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Saponin and Sapogenol. XIII.[1] Structures of Three Soybean Saponins: Soyasaponin I, Soyasaponin II, and Soyasaponin III

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Soyasaponin I (major), II and III, all of which possess soyasapogenol B (**2**) as the common aglycone, have been isolated from the MeOH extractive of soybean (*Glycine max* Merrill, Leguminosae). On the basis of chemical and physicochemical evidence, the structure of soyasaponin I has been elucidated to be 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**7**) and the structures of soyasaponin II and III to be 3-O-[α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl (1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**8**) and 3-O-[β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**9**), respectively.

Since the toxic principle of alfalfa (*Medicago sativa* L.), which has been known as the important feed for farm animals, was announced to be saponin, the saponins of soybean (*Glycine max* Merrill) have also evoked considerable attention, and several physiological activities of the soybean saponins (mixture) such as hemolytic, piscicidal, and insecticidal properties, have been reported.[3,4] As for the chemical studies on the soybean saponins, five sapogenols named soyasapogenol A (**1**),[5,6] B (**2**),[5,6] C (**3**),[5,6] D (**4**),[5] and E (**5**)[7] have been elucidated. However, no report on the structure elucidation of the saponins has been provided, but only the carbohydrate ingredients of saponin mixture have been revealed to be glucuronic acid, galactose, glucose, arabinose, xylose, and rhamnose.[3,8]

Recently, we reported the isolation of three soybean saponins designated as soyasaponin I (major), II, and III[9] and the structure elucidation of a prosapogenol (**6a**) which was obtained by mild acid hydrolysis of soyasaponin I.[10] In the same report, we described that both **6a** and soyasaponin I liberated soyasapogenol B (**2**) upon ultraviolet light irradiation in a quartz tube, and also reported that the similar photochemical cleavage was also observed for desacyljegosaponin which possesses in the oligosaccharide portion a glucuronopyranoside moiety directly attached to the sapogenol (barringtogenol C).[11] The present paper deals with the full account on the structure elucidation of soyasaponin I (**7**), II (**8**), and III (**9**).[12]

1) Part XII: I. Kitagawa, A. Inada, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 2268 (1975).
2) Location: *133-1, Yamada-kami, Suita, Osaka, 565, Japan.*
3) I.E. Liener, "Toxic Constituents of Plant Foodstuffs," Academic Press, New York, 1969, p. 169.
4) *a*) I. Ishaaya, Y. Birk, A. Bondi, and Y. Tencer, *J. Sci. Food Agr.*, **20**, 433 (1969) [*C. A.*, **71**, 89173 (1969)]; *b*) H.C.F. Su, R.D. Speirs, and P.G. Mahany, *J. Econ. Entomol.*, **65**, 844 (1972) [*C. A.*, **77**, 84473 (1972)].
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8) *a*) A.C. Eldridge and W.J. Wolf, *Cereal Chem.*, **46**, 344 (1969) [*C. A.*, **71**, 87767 (1969)]; *b*) W.J. Wolf and B.W. Thomas, *J. Amer. Oil Chem. Soc.*, **47**, 86 (1970) [*C. A.*, **72**, 118305 (1970)]; *c*) W.J. Wolf and B.W. Thomas, *J. Chromatog.*, **56**, 281 (1971).
9) Muramatsu designated the crude soybean saponin as hispidic acid: S. Muramatsu, *Nippon Kagaku Zasshi*, **44**, 1035 (1923).
10) *a*) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, **1973**, 3997; *b*) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1339 (1974).
11) I. Kitagawa, Y. Imakura, T. Hayashi, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 1675, 3009 (1974).
12) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 3010 (1974) (preliminary report).

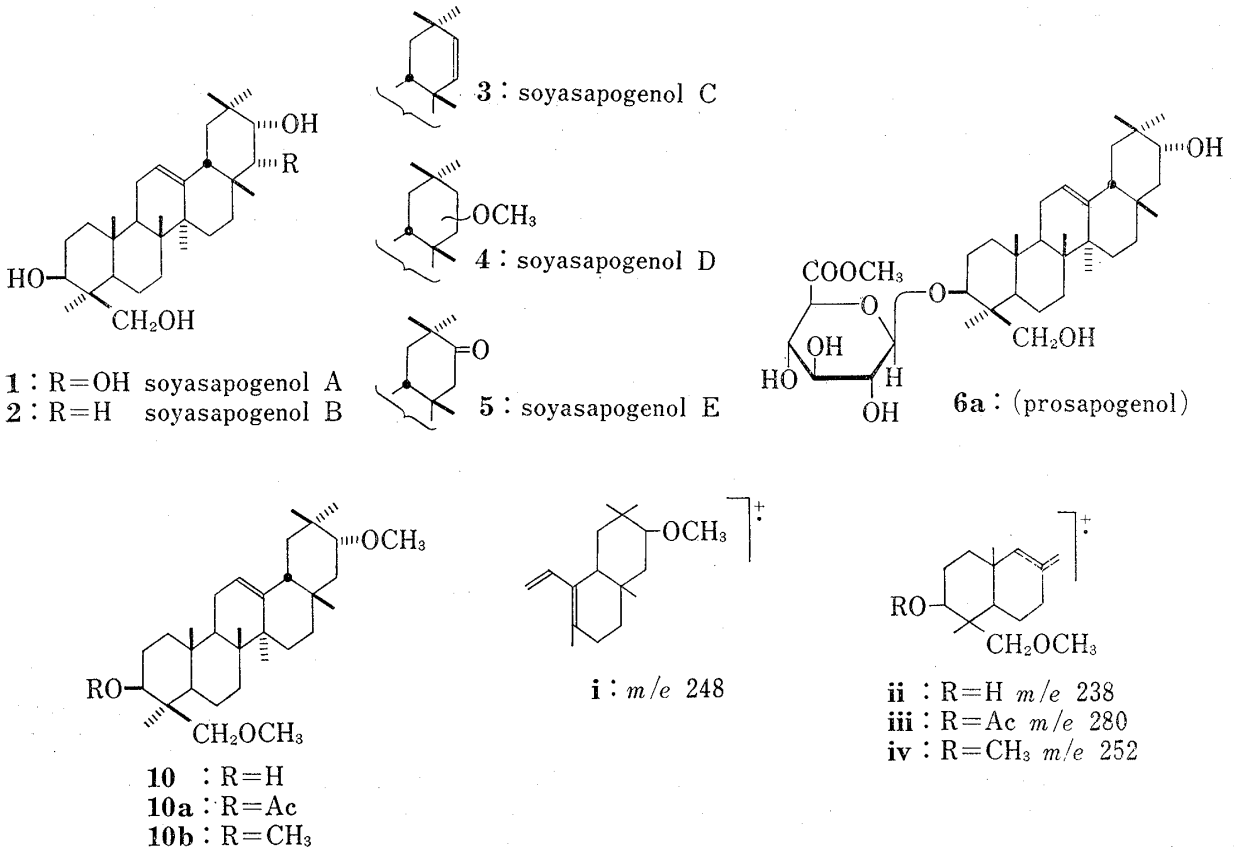


Chart 1

Soyasaponin I (7)

Due to the complicated composition, the isolation of pure soybean saponins had not yet been effected before our recent work[10] although several attempts by paper or column (using alumina or ionic resin) chromatography had been made.[3,8] On acid hydrolysis, the saponin mixture, which was isolated from the MeOH extractive of soybean as reported previously,[10] furnished soyasapogenol A (**1**), B (**2**, major), C (**3**), D (**4**, minor), and E (**5**, minor),[13] while brief treatment of the saponin mixture with *n*-BuOH and weak alkali followed by silica gel column chromatography afforded soyasaponin I (**7**), II (**8**), and III (**9**), all of which carry soyasapogenol B (**2**) as the common aglycone.[10] Since soyasaponin I thus obtained was still contaminated with the carboxylate form (infrared (IR) spectrum (Nujol): 1610 cm^{-1}), the pure sample of soyasaponin I was obtained by treatment with weakly acidic MeOH followed by recrystallization from MeOH.[14]

The IR spectrum (Nujol) of soyasaponin I (**7**) shows the presence of hydroxyl (3400 (br) cm^{-1}) and carboxyl (1710 cm^{-1}). Acid hydrolysis of soyasaponin I yielded soyasapogenol B (**2**), rhamnose, galactose, and an uronic acid, the latter being confirmed to be glucuronic acid by the evidence described below.

Methylation of **7** with diazomethane gave soyasaponin I methyl ester (**7a**), which possesses the hydroxyl (IR (Nujol): 3300 (br) cm^{-1}) and the methoxycarbonyl function (IR: 1740 cm^{-1}; proton magnetic resonance (PMR) spectrum: δ 3.73 (d_5-pyridine)). Reduction of **7a** with

13) As for the genuineness of soyasapogenol C, D, and E, the further examination seems to be needed.

14) In the previous paper,[10] the brief treatment with weakly acidic MeOH was undertaken prior to silica gel column chromatography (giving soyasaponin I, II, and III). However, in order to avoid the ambiguity on the genuineness of soyasaponin III (**9**), which is closely related to one of the partial hydrolysates of soyasaponin I (**7**), the acid treatment was done after separating soyasaponin I, II, and III by column chromatography.

$NaBH_4$ gave **7b** (IR: no ester carbonyl), which on subsequent acid hydrolysis liberated **2**, rhamnose, galactose, and glucose.

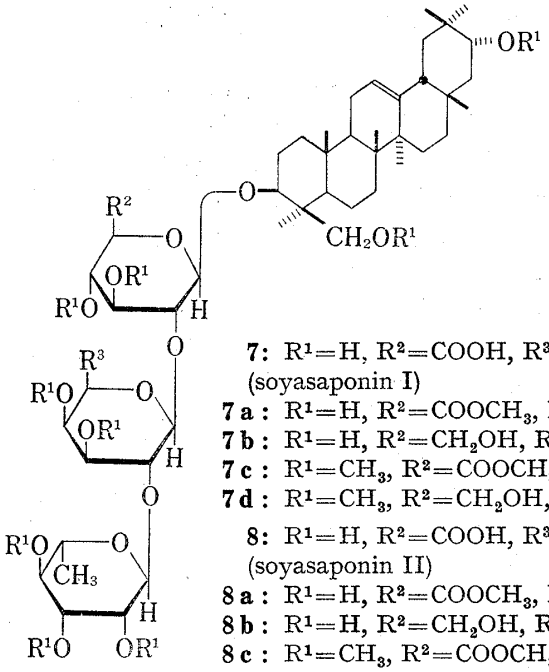
Methylation of **7** with CH_3I/DMSO/NaH[15] gave the undeca-O-methyl derivative (**7c**), which possesses no hydroxyl but the ester carbonyl function (IR (CCl_4): 1756 cm^{-1}). The PMR spectrum of **7c** (C_6D_6) shows three anomeric proton signals at δ 4.44 (d, J=7 Hz), δ 4.89 (d, J=7 Hz), and δ 5.68 (s), which suggest that glucuronic acid and galactose in **7** connect with β-orientation (Cl form). $LiAlH_4$ reduction of **7c** gave **7d** (IR (CCl_4): 3590 (w) cm^{-1}, no ester carbonyl), which on methanolysis liberated methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, and methyl 3,4-di-O-methyl-glucopyranoside as identified by gas-liquid and thin-layer chromatography (GLC and TLC). Concomitantly yielded methylated aglycone (**10**) possesses two methoxyls as shown by the PMR spectrum (δ 3.26 and δ 3.31) and gives in the mass spectrum the prominent fragment ion peaks at *m/e* 248 (**i**, base peak) and *m/e* 238 (**ii**), both of which are derived through the characteristic reverse Diels-Alder type fragmentation of the ring C.[16,17] The monoacetate (**10a**), prepared by acetylation of **10**, shows no hydroxyl absorption band in its IR spectrum (in CCl_4, acetate: 1730, 1240 cm^{-1}). The PMR spectrum of **10a** shows a triplet-like signal at δ 4.56 due to 3α-H geminal to 3β-OAc.[17] The mass spectrum of **10a** gives the base peak **i** and the prominent fragment ion peak **iii** (*m/e* 280), while that of 3, 16, 24-tri-O-methyl-soyasapogenol B (**10b**), prepared from **2** by methylation, gives the same base peak **i** and the prominent fragment ion peak **iv** (*m/e* 252).[18] Consequently, the methylated aglycone obtained by methanolysis of **7d** is now disclosed to be 21,24-di-O-methyl-soyasapogenol B (**10**) and the carbohydrate portion of soyasaponin I has been shown to be linked to 3β-OH of soyasapogenol B (**2**) in the order of glucuronic acid, galactose, and rhamnose (nonreducing terminal end).

On the other hand, the PMR signal observed at δ 2.81 (d.d, J=3.5 and 6.0 Hz) in **10** or at δ 2.82 (d.d, J=3.5 and 6.0 Hz) in **10a** supports spectroscopically the 21α-OH configuration in soyasapogenol B (**2**), which was hitherto estimated from the chemical evidence.[6]

Mild methanolic acid hydrolysis of soyasaponin I (**7**) yielded **2**, **6a**, and another prosapogenol (**9a**) which possesses the hydroxyl (IR (Nujol): 3350 (br) cm^{-1}) and the methoxycarbonyl function (IR: 1730 cm^{-1}; PMR (d_5-pyridine): δ 3.75), the latter being formed as in **6a** during the methanolic acid treatment. Acid hydrolysis of **9a** gave **2**, galactose and glucuronic acid (further confirmed by acid hydrolysis of **9b**). The nona-O-methyl derivative (**9c**), prepared by methylation of **9a**, shows no hydroxyl but the ester carbonyl absorption band (1754 cm^{-1}) in its IR spectrum (CCl_4). In the PMR spectrum of **9c**, are observed two anomeric proton signals at δ 4.39 and δ 4.59 (each d, J=7 Hz). $LiAlH_4$ reduction of **9c** gave **9d**, which shows no ester carbonyl but the weak hydroxyl absorption band (3640 cm^{-1}) in its IR spectrum (CCl_4) and liberated, on methanolysis, **10**, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, and methyl 3,4-di-O-methyl-glucopyranoside (GLC, TLC).

The accumulated evidence described above has led us to express soyasaponin I as 3-O-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**7**) in which the terminal α-L-rhamnopyranoside linkage has been substantiated by application of the Klyne's rule[19]: $[M]_D$ (**7a**)$-[M]_D$(**9a**) $=-145.1°$[20]; $[M]_D$ (methyl α-L-rhamno-

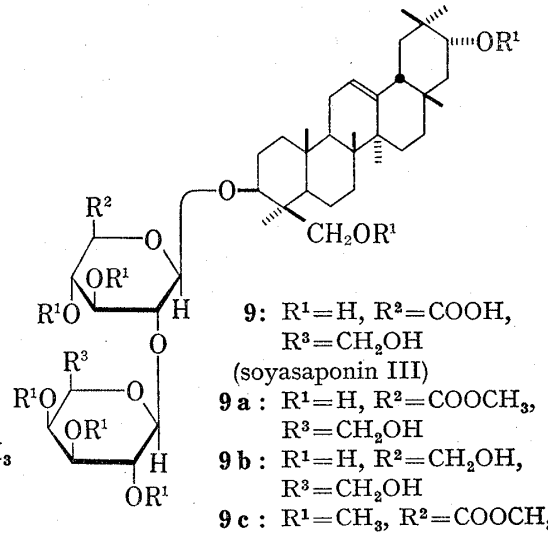
15) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
16) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 121.
17) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).
18) The ordinary reverse Diels-Alder type fragmentation at ring C of **10**, **10a**, or **10b** is expected to give the ion peak at *m/e* 237 (**ii**-H), *m/e* 279 (**iii**-H), or *m/e* 251 (**iv**-H), however, in the mass spectrum of **10**, **10a**, or **10b**, is observed the ion peak **ii**, **iii**, or **iv** as in the case of protobassic acid: I. Yosioka, A. Inada, and I. Kitagawa, *Tetrahedron*, **30**, 707 (1974).
19) W. Klyne, *Biochem. J.*, **47**, xli (1950).
20) The value was reported as $-538°$ in our preliminary report,[12] however, we wish to reform it as given here with cordial apology.



7: R^1=H, R^2=COOH, R^3=CH_2OH (soyasaponin I)
7a: R^1=H, R^2=$COOCH_3$, R^3=CH_2OH
7b: R^1=H, R^2=CH_2OH, R^3=CH_2OH
7c: R^1=CH_3, R^2=$COOCH_3$, R^3=CH_2OCH_3
7d: R^1=CH_3, R^2=CH_2OH, R^3=CH_2OCH_3

8: R^1=H, R^2=COOH, R^3=H (soyasaponin II)
8a: R^1=H, R^2=$COOCH_3$, R^3=H
8b: R^1=H, R^2=CH_2OH, R^3=H
8c: R^1=CH_3, R^2=$COOCH_3$, R^3=H
8d: R^1=CH_3, R^2=CH_2OH, R^3=H

Chart 2



9: R^1=H, R^2=COOH, R^3=CH_2OH (soyasaponin III)
9a: R^1=H, R^2=$COOCH_3$, R^3=CH_2OH
9b: R^1=H, R^2=CH_2OH, R^3=CH_2OH
9c: R^1=CH_3, R^2=$COOCH_3$, R^3=CH_2OCH_3
9d: R^1=CH_3, R^2=CH_2OH, R^3=CH_2OCH_3
11a: R^1=H, R^2=$COOCH_3$, R^3=H

Chart 3

pyranoside)=−109°; $[M]_D$ (methyl β-L-rhamnopyranoside)=+169°.[21]

Soyasaponin II (8)

Since the soyasaponin II fraction obtained after silica gel column chromatography (*vide supra*) yet contained the carboxylate counterpart (IR), it was treated with ionic resin (H^+) before recrystallization. The IR spectrum (Nujol) of soyasaponin II (**8**) shows the hydroxyl (3400 (br) cm^{-1}) and carboxyl (1720 cm^{-1}) absorption bands. Acid hydrolysis of soyasaponin II gave soyasapogenol B (**2**), rhamnose, arabinose,[22] and an uronic acid, whereas acid hydrolysis of the reduction product (**8b**), which was obtained by $NaBH_4$ treatment of soyasaponin II methyl ester (**8a**), furnished **2**, rhamnose, arabinose, and glucose, thus the presence of glucuronic acid in soyasaponin II (**8**) being confirmed.

Deca-O-methyl derivative (**8c**), obtained by CH_3I/DMSO/NaH treatment of **8**, shows no hydroxyl but the ester carbonyl absorption band (1760 cm^{-1}) in its IR spectrum (CCl_4). The PMR spectrum (C_6D_6) of **8c** indicates three anomeric proton signals at δ 4.47 (d, J=8 Hz), δ 4.88 (d, J=6 Hz), and δ 5.44 (s) and suggests the presence of β-D-glucuronopyranoside and α-L-arabinopyranoside linkages in soyasaponin II (**8**). $LiAlH_4$ reduction of **8c** gave **8d** (IR (CCl_4): no ester carbonyl, 3620 (w) cm^{-1}), which on subsequent methanolysis liberated 21,24-di-O-methyl-soyasapogenol B (**10**), methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 3,4-di-O-methyl-arabinopyranoside, and methyl 3,4-di-O-methyl-glucopyranoside (identified as above).

Mild treatment of soyasaponin II (**8**) with methanolic sulfuric acid afforded **2**, **6a**, and another prosapogenol (**11a**) which carries the hydroxyl and ester function (IR (Nujol): 3350 (br), 1743 cm^{-1}). Acid hydrolysis of **11a** yielded **2**, arabinose, and an uronic acid (supposedly glucuronic acid).

Therefore, the structure of soyasaponin II is expressed as 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**8**). The terminal α-L-rhamnopyranoside linkage has been supported by the molecular rotation comparison as for soyasaponin I (**7**): $[M]_D$ (**8a**)−$[M]_D$ (**11a**)=−149°.

21) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1972).
22) Confirmed to be L by measuring the specific rotation.

Soyasaponin III (9)

Soyasaponin III (**9**) (final purification being made as for soyasaponin II (**8**)) shows the hydroxyl (3350 (br) cm^{-1}) and carboxyl (1710 cm^{-1}) absorption bands in its IR spectrum (Nujol). Acid hydrolysis of **9** furnished soyasapogenol B (**2**), galactose, and an uronic acid. Nona-O-methyl derivative prepared by methylation of **9** exhibits the identical IR and PMR spectra with those of **9c**: two anomeric proton signals at δ 4.60 and δ 4.40 (each d, J=7 Hz), whereas alkaline treatment of **9a** (a prosapogenol of soyasaponin I (**7**)) gave soyasaponin III (**9**). $LiAlH_4$ treatment followed by methanolysis of the above nona-O-methyl derivative liberated 21,24-di-O-methyl-soyasapogenol B (**10**), methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, and methyl 3,4-di-O-methyl-glucopyranoside.

Consequently, soyasaponin III is expressed as 3-O-[β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-soyasapogenol B (**9**), the methyl ester of which corresponds to one of the prosapogenols (**9a**) of soyasaponin I (**7**).

Three soybean saponins elucidated here possess a glucuronopyranoside moiety which attaches directly to 3β-OH of the aglycone soyasapogenol B (**2**). In connection with the previously reported photochemical cleavage of soybean saponins,[10] the present structure elucidation also shows utility of the photochemical cleavage especially to examine the presence of uronic acid moiety in the oligosaccharide portion of saponin which carries the photochemically stable aglycone.

Experimental[23]

Isolation of Soyasaponin I (7), II (8), and III (9)——The MeOH extractive (1.4 kg), obtained from soybean (10 kg) as described previously,[10b] was partitioned in *n*-BuOH–water (1: 1) mixture. The isoflavone mixture (*ca.* 30 g) was separated out as the light yellow precipitate during the procedure. Repeated fractional recrystallization from MeOH of the isoflavone mixture gave genistin of mp 253—255° and daidzin of mp 234—236° (both identified by comparison of IR and PMR data). The *n*-BuOH layer was evaporated under reduced pressure to give the residue which was dissolved in a small amount of MeOH and poured into large quantity of ether. The precipitated crude saponin was collected by filtration and passed through a column of charcoal (200 g, Tokusei-Shirasagi, Takeda Chem. Ind.)- Celite 535 (200 g, Wako Pure Chem. Ind.) with the aid of MeOH to give a saponin mixture (30 g), which was washed with *n*-BuOH and weak aq. alkali. The white insoluble portion (10 g) collected with a centrifuge was then chromatographed on silica gel (500 g) eluting with $CHCl_3$–MeOH–water (7: 3: 1, lower layer) to give soyasaponin I (**7**) (5.5 g), II (**8**) (0.5 g), III (**9**) (0.1 g), and a mixture of **7** and **8** (2 g).

Complete Acid Hydrolysis of Saponin Mixture giving Soyasapogenols——A solution of soyasaponin mixture (5 g) in aq. 10% H_2SO_4–MeOH (1: 1, 400 ml) was refluxed for 20 hr, concentrated under reduced pressure to remove MeOH, and added with cold water to give the white precipitate (2 g) which was collected by filtration. The precipitate was mixed with silica gel (10 g) with the aid of MeOH, dried, and put on a column of silica gel (200 g) and the column was eluted with benzene, benzene–$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH successively. A minor sapogenol mixture eluted earlier with benzene–$CHCl_3$ (1: 5) was acetylated with Ac_2O–pyridine (1: 1, 2 ml) and purified by preparative TLC ($CHCl_3$–benzene=5: 1) to give soyasapogenol D diacetate (4 mg) and E diacetate (3 mg) which were identified with the authentic specimens by TLC ($CHCl_3$–benzene=1: 1). Soyasapogenol E diacetate was further confirmed by hydrolysis with aq. 1% NaOH–MeOH to give soyasapogenol E (**5**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1695 (CO). Later eluate with benzene–$CHCl_3$ (1: 5) was recrystallized from $CHCl_3$–MeOH to give colorless needles (15 mg) of mp 239—241° which was identified

23) The following instruments were used for obtaining the physical data. Melting points: Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and recorded uncorrected; Specific rotations: Rex Photoelectric Polarimeter NEP-2 (l=1 dm); IR spectra: Hitachi IR Spectrometer EPI-S2 or EPI-G3; PMR spectra (tetramethylsilane as the internal standard): Hitachi R-22 (90 MHz) NMR Spectrometer; Mass spectra: Hitachi RMU-6D Mass Spectrometer (direct inlet, at 70 eV).

For chromatography, silica gel (Merck, 70—230 mesh) was used for column and silica gel (Camag D-5) for TLC on which detection was made by spraying 1% $Ce(SO_4)_2$-10% H_2SO_4 solution followed by heating. For preparative TLC, detection was made by spraying water or by keeping the developed plate in the I_2 chamber. For GLC, Hitachi Gas Chromatograph Model 063 with FID was used, and Toyo Filter Paper no. 50 was used for paper partition chromatography (PPC) and detection was made by spraying aniline hydrogen phthalate followed by heating.

with soyasapogenol C (3) by mixed mp, IR (KBr), and TLC. Acetylation of the crystals gave soyasapogenol C diacetate of mp 202—203° (colorless needles from $CHCl_3$–MeOH) (in lit.[6]: mp 202—203°). The combined eluate of $CHCl_3$ and $CHCl_3$–MeOH (300: 1) was recrystallized from $CHCl_3$–MeOH to give colorless needles (310 mg) of mp 262—264°, which were identified with soyasapogenol B (2) by mixed mp, IR (KBr), and TLC (in lit.[6]: mp 260—261°). Triacetate (colorless needles of mp 181—182° from $CHCl_3$–MeOH) obtained by ordinary acetylation of the crystals showed the physicochemical properties (IR (KBr) and PMR) which are compatible with soyasapogenol B triacetate (in lit.[6]: mp 177—178°). Elution with $CHCl_3$–MeOH (100: 1) followed by recrystallization from $CHCl_3$–MeOH gave colorless needles (100 mg) of mp 305—312°, which were identified with soyasapogenol A (1) by mixed mp, IR (KBr), and TLC (in lit.[6]: mp 310—313°). Tetraacetate of mp 229—230° (colorless needles from $CHCl_3$–MeOH) showed the IR (KBr) and PMR spectra being compatible with soyasapogenol A tetraacetate. Successive elution with $CHCl_3$–MeOH (50: 1) and MeOH gave a mixture of soyasapogenol A and B (1.02 g).

Soyasaponin I (7)——Soyasaponin I (1.2 g) obtained above (IR ν_{max}^{Nujol} cm^{-1}: 3350 (br, OH), 1720 (br, COOH), 1610 (br, COO^-), which was hardly soluble in MeOH and EtOH, was dissolved in warm weakly acidic aq. MeOH (50 ml, pH 3—4) and filtered to remove the insoluble portion.[24] The crystals (0.95 g) separated out from the filtrate were recrystallized from MeOH to give colorless needles of pure soyasaponin I (7), mp 238—240°, $[\alpha]_D^{14}$ −8.5° (c=1.0, MeOH). *Anal.* Calcd. for $C_{48}H_{78}O_{18}\cdot 2H_2O$: C, 58.88; H, 8.44. Found: C, 58.98; H, 8.55. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br, OH), 1710 (br, COOH); ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1735 (br, COOH). PMR (d_5-pyridine) δ: 0.70 (3H), 0.97 (6H), 1.29 (3H), 1.37 (6H), 1.51 (3H) (all s, seven methyls), 1.82 (3H, d, J=6 Hz, >CH–C<u>H</u>$_3$ in rhamnose).

Complete Acid Hydrolysis of Soyasaponin I (7)——A solution of 7 (20 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 10 ml) was heated under reflux for 6 hr, concentrated under reduced pressure to remove MeOH, and diluted with cold water to give the precipitate which was collected by filtration and purified by preparative TLC ($CHCl_3$–MeOH=30: 1) to give colorless needles (4 mg) of mp 258—260° being identical with soyasapogenol B (2) by mixed mp, IR (KBr), and TLC. The aqueous filtrate was concentrated under reduced pressure while adjusting to pH 5—6 with aq. satur. $Ba(OH)_2$ and centrifuged. The supernatant was further concentrated under reduced pressure and subjected to PPC (iso-PrOH–*n*-BuOH–water=7: 1: 2, ascending for 24 hr) to identify with rhamnose (Rf=0.60), galactose (Rf=0.37) and glucuronic acid (Rf=0.10).

Soyasaponin I Methyl Ester (7a)——To a solution of 7 (0.8 g) in MeOH (10 ml) was added excess ethereal diazomethane and the reaction mixture was left standing at room temperature overnight and evaporated to dryness to give a product (0.8 g) which was recrystallized from MeOH to furnish soyasaponin I methyl ester (7a), mp 270—274° (colorless prisms), $[\alpha]_D^{27}$ −6.8° (c=1.0, MeOH). *Anal.* Calcd. for $C_{49}H_{80}O_{18}$: C, 61.48; H, 8.43. Found: C, 61.21; H, 8.15. IR ν_{max}^{Nujol} cm^{-1}: 3300 (br, OH), 1740 (br, $COOCH_3$). PMR (d_5-pyridine) δ: 0.72 (3H), 0.96 (6H), 1.18 (3H), 1.22 (6H), 1.40 (3H) (all s, seven methyls), 1.69 (3H, d, J=6 Hz, >CH–C<u>H</u>$_3$ in rhamnose), 3.73 (3H, s, OCH_3).

$NaBH_4$ Reduction of 7a followed by Complete Acid Hydrolysis——A stirred solution of **7a** (180 mg) in EtOH (10 ml) was treated with a suspension of $NaBH_4$ (200 mg) in EtOH (2 ml) at room temperature for 2 hr and added with acetone to decompose excess $NaBH_4$. The reaction mixture was treated with Dowex 50w×8 (H^+, 15 g) and stirred for 30 min and filtered to remove resin. The filtrate was then added with Amberlite IRA-400 (OH^-, 15 g) with stirring for 30 min, filtered, and evaporated under reduced pressure to give white powder of **7b** (160 mg), IR ν_{max}^{Nujol} cm^{-1}: 3360 (br, OH), no ester carbonyl. A solution of **7b** (20 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 10 ml) was refluxed for 5 hr and treated as for acid hydrolysis of 7 to give 2 (6 mg, identified by IR (KBr) and TLC). The aqueous layer was neutralized with Amberlite IR-45 (10 g) with stirring for 2 hr, concentrated under reduced pressure, and subjected to PPC (as in 7) to identify with rhamnose (Rf=0.50), glucose (Rf=0.30), and galactose (Rf=0.25).

Undeca-O-methyl Derivative of Soyasaponin I (7c)——a) Dimsyl Carbanion: A solution of NaH (1 g, washed with *n*-hexane beforehand) in DMSO (20 ml) was heated under N_2 atmosphere at 60—70° for 2 hr to give a slightly greenish solution.

b) To a solution of 7 (330 mg) in DMSO (10 ml) was added the above prepared dimsyl carbanion solution (10 ml) and the total solution was stirred under N_2 atmosphere for 1 hr, treated with CH_3I (10 ml) in the dark while keeping the reaction temperature below 20°, left standing in the dark overnight, poured into ice-water, and extracted with ether. The ether extract was washed with aq. 10% $Na_2S_2O_3$ and water, and evaporated to dryness. A syrup thus obtained was crystallized from MeOH to give colorless needles of **7c** (230 mg) which was recrystallized from acetone–MeOH to furnish the analytical sample of 7c, mp 211—214°, $[\alpha]_D^{29}$ −8.2° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{59}H_{100}O_{18}$: C, 64.57; H, 9.19. Found: C, 64.33; H, 9.32. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1756 ($COOCH_3$). PMR (C_6D_6) δ: 0.93 (3H), 1.09 (9H), 1.14, 1.22, 1.42 (3H each) (all s, seven methyls), 1.43 (3H, d, J=6 Hz, >CH–C<u>H</u>$_3$ in rhamnose), 3.09, 3.17, 3.22, 3.35 (3H each), 3.37, 3.41 (6H each), 3.43, 3.50, 3.84 (3H each) (all s, $OCH_3\times 11$), 4.44, 4.89 (1H each, d, J=7 Hz, anomeric protons of glucuronide and galactoside), 5.26 (1H, br.s, $W_{h/2}$=7 Hz, 12-H), 5.68 (1H, s, anomeric proton of

24) TLC examination before and after acidic MeOH treatment showed the identical chromatogram in regard to saponin, except the change of the carboxylate form to the acid form.

rhamnoside). PMR ($CDCl_3$) δ: 0.86, 0.90, 0.96 (3H each), 1.01 (6H), 1.09, 1.18 (3H each) (all s, seven methyls), 1.24 (3H, d, J=6 Hz, >CH-C<u>H</u>$_3$ in rhamnose), 3.26, 3.28, 3.37 (3H each), 3.45 (6H), 3.47 (9H), 3.52, 3.66, 3.79 (3H each) (all s, $OCH_3\times11$), 4.28, 4.65 (1H each, d, J=7 Hz, anomeric protons of glucuronide and galactoside), 5.22 (2H, br.s, $W_{h/2}$=6 Hz, 12-H and anomeric proton of rhamnoside).

$LiAlH_4$ Reduction followed by Methanolysis of 7c——To a solution of **7c** (200 mg) in dry ether (20 ml) was added a suspension of $LiAlH_4$ (200 mg) in dry ether (5 ml) and the reaction mixture was stirred at room temperature for 1 hr, treated with a few drops of aqueous ether, acidified with aq. 20% H_2SO_4, and extracted with ether. The ether extract after usual work-up gave **7d** as white powder (180 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3590 (w, OH), no ester carbonyl. A solution of **7d** (180 mg) in anhydrous 6% HCl–MeOH (10 ml) was heated under reflux for 1 hr, neutralized with Ag_2CO_3 (powder), and filtered. The filtrate was concentrated and left standing to give a precipitate which was collected by filtration and recrystallized from $CHCl_3$–MeOH to furnish **10** (60 mg), colorless needles of mp 194—195°, $[\alpha]_D^{29}$ +41.1° (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 79.03; H, 11.36. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3350 (OH). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.91, 0.93, 0.99, 1.10, 1.21 (3H each) (all s, seven methyls), 2.81 (1H, d.d, J=3.5 & 6.0 Hz, 21β-H), 3.26, 3.31 (3H each, all s, $OCH_3\times2$), 3.15—3.26 (1H, 24-H),[25] 3.82—3.96 (2H, m, 3α-H, 24-H), 5.22 (1H, t-like, 12-H). Mass Spectrum m/e (%): 486 (M^+, 9), 248 (**i**, base peak), 238 (**ii**, 15), 237 (**ii**–H, 7), 233 (**i**–CH_3, 61), 216 (**i**–MeOH, 14). The filtrate after removing **10** gave methylated monosaccharides which were identified with Me 2,3,4-tri-O-methyl-rhamnopyranoside [I], Me 3,4,6-tri-O-methyl-galactopyranoside [II], and Me 3,4-di-O-methyl-glucopyranoside [III] by GLC and TLC. GLC: i) Column: 15% polyneopentyl glycol succinate on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 205°; carrier gas: N_2; flow rate: 20 ml/min; t_R (min): I, 2′33″ (major), 3′10″ (minor); II, 10′26″ (major), 14′25″ (minor); III, 17′15″ (major), 19′10″ (minor); ii) Column: 15% ethylene glycol succinate polyester on uniport B (80—100 mesh), 3 mm×1 m; column temp.: 185°; carrier gas: N_2; flow rate: 20 ml/min; t_R (min): I, 2′40″ (major); 4′10″ (minor); iii) Column: 3% SE-30 on chromosorb WAW (80—100 mesh) 3 mm×2 m; column temp.: 145°; carrier gas: N_2; flow rate: 20 ml/min; t_R (min): III, 4′34″. TLC (Rf): i) Benzene–acetone (1:1): I, 0.75; II, 0.40; III, 0.30; ii) Benzene–MeOH (15:1): I, 0.44 (major), 0.28 (minor); iii) Benzene–MeOH (5:1): II, 0.45 (major), 0.38 (minor).

Acetylation of 10 giving Monoacetate (10a)——A solution of **10** (60 mg) in pyridine (3 ml) was treated with Ac_2O (3 ml), left standing at room temperature overnight, and treated as usual to give a product (60 mg), which was crystallized from $CHCl_3$–MeOH to furnish **10a** as colorless needles of mp 178—179°, $[\alpha]_D^{16}$ +63.9° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{56}O_4$: C, 77.22; H, 10.67. Found: C, 77.41; H, 10.42. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1730, 1240 (OAc). PMR ($CDCl_3$) δ: 0.86, 0.89, 0.96 (3H each), 1.00 (9H), 1.10 (3H) (all s, seven methyls), 2.04 (3H, s, OAc), 2.82 (1H, d.d, J=3.5 & 6.0 Hz, 21β-H), 3.29 (6H, s, $OCH_3\times2$), 3.15, 3.71 (2H, ABq, J_{AB}=10 Hz, 24-H_2), 4.56 (1H, t-like, 3α-H), 5.21 (1H, br.s, $W_{h/2}$=7 Hz, 12-H). Mass Spectrum m/e (%): 528 (M^+, 5), 280 (**iii**, 7), 279 (**iii**–H, 4), 248 (**i**, base peak), 233 (**i**–CH_3, 57), 216 (**i**–MeOH, 12).

Methylation of Soyasapogenol B (2) giving 10b——A solution of **2** (200 mg) in DMSO (5 ml) was treated with dimsyl carbanion solution (10 ml, prepared as above), kept stirring at room temperature for 1 hr, treated with CH_3I (5 ml), and kept stirring in the dark overnight. The ether extractive obtained after usual work-up was purified by preparative TLC (benzene–acetone=10:1) and recrystallized from $CHCl_3$–acetone to give colorless needles of **10b** (110 mg), mp 199—200°, $[\alpha]_D^{16}$ +62.0° (c=0.7, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{56}O_3$: C, 79.14; H, 11.27. Found: C, 78.94; H, 11.23. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH. PMR ($CDCl_3$) δ: 0.87, 0.90, 0.97 (3H each), 1.00, 1.11 (6H each) (all s, seven methyls), 2.62—2.85 (2H, m, 3α-H, 21β-H), 3.24, 3.27, 3.34 (3H each, all s, $OCH_3\times3$), 3.24—3.34 (1H, 24-H),[25] 3.56 (1H, d, J=10 Hz, 24-H), 5.23 (1H, t-like, 12-H). Mass Spectrum m/e (%): 500 (M^+, 3), 252 (**iv**, 20), 251 (**iv**–H, 10), 248 (**i**, base peak), 233 (**i**–CH_3, 75), 216 (**i**–MeOH, 17).

Partial Acid Hydrolysis of Soyasaponin I (7) giving 9a——A solution of **7** (2.2 g) in aq. 10% H_2SO_4–MeOH (1:2, 30 ml) was heated under reflux for 14 hr, concentrated under reduced pressure to remove MeOH, and diluted with cold water. The white precipitate (1.5 g) was collected by filtration and chromatographed on silica gel (90 g) eluting with $CHCl_3$–MeOH mixture. The elution with $CHCl_3$–MeOH (400:1) gave soyasapogenol B (2) (180 mg) and elution with $CHCl_3$–MeOH (40:1—30:1) gave prosapogenol **6a** (200 mg). Prosapogenol **9a** (500 mg) was eluted with $CHCl_3$–MeOH (10:1) and a mixture of **9a**, **7a**, and **7** (300 mg) was obtained from the eluate with $CHCl_3$–MeOH (1:1). Prosapogenol **9a**, mp 262—263° (colorless fine crystals from $CHCl_3$–MeOH), $[\alpha]_D^{19}$ +9.9° (c=1.0, MeOH). *Anal.* Calcd. for $C_{43}H_{70}O_{14}\cdot2H_2O$: C, 60.97; H, 8.81. Found: C, 61.13; H, 8.91. IR ν_{max}^{Nujol} cm^{-1}: 3350 (br, OH), 1730 ($COOCH_3$); ν_{max}^{KBr} cm^{-1}: 3390 (br, OH), 1744 ($COOCH_3$). PMR (d_5-pyridine) δ: 0.76 (3H), 0.98 (6H), 1.18, 1.23, 1.25, 1.34 (3H each) (all s, seven methyls), 3.75 (3H, s, OCH_3).

Complete Acid Hydrolysis of 9a——A solution of **9a** (10 mg) in aq. 20% H_2SO_4–MeOH (1:1, 4 ml) was heated under reflux for 10 hr and worked up as for acid hydrolysis of **7** to give **2** (3 mg, identified by TLC). Aqueous layer furnished galactose and glucuronic acid (identified by PPC).

$NaBH_4$ Reduction of 9a giving 9b——A stirred solution of **9a** (90 mg) in EtOH (10 ml) was treated with a mixture of $NaBH_4$ (50 mg) in EtOH (2 ml) at room temperature for 30 min. The reaction mixture was

25) The precise δ value and coupling pattern were unclear due to the overlap of OCH_3 signal.

then treated as in case of **7a** with Dowex 50w×8 (H^+, 5 g) and Amberlite IRA-400 (OH^-, 5 g) successively. Recrystallization of the product from MeOH furnished **9b** (70 mg), colorless needles of mp 268—269°, $[\alpha]_D^{29}$ +7.5° (c=0.5, MeOH). *Anal.* Calcd. for $C_{42}H_{70}O_{13}\cdot 2H_2O$: C, 61.59; H, 9.11. Found: C, 61.85; H, 9.36. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), no ester carbonyl. PMR (d_5-pyridine) δ: 0.76 (3H), 0.97 (6H), 1.17, 1.22, 1.25, 1.34 (3H each) (all s, seven methyls).

Complete Acid Hydrolysis of 9b——A solution of **9b** (10 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 10 ml) was refluxed for 6 hr. Working up of the reaction mixture as above gave **2** (3 mg, identified by TLC). Aqueous layer was neutralized with Amberlite IR-45 (5 g) and analyzed by PPC (iso-PrOH–*n*-BuOH–water=7: 1: 2, developing for 24 hr twice) to identify with glucose (Rf=0.54) and galactose (Rf=0.48).

Nona-O-methyl Derivative (9c) of 9a——A solution of **9a** (100 mg) in DMSO (4 ml) was treated with dimsyl carbanion solution (4 ml, prepared as above) under N_2 atmosphere with stirring at room temperature for 1 hr, added with CH_3I (5 ml), and kept stirring in the dark overnight. The ether extractive obtained after working up as above was purified by preparative TLC (benzene–MeOH=30: 1) to furnish **9c** (66 mg, white powder, crystallization being failed), $[\alpha]_D^{16}$ +21.3° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{86}O_{14}$: C, 66.35; H, 9.39. Found: C, 66.39; H, 9.65. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1754 ($COOCH_3$). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.95 (3H each), 1.00 (6H), 1.07, 1.16 (3H each) (all s, seven methyls), 3.25, 3.27, 3.35 (3H each), 3.49 (9H), 3.57, 3.61, 3.77 (3H each) (all s, $OCH_3\times 9$), 4.39, 4.59 (1H each, d, J=7 Hz, anomeric protons of glucuronide and galactoside), 5.19 (1H, br.s, $W_{h/2}$=6 Hz, 12-H).

$LiAlH_4$ Reduction followed by Methanolysis of 9c——A solution of **9c** (90 mg) in dry ether (15 mg) was treated with $LiAlH_4$ (50 mg)-dry ether (5 ml) mixture, kept stirring for 4 hr, and treated as above to give **9d** (75 mg) as white powder, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3640 (w, OH), no ester carbonyl. A solution of **9d** (75 mg) in anhydrous 2N HCl–MeOH (10 ml) was refluxed for 1 hr and neutralized with Ag_2CO_3 (powder). The crystalline product (31 mg) obtained by concentration of the filtrate was recrystallized from $CHCl_3$–MeOH to give colorless needles of **10**, mp 194—195° (identified by mixed mp, IR (KBr), and TLC). The mother layer gave methylated monosaccharides which were identified with Me 2,3,4,6-tetra-O-methyl-galactopyranoside [IV] and Me 3,4-di-O-methyl-glucopyranoside [III] by GLC and TLC. GLC: i) Column: 15% ethylene glycol succinate polyester on uniport B, 3 mm×1 m; column temp.: 210°; carrier gas: N_2; flow rate: 20 ml/min; t_R (min): IV, 5′04″ (major), 7′40″ (minor); ii) Column: 15% polyneopentyl glycol succinate on chromosorb WAW, 3 mm×2 m; column temp.: 200°; carrier gas: N_2; flow rate: 30 ml/min; t_R (min): III, 12′29″ (major), 14′06″ (minor). TLC (Rf, benzene–acetone=1: 2): IV, 0.60; III, 0.35, 0.39.

Soyasaponin II (8)——Soyasaponin II (100 mg) obtained by silica gel column chromatography (*vide supra*) was dissolved in MeOH (50 ml) and passed through a column of Dowex 50w×8 (H^+, 20 g) and recrystallized from MeOH to give pure soyasaponin II (**8**) as colorless fine crystals of mp 212—215°, $[\alpha]_D^{29}$ −9.6° (c=0.5, MeOH). *Anal.* Calcd. for $C_{47}H_{76}O_{17}\cdot 3H_2O$: C, 58.37; H, 8.55. Found: C, 58.78; H, 8.36. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br, OH), 1720 (br, COOH); ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1733 (br, COOH). PMR (d_5-pyridine) δ: 0.70 (3H), 0.95 (6H), 1.19 (9H), 1.35 (3H) (all s, seven methyls).

Complete Acid Hydrolysis of Soyasaponin II (8)——A solution of **8** (10 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 6 ml) was heated under reflux for 6 hr. A white product obtained by working up as for **7** was recrystallized from $CHCl_3$–MeOH to give colorless needles of mp 245—249° (3 mg), being identical with soyasapogenol B (**2**) by mixed mp, IR (KBr), and TLC. PPC of the aqueous layer identified rhamnose (Rf=0.50), arabinose (Rf=0.34), and glucuronic acid (Rf=0.04). Arabinose was further confirmed to be L by examining the specific rotation[1] of the product obtained from 200 mg of soyasaponin II through the same procedure as above (final purification by preparative PPC).

Soyasaponin II Methyl Ester (8a)——To a solution of **8** (25 mg) in MeOH (5 ml) was added excess ethereal diazomethane and the total solution was left standing overnight and evaporated to give a product (25 mg), which was recrystallized from MeOH to give soyasaponin II methyl ester (**8a**) of mp 226—229° (colorless prisms), $[\alpha]_D^{22}$ +2.6° (c=0.3, MeOH). *Anal.* Calcd. for $C_{48}H_{78}O_{17}$: C, 61.52; H, 8.39. Found: C, 61.58; H, 8.33. IR ν_{max}^{Nujol} cm^{-1}: 3400 (br, OH), 1740 ($COOCH_3$).

$NaBH_4$ Reduction of 8a followed by Complete Acid Hydrolysis——A solution of **8a** (10 mg) in EtOH (5 ml) was treated with $NaBH_4$ (20 mg)–EtOH (1 ml) as for **7a** and the product (**8b**, 8 mg) was dissolved in aq. 20% H_2SO_4–MeOH (1: 1, 10 ml), heated under reflux for 5 hr, and worked up as above. White powder (3 mg) thus obtained was crystallized from $CHCl_3$–MeOH and identified with **2** by IR (KBr) and TLC. The saccharides obtained from the aqueous layer were identified with rhamnose (Rf=0.60), arabinose (Rf=0.50), and glucose (Rf=0.45) by PPC.

Deca-O-methyl Derivative (8c) of Soyasaponin II (8)——A solution of **8** (120 mg) in DMSO (5 ml) was treated with dimsyl carbanion solution (5 ml) under N_2 atmosphere with stirring, added with CH_3I (5 ml), kept stirring in the dark overnight. Working up of the reaction mixture as above followed by preparative TLC (benzene–MeOH=15: 1) gave **8c** (80 mg) as white powder (crystallization being failed), $[\alpha]_D^{12}$ −2.6° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{57}H_{96}O_{17}$: C, 64.99; H, 9.19. Found: C, 65.29; H, 9.09. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1760 ($COOCH_3$). PMR (C_6D_6) δ: 0.94 (3H), 1.06 (9H), 1.14, 1.22, 1.42 (3H each) (all s, seven methyls), 1.44 (3H, d, J=6 Hz, >CH–C$\underline{H}_3$ in rhamnose), 3.12 (6H), 3.14, 3.31, 3.37 (3H each), 3.39 (6H), 3.42, 3.49, 3.82 (3H each) (all s, $OCH_3\times 10$), 4.47 (1H, d, J=8 Hz), 4.88 (1H, d, J=6 Hz) (anomeric protons of glucuronide and arabinoside), 5.25 (1H, br.s, $W_{h/2}$=6 Hz, 12-H), 5.44 (1H, s, anomeric proton of rhamnoside).

$LiAlH_4$ Reduction followed by Methanolysis of 8c——A stirred solution of **8c** (40 mg) in dry ether (5 ml) was treated with $LiAlH_4$ (40 mg)-dry ether (5 ml) mixture at room temperature for 30 min. Working up as for **7c** gave **8d** (38 mg) as white powder, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (w, OH), no ester carbonyl. A solution of **8d** (38 mg) in anhydrous 12% HCl–MeOH (5 ml) was refluxed for 30 min and worked up as above to give a white product (12 mg) which was crystallized from $CHCl_3$–MeOH. Colorless needles of mp 188—192° thus obtained was identified with 21,24-di-O-methyl-soyasapogenol B (**10**) by mixed mp, IR (CCl_4), and TLC. Methylated monosaccharides obtained from the filtrate were identified with Me 2,3,4-tri-O-methyl-rhamnopyranoside [I], Me 3,4-di-O-methyl-arabinopyranoside [V], and Me 3,4-di-O-methyl-glucopyranoside [III] by GLC and TLC. GLC: i) Column: 15% ethylene glycol succinate polyester on uniport B (80—100 mesh), 3 mm×1 m; column temp.: 170°; carrier gas: N_2; flow rate: 40 ml/min; t_R (min): I, 1′17″ (major), 1′59″ (minor); V, 6′18″; ii) Column: 15% polyneopentyl glycol succinate on chromosorb WAW (80—100 mesh), 3 mm×2 m; column temp.: 200°; carrier gas: N_2; flow rate: 30 ml/min; t_R (min): V, 4′13″; III, 12′30″ (major), 14′10″ (minor). TLC (*Rf*, benzene–acetone=1: 2): I, 0.60; V, 0.40; III, 0.25.

Partial Acid Hydrolysis of Soyasaponin II (8) giving 11a——A solution of **8** (200 mg) in aq. 20% H_2SO_4–MeOH (1: 4, 25 ml) was heated under reflux for 5 hr, concentrated under reduced pressure to remove MeOH, and diluted with cold water. The white product (150 mg) obtained by filtration was purified by preparative TLC ($CHCl_3$–MeOH=5: 1) to afford soyasapogenol B (**2**) (16 mg), **6a** (61 mg, colorless needles of mp 242—246° from MeOH, identified by mixed mp, IR (KBr), and TLC), and another prosapogenol (**11a**) (11 mg). Recrystallization from $CHCl_3$–MeOH gave a pure sample of **11a**, mp 260—265° (colorless fine crystals), $[\alpha]_D^{29}$ +7.9° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{42}H_{68}O_{13}\cdot 3H_2O$: C, 60.41; H, 8.93. Found: C, 60.72; H, 8.74. IR ν_{max}^{Nujol} cm^{-1}: 3350 (br, OH), 1743 ($COOCH_3$).

Complete Acid Hydrolysis of 11a——A solution of **11a** (5 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 5 ml) was refluxed for 6 hr. Working up as for **7** gave **2** (1 mg, identified by TLC), arabinose (*Rf*=0.40) and glucuronic acid (*Rf*=0.05) (identified by PPC).

Soyasaponin III (9)——Soyasaponin III (50 mg) obtained by silica gel column chromatography (*vide supra*) was dissolved in MeOH and passed through a column of Dowex 50w×8 (H^+, 10 g) and recrystallized from MeOH to give a pure sample of soyasaponin III (**9**) as colorless needles of mp 215—216°, $[\alpha]_D^{29}$ +15.0° (*c*=0.5, MeOH). *Anal.* Calcd. for $C_{42}H_{68}O_{14}\cdot 2H_2O$; C, 60.56; H, 8.71. Found: C, 60.88; H, 8.55. IR ν_{max}^{Nujol} cm^{-1}: 3350 (br, OH), 1710 (br, COOH); ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1734 (br, COOH).

Complete Acid Hydrolysis of Soyasaponin III (9)——A solution of **9** (10 mg) in aq. 20% H_2SO_4–MeOH (1: 1, 10 ml) was heated under reflux for 8 hr, and treated as for **7** to give a product (3 mg) which was identified with **2** by IR (KBr) and TLC. The aqueous layer was examined by PPC to identify with galactose (*Rf*=0.30) and glucuronic acid (*Rf*=0.10).

Methylation of Soyasaponin III (9)——A solution of **9** (20 mg) in DMSO (5 ml) was treated with dimsyl carbanion solution (5 ml) and CH_3I (5 ml) as above. The ether extractive was purified by preparative TLC (benzene–MeOH=30: 1) to give nona-O-methyl derivative (12 mg, white powder), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1756 ($COOCH_3$). PMR ($CDCl_3$) δ: 0.85, 0.89, 0.95 (3H each), 1.00 (6H), 1.08, 1.16 (3H each) (all s, seven methyls), 3.26, 3.28, 3.36 (3H each), 3.49 (9H), 3.58, 3.62, 3.78 (3H each) (all s, $OCH_3\times 9$), 4.40, 4.60 (1H each, d, *J*=7 Hz) (anomeric protons of glucuronide and galactoside), 5.20 (1H, br.s, $W_{h/2}$=7 Hz, 12-H). The nona-O-methyl derivative was identified with **9c** by TLC (benzene–acetone=10: 1; benzene–MeOH=30: 1), IR, and PMR.

$LiAlH_4$ Reduction followed by Methanolysis of Nona-O-methyl Derivative of Soyasaponin III (=9c)——A stirred solution of nona-O-methyl derivative of soyasaponin III (20 mg) in dry ether (5 ml) was treated with $LiAlH_4$ (20 mg)-dry ether (5 ml) suspension at room temperature for 30 min. The product (17 mg, white powder, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (w, OH), no ester carbonyl) obtained by usual work-up was dissolved in anhydrous 12% HCl–MeOH (5 ml) and heated under reflux for 20 hr. Working up as above gave a product (7 mg, white precipitate) which was identified with **10** by IR (CCl_4) and TLC. Methylated monosaccharides thus obtained were identified with Me 2,3,4,6-tetra-O-methyl-galactopyranoside [IV] and Me 3,4-di-O-methyl-glucopyranoside [III] by GLC and TLC. GLC: i) Column: 3% SE-30 on chromosorb WAW (80—100 mesh), 3 mm×1 m; column temp.: 160°; carrier gas: N_2; flow rate: 20 ml/min; t_R (min): IV, 2′30″ (minor), 3′56″ (major); III, 4′15″; ii) Column: 15% polyneopentyl glycol succinate on chromosorb WAW (80—180 mesh), 3 mm×2 m; column temp.; 200°; carrier gas: N_2; flow rate: 30 ml/min; t_R (min): III, 12′29″ (major), 14′07″ (minor). TLC (*Rf*, benzene–acetone=1: 1): IV, 0.68 (minor), 0.60 (major); III, 0.13.

Alkaline Treatment of 9a giving Soyasaponin III (9)——A solution of **9a** (50 mg) in MeOH (20 ml) was treated with aq. 1N NaOH (3 ml) under reflux for 4.5 hr and concentrated under reduced pressure to give a precipitate which was collected by filtration and dissolved in MeOH and passed through a column of Dowex 50w×8 (H^+, 20 g). The product was then recrystallized from MeOH to give colorless needles (45 mg) of mp 216—218° being identical with **9** by mixed mp, IR (KBr), and TLC.

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