Novel Isoflavone, Cinnamic Acid, and Triterpenoid Glycosides in Soybean Molasses

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Seven known isoflavones, genistein (**4**), daidzein (**5**), glycitein (**6**), formononetin (**7**), genistin (**8**), daidzin (**9**), and glycitein 7-*O*-*β*-D-(6″-*O*-acetylglucopyranoside) (**10**), ferulic acid, and two known saponin glycosides, soysaponin I (**14**) and soysaponin A2 (**15**), were isolated from soybean molasses. Several new compounds were also isolated and identified, including three isoflavones (**1**−**3**), two cinnamic acid ester glycosides (**11**) and (**12**), and a new saponin hexaglycoside (**13**). The structures of the new compounds were established on the basis of spectral data interpretation.

Soybeans are among the world's most valuable crops. They are used as animal feed, as sources of dietary protein and oil, and for other industrial products.[1,2] The three major species of soybean are *Glycine ussuriensis* Regel & Maack wild, *Glycine max* cultivated, and *Glycine gracilis* Skvortzow intermediate. *Glycine max* (L.) Merr. (Leguminosae), which is commonly grown throughout the world, belongs to the subgenus *Soja*.[1,2] Soybean cotyledons, hypocotyls, toasted defatted soyflakes, and leaves accumulate known isoflavones including daidzein,[3] glycitein,[3] genistein,[3] daidzin,[3] glycitin,[3] genistin,[3] formononetin,[4] isoformononetin,[5] 6,7,4′-trihydroxyisoflavone,[6] 6″-*O*-acetylgenistin,[7] 6″-*O*-acetyldaidzin,[7] glycitein 7-*O*-*β*-D-6″-*O*-acetylglucopyranoside,[8] 6″-*O*-malonylgenistin,[9] 6″-*O*-malonyldaidzin,[9] and 6″-*O*-malonylglycitin.[9] Numerous biological activities have been attributed to isoflavones including antioxidant,[9,10] antihemolytic,[10] antifungal,[11] antibacterial,[12] cancer chemoprotectant,[13] cardiovascular,[14] and estrogenic.[15] Soybean saponins identified so far include soyasapogenols and soyasaponins,[16−18] acetylsoyasaponins,[19] and group B saponins.[20] These soyasaponins possess antineoplastic,[13] antioxidant,[21] goitorogenic,[18] hemolytic,[20] or hypolipidemic[22] properties. Several antioxidant phenolic acids from soybeans include chlorogenic, isochlorogenic, caffeic, ferulic, *p*-coumaric, syringic, vanillic, and *p*-hydroxybenzoic acids.[23,24]

The increasing use of soybean products in foods has resulted in the accumulation of byproducts obtained during soybean processing. This study was undertaken to identify and recover potentially valuable new compounds from soybean extracts.

Results and Discussion

The water-soluble portion of soy molasses was concentrated and partitioned into EtOAc and *n*-BuOH. Chromatographic resolution of the EtOAc-soluble fraction furnished ferulic acid and 10 isoflavones (**1**−**10**), three of which (**1**−**3**) were new compounds. Chromatographic purification of the *n*-BuOH-soluble fraction furnished two new cinnamic acid glycoside esters (**11** and **12**) and one new (**13**) and two known soysaponins (**14** and **15**) (Chart 1).

Spectra (UV, IR, ^{1}H and ^{13}C NMR, and FABMS) measured for the isolated isoflavones of known structure were in good agreement with reported data for genistein (**4**), daidzein (**5**), glycitein (**6**), formononetin (**7**), genistin (**8**), daidzin (**9**), and glycitein 7-*O*-*β*-D-6″-*O*-acetylglucopyranoside (**10**).[3,4,8] Spectra (1D and 2D NMR, FABMS) for saponins **14** and **15** confirmed their structures as soysaponin I[17,25] 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl] soyasapogenol B (**14**) and soysaponin A2[18,26] 3-*O*-[*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-22-*O*-[*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl] soyasapogenol A (**15**).

Compound **1** gave *m*/*z* 573.1617 for $C_{28}H_{29}O_{13}$ by HR-FABMS and on TLC gave greenish brown and yellow with $FeCl_3$ and Pauly's reagents, respectively. Bands for hydroxyl and chelated carbonyl functional groups were suggested by IR spectroscopy, and the UV absorption at 278 nm was typical for an isoflavone and very similar to that of **7**. A bathochromic shift induced by NaOMe (+18 nm) indicated the presence of a free 4′-hydroxyl group,[27,28] which was also evident by a fragment ion at *m*/*z* 118 in the EIMS. No UV bathochromic shifts were observed with $AlCl_3$ and NaOAc, and this finding suggested the absence of free C-5 or C-7 hydroxyl groups in **1**.[27,28] ^{1}H and ^{13}C NMR resonances, assigned by 1D and 2D NMR techniques, were similar to those for **7**, except for the sugar moiety. The ^{1}H NMR spectrum of **1** contained a singlet for H-2 (*δ* 8.22), signals for six additional aromatic protons including a set of *ortho*-coupled (J = 8.7 Hz) doublets (*δ* 6.80 and 7.46, each 2H), a singlet for H-5 (*δ* 7.55), and a singlet (*δ* 7.38) for H-8. Signals observed for a 6-*O*-methyl group (*δ* 3.82), an *O*-linked glycosyl unit, and three acetyl methyl group singlets (*δ* 1.96, 2.07, and 2.11) all indicated that **1** was an acylated derivative of **6**. The acetyl moieties were attached to positions 2″, 4″, and 6″, based on ^{1}H NMR shifts of protons attached to these positions versus free glucose.[29] Cross-peaks between the glucosyl anomeric proton (*δ* 5.06) and H-8 in the NOESY spectrum of **1** indicated that glucose was linked at C-7. HMBC NMR cross-peaks between the glucosyl anomeric proton and the quaternary aromatic carbon at *δ* 152.04 confirmed the C-7 glucose linkage. Three-bond correlation of the 6-*O*-methyl group signal (*δ* 3.82) with C-6 (*δ* 146.78) confirmed the position of the methoxyl group. Thus, compound **1** was assigned as glycitein 7-*O*-*β*-D-(2″,4″,6″-*O*-triacetyl)glucopyranoside.

Compound **2** gave *m*/*z* 519.1874 for $C_{26}H_{31}O_{11}$ by HR-FABMS, and by TLC the spots gave positive $FeCl_3$ and Pauly's color tests, as for **1**. The identity of **2** as a

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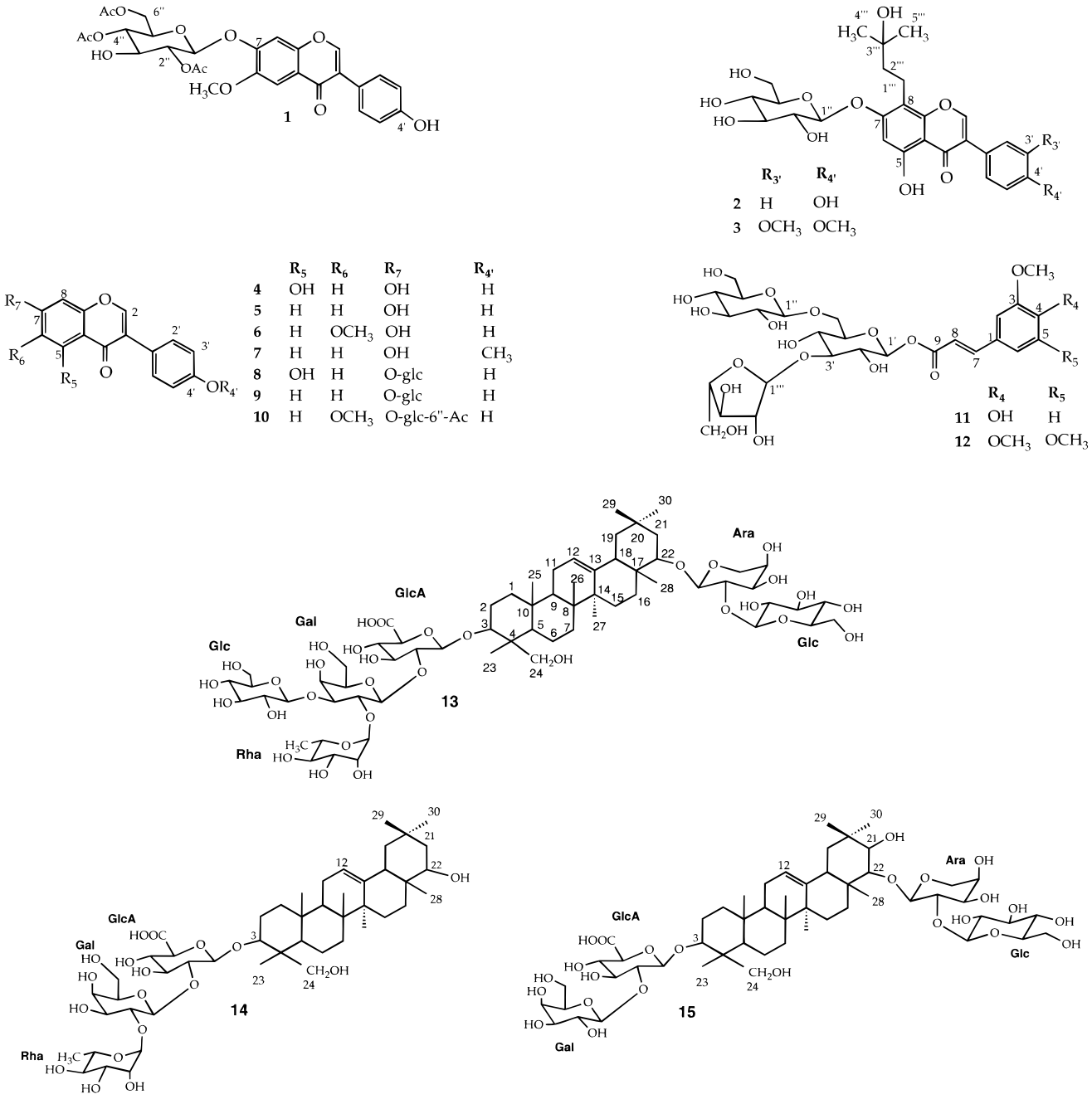
Chart 1



5-hydroxyisoflavone, with genistein-like B- and C-rings, was deduced from its IR (OH, chelated carbonyl) and UV spectra (λ_{max} 286 nm), with the latter shifting bathochromically with $AlCl_3$ ($\Delta\lambda = 8$ nm) and HCl ($\Delta\lambda = 15$ nm).[28] The UV λ_{max} in MeOH was unchanged with NaOAc, indicating a substitution of the HO-7 group. ^1H and ^{13}C NMR spectra were similar to those for genistin (**8**). Signals were observed for a γ-hydroxy-γ,γ-dimethylpropyl unit, including 2-H multiplets for H-1‴ (δ 2.76) and H-2‴ (δ 1.66) and methyl group singlets for H-4‴ (δ 1.25) and H-5‴ (δ 1.30). EIMS confirmed the location of the five-carbon unit on the isoflavone ring by a fragment at *m*/*z* 357 [aglycon].[30] Characteristic signals for C-7 and C-8 were absent in the ^1H NMR spectrum of **2**.[27,28] HMBC NMR correlations between H-2 (δ 8.40) and C-9 (δ 157.70), H-1‴ (δ 2.76) and C-9 (δ 157.70), H-1‴ (δ 2.76) and C-7 (δ 162.30), and H-1‴ (δ 2.76) and C-8 (δ 104.75) confirmed the linkage of the five-carbon unit to C-8. Moreover, correlations between H-1″ (δ 4.92) and C-7 (δ 162.30) confirmed that the β-glucosyl moiety was at the C-7 position. The structure of compound **2** was assigned as 8-(γ-hydroxy-γ,γ-dimethylpropyl)genistein 7-*O*-β-D-glucopyranoside.

Compound **3** gave *m*/*z* 563.2139 for $C_{28}H_{35}O_{12}$ by HR-FABMS and an IR spectrum similar to that of **2**. From their UV, ^1H, and ^{13}C NMR spectra, **2** and **3** were almost identical, except for the presence of two methoxyl signals at δ 3.89 (δ 55.92) and 3.80 (δ 55.98) in **3**. By EIMS the fragment at *m*/*z* 162 indicated that the two methoxyl groups were in ring B. Aromatic protons in ring B gave rise to an ABX pattern with H-6′ coupled to H-5′ ($J = 8.5$ Hz) and H-2′ ($J = 2.0$ Hz). In the HMBC NMR spectrum, the methoxyl proton signals were correlated with C-3′ (δ 147.91) and C-4′ (148.96). The ^{13}C NMR spectrum of the aglycon of **3** was comparable to that of formononetin[4] (**6**), except for the C-3′ signal, which was shifted downfield to

δ 147.91 ($\Delta\delta$ −34.51). These data permitted the identification of **3** as 5-hydroxy-8-(γ-hydroxy-γ,γ-dimethylpropyl)-3′,4′-dimethoxyisoflavone-7-*O*-β-D-glucopyranoside.

Compound **11** gave *m*/*z* 651.2115 for $C_{27}H_{39}O_{18}$ by HRFABMS. Acid hydrolysis of **11** gave glucose, arabinose, and ferulic acid as identified by TLC and GC−MS. ^{1}H NMR signals were evident for a *trans*-feruloyl moiety with olefinic protons at δ 6.54 and 7.65 coupled by 16.2 Hz. ^{1}H−^{1}H COSY NMR revealed two sets of *trans*-vicinal β-D-glucopyranosyl signals (H-1′/2′ and H-1″/2″), as well as an anomeric proton signal for H-1‴ of an α-L-arabinofuranosyl unit (br s, δ 4.96).[31] Significant downfield shifts of methine and methylene protons at positions H-3′ (δ 5.15) and H-6′ (δ 4.20 and 4.48) versus glucose suggested a 1′, 3′, and 6′ substituted glucose moiety. By ^{13}C NMR, C-3′ (δ 82.18) and C-6′ (δ 66.83) of the substituted glucose were observed at lower field than glucose itself, suggesting these as the sites of glycosidation. A NOE between H-6′ and H-1″ indicated a C-6′, C-1″ glucose linkage, and a NOE between H-3′ and H-1‴ showed an arabinose-to-glucose linkage as C-1‴, C-3′. HMBC NMR correlations of H-1′ (δ 5.0, d, J = 7.7 Hz) with the ferulic acid carbonyl signal (δ 167.10) and the HMQC NMR correlation between H-1′ (δ 5.0) and C-1′ (δ 100.15) proved the β-glycosidic ester linkage with ferulic acid. HMBC correlations between H-1″ and glucosyl C-6′ and between arabinosyl H-1‴ and glucosyl C-3′ confirmed the linkage points of the sugar moieties and the structure of compound **11** as 1-*O*-(*E*)-feruloyl[α-L-arabinofuranosyl-(1→3)][β-D-glucopyranosyl(1→6)]β-D-glucopyranose.

Compound **12** gave *m*/*z* 695.2373 for $C_{29}H_{43}O_{19}$ by HRFABMS and UV, IR, and NMR spectral data nearly identical to **11**, except for those of the cinnamoyl moiety. Compound **12** contained a trimethoxycinnamoyl moiety versus the feruloyl moiety in **11**. The large coupling constant (J_{AB} = 15.9 Hz) at δ 6.52 and 7.70 proved *E*-geometry of the double bond. Signals for three methoxyl groups were at δ 3.93 (6H) and δ 3.82, (3H). The more deshielded methoxyl-attached carbon C-5 was assigned a chemical shift of δ 155.15, and C-4, which is di-*ortho*-substituted by methoxyl groups, was considered to be more shielded at δ 141.57. The remaining signal was attributed to C-3 (δ 153.45). By use of a combination of COSY, HMQC, and HMBC NMR techniques, this compound was identified as 1-*O*-(*E*)-3,4,5-trimethoxycinnamoyl[α-L-arabinofuranosyl(1→3)-*O*-β-D-glucopyranosyl(1→6)]β-D-glucopyranose.

Saponin **13** gave *m*/*z* 1399.6976 for $C_{65}H_{107}O_{32}$ by HR-FABMS. The structure of **13** contained aglycon and sugar components similar to those found in the structures of known soysaponin B glycosides such as soysaponin I (**14**). For **13**, negative-ion FABMS indicated the loss of three hexoses, one deoxyhexose, one deoxypentose, and one glucuronic acid residue. Acid hydrolysis of **13** afforded glucose, galactose, arabinose, rhamnose, and glucuronic acid as the sugar components by TLC and GC−MS. The aglycon, soysapogenol B, gave *m*/*z* $C_{30}H_{50}O_3$ by EIMS as with **14**. ^{1}H and ^{13}C NMR spectra (Table 1) indicated that **13** was a hexasaccharide saponin with soysapogenol B as the aglycon.[16−23,32−34] The seven tertiary methyl groups were assigned by HMBC NMR spectroscopy, showing relationships between H-3α and Me-23 as well as between H-18β and Me-29, and by literature comparison.[16−23,32−34] Glycosidic substitutions for C-3 and C-22 were suggested by downfield shifts in the ^{13}C NMR spectrum for C-3 (δ 90.38) and C-22 (δ 88.65) relative to the corresponding signals in soyasapogenol B.[32] The ^{1}H NMR spectrum of **13** contained signals for six anomeric protons at δ 5.05, 5.29, 5.17, 5.13, 5.10, and 4.92 (Table 1). Signal multiplicities, chemical shifts, and coupling constants in the ^{1}H NMR spectrum indicated anomeric β-configurations for glucuronopyranosyl (J_{H1-H2} = 7.5 Hz), galactopyranosyl (J_{H1-H2} = 8.0 Hz), glucopyranosyl (J_{H1-H2} = 8.0 Hz), and glucopyranosyl (J_{H1-H2} = 7.9), and anomeric α-configurations for rhamnopyranosyl (J_{H1-H2} = 1.9 Hz) and arabinopyranosyl (J_{H1-H2} = 6.3 Hz) units. The COSY NMR spectrum showed connectivities between H-3α (δ 3.32 dd, J = 12.0, 4.5 Hz) and H-2 (δ 2.18 and 1.95). The 2D ^{1}H−^{1}H ROESY NMR spectrum showed connectivities between the H-3α (δ 3.32) and H-5α (δ 0.82) protons. Ambiguities in the assignments of the H-6, H-7, H-15, and H-16 methylene protons were resolved by observations of couplings between H-6β and Me-26 and between H-15β (axial) and Me-27. In the HMBC spectrum, C-27 was correlated with a single methylene, H-15, and with two quaternary carbons at C-8 and C-13. These latter two resonances permitted the recognition of Me-26, which also gave cross-peaks with H-7 and H-9. Methine H-9 also allowed for the identification of Me-26 and thus the location of quaternary carbon atoms at C-1, C-5, and C-10. The geminal methyl groups, Me-29 and Me-30, were easily distinguished because they shared three correlations: quaternary C-20 and methylenes C-19 and C-21. Compound **13** has the same sugar moieties as attached to ring E in soysaponin A2 (**15**) and a glucose moiety attached to ring A as in **14** (Table 1). The connectivities of sugar units in **13** were established by careful study of the COSY, ROESY, HMBC, and HMQC spectral results (Table 1). Similarly, the detection of all possible two- and three-bond, interresidue and intraresidue correlations of the anomeric protons confirmed unambiguously the glycosidic linkages and assignments for most ^{1}H and ^{13}C NMR resonances. Analysis of cross correlations by HMBC established the interglycosidic connectivities, showing correlations between H-1 (δ 5.05) of the β-D-glucuronic acid unit and C-3 of the aglycon (δ 90.38), H-1 (δ 5.10) of the α-L-arabinose unit and C-22 (δ 88.65) of the aglycon; C-2 (δ 79.12) of the β-D-glucuronic acid unit and H-1 (δ 5.29) of the β-D-galactose moiety; C-2 (δ 77.98) of the β-D-galactose and H-1 (δ 5.17) of the α-L-rhamnose; C-3 (δ 85.10) of the β-D-galactose and H-1 (δ 5.13) of the β-D-glucose unit; C-2 (δ 83.47) of the α-L-arabinose and H-1 (δ 4.92) of the β-D-glucose. The observed ROESY interaction between H-1 of β-D-glucuronic acid and H-3 of the aglycon and between H-1 of α-L-arabinose and H-22 of the aglycon indicated the attachments of the terminal sugar moieties to the aglycon. The sequence and linkage sites of the disaccharide units linked at C-22 were solved using a similar approach. Thus, the structure of **13** was established as 3-*O*-{[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)]β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl}-22-*O*-[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl] 3β,22β,24-trihydroxyolean-12-ene.

Experimental Section

General Experimental Procedures. Flash column liquid chromatography was performed using J. T. Baker glassware with 40-μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phases. TLC was carried out on precoated Si gel 60 F_{254} (Merck) plates; 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 the following solvent systems: A ($CHCl_3$−MeOH−H_2O, 80:20:2), B (EtOAc−MeOH−H_2O, 100:16.5:13.5), and C ($CHCl_3$−MeOH−H_2O, 61:32:7). Optical rotations were determined on an Autopol III automatic polarimeter (Rudolph Research Co., Flanders, NJ). UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were obtained using a

Table 1. 1D and Selected 2D NMR Chemical Shift Assignments of Aglycon and Sugar Moieties of Saponin **13** (CD_3OD, 600 MHz for ^1H and 90.56 MHz for ^{13}C NMR)

position	^1H (*J* in Hz)	^{13}C	DEPT	COSY	ROESY	HMBC
aglycon						
1α	1.07 m	38.63	CH_2	H-1*β*, 2*β*	H-1*β*, 9α	C-25
1*β*	1.76 dt (11.5, 4.5)			H-1α, 2α, 2*β*	H-11, Me-25	C-25
2α	2.18 dt (11.5, 4.5)	25.15	CH_2	H-1α, 1*β*, 2*β*, 3α	H-2*β*	*a*
2*β*	1.95 dq (4.5, 11.5)			H-1*β*, 2α, 3α	Me-25	
3α	3.32 dd (12.0, 4.5)	90.38	CH	H-2α, 2*β*	H-5α, Me-23, GlcA-1	C-1, 2, 4, 5, 23, 24, GlcA-1
4		42.80	C			
5α	0.82 d (12.0)	55.40	CH	H-5α, 6*β*, 7α, 7*β*	6α, 9α, Me-23	C-3, 23, 24, 25
6α	1.86 m	19.23	CH_2	H-6*β*, 7α, 7*β*	Me-23	C-4, 8, 10
6*β*	1.30 m			H-6α, 7*β*	H-7*β*, Me-26	C-5, 26
7α	1.57 dt (12.0, 3.0)	32.92	CH_2	H-6α, 6*β*, 7*β*	H-7*β*	C-5, 26
7*β*	1.24 d (12.0)			H-6α, 7α	H-7α,	C-8, 14, 26
8		40.30	C			
9α	1.42 dd (13.0, 8.1)	48.16	CH	H-12	H-1α, 11, Me-27	C-5, 7, 8, 10, 11, 12, 14, 25, 26
10		36.60	C			
11	1.82 m	23.85	CH_2	H-12	H-1*β*, 9α, Me-25	C-8, 9, 10, 13
12	5.27 br s	122.0	CH	H-9α, 11, 18*β*	H-19*β*	C-9, 14, 18
13		144.12	C			
14		42.09	C			
15α	1.68 m	26.55	CH_2	H-15*β*, 16*β*	H-15*β*, 16*β*	C-13, 17, 18, Me-27
15*β*	1.21 m			H-15α, 16*β*	H-15α, 16*β*, Me-26, 28	C-17, Me-26, Me-27
16α	1.95 m	29.35	CH_2	*a*	*a*	*a*
16*β*	1.80			15α, 15*β*	15α, 15*β*, Me-28	C-14, Me-28
17		37.14	C			
18*β*	2.11 t (13.0)	45.77	CH	H-19α, 19*β*	H-12, Me-28, 30	C-14, 20, Me-28, Me-29 or Me-30
19α	1.69[a]	46.80	CH_2	19*β*	19*β*, Me-29	C-13, 17, 18, 20, Me-29, Me-30
19*β*	1.05 m			H-19α, 21	H-19α, Me-28, 30	C-17, 18, 20, 21, 29 or 30
20		31.11	C			
21α	1.67 m	36.70	CH_2	H-21*β*, 22*β*	H-21*β*, 29	*a*
21*β*	1.45 m			H-21α, 22α	H-21α, 22α, 28	*a*
22α	4.20 m	88.65	CH	21α, 21*β*	H-18*β*, 21α, 21*β*, ara-1	C-16, 18, 20, 28, ara-1
23	0.95 s	23.10	CH_3		H-3α, 5α, 6α, 24	C-3. 4. 5. 24
24	4.38 d (3.6)	64.51	CH_2	H-24	H-5α, 6α, 23	C-3, 4, 5, 23
	4.52 d (3.6)					
25	1.10 s	15.60	CH_3		H-1*β*	C-1, 5, 10
26	0.98 s	16.85	CH_3		H-11, 6*β*, 15*β*	C-7, 8, 9, 10, 14
27	1.13 s	26.20	CH_3		H-9α	C-8, 13, 14, 15
28	0.88 s	22.02	CH_3		H-15*β*, 19*β*, 21*β*	C-16, 17, 18, 22
29	1.07 s	29.30	CH_3		H-19α, 21α	C-19, 20, 21, 30
30	0.90 s	27.00	CH_3		H-18*β*, 19*β*, 21*β*	C-19, 20, 21, 29
sugar moiety						
C-3						
glcA-1	5.05 d (7.5)	104.89	CH	glcA-2	H-3α, Me-23	C-3
glcA-2	4.61 t (7.5)	79.12	CH	glcA-3, Gal-1	gal-1	gal-1
gal-1	5.29 d (8.0)	101.45	CH	glcA-2	glcA-2	glcA-2
gal-2	4.69 dd (9.8, 8.0)	77.98	CH	rha-1	rha-1	rha-1
gal-3	4.83 dd (9.8, 3.5)	85.10	CH	glc-1	glc-1	glc-1
rha-1	5.17 d (1.9)	101.79	CH	gal-2	gal-2	gal-2
rha-6	1.32 d (6.2)	18.25	CH_3			
glc-1	5.13 d (8.0)	105.15	CH	gal-3	gal-3	gal-3
C-22						
ara-1	5.10 d (6.3)	107.20	CH	ara-2	H-22, Me-28	C-22
ara-2	4.76 dd (9.0, 3.5)	83.47	CH	glc-1	glc-1	glc-1
glc-1	4.92 d (7.9)	102.60	CH	ara-2	ara-2	ara-2

[a] Data could not be clearly resolved.

Nicolet 205 FT-IR spectrometer connected to a Hewlett−Packard ColorPro plotter. ^1H and ^{13}C NMR spectra were obtained with a Bruker NM 360 spectrometer operating at 360 MHz (for ^1H) and 90.56 MHz (for ^{13}C). All NMR spectra were obtained in CD_3OD and pyridine-d_5 using TMS as internal standard, with chemical shifts expressed in δ and coupling constants (*J*) in Hertz. COSY, NOESY, ROESY, HMBC, and HMQC NMR experiments were carried out using a Bruker AMX-600 highfield spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1. HR-FABMS 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.

Plant Material. Soy molasses, also known as soy solubles from the processing of *G. max* cultivated,[35] was obtained from Archer Daniels Midland, Inc., Decatur, IL.

Extraction and Isolation. Soy molasses (3 L) was concentrated in vacuo at 40 °C to near dryness (208 g). The concentrated extract was mixed with 100 mL H_2O and filtered through Celite 545 (Fisher Scientific). The filtrate and H_2O washings (400 mL) were combined and washed with petroleum ether (3 × 100 mL). The concentrated crude aqueous extract (32 g) was partitioned first with EtOAc (3 × 1L) and then with *n*-BuOH (3 × 1L). The EtOAc extract (9.2 g) was fractionated by Si gel flash column chromatography (2.5 × 62 cm) using, in sequence, CH_2Cl_2, CH_2Cl_2−EtOAc, EtOAc, and EtOAc−MeOH. Eight fractions were combined based on TLC using solvent systems A, B, and C. Subsequent purification of TLC−similar chromatographic fractions was accomplished by Sephadex LH-20 column chromatography using gradients of CH_3OH in CH_2Cl_2 ranging from 20 to 60% or *n*-hexane−CH_2Cl_2−MeOH, 4:5:1. Alternatively, fractions were resolved by reversed-

phase Sepralyte C_{18}, Si gel flash column chromatography (1.5 × 50 cm), using a H_2O−MeOH gradient solvent system (10→55%, 4 mL min). Final sample purification was carried out with Sephadex LH 20 column chromatography eluted with MeOH to afford compounds **1** (9 mg), **2** (12 mg), **3** (11 mg), **4** (61 mg), **5** (38 mg), **6** (19 mg), **7** (15 mg), **8** (18 mg), **9** (12 mg), and **10** (14 mg).

The *n*-BuOH extract was concentrated in vacuo at 40 °C, and the residue (8.45 g) was dissolved in MeOH−H_2O (1:1, 5 mL) and chromatographed over a polyamide column (3.5 × 90 cm) eluted with H_2O initially, with increasing concentrations of MeOH, while being monitored by TLC solvent system C. Fractions were grouped as A (H_2O, 875 mg), B (20% MeOH−H_2O, 423 mg), C (30% MeOH−H_2O, 145 mg), or D (50% MeOH−H_2O, 150 mg). Fraction A (875 mg) was rechromatographed over polyamide, and fractions eluting with 20 and 30% aqueous MeOH were combined separately for further Si gel flash column chromatography using ($CHCl_3$−MeOH−H_2O, 70:30:3→50:50:10) and repeated reversed-phase Sepralyte C_{18} flash column chromatography, using a H_2O−MeOH gradient solvent system (0→30%, 3 mL min) to afford compounds **13** (27 mg), **14** (19 mg), and **15** (25 mg), respectively. Fraction B was chromatographed again over a Si gel flash column (1.5 × 60 cm) using ($CHCl_3$−MeOH−H_2O 80:20:1→60:40:4) to yield two further fractions, B1 and B2. Fraction B1 (156 mg) was subjected to reversed-phase Sepralyte C_{18} flash column chromatography using a H_2O−MeOH gradient solvent system (10→30%, 4 mL min) to afford compound **11** (18 mg). Similarly, fraction C (145 mg) was subjected to reversed-phase column chromatography with the same solvent as for **11** and further purified by Sephadex LH-20 eluted with MeOH to afford compound **12** (14 mg).

Compound 1: obtained as a yellow, amorphous powder (9 mg); $[\alpha]^{20}_D$ +9.3° (*c* 0.55, pyridine−H_2O, 1:1); UV (MeOH) λ_{max} (log ϵ) 238 (4.12), 278 (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 (pyridine-d_5, 360 MHz) aglycon δ 8.22 (1H, s, H-2), 7.55 (1H, s, H-5), 7.38 (1H, s, H-8), 7.46 (2H, d, J = 8.7 Hz, H-2′,6′), 6.80 (2H, d, J = 8.7 Hz, H-3′,5′), 3.82 (3H, s, MeO-6), 8.51 (1H, s, HO-4′); glucosyl moiety δ 5.06 (1H, d, J = 8.1 Hz, H-1″), 4.88 (1H, dd, J = 8.1, 9.6 Hz, H-2″), 4.13 (1H, t, J = 9.6 Hz, H-3″), 4.94 (1H, t, J = 9.5 Hz, H-4″), 4.21 (1H, m, H-5″), 4.62 (1H, dd, J = 11.3, 2.5 Hz, H-6″A), 4.38 (1H, dd, J = 11.3, 6.0 Hz, H-6″B), 1.96 (3H, s, AcO-2″), 2.07 (3H, s, AcO-4″), 2.11 (3H, s, AcO-6″); ^{13}C NMR (pyridine-d_5, 90.56 MHz) aglycon δ 152.55 (C-2), 123.82 (C-3), 175.86 (C-4) 105.15 (C-5), 146.78 (C-6), 152.04 (C-7), 102.82 (C-8), 151.90 (C-9) 117.73 (C-10), 122.30 (C-1′), 130.00 (C-2′, 6′), 157.44 (C-4′), 115.58 (C-3′, 5′); glucosyl moiety δ 98.95 (C-1″), 76.76 (C-2″), 72.18 (C-3″), 72.92 (C-4″), 69.61 (C-5″), 64.97 (C-6″), 172.30, 20.18 (AcO-2″), 171.73, 20.69 (AcO-4″), 172.54, 20.80 (AcO-6″), 56.24 (MeO-6); HRFABMS, *m/z* 573.1617 $[M + H]^+$ (calcd for $C_{28}H_{29}O_{13}$, 573.1608).

Compound 2: obtained as a yellow, amorphous powder (12 mg); $[\alpha]^{20}_D$ +5.9° (*c* 0.75, pyridine−H_2O, 1:1); UV (MeOH) λ_{max} (log ϵ) 236 (4.09), 286 (4.28), + $AlCl_3$ 294, + HCl 301, + NaOAc 282 nm; IR (KBr) ν_{max} 3430 (OH), 1700 (C=O), 1590 (aromatic ring) cm^{-1}; ^1H NMR (pyridine-d_5, 360 MHz) aglycon δ 8.40 (1H, s, H-2), 6.55 (1H, s, H-6), 7.47 (2H, d, J = 8.6 Hz, H-2′,6′), 6.92 (2H, d, J = 8.6 Hz, H-3′,5′) 8.66 (1H, br s, HO-4′); 12.57 (1H, br s, HO-5) 2.76 (2H, m, H-1‴), 1.66 (2H, m, H-2‴), 1.25 (3H, s, H-4‴), 1.30 (3H, s, H-5‴); glucosyl moiety δ 4.92 (1H, d, J = 7.8 Hz, H-1″), 3.34 (1H, dd, J = 7.8, 9.6 Hz, H-2″), 3.60 (1H, t, J = 9.6 Hz, H-3″), 3.46 (1H, t, J = 9.6 Hz, H-4″), 3.38 (1H, m, H-5″), 3.62 (1H, dd, J = 12.5, 3.8 Hz, H-6″A), 3.80 (1H, dd, J = 12.5, 6.2 Hz, H-6″B); ^{13}C NMR (pyridine-d_5, 90.56 MHz) aglycon δ 154.62 (C-2), 122.90 (C-3), 179.47 (C-4) 162.49 (C-5), 98.67 (C-6), 162.30 (C-7), 104.75 (C-8), 157.70 (C-9) 106.49 (C-10), 43.16 (C-1‴), 17.89 (C-2‴), 67.82 (C-3‴), 30.15 (C-4‴), 28.74 (C-5‴), 122.00 (C-1′), 131.10 (C-2′, 6′), 57.76 (C-4′), 115.64 (C-3′, 5′); glucosyl moiety δ 100.76 (C-1″), 74.45 (C-2″), 76.82 (C-3″), 69.95 (C-4″), 77.68 (C-5″), 61.30 (C-6″); HRFABMS *m/z* 519.1874 $[M + H]^+$, 541 $[M + Na]^+$ (calcd for $C_{26}H_{31}O_{11}$, 519.1866).

Compound 3: obtained as a yellow, amorphous powder (11 mg); $[\alpha]^{20}_D$ +6.1° (*c* 0.54, pyridine−H_2O 1:1); UV (MeOH) λ_{max} (log ϵ) 234 (4.51), 282 (4.33), + NaOMe 296, + NaOAc 280, + NaOAc/ H_3BO_3 276 nm; IR (KBr) ν_{max} 3445 (OH), 1712 (C=O), 1593 (aromatic ring) cm^{-1}; ^1H NMR (pyridine-d_5, 360 MHz) aglycon δ 8.37 (1H, s, H-2), 6.59 (1H, s, H-6), 6.88 (1H, d, J = 2.0 Hz, H-2′), 6.95 (1H, d, J = 8.5 Hz, 5′), 7.63 (1H, dd, J = 2.0, 8.5), 3.89 (3H, s, MeO-3′), 3.80 (3H, s, MeO-4′), 12.55 (1H, br s, HO-5) 2.73 (2H, m, H-1‴), 1.60 (2H, m, H-2‴), 1.21 (3H, s, H-4‴), 1.26 (3H, s, H-5‴); glucosyl moiety δ 4.89 (1H, d, J = 7.9 Hz, H-1″), 3.28 (1H, dd, J = 7.9, 9.3 Hz, H-2″), 3.51 (1H, t, J = 9.3 Hz, H-3″), 3.40 (1H, t, J = 9.3 Hz, H-4″), 3.32 (1H, m, H-5″), 3.70 (1H, dd, J = 11.5, 3.5 Hz, H-6″A), 3.84 (1H, dd, J = 11.5, 6.5 Hz, H-6″B); ^{13}C NMR (pyridine-d_5, 90.56 MHz) aglycon δ 155.10 (C-2), 122.40 (C-3), 181.05 (C-4) 161.62 (C-5), 97.59 (C-6), 161.85 (C-7), 105.20 (C-8), 156.77 (C-9) 105.70 (C-10), 43.53 (C-1‴), 17.66 (C-2‴). 68.94 (C-3‴), 31.00 (C-4‴), 29.13 (C-5‴), 124.45 (C-1′), 111.83 (C-2′), 147.91 (C-3′), 148.96 (C-4′), 111.31 (C-5′), 120.52 (C-6′); glucosyl moiety δ 99.60 (C-1″), 73.49 (C-2″), 77.90 (C-3″), 70.95 (C-4″), 78.14 (C-5″), 62.10 (C-6″), 55.92 (MeO-3′), 55.98 (MeO-4′); HRFABMS, *m/z* 563.2139 $[M + H]^+$, 585 $[M + Na]^+$ (calcd for $C_{28}H_{35}O_{12}$, 563.2130).

Compounds **4**−**10** gave UV, IR, ^1H and ^{13}C NMR, and FABMS data for $[M + H]^+$ ions in good agreement with reported data for genistein (**4**) ($C_{15}H_{11}O_5$, $[M + H]^+$ 271),[3,36] daidzein (**5**) ($C_{15}H_{11}O_4$, $[M + H]^+$ 255),[3,36] glycitein (**6**) ($C_{16}H_{13}O_5$, $[M + H]^+$ 285),[3,36] formononetin (**7**) ($C_{16}H_{13}O_4$, $[M + H]^+$ 269),[4] genistin (**8**) ($C_{21}H_{21}O_{10}$, M^+ 433),[3,36] daidzin (**9**) ($C_{21}H_{21}O_9$, M^+ 417),[3,36] and glycitein 7-*O*-β-D-6″-*O*-acetylglucopyranoside (**10**) ($C_{24}H_{25}O_{11}$, M^+ 489).[8]

Compound 11: obtained as a white, amorphous powder (18 mg); $[\alpha]^{25}_D$ −23.8° (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.90), 275 (4.15) nm; IR (KBr) ν_{max} 3415 (OH), 1695 (C=O), 1590 (aromatic ring) cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) feruloyl δ 7.28 (1H, d, J = 2.0 Hz, H-2), 6.82 (1H, d, J = 8.3 Hz, H-5), 7.12 (1H, dd, J = 8.3, 2.0 Hz, H-6), 7.65 (1H, d, J = 16.2 Hz, H-7), 6.54 (1H, d, J = 16.2 Hz, H-8), 3.92 (3H, s, MeO-3); β-D-glucopyranosyl moieties δ 5.00 (1H, d, J = 7.7 Hz, H-1′), 3.62 (1H, dd, J = 9.5, 7.7 Hz, H-2′), 5.15 (1H, t, J = 9.5 Hz, H-3′), 3.57 (1H, dd, J = 9.5, 9.1 Hz, H-4′), 3.80 (1H, m, H-5′), 4.20 (1H, dd, J = 12.0, 2.5 Hz, H-6′A), 4.48 (1H, dd, J = 12.0, 6.0 Hz, H-6′B), 5.10 (1H, d, J = 7.8 Hz, H-1″), 3.32 (1H, dd, J = 9.5, 7.5 Hz, H-2″), 3.22 (1H, t, J = 9.5 Hz, H-3″), 3.35 (1H, t, J = 9.5 Hz, H-4″), 3.41 (1H, m, H-5″), 3.66 (1H, dd, J = 12.0, 3.5 Hz, H-6″A), 3.78 (1H, dd, J = 12.0, 5.8 Hz, H-6″B); α-L-arabinofuranosyl moiety δ 4.96 (1H, br s, H-1‴), 4.10 (1H, d, J = 3.0 Hz, H-2‴), 3.86 (1H, d, J = 5.5 Hz, H-3‴) 4.03 (1H, d, J = 5.7 Hz, H-4‴), 3.79 (1H, d, J = 5.5 Hz, H-5‴A), 3.87 (1H, dd, J = 11.4, 5.5 Hz, H-5‴B); ^{13}C NMR (CD_3OD, 90.56 MHz) feruloyl δ 127.56 (C-1), 111.73 (C-2), 148.51 (C-3), 149.32 (C-4), 115.74 (C-5), 124.38 (C-6), 147.20 (C-7), 114.84 (C-8), 167.10 (C-9), 56.27 (MeO-3); β-D-glucopyranosyl moieties δ 100.15 (C-1′), 76.83 (C-2′), 82.18 (C-3′), 69.05 (C-4′), 75.42 (C-5′), 66.83 (C-6′), 102.41 (C-1″), 75.44 (C-2″), 77.03 (C-3″), 71.15 (C-4″), 78.10 (C-5″), 62.45 (C-6″); α-L-arabinofuranosyl moiety δ 107.41 (C-1‴), 82.50 (C-2‴), 77.10 (C-3‴), 82.86 (C-4‴), 63.65 (C-5′); HRFABMS *m/z* 651.2115 $[M + H]^+$, 673 $[M + Na]^+$ (calcd for $C_{27}H_{39}O_{18}$, 651.2109).

Compound 12: obtained as a white, amorphous powder (14 mg); $[\alpha]^{25}_D$ −19.7° (*c* 0.87, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (4.18), 283 (4.36) nm; IR (KBr) ν_{max} 3420 (OH), 1710 (C=O), 1595 (aromatic ring) cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) 3,4,5-trimethoxycinnamoyl δ 6.91 (2H, br s, H-2, 6), 7.70 (1H, d, J = 15.9 Hz, H-7), 6.52 (1H, d, J = 15.9 Hz, H-8), 3.93 (6H, s, MeO-3, 5), 3.82 (3H, s, MeO-4); β-D-glucopyranosyl moieties δ 5.12 (1H, d, J = 8.0 Hz, H-1′), 3.51 (1H, dd, J = 9.2, 8.0 Hz, H-2′), 4.93 (1H, t, J = 9.2 Hz, H-3′), 3.48 (1H, dd, J = 9.2, 9.2 Hz, H-4′), 3.83 (1H, m, H-5′), 4.27 (1H, dd, J = 11.9, 2.6 Hz, H-6′A), 4.41 (1H, dd, J = 11.9, 6.5 Hz, H-6′B), 4.98 (1H, d, J = 7.9 Hz, H-1″), 3.29 (1H, dd, J = 9.0, 7.9 Hz, H-2″), 3.50 (1H, t, J = 9.0 Hz, H-3″), 3.38 (1H, t, J = 9.0 Hz, H-4″), 3.60 (1H, m, H-5″), 3.70 (1H, dd, J = 12.0, 3.6 Hz, H-6″A), 3.89 (1H, dd, J = 12.0, 6.5 Hz, H-6″B); α-L-arabinofuranosyl moiety δ 5.18 (1H, d, J = 1.6 Hz, H-1‴), 4.08 (1H, d, J = 2.7 Hz, H-2‴), 3.90 (1H, d, J = 5.2 Hz, H-3‴) 4.13 (1H, d, J = 5.5 Hz, H-4‴), 3.72

(1H, d, J = 6.0 Hz, H-5‴A), 3.95 (1H, dd, J = 12.0, 6.0 Hz, H-5‴B); ^{13}C NMR (CD_3OD, 90.56 MHz) 3,4,5-trimethoxycinnamoyl δ 129.92 (C-1), 107.48 (C-2, C-6), 157.45 (C-3 or C-5), 141.57 (C-4), 155.15 (C-5 or C-3), 147.10 (C-7), 117.00 (C-8) 168.73 (C-9), 56.40 (MeO-3), 57.10 (MeO-4), 57.55 (MeO-5); β-D-glucopyranosyl moieties δ 100.65 (C-1′), 75.81 (C-2′), 81.72 (C-3′), 68.70 (C-4′), 75.19 (C-5′), 67.34 (C-6′), 102.95 (C-1″), 75.20 (C-2″), 78.10 (C-3″), 71.28 (C-4″), 78.32 (C-5″), 62.33 (C-6″); α-L-arabinofuranosyl moiety δ 106.70 (C-1‴), 82.61 (C-2‴), 77.28 (C-3‴), 83.23 (C-4‴), 63.50 (C-5′; HRFABMS m/z 695.2373 $[M + H]^+$, 717 $[M + Na]^+$ (calcd for $C_{29}H_{43}O_{19}$, 695.2371).

Compound 13: obtained as a white, amorphous powder (27 mg); $[\alpha]^{25}_D$ −16.36° (c 0.51, MeOH); IR (KBr) ν_{max} 3475 (OH), 1635 (C═C) cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); negative-ion FABMS m/z 1235 [M − H − hexose]$^-$, 1103 [M − H − (hexose + pentose)]$^-$, 1251 [M − H − deoxyhexose]$^-$, 1089 [M − H − (deoxy − hexose + hexose)]$^-$, 927 [M − H − (deoxyhexose + 2 × hexose)]$^-$, and 751 [M − H − (deoxyhexose + 2 × hexose + glucuronic acid)]$^-$; HRFABMS m/z 1399.6976 $[M + H]^+$, 1421 $[M + Na]^+$ (calcd for $C_{65}H_{109}O_{32}$, 1399.6953).

Compound 14: obtained as a white amorphous powder (19 mg); IR (KBr) 3475 (OH), 1638 (C═C) cm^{-1}; FABMS, m/z 943 $[M + H]^+$ 965 $[M + Na]^+$ (calcd for $C_{48}H_{79}O_{18}$, 943). It was identified by literature comparison as soysaponin I.[17,25]

Compound 15: obtained as a white amorphous powder (25 mg); IR (KBr) 3455 (OH), 1630 (C═C) cm^{-1}; FABMS m/z 1107 $[M + H]^+$ 1129 $[M + Na]^+$ (calcd for $C_{53}H_{87}O_{24}$, 1107); identified by literature comparison as soysaponin A2.[18,25]

Acid Hydrolysis of Compounds 11 and 13. A 5-mg quantity of each compound was refluxed with 2 M HCl in MeOH (3 mL) for 4 h. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (10 mL) was extracted with Et_2O (3 × 20 mL). The ether extracts were evaporated to afford the aglycons, which were identified as ferulic acid from **11** (by TLC) and soysapogenol B from **12** (by EIMS).[32−34] The aqueous layer was neutralized with 2N KOH solution and concentrated to 5 mL under reduced pressure. The concentrate was divided into two portions, one of which was compared with standard sugars by TLC ($CHCl_3$−MeOH−H_2O, 30:12:4, 9 mL of lower layer and 1 mL of HOAc).[37] From **11**, the sugars were glucose (R_f 0.49) and arabinose (R_f 0.67). From **13** the sugars were glucuronic acid (R_f 0.12), galactose (R_f 0.43), glucose (R_f 0.49), rhamnose (R_f 0.71), and arabinose (R_f 0.67). The second portion was reacted with $NaBH_4$ (5 mg) at room temperature for 2 h. After addition of glacial HOAc to eliminate excess $NaBH_4$, the mixture was concentrated to dryness and HOAc removed azeotropically with MeOH (3 × 5 mL). The resulting residue was acetylated by refluxing for 10 h with Ac_2O−pyridine (1:1, 2 mL). After that time, 5 mL of H_2O was added, and the acetylated derivatives were extracted with $CHCl_3$ and identified by GC−MS[38] as peracetylarabinose (t_R 14.7 min, m/z 259) and peracetylglucose (t_R 20.2 min, m/z 390) in **11** and peracetylrhamnose (t_R 11.6 min, m/z 332), peracetylarabinose (t_R 14.7 min, m/z 259), peracetylglucose (t_R 20.1 min, m/z 390), and peracetylgalactose (t_R 23.5 min, m/z 390) in **13**.

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