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Saponin and Sapogenol. XLIII.[1] Acetyl-soyasaponins A_4, A_5, and A_6, New Astringent Bisdesmosides of Soyasapogenol A, from Japanese Soybean, the Seeds of *Glycine max* MERRILL.

TOSHIO TANIYAMA, YOSHIKO NAGAHAMA, MASAYUKI YOSHIKAWA, and ISAO KITAGAWA*

Faculty of Pharmaceutical Sciences, Osaka University, 1–6, Yamada-oka, Suita, Osaka 565, Japan

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In addition to soyasaponins I, II, and III, three new bitter and astringent bisdesmosides named acetyl-soyasaponin A_4(**2**), acetyl-soyasaponin A_5 (**3**), and acetyl-soyasaponin A_6 (**4**) which are characterized by having a fully acetylated terminal xylosyl moiety, have been isolated from a Japanese species of soybean (Tamanishiki strain), the seeds of *Glycine max* MERRILL. cultivated in Hokkaido Prefecture. By means of photochemical degradation (a selective cleavage method for the glucuronide linkage), and on the basis of physicochemical evidence including ^{1}H- and ^{13}C-nuclear magnetic resonance analysis, the structures of acetyl-soyasaponins A_4, A_5, and A_6 have been elucidated as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**2**), 3-*O*-[β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**3**), and 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl]-22-*O*-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**4**), respectively.

Keywords——*Glycine max*; soybean; acetyl-soyasaponin A_4; acetyl-soyasaponin A_5; acetyl-soyasaponin A_6; soyasaponin A_4; soyasaponin A_5; soyasaponin A_6; oligoglycoside ^{13}C-NMR; glucuronide photolysis

As a part of our chemical studies on biologically active constituents in leguminous naturally occurring drugs, we have been working on the saponin constituents of various kinds of soybeans, the seeds of *Glycine max* MERRILL. We have isolated five saponins named soyasaponins I, II, III,[2,3] A_1,[4] and A_2,[5] which exhibit various biological activities,[6] from a soybean species (sousei strain) cultivated in Akita Prefecture, Japan, and have elucidated their chemical structures. Afterwards, we found that the composition of saponins in soybeans varies significantly depending upon the region of production and the variety.[7] We have also isolated partially acetylated soyasaponins, acetyl-soyasaponins A_1, A_2, and A_3, from an American variety of soybean and have reported the elucidation of their chemical structures in the preceding paper.[1] Those acetyl-soyasaponins are bitter and astringent, and may be responsible for the unpleasant taste of soybeans.[1] In a continuing study, we have investigated the saponin constituents in a Japanese variety (tamanishiki strain) cultivated in Hokkaido Prefecture and have isolated three new saponins named acetyl-soyasaponin A_4 (**2**), acetyl-soyasaponin A_5 (**3**), and acetyl-soyasaponin A_6 (**4**). This paper deals with the structure elucidation of these bitter and astringent saponins.[8]

As in the case of an American variety of soybean reported previously,[1] the methanolic extract of defatted soybean cultivated in Hokkaido Prefecture was partitioned into a mixture of 1-butanol and water. The 1-butanol-soluble portion was subjected to successive column chromatographic separations using reversed-phase and ordinary-phase silica gel and then to

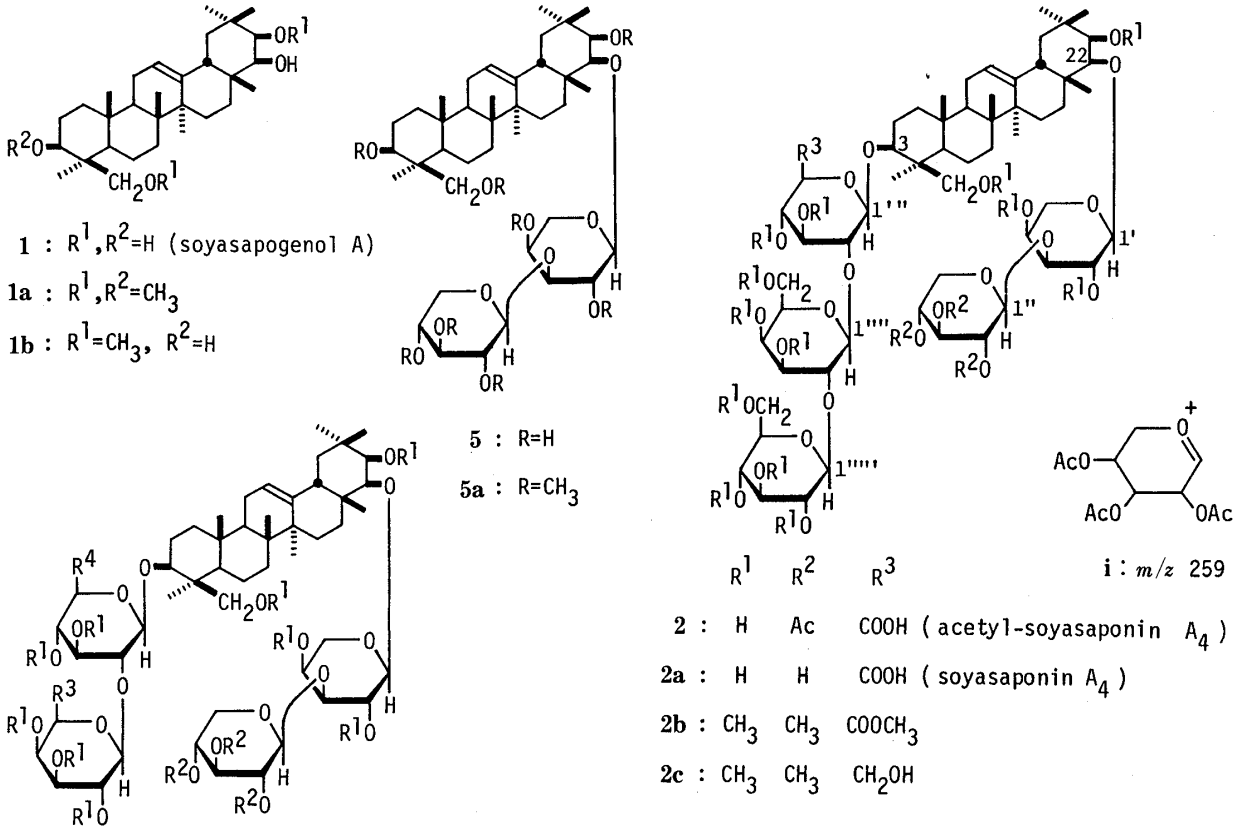

Chart 1

high-performance liquid chromatography (HPLC) to afford soyasaponins I, II, and III and acetyl-soyasaponins A_4 (**2**), A_5 (**3**), and A_6 (**4**).[9]

Acetyl-soyasaponin A_4 (2)

The infrared (IR) spectrum of acetyl-soyasaponin A_4 (**2**) showed absorption bands due to hydroxyl and ester functions. Alkaline hydrolysis of **2** yielded a new saponin soyasaponin A_4 (**2a**) and acetic acid, which was identified by gas-liquid chromatography (GLC). The IR spectrum of soyasaponin A_4 (**2a**) showed hydroxyl and carboxyl absorption bands. Methanolysis of **2a** with 9% hydrogen chloride in dry methanol liberated soyasapogenol A (**1**) together with methyl L-arabinoside, methyl D-xyloside, methyl D-galactoside, methyl D-glucuronide, and methyl D-glucoside in 1 : 1 : 1 : 1 : 1 ratio.[10] The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2a** showed signals due to five anomeric carbons (δ_C 101.4,

102.7, 104.4, 104.5, 107.0), whereas the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2a** showed signals assignable to five anomeric protons at δ 4.85 (1H, d, J= 7.9 Hz), 5.04 (2H, d, J=7.3 Hz), 5.22 (1H, d, J=7.9 Hz), and 5.45 (1H, d, J=7.6 Hz) (Table I). Comparison of these anomeric carbon chemical shifts with those of various methyl glycosides,[11] and the J values for anomeric proton signals, indicated that the arabinoside linkage in **2a** is in α-orientation while all the other glycoside linkages are in β-orientation. In addition, comparison of the ^{13}C-NMR data for **2a** with those for soyasaponin A_1[1] has led us conclude that soyasaponin A_4 (**2a**) is a bisdesmoside of soyasapogenol A (**1**) having a β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl moiety and a β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl moiety attached to the hydroxyl moieties at C-3 and C-22.

In order to accumulate chemical evidence on the structure of the carbohydrate moieties, soyasaponin A_4 (**2a**) was subjected to photochemical dergradation,[12] which is a selective cleavage method for the glucuronide linkage in oligoglycosides such as glucuronide-saponins. Thus, irradiation of a methanolic solution of **2a** with a 500 W high-pressure mercury lamp furnished a prosapogenol (**5**). The IR spectrum of **5** showed hydroxyl absorption bands but lacked the carboxyl absorption band. Methanolysis of **5** with 9% hydrogen chloride in dry methanol yielded soyasapogenol A (**1**) and methyl L-arabinoside and methyl D-xyloside in 1 : 1 ratio. The ^{13}C-NMR spectrum of **5** showed two anomeric carbon signals (δ_C 105.4, 107.6), whereas the ^{1}H-NMR spectrum of **5** showed two anomeric proton signals at δ 4.74 (d, J= 7.9 Hz, α-arabinopyranosyl) and 4.93 (d, J=7.3 Hz, β-xylopyranosyl). From a detailed comparison of these NMR data with those for the prosapogenol[1] which was obtained by photolysis of soyasaponin A_1,[1] **5** was presumed to be 22-*O*-[β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A.

Methylation of the prosapogenol (**5**) with methyl iodide (CH_3I)–dimethyl sulfoxide (DMSO)–sodium hydride (NaH)[13] yielded the octa-*O*-methyl derivative (**5a**). The IR spectrum of **5a** lacked a hydroxyl absorption band, while the ^{1}H-NMR spectrum showed signals due to eight methoxyl functions. Methanolysis of **5a** provided 3,21,24-tri-*O*-methylsoyasapogenol A (**1a**)[5] from the sapogenol part and methyl 2,4-di-*O*-methylarabinopyranoside (**a**) and methyl 2,3,4-tri-*O*-methylxylopyranoside (**b**) from the sugar part. Thus, the structure of the prosapogenol (**5**) has been elucidated as 22-*O*-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl]soyasapogenol A. The structure is further supported by the glycosidation shifts[14] observed in the ^{13}C-NMR data for C-22 and C-3′ (Table II).

Methylation of soyasaponin A_4 (**2a**) with CH_3I–DMSO–NaH furnished the heptadeca-*O*-methyl derivative (**2b**), which lacked a hydroxyl absorption band but showed ester absorption bands in its IR spectrum. Lithium aluminum hydride ($LiAlH_4$) reduction of **2b** gave **2c**, which showed hydroxyl but not ester absorptions in its IR spectrum. Methanolysis of **2c** afforded 21,24-di-*O*-methylsoyasapogenol A (**1b**),[5] methyl 2,4-di-*O*-methylarabinopyranoside (**a**), methyl 2,3,4-tri-*O*-methylxylopyranoside (**b**), methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**c**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**d**), and methyl 3,4-di-*O*-methylglucopyranoside (**e**). Based on this chemical evidence combined with the ^{13}C- and ^{1}H-NMR data for **2**, **2a**, and **2c**, the structure of soyasaponin A_4 has been determined as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**2a**).

The secondary ion mass spectrum (SIMS) of acetyl-soyasaponin A_4 (**2**) gave an ion peak of m/z 1387 $(M+Na)^+$, while an ion peak of m/z 1403 $(M+K)^+$ was observed when the spectrum was taken with addition of potassium chloride. In the fast atom bombardment mass spectrum (FAB-MS) of **2**, a fragment ion peak of m/z 259 (**i**) derived from the 2,3,4-tri-*O*-acetylxylopyranosyl moiety was observed. Thus, acetyl-soyasaponin A_4 (**2**) has been shown to be a triacetyl derivative of soyasaponin A_4 (**2a**). The ^{1}H-NMR spectrum of **2** showed five one-

TABLE I. ^{1}H-NMR Data for Acetyl-soyasaponins A_4 (**2**), A_5 (**3**), and A_6 (**4**), and Soyasaponins A_4 (**2a**), A_5 (**3a**), and A_6 (**4a**), and Prosapogenol (**5**)[a]

		2a	**2**	**3a**	**3**	**4a**	**4**	**5**
Sapogenol moiety	12-H	5.21 (br s, $W_{h/2}=9.0$)	5.24 (br s, $W_{h/2}=9.0$)	5.26 (br s, $W_{h/2}=9.0$)	5.27 (br s, $W_{h/2}=9.0$)	5.22 (br s, $W_{h/2}=9.0$)	5.26 (br s, $W_{h/2}=9.0$)	5.13 (br s, $W_{h/2}=9.0$)
3-*O*-*β*-D-Glucurono-pyranosyl moiety	1‴-H	5.22 (d, $J=7.9$)	5.19 (d, $J=7.6$)	4.88 (d, $J=7.0$)	4.85 (d, $J=7.0$)	4.85 (d, $J=7.0$)	4.83 (d, $J=7.6$)	
2‴-*O*-*β*-D-Galacto-pyranosyl moiety	1⁗-H	5.45 (d, $J=7.6$)	5.47 (d, $J=7.6$)	5.42 (d, $J=7.6$)	5.40 (d, $J=7.0$)			
2‴-*O*-α-L-Arabino-pyranosyl moiety	1⁗-H					5.23 (d, $J=7.3$)	5.24 (d, $J=7.6$)	
2⁗-*O*-*β*-D-Gluco-pyranosyl moiety	1⁗′-H	5.04 (d, $J=7.3$)	5.02 (d, $J=7.6$)					
22-*O*-α-L-Arabino-pyranosyl moiety	1′-H	4.85 (d, $J=7.9$)	4.84 (d, $J=7.6$)	4.88 (d, $J=7.0$)	4.88 (d, $J=7.0$)	4.87 (d, $J=7.0$)	4.88 (d, $J=7.6$)	4.74 (d, $J=7.9$)
3′-*O*-*β*-D-Xylo-pyranosyl moiety	1″-H	5.04 (d, $J=7.3$)	5.37 (d, $J=7.9$)	5.07 (d, $J=7.6$)	5.41 (d, $J=7.0$)	5.06 (d, $J=7.6$)	5.40 (d, $J=7.6$)	4.93 (d, $J=7.3$)
	2″-H		5.35 (dd, $J=7.9$, 8.5)		5.38 (dd, $J=7.0$, 8.5)		5.37 (dd, $J=7.6$, 8.2)	
	3″-H		5.55 (dd, $J=8.5$, 8.5)		5.57 (dd, $J=8.2$, 8.5)		5.57 (dd, $J=8.2$, 8.6)	
	4″-H		5.18 (ddd, $J=5.5$, 8.2, 8.5)		5.19 (ddd, $J=5.5$, 8.2, 8.5)		5.19 (ddd, $J=5.2$, 8.6, 8.9)	
	5″-H		3.74[b] 4.35[b]		3.78[b] 4.38[b]		3.78[b] 4.40[b]	

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

proton doublets with $J=7.6$—7.9 Hz at δ 4.84, 5.02, 5.19, 5.37, and 5.47 (assignable to anomeric protons), three acetoxyl signals, and three methine proton signals at δ 5.18 (ddd, $J=$ 5.5, 8.2, 8.5 Hz), 5.35 (dd, $J=7.9$, 8.5 Hz), and 5.55 (dd, $J=8.5$, 8.5 Hz), assignable to protons geminal to an acetoxyl residue. Based on detailed decoupling experiments on **2** together with a comparison of the ^{1}H-NMR data for **2** with those for the above-described prosapogenol (**5**) and soyasaponin A_4 (**2a**), these three proton signals have been assigned as given in Table I, which shows that three acetyl residues in **2a** are attached to the terminal xylopyranosyl residue in the C-22 sugar moiety. In addition, the ^{13}C-NMR spectrum of **2** showed signals due to five anomeric carbons (δ_C 101.2, 101.5, 102.9, 104.5, 107.1). Comparison of the ^{13}C-NMR data for **2** with those for soyasaponin A_1,[4] **5**, and **2a**, has led us to assign the carbon signals as given in Table II, which clearly demonstrates that signals due to the terminal xylosyl carbons suffer acetylation shifts.[15] Consequently, the structure of acetyl-soyasaponin A_4 (**2**) has been determined to be as shown.

Acetyl-soyasaponin A_5 (3)

The IR spectrum of acetyl-soyasaponin A_5 (**3**) is very similar to that of acetyl-soyasaponin A_4 (**2**). It showed hydroxyl and ester absorption bands. Alkaline hydrolysis of **3** afforded a new saponin soyasaponin A_5 (**3a**) and acetic acid. The IR spectrum of **3a** showed hydroxyl and carboxyl absorption bands. Methanolysis of **3a** with 9% hydrogen chloride in dry methanol yielded soyasapogenol A (**1**) and methyl L-arabinoside, methyl D-xyloside, methyl D-galactoside, and methyl D-glucuronide in 1 : 1 : 1 : 1 ratio.

The ^{13}C-NMR spectrum of soyasaponin A_3 (**3a**) showed four anomeric carbon signals [δ_C 103.3 (2C), 104.9, 106.9], whereas the ^{1}H-NMR spectrum showed four anomeric proton

TABLE II. ^{13}C-NMR Data for Acetyl-soyasaponins A_4 (**2**), A_5 (**3**), and A_6 (**4**), and Soyasaponins A_4 (**2a**), A_5 (**3a**), and A_6 (**4a**), and Prosapogenol (**5**)[a]

		2a	**2**	**3a**	**3**	**4a**	**4**	**5**
Sapogenol moiety	C-3	89.4	89.4	89.5	89.5	89.4	89.5	79.3
	C-12	121.3	121.4	121.3	121.3	121.3	121.5	121.8
	C-13	142.9	143.0	142.9	142.8	142.9	143.1	143.5
	C-21	74.5	74.4	74.5	74.2	74.5	74.5	74.9
	C-22	91.3	91.1	91.4	91.0	91.3	91.3	91.9
	C-24	62.2	62.3	62.2	62.1	62.0	62.2	63.7
3-*O*-*β*-D-Glucurono-pyranosyl moiety	C-1‴	102.7	102.9	103.3	103.0	102.9	103.2	
	C-2‴	79.1	79.2	78.9	78.8	78.8	79.0	
	C-3‴	75.2[b]	75.3[b]	75.7[b]	75.1[b]	75.8[b]	75.5[b]	
	C-4‴	71.1	71.2	71.9	71.7	71.6	72.0	
	C-5‴	75.8[b]	76.0[b]	76.7[b]	76.6[b]	76.4[b]	76.7[b]	
	C-6‴	174.0	174.0	172.9	174.7	173.8	174.6	
2‴-*O*-*β*-D-Galacto-pyranosyl moiety	C-1⁗	101.4	101.5	103.3	103.2			
	C-2⁗	81.4	81.6	71.6	71.6			
	C-3⁗	72.9	73.0	73.7	73.5			
	C-4⁗	68.7	68.8	69.4	69.2			
	C-5⁗	77.1	77.2	76.2	75.5			
	C-6⁗	60.7	60.8	60.9	60.9			
2‴-*O*-*α*-L-Arabino-pyranosyl moiety	C-1⁗					103.3	103.6	
	C-2⁗					71.1	71.8	
	C-3⁗					72.9	73.3	
	C-4⁗					68.5	68.8	
	C-5⁗					66.0	66.1	
2⁗-*O*-*β*-D-Gluco-pyranosyl moiety	C-1⁗′	104.4	104.5					
	C-2⁗′	74.5	74.7					
	C-3⁗′	75.8[b]	76.0[b]					
	C-4⁗′	69.4	69.5					
	C-5⁗′	76.0[b]	76.1[b]					
	C-6⁗′	60.7	60.7					
22-*O*-*α*-L-Arabino-pyranosyl moiety	C-1′	107.0	107.1	106.9	107.1	106.6	107.3	107.6
	C-2′	71.4	71.4	71.3	71.0	71.5	71.3	71.7
	C-3′	82.8	82.5	83.1	82.5	82.7	82.7	83.6
	C-4′	67.8	67.8	67.9	67.7	67.8	68.0	68.4
	C-5′	65.8	66.0	65.9	65.9	65.7	66.1	66.4
3′-*O*-*β*-D-Xylo-pyranosyl moiety	C-1″	104.5	101.2	104.9	101.3	104.4	101.4	105.4
	C-2″	73.5	70.7	73.7	70.7	73.4	70.9	74.2
	C-3″	75.8	71.1	75.7	71.1	75.8	71.2	76.7
	C-4″	69.2	68.3	69.4	68.2	69.1	68.5	69.0
	C-5″	65.2	61.1	65.5	61.0	65.3	61.2	66.0

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

signals [δ 4.88, 2H, d, J=7.0 Hz; 5.07, 1H, d, J=7.6 Hz; 5.42, 1H, d, J=7.6 Hz]. Comparison of these carbon data with those for various methyl glycosides,[11] and the J values of anomeric proton signals, indicated that the L-arabinosyl residue is in α orientation while the D-xylosyl, D-galactosyl, and D-glucuronyl residues are in β. Furthermore, comparison of the ^{13}C-NMR data for **3a** with those for soyasaponin A_4 (**2a**) and soyasaponin A_2[5] has led us to presume that **3a** contains a β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl residue and a β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl residue attached to the 3-OH and 22-OH functions of soyasapogenol A(**1**). In order to clarify the locations of these disaccharide moieties, **3a** was subjected to photolysis[12] as carried out for soyasaponin A_4 (**2a**) to provide the same

prosapogenol (**5**) as obtained from **2a**. Thus, the 22-*O*-[β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl] residue in **3a** has been confirmed.

Methylation of soyasaponin A_5 (**3a**) with CH_3I–DMSO–NaH furnished the tetradeca-*O*-methyl derivative (**3b**), which lacked hydroxyl but showed ester absorption bands in its IR spectrum. $LiAlH_4$ reduction of **3b** gave **3c**, which showed hydroxyl but not ester absorption bands in its IR spectrum. Methanolysis of **3c** liberated 21,24-di-*O*-methylsoyasapogenol A (**1b**), methyl 2,4-di-*O*-methylarabinopyranoside (**a**), methyl 2,3,4-tri-*O*-methylxylopyranoside (**b**), methyl 3,4-di-*O*-methylglucopyranoside (**e**), and methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside (**f**).

Based on the above-mentioned chemical and spectral evidence, the structure of soyasaponin A_5 has been determined as 3-*O*-[β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**3a**).

The SIMS of acetyl-soyasaponin A_5 (**3**) showed ion peaks of m/z 1225 $(M+Na)^+$ and m/z 1241 $(M+K)^+$, respectively as observed in the case of acetyl-soyasaponin A_4 (**2**). Here again, **3** has been shown to be a triacetate of soyasaponin A_5 (**3a**). The ^{1}H-NMR spectrum of **3** showed signals ascribable to four anomeric protons at δ 4.85, 4.88, 5.40, and 5.41 (all d, J= 7.0 Hz), three acetoxyls, and three methine protons (δ 5.19, ddd, J=5.5, 8.2, 8.5 Hz; 5.38, dd, J=7.0, 8.5 Hz; 5.57, dd, J=8.2, 8.5 Hz). Comparison of these ^{1}H-NMR data with those for **2** and **3a** has led to the assignment as given in Table I, and decoupling experiments on **3** have shown that the terminal xylopyranosyl residue is fully acetylated. The ^{13}C-NMR spectrum of **3** showed signals due to four anomeric carbons (δ_C 101.3, 103.0, 103.2, 107.1) and other carbons which are assigned as shown in Table II from a comparison of the ^{13}C-NMR data with those for **2** and **3a**. Thus, the structure of acetyl-soyasaponin A_5 (**3**), having a fully acetylated xylopyranosyl moiety, has been determined.

Acetyl-soyasaponin A_6 (4)

The structure of acetyl-soyasaponin A_6 (**4**) has been determined in the same manner as described for acetyl-soyasaponins A_4 (**2**) and A_5 (**3**) described above. The IR spectrum of **4** was very similar to the spectra of **2** and **3**. Alkaline hydrolysis of **4** provided a new saponin soyasaponin A_6 (**4a**) and acetic acid.

The IR spectrum of **4a** showed the presence of hydroxyl and carboxyl functions. Methanolysis of **4a** with 9% hydrogen chloride in dry methanol afforded soyasapogenol A (**1**) and methyl L-arabinoside, methyl D-xyloside, and methyl D-glucuronide in 2 : 1 : 1 ratio. The ^{13}C- and ^{1}H-NMR spectra of **4a** showed signals due to four anomeric carbons (δ_C 102.9, 103.3, 104.4, 106.6) and four anomeric protons (δ 4.85, d, J=7.0 Hz; 4.87, d, J=7.0 Hz; 5.06, d, J=7.6 Hz; 5.23, d, J=7.3 Hz). Comparison of these ^{13}C- and ^{1}H-NMR data with those for various methyl glycosides,[11] **2a**, **3a**, and soyasaponin A_3[1] has led us to presume that soyasaponin A_6 (**4a**) has an α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl residue and a β-D-xylopyranosyl(1→3)-α-L-arabinopyranosyl residue attached to the 3-OH and the 22-OH groups, respectively, of soyasapogenol A (**1**). This presumption has been verified by photolysis[12] of **4a**, which gave the above-described prosapogenol (**5**).

Methylation of soyasaponin A_6 (**4a**) with CH_3I–DMSO–NaH furnished the trideca-*O*-methyl derivative (**4b**) which, on $LiAlH_4$ reduction, was converted to **4c**. The IR spectrum of **4c** showed hydroxyl absorption bands, but lacked any ester absorption band. Methanolysis of **4c** liberated 21,24-di-*O*-methylsoyasapogenol A (**1b**), methyl 2,4-di-*O*-methylarabinopyranoside (**a**), methyl 2,3,4,-tri-*O*-methylxylopyranoside (**b**), methyl 3,4-di-*O*-methylglucopyranoside (**e**), and methyl 2,3,4-tri-*O*-methylarabinopyranoside (**g**). Based on the chemical and ^{13}C- and ^{1}H-NMR spectral evidence, the structure of soyasaponin A_6 has been elucidated as 3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-xylopyranosyl-(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**4a**).

The SIMS of acetyl-soyasaponin A_6 (**4**), giving ion peaks of *m/z* 1195 $(M+Na)^+$ and *m/z* 1211 $(M+K)^+$, indicated **4** to be a triacetate of soyasaponin A_6 (**4a**). The ^{1}H-NMR spectrum of **4** supported the formulation, with signals assignable to four anomeric protons (δ 4.83, 4.88, 5.24, 5.40, all d of $J=7.6$ Hz) and three methine protons (δ 5.19, ddd, $J=5.2$, 8.6, 8.9 Hz; 5.37, dd, $J=7.6$, 8.2 Hz; 5.57, dd, $J=8.2$, 8.6 Hz) which are geminal to an acetoxyl residue. Comparison of ^{1}H-NMR data (Table I) together with decoupling experiments has indicated the presence of a fully acetylated terminal xylopyranosyl residue in the 22-*O*-sugar moiety. Finally, the ^{13}C-NMR data for **4** as assigned in Table II proved the structure of acetyl-soyasaponin A_6 (**4**) to be expressed as shown.

Acetyl-soyasaponins A_4 (**2**), A_5 (**3**), and A_6 (**4**) are bisdesmosides of soyasapogenol A (**1**) having a 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl (1→3)-α-L-arabinopyranosyl residue as a common disaccharide chain attached to the 22-OH function of the aglycone. It is noteworthy that aqueous solutions (0.1—0.05 w/v%) of these acetyl-soyasaponins (**2**, **3**, **4**) showed significantly bitter and astringent tastes. However, their deacetylated derivatives, soyasaponins A_4 (**2a**), A_5 (**3a**), and A_6 (**4a**), did not show such tastes.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1)]

Isolation of Soyasaponins I, II and III, and Acetyl-soyasaponins A_4 (2), A_5 (3), and A_6 (4) from Japanese Soybean——Powdered soybeans (1.8 kg, Tamanishiki strain cultivated in Hokkaido Prefecture in 1985, purchased from Fujita Shushi-ten, Osaka) were defatted with AcOEt three times (3 l each, with heating under reflux for 3 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 (160 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 (70 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 (52.6 g, sugars, amino acids, *etc.*), fr. 2 (5.5 g, flavonoids), fr. 3 (2.2 g, acetyl-soyasaponins), fr. 4 (3.0 g, soyasaponins I, II and III fractions), and fr. 5 (15.1 g, lipids). Fraction 3 was purified by column chromatography (SiO_2 200 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower phase) and by subsequent treatment with Dowex 50 W × 8 (H^+ form) with stirring at room temperature (25 °C) for 2 h. After removal of the resin by filtration, the solvent was evaporated off under reduced pressure and the residue was purified by HPLC (Shimadzu LC-6A, Shimpak ODS, 20 mm × 25 cm, H_2O : CH_3CN = 6 : 4 → 1 : 1) to afford acetyl-soyasaponins A_4 (**2**, 680 mg), A_5 (**3**, 160 mg), and A_6 (**4**, 90 mg). Fraction 4 (1 g) was subjected to 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) → $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower phase)] and each fraction of soyasaponins was treated with Dowex 50 W × 8 (H^+ form) to afford soyasaponins I (570 mg), II (250 mg), and III (25 mg).

Acetyl-soyasaponin A_4 (**2**): mp 255—258 °C (colorless fine crystals from EtOH), $[\alpha]_D^{16}$ +13.5° ($c=0.1$, MeOH). *Anal.* Calcd for $C_{64}H_{100}O_{31}\cdot 2H_2O$: C, 54.85; H, 7.48. Found: C, 54.59; H, 7.33. IR ν_{max}^{KBr} cm^{-1}: 3420, 2921, 1739, 1228. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.65, 0.84, 1.18, 1.20, 1.25, 1.26, 1.43 (3H each) (all s, *tert*-CH_3 × 7), 2.06, 2.09, 2.11 (3H each) (all s, OAc × 3), 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, 19.6 (2C), 170.0 (2C), 170.1 (acetoxyl groups), and other signals as given in Table II. SIMS, FAB-MS: as given in the text.

Acetyl-soyasaponin A_5 (**3**): mp 260—264 °C (colorless fine crystals from EtOH), $[\alpha]_D^{16}$ +9.7° ($c=0.7$, MeOH). *Anal.* Calcd for $C_{58}H_{90}O_{26}\cdot 4H_2O$: C, 54.62; H, 7.74. Found: C, 54.42; H, 7.76. IR ν_{max}^{KBr} cm^{-1}: 3380, 2921, 1749, 1228. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.68, 0.88, 1.21, 1.22, 1.28, 1.30, 1.35 (3H each) (all s, *tert*-CH_3 × 7), 2.10, 2.13, 2.14 (3H each) (all s, OAc × 3), and other signals as given in Table I. ^{13}C-NMR (125 MHz, d_5-pyridine : D_2O = 5 : 1, 25 °C), δ_C: 19.7, 19.8 (2C), 169.9 (2C), 170.0 (acetoxyl groups), and other signals as given in Table II. SIMS: as given in the text.

Acetyl-soyasaponin A_6 (**4**): mp 240—244 °C (colorless fine crystals from EtOH), $[\alpha]_D^{16}$ +14.2° ($c=0.2$, MeOH). *Anal.* Calcd for $C_{57}H_{88}O_{25}\cdot 3H_2O$: C, 55.78; H, 7.72. Found: C, 55.51; H, 7.77. IR ν_{max}^{KBr} cm^{-1}: 3380, 2908, 1745, 1229. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.68, 0.87, 1.21, 1.22, 1.28, 1.29, 1.35 (3H each) (all s, *tert*-CH_3 × 7), 2.10, 2.14, 2.15 (3H each) (all s, OAc × 3), and other signals as given in Table I. ^{13}C-NMR (125 MHz, d_5-pyridine : D_2O = 5 : 1, 25 °C), δ_C: 19.6, 19.7 (2C), 169.9, 170.0, 170.1 (acetoxyl groups), and other signals as given in Table II. SIMS: as given in the text.

Alkaline Hydrolysis of Acetyl-soyasaponin A_4 (2) Giving Soyasaponin A_4 (2a)——A solution of **2** (230 mg) was treated with 5% KOH–MeOH (6 ml) and the whole mixture was heated under reflux for 1 h. The reaction mixture was neutralized with Dowex 50 W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to yield a suspension (about 2 ml), from which a crystalline precipitate was obtained by addition of EtOH. The precipitate was collected by filtration and crystallized from EtOH to furnish soyasaponin A_4 (**2a**, 216 mg). The filtrate was subjected to GLC analysis to identify acetic acid. GLC: 1) 15% free fatty acid polyester (FFAP) on Chromosorb GAW DMCS (100—200 mesh); 3 mm × 1 m glass column; column temp. 140 °C; N_2 flow rate 25 ml/min; t_R, 5 min 15 s.

Soyasaponin A_4 (**2a**): mp 281—285 °C (colorless fine crystals), $[\alpha]_D^{16}$ +21.3° (c=0.3, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{28}\cdot 2H_2O$: C, 54.62; H, 7.74. Found: C, 54.32; H, 7.63. IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 1720, 1608, 1070. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.65, 0.82, 1.15, 1.18, 1.23, 1.26, 1.45 (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_4 (2a)——A solution of **2a** (15 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 $CHCl_3$–MeOH furnished soyasapogenol A (**1**, 5 mg), which was shown to be identical with an authentic sample[5] 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) and treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) for 1 h. The product was then analyzed by GLC to identify trimethylsilyl (TMS) derivatives of methyl arabinoside, methyl xyloside, methyl galactoside, methyl glucuronide, and methyl glucoside. The composition of these five methyl glycosides was determined from the GLC peak areas. GLC: 2) 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 xyloside 5 min 25 s, 5 min 57 s, TMS-methyl galactoside 10 min 42 s, 12 min 15 s, 14 min 20 s, TMS-methyl glucuronide 7 min 22 s, 16 min 41 s, TMS-methyl glucoside 14 min 54 s, 18 min 5 s. 3) 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 xyloside 5 min 45 s, 6 min 15 s, TMS-methyl galactoside 11 min 30 s, 13 min 12 s, 15 min 28 s, TMS-methyl glucuronide 7 min 43 s, 18 min 9 s, TMS-methyl glucoside 16 min 25 s, 19 min 42 s.

Photolysis of Soyasaponin A_4 (2a)——A solution of **2a** (190 mg) in MeOH (30 ml) in a Vycor tube was irradiated externally with a 500 W high-pressure mercury lamp (Eikosha, PIH-500) for 5 h while keeping the solution temperature below 10 °C. The reaction mixture was neutralized with 10% aqueous K_2CO_3, then 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 10 g, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1, lower phase) and crystallized from aqueous MeOH to furnish the prosapogenol (**5**, 45 mg). **5**: mp 245—248 °C (colorless fine crystals), $[\alpha]_D^{16}$ +16.9° (c=0.2, MeOH). *Anal.* Calcd for $C_{40}H_{66}O_{12}\cdot 2H_2O$: C, 61.99; H, 9.10. Found: C, 61.10; H, 8.80. IR ν_{max}^{KBr} cm^{-1}: 3390, 2910, 1074. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.55, 0.74, 1.08, 1.10, 1.15, 1.16, 1.33 (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 Prosapogenol (5)——A solution of **5** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. The aglycone, which was obtained by work-up of the reaction mixture as described above for the methanolysis of **2a**, was shown to be identical with soyasapogenol A (**1**) by TLC comparison (as described above). The sugar portion was worked up and analyzed by GLC (as TMS derivatives), as described above for the methanolysis product of **2**, to identify methyl arabinoside and methyl xyloside in 1 : 1 ratio.

Methylation of Prosapogenol (5)——A solution of **5** (15 mg) in DMSO (2 ml) was treated with dimsyl carbanion[13] (2 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, *n*-hexane : acetone = 4 : 1) and by crystallization from MeOH to furnish **5a** (13 mg). **5a**: mp 172—175 °C (colorless fine crystals), $[\alpha]_D^{16}$ +21.2° (c=0.2, $CHCl_3$). *Anal.* Calcd for $C_{48}H_{82}O_{12}$: C, 67.73; H, 9.71. Found: C, 67.71; H, 10.01. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2920, 1455, 1315, 1096. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.98 (12H), 1.12 (6H), 1.26 (3H) (all s, *tert*-CH_3 × 7), 3.26 (3H), 3.34, 3.46 (6H each), 3.54 (3H), 3.60 (6H) (all s, OCH_3 × 8), 4.56 (1H, d, J=7.0 Hz, anomeric H),[16] 5.22 (1H, br s, $W_{h/2}$=7.0 Hz, 12-H).

Methanolysis of 5a——A solution of **5a** (7 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. After cooling, the precipitate was collected by filtration and crystallized from $CHCl_3$–MeOH to afford 3,21,24-tri-*O*-methylsoyasapogenol A (**1a**, 3 mg), which was shown to be identical with an authentic sample[5] by TLC [benzene : acetone = 15 : 1, *n*-hexane : acetone = 8 : 1, *n*-hexane : AcOEt = 4 : 1], mixed melting point determination, and IR ($CHCl_3$) spectral comparison. The filtrate was neutralized with Ag_2CO_3 powder and the inorganic precipitate was

removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the product was subjected to TLC and GLC analyses to identify methyl 2,4,-di-*O*-methylarabinopyranoside (**a**) and methyl 2,3,4-tri-*O*-methylxylopyranoside (**b**). TLC: benzene : acetone = 3 : 1, *n*-hexane : acetone = 2 : 1, *n*-hexane : AcOEt = 1 : 2. GLC: 4) 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** 5 min 55 s, 6 min 16 s (major), **b** 1 min 31 s, 1 min 48 s (major); 5) 15% neopentyl glycol succinate (NPGS) 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** 7 min 21 s, **b** 2 min 16 s, 2 min 48 s (major).

Methylation of Soyasaponin A_4 (2a)——A solution of **2a** (15 mg) in DMSO (2 ml) was treated with dimsyl carbanion (2 ml) and the whole mixture was stirred at 20 °C under a nitrogen atmosphere for 1 h. The reaction mixture was then treated with CH_3I (2 ml) with stirring in the dark for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract as described above for the methylation of **5**, the product was purified by column chromatography (SiO_2 3 g, *n*-hexane : acetone = 4 : 1) and subsequently by crystallization from MeOH to furnish **2b** (13 mg). **2b**: mp 114—116 °C (colorless fine crystals), $[\alpha]_D^{16}$ −5.1 ° (c = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{75}H_{128}O_{28}$: C, 60.96; H, 8.73. Found: C, 60.59; H, 8.96. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2926, 1750, 1086. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.94 (12H), 1.08 (6H), 1.15 (3H) (all s, *tert*-CH_3 × 7), 3.29 (3H), 3.37, 3.43 (6H each), 3.45 (9H), 3.48 (6H), 3.52, 3.55 (3H each), 3.59 (6H), 3.62, 3.63 (3H each) (all s, OCH_3 × 16), 3.78 (3H, s, $COOCH_3$), 4.33, 4.55 (1H each), 4.62 (2H) (all d, J = 7.0 Hz, anomeric H × 4)[16] 5.23 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

$LiAlH_4$ Reduction of 2b——A solution of **2b** (12 mg) in dry ether (3 ml) was treated with a suspension of $LiAlH_4$ (20 mg) in dry ether (2 ml) and the whole mixture was stirred at 16 °C for 30 min. After decomposition of excess $LiAlH_4$ with wet ether, the reaction mixture was made weakly acidic with 5% aqueous H_2SO_4 and the whole was extracted with ether. Work-up of the ether extract in the usual manner and removal of the solvent under reduced pressure furnished **2c** (11 mg). **2c**: a white powder, $[\alpha]_D^{20}$ −3.3 ° (c = 0.6, $CHCl_3$). *Anal.* Calcd for $C_{74}H_{128}O_{27}$: C, 61.31; H, 8.90. Found: C, 61.19; H, 9.13. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3602, 2922, 1076. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.98 (12H), 1.09 (6H), 1.19 (3H) (all s, *tert*-CH_3 × 7), 3.30 (3H), 3.38 (6H), 3.40, 3.43 (3H each), 3.46 (9H), 3.49 (3H), 3.53 (6H), 3.56 (3H), 3.60 (6H), 3.63, 3.66 (3H each) (all s, OCH_3 × 16), 4.28, 4.55 (1H each), 4.66 (2H) (all d, J = 7.0 Hz, anomeric H × 4),[16] 5.22 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

Methanolysis of 2c——A solution of **2c** (10 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h, then cooled. The precipitate was collected by filtration and purified by crystallization from MeOH to furnish 21,24-di-*O*-methylsoyasapogenol A (**1c**, 3 mg), which was shown to be identical with an authentic sample[5] by TLC [benzene : acetone = 3 : 1, *n*-hexane : acetone = 2 : 1, *n*-hexane : AcOEt = 2 : 1], mixed melting point determination, and IR ($CHCl_3$) spectral comparison. The filtrate was neutralized with Ag_2CO_3 powder and worked up as described above. The product was subjected to TLC (as described for **5a**) and GLC [conditions 4 and 5] analyses to determine the composition as **a**, **b**, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**c**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**d**), and methyl 3,4-di-*O*-methylglucopyranoside (**e**). GLC: 4) t_R: **a**, **b** (as described for **5a**), **c** 2 min 33 s, 3 min 22 s (major), **d** 9 min 43 s (major), 15 min 30 s, **e** 19 min 22 s (major), 23 min 46 s. 5) t_R: **a**, **b** (as described for **5a**), **c** 4 min 11 s, 5 min 29 s (major), **d** 12 min 34 s (major), 18 min 30 s, **e** 21 min 42 s (major), 24 min 50 s.

Alkaline Hydrolysis of Acetyl-soyasaponin A_5 (3) Giving Soyasaponin A_5 (3a)——A solution of **3** (120 mg) in a mixture of H_2O (1 ml) and 5% KOH–MeOH (4 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Dowex 50 W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was worked up as described above for the alkaline hydrolysis of **2**. The product, obtained from the precipitate, was crystallized from EtOH to afford soyasaponin A_5 (**3a**, 116 mg). The filtrate was analyzed by GLC (condition 1) to identify acetic acid.

Soyasaponin A_5 (**3a**): mp 276—279 °C (colorless fine crystals), $[\alpha]_D^{16}$ +19.6 ° (c = 0.4, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{23} \cdot 3H_2O$: C, 55.21; H, 8.02. Found: C, 55.29; H, 7.71. IR ν_{max}^{KBr} cm^{-1}: 3380, 2900, 1716, 1592, 1042. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C) δ: 0.69, 0.87, 1.18, 1.22, 1.28, 1.29, 1.36 (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_5 (3a)——A solution of **3a** (15 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1 h. The aglycone, which was obtained by work-up of the reaction mixture as described above for the methanolysis of **2a**, was shown to be identical with soyasapogenol A(**1**, 4 mg) by TLC (as described for **2a**), mixed melting point determination, and IR (KBr) spectral comparison. The sugar portion was worked up and analyzed by GLC as the TMS derivatives (conditions 2 and 3), as described above for the methanolysis products of **2a**, to identify methyl arabinoside, methyl xyloside, methyl galactoside, and methyl glucuronide in 1 : 1 : 1 : 1 ratio.

Photolysis of Soyasaponin A_5 (3a)——A solution of **3a** (60 mg) in MeOH (20 mg) in a Vycor tube was irradiated externally with a 500 W high-pressure mercury lamp for 4 h and worked up as described above for the photolysis of **2a**. The reaction product was partitioned into 1-BuOH : H_2O (1 : 1). Work-up of the 1-BuOH phase in the usual manner gave the product, which was purified by column chromatography (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1, lower phase) to furnish **5** (15 mg). **5** thus obtained was shown to be identical with an authentic sample which was obtained above by photolysis of **2a**, by TLC [$CHCl_3$: MeOH : H_2O = 10 : 3 : 1 (lower phase), 1-BuOH : AcOEt : H_2O = 1 : 1 : 1 (upper phase)], mixed melting point determination, and IR (KBr) spectral comparison.

Methylation of Soyasaponin A_5 (3a)——A solution of **3a** (30 mg) in DMSO (3 ml) was treated with dimsyl

carbanion (3 ml) and the whole mixture was stirred at 20 °C under a nitrogen atmosphere for 1 h, then treated with CH_3I (3 ml) with stirring in the dark for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above for the methylation of **5** gave the product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane : acetone = 3 : 1) and subsequently by crystallization from MeOH to furnish **3b** (17 mg). **3b**: mp 125—128 °C (colorless fine crystals), $[\alpha]_D^{16}$ −0.3° (*c* = 0.8, $CHCl_3$). *Anal.* Calcd for $C_{64}H_{112}O_{23}$: C, 61.52; H, 9.03. Found: C, 61.29; H, 9.28. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2924, 1746, 1093. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.94 (12H), 1.08 (6H), 1.16 (3H) (all s, *tert*-CH_3 × 7), 3.28 (3H), 3.36, 3.46 (6H each), 3.49 (9H), 3.53 (3H), 3.59 (9H), 3.62 (3H) (all s, OCH_3 × 13), 3.78 (3H, s, $COOCH_3$), 4.39, 4.55, 4.62 (1H each, all d, *J* = 7.0 Hz, anomeric H × 3),[16] 5.21 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

$LiAlH_4$ Reduction of 3b——A solution of **3b** (15 mg) in dry ether was treated with a suspension of $LiAlH_4$ (30 mg) in dry ether (2 ml) and the whole mixture was stirred at 18 °C for 30 min. After quenching of the reaction with wet ether, the reaction mixture was worked up as described above for the reduction of **2b** to furnish **3c** (13 mg). **3c**: a white powder, $[\alpha]_D^{18}$ +4.7° (*c* = 0.8, $CHCl_3$). *Anal.* Calcd for $C_{63}H_{112}O_{22}$: C, 61.94; H, 9.24. Found: C, 61.61; H, 9.29. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3601, 2925, 1095. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.95 (9H), 1.01 (3H), 1.08 (6H), 1.14 (3H) (all s, *tert*-CH_3 × 7), 3.29 (3H), 3.36, 3.46, 3.50, 3.54 (6H each), 3.60 (9H), 3.64 (3H) (all s, OCH_3 × 13), 4.35, 4.56, 4.64 (1H each, all d, *J* = 7.0 Hz, anomeric H × 3),[16] 5.22 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

Methanolysis of 3c——A solution of **3c** (10 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. The reaction mixture was worked up as described above for the methanolysis of **2c**. The product, obtained as a precipitate, was crystallized from MeOH to afford 21,24-di-*O*-methylsoyasapogenol A (**1b**, 4 mg) which was shown to be identical with an authentic sample[5] by TLC (as described for **2c**), mixed melting point determination, and IR ($CHCl_3$) spectral comparison. The other product, obtained from the filtrate, was proved to comprise **a**, **b**, **e**, and methyl 2,3,4,6-tetra-*O*-methylgalactopyranoside (**f**) by TLC (as described for **2c**) and GLC [conditions 4 and 5] analyses. GLC: 4) t_R: **a**, **b**, **e** (as described for **2c**), **f** 4 min 20 s. 5) **a**, **b**, **e** (as described for **2c**), **f** 6 min 20 s.

Alkaline Hydrolysis of Acetyl-soyasaponin A_6 (4) Giving Soyasaponin A_6 (4a)——A solution of **4** (80 mg) in a mixture of H_2O (1 ml) and 5% KOH–MeOH (3 ml) was heated under reflux for 1 h. The reaction mixture was worked up as described above for the alkaline hydrolysis of **2**. The product, obtained as a precipitate, was purified by crystallization from EtOH to afford soyasaponin A_6 (**4a**, 75 mg). The filtrate was analyzed by GLC [condition 1] to identify acetic acid.

Soyasaponin A_6 (**4**): mp 282—285 °C (colorless fine crystals), $[\alpha]_D^{16}$ +20.2° (*c* = 0.3, MeOH). *Anal.* Calcd for $C_{51}H_{82}O_{22} \cdot 3H_2O$: C, 55.64; H, 8.05. Found: C, 55.30; H, 7.74. IR ν_{max}^{KBr} cm^{-1}: 3407, 2911, 1719, 1609, 1070. ^{1}H-NMR (500 MHz, d_5-pyridine : D_2O = 10 : 1, 25 °C), δ: 0.69, 0.85, 1.17, 1.20, 1.25, 1.28, 1.38 (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_6 (4a)——A solution of **4a** (12 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1 h. Work-up of the reaction mixture as described above for the methanolysis of **2a** furnished soyasapogenol A (**1**, 3 mg) which was shown to be identical with an authentic sample[3] by TLC (as described above for **2a**), mixed melting point determination, and IR (KBr) spectral comparison. The sugar portion was worked up and analyzed by GLC [conditions 2 and 3] as the TMS derivatives, as described above for the methanolysis products of **2a**, and the products were identified as methyl arabinoside, methyl xyloside, and methyl glucuronide in 2 : 1 : 1 ratio.

Photolysis of Soyasaponin A_6 (4a)——A solution of **4a** (30 mg) in MeOH (10 ml) in a Vycor tube was irradiated and worked up as described above for the photolysis of **2a**. The reaction product was purified by column chromatography (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1, lower phase) to furnish **5** (6 mg) which was shown to be identical with an authentic sample by TLC (as described above for **3a**), mixed melting point determination, and IR (KBr) spectral comparison.

Methylation of Soyasaponin A_6 (4a)——A solution of **4a** (20 mg) in DMSO (2 ml) was treated with dimsyl carbanion (2 ml) and the whole mixture was stirred at 20 °C under a nitrogen atmosphere for 1 h. The reaction mixture was then treated with CH_3I (2 ml) with stirring in the dark for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above for the methylation of **2a** gave the product, which was purified by column chromatography (SiO_2 10 g, benzene : acetone = 5 : 1) to furnish **4b** (10 mg). **4b**: a white powder, $[\alpha]_D^{16}$ +6.2° (*c* = 0.3, $CHCl_3$). *Anal.* Calcd for $C_{62}H_{108}O_{22}$: C, 61.77; H, 9.03. Found: C, 61.49; H, 9.13. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2923, 1748, 1098. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.93 (12H), 1.08 (6H), 1.17 (3H) (all s, *tert*-CH_3 × 7), 3.23, 3.37 (6H each), 3.46 (9H), 3.48, 3.54 (3H each), 3.58 (6H, 3.60 (3H) (all s, OCH_3 × 12), 3.79 (3H, s, $COOCH_3$), 4.39, 4.56, 4.61 (1H each) (all d, *J* = 7.0 Hz, anomeric H × 3),[16] 5.21 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

$LiAlH_4$ Reduction of 4b——A solution of **4b** (10 mg) in dry ether (3 ml) was treated with a suspension of $LiAlH_4$ (30 mg) in dry ether (2 ml) and the whole mixture was stirred at 18 °C for 30 min. After quenching of the reaction with wet ether, the reaction mixture was worked up as described above for the reduction of **2b** to furnish **4c** (9 mg). **4c**: a white powder, $[\alpha]_D^{20}$ +5.4° (*c* = 0.8, $CHCl_3$). *Anal.* Calcd for $C_{61}H_{108}O_{21}$: C, 62.22; H, 9.24. Found: C, 62.44; H, 9.11. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 2927, 1089. ^{1}H-NMR (90 MHz, $CDCl_3$), δ: 0.93 (12H), 1.01 (3H), 1.08 (6H) (all s, *tert*-CH_3 × 7), 3.24, 3.56 (6H each), 3.46 (9H), 3.48, 3.54 (3H each), 3.58 (6H), 3.61 (3H) (all s, OCH_3 × 12), 4.37, 4.54, 4.60 (1H each) (all d, *J* = 7.0 Hz, anomeric H × 3),[16] 5.21 (1H, br s, $W_{h/2}$ = 7.0 Hz, 12-H).

Methanolysis of 4c——A solution of **4c** (8 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h. Work-up of the reaction mixture as described above for the methanolysis of **2c** provided **1b** (2 mg) as colorless needles. **1b** was shown to be identical with an authentic sample[5] by TLC (as described above for **2c**) and mixed melting point determination. The mother liquor, after removal of the aglycone by filtration, furnished **a**, **b**, **e** and methyl 2,3,4,-tri-*O*-methyl-arabinopyranoside (**g**) as the methylated sugars, which were identified by TLC (as described for **2c**) and GLC [conditions 4 and 5]. GLC: 4) t_R: **a**, **b**, **e** (as described for **2c** and **3c**), **g** 2 min 37 s, 2 min 59 s (major). 5) t_R: **a**, **b**, **e** (as described for **2c** and **3c**), **g** 3 min 59 s.

References and Notes

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9) *a*) In the 1-butanol-soluble portion, a part of soyasaponins I, II, and III was noticed on TLC to be in partially acetylated forms. However, they were deacetylated readily during the isolation procedure. *b*) TLC and HPLC indicated the presence of a minor quantity of soyasaponins IV and V[7] and partially deacetylated acetyl-soyasaponins A_4 (**2**), A_5 (**3**), and A_6 (**4**). Although soyasaponins IV and V were characterized later, the partially deacetylated **2**, **3**, and **4** were not isolated. *c*) Since acetyl-soyasaponins were recognized to be partially in the carboxylate forms from their IR (KBr) spectra, the treatment with Dowex 50W × 8 (H^+ form) was carried out.
10) The D or L form of these methyl glycosides was assigned to be the same as in hitherto elucidated soyasaponins.[2-5]
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16) One anomeric proton signal was missing due to overlapping with other signals.