New Isoflavone and Triterpene Glycosides from Soybeans

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Chemical investigation of a soybean phytochemical concentrate resulted in the isolation and identification of two new isoflavanones, dihydrodaidzin (**1**) and dihydrogenistin (**2**), a new isoflavone, 2″,6″-*O*-diacetyloninin (**3**), and two new triterpenoid saponins (**13** and **14**). Nine known isoflavonoids (**4**−**12**) and three known saponins (**15**−**17**) were also identified. Structures of the new compounds were established by a combination of extensive NMR (DEPT, DQF-COSY, HMBC, HMQC, and ROESY) studies and chemical degradation. Cytotoxic activities (ED_{50}) of various extracts and selected isoflavonoids and saponins were measured against human stomach carcinoma (Hs 740.T, Hs 756 T), breast adenocarcinoma (Hs 578 T, Hs 742.T), and prostate carcinoma (DU 145, LNCaP-FGC) cell lines. Isoflavonoids **3** and **5** were more active than **1** and **2** versus at least one of the three cell lines examined, indicating the importance of the 2,3-double bond in cytotoxicity. Saponins **13**, **14**, and **15** were slightly more active than saponins **16** and **17**, indicating that sugar attachments at position-22 enhance cytotoxic activity.

Soybeans and processed soy products containing isoflavonoids and saponins are of wide interest for their multifaceted biological effects.[1−4] In a preceding paper[5] we described the isolation and characterization of several new and known isoflavone, cinnamic acid, and triterpenoid glycosides from soybean molasses, a concentrate of the aqueous extract prepared during the processing of soybeans. In our continuing studies of bioactive constituents in soybeans, we have now obtained several additional new and known isoflavonoid and triterpenoid components from a soybean phytochemical concentrate.[6]

Soybean phytochemical concentrate (SPC) is a powdered proprietary concentrate prepared from *Glycine max* (L.) Merr. (Fabaceae) by Archer Daniel Midland (ADM).[6] SPC contains 40−50% (w/w) of soy isoflavones with a profile of components that matches those found in tofu, the most commonly consumed soy food.[7] The principal known isoflavones in SPC are daidzein-7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**11**) and genistein-7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**12**) along with daidzein, genistein, glycitein, and their respective β-glucosides daidzin, genistin, and glycitin. While the overall composition of SPC is similar to that of soybean molasses, SPC also contains different isoflavonoid components.[5,6] SPC also contains soy saponins, plus a variety of very minor components, all of which are found naturally in soybeans.

Since SPC is prepared in a manner that concentrates soy isoflavonoids and saponins, this study was undertaken to identify and recover potentially valuable new compounds and to evaluate their cytotoxic activities.

Results and Discussion

HPLC analysis of SPC (Figure 1) revealed an abundance of known isoflavones together with minor eluting peaks representing unknown isoflavonoids.[5] The predominant isoflavonoids in SPC were **4** (1.2 mg/g), **5** (1.6 mg/g), **6** (0.1 mg/g), **7** (0.2 mg/g), **8** (0.5 mg/g), **9** (4.3 mg/g), **11** (3.1 mg/g), and **12** (4.6 mg/g) of the total extract. Concentrations of known isoflavonoids (Figure 2) in our extract were comparable to those reported previously.[6] New compounds eluting at retention volumes of 13.89, 17.34, and 21.14 mL were isolated and characterized in this study.

To isolate compounds for spectral and chemical characterization, SPC was first suspended in water, and the water-soluble fraction was concentrated and partitioned separately into *n*-hexane, EtOAc, and *n*-BuOH. Chromatographic resolution of the EtOAc-soluble fraction furnished 12 isoflavonoids (**1**−**12**), three of which (**1**−**3**) were new compounds (Figures 2 and 3). Chromatographic purification of the *n*-BuOH-soluble fraction furnished two new (**13**−**14**) and three known soy saponins (**15**−**17**). Spectra (UV, IR, ^{1}H and ^{13}C NMR, and FABMS) for isolated isoflavones were in good agreement with reported data for daidzein[5] (**4**), genistein[5] (**5**), glycitein[5] (**6**), biochanin A (**7**),[8] daidzin (**8**),[5] genistin (**9**),[5] glycitin (**10**),[8] daidzein-7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**11**),[9] and genistein-7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**12**).[9] Spectra (1D and 2D NMR) for saponins (**15**−**17**) confirmed their structures as soysaponin A2, or 3-*O*-{[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]}-22-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**15**),[10] soysaponin V, or 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]soyasapogenol B (**16**),[11] and soyasapogenol B monoglucuronide, or 3-*O*-[β-D-glucuronopyranosyl]soyasapogenol B (**17**).[12]

Of the new compounds, isoflavanone **1** gave $C_{21}H_{23}O_9$ by HRFABMS. Bands for hydroxyl and chelated carbonyl functional groups were suggested by IR, and the UV absorptions at 276 and 310 nm were typical for an isoflavanone.[13] The 310 nm absorbance shifted to 318 nm after addition of NaOMe reagent, indicating a free hydroxyl group at C-4′, which was also evident by a *m*/*z* 120 fragment ion in the EIMS. Lack of UV bathochromic shifts with $AlCl_3$ or NaOAc suggested the absence of free C-5 or C-7 hydroxyl groups in **1**. The proton singlet typically observed between δ 8.2−8.5 for H-2 in isoflavones (**4**−**12**) was absent in **1**. An AMX system with signals at δ 4.86 (1H, dd, J = 4.9, 12.2 Hz, H-2a), 4.97 (1H, dd, J = 7.2, 12.2 Hz, H-2b), and 3.91 (1H, t, J = 4.9 Hz, H-3) indicated a flavanone structure for **1**. These protons were correlated with carbon signals for C-2 and C-3 at δ 71.34 and 47.19 respectively, while a signal at δ 198.40 was assigned to a

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Figure 1. HPLC analysis of soybean phytochemical methanol extract. Identified samples are peak 1, dihydrodaidzin (**1**), peak 2, daidzin (**8**), peak 3, genistin (**9**), peak 4, daidzein 7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**11**), peak 5, dihydrogenistin (**2**), peak 6, glycitin (**10**), peak 7, genistein 7-O-β-D-(6″-*O*-acetylglucopyranoside) (**12**), peak 8, unknown, peak 9, unknown, peak 10, 2″,6″-*O*-diacetylononin (**3**), peak 11, glycitein (**6**), peak 12, daidzein (**4**), peak 13, genistein (**5**), and peak 14, biochanin A (**7**).

Figure 2. Structures of new isoflavonoids and saponins isolated from soybean phytochemical extract.

C-4 isoflavanone ketone.[14] An ABX spin system of δ 7.38 (1H, d, J = 8.6 Hz), 6.69 (1H, dd, J = 2.2, 8.6 Hz), and 6.51 (1H, d, J = 2.2 Hz) was attributed to H-5, H-6, and H-8, while the four-proton AA′BB′ spin system was assigned to a 4′-substituted ring B.

Signals observed for five *trans* diaxial oxymethine protons (J = 7.8 Hz) and one oxymethylene group indicated the presence of a β-D-glucopyranosyl moiety for **1**. HMBC correlations confirmed assignments of C-5, C-6, and C-8. Thus, the signal for C-5 (δ 129.00) was correlated with H-6 (δ 6.69, dd, J = 2.2, 8.6 Hz), while both H-6 and H-8 (δ 6.51 d J = 2.2 Hz) were correlated with C-7 (δ164.60), and H-8 was correlated with C-9 (δ 155.20). The glucosyl residue was located at the 7-*O*-position of **1** by HMBC correlations between C-7 and the anomeric H-1″. The absolute configuration at C-3 of isoflavanones remains poorly defined in the literature because of keto−enol tautomerism that compromises the isomeric composition of these compounds.[15] The optical rotation of **1** as $[\alpha]^{22}_{D}$ −12.9° is similar to that reported for isoflavanone sophorol

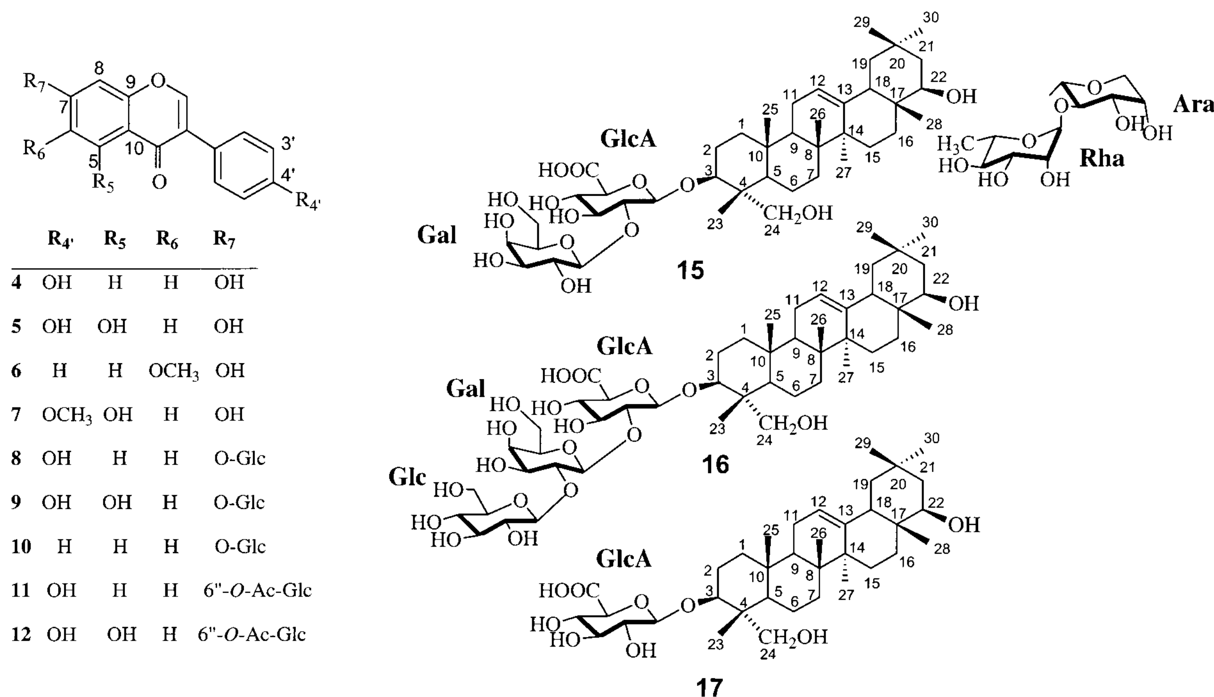

Figure 3. Structures of known isoflavones and saponins in soybean phytochemical extract.

([α]$_D$ −13.6°).[15] Thus, **1** is (−)-dihydrodaidzin or 7-O-β-D-glucopyranosyl-4′-hydroxyisoflavanone.

Isoflavanone **2** gave $C_{21}H_{23}O_{10}$ by HRFABMS, indicating the presence of one more oxygen atom than **1**. The UV, IR, and ^{1}H and ^{13}C NMR spectra of **1** and **2** were almost identical, except for the presence of a hydroxyl group singlet at δ 13.98 in **2**, which disappeared on deuterium exchange.[16] This functionality was further confirmed by a bathochromic shift of 9 nm in the UV spectrum in the presence of $AlCl_3$. Two, *meta*-coupled doublets at δ 6.19 and 5.97 (2H, d, J = 2.0 Hz) represented H-6 and H-8, respectively. ^{1}H−^{1}H DQF-COSY analysis showed three spin-coupled systems resulting from the presence of substitutions at positions C-5, C-7, and C-4′. Thus, **2** is (−)-dihydrogenistin or 7-*O*-β-D-glucopyranosyl-5,7,4′-trihydroxyisoflavanone.

Dihydrodaidzin (**1**) and dihydrogenistin (**2**) are previously undescribed isoflavone glycosides. The isoflavanone aglycons of **1** and **2** were previously identified as products of biotransformation by human intestinal bacteria. In the gut, the isoflavone glycosides daidzin (**8**) and genistin (**9**) are cleaved by intestinal glycosylases to daidzein (**4**) and genistein (**5**), and the aglycones are subsequently reduced by the gut microflora to form the free isoflavanone aglycones of **1** and **2**.[17,18] Although **1** was reported in the literature,[19] no corresponding spectral data for this compound has been presented.

Isoflavone **3** gave $C_{26}H_{27}O_{11}$ by HRFABMS. The UV absorption at 272 nm was typical for an isoflavone.[12,13] ^{1}H NMR revealed the characteristic chemical shifts and coupling patterns for a 7,4′-oxygenated isoflavone. The most downfield signal δ 7.98 (1H, d, J = 8.7 Hz) was for H-5, which is *peri* to the C-4 carbonyl, while signals for H-6 and H-8 partially overlapped to appear as a multiplet centered at δ 6.97. Signals for a 4′-*O*-methyl group (δ 3.94), a 7-*O*-linked glucosyl unit, and two acetyl methyl group singlets (δ 1.95 and 2.07) all indicated that **3** was an acylated derivative of oninin (formononetin-7-*O*-β-D-glucopyranoside).[20] The acetyl moieties were attached to positions 2″ and 6″, on the basis of the ^{1}H NMR shifts of protons attached to these positions versus free glucose.[21] Characteristic correlations observed between the glucosyl anomeric proton (δ 5.05) and H-8 in the DQF-COSY spectrum and the quaternary aromatic carbon at (δ 164.50) by HMBC confirmed the C-7 glucose linkage. The methoxyl group singlet (δ 3.94) of **3** was correlated with the C-4′ carbon at δ 161.00 by HMBC. HMBC correlations between the two acetyl moieties and the oxygenated H-2 and H-6 of the glucose core (^{1}H/^{13}C/^{1}H: δ 1.95/170.40/4.36, C-2″ acetyl; and δ 2.07/172.80/4.66/4.83, C-6″ acetyl) confirmed the sites of acylations. Therefore, **3** was identified as the new compound, formononetin-7-*O*-(2″,6″-*O*-diacetyl)glucopyranoside (2″,6″-*O*-diacetyloninin).

Saponin **13** gave *m*/*z* 1243.6110 for $C_{59}H_{96}NaO_{26}$ by HRFABMS. Negative-ion FABMS indicated the loss of one pentose, one hexose, two deoxyhexoses, and one glucuronic acid residue. Acid hydrolysis of **13** furnished the aglycone and monosaccharide components, glucuronic acid, galactose, rhamnose, and arabinose by TLC analysis. The sugars of **13** were evidenced by five anomeric proton and carbon signals in ^{1}H and ^{13}C NMR spectra (Table 1). By ^{13}C NMR, the two methyl carbon signals at δ 18.80 and 18.00 and proton signals at δ 1.24 and 1.28 (3H each, d, J = 6.5 Hz) indicated that **13** contained two 6-deoxy sugars. By ^{13}C NMR, all of the monosaccharides were pyranoses. The anomeric configurations were fully defined from their $^3J_{H1-H2}$ coupling constants as well as from NOE correlations observed in the ROESY spectrum.[5] The β-anomeric configurations for the glucuronic acid and galactose residues were evident from correlations between H-1 and H-5. The J_{H-H} values of 7.8 and 8.2 Hz for glucuronic acid and galactose, respectively, provided further evidence for their β-configurations. The small $^3J_{H-1-H-2}$ coupling constants of 1.5 and 1.6 Hz, the three-bond HMBC couplings between the anomeric protons and C-3 and C-5, and NOE relationships between H-1 and H-3, H-1 and H-5 in ROESY spectra all indicated α-orientations for the two rhamnoses. The presence of an α-L-arabinopyranoside was shown by a $J_{H-1-H-2}$ coupling constant of 6.5 Hz and the ROESY spectrum, which showed NOEs from H-1 to H-2, and H-3

Table 1. 1D and Selected 2D NMR Chemical Shift Assignments of Aglycon and Sugar Moieties for Saponins **13** and **14** (CD_3OD), 600 MHz for ^{1}H and 100 MHz for ^{13}C NMR

position	**13**		**14**					
	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	DEPT	DQF-COSY	ROESY	HMBC
aglycon								
1α	1.12 dt, (12.5, 4.5)	39.22	1.09 dt, (11.5, 4.0)	38.81	CH_2	H-1β, 2α, 2β	H-1β, 9α	Me-25
1β	1.84 m		1.72 m			H-1α, 2α, 2β	H-11, Me-25	Me-25
2α	2.31 dt, (12.5, 4.5)	26.20	2.25 dt, (11.5, 4.0)	26.75	CH_2	H-1α, 1β, 2β, 3α	H-2β	C-4, 10
2β	2.11 dq, (4.5, 12.5)		2.08 dq, (4.0, 11.5)			H-1β, 2α, 3α	Me-25	C-4
3α	3.78 dd, (12.5, 4.5)	89.30	3.65 dd (11.5, 4.0)	88.58	CH	H-2α, 2β	H-5α, Me-23, glcA-1	C-1, 2, 4, 5, Me-23, 24 glcA-1
4		41.83		41.80	C			
5α	1.44 d, (12.5)	55.14	1.62 d, (11.5)	55.48	CH	H-6β, 7α, 7β	6α, 9α, Me-23	C-3, Me-23, 24, Me-25
6α	1.90 d, (12.5)	18.80	1.75 m	19.15	CH_2	H-6β, 7α, 7β	Me-23	C-4, 8, 10
6β	1.35 m		1.49 m			H-5α, 6α, 7α, 7β	H-7β, Me-26	C-5, Me-26
7α	1.60 dt, (12.0, 3.8)	32.12	1.67 dt, (11.5, 4.0)	33.00	CH_2	H-5α, 6α, 6β, 7β	H-7β	C-5, Me26
7β	1.21 d, (12.0)		1.28 d, (11.5)			H-5α, 6α, 6β, 7α	H-7α,	C-8, 14, Me26
8		40.75		40.66	C			
9α	2.16 dd (13.0, 8.0)	47.64	2.13 dd, (13.2, 8.0)	48.26	CH	H-12,	H-1α, 11, Me-27	C-5, 7, 8, 10, 11, 12, 14, Me-25, Me-26
10		37.60		37.28	C			
11	1.86 m	24.11	1.88 m	24.45	CH_2	H-12	H-1β, 9α, Me-25	C-8, 9, 10, 13
12	5.66 s	122.83	5.39 s	122.56	CH	H-9α, 11, 18β	H-19β	C-9, 14, 18
13		144.30		144.54	C			
14		42.30		42.15	C			
15α	1.78 m	26.95	1.83 m	26.64	CH_2	H-15β, 16β	H-15β, 16β	C-13, 17, 18, Me-27
15β	1.39 m		1.42 m			H-15α, 16β	H-15α, 16β, Me-26, Me-28	C-17, Me-26, Me-27
16α	2.05 m	28.17	1.91 m	27.76	CH_2			
16β	1.90 m		1.86 m			H-15α, 15β	H-15α, 15β, Me-28	C-14, Me-28
17		39.32		38.25	C			
18β	2.42 dd, (14.0, 4.5)	45.77	2.38 dd, (13.5, 4.0)	43.86	CH	H-19α, 19β	H-12, Me-28, Me-30	C-14, 20, Me-28, Me-29 or Me-30
19α	1.95 d, (12.0)	45.18	2.00 d, (13.5)	46.28	CH_2	H-18β, 19β	H-19β, Me-29	C-13, 17, 18, 20, Me-29, Me-30
19β	1.64 d, (12.0)		1.73 d, (13.5)			H-18β, 19α, 21	H-19α, Me-28, Me-30	C-17, 18, 20, 21, Me-29 or Me-30
20		30.92		30.75	C			
21α	1.80 m	37.24	1.77 m	36.63	CH_2	H-21β, 22α	H-21β, Me-29	
21β	1.55 m		1.62 m			H-21α, 22α	H-21α, 22α, Me-28	
22α	4.43 m	84.95	4.30 m	86.44	CH	21α, 21β	H-18β, 21α, 21β, ara-1	C-16, 18, 20, Me-28, ara-1
23	1.12 s	22.54	1.16 s	22.00	CH_3		H-3α, 5α, 6α, 24	C-3, 4, 5, 24
24	4.11 d, (11.0) 4.58 d, (11.0)	63.67	4.27 d, (10.5) 4.40 d, (10.5)	64.40	CH_2	H-24	H H-5α, 6α, Me-23	C-3, 4, 5, Me-23
25	0.86 s	15.70	0.80 s	16.29	CH_3		H-1β	C-1, 5, 10
26	0.93 s	16.53	0.95 s	17.18	CH_3		H-11, 6β, 15β	C-7, 8, 9, 10, 14
27	1.18 s	25.50	1.25 s	25.65	CH_3		H-9α	C-8, 13, 14, 15
28	0.89 s	21.15	0.85 s	21.00	CH_3		H-15β, 19β, 21β	C-16, 17, 18, 22
29	1.03 s	31.11	1.00 s	32.20	CH_3		H-19α, 21α	C-19, 20, 21, Me-30
30	0.98 s	27.85	0.98 s	27.12	CH_3		H-18β, 19β, 21β	C-19, 20, 21, Me-29
sugar moiety								
C-3								
glcA-1	5.12 d, (7.8)	105.30	5.10 d, (8.0)	104.85	CH	glcA-2	H-3α, Me-23	C-3
glcA-2	4.65 dd, (7.8, 9.0)	80.95	4.54 dd, (8.0, 9.2)	79.42	CH	glcA-3, gal-1	gal-1	gal-1
glcA-3	3.57 m	76.17	3.50 m	76.62	CH	glcA-2	glcA-2, glcA-4	glcA-1, glcA-5
gal-1	5.30 d, (8.2)	102.73	5.20 d, (8.0)	103.20	CH	glcA-2	glcA-2	glcA-2
gal-2	4.76 dd, (8.2, 8.0)	78.57	4.62 dd, (8.0, 9.5)	78.00	CH	rha-1	rha-1	rha-1
rha-1	5.22 d, (1.6)	103.25	5.15 d, (2.1)	103.50	CH	gal-2	gal-2	gal-2
rha-6	1.24 d, (6.5)	18.80	1.22 d, (6.3)	18.24	CH_3			
C-22								
ara-1	5.18 d, (6.5)	108.10			CH	ara-2	H-22, Me-28	C-22
ara-2	4.60 dd, (8.6, 6.5)	82.70			CH	rha-1	rha-1	rha-1
glc-1			4.98 d, (7.7)	105.00	CH	rha-2	H-22, Me-28	C-22
glc-2			4.18 dd, (7.7, 9.5)	80.11	CH	rha-1	rha-1	rha-1
rha-1	5.37 d, (1.5)	102.92	5.18 d, 2.0)	102.33	CH	glc-2	glc-2	glc-2
rha-2	3.52 dd (3.5. 1.5)	72.57	3.58 dd (3.1, 2.0)	72.73	CH	rha-2	ara-2, rha-4	ara-2, rha-4
rha-6	1.28 d, (6.5)	18.00	1.33 d, (6.0)	17.80	CH_3			

to H-5, as expected for an α-L-arabinopyranoside in rapid $^4C_1 \leftrightarrow {}^1C_4$ conformational exchange.[22,23]

DEPT NMR analysis indicated 30 methines, 12 methylenes, 9 methyl groups, and by difference from the broadband spectrum, 8 quaternary carbon atoms for **13** (Table 1). Twenty eight sp^3 and two sp^2 carbon signals by ^{13}C NMR and seven methyl group singlets and a broad triplet vinyl proton by ^{1}H NMR confirmed that the aglycon possessed an olean-12-ene skeleton.[5] EIMS and detailed 2D NMR analyses using DQF-COSY, ROESY, HMQC, and HMBC

identified the aglycon as soysapogenol B (Table 1), a common aglycon of soybean triterpenoid glycosides,[11,12] including one compound previously identified by us from soy molasses.[5] Downfield shifts in ^{13}C NMR for C-3 (δ 89.30) and C-22 (δ 84.95) versus soyasapogenol B suggested **13** was a bisdesmosidic glycoside containing sugars at both of these positions.

The position of the trisaccharide moiety was unambiguously defined by HMBC and ROESY experiments. A significant cross-peak due to long-range correlation between C-3 (δ 89.30) of the aglycon and H-1 (δ 5.12) of glucuronic acid indicated that the glucuronic acid residue was linked to C-3 of the aglycon. A cross-peak between H-1 (δ 5.30) of galactose and C-2 (δ 80.95) of glucuronic acid and a cross-peak between H-1 (δ 5.22) of rhamnose and C-2 (δ 78.57) of galactose identified the trisaccharide as the known β-fabatriosyl.[24] Similarly, the sequence of the disaccharide chain at C-22 was deduced from long-range correlations of H-1 (δ 5.37) of rhamnose with C-2 (δ 82.70) of arabinose. Correlation between C-22 (δ 84.95) of the aglycon and H-1 (δ 5.18) of arabinose indicated the attachments of terminal sugar moieties to the aglycon. The two sugar chains of **13** were also deduced from the following key nuclear Overhauser effect (NOE) correlations observed in the ROESY spectrum: H-1 (δ 5.22) of rhamnose with H-2 (δ 4.76) of galactose, H-1 (δ 5.30) of galactose with H-2 (δ 4.65) of glucuronic acid, and H-1 (δ 5.37) of rhamnose with H-2 (δ 4.60) of arabinose. Thus, the structure of saponin **13** was established as 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl}-22-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-3β,22β,24-trihydroxyolean-12-ene.

Saponin **14** gave *m/z* 1273.6189 for $C_{60}H_{98}NaO_{27}$ by HRFABMS. 2D NMR spectral data (Table 1) were nearly identical to that for **13**, except for the disaccharide chain at C-22 of the aglycon. A β-D-gluopyranosyl unit was present in **14** versus an α-L-arabinopyranosyl unit in **13**. By use of a combination of DQF-COSY, HMQC, HMBC, and ROESY (Table 1), the structure of saponin **14** was established as 3-*O*-{[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl}-22-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-3β,22β,24-trihydroxyolean-12-ene. Both **13** and **14** are new natural products.

Several investigations have shown that soy isoflavonoids, particularly genistein, inhibit the proliferation of transformed cells in culture.[25] Some in vivo studies[26] report that pure isoflavones or dietary soy protein inhibits tumorigenesis in rodent models.[26] Different mechanisms may be implicated in such activities including agonist/antagonist effects on estrogen receptors,[27] stimulation of sex hormone-binding globulin synthesis,[27] inhibition of growth factor-associated tyrosine-kinase signal transduction,[28] antioxidant properties,[29] and inhibition of DNA topoisomerase.[30] Although such studies are highly suggestive of the beneficial effects of soy consumption with regard to cancer prevention, definitive intervention trials have not been completed. In contrast, others have shown that soybean components do not inhibit tumorigenesis.[31] Consumption of a soybean-based diet[32] or administration of genistein[25] resulted in increasing colon tumor incidence and tumor burden in rats treated by chemical carcinogens. Further, it has been hypothesized that the estrogenic properties of soy isoflavones may actually stimulate breast tumor growth under some conditions.[33]

Many types of cell lines have been used to display the growth inhibitory properties of soybean isoflavonoids and soy saponins. These have included human cancer cell lines from prostate (LNCaP, DU 145, PC-3), breast carcinoma (MDA-468, MCF-7, MCF-7-D-40, MDA-MB-231), stomach (HSC-41E6, HSC-45M2, SH101−P4), colon (HT29 clone 19A), bladder (HT-1376, UM-UC-3, RT-4, J82, TCCSUP), melanoma (B16F-10), leukemia (HL-60), carcinoma (HCT-15), and rat prostate (MAT-LyLu).[6,33−41] For this work, we used human stomach carcinoma (Hs 740.T, Hs 746 T),[42] breast adenocarcinoma (Hs 578 T, Hs 742.T),[43] and prostate carcinoma (DU 145[44] and LNCaP-FGC[45]) cell lines to evaluate the cytotoxic activities of extracts and some of the compounds obtained in this work. The 50% effective doses (ED_{50}) obtained by measuring growth inhibition with MTT (3-[4,5-dimethlthiazol-2-yl]-2,5-diphenyltetrazolium bromide])[46,47] are shown in Table 2.

Cell lines LNCAP-FGC and HS742.T were the least sensitive of all lines examined to the activities of these compounds. The activities observed with crude extracts were surprising. While none gave activities surpassing those of pure compounds, these extracts contain mixtures of compounds that could function together synergistically in displaying cytotoxicity. Of the isoflavonoids, less polar isoflavones **3** and **5** were generally more potent than isoflavanones **1** and **2**. This suggests the importance of the C_2-C_3 double bond for cytotoxicities in these cell lines. In general soy saponins **13**−**17** were more cytotoxic than the isoflavonoids **1**−**3** and **5** with these cell lines. Soy saponins **13**, **14**, and **15** were more cytotoxic versus both stomach carcinomas, the Hs 578 T breast adenocarcinoma, and the DU 145 prostate carcinoma lines. All three compounds are C-22-glycosides, whereas the less active **16** and **17** glycosides possess free hydroxyl groups at C-22. The results suggest a slight enhancement of cytotoxicity when this position is glycosylated. Relatively limited information exists on the structure−activity relationships for the cytotoxicities of these classes of compounds. Although it is impossible to establish structure−activity relationships with the small number of compounds evaluated here, the results provide some insights as to structural moieties that modulate cytotoxic activity and add to existing knowledge on the cytotoxicities of these classes of compounds. In summary, our findings confirm the work of others[6,25,26,33−41] in showing that soy phytochemical concentrate extracts, isoflavonoids, and soy saponins have direct effects on human stomach, breast, and prostate carcinoma cell lines in vitro.

Experimental Section

General Experimental Procedures. Flash column liquid chromatography was performed using J. T. Baker glassware with 40 μm silica gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by fluorescence quenching under 245 nm UV light and by spraying with diazotized sulfanilic acid spray reagent. The reagent was freshly prepared by mixing equal volumes of 0.5% $NaNO_2$ and 0.5% sulfanilic acid in 2% HCl. Plates were sprayed with this mixture, followed by spraying with 5% NaOH in 50% ethanol and warming with a heat gun for 3 min for isoflavonoids. For saponins, chromatograms were visualized by spraying developed plates with 1% vanillin/H_2SO_4, followed by heating at 100 °C for 5 min. TLC plates were developed with solvent systems A ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v), B (EtOAc/MeOH/H_2O, 100:16.5:13.5), C ($CHCl_3$/MeOH/H_2O, 61:32:7).

The HPLC system (Shimadzu, Columbia, MD) consisted of a solvent delivery system equipped with dual pumps (LC-10 AD), an in-line degasser (DGU-14A), a quaternary solvent mixer (FCV-10A), a photodiode array (PDA) ultraviolet-visible

Table 2. Cytotoxicity of Soybean Extracts and Isolated Compounds against Breast, Prostate, and Stomach Tumor Cell Lines[a]

extract compound	ED$_{50}$ (μg/mL) stomach carcinoma		breast adenocarcinoma		prostate carcinoma	
	Hs 740.T	Hs 746 T	Hs 578 T	HS 742.T	DU 145	LNCAp-FGC
MeOH	12.32 (± 0.27)	8.45 (± 0.18)	17.11 (± 0.38)	45.29 (± 0.73)	7.15 (± 0.12)	42.18 (± 0.72)
EtOAc	10.78 (± 0.24)	11.57 (± 0.25)	8.84 (± 0.17)	38.19 (± 0.61)	3.94 (± 0.07)	37.77 (± 0.58)
n-BuOH	8.89 (± 0.19)	9.43 (± 0.15)	5.22 (± 0.09)	29.15 (± 0.52)	4.59 (± 0.09)	30.53 (± 0.55)
isoflavonoids						
1	17.37 (± 0.43)	14.92 (± 0.30)	28.23 (± 0.52)	33.57 (± 0.55)	9.16 (± 0.17)	32.45 (± 0.59)
2	15.12 (± 0.34)	12.24 (± 0.28)	15.36 (± 0.30)	30.80 (± 0.50)	10.25 (± 0.20)	41.58 (± 0.68)
3	7.61 (± 0.10)	8.89 (± 0.18)	5.44 (± 0.10)	25.53 (± 0.44)	4.18 (± 0.06)	22.12 (± 0.37)
5	4.38 (± 0.07)	5.82 (± 0.09)	3.50 (± 0.05)	14.88 (± 0.22)	2.39 (0.04)	25.45 (± 0.39)
saponins						
13	3.53 (± 0.05)	2.47 (± 0.04)	2.39 (± 0.03)	17.51 (±0.29)	3.12 (± 0.05)	27.50 (± 0.0.43)
14	4.10 (± 0.05)	3.94 (± 0.05)	2.12 (± 0.02)	14.63 (± 0.25)	3.25 (± 0.05)	24.10 (±0.35)
15	3.15 (± 0.04)	3.22 (± 0.05)	4.84 (± 0.09)	30.10 (± 0.53)	2.11 (± 0.02)	30.70 (± 0.50)
16	8.97 (± 0.11)	7.36 (±0.13)	9.87 (± 0.15)	31.55 (± 0.58)	5.75 (± 0.10)	40.68 (± 0.62)
17	9.61 (± 0.15)	9.59 (±0.12)	8.77 (± 0.10)	28.68 (± 0.48)	9.13 (± 0.16)	37.29 (± 0.60)

[a] Values are presented as mean ± SE of two test sample observations, compared with that of control group ($p < 0.05$) for all values.

detector (SPD-M6A), and a sample autoinjector (SIL-10A), connected to a system controller (SCL-10A *VP*). Data acquisition and processing were controlled by a Shimadzu SPD-M6A program (Version 2.0) running on an IBM Aptiva C3E Pentium computer (IBM, Stamford, CT) attached to a Cannon BJC-620 color bubble jet printer (Costa Mesa, CA). Standard compounds and sample mixtures were resolved over a 250 mm × 4.6 mm i.d. Econosil C_{18} (particle size 10 μm) column and eluted at a flow rate 1.0 mL/min when analyzing standards and SPC mixtures. The following gradients were used for all separations: solvent A was $CH_3CN/H_2O/HCOOH$ (10:90:5, v/v/v), and solvent B was composed of $H_2O/CH_3CN/HCOOH$ (10:90:5, v/v/v). Gradient conditions were 0−3 min 0% B; 3−4 min 0−17% B; 4−22 min 17−28% B; 22−23 min 28−50% B; 23−45 min 50% B. HPLC samples were detected at a wavelength of 260 nm. Sample injection volumes varied between 2.5 and 20 μg depending upon sample concentrations.

The HPLC retention volumes (R_V) in milliliters for isoflavone standards[5] and isolated compounds were as follows: dihydrodaidzin (**1**) (13.89), daidzin (**8**) (15.28), genistin (**9**) (15.64), daidzein-7-*O*-*β*-D-(6″-*O*-acetylglucopyranoside (**11**) (16.18), dihydrogenistin (**2**) (16.92), glycitin (**10**) (17.34), genistein-7-*O*-*β*-D-(6″-*O*-acetylglucopyranoside (**12**) (18.31), ononin (Indofine Chemical Company, Inc.) (18.85), formononetin-7-*O*-(2″,6″-*O*-diacetyl) glucopyranoside (**3**) (21.14), glycitein (**6**) (22.04), sissotrin (Indofine Chemical Company, Inc.) (25.92), daidzein (**4**) (26.47), genistein (**5**) (31.08), formononetin (31.39), biochanin A (**7**) (39.88).

Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra (cm^{-1}) were obtained using a Nicolet 205 FT-IR spectrometer connected to a Hewlett-Packard ColorPro plotter. High-resolution fast atom bombardment mass spectra spectra (HRFABMS) were taken on a VG-ZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.).

NMR spectra were obtained in DMSO-d_6 or CD_3OD using TMS as the internal standard, with chemical shifts expressed in δ and coupling constants (J) in Hz. Routine ^{1}H and ^{13}C NMR spectra were obtained with a Bruker NMR 400 (Bruker Instruments, Billerica, MA), operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). DQF-COSY, ROESY, HMBC, and HMQC NMR experiments were carried out using a Bruker AMX-600 high-field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1.

The following materials and reagents were used for cell culture and cytotoxic assays. Human stomach carcinoma (Hs 740.T, Hs 756 T), breast adenocarcinoma (Hs 578 T, Hs 742.T), and prostate carcinoma (DU 145, LNCaP-FGC) cell lines were purchased from the American Type Culture Collection ATCC. Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY), Eagle minimum essential medium (EMEM), and Rosewell Park Memorial Institute (RPMI) 1640 medium were from Nissui Pharm. Co., Ltd., Tokyo, Japan. Flat-bottom, 96-well plates were from Iwaki Glass Co., LTD (Funabashi-Chiba-Ken, Japan). The 3-[4,5-dimethlthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay[46,47] was from Sigma (St. Louis, MO), and 10% fetal bovine serum FBS was from Gibco BRL (Rockville, MD). All other chemicals used were of analytical reagent grade.

Plant Material. Soy phytochemical concentrate (SPC) was provided as a free-flowing powder by Archer Daniels Midland, Inc., Decatur, IL. SPC was prepared as described previously.[6] As described by Zhou et al., soybeans (*Glycine max* cultivated)[6,48] were cracked, dehulled, and flaked by standard procedures followed by hexane extraction to remove the majority of lipid. The resulting defatted soy flour was extracted with aqueous ethanol (60% v/v) to produce a mixture containing carbohydrates (0.6−0.7 g/g), isoflavonoids (0.02 g/g), fat 0.12 g/g), ash (0.04 g/g), and protein (0.05 g/g).[6] A proprietary solid phase, hydrophobic extraction procedure was accomplished by passing the aqueous alcoholic extract over Amberlite XAD. After washing the column with water to remove carbohydrates and other water-soluble materials, the hydrophobic column was eluted with 95% ethanol, and the eluant was spray-dried to form a powder called SPC.

HPLC analysis of the SPC powder was accomplished as follows. A sample of 1 g of SPC was defatted by extraction twice with 10 mL volumes of hexane. After centrifugation, the pellet was extracted three times with 10 mL of MeOH. The MeOH extracts were combined and concentrated to give 238 mg of crude extract. A 1 mg/mL solution of the crude extract

in MeOH was analyzed by HPLC. Standard curves were prepared from each of the available isoflavones.

Extraction and Isolation. SPC powder (725 g) was exhaustively extracted at room temperature with MeOH (3 × 5 L). The combined MeOH extracts were concentrated in vacuo at 30 °C to a brown residue (272 g). The concentrated extract was suspended in H_2O (150 mL) and filtered through Celite 545 (Fisher Scientific). The filtrate and H_2O washings (500 mL) were combined and washed with *n*-hexane (3 × 500 mL). The aqueous fraction (435 mL) was concentrated in vacuo to give a crude extract of 186 g that was partitioned first with EtOAc (3 × 2 L) and then with *n*-BuOH (3 × 2 L). The EtOAc extract (64 g) was fractionated by Si gel flash column chromatography (2.5 × 62 cm) using in sequence CH_2Cl_2/MeOH (90:10 → 50:50). Twelve fractions were combined on the basis of TLC using solvent systems A, B, and C. Subsequent purification of chromatographically similar fractions was accomplished by Sephadex LH-20 column chromatography using gradients of CH_3OH in CH_2Cl_2 ranging from 30 to 70%. Alternatively, fractions were resolved by reversed-phase Sepralyte C_{18} flash column chromatography (1.5 × 50 cm), using a H_2O/MeOH gradient solvent system (20 → 50%) at a flow rate of 3 mL/min. Final sample purifications were carried out with Sephadex LH-20 (25−150 μm, Pharmacia Fine Chemical Co.) columns eluted with MeOH to afford compounds **1** (58 mg), **2** (92 mg), **3** (28 mg), **4** (42 mg), **5** (95 mg), **6** (18 mg), **7** (13 mg), **8** (54 mg), **9** (83 mg), **10** (15 mg), **11** (264 mg), and **12** (285 mg).

The *n*-BuOH extract was concentrated in vacuo at 30 °C, and the residue (26 g) was dissolved in 10 mL of H_2O and chromatographed over a Diaion HP-20 (Mitsubishi Kasei Co., Ltd., Tokyo, Japan) column (3.5 × 90 cm) eluted with H_2O initially, with increasing concentrations of MeOH while being monitored by TLC (solvent systems B and C). Fractions were grouped as A (H_2O, 5.170 g), B (25% MeOH/H_2O, 3.785 g), C (50% MeOH/H_2O, 2.550 g), and D (75% MeOH/H_2O, 1.620 g). Fraction A (5.170 g) was rechromatographed over polyamide (SC6, Machery-Nagel, Düren, Germany 3.5 × 90 cm), and fractions eluting with 20 and 30% aqueous MeOH were combined separately for further Si gel flash column chromatography using (CH_2C1_2/MeOH/H_2O, 90:10:1 → 60:40:10) and repeated reversed-phase Sepralyte C_{18} flash column chromatography, using a H_2O/MeOH gradient solvent system (10 → 40%, 3 mL min) to afford **13** (28 mg) and **14** (37 mg). Fractions B and C were chromatographed again over a Si gel flash column (2.5 × 62 cm) using ($CHCl_3$/MeOH/H_2O, 80:20:1 → 60:40:4) to yield three further fractions, B1, B2 from the B fraction, and C-1 from C. Fraction B1 (525 mg) was subjected to Sepralyte C_{18} flash column chromatography using a H_2O/MeOH gradient solvent system (10 → 30%, 3 mL min) to afford **15** (35 mg). Similarly, fraction B-2 (440 mg) and fraction C-1 (378 mg) were separately subjected to Sepralyte C_{18} column chromatography with the same solvent as for **15** and further purified by Sephadex LH-20 eluted with MeOH to afford **16** (26 mg) and **17** (16 mg).

Compound 1: yellow, amorphous powder (58 mg); $[\alpha]^{22}_D$ −12.9° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) *276* (4.27), 310 (4.08), + NaOMe 318, + $AlCl_3$ 308, + HCl 308, + NaOAc 277 nm; IR (KBr) ν_{max} 3420, (OH), 1710 (C=O), 1590 (aromatic ring) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) aglycon δ 4.86 (1H, dd, J = 4.9, 12.2 Hz, H-2a), 4.97 (1H, dd, J = 7.2, 12.2 Hz, H-2b), 3.91 (1H, t, J = 4.9 Hz, H-3), 7.38 (1H, d, J = 8.6 Hz, H-5), 6.69 (1H, dd, J = 2.2, 8.6 Hz, H-6), 6.51 (1H, d, J = 2.2 Hz, H-8), 7.15 (2H, d, J = 8.6 Hz, H-2′, 6′), 6.82 (2H, d, J = 8.6 Hz, H-3′, 5′); glucosyl moiety, δ 5.12 (1H, d, J = 7.8 Hz, H-1″), 3.45 (1H, dd, J = 7.8, 9.2 Hz, H-2″), 3.54 (1H, t, J = 9.2 Hz, H-3″), 3.50 (1H, t, J = 9.2 Hz, H-4″), 3.39 (1H, m, H-5″), 3.72 (1H, dd, J = 12.0, 3.4 Hz, H-6″A), 3.86 (1H, dd, J = 12.0, 5.6 Hz, H-6″B); ^{13}C NMR (DMSO-d_6, 100 MHz) aglycon δ 71.34 (C-2), 47.19 (C-3), 198.40 (C-4) 129.00 (C-5), 116.30 (C-6), 164.60 (C-7), 104.90 (C-8), 155.20 (C-9) 117.80 (C-10), 128.70 (C-1′), 131.40 (C-2′, 6′), 159.20 (C-4′), 116.20 (C-3′, 5′); glucosyl moiety, δ 101.20 (C-1″), 74.60 (C-2″), 77.70 (C-3″), 71.11 (C-4″), 78.22 (C-5″), 62.31 (C-6″); HRFABMS, *m/z* 419.1346 $[M + H]^+$ (calcd for $C_{21}H_{23}O_9$, 419.1342).

Compound 2: yellow, amorphous powder (92 mg); $[\alpha]^{25}_D$ −23.7° (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.36), 308 (3.95), + NaOMe 314, + $AlCl_3$ 317, + HCl 319, + NaOAc 271 nm; IR (KBr) ν_{max} 3435, (OH), 1695 (C=O), 1595 (aromatic ring) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) aglycon, δ 4.77 (lH, dd, J = 5.1, 12.1 Hz, H-2a), 4.88 (lH, dd, J = 7.0, 12.1 Hz, H-2b), 3.90 (lH, t, J = 5.1 Hz, H-3), 6.19 (lH, d, J = 2.0 Hz, H-6), 5.98 (lH, d, J = 2.0 Hz, H-8), 7.43 (2H, d, J = 8.6 Hz, H-2′, 6′), 6.89 (2H, d, J = 8.6 Hz, H-3′, 5′), 13.98 (lH, br s, HO-5); glucosyl moiety, δ 5.08 (lH, d, J = 7.8 Hz, H-l″), 3.39 (1H, dd, J = 7.8, 9.1 Hz, H-2″), 3.52 (lH, t, J = 9.1 Hz, H-3″), 3.48 (lH, t, J = 9.2 Hz, H-4″), 3.42 (lH, m, H-5″), 3.65 (lH, dd, J = 11.9, 3.5 Hz, H-6″A), 3.79 (lH, dd, J = 11.9, 6.2 Hz, *H*-6″B); ^{13}C NMR (DMSO-d_6,100 MHz) aglycon, δ 71.26 (C-2), 47.10 (C-3), 197.86 (C-4) 164.50 (C-5), 102.10 (C-6), 169.00 (C-7), 96.35 (C-8), 165.40 (C-9) 103.10 (C-10), 128.60 (C-l′), 133.10 (C-2′, 6′), 159.00 (C-4′), 117.50 (C-3′, 5′); glucosyl moiety, δ 98.29 (C-1″), 74.76 (C-2″), 77.67 (C-3″), 71.02 (C-4″), 78.11 (C-5″), 62.43 (C-6″); HRFABMS, *m/z* 435.1297 $[M + H]^+$ (calcd for $C_{21}H_{23}O_{10}$, 435.1291).

Compound 3: yellow, amorphous powder (28 mg); $[\alpha]^{25}_D$ + 9.3° (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (4.12), 272 (4.48), + NaOMe 296, + $AlCl_3$ 276, + HCl 278, + NaOAc 279 nm; IR (KBr) ν_{max} 3400, (OH), 1735 (acetyl), 1710 (C=O), 1595 (aromatic ring) cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) aglycon, δ 8.20 (1H, s, H-2), 7.98 (lH, d, J = 8.7 Hz, H-5), 6.97 (2H, m, H-6, 8), 7.38 (2H, d, J = 8.7 Hz, H-2′, 6′), 7.08 (2H, d, J = 8.7 Hz, H-3′, 5′), 3.94 (3H, s, MeO-4′); glucosyl moiety, δ 5.05 (lH, d, J = 7.7 Hz, H−l″), 4.36 (1H, dd, J = 7.7, 9.1 Hz, H-2″), 3.58 (lH, t, J = 9.1 Hz, H-3″), 3.40 (lH, t, J = 9.1 Hz, H-4″), 3.72 (lH, m, H-5″), 4.66 (lH, dd, J = 11.9, 3.2 Hz, H-6″ A), 4.83 (lH, dd, J = 11.9, 6.4 Hz, *H*-6″B*)*, 1.95 (3H, s, AcO-2″), 2.07 (3H, s, AcO-6″); ^{13}C NMR (DMSO-d_6,100 MHz) aglycon, δ 153.30 (C-2), 123.60 (C-3), 176.70 (C-4) 128.72 (C-5), 111.20 (C-6), 164.50 (C-7), 104.90 (C-8), 158.00 (C-9) 117.25 (C-10), 123.20 (C−l′), 131.36 (C-2′, 6′), 161.00 (C-4′), 114.58 (C-3′, 5′); glucosyl moiety, δ 101.20 (C-1″), 77.84 (C-2″), 72.55 (C-3″), 73.05 (C-4″), 71.77 (C-5″), 64.33 (C-6″), 170.40, 20.25 (AcO-2″), 172.80, 20.90 (AcO-6″), 56.75 (MeO-4′); HRFABMS, *m/z* 515.1550 $[M + H]^+$ (calcd for $C_{26}H_{27}O_{11}$, 515.1553).

Compounds 4−12 gave UV, IR, ^1H and ^{13}C NMR, and FABMS data for $[M + H]^+$ ions in good agreement with reported data for daidzein (**4**) ($C_{15}H_{10}O_4$ $[M + H]^+$, 254);[5] genistein (**5**) ($C_{15}H_{10}O_5$, $[M + H]^+$, 270);[5] glycitein (**6**) $C_{16}H_{13}O_5$, $[M + H]^+$, 285);[5] biochanin A(**7**) ($C_{16}H_{13}O_5$ $[M + H]^+$, 285);[8] daidzin (**8**) ($C_{21}H_{21}O_9[M + H]^+$, 417);[5] genistin (**9**) ($C_{21}H_{21}O_{10}$ $[M + H]^+$, 433);[5] glycitin (**10**) ($C_{22}H_{23}O_{10}$ $[M + H]^+$, 447);[8] daidzein 7-*O*-β-D-(6″-*O*-acetylglucopyranoside) (**11**) ($C_{23}H_{23}O_{10}$ $[M + H]^+$, 459);[9] and genistein 7-*O*-β-D-(6"-*O*-acetylglucopyranoside) (**12**) ($C_{23}H_{23}O_{11}$ $[M + H]^+$, 475).[9]

Compound 13: white amorphous powder (28 mg); $[\alpha]^{25}_D$ −14.20° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3395, (OH), 1632 (C=C) cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRFABMS *m/z* 1243.6110 $[M + Na]^+$ (calcd for $C_{59}H_{96}NaO_{26}$, 1243.6107); negative ion FABMS *m/z* 1087 $[M - H - \text{arabinose}]^-$, 1073 $[M - H - \text{rhamnose}]^-$, 1057 $[M - H - \text{galactose}]^-$, 911 $[M - H - \text{rhamnose} - \text{galactose}]^-$, 735 $[M - H - \text{rhamnose} - \text{galactose} - \text{glucuronic acid}]^-$.

Compound 14: white amorphous powder (37 mg); $[\alpha]^{25}_D$ −23.00° (*c* 0.53, MeOH); IR (KBr) ν_{max} 3450, (OH), 1638(C=C) cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRFABMS *m/z* 1273.6189 $[M + Na]^+$ (calcd for $C_{60}H_{98}NaO_{27}$, 1273.6193); negative ion FABMS *m/z* 1103 $[M - H - \text{rhamnose}]^-$, 1087 $[M - H - \text{glucose}]^-$, 941 $[M - H - \text{rhamnose} - \text{galactose}]^-$, 765 $[M - H - \text{rhamnose} - \text{galactose} - \text{glucuronic acid}]^-$.

Compound 15: white amorphous powder (35 mg); IR (KBr), 3455 (OH), 1635(C=C) cm^{-1}; FABMS, *m/z* 1107 $[M + H]^+$ 1129 $[M + Na]^+$ (calcd for $C_{53}H_{87}O_{24}$ 1107). By these and ^1H and ^{13}C NMR, **15** was identified as soysaponin A2.[5,9]

Compound 16: white amorphous powder (26 mg); IR (KBr), 3450 (OH), 1635 (C=C) cm-1; FABMS, *m/z* 959 $[M + H]^+$, 981 $[M + Na]^+$ (calcd for $C_{48}H_{79}O_{19}$, 959*)*. By these and ^1H and ^{13}C NMR, **16** was identified as soysaponin V.[10]

Compound 17: white amorphous powder (16 mg); IR (KBr), 3455 (OH), 1632(C=C) cm^{-1}; FABMS, *m/z* 635 $[M + H]^+$, 657 $[M + Na]^+$ (calcd for $C_{63}H_{59}O_9$, 635). By these and ^1H and ^{13}C NMR, **17** was identified as soyasapogenol B monoglucuronoid.[11]

Acid Hydrolysis of 13 and 14. A 5 mg quantity of each compound was refluxed with 1 mL of 2 M HCl in 60% aqueous dioxane (5 mL) for 2 h. The reaction mixture was evaporated, and the hydrolysate after dilution with water (10 mL) was extracted with ether (3 × 10 mL). The ether extracts were evaporated to afford the aglycons, which were identified as soysapogenol B (*m/z* $C_{30}H_{50}O_3$ by EIMS).[5,11,12] The aqueous layer was neutralized with Ag_2CO_3 and filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars by cellulose TLC [pyridine/EtOAc/HOAc/H_2O, 36:36:7:21)], which indicated the sugars to be glucuronic acid, galactose, rhamnose, and arabinose in **13** and glucuronic acid, galactose, rhamnose, and glucose in **14**.

Cytotoxicity Assay.[46,47] Cell lines Hs 740.T, Hs 746 T, Hs 578 T, and Hs 742.T were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS). The DU 145 cells were cultured in Eagle minimum essential medium (EMEM) containing Earle's salts and supplemented with amino acids and 10% heat-inactivated FBS. The LNCaP-FGC cell lines were maintained in Rosewell Park Memorial Institute (RPMI) 1640 medium containing 10% heat-inactivated FBS. All cell lines were cultivated in an incubator at 37 °C in humidified air containing 5% CO_2. For routine cytotoxicity assays, all six cell lines were adapted to one single medium, RPMI 1640 medium supplemented with 10% FBS, 2 mM l-glutamine, penicillin (100 units/mL), and streptomycin (100 μg/mL). For the microassay, the growth medium was supplemented with 10 mM HEPES (1-(2-hydroxyethyl) piperazine-4-ethanesulfonic acid) buffer (pH 7.3) and incubated at 37 °C in a CO_2 incubator. These methods are similar to those used by Goren et al. and Rubinstein et al.[46,47]

Cellular viability in the presence and absence of experimental agents was determined using the standard MTT (3-[4,5-dimethlthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay.[46,47] The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically.[46,47] In brief, exponentially growing cells were harvested, and 200 μL cell suspensions were seeded in 96-well microplates and preincubated for 24 h at 37 °C under 5% CO_2 to allow cell attachment. After attachment, 10 μL of an EtOH/H_2O (1:1 solution) containing varying concentrations of test samples was added to wells in duplicate, and 10 μL of EtOH/H_2O (1:1) was added into wells as a control. Sample-containing microplates were further incubated for 6 days. Cell survival was evaluated by adding 10 μL of 5 mg/mL MTT in 0.1 mM, pH 7.4 phosphate buffered saline to each well and reincubating plates in 5% CO_2/air for 4 h at 37 °C. Plates were then centrifuged at 1500*g* for 5 min to precipitate cells and MTT formazan. An aliquot of 100 μL of the supernatant was removed, and DMSO (100 μL) was added to dissolve the precipitated, reduced MTT. The plate was mixed on a microshaker for 10 min, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (Dynex MR 5000, Chantilly VA). The ED_{50} value, which reduces the viable cell number, was defined as the concentration of test samples resulting in a 50% reduction of absorbance compared to untreated controls.[46,47]

Statistical Analysis. All cytotoxic data were expressed as mean ± SE. Student's *t*-test[49] was applied for detecting the significance of difference between each sample. $p < 0.05$ was taken as the level of significance.

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