Medicinal Foodstuffs. VI.[1] Histamine Release Inhibitors from Kidney Bean, the Seeds of *Phaseolus vulgaris* L.: Chemical Structures of Sandosaponins A and B

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Two new olean-12-ene-type triterpene oligoglycosides, named sandosaponins A and B, were isolated from kidney bean, the seed of *Phaseolus vulgaris* L., together with three known saponins, soyasaponins I and V and dehydrosoyasaponin I. The structures of sandosaponins A and B were determined on the basis of chemical and physicochemical evidence, which included the chemical derivation of sandosapogenol from a known sapogenol, soyasapogenol B. Five saponins obtained from kidney bean were found to inhibit histamine release from rat exudate cells induced by an antigen–antibody reaction and, among them, sandosaponins A and B showed the most potent inhibitory activity.

Key words sandosaponin; *Phaseolus vulgaris*; kidney bean; histamine release inhibitor; medicinal foodstuff; antiallergic glycoside

Phaseolus vulgaris L. (Leguminosae, Japanese names: sandomame, ingenmame, and saitou) has been widely cultivated as a vegetable or a staple food in Asian, African, Middle and South American countries. The seeds of this plant have many common names such as kidney bean, apricot bean, french bean, navy bean, and common bean. In Chinese traditional medicine, the seeds of *Phaseolus vulgaris* L. have been prescribed for nutritive, antipyretic and diuretic purposes. In regard to the chemical constituents of the seeds of *Phaseolus vulgaris* L., two oleanene-type triterpene glycosides (soyasaponin V and soyasapogenol B-glucoside),[2] amino acids,[3] and glycoproteins[4] have been isolated, while several flavonoids have been reported as phytoalexins from the fungal-infected seeds.[5]

As part of our search for bioactive constituents in medicinal foodstuffs,[1,6] we have isolated two new olean-12-ene-type oligoglycosides, named sandosaponins A (**1**) and B (**2**), together with soyasaponins I (**3**)[7] and V (**5**)[8] and dehydrosoyasaponin I (**4**)[9] from the seeds of Japanese *Phaseolus vulgaris* L. Recently, we have isolated four methyl-migrated 16,17-*seco*-dammarane-type triterpene glycosides named hoveidulciosides A_1, A_2, B_1, and B_2 from the seeds and fruits of *Hovenia dulcis*, and these triterpene glycosides were found to show inhibitory activity on histamine release from rat peritoneal exudate cells.[10] As an extension of this work, we have continued to examine the antiallergic activity of various saponin constituents. In this paper, we provide the structural elucidation of sandosaponins A (**1**) and B (**2**) and the inhibitory activity of the olean-12-ene-type triterpene saponins (**1**—**5**) from the seeds of *Phaseolus vulgaris* L. on the histamine release from rat exudate cells induced by an antigen–antibody reaction.[11]

The methanolic extract of the seeds of *Phaseolus vulgaris* L., cultivated in Hokkaido Prefecture, was partitioned into a mixture of 1-butanol and water, and the 1-butanol-soluble portion was subjected to repeated chromatographic separation to afford sandosaponins A (**1**, 0.0030%) and B (**2**, 0.0005%), soyasaponins I (**3**, 0.0043%) and V (**5**, 0.014%), and dehydrosoyasaponin I (**4**, 0.0004%).

Sandosaponin A (1) Sandosaponin A (**1**) was obtained as colorless fine crystals, mp 200—201 °C, from aqueous methanol. The IR spectrum of **1** showed absorption bands at 1736 and 1701 cm^{-1} ascribable to ketone and carboxyl functions by the following additional evidence, as well as broad bands at 3411 and 1076 cm^{-1} suggestive of a glycosidic structure. In the negative and positive-mode FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 955 $(M-H)^-$ and 979 $(M+Na)^+$, respectively, and a fragment ion peak at *m/z* 793 $(M-C_6H_{11}O_5)^-$ was observed in the negative FAB-MS. High-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **1** to be $C_{48}H_{76}O_{19}$. Enzymatic hydrolysis of **1** with glycyrrhizinic acid hydrolase[12] furnished soyasapogenol E (**7**). Carbon signals due to the oligosaccharide moiety in the ^{13}C-NMR spectrum (Table 1)[13] of **1** were superimposable on those of soyasaponin V (**5**),[8] while carbon signals due to the sapogenol moiety were superimposable on those of dehydrosoyasaponin I (**4**),[9] having soyasapogenol E (**7**)[7] as the aglycone. The ^1H-NMR (pyridine-d_5)[13] of **1** showed signals due to the soyasapogenol E moiety [δ 2.15, 2.60 (1H each, both d, $J=7.6$ Hz, 21-H_2), 3.41 (1H, dd, $J=3.7$, 12.2 Hz, 3-H)], and the trisaccharide moiety consisted of β-D-glucuronopyranosyl [δ 5.10 (1H, d, $J=7.6$ Hz, 1′-H)], β-D-galactopyranosyl [δ 5.65 (1H, d, $J=7.6$ Hz, 1″-H)], and β-D-glucopyranosyl [δ 5.20 (1H, d, $J=7.6$ Hz, 1‴-H)] parts. The trisaccharide structure bonding to the 3-position of soyasapogenol E part was also characterized by an heteronuclear multiple bond connectivity (HMBC) experiment. Namely, long-range correlations were observed between the 1‴-proton of the β-D-glucopyranosyl part and the 2″-carbon of the β-D-galactopyranosyl part,

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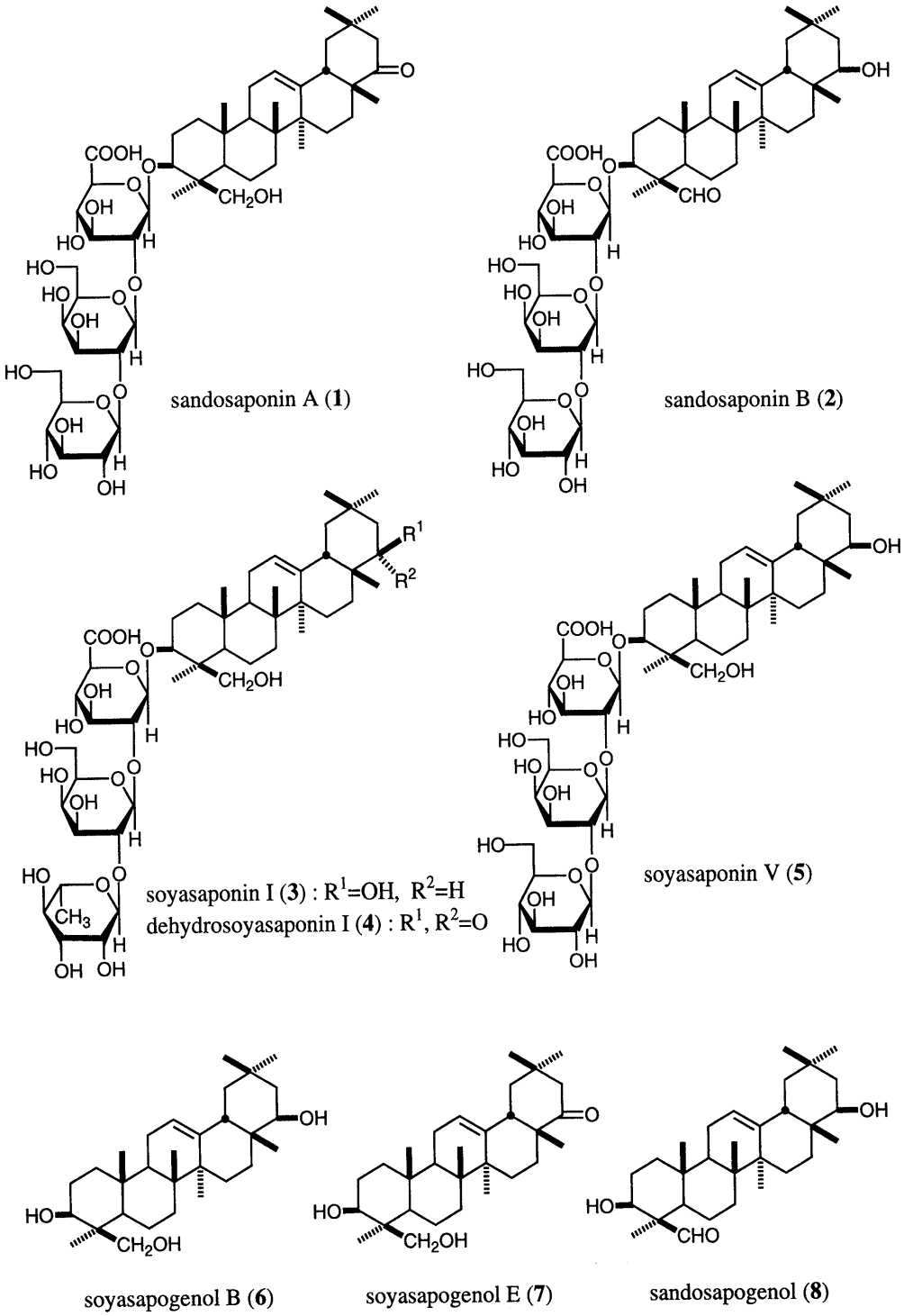

Chart 1

between the 1″-proton of the β-D-galactopyranosyl part and the 2′-carbon of the β-D-glucuronopyranosyl part, and between the 1′-proton of the β-D-glucuronopyranosyl part and the 3-carbon of the soyasapogenol E part. Finally, sandosaponin A (**1**) was derived from soyasaponin V (**5**) by partial oxidation[8] with chromium trioxide in pyridine at 0 °C. Consequently, the structure of sandosaponin A was determined to be soyasapogenol E 3-*O*-β-D-glucopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucopyranosiduronic acid (**1**).

Sandosaponin B (2) Sandosaponin B (**2**) was also isolated as colorless fine crystals, mp 212—213 °C, from aqueous methanol, and its IR spectrum showed absorption bands at 3410, 1736, 1725, and 1076 cm^{-1}, suggestive of glycosidic, aldehyde, and carboxyl functions. The molecular formula $C_{48}H_{76}O_{19}$, which was the same as that of sandosaponin A (**1**), was determined from the negative-mode FAB-MS [*m/z* 955 $(M-H)^-$] and by high-resolution MS measurement. Furthermore, fragment ion peaks at *m/z* 793 $(M-C_6H_{11}O_5)^-$ and 631 $(M-C_{12}H_{21}O_{10})^-$ were observed in the negative-mode FAB-MS of **2**. Enzymatic hydrolysis of **2** with glycyrrhizinic acid hydrolase furnished a new triterpene sapogenol named sandosapogenol (**8**). The structure of **8**

was clarified by chemical derivations from soyasapogenol B (**6**), whose absolute stereostructure was determined by X-ray analysis,[7] as shown in Chart 2. Namely, **6** was subjected to tritylation with *p*-methoxyphenyldiphenylmethyl chloride (MMTrCl) in pyridine, followed by silylation with *tert*-butyldimethylsilyl chloride (TBDMSCl) to give **9** in 86% yield. Removal of the trityl group in **9** with perchloric acid ($HClO_4$) furnished **10** in 53% yield. Treatment of **10** with chromium trioxide in pyridine, and finally, desilylation with 80% trifluoroacetic acid yielded sandosapogenol (**8**, 3,22-dihydroxyolean-12-en-24-al) in an unsatisfactory overall yield. On the other hand, **6** was converted to **9a** in 72% yield by tritylation with triphenyl chloride (TrCl) and subsequent benzylation with benzyl bromide (BnBr) in the presence of sodium hydride (NaH) and tetrabutylammonium iodide (*n*-Bu_4NI). Detritylation of **9a** with hydrochloric acid furnished **10a** (75% yield), which was subjected to Swern oxidation[14] followed by debenzylation with hydrogenation to provide **8** in 75% yield.

Table 1. ^{13}C-NMR Data for Sandosaponins A (**1**) and B (**2**) and Sandosapogenol (**8**) (125 MHz, δ)

	1[a]	2[a]	8[b]		1[a]	2[a]
1	38.5	38.0	38.5	GluA-1′	104.8	104.5
2	27.4	27.1	28.0	GluA-2′	80.9	84.0
3	90.7	86.7	77.3	GluA-3′	77.5	77.2
4	43.9	53.2	52.7	GluA-4′	72.5	71.9
5	56.1	57.2	56.4	GluA-5′	77.9	77.4
6	18.6	19.1	18.5	GluA-6′	172.4	172.3
7	33.0	33.1	33.1	Gal-1″	103.0	104.4
8	39.8	39.7	39.5	Gal-2″	84.7	84.5
9	47.8	46.6	46.5	Gal-3″	74.7	74.9
10	36.4	36.8	37.2	Gal-4″	70.5	69.2
11	24.0	24.2	23.9	Gal-5″	76.6	76.4
12	122.8	122.3	122.4	Gal-6″	62.5	61.6
13	141.8	144.9	143.9	Glu-1‴	106.9	106.6
14	42.0	42.4	42.2	Glu-2‴	76.7	76.6
15	26.6	26.3	25.3	Glu-3‴	78.0	77.8
16	27.4	28.6	28.2	Glu-4‴	71.6	71.1
17	47.8	38.0	37.4	Glu-5‴	79.1	79.2
18	47.6	45.4	44.9	Glu-6‴	62.9	62.6
19	46.6	46.8	46.1			
20	34.1	30.9	30.5			
21	50.9	42.2	41.5			
22	215.6	75.5	76.8			
23	22.9	22.0	20.0			
24	63.6	206.9	208.0			
25	15.7	16.4	14.8			
26	16.7	17.0	17.0			
27	25.5	25.6	25.1			
28	21.0	21.1	19.2			
29	31.9	33.1	32.7			
30	25.3	28.6	28.2			

a) Measured in pyridine-d_5 solution. *b*) Measured in $CDCl_3$ solution.

The ^1H-NMR (pyridine-d_5) of **2** showed signals due to the sandosapogenol moiety [δ 3.53 (1H, dd, J=5.2, 11.9 Hz, 3-H), 3.71 (1H, dd, J=3.5, 6.6 Hz, 22-H), 10.44 (1H, s, 24-CHO)] and the trisaccharide moiety [δ 5.08 (1H, d, J=7.9 Hz, 1′-H), 5.31 (1H, d, J=7.6 Hz, 1″-H), 5.22 (1H, d, J=7.3 Hz, 1‴-H)]. The carbon signals of the trisaccharide moiety in the ^{13}C-NMR (Table 1) of **2** was similar to those of **1** and **5**, except for the signals due to the 2′ and 1″-carbons, which were influenced by the steric effect of the 24-functional group.[15] The trisaccharide structure of **2** was confirmed by an HMBC experiment, in which was observed long-range correlations between the following protons and carbons: 1‴-H and 2″-C; 1″-H and 2′-C; 1′-H and 3-C. Finally, the reduction of **2** with sodium borohydride ($NaBH_4$) in methanol furnished **5**. On the basis of the above mentioned evidence, the structure of sandosaponin B was determined as sandosapogenol (3,22-dihydroxyolean-12-en-24-al)

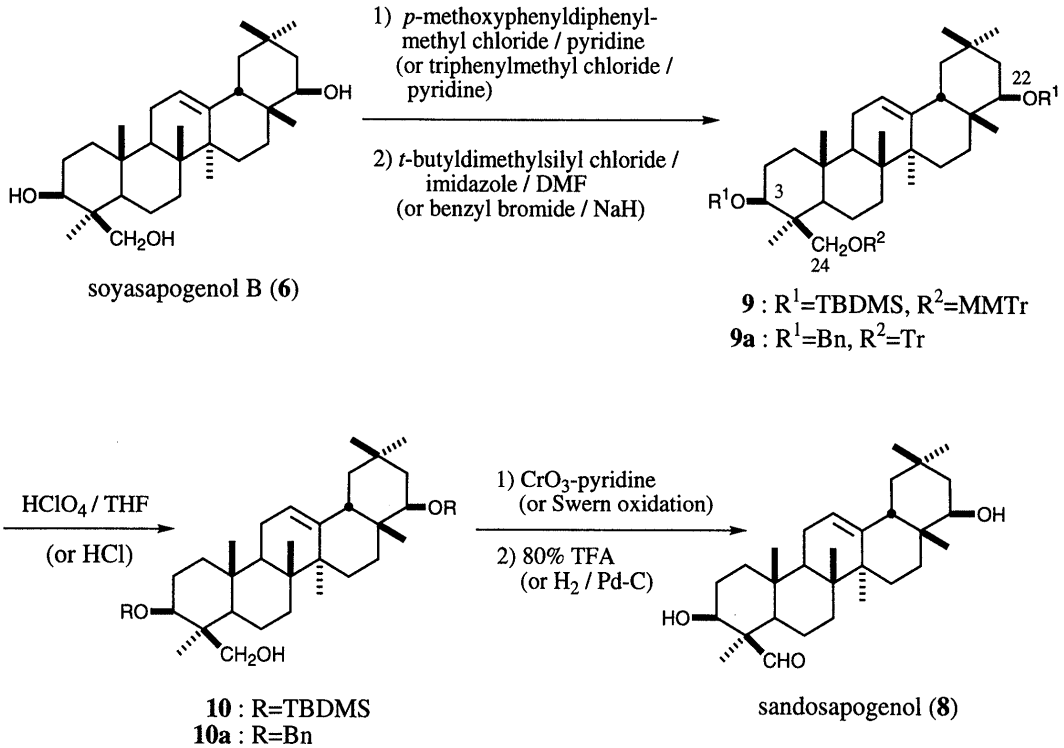

Chart 2

Table 2. Inhibitory Effects of Saponins (**1**—**5**) on the Histamine Release from Rat Exudate Cells Induced by an Antigen–Antibody Reaction

	Concentration (M)	n	Inhibition (%)
Sandosaponin A (**1**)	10^{-6}	4	36.7±14.1
	10^{-5}	4	58.2± 4.1
	10^{-4}	4	90.9± 7.5
Sandosaponin B (**2**)	10^{-6}	4	43.0± 8.4
	10^{-5}	4	59.4± 6.7
	10^{-4}	4	66.8± 1.4
Soyasaponin I (**3**)	10^{-6}	4	4.6± 9.1
	10^{-5}	4	11.2± 4.7
	10^{-4}	4	62.3± 5.0
Dehydrosoyasaponin I (**4**)	10^{-6}	4	8.8± 8.6
	10^{-5}	4	15.9±10.6
	10^{-4}	4	42.9±12.5
Soyasaponin V (**5**)	10^{-6}	4	6.0±12.8
	10^{-5}	4	10.7± 5.1
	10^{-4}	4	19.3± 3.5

Means±S.E.

3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-galactopyranosyl (1→2)-*β*-D-glucopyranosiduronic acid (**2**).

Inhibitory Effects of the Saponin Constituents from the Seeds of *Phaseolus vulgaris* on the Histamine Release from Rat Peritoneal Exudate Cells Recently, soyasaponin I (**3**) and dehydrosoyasaponin I (**4**) were isolated from a Ghanaian traditional medicine, *Desmodium adscendence*, which was used as a treatment for asthma. The extract of this plant was found to inhibit contractions of the guinea pig ileum caused by electrical field stimulation, and contractions of sensitized guinea pig airway smooth muscle induced by antigen, arachidonic acid, or leukotriene D_4, and soyasaponins showed inhibitory activity on the binding of monoiodotyrosine charybdotoxin to large-conductance calcium-dependent potassium channels in smooth muscle membranes.[16] This evidence led us to expect that the saponin constituents (**1**—**5**) from the seeds of *Phaseolus vulgaris* showed antiallergic activity. As shown in Table 2, all saponins (**1**—**5**) were found to inhibit histamine release from rat peritoneal exudate cells induced by an antigen–antibody reaction. Particularly, the new sandosaponins A (**1**) and B (**2**) showed more potent inhibitory activity than soyasaponins I (**3**) and V (**5**). It is noteworthy that replacing the 22 or 24-hydroxyl group in **5** with a carbonyl group significantly increased the activity.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were as described in our previous paper.[17]

Bioassay Reagents: Mouse monoclonal anti-2,4-dinitrophenyl immunoglobulin E (anti-DNP IgE, PCA titer 100000, Seikagaku Corporation), and 2,4-dinitrophenylated bovine serum albumin (DNP-BSA, Cosmo Bio Co., Ltd., Tokyo). Other reagents were purchased from Wako Pure Chemical Industries.

Extraction and Isolation The air-dried seeds of *Phaseolus vulgaris* L. (10 kg, cultivated in Hokkaido and purchased from Tokiwa Kanpo Pharmaceutical Co., Ltd., Osaka) was minced and extracted three times with MeOH under reflux. Evaporation of the solvent from the extract solution under reduced pressure gave the MeOH extract (392 g). This extract (392 g) was partitioned into an AcOEt–H_2O (1:1) mixture. The aqueous layer was further extracted with 1-butanol. Removal of the solvent under reduced pressure from the AcOEt-soluble portion and 1-butanol-soluble portion yielded 35.7 and 84.9 g, respectively. The 1-butanol extract (84.9 g) was separated by normal-phase silica-gel column chromatography (1.0 kg, $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O) to afford eleven fractions [fr. 1 (7.9 g), fr. 2 (3.6 g), fr. 3 (10.6 g), fr. 4 (1.6 g), fr. 5 (3.5 g), fr. 6 (21.0 g), fr. 7 (11.6 g), fr. 8 (8.9 g), fr. 9 (2.6 g), fr. 10 (5.3 g), fr. 11 (8.3 g)]. Fraction 7 (11.6 g) was further separated by normal-phase silica-gel column chromatography [580 g, $CHCl_3$–MeOH–H_2O (10:3:1, 7:3:1, 65:35:10, all lower layer)] to give five fractions (fr. 7-1—7-5). Fraction 7-4 (5.5 g) was subjected successively to reversed-phase silica-gel column chromatography [130 g, 50%→80% MeOH], and then to HPLC (YMC-pack octadecyl selica (ODS), 80% MeOH–1% AcOH), to give sandosaponin A (**1**, 75 mg), soyasaponins I (**3**, 32 mg) and V (**5**, 85 mg), and dehydrosoyasaponin I (**4**, 36 mg). Fraction 8 (4.3 g) was subjected to reversed-phase silica-gel column chromatography (40 g, H_2O–MeOH) and then HPLC (YMC-pack ODS, 75% MeOH–1% AcOH) to furnish **1** (111 mg), **3** (193 mg), and **5** (512 mg). Fraction 9 (2.6 g) was separated by reversed-phase silica-gel column chromatography (100 g, H_2O–MeOH) and HPLC (YMC-pack ODS, 80% MeOH–1% AcOH) to give sandosaponin B (**2**, 48 mg) and **5** (250 mg). The known compounds (**3**—**5**) were identified by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5) spectral comparisons with authentic samples.[6-8]

Sandosaponin A (**1**): Colorless fine crystals from H_2O–MeOH, mp 200—201 °C, $[\alpha]_D^{23}$ −5.8° (c=0.8, MeOH). High-resolution FAB-MS: Calcd for $C_{48}H_{76}NaO_{19}$ $(M+Na)^+$: 979.4878. Found: 979.4874. IR (KBr) cm^{-1}: 3411, 2926, 1736, 1701, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.85, 0.86, 0.96, 1.17, 1.28, 1.43 (3H each, all s, 25, 30, 26, 29, 28, 27, 23-CH_3), 2.15 (1H, d, J=13.7 Hz), 2.60 (1H, d, J=13.7 Hz, 21-H_2), 3.41 (dd, J=3.7, 12.2 Hz, 3-H), 5.10 (1H, d, J=7.6 Hz, 1′-H), 5.20 (1H, d, J=7.6 Hz, 1‴-H), 5.23 (1H, br s, 12-H), 5.65 (1H, d, J=7.6 Hz, 1″-H). ^{13}C-NMR: see Table 1. Negative-mode FAB-MS m/z: 955 $(M-H)^-$, 793 $(M-C_6H_{11}O_5)^-$. Positive-mode FAB-MS m/z: 979 $(M+Na)^+$.

Sandosaponin B (**2**): Colorless fine crystals from H_2O–MeOH, mp 212—213 °C, $[\alpha]_D^{28}$ +34.8° (c=0.3, MeOH). High-resolution FAB-MS: Calcd for $C_{48}H_{76}NaO_{19}$ $(M+Na)^+$: 979.4878. Found: 979.4874. IR (KBr) cm^{-1}: 3410, 2924, 1736, 1725, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.73, 0.91, 0.97, 1.18, 1.21, 1.26, 1.62 (3H each, all s, 25, 26, 29, 28, 27, 30, 23-CH_3), 3.53 (dd, J=5.2, 11.9 Hz, 3-H), 3.71 (1H, dd, J=3.5, 6.6 Hz, 22-H), 5.08 (1H, d, J=7.9 Hz, 1′-H), 5.22 (1H, d, J=7.3 Hz, 1‴-H), 5.27 (1H, br s, 12-H), 5.31 (1H, d, J=7.6 Hz, 1″-H), 10.44 (1H, s, 24-CHO). ^{13}C-NMR: see Table 1. Negative-mode FAB-MS m/z: 955 $(M-H)^-$, 793 $(M-C_6H_{11}O_5)^-$, 631 $(M-C_{12}H_{21}O_{10})^-$. Positive-mode FAB-MS m/z: 979 $(M+Na)^+$.

Enzymatic Hydrolysis of Sandosaponin A (1) A solution of **1** (5 mg) in acetate buffer (pH 4.4, 2 ml) was treated with glycyrrhizinic acid hydrolase (0.5 mg), and the entire solution was left standing at 40 °C for 3 h. The reaction solution evaporated to dryness under reduced pressure and the residue was separated by silica-gel column chromatography (1 g, *n*-hexane–AcOEt=1:1) to give soyasapogenol E (**7**, 2.3 mg), which was identified by comparison of TLC, ^{1}H-NMR ($CDCl_3$), and ^{13}C-NMR ($CDCl_3$) spectra with an authentic sample.[6]

Partial Oxidation of Soyasaponin V (5) with CrO_3 Giving Sandosaponin A (1) A solution of **5** (150 mg) in pyridine (5 ml) was treated with CrO_3 (200 mg)–pyridine (5 ml), and the mixture was stirred at 0 °C for 4 h. The reaction solution was treated with isopropyl alcohol (5 ml) to quench excess CrO_3, and the whole mixture was stirred for 0.5 h. After removal of the insoluble part by filtration, the filtrate was evaporated under reduced pressure to give a residue which was purified by silica-gel column chromatography [20 g, ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower layer)] to furnish **1** (35 mg). Thus obtained **1** was identified by TLC, IR (KBr), ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) with authentic sandosaponin A.

Enzymatic Hydrolysis of Sandosaponin B (2) Giving Sandosapogenol (8) A solution of **2** (1 mg) in acetate buffer (pH 4.4, 0.2 ml) was treated with glycyrrhizinic acid hydrolase (0.1 mg), and the whole mixture was left standing at 40 °C for 3 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue was separated by silica-gel column chromatography (1 g, *n*-hexane : AcOEt = 7 : 1) to yield sandosapogenol (**8**, 0.3 mg, 63%).

Sandosapogenol (**8**): A white powder, $[\alpha]_D^{24}$ +61.2° (c=1.38, $CHCl_3$), High-resolution FAB-MS: Calcd for $C_{30}H_{48}NaO_3$ $(M+Na)^+$: 479.3501. Found: 479.3478. IR (KBr) cm^{-1}: 3535, 2954, 1637. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H), 0.91, 1.00, 1.04, 1.13, 1.29 (3H each, all s, *tert*-CH_3 × 7), 3.10 (1H, br s, OH), 3.20 (1H, m, 3-H), 3.44 (1H, dd, J=5.0, 5.0 Hz,

22-H), 5.26 (1H, t-like, 12-H), 9.76 (1H, d, J=2.5 Hz, 24-CHO). ^{13}C-NMR: see Table 1. Positive-mode FAB-MS m/z: 479 $(M+Na)^+$. Negative-mode FAB-MS m/z: 455 $(M-H)^-$.

Tritylation of Soyasapogenol B (6) Followed by Silylation A solution of **6** (38 mg, 0.083 mmol) in pyridine (2 ml) was treated with *p*-methoxyphenyldiphenylmethyl chloride (230 mg, 0.74 mmol), and the whole mixture was stirred at room temperature under an N_2 atmosphere for 2.5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. After removal of the solvent under reduced pressure, a residue was purified by silica-gel column chromatography (5 g, *n*-hexane : AcOEt = 7 : 1→3 : 1) to yield the 24-trityl derivative (54 mg, 86%) : a white powder. ^{1}H-NMR ($CDCl_3$) δ: 0.38, 0.81, 0.83, 0.90, 1.02, 1.07, 1.53 (3H each, all s, *tert*-CH_3 × 7), 3.13, 4.11 (1H each, both d, J=9.6 Hz, 24-H_2), 3.39 (2H, m, 3, 22-H), 3.80 (3H, s, OCH_3), 5.19 (1H, t-like, 12-H), 6.83—6.87 (2H, m), 7.22—7.46 (12H, m, aromatic protons).

A solution of the 24-trityl derivative (54 mg, 0.071 mmol) in *N*,*N*-dimethylformamide (DMF) (0.5 ml) was treated with *tert*-butyldimethylsilyl chloride (172 mg, 1.14 mmol) and imidazole (78 mg, 1.14 mmol) in DMF (0.5 ml), and the whole mixture was stirred at room temperature for 5 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with 5% aqueous HCl and saturated $NaHCO_3$ and brine, and then dried over $MgSO_4$ powder and filtered. Evaporation of the filtrate under reduced pressure yielded a residue, which was purified by silica-gel column chromatography (10 g, *n*-hexane : AcOEt = 30 : 1) to give **9** (68 mg, quant.). ^{1}H-NMR ($CDCl_3$) δ: 0.01 , 0.02 (6H each, both s, $Si(CH_3)_2$), 0.27, 0.79, 0.89, 1.00 1.08, 1.27, 1.55 (3H each, all s, *tert*-CH_3 × 7), 0.86 (18H, s, *tert*-Bu), 3.15, 3.44 (1H each, both d, J=9.6 Hz, 24-H_2), 3.78 (3H, s, OCH_3), 5.17 (1H, t, J=3.8 Hz, 12-H), 6.76—6.84 (2H, m), 7.14—7.52 (12H, m, aromatic protons).

Detritylation of 9, Giving 10 A solution of **9** (68m g, 0.071 mmol) in dry tetrahydrofuran (THF) (5 ml) was treated with 70% $HClO_4$–THF (1 ml), and the whole mixture was stirred at 0 °C for 10 min and then at room temperature for 1.5 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$ powder. After removal of the desiccant by filtration, the filtrate was evaporated under reduced pressure to yield a product which was purified by silica-gel column chromatography (65 g, *n*-hexane : AcOEt = 5 : 1) to give **10** (26 mg, 53%). ^{1}H-NMR ($CDCl_3$) δ: 0.01, 0.03 (6H each, both s, $Si(CH_3)_2$), 0.86 (18H, s, *tert*-Bu), 0.80, 0.88, 0.89, 0.94, 1.01, 1.11, 1.25 (3H each, all s, *tert*-CH_3 × 7), 4.21 (1H, d, J=10.9 Hz, 24-H), 5.23 (1H, t, J=3.8 Hz, 12-H).

Oxidation of 10 with CrO_3 Followed by Desilylation A solution of **10** (20 mg, 0.03 mmol) in pyridine (2 ml) was treated with CrO_3 (18 mg, 0.17 mmol) in pyridine (1.5 ml), and the whole solution was stirred at room temperature under an N_2 atmosphere 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 furnished a product which was separated by silica-gel column chromatography (2 g, *n*-hexane : AcOEt = 7 : 1) to give the 24-aldehyde derivative (4.6 mg, 23%). ^{1}H-NMR ($CDCl_3$) δ: 0.02, 0.03 (6H each, both s, $Si(CH_3)_2$), 0.86 (18H, s, *tert*-Bu), 0.81, 0.88, 0.89, 0.99, 1.01, 1.12, 1.29 (3H each, all s, *tert*-CH_3 × 7), 5.24 (1H, t, J=3.6 Hz, 12-H), 9.76 (1H, d, J=2.0 Hz, 24-CHO).

A solution of the 24-aldehyde derivative (4.6 mg) in 80% trifluoroacetic acid (TFA) (0.5 ml) was stirred at room temperature for 30 min. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by silica-gel column chromatography (1 g, *n*-hexane : AcOEt = 7 : 1) to furnish **8** (1.1 mg), which was identified on the basis of TLC, IR (KBr) and ^{1}H-NMR ($CDCl_3$) spectral comparisons with authentic sandosapogenol.

Tritylation of Soyasapogenol B (6) Followed by Benzylation A solution of **6** (69 mg, 0.15 mmol) in pyridine (3 ml) was treated with triphenylmethyl chloride (126 mg, 0.45 mmol), and the whole mixture was heated under reflux for 1.5 h. The reaction mixture was poured into ice-water and the resulting precipitate was collected by filtration, then purified by silica-gel column chromatography (1 g, *n*-hexane–AcOEt, $CHCl_3$) to give the 24-trityl derivative (97 mg, 92%).

A solution of the 24-trityl derivative (97 mg, 0.14 mmol) in benzyl bromide (0.8 ml, 6.9 mmol) and THF–*N*,*N*-dimethylformamide (DMF) (1 : 1, 6 ml) was treated with 60% NaH (277 mg, 6.9 mmol) and *n*-Bu_4NI (51 mg, 0.14 mmol), and the whole mixture was stirred at 60—70 °C for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract, the product was purified by ODS (85% aqueous MeOH, MeOH, $CHCl_3$) and silica-gel (*n*-hexane : AcOEt = 15 : 1) column chromatography to give **9a** (95 mg, 78%).

9a: A white powder. High-resolution FAB-MS: Calcd for $C_{63}H_{76}NaO_3$ $(M+Na)^+$: 903.5692. Found: 903.5743. ^{1}H-NMR ($CDCl_3$) δ: 0.33, 0.82, 0.88, 0.92, 1.03, 1.09, 1.34 (3H each, all s, *tert*-CH_3 × 7), 2.95 (1H, dd, J=3.6, 11.6 Hz, 3-H), 3.06 (1H, dd, J=3.0, 5.4 Hz, 22-H), 3.17, 3.53 (1H each, both d, J=9.6 Hz, 24-H_2), 4.32, 4.38, 4.61, 4.63 (1H each, all d, J=11.9 Hz, benzyl methylene × 2), 5.17 (1H, t-like, 12-H), 7.10—7.60 (25H, m, aromatic protons). Positive-mode FAB-MS m/z: 903 $(M+Na)^+$.

Detritylation of 9a, Giving 10a A solution of **9a** (95 mg, 0.11 mmol) in MeOH–acetone (5 : 1, 6 ml) was treated with conc. HCl (1 drop), and the whole mixture was heated under reflux for 5 min. After cooling, the reaction solution was neutralized with 5% aqueous NaOH and the whole was extracted with AcOEt. Work-up of the AcOEt extract gave a residue, which was separated by silica-gel column chromatography (1 g, *n*-hexane : AcOEt = 15 : 1) to give **10a** (51 mg, 75%).

10a: A white powder, $[\alpha]_D^{26}$ +51.5° (c=0.64, $CHCl_3$). High-resolution FAB-MS: Calcd for $C_{44}H_{62}NaO_3$ $(M+Na)^+$: 661.4597. Found: 661.4577. UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 242.0 (3.00), 254.5 (3.02), 286.0 (2.65). IR (KBr) cm^{-1}: 3452, 2949, 2924, 2857, 1460, 1075, 1028. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H), 0.93, 0.94, 1.05, 1.11, 1.21 (3H each)(all s, *tert*-CH_3 × 7), 3.07 (1H, dd, J=3.0, 5.9 Hz, 22-H), 3.18 (1H, m, 3-H), 3.21 (1H, br s, OH), 3.22, 4.16 (1H each, both d, J=9.6 Hz, 24-H_2), 4.32, 4.40, 4.61, 4.67 (1H each, all d, J=11.2 Hz, benzyl methylene × 2), 5.22 (1H, t-like, 12-H), 7.20—7.50 (10H, m, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ_C: 38.3, 22.7, 88.6, 43.1, 56.2, 18.5, 33.0, 39.7, 47.6, 36.6, 23.8, 122.0, 144.2, 41.9, 25.9, 28.2, 37.7, 44.8, 46.2, 30.6, 36.2, 84.0, 22.7, 64.1, 16.1, 16.8, 25.6, 20.7, 33.1, 27.6, (C-1—C-30), 71.8, 71.3 (benzyl methylene-C). Positive-mode FAB-MS m/z: 661 $(M+Na)^+$.

Swern Oxidation of 10a Followed by Debenzylation Dimethyl sulfoxide (DMSO) (0.11 ml, 1.6 mmol) was added dropwise to a solution of oxalyl chloride (0.07 ml, 0.8 mmol) in CH_2Cl_2 (1 ml) at −78 °C and the whole was stirred at −78 °C for 5min. A solution of **10a** (51.4 mg, 0.08 mmol) in CH_2Cl_2 (0.5 ml) was added to the reaction mixture. Stirring was continued at −78 °C for 3 h, then Et_3N (0.4 ml) in CH_2Cl_2 (0.5 ml) was added. The reaction mixture was further stirred for 15 min and warmed to room temperature, then poured into ice-water. The whole mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous saturated NaCl, then dried over $MgSO_4$ powder and filtered. After removal of the solvent under reduced pressure, the residue was purified by silica-gel column chromatography (1 g, *n*-hexane : AcOEt = 20 : 1) to give the 24-aldehyde derivative (43 mg, 83%). A white powder. ^{1}H-NMR ($CDCl_3$) δ: 0.83, 0.89, 1.05, 1.11, 1.21 (3H each), 0.93 (6H) (all s, *tert*-CH_3 × 7), 3.07 (1H, m, 22-H), 3.18 (1H, dd, J=5.0, 11.6 Hz, 3-H), 4.31, 4.71 (1H each), 4.44 (2H), (all d, J=11.9 Hz, benzyl methylene × 2), 5.23 (1H, t-like, 12-H), 7.10—7.50 (10H, m, aromatic protons), 10.06 (1H, s, 24-CHO).

A solution of the 24-aldehyde derivative (43 mg, 0.067 mmol) in EtOH (3 ml) was hydrogenated in the presence of 20% Pd–C (4 mg) at room temperature for 1 h. The catalyst was filtered off, and the solvent of the filtrate was evaporated under reduced pressure to give a residue, which was purified by silica-gel column chromatography (1 g, $CHCl_3$) to provide **8** (28 mg, 90%). Thus obtained **8** was identified on the basis of TLC, $[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, and MS comparisons with natural sandosapogenol.

$NaBH_4$ Reduction of Sandosaponin B (2) A solution of **2** (5 mg) in MeOH (0.5 ml) was treated with $NaBH_4$ (5 mg), and the whole mixture was left standing at room temperature for 5 min. The reaction mixture was treated with acetone (0.2 ml) to quench excess $NaBH_4$, and the whole mixture was neutralized with Dowex HCR-W2 (H^+ form). After removal of the resin by filtration, the filtrate was evaporated under reduced pressure to yield a crude reduction product. The crude reduction product was separated by silica-gel column chromatography (1 g, $CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to furnish **5** (5 mg, quant.), which was identified by comparison of TLC (80% MeOH–1% AcOH, 50% CH_3CN–1% AcOH), HPLC (YMC-pack ODS, 80% MeOH–1% AcOH), and ^{1}H-NMR and ^{13}C-NMR data with an authentic soya-

saponin V.

Histamine Release from Rat Peritoneal Exudate Cells The method of bioassay testing was basically as the same as described in the previous report.[18] Male Wistar rats (Kiwa Laboratory Animals, Ltd.) weighing 350—500 g were exsanguinated to death and injected intraperitoneally with 10 ml of physiological solution consisting of NaCl (150 mM), KCl (2.7 mM), $CaCl_2$ (0.9 mM), glucose (5.6 mM) and HEPES (5 mM) (pH 7.4). The abdominal region was gently massaged for 2 min and then the peritoneal exudate was collected. The cell suspension was centrifuged ($100 \times g$, 4 °C, 10 min) and washed several times with the physiological solution. The peritoneal exudate cells were sensitized with diluted anti-DNP IgE ($\times 100$) at 37 °C for 1 h. The cell suspension (10^4/1.62 ml) and 180 μl of test compound were preincubated for 15 min; 200 μl of phosphatidyl-L-serine (1 mg/ml) and 222 μl of DNP-BSA (1 mg/ml) were added at the same time. The incubation was continued for 20 min. The test tube was dropped into an ice-cold bath to stop the reaction. The supernatant was obtained by centrifugation for 10 min at $100 \times g$, 4 °C, and the histamine concentration was measured by the method of Imada *et al.*[19]

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