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New Derivatives of Saikosaponins

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In the course of studies on the metabolism of saikosaponins, which are the main constituents of *Bupleurum falcatum* L., five new compounds derived from saikosaponins a, c and d were isolated after incubation with acid and snail enzyme. On incubation of saikosaponins a and c at 60 °C in a 1 N sulfuric acid–dioxane solution, saikosaponins g and i possessing a homoannular diene moiety at C-9(11),12 were isolated, and their structures were elucidated as 3β,16β,23,28-tetrahydroxyoleana-9(11),12-diene 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside and 3β,16β,28-trihydroxyoleana-9(11),12-diene 3-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside, respectively. Furthermore, on incubation of saikosaponins b_1, g and b_2 with snail enzyme for 12 h at 37 °C, prosaikogenins A, H, and D were formed; they were identified as the 3-β-D-fucopyranosides of saikogenins A, H and D.

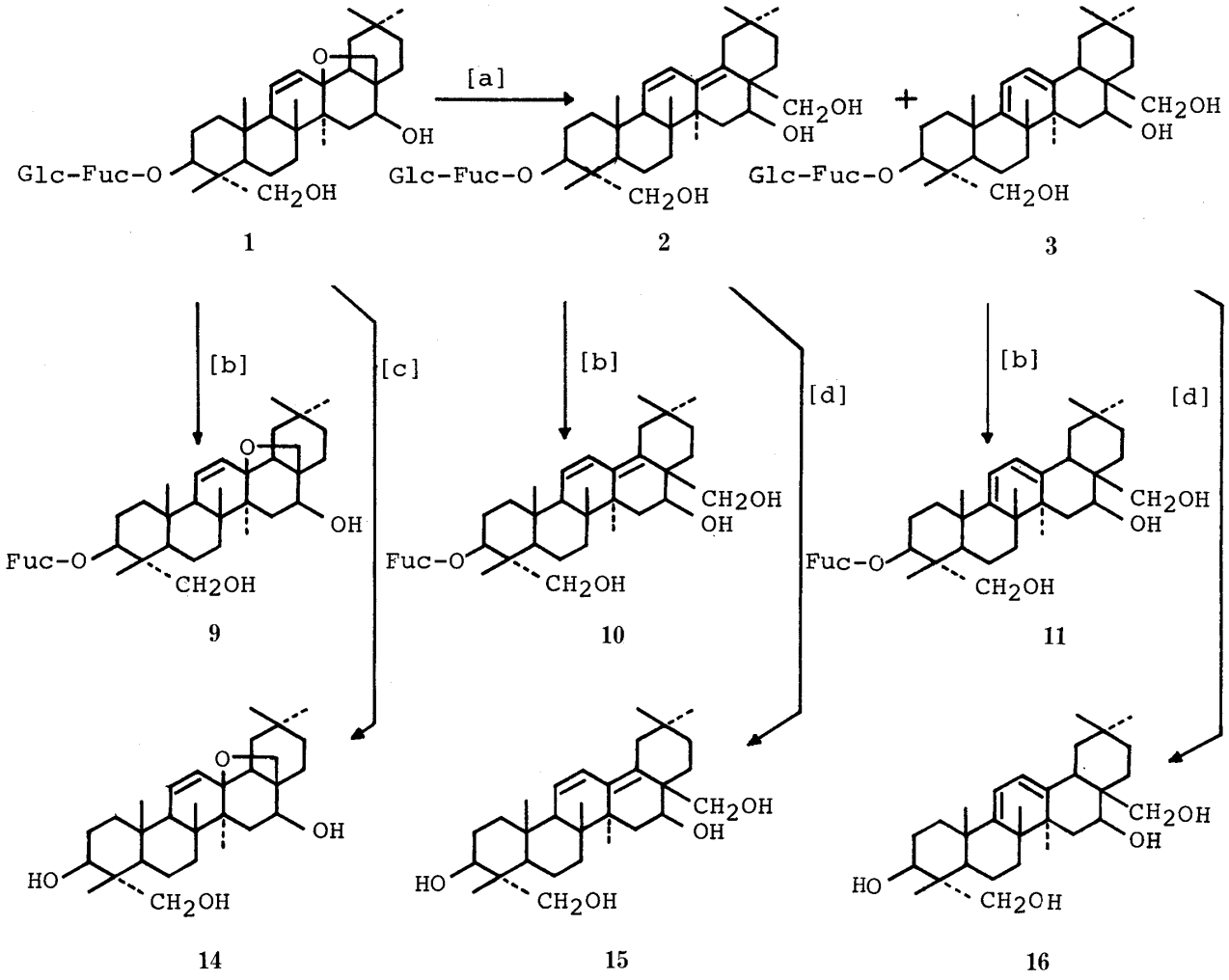
Keywords——saikosaponin; prosaikogenin; saikogenin; snail enzyme; intestinal flora

Studies on the triterpenoidal saponins of the roots of *Bupleurum falcatum* L. were originated by Shibata *et al.*[1,2] and Kubota *et al.*[3] who reported chemical studies on saikogenins A, C and D. Then, Kubota *et al.*[4,5] identified the genuine genins, saikogenins E, F and G, and glycosides, saikosaponins a, b, c and d. Later, Shimaoka *et al.*[6] reinvestigated the fraction of saikosaponins a and b, and showed that so called saikosaponins a and b were a mixture of saikosaponins a, b_1 and b_3 and a mixture of saikosaponins b_2 and b_4, respectively. Recently, Ishii *et al.*[7] reported the isolation of saikosaponins e and f, and five monoacetyl saikosaponins. He also reported comparative carbon-13 nuclear magnetic resonance (^{13}C-NMR) studies on 23 related compounds excluding saponins possessing a homoannular diene moiety. The structure of saikosaponin h was clarified by Kimata *et al.*,[8] but the spectral data were not given. As to the structures of saikogenins possessing a homoannular diene, saikogenin B was reported by Kubota and Tonami[9] and saikogenin H by Takeda,[10] but no detailed spectral data for saikogenin H were presented. As to the report on ^{13}C-NMR studies of triterpenoid possessing a homoannular diene moiety, only Diaz *et al.* reported[11] about 3β-acetoxyoleana-9,12-diene.

This paper reports the isolation and structural identification of eighteen derivatives, including five new compounds, which were formed by pseudo-metabolic reactions, as well as comparative ^{13}C-NMR studies of the compounds obtained, which include derivatives possessing a homoannular diene moiety.

Results and Discussion

Treatment of saikosaponin a (**1**) with 1 N sulfuric acid–dioxane (1 : 1) for 1 h at 60 °C, followed by separation by reversed-phase high performance liquid chromatography (HPLC) or on a Lobar column afforded known saikosaponin b_1 (**2**), identical with an authentic sample, and a new saikosaponin g (**3**) (resulting ratio, 3 : 1). Compound **3** was suggested to have a homoannular diene moiety at C-9(11),12 on the basis of the observation of a

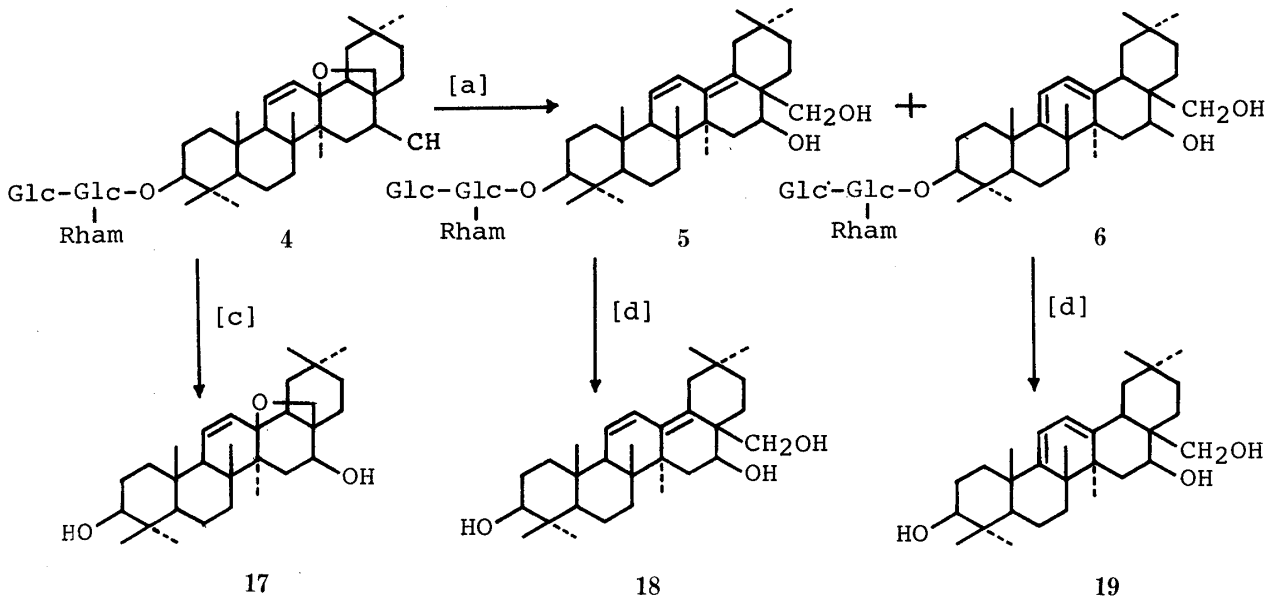


reagents: [a] 1 N H_2SO_4 : 1,4-dioxane (1 : 1)
[b] snail enzyme
[c] intestinal flora
[d] 2 N H_2SO_4 : 1,4-dioxane (1 : 1)

Chart 1

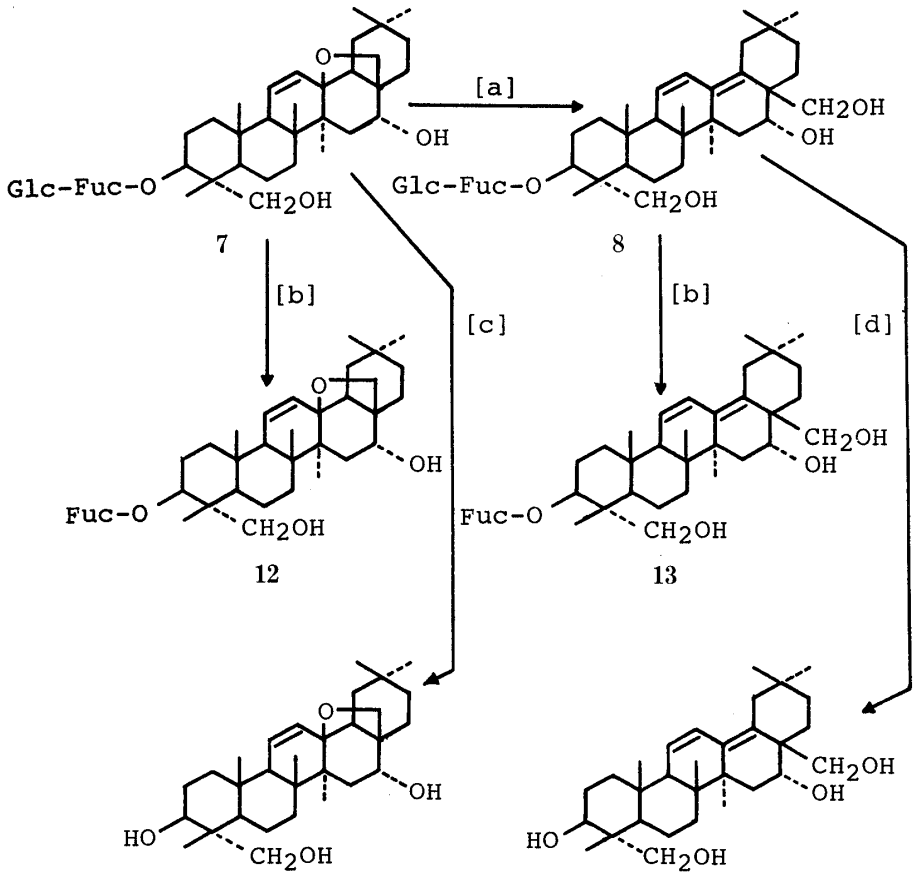
ultraviolet (UV) absorption maximum at 280 nm. This was supported by the singlet signal due to two olefinic protons at δ 5.62 in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the peracetate (**22**), prepared by acidic hydrolysis of **3** and acetylation of the resulting saikogenin H (**16**). The ^{13}C-NMR signals due to the sugar moiety of **3** were identical with those of **1** (Chart 1 and Table I). Thus, the structure of **3** was determined to be 3β,16β,23,28-tetrahydroxyoleana-9(11),12-diene 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside. Saikosaponin c (**4**) was also treated by the same procedure as described for **1** to give saikosaponin h (**5**) and a new compound, saikosaponin i (**6**). The structure of **6** was determined to be 3β,16β,28-trihydroxyoleana-9(11),12-diene 3-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl- (1→4)]-β-D-glucopyranoside mainly based on the ^{13}C-NMR spectral analysis (Chart 2 and Table I). On acidic hydrolysis of **5** and **6**, saikogenin C (**18**) and saikogenin B (**19**) were obtained, respectively. Saikosaponin d (**7**) was treated with 1 N sulfuric acid–dioxane (1 : 1) for 30 min at 30 °C to give known saikosaponin b_2 (**8**) only, no saponin possessing a homoannular diene moiety being detected in this case (Chart 3).

Compounds, **1**, **2**, **3**, **7**, and **8**, were incubated with snail enzyme for 12 h at 37 °C and prosaikogenins F (**9**), A (**10**), H (**11**), G (**12**) and D (**13**), respectively, were formed by the



reagents: [a] 1 N H_2SO_4 : 1,4-dioxane (1 : 1)
[c] intestinal flora
[d] 2 N H_2SO_4 : 1,4 dioxane (1 : 1)

Chart 2



reagents: [a] 1 N H_2SO_4 : 1,4-dioxane (1 : 1)
[b] snail enzyme
[c] intestinal flora
[d] 2 N H_2SO_4 : 1,4-dioxane (1 : 1)

Chart 3

TABLE I. ^{13}C-NMR Spectral Data for Saikosaponins

	1	**9**	**14**	**7**	**12**	**20**	**4**	**17**[a]	**2**[a]	**10**
C-1	38.7	38.6	38.6	38.5	38.7	38.6	38.4	38.8	38.7	38.4
C-2	26.0[b]	26.0[b]	27.5	26.0	26.1	27.6	26.5	27.9	25.8	26.0
C-3	81.6	81.7	73.2	81.7	81.8	73.4	88.9	78.0	82.4	81.6
C-4	43.7	43.7	43.1	43.7[d]	43.8[d]	43.1	39.6	39.5	43.7	43.6
C-5	47.3	47.4	49.7	47.3	47.6	48.4	55.8	55.3	48.1	47.4
C-6	17.6	17.4	18.0	17.6	17.7	18.0	18.4	18.2	18.8	18.9
C-7	31.5	31.6	31.6	31.5	31.6	31.6	31.5	31.9	32.2	32.2[h]
C-8	42.2	42.2	42.2	41.9	42.0	41.9	42.1	42.2	40.8	40.5
C-9	53.0	53.1	53.1	53.0	53.1	53.0	52.7	53.0	54.7	54.5
C-10	36.2	36.3	36.6	36.3	36.4	36.6	36.2	36.7	36.9	36.6
C-11	132.2	132.2	132.2	131.9	132.0	132.0	132.0	132.1	127.1	127.1
C-12	131.1	131.2	131.2	131.9	132.0	132.0	131.0	131.2	125.7	125.7
C-13	83.9	84.0	84.0	84.9	84.9	85.0	83.9	84.0	136.8	136.6
C-14	45.6	45.7	45.7	43.6[d]	43.7[d]	43.6	45.6	45.6	44.6[f]	44.3[f]
C-15	36.2	36.1	36.1	35.4	35.5	35.4	36.2	36.3	35.1[g]	34.9[g]
C-16	64.0	64.0	64.0	77.1	77.2	77.2	64.0	64.0	76.5	76.5
C-17	46.9	47.0	47.0	45.3	45.4	45.4	46.9	47.0	44.8[f]	44.8[f]
C-18	52.1	52.1	52.1	51.3	51.4	51.4	52.0	52.2	133.6	133.6
C-19	37.7	37.8	37.8	38.5	38.5	38.5	38.1	37.8	38.7	38.4
C-20	31.5	31.6	31.6	31.9	32.0	31.9	31.5	31.6	32.8	32.7
C-21	34.7	34.7	34.7	36.8	36.9	36.8	34.8	34.7	35.4[g]	35.1[g]
C-22	25.7[b]	25.7[b]	25.7	31.2	31.3	31.3	25.7	25.7	30.2	30.0
C-23	64.0	64.3	67.5	64.1	64.5	67.8	27.8	28.4	65.2	64.3
C-24	13.0	13.0	12.5	13.0	13.1	12.5	16.3	15.9	12.9	12.9
C-25	18.7	18.8	18.6	18.8	18.9	18.7	18.1	18.2	18.5	18.2
C-26	20.0	20.0	20.0	19.5	19.6	19.5	19.9	20.0	17.3	17.4
C-27	20.8	20.9	20.9	18.1	18.2	18.0	20.9	20.9	22.1	21.9
C-28	73.0	72.8	73.0	77.7	77.9	77.8	72.9	73.0	64.1	63.9
C-29	33.6	33.6	33.6	33.7	33.8	33.8	33.6	33.7	25.0	24.8
C-30	23.8	23.8	23.8	24.4	24.5	24.4	23.8	23.8	32.8	32.3[h]
C-1′	105.9	106.2		105.9	106.3		106.4		105.5	106.3
C-2′	71.5	73.0		71.5[e]	73.1		75.4		71.8	73.0
C-3′	85.1	75.5		85.1	75.5		76.7		85.2	75.5
C-4′	71.7	72.8		71.7[e]	72.9		79.7		72.1	72.8
C-5′	70.9	71.3		71.0	71.4		75.4		71.0	71.3
C-6′	17.2	17.3		17.2	17.5		68.9		17.0	17.1
C-1″	106.4			106.5			102.7		106.0	
C-2″	75.7			75.7			72.5		75.7	
C-3″	78.6[c]			78.6[c]			72.5		78.3	
C-4″	72.1			72.1[e]			73.7		72.1	
C-5″	78.3[c]			78.3[c]			70.5		78.3	
C-6″	62.6			62.7			18.1		63.1	
C-1‴							104.9			
C-2‴							74.9			
C-3‴							78.2			
C-4‴							71.3			
C-5‴							78.2			
C-6‴							62.4			

^{13}C-NMR Spectra were observed at 25 °C. *a*) Taken from ref. 6. *b*—*l*) Assignments may be reversed in each column.

removal of the terminal glucose from the starting saponins (Charts 1 and 3). Compounds **10** and **13** were shown to be the 3-*O*-*β*-D-fucopyranosides of saikogenin A (**15**) and saikogenin D (**21**), respectively, based on observation of a UV absorption maximum at 254 nm, indicative of

and Their Derivatives in Pyridine-d_5

15[a]	**8**[a]	**13**	**21**	**5**	**18**[a]	**3**	**11**	**16**	**6**	**19**
38.4	38.7	38.4	38.4	38.6	38.8	37.7	37.6	37.6	37.4	37.8
27.6	25.9	26.0	27.6	26.5	28.1	26.9	26.8	28.4	27.3	28.8
73.1	82.6	81.8	73.3	89.0	78.5	81.6	81.7	73.0	88.8	77.7
43.1	43.7	43.7	43.0	39.6	39.6	43.8	43.7	43.2	39.7	39.6
48.2	48.2	47.5	48.4	55.3	55.7	43.8	43.7	44.6	51.8	51.8
18.6	18.8	18.9	18.7	18.5	19.0	18.0	18.1	18.5	18.5	18.7
32.4[h]	32.5	32.4	31.9	32.7	33.1	32.1	32.2	32.2	32.4	32.5
40.5	41.4	41.1	41.0	40.4	40.7	43.2[k]	43.2[k]	43.2	43.1[k]	43.1[k]
54.5	54.2	54.0	54.0	54.2	54.6	155.0	155.0	155.0	154.7	154.9
36.8	36.9	36.6	36.8	36.5	37.3	38.7	38.7	39.0	38.7	39.1
127.1	126.3	126.2	126.2	127.0	127.1	116.1	116.1	116.1	116.1	116.1
125.7	126.3	126.2	126.2	125.7	125.8	121.2	121.2	121.2	121.2	121.2
136.4	137.1	136.1	136.0	135.8	136.7	145.3	145.3	145.4	145.3	145.4
44.4	42.2	41.9	41.9	44.3[f]	44.6[f]	43.3[k]	43.3[k]	43.2	43.2[k]	43.3[k]
34.8[g]	32.8	32.6	32.8	34.8[g]	35.1[g]	36.2	36.2	36.2	36.1	36.1
76.5	68.8	67.7	67.6	76.6	76.5	66.8	66.8	66.8	66.8	66.8
44.4	45.4	45.3	45.3	44.4[f]	44.8[f]	40.6	40.6	40.6	40.6	40.6
133.3	133.0	133.1	133.0	133.4	133.7	42.7	42.7	42.7	42.7	42.7
38.4	39.2	39.0	39.0	38.5	38.7	47.0	47.0	47.0	47.1	47.1
32.7	32.8	32.6	32.6	32.7	32.8	31.0	31.0	31.0	31.0	31.0
35.1[g]	35.8	35.4	35.4	35.1[g]	35.4[g]	34.1	34.1	34.2	34.1	34.1
29.9	24.9	24.4	24.4	30.0	30.2	26.2	26.2	26.1	26.1	26.1
67.4	65.3	64.7[i]	64.7[i]	28.0	28.5	64.4	64.7	67.7	28.4	28.9
12.6	12.9	12.6	12.6	16.4	15.8	13.7	13.6	13.2	17.1	16.6
18.6	18.6	18.7	18.7	18.5	18.3	21.1[l]	21.1[l]	21.1[l]	21.1[l]	21.0[l]
17.1	17.5	17.3	17.3	17.0	17.2	21.3[l]	21.3[l]	21.3[l]	21.2[l]	21.3[l]
22.0	22.3	21.9	21.9	22.0	22.1	26.2	26.2	26.1	25.4	25.6
64.0	65.6	64.4[i]	64.4[i]	63.9	64.1	69.5	69.4	69.4	69.3	69.4
24.8	25.3	25.1	25.1	24.8	25.0	33.2	33.2	33.2	33.2	33.2
32.3[h]	32.5	32.4	32.4	32.3	32.3	24.1	24.1	24.1	24.0	24.0
	105.5	106.3		106.7		106.0	106.3		106.7	
	71.8	73.0		75.2		71.6	73.0		75.1	
	85.2	75.5		76.8		85.3	75.5		76.8	
	72.1	72.9		79.9		71.8	72.8		79.8	
	71.0	71.3		75.5		71.0	71.3		75.5	
	17.0	17.3		69.1		17.3	17.4		69.0	
	105.9			102.9		106.7			102.9	
	75.6			72.5[j]		75.8			72.5	
	78.2			72.6[j]		78.8			72.6	
	72.1			73.8		72.1			73.8	
	78.1			70.6		78.4			70.6	
	63.1			18.2		62.7			18.5	
				105.1					105.1	
				74.8					74.8	
				78.4					78.5	
				71.4					71.4	
				78.4					78.4	
				62.6					62.5	

the existence of a heteroannular diene moiety at C-11,13(18), as well as on the ^{13}C-NMR spectrum which exihibited signals due to carbons of the fucose moiety. Compound **11** was also characterized as the 3-*O*-β-D-fucopyranoside of **16** based on analysis of the ^{13}C-NMR data.

When treated by the same procedure as mentioned above, **4**, **5** and **6** remained unchanged and the starting materials were recovered.

Treatment of **1** with 2 N sulfuric acid–dioxane (1 : 1) for 2 h at 100 °C gave a mixture of **15** and **16**, which were separated on a Lobar column. Treatment of **7** by the same procedure as mentioned above gave saikogenin D (**21**) only. Saikogenin C (**18**) and **19** were obtained by the treatment of **4** with 2 N sulfuric acid–dioxane (1 : 1) for 6 h at 100 °C, followed by purification on a Lobar column.

Saikogenins F (**14**), E (**17**) and G (**20**) were derived from **1**, **4** and **7**, respectively, by incubation with intestinal flora of mouse for 4 h at 37 °C (Charts 1, 2 and 3).

As described above, we isolated 18 kinds of derivatives from saikosaponins a (**1**), c (**4**) and d (**7**) under pseudo-physiological and chemical conditions. The detection of these compounds in feces, urine and blood after the oral administration of saikosaponins is being attempted.

Experimental

All melting points were measured on a Yanagimoto microscope hot plate and are uncorrected. Infrared (IR) spectra were determined on a JASCO IR spectrometer. Optical rotations were measured with a DIP-140 degital polarimeter. UV spectra were taken with a Shimadzu UV-240 spectrometer. ^{1}H-NMR spectra were measured on a JEOL JNM-MH-100 spectrometer and ^{13}C-NMR spectra were measured on a JEOL FX-100 spectrometer with tetramethylsilane (TMS) as an internal standard. The ^{13}C-NMR signal assignments were carried out by means of known chemical shift rules, off-resonance decoupling studies and also by comparison with the reported data for known compounds.[1-11] The chemical shifts are given in δ values. Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck) and RP-18 F_{254} (Merck). For column chromatography, Kieselgel 60 (Merck) and LiChroprep RP-18 (40—60 μm) (Merck) were used. Saikosaponins a, c and d used in these experiments were supplied by Takeda Chemical Industries Co., Ltd.

Acidic Treatment of Saikosaponins a (1), c (4) and d (7)——Compound **1** (200 mg) was dissolved in dioxane (20 ml) and 1 N sulfuric acid (20 ml) was added. The mixture was incubated for 1 h at 60 °C with stirring. After cooling, the solution was neutralized with 10% sodium hydroxide and extracted with *n*-butanol. The butanol layer was washed with water and evaporated to dryness. The residue (170 mg) was separated into saikosaponin b_1 (**2**) (122 mg) and saikosaponin g (**3**) (40 mg) on a LiChroprep RP-18 column. The solvent system employed was methanol–water (6 : 1). Compound **3** was obtained as a white powder, mp 239—245 °C. $[\alpha]_D^{25}$ +92.3° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 281 (6600). *Anal.* Calcd for $C_{42}H_{68}O_{13}\cdot H_2O$: C, 63.12; H, 8.84. Found: C, 62.80; H, 9.03.

Compound **4** (200 mg) was treated as mentioned above and the reaction product (182 mg) was separated into saikosaponin h (**5**) (135 mg) and saikosaponin i (**6**) (42 mg) on a LiChroprep RP-18 column. The solvent system employed was methanol–water (4 : 1). Compound **5** was obtained as a white powder, mp 204—208 °C. $[\alpha]_D^{25}$ −63.3° (c=0.5 in methanol). UV λ_{max}^{MeOH} nm (ε): 242 (18600), 250 (20600), 259 (12900). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot 2H_2O$: C, 59.84; H, 8.59. Found: C, 59.82; H, 8.50. Compound **6** was obtained as a white powder, mp 201—204 °C. $[\alpha]_D^{25}$ +52.6° (c=0.5 in methanol). UV λ_{max}^{MeOH} nm (ε): 280 (6480). *Anal.* Calcd for $C_{48}H_{78}O_{17}\cdot 3H_2O$: C, 58.74; H, 8.33. Found: C, 59.22; H, 8.46.

Compound **7** (50 mg) was dissolved in dioxane (5 ml), and 1 N sulfuric acid (5 ml) was added. After incubation for 1 h at 30 °C, the solution was neutralized with 10% sodium hydroxide and extracted with *n*-butanol. The extract was washed with water and evaporated to dryness. The residue (48 mg) was purified by silica gel column chromatography to afford saikosaponin b_2 (**8**) (45 mg). The solvent system was the lower layer of chloroform–methanol–water (65 : 35 : 10).

Partial Hydrolysis of 1, 7, 2, 3 and 8 by Snail Enzyme——Compounds, **1**, **7**, **2**, **3** and **8** (each 50 mg) were each dissolved in 10% ethanol solution (300 ml) and the snail enzyme (100 mg) was added. After incubation for 12 h at 37 °C, each solution was extracted with ether. The ether layer was washed with water, dried with sodium sulfate and evaporated to dryness. Each residue was chromatographed on silica gel [solvent system: the lower layer of chloroform–methanol–water (65 : 23 : 10)] to afford prosapogenins F (**9**) (30 mg), G (**12**) (16 mg), A (**10**) (28 mg), H (**11**) (23 mg) and D (**13**) (30 mg), respectively. Compound **10** was obtained as a white powder, mp 209—212 °C $[\alpha]_D^{25}$ −63.3° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 242 (20620), 254 (23140), 259 (14810). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot 2H_2O$: C, 67.88; H, 9.50. Found: C, 67.36; H, 9.26. Compound **11** was obtained as a white powder, mp 216—221 °C. $[\alpha]_D^{25}$ +129.2° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 282 (7880). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot 1/2\,H_2O$: C, 68.85; H, 9.48. Found: C, 68.64; H, 8.99. Compound **13** was obtained as a white powder, mp 202—205 °C. $[\alpha]_D^{25}$ −37.0° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 242 (20020), 254 (22250), 260 (14580). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot H_2O$: C, 67.88; H, 9.50. Found: C, 67.10; H, 8.81.

Hydrolysis of 1, 4 and 7 by Mouse Intestinal Flora——Intestinal flora obtained from fifty male mice (ddY) was added to 500 ml of Davis' medium ($K_2HPO_4 \cdot 2H_2O$ 7 g, KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $(NH_4)_2SO_4$ 1 g, $Na_3C_6H_5O_7 \cdot 2H_2O$ and glucose 2 g were made up to 1000 ml and the pH was adjusted to 7.0), and the mixture was filtered through cotton. Compound **1**, **4** or **7** (each 50 mg) in ethanol (10 ml) was added to the medium and incubated for 12 h at 37 °C. Each solution was extracted with ether and the ether layer was washed with water. After evaporation of the organic layer, the residue was chromatographed on silica gel [solvent system: chloroform–methanol (8 : 1 for **14** and **20**, 10 : 1 for **17**)] to afford saikogenin F (**14**) (20 mg), saikogenin E (**17**) (12 mg) or saikogenin G (**20**) (11 mg), respectively.

Acidic Hydrolysis of 1, 4 and 7——Compound **1** (100 mg) was dissolved in dioxane (20 ml), and 1 N sulfuric acid (20 ml) was added. The mixture was refluxed for 3 h at 120 °C. After cooling, the solution was extracted with chloroform. The organic layer was washed with water and evaporated to dryness. The residue was separated into saikogenin A (**15**) (39 mg) and saikogenin H (**16**) (12 mg) on a LiChroprep RP-18 column [solvent system: methanol–water (8 : 1)]. Compound **16** was obtained as a white powder, mp >300 °C. $[\alpha]_D^{25}$ +209.8° ($c=0.5$ in methanol). UV λ_{max}^{MeOH} nm (ε): 282 (9060). *Anal.* Calcd for $C_{30}H_{48}O_4 \cdot 1/2\,H_2O$: C, 74.79; H, 10.26. Found: C, 74.59; H, 10.02. The peracetate (**22**) of **16** was obtained by dissolving **16** (2 mg) in pyridine (1 ml) and acetic anhydride (1 ml) for 12 h at room temperature. Compound **22** was obtained as a white powder, mp 197—201 °C. $[\alpha]_D^{25}$ +218° ($c=0.4$ in chloroform). IR ν_{max}^{KBr} cm^{-1}: 1740, 1240, 1032. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 282 (7870). ^{1}H-NMR ($CDCl_3$): 3.69 (1H, d, $J=$ 12 Hz), 3.98 (1H, d, $J=12$ Hz), 4.03 (1H, d, $J=12$ Hz), 4.21 (1H, d, $J=12$ Hz), 4.70—4.90 (1H, m), 5.42—5.64 (1H, m), 5.62 (2H, s). *Anal.* Calcd for $C_{38}H_{56}O_8$: C, 71.21; H, 8.81. Found: C, 70.64; H, 8.92.

Compound **4** (200 mg) was dissolved in dioxane (20 ml), and 1 N sulfuric acid (20 ml) was added. The mixture was refluxed for 6 h at 120 °C. After cooling, the solution was extracted with chloroform, washed with water and evaporated to dryness. The residue was separated into saikogenin C (**18**) (58 mg) and saikogenin B (**19**) (20 mg) on a LiChroprep RP-18 column [solvent system: methanol–water (15 : 1)].

Compound **7** (100 mg) was dissolved in dioxane (20 ml) and 1 N sulfuric acid (20 ml) was added. The mixture was refluxed for 2 h at 120 °C. After cooling, the solution was extracted with chloroform, washed with water and evaporated to dryness. The residue was chromatographed on silica gel to afford saikogenin D (**21**) (53 mg) [solvent system: chloroform–methanol (8 : 1)].

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