Chem. Pharm. Bull.
33(3)1069—1076(1985)

Saponin and Sapogenol. XXXIX.[1] Structure of Soyasaponin A_1, a Bisdesmoside of Soyasapogenol A, from Soybean, the Seeds of *Glycine max* MERRILL

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(Received July 12, 1984)

Five bioactive triterpene-oligoglycosides, named soyasaponins I, II, III, A_1 (**3**), and A_2 (**2**), were isolated from soybean, the seeds of *Glycine max* MERRILL (Leguminosae). By employing a photochemical degradation method and a lead tetraacetate degradation method, which are two of four selective cleavage methods available for the glucuronide linkage in oligoglycosides, the structure of soyasaponin A_1 was elucidated to be 3-*O*[β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl-22-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]-soyasapogenol A (**3**).

Keywords——soybean; *Glycine max*; soyasaponin A_1; oleanene-bisdesmoside; glucuronide linkage selective cleavage; glucuronide linkage photolysis; glucuronide linkage lead tetraacetate degradation; oleanene-bisdesmoside ^{13}C-NMR; triterpene-oligoglycoside biological activity

As a continuation of our work on the chemical elucidation of bioactive constituents in Leguminous naturally occurring drug materials, we have isolated five saponins from soybean, the seeds of *Glycine max* MERRILL, *i.e.* soyasaponins I, II, and III, which possess soyasapogenol B as the common aglycone, and soyasaponins A_1 and A_2 having soyasapogenol A (**1**) as their aglycone. We have so far reported the structures of soyasaponins I, II, III,[2] and A_2 (**2**).[1] In this paper, we describe the structure elucidation of soyasaponin A_1 (**3**)[3] by employing two selective cleavage methods for the glucuronide linkage in oligoglycosides,[4] *i.e.* a photochemical degradation method[4,5] and a lead tetraacetate degradation method.[4,6]

The infrared (IR) spectrum of soyasaponin A_1 (**3**) showed the presence of hydroxyl and carbonyl functions in the structure. Acidic hydrolysis of **3** yielded soyasapogenol A (**1**) as the aglycone, whereas acidic methanolysis of **3** furnished, in addition to **1**, methyl L-arabinoside, methyl D-galactoside, methyl D-glucoside, and methyl D-glucuronide in 1 : 1 : 2 : 1 ratio. Since it was found that soyasaponin A_1 (**3**) contains D-glucuronic acid as one of the carbohydrate components, **3** was subjected to the above-mentioned photochemical and lead tetraacetate degradations.

External irradiation of a methanolic solution of soyasaponin A_1 (**3**) in a Vycor tube with a 500 W high-pressure mercury lamp liberated a prosapogenol (**4**)[1] and a carbohydrate mixture, from which an octa-*O*-acetyl disaccharide (**5**) was identified after acetylation.[7] The IR spectrum of **5** lacked hydroxyl absorption bands but showed strong absorption bands due to acetoxyl functions. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals assignable to eight acetoxyl groups, whereas the field desorption mass spectrum (FD-MS) showed the molecular ion peak at *m/z* 678 together with a deacetylated ion peak at *m/z* 619. Deacetylation followed by methylation with methyl iodide and dimsyl carbanion[8] provided a fully methylated product, which, upon methanolysis, liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4,6-tri-*O*-methylgalactopyranoside (**b**).[9]

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Thus, soyasaponin A_1 (**3**) was found to be a glucuronide of a prosapogenol (**4**) having a D-glucopyranosyl(1→2)-D-galactopyranosyl residue connected to the glucuronide moiety.

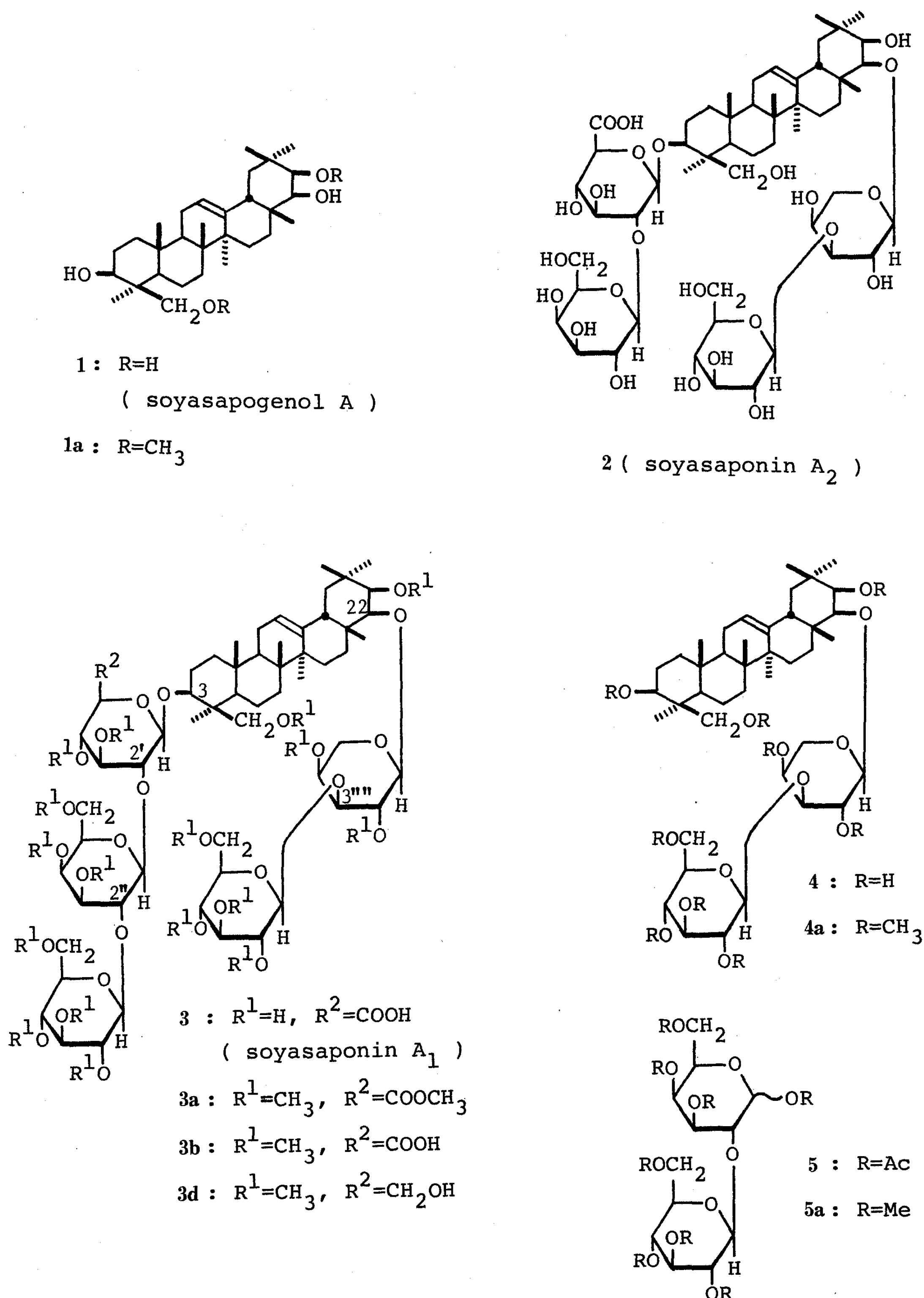

Chart 1

In order to employ the lead tetraacetate degradation method, soyasaponin A_1 (**3**) was methylated[8] to afford an octadeca-*O*-methyl derivative (**3a**). The IR spectrum of **3a** showed ester absorption bands but lacked absorption bands due to hydroxyl groups. The ^{1}H-NMR

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spectrum (taken in hexadeuteroacetone) of **3a** showed signals due to four of five anomeric protons, but the signal of the remaining one anomeric proton was not observed due to overlap of with other proton signals.

After hydrolysis of **3a** with aqueous potassium carbonate, the resulting carboxylic acid (**3b**) was treated with lead tetraacetate in benzene under reflux to provide a 5′-epimeric mixture of decarboxylative acetoxylated derivatives (**3c**). The structure of **3c** was supported by its IR spectrum, which showed acetoxyl absorption bands, and by its ^{1}H-NMR spectrum, which showed signals due to 5′α and 5′β acetoxyl groups at δ 2.07 and δ 2.10 (total intensity corresponding to 3H) and 5′α and 5′β protons at δ 5.57 (d, $J=8$ Hz) and δ 6.25 (d, $J=4$ Hz) (total 1H).

Treatment of **3c** with methanolic sodium methoxide yielded three products, which were acetylated to facilitate the isolation, furnishing a monoacetyl-octa-*O*-methyl prosapogenol (**4b**), a monoacetyl-hepta-*O*-methyl disaccharide (**5b**), and a dienic compound (**6**).[4,10] The IR spectrum of **4b** showed acetoxyl absorption bands, whereas the ^{1}H-NMR spectrum showed signals ascribable to one acetoxyl and eight methoxyl groups, two anomeric protons, and the 3α proton (δ 4.55, m). Deacetylation of **4b** followed by methanolysis furnished 21,24-di-*O*-methylsoyasapogenol A (**1a**),[1] methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), and methyl 2,4-di-*O*-methylarabinopyranoside (**c**). Furthermore, deacetylation of **4b** followed by methylation gave a nona-*O*-methyl prosapogenol (**4a**).[1] Thus, the structure of **4b** was confirmed.

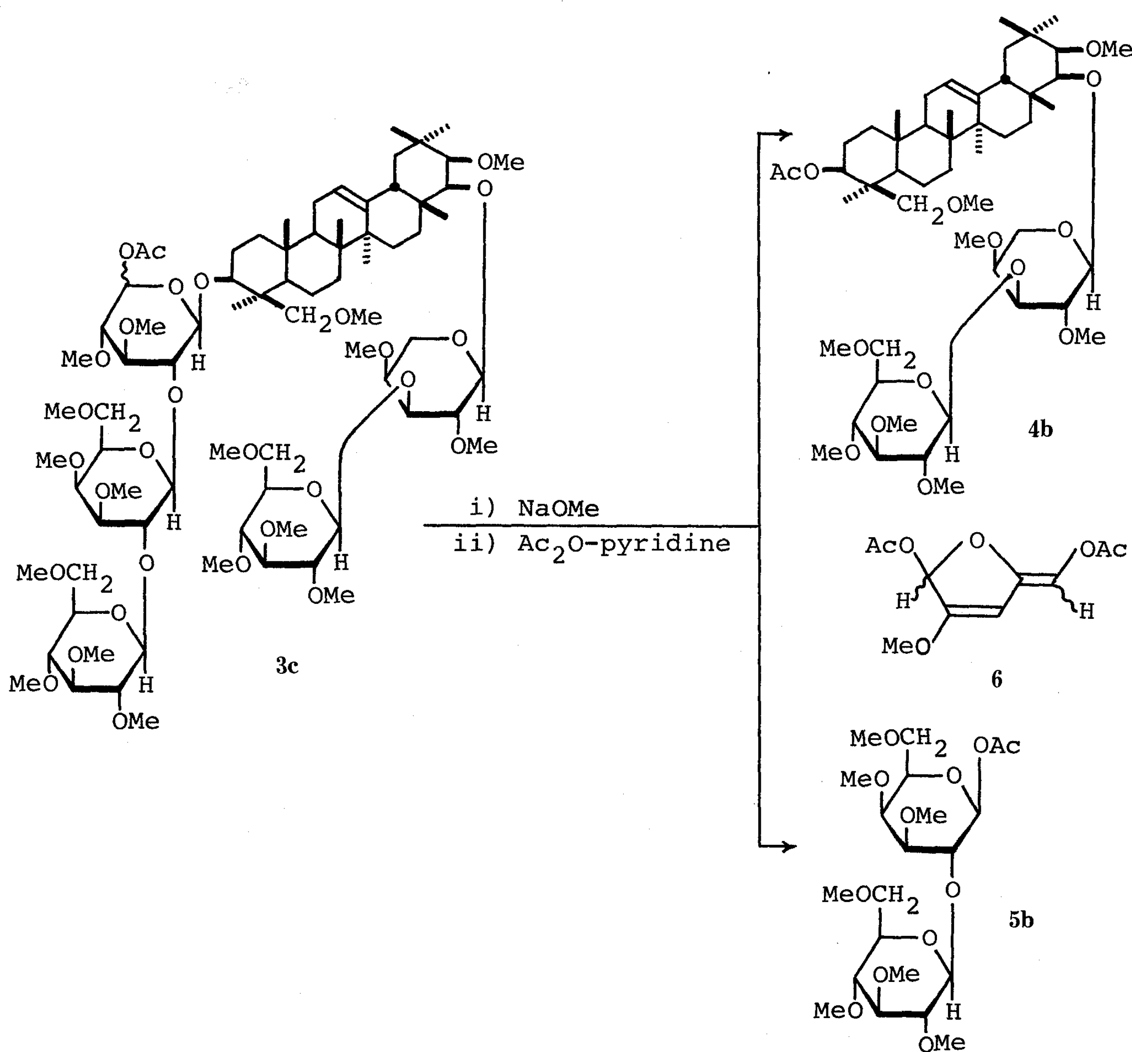

Chart 2

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The FD-MS of the monoacetyl-hepta-*O*-methyl prosapogenol (**5b**) gave a molecular ion peak at *m/z* 482 and a deacetylated ion peak at *m/z* 423. The IR spectrum of **5b** showed acetoxyl absorption bands, whereas the ^{1}H-NMR spectrum showed signals due to one acetoxyl and seven methoxyl groups and two anomeric protons, one in the β-glucopyranoside and the other in the β-galactopyranoside moieties. Methanolysis of **5b** yielded methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4,6-tri-*O*-methylgalactopyranoside (**b**). Thus, the structure of **5b** was elucidated.

On the other hand, lithium aluminum hydride reduction of **3a** provided **3d**, which, upon methanolysis, liberated **1a** as the aglycone and four methyl glycosides, *i.e.* methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**b**), methyl 2,4-di-*O*-methylarabinopyranoside (**c**), and methyl 3,4-di-*O*-methylglucopyranoside (**d**) in 2 : 1 : 1 : 1 ratio.

Based on the above-mentioned evidence, soyasaponin A_1 (**3**) was concluded to be a 2″-β-D-glucopyranoside of soyasaponin A_2 (**2**), and this was supported by an enzymatic hydrolysis of **3** with almond emulsin, giving **2** in high yield. Furthermore, a detailed comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data for soyasapogenol A (**1**), soyasaponin A_2 (**2**), and a prosapogenol (**4**)[1] with those for soyasaponin A_1 (**3**) (Table) supported the structure assignment.

Consequently, the structure of soyasaponin A_1 was determined as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucopyranosyl(1→3)-α-L-arabinopyranosyl]soyasapogenol A (**3**).

We have thus isolated five saponins, soyasaponins I, II, III, A_1 (**3**), and A_2 (**2**), from soybeans and elucidated their structures by various methods including two selective cleavage methods for the glucuronide linkage. Since the various biological activities reported for soybean saponins included some unfavorable ones,[13] we subjected a mixture of soybean saponins (named total soyasaponin), which retained the naturally occurring ratio of the

TABLE. ^{13}C-NMR Data for Soyasaponin A_1 (**3**) (25 MHz, in d_5-pyridine, δc)[a]

Sapogenol moiety		3-*O*-β-D-Glucuronopyranosyl moiety		2′-*O*-β-D-Galactopyranosyl moiety	
C-3	90.5 (d)	C-1′	104.5 (d)	C-1″	102.9 (d)
C-12	122.5 (d)	C-2′	80.7 (d)	C-2″	84.2 (d)
C-13	144.0 (s)	C-3′	76.3 (d)	C-3″	74.4 (d)
C-21	72.6 (d)	C-4′	72.4 (d)	C-4″	70.3 (d)
C-22	92.6 (d)	C-5′	76.3 (d)	C-5″	77.6 (d)
C-24	63.5 (t)	C-6′	172.1 (s)	C-6″	62.3 (t)

2‴-*O*-β-D-Glucopyranosyl moiety		22-*O*-α-L-Arabinopyranosyl moiety		3⁗′-*O*-β-D-Glucopyranosyl moiety	
C-1‴	106.6 (d)	C-1⁗	108.1 (d)	C-1⁗′	105.8 (d)
C-2‴	75.3 (d)	C-2⁗	69.0 (d)	C-2⁗′	75.3 (d)
C-3‴	78.1 (d)	C-3⁗	85.0 (d)	C-3⁗′	78.1 (d)
C-4‴	71.2 (d)	C-4⁗	67.1 (d)	C-4⁗′	71.2 (d)
C-5‴	78.8 (d)	C-5⁗	63.5 (t)	C-5⁗′	78.1 (d)
C-6‴	62.3 (t)			C-6⁗′	62.3 (t)

a) The off-resonance patterns of the signals are given in parentheses with abbreviations: d = doublet, s = singlet and t = triplet. The carbon signals affected by glycosidation shifts are underlined.

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above-mentioned five soyasaponins, to several biological activity tests.[14] So far, it has been found that total soyasaponin shows various interesting activities: inhibitory effects on lipid oxidation,[15] suppression of lipid-peroxide formation induced in mice by the application of adriamycin,[16] suppression of liver lesion generation in rats,[15] and some improvement in human serum lipid composition.[17] Among the five soyasaponins, soyasaponin A_1 (**3**) was shown to have a significant anti-oxidant action on lipid.

Experimental[18]

Soyasaponin A_1 (3)——Soyasaponin A_1 (**3**, 0.16 g) was isolated from soybean (667 g) as reported in our previous paper.[1] **3**, mp 240—242 °C (colorless fine crystals from aq. MeOH), $[\alpha]_D^{26}$ +23.2° (c=0.91, MeOH). *Anal.* Calcd for $C_{59}H_{96}O_{29} \cdot 4H_2O$: C, 52.82; H, 7.81. Found: C, 52.91; H, 7.70. IR ν_{max}^{KBr} cm^{-1}: 3390, 2918, 1737, 1074. ^{13}C-NMR (d_5-pyridine): Table.

Acidic Hydrolysis of Soyasaponin A_1 (3)——A solution of **3** (20 mg) in MeOH (5 ml) was treated with 20% aq. H_2SO_4 (5 ml) and the whole mixture was heated under reflux for 2 h. After removal of the MeOH under reduced pressure, the reaction mixture was diluted with water and the whole solution was extracted with AcOEt. The AcOEt extract was washed with sat. aq. $NaHCO_3$ and water, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by preparative thin-layer chromatography (TLC) ($CHCl_3$–MeOH = 20 : 1) followed by crystallization from $CHCl_3$–MeOH to furnish soyasapogenol A (**1**, 6 mg). Soyasapogenol A (**1**) thus obtained was shown to be identical with an authentic sample[2] by mixed mp determination and by TLC ($CHCl_3$–MeOH = 20 : 1, benzene–acetone = 5 : 1, n-hexane–AcOEt = 2 : 1), and IR(KBr) comparisons.

Methanolysis of Soyasaponin A_1 (3)——A solution of **3** (3 mg) in 9% HCl–dry MeOH (0.9 ml) was heated under reflux for 2 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. After identification of **1** in the filtrate by TLC (as described above), the solvent was removed from the filtrate under reduced pressure to give a product. The product was dissolved in pyridine (0.1 ml) and treated with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) for 10 min. The trimethylsilylated (TMS) product was then subjected to gas-liquid chromatography (GLC) to identify the TMS derivatives of methyl arabinoside, methyl galactoside, methyl glucoside, and methyl glucuronide. GLC: 1) 3% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh); 3 mm × 1 m glass column; column temp. 140 °C; N_2 flow rate 36 ml/min; t_R, TMS-methyl arabinoside 2′50″, 3′16″, TMS-methyl galactoside 9′48″, 11′08″, 12′56″, TMS-methyl glucuronide 6′51″, 15′43″, TMS methyl glucoside 14′15″, 16′02″. 2) 5% silicone SE-52 on Chromosorb WAW DMCS (80—100 mesh), 3 mm × 2 m glass column; column temp. 170 °C; N_2 flow rate 38 ml/min; t_R, TMS-methyl arabinoside 2′56″, 3′19″, TMS-methyl galactoside 8′03″, 9′18″, 10′36″, TMS-methyl glucuronide 7′08″, 14′35″, TMS-methyl glucoside 11′50″, 12′55″. The composition of the four methyl glycosides was determined from the GLC peak areas.

In another experiment, a solution of soyasaponin A_1 (300 mg) in 9% HCl–dry MeOH (5 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Ag_2CO_3 powder and filtered. Removal of the solvent from the filtrate under reduced pressure gave a product, which was purified by column chromatography (SiO_2 10 g, $CHCl_3$–MeOH = 10 : 1) to furnish methyl arabinoside (18 mg). The methyl arabinoside (18 mg) was dissolved in 5% aq. HCl (1 ml) and the whole mixture was heated at 100 °C for 30 min. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Removal of the water from the filtrate under reduced pressure furnished arabinose (15 mg), which was concluded to be of L form from its $[\alpha]_D^{18}$ +95.4° (c=1.4, H_2O, 2 h after preparing the solution).

Photolysis of Soyasaponin A_1 (3)——A solution of **3** (300 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. During the irradiation, the temperature of the reaction mixture was kept below 20 °C by cooling. The reaction mixture was neutralized with 10% aq. K_2CO_3 and the solvent was removed under reduced pressure. The product was then partitioned into an n-BuOH–H_2O (1 : 1) mixture. Removal of the solvent from the n-BuOH phase under reduced pressure yielded a product, which was purified by preparative TLC ($CHCl_3$–MeOH–H_2O = 7 : 3 : 1, lower phase) and by crystallization from EtOH to furnish a prosapogenol (**4**, 64 mg), mp 284—286 °C. **4** was shown to be identical with an authentic sample[1] by mixed mp determination and TLC [$CHCl_3$–MeOH–H_2O = 7 : 3 : 1 (lower phase), $CHCl_3$–MeOH–AcOEt–H_2O = 9 : 15 : 23 : 3, n-BuOH–AcOEt–H_2O = 4 : 1 : 5 (upper phase), $CHCl_3$–MeOH = 6 : 1], IR(KBr), and ^{1}H-NMR (d_5-pyridine) comparisons. Removal of the solvent from the H_2O phase under reduced pressure gave a product, which was dried and treated with Ac_2O–pyridine (1 : 1, 6 ml) at 18 °C for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by column chromatography (SiO_2 15 g, benzene–acetone = 20 : 1) to furnish **5** (27 mg). **5**, colorless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1759, 1218. ^{1}H-NMR ($CDCl_3$, δ): 1.97—2.18 (total 24H, OAc × 8), 5.62 (*ca.* 1/2H, d, J=8 Hz), 6.29 (*ca.* 1/2H, d, J=4 Hz) (anom. H of galactoside moiety). FD-MS (m/z): 678 (M^+), 619 (M^+ −OAc).

Deacetylation of 5 Followed by Methylation and Methanolysis——A solution of **5** (20 mg) in MeOH (1 ml) was

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treated with 10% NaOMe–MeOH (1 ml) and the whole mixture was stirred at 19 °C for 2 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and filtered. Removal of the solvent from the filtrate under reduced pressure gave a product which was dissolved in DMSO (1 ml) and treated with dimsyl carbanion (1 ml)[1,8] with stirring under an N_2 atmosphere at 19 °C for 1 h. The reaction mixture was then treated with CH_3I (2 ml) in the dark and stirred for a further 12 h, then poured into ice-water The whole mixture was extracted with AcOEt and the AcOEt extract was washed with 10% aq. $Na_2S_2O_3$ and water, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a syrupy product, which was purified by column chromatography (SiO_2 3 g, benzene–acetone = 8 : 1) to furnish **5a** (10 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2925, 1100. **5a** (5 mg) was dissolved in 9% HCl–dry MeOH (1 ml) and the solution was heated under reflux for 2 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a product, which was examined by TLC (benzene–acetone = 5 : 1, *n*-hexane–acetone = 2 : 1, *n*-hexane–AcOEt = 1 : 1) and GLC to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4,6-tri-*O*-methyl-galactopyranoside (**b**). GLC: 3) 15% ethylene glycol succinate on Chromosorb WAW (80—100 mesh); 3 mm × 1 m glass column; column temp. 150 °C; N_2 flow rate 28 ml/min; t_R, **a** 3′24″, 5′00″, **b** 19′00″, 32′48″. 4) 15% polyneopentyl glycol succinate on Chromosorb WAW (80—100 mesh); 3 mm × 2 m glass column; column temp. 170 °C; N_2 flow rate 35 ml/min; t_R, **a** 4′51″, 6′27″, **b** 15′22″, 23′00″.

Complete Methylation of Soyasaponin A_1 (3)——A solution of **3** (200 mg) in DMSO (20 ml) was treated with dimsyl carbanion (10 ml) and the whole mixture was stirred at 19 °C under an N_2 atmosphere for 1 h. The reaction mixture was then treated with CH_3I (10 ml) in the dark and stirred for a further 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a syrupy product, which was purified by column chromatography (SiO_2 30 g, benzene–acetone = 20 : 1—10 : 1) to furnish **3a** (155 mg). Octadeca-*O*-methylsoyasaponin A_1 (**3a**), mp 130—132 °C (colorless plates, MeOH), $[\alpha]_D^{27}$ +3.7° (c = 0.87, $CHCl_3$). *Anal.* Calcd for $C_{77}H_{132}O_{29}$: C, 60.77; H, 8.74. Found: C, 60.63; H, 8.60. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 2936, 1760, 1102. ^{1}H-NMR ($CDCl_3$, δ): 0.92 (6H), 0.96, 0.98 (3H each), 1.08 (6H), 1.11 (3H) (all s, *tert*-CH_3 × 7), 3.24 (9H), 3.33, 3.37, 3.39, 3.44 (6H each), 3.46 (9H), 3.49 (3H), 3.54 (12H) (all s, OCH_3 × 17), 3.78 (3H, s, $COOCH_3$), 4.31, 4.50 (1H each, both d, J = 7 Hz), 4.60 (2H, d, J = 7 Hz) (anom. H × 4), 5.20 (1H, br s, $W_{h/2}$ = 7 Hz, 12-H): (d_6-acetone, δ): 0.94, 0.96 (3H each), 0.99 (9H), 1.04, 1.10 (3H each) (all s, *tert*-CH_3 × 7), 3.29 (3H), 3.33, 3.39, 3.42, 3.45 (6H each), 3.50 (9H), 3.57 (12H), 3.63 (3H) (all s, OCH_3 × 17), 3.74 (3H s, $COOCH_3$), 4.42, 4.52, 4.60, 4.69 (1H each, all d, J = 7 Hz, anom. H × 4), 5.23 (1H, br s, $W_{h/2}$ = 8 Hz, 12-H).

Alkaline Hydrolysis of 3a Giving 3b——A solution of **3a** (57 mg) in MeOH (3 ml) was treated with 10% aq. K_2CO_3 (5 ml) and the whole mixture was heated under reflux for 2 h. The reaction mixture was made weakly acidic with 10% aq. HCl and the MeOH was removed under reduced pressure. The whole mixture was then extracted with AcOEt and the AcOEt extract was washed with water, then dried over $MgSO_4$. Removal of the AcOEt under reduced pressure gave **3b** (53 mg), white powder,[19] $[\alpha]_D^{18}$ +5.4° (c = 0.92, $CHCl_3$). *Anal.* Calcd for $C_{76}H_{130}O_{29}$: C, 60.54; H, 8.69. Found: C, 60.23; H, 8.84. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2925, 1748, 1732, 1090. ^{1}H-NMR ($CDCl_3$, δ): 0.96 (9H), 1.08, 1.15 (3H each), 1.25 (6H) (all s, *tert*-CH_3 × 7), 3.27 (3H), 3.36 (6H), 3.40, 3.45 (3H each), 3.51, 3.52, 3.54, 3.60 (9H each) (all s, OCH_3 × 17), 4.41—4.66 (4H, anom. H × 4), 4.97 (1H, br s, $W_{h/2}$ = 9 Hz, exchangeable with D_2O, COOH), 5.20 (1H, br s, $W_{h/2}$ = 6 Hz, 12-H).

Lead Tetraacetate Oxidation of 3b——A solution of **3b** (53 mg) in benzene (5 ml) was treated with $Pb(OAc)_4$ (100 mg) and the whole mixture was heated under reflux for 3.5 h. After cooling, the reaction mixture was dilute with AcOEt and the whole mixture was washed with water, then dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure furnished **3c** (50 mg), white powder. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2937, 1758, 1225, 1100. ^{1}H-NMR ($CDCl_3$, δ): 0.98 (9H), 1.08, 1.13 (3H each), 1.36 (6H) (all s, *tert*-CH_3 × 7), 2.07, 2.10 (total 3H, both s, α- and β-OAc), 3.28 (3H), 3.36 (9H), 3.41, 3.46 (3H each), 3.51 (18H), 3.61 (12H) (all s, OCH_3 × 17), 4.42—4.66 (4H, anom. H × 4), 5.20 (1H, br s, $W_{h/2}$ = 7 Hz, 12-H), 5.57 (*ca.* 1/2H, d, J = 8 Hz, 5′α-H), 6.23 (*ca.* 1/2H, d, J = 4 Hz, 5′β-H).

Alkaline Degradation of 3c Followed by Acetylation——A solution of **3c** (120 mg) in 2% NaOMe–MeOH (5 ml) was stirred at 26 °C for 30 min. The reaction mixture was neutralized with 9% HCl–dry MeOH and the solvent was removed under reduced pressure. The product thus obtained was treated with Ac_2O–pyridine (1 : 1, 4 ml) and the whole mixture was allowed to stand at 25 °C for 9 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner followed by removal of the solvent under reduced pressure gave a product, which was purified by preparative TLC (benzene–acetone = 5 : 2) to furnish **4b** (55 mg), **5b** (11 mg), and **6** (7 mg). **4b**, mp 151—152 °C (colorless needles from acetone–H_2O), $[\alpha]_D^{18}$ +13.3° (c = 0.92, $CHCl_3$). *Anal.* Calcd for $C_{51}H_{86}O_{14}$: C, 66.35; H, 9.39. Found: C, 66.33; H, 9.42. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1737, 1244. ^{1}H-NMR ($CDCl_3$, δ): 0.93, 0.97 (3H each), 1.00 (6H), 1.09 (3H), 1.25 (6H) (all s, *tert*-CH_3 × 7), 2.03 (3H, s, OAc), 3.27 (3H), 3.36 (6H), 3.45, 3.50, 3.52 (3H each), 3.60 (6H) (all s, OCH_3 × 8), 4.49 (2H, d, J = 7 Hz, anom. H × 2), 4.55 (1H, m, 3-H), 5.21 (1H, br s, $W_{h/2}$ = 8 Hz, 12-H). **5b**, white powder, $[\alpha]_D^{18}$ +2.6° (c = 0.31, $CHCl_3$). FD-MS (m/z): 482 (M^+), 423 (M^+ − OAc). High-resolution MS: Calcd for $C_{19}H_{35}O_{10}$ (M^+ − OAc) 423.225. Found: 423.224. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755, 1230. ^{1}H-NMR ($CDCl_3$, δ): 2.08 (3H, s, OAc), 3.34, 3.38 (3H each), 3.52, 3.56 (6H each), 3.61 (3H) (all s, OCH_3 × 7), 4.57 (1H, d, J = 8 Hz, anom. H of glucoside moiety), 5.63 (1H, d, J = 8 Hz, anom. H of galactoside moiety). **6** obtained

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here was shown to be identical with an authentic sample[4,10] by mixed mp determination and TLC (benzene–acetone = 5 : 2, *n*-hexane–AcOEt = 1 : 1, benzene–MeOH = 5 : 1), IR (CCl_4), and ^{1}H-NMR ($CDCl_3$) comparisons.

Deacetylation of 4b Followed by Methanolysis——A solution of **4b** (38 mg) in 2% NaOMe–MeOH (5 ml) was stirred at 37 °C for 2 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a product, which was dissolved in 9% HCl–dry MeOH (2 ml), and the whole solution was heated under reflux for 1.5 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the inorganic precipitate was removed by filtration. Removal of the solvent from the filtrate under reduced pressure yielded a crystalline product, which was collected by filtration and recrystallized from $CHCl_3$–MeOH to furnish **1a** (9 mg). **1a** was shown to be identical with an authentic sample[1] by mixed mp determination and TLC (benzene–acetone = 5 : 2, *n*-hexane–AcOEt = 1 : 1, benzene–MeOH = 5 : 1), IR ($CHCl_3$), and ^{1}H-NMR ($CDCl_3$) comparisons. After separation of **1a**, the filtrate was examined by TLC (benzene–MeOH = 5 : 1, benzene–acetone = 5 : 2, *n*-hexane–AcOEt = 1 : 1) and GLC to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 2,4-di-*O*-methylarabinopyranoside (**c**). GLC: 5) 15% polyneopentyl glycol succinate on Chromosorb WAW (80—100 mesh); 3 mm × 2 m glass column; column temp. 180 °C; N_2 flow rate 38 ml/min; t_R, **a** 3′05″, 4′01″, **c** 4′55″. 6) 5% butane-1,4-diol succinate on Uniport B (80—100 mesh); 3 mm × 2 m glass column; column temp. 160° C; N_2 flow rate 37 ml/min: t_R, **a** 5′31″, 7′48″, **c** 11′25″, 12′03″.

Conversion from 4b to 4a——**4b** (20 mg) was deacetylated with 2% NaOMe–MeOH as described above. The deacetylated product was dissolved in DMSO (5 ml) and treated with dimsyl carbanion (5 ml) and the whole mixture was stirred under an N_2 atmosphere at 20 °C for 1 h, then treated with CH_3I (3 ml) in the dark and stirred for a further 5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner and removal of the solvent under reduced pressure yielded a syrupy product, which was purified by preparative TLC (benzene–acetone = 3 : 1) to furnish **4a** (18 mg). **4a** obtained here was shown to be identical with an authentic sample[1] by mixed mp determination and TLC (benzene–acetone = 3 : 1, *n*-hexane–AcOEt = 1 : 2, benzene–MeOH = 4 : 1), and IR (CCl_4) comparisons.

Methanolysis of 5b——A solution of **5b** (2 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1.5 h. The reaction mixture was worked up as described above for the methanolysis of **5a** and the resulting product was examined by TLC (as above) and GLC [conditions 3) and 4)] to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**) and methyl 3,4,6-tri-*O*-methylgalactopyranoside (**b**).

$LiAlH_4$ Reduction of 3a Giving 3d——A solution of **3a** (80 mg) in ether (20 ml) was treated with a suspension of $LiAlH_4$ (100 mg) in ether (5 ml) and the whole mixture was stirred at 26 °C for 1 h. The reaction was quenched by adding wet ether. The reaction mixture was weakly acidified with 10% aq. H_2SO_4 and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner and removal of the solvent under reduced pressure furnished **3d** (66 mg). **3d**, white powder, $[\alpha]_D^{18}$ +2.8° (c = 0.22, $CHCl_3$). *Anal.* Calcd for $C_{76}H_{132}O_{28}$: C, 61.10; H, 8.91. Found: C, 59.91; H, 8.84. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600. ^{1}H-NMR ($CDCl_3$, δ): 0.93 (3H), 0.97 (9H), 1.02, 1.09, 1.19 (3H each) (all s, *tert*-CH_3 × 7), 3.29 (3H), 3.36 (9H), 3.42, 3.44 (3H each), 3.50 (6H), 3.51, 3.52, 3.60 (9H each) (all s, OCH_3 × 17), 4.28, 4.52, 4.63, 4.64 (1H each, all d, J = 7 Hz, anom. H × 4), 5.22 (1H, br s, $W_{h/2}$ = 6 Hz, 12-H).

Methanolysis of 3d——A solution of **3d** (22 mg) in 9% HCl–dry MeOH (2 ml) was heated under reflux for 1.5 h. The reaction mixture was neutralized with Ag_2CO_3 powder and the filtrate was worked up as described above for the methanolysis of **4b** to afford **1a** as a crystalline product. **1a** (6 mg) was collected by filtration and was shown to be identical with an authentic sample[1] by mixed mp determination and TLC (as above), IR ($CHCl_3$), and ^{1}H-NMR ($CDCl_3$) comparisons. The filtrate was examined by GLC to identify methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (**a**), methyl 3,4,6-tri-*O*-methylgalactopyranoside (**b**), methyl 2,4-di-*O*-methylarabinopyranoside (**c**), and methyl 3,4-di-*O*-methylglucopyranoside (**d**). GLC: 7) the same conditions as for 3) except column temp. 165 °C and N_2 flow rate 25 ml/min; t_R, **a** 1′34″, 2′11″, **b** 7′06″, 11′42″, **c** 4′04″, 4′22″, **d** 14′32″, 17′40″; 8) the same conditions as for 4) except column temp. 190 °C and N_2 flow rate 43 ml/min; t_R, **a** 2′17″, 2′53″, **b** 6′17″, 9′04″, **c** 5′10″, **d** 10′36″, 13′05″.

Enzymatic Hydrolysis of Soyasaponin A_1 (3) Giving Soyasaponin A_2 (2)——A solution of **3** (240 mg) in a buffer solution (Wako, pH 5.0, 150 ml) was treated with almond emulsin (Sigma, 300 mg) and the whole mixture was stirred at 37 °C for 6 d. The reaction mixture was extracted with *n*-BuOH and removal of the *n*-BuOH under reduced pressure gave a product, which was purified by column chromatography (SiO_2 20 g, $CHCl_3$–MeOH–H_2O = 7 : 3 : 1, lower phase) to furnish **2** (120 mg) and **3** (60 mg, recovered). **2** was crystallized from aq. MeOH and was shown to be identical with soyasaponin A_2 (**2**) by mixed mp determination and TLC ($CHCl_3$–MeOH–H_2O = 6 : 4 : 1; *n*-BuOH–AcOH–H_2O = 4 : 1 : 5, upper phase; $CHCl_3$–MeOH = 2 : 1), IR (KBr), and ^{13}C-NMR (d_5-pyridine) comparisons.

Acknowledgement The authors are grateful to the Ministry of Education, Science and Culture of Japan for a Grant-in-Aid for Scientific Research (No. 57430028).

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