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Saponin and Sapogenol. XXXI.[1] Chemical Constituents of the Seeds of *Vigna angularis* (WILLD.) OHWI *et* OHASHI. (1). Triterpenoidal Sapogenols and 3-Furanmethanol β-D-Glucopyranoside

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3-Furanmethanol β-D-glucopyranoside (**1**) and (+)-catechin 7-*O*-β-D-glucopyranoside (**3**) were isolated from azuki beans, the seeds of *Vigna angularis* (WILLD.) OHWI *et* OHASHI (Leguminosae) and their structures were substantiated. The saponin mixture designated as total azukisaponin was also isolated from azuki beans. By means of enzymatic hydrolysis, acidic hydrolysis, and photochemical degradation, four genuine sapogenols, which included three known sapogenols, sophoradiol (**7**), soyasapogenol B (**8**), gypsogenic acid (**9**), and a new sapogenol named azukisapogenol (**10**), were isolated from total azukisaponin and the structure of **10** was elucidated. As one of the artifact sapogenols which were liberated by acidic hydrolysis of total azukisaponin, a new sapogenol named anhydrosophoradiol (**5**) was isolated and its structure was elucidated.

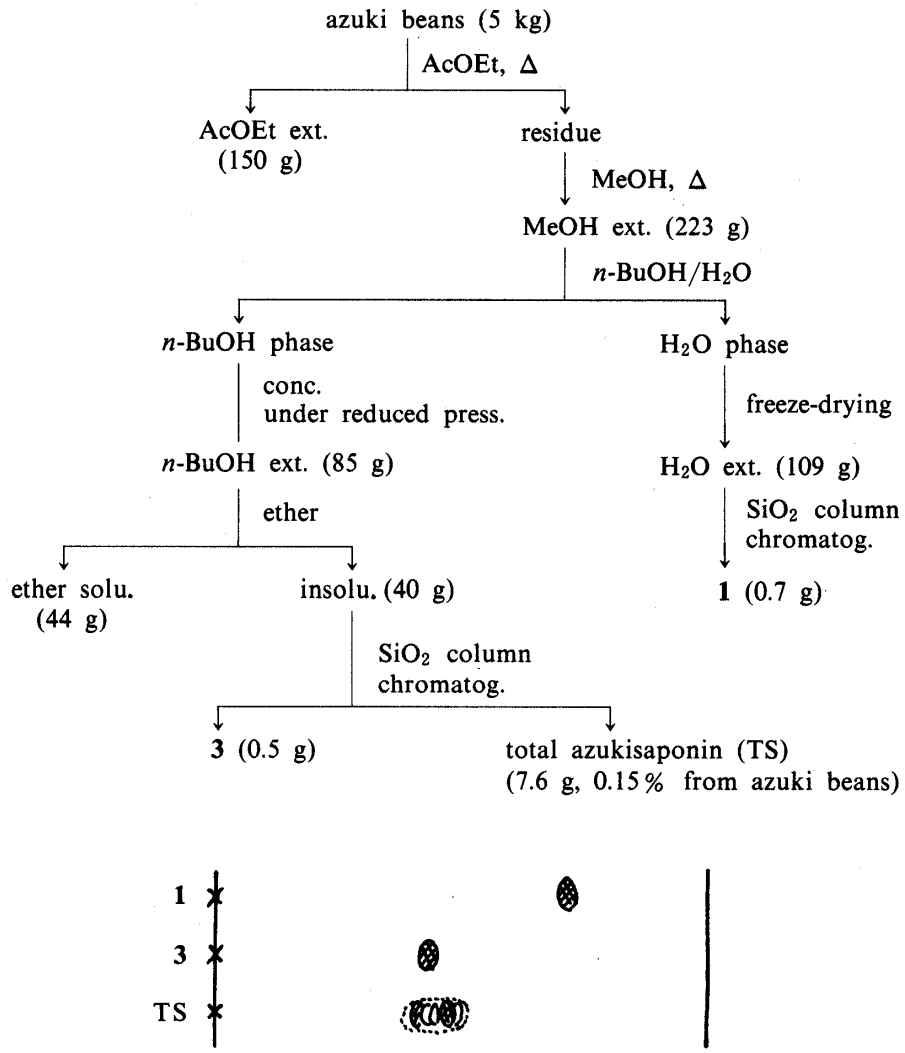
Keywords——azuki bean; *Vigna angularis*; 3-furanmethanol β-D-glucopyranoside; glucuronide linkage; photochemical degradation; enzymatic hydrolysis; genuine sapogenol; artifact sapogenol; azukisapogenol; anhydrosophoradiol

Azuki beans, the seeds of *Vigna angularis* (WILLD.) OHWI *et* OHASHI (Leguminosae), are a very important food material. In Chinese traditional medicine, azuki beans are called "chi xiao dou" (Phaseoli Semen) and have been used for various purposes, *e.g.* diuretic, antidote, and a remedy for dropsy and beriberi.[2]

In order to clarify the chemical constituents of azuki beans, many investigations have been carried out to identify monosaccharide,[3] oligosaccharide,[3] polysaccharide,[4] glycoside,[5] lipid,[6] amino acid,[7] and protein[8] constituents. In addition, the presence of saponin in azuki beans has been known for a long time.[9] However, the chemical nature of the saponin ingredients has not yet been fully characterized; it was only reported that acidic hydrolysis of the saponin mixture liberated soyasapogenol C (**6**) as the sapogenol and L-arabinose, D-glucose, D-glucuronic acid, and L-rhamnose as the carbohydrates.[10]

Recently, we isolated five saponins from soybeans,[11] the seeds of *Glycine max* MERRILL (Leguminosae), and found that these saponins showed several interesting biological activities.[12] As a continuing study on the bioactive constituents of naturally occurring drug materials from leguminous plants, we have been investigating the chemical constituents of azuki beans and isolated 3-furanmethanol β-D-glucopyranoside (**1**), (+)-catechin 7-*O*-β-D-glucopyranoside (**3**)[13] and six new saponins named azukisaponins I, II, III, IV, V, and VI.[14] This paper deals with the chemical elucidation of **1**, **3**, and triterpenoid sapogenols which were liberated from the saponin mixture of azuki beans (now named total azukisaponin) by various degradation methods.[15]

The methanolic extract of azuki beans, which were defatted with ethyl acetate beforehand, was subjected to solvent-fractionation as shown in Chart 1. After silica gel column chromatography, the water-soluble portion gave 3-furanmethanol β-D-glucopyranoside (**1**), while the *n*-butanol-soluble portion gave (+)-catechin 7-*O*-β-D-glucopyranoside (**3**) and total azukisaponin, which was a mixture of azukisaponins I, II, III, IV, V, and VI.



TLC: pre-coated silica gel 60 F-254 (Merck)
solvent: $CHCl_3$: MeOH : H_2O=6 : 4 : 1

Chart 1

3-Furanmethanol β-D-Glucopyranoside (1) and (+)-Catechin 7-*O*-β-D-Glucopyranoside (3)

The infrared (IR) spectrum of **1** showed the presence of a furan ring, which was further supported by the fragment ion peak at *m/z* 81 (**i**) in the mass spectrum (MS). The carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** also showed the presence of a furan ring together with a methyleneoxy moiety attached to C-3 of the furan ring.[16] The anomeric carbon signal of **1** was observed at δc 103.6 as a doublet of J_{CH}=161 Hz, and thus the β-glucosidic linkage was corroborated.

Ordinary acetylation of **1** furnished the crystalline tetraacetate (**1a**), whose proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals ascribable to the 2,3,4,6-tetra-*O*-acetylglucopyranoside moiety along with signals due to the furan-ring protons: δ 6.32, 1H, t-like, due to 4-H, and δ 7.39, 2H, m, due to 2,5-H. Based on these data, **1** was presumed to be 3-furanmethanol β-D-glucopyranoside.

This presumption was verified by the following evidence. Thus, methanolysis of **1** with 9% hydrogen chloride in methanol liberated methyl glucopyranoside as a sole product and no

product was isolated from the aglycone part. On the other hand, enzymatic hydrolysis of **1** with almond emulsin furnished 3-furanmethanol (**2**)[17] as the aglycone. Finally, treatment of **2** in benzene–nitromethane with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric cyanide furnished **1a** in high yield. 3-Furanmethanol β-D-glucopyranoside (**1**) seems to be the first example of a naturally occurring glucoside of a simple furan derivative.

The chemical behavior (*e.g.* upon methanolysis and/or enzymatic hydrolysis) of **3** and its tri-*O*-methyl derivative (**3a**) and examination of the physicochemical properties of **3a** led us to assume that **3** was identical with (+)-catechin 7-*O*-β-D-glucopyranoside which was previously isolated from the wooden part of *Schizandra nigra* MAX. (Magnoliaceae).[13] The structure of the sugar moiety of the glucoside was not yet definitely proved, but the correctness of the presumed β-glucopyranosidic nature was confirmed by the following evidence. Thus, in the ^{1}H-NMR spectrum of the octa-*O*-methyl derivative (**3b**), the anomeric proton signal was observed at δ 4.85 as a doublet of J=7 Hz, whereas methanolysis of **3b** liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside.

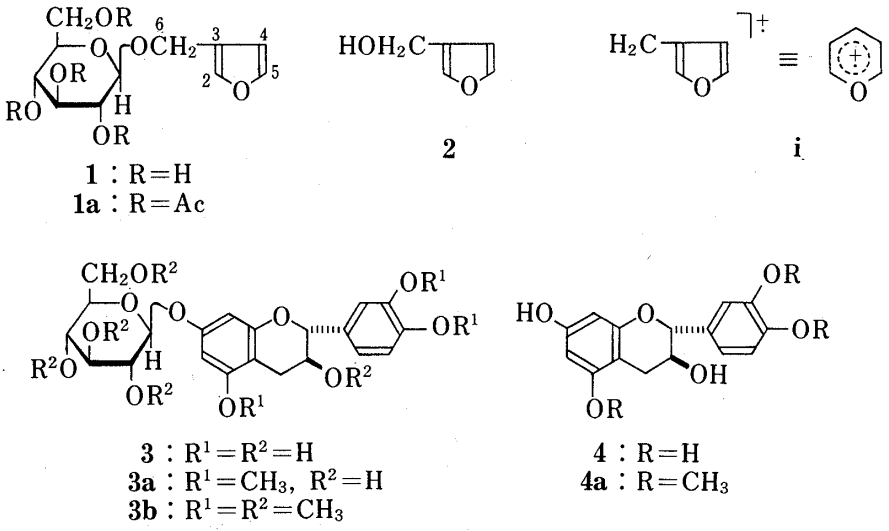


Chart 2

Sapogenols of Total Azukisaponin

As mentioned above, soyasapogenol C (**6**) was the only hitherto-reported sapogenol of azuki beans. However, this sapogenol seemed to be an artifact sapogenol secondarily formed during the acidic hydrolysis of the saponin mixture, so that the sapogenol composition of total azukisaponin was investigated.

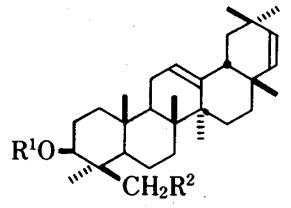
Enzymatic hydrolysis of total azukisaponin with crude hesperidinase liberated sophoradiol (**7**,)[11b,18] soyasapoganol B (**8**),[11b] gypsogenic acid (**9**)[19] and a new sapogenol named azukisapogenol (**10**). On the other hand, acidic hydrolysis of total azukisaponin with 20% aq. sulfuric acid–methanol (2:5) yielded **6**, **7**, **8**, azukisapogenol methyl ester (**11**),[20] and a new sapogenol named anhydrosophoradiol (**5**).[21]

Since D-glucuronic acid is known to be one of the carbohydrate ingredients of total azukisaponin, the total saponin mixture was subjected to photochemical degradation,which is one of selective cleavage method for the glucuronide linkage in glucuronide-saponin.[22] Photolysis of the methanolic solution of total azukisaponin by direct irradiation with a 500W high pressure mercury lamp for 1 h yielded **7** and **8**.[23]

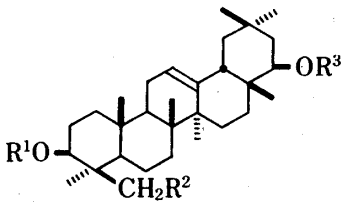
In order to shed light on the genuineness of these sapogenols which were liberated by the different degradation methods, we next carried out the structural elucidation of two new sapogenols: anhydrosophoradiol (**5**) and azukisapogenol (**10**).

The ^{1}H-NMR spectrum of **5** showed signals due to eight tertiary methyl groups, 3α-proton geminal to the 3β-hydroxyl group, and three olefinic protons. Ordinary acetylation of **5** gave the monoacetate (**5a**). Observation of the base peak (**ii**)[24] in MS of both **5** and **5a** and detailed comparisons of physicochemical properties of **5** and **5a** with those of **6** and **6a**[11a] led us to presume the structure **5** which was derived by dehydration of the 22β-hydroxyl (axial) group of sophoradiol (**7**). This presumption was verified by the two following observations. Thus, methanolic sulfuric acid treatment of **7** under reflux yielded **5**, while phosphorus oxychloride treatment of 3-*O*-acetylsophoradiol (**7a**)[18] afforded **5a**, both in high yield. Consequently, it become clear that anhydrosophoradiol (**5**) was an artifact sapogenol which was secondarily formed from sophoradiol (**7**) during the acidic hydrolysis.

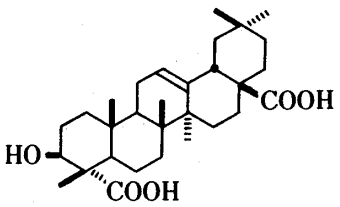
The IR spectrum of azukisapogenol (**10**) showed the presence of the hydroxyl group and carboxylic function. The ^{1}H-NMR spectrum of **10** showed signals ascribable to six tertiary



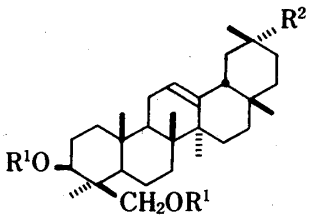
5 : $R^1=R^2=H$ (anhydrosophoradiol)
5a : R^1=Ac, $R^2=H$
6 : $R^1=H$, R^2=OH (soyasapogenol C)
6a : R^1=Ac, R^2=OAc



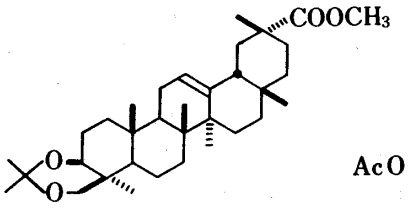
7 : $R^1=R^2=R^3=H$ (sophoradiol)
7a : R^1=Ac, $R^2=R^3=H$
7b : $R^1=R^2=H$, R^3=Ac
8 : $R^1=H$, R^2=OH, $R^3=H$ (soyasapogenol B)



9 : gypsogenic acid



10 : $R^1=H$, R^2=COOH (azukisapogenol)
10a : R^1=Ac, R^2=COOH
11 : $R^1=H$, R^2=$COOCH_3$
11a : R^1=Ac, R^2=$COOCH_3$
12 : R^1=Ac, R^2=CH_2OAc



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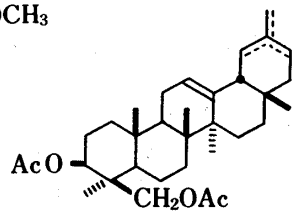

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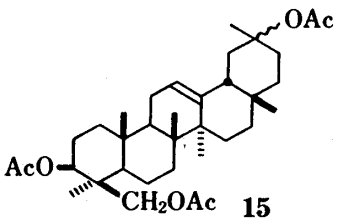

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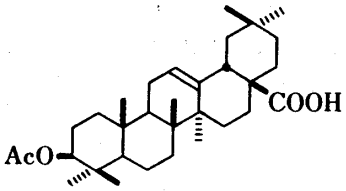

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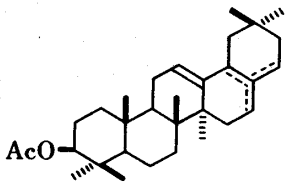

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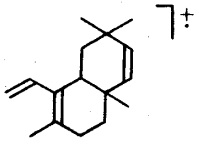

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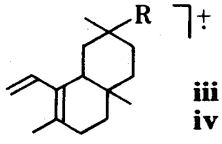
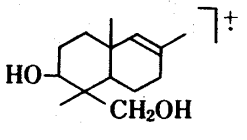


iii : R=COOH
iv : R=$COOCH_3$
v : R=CH_2OAc



vi

Chart 3

methyl groups, a 3α-proton which is characteristic of 3β-hydroxy-olean-12-ene triterpenes,[25] one axial hydroxymethyl function attached to a quaternary carbon, and one olefinic proton. Here again, the MS of **10** gave information on the location of substituents. The base peak (**iii**) and the fragment ion peak (**vi**), both derivable *via* retro Diels-Alder fragmentation at the *C* ring, suggested that **10** was an olean-12-ene triterpenoid possessing a carboxylic function in the *D*/*E* ring and a 3β-hydroxyl and one primary carbinol moiety in the *A*/*B* ring.

Methanolic sulfuric acid treatment of **10** gave in high yield the monomethyl ester (**11**), which was identical with the one obtained by acidic hydrolysis of total azukisaponin as described above. Therefore, **11** was shown to be an artifact sapogenol produced during the methanolic acid treatment of the total saponin mixture. In addition, C-17 was excluded for the location of the carboxylic moiety in azukisapogenol, since the carboxyl group at C-17 was not easily methylated.[26]

Acetylation of **10** and **11** gave the respective diacetates **10a** and **11a**. Although syntheses of **10a**, **11**, and **11a** were reported in the structural study of 24-hydroxyliquiritic acid,[20] a sapogenol of the root of *Glycyrrhiza glabra* (Leguminosae), some inconsistency was noticed among the reported physical data. Thus, some additional structural analyses of **10a**, **11**, and **11a** were carried out.

Lithium aluminum hydride reduction of **11** followed by acetylation furnished the triacetate **12**. The ^{1}H-NMR spectra of **10a**, **11a** and **12** showed that **10a** and **11a** possessed one axial acetoxymethyl moiety[27] while **12** contained one axial and one newly formed equatorial acetoxymethyl moiety[27] in the molecule. Next, **10a** was subjected to decarboxylation by use of lead tetraacetate.[26] The products were a mixture of dienic compounds (**14**)[20] and an epimeric mixture of acetoxylated compounds (**15**).[26] When 3-*O*-acetyloleanolic acid (**16**) was subjected to the same decarboxylation reaction, a mixture of dienic compounds (**17**), which included a conjugated diene as shown by its ultraviolet (UV) spectrum,[28] was formed.

The accumulated evidence together with the MS fragmentation patterns of **10**, **11**, and **12** (giving **iii**, **iv**, **v**, and **vi**, *etc.*) led us to locate the carboxyl moiety in azukisapogenol (**10**) at C-20α (equatorial). Furthermore, the physicochemical properties of the monoacetonide (**13**), which was prepared from **11**, were consistent with the location of two hydroxyl groups in **11** at C-3β and C-24 (axial). Thus, the structures of **10a**, **11**, **11a** were reconfirmed, and the structure of azukisapogenol (**10**) was substantiated.

Based on the above-described details and a consideration of the features of the three degradation methods applied to the saponin mixture, the genuine sapogenols of total azukisaponin were concluded to be sophoradiol (**7**), soyasapogenol B (**8**), gypsogenic acid (**9**), and azukisapogenol (**10**). It was also shown that soyasapogenol C (**6**) and two newly isolated sapogenols, anhydrosophoradiol (**5**) and azukisapogenol methyl ester (**11**), were artifact sapogenols.

Experimental[29]

Isolation of Glucosides (1, 3) and Total Azukisaponin——Powdered azuki beans ("Dainagon", cultivated at Obihiro in Hokkaido,5 kg) were defatted with AcOEt three times (10 l each, with heating under reflux for 5 h). The powder was then extracted with MeOH three times (10 l each, with heating under reflux for 5 h). Removal of the solvent from each extract under reduced pressure gave the AcOEt extract (150 g) and the MeOH extract (223 g). The MeOH extract was partitioned into *n*-BuOH–H_2O (1:1). Freeze-drying of the water phase yielded the H_2O extract (109 g), whereas removal of the solvent from the *n*-BuOH phase under reduced pressure yielded the *n*-BuOH extract (85 g). Separation of the H_2O extract by column chromatography (SiO_2 1.2 kg, $CHCl_3$–MeOH–H_2O=6:4:1) furnished 3-furanmethanol β-D-glucopyranoside (**1**, 0.7 g). The *n*-BuOH extract (85 g) was dissolved in a small amount of MeOH and the solution was poured dropwise into ether (1.5 l) with stirring. The precipitate (40 g) was collected by filtration and dried. Column chromatography (SiO_2 2 kg, *n*-BuOH–AcOEt–H_2O=4:1:5, upper phase) of the resulting powder furnished (+)-catechin 7-*O*-β-D-glucopyranoside (**3**, 0.5 g) and total azukisaponin (7.6 g).

1, hygroscopic powder, $[\alpha]_D^{26}$ +40.0° (c=1.1, MeOH). High resolution MS: Found 260.089, 82.042, 81.034. Calcd for $C_{11}H_{16}O_7$ (M^+): 260.089, C_5H_6O (**i**+H): 82.042, C_5H_5O (**i**): 81.035. IR ν_{max}^{KBr} cm^{-1}: 3374 (br, OH), 1070, 1017, 877 (furan). ^{1}H-NMR (d_5-pyridine, δ): 6.51 (1H, d-like), 7.47 (1H, t-like), 7.59 (1H, m). ^{13}C-NMR (CD_3OD, δ_c, off-resonance pattern): 63.6 (t, 6'-C) 64.0 (t, 6-C), 72.5, 75.7, 78.5, 78.8 (all d, 4', 2', 5', 3'-C), 103.6 (d, 1'-C), 112.3 (d, 4-C), 123.8 (s, 3-C), 143.1 (d, 2-C), 145.1 (d, 5-C). MS m/z (%): 260 (M^+, <1), 82 (100), 81 (56).

3, colorless needles, mp 213—215°C ($CHCl_3$-MeOH), $[\alpha]_D^{25}$ −50.4° (c=0.3, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3450, 1620, 1030. [lit.[13] colorless needles, mp 214—216°C, $[\alpha]_D^{32}$ −33.4° (c=1.0, MeOH)].

Acetylation of 1———A solution of **1** (24 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the mixture was allowed to stand at 36°C for 13 h. The reaction mixture was poured into ice-water and the whole mixture was extracted with AcOEt. The AcOEt extract was washed successively with dil. aq. HCl, sat. aq. $NaHCO_3$, and H_2O, and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the product, which was crystallized from ether to furnish **1a** (38 mg), mp 110—111°C (colorless needles), $[\alpha]_D^{22}$ −38.1° (c=0.7, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{24}O_{11}$: C, 53.27; H, 5.65. Found: C, 53.20; H, 5.61. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1745, 1200, 1060, 1033, 870. ^{1}H-NMR ($CDCl_3$, δ): 1.98 (6H, s), 2.00, 2.08 (3H each, both s), and other signals as given in the text. MS m/z (%): 428 (M^+, 1), 139 (100), 81 (96).

Methanolysis of 1———A solution of **1** (2 mg) in 9% HCl-MeOH (2 ml) was heated under reflux for 30 min. The reaction mixture was neutralized ith 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 dissolved in pyridine (0.1 ml) and treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) for 10 min. The product was then subjected to gas-liquid chromatography (GLC) analyses (a, b) and shown to be identical with the TMS derivative of methyl glucopyranoside. a) 5% silicon SE-30 on Chromosorb WAW DMCS (80—100 mesh) 3 mm×2 m glass column; column temp., 170°C; carrier gas, N_2; flow rate, 38 ml/min; t_R, 10'33" (major), 11'28". b) 15% ethyleneglycol succinate polyester on Uniport B (80—100 mesh), 3 mm×1 m glass column; column temp., 130°C; carrier gas, N_2; flow rate, 35 ml/min; t_R, 13'02" (major), 14'28".

Enzymatic Hydrolysis of 1———A solution of **1** (15 mg) in H_2O (5 ml) was treated with almond emulsin (Sigma, 1 mg) with stirring at 37°C for 24 h. The reaction mixture was extracted with ether and the ether extract was dried over $MgSO_4$ then evaporated to dryness under reduced pressure to yield the aglycone (5 mg). The aglycone was shown to be identical with 3-furanmethanol (**2**) by thin layer chromatography (TLC) comparisons [benzene-acetone=5:1, n-hexane-AcOEt=1:1, $CHCl_3$ (double development)] and GLC analyses (c, d). c) 5% butane-1,4-diol succinate on Uniport B (80—100 mesh), 3 mm×2 m glass column; column temp., 95°C; carrier gas, N_2; flow rate, 30 ml/min; t_R, 10'52". d) 15% polyneopentylglycol succinate on Chromosorb WAW (80—100 mesh), 3 mm×2 m glass column; column temp., 150°C; carrier gas, N_2; flow rate, 30 ml/min; t_R, 5'03".

Synthesis of 1a———A solution of **2** (1.1 g) in benzene-nitromethane (1:1, 10 ml) was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (3.3 g) and $Hg(CN)_2$ (3.4 g) and the whole mixture was stirred at 19°C for 24 h. After dilution of the reaction mixture with benzene, the whole solution was washed with aq. sat. $NaHCO_3$ and water, and dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure yielded the product (2.7 g), which was purified by centrifugal liquid chromatography (CLC) (KT gel 80 g, $CHCl_3$) to furnish the glucoside (1.25 g). The glucoside thus obtained was shown to be identical with **1a** by mixed mp determination, and $[\alpha]_D$, TLC [benzene-acetone=5:1, n-hexane-AcOEt=1:1, $CHCl_3$ (double development)], IR ($CHCl_3$), and ^{1}H-NMR ($CDCl_3$) comparisons.

Methanolysis of 3———A solution of **3** (5 mg) in 9% HCl-dry MeOH (0.5 ml) was heated under reflux for 2 h. The reaction mixture was worked up as described in the case of **1** and the product, after trimethylsilylation, was subjected to GLC analyses (a, b) and shown to be identical with the TMS derivative of methyl glucopyranoside.

Enzymatic Hydrolysis of 3———A solution of **3** (50 mg) in the buffer solution (Wako "standard", pH 6.86, 1 ml) was treated with almond emulsin (Sigma, 50 mg) and incubated with stirring at 34°C for 24 h. The reaction mixture was worked up as in the case of **1** to yield (+)-catechin (4, 20 mg) which was shown to be identical with an authentic sample by TLC [$CHCl_3$-MeOH-H_2O=7:3:1 (lower phase), n-BuOH-AcOEt-H_2O=4:1:5 (upper phase)], $[\alpha]_D$, IR (KBr), and ^{1}H-NMR (d_6-acetone) comparisons.

Methylation of 3———A solution of **3** (130 mg) in MeOH (10 ml) was treated with excess CH_2N_2-ether and the yellow solution was allowed to stand at 12°C for 12 h. Removal of the solvent gave the tri-*O*-methyl derivative (127 mg), whose physical data (mp, $[\alpha]_D$, ^{1}H-NMR) were in good agreement with those reported for **3a**.[13] Enzymatic hydrolysis of the tri-*O*-methyl derivative (**3a**) (91 mg) with almond emulsin as described above furnished 5,3',4'-tri-*O*-methyl-(+)-catechin (**4a**, 45 mg), which was identical with an authentic sample by mixed mp determination, and TLC ($CHCl_3$-MeOH=20:1, benzene-acetone=1:1, benzene-MeOH=10:1), $[\alpha]_D$, and IR (KBr) comparisons.

A solution of **3a** (500 mg) in dimethylformamide (7 ml) was treated with Ag_2O (500 mg) and CH_3I (7 ml) and the whole mixture was stirred in the dark at 28°C for 24 h. The reaction mixture was extracted with AcOEt and work-up of the AcOEt extract in the usual manner gave the product. Purification of the product by column chromatography (SiO_2 25 g, benzene-acetone=25:2) furnished **3b** (350 mg), white powder,

$[\alpha]_D^{27}$ −21.6° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{40}O_{11}$: C, 61.68; H, 7.15. Found: C, 61.77; H, 7.27. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1610, 1590, 1125, 1095. ^{1}H-NMR ($CDCl_3$, δ): 2.58 (1H, dd, J=8, 16 Hz), 2.95 (1H, dd, J=6, 16 Hz) (4-H_2), 3.22, 3.25, 3.52 (all 3H), 3.61 (6H), 3.77 (3H), 3.85 (6H) (all s, OCH_3×8), 4.87 (2H, d, J=7 Hz, 1′, 2-H), 6.25 (2H, s), 6.90 (3H, m) (phenyl H_5); (d_6-acetone, δ): 4.81 (1H, d, J=7 Hz), 4.85 (1H, d, J=7 Hz) (1′, 2-H).

Methanolysis of 3b——A solution of **3b** (1 mg) in 9% HCl-dry MeOH (1 ml) was heated under reflux for 1 h. The product obtained by work-up as described above was subjected to TLC (benzene–acetone=2:1, *n*-hexane–MeOH=5:1, $CHCl_3$–MeOH=15:1) and GLC (e, f) analyses, and shown to be identical with methyl 2,3,4,6-tetra-*O*-methylglucopyranoside. e) 15% polyneopentylglycol succinate on Chromosorb WAW (80—100 mesh), 3 mm×2 m glass column; column temp., 190°C; carrier gas, N_2; flow rate, 35 ml/min; t_R, 5′53″ (major), 7′43″. f) 15% diethylene glycol succinate on Chromosorb WAW (80—100 mesh), 3 mm×2 m glass column; column temp., 170°C; carrier gas, N_2; flow rate, 35 ml/min; t_R, 5′42″ (major), 8′07″.

Enzymatic Hydrolysis of Total Azukisaponin——A solution of total azukisaponin (4 g) in H_2O (200 ml) was treated with crude hesperidinase (2 g) and the whole mixture was incubated with stirring at 35°C for 14 h. The reaction mixture was extracted with $CHCl_3$–MeOH (5:1), the extract was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Separation of the product by column chromatography (SiO_2 100 g, eluting with $CHCl_3$ and $CHCl_3$–MeOH mixtures of increasing polarity) furnished sophoradiol (**7**, 310 mg), soyasapogenol B (**8**, 460 mg), gypsogenic acid (**9**, 20 mg), and azukisapogenol (**10**, 120 mg). Compounds **7**, **8**, and **9** were identified by direct comparisons with authentic samples by mixed mp determinations, and TLC (for **7** and **8**, $CHCl_3$–MeOH=15:1, benzene–acetone=4:1, *n*-hexane–AcOEt=1:1, benzene–MeOH=20:1; for **9**, $CHCl_3$–MeOH=10:1, benzene–MeOH=10:1, benzene–acetone=1:1), and IR (KBr) comparisons.

Azukisapogenol (**10**), mp 286—287°C (colorless needles, $CHCl_3$–MeOH). $[\alpha]_D^{13}$ +38.5° (c=1.2, pyridine). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.01; H, 10.28. High resolution MS: Found 472.355, 248.178, 224.176. Calcd for $C_{30}H_{48}O_4$ (M^+): 472.355, $C_{16}H_{24}O_2$ (**iii**): 248.179, $C_{14}H_{24}O_2$ (**vi**) 224.174. IR ν_{max}^{KBr} cm^{-1}: 3370 (br), 1690, 1030. ^{1}H-NMR (d_5-pyridine, δ): 0.96 (6H), 0.98 1.21, 1.48, 1.54 (3H each) (all s, *tert*-CH_3×6), 3.76 (1H, t-like, 3-H), 3.59, 4.52 (2H, ABq, J=11 Hz, 24-H_2), 5.32 (1H, br s, 12-H). MS m/z (%): 472 (M^+, 1), 248 (**iii**, 100), 224 (**vi**, 9).

Acidic Hydrolysis of Total Azukisaponin——A solution of total azukisaponin (15 g) in MeOH (500 ml) was treated with 20% aq. H_2SO_4 (200 ml) and the whole mixture was heated under reflux for 20 h. After removal of the MeOH under reduced pressure, the reaction mixture was diluted with water and the resulting precipitate was collected by filtration. Purification of the product by column chromatography (SiO_2 500 g, $CHCl_3$–MeOH=200:1→100:1) furnished anhydrosophoradiol (**5**, 0.25 g), sophoradiol (**7**, 0.88 g), a mixture (0.61 g) of soyasapogenol C (**6**) and azukisapogenol methyl ester (**11**), and soyasapogenol B (**8**, 1.37 g). The mixture of **6** and **11** was acetylated with Ac_2O–pyridine (1:1, 5 ml) and the product was subjected to preparative TLC ($CHCl_3$–MeOH=10:1) to furnish **6a** (0.23 g) and **11a** (0.49 g). Treatment of **6a** and **11a** separately with 1% NaOMe–MeOH at 25°C with stirring for 1 h yielded **6** (0.19 g) and **11** (0.41 g), respectively. Compounds **6**, **6a**, **7**, and **8** were identified by mixed mp determinations, and TLC (for **6**, $CHCl_3$–MeOH=15:1, *n*-hexane–AcOEt=1:1, benzene–acetone=4:1; for **6a**, benzene–$CHCl_3$=1:5, benzene–acetone=15:1, *n*-hexane–AcOEt=2:1; for **7** and **8**, as described above), and IR (KBr) comparisons with the respective authentic samples.

Anhydrosophoradiol (**5**), mp 180—181°C (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{13}$ +54.8° (c=0.7, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.56; H, 11.44. High resolution MS: Found 424.370. Calcd for $C_{30}H_{48}O$ (M^+): 424.370. IR ν_{max}^{KBr} cm^{-1}: 3300, 1035. ^{1}H-NMR ($CDCl_3$, δ): 0.79, 0.86, 1.11, 1.25 (3H each), 0.93, 0.95 (6H each) (all s, *tert*-CH_3×8), 3.21 (1H, dd, J=4, 8 Hz, 3-H), 5.24 (3H, m, 12, 21, 22-H). MS m/z (%): 424 (M^+, 9), 216 (**ii**, 100), 208 (7).

Azukisapogenol methyl ester (**11**), mp 262—263°C (colorless needles from $CHCl_3$–ligroin), $[\alpha]_D^{13}$ +56.4° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3250 (br), 1730, 1110, 1050. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1715. ^{1}H-NMR ($CDCl_3$, δ): 0.84, 0.88, 0.93, 1.13, 1.19, 1.24 (3H each, all s, *tert*-CH_3×6), 3.40 (1H, t-like, 3-H), 3.33, 4.20 (2H, ABq, J=11 Hz, 24-H_2) 3.65 (3H, s, $COOCH_3$), 5.21 (1H, t-like, 12-H). MS m/z (%): 486 (M^+, 3) 262 (**iv**, 100), 224 (**vi**, 13). [lit.[20] mp 254—255°C ($CHCl_3$–ligroin), $[\alpha]_D^{20}$ +58.5° (c=1.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1725, ^{1}H-NMR ($CDCl_3$, δ): 0.86, 0.90, 0.95, 1.15, 1.21, 1.26 (3H each, all s, *tert*-CH_3×6), 3.40 (1H, m), 3.36, 4.25 (1H each, both d, J=12 Hz), 3.68 (3H, s), 5.26 (1H, m)]. **11a**, mp 225—227°C (colorless needles from $CHCl_3$–ligroin), $[\alpha]_D^{13}$ +54.7° (c=1.8, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{54}O_6$: C, 73.65; H, 9.54. Found: C, 73.53; H, 9.75. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1726, 1255, 1025. ^{1}H-NMR ($CDCl_3$, δ): 0.86 (3H), 0.98 (6H), 1.03, 1.14, 1.20 (3H each) (all s, *tert*-CH_3×6), 2.02, 2.04 (3H each, both s, OAc×2), 3.66 (3H, s, OCH_3), 4.14, 4.38 (2H, ABq, J=13 Hz, 24-H_2), 4.60 (1H, t-like, 3-H), 5.24 (1H, t-like, 12-H). MS m/z (%): 570 (M^+, 2), 262 (**iv**, 100). [lit.[20] mp 215—216°C ($CHCl_3$–ligroin), $[\alpha]_D^{20}$ +54° (c=1.5), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1250].

Photolysis of Total Azukisaponin——A solution of total azukisaponin (1 g) in MeOH (500 ml) was irradiated internally with a 500 W high pressure mercury lamp (Eikosha, PIH-500) for 1 h with cooling to keep the solution temperature at 5—10°C. The reaction mixture was neutralized with 10% aq. K_2CO_3 and the solvent was evaporated off under reduced pressure. Purification of the product by column chromatography (SiO_2 40 g, $CHCl_3$–MeOH=100:1→50:1) furnished sophoradiol (**7**, 75 mg) and soyasapogenol B (**8**, 96 mg),

which were identified by comparison with authentic samples as described above.

Acetylation of Anhydrosophoradiol (5)———A solution of **5** (20 mg) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) at 21°C for 14 h. Work-up of the product in the usual manner furnished **5a** (21 mg), mp 233—234°C (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{13}$ +66.1° (c=0.9, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{52}O_2$: C, 82.35; H, 10.80. Found: C, 82.13; H, 10.64. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1740, 1245. ^{1}H-NMR ($CDCl_3$, δ): 0.87, 0.98 (9H each), 1.11, 1.26 (3H each) (all s, *tert*-CH_3×8), 2.03 (3H, s, OAc), 4.55 (1H, dd, J=4, 8 Hz, 3-H), 5.22 (3H, m, 12, 21, 22-H). MS m/z (%): 466 (M^+, 1), 216 (**ii**, 100).

Acid Treatment of Sophoradiol (7)———A solution of **7** (20 mg) in conc. H_2SO_4–MeOH (1:4, 5 ml) was heated under reflux for 4 h. The reaction mixture was worked up as described for the hydrolysis of total azukisaponin. The product was then purified by preparative TLC ($CHCl_3$) to furnish **5** (7 mg), which was shown to be identical with an authentic sample by mixed mp determination, and TLC ($CHCl_3$–MeOH=30:1, benzene–acetone=5:1, *n*-hexane–AcOEt=2:1), and IR (KBr) comparisons.

Acetylation of Sophoradiol (7) and $POCl_3$ Treatment of 3-*O*-Acetylsophoradiol (7a)———A solution of **7** (150 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the whole mixture was allowed to stand at 0°C for 10 h. Work-up of the reaction mixture in the usual manner and purification of the product by preparative TLC ($CHCl_3$–MeOH=100:1, double development) furnished 3,22-di-*O*-acetylsophoradiol (91 mg), 3-*O*-acetylsophoradiol (**7a**, 40 mg), 22-*O*-acetylsophoradiol (**7b**, 26 mg), and **7** (10 mg, recovered). **7a**, mp 225—226°C (colorless needles from MeOH), $[\alpha]_D^{13}$ +97.8° (c=0.3, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{52}O_3$: C, 79.29; H, 10.81. Found: C, 79.30; H, 10.69. IR ν_{max}^{KBr} cm^{-1}: 3540, 1705, 1250. ^{1}H-NMR ($CDCl_3$, δ): 0.89 (9H), 0.93 (3H), 0.99 (6H), 1.05, 1.13 (3H each) (all s, *tert*-CH_3×8), 2.05 (3H, s, OAc), 3.45 (1H, t, J=5 Hz, 22-H), 4.51 (1H, dd, J=4, 8 Hz, 3-H), 5.26 (1H, t, J=4 Hz, 12-H). MS m/z (%): 484 (M^+, 3), 234 (100). [lit.[18] mp 226—228°C (MeOH), $[\alpha]_D^{28}$ +80.9°]. **7b**, mp 264—265°C (colorless needles, from $CHCl_3$–MeOH), $[\alpha]_D^{13}$ +71.0° (c=1.9, $CHCl_3$). High resolution MS: Found 484.390. Calcd for $C_{32}H_{52}O_3$ (M^+): 484.391. IR ν_{max}^{KBr} cm^{-1}: 3520, 1700, 1260. ^{1}H-NMR ($CDCl_3$, δ): 0.79, 0.82, 0.89, 0.94 (3H each), 1.00 (9H), 1.14 (3H) (all s, *tert*-CH_3×8), 2.01 (3H, s, OAc), 3.21 (1H, dd, J=4, 8 Hz, 3-H), 4.63 (1H, t, J=4 Hz, 22-H), 5.27 (1H, t, J=3 Hz, 12-H). MS m/z (%): 484 (M^+, 2), 276 (12), 216 (100).

A solution of **7a** (40 mg) in pyridine (1 ml) was treated with $POCl_3$ (1 ml) with stirring at 18°C for 20 h. The reaction mixture was then poured into ice-water and the whole mixture was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner furnished **5a** (35 mg), which was shown to be identical with an authentic sample by mixed mp determination, and TLC (as described for **6a**), and IR (KBr) comparisons.

Methanolic Sulfuric Acid Treatment of Azukisapogenol (10)———A solution of **10** (10 mg) in conc. H_2SO_4–MeOH (1:4, 5 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the solvent was evaporated off under reduced pressure to furnish **11** (10 mg), which was shown to be identical with an authentic sample by mixed mp determination, and IR (KBr), and TLC (as described for **6**) comparisons.

Acetylation of 10———A solution of **10** (20 mg) in pyridine (0.5 ml) was acetylated with Ac_2O (0.5 ml) in the usual manner to furnish **10a** (22 mg), mp 246—247°C (colorless fine crystals from MeOH), $[\alpha]_D^{13}$ +53.5° (c=1.4, $CHCl_3$, 8). High resolution MS: Found 556.377. Calcd for $C_{34}H_{52}O_6$ (M^+): 556.377. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1720, 1220. ^{1}H-NMR ($CDCl_3$,δ): 0.86 (3H), 0.97 (6H), 1.02, 1.04, 1.22 (3H each) (all s, *tert*-CH_3×6), 2.01, 2.04 (3H each, both s, OAc×2), 4.12, 4.36 (2H, ABq, J=13 Hz, 24-H_2), 4.59 (1H, t-like, 3-H), 5.22 (1H, br s, 12-H), 10.51 (1H, br s, COOH, disappeared on D_2O addition). MS m/z (%): 556 (M^+, 2), 248 (**iii**, 100). [lit.[20] mp 213—215°C (MeOH), $[\alpha]_D^{20}$ +52.5°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1730, 1710].

$Pb(OAc)_4$ Decarboxylation of 10a———A solution of **10a** (60 mg) in benzene (2.5 ml) was treated with $Pb(OAc)_4$ (150 mg) and the whole mixture was heated under reflux for 2.5 h. The reaction mixture was then diluted with AcOEt and filtered to remove inorganic material. The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the product. The product was purified by preparative TLC ($CHCl_3$) to furnish a mixture of dienic compounds (**14**, 13 mg) and a mixture of acetoxylated compounds (**15**, 12 mg). **14**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1720, 1220. ^{1}H-NMR ($CDCl_3$, δ): 1.63 (*ca.* 5/2H, s, vinyl methyl), 2.04, 2.06 (3H each, both s, OAc×2), 4.17, 4.39 (2H, ABq, J=12 Hz, 24-H_2), 4.62 (1H, t-like, 3-H), 5.10 (*ca.* 3/2H, br s), 5.26 (*ca.* 1/2H, m) (olefinic proton). MS m/z (%): 510 (M^+, 11), 202 (100). **15**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1720, 1225. ^{1}H-NMR ($CDCl_3$, δ): 0.86 (3H), 0.97 (6H), 1.02 (3H), 1.10, 1.14 (3/2H each), 1.42, 1.49 (3/2H each) (all s) (*tert*-CH_3), 1.94, 1.99 (3/2H each), 2.02 (3H), 2.04 (3H) (all s, OAc), 4.12, 4.38 (2H, ABq, J=12 Hz, 24-H_2), 4.58 (1H, t-like, 3-H), 5.23 (1H, br s, 12-H). MS m/z (%): 510 (M^+—AcOH, 10), 202 (100).

$Pb(OAc)_4$ Decarboxylation of 3-*O*-Acetyloleanolic Acid (16)———A solution of **16** (50 mg) in benzene (5 ml) was treated with $Pb(OAc)_4$ (150 mg) and the whole mixture was heated under reflux for 8 h. Work-up of the reaction mixture as described above gave the product, which was purified by preparative TLC ($CHCl_3$) to furnish a mixture of dienic compounds (**17**, 11 mg). **17**, UV λ_{max}^{EtOH} nm(ϵ): 238 (1900), 245 (2000), 253 (1500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: no OH, 1715, 1250. ^{1}H-NMR ($CDCl_3$, δ): 0.90 (9H), 0.93, 1.01, 1.04, 1.09 (3H each) (all s, *tert*-CH_3×7) 2.06 (3H, s, OAc), 4.52 (1H, dd, J=4, 8 Hz, 3-H), 5.39 (*ca.* 1.8H, br s, 12-H, 16-H, and/or 22-H). MS m/z(%): 452 (M^+, 63), 173 (100). High resolution MS: Found 452.366. Calcd for $C_{31}H_{48}O_2$ (M^+): 452.366.

$LiAlH_4$ Reduction of 11 followed by Acetylation——A solution of **11** (40 mg) in ether (5 ml) was treated with a suspension of $LiAlH_4$ (40 mg) in ether (5 ml) and the whole mixture was stirred at 19°C for 1.5 h. After treatment of the reaction mixture with aq. ether, the whole mixture was made weakly acidic with aq. 10% H_2SO_4 and extracted with AcOEt. Work-up of the AcOEt extract in the usual manner yielded the product which, after drying, was acetylated with Ac_2O–pyridine (1:1, 2 ml) at 37°C for 20 h to furnish **12** (39 mg), mp 217—218°C (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{13}$ +62.8° (c=1.0, $CHCl_3$). High resolution MS: Found 584.408. Calcd for $C_{36}H_{56}O_6$ (M^+): 584.409. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1740, 1240. ^{1}H-NMR ($CDCl_3$, δ): 0.84, 0.94 (3H each), 0.96 (6H), 1.02, 1.11 (3H each) (all s, *tert*-CH_3×6), 2.01 (3H), 2.04 (6H) (both s, OAc×3), 3.68, 3.77 (2H, ABq, J=10 Hz, 29-H_2), 4.13, 4.37 (2H, ABq, J=13 Hz, 24-H_2), 4.58 (1H, t-like, 3-H), 5.21 (1H, t-like, 12-H). MS m/z (%): 584 (M^+, 3), 276 (v, 100).

Acetonidation of 11——A solution of **11** (50 mg) in acetone (10 ml) was treated with 2,2-dimethoxypropane (0.05 ml) and p-TsOH·H_2O (2 mg) and the whole mixture was stirred at 18°C for 1 h. After treatment of the reaction mixture with pyridine (0.1 ml), the solvent was evaporated off under reduced pressure. The product was then treated with water and extracted with AcOEt. Work-up of the AcOEt extract in the usual manner yielded the product, which was purified by preparative TLC (Merck Al_2O_3 150 neutral, type T, n-hexane–AcOEt=10:1) to furnish **13** (45 mg), mp 245—247°C (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{13}$ +62.8° (c=1.0, $CHCl_3$). High resolution MS: Found 526.399. Calcd for $C_{34}H_{54}O_4$ (M^+): 526.402. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1730, 1230. ^{1}H-NMR ($CDCl_3$, δ): 0.86, 0.99 (3H each), 1.15, 1.20 (6H each) (all s, *tert*-CH_3×6), 1.35, 1.42 (3H each, both s, isopropylidene), 3.59 (1H, dd, J=4, 8 Hz, 3-H), 3.65 (3H, s, OCH_3), 3.21, 4.03 (2H, ABq, J=12 Hz, 24-H_2), 5.22 (1H, t-like, 12-H). MS m/z (%): 526 (M^+, 3), 264 (10), 262 (100).

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16) The assignments for the furan ring carbons were made on the basis of ^{1}H-gated decoupling experiments: δc 143.1 (2-C, dddt, J_{CH}=201.8, 6.0, 6.0, 5.5 Hz) and δc 145.1 (5-C, ddd, J_{CH}=202.3, 10.4, 7.1 Hz).
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21) From the acidic hydrolysate of total azukisaponin, we could isolate neither gypsogenic acid (**9**) nor a secondarily modified product, presumably due to complex mixture formation with unidentified prosapogenols.
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23) As will be reported in detail in the following papers (Part XXXII and Part XXXIII), azukisaponin IV, which contained gypsogenic acid (**7**) as the aglycone, did not possess D-glucuronic acid in the carbohydrate moiety, so that it did not yield the sapogenol upon photolysis. On the other hand, azukisaponins III and VI contained D-glucuronic acid in their carbohydrate moieties. However, azukisaponin VI was a bisdesmoside, so that it also did not liberate the sapogenol but furnished the prosapogenol upon photolysis. Furthermore, azukisapogenol (**10**) was photo-labile. For these reasons,upon photolysis of total azukisaponin, only sophoradiol (**7**) and soyasapogenol B (**8**), both of which were the sapogenols of glucuronide saponins such as azukisaponins I, II, and V, were liberated.
24) *a*) 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; *b*) I. Yosioka, A. Inada, and I. Kitagawa, *Tetrahedron,* **30**, 707 (1974).
25) I. Yosioka, T. Nishimura, A.Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.,* **18**, 1610 (1970).
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29) The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus and Ishii high-melting point apparatus (values are uncorrected); specific rotations, JASCO DIP-181 digital polarimeter (l=0.5 or 1 dm); IR spectra, Hitachi 260-30 infrared spectrometer; UV spectra, Hitachi 330 spectrophotometer; ^{1}H-NMR spectra, Hitachi R-22 (90 MHz) NMR spectrometer (with TMS as an internal standard); ^{13}C-NMR spectra, JEOL JNM-FX90Q FT-NMR spectrometer (pulse width, 12 μs; spectral width, 4504 Hz; data points, 8192; number of pulses, 6000; temp. 50°C, measured in CD_3OD); MS, JEOL JMS-new D-300 mass spectrometer; high resolution MS, JEOL JMS-new D-300 or JEOL JMS-01SG mass spectrometer. The following experimental conditions were used for chromatography: GLC, Hitachi gas chromatograph model 663-50 with FID; CLC, Hitachi centrifugal liquid chromatograph model CLC-5 with KT-gel 2061 (Fuji gel) as the adsorbent; column chromatography, Silica gel 60 (Merck 60—230 mesh) as the adsorbent; preparative TLC, with silica gel Camag D-5, detection by spraying with water or by exposure to I_2 vapor; TLC, pre-coated TLC plates silica gel 60F-254 (0.25 mm), detection by spraying with 1% $Ce(SO_4)_2$–10% aq. H_2SO_4 followed by heating.