Novel Synthesis of the Isoflavone Genistein

Jeffrey D. St. Denis, James S. Gordon IV, Vincent M. Carroll, Ronny Priefer*

Department of Chemistry, Biochemistry, and Physics, Niagara University, Niagara, NY 14109, USA
Fax +1(716)2868254; E-mail: rpriefer@niagara.edu

Received 9 December 2009; revised 27 February 2010

Abstract: Genistein was efficiently synthesized from (2,4,6-trihydroxyphenyl)ethanone by a novel five-step procedure involving the formation of an enamino ketone, followed by ring closure and a Suzuki coupling reaction using palladium acetate and poly(ethylene glycol).

Key words: ring closure, coupling, heterocycles, genistein, isoflavone, green chemistry

Isoflavones are found naturally in many plant species, particularly those of the legume family. Isoflavones are often produced by the plant as a defense mechanism to ward off microbes or other pathogens, and to reduce oxidative stress caused by reactive-oxygen species (ROS). The soy isoflavones consist of three compounds: genistein, daidzein, and, less commonly, glycitein (Figure 1). These compounds are usually O-glycosylated within the plant and are metabolized into the active aglycone forms by bacterial or digestive action.[1]

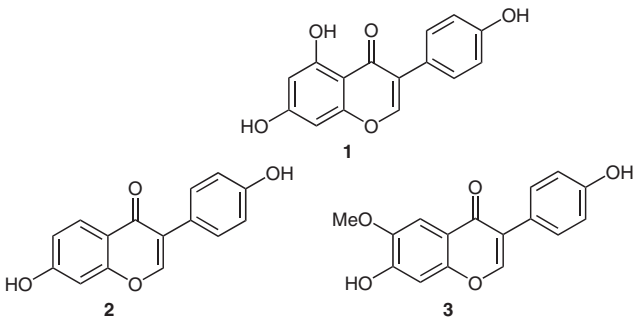


Figure 1 Soy isoflavones: genistein (**1**), daidzein (**2**), and glycitein (**3**)

In vitro studies have shown that isoflavones can be potent inhibitors of cancer metastasis.[1,2] In 1994, genistein was found to inhibit the metastatic breast cancer line BALB/c 410.4, with an EC_{50} of less than 1 μM. Other soy isoflavonoids have shown much lower activities against this cell line.[3] It has also been shown that genistein is an effective estrogenic agent[4] and, interestingly, it exhibits its estrogenic activity in estrogen-receptor-negative human breast carcinoma cell lines, as well as in normal breast-cancer cell lines, suggesting that it has an independent mode of action in relation to estrogen.[4]

SYNTHESIS 2010, No. 10, pp 1590–1592
Advanced online publication: 06.04.2010
DOI: 10.1055/s-0029-1219757; Art ID: M06309SS

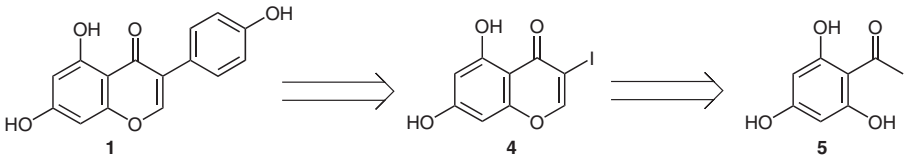
Genistein has also been examined as a treatment for prostate cancer. In vivo studies showed that an intake of genistein can significantly reduce tumor growth and lymph node metastases.[5] Studies have also suggested that genistein shows efficacy in vitro against the fibrosarcoma cell line HT1080,[6] lung carcinoma LL2,[7] and cervical cancer cell lines.[8]

One of the primary mechanisms through which bioflavonoids exert antiproliferative properties is by suppression of angiogenesis. Bioflavonoids are effective at preventing this crucial vascularization by inhibiting the expression of carcinomal growth factors that result from a rise in ROS. Cancer cells produce a number of compounds, such as vascular endothelial growth factors, basic fibroblast growth factors, and platelet-derived endothelial growth factors, that stimulate the generation of new blood vessels.[1] By suppressing ROS, genistein effectively prevents the cascade of events that lead to the production of carcinomal growth factors.

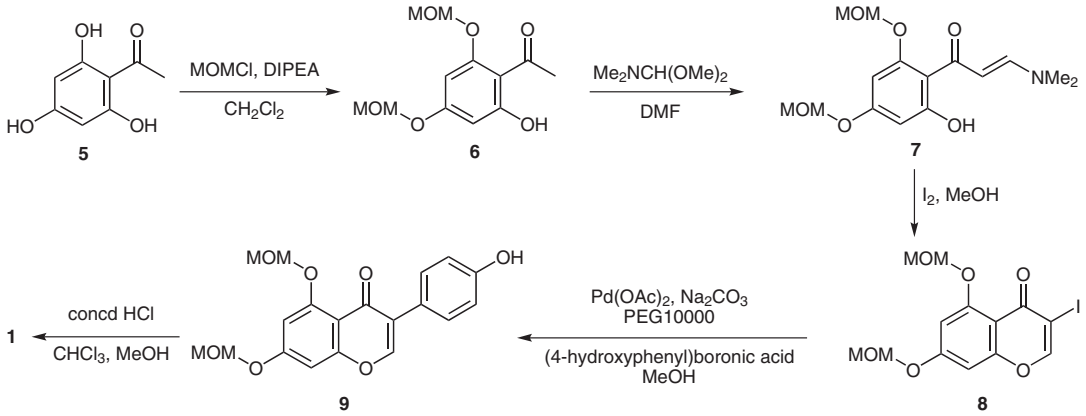
Genistein was first isolated from *Genista trictoria* (dyer's broom) in 1899,[9] and successfully synthesized for the first time in 1928 by Baker.[10] The pyrone ring was formed by treatment of the corresponding deoxybenzoin by various anhydrides, with subsequent decarboxylation. In 1945, Shriner and Hull performed an analogous synthesis by using ethyl formate, which eliminated the need for the decarboxylation step.[11] The use of mesyl chloride[12,13] or (dimethoxymethyl)dimethylamine (*N*,*N*-dimethylformamide dimethyl acetal)[14] with boron trifluoride etherate has also proved successful in the case of many isoflavone derivatives synthesized from the corresponding deoxybenzoins. Oxidative rearrangement of chalcones with thallium(III) nitrate has shown promise for the synthesis of the isoflavone scaffold,[15,16] but there are obvious concerns regarding the toxicity of this reagent.

Here, we describe a novel synthesis of genistein in which a phosphine-free palladium acetate-catalyzed Suzuki reaction is used to attach the final ring to 3-iodo-4*H*-1-benzopyran-4-one (**4**), which is obtained from the commercially available (2,4,6-trihydroxyphenyl)ethanone (**5**) by using (dimethoxymethyl)dimethylamine to form an initial enamino ketone that undergoes subsequent ring closure and iodination (Scheme 1).

Our synthesis (Scheme 2) began from commercially available (2,4,6-trihydroxyphenyl)ethanone (**5**). However initial attempts to form an enamino ketone were not successful, presumably because (dimethoxymethyl)dimethylamine is capable of reacting with phenols.[17] We therefore



Scheme 1 Retrosynthetic analysis



Scheme 2 Synthesis of genistein

considered it necessary to protect two of the hydroxy substituents in the triol **5**.

Initially, triol **5** was protected as its dibenzyl derivative, albeit in moderate yield (64%). However, this protection did not permit the subsequent ring closure and halogenation reactions.[1] Protection as the methoxymethyl (MOM) ether by treatment with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine in dichloromethane gave the desired protected acetophenone **6** (94%) without additional purification. This was subsequently treated with (dimethoxymethyl)dimethylamine[18] to form the enamino ketone **7** in 99% yield after column chromatography. On stirring in methanol with excess diiodine,[1,18] the MOM-protected enamino ketone **7** underwent tandem cyclization and iodination to afford 3-iodo-5,7-bis(methoxymethoxy)-4*H*-1-benzopyran-4-one (**8**). During this reaction, two distinct bands were observed by thin-layer chromatography. The blue fluorescent band was isolated and shown, by means of NMR spectroscopy, to contain the non-iodinated ring product. The upper band contained the required iodinated product **8**. This provided evidence that the halogenation reaction succeeded the ring-closure reaction.

A Suzuki coupling reaction was next used to attach the final ring of the isoflavone. A green approach to this procedure has recently been demonstrated that uses poly(ethylene glycol) (PEG 4000), methanol, sodium carbonate, and palladium diacetate as a source of palladium at a mild temperature of 50 °C.[19] We replaced PEG 4000 with PEG 10000 in our reaction, which gave 5,7-bis(methoxymethoxy)-3-[4-(methoxymethoxy)phenyl]-4*H*-1-benzopyran-4-one (**9**) in 88% yield. The PEG and palladium diacetate could be reused without further addition of a palladium source.

Although a variety of dimers formed during the Suzuki-coupling reaction, the major product was the desired MOM-protected genistein (**9**). We also observed the formation of a phenolic dimer and the coupling product of two molecules of the ring-closed product **8**. The subsequent deprotection to give genistein (**1**) could be coupled with the Suzuki reaction, thereby eliminating a purification step. To increase the solubility of genistein during chromatography, ethanol was added to the eluting solvent.

In conclusion, we have demonstrated a novel synthetic route for the total synthesis of the isoflavone genistein. This five-step pathway, with an overall yield of 63%, also permits the preparation of numerous derivatives of genistein. The use of PEG introduces an aspect of green chemistry into the synthesis.

Purchased chemicals were reagent grade. Melting points were obtained on a Gallenkamp apparatus and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian instrument in the solvent indicated, and they are referenced to TMS or residual undeuterated solvent. HRMS were recorded on a ThermoFinnigan MAT 95XL with ESI II source for electrospray ionization.

1-[2-Hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (6)

A flame-dried flask was charged with CH_2Cl_2 (30 mL) and ketone **5** (1.01 g, 6.07 mmol) under argon. The mixture was cooled to 0 °C, and DIPEA (3 mL, 17.2 mmol) was slowly added. After 20 min, MOMCl (1.15 g, 14.3 mmol) was added dropwise. The mixture was maintained at 0 °C for 20 min, then brought to r.t. The reaction was quenched with H_2O (40 mL). The organic layer was removed, and the aqueous layer was extracted with $CHCl_3$ (3 × 40 mL). The combined organic layers were dried ($MgSO_4$) and concentrated; yield: 1.46 g (94%); mp 39–42 °C.

^{1}H NMR ($CDCl_3$): δ = 13.69 (s, 1 H, COH), 6.27 (s, 1 H, Ar-H), 6.24 (s, 1 H, Ar-H), 5.25 (s, 2 H, OCH_2OCH_3), 5.16 (s, 2 H,

OCH_2OCH_3), 3.51 (s, 3 H, OCH_2OCH_3), 3.46 (s, 3 H, OCH_2OCH_3), 2.65 (s, 3 H, $COCH_3$).

^{13}C NMR ($CDCl_3$): δ = 203.3, 190.0, 166.9, 163.6, 160.5, 107.0, 97.3, 94.6, 94.1, 56.8, 56.5, 33.1.

3-(Dimethylamino)-1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]prop-2-en-1-one (7)

Ketone **2** (1.55 g, 6.05 mmol) was dissolved in DMF (60 mL) and the soln was warmed to 74 °C in an oil bath. $Me_2NCH(OMe)_2$ (3.50 g, 29.4 mmol) was then added dropwise to the flask. The mixture was stirred for 4.5 h then cooled to r.t. The reaction was quenched with H_2O (100 mL), and the mixture was extracted with EtOAc (5 × 100 mL). The extracts were washed with H_2O (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a thick yellow oil that was purified by column chromatography (EtOAc) to give cubic yellow crystals; yield: 1.86 g (99%); mp 89–91 °C.

^1H NMR ($CDCl_3$): δ = 7.90 (d, *J* = 12.8 Hz, 1 H, olefinic CH), 6.27 (d, *J* = 12.8 Hz, 1 H, olefinic CH) 6.25 (s, 1 H, Ar-H), 6.16 (s, 1 H, Ar-H), 5.20 (s, 2 H, OCH_2OCH_3), 5.13 (s, 2 H, OCH_2OCH_3), 3.50 (s, 3 H, OCH_2OCH_3), 3.45 (s, 3 H, OCH_2OCH_3), 3.14 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3).

^{13}C NMR ($CDCl_3$): δ = 191.5, 190.0, 166.9, 161.3, 158.9, 154.6, 107.0, 97.9, 96.9, 95.2, 94.5, 94.1, 56.8, 56.4, 29.8.

HRMS (ESI); *m/z* $[M + H]^+$ calcd for $C_{15}H_{22}O_6N$: 312.1442; found: 312.1430.

3-Iodo-5,7-bis(methoxymethoxy)-4*H*-1-benzopyran-4-one (8)

A soln of amino ketone **3** (1.23 g, 3.95 mmol) and I_2 (1.44 g, 5.67 mmol) in MeOH (100 mL) was stirred at r.t. for 10.5 h, then concentrated in vacuo to give a red-black residue. To remove residual I_2, the residue was treated with sat. aq $NaSO_3$ until the mixture became clear. The mixture was then extracted with $CHCl_3$ (3 × 40 mL), and the extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting off-white solid was purified by chromatography [silica gel, EtOAc–hexanes (1:1)] to give a white solid; yield: 1.28 g (83%); mp 137–140 °C.

^1H NMR ($CDCl_3$): δ = 8.09 (s, 1 H, olefinic CH), 6.75 (s, 1 H, Ar-H), 6.72 (s, 1 H, Ar-H), 5.30 (s, 2 H, OCH_2OCH_3), 5.22 (s, 2 H, OCH_2OCH_3), 3.54 (s, 3 H, OCH_2OCH_3), 3.49 (s, 3 H, OCH_2OCH_3).

^{13}C NMR ($CDCl_3$): δ = 171.3, 161.6, 159.3, 158.2, 155.7, 108.8, 102.1, 96.8, 95.5, 94.4, 89.5, 56.8, 56.6.

HRMS (ESI); *m/z* $[M + Na]^+$ calcd for $C_{13}H_{13}O_6INa$: 414.9649; found: 414.9648.

3-(4-Hydroxyphenyl)-5,7-bis(methoxymethoxy)-4*H*-1-benzopyran-4-one (9)

PEG 10000 (21.0 g), ground to a fine consistency in a mortar, and $Pd(OAc)_2$ (0.025 g, 0.11 mmol) were added to a stirred mixture of MeOH (25 mL) and Na_2CO_3 (0.548 g, 5.17 mmol). The reaction flask was fitted with a condenser, and the mixture was warmed to 50 °C in a water bath. Once the mixture had turned black, the iodo compound **8** (0.852 g, 2.17 mmol) and (4-hydroxyphenyl)boronic acid (0.746 g, 5.41 mmol) were added, and the mixture was stirred for 3 h. The resulting mixture was emptied into a vacuum funnel and washed with Et_2O (100 mL). The ethereal extract was concentrated in vacuo to give a white solid that was used in the next reaction without further purification; yield: 0.685 g (88%); mp 109–110 °C.

^1H NMR ($CDCl_3$): δ = 7.79 (s, 1 H, olefinic CH), 7.34 (d, *J* = 7.2 Hz, 2 H, Ph-H), 7.01 (d, *J* = 7.2 Hz, 2 H, Ph-H), 6.36 (s, 1 H, Ar-H), 6.29 (s, 1 H, Ar-H), 5.32 (s, 2 H, OCH_2OCH_3), 5.24 (s, 2 H, OCH_2OCH_3), 3.54 (s, 3 H, OCH_2OCH_3), 3.51 (s, 3 H, OCH_2OCH_3).

^{13}C NMR ($CDCl_3$): δ = 175.8, 161.2, 159.2, 158.5, 158.4, 150.9, 130.2, 127.3, 124.2, 114.5, 110.6, 101.2, 96.7, 94.9, 94.1, 56.3, 56.2.

Genistein (1)

A mixture of the benzopyranone **9** (0.650g, 1.81 mmol), $CHCl_3$ (5 mL), MeOH (5 mL), and concd HCl (1 mL) was refluxed for 1 h. The reaction was quenched with H_2O, and the mixture was extracted with $CHCl_3$ (2 × 10 mL). The extracts were washed with H_2O (10 mL) and purified by column chromatography [EtOAc–hexanes–4% EtOH (1:2.5)]; yield: 0.451 g (92%); mp 292–297 °C (Lit.[20] 291–296 °C).

^1H NMR (DMSO-d_6): δ = 13.03 (s, 1 H, Ar-OH), 10.90 (s, 1 H, Ar-OH), 9.61 (s, 1 H, Ar-OH), 8.33 (s, 1 H, olefinic CH), 7.36 (d, *J* = 7.6 Hz, 2 H, Ph-H), 6.80 (d, *J* = 7.6 Hz, 2 H, Ph-H), 6.38 (s, 1 H, Ar-H), 6.22 (s, 1 H, Ar-H).

^{13}C NMR (DMSO-d_6): δ = 180.3, 164.4, 162.1, 157.7, 157.5, 154.1, 130.3, 122.4, 121.3, 115.2, 104.5, 99.1, 93.8.

HRMS (ESI); *m/z* $[M + H]^+$ calcd for $C_{15}H_{11}O_5$: 271.0601; found: 271.0602.

Acknowledgment

The authors thank the Niagara University Academic Center for Integrated Science for financial support.

References

(1) Vasselin, D. A.; Westwell, A. D.; Matthews, C. S.; Bradshaw, T. D.; Stevens, M. F. G. *J. Med. Chem.* **2006**, *49*, 3973.
(2) Setchell, K. D.; Brown, N. M.; Desai, P.; Zimmer-Nechemias, L.; Wolfe, B. E.; Brashear, W. T.; Kirschner, A. S.; Cassidy, A.; Heubi, J. E. *J. Nutr.* **2001**, *131*, 1362.
(3) Scholar, E. M.; Toews, M. L. *Cancer Lett. (Shannon, Irel.)* **1994**, *2*, 159.
(4) Merlino, G. T.; Xu, Y. H.; Ishii, S.; Clark, A. J.; Semba, K.; Toyoshima, K.; Yamamoto, T.; Pastan, I. *Science* **1984**, *224*, 417.
(5) Messina, M. J.; Persky, V.; Setchell, K. D.; Barnes, S. *Nutr. Cancer* **1994**, *21*, 114.
(6) Zhou, J.-R. *Nutr. Cancer Prev.* **2006**, 325.
(7) Wietrzyk, J.; Opolski, A.; Madej, J.; Radzikowski, C. *In Vivo* **2000**, *2*, 357.
(8) Wang, S. Y.; Yang, K. W.; Hsu, Y. T.; Chang, C. L.; Yang, Y. C. *Neoplasma* **2001**, *3*, 227.
(9) Perkin, A. G.; Newbury, F. G. *J. Chem. Soc., Trans.* **1899**, *75*, 830.
(10) Baker, W.; Robinson, R. *J. Chem. Soc.* **1928**, 3115.
(11) Shriner, R. L.; Hull, C. J. *J. Chem. Soc.* **1945**, 228.
(12) Bass, R. J. *J. Chem. Soc., Chem. Commun.* **1976**, 78.
(13) Chang, Y.-C.; Nair, M. G.; Santell, R. C.; Helferich, W. G. *J. Agric. Food. Chem.* **1994**, *42*, 1869.
(14) Pelter, A.; Ward, R. S.; Ashdown, D. H. J. *Synthesis* **1978**, 843.
(15) Farkas, L.; Gottsegen, À.; Nógrádi, M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 305.
(16) Sekizaki, H.; Yokosawa, R. *Chem. Pharm. Bull.* **1988**, *36*, 4876.
(17) Sinkevich, Y.; Shchekotikhin, A.; Luzikov, Y. *Chem. Heterocycl. Compd.* **2007**, *43*, 1252.
(18) Gammill, R. B. *Synthesis* **1979**, 901.
(19) Liu, L.; Zhang, Y.; Wang, Y. *J. Org. Chem.* **2005**, *70*, 6122.
(20) Baker, W.; Chadderton, J.; Harborne, J. B.; Ollis, W. D. *J. Chem. Soc.* **1953**, 1852.