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New Derivatives of Saikosaponin c

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In the course of studies on the metabolism of saikosaponins, which are the main constituents of *Bupleurum falcatum* L., nine new compounds derived from saikosaponin c were isolated. On cleavage of the glycosidic bond of saikosaponin c at 80 °C in alcoholic alkali metal solution, prosaikogenins E-1, E-2, and E-3 were isolated, and their structures were elucidated as 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of saikogenin E, 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin E and 3-*O*-β-D-glucopyranoside of saikogenin E, respectively. Furthermore, on the treatment of prosaikogenins E-1, E-2, and E-3 with 1 N sulfuric acid–dioxane solution for 4 h at 60 °C, prosaikogenins C-1, C-2, and C-3 were formed [elucidated as 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of saikogenin C, 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin C, and 3-*O*-β-D-glucopyranoside of saikogenin C, respectively, possessing a heteroannular diene moiety at C-11, 13(18)], together with prosaikogenins B-1, B-2, and B-3 [elucidated as 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of saikogenin B, 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin B, and 3-*O*-β-D-glucopyranoside of saikogenin B, respectively, possessing a homoannular diene moiety at C-9(11), 12].

Keywords saikosaponin; prosaikogenin; saikogenin; alcoholic alkali metal degradation; acidic hydrolysis; heteroannular diene; homoannular diene; ^{13}C-NMR spectrum

Introduction

Triterpenoidal saponins of the root of *Bupleurum falcatum* L. have been examined chemically by many investigators[1-10] and their pharmacological actions have also been reported.[11,12] However, the metabolism of saikosaponins has not been investigated. In our previous papers,[13,14] we reported the cleavage of the ether ring of saikosaponins a and d by rat gastric juice to produce saikosaponins b_1 and b_2, possessing a heteroannular diene moiety. Saikosaponin g, possessing a homoannular diene, was also obtained from saikosaponin a by the same treatment. Furthermore, the hydrolysis of saponins obtained in gastric juice by mouse intestinal flora produced monofucosides, prosaikogenins F, A, H, G, and D, and their aglycones, saikogenins F, A, H, G, and D. As regards saikosaponin c, we reported[14] the cleavage of the ether ring by rat gastric juice to produce saikosaponin h (possessing a heteroannular diene) and saikosaponin i (possessing a homoannular diene), and their hydrolysis to saikogenins E, C, and B by intestinal flora. However, the prosaikogenins could not be isolated in the incubation mixture of intestinal flora, since the amounts involved were too small. On the other hand, we found a new alcoholic alkali metal degradation to cleave the glycosidic bonds.[15-17] This new reaction is effective to obtain partial hydrolysis products of glycosides. By this method, we have obtained the prosaikogenins from saikosaponins a and d in satisfactory yields.

This paper reports the isolation and structural identification of nine prosaikogenins from saikosaponin c using alcoholic alkali metal solution and acid, as well as comparative corbon-13 nuclear magnetic resonance (^{13}C-NMR) studies of the compounds obtained.

Results and Discussion

Treatment of saikosaponin c (1) with alcoholic alkali metal solution (a mixture of *n*-butanol and sodium metal) for 6 h at 80 °C, followed by the separation on a silica gel column, gave the known saikogenin E (5) and three new prosaikogenins E-1 (2), E-2 (3), E-3 (4) by the removal of terminal glucose or/and rhamnose as shown in Chart 1. Compound 2 was suggested to have a two-glucose moiety at C3 of saikogenin E on the basis of the observation of the two anomeric proton signals at δ 5.08 and 4.69 in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. The ^{13}C-NMR signals due to the sugar moiety of 2 were also identical with those of the two glucoses of 1, as shown in Table I. Thus, the structure of 2 was determined to be 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of sai-

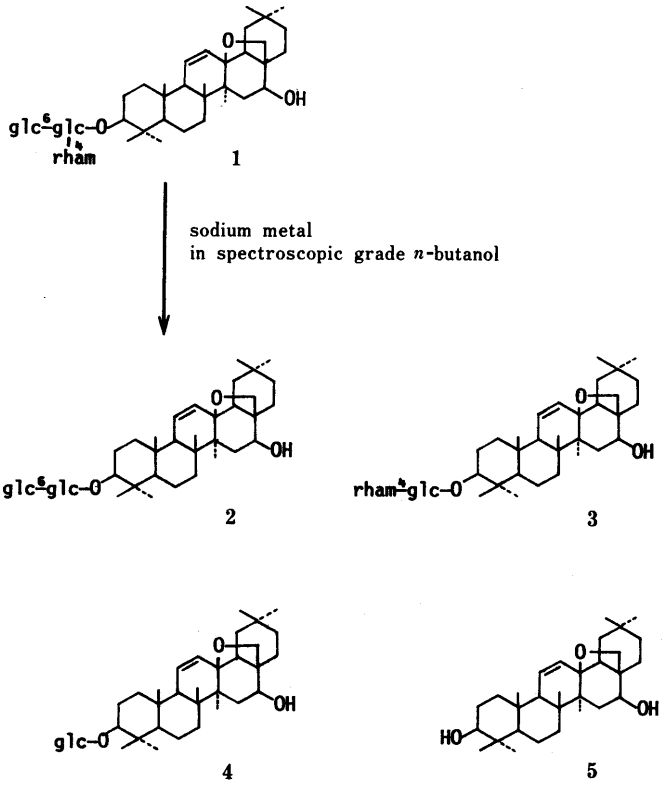


Chart 1

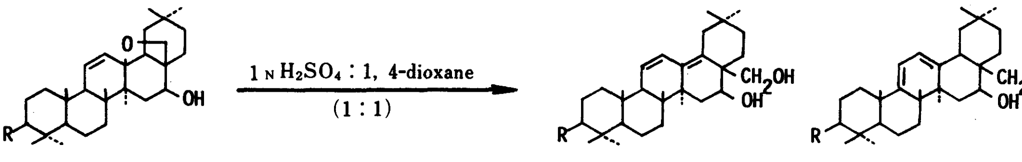
TABLE I. ^{13}C-NMR Spectral Data for Saikosaponin c and Its Derivatives in Pyridine-d_5

Carbon No.	**1**[a]	**2**	**3**	**4**	**5**[a]	**6**[a]	**7**	**8**	**9**	**10**[b]	**11**[a]	**12**	**13**	**14**	**15**[a]
1	38.4	38.5	38.5	38.6	38.8	38.6	38.4	38.4	38.5	38.8	37.4	37.5	37.5	37.6	37.8
2	26.5	26.6	26.4	26.6	27.9	26.5	26.6	26.4	26.5	28.1	27.3	27.3	27.1	27.2	28.8
3	88.9	88.9	88.9	88.8	78.0	89.0	88.9	88.9	88.8	78.5	88.8	88.8	88.7	88.7	77.7
4	39.6	39.7	39.6	39.7	39.5	39.6	39.6	39.4	39.6	39.6	39.7	39.7	39.6	39.7	39.6
5	55.8	55.2	55.3	55.3	55.3	55.3	55.2	55.3	55.3	55.7	51.8	51.8	51.9	51.9	51.8
6	18.4	17.9[c]	17.9[c]	17.9[c]	18.2	18.5	18.5	18.5	18.5	19.0	18.5	18.4	18.5	18.5	18.7
7	31.5	31.6[d]	31.9[d]	31.9[d]	31.9	32.7	32.7	32.7	32.7	33.1	32.4	32.4	32.4	32.5	32.5
8	42.1	42.2	42.2	42.3	42.2	40.4	40.4	40.5	40.5	40.7	43.1[h]	43.1[h]	43.1	43.2	43.1
9	52.7	52.8	52.8	52.9	53.0	54.2	54.2	54.2	54.3	54.6	154.7	154.7	154.6	154.6	154.9
10	36.2	36.3	36.3	36.4	36.7	36.5	36.5	36.6	36.6	37.3	38.7	38.8	38.7	38.8	39.1
11	132.0	132.1	132.0	132.1	132.1	127.0	127.0	126.9	126.9	127.1	116.1	116.1	116.1	116.1	116.1
12	131.0	131.1	131.3	131.3	131.2	125.7	125.6	125.7	125.7	125.8	121.2	121.2	121.2	121.2	121.2
13	83.9	84.0	84.0	84.0	84.0	135.8	135.6	135.5	135.5	136.7	145.3	145.2	145.4	145.4	145.4
14	45.6	45.6	45.7	45.7	45.6	44.3[e]	44.3[e]	44.0[e]	44.3[e]	44.6[e]	43.2[h]	43.2[h]	43.1	43.3	43.3
15	36.2	36.1	36.1	36.2	36.3	34.8[f]	34.8[f]	34.8	34.8	35.1[f]	36.1	36.1	36.1	36.1	36.1
16	64.0	64.0	64.0	64.1	64.0	76.6	76.6	76.5	76.6	76.5	66.8	66.8	66.8	66.8	66.8
17	46.9	47.0	47.0	47.1	47.0	44.4[e]	44.4[e]	44.3[e]	44.4[e]	44.8[e]	40.6	40.6	40.6	40.6	40.6
18	52.0	52.1	52.1	52.2	52.2	133.4	133.3	133.5	133.4	133.7	42.7	42.7	42.7	42.7	42.7
19	38.1	37.9	37.8	37.8	37.8	38.5	38.2	38.2	38.2	38.7	47.1	47.0	47.0	47.1	47.1
20	31.5	31.6[d]	31.6[d]	31.7[d]	31.6	32.7	32.7	32.7	32.7	32.8	31.0	31.0	31.0	31.1	31.0
21	34.8	34.7	34.7	34.7	34.7	35.1[f]	35.1[f]	35.1	35.1	35.4[f]	34.1	34.2	34.2	34.2	34.1
22	25.7	25.8	25.7	25.8	25.7	30.0	30.0	29.9	30.0	30.2	26.1	26.2	26.2	26.1	26.1
23	27.8	27.9	27.9	28.0	28.4	28.0	27.9	28.0	28.0	28.5	28.4	28.3	28.4	28.4	28.9
24	16.3	16.4	16.4	16.4	15.9	16.4	16.5	16.4	16.5	15.8	17.1	17.1	17.1	17.1	16.6
25	18.1	18.2[c]	18.2[c]	18.2[c]	18.2	18.5	18.3	18.5	18.3	18.3	21.1[i]	21.1[i]	21.1	21.0	21.0
26	19.9	20.0	20.0	20.0	20.0	17.0	17.0	17.0	17.0	17.2	21.2[i]	21.2[i]	21.2	21.3	21.3
27	20.9	20.9	20.9	21.0	20.9	22.0	22.0	22.0	22.0	22.1	25.4	25.5	25.4	25.5	25.6
28	72.9	73.0	73.1	73.1	73.0	63.9	63.9	63.9	64.0	64.1	69.3	69.4	69.3	69.3	69.4
29	33.6	33.7	33.7	33.7	33.7	24.8	24.9	24.8	24.8	25.0	33.2	33.2	33.2	33.2	33.2
30	23.8	23.8	23.8	23.8	23.8	32.3	32.3	32.3	32.3	32.3	24.0	24.0	24.0	24.1	24.0
1′	106.4	106.9	106.7	106.9		106.7	107.0	106.7	107.0		106.7	106.9	106.6	106.9	
2′	75.4	75.6	75.9	75.8		75.2	75.6	75.9	75.8		75.1	75.6	75.9	75.8	
3′	76.7	77.0	77.0	78.8		76.8	77.0	77.1	78.8		76.8	76.9	76.9	78.8	
4′	79.7	71.7	78.5	71.9		79.9	71.7	78.5	71.8		79.8	71.5[j]	78.4	71.8	
5′	75.4	78.5	76.9	78.4		75.5	78.4	76.8	78.3		75.5	78.5	76.9	78.2	
6′	68.9	70.5	61.8	63.1		69.1	70.5	61.8	63.1		69.0	70.5	61.7	63.1	
1″	102.7	105.5	102.7			102.9	105.4	102.7			102.9	105.5	102.7		
2″	72.5	75.2	72.6			72.5[g]	75.2	72.6			72.5	75.2	72.6		
3″	72.5	78.5	72.8			72.6[g]	78.4	72.8			72.6	78.5	72.8		
4″	73.7	71.7	73.9			73.8	71.7	74.0			73.8	71.7[j]	74.0		
5″	70.5	78.5	70.3			70.6	78.4	70.3			70.6	78.5	70.3		
6″	18.1	62.8	18.5			18.2	62.7	18.3			18.5	62.7	18.5		
1‴	104.9					105.1					105.1				
2‴	74.9					74.8					74.8				
3‴	78.2					78.4					78.5				
4‴	71.3					71.4					71.4				
5‴	78.2					78.4					78.4				
6‴	62.4					62.5					62.5				

^{13}C-NMR spectra were observed at 25 °C. *a*) Taken from ref. 14. *b*) Taken from ref. 7. *c*—*j*) Assignments may be reversed in each column.

kogenin E. On the other hand, compound **3** was suggested to have a glucose and rhamnose moiety at C3 on the basis of the observation of two anomeric proton signals at δ 5.90 and 4.85 in the ^{1}H-NMR spectrum and two anomeric carbon signals at δ 106.7 and 102.7 in the ^{13}C-NMR spectrum, and was concluded to be 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin E. Compound **4** was determined to be 3-*O*-β-D-glucopyranoside of saikogenin E on the basis of the observation of the anomeric proton signal at δ 4.92 in the ^{1}H-NMR spectrum and the anomeric carbon signal at δ 106.9 in the ^{13}C-NMR spectrum.

Treatment of **1** with 1 N sulfuric acid–dioxane (1 : 1) for 4 h at 60 °C, followed by separation on a LiChroprep RP-18 column, afforded saikosaponins h (**6**) and i (**11**) as reported in our previous paper[14] as shown in Chart 2. They were identical with authentic samples. Similarly, compound **2** gave prosaikogenin C-1 (**7**) and prosaikogenin B-1 (**12**) by the cleavage of the ether ring. Compound **7** was shown to be 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of saikogenin C based on the observation of an ultraviolet (UV) absorption maximum at 250 nm, as well as two olefinic proton signals at δ 6.40 and 5.56 in the ^{1}H-NMR spectrum and four olefinic carbon signals at δ 125.6, 127.0, 133.3, and 135.6 in the ^{13}C-NMR spectrum, indicative of the existence of a heteroannular diene moiety at C11, 13(18). Compound **12** was also characterized as 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of saikogenin B based on the observation of a UV absorption maximum at 280 nm, as well as two olefinic proton signals at δ 5.62



R
1: glc^{6}—glc$_{4}$—O—
rham
2: glc^{6}—glc—O—
3: rham^{4}—glc—O—
4: glc—O—
5: HO—

R
6: glc^{6}—glc$_{4}$—O—
rham
7: glc^{6}—glc—O—
8: rham^{4}—glc—O—
9: glc—O—
10: HO—

R
11: glc^{6}—glc$_{4}$—O—
rham
12: glc^{6}—glc—O—
13: rham^{4}—glc—O—
14: glc—O—
15: HO—

Chart 2

in the ^{1}H-NMR spectrum and four olefinic carbon signals at δ 116.1, 121.2, 145.2 and 154.7 in the ^{13}C-NMR spectrum, indicative of the existence of a homoannular diene moiety at C9 (11), 12. Treatment of **3** and **4** with 1 N sulfuric acid–dioxane (1 : 1) for 3.5 h at 60 °C, followed by separation on a LiChroprep RP-18 column, gave prosaikogenins C-2 (**8**) and C-3 (**9**), and prosaikogenins B-2 (**13**) and B-3 (**14**), respectively. Compounds **8** and **9** were concluded to be 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin C and 3-*O*-β-D-glucopyranoside of saikogenin C, respectively, possessing a heteroannular diene moiety at C11, 13(18), based on analysis of the UV spectrum and ^{1}H- and ^{13}C-NMR spectra. Moreover, compounds **13** and **14** were elucidated as 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside of saikogenin B and 3-*O*-β-D-glucopyranoside of saikogenin B, respectively, possessing a homoannular diene moiety at C-9 (11), 12. Saikogenins C (**10**) and B(**15**) were derived from **5** by similar acidic treatment.

As described above, we isolated 14 kinds of derivatives of **1** by the alcoholic alkali metal treatment and usual acidic treatment. Saikosaponin c is expected to be converted into these compounds in the alimentary tract and so these compounds might be absorbed into the blood after oral administration.

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 digital polarimeter. UV spectra were taken with a Shimadzu UV-240 spectrometer. ^{1}H-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 applying known chemical shift rules and also by comparison with the reported data for known compounds. The chemicals shifts are given in δ values. Thin layer chromatography (TLC) was performed on Kieselgel 60 (Merck) and LiChroprep RP-18 (40—60 μm) (Merck) was used. Saikosaponin c used in these experiments was supplied by Takeda Chemical Industries Co., Ltd.

Alcoholic Alkali Metal Treatment of Saikosaponin c (1) Compound **1** (1.0 g) was dissolved in *n*-butanol (150 ml) and sodium metal (5.0 g) was added. This mixture was allowed to react for 6 h at 80 °C. Then water was added to stop the reaction. The butanol layer was washed with water three times and evaporated to dryness. The residue (790 mg) was purified on silica gel with the lower layer of chloroform–methanol–water (65 : 35 : 10) to afford recovered **1** (272.0 mg), prosaikogenins E-1 (**2**) (92.0 mg), E-2 (**3**) (158.9 mg) and E-3 (**4**) (62.3 mg), and saikogenin E (**5**) (161.6 mg). Compound **2** was obtained as a white powder, mp 221.0—222.5 °C, $[\alpha]_D^{25}$ +25.3° (c=0.2 in methanol). *Anal.* Calcd for $C_{42}H_{68}O_{13}\cdot 3H_2O$: C, 60.41; H, 8.93. Found: C, 60.77; H, 8.58. Compound **3** was obtained as a white powder, mp 200.5—202.5 °C, $[\alpha]_D^{25}$ +24.7° (c=0.2 in methanol). *Anal.* Calcd for $C_{42}H_{68}O_{12}\cdot 3/2H_2O$: C, 63.69; H, 9.04. Found: C, 63.54; H, 8.83. Compound **4** was obtained as a white powder, mp 213.5—214.0 °C, $[\alpha]_D^{25}$ +42.5° (c=0.2 in methanol). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot H_2O$: C, 67.90; H. 9.50. Found: C, 67.54; H, 9.45.

Acidic Treatment of 2, 3 and 4 Compound **2** (92.0 mg) was dissolved in dioxane (10 ml) and 1 N sulfuric acid (10 ml) was added. The mixture was stirred at 60 °C for 4 h. After cooling to room temperature, the mixture was neutralized with 10% sodium hydroxide solution and extracted with *n*-butanol. The *n*-butanol layer was washed with water and evaporated to dryness. The residue (56.5 mg) was separated into prosaikogenin C-1 (**7**) (30.0 mg) and prosaikogenin B-1 (**12**) (14.0 mg) on a LiChroprep RP-18 column. The solvent system employed was metanol–water (3 : 1). Compound **7** was obtained as a white powder, mp 202.5—205.0 °C, $[\alpha]_D^{25}$ −52.5° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 242 (28580), 250 (31620), 258 (19980). *Anal.* Calcd for $C_{42}H_{68}O_{13}\cdot 3/2H_2O$: C, 62.43; H. 8.86. Found: C, 62.45; H, 8.85. Compound **12** was obtained as a white powder, mp 198—201.5 °C, $[\alpha]_D^{25}$ +67.9° (c=0.2 in methanol). UV λ_{max}^{MeOH}nm (ε): 280 (7530). *Anal.* Calcd for $C_{42}H_{68}O_{13}\cdot 4H_2O$: C, 59.14; H. 8.98. Found: C, 59.89; H, 8.62.

Compound **3** (158.9 mg) was dissolved in dioxane (10 ml) and 1 N sulfuric acid (10 ml) was added. The mixture was stirred at 60 °C for 3.5 h. After cooling to room temperature, the mixture was neutralized with 10% sodium hydroxide and extracted with *n*-butanol. The butanol layer was evaporated in dryness and the residue (87.6 mg) was separated into prosaikogenin C-2 (**8**) (32.8 mg) and prosaikogenin B-2 (**13**) (13.2 mg) on a LiChroprep RP-18 column. The solvent system employed was methanol–water (5 : 1). Compound **8** was obtained as a white powder, mp 218.5—220.0 °C, $[\alpha]_D^{25}$ −46.7° (c=0.2 in methanol). UVλ_{max}^{MeOH} nm (ε): 242 (26220), 250 (29190), 259 (18700). *Anal.* Calcd for $C_{42}H_{68}O_{12}\cdot H_2O$: C, 64.43; H, 9.01. Found: C, 64.34; H, 9.16. Compound **13** was obtained as a white powder, mp 200.5—203.0 °C, $[\alpha]_D^{25}$ +68.1° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 281 (6970). *Anal.* Calcd for $C_{42}H_{68}O_{12}\cdot 2H_2O$: C, 62.98; H, 9.06. Found: C, 62.88; H, 9.27.

Compound **4** (62.3 mg) was dissolved in dioxane (10 mg) and 1 N sulfuric acid (10 ml) was added. The mixture was stirred at 60 °C for 3.5 h. After cooling to room temperature, the mixture was neutralized with 10% sodium hydroxide and extracted with *n*-butanol. The organic layer was washed with water and evaporated to dryness. The residue (39.5 mg) was separated into prosaikogenin C-3 (**9**) (18.2 mg) and prosaikogenin B-3 (**14**) (6.4 mg) on a LiChroprep RP-18 column. The solvent system employed was methanol–water (7 : 1). Compound **9** was obtained as a white powder, mp 199.0—200.5 °C, $[\alpha]_D^{25}$ −35.7° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 242 (24020), 250 (26980), 259 (17190). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot H_2O$: C, 67.90; H, 9.50. Found: C, 67.24; H, 9.39. Compound **14** was obtained as a white powder, mp 186.5—190.5 °C, $[\alpha]_D^{25}$ +106.8° (c=0.2 in methanol). UV λ_{max}^{MeOH} nm (ε): 280 (6780). *Anal.* Calcd for $C_{36}H_{58}O_8\cdot 2H_2O$: C, 66.03; H, 9.54. Found: C, 66.07; H, 9.81.

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