Microwave-Mediated Synthesis of Anticarcinogenic Isoflavones from Soybeans

Yu-Chen Chang,† Muraleedharan G. Nair,*,† Ross C. Santell,‡ and William G. Helferich‡

Bioactive Natural Product Laboratory, Department of Horticulture, and Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

Soybean isoflavonoids, 7,4′-dihydroxyisoflavone (daidzein), 7-hydroxy-4′-methoxyisoflavone (formononetin), 5,7,4′-trihydroxyisoflavone (genistein), and 5,7-dihydroxy-4′-methoxyisoflavone (biochanin A), were synthesized in high yields by cyclization of their corresponding ketones in a conventional microwave oven.

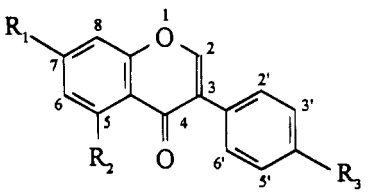
Keywords: *Microwave synthesis; isoflavones; daidzein; genistein; formononetin; biochanin A*

INTRODUCTION

Soybean isoflavonoids, daidzein, formononetin, genistein, and biochanin A (Figure 1), are reported to have important biological activities, such as being the signal molecules for vesicular-arbuscular mycorrhiza infection on host plants (Nair et al., 1991; Siqueira et al., 1991a; Safir et al., 1992), a herbicide safening effect (Siqueira et al., 1991b), and estrogenic and anticarcinogenic activities in sheep and rats (Braden et al., 1967; Troll et al., 1980), and are implicated in the inhibition of growth of human breast cancer cells (Peterson and Barnes, 1991). Epidemiology studies showed that the incidence of breast cancer is high in North America and northwestern Europe (Drasar and Irving, 1973). Diets are normally considered an important factor, for breast cancer is highly correlated with a high fat and animal protein diet (Drasar and Irving, 1973; Lee et al., 1991). It is reported that women consuming high levels of isoflavonoid-containing diets have lower breast cancer incidence than women with a low intake of such diets (Aldercreutz et al., 1988; Aldercreutz, 1990; Lee et al., 1991; Messina and Messina, 1991; Messina and Barnes, 1991; Setchell et al., 1984). However, the preclinical studies of these important compounds have not been adequately evaluated due to the limited quantities of isoflavonoids. Most of the soybean isoflavonoids are very expensive, which seriously limited their evaluation as potential anticarcinogens. Therefore, the source of these isoflavones became our first priority to investigate them as anticarcinogens.

The reported syntheses of many soybean isoflavonoids including genistein, biochanin A, daidzein, and formononetin are time-consuming (Bass, 1976; Baker et al., 1953; Farkas et al., 1971; Pelter and Foot, 1976; Shriner and Hull, 1945; Yoder et al., 1954). The use of microwave energy in organic synthesis is now popular (Abramovitch, 1991). It has been shown that commercial microwave ovens dramatically reduced the reaction times of many organic reactions such as Diels–Alder and Claisen reactions (Giguere et al., 1986) and α-vinyl β-lactam synthesis (Banik et al., 1992). In this paper, we describe an effective and rapid synthesis of daidzein, formononetin, genistein, and biochanin A using an unmodified microwave oven by cyclizing their corresponding ketones.

† Bioactive Natural Product Laboratory.
‡ Food Science and Human Nutrition.



R_1= OH,	R_2= H,	R_3= OH	Daidzein
R_1= O-Glu,	R_2= H,	R_3= OH	Daidzin
R_1= OH,	R_2= H,	R_3= OMe,	Formononetin
R_1=OH,	R_2=OH,	R_3=OH	Genistein
R_1= O-Glu,	R_2= OH,	R_3=OH	Genistin
R_1= OH,	R_2= OH,	R_3= OMe,	Biochanin A

Figure 1. Isoflavonoids reported from soybeans.

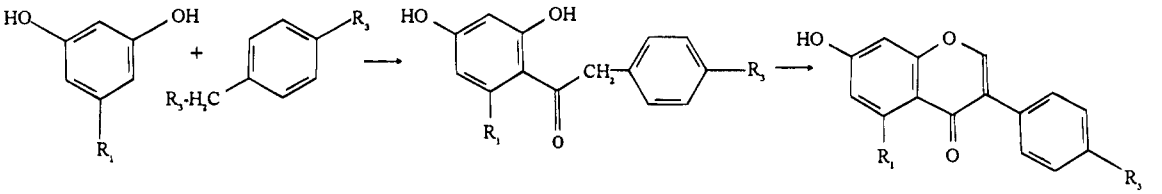
RESULTS AND DISCUSSION

4-Hydroxybenzyl 2,4-dihydroxyphenyl ketone (**1**), as needle-like crystals (88%), was synthesized by refluxing resorcinol and 4-hydroxyphenylacetic acid with boron trifluoride etherate (BF_3·Et_2O) (Scheme 1). The ABX pattern in the A ring of **1** and a singlet of 2 protons of the benzylic methylene group at 4.09 ppm were confirmed by the ^{1}H-NMR spectrum. The synthesis of this ketone was reported by Shriner and Hull (1945) and Yoder et al. (1954) by saturating a solution of resorcinol and 4-hydroxyphenylacetonitrile in ether with dry HCl over a period of 3 days. Pelter and Foot (1976) reported that the cyclization of **1** to daidzein can be achieved by refluxing **1** with *N*,*N*-dimethylformamide dimethyl acetal in DMF for 3 h, affording 76% of daidzein. Using *N*,*N*-dimethylformamide dimethyl acetal and THF as the solvent, daidzein was obtained (71%) from **1** under medium microwave energy for 2 min, which gave the overall yield of 57%. To prevent the evaporation of *N*,*N*-dimethylformamide dimethyl acetal during the cyclization of **1**, the reaction was carried out in a sealed vial. The resulting product, after recrystallization, did not show the $-CH_2-$ signal which appeared at 4.09 ppm in ketone **1**. The H-2 signal was at δ 8.2, as a singlet, in its ^{1}H-NMR spectrum. Both ^{1}H- and ^{13}C-NMR spectra of the product were identical to an authentic sample of daidzein.

Similarly, 4-methoxybenzyl 2,4-dihydroxyphenyl ketone (**2**) was synthesized (51%) as in the case of compound **1** by replacing the starting material, 4-hydroxyphenylacetic acid, with 4-methoxyphenylacetic acid. Using *N*,*N*-dimethylformamide dimethyl acetal

0021-8561/94/1442-1869$04.50/0

Scheme 1



R_1	R_2	R_3	Ketones	Isoflavones
H	COOH	OH	1	Daidzein
H	COOH	OMe	2	Formononetin
OH	CN	OH	3	Genistein
OH	CN	OMe	4	Biochanin A

and THF as the solvent, formononetin was produced (91%, overall 45%) by the cyclization of **2** under medium microwave energy for 1 min as in the case of daidzein. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of 1 and contained the signal for a methoxyl group. The ^{1}H- and ^{13}C-NMR spectra of formononetin were identical to the published data (Nair et al., 1991).

Syntheses of ketones **3** and **4**, for genistein and biochanin A syntheses, respectively, were conducted by the modification of a reported procedure (Yoder et al., 1954). The 4-hydroxybenzyl 2,4,6-trihydroxyphenyl ketone (**3**) was produced by bubbling dry HCl into a solution of phloroglucinol and *p*-hydroxyphenylacetonitrile in dry ether in 46% yield. Compound **3** gave distinct singlets of two protons each at δ 6.69 and 4.26 for H-2′ and H-6′ and the methylene protons, respectively, in the ^{1}H-NMR spectrum. The cyclization of **3** with BF_3·Et_2O in DMF and methanesulfonyl chloride (Bass, 1976) was also accomplished in a microwave oven for 2 min at low energy and afforded genistein in high purity (80%, overall 36%). The structure of genistein was confirmed by ^{1}H- and ^{13}C-NMR spectra.

Using identical procedures used for the synthesis of **3**, 4-methoxybenzyl 2,4,6-trihydroxyphenyl ketone (**4**), for biochanin A synthesis, was prepared (47%) from *p*-methoxyphenyl acetonitrile and phloroglucinol as the starting materials. Similarly, the cyclization of **4** to biochanin A (86%, overall 40%) was carried out in a microwave using the same conditions as in the case of genistein. The structures of **4** and biochanin A were confirmed by their ^{1}H- and ^{13}C-NMR spectra.

Microwave conversion of ketones **2**, **3**, and **4**, to formononetin, genistein, and biochanin A, afforded yields superior to the reported values of 85 (Pelter and Foot, 1976), 74 (Yoder et al., 1954), and 65% (Bass, 1976), respectively. The microwave conversion of the ketone **1** to daidzein in 71% yield is comparable to the yield reported by Pelter and Foot (1976). The overall yields for the isoflavones are not available from published reports. However, the overall yield of isoflavones daidzein, formononetin, genistein, and biochanin A under microwave condition were 57, 40, 36, and 40%, respectively. Our synthesis of these isoflavones has the advantages of reduced cost and time consumption. For example, the 4-hydroxyphenylacetic acid used for the synthesis of **1** is considerably cheaper than its corresponding nitrile. The syntheses of these soybean isoflavonoids, especially genistein and daidzein, in substantial quantities using less expensive reagents in a very short time facilitate their in vivo evaluation as anticarcinogens in humans. Also, in vitro studies on human gut metabolism of genistein and daidzein will help us understand whether these isoflavonoids, once ingested, are absorbed directly or converted to various metabolites in the human gut prior to their participation in breast cancer prevention.

MATERIALS AND METHODS

Instruments. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian VXR 300- and 500-MHz spectrometers, respectively, in CD_3OD or DMSO-d_6 solution at ambient temperature. The melting points were recorded on a Thomas Model 40 micro hot-stage apparatus and are not corrected.

4-Hydroxybenzyl 2,4-Dihydroxyphenyl Ketone (1). Resorcinol (2.9 g) was added to a mixture containing 4-hydroxyphenylacetic acid (2 g) and BF_3·Et_2O (4.5 mL). The reaction mixture was refluxed for 10 min, cooled, and treated with saturated aqueous NaOAc (30 mL) and $NaHCO_3$ (15 mL), respectively. The precipitate formed was filtered off and washed with water, dried, and then washed with $CHCl_3$ to give yellow needle-like crystals (2.8 g, 88%): mp 188–190 °C; ^{1}H NMR (CD_3OD) δ 7.82 (1H, d, J = 8.7 Hz, H-6), 7.08 (2H, dd, J = 6.6, 2.1 Hz, H-2′, 6′), 6.72 (1H, dd, J = 6.6, 2.1 Hz, H-3′, 5′), 6.35 (1H, dd, J = 8.7, 2.4 Hz, H-5), 6.24 (1H, d, J = 2.1 Hz, H-3), 4.09 (2H, s, $-CH_2-$); ^{13}C NMR (CD_3OD) δ 202.66 (CO), 164.80 (C-2), 164.52 (C-4), 155.26 (C-4′), 132.65 (C-1′), 129.51 (C-2′, C-6′), 125.38 (C-6), 114.58 (C-3′, C-5′), 111.72 (C-1), 107.43 (C-5), 101.87 (C-3), 42.83 ($-CH_2-$).

Daidzein. *N*,*N*-Dimethylformamide dimethyl acetal (0.5 mL) and THF (0.5 mL) were added to a pressure-resistant vial containing compound **1** (40.9 mg). The reaction mixture was heated in a microwave for 2 min at medium energy and afforded a red solution. Methanol (2 mL) was added into the reaction product and evaporated to dryness in vacuo. The crude product thus obtained was purified by preparative TLC ($CHCl_3$/MeOH 9:1) and recrystallized from aqueous methanol to give daidzein (27.8 mg, 71%): mp 290 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 8.27 (1H, s, H-2), 7.95 (1H, d, J = 9.0 Hz, H-5), 7.36 (2H, d, J = 6.6, 1.8 Hz, H-2′, 6′), 6.92 (1H, dd, J = 8.7, 2.4 Hz, H-6), 6.84 (1H, d, J = 2.1 Hz, H-8), 6.79 (1H, d, J = 6.6, 1.8 Hz, H-3′ 5′); ^{13}C NMR (DMSO-d_6) δ 174.64 (C-4), 162.44 (C-4′), 157.38 (C-8a), 157.11 (C-7), 130.00 (C-2′, C-6′), 127.22 (C-5), 123.46 (C-3), 122.52 (C-1′), 116.62 (C-4a), 115.05 (C-6), 114.90 (C-3′, C-5′), 102.04 (C-8).

4-Methoxybenzyl 2,4-Dihydroxyphenyl Ketone (2). The same synthetic procedure was employed as in compound **1** by substituting 4-methoxyphenylacetic acid (2.2 g) instead of 4-hydroxyphenylacetic acid. The corresponding ketone was obtained as yellow needle-like crystals (2.47 g, 51%): mp 159–163 °C; ^{1}H NMR (CD_3OD) δ 7.94 (1H, d, J = 9 Hz, H-6), 7.20 (2H, d, J = 8.4 Hz, H-2′, 6′), 6.87 (2H, d, J = 8.7 Hz, H-3′, 5′), 6.33 (2H, dd, J = 8.8, 2.7 Hz), 6.25 (1H, d, J = 2.1 Hz, H-3), 4.20 (2H, s, $-CH_2-$), 3.71 (3H, s, $-OMe$); ^{13}C NMR (CD_3OD) δ 202.40 (CO), 164.89 (C-2), 164.64 (C-4), 157.99 (C-4′), 133.49 (C-1), 130.43 (C-2′, C-6′), 126.93 (C-6′), 126.93 (C-6), 113.79 (C-3′, C-5′), 112.40 (C-1), 108.20 (C-5), 102.44 (C-3), 54.94 ($-OMe$), 43.16 ($-CH_2-$).

Formononetin. Using the same procedure as for daidzein, compound **2** (40 mg) was converted to formononetin. The crude precipitate was recrystallized from aqueous methanol to give formononetin (32.3 mg, 91%): ^{1}H NMR (DMSO-d_6) δ 8.30 (1H, s, H-2), 7.95 (1H, d, J = 9 Hz, H-5), 7.49 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.97 (2H, d, J = 8.7 Hz, H-3′, H-5′), 6.92 (1H, dd, J = 8.7, 2.1 Hz, H-6), 6.84 (1H, d, J = 1.8 Hz, H-8), 3.76 (3H, s, −OMe); ^{13}C NMR (DMSO-d_6) δ 174.50 (C-4), 163.67 (C-4′), 158.89 (C-8a), 157.57 (C-7), 152.87 (C-2), 130.00 (C-2′, C-6′), 127.09 (C-5), 124.34 (C-3), 123.04 (C-1′), 116.02 (C-4a), 115.56 (C-6), 113.54 (C-3′, C-5′), 102.00 (C-8), 55.10 (−OMe).

4-Hydroxybenzyl 2,4,6-Trihydroxyphenyl Ketone (3). Phloroglucinol (1.0 g) and 4-hydroxyphenylacetonitrile (1.1 g) in ether (10 mL) were cooled in an ice bath and saturated with a stream of HCl gas (HCl was produced by reacting NaCl and concentrated H_2SO_4). The reaction mixture was refrigerated for 12 h, saturated again with HCl gas, and refrigerated for another 12 h. The precipitate after the ether was decanted was washed further with ether. The white precipitate thus obtained was refluxed with 2% aqueous HCl (20 mL) for 3 h and cooled. The solution was extracted twice with ether (50 mL each), and the organic layer was neutralized with saturated $NaHCO_3$ solution. The ether was removed in vacuo to give yellow needle-like crystals (1.0 g, 46.5%): mp 258−262 °C; ^{1}H NMR (CD_3OD) δ 7.05 (2H, d, J = 8.1 Hz, H-3′, H-5′), 6.69 (2H, d, J = 8.1, H-2′, H-6′), 5.80 (2H, s, H-3, H-5), 4.26 (2H, s, −CH2−); ^{13}C NMR (CD_3OD) δ 205.08 (CO), 166.24 (C-2, C-6), 164.80 (C-4), 156.90 (C-4′), 131.66 (C-2′, C-6′), 128.20 (C-1′), 115.98 (C-3′, C-5′), 95.83 (C-3, C-5), 49.54 (−CH_2−).

Genistein. BF_3·Et_2O (1 mL) was added to a solution containing DMF (2 mL) and compound **3** (50 mg) in a beaker. The reaction mixture was heated in a microwave for 15 s using low energy followed by the addition of methanesulfonyl chloride (CH_3SO_2Cl, 1 mL). The resulting product was heated in a microwave again for 1 min at low energy. A light yellowish precipitate, obtained by the addition of water (100 mL) into the reaction mixture, was centrifuged, washed with water (10 mL × 3), and recrystallized from aqueous methanol to give genistein (43 mg, 80%): mp 291−296 °C; ^{1}H NMR (DMSO-d_6) δ 8.30 (1H, s, H-2), 7.35 (2H, dd, J = 6.6, 1.8 Hz, H-2′, H-6′), 6.80 (2H, dd, J = 6.6, 1.8 Hz, H-8), 6.21 (1H, d, J = 1.8 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ 180.68 (C-4), 164.77 (C-4′), 162.47 (C-5), 158.06 (C-8a), 157.86 (C-7), 154.44 (C-2), 130.64 (C-2′, C-6′), 122.75 (C-3), 121.69 (C-1′), 115.51 (C-3′, 5′), 104.93 (C-4a), 99.43 (C-8), 94.13 (C-6).

4-Methoxybenzyl 2,4,6-Trihydroxyphenyl Ketone (4). Using 4-hydroxyphenylacetonitrile (1 g) and phloroglucinol (1 g), a similar synthetic procedure as for compound **3** was employed to synthesize compound **4** as yellow plate-like crystals (0.88 g, 47%): mp 195−197 °C; ^{1}H NMR (CD_3OD) δ 7.15 (2H, d, J = 8.7 Hz, H-2′, H-6′), 6.83 (2H, d, J = 8.4 Hz, H-3′, H-5′), 5.80 (2H, s, H-3, H-5), 4.89 (2H, s, −CH_2−), 3.76 (3H, s, −OMe); ^{13}C NMR (CD_3OD) δ 205.0 (CO), 165.80 (C-2, C-6), 165.75 (C-4), 159.77 (C-4′), 131.67 (C-2′, C-6′), 130.22 (C-1′), 114.71 (C-3′, C-5′), 105.29 (C-1), 95.87 (C-3, C-5), 49.84 (−CH_2−).

Biochanin A. Biochanin A (45.2 mg, 86%) was synthesized from compound **4** (50.5 mg) using the same procedure as for genistein: mp 180−184 °C; ^{1}H NMR (DMSO-d_6, 300, MHz) δ 8.36 (1H, s, H-2), 7.48 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.99 (2H, d, J = 8.7 Hz, H-3′, H-5′), 6.38 (1H, d, J = 2.4 Hz, H-8), 6.22 (1H, d, J = 2.1 Hz, H-6), 3.77 (3H, −OMe); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 180.07 (C-4), 164.29 (C-4′), 161.97 (C-5), 159.15 (C-8a), 157.56 (C-7), 154.17 (C-2), 130.15 (C-2′, C-6′), 122.90 (C-3), 121.95 (C-1′), 113.69 (C-3′, C-5′), 104.45 (C-4a), 98.99 (C-8), 93.67 (C-6), 55.135 (−OMe).

LITERATURE CITED

Abramovitch, R. A. Applications of Microwave Energy in Organic Chemistry. A Review. *Org. Prep. Procedures Int.* **1991**, *23* (6), 683−711.

Aldercreutz, H. Western Diet and Western Diseases: Some Hormonal and Biochemical Mechanisms and Associations. *Scand. J. Clin. Lab. Invest.* **1990**, *50* (Suppl. 201), 3−23.

Aldercreutz, H.; Honjo, H.; Higashi, A.; Fotsis, T.; Hamaiainen, E.; Hasegawa, T.; Okada, H. Lignan and Phytoestrogen Excretion in Japanese Consuming Traditional Diet. *Scand. J. Clin. Lab. Invest.* **1988**, *48* (Suppl. 190), 190.

Baker, W.; Chaderton, J.; Harborne, J. B.; Ollis, W. D. A New Synthesis of Isoflavones. Part I. *J. Chem. Soc.* **1953**, 1852−1860.

Banik, B. K.; Manhas, M. S.; Kaluza, Z. K.; Barakat, K. J.; Bose, A. K. Microwave-Induced Organic Reaction Enhancement chemistry. Convenient Synthesis of Enantiopure α-Hydroxy-β-Lactams. *Tetrahedron Lett.* **1992**, *33* (25), 3603−3606.

Bass, R. J. Synthesis of Chromones by Cyclization of 2-Hydroxyphenyl Ketone with Boron Trifluoride-Diethyl and Methanesulphonyl Chloride. *J. Chem. Soc., Chem. Commun.* **1976**, 78−79.

Braden, A. W. H.; Hart, N. K.; Lamberton, J. A. The Oestrogenic Activity and Metabolism of Certain Isoflavones in Sheep. *Aust. J. Agric. Res.* **1967**, *18*, 335−348.

Drasar, B. S.; Irving, D. Environmental Factors and Cancer of the Colon and Breast. *Br. J. Cancer* **1973**, *27*, 167−172.

Farkas, L.; Gottsegen, A.; Norgradi, M. Synthesis of the Natural Isoflavonones Ferreirin, Dalbergiodin, and Ougenin. *J. Chem. Soc. (C)* **1971**, 1994−2000.

Giguere, R. J.; Bray, T. L.; Duncan, S. M. Application of Commercial Microwave Ovens to Organic synthesis. *Tetrahedron Lett.* **1986** *27* (27), 4945−4948.

Lee, H. P.; Gourley, L.; Duffy, S. W.; Esteve, J.; Lee, J.; Day, N. E. Dietary Effect on Breast-cancer Risk in Singapore. *Lancet* **1991**, *337*, 1197−1200.

Messina, M.; Barnes, S. The Role of Soy Products in Reducing Risk of Cancer. *J. Natl. Cancer Inst.* **1991**, *83*, 541−546.

Messina, M.; Messina, V. Increasing Use of Soyfoods and Their Potential Role in Cancer Prevention. *J. Am. Diet. Assoc.* **1991**, *91*, 836−840.

Nair, M. G.; Safir, G. R.; Siqueira, J. O. Isolation and Identification of Vesicular-Arbuscular Mycorrhiza-Stimulatory Compounds from Clover (*Trifolium repens*) Roots. *Appl. Environ. Microbiol.* **1991**, *57* (2), 434−439.

Pelter, A.; Foot, S. A New Convenient Synthesis of Isoflavones. *Synthesis* **1976**, May, 326.

Peterson, G.; Barnes, S. Genistein Inhibition of the Growth of Human Breast Cancer Cells: Independence from Estrogen Receptors and the Multi-Drug Resistance Gene. *Biochem. Biophys. Res. Commun.* **1991**, *179* (1), 661−667.

Safir, G. R.; Nair, M. G.; Siqueira, J. O. Method and Compositions for Stimulating Vesicular-Arbuscular Mycorrhizal Fungi. U.S. Pat. 5,002,603, 1991; U.S. Pat. 5,085,682, 1992; U.S. Pat. 5,125,955, 1992; Taiwan Pat. 60604, 1993.

Setchell, K. D. R.; Borriello, S. P.; Hulme, P.; Kirk, D. N.; Axelson, M. Nonsteroidal Estrogens of Dietary Origin: Possible Role in Hormone-dependent Disease. *Am. J. Clin. Nutr.* **1984**, *40*, 569−578.

Shriner, R. L.; Hull, C. J. Isoflavones. III. The structure of Prunetin and A New Synthesis of Genistein. *J. Org. Chem.* **1945**, *10*, 288−291.

Siqueira, J. O.; Safir, G. R.; Nair, M. G. Stimulation of Vesicular-Arbuscular Mycorrhiza formation and Growth of White Clover by Flavonoid Compounds. *New Phytol.* **1991a**, *118*, 87−93.

Siqueira, J. O.; Safir, G. R.; Nair, M. G. VA-Mycorrhiza and Mycorrhiza Stimulating Isoflavonoid Compounds Reduce Plant Herbicide Injury. *Plant Soil* **1991b**, *134*, 233−242.

Troll, W.; Wiesner, R.; Shellabarger, C. J.; Holtzman, S.; Stone, J. P. Soybean Diet Lowers Tumor Incidence in Irradiation Rats. *Carcinogenesis* **1980**, *I*, 469−472.

Yoder, L.; Cheng, E.; Burroughs, W. Synthesis of Estrogenic Isoflavone Derivatives. *Iowa Acad. Sci.* **1954**, *61*, 271−276.

Received for review March 16, 1994. Accepted June 13, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1994.