Medicinal Foodstuffs. XXII.[1] Structures of Oleanane-Type Triterpene Oligoglycosides, Pisumsaponins I and II, and Kaurane-Type Diterpene Oligoglycosides, Pisumosides A and B, from Green Peas, the Immature Seeds of *Pisum sativum* L.

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Two new oleanane-type triterpene oligoglycosides, pisumsaponins I and II, and two new kaurane-type diterpene oligoglycosides, pisumosides A and B, were isolated from the immature seeds (green peas) of *Pisum sativum* L. together with soyasaponin I, bersimoside I, dehydrosoyasaponin I, and their 6′-methyl esters. The structures of pisumsaponins and pisumosides were determined on the basis of chemical and physicochemical evidence as 22-*O*-malonylsoyasapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucopyranosiduronic acid (22-*O*-malonylsoyasaponin I), sandosapogenol 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucopyranosiduronic acid, 17-*O*-β-D-glucopyranosyl-6β,7β,13β,17-tetrahydroxy-19-kauranoic acid 19-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside, and 6β,7β,13β,17-tetrahydroxy-19-kauranoic acid 19-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside, respectively.

Key words green pea; *Pisum sativum*; pisumsaponins A and B; pisumosides A and B; oleanane-type triterpene saponin; kaurane-type diterpene oligoglycoside

The Leguminosae plant *Pisum* (*P.*) *sativum* L. has been widely cultivated from ancient times and is classified into two groups differing in their edible parts as immature seeds or young pods. In Chinese traditional medicine, the seeds of this plant have been prescribed for diuretic, antiinflammatory, and stomachic purposes. As the chemical constituents of *P. sativum*, the plant hormone, chlorine-containing tryptophans, was reported from the immature seeds, while an oleanane-type triterpene saponin, chromosaponin I, and flavonol glycosides were isolated from the seedlings and roots, respectively.[2] The immature seeds of *P. sativum*, which go by the name of green peas, has been used world wide as a vegetable, but its pharmacologically active constituent remained uncharacterized. As a part of our characterizetion studies on the chemical constituents of medicinal foodstuffs,[3] we have characterized bioactive steroid and triterpene saponins from Leguminous edible beans [*e.g.*, fenugreek (*Trigonella foenum-graecum*),[4] kidney bean (*Phaseolus vulgaris*),[5] hyacinth bean (*Dolichos lablab*),[6] and sword bean (*Canavalia gladiata*)[7]]. In a continuing study, we have isolated two new oleanane-type triterpene oligoglycosides and two new kaurane-type diterpene oligoglycosides called pisumsaponins I (**1**) and II (**2**) and pisumosides A (**4**) and B (**5**). In this paper, we describe the isolation and structure elucidation of these glycosides.

The constituents from the immature seeds of *P. sativum* L. were separated by the procedures shown in Chart 1. Namely, the methanolic extract from the immature seeds was partitioned into an ethyl-acetate and water mixture to furnish the ethyl-acetate soluble portion and the water phase. The water phase was subjected by Diaion HP-20 column chromatography to give the H_2O eluate, MeOH eluate, and $CHCl_3$ eluate. The MeOH eluate was separated by normal- and reversed-phase silica gel column chromatography and HPLC to give **1** (0.00026%) and **2** (0.00044%) and **4** (0.00083%) and **5** (0.00018%) together with soyasaponins I (**7**,[8] 0.016%) and I methyl ester (**8**,[8,9] 0.00046%), bersimosides I (**9**,[10] 0.0017%) and I methyl ester (**10**,[11] 0.00027%), and dehydrosoyasaponin I (**11**,[9] 0.00091%).

Pisumsaponins I (1) and II (2) Pisumsaponin I (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{24}$ +5.8°) and its IR spectrum showed absorption bands at 3453, 1719, and 1076 cm^{-1}, suggestive of glycosidic and carbonyl functions. The molecular formula $C_{51}H_{80}O_{21}$ of **1** was determined from the negative- and positive-ion FAB-MS [*m/z* 1027 $(M-H)^-$, *m/z* 1029 $(M+H)^+$, *m/z* 1051 $(M+Na)^+$] and by high-resolution MS measurement. Furthermore, fragment ion peaks at *m/z* 881 $(M-C_6H_{11}O_4)^-$ and *m/z* 719 $(M-C_{12}H_{21}O_9)^-$ were observed in the negative-ion FAB-MS of **1**. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1, v/v) provided **7**[8] and malonic acid. Malonic acid was derived as *p*-nitrobenzyl ester,[12] which was identified by HPLC analysis. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[13] of **1** showed signals assignable to a malonyl moiety [δ 3.78 (s, 2‴-H_2)] together with a soyasaponin I moiety [δ 2.34 (dd-like, 18-H), 3.22, 4.22 (both d, *J*=11.3 Hz, 24-H2), 3.42 (dd, *J*=7.6, 11.6 Hz, 3-H), 4.97 (d, *J*=7.3 Hz, 1′-H), 5.02 (dd-like, 22-H), 5.20 (br s, 12-H), 5.75 (d, *J*=7.6 Hz, 1″-H), 6.26 (br s, 1‴-H)]. The positions of the malonyl groups in **1** were determined by an HMBC experiment, which showed a long-range correlation between the 22-proton of the soyasaponin I moiety and the carbonyl carbon of the malonyl group. Consequently, the structure of pisumsaponin I was determined to be 22-*O*-malonylsoyasapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucopyranosiduronic acid (22-*O*-malonylsoyasaponin I, **1**).

Pisumsaponin II (**2**) was also obtained as a white powder with a negative optical rotation ($[\alpha]_D^{24}$ −2.2°). The IR spectrum of **2** showed absorption bands at 1713 and 1645 cm^{-1} ascribable to formyl and carboxyl functions by the following additional evidence, as well as broad bands at 3454, 2928, and 1076 cm^{-1} suggestive of a glycosidic structure. In the negative- and positive-ion FAB-MS of **2**, quasimolecular ion

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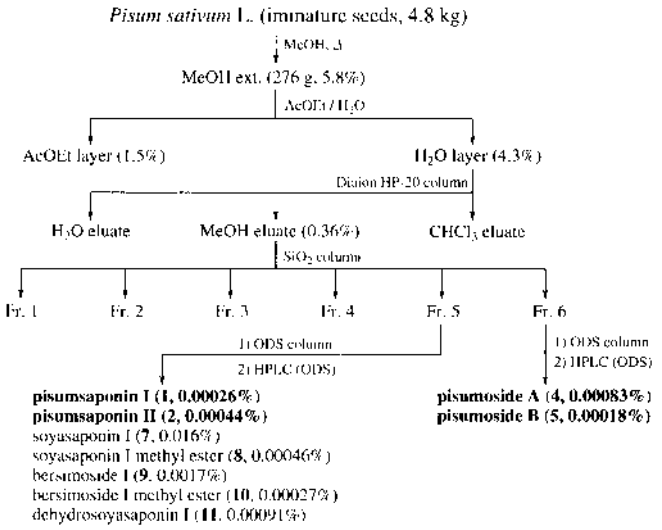


Chart 1

peaks were observed at *m/z* 939 $(M-H)^-$ and *m/z* 941 $(M+H)^+$, respectively, and fragment ion peaks at *m/z* 793 $(M-C_6H_{11}O_4)^-$ and *m/z* 631 $(M-C_{12}H_{21}O_9)^-$ were observed in the negative-ion FAB-MS. High-resolution MS analysis of the quasimolecular ion peak $(M+H)^+$ revealed the molecular formula of **2** to be $C_{48}H_{76}O_{18}$. Enzymatic hydrolysis of **2** with glycyrrhizinic acid hydrolase[14] furnished sandosapogenol (**3**).[5] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[13] of **2** showed signals due to a sandosapogenol moiety [δ 2.34 (dd-like, 18-H), 3.57 (dd, J=5.2, 12.2 Hz, 3-H), 3.71 (dd-like, 22-H), 5.30 (br s, 12-H), 10.43 (s, 24-H)] and a trisaccharide moiety [δ 5.05 (d, J=7.6 Hz, 1′-H), 5.58 (d, J=7.6 Hz, 1″-H), 6.25 (br s, 1‴-H)]. The carbon signals of the trisaccharide moiety in the ^{13}C-NMR (Table 1) of **2** were similar to those of **7**, except for the signals due to the 2′- and 1″-carbons, which were influenced by the steric effect of the 24-hydroxyl group.[15] The trisaccharide structure of **2** was confirmed by an HMBC experiment, in which was observed long-range correlations between the

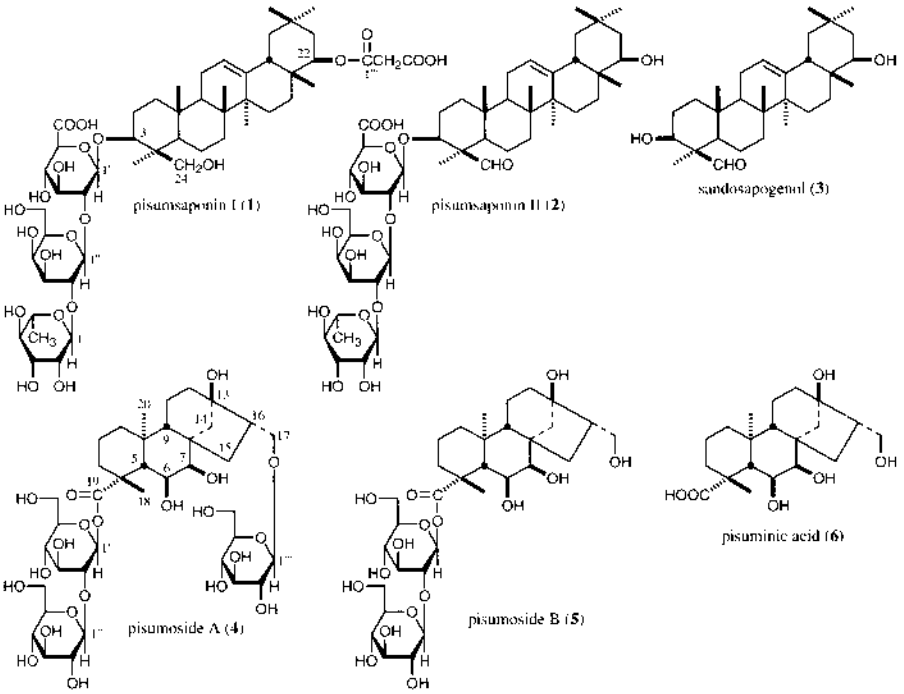


Chart 2

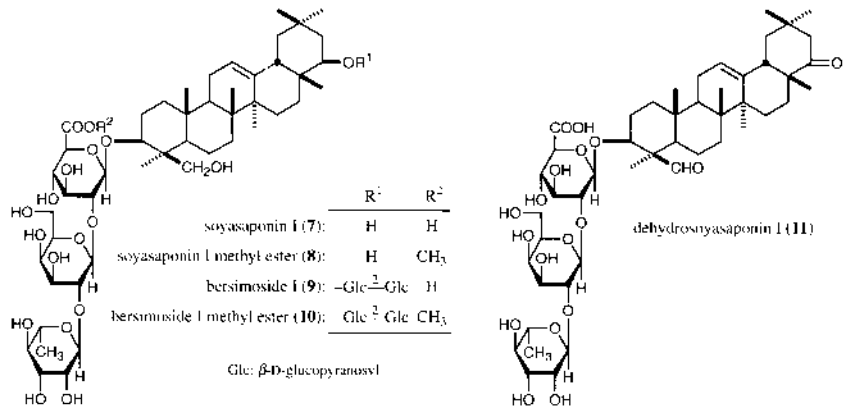


Chart 3

Table 1. ^{13}C-NMR Data of Pisumsaponins I (**1**) and II (**2**), Pisumosides A (**4**) and B (**5**), and Pisuminic Acid (**6**)

	1	2	4	5	6		1	2	4	5
C-1	38.7	38.1	40.8	40.8	41.2	27	26.2	25.6		
2	26.7	27.1	19.6	19.6	19.8	28	21.0	21.1		
3	91.3	87.2	40.4	40.3	40.6	29	33.5	33.3		
4	44.0	53.6	44.9	44.9	44.7	30	27.3	28.7		
5	56.1	57.4	52.3	52.3	52.1	1′	105.5	105.0	94.2	94.2
6	18.6	19.2	71.2	71.3	72.1	2′	78.5	79.5	79.8	79.6
7	33.0	33.2	82.5	82.6	82.7	3′	76.9	77.5	79.0	78.9
8	40.1	39.9	49.1	49.1	49.4	4′	73.8	73.3	70.9	70.9
9	47.8	46.7	49.8	49.9	50.2	5′	77.7	78.5	79.2	79.1
10	36.5	36.8	41.1	41.1	41.2	6′	172.3	172.4	62.2	62.2
11	24.1	24.3	18.5	18.7	18.7	1″	101.9	102.2	105.1	105.0
12	122.9	122.4	27.3	27.2	27.4	2″	77.8	77.0	75.8	75.7
13	144.1	144.9	80.4	81.2	81.3	3″	76.6	76.5	78.5	78.4
14	42.0	42.5	50.3	50.9	51.3	4″	71.3	70.7	72.2	72.2
15	26.7	26.4	36.6	36.8	37.0	5″	76.5	76.3	78.6	78.4
16	27.4	28.8	46.1	45.6	45.9	6″	61.8	61.8	62.9	62.9
17	36.9	38.1	75.5	66.4	66.5	1‴	102.4	103.0	106.5	
18	44.9	45.5	32.3	32.3	33.2	2‴	72.4	72.3	75.5	
19	46.3	46.8	177.4	177.4	181.2	3‴	72.8	72.8	78.6	
20	30.6	30.9	17.3	17.3	17.2	4‴	74.5	74.4	71.8	
21	38.7	42.4				5‴	69.5	69.6	78.4	
22	79.5	75.6				6‴	19.0	18.9	62.9	
23	23.1	22.3				1⁗	167.2			
24	63.6	207.2				2⁗	43.3			
25	15.8	16.4				3⁗	169.6			
26	16.8	17.1								

125 MHz, pyridine-d_5.

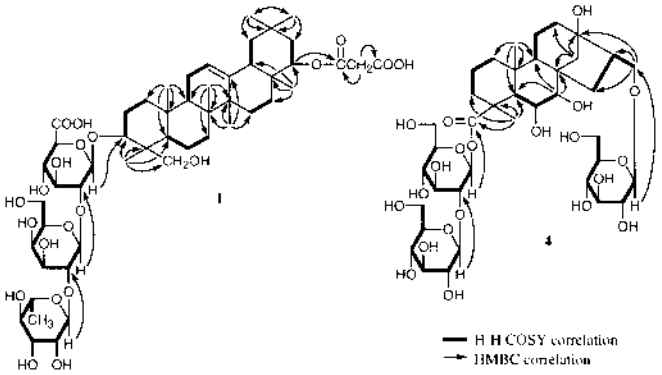
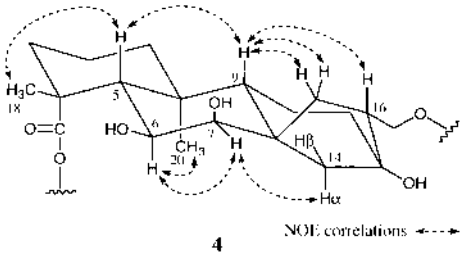


Fig. 1

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 **7**. On the basis of this evidence, the structure of pisumsaponin II was determined as sandosapogenol 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucopyranosiduronic acid (**2**).

Pisumosides A (4) and B (5) Pisumoside A (**4**) was obtained as a white powder and its IR spectrum showed absorption bands at 3453, 2928, 1739, and 1076 cm^{-1} suggestive of glycosidic and carbonyl functions. The molecular formula $C_{38}H_{62}O_{21}$ was determined from the negative- and positive-ion FAB-MS [*m/z* 853 $(M-H)^-$, *m/z* 855 $(M+H)^+$, *m/z* 877 $(M+Na)^+$] and by high-resolution MS measurement. Furthermore, a fragment ion peak at *m/z* 529 $(M-C_{12}H_{21}O_{10})^-$ was observed in the negative-ion FAB-MS of **4**. Acid hydrolysis of **4** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.[16] On the other hand, enzymatic hydrolysis of **4** with naringinase liberated an aglycone called pisuminic acid (**6**). The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[13] of **4** showed signals due to two methyls [δ 1.09, 1.85 (both s, 20, 18-H_3)], a hydroxymethyl [δ 3.93, 4.47 (both m, 17-H_2)] and two methines bearing hydroxyl group [δ 4.12 (d, J=2.1 Hz, 7-H), 4.96 (dd, J=2.1, 11.0 Hz, 6-H)] together with a carbonyl and three β-D-glucopyranosyl moieties [δ 4.96 (d, J=7.6 Hz, 1‴-H), 5.67 (d, J=7.9 Hz, 1″-H), 6.16 (d, J=7.9 Hz, 1′-H)]. The plane structures of **4** and **6** were constructed on the basis of the H–H COSY and HMBC experiments shown in Fig. 1. Thus, the H–H COSY experiments on **4** and **6** indicated the presence of partial structures in bold lines (from C-1—C-3, from C-5—C-7, from C-9—C-12, and from C-15—C-17). In the HMBC experiment, long-range correlations were observed between the following protons and carbons: 18-H_3 and 3, 4, 5, 19-C; 20-H_3 and 1, 5, 9, 10-C; 17-H_2 and 13, 15, 16-C; 14-H_2 and 9, 13, 15-C, so that the connectivities of the quaternary carbons in **4** and **6** were identified. By comparison of the ^{13}C-NMR data for **4** with those for **6**, a glycosylation shift was observed around the 17- and 18-carbons of **4**. In addition, since the HMBC experiment on **4** showed long-range correlations between the 1′-proton and 19-carbon, between the 1″-proton and 2′-carbon, and between the 1‴-proton and the 17-carbon, three β-D-glucopyranosyl moieties were found to be linked at the 19-carboxyl, 2′-hydroxyl, and 17-hydroxyl groups of **4**. Furthermore, the relative stereostructure of **4** and **6** was characterized by a difference nuclear Overhauser effect (dif NOE) experiment, which showed NOE correlations between the following protons: 5-H and 18-H_3, 9-H; 6-H and 7-H, 20-H_3; 7-H and 14α-H; 17-H_2 and 9-H (Fig. 2). These findings and comparisons of the ^{1}H- and ^{13}C-NMR



4

Fig. 2

spectra of **4** with those of known kaurane-type diterpene glycosides[17] led us to formulate the 17-*O*-β-D-glucopyranosyl-6β,7β,13β,17-tetrahydroxy-19-kauranoic acid 18-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside (**4**).

Pisumoside B (**5**) was also isolated as a white powder and its IR spectrum showed absorption bands due to hydroxyl and carbonyl functions. In the positive-ion FAB-MS of **5**, a quasimolecular ion peak was observed at *m/z* 715 $(M+Na)^+$ and high-resolution MS analysis of a quasimolecular ion peak revealed the molecular formula of **5** to be $C_{32}H_{52}O_{16}$. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[13] of **5** indicated the presence of two β-D-glucopyranosyl moieties [δ 5.69 (d, J=7.9 Hz, 1″-H), 6.17 (d, J=7.9 Hz, 1′-H)] and a pisuminic acid moiety [δ 1.14, 1.86 (both s, 20, 18-H_3), 1.76 (d, J=7.9 Hz, 9-H), 2.02 (dd-like, 15α-H), 2.05 (d, J=14.8 Hz, 14β-H), 2.30 (d, J=11.0 Hz, 5-H), 2.32 (dd-like, 15β-H), 2.51 (d, J=14.8 Hz, 14α-H), 4.05, 4.12 (both d, J=11.0 Hz, 17-H_2), 4.17 (d, J=2.1 Hz, 7-H), 5.00 (dd, J=2.1, 11.0 Hz, 6-H)]. Comparison of the ^{1}H- and ^{13}C-NMR data for **5** with those for **4** allowed us to elucidate structure **5**, which lacked the 17-*O*-β-D-glucopyranosyl moiety of **4**. Finally, **5** was derived from **4** by partial enzymatic hydrolysis with cellulase. On the basis of this evidence, the structure of **5** was determined to be as shown.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[3]

Isolation of Pisumsaponins I (1) and II (2) and Pisumosides A (4) and B (5) from the Immature Seeds of *P. sativum* L. The immature seeds of *P. sativum* L. (4.8 kg, cultivated at Hokkaido, Japan and purchased from Tokiwa Kampo Pharmaceutical Co., Ltd., Osaka) were crushed and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (276 g, 5.8%), and this extract (270 g) was partitioned in an AcOEt–H_2O (1 : 1, v/v) mixture. Removal of the solvent from the AcOEt-soluble and H_2O-soluble fractions under reduced pressure yielded the AcOEt extract (73 g, 1.5%) and H_2O phase (197 g, 4.1%). The H_2O phase (190 g) was subjected to Diaion HP-20 column chromatography [Diaion HP-20 (Nippon Rensou, 3 kg), H_2O→MeOH→$CHCl_3$] to give the H_2O eluate, MeOH eluate (16.3 g, 0.36%), and $CHCl_3$ eluate. Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia, Ltd., 480 g), $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer→65 : 35 : 10, lower layer→6 : 4 : 1, v/v)→MeOH] of the MeOH eluate (16.3 g) gave seven fractions. Fraction 5 (7.4 g) was separated by reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia, Ltd., 150 g), MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] and HPLC [YMC-Pack ODS-A (250×20 mm i.d., YMC Co., Ltd.), MeOH–1% aq. TFA (80 : 20, v/v)] to give **1** (12.3 mg, 0.00026%) and **2** (21.1 mg, 0.00044%), soyasaponins I (**7**, 749.7 mg, 0.016%) and I methyl ester (**8**, 23.7 mg, 0.00046%), and dehydrosoyasaponin I (**11**, 43.9 mg, 0.00091%). Fraction 6 (4.0 g) was separated by reversed-phase silica gel column chromatography [Cosmosil $^{75}C_{18}$-OPN (Nacalai Tesque, 120 g), MeOH–H_2O (30 : 70→40 : 60, v/v)] to give four fractions. Fraction 6-2 (541.4 mg) was purified by HPLC [MeOH–1% aq. TFA (40 : 60, v/v)] to give pisumosides A (**4**, 40.0 mg, 0.00083%) and B (**5**, 9.1 mg, 0.00018%). Fraction 6-3 (257.7 mg) was purified by HPLC [MeOH–1% aq. TFA (80 : 20, v/v)] to yield bersimosides I (**9**, 82.9 mg, 0.0017%) and I methyl ester (**10**, 13.0 mg, 0.00027%). The known compounds (**7**—**11**) were identified by comparison of their physical date ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[8—11]

Pisumsaponin I (**1**): A white powder, $[\alpha]_D^{24}$ +5.8° (c=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{51}H_{80}O_{21}Na$ $(M+Na)^+$: 1051.5100. Found: 1051.5090. IR (KBr): 3453, 2948, 1719, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.70, 0.87, 0.91, 1.05, 1.15, 1.23, 1.44 (3H each, all s, 25, 26, 30, 28, 29, 27, 23-H_3), 2.34 (1H, dd-like, 18-H), 3.22, 4.22 (1H each, both d, J=11.3 Hz, 24-H_2), 3.42 (1H, dd, J=7.6, 11.6 Hz, 3-H), 3.78 (2H, s, 2″″-H_2), 4.97 (1H, d, J=7.3 Hz, 1′-H), 5.02 (1H, dd-like, 22-H), 5.20 (1H, br s, 12-H), 5.75 (1H, d, J=7.6 Hz, 1″-H), 6.26 (1H, br s, 1‴-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 1027 $(M-H)^-$, 881 $(M-C_6H_{11}O_4)^-$, 719 $(M-C_{12}H_{21}O_9)^-$. Positive-ion FAB-MS: *m/z* 1029 $(M+H)^+$, 1051 $(M+Na)^+$.

Pisumsaponin II (**2**): A white powder, $[\alpha]_D^{24}$ −2.2° (c=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{48}H_{77}O_{18}$ $(M+H)^+$: 941.5115. Found: 941.5110. IR (KBr): 3454, 2928, 1713, 1645, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.75, 0.94, 1.00, 1.19, 1.23, 1.28, 1.67 (3H each, all s, 25, 26, 30, 28, 27, 29, 23-H_3), 2.34 (1H, dd-like, 18-H), 3.57 (1H, dd, J=5.2, 12.2 Hz, 3-H), 3.71 (1H dd-like, 22-H), 5.05 (1H, d, J=7.6 Hz, 1′-H), 5.30 (1H, br s, 12-H), 5.58 (1H, d, J=7.6 Hz, 1″-H), 6.25 (1H, br s, 1‴-H), 10.43 (1H, s, 24-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 939 $(M-H)^-$, 793 $(M-C_6H_{11}O_4)^-$, 631 $(M-C_{12}H_{21}O_9)^-$. Positive-ion FAB-MS: *m/z* 941 $(M+H)^+$.

Pisumoside A (**4**): A white powder, $[\alpha]_D^{26}$ −18.8° (c=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{38}H_{63}O_{21}$ $(M+H)^+$: 855.3859. Found: 855.3862. IR (KBr): 3453, 2928, 1739, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.09, 1.85 (3H each, both s, 20, 18-H_3), 1.70 (1H, d, J=6.7 Hz, 9-H), 1.93 (1H, d, J=15.0 Hz, 14β-H), 1.94 (1H, dd-like, 15α-H), 2.23 (1H, dd-like, 15β-H), 2.28 (1H, d, J=11.0 Hz, 5-H), 2.45 (1H, d, J=15.0 Hz, 14α-H), 3.93, 4.47 (1H each, both m, 17-H_2), 4.12 (1H, d, J=2.1 Hz, 7-H), 4.96 (1H, dd, J=2.1, 11.0 Hz, 6-H), 4.96 (1H, d, J=7.6 Hz, 1‴-H), 5.67 (1H, d, J=7.9 Hz, 1″-H), 6.16 (1H, d, J=7.9 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Negative-ion FAB-MS: *m/z* 853 $(M-H)^-$, 529 $(M-C_{12}H_{21}O_{10})^-$. Positive-ion FAB-MS: *m/z* 855 $(M+H)^+$, 877 $(M+Na)^+$.

Pisumoside B (**5**): A white powder, $[\alpha]_D^{25}$ −31.5° (c=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{52}O_{16}Na$ $(M+Na)^+$: 715.3168. Found: 715.3153. IR (KBr): 3432, 2926, 1744, 1075 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.14, 1.86 (3H each, both s, 20, 18-H_3), 1.76 (1H, d, J=7.9 Hz, 9-H), 2.02 (1H, dd-like, 15α-H), 2.05 (1H, d, J=14.8 Hz, 14β-H), 2.30 (1H, d, J=11.0 Hz, 5-H), 2.32 (1H, dd-like, 15β-H), 2.51 (1H, d, J=14.8 Hz, 14α-H), 4.05, 4.12 (1H each, both d, J=11.0 Hz, 17-H_2), 4.17 (1H, d, J=2.1 Hz, 7-H), 5.00 (1H, dd, J=2.1, 11.0 Hz, 6-H), 5.69 (1H, d, J=7.9 Hz, 1″-H), 6.17 (1H, d, J=7.9 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: *m/z* 715 $(M+Na)^+$.

Alkaline Hydrolysis of 1 A solution of **1** (1.0 mg) in 50% aqueous dioxane (2 ml) was treated with 10% aqueous KOH (2 ml) and the whole was stirred at 37 °C for 1 h. After removal of the solvent from a part (0.1 ml) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (1 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (5 mg), then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl ester of malonic acid. HPLC conditions: column, YMC-Pack ODS-A (YMC Co., Ltd.), 250×4.6 mm (i.d.); solvent, MeOH–H_2O (70 : 30, v/v); flow rate, 1.0 ml/min; t_R, 19.2 min.

The rest of the reaction mixture was neutralized with Dowex HCR W2 (H^+ from) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product, which was subjected to normal-phase silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer, v/v)] to give soyasaponin I (**7**, 0.8 mg, 75%), which was identified by comparison of its physical data ($[\alpha]_D$, IR, ^{1}H-NMR) with an authentic sample.

Enzymatic Hydrolysis of 2 A solution of **2** (3.0 mg) in 0.1 M acetate buffer (pH 4.4, 0.2 ml) was treated with glycyrrhizinic acid hydrolase (Maruzen Co., Ltd., 0.2 ml) and the reaction mixture was stirred at 44 °C for 3 h. The mixture was then poured into EtOH and the whole was removed under reduced pressure. The residue was purified by reversed-phase [500 mg, H_2O→MeOH] and normal-phase silica gel column chromatography [600 mg, *n*-hexane–AcOEt (1 : 1, v/v)] to give sandosapogenol (**3**,

0.3 mg), which was identified by a comparison of physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

Conversion from 2 to 7 A solution of **2** (2.0 mg) in dry MeOH was treated with $NaBH_4$ (2.0 mg) and the reaction mixture was stirred at room temperature (24 °C) for 10 min. The mixture was poured into acetone (0.2 ml), then neutralized with Dowex HCR W2 (H^+ form) and an insoluble portion was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the crude product was purified by normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer, v/v)] to give **7** (2.0 mg), which was identified by a comparison of physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

Enzymatic Hydrolysis of 4 A solution of **4** (8.7 mg) in 0.1 M acetate buffer (pH 4.0, 2.0 ml) was treated with naringinase (Sigma Chemical Co., 20.0 mg) and the reaction mixture was stirred at 40 °C for 20 h, then poured into EtOH and the whole was removed under reduced pressure. The residue was purified by reversed-phase [1.0 g, H_2O→MeOH] and normal-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower layer, v/v)] to give pisuminic acid (**6**, 2.3 mg, 63.0%).

Pisuminic acid (**6**): A white powder, $[\alpha]_D^{25}$ −30.5° (c=0.1, MeOH). High-resolution EI-MS: Calcd for $C_{20}H_{32}O_6$ (M^+): 368.2199. Found: 368.2188. IR (KBr): 3453, 2926, 1645 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.28, 1.85 (3H each, both s, 20, 18-H_3), 1.82 (1H, d, J=7.9 Hz, 9-H), 1.99 (1H, br d, 15α-H), 2.10 (1H, d, J=14.6 Hz, 14β-H), 2.21 (1H, m, 15β-H), 2.56 (1H, d, J=14.6 Hz, 14α-H), 2.38 (1H, d, J=10.7 Hz, 5-H), 4.15 (1H, d, J=1.8 Hz, 7-H), 4.94 (1H dd, J=1.8, 10.7 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. EI-MS (%, 20 eV): m/z 368 (M^+, 2), 355 (M^+-H_2O, 2), 45 (100).

Acid Hydrolysis of 4 A solution of **4** (2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 2 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and residue was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was transferred to a Sep-Pak C_{18} cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (3 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose: GLC conditions: Supeluco STB^{TM}-1, 30 m×0.25 mm (i.d.) capillary column, column temperature 230 °C, He flow rate 15 ml/min, t_R: 24.1 min.

Enzymatic Hydrolysis of 4 Giving 5 A solution of **4** (1.0 mg) in 0.1 M acetate buffer (pH 5.0, 1.0 ml) was treated with cellulase (Sigma Chemical Co., 2.0 mg) and the reaction mixture was stirred at 40 °C for 3 d. The reaction mixture was poured into EtOH and the whole was removed under reduced pressure. The residue was purified by reversed-phase [500 mg, H_2O→MeOH] and normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (6 : 4 : 1, v/v)] to give **5** (0.6 mg, 75%), which was identified by a comparison of physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

References and Notes

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