ISOLATION OF NEW SAPONINS FROM THE ROOT OF *BUPLEURUM FALCATUM* L.

Naobumi EBATA,* Kaoru NAKAJIMA, Heihachiro TAGUCHI, and Hiroshi MITSUHASHI

Research Institute for Biology & Chemistry, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan

Two new malonylated oleanane-type triterpene oligoglycosides, named malonylsaikosaponins a (1) and d (2), were isolated from Bupleuri Radix, the root of *Bupleurum falcatum* L. Their structures were elucidated on the basis of the chemical and spectroscopic studies. Quantitative analysis of various Bupleuri Radixes by HPLC suggested that these malonylsaikosaponins are genuine saponins of this crude drug.

KEYWORDS Bupleuri Radix ; *Bupleurum falcatum* ; Umbelliferae ; malonylsaikosaponin ; malonic acid ; saikosaponin ; oleanane-type triterpene oligoglycoside

Bupleuri Radix, the root of *Bupleurum falcatum* L. and its varieties (Umbelliferae), is one of the best known crude drugs in Japan, and has been the subjects of many investigations. Especially the triterpene oligoglycosides, saikosaponins which are the principal ingredients of Bupleuri Radix, have been investigating as a major bioactive component of this crude drug.[1)]

In the course of our quantitative analysis of saikosaponins in this crude drug, we have found that the MeOH extract of the Bupleuri Radix contains several polar compounds which give saikosaponins on alkaline hydrolysis. Isolation of the MeOH extract gave two new acylated saikosaponins named malonylsaikosaponins a (1) and d (2). Quantitative analysis of various Bupleuri Radixes and the fresh plant showed that these compounds occur in larger amounts than the non-acylated ones. This paper deals with the structural elucidation of two new compounds and the result of quantitative analysis.

The MeOH extract of Bupleuri Radix was partitioned between BuOH and H_2O, and then the BuOH- soluble portion was washed with ether. Separation of the BuOH- soluble portion by silica gel column chromatography (AcOEt-EtOH-H_2O) furnished a mixture of polar compounds. Further purification of the mixture by reversed-phase and normal-phase silica gel column chromatographies gave 1 (0.004%) and 2 (0.006%), together with three known compounds, saikosaponins a (3), c, and d (4).

Malonylsaikosaponin a (1), white amorphous powder, $[\alpha]_D^{26}$ +42.8° (*c*=0.1, MeOH), FAB-MS *m/z* : 905 $(M+K)^+$ [high resolution FAB-MS (HR-FAB-MS) Calcd. for $C_{45}H_{70}O_{16}K$: 905.4301. Found 905.4316], 889 $(M+Na)^+$, 861, 455, 437, IR (KBr) : 3416 (OH), 2944, 1730 (C=O), 1592, 1386, 974, 906 cm^{-1}, ^1H-NMR (500 MHz, CD_3OD) δ : 0.70 (3H, s), 0.92 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.04 (3H, s), 1.09 (3H, s), 1.28 (3H, d, *J* =6.4 Hz), 4.25 (1H, dd, *J* =12.0, 6.1 Hz), 4.47 (1H, dd, *J* =12.0, 2.0 Hz), 5.38 (1H, dd, *J* =10.5, 3.0 Hz), 5.95 (1H, d, *J* =10.5 Hz). These spectral data suggested that 1 was composed of some saikosaponin and acid. Alkaline hydrolysis of 1 with KOH gave saikosaponin a (3). Methylation of 1 with ethereal CH_2N_2 at -50°C in MeOH provided the monomethyl ester of 1, FAB-MS *m/z* : 903 $(M+Na)^+$, 455. On methylation at room temperature, 1 scarcely gave the monomethyl ester of 1 but yielded 3 and dimethyl malonate, EI-MS *m/z* : 132 (M^+), 101(base peak). The recent publications[2,3)] of ^{13}C assignment of saikosaponins enabled us to distinguish the signals of the glucosyl, fucosyl, and aglycone moieties in 1 (Table I). By comparing the ^{13}C chemical shifts of 1 with those of 3, the C-6" signal of glucose was displaced downfield by 2.6 ppm and the neighboring C-5" signal was shifted upfield by 2.6 ppm. This acylation shift [3)] showed the malonyl group to be present at C-6" of the glucosyl moiety. The structure of malonylsaikosaponin a was thus formulated as 1.

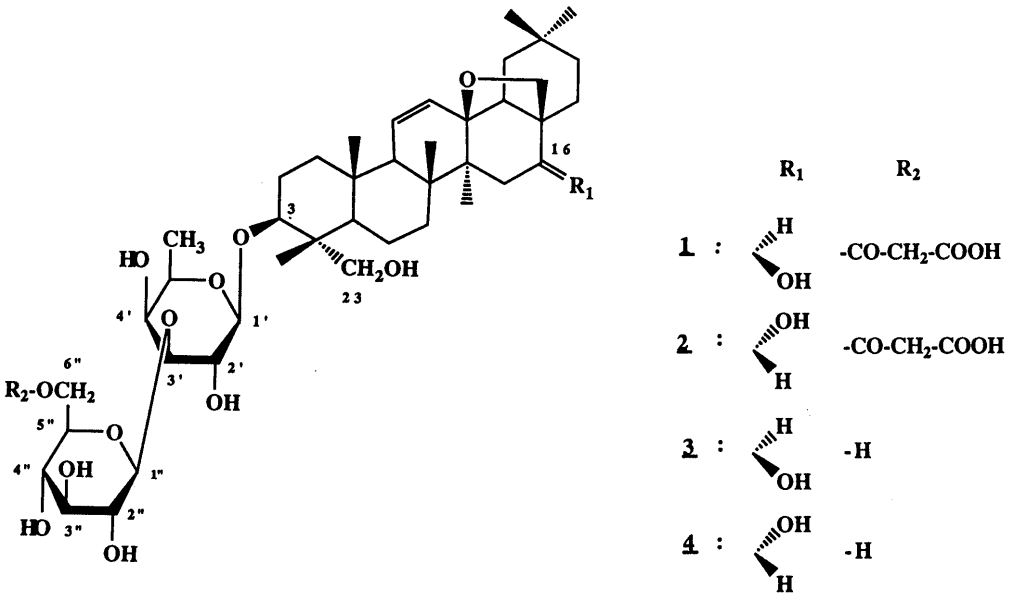
Malonylsaikosaponin d (2), white amorphous powder, $[\alpha]_D^{25}$ +29.6° (*c*=0.1, MeOH), FAB-MS *m/z* : 905 $(M+K)^+$ [HR-FAB-MS Calcd. for $C_{45}H_{70}O_{16}K$: 905.4301. Found 905.4268] , 889 $(M+Na)^+$, 862, 455, IR (KBr) : 3416 (OH), 2948, 1730 (C=O), 1596, 1384, 910, 890 cm^{-1}, ^1H-NMR (500 MHz, CD_3OD) δ : 0.70 (3H, s), 0.92 (3H, s), 0.94 (3H, s), 0.95 (3H, s), 1.04 (3H, s), 1.27 (3H, d, *J* =6.4 Hz), 1.30 (3H, s), 4.22 (1H, dd, *J* =11.8, 6.4 Hz), 4.46 (1H, dd, *J* =11.8, 1.8 Hz), 5.36 (1H, dd, *J* =10.5, 3.0 Hz), 5.94 (1H, d, *J* =10.5 Hz). Compound 2 seemed to be similar to 1. Alkaline hydrolysis with KOH of 2 gave saikosaponin d (4). Methylation of 2 with CH_2N_2 at room temperature gave 4 and dimethyl malonate. When the ^{13}C-NMR spectral data of 2 were compared with those of 1, the ^{13}C chemical shift values for the glucosyl moiety in 2 were in good agreement with those of 1. Thus, the structure of 2 was elucidated as 6"-*O*-malonylsaikosaponin d.

We have analyzed various Bupleuri Radixes and the fresh roots of *Bupleurum falcatum* by HPLC. Since these malonylsaikosaponins were found to be unstable, we extracted Bupleuri Radix with 75%MeOH at 0°C for 1 h. Simultaneous analysis

Table I. ^{13}C-NMR Data for Saikosaponins (δ in CD_3OD)

		1	3	2	4
Aglycone moiety	C-3	83.2	83.1	83.4	83.3
	C-16	65.4	65.3	77.5	77.7[c]
	C-23	64.9[d]	64.8	65.0	65.0
3-*O*-β-D-Fucopyranosyl moiety	C-1'	105.3[a]	105.6	105.3[a]	105.6[a]
	C-2'	72.0	71.8	71.5[b]	71.4[b]
	C-3'	84.9	85.1	85.3	85.2
	C-4'	72.4	72.3	72.4	72.4
	C-5'	71.4[b]	71.2[a]	71.4[b]	71.3[b]
3'-*O*-β-D-Glucopyranosyl moiety	C-1"	105.6[a]	105.6	105.6[a]	105.7[a]
	C-2"	75.4[c]	75.3	75.4[c]	75.4
	C-3"	77.6	77.7[b]	78.0	78.0[c]
	C-4"	71.6[b]	71.3[a]	71.9	71.9
	C-5"	75.3[c]	77.9[b]	75.3[c]	77.9[c]
	C-6"	65.0[d]	62.4	65.0	62.5

a) - d) Assignments may be interchangeable within the same column.



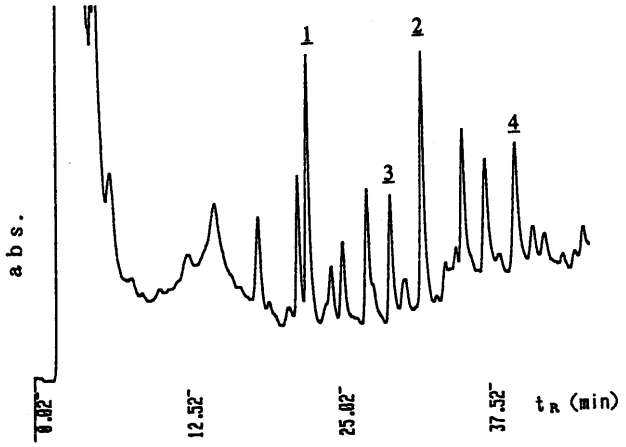


Fig. 1. HPLC of MeOH Extract of Bupleuri Radix

Condition

Column : LiChrosorb RP-18 (φ4 × 250mm ; particle size, 7μm)

Eluent :

CH_3CN : 0.05M $(NH_4)H_2PO_4$ (3 : 7) —45 min, linear grad.→ CH_3CN : 0.07M $(NH_4)H_2PO_4$ (1 : 1)

Flow rate : 1.0 ml/min, detection : 205 nm, oven temp. : 40 °C.

of malonylsaikosaponins and non-acylated saikosaponins has been achieved by reversed-phase HPLC with aqueous CH_3CN containing 35 mM $(NH_4)H_2PO_4$ as a mobile phase (Fig. 1). The quantitative analysis indicated that malonylsaikosaponins are widely distributed in Bupleuri Radix, and that the fresh plant contains a larger amount of malonylsaikosaponins than their correlated saikosaponins. Moreover, a trace amount of these acylated compounds was detected in the stale crude drug. So the malonylsaikosaponins seemed to be genuine saponins of this crude drug.

REFERENCES AND NOTES

1) K. Takeda, *Metabolism and Disease*, 10 (Special Issue for Wakan-Yaku), 676 (1973); A. Akahori, *J. Traditional Sino-Japanese Medicine*, 1, 45 (1980); A. Akahori, *Pharm Tech Japan*, 3, 1153 (1987).

2) T. Kubota, and H. Hinoh, *Tetrahedron Lett.*, 303 (1968); H. Ishii, M. Nakamura, S. Seo, K. Tori, T. Tozyo, and Y. Yoshimura, *Chem. Pharm. Bull.*, 28, 2367 (1980).

3) D. E. Dorman, D. Bauer, and J. D. Roberts, *J. Org. Chem.*, 40, 3729 (1975); S. E. Pelletier, Z. Djurmati, and C. Pape, *Tetrahedron*, 32, 995 (1976); Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Lett.*, 621 (1976); K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, and O. Tanaka, *Tetrahedron Lett.*, 1231 (1977); I. Kitagawa, T. Taniyama, T. Hayashi, and M. Yoshikawa, *Chem. Pharm. Bull.*, 31, 3353 (1983).

(Received February 13, 1990)