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Soyasaponins from Soybean Flour Medium for the Liquid Culture of *Ganoderma applanatum*

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Two new unusual soyasaponins named 6"-*O*-methyldehydrosoyasaponin I (**7**) and desglucosylsoyasaponin A_1 (**10**) along with eight known saponins, dehydrosoyasaponin IV (**1**), dehydrosoyasaponin III (= impatienoside A) (**2**), soyasaponin III (**3**), dehydrosoyasaponin II (= soyasaponin Bg) (**4**), soyasaponin II (**5**), dehydrosoyasaponin I (= soyasaponin Be) (**6**), soyasaponin I (**8**), and kudzusaponin SA_3 (**9**), were isolated as their methyl esters and identified from the liquid culture of *G. applanatum*. Their structures were determined by chemical and spectroscopic analyses including 1D- and 2D-NMR as well as by comparison of their spectroscopic data with those of the reported in literatures. Although dehydrosoyasaponin IV was identified by LC-MS/MS method from soy protein isolate, this is the first report of the isolation of this compound. Dehydrosoyasaponin III (**2**) and kudzusaponin SA_3 (**9**) were also isolated for the first time from soybean. The presence of soyasaponins in *Ganoderma* species seems to be unusual feature. Thus, we presumed that compounds **1**-**10** might all be derived from the defatted soybean flour which was added to the culture medium as a nitrogen source.

Key Words : *Ganoderma applanatum*, Soyasaponins, Liquid culture, Soybean flour

Introduction

As part of a program to study the chemical diversity of the medicinal fungus and its biological effects, we investigated cultures of the basidiomycete *Ganoderma applanatum*.[1] In this paper, we describe the isolation and structure elucidation of two new unusual soyasaponins, named 6"-*O*-methyldehydrosoyasaponin I (**7**) and desglucosylsoyasaponin A_1 (**10**) along with 8 known ones (**1**-**6**, **8** and **9**), as their methyl esters from a scaled-up liquid culture of the basidiomycete *G. applanatum*. Soyasaponins have been shown to have many biological activities including chemoprotective, hypocholesterolemic, immunostimulatory, antiviral, and anticarcinogenic activities.[2,3] The isolation of soyasaponins was limited to soy and other leguminous plants[2,3] and *Impatiens siculifer*[4] (Balsaminaceae) to date. Therefore, the presence of soyasaponins in the liquid cultured mycelia of *G. applanatum* seems to be an unusual feature. Thus, we presumed that all the soyasaponins might be derived from the defatted soybean flour which was added to the culture medium as a nitrogen source.

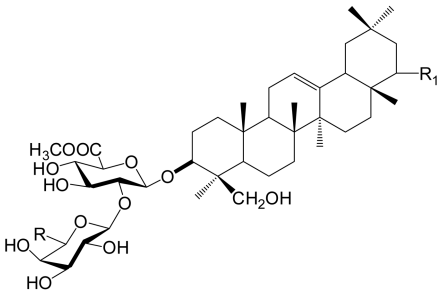
Experimental Section

General Procedures. Optical rotations were obtained with a JASCO P-1020 polarimeter. IR spectra were obtained with a JASCO FT/IR-300E spectrometer and UV spectra were recorded on a Hitachi U-3010. EI and FAB mass spectra were obtained with a JEOL JMS-700 spectrometer. NMR spectra were measured with a Bruker AVANCE 400 (400 MHz), a Bruker AVANCE 500 (500 MHz) or a Bruker AVANCE 600 (600 MHz) spectrometer using the solvent signal as an internal reference. TLC was carried out on silica gel 60 F_{254} (Merck, art. no. 5715), C18 (Merck, art. no. 5685) and cellulose (Merck, art. no. 5716) plates. Spots on the plates were visualized by spraying silica gel and cellulose plates with 20% H_2SO_4 in H_2O (v/v) and aniline phthalate, respectively, followed by heating.

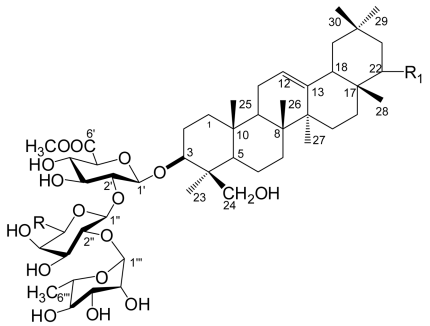
Organism. The fruiting bodies of *G. applanatum* were provided by St. Clair Milk and Grocery (Niagara Falls, Canada) in March 2002. The botanical identification was made by Mr. Gregory J. Belmore (Ministry of Natural Resources, Ontario, Canada). A voucher specimen (No. 2002-02) was deposited in the herbarium of our institute. The producing organism was isolated from the fruiting bodies and was maintained on potato dextrose agar medium.

Fermentation. The lyophilized culture broth was donated by MIRACLE D.K.C. LIFE Inc. For fermentation, the fungal strain was grown in a fermenter containing 200 L of medium consisting of 2 kg of soybean flour (Yoosung Food Co., Ltd.), 0.6 kg of starch, 3 kg of sucrose, and 50 mL of antifoam (Dow Corning Korea Ltd., LS 303). As an inoculum, a well-grown culture in the same medium was used. Fermentation was carried out at 22-25 °C with aeration and agitation. After 7 days, the culture was harvested. The culture broth was lyophilized to yield a white amorphous powder (2.25 kg), which was then extracted six times with MeOH at room temperature to yield a MeOH extract (638 g).

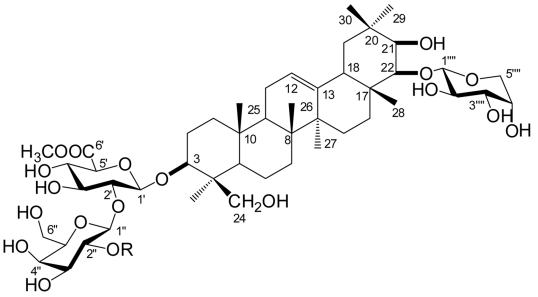
Extraction and Isolation. The MeOH extract (638 g) was suspended in water and successively partitioned with *n*-



1 Dehydrosoyasaponin IV methyl ester R = H, R_1 = O
2 Dehydrosoyasaponin III methyl ester R = CH_2OH, R_1 = O
3 Soyasaponin III methyl ester R = CH_2OH, R_1 = β-OH



4 Dehydrosoyasaponin II (soyasaponin Bg) methyl ester R = H, R_1 = O
5 Soyasaponin II methyl ester R = H, R_1 = β-OH
6 Dehydrosoyasaponin I (soybean saponin Be) methyl ester R = CH_2OH, R_1 = O
7 6"-*O*-Methyldehydrosoyasaponin I methyl ester R = CH_2OCH_3, R_1 = O
8 Soyasaponin I methyl ester R = CH_2OH, R_1 = β-OH



9 Kudzusaponin SA_3 methyl ester R = Rhamnose
10 Desglucosylsoyasaponin A_1 methyl ester R = Glucose

Figure 1. Structural formulas of soyasaponins (**1**-**10**)

hexane, dichloromethane, and BuOH to yield 32.9, 7.6, and 80.5 g fractions, respectively. A portion (80.0 g) of the *n*-BuOH fraction was separated by silica gel column chromatography with CH_2Cl_2:MeOH:H_2O [7:1:0.5 (500 mL) → 7:1.5:0.5 (3 L) → 7:1.8:0.5 (2 L) → 7:2:0.5 (1 L) → 7:2.5:0.5 (2 L)] and divided into subfractions B and D. Subfraction B was separated by silica gel column chromatography with CH_2Cl_2:MeOH:H_2O (7:1.5:0.5, 2 L), (7:1.8:0.5, 2 L), and (7:2:0.5, 2 L) to yield subfractions B1-B20. Fraction B20 was chromatographed on an RP-18 column with MeOH:H_2O (3:1, 2 L) to yield fraction B20-8, which was rich in saponin; fraction B20-8 was then methylated with CH_2N_2 to yield methylated saponin fraction MB20-8. Fraction B20-12 was treated the same as fraction B20-8 to give fraction MB20-12. Both methylated saponin fractions MB20-8 and MB20-12 were purified by crystallization to yield compounds **1** (4 mg) and **2** (4 mg), respectively. Fractions B20-16, 23, and 26 were separately methylated with CH_2N_2 to give fractions MB20-16, 23, and 26, respectively. Fraction MB20-16 and MB20-23 were separately chromatographed on a silica gel column with $CHCl_3$:MeOH:H_2O (7:1:0.5, 4 L) to afford compounds **3** (1 mg) and **4** (19 mg) from MB20-16 and compounds **5** (8 mg), **6** (12 mg) and **7** (2 mg) from MB20-23, respectively. MB20-26 was chromatographed on a silica gel column with $CHCl_3$:MeOH:H_2O (7:1.5:0.5, 3.4 L) to yield compound **8** (5 mg). Subfraction D was methylated with CH_2N_2 and then subjected to column chromatography on RP-18 eluted with MeOH:H_2O (2:1, 5 L) to give subfractions D1 and D2, which were separately subjected to column chromatography on Sephadex LH-20 eluted with MeOH to give compounds **9** (5 mg) and **10** (10 mg), respectively.

6"-*O*-Methyldehydrosoyasaponin I methyl ester (**7**): $[\alpha]_D^{23}$ –25.5° (c 0.5, MeOH); (+)-FAB-MS *m/z* 991 $[M + Na]^+$, 969 $[M + H]^+$, 845 $[(M + Na) - 146]^+$, 669 $[(M + Na) - 146 - 176]^+$; HR-FAB-MS *m/z* 991.5217 $[M + Na]^+$ (calcd. $C_{50}H_{80}O_{18}Na$ 991.5242).

Desglucosylsoyasaponin A_1 methyl ester (**10**): $[\alpha]_D^{26}$ +25.8° (c 1.0, MeOH); (+)-FAB-MS *m/z* 1143 $[M + Na]^+$; HR-FAB-MS *m/z* 1143.5536 $[M + Na]^+$ (calcd. $C_{54}H_{88}O_{24}Na$ 1143.5563).

Acid Hydrolysis. Compound **10** (3 mg) was refluxed with 5% HCl in 60% aqueous dioxane (10 mL) for 2 h. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The ether extract was evaporated to yield the aglycon, soyasapogenol A, which was identified by direct comparison with authentic sample. The H_2O layer was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars using cellulose TLC [pyridine-EtOAc-HOAc-H_2O (36:36:7:21)], and the sugars were identified as arabinose, glucose, galactose and glucuronic acid. Compound **7** was treated in the same manner as described above and identified soyasapogenol E as an aglycon and rhamnose, 6-*O*-methylgalactose and glucuronic acid as sugars.

Determination of the Absolute Configuration of Sugars. The absolute configuration of glucuronic acid was determined after $NaBH_4$ reduction as described in the literature.[9] To a solution of sugar mixture (1 mg) in MeOH was added $NaBH_4$, and the mixture was kept at room temperature for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was treated as above, and the dried sugar mixture was dissolved in pyridine (0.1 mL), and then the solution was added to a pyridine solution (0.1 mL) of L-cysteine methyl ester hydrochloride (2 mg) and warmed at 60 °C for 1 h. The solvent was evaporated under a N_2 stream and dried *in vacuo*. The residue was trimethyl-

Table 1. ^{1}H-NMR data of **7** and **10** in pyridine-d_5

No.	**7**[a]	**10**[b]	No.	**7**[a]	**10**[b]
3	3.39 (dd, 4.0, 12.0)	3.36 (t, 9.5)	1"	5.70 (d, 7.5)	5.68 (d, 7.6)
12	5.23 (t-like)	5.32 (br s)	2"	4.47 (t, 9.0)	4.58 (t, 8.0)
18	2.36 (dd, 3.9, 13.5)	2.49 (dd, 3.2, 13.3)	3"	4.04 (dd, 2.7, 9.6)	4.21 (dd, 2.8, 9.0)
21	2.12 (br d, 14.0)	3.93*	4"	4.15 (br d, 2.7)	4.49 (d, 2.8)
	2.56 (d, 14.0)		5"	3.85 (t, 6.0)	3.94*
22	–	3.80 (d, 2.8)	6"	3.77 (dd, 2.9, 9.5)	4.37 (dd, 5.1, 11.6)
23	1.41 (s)	1.41 (s)		3.97 (dd, 6.3, 9.5)	4.44 (dd, 5.6, 11.6)
24	3.26 (d, 11.3)	3.37 (d, 11.4)	OCH_3	3.29 (s)	
	4.30 (d, 11.3)	4.29 (d, 11.4)			
25	0.74 (s)	0.69 (s)	1"'	6.23 (br s)	5.20 (d, 7.7)
26	0.86 (s)	0.90 (s)	2"'	4.76 (br d, 3.4)	4.12 (t, 9.2)
27	1.27 (s)	1.27 (s)	3"'	4.62 (dd, 3.4, 9.3)	4.21 (t, 9.0)
28	1.15 (s)	1.40 (s)	4"'	4.30 (t, 9.2)	4.07 (t, 9.0)
29	0.94 (s)	1.26 (s)	5"'	4.95*	3.95*
30	0.83 (s)	1.45 (s)	6"'	1.75 (d, 6.2)	4.24*
					4.61*
1'	4.96 (d, 8.0)	5.08 (d, 7.8)			
2'	4.51 (t, 9.1)	4.25 (t, 7.9)	1""		4.92 (d, 7.6)
3'	4.53 (t, 9.1)	4.71 (t, 9.2)	2""		4.54 (t, 8.6)
4'	4.34 (t, 9.1)	4.41 (t, 9.8)	3""		4.13 (br d, 9.0)
5'	4.51 (d, 9.3)	4.61 (d, 9.7)	4""		4.23 (m)
6'	–	–	5""		3.73 (br d, 10.9)
$COOCH_3$	3.72 (s)	3.74 (s)			4.32 (d, 10.9)

*Overlapping signal. [a]400 MHz. [b]600 MHz

silylated with TMS-HT (0.5 mL) at 60 °C for 30 min. After the addition of hexane and water, the hexane layer was removed and checked by GC. The retention times (t_R) of the peaks were 18.24 min (L-arabinose), 23.73 min (L-rhamnose), 37.87 min (D-glucose), and 38.23 min (D-galactose).

Results and Discussion

The lyophilized powder of the culture broth of *G. applanatum* was extracted and partitioned as described in the Experimental section. Methylation of the *n*-BuOH fraction with CH_2N_2, followed by successive chromatography using silica, Sephadex LH-20, and reverse-phase C_{18} afforded compounds **1-10** as their methyl esters. The structures of these isolated compounds were established through analysis of their NMR and FABMS spectra, which enabled the characterization of compounds **7** and **10** as novel and compounds **1-6**, **8** and **9** as known. Spectra for known saponins confirmed their structures to be dehydrosoyasaponin IV methyl ester (**1**),[4] dehydrosoyasaponin III (impatienoside A) methyl ester (**2**),[4] soyasaponin III methyl ester (**3**),[5] dehydrosoyasaponin II (soyasaponin Bg) methyl ester (**4**),[4] soyasaponin II methyl ester (**5**),[6] dehydrosoyasaponin I (soybean saponin Be) methyl ester (**6**),[7,8] soyasaponin I methyl ester (**8**),[7,8] and kudzusaponin SA_3 methyl ester (**9**).[9,10]

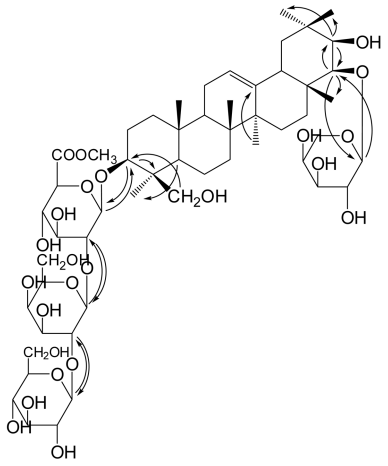
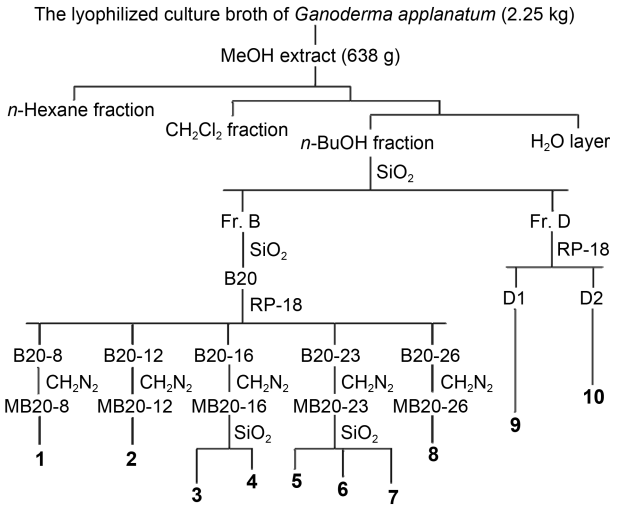


Figure 2. Key HMBC correlations of desglucosylsoyasaponin A_1 methyl ester (**10**).

6"-*O*-Methyldehydrosoyasaponin I methyl ester (**7**) was shown to have the molecular formula $C_{50}H_{80}O_{18}$ on the basis of the (+)-HRFABMS, indicating a molecular weight of 968, 14 mass units higher than that of **6**. Acid hydrolysis of **7** afforded rhamnose, glucuronic acid and 6-*O*-methylgalactose as the sugar components identified on TLC analysis by comparison with authentic samples. Absolute configurations for sugars were determined to be the D-form except for rhamnose (L-form), according to the our previous procedure.[9] The ^{1}H and ^{13}C NMR spectra of **7** suggested the presence of a triterpenoid aglycon bonded to a trisaccharide chain, which were similar to those of **6**, with a major difference due to the presence of one methoxyl proton and carbon signals at δ 3.29 and 59.1, respectively. The positive

ion mode FABMS of **7**, *m/z* 991 $[M + Na]^+$, 969 $[M + H]^+$, 845 $[(M + Na) - 146]^+$, and 669 $[(M + Na) - 146 - 176]^+$, along with the presence of a methoxyl singlet at δ 3.29 and 59.1 in the ^1H and ^{13}C NMR spectra, respectively, indicated that **7** contained a hexosyl residue containing one methoxyl group (176 mass unit).[11] The ring protons of the *O*-methyl hexose residue were assigned, starting from the readily identifiable anomeric proton, by means of COSY, HMQC, and HMBC experiments. The chemical shifts and the splitting patterns of the hexosyl residue containing one methoxyl group were close similar to those of the galactopyranose moiety in **6**. The upfield shift of H-6" to δ 3.77 (1H, dd, J = 2.9, 9.5 Hz) and 3.97 (1H, dd, J = 6.3, 9.5 Hz) [δ 4.32 (1H, d, J = 11.3 Hz) and 4.43 (1H, d, J = 11.3 Hz) in the galactopyranosyl signals in **6**] suggested the location of the methoxy group at C-6" of the galactose residue.[11] A triplet at δ 3.85 (1H, t, J = 6.0 Hz, H-5") was correlated with a pair of double doublets at δ 3.77 (1H, dd, J = 2.9, 9.5 Hz, H-6"a) and 3.97 (1H, dd, J = 6.3, 9.5 Hz, H-6"b) in the ^1H-^1H COSY spectrum, which were unambiguously correlated by HMQC experiment to the corresponding carbon resonances at δ 74.2 (C-5") and 71.5 (C-6"), respectively. Comparison of the ^{13}C NMR data of the galactose moiety of **7** with those of **6** showed that the signals for C-5" and C-6" of **7** were significantly shifted upfield by –2.4 and downfield by +9.9 ppm, respectively, due to a methylation shift.[12] In the HMBC spectrum, the cross-peaks between H-1" (δ 5.70, d, J = 7.5 Hz) and C-2' (δ 78.2) of inner glucuronic acid, H-1"' (δ 6.23, br s) of terminal rhamnose and C-2" (δ 77.7) of 6-*O*-methyl-galactose, and OCH_3 (δ 3.29) and C-6" of 6-*O*-methyl-galactose (δ 71.5) allowed us to establish α-L-rhamnopyranosyl(1→2)-6-*O*-methyl-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl soyasapogenol E. The long-range correlation between the C-3 resonance at δ 91.1 and the anomeric proton signal of the glucuronosyl unit at δ 4.96 (d, J = 8.0 Hz) confirmed the attachment of the β-glucuronosyl moiety at C-3 of the aglycon. Consequently, the structure of **7** was established as 3-*O*-α-L-rhamnopyranosyl(1→2)-6-*O*-methyl-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl soyasapogenol E. It should be noted that the limited number of triterpenoid saponins carrying *O*-methyl sugar(s) have been reported from higher plants such as *Rosa laevigata*[13] and *Rubus pungens* var. *oldhemii*[14] but are common from marine organisms.[15]



Scheme 1. Fractionation and isolation of compounds **1-10** from the MeOH extract of the lyophilized culture broth of *G. applanatum*.

Desglucosylsoyasaponin A_1 methyl ester (**10**) was obtained as an amorphous powder. High resolution FABMS gave the composition $C_{54}H_{88}O_{24}$. Acid hydrolysis of **10** gave sugars identified as D-glucose, L-arabinose, D-glucuronic acid, and D-galactose as described in **7**. An inspection of the ^1H and ^{13}C NMR spectra of the compound readily indicated the presence of four monosaccharide units through easily identifiable signals for anomeric protons and carbons. The FAB mass spectrum of **10** exhibited an $[M + H]^+$ ion at *m/z* 1143, which is consistent with a tetrasaccharide glycoside carrying two hexoses, one arabinose, one glucuronic acid methyl ester, and an aglycon, soyasapogenol A, with a molecular mass of 474. Comparison of the ^{13}C NMR data of the aglycon moiety of **10** with soyasapogenol A[10] showed that the signals for C-3 and C-22 of soyasapogenol A were significantly shifted downfield by +10.7 and +13.2 ppm,

Table 2. ^{13}C-NMR data of **6**, **7**, **9**, and **10** in pyridine-d_5

No.	**6**[a]	**7**[b]	**9**[c]	**10**[c]	No.	**6**[a]	**7**[b]	**9**[c]	**10**[c]
1	38.6	38.7	38.6	38.5	1'	105.7	105.5	105.5	104.9
2	26.7	26.6	26.7	26.5	2'	78.4	78.2	78.4	80.3
3	91.3	91.1	91.3	90.8	3'	76.5	76.4	76.4	77.3
4	43.9	43.9	43.9	43.9	4'	73.7	73.7	73.7	72.4
5	56.1	56.1	56.0	56.0	5'	77.0	77.0	77.0	77.1
6	18.5	18.5	18.5	18.6	6'	170.4	170.3	170.4	170.4
7	33.0	33.0	32.8	32.8	OCH_3	52.1	52.1	52.2	52.1
8	39.8	39.8	40.2	40.1					
9	47.7	47.7	47.8	47.7	1"	101.9	101.6	101.8	102.8
10	36.5	36.5	36.4	36.4	2"	77.8	77.7	77.8	84.8
11	24.0	24.0	24.1	24.1	3"	76.7	76.4	76.7	74.7
12	124.1	124.4	122.5	122.5	4"	71.2	70.9	71.2	70.5
13	141.9	141.8	144.4	144.4	5"	76.6	74.2	76.6	76.6
14	42.0	42.0	41.8	41.8	6"	61.6	71.5	61.6	62.5
15	25.4	25.4	26.7	26.6	OCH_3		59.1		
16	27.4	27.3	27.8	27.8					
17	47.8	47.8	39.3	39.3	1"'	102.7	102.4	102.6	107.1
18	47.9	47.9	44.4	44.4	2"'	72.4	72.4	72.5	76.8
19	46.7	46.6	47.2	47.2	3"'	72.9	72.8	72.9	78.1
20	34.1	34.1	36.4	36.9	4"'	74.4	74.4	74.4	71.7
21	51.0	50.9	75.8	75.9	5"'	69.5	69.4	69.5	79.2
22	215.6	215.5	92.8	92.8	6"'	19.0	19.0	19.1	63.0
23	23.1	23.0	23.0	22.8					
24	63.6	63.8	63.6	63.6	1""			108.8	108.8
25	15.8	15.8	15.8	15.7	2""			73.9	73.9
26	16.7	16.7	16.7	16.7	3""			75.5	75.5
27	25.5	25.4	26.7	26.7	4""			70.0	70.0
28	21.0	20.9	23.2	23.2	5""			67.8	67.8
29	31.9	31.8	31.6	31.6					
30	25.3	25.2	21.4	21.4					

[a]125.8 MHz. [b]100 MHz. [c]150.9 MHz

respectively, due to a glycosidation shift.[16] The ^{13}C NMR spectrum of **10** was superimposable on that of kudzusaponin SA_3 methyl ester (**9**), except for the rhamnopyranosyl signals at C-2" of the inner galactosyl moiety, which was substituted for by the glucopyranosyl moiety in **10** as shown in Table 2. From these observations, we deduced that **10** was a 3,22-bisdesmoside of soyasapogenol A with four units of sugar. The ^1H-^1H COSY experiment for **10** allowed the sequential assignments of four monosaccharides. Their multiplet patterns and coupling constants allowed the identification of an α-L-arabinopyranosyl, β-D-glucopyranosyl, β-D-galactopyranosyl, and β-D-glucuronopyranosyl unit. The arabinosyl unit was shown to be directly attached at C-22 of the aglycon by an HMBC correlation between the signals of the anomeric proton of the arabinosyl unit at δ 4.92 and C-22 of the aglycon at δ 92.8. The anomeric proton of the glucosyl moiety at δ 5.20 showed a $^3J_{C,H}$ correlation with C-2" of the galactosyl residue at δ 84.8, the anomeric proton of which at δ 5.68 in turn showed a long-range correlation with C-2' of the glucuronosyl moiety at δ 80.3. The long-range correlation between the C-3 resonance at δ 90.8 and the anomeric proton signal of the glucuronosyl unit at δ 5.08 confirmed the attachment of the glucuronosyl moiety at C-3 of the aglycon. In light of the above observations, the structure of the desglucosylsoyasaponin A_1 (**10**) was assigned as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside.

Generally, soyasaponins are classified into four major groups on the basis of their aglycon structures: groups A, B, E, and DDMP. There are about 40 soyasaponins identified in soybean.[17] Group B soyasaponins have been reported to be the most abundant group of soyasaponins, whereas group E soyasaponins are the least abundant of the four groups. The C-22 hydroxyl group of group A soyasaponins consists of two sugar residues, starting with an arabinosyl. The terminal sugar moiety is a xylosyl or glucosyl residue that can be acetylated at three or four positions, respectively.[17,18] However, isolated saponins (**9**, **10**) carried only one sugar residue, arabinosyl, at C-22 with no acetoxyl group(s). The DDMP-conjugated soyasaponins were the primary group B soyasaponins detected in the raw soybean flour, but could not be isolated from our samples.[19] Interestingly, we isolated only three group B soyasaponins as minor components, whereas four group E and two deglycosylated group A soyasaponins were isolated as major ones. These controversial discrepancies could well be due to decomposition of group B soyasaponins to group E ones through processing or biotransformation during the cultivation of *G. applanatum* in liquid culture medium. Although six group E soyasaponins, including dehydrosoyasaponins I (soybean saponin Be, **6**), II (soyasaponin Bg, **4**), III (**2**), IV (**1**), soyasaponin Bd and unidentified dehydrosoyasaponin, were identified in soy protein isolate by LC-MS/MS, only four soyasaponins Bd, Be (**6**), Bf, and Bg (**4**) were isolated and identified from soybean to date.[18,20] Dehydrosoyasaponin IV (**1**), dehydrosoyasaponin III (impatienoside A) (**2**), and kudzusaponin SA_3 (**9**) were isolated for the first time from soybean. Overall, it remains to be investigated whether group E soyasaponins were present in the original sample as major components, or whether group E soyasaponins were biotransformed during the cultivation.

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