varieties of soybeans were analyzed for their content of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin.

INTRODUCTION

Soybeans contain the estrogenic isoflavones genistein, daidzein, and their glucosides, genistin and daidzin (Naim et al., 1974; Eldridge, 1982a; Murphy, 1981). Gyorgy et al. (1964) reported the presence of 6,7,4′-trihydroxyisoflavone in fermented soybeans (tempeh), which was a product of the fermentation process. Naim et al. (1973) reported the presence of the isoflavones, glycitin and glycitein. The compounds are the 6-methoxy derivatives of daidzin and daidzein, respectively. Eldridge (1982a,b) and Eldridge and Kwolek (1983) also reported the presence of glycitin and glycitein in a variety of soybean products analyzed by high-performance liquid chromatography. Other researchers (Ohta et al., 1979, 1980) reported that glycitin and glycitein were not detected in their extracts. Instead 6″-*O*-acetylgenistin and 6″-*O*-acetylydaidzin were detected. The structures of the isoflavone glucosides found in soybeans are shown in Figure 1. Earlier reports from our laboratory (Murphy, 1982) have drawn attention to two additional unidentified peaks present in chromatograms of several soy protein-based foods analyzed by high-performance liquid chromatography.

To date, there has been only one report (Eldridge, 1982b) that gives quantitative data on glycitin and glycitein in soybeans and soy protein products. No quantitative data exist for 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin. Bioactivities for glycitein, glycitin, and the two acetyl isoflavones as estrogens have not been reported.

In view of the differences observed among researchers concerning the natural occurrence of soy isoflavones, it was of interest to isolate and characterize the compounds contained in peaks 3 and 4 (Figure 2) observed in our chromatograms. Four soybean varieties were analyzed for their content of isoflavones. Two solvent systems were compared for extraction efficiency of the two isoflavones isolated from soyflakes.

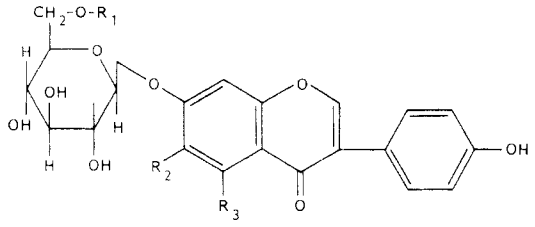
MATERIALS AND METHODS

Extraction and Isolation. Toasted defatted soyflakes were obtained from A. E. Staley and Co. (Des Moines, IA). Soyflakes (2 kg) was extracted by stirring with acetone and 0.1 N HCl overnight in the ratio of 5:1 (mL/g of flakes). After filtration through Whatman filter paper number 1, the extract was concentrated on a steam bath and then dried by using a rotary evaporator at 40 °C. The crude extract was suspended in chloroform–methanol (9:1) and chromatographed through a 4 × 50 cm silica gel column 60–80 mesh, J. T. Baker, Knoxville, TN). The column was washed with 400 mL of chloroform–methanol (9:1). The compounds under investigation were eluted from the column with 250 mL of chloroform–methanol (85:15). After evaporation of the solvent, the residue was dissolved in 50% water-saturated ethyl acetate and chromatographed on a 2.5 × 50 cm silica gel column (60–80 mesh) according to the method of Ohta et al. (1979). Partial separation of the two new isoflavones was achieved at this point. Further fractionation to the individual compounds was achieved by chromatography on a 2.5 × 50 cm Sephadex LH-20 column with 100% ethanol (Ohta et al., 1979). Purification of the compound contained in peak 3 was necessary at this point to remove impurities, mainly daidzin. This was accomplished by repeated chromatography through a silica gel column equilibrated with 50% water-saturated ethyl acetate (Ohta et al., 1979). The compound in peak 3 (6″-*O*-acetyldaidzin) was precipited twice from 90% methanol. The compound in peak 4 (6″-*O*-acetylgenistin) was precipitated twice from 25% methanol and once from 20% ethanol.

Ultraviolet (UV) spectra (in methanol) were measured in a Beckman DK-2 spectrophotometer. The effects of $AlCl_3$–HCl, sodium methoxide, and sodium acetate on the UV absorption maxima were carried out according to Mabry et al. (1970). Infrared spectra (on KBr disk) were performed in a Beckman IR-2 spectrophotometer. The proton magnetic resonance (^1H NMR) spectrum of the trimethylsilated derivative (Mabry et al., 1970) of 6″-*O*-acetylgenistin was performed on a Brucker WM-300 spectrophotometer with tetramethylsilane (Me_4Si) as an internal standard. Electron impact mass spectra (ms) were recorded on a Finnigan Model 3680A and AE1 MS902 for exact mass. Chemical ionization mass spectrometry with isoctane was performed on a Finnigan Model 6817A. Analytical thin-layer chromatography (TLC) was performed on silica gel G (Fisher Scientific Co.) with the following solvent systems: A, chloroform–methanol (9:1) (Beck, 1964); B, benzene–ethyl acetate–petroleum ether (bp 40–60 °C)–methanol (6:4:3:1) (Barz, 1969); C, ether–petroleum ether (bp 40–60 °C) (7:3) (Barz, 1969); D, chloroform–methanol–water (65:25:4) (Wang 1971). Spots were visualized with the aid of a UV lamp at 366 nm (Beck, 1964). Enzymatic hydrolysis with almond β-glucosidase (Sigma Chemical Co., St. Louis, MO) and acidic hydrolysis with 6% HCl was carried out according to Mabry et al. (1970). Elemental analysis was performed by Gailbraith Laboratories (Knoxville, TN). Analysis of the carbohydrate moiety was performed by analytical thin-layer chromatography on Kieselguhr G (Merck Co) impregnated with phosphate buffer, pH 5 (Lewis and Smith, 1969). The solvent system used was butanol–acetone–sodium phosphate buffer, pH 5 (40:50:10) (Waldi, 1965).

Department of Food Technology, Iowa State University, Ames, Iowa 50011.

0021-8561/85/1433-0385$01.50/0



R_1-H;	R_2-H;	R_3-H;	Daidzin
R_1-H;	R_2-OCH_3;	R_3-H;	Glycitin
R_1-H;	R_2-H	R_3-OH;	Genistin
R_1-$COCH_3$	R_2-H	R_3 H;	6"-O-Acetyldaidzin
R_1-$COCH_3$	R_2-H	R_3-OH	6"-O-Acetylgenistin

Figure 1. The structures of isoflavonoid glucosides.

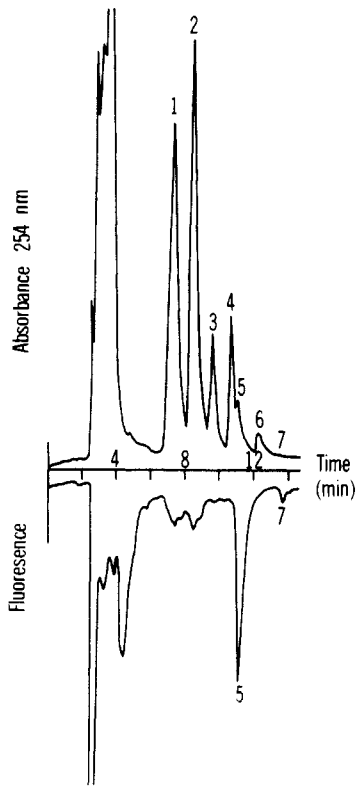


Figure 2. HPLC chromatogram of toasted defatted soyflakes extracted with acetone–HCl. Peaks: 1 = daidzin; 2 = genistin; 3 = 6″-*O*-acetyldaidzin; 4 = 6″-*O*-acetylgenistin; 5 = daidzein; 6 = genistein; 7 = coumestrol.

Comparison of Extraction Methods. Samples from the same batch of toasted defatted soyflakes were extracted with (a) acetone and 0.1 N HCl (Murphy, 1981) and (b) with 80% methanol (Eldridge, 1982b). The extracts obtained were analyzed by high-performance liquid chromatography (HPLC) according to Murphy (1981, 1982).

Identification of Compounds. Purity of the compounds was determined by melting points, thin-layer chromatography, and high-performance liquid chromatography. Melting points were not corrected.

Analysis of Soybean Samples. Soybean samples were obtained from the Agronomy Department at Iowa State University. Four samples of Amsoy 71 and two samples of Vinton 81 varieties grown in different counties in Iowa, in different years, were analyzed. The year and location of growth for Strayer and Weber soybean varieties analyzed were unknown. Defatted samples were extracted with acetone–HCl. The extracts obtained were analyzed by high-performance liquid chromatography (Murphy, 1981, 1982).

Table I. R_f Values for Soybean Isoflavones on Silica Gel G

solvent[a]	acetyl daidzin	acetyl genistin	daidzin	genistin	daidzein	genistein
A	0.19	0.30	0.05	0.13	0.69	0.76
B	0.13	0.20	0.06	0.08	0.59	0.73
C	0	0	0	0	0.12	0.41
D	0.74	0.78	0.46	0.50	0.90	0.92

[a] Solvent compositions appear in Materials and Methods.

Table II. UV Absorption Maxima (nm) of Acetylgenistin and Acetyldaidzin

reagent	6″-*O*-acetyl-genistin	6″-*O*-acetyl-daidzin
methanol	260	256
methanol/Na methoxide	276	258
methanol/$AlCl_3$:HCl	272	256
methanol/Na acetate	260	256

Table III. R_f Values for Sugar Standards and Unknown Sugars

sugar	R_f value
glucose	0.40
fructose	0.47
xylose	0.52
ribose	0.64
rhamnose	0.68
sugar (obtained by acid hydrolysis)	0.39
sugar (obtained by enzyme hydrolysis)	0.00

RESULTS AND DISCUSSION

Identification of Compounds. The purity of the compounds isolated was confirmed by TLC, HPLC, and melting points (mp 186 ± 2 °C, 6″-*O*-acetyldaidzin; mp 196 ± 2 °C, 6″-*O*-acetylgenistin). TLC R_f values of the compounds in the various solvents appear in Table I. The compounds were less polar than the glucosides, genistin and daidzin, but more polar than genistein and daidzein as shown by TLC and HPLC analysis. This indicated that the two new compounds had fewer hydroxyl groups than the glucosides (genistin and daidzin) but more hydroxyl groups than the aglucones (genistein and daidzein).

Ultraviolet absorption maxima of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin in methanol and with the various reagents appear appear in Table II. Addition of Na methoxide caused a bathochromic shift in the spectrum of 6″-*O*-acetylgenistin, indicating the presence of a hydroxyl group in the A ring. Addition of $AlCl_3$:HCl indicated the presence of a 5-hydroxyl group in 6″-*O*-acetylgenistin. The UV absorption maxima of both compounds did not show any change with the addition of Na acetate, indicating a bound 7-OH group.

The structures of 6″-*O*-acetyldaidzin and 6″-*O*-acetylgenistin were assigned to the compounds corresponding to peaks 3 and 4 (Figure 2), respectively, on the basis of the following experimental results.

Enzymatic hydrolysis of the compounds with β-glucosidase was not complete and yielded a mixture of the original isoflavonoid and (a) genistein when 6″-*O*-acetylgenistin was used or (b) daidzein when 6″-*O*-acetyldaidzin was used. In contrast, acid hydrolysis yielded mixtures of genistein or daidzein with genistin or daidzin, rather than the original isoflavonoid, respectively. These results, combined with those obtained by UV spectroscopy (Table II), indicated that the compounds were derivatives of genistin and daidzin. Analysis of the carbohydrate moiety (Table III) obtained by enzymatic hydrolysis yielded a

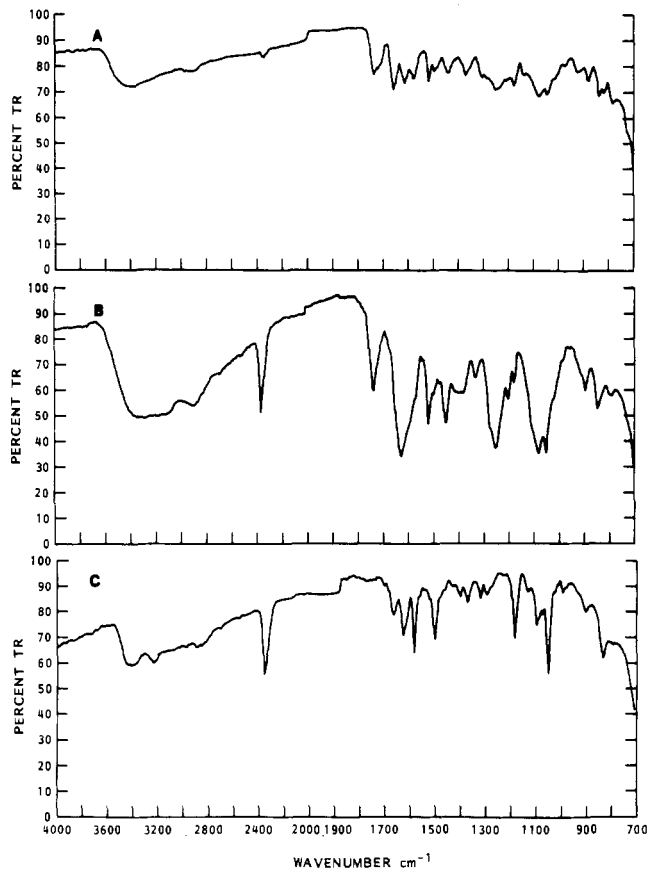


Figure 3. Infrared spectrum of (A) 6″-*O*-acetylgenistin, (B) 6″-*O*-acetyldaidzin, and (C) genistin.

sugar that did not correspond to any of the standard sugars used. Analysis of the carbohydrate moiety obtained through acid hydrolysis revealed the presence of D-glucose. The difference in the identity of the carbohydrate moiety observed between the enzymatic and acidic hydrolysis suggested that the sugar moiety attached to the isoflavonoid nucleus was modified in some way. Enzymatic hydrolysis of the bond linking the carbohydrate moiety to the isoflavonoid nucleus would yield genistein or daidzein from the compounds and a modified sugar molecule. Acid hydrolysis produces genistin or daidzin and glucose.

The infrared spectra of 6″-*O*-acetylgenistin (Figure 3 part A) and 6″-*O*-acetyldaidzin (Figure 3 part B) show absorption at 1735 cm^{-1} that was absent from the spectrum of genistin (Figure 3 part C) and daidzin (spectrum not shown), respectively. The peak at 1735 cm^{-1} was assigned to an aliphatic acetoxyl group (Harborne et al., 1975). Infrared peaks were assigned as follows: IR ν_{max} 3400 (KB) (OH), 1735 (CH_3COOR), 1625 (C=O), 1605, 1570, 1515 (C=C), 1260 (aromatic C–O).

The ^1H NMR spectrum of 6″-*O*-acetylgenistin (60 MHz in CCl_4) showed the signal of a doublet at δ 5.1. This was assigned to the anomeric proton of the glucose molecule, indicating a β-configuration of the glucosidic linkage (Mabry et al., 1970). The signals of the sugar protons between 3.6 and 3.9 were assigned to the protons at 2″, 3″, and 4″ of the glucose molecule. The signals between 4.1 and 4.5 were assigned to 5″ and 6″-protons, seemingly because of the downfield shifts due to the acetyl group (Higuchi and Donnely, 1978). The signals for the aromatic protons were observed at δ 7.4 (2′-H, 6′-H), 6.69–6.95 (3′-H, 5′-H, 8′-H), 6.4 (6-H), 2.1 (acetyl group), 7.8 (2-H).

The molecular formula $C_{23}H_{22}O_{11}$ was assigned to 6″-*O*-acetylgenistin by mass spectrum, *m/e* 474 (M^+), and by elemental analysis. Anal. Found: C, 57.9; H, 4.5; O, 37.5.

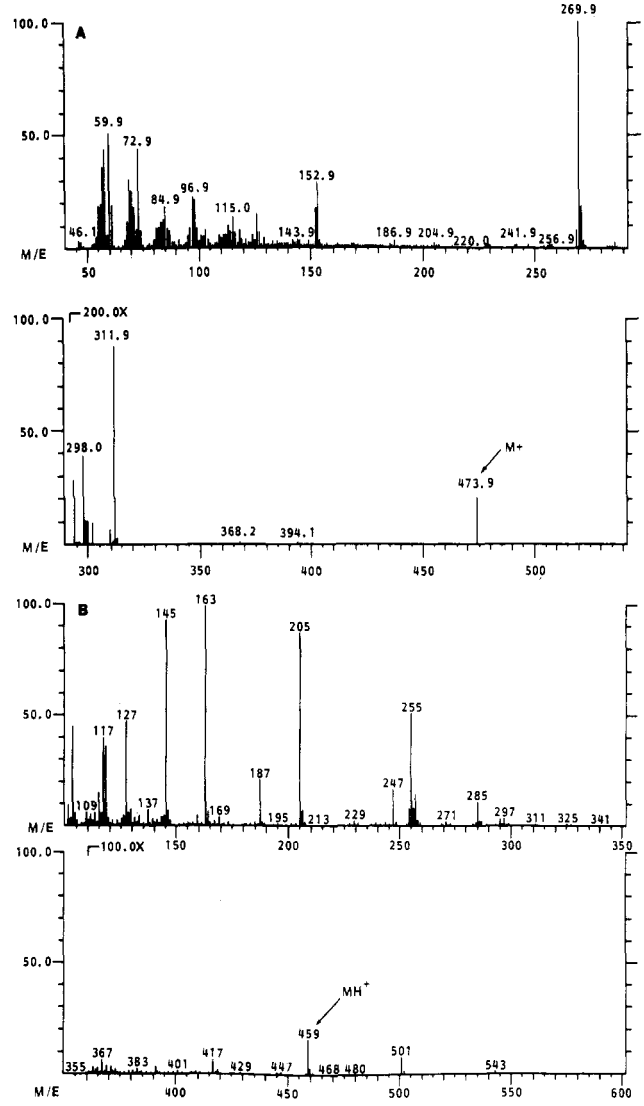


Figure 4. Mass spectrum of (A) 6″-*O*-acetylgenistin and (B) 6″-*O*-acetyldaidzin.

Calcd: C, 58.2; H, 4.6; O, 37.1. The molecular formula $C_{23}H_{22}O_{10}$ was assigned to 6″-*O*-acetyldaidzin by mass spectrum, *m/e* 459 (MH^+). Both mass spectra showed high concentrations of the ions corresponding to the aglucones, genistein (*m/e* 270 (M^+), Figure 4 part A) and daidzein (*m/e* 255 (MH^+), Figure 4 part B). The results obtained by ^1H NMR spectroscopy and MS also indicated that the additional acetyl group present in the two new compounds was attached to the glucose molecule rather than to the parent isoflavonoid nucleus. The data obtained by ^1H NMR, IR, UV, and MS agree with those reported by Ohta et al. (1979, 1980) for these compounds and confirm the presence of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin in toasted defatted soyflakes.

Comparison of Extraction Methods. The chromatograms obtained by HPLC analysis of the soyflake extracts with (a) acetone–HCl and (b) 80% methanol are shown in Figures 2 and 5, respectively. A marked difference is observed between the two chromatograms. In Figure 5, peaks corresponding to 6″-*O*-acetyldaidzin and 6″-*O*-acetylgenistin (peak numbers 3 and 4 in Figure 2) are almost nonexistant. Toasted defatted soyflakes contained 52 ppm of 6″-*O*-acetyldaidzin and 88 ppm of 6″-*O*-acetylgenistin. The concentrations of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin in the 80% methanol extract (figure 5) were 15% and 9%, respectively, of the concentrations present in the acetone–HCl extract (Figure 2).

Table IV. Concentration of 6″-O-Acetyldaidzin, 6″-O-Acetylgenistin, Daidzin, and Genistin in Soybeans

soybeans	location[a]	year	6″-O-acetyl-daidzin, ppm	daidzin, ppm	6″-O-acetyl-genistin, ppm	genistin, ppm
Amsoy 71	Pilot Mount	1982	58	301	126	832
Amsoy 71	Ames	1982	8	129	81	522
Amsoy 71	Hudson	1981	b	345	17	825
Amsoy 71	Beaman	1982	b	194	81	660
Vinton 81	Ames	1982	32	315	64	666
Vinton 81	Hudson	1982	11	352	36	664
Strayer	unknown	unknown	b	392	17	852
Weber	unknown	unknown	80	330	145	791

[a] Location in Iowa where soybeans were grown. [b] Not detected.

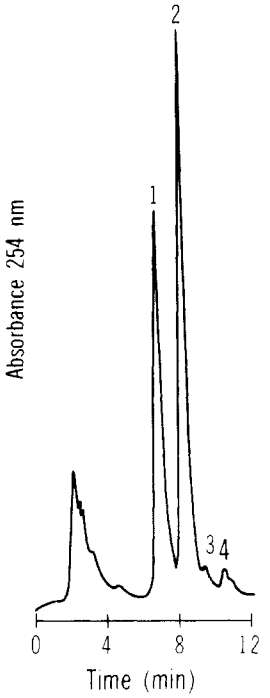


Figure 5. HPLC chromatogram of toasted defatted soyflakes extracted with 80% methanol. Peaks: 1 = daidzin; 2 = genistin; 3 = 6″-O-acetyldaidzin; 4 = 6″-O-acetylgenistin.

The concentrations of genistin and daidzin extracted with both solvents were essentially the same. Obviously, there is selective extraction of the isoflavones with 80% methanol.

Eldridge (1982a,b) did not report the presence of the acetyl isoflavones in 80% methanol extracts of a number of soy products. The difference between the two extraction solvents observed in this experiment can explain why 6″-O-acetyldaidzin and 6″-O-acetylgenistin were not detected by Eldridge (1982a,b). Glycitein and glycitin were reported to be present in a variety of soybean samples extracted with 80% methanol. The compounds were not detected in our extracts obtained with (a) acetone–HCl or (b) 80% methanol. The isoflavone content of soybeans is known to be affected by environmental conditions (Eldridge, 1983); thus, it is possible that glycitein and/or glycitin were not present in our samples.

Naim et al. (1973) were the first to report glycitin and glycitein in soybeans. Extraction of glycitin and glycitein was performed by using ether followed by absolute methanol in a Soxhlet apparatus (Naim et al., 1974). Although a direct comparison of our method of extraction with that used by Naim et al. (1974) cannot be made, the important difference between the two methods was a cold extraction solvent used in this study in contrast to a hot extraction solvent used by Naim et al. (1974). The hot extraction conditions could modify the isoflavonoid constituents. Therefore, a comprehensive comparison of extraction solvents used by the various researchers should be carried out.

The concentrations of 6″-O-acetylgenistin and 6″-O-acetyldaidzin along with the concentrations of daidzin and genistin present in the four soybean varieties analyzed, appear in Table IV. It is known that environmental factors (Alexander and Watson, 1951; Eldridge and Kwolek, 1983), varietal differences (Eldridge and Kwolek, 1983; Murphy, 1982), and infections of the plants by bacteria, fungi, or insects (Loper and Hanson, 1964; Francis and Millington, 1971) affect the isoflavonoid content of soybeans. The data presented in Table IV clearly show that variety and environment (location) affect the concentrations of daidzin and genistin as well as the concentrations of the two new isoflavones. It is unlikely that the acetyl isoflavones isolated in this experiment are produced during extraction. The variation in the concentrations of the acetyl isoflavones that occurs among different soybean varieties analyzed shows that the compounds are natural isoflavonoid constituents of soybeans. Glycitein and/or glycitin have not been isolated nor chemically identified since the original report (Naim et al., 1973). Thus, the natural occurrence of glycitin and glycitein requires further substantiation.

The estrogenic activity of glycitein, glycitin, and the two acetyl isoflavones has not been investigated. Further experimentation is required to determine the importance of these compounds in soybeans and/or soybean products intended for human use.

Registry No. 6″-O-Acetylgenistin, 73566-30-0; 6″-O-acetyldaidzin, 71385-83-6; daidzin, 552-66-9; genistin, 529-59-9.

LITERATURE CITED

Alexander, G.; Watson, R. H. *Aust. J. Agric. Res.* **1951**, *2*, 257.
Barz, W. Z. *Z. Naturforch. B* **1969**, *B24*, 234.
Beck, A. B. *Aust. J. Agric. Res.* **1964**, *15*, 223.
Eldridge, A. C. *J. Chromatogr.* **1982a**, *234*, 241.
Eldridge, A. C. *J. Agric. Food Chem.* **1982b**, *30*, 353–355.
Eldridge, A. C.; Kwolek, W. F. *J. Agric. Food Chem.* **1983**, *31*, 394.
Francis, C. M.; Millington, A. J. *Aust. J. Agric. Res.* **1971**, *22*, 75–80.
Gyorgy, P.; Murata, K.; Ikehata, H. *Nature (London)* **1964**, *203*, 870.
Harborne, J. B.; Mabry, T. J.; Mabry, H. "The Flavonoids", 2nd ed.; Chapman and Hall: London, 1975; p 75.
Higuchi, R.; Donnely, D. M. X. *Phytochemistry* **1978**, *17*, 787.
Lewis, B. A.; Smith, F. In "Thin Layer Chromatography", 2nd ed.; Stahl, E., Ed.; Springer-Verlag: New York, 1969; Chapter 10.
Loper, G. M.; Hanson, C. H. *Crop Sci.* **1964**, *4*, 480–482.
Mabry, T. J.; Markham, K. R.; Thomas, M. B. "The Systematic Identification of Flavonoids", 1st ed.; Springer-Verlag: New York, 1970; p 36.
Murphy, P. A. *J. Chromatogr.* **1981**, *211*, 166–169.

Murphy, P. A. *Food Technol.* **1982**, *1*, 60–64.
Naim, M.; Gestetner, B.; Kirson, I.; Birk, Y.; Bondi, A. *Phytochemistry* **1973**, *12*, 169–170.
Naim, M.; Gestetner, B.; Zilkah, S.; Birk, Y.; Bondi, A. *J. Agric. Food Chem.* **1974**, *22*, 806–810.
Ohta, N.; Kuwata, G.; Akahori, H.; Watanabe, T. *Agric. Biol. Chem.* **1979**, *43*, 1415.
Ohta, N.; Kuwata, G.; Akahori, H.; Watanabe, T. *Agric. Biol. Chem.* **1980**, *44*, 469.
Pieterse, P. J. S.; Andrews, F. N. *J. Anim. Sci.* **1956**, *15*, 25.
Waldi, D. *J. Chromatogr.* **1965**, *18*, 417.
Wang, L. C. *Anal. Biochem.* **1971**, 42, 296–298.

Received for review September 24, 1984. Accepted January 28, 1985. The project was supported by the Iowa Agriculture and Home Economics Experiment Station. The article is Journal Paper No. J-11565 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 2433 and 2164, the latter being a contributing project to North Central Regional Project NC-136.

Bowman–Birk Proteinase Isoinhibitor Complements of Soybean Strains

Anna L. Tan-Wilson,* Sarah E. Cosgriff, Michelle C. Duggan, R. Scott Obach, and Karl A. Wilson

When immunological tests to identify proteins that show cross-reaction with antiserum from rabbits immunized with Bowman–Birk soybean trypsin inhibitor were used, chromatographic and electrophoretic analyses of the Bowman–Birk proteinase inhibitors in eight soybean strains reveal four to seven isoinhibitor species in each strain. There were ten different isoinhibitors altogether. These were designated BBSTI-A, A′, A″, B, B′, C, C′, D, E, and E′ from the slowest to the fastest migrating on 10% polyacrylamide disc gel electrophoresis in the Davis system.

The utilization of the rich protein resource in soybean is limited in part by antinutritional components. Unless partially destroyed by moist heat, these components cause growth inhibition, pancreatic hypertrophy, and inability to utilize the bean protein in nonruminant animals (Rackis, 1981; Rackis and Gumbmann, 1981). A significant portion of this antinutritional toxicant effect is due to the proteinase inhibitors, proteins which form complexes with mammalian pancreatic serine proteinases and inhibit their enzymatic activity. There are two classes of proteinase inhibitors, the Kunitz (KSTI) and the Bowman–Birk (BBSTI) inhibitors. Within each class, there are isoinhibitor forms (Wilson, 1981; Laskowski and Kato, 1981; Orf and Hymowitz, 1979).

Three isoinhibitors of the Kunitz class have been described. These have been designated Ti^a, Ti^b, and Ti^c (Orf and Hymowitz, 1979). Equilibrium constants for the complex formation of the Kunitz isoinhibitors with bovine trypsin differ by as much as three orders of magnitude (Freed and Ryan, 1980). Soybean strains differ in the type of Kunitz isoinhibitors present in the seed. An extensive study of this has been done by Orf and Hymowitz (1979).

The identification of Bowman–Birk isoinhibitors in soybeans has not been as extensive. Odani and Ikenaka (1977a) purified five Bowman–Birk inhibitors from a Japanese strain of soybeans Sode-furi. These were designated as inhibitors A, B, C-II, D-II, and E-I. Inhibitor A, the major component, was the same as the classical Bowman–Birk inhibitor purified by Bowman and Birk and whose amino acid sequence was elucidated by Odani et al. (1972) and by Odani and Ikenaka (1972). This inhibitor inhibited one molecule of bovine trypsin and one molecule of bovine chymotrypsin simultaneously. Species B was found to be identical with species A, except for the deamidation of one or more asparaginyl or glutaminyl residues. Species C-II sequenced by Odani and Ikenaka (1977b) was double-headed also, having one reactive site for either bovine trypsin or bovine chymotrypsin, the other active against bovine elastase. Inhibitor D-II inhibited only bovine trypsin and very weakly at that. Inhibitor E-I, having essentially the same inhibitor activity as D-II, was found to be a variant of D-II, lacking nine amino acid residues at the N-terminal region (Odani and Ikenaka, 1978).

Hwang et al. (1977) found five Bowman–Birk inhibitors in soybean cultivar Tracy. PI-V, the major component, was the same as the classical Bowman–Birk inhibitor. PI-I to PI-IV belonged to another set. Three of these are thought to be derived from the fourth by proteolysis. Unlike PI-V, none of these four inhibits bovine chymotrypsin and their inhibition of trypsin was weaker than that of PI-V. Recently, Foard et al. (1982) mentioned that the amino acid sequence of PI-II and PI-IV matched the sequence of E-I and D-II (Odani and Ikenaka, 1978), respectively.

Stahlhut and Hymowitz (1983) found five isoinhibitors in strain Amsoy 71 and named these I, II, III, IV, and V. Inhibitor III was found to be the classical Bowman–Birk inhibitor. They classified 470 strains of soybean according to the presence or absence of inhibitors III and V.

In an earlier report, we studied the isoinhibitors in cultivar Fiskeby V (Tan-Wilson et al., 1982). The classical Bowman–Birk inhibitor was designated BBSTI-E. Another inhibitor which was present in trace amounts in seed but increased in proportion to BBSTI-E in the cotyledon of the day 4 to day 6 seedlings was BBSTI-D. In addition, there were other isoinhibitors which eluted prior to BBSTI-E from a DEAE-cellulose column in a pH gradient from pH 6.5 to pH 5.

Although complete amino acid composition and sequence analysis for the isoinhibitors in these four strains of soybeans are not available, a cursory survey seems to point toward some isoinhibitors being common to all four strains and some isoinhibitors being in one but not another soybean strain. Since the studies were all conducted by separate research groups with different separation schemes and electrophoretic systems, we do not know just how extensive the number and variation of Bowman–Birk

Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901.

0021-8561/85/1433-0389$01.50/0