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SAPONINS FROM THE ROOT OF *BUPLEURUM FALCATUM*

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Key Word Index—*Bupleurum falcatum*; Umbelliferae; Bupleuri Radix; malonylsaikosaponin; acetylsaikosaponin; hydroxysaikosaponin; saikosaponin.

Abstract—Three new saponins and nine known saponins were isolated from the dried roots of *Bupleurum falcatum*. On the basis of chemical and spectral analyses, the structures of new compounds, named 4″-*O*-acetylsaikosaponin d and hydroxysaikosaponins a and c, were established. In aqueous acidic conditions, saikosaponins a and d were converted into not only known compounds, saikosaponins b_1 and b_2, but also hydroxysaikosaponins a and d, respectively. Furthermore, quantitative analysis of the decoction of Bupleuri Radix itself by HPLC exhibited that it contained saikosaponins a, c and d, and hydroxysaikosaponins a, c and d.

INTRODUCTION

Bupleuri Radix is the root of *Bupleurum falcatum* L. and its varieties (Umbelliferae). It is one of the best known crude drugs of KAMPO medicine in Japan and has been the subject of many investigations. In particular, saikosaponins, which are the principal ingredients of Bupleuri Radix, have been investigated as major biologically active substances in this crude drug [1–7].

In the preceding communication [8], we reported the presence of two new saponins, named malonylsaikosaponins a (**1**) and d (**2**) in fresh Bupleuri Radix, which contains a larger amount of **1** and **2** than their correlated saikosaponins a and d. Further, in continuing the chemical investigation on constituents of this plant, we isolated three new saponins (**9**, **11** and **12**), together with seven known saponins (**3**–**8** and **10**). This paper deals with the isolation and structural elucidation of these new compounds.

RESULTS AND DISCUSSION

The saponin fraction of the methanolic extract of this plant was separated by using a combination of reversed-phase and normal-phase silica gel column chromatography to afford 12 compounds (**1**–**12**). Among them, **9**, **11** and **12** were novel compounds, identified as 4″-*O*-acetylsaikosaponin d, hydroxysaikosaponin a and hydroxysaikosaponin c, respectively.

Compounds **1**–**8** and **10** from the root of *B. falcatum* and related genus were identified as 6″-*O*-malonyl-saikosaponin a (**1**), 6″-*O*-malonylsaikosaponin d (**2**), 2″-*O*-acetylsaikosaponin a (**3**), 3″-*O*-acetylsaikosaponin a (**4**), 4″-*O*-acetylsaikosaponin a (**5**), 6″-*O*-acetylsaikosaponin a (**6**), 2″-*O*-acetylsaikosaponin d (**7**), 3″-*O*-acetylsaikosaponin d (**8**) and 6″-*O*-acetylsaikosaponin d (**10**), respectively, by their chemical data and comparing physicochemical and spectral data with those described in the literature [9–13].

Compound **9**, $C_{44}H_{70}O_{14}$, FAB mass spectrum *m/z* 823 $[M + H]^+$, was obtained as a white amorphous powder. Its ^1H NMR spectrum showed eight methyl signals, two anomeric proton signals and two olefinic proton signals. Infrared bands at 1740 and 1248 cm^{-1}, and the ^{13}C NMR spectrum at δ 20.9 (*s*) and 172.1 (*q*), showed an acetyl group. On alkaline hydrolysis with KOH, it afforded saikosaponin d, which was identified by HPLC and TLC.

These spectral and chemical data suggested that **9** was composed of saikosaponin d esterified with acetic acid. The recent publications [10–13] on the ^{13}C NMR assignment of saikosaponins enabled us to distinguish the signals of the glucosyl, fucosyl and aglycone moieties in **9**. By comparing the ^{13}C chemical shifts of **9** with those of saikosaponin d, the C-4″ signal of glucose shifted downfield by 1.0 ppm and the neighbouring C-3″ and C-5″ signals shifted upfield by 2.1 and 2.3 ppm, respectively. These acylation shifts showed the acetyl group to be present at C-4″ of the glucosyl moiety. Comparison of the ^{13}C chemical shifts of the glucosyl moiety of **9** and **5** showed the same acylation shifts (Table 1).

Further, the position of the acetyl group in **9** was confirmed by ^1H–^1H shift correlation spectroscopy (COSY) and the homonuclear Hartman–Hahn spectrum (HOHAHA). Thus, the structure of **9** was elucidated as 4″-*O*-acetylsaikosaponin d.

Compound **11**, $C_{42}H_{70}O_{14}$, FAB mass spectrum *m/z* 797 $[M - H]^-$, was obtained as a white amorphous powder. Its ^{13}C NMR spectrum was similar to that of

Table 1. ^{13}C NMR data for acetylsaikosaponins (CD_3OD)

	3	**7**	**4**	**8**	**5**	**9**	**6**	**10**
C-1	39.3	39.3	39.2	39.2	39.2	39.2	39.3	39.3
C-2	26.1	26.2	26.1	26.1	26.1	26.1	26.2	26.1
C-3	82.9	83.0	83.1	83.3	83.1	83.3	83.1	83.3
C-4	44.1	44.3	44.0	44.3	44.0	44.3	44.1	44.3
C-5	47.9	48.0	48.0	48.1	48.1	48.1	48.1	48.2
C-6	18.2	18.2	18.2	18.2	18.2	18.2	18.2	18.2
C-7	32.1	32.1	32.1	32.1	32.1	32.1	32.2	32.2
C-8	43.0	42.6	43.0	42.6	43.0	42.6	43.0	42.6
C-9	54.0	53.9	54.0	53.9	54.0	53.9	54.0	53.9
C-10	37.0	37.1	37.1	37.1	37.1	37.1	37.1	37.1
C-11	134.2	134.0	134.2	133.9	134.2	134.0	134.2	133.9
C-12	130.6	131.5	130.6	131.5	130.6	131.5	130.6	131.5
C-13	85.7	86.7	85.7	86.7	85.7	86.7	85.7	86.7
C-14	46.5	44.1	46.5	44.1	46.5	44.1	46.5	44.1
C-15	36.0	35.5	36.0	35.5	36.0	35.5	36.0	35.5
C-16	65.4	78.0	65.4	78.0	65.4	78.0	65.4	78.0
C-17	47.5	46.1	47.5	46.1	47.5	46.1	47.6	46.1
C-18	53.1	52.1	53.1	52.1	53.1	52.1	53.1	52.1
C-19	38.5	39.2	38.5	39.2	38.5	39.2	38.6	39.2
C-20	32.3	32.5	32.3	32.5	32.3	32.5	32.3	32.5
C-21	35.2	37.4	35.2	37.4	35.2	37.4	35.3	37.4
C-22	26.2	31.6	26.1	31.6	26.1	31.6	26.1	31.6
C-23	64.5	64.6	64.8	65.0	64.8	64.9	64.8	65.0
C-24	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8
C-25	18.9	19.0	19.0	19.0	19.0	19.0	19.0	19.0
C-26	20.2	19.7	20.2	19.8	20.2	19.8	20.3	19.8
C-27	21.3	18.5	21.3	18.6	21.3	18.6	21.2	18.6
C-28	73.4	78.5	73.4	78.5	73.4	78.5	73.4	78.5
C-29	34.0	34.0	34.0	34.0	34.0	34.0	34.0	34.0
C-30	24.1	24.6	24.1	24.7	24.1	24.7	24.1	24.6
(Fucosyl moiety)								
C-1′	106.2	106.2	105.6	105.6	105.6	105.6	105.6	105.6
C-2′	71.6	71.6	71.8	71.9	71.8	71.9	71.8	71.8
C-3′	84.7	84.8	85.1	85.1	85.1	85.2	85.2	85.3
C-4′	72.4	72.5	72.4	72.4	72.4	72.4	72.3	72.3
C-5′	71.2	71.2	71.3	71.4	71.4	71.4	71.3	71.3
C-6′	16.9	16.9	16.9	16.9	16.9	17.0	17.0	17.0
(Glucosyl moiety)								
C-1″	103.7	103.7	105.4	105.4	105.6	105.6	105.4	105.3
C-2″	76.0	76.0	73.6	73.6	75.4	75.4	75.2	75.2
C-3″	75.5	75.5	78.6	78.7	75.8	75.8	77.6	77.6
C-4″	71.3	71.4	69.4	69.4	72.4	72.4	71.7	71.7
C-5″	77.9	78.0	77.7	77.7	75.4	75.4	75.4	75.4
C-6″	62.3	62.4	62.1	62.1	62.1	62.1	64.8	64.8
(Acetyl)								
C=O	172.6	172.6	172.7	172.7	172.1	172.1	172.7	172.7
CH_3	21.2	21.2	21.2	21.1	20.9	20.9	20.8	20.8

saikosaponin b_3 (**14**). However, the signal of a methoxyl group was detected in saikosaponin b_3, but not in **11**. By comparing the ^{13}C chemical shifts of **11** with those of saikosaponin b_3, the C-11 signal of the aglycone shifted upfield by 9.3 ppm and neighbouring C-9 and C-12 signals shifted downfield by 2.9 and 4.9 ppm (Table 2), respectively. These spectral data suggested that **11** possesses not a methoxyl group, but a hydroxyl group at C-11 of the aglycone moiety of saikosaponin b_3. On acid treatment with acetic acid–H_2O, compound **11** afforded saikosaponin a. Conversely, saikosaponin a afforded **11** under similar conditions. Thus, the structure of **11** was elucidated as de-11-*O*-methylsaikosaponin b_3, named hydroxysaikosaponin a.

Table 2. ^{13}C NMR data for hydroxysaikosaponins (CD_3OD)

	14	**11**	**12**	**15**	**13**
C-1	40.7	41.5	42.0	40.7	41.6
C-2	26.4	26.4	27.3	26.4	26.4
C-3	83.3	83.4	90.5	83.4	83.5
C-4	44.5	44.8	40.5	44.3	44.6
C-5	48.4	48.5	57.2	48.5	48.6
C-6	18.9	19.0	19.5	18.9	19.0
C-7	33.8	34.1	34.6	34.1	34.3
C-8	41.5	41.6	41.6	41.1	41.2
C-9	52.6	55.5	55.6	52.3	55.3
C-10	38.8	38.8	39.0	39.0	39.0
C-11	77.4	68.1	68.1	77.6	68.3
C-12	123.1	128.0	128.0	122.8	127.6
C-13	149.0	146.5	146.6	150.6	148.0
C-14	44.1	44.2	44.7	42.7	42.7
C-15	36.7	36.7	36.7	37.5	37.5
C-16	67.3	67.5	67.5	74.6	74.7
C-17	44.7	44.8	44.9	44.1	44.2
C-18	44.6	44.4	44.4	43.0	42.8
C-19	47.6	47.2	47.3	48.7	48.3
C-20	31.7	31.7	31.7	31.7	31.6
C-21	34.7	34.8	34.8	35.0	35.0
C-22	25.9	26.0	25.9	30.7	30.6
C-23	65.2	65.3	28.6	65.2	65.4
C-24	13.4	13.4	17.0	13.4	13.4
C-25	18.1	17.9	17.4	18.1	17.9
C-26	18.8	18.7	18.7	18.8	18.8
C-27	26.6	27.1	27.1	26.6	27.1
C-28	68.8	68.9	68.8	71.1	71.1
C-29	33.7	33.7	33.7	33.5	33.5
C-30	24.3	24.3	24.3	24.8	24.9
OCH_3	54.4	—	—	54.1	—
	(Fucosyl)	(Fucosyl)	(Glucosyl)	(Fucosyl)	(Fucosyl)
C-1′	105.5	105.6	106.6	105.6	105.5
C-2′	71.8	71.9	75.6	71.9	71.9
C-3′	85.1	85.2	77.0	85.2	85.3
C-4′	72.3	72.4	80.6	72.4	72.4
C-5′	71.8	71.3	75.7	71.2	71.3
C-6′	16.9	16.9	69.5	17.0	17.0
(Glucosyl moiety)					
C-1″	105.6	105.7	104.9	105.7	105.7
C-2″	75.3	75.4	75.0	75.4	75.4
C-3″	77.7	77.7	78.1	77.9	78.0
C-4″	71.2	71.4	71.6	71.4	71.4
C-5″	77.9	77.9	78.0	77.7	77.8
C-6″	62.4	62.5	62.7	62.4	62.4
(Rhamnosyl moiety)					
C-1‴			103.0		
C-2‴			72.2		
C-3‴			72.4		
C-4‴			73.8		
C-5‴			70.8		
C-6‴			17.9		

Compound **12**, $C_{48}H_{80}O_{18}$, FAB mass spectrum *m/z* 943 $[M - H]^-$, was obtained as a white amorphous powder. Its ^{13}C NMR spectrum was similar to that of saikosaponin c. Chemical shifts of C-9, C-11, C-12 and C-13 of the aglycone moiety of **12** were similar to those of **11** (Table 2). These spectral data suggested that the structure of **12** has a hydroxyl group at C-11 of the aglycone moiety of saikosaponin f. Compound **12** was obtained by

acid treatment of saikosaponin c with acetic acid–H_2O. Thus, the structure of **12** was elucidated as 11-hydroxysaikosaponin f, named hydroxysaikosaponin c.

In the nuclear Overhauser and exchange spectroscopy (NOESY) of **11** and **12**, the H-11 signal showed correlation with the C-25 and C-26 methyl groups with a 1,3 diaxial relation. Thus, the hydroxyl group at C-11 of **11** and **12** was in the α-configuration. This is the first report of the isolation of compounds **9**, **11** and **12**.

Compound **13**, $C_{42}H_{70}O_{14}$, FAB mass spectrum *m/z* 797 $[M - H]^-$, was obtained as a white amorphous powder. Its ^{13}C NMR spectrum was similar to that of saikosaponin b_4 (**15**), but the signal of a methoxyl group was detected in saikosaponin b_4, but not in **13**. By comparing the ^{13}C chemical shifts of **13** with those of saikosaponin b_4, the C-11 signal of the aglycone shifted upfield by 9.3 ppm and neighbouring C-9 and C-12 signals shifted downfield by 3.0 and 4.9 ppm (Table 2), respectively. These spectral data suggested that **13** was similar to saikosaponin b_4, but with a hydroxyl group connected at C-11 of the aglycone moiety. Thus, the structure of **13** was elucidated as de-11-*O*-methylsaikosaponin b_4, named hydroxysaikosaponin d. On the basis of this result, saikosaponin d was also converted into compound **13** by carefully controlled acid conditions.

Moreover, we elucidated that saikosaponin a and **11** were interconvertible under acidic conditions in water. These results suggested that saikosaponin a and **11** reach an equilibrium state and gradually convert into saikosaponin b_1 under the acidic conditions in water. Therefore, other saikosaponins, e.g. saikosaponins c and d, acetylsaikosaponins a and d, malonylsaikosaponins a and d, also might undergo a similar reaction under the acidic conditions.

Generally, the decoction of crude drugs is found to be weakly acidic. Therefore, it is possible that the above reactions can occur during preparation of the decoction. Therefore, a decoction of Bupleuri Radix was prepared and subjected to detailed analysis by HPLC. The decoction contained saikosaponins a, c and d, hydroxysaikosaponins a, c and d, and saikosaponin b_2. This result suggested that the KAMPO decoction including Bupleuri Radix contains not only saikosaponins, but also hydroxysaikosaponins.

In the preceding communication [8], we reported that Bupleuri Radix contains a larger amount of malonylsaikosaponins a and d than their corresponding saikosaponins. However, we were not able to detect these malonylsaikosaponins a and d in the decoction of Bupleuri Radix. This result suggested that malonylsaikosaponins a and d were hydrolysed to saikosaponins or converted into other compounds by heat and/or acid during the decocting process.

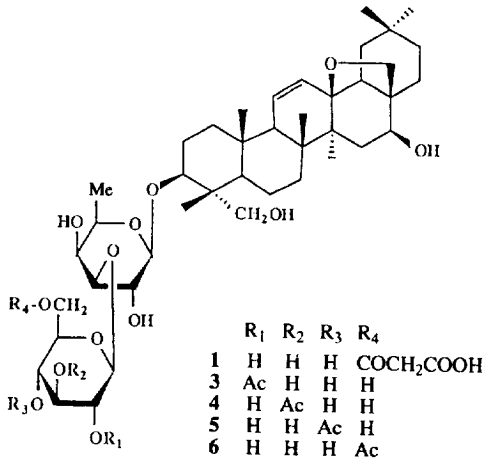
EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR: 400 and 500 MHz, respectively, with TMS as int. standard. TLC: precoated Silica gel 60 F_{254} plate, spots visualized by dil. H_2SO_4. Prep. HPLC: YMC packed column R-354 (ODS) and CIG Si-10 column (1.5 cm i.d. × 20 cm). HPLC analyses were performed on a TRIROTAR-III HPLC system. HPLC conditions: column, YMC packed column A-212 (6 mm i.d. × 150 mm); solvent, 40% AcCN; detection, 205 nm. Plant material: *B. falcatum* L. was cultivated in Nagano Prefecture (Japan), and roots were collected in October 1988.

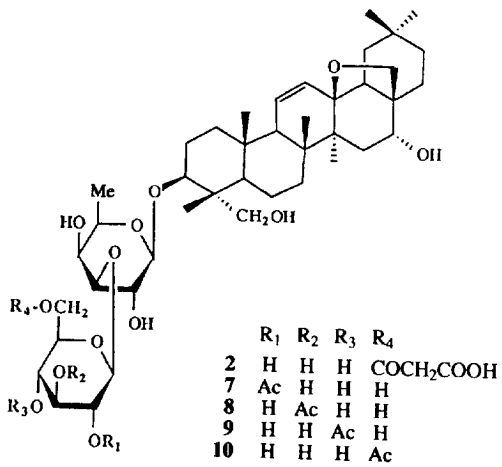
Extraction and isolation. The dried root of *B. falcatum* (9 kg) was extracted with MeOH (36 l) at room temp. The MeOH extract (1.4 kg) was partitioned between BuOH and H_2O. The BuOH extract (645 g) was dissolved in MeOH and then added dropwise to Et_2O. The ppt. (99 g) of the saponin mixt. was sepd from the soln by filtration. Sepn of the ppt. by silica gel CC (EtOAc–EtOH–H_2O, 10:8:1) furnished 3 saponin frs; fr. A (7.0 g), B (47.3 g) and C (6.5 g). Fr. A was subjected to reversed-phase CC with H_2O–MeOH (3:8) to give 4 further frs; fr. A-1, A-2, A-3 and A-4. Rechromatography of fr. A-1–A-4 on silica gel with EtOAc–EtOH–H_2O (25:2:1) and EtOAc–Me_2CO–H_2O (25:2:1) afforded **3** (75 mg), **4** (131 mg), **5** (13 mg), **6** (76 mg), **7** (29 mg), **8** (148 mg), **9** (15 mg) and **10** (141 mg). Further purification of fr. C by combination of reversed- and normal-phase silica gel CC gave **1** (*ca* 400 mg) and **2** (*ca* 500 mg), and fr. B gave **11** (40 mg) and **12** (18 mg), together with saikosaponins a, c and d.

6″-O-*Malonylsaikosaponin a* (**1**). Amorphous powder, $[\alpha]_D^{26}$ + 42° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3416, 2944, 1730, 1592, 1386, 974, 906. ^1H NMR (CD_3OD): δ 0.70, 0.92, 0.95, 0.98, 1.04, 1.09 (each 3H, *s*, -CH_3), 1.28 (3H, *d*, *J* = 6.4 Hz, Fuc H-6), 1.90 (1H, *br s*, H-9), 3.04 (1H, *d*, *J* = 7.3 Hz, H-28), 3.84 (1H, *d*, *J* = 2.5 Hz, Fuc H-4), 3.90 (1H, *d*, *J* = 7.3 Hz, H-28), 4.17 (1H, *dd*, *J* = 9.8, 6.1 Hz, H-16), 4.25 (1H, *dd*, *J* = 12.0, 6.1 Hz, Glc H-6), 4.40 (1H, *d*, *J* = 7.2 Hz, Fuc H-1), 4.47 (1H, *dd*, *J* = 12.0, 2.0 Hz, Glc H-6), 4.56 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.38 (1H, *dd*, *J* = 10.5, 3.0 Hz, H-12), 5.95 (1H, *d*, *J* = 10.5 Hz, H-11). ^{13}C NMR (CD_3OD): δ 12.8 (*q*, C-24), 17.1 (*q*, Fuc C-6), 18.3 (*t*, C-6), 19.0 (*q*, C-25), 20.3 (*q*, C-26), 21.3 (*q*, C-27), 24.1 (*q*, C-30), 26.1 (*t*, C-22), 26.2 (*t*, C-2), 32.2 (*t*, C-7), 32.3 (*s*, C-20), 34.0 (*q*, C-29), 35.3 (*t*, C-21), 36.1 (*t*, C-15), 37.2 (*s*, C-10), 38.6 (*t*, C-19), 39.3 (*t*, C-1), 43.0 (*s*, C-8), 44.1 (*s*, C-4), 46.6 (*s*, C-14), 47.6 (*s*, C-17), 48.2 (*d*, C-5), 53.1 (*d*, C-18), 54.0 (*d*, C-9), 65.0 (*t*, C-23), 65.1 (*t*, Glc C-6), 65.4 (*d*, C-16), 71.4 (*d*, Fuc C-5), 71.6 (*d*, Glc C-4), 71.9 (*d*, Fuc C-2), 72.4 (*d*, Fuc C-4), 73.4 (*t*, C-28), 75.3 (*d*, Glc C-5), 75.4 (*d*, Glc C-2), 77.6 (*d*, Glc C-3), 83.2 (*d*, C-3), 85.0 (*d*, Fuc C-3). 85.7 (*s*, C-13), 105.4 (*d*, Fuc C-1), 105.7 (*d*, Glc C-1), 130.7 (*d*, C-12), 134.2 (*d*, C-11), 171.5 (*s*, Malonyl C=O). FABMS *m/z*: 905 $[M + K]^+$, 889 $[M + Na]^+$, 861, 455. HRFABMS *m/z*: 905.4316 $[M + K]^+$ (calc. for $C_{45}H_{70}O_{16}K$ 905.4301).

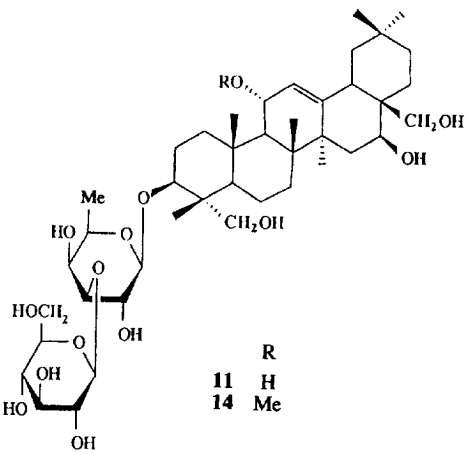
6″-O-*Malonylsaikosaponin d* (**2**). Amorphous powder, $[\alpha]_D^{25}$ + 29.6° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3416, 2948, 1730, 1596, 1384, 910, 890. ^1H NMR (CD_3OD): δ 0.70, 0.92, 0.94, 0.95, 1.04 (each 3H, *s*, -CH_3), 1.27 (3H, *d*, *J* = 6.4 Hz, Fuc H-6), 1.30 (3H, *s*, -CH_3), 1.97 (1H, *br s*, H-9), 3.11 (1H, *d*, *J* = 7.2 Hz, H-28), 3.45 (1H, *d*, *J* = 7.2 Hz, H-28), 3.84 (3H, *d*, *J* = 2.3 Hz, Fuc H-4), 3.90



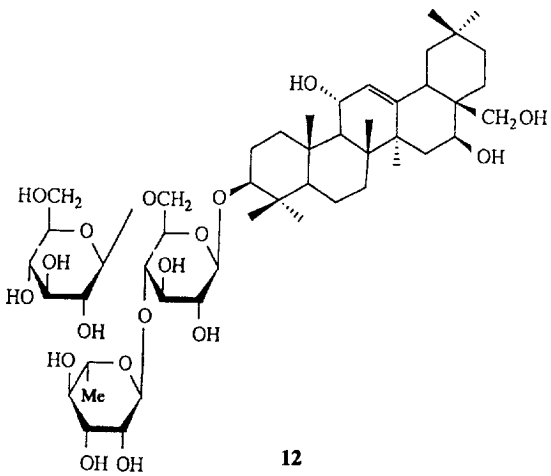
	R_1	R_2	R_3	R_4
1	H	H	H	$COCH_2COOH$
3	Ac	H	H	H
4	H	Ac	H	H
5	H	H	Ac	H
6	H	H	H	Ac



	R_1	R_2	R_3	R_4
2	H	H	H	$COCH_2COOH$
7	Ac	H	H	H
8	H	Ac	H	H
9	H	H	Ac	H
10	H	H	H	Ac



	R
11	H
14	Me



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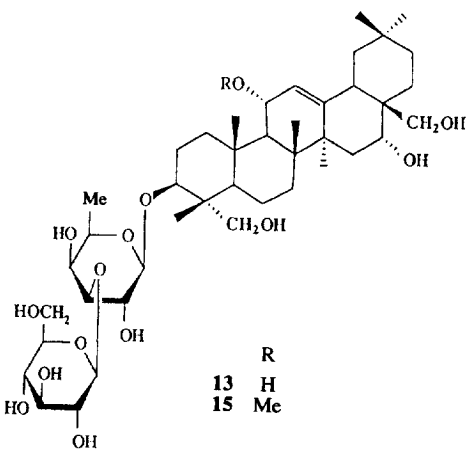

	R
13	H
15	Me

(1H, *d*, J = 4.8 Hz, H-16), 4.22 (1H, *dd*, J = 11.8, 6.4 Hz, Glc H-6), 4.40 (1H, *d*, J = 7.3 Hz, Fuc H-1), 4.46 (1H, *dd*, J = 11.8, 1.8 Hz, Glc H-6), 4.56 (1H, *d*, J = 7.7 Hz, Glc H-1), 5.36 (1H, *dd*, J = 10.5, 3.0 Hz, H-12), 5.94 (1H, *d*, J = 10.5 Hz, H-11). ^{13}C NMR (CD_3OD): δ12.8 (*q*, C-24), 17.0 (*q*, Fuc C-6), 18.2 (*t*, C-6), 18.5 (*q*, C-27), 19.0 (*q*, C-25), 19.8 (*q*, C-26), 24.6 (*q*, C-30), 26.1 (*t*, C-2), 31.6 (*t*, C-22), 32.1 (*t*, C-7), 32.5 (*s*, C-20), 33.9 (*q*, C-29), 35.5

(*t*, C-15), 37.1 (*t*, C-21), 37.4 (*s*, C-10), 39.2 (*t*, C-1), 39.2 (*t*, C-19), 42.6 (*s*, C-8), 44.1 (*s*, C-14), 44.3 (*s*, C-4), 46.1 (*s*, C-17), 48.2 (*d*, C-5), 52.1 (*d*, C-18), 53.9 (*d*, C-9), 65.0 (*t*, C-23), 65.0 (*t*, Glc C-6), 71.4 (*d*, Fuc C-5), 71.5 (*d*, Fuc C-2), 71.9 (*d*, Glc C-4), 72.4 (*d*, Fuc C-4), 75.3 (*d*, Glc C-5), 75.4 (*d*, Glc C-2), 77.5 (*d*, C-16), 78.0 (*d*, Glc C-3), 78.5 (*t*, C-28), 83.4 (*d*, C-3), 85.3 (*d*, Fuc C-3), 86.7 (*s*, C-13), 105.3 (*d*, Fuc C-1), 105.6 (*d*, Glc C-1), 131.4 (*d*, C-12), 134.0 (*d*, C-11), 171.1 (*s*, Malonyl C=O). FABMS *m/z*: 905 $[M + K]^+$, 889 $[M + Na]^+$, 862, 455. HRFABMS *m/z*: 905.4268 $[M + K]^+$ (calc. for $C_{45}H_{70}O_{16}K$ 905.4301).

4″-O-Acetylsaikosaponin d (**9**). Amorphous powder, $[\alpha]_D^{27}$ + 42.6° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3416, 2948, 1740, 1248, 908, 890. ^1H NMR (CD_3OD): δ0.71, 0.93, 0.95, 0.96, 1.05 (each 3H, *s*, -CH_3), 1.27 (3H, *d*, *J* = 6.4 Hz, Fuc H-6), 1.30 (3H, *s*, -CH_3), 2.09 (3H, *s*, -OAc), 3.11 (1H, *d*, *J* = 7.4 Hz, H-28), 3.45 (1H, *d*, *J* = 7.4 Hz, H-28), 3.91 (1H, *d*, *J* = 4.8 Hz, H-16), 4.39 (1H, *d*, *J* = 7.2 Hz, Fuc H-1), 4.59 (1H, *d*, *J* = 7.6 Hz, Glc H-1), 5.37 (1H, *dd*, *J* = 10.4, 2.4 Hz, H-12), 5.94 (1H, *d*, *J* = 10.4 Hz, H-11). ^{13}C NMR: Table 1. FABMS *m/z*: 845 $[M + Na]^+$, 822 $[M]^+$, 455. HRFABMS *m/z*: 823.48690 $[M + H]^+$ (calc. for $C_{44}H_{71}O_{14}$ 823.48438).

Alkaline hydrolysis of **9**. Compound **9** (1 mg) was dissolved in 0.2% NaOH–MeOH (0.5 ml) and kept at room temp. for 1 hr. The reaction mixt. yielded saikosaponin d, which was identified with authentic sample on TLC, HPLC and FABMS.

Hydroxysaikosaponin a (**11**). Amorphous powder, $[\alpha]_D^{28}$ + 4.4° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3404, 2944, 1658, 1462, 1454, 1384, 1366, 1162, 1076, 902. ^1H NMR (CD_3OD): δ0.74, 0.91, 0.94, 1.08, 1.13 (each 3H, *s*, -CH_3), 1.26 (3H, *d*, *J* = 6.4 Hz, Fuc H-6), 1.35 (3H, *s*, -CH_3), 1.64 (1H, *d*, *J* = 8.8 Hz, H-9), 2.22 (1H, *dd*, *J* = 13.6, 4.4 Hz, H-18), 3.27 (1H, *d*, *J* = 10.6 Hz, H-28), 3.81 (1H, *d*, *J* = 10.6 Hz, H-28), 3.84 (1H, *dd*, *J* = 11.8, 1.8 Hz, Glc H-6), 3.85 (1H, *d*, *J* = 2.0 Hz, Fuc H-4), 4.17 (1H, *dd*, *J* = 8.8, 3.6 Hz, H-11), 4.27 (1H, *dd*, *J* = 11.6, 4.8 Hz, H-16), 4.38 (1H, *d*, *J* = 7.6 Hz, Fuc H-1), 4.54 (1H, *d*, *J* = 7.6 Hz, Glc H-1), 5.21 (1H, *d*, *J* = 3.6 Hz, H-12). ^{13}C NMR: Table 2. FABMS *m/z*: 797 $[M - H]^-$. HRFABMS *m/z*: 797.46828 $[M - H]^-$ (calc. for $C_{42}H_{69}O_{14}$ 797.46873).

Hydroxysaikosaponin c (**12**). Amorphous powder, $[\alpha]_D^{26}$ − 30.8° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3396, 2944, 1644, 1454, 1388, 1368, 1166, 1036, 984, 920. ^1H NMR (CD_3OD): δ0.88, 0.91, 0.94, 1.08, 1.08, 1.10 (each 3H, *s*, -CH_3), 1.27 (3H, *d*, *J* = 6.4 Hz, Rha H-6), 1.33 (3H, *s*, -CH_3), 1.60 (1H, *d*, *J* = 8.8 Hz, H-9), 2.23 (1H, *dd*, *J* = 13.6, 4.4 Hz, H-18), 3.23 (1H, *d*, *J* = 9.8 Hz, H-28), 3.63 (1H, *dd*, *J* = 9.4, 3.4 Hz, Rha H-2), 3.68 (1H, *dd*, *J* = 12.0, 5.6 Hz, Glc′ H-6), 3.81 (1H, *d*, *J* = 9.8 Hz, H-28), 3.93 (1H, *dd*, *J* = 9.6, 6.0 Hz, Rha H-5), 4.07 (1H, *brd*, *J* = 11.2 Hz, Glc H-6), 4.17 (1H, *dd*, *J* = 8.8, 3.6 Hz, H-11), 4.27 (1H, *dd*, *J* = 11.4, 5.0 Hz, H-16), 4.34 (1H, *d*, *J* = 8.0 Hz, Glc′ H-1), 4.36 (1H, *d*, *J* = 8.0 Hz, Glc H-1), 4.89 (1H, *d*, *J* = 1.6 Hz, Rha H-1), 5.22 (1H, *d*, *J* = 3.6 Hz, H-12). ^{13}C NMR: Table 2. FABMS *m/z*: 943 $[M - H]^-$. HRFABMS *m/z*: 943.52534 $[M - H]^-$ (calc. for $C_{48}H_{79}O_{18}$ 943.52664).

Acid treatment of **11**. Compound **11** (5 mg) was dissolved in 10% AcOH (50 ml) and kept at room temp. for 1 hr. The reaction mixt. yielded saikosaponin a, which was identified with authentic sample on TLC, HPLC and FABMS.

Compounds **11**–**13** *derived from saikosaponins a, c or d*. Each compound (50 mg) was dissolved in 10% AcOH (500 ml) and kept at room temp. for 1 hr. The reaction mixt. yielded **11**, **12** or **13**, respectively. Compounds **11** and **12** were in good agreement with authentic samples on TLC, HPLC, FABMS, ^1H and ^{13}C NMR.

Hydroxysaikosaponin d (**13**). Amorphous powder, $[\alpha]_D^{23}$ − 0.5° (MeOH; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3404, 2940, 1644, 1442, 1384, 1160, 1068, 998, 904. ^1H NMR (CD_3OD): δ0.74, 0.88, 0.93, 1.00, 1.13 (each 3H, *s*, -CH_3), 1.26 (3H, *d*, *J* = 6.0 Hz, Fuc H-6), 1.52 (3H, *s*, -CH_3), 1.74 (1H, *d*, *J* = 8.4 Hz, H-9), 2.05 (1H, *dd*, *J* = 13.6, 4.0 Hz, H-18), 3.08 (1H, *d*, *J* = 11.2 Hz, H-28), 3.26 (1H, *d*, *J* = 11.2 Hz, H-28), 3.84 (1H, *dd*, *J* = 12.4, 2.0 Hz, Glc H-6), 3.86 (1H, *d*, *J* = 2.4 Hz, Fuc H-4), 3.99 (1H, *brs*, H-16), 4.17 (1H, *dd*, *J* = 9.2, 3.2 Hz, H-11), 4.39 (1H, *d*, *J* = 7.6 Hz, Fuc H-1), 4.54 (1H, *d*, *J* = 7.6 Hz, Glc H-1), 5.23 (1H, *d*, *J* = 3.6 Hz, H-12). ^{13}C NMR: Table 2. FABMS *m/z*: 797 $[M - H]^-$. HRFABMS *m/z*: 797.46922 $[M - H]^-$ (calc. for $C_{42}H_{69}O_{14}$ 797.46873).

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