[Chem. Pharm. Bull. 30(12)4373—4377(1982)]

Saponins of Juk-Siho and Roots of *Bupleurum longeradiatum* Turcz.

Hiroko Kimata, Ryoji Kasai and Osamu Tanaka*

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734, Japan

(Received July 5, 1982)

In connection with our studies on the chemical evaluation of commercial Bupleuri Radix, isolation and identification of saponins of Juk-Siho (a Korean Bupleuri Radix) and roots of *Bupleurum longeradiatum*, the source plant of this crude drug, were carried out.

From Juk-Siho, five saponins **1**—**5** were isolated. The saponins **1**—**3** were identified as saikosaponins-a(**1**),-c(**2**) and -d(**3**), respectively, all of which have already been isolated from roots of *Bupleurum falcatum*. The structures of the new saponins, named chikusaikosides-I (**4**) and -II (**5**), were established as 3-*O*-β-xylopyranosyl(1→2)-β-glucopyranosyl-(1→3)-β-fucopyranoside of saikogenin F (**6**) and 3-*O*-β-glucopyranosyl(1→6)-(α-rhamnopyranosyl(1→4))-β-glucopyranoside of **6**, respectively. From roots of *B. longeradiatum*, saponins **1**—**4** were isolated and identified.

During the course of the structure study of **4** by ^{13}C-nuclear magnetic resonance (^{13}C-NMR), an anomalous glycosylation shift for the 1,2-linked glycoside was encountered.

Keywords——Juk-Siho; Bupleuri Radix; *Bupleurum longeradiatum*; Umbelliferae; saikosaponins; chikusaikosides-I, -II; oleanane-saponin; ^{13}C-NMR; 1,2-linked oligosaccharide

Bupleuri Radix is a very important crude drug in traditional Oriental medicine. In Japan, the roots of *Bupleurum falcatum* L. (Japanese name, Mishima Saiko; Umbelliferae) have been used as a source of this crude drug, from which oleanane saponins, saikosaponins-a (**1**), -c (**2**) and -d (**3**) and several other minor saponins have been isolated.[1] In Japan, a recent increase in the consumption of this crude drug has resulted in the import of many kinds of Bupleuri Radix from China and Korea. For the purpose of the chemical evaluation of this crude drug from different sources, we have carried out quantitative analysis of pharmacologically active representative components of this drug, **1** and **3**, by high performance liquid chromatography (HLC)[2] and by thin layer chromatogram (TLC) densitometry.[3] In these analytical studies, we found that the HLC pattern of the saponin fraction of one of the samples of Korean Bupleuri Radix, "Juk-Siho" (Japanese name: Chiku Saiko) was significantly different from samples from other sources, (Chinese Bupleuri Radix, Japanese and Korean *B. falcatum*). Previously, Konoshima *et al.* reported that the source plant of Juk-Siho must be *B. longeradiatum* Turcz. (Japanese name: Hotaru Saiko),[4] and this argument was supported by the similarity of TLC and HLC patterns of the saponin fraction of this crude drug to those of roots of this plant collected in Japan in our analytical study.[2] Although a TLC comparison of the saponin fraction of the roots of this plant with those of *B. falcatum* and other *Bupleurum* spp. was reported by Shibata *et al.*,[5] isolation and unambiguous identification of the saponins of *B. longeradiatum* have not been reported in the literature. The present paper deals with the isolation and identification of saponins of Korean Juk-Siho and the roots of *B. longeradiatum*.

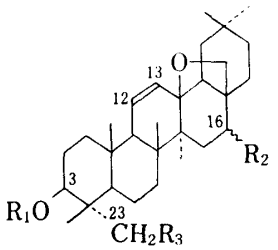
Since the allyl ether linkage of the aglycones of saikosaponins is known to be readily opened even by treatment with boiling methanol, Juk-Siho imported from Korea was extracted with methanol at room temperature. After evaporation of the solvent *in vacuo*, a suspension of the methanolic extract in water was washed with ether and then extracted with 1-butanol saturated with water. The butanolic layer was concentrated to dryness *in vacuo* to give a crude saponin fraction (yield: 3.4%), which was subjected to repeated chromatography,

affording three known saponins, **1**, **2** and **3** (yields: 0.16, 0.16 and 0.08%, respectively) and two new saponins, named chikusaikosides-I (**4**) and -II (**5**), in yields of 0.09% each.

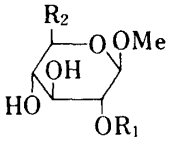
In a comparison of the ^{13}C-NMR spectrum of **4** with those of known saikosaponins,[1] all of the signals due to the aglycone moiety of **4** were almost superimposable on those of **1**, indicating that the genuine aglycone of **4** is the same as that of **1** (saikogenin F (**6**)) and that its 3-hydroxyl group carries a sugar moiety (Table I). Mineral acid hydrolysis of **4** yielded fucose, glucose and xylose. Permethylation of **4** by Hakomori's procedure[6] followed by methanolysis gave methyl 2,3,4-tri-*O*-methyl-xylopyranoside, methyl 3,4,6-tri-*O*-methyl-glucopyranoside and methyl 2,4-di-*O*-methyl-fucopyranoside. The mass spectrum (MS) of acetylated **4** exhibited fragment ions at *m/z* 254 (terminal xylose) and 547 (xylose–glucose). The coupling constants of all of the glycosyl linkages, δ 4.92 (2H, d, J=8 Hz) and δ 5.14 (1H, d, J=8 Hz) in pyridine-d_5, indicated the β-configuration. It follows that **4** is the 3-*O*-β-xylopyranosyl(1→2)-β-glucopyranosyl(1→3)-β-fucopyranoside of **6**.

^{13}C-NMR spectroscopy is a very powerful tool for the structure determination of glycosides, affording conclusive evidence of the location and configuration of glycosyl linkages (regularity of glycosylation shift).[7,8] However, we encountered anomalous glycosylation shift values for the 1,2-linked oligosaccharide moiety of **4**. It has been reported that on going from methyl β-D-glucoside (**7**) to methyl β-sophoroside (**8**, 1,2-β-linked glucobioside), the signal due to C-2, which participates in the glycosyl linkage, is deshielded by 8.2 ppm and the anomeric carbon signal of the inner glucosyl moiety is displaced upfield by 2.1 ppm, while all of the other carbon signals including the anomeric carbon resonance of the terminal glucosyl moiety, remain almost unshifted (in pyridine-d_5). Such a glycosylation shift has been applied to the structure determination of a variety of glycosides including 1,2-linked β-xylosyl, β-galactosyl or α-arabinosyl glucosides, *etc.* However, the C-2 resonance of the glucosyl moiety of **4** appears at δ 87.6, being anomalously deshielded by 11.8 ppm, and the signal due to the anomeric carbon of the terminal xylosyl moiety is also deshielded by 1.8 ppm, as shown in Table II. Another type of exceptional shift for 1,2-linked glycosides was reported recently for notoginsenosides-R1 and -R2 (saponins of the roots of *Panax notoginseng*) and ginsenosides-Rf and -Re (Ginseng saponins),[9] the signal assignments of which were substantiated by means of the selective deuteration.[10] This effect can be explained in terms of conformational change of the 1,2-linked glycosyl bonding due to stereostructural interaction around the glycoside linkage.

On acid hydrolysis, another new saponin, chikusaikoside-II (**5**) yielded glucose and rhamnose, and the mass spectrum of its trimethylsilyl ether exhibited ions at *m/z* 451 (terminal



1: saikosaponin a, R_1=-fuc$\frac{3}{}$glc, R_2=β-OH, R_3=OH
2: saikosaponin c, R_1=-glc$\frac{4}{}$rha, R_2=β-OH, R_3=H
(6 glc)
3: saikosaponin d, R_1=-fuc$\frac{3}{}$glc, R_2=α-OH, R_3=OH
4: chikusaikoside I, R_1=-fuc$\frac{3}{}$glc $\frac{2}{}$xyl, R_2=β-OH, R_3=OH
5: chikusaikoside II, R_1=-glc$\frac{4}{}$rha, R_2=β-OH, R_3=OH
(6 glc)
6: saikogenin F, R_1=H, R_2=β-OH, R_3=OH



7: Me β-glucopyranoside, R_1=H, R_2=CH_2OH
8: Me β-sophoroside, R_1=glc, R_2=CH_2OH
9: Me β-xylopyranoside, R_1=H, R_2=H

fuc: β-fucopyranosyl, glc: β-glucopyranosyl,
rha: α-rhamnopyranosyl, xyl: β-xylopyranosyl.

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moiety

	1	2	4	5
C-1	38.6[a]	38.3[a]	38.5[a]	38.4[a]
C-2	26.0	26.3	25.7	25.9
C-3	81.6	88.9	81.5	81.7
C-4	43.7	39.5	43.6	43.6
C-5	47.4	55.2	47.3	47.2
C-6	17.6	18.3[b]	17.2	17.5
C-7	31.5	31.7	31.5	31.5
C-8	42.2	42.0	42.1	42.1
C-9	53.1	52.7	53.0	52.9
C-10	36.3	36.2	36.2	36.2
C-11	132.1[c]	132.0[c]	132.1[c]	132.1[c]
C-12	131.1[c]	131.0[c]	131.0[c]	131.0[c]
C-13	84.0	83.9	83.9	84.0
C-14	45.6	45.5	45.5	45.6
C-15	36.3	36.0	36.2	36.2
C-16	64.1	64.0	64.1	64.1
C-17	47.0	46.9	46.9	46.9
C-18	52.1	52.0	52.0	52.1
C-19	37.8[a]	37.8[a]	37.8[a]	37.7[a]
C-20	31.5	31.5	31.5	31.5
C-21	34.7	34.7	34.7	34.7
C-22	25.7	25.7	25.7	25.7
C-23	64.1	27.9	64.1	64.1
C-24	13.0	16.3	12.9	12.9
C-25	18.7	18.1[b]	18.7	18.6
C-26	20.0	19.9	20.0	20.0
C-27	20.9	20.8	20.9	20.8
C-28	73.0	72.9	73.0	73.0
C-29	33.6	33.6	33.6	33.6
C-30	23.8	23.8	23.8	23.8

δ ppm from internal TMS in C_5D_5N at 25°C.
a, b, c) Values in any column may be reversed, though those given here are preferred.

TABLE II. ^{13}C-NMR Chemical Shifts of the Sugar Moiety

1	4	7	8	9	2	5
f-1 105.9[a]	f-1 104.9[a]				g-1 105.6	106.6
f-2 71.1[b]	f-2 71.2[b]				g-2 75.0[a]	75.0[a]
f-3 85.1	f-3 85.7				g-3 76.7	76.7
f-4 72.1	f-4 71.6[b]				g-4 79.6	79.7
f-5 71.0[b]	f-5 70.9[c]				g-5 75.4[a]	75.4[a]
f-6 17.2	f-6 17.2				g-6 68.9	68.9
g-1 106.5[a]	g-1 104.4[a]	g-1 105.5	103.4(−2.1)		g′-1 104.9	104.9
g-2 75.7	g-2 86.7(+11.8)	g-2 74.9	83.1(+8.2)		g′-2 74.6	74.6
g-3 78.3	g-3 77.6[d]	g-3 78.3	78.0		g′-3 78.2	78.2
g-4 71.5[b]	g-4 70.9[b]	g-4 71.6	71.1		g′-4 71.4	71.3
g-5 78.3	g-5 78.1[d]	g-5 78.3	78.0		g′-5 78.2	78.2
g-6 62.7	g-6 62.1	g-6 62.7	62.4		g′-6 62.5	62.4
	x-1 107.7[+1.8]	g′-1	105.6(+0.1)	x-1 105.9	r-1 102.8	102.8
	x-2 76.0	g′-2	76.0	x-2 74.6	r-2 72.4	72.4
	x-3 77.6[d]	g′-3	78.0	x-3 78.0	r-3 72.4	72.4
	x-4 70.3[c]	g′-4	71.4	x-4 70.8	r-4 73.7	73.7
	x-5 67.5	g′-5	78.3	x-5 66.8	r-5 70.5	70.5
		g′-6	62.4		r-6 18.3	18.1
		OMe 56.7	56.5	56.6		

f: β-fucopyranosyl, g: β-glucopyranosyl, x: β-xylopyranosyl, r: α-rhamnopyranosyl, g′: terminal β-glucopyranosyl.
δ ppm from internal TMS in C_5D_5N at 25°C. **9**: Me β-xylopyranoside.
a, b, c, d) Values in any column may be reversed, though those given here are preferred.
( ): δ (**4** or **8**)−δ **7**, [ ]: δ**4**−δ**9**.

glucose) and 363 (terminal rhamnose). In a comparison of the ^{13}C-NMR spectrum of **5** with those of **1** and **2**,[1] signals due to the aglycone moiety of **5** appeared at almost the same positions as those of **1** and those due to the sugar moiety of **5** were almost superimposable on those of **2** (Tables I and II). It follows that **5** can be formulated as 3-*O*-β-glucopyranosyl-(1→6)-(α-rhamnopyranosyl(1→4))-β-glucopyranoside of saikogenin-F (**6**).

The saponin fraction of roots of *B. longeradiatum* was subjected to column chromatographic separation in the same way as in the case of Juk-Siho, affording **1**, **2**, **3** and **4** in yields of 0.16, 0.48, 0.17 and 0.12%, respectively. Although the presence of **5** in the roots was demonstrated by TLC, a sufficient amount of this saponin for definite identification could not be obtained due to its low content and the limited amount of the plant material available.

Experimental

General Procedures——NMR spectra were taken on a JEOL PFT-100 spectrometer in C_5D_5N using TMS as an internal standard (^1H-NMR at 100 MHz and ^{13}C-NMR at 25.15 MHz). MS were taken at 75 eV on a JEOL 01-SG-2 spectrometer by the direct inlet method; ionization current, 200 μA; accelerating voltage, 6—8 kV.

Trimethylsilylation for MS: A sample of saponin (1—2 mg) was heated with *N*-trimethyl-silylimidazole (5 drops) in a sealed micro-tube at 80°C for 2—3 h. The reaction mixture was diluted with H_2O and then extracted with *n*-C_6H_{14}. The C_6H_{14} layer was washed with H_2O and concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was subjected to MS.

Acetylation for MS: A solution of a saponin (1—2 mg) in C_5H_5N (5—6 drops) and Ac_2O (2—3 drops) was allowed to stand at room temperature overnight. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature and the residue was subjected to MS.

Identification of the Known Saponins——Each saponin isolated in the present study was identified by comparison with authentic samples. The methods used were the identification of monosaccharides formed by acid hydrolysis (*vide infra*), TLC comparison on Kieselgel $60F_{254}$ (Merck) using EtOAc–EtOH–H_2O (8: 2: 1, homogeneous) with H_2SO_4 detection ^1H-NMR and ^{13}C-NMR spectroscopy, MS of the acetates or trimethylsilyl ethers, and optical rotation measurement.

Hydrolysis of a Saponin and Identification of the Resulting Monosaccharides——A saponin (a few mg) was heated with 2 N HCl in H_2O–dioxane (1: 1) in a sealed micro-tube at 90°C for 4 h. The reaction mixture was diluted with H_2O and washed with $CHCl_3$. The aqueous layer was neutralized with Amberlite MB3, concentrated and subjected to TLC on Kieselgel (*vide supra*) with $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer), using 2,3,5-triphenyltetrazolium chloride detection. For gas liquid chromatography (GLC), the above aqueous layer was concentrated to dryness and the residue was trimethylsilylated by the same procedure as used for MS (*vide supra*). GLC was carried out on a Shimadzu GC-4A gas chromatograph (glass column, 4 mm × 1.5 m, 1.5% SE-30 on Chromosorb W, detector, FID; injection temperature, 200°C; column temperature, 180°C; carrier gas, N_2 at 1 kg/cm^2). Retention times (min): glucose, 7.4 and 11.4; xylose, 2.6, 3.6 and 4.2; rhamnose, 2.8; fucose, 3.0.

Separation of Saponins of Juk-Siho——The crude drug (190 g) imported from Korea was extracted three times with MeOH (2 l) at room temperature for 3 min under vigorous stirring in a mechanical mixer (Solid State MX-1200S, Toshiba). The MeOH solution was concentrated to dryness *in vacuo* and a suspension of the residue (yield: 15.3%) in H_2O was washed with Et_2O and then extracted three times with 1-BuOH saturated with H_2O. The BuOH layer was concentrated to dryness *in vacuo*, affording a saponin fraction (yield 3.4%), which was subjected to column chromatography on silica gel with EtOAc–EtOH–H_2O (15: 4: 1→40: 11: 2.5, homogeneous), yielding five fractions, Fr. 1—5, in increasing order of polarity.

Fr. 1 was homogeneous and afforded **3** in a yield of 0.08%, white powder, $[\alpha]_D^{23}$ +36.8° (c=1.9, EtOH).

Fr. 2 was further chromatographed on silica gel with EtOAc–EtOH–H_2O (40: 4: 1, homogeneous) and then on the same adsorbent with EtOAc–EtOH–H_2O (20: 5: 1, homogeneous) to give **1** in a yield of 0.16%, white powder, $[\alpha]_D^{25}$ +36.1° (c=0.33, EtOH).

Fr. 3 was further purified by column chromatography on silica gel with EtOAc–EtOH–H_2O (12: 2: 1, homogeneous) or by preparative HLC on a reverse phase column of TSK-gel LS-410 (7.5 mm × 30 cm; mobile phase, 70% MeOH; flow rate, 1.4 ml/min) to give **4** in a yield of 0.09%, white powder, $[\alpha]_D^{14}$ +51.7° (c=1.26, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{17}\cdot 3H_2O$: C, 58.37; H, 8.24. Found: C, 58.03; H, 8.55.

A mixture of NaH (50 mg) and DMSO (1 ml) was heated at 65°C for 1 h under N_2, then a solution of **4** (10 mg) in DMSO (1 ml) was added. The whole was heated for 15 min at 65°C again, then MeI (3 ml) was added and the mixture was allowed to stand at room temperature overnight. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and concentrated to dryness. A solution of the resulting permethylated saponin in 6.7% HCl/MeOH (2 ml) was refluxed for 4 h. The reaction mixture was neutralized with Ag_2CO_3. After removal of the precipitate by filtration, the filtrate

was concentrated to dryness and the residue was subjected to GLC analysis on a Shimadzu GC-4A gas chromatograph, (glass column, 4 mm × 2 m, 5% NPGS on Chromosorb W; detector, FID; injection temperature, 200°C; column temperature, 170°C; carrier gas, N_2 1.0 kg/cm^2. Methyl 2,3,4-tri-*O*-methyl-xylopyranoside, methyl 3,4,6-tri-*O*-methyl-glucopyranoside and methyl 2,4-di-*O*-methyl-fucopyranoside were identified by the comparison of the retention times with those of authentic samples.

Fr. 4 was further chromatographed on silica gel with EtOAc–EtOH–H_2O (10: 2: 1, homogeneous) to give **2** in a yield of 0.16%, white powder, $[\alpha]_D^{25}$ +11.2° (c=1.01, EtOH).

Fr. 5 was rechromatographed on silica gel with EtOAc–EtOH–H_2O (8: 2: 1, homogeneous) to give **5** in a yield of 0.09%, $[\alpha]_D^{21}$ −5.6° (c=0.89, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{18}\cdot 4H_2O$: C, 56.79; H, 8.54. Found: C, 57.00; H, 8.27.

Separation of Saponins of Roots of *Bupleurum longeradiatum*——The roots (350 g) collected at Nobeyama, Nagano-ken, in August 1979 were extracted and fractionated in the same way as described for Juk-Siho to give a saponin fraction (yield: 3.5%), which was separated by column chromatography under the same conditions as used for Juk-Siho, affording **3**, **1**, **4** and **2** in yields of 0.17, 0.16, 0.12 and 0.48%, respectively.

Acknowledgement The authors are grateful to Dr. H. Kohda, Miss K. Itano and Mr. T. Tanaka of this Institute for the collection of wild *B. longeradiatum* and to Nihon Funmatsu Co., Ltd., Osaka for the kind supply of imported Juk-Siho.

References

1) H. Ishii, M. Nakamura, S. Seo, K. Tori, T. Tozyo and Y. Yoshimura, *Chem. Pharm. Bull.*, **28**, 2367 (1980); and references cited therein.
2) H. Kimata, C. Hiyama, S. Yahara, O. Tanaka, O. Ishikawa and M. Aiura, *Chem. Pharm. Bull.*, **27**, 1836 (1979).
3) H. Kimata, N. Fujioka, O. Tanaka and Y. Miyazaki, *Shoyakugaku Zasshi*, **34**, 311 (1980).
4) M. Konoshima, T. Miyagawa and M. Takigami, *Shoyakugaku Zasshi*, **28**, 161 (1974).
5) *a*) S. Shibata, I. Kitagawa, R. Takahashi and H. Fujimoto, *Yakugaku Zasshi*, **86**, 1132 (1966); *b*) H. Otsuka, S. Kobayashi and S. Shibata, *Planta Medica*, **33**, 152 (1978).
6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
7) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimula, H. Sugiyama and S. Seto, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 2425.
8) *a*) R. Kasai, M. Suzuo, J. Asakawa and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; K. Tori, S. Seo, Y. Yoshimura, H. Arita and Y. Tomita, *ibid.*, **1977**, 179; S. Seo, Y. Tomita, K. Tori and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978); *b*) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979); *c*) K. Mizutani, R. Kasai and O. Tanaka, *Carbohydrate Research*, **87**, 19 (1980).
9) J. Zhou, M. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari and T. Fuwa, *Chem. Pharm. Bull.*, **29**, 2844 (1981).
10) K. Mizutani, H. Kajita, T. Tashima and O. Tanaka, *Nippon Kagaku Kaishi*, **10**, 1595 (1982).