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Saponin and Sapogenol. XLII.[1] Structures of Acetyl-soyasaponins A_1, A_2, and A_3, Astringent Partially Acetylated Bisdesmosides of Soyasapogenol A, from American Soybean, the Seeds of *Glycine max* MERRILL.

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By means of high performance liquid chromatography and other methods, three new partially acetylated soyasaponins, named acetyl-soyasaponin A_1 (**6a**), acetyl-soyasaponin A_2 (**7a**), and acetyl-soyasaponin A_3 (**8a**), were isolated from American soybeans, the seeds of *Glycine max* MERRILL., together with hitherto known soyasaponins I (**3**), II (**4**), and III (**5**). Acetyl-soyasaponins A_1, A_2, and A_3 are noteworthy due to their bitter and astringent tastes, although their parent saponins, soyasaponins A_1 (**6**), A_2 (**7**), and A_3 (**8**), do not show such tastes.

By virtue of the photochemical degradation method for the glucuronide linkage and on the basis of ^{1}H- and ^{13}C-nuclear magnetic resonance and secondary ion mass spectrum analyses, structures of acetyl-soyasaponins A_1, A_2, and A_3 have been elucidated as 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**6a**), 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**7a**), and 3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**8a**), respectively.

Keywords——*Glycine max*; soybean; acetyl-soyasaponin A_1; acetyl-soyasaponin A_2; acetyl-soyasaponin A_3; soyasaponin A_3; oligoglycoside SIMS; oligoglycoside ^{13}C-NMR; glucuronide photolysis

As part of our search for biologically active substances in foodstuffs, we have been engaged in chemical studies of saponins in soybeans, the seeds of *Glycine max* MERRILL. We have so far isolated five saponins, *i.e.*, soyasaponins I (**3**),[1,2] II (**4**), [1,2] and III (**5**)[1,2] (having soyasapogenol B (**2**) as the aglycone) and soyasaponins A_1 (**6**)[3] and A_2 (**7**)[4] (having soyasapogenol A (**1**) as the aglycone), from soybeans cultivated in Akita Prefecture, Japan and elucidated their structures. These soyasaponins have been shown to exhibit various biological activities, *e.g.*, an inhibitory effect on lipid-oxidation and liver-lesion generation and an improving effect on hyper-cholesteremia.[5] By means of gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC), we have developed two quantitative analytical methods for soyasaponins contained in various kinds of soybeans and soybean products.[6] We have also reported that soyasaponins are contained in partially acetylated forms in various kinds of soybeans, either domestic or foreign.[6] Afterwards, it was reported that those partially acetylated soyasaponins may be responsible for the bitter and astringent taste of soybeans,[7] so that we have resumed chemical investigations of saponins in soybeans of different origins and kinds. In this paper, we report the results of our recent study

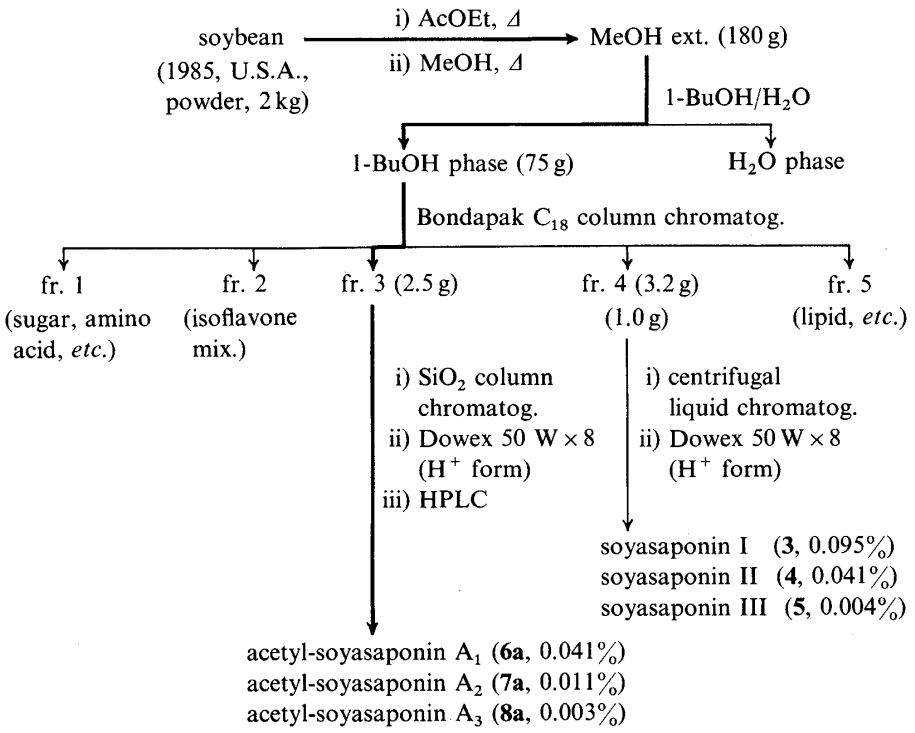


Chart 1

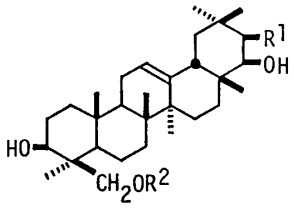
on soyasaponins in soybeans imported from Ohio, U.S.A., these soybeans are currently consumed on a large scale in Japan. We have isolated, in addition to soyasaponins I (**3**), II (**4**), and III (**5**), three new partially acetylated soyasaponins, named acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**), having bitter and astringent tastes, and have elucidated their chemical structures.[8]

The methanolic extract of defatted soybeans was partitioned into a mixture of 1-butanol and water and the 1-butanol-soluble portion was subjected to repeated chromatographic separation as shown in Chart 1 to afford soyasaponins I (**3**), II (**4**), and III (**5**), and acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**).[9-11]

Acetyl-soyasaponin A_1 (6a)

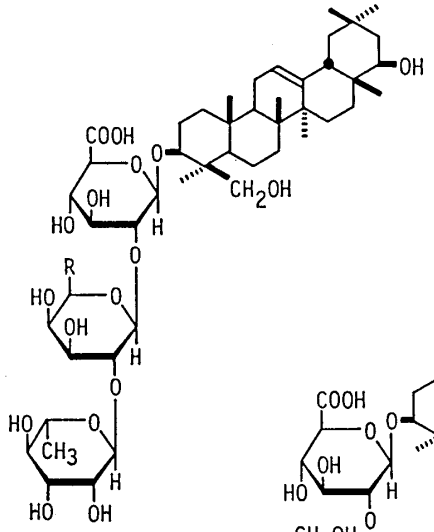
The infrared (IR) spectrum of acetyl-soyasaponin A_1 (**6a**) showed absorption bands ascribable to hydroxyl, ester and carboxyl functions. Alkaline treatment of **6a** yielded soyasaponin A_1 (**6**) and acetic acid. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **6a** showed five anomeric proton signals at δ 4.88, 5.06, 5.19, 5.52, and 5.55, all of which were observed as doublets of J=7.3—8.6 Hz. It also showed signals due to four acetoxyl groups and three of a methine proton geminal to an acetoxyl group on the terminal glucosyl residue at δ 5.44 (dd, J=9.4, 9.7 Hz, 4″-H), 5.45 (dd, J=7.9, 9.4 Hz, 2″-H), and 5.73 (dd, J=9.4, 9.4 Hz, 3″-H). These glucosyl proton signals were observed by taking the spectrum at 40 °C; they overlapped with the H_2O signal when measured at 25 °C. Detailed decoupling experiments, a ^{1}H–^{1}H two-dimensional correlation (COSY) study of **6a**, and a comparison of ^{1}H-NMR data for **6a** with those for soyasaponins A_1 (**6**), A_2 (**7**), and prosapogenol (**9**),[4] have led us to assign the ^{1}H-NMR data for **6a** as summarized in Table I. Thus, the terminal glucosyl moiety in **6a** has been proved to be fully acetylated.

In the secondary ion mass spectrum (SIMS, glycerol matrix) of **6a**, an ion peak of m/z 1459 $(M+Na)^+$ was observed, while an ion peak of m/z 1475 $(M+K)^+$ was observed when the spectrum was taken with potassium chloride. The SIMS also showed fragment ion peaks at m/z 331 (**i**), 371 (**ii**+Na), 311 (**ii**−AcOH+Na), 387 (**ii**+K), 327 (**ii**−AcOH+K), which are



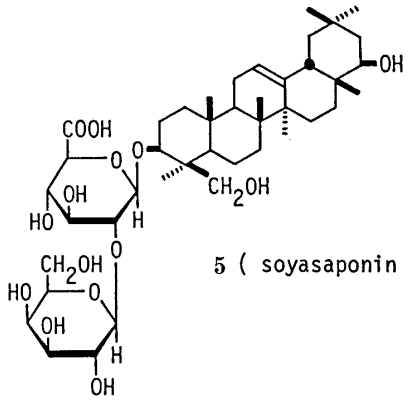
1 : R^1 = OH, R^2 = H (soyasapogenol A)
1a : R^1 = OCH_3, R^2 = CH_3

2 : R^1, R^2 = H (soyasapogenol B)

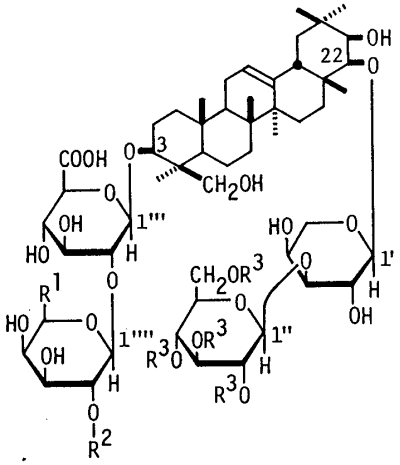


3 : R = CH_2OH
(soyasaponin I)

4 : R = H
(soyasaponin II)

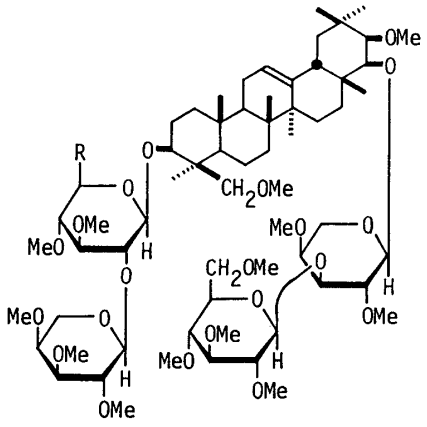


5 (soyasaponin III)



	R^1	R^2	R^3	
6 :	CH_2OH	β-D-glu.	H	(soyasaponin A_1)
6a :	CH_2OH	β-D-glu.	Ac	(acetyl-soyasaponin A_1)
7 :	CH_2OH	H	H	(soyasaponin A_2)
7a :	CH_2OH	H	Ac	(acetyl-soyasaponin A_2)
8 :	H	H	H	(soyasaponin A_3)
8a :	H	H	Ac	(acetyl-soyasaponin A_3)

β-D-glu. = β-D-glucopyranosyl



8b : R = $COOCH_3$

8c : R = CH_2OH

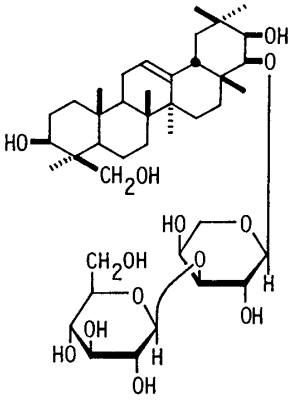


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Chart 2

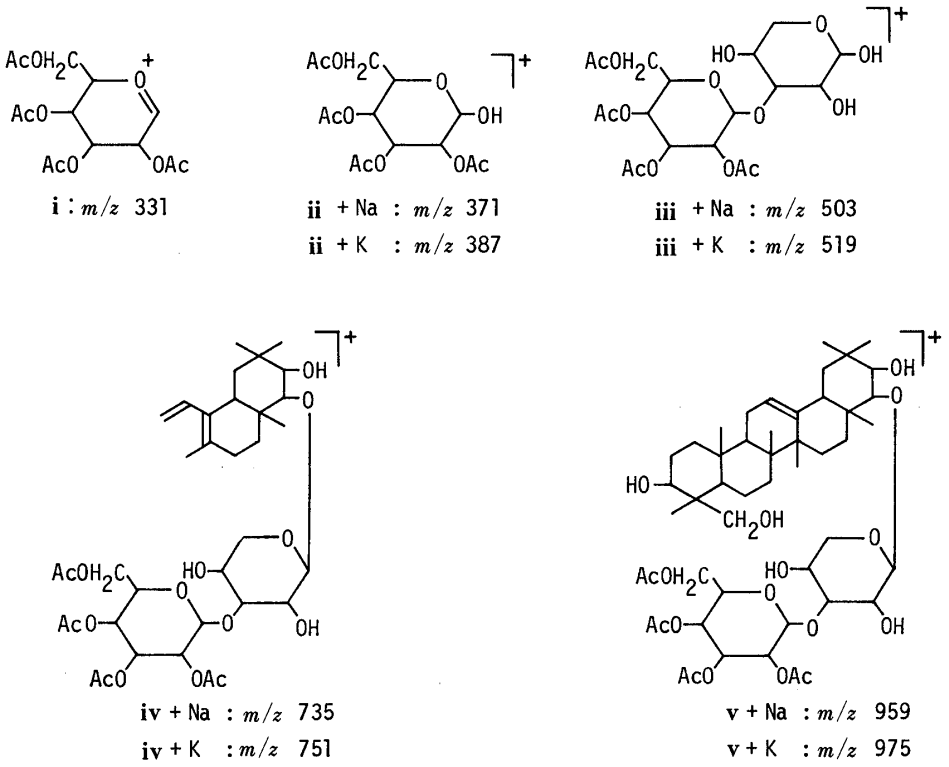

Chart 3

derivable from the 2,3,4,6-tetra-*O*-acetylglucosyl moiety. Furthermore, fragment ion peaks, assigned to **iii**+Na, **iii**+K, **iv**+Na, **iv**+K, **v**+Na, and **v**+K, have shown that the terminal glucosyl residue in the disaccharide moiety attached to 22-OH of the aglycone (soyasapogenol A) in **6a** is fully acetylated. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **6a** showed five anomeric carbon signals at δ_C 101.1, 101.5, 102.8, 104.5, and 107.1. Detailed comparison of the ^{13}C-NMR data for **6a** with those for soyasaponins A_1 (**6**), A_2 (**7**), and prosapogenol (**9**), have led us to assign all carbon signals (assignments of major signals are given in Table II). Thus, the fully acetylated terminal glucosyl residue in the sugar moiety attached to 22-OH of the aglycone has been confirmed. Consequently, the structure of acetyl-soyasaponin A_1 (**6a**) has been determined.

Acetyl-soyasaponin A_2 (7a)

The IR spectrum of acetyl-soyasaponin A_2 (**7a**) showed absorption bands ascribable to hydroxyl, ester and carboxyl groups. Alkaline hydrolysis of **7a** provided soyasaponin A_2 (**7**) and acetic acid. The ^{1}H-NMR spectrum of **7a** showed signals due to four anomeric protons (δ 4.84, 4.90, 5.39, 5.52, all d, J=7.0—8.2 Hz), four acetoxyl functions, and three methine protons on the acetylated terminal glucosyl residue at δ 5.40 (dd, J=9.4, 9.8 Hz, 4″-H), 5.43 (dd, J=8.2, 9.4 Hz, 2″-H), and 5.67 (dd, J=9.4, 9.4 Hz, 3″-H). Decoupling experiments and a ^{1}H–^{1}H COSY study of **7a**, together with a ^{1}H-NMR data comparison of **6a**, **7**, **7a**, and **9**, have led us to the ^{1}H-NMR data assignments for **7a** as shown in Table I. Here again, the terminal glucosyl moiety has turned out to be fully acetylated. The structure has been further supported by the SIMS of **7a**. Thus, a prominent ion $(M+Na)^+$ was observed at m/z 1297, while fragment ions (**i**, **ii**, **iii**, **iv**, and **v**) relevant to the terminal 2″,3″,4″,6″-tetra-*O*-acetylglucosyl moiety are also observed, as in the SIMS of acetyl-soyasaponin A_2 (**6a**). The ^{13}C-NMR data for **7a** have been assigned as shown in Table II in the same manner as in the case of **6a**. Based on these findings, the structure of acetyl-soyasaponin A_2 has been determined as **7a**, in which the terminal glucosyl residue in the 22-*O*-sugar moiety is fully

TABLE I. ^{1}H-NMR Data for Soyasaponins A_1 (**6**), A_2 (**7**), and A_3 (**8**), Acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**), and Prosapogenol (**9**)

		6[a]	**6a**[b]	**7**[a]	**7a**[a]	**8**[a]	**8a**[a]	**9**[a]
Sapogenol moiety	12-H	5.30 (br s, $W_{h/2}=9.0$)	5.30 (br s, $W_{h/2}=9.0$)	5.26 (br s, $W_{h/2}=9.5$)	5.26 (br s, $W_{h/2}=9.5$)	5.27 (br s, $W_{h/2}=9.0$)	5.26 (br s, $W_{h/2}=9.0$)	5.22 (br s, $W_{h/2}=9.0$)
3-*O*-*β*-D-Glucurono-pyranosyl moiety	1‴-H	5.19 (d, $J=7.3$)	5.19 (d, $J=8.6$)	4.88 (d, $J=7.0$)	4.84 (d, $J=7.0$)	4.87 (d, $J=7.9$)	4.82 (d, $J=7.6$)	
2‴-*O*-D-Galacto-pyranosyl moiety	1⁗-H	5.59 (d, $J=7.6$)	5.55 (d, $J=7.9$)	5.44 (d, $J=7.6$)	5.39 (d, $J=7.6$)			
2‴-*O*-*α*-L-Arabino-pyranosyl moiety	1⁗-H					5.15 (d, $J=7.9$)	5.24 (d, $J=7.6$)	
2⁗-*O*-*β*-D-Gluco-pyranosyl moiety	1⁗′-H	5.12 (d, $J=7.6$)	5.06 (d, $J=7.3$)					
22-*O*-*α*-L-Arabino-pyranosyl moiety	1′-H	4.87 (d, $J=7.6$)	4.88 (d, $J=7.6$)	4.90 (d, $J=7.0$)	4.90 (d, $J=7.6$)	4.91 (d, $J=7.6$)	4.90 (d, $J=7.6$)	4.77 (d, $J=7.6$)
3′-*O*-*β*-D-Gluco-pyranosyl moiety	1″-H	5.20 (d, $J=7.6$)	5.52 (d, $J=7.9$)	5.19 (d, $J=8.2$)	5.52 (d, $J=8.2$)	5.18 (d, $J=7.6$)	5.52 (d, $J=8.0$)	5.09 (d, $J=7.6$)
	2″-H		5.45 (dd, $J=7.9, 9.4$)		5.43 (dd, $J=8.2, 9.4$)		5.42 (dd, $J=8.0, 9.4$)	
	3″-H		5.37 (dd, $J=9.4, 9.4$)		5.67 (dd, $J=9.4, 9.4$)		5.67 (dd, $J=9.4, 9.4$)	
	4″-H		5.44 (dd, $J=9.4, 9.7$)		5.40 (dd, $J=9.4, 9.8$)		5.40 (dd, $J=9.4, 9.7$)	
	5″-H		4.65[c]		4.60[c]		4.59[c]	
	6″-H		4.23[c] 4.48[c]		4.25[c] 4.43[c]		4.27[c] 4.43[c]	

a, b) Measured at 500 MHz in d_5-pyridine : D_2O (10 : 1) at 25 °C[a] or at 40 °C[b]. Chemical shifts are in δ and coupling constants (J) in Hz. *c*) The coupling patterns are unclear due to overlapping with other signals.

acetylated.

Acetyl-soyasaponin A_3 (8a) and Soyasaponin A_3 (8)

The presence of hydroxyl, ester, and carboxyl functions in acetyl-soyasaponin A_3 (**8a**) was apparrent in its IR spectrum. Alkaline hydrolysis of **8a** liberated acetic acid and a new saponin soyasaponin A_3 (**8**). The IR spectrum of **8** showed absorption bands ascribable to hydroxyl and carboxyl groups, but it lacked ester absorption bands. Upon methanolysis with 9% hydrogen chloride in dry methanol, soyasaponin A_3 (**8**) provided soyasapogenol A (**1**) together with methyl arabinoside, methyl glucoside, and methyl glucuronide in 2 : 1 : 1 ratio. The ^{13}C-NMR spectrum of **8** showed four anomeric carbon signals at δ_C 103.2, 103.4, 103.9, and 106.8, while the ^{1}H-NMR spectrum showed signals due to four anomeric protons at δ4.87 (d, $J=7.9$ Hz), 4.91 (d, $J=7.6$ Hz), 5.15 (d, $J=7.9$ Hz), and 5.18 (d, $J=7.6$ Hz). Comparison of these ^{13}C-NMR data for anomeric carbons with those for various methyl glycosides,[12] and the J values of anomeric proton signals, suggested that two L-arabinopyranosyl moieties in soyasaponin A_3 (**8**) are in α-orientation whereas one D-glucopyranosyl moiety and one D-glucuronopyranosyl moiety are in β-orientation. In addition, detailed comparison of the ^{13}C-NMR data for **8** with those for soyasaponins A_1 (**6**) and A_2 (**7**) has led us to presume that soyasaponin A_3 (**8**) is a bisdesmoside of soyasapogenol A (**1**) in which an α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl moiety and a β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl moiety are attached to the 3-OH and 22-OH functions (or *vice versa*), respectively.[13]

In order to determine chemically the locations of the sugar moieties, soyasaponin A_3 (**8**) was subjected to photodegradation for selective cleavage of the glucuronide linkage.[14]

TABLE II. ^{13}C-NMR Data for Soyasaponins A_1 (**6**), A_2 (**7**), and A_3 (**8**), Acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**), and Prosapogenol (**9**)[a]

		6	**6a**	**7**	**7a**	**8**	**8a**	**9**
Sapogenol moiety	C-3	89.6	89.4	89.7	89.8	89.6	89.5	79.4
	C-12	121.4	121.3	121.4	121.4	121.4	121.5	121.9
	C-13	143.2	143.0	143.1	143.0	143.1	143.1	143.5
	C-21	74.6	74.4	74.6	74.3	74.6	74.4	75.0
	C-22	91.5	91.1	91.5	91.2	91.4	91.2	92.0
	C-24	62.4	62.3	62.3	62.1	62.2	62.1	63.8
3-*O*-*β*-D-Glucurono-pyranosyl moiety	C-1‴	103.2	102.8	103.4	103.0	103.2	103.2	
	C-2‴	79.4	79.2	78.9	78.7	78.9	78.9	
	C-3‴	75.5[b]	75.2[b]	75.7[b]	75.4[b]	76.0[b]	75.4[b]	
	C-4‴	71.3	71.4	71.9	71.6	71.8	71.9	
	C-5‴	76.2[b]	75.9[b]	76.7[b]	76.5[b]	76.4[b]	76.6[b]	
	C-6‴	172.5	174.0	172.7	174.9	173.6	174.8	
2‴-*O*-*β*-D-Galacto-pyranosyl moiety	C-1⁗	101.7	101.5	103.4	103.0			
	C-2⁗	82.1	81.6	71.6	71.6			
	C-3⁗	73.2	72.9	73.7	73.4			
	C-4⁗	68.9	68.8	69.3	69.2			
	C-5⁗	77.5	77.1	76.0	75.4			
	C-6⁗	60.8[c]	60.8[c]	60.9	61.3[c]			
2‴-*O*-*α*-L-Arabino-pyranosyl moiety	C-1⁗					103.4	103.5	
	C-2⁗					71.2	71.4	
	C-3⁗					73.0	73.2	
	C-4⁗					68.6	68.6	
	C-5⁗					66.2	66.1	
2⁗-*O*-*β*-D-Gluco-pyranosyl moiety	C-1⁗′	105.0	104.5					
	C-2⁗′	75.0	74.6					
	C-3⁗′	76.2[b]	75.9[b]					
	C-4⁗′	69.8	69.5					
	C-5⁗′	76.2[b]	76.1[b]					
	C-6⁗′	61.2[c]	61.2[c]					
22-*O*-*α*-L-Arabino-pyranosyl moiety	C-1′	107.0	107.1	106.9	107.2	106.8	107.3	107.5
	C-2′	71.3	71.2	71.4	71.3	71.6	71.8	71.8
	C-3′	83.5	83.4	83.4	83.4	83.3	83.5	84.1
	C-4′	67.8	67.7	67.8	67.9	67.7	67.9	68.3
	C-5′	66.0	65.9	65.9	65.9	65.9	66.1	66.4
3′-*O*-*β*-D-Gluco-pyranosyl moiety	C-1″	104.2	101.1	104.1	101.1	103.9	101.2	104.8
	C-2″	74.0	70.7	73.9	70.8	73.7	70.9	75.0
	C-3″	76.5[d]	71.0	76.4[d]	71.0	76.1[d]	71.1	77.1[d]
	C-4″	70.0	67.7	69.9	67.9	69.7	67.9	70.5
	C-5″	76.8[d]	72.6	76.7[d]	72.6	76.4[d]	72.7	77.4[d]
	C-6″	61.1[c]	60.7[c]	60.9	61.0[c]	60.8	61.4	61.6

a) Measured at 125 MHz in d_5-pyridine : D_2O (5 : 1) at 25 °C. Chemical shifts are in δ_C. *b*—*d*) Assignments may be interchangeable within the same column.

Irradiation of a methanolic solution of **8** with a 500 W high-pressure mercury lamp provided a known prosapogenol (**9**).[4] Thus, the *β*-D-glucopyranosyl(1→3)-*α*-L-arabinopyranosyl residue has been alloted to the 22-OH function of the aglycone.

Complete methylation of soyasaponin A_3 (**8**) with methyl iodide and dimsyl carbanion[15] afforded the tetradeca-*O*-methyl derivative (**8b**), which, on lithium aluminum hydride reduction, was derivatized to **8c**. The IR spectrum of **8c** showed hydroxyl absorption bands, but lacked ester absorption bands. Methanolysis of **8c** liberated 21,24-di-*O*-methylsoyasapogenol A (**1a**),[4] methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), methyl 2,3,4,-tri-*O*-methylarabinopyranoside (**b**), methyl 2,4-di-*O*-methylarabinopyranoside (**c**), and

methyl 3,4-di-*O*-methylglucopyranoside (**d**). Based on the above-mentioned combined evidence, the structure of soyasaponin A_3 has been determined as 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-soyasapogenol A (**8**).

The ^{1}H-NMR spectrum of acetyl-soyasaponin A_3 (**8a**) showed four doublets (J=7.6—8.0 Hz) assigned to four anomeric protons observed at δ 4.82, 4.90, 5.24, and 5.52. It also showed signals due to four acetoxyl functions and three methine protons on the fully acetylated terminal glucosyl residue (δ 5.40, dd, J=9.4, 9.7 Hz, 4″-H; 5.42, dd, J=8.0, 9.4 Hz, 2″-H; 5.67, dd, J=9.4, 9.4 Hz, 3″-H), as were observed in the spectra of acetyl-soyasaponins A_1 (**6a**) and A_2 (**7a**). Decoupling experiments and a ^{1}H–^{1}H COSY study of **8a** and a detailed comparison of the ^{1}H-NMR data for **8a** with those for soyasaponin A_3 (**8**), **6a**, and **7a**, have led us to assign the main ^{1}H-NMR data for **8a** as given in Table I. Here again, the terminal glucosyl moiety in **8a** is presumed to be fully acetylated at the 2″-, 3″-, 4″-, and 6″-hydroxyl residues.

The SIMS of **8a** showed a prominent ion peak at m/z 1267 $(M+Na)^+$ and fragment ions (**i**, **ii**, **iii**, **iv**, **v**) relevant to the 2,3,4,6-tetra-*O*-acetylglucosyl moiety, as were observed in the SIMS of **6a** and **7a**. The ^{13}C-NMR spectrum of **8a** showed four anomeric carbon signals at δ_C 101.2, 103.2, 103.5, and 107.3. Comparison of the ^{13}C-NMR data for **8a** with those for soyasaponin A_3 (**8**) and acetyl-soyasaponins A_1 (**6a**) and A_2 (**7a**) allowed in assignment of the main carbon signals as given in Table II. Consequently, the structure of acetyl-soyasaponin A_3 (**8a**) has been elucidated to be as shown, in which the terminal fully acetylated glucopyranosyl residue in the disaccharide moiety is attached to the 22-OH function of the aglycone.

In conclusion, during the course of our investigations on the oligoglycosidic constituents in soybeans imported from the U.S.A., we have characterized three partially acetylated soyasaponins named acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**). They are bisdesmosides of soyasapogenol A (**1**) having two oligosaccharide moieties attached to the 3-OH and 22-OH functions, of which the 22-*O*-[2″,3″,4″,6″-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl] residue is common in these acetyl-soyasaponins. Finally, it is interesting from the viewpoint of the chemical structure-taste relationship that acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**) are bitter and astringent, whereas soyasaponins I (**3**), II (**4**), III (**5**), A_1 (**6**), A_2 (**7**), and A_3 (**8**) are not.[16]

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper[1] except for the following. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL FX-500 FT-NMR spectrometer at 500 MHz for ^{1}H and at 125 MHz for ^{13}C. The SIMS spectra were taken with a Hitachi M-68 mass spectrometer.

Isolation of Soyasaponins I (3), II (4), and III (5), and Acetyl-soyasaponins A_1 (6a), A_2 (7a), and A_3 (8a) from American Soybeans——Powdered soybeans (cultivated in Ohio, U.S.A. in 1985, 2 kg, purchased from Fujita Shushiten, Osaka) were defatted with AcOEt three times (3 l each, with heating under reflux for 5 h). The defatted powder was then extracted with MeOH three times (3 l each, with heating under reflux for 5 h). Removal of the solvent from the combined MeOH solutions under reduced pressure gave the MeOH extract (180 g). The MeOH extract was partitioned into 1-BuOH–H_2O (2:1, 1.5 l) and removal of the solvent from the 1-BuOH phase under reduced pressure provided the 1-BuOH extract (75 g). Column chromatography of the 1-BuOH extract over reversed-phase silica gel (Bondapak C_{18} 500 g, H_2O : MeOH = 5 : 1→1 : 3) furnished five fractions after removal of the solvent under reduced pressure: fr. 1 (42.1 g), fr. 2 (6.0 g), fr. 3 (2.5 g), fr. 4 (3.2 g), and fr. 5 (18.0 g). Fraction 3 (2.5 g) was purified by column chromatography (SiO_2 200 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower phase) and the product was dissolved in MeOH and treated with Dowex 50 W×8 (H^+ form). After removal of the resin by filtration, the solvent was evaporated off under reduced pressure and the residue was subjected to HPLC [Shimadzu LC-6A, Shimpak ODS, H_2O : CH_3CN = 6 : 4→1 : 1] to furnish acetyl-soyasaponins A_1 (**6a**, 820 mg), A_2 (**7a**, 220 mg) and A_3 (**8a**, 60 mg). Fraction 4 (1 g) was purified by centrifugal liquid chromatography (CLC) [Hitachi centrifugal liquid chromatograph

model CLC-5, Fuji gel KT 2061 80 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)→65 : 35 : 10 (lower phase)] and by subsequent treatment with Dowex 50 W × 8 (H^+ form) to furnish soyasaponins I (**3**, 593 mg), II (**4**, 256 mg), and III (**5**, 25 mg).[9]

Acetyl-soyasaponin A_1 (**6a**): mp 263—265 °C (colorless fine crystals from EtOH), $[\alpha]_D^{16}$ +17.0° (c = 0.6, MeOH). *Anal.* Calcd for $C_{67}H_{104}O_{33} \cdot 4H_2O$: C, 53.31; H, 7.48. Found: C, 53.05; H, 7.25. IR ν_{max}^{KBr} cm^{-1}: 3420 (br), 2923, 1746, 1607, 1229. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 40 °C), δ: 0.67, 0.89, 1.23, 1.26 (3H each), 1.32 (6H), 1.43 (3H) (all s, *tert*-CH_3 × 7), 1.98, 2.06 (3H each), 2.10 (6H) (all s, OAc × 4), and other signals as given in Table I. ^{13}C-NMR (125 MHz, d_5-pyridine : D_2O = 5 : 1, 25 °C), δ_C: 19.5 (2C), 19.6 (2C), 169.1, 170.0, 170.1, 170.6 (acetoxyl groups), and other signals as given in Table II. SIMS: as given in the text.

Acetyl-soyasaponin A_2 (**7a**): mp 276—278 °C (colorless fine crystals from EtOH), $[\alpha]_D^{18}$ +18.8° (c = 0.3, MeOH). *Anal.* Calcd for $C_{61}H_{94}O_{28} \cdot 3H_2O$: C, 55.11; H, 7.68. Found: C, 55.13; H, 7.70. IR ν_{max}^{KBr} cm^{-1}: 3401 (br), 2921, 1745, 1609, 1226. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.68, 0.87, 1.22, 1.23, 1.28, 1.30, 1.34 (3H each) (all s, *tert*-CH_3 × 7), 2.09, 2.13, 2.17, 2.18 (3H each) (all s, OAc × 4), and other signals as given in Table I. ^{13}C-NMR (125 MHz, d_5-pyridine : D_2O = 5 : 1, 25 °C), δ_C: 19.5 (2C), 19.7 (2C), 169.6, 169.9, 170.0, 170.4 (acetoxyl groups), and other signals as given in Table II. SIMS: as given in the text.

Acetyl-soyasaponin A_3 (**8a**): mp 257—260 °C (colorless fine crystals from EtOH), $[\alpha]_D^{20}$ +23.6° (c = 0.5, MeOH). *Anal.* Calcd for $C_{60}H_{92}O_{27} \cdot 5H_2O$: C, 53.93; H, 7.70. Found: C, 54.03; H, 7.55. IR ν_{max}^{KBr} cm^{-1}: 3404 (br), 2924, 1743, 1606, 1223. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.67, 0.87, 1.22, 1.23, 1.28, 1.30, 1.35 (3H each) (all s, *tert*-CH_3 × 7), 2.08, 2.13, 2.17, 2.18 (3H each) (all s, OAc × 4), and other signals as given in Table I. ^{13}C-NMR (125 MHz, d_5-pyridine : D_2O = 5 : 1, 25 °C), δ_C: 19.5 (2C), 19.6 (2C), 169.1, 170.0, 170.1, 170.6 (acetoxyl groups), and other signals as given in Table II. SIMS: as given in the text.

Alkaline Hydrolysis of Acetyl-soyasaponin A_1 (6a) Giving Soyasaponin A_1 (6)——A solution of **6a** (40 mg) in H_2O (1 ml) was treated with 5% KOH–MeOH (4 ml). The solution was then heated under reflux for 1 h, neutralized with Dowex 50 W × 8 (H^+ form) and filtered. Concentration of the filtrate under reduced pressure yielded a suspension (about 1 ml), from which the precipitate was collected by filtration. Crystallization of the precipitate from EtOH furnished soyasaponin A_1 (**6**, 36 mg). The filtrate was subjected to GLC to identify acetic acid [15% free fatty acid polyester (FFAP) on Chromosorb GAW DMCS (100—120 mesh), 2 mm × 1 m glass column; column temp. 140 °C; N_2 flow rate 25 ml/min; t_R 5 min 15 s]. The product (**6**) was shown to be identical with an authentic sample[3] by thin-layer chromatography (TLC) [$CHCl_3$: MeOH : H_2O = 6 : 4 : 1; 1-BuOH : AcOH : H_2O = 4 : 1 : 5, upper phase], mixed melting point determination, and IR (KBr) and ^{13}C-NMR (d_5-pyridine : D_2O = 5 : 1) spectral comparisons.

Alkaline Hydrolysis of Acetyl-soyasaponin A_2 (7a) Giving Soyasaponin A_2 (7)——A solution of **7a** (30 mg) in H_2O (1 ml) was treated with 5% KOH–MeOH (3 ml). The solution was then heated under reflux for 1 h, neutralized with Dowex 50 W × 8 (H^+ form) and filtered. Work-up of the filtrate as described above for the deacetylation of **6a**, gave the product, which was crystallized from EtOH to furnish soyasaponin A_2 (**7**, 27 mg). This product (**7**) was shown to be identical with an authentic sample[4] by TLC, mixed melting point determination, and IR and ^{13}C-NMR spectral comparisons as described above. From the filtrate, acetic acid was identified by GLC comparison as described above.

Alkaline Hydrolysis of Acetyl-soyasaponin A_3 (8a) Giving Soyasaponin A_3 (8)——A solution of **8a** (40 mg) in H_2O (1 ml) was treated with 5% KOH–MeOH (4 ml). The solution was then heated under reflux for 1 h, neutralized and worked up as described above for the deacetylation of **6a**. The product, obtained as a precipitate, was crystallized from aqueous EtOH to furnish soyasaponin A_3 (**8**, 34 mg). From the filtrate, acetic acid was identified by GLC comparison as described above. Soyasaponin A_3 (**8**), mp 250—255 °C (colorless fine crystals), $[\alpha]_D^{20}$ +21.9° (c = 0.2, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{23} \cdot 4H_2O$: C, 54.34; H, 8.07. Found: C, 54.19; H, 7.74. IR ν_{max}^{KBr} cm^{-1}: 3386 (br), 2911, 1722, 1070. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.69, 0.87, 1.17, 1.24, 1.27, 1.29, 1.37 (3H each) (all s, *tert*-CH_3 × 7), and other signals as given in Table I. ^{13}C-NMR: as given in Table II.

Methanolysis of Soyasaponin A_3 (8)——A solution of **8** (10 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. Concentration of the filtrate under reduced pressure yielded a suspension, from which the precipitate was collected by filtration. Crystallization of the precipitate from MeOH furnished soyasapogenol A (**1**, 3 mg), which was identified with an authentic sample[3] by TLC [$CHCl_3$: MeOH = 15 : 1, benzene : acetone = 2 : 1, *n*-hexane : acetone = 1 : 1], mixed melting point determination, and IR (KBr) spectral comparison. Removal of the solvent from the filtrate under reduced pressure gave a methyl glycoside mixture. The mixture was dissolved in pyridine (0.1 ml), treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) and left standing for 1 h. The product was then analyzed by GLC to identify the trimethylsilyl (TMS) derivatives of methyl arabinoside, methyl glucoside, and methyl glucuronide. The composition of these three methyl glycosides was determined from the GLC peak areas. GLC: 1) 1.5% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh); 3 mm × 1 m glass column; column temp. 150 °C; N_2 flow rate 35 ml/min; t_R, TMS-methyl arabinoside 3 min 10 s, 3 min 22 s, TMS-methyl glucoside 14 min 54 s, 18 min 5 s, TMS-methyl glucuronide 7 min 22 s, 16 min 41 s. 2) 1.5% silicone OV-1 on Chromosorb WAW DMCS (80—100 mesh); 3 mm × 1 m glass column; column temp. 150 °C; N_2 flow rate 35 ml/min; t_R, TMS-methyl arabinoside 3 min 15 s, 3 min 24 s, TMS-methyl glucoside 16 min 25 s, 19 min 42 s, TMS-methyl glucuronide 7 min 43 s, 18 min 9 s.

Photolysis of Soyasaponin A_3 (8)——A solution of **8** (50 mg) in MeOH (30 ml) in a Vycor tube was irradiated

externally with a 500 W high-pressure mercury lamp (Eikosha, PIH-500) for 4 h while keeping the solution temperature below 10 °C. The reaction mixture was neutralized with 10% aqueous K_2CO_3 and the solvent was evaporated off under reduced pressure. The product was partitioned into 1-BuOH–H_2O (1 : 1). The product, obtained after removal of the solvent from the 1-BuOH phase under reduced pressure, was purified by column chromatography (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1, lower phase) and crystallized from aqueous MeOH to furnish the prosapogenol (**9**, 15 mg). This product (**9**) was shown to be identical with an authentic sample[4] by TLC [$CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase); 1-BuOH : AcOEt : H_2O = 1 : 1 : 1 (upper phase)], mixed melting point determination, and IR (KBr) and ^{13}C-NMR (d_5-pyridine) spectral comparisons.

Methylation of Soyasaponin A_3 (8)——A solution of **8** (25 mg) in DMSO (2 ml) was treated with dimsyl carbanion (5 ml) and the whole mixture was stirred at 18 °C under a nitrogen atmosphere for 1 h. The reaction mixture was then treated with CH_3I (2 ml), stirred at room temperature in the dark for a further 3 h, and poured into ice-water. The whole mixture was extracted with AcOEt, and the AcOEt extract was washed with 10% aqueous $Na_2S_2O_3$ and water, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the product, which was purified by column chromatography (SiO_2 5 g, benzene : acetone = 6 : 1) and by crystallization from MeOH to furnish **8b** (18 mg). **8b**, mp 162—165 °C (colorless needles), $[\alpha]_D^{20}$ +20.5° (c = 0.4, $CHCl_3$). *Anal.* Calcd for $C_{64}H_{112}O_{23}$: C, 61.52; H, 9.03. Found: C, 61.69; H, 9.08. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no-OH, 2920, 1748, 1090. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.94 (9H), 1.09 (6H), 1.17, 1.25 (3H each) (all s, *tert*-CH_3 × 7), 3.25 (6H), 3.37 (12H), 3.46 (3H), 3.48 (6H), 3.52, 3.58 (3H each), 3.61 (6H) (all s, OCH_3 × 13), 3.79 (3H, s, $COOCH_3$), 4.40, 4.41, 4.59 (1H each, all d; J = 7 Hz, anomeric H × 3),[17] 5.21 (1H, br s, $W_{h/2}$ = 7 Hz, 12-H).

$LiAlH_4$ Reduction of 8b——A solution of **8b** (30 mg) in dry ether was treated with a suspension of $LiAlH_4$ (50 mg) in dry ether (3 ml) and the mixture was stirred at 25 °C for 1 h. After quenching of the reaction with wet ether, the reaction mixture was made weakly acidic with 5% aqueous H_2SO_4 and the whole was extracted with ether. The ether extract was washed with saturated aqueous $NaHCO_3$ and water, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave **8c** (28 mg). **8c**, a white powder, $[\alpha]_D^{20}$ +20.6° (c = 0.9, $CHCl_3$). *Anal.* Calcd for $C_{63}H_{112}O_{22}$: C, 61.94; H, 9.24. Found: C, 61.46; H, 9.20. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3601, 2928, 1094. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.95 (12H), 1.01, 1.09, 1.21 (3H each) (all s, *tert*-CH_3 × 7), 3.25 (3H), 3.37 (6H), 3.39, 3.45, 3.48, 3.52 (3H each), 3.54 (6H), 3.59 (3H), 3.61 (6H), 3.65 (3H) (all s, OCH_3 × 13), 4.38, 4.52, 4.62 (1H each, all d, J = 7 Hz anomeric H × 3),[17] 5.22 (1H, br s, $W_{h/2}$ = 7 Hz, 12-H).

Methanolysis of 8c——A solution of **8c** (20 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was worked up as described above for the methanolysis of **8**. The product, obtained as a precipitate, was crystallized from MeOH to afford 21,24-di-*O*-methylsoyasapogenol A (**1a**, 7 mg), which was shown to be identical with an authentic sample[4] by TLC [benzene : acetone = 5 : 1, *n*-hexane : acetone = 3 : 1, *n*-hexane : AcOEt = 2 : 1], mixed melting point determination, and IR ($CHCl_3$) spectral comparison. The other products, obtained from the filtrate after neutralization with Ag_2CO_3 powder, were identified as methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), methyl 2,3,4-tri-*O*-methylarabinopyranoside (**b**), methyl 2,4-di-*O*-methylarabinopyranoside (**c**), and methyl 3,4-di-*O*-methylglucopyranoside (**d**) by TLC and GLC. TLC: benzene : acetone = 3 : 1, *n*-hexane : acetone = 2 : 1, *n*-hexane : AcOEt = 1 : 2. GLC: 3) 15% polyethylene glycol succinate (PEGS) on Chromosorb WAW (80—100 mesh); 3 mm × 2 m glass column; column temp. 200 °C; N_2 flow rate 35 ml/min; t_R, **a** 2 min 33 s, 3 min 22 s (major peak); **b** 2 min 37 s, 2 min 59 s (major); **c** 5 min 55 s, 6 min 13 s (major); **d** 19 min 22 s (major), 23 min 46 s. 4) 15% polyneopentyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh); 3 mm × 2 m glass column; column temp. 200 °C; N_2 flor rate 35 ml/min; t_R, **a** 4 min 11 s, 5 min 29 s (major peak); **b** 3 min 59 s; **c** 7 min 21 s; **d** 21 min 43 s (major), 24 min 50 s.

References and Notes

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8) M. Yoshikawa, T. Taniyama, Y. Nagahama, I. Kitagawa, K. Okubo, M. Shiraiwa, S. Shimoyamada, and F. Yamauchi, presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, April 1987, Kyoto, Abstract, p. 337.
9) *a*) TLC examination has shown the presence of minor quantities of partially acetylated soyasaponins I, II, and III in the 1-butanol soluble portion. However, those acetates were readily deacetylated to provide soyasaponins

I, II, and III during subsequent isolation procedures; *b*) We have also detected by TLC and HPLC the presence of soyasaponins IV[10] and V[11] and the partially deacetylated derivatives of acetyl-soyasaponins A_1 (**6a**), A_2 (**7a**), and A_3 (**8a**). Although their isolations were not accomplished in this work, soyasaponins IV and V were isolated in pure forms afterwards;[10,11] *c*) Since a part of the soyasaponins was in the carboxylate forms as judged from the IR (KBr) spectra, the treatment with Dowex (H^+) was carried out.

10) *a*) Recently, a minor saponin, characterized as 3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl]-soyasapogenol B, was isolated and named soyasaponin IV[10b] although it corresponded to one of the prosapogenols of soyasaponin II; *b*) J. C. Burrows, K. R. Prince, and G. R. Fenwick, *Phytochemistry*, **26**, 1214 (1987).
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13) The D or L configuration of the constituent monosaccharides has been assigned to be the same as in hitherto known soyasaponins.[1-4]
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15) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
16) Aqueous solutions (0.1—0.05% (w/v)) of **6a**, **7a**, and **8a** showed very bitter and astringent taste.
17) The one remaining proton signal was overlapped by other signals.