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Chemical Evaluation of *Bupleurum* Species Collected in Yunnan, China

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Saponins were isolated from roots of *Bupleurum marginatum* (Zhuye-Chaihu), *B. marginatum* var. *stenophyllum* (Zezhuye-Chaihu) and *B. rockii* (Lijiang-Chaihu) collected in Yunnan, China, and identified. For the evaluation of these plants as a medicine, quantitative analysis of pharmacologically active saponins, saikosaponins-a (**1**) and -d (**3**), in the plants was also conducted in comparison with *B. falcatum* and *B. chinensis*.

Keywords——Chinese Bupleuri Radix; Saiko; Chaihu; *Bupleurum marginatum*; *B. marginatum* var. *stenophyllum*; *Bupleurum rockii*; Umbelliferae; Yunnan medicinal plant; saikosaponin; acetylsaikosaponin

Bupleuri Radix (roots of *Bupleurum* spp., Umbelliferae, Chinese name: Chaihu, Japanese name: Saiko) is a well-known and very important crude drug in the prescriptions of traditional oriental medicine. In Japan, the roots of *Bupleurum falcatum* L. (Japanese name: Mishima-Saiko) have been used as a source of this crude drug. However, because of the increasing demand for this crude drug as well as a shortage of domestic supply in Japan, the roots of closely related Chinese *Bupleurum* spp., *B. chinensis* DC. (Chinese name: Pei-Chaihu) which grows wild in north and central provinces of China, are currently exported from China to Japan.

Thirty-six species and seventeen varieties of *Bupleurum* have been found in China and of these, eight species and five varieties grow wild in the south-west region of China (Yunnan, *etc.*) as shown in Table I.[1] As a part of our cooperative studies on medicinal plants of Yunnan, the present paper reports the chemical evaluation of roots of *Bupleurum* spp. collected in Yunnan which have not so far been exported to Japan.

From roots of *B. falcatum* and *B. chinensis*, several oleanane saponins have been isolated, and extensive studies on the pharmacological activities of these saponins have been reported.[2]

Bupleurum marginatum WALL. *ex* DC. var. *stenophyllum* (WOLFF) SHAN *et* Y. LI (Chinese name: Zezhuye-Chaihu) grows wild throughout the south-west region of China and has been used as a folk medicine. The methanolic extract of roots of this plant was separated by repeated chromatography as shown in Fig. 1, affording fifteen saponins **1**—**15**. Saponins **1**, **2** and **3** were identified as saikosaponins-a, -c and -d, respectively, which are known as the major saponins of Japanese Mishima-Saiko[3] and Chinese Pei-Chaihu. Saponins **4** and **5** were identified as chikusaikosides I and II, respectively. Both the saponins, **4** and **5**, were recently isolated from a kind of Korean Bupleuri Radix (Korean name: Juk-Siho, Japanese name: Chiku-Saiko; source plant: *B. longeradiatum* TURCZ. distributed in Korea and Japan).[4]

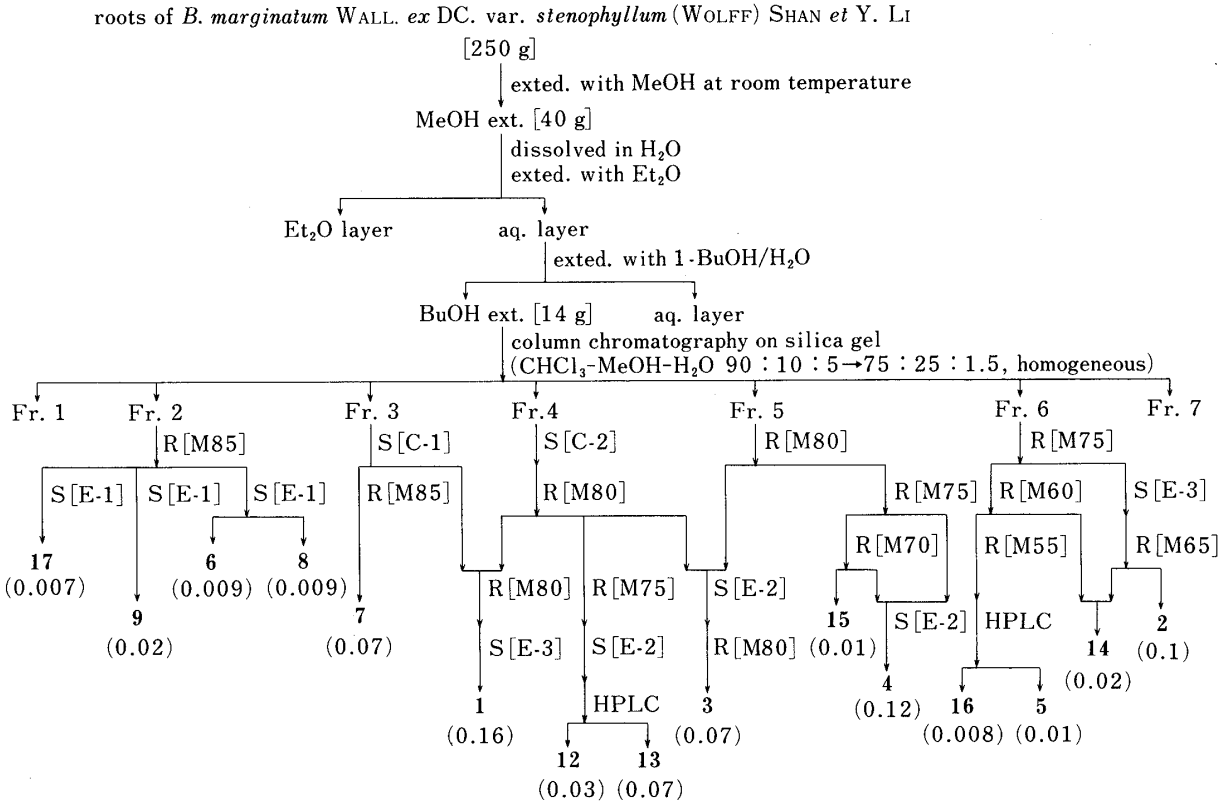


Fig. 1. Separation of Saponins of *B. marginatum* WALL. *ex* DC. var. *stenophyllum* (WOLFF) SHAN *et* Y. LI

R: column chromatography on LiChroprep RP-8.
S: column chromatography on silica gel.
[M85], 85% aq. MeOH; [M80], 80% aq. MeOH; [M75], 75% aq. MeOH; [M70], 70% aq. MeOH; [M65], 65% aq. MeOH; [M60], 60% aq. MeOH; [M55], 55% aq. MeOH.
[E-1], EtOAc–EtOH–H_2O 12:2:1; [E-2], EtOAc–EtOH–H_2O 10:2:1; [E-3], EtOAc–EtOH–H_2O 8:2:1.
[C-1], $CHCl_3$–MeOH–H_2O 9:1:0.05; [C-2], $CHCl_3$–MeOH–H_2O 7:3:0.1.
HPLC: column, TSK-gel ODS-120A (7.5 mm × 30 cm); mobile phase, 55% aq. MeOH; flow rate, 0.8 ml/min; detection, RI.
Solvents are all homogeneous.
( ): yield %.

Saponins **4** and **5** are absent in Japanese Mishima-Saiko and Chinese Pei-Chaihu, while the contents of **1** and **3** in Juk-Siho have been found to be extremely low. The isolation of **4** and **5** as well as **1**, **2** and **3** from roots of *B. marginatum* var. *stenopyllum* seems significant from a taxonomical viewpoint. Saponins **6**, **7** and **8** were identical with 3″-*O*-acetylsaikosaponin-d, 6″-*O*-acetylsaikosaponin-a and 6″-*O*-acetylsaikosaponin-d, respectively, which have already been isolated from Japanese Mishima-Saiko as minor saponins.[5,6]

A new saponin **9** exhibited a proton signal at δ 1.98 (3H, s) and carbon signals at δ 21.2 and 170.8 which indicated the presence of an acetoxyl group. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **9**, resonances due to the sugar moiety were almost superimposable over those of **6** and the aglycone signals were observed at almost the same positions as those of **1**. It follows that **9** can be formulated as 3″-*O*-acetylsaikosaponin-a. The electron impact-mass spectra (EI-MS) of the trimethylsilyl (TMSi) ether of **9** showed fragment ions at *m/z* 711 [(Fuc-Glc) $(TMSi)_5Ac$], 421 [(Glc)$(TMSi)_3Ac$] and 361 (421 −AcOH), supporting the allocation of the acetoxyl group to the terminal glucosyl unit.

It has been found that the allyl ether part of the sapogenin moiety of saponins of *Bupleurum* spp. is unstable to acid, being readily converted into a diene system (type **10**). The compounds having a methoxyl group (type **11**) are also formed from **1**, **2** and **3** on treatment

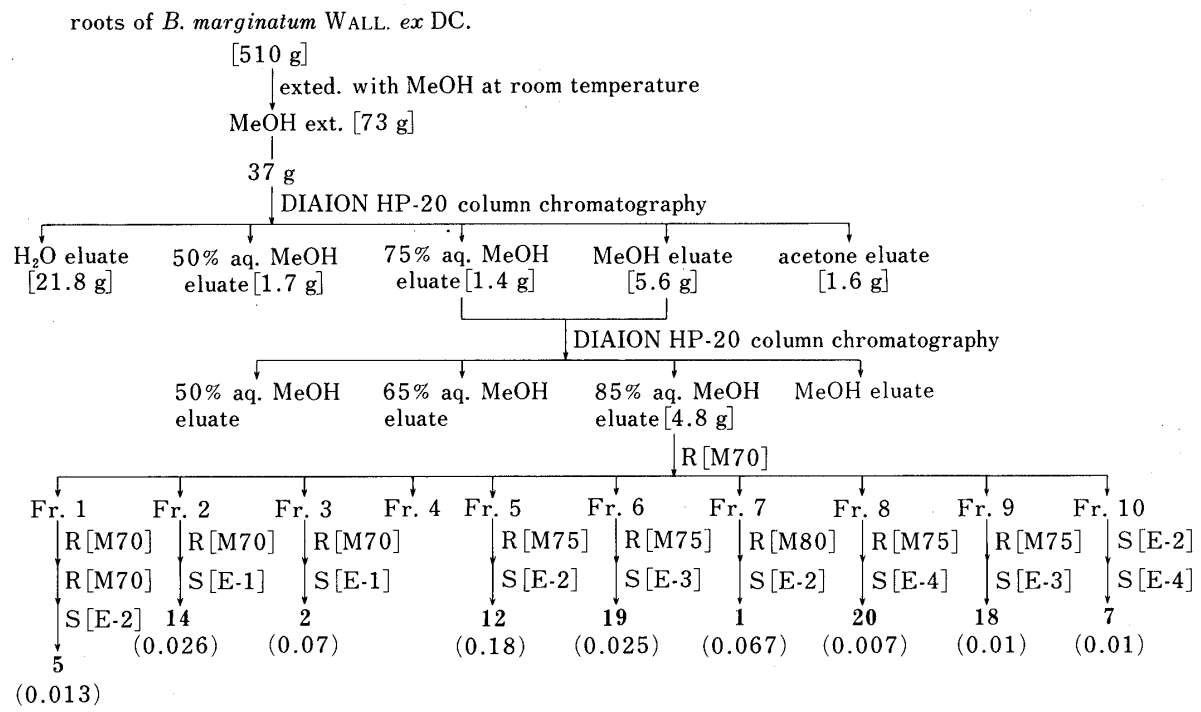


Fig. 2. Separation of Saponins of *B. marginatum* WALL. *ex* DC.

R: column chromatography on LiChroprep RP-8.
S: column chromatography on silica gel.
[M70], 70% aq. MeOH; [M75], 75% aq. MeOH; [M80], 80% aq. MeOH.
[E-1], EtOAc–EtOH–H_2O 8:2:1; [E-2], EtOAc–EtOH–H_2O 10:2:1; [E-3], EtOAc–EtOH–H_2O 12:2:1; [E-4], EtOAc–EtOH–H_2O 15:2:1.
Solvents are all homogeneous.
( ): yield %.

with acidic methanol (Chart 2).[7,8] Partial conversion into artifacts of these types has also been observed even during the process of extraction or separation. Saponins **12** and **13** were found to be identical with the known compounds of type **11**, saikosaponins-b_3 and -b_4, respectively, which were formed from **1** and **3** during the extraction. The minor saponins **14**—**17** were also assumed to be artifacts of type **11** by comparison of the ^{13}C-NMR spectra of these compounds with those of **2**, **4**—**6**, as well as **12** and **13** (Table II).

Bupleurum marginatum WALL. *ex* DC. (Chinese name: Zhuye-Chaihu) is also abundantly distributed in the south-west region of China and is used as a folk medicine. The extract of the roots collected in Yunnan was separated as shown in Fig. 2, affording saponins, **1**, **2**, **5**, **7** and saikosaponin-3 (**18**, a minor saponin of Japanese Mishima-Saiko[9]) together with the artifacts, **12** and **14**. Another artifact **19** was also obtained and its structure was proposed to be as shown in Chart 2 by comparison of its ^{13}C-NMR spectrum with those of **7** and **12** (Tables II and III).

Besides these saponins, a new minor saponin (**20**) was isolated from Zhuye-Chaihu. Acid hydrolysis of **20** afforded fucose. The ^{13}C-NMR signals of **20** were found to consist of those due to the aglycone moiety of **1** (3-*O*-glycosylsaikogenin F) as well as the signals expected for β-D-fucopyranoside linked with the 3β-hydroxyl group of an oleanane-type triterpene. Based on this evidence, **20** can be formulated as 3-*O*-β-D-fucopyranosylsaikogenin F (=desgluco-saikosaponin-a). Recently, formation of **20** from **1** by enzymatic partial hydrolysis was observed.[10]

Bupleurum rockii WOLFF (Chinese name: Lijiang-Chaihu) is distributed in Yunnan, Szechwan and Tibet, being less abundant than Zhuye-Chaihu and Zezhuye-Chaihu. The extract of roots of this plant was separated by chromatography as shown in Fig. 3 to give **1**—

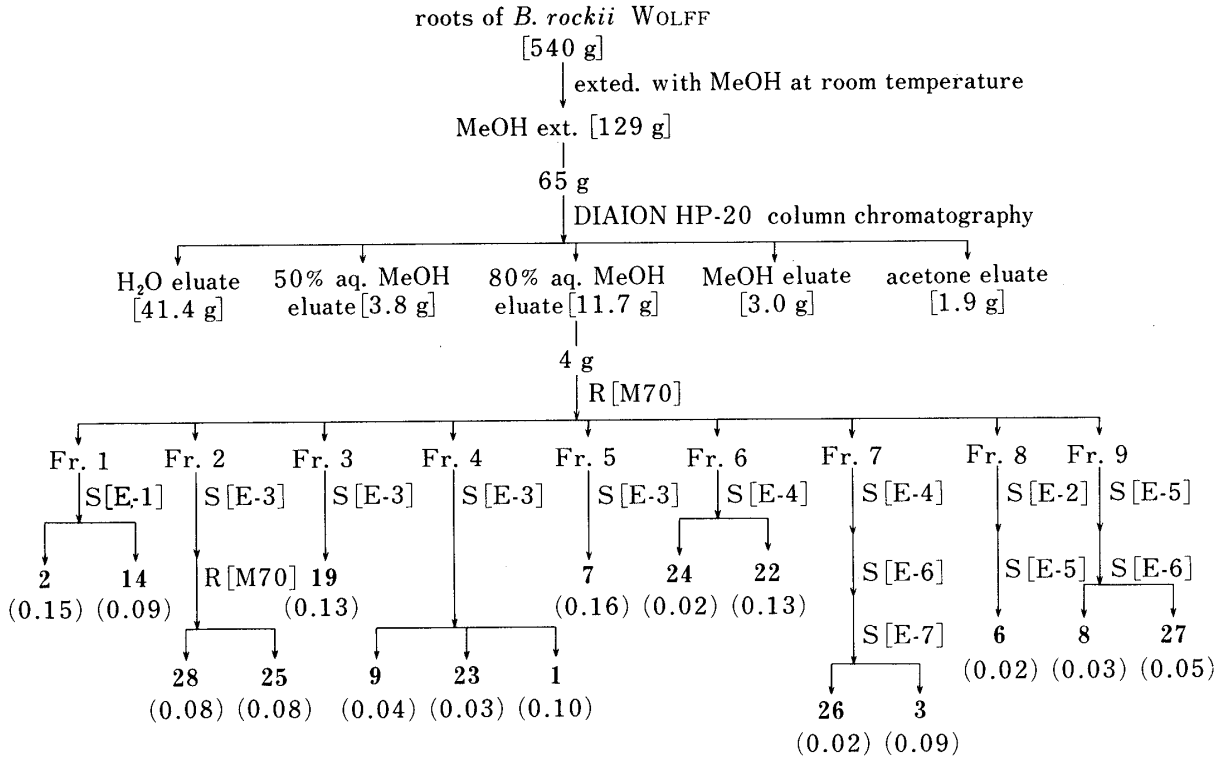


Fig. 3. Separation of Saponins of *B. rockii* WOLFF

R: column chromatography on LiChroprep RP-8.
S: column chromatography on silica gel.
[M70], 70% aq. MeOH.
[E-1], EtOAc–EtOH–H_2O 9:2:1; [E-2], EtOAc–EtOH–H_2O 10:2:1; [E-3], EtOAc–EtOH–H_2O 12:2:1; [E-4], EtOAc–EtOH–H_2O 13:2:1; [E-5], EtOAc–EtOH–H_2O 14:2:1; [E-6], EtOAc–EtOH–H_2O 18:2:1; [E-7], EtOAc–EtOH–H_2O 20:2:1.
Solvents are all homogeneous.
( ): yield %.

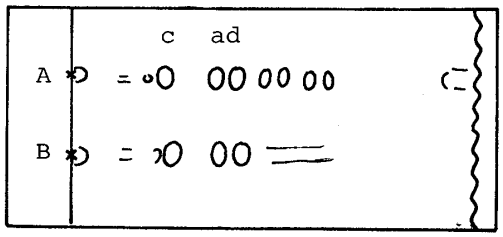


Fig. 4. Thin-Layer Chromatogram of *B. rockii* WOLFF

A: MeOH ext. of fresh roots.
B: MeOH ext. of dried roots (after one year).
a, c, d: saikosaponin-a, -c, -d.
Adsorbent: Silica gel HF_{254} (Merck).
Solvent: EtOAc–EtOH–H_2O (8:2:1).
Detection: 10% H_2SO_4, heat.

TABLE I. *Bupleurum* spp. Distributed in Yunnan Province

Bupleurum marginatum WALL. *ex* DC. (竹叶柴胡)[a]
B. marginatum WALL. *ex* DC. var. *stenophyllum* (WOLFF) SHAN *et* Y.LI (窄竹叶柴胡)[a]
B. yunnanense FRANCH. (雲南柴胡)[a]
B. candollei WALL. *ex* DC. (川滇柴胡)[a]
B. candollei WALL. *ex* DC. var. *atropurpureum* C. Y. WU (紫紅川滇柴胡)
B. tenue BUCH.-HAM. *ex* D. DON (小柴胡)[a]
B. tenue BUCH.-HAM. *ex* D. DON var. *humile* FRANCH. (矮小柴胡)
B. longicaule WALL. *ex* DC. var. *franchetii* de BOISS. (空心柴胡)[a]
B. longicaule WALL. *ex* DC. var. *amplexicaule* C. Y. WU (抱茎柴胡)
B. commelynoideum de BOISS. (紫花鴨跖柴胡)[a]
B. rockii WOLFF (麗江柴胡)
B. dalhousieanum (CLARKE) K.-POL. (葡枝柴胡)
B. petiolulatum FRANCH. (有柄柴胡)

a) Used as medicinal plants in Yunnan Province, China.

TABLE IIa. ^{13}C-NMR Chemical Shifts of Aglycone Moieties (Genuine Saponins) in C_5D_5N

	1	2	3	4	5	6	7	8	9	18	20	22
C-1	38.6	38.4	38.6[b]	38.5	38.4	38.6[b]	38.6	38.5[b]	38.6	38.6	38.7	38.6
C-2	26.0[a]	26.4	26.1	25.7	25.8	26.1	25.8	26.0	26.1[a]	26.6	26.0[a]	26.0[a]
C-3	81.6	89.0	81.7	81.5	82.0	81.7	81.6	81.7	81.6	88.7	81.7	81.5
C-4	43.7	39.5	43.7[c]	43.5	43.6	43.7[c]	43.6	43.6[c]	43.7	39.8	43.5	43.6
C-5	47.2	55.2	47.3	47.2	47.2	47.3	47.2	47.2	47.2	55.4	47.5	47.2
C-6	17.5	18.4	17.3	17.2	17.5	17.2	17.3	17.3	17.2	18.2	17.6	17.2
C-7	31.6	31.5	31.9	31.5	31.5	31.9	31.5	31.8	31.5	31.9	31.6	31.5
C-8	42.2	42.1	41.8	42.1	42.1	41.9	42.1	41.8	42.2	42.2	42.2	42.1
C-9	53.0	52.7	53.0	53.0	52.9	53.0	53.0	52.9	53.0	52.9	53.1	53.0
C-10	36.2	36.2	36.2	36.2	36.2	36.3	36.2	36.2	36.2	36.4	36.4	36.2
C-11	132.2	132.1	132.0[d]	132.2	132.2	132.0	132.2	131.9[d]	132.2	132.0	132.2	132.1
C-12	131.1	131.1	131.9[d]	131.1	131.0	132.0	131.1	131.8[d]	131.1	131.3	131.2	131.1
C-13	84.0	83.9	84.9	83.9	83.9	84.9	83.9	84.8	84.0	84.0	84.0	83.9
C-14	45.6	45.5	43.5[c]	45.6	45.6	43.6[c]	45.5	43.5[c]	45.6	45.7	45.5	45.6
C-15	36.2	35.9	35.4	36.2	36.2	35.4	36.2	35.3	36.2	36.1	36.2	36.2
C-16	64.0	64.1	77.0	64.0	64.0	77.1	64.0	77.0	64.0	64.0	64.1	64.0
C-17	46.9	46.9	45.3	46.9	46.9	45.3	46.9	45.2	47.0	47.0	47.0	46.9
C-18	52.1	52.1	51.3	52.1	52.1	51.3	52.1	51.3	52.1	52.1	52.2	52.1
C-19	37.7	37.7	38.4[b]	37.7	37.7	38.4[b]	37.9	38.3[b]	37.7	37.8	37.8	37.7
C-20	31.6	31.5	31.9	31.5	31.5	31.9	31.5	31.8	31.5	31.6	31.6	31.5
C-21	34.7	34.7	36.7	34.6	34.7	36.8	34.6	36.7	34.7	34.7	34.7	34.6
C-22	25.7[a]	25.7	31.2	25.7	25.8	31.3	25.8	31.2	25.7[a]	25.8	25.8[a]	25.7[a]
C-23	64.0	27.8	64.0	64.0	64.0	63.9	64.0	64.0	64.0	27.8	64.4	64.0
C-24	13.0	16.3	13.0	12.9	13.0	13.0	12.9	13.0	13.0	16.3	13.0	12.9
C-25	18.7	18.1	18.8	18.7	18.7	18.8	18.7	18.8	18.7	18.1	18.8	18.7
C-26	20.0	19.9	19.5	20.0	20.0	19.5	20.0	19.5	20.0	20.0	20.1	20.0
C-27	20.9	20.9	18.1	20.8	20.8	18.1	20.8	18.0	20.8	20.9	20.9	20.8
C-28	73.0	72.9	77.8	73.0	72.9	77.8	73.0	77.9	73.0	73.0	73.0	73.0
C-29	33.6	33.7	33.7	33.6	33.6	33.7	33.6	33.7	33.6	33.7	33.7	33.6
C-30	23.8	23.8	24.4	23.8	23.8	24.4	23.8	24.4	23.6	23.8	23.8	23.8

^{13}C-NMR spectra were observed at 22.5 °C. *a—d*) Assignments may be reversed in each column.

3, **6**—**9** and a new saponin (**22**). The presence of an acetoxyl group in **22** was shown by a proton signal at δ 1.95 (3H, s) and carbon signals at δ 21.1 and 170.5. On mild alkaline treatment, **22** afforded **1**. The EI-MS of the TMSi ether of **22** exhibited fragment ions at *m/z* 711 [(Fuc-Glc)(TMSi)$_5$Ac], 421 [(Glc)(TMSi)$_3$Ac] and 361 (421 − AcOH), indicating the location of the acetoxyl group on the terminal glucosyl unit of **1**. The carbon signals due to the aglycone moiety and fucosyl unit of **1** appeared at almost the same positions in the spectrum of **22**, while with regard to the carbon resonances due to the terminal glucosyl unit, the signal due to C-2″ was displaced downfield and those due to C-1″ and -3″ were shielded on going from **1** to **22**. These results led to the formulation of **22** as 2″-*O*-acetylsaikosaponin-a.

Besides these saponins, **14**, **21** and several other artifacts of the acetylated saponins (**23**—**27**) were also isolated and their structures were deduced by comparison of the carbon signals with those of the corresponding genuine saponins, **22**, **6**, **8** and **9**, as well as the known artifact, **21** (Table III, Chart 2).

A variety of pharmacological activities were reported for **1** and **3**, while no activity was observed for the other major saponin, **2**.[2] Accordingly, the chemical evaluation of Bupleuri Radix as a medicine has been conducted by quantitative analysis of both the active major saponins, **1** and **3**. Separative analysis of these saponins by high-performance liquid chromatography (HPLC) or thin layer chromatogram densitometry after converting them

TABLE IIb. ^{13}C-NMR Chemical Shifts of Sugar Moieties (Genuine Saponins) in C_5D_5N

	1	2	3	4	5	6	7	8	9	18	20	22
C-1′	105.9[a]	106.6	105.9[a]	104.9[a]	105.7[a]	106.1[a]	105.8	105.9[a]	106.1	106.8[a]	106.3	106.0
C-2′	71.4	75.0	71.4	71.2[b]	75.0	71.6	71.4	71.4	71.5	71.5	72.4	71.4
C-3′	85.0	76.7	85.0	85.7	76.7	84.9	85.1	85.2	84.8	85.1	75.5	84.2
C-4′	72.0	79.5	72.0	71.6[b]	79.7	72.1	71.4	71.4	72.1	71.9	73.0	71.4
C-5′	71.0	75.4	70.9	70.8[c]	75.4	71.0	70.7	70.8	70.9	71.0	71.3	70.6
C-6′	17.3	69.0	17.3	17.2	68.7	17.2	17.3	17.4	17.2	17.3	17.5	17.2
C-1″	106.3[a]	104.9	106.3[a]	104.3[a]	105.0[a]	106.2[a]	105.8	106.0[a]	106.1	106.6[a]		103.4
C-2″	75.6	74.6	75.6	86.7	74.6	73.5	75.2	75.1	73.5	75.8		76.0
C-3″	78.3	78.2	78.6	77.6[d]	78.2	79.2	77.9	77.9	79.1	78.4		75.4
C-4″	71.7	71.4	71.7	70.8[b]	71.4	69.2	71.8	71.9	69.2	72.1		71.7
C-5″	78.3	78.2	78.2	78.1[d]	78.3	78.5	75.2	75.3	78.4	78.7		78.5
C-6″	62.6	62.4	62.6	62.1	62.5	62.0	64.6	64.6	62.0	62.7		62.3
C-1‴		102.7		107.6	102.8							
C-2‴		72.4		76.0	72.4							
C-3‴		72.4		77.6[d]	72.4							
C-4‴		73.6		70.3[c]	73.7							
C-5‴		70.4		67.4	70.5							
C-6‴		18.4			18.4							
$\underline{C}OOCH_3$						170.8	170.9	170.8	170.8			170.5
$COO\underline{C}H_3$						21.2	20.8	20.7	21.2			21.1

^{13}C-NMR spectra were observed at 22.5 °C. C-1′—-6′; **1**, **3**, **4**, **6**—**9**, **18**, **20**, **22**, β-D-fucopyranosyl; **2**, **5**, inner β-D-glucopyranosyl. C-1″—-6″; **1**, **3**, **4**, **6**—**9**, **18**, **22**, β-D-glucopyranosyl; **2**, **5**, terminal β-D-glucopyranosyl. C-1‴—-6‴; α-L-rhamnopyranosyl, C-1‴—-5‴; β-D-xylopyranosyl. *a*—*d*) Assignments may be reversed in each column.

into the **10**-type diene saponins have been reported.[2,11] Contents of **1** and **3** in the roots were determined in the present study by means of HPLC and were compared with those of Japanese Mishima-Saiko, Chinese Pei-Chaihu and several other types of commercial Bupleuri Radix (see Table IV). The contents of both the saponins in Zuzhuye-Chaihu are similar to those of Mishima-Saiko and Pei-Chaihu, indicating that this plant should have medicinal value. Zhuye-Chaihu contains a large amount of **1**, while only a trace of **3** is present in this plant. The high contents of both saponins in Lijiang-Chaihu indicate that it should have excellent medicinal value, though it has not been utilized as a medicine because it is rather rare in nature.

It is noteworthy that the contents of the acetylated saponins in the plants of the present study are relatively higher than those in Mishima-Saiko and Pei-Chaihu. It is also notable that these acetylated saponins were gradually deacetylated during the storage of the dried roots. This was observed in the case of Lijiang-Chaihu by comparison of the thin layer chromatogram of the extract prepared from the dried roots just after collection with that after storage for more than one year (Fig. 4).

Experimental

General Procedures——NMR spectra were taken on JEOL FX-100 (^{1}H-NMR at 99.55 MHz and ^{13}C-NMR at 25.00 MHz) and JEOL GX-270 (^{1}H-NMR at 270 MHz and ^{13}C-NMR at 67.80 MHz) spectrometers in C_5D_5N and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectrum (MS) were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Trimethylsilylation for MS: see previous paper.[4]

Identification of the Known Saponins——Each saponin isolated in the present study was identified by comparison of the ^{1}H- and ^{13}C-NMR spectra, MS of the trimethylsilyl esters and optical rotation with those of an authentic sample.

TABLE IIIa. ^{13}C-NMR Chemical Shifts of Aglycone Moieties (Artificial Saponins) in C_5D_5N

	12	13	14	15	16	17	19	21	23	24	25	26	27
C-1	40.0	40.1	39.8	40.1	39.9	40.1	40.1	38.4	38.4	39.9	38.4	38.4	40.0
C-2	26.2	26.4	26.3	26.3	26.3	26.4	25.8	26.0	26.1	26.2	26.1	26.0	26.2
C-3	81.7	81.9	88.9	81.7	82.0	81.9	81.8	81.7	81.7	81.8	81.7	81.7	81.8
C-4	43.6	43.7	39.8	43.7	43.6	43.8	43.6	43.6	43.6	43.5	43.6	43.6	43.6
C-5	47.5	48.3	55.7	47.6	47.6	48.4	47.6	47.3	47.3	47.5	47.3	47.3	47.5
C-6	18.3	18.4	18.3	18.3	18.4	18.6	18.3	18.2	18.2	18.3	18.2	18.2	18.3
C-7	33.2	33.4	33.3	33.2	33.3	33.8	33.3	31.8	31.8	33.2	31.8	31.8	33.2
C-8	40.9	40.6	41.0	41.0	40.9	40.7	40.9	41.0	41.0	40.8	41.0	41.0	40.8
C-9	52.0	51.6	52.0	52.1	52.0	51.7	52.0	54.0	54.0	51.9	54.0	54.0	51.9
C-10	38.0	38.2	38.1	38.1	38.1	38.2	38.0	36.5	36.5	38.1	36.5	36.5	38.0
C-11	75.9	76.0	76.0	76.1	76.0	76.0	75.9	126.2	126.2	75.9	126.2	126.2	75.9
C-12	122.5	122.6	122.6	122.5	122.5	122.5	122.5	126.2	126.2	122.5	126.2	126.2	122.6
C-13	148.2	149.8	148.1	148.3	148.2	149.8	148.2	136.0	136.0	148.2	136.0	136.0	148.2
C-14	43.8[a)]	41.9	43.8[a)]	43.8[a)]	43.8[a)]	42.0	43.8[a)]	41.8	41.8	43.8[a)]	41.8	41.8	43.7[a)]
C-15	36.7	37.2	36.7	36.7	36.7	37.2	36.7	32.6	32.6	36.6	32.6	32.6	36.7
C-16	66.2	74.1	66.2	66.2	66.2	74.1	66.2	67.6	67.6	66.2	67.6	67.6	66.2
C-17	43.6[a)]	43.4	43.5[a)]	43.7[a)]	43.6[a)]	43.4	43.6[a)]	45.2	45.2	43.5[a)]	45.2	45.2	43.6[a)]
C-18	43.9[a)]	41.9	43.8[a)]	43.8[a)]	43.9[a)]	42.0	43.8[a)]	133.0	133.0	43.8[a)]	133.0	133.0	43.7[a)]
C-19	46.9	47.7	46.9	47.0	46.9	47.7	46.9	39.0	39.0	46.8	38.9	39.0	46.9
C-20	31.0	31.3	31.1	31.1	31.1	31.3	31.0	32.3	32.2	31.0	32.3	32.2	31.0
C-21	34.2	34.9	33.6	34.2	34.2	35.0	34.2	35.4	35.4	34.0	35.4	35.4	34.2
C-22	25.9	30.9	26.3	25.9	25.9	30.9	25.9	24.4	24.4	25.8	24.3	24.3	25.9
C-23	64.1	64.3	28.2	64.4	64.4	64.2	64.2	64.0	64.0	64.1	64.0	64.1	64.0
C-24	13.5	13.6	17.0	13.5	13.6	13.6	13.5	13.1	13.0	13.5	13.1	13.1	13.5
C-25	17.9	17.9	17.3	17.9	17.9	17.9	17.9	18.8	18.8	17.8	18.8	18.8	17.9
C-26	18.3	18.4	18.5	18.3	18.4	18.3	18.3	17.2	17.2	18.3	17.2	17.3	18.3
C-27	26.2	26.3	26.3	26.3	26.3	26.4	26.2	21.8	21.8	26.2	21.8	21.8	26.2
C-28	68.6	70.1	69.1	68.5	68.5	70.1	68.6	64.6	64.7	68.6	64.7	64.6	68.7
C-29	33.2	33.4	33.3	33.2	33.3	33.4	33.3	25.1	25.1	33.2	25.1	25.1	33.2
C-30	24.0	24.6	24.0	24.0	24.0	24.6	24.0	32.6	32.6	24.0	32.6	32.6	24.0
OCH_3	54.0	53.7	54.2	54.1	54.1	53.8	54.0			53.9			53.9

^{13}C-NMR spectra were observed at 22.5 °C. *a*) Assignments may be reversed in each column.

Hydrolysis of saponin and identification of the resulting monosaccharides: see previous paper.[4)]

Separation of Saponins of *B. marginatum* WALL *ex* DC. var. *stenophyllum* (WOLFF) SHAN *et* Y. LI——The roots (250 g) collected at Qiaojia, Yunnan, China, in October 1983, were extracted five times with MeOH at room temperature. The MeOH solution was concentrated to dryness *in vacuo* and a suspension of the residue (yield 16%) in H_2O was washed with Et_2O and then extracted with 1-BuOH saturated with H_2O. The BuOH layer was concentrated to dryness *in vacuo*, affording a saponin fraction (yield 5.6%), which was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (90 : 10 : 0.5→75 : 25 : 1.5, all homogeneous), yielding seven fractions, Fr. 1—7, in increasing order of polarity. These seven fractions were separated by repeated column chromatography on silica gel, by reverse-phase column chromatography on LiChroprep RP-8 (Merck) and finally by HPLC on TSK-Gel ODS-120A (see Fig. 1), affording **1**—**9**, **12**—**17**.

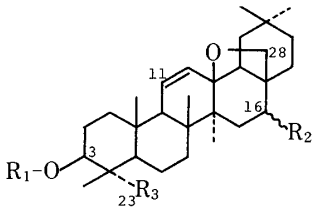
3″-*O*-Acetylsaikosaponin-a (**9**): A white powder, $[\alpha]_D^{18}$ +63.7° (*c*=2.0, MeOH). *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot 2H_2O$: C, 61.52; H, 8.68. Found: C, 61.22; H, 8.42. **14**: A white powder, $[\alpha]_D^{24}$ −33.0° (*c*=0.94, MeOH). **15**: A white powder, $[\alpha]_D^{24}$ +2.4° (*c*=2.5, MeOH). **16**: A white powder, $[\alpha]_D^{24}$ −26.9° (*c*=1.3, MeOH). **17**: A white powder, $[\alpha]_D^{24}$ 0° (*c*=1.0, MeOH).

Separation of Saponins of *B. marginatum* WALL *ex* DC.——The roots (510 g) collected at Kunming, Yunnan, China, in August 1983, were extracted six times with MeOH at room temperature. After removal of the solvent by evaporation, the MeOH ext. (yield 14.4%) was chromatographed on highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind., Tokyo, Japan) (10%, 50%, 75% aq. MeOH, MeOH and finally acetone), affording a crude saponin fraction from the eluates with 75% aq. MeOH and MeOH. The 75% aq. MeOH eluate and MeOH eluate were combined and further separated on DIAION HP-20 (50%, 65%, 85% aq. MeOH and MeOH), affording a crude saponin fraction from the eluate with 85% aq. MeOH (yield 1.9%). This fraction was separated by repeated column

TABLE IIIb. ^{13}C-NMR Chemical Shifts of Sugar Moieties (Artificial Saponins) in C_5D_5N

	12	**13**	**14**	**15**	**16**	**17**	**19**	**21**	**23**	**24**	**25**	**26**	**27**
C-1′	105.9[a]	105.8[a]	106.6	105.1[a]	105.7[a]	106.1[a]	106.0	105.8[a]	106.3	105.9	106.0	105.9[a]	105.9
C-2′	71.7	71.4	75.1	71.3[b]	75.0	71.5	71.4	71.4	71.7	71.5	71.5	71.4	71.5
C-3′	85.1	84.9	76.7	85.8	76.7	84.9	85.2	85.0	84.2	84.2	84.8	85.2	84.8
C-4′	71.7	72.0	79.8	71.7[b]	79.7	72.1	71.4	72.0	71.7	71.5	72.0	71.4	71.9
C-5′	70.9	70.8	75.4	70.9[c]	75.4	71.0	70.9	70.9	70.6	70.5	70.9	70.9	70.8
C-6′	17.1	17.2	68.5	17.3	68.7	17.3	17.3	17.2	17.2	17.2	17.2	17.3	17.2
C-1″	106.3[a]	106.2[a]	105.1	104.4[a]	105.2[a]	106.2[a]	106.0	106.2[a]	103.4	103.3	106.0	106.0[a]	105.9
C-2″	75.6	75.6	74.7	86.8	74.7	73.6	75.2	75.6	76.1	75.9	73.4	75.1	73.4
C-3″	78.2	78.2	78.2	77.5[d]	78.2	79.2	78.0	78.5	75.4	75.3	79.1	77.9	79.0
C-4″	72.0	72.0	71.3	70.9[b]	71.4	69.2	71.9	71.7	71.7	71.5	69.2	71.9	69.1
C-5″	78.6	78.5	78.2	78.3[d]	78.3	78.4	75.3	78.2	78.5	78.3	78.3	75.3	78.2
C-6″	62.6	62.5	62.5	62.1	62.5	62.0	64.7	62.5	62.3	62.2	61.9	64.6	61.9
C-1‴			102.8	107.8	102.9								
C-2‴			72.4	75.9	72.5								
C-3‴			72.4	77.7[d]	72.5								
C-4‴			73.7	70.4[c]	73.7								
C-5‴			70.5	67.5	70.5								
C-6‴			18.2		18.4								
$\underline{C}OOCH_3$						170.8	170.8		170.5	170.2	170.8	170.8	170.8
$COO\underline{C}H_3$						21.2	20.8		21.5	21.5	21.2	20.7	21.2

^{13}C-NMR spectra were observed at 22.5 °C. C-1′—-6′; **12**, **13**, **15**, **17**, **19**, **21**, **23**—**27**, β-D-fucopyranosyl; **14**, **16**, inner β-D-glucopyranosyl. C-1″—-6″; **12**, **13**, **15**, **17**, **19**, **21**, **23**—**27**, β-D-glucopyranosyl; **14**, **16**, terminal β-D-glucopyranosyl. C-1‴—-6‴; α-L-rhamnopyranosyl; C-1‴—-5‴; β-D-xylopyranosyl. *a*—*d*) Assignments may be reversed in each column.



	R_1	R_2	R_3
1:	–Fuc-(3)-Glc	–β-OH	$-CH_2OH$
2:	–Glc-(4,6)-Rha, Glc	–β-OH	$-CH_3$
3:	–Fuc-(3)-Glc	–α-OH	$-CH_2OH$
4:	–Fuc-(3)-Glc-(2)-Xyl	–β-OH	$-CH_2OH$
5:	–Glc-(4,6)-Rha, Glc	–β-OH	$-CH_2OH$
6:	–Fuc-(3)-Glc-(3)-Ac	–α-OH	$-CH_2OH$
7:	–Fuc-(3)-Glc-(6)-Ac	–β-OH	$-CH_2OH$
8:	–Fuc-(3)-Glc-(6)-Ac	–α-OH	$-CH_2OH$
9:	–Fuc-(3)-Glc-(3)-Ac	–β-OH	$-CH_2OH$
18:	–Fuc-(3)-Glc	–β-OH	$-CH_3$
20:	–Fuc	–β-OH	$-CH_2OH$
22:	–Fuc-(3)-Glc-(2)-Ac	–β-OH	$-CH_2OH$

Fuc: β-D-fucopyranosyl Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl Xyl: β-D-xylopyranosyl
Ac: acetyl

Chart 1

chromatography on silica gel, and by reverse-phase column chromatography on RP-8 (see Fig. 2), affording **1**, **2**, **5**, **7**, **12**, **14**, **18**—**20**.

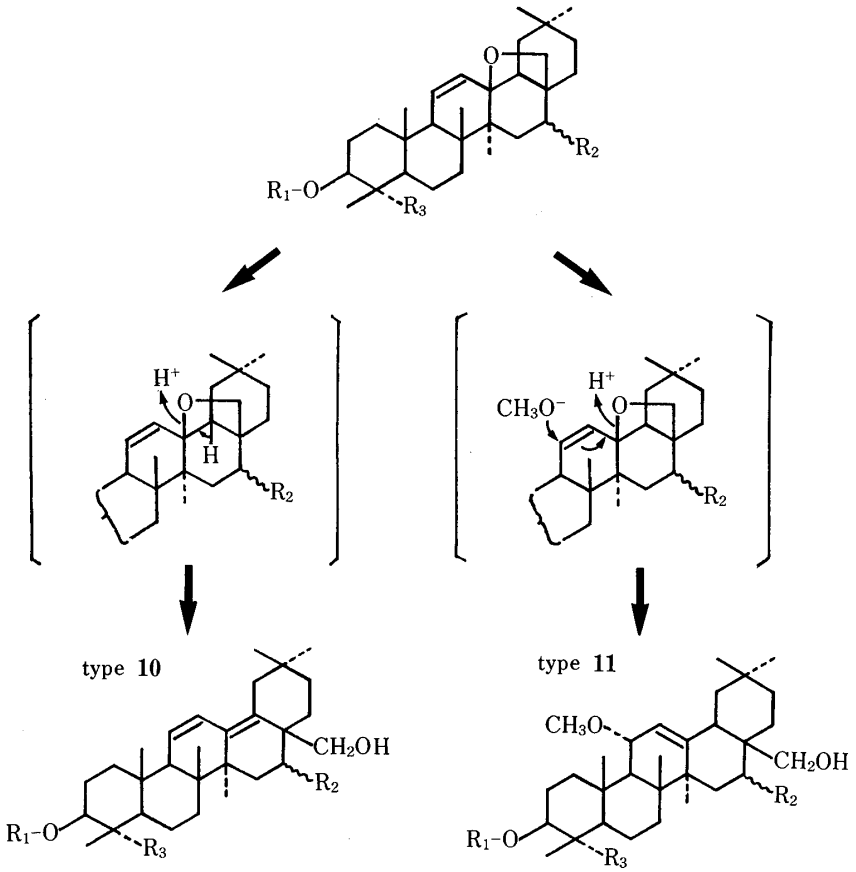
3-*O*-Fucopyranosylsaikogenin F (**20**): A white powder, $[\alpha]_D^{18}$ +68.3° (*c*=1.2, MeOH). *Anal.* Calcd for $C_{36}H_{58}O_8 \cdot 5/2H_2O$: C, 65.13; H, 9.56. Found: C, 65.02; H, 9.25. **19**: A white powder, $[\alpha]_D^{18}$ 0° (*c*=3.0, MeOH).

Separation of Saponins of *B. rockii* WOLFF——The roots (540 g) collected at Qiaojia, Yunnan, China, in October 1983, were extracted five times with MeOH at room temperature. After removal of the solvent by evaporation, the MeOH ext. (yield 23.8%) was chromatographed on DIAION HP-20 (H_2O, 50%, 80% aq. MeOH, MeOH and finally acetone), affording a crude saponin fraction from the eluate with 80% aq. MeOH (yield 4.3%). This fraction was

TABLE IV. Contents of Saikosaponin-a (**1**) and -d (**3**) (by HPLC Analysis[2])

	Bupleuri Radix			**1** (%)	**3** (%)
1	Mishima-Saiko, *B. falcatum*	(Japan)	Cultivated	0.38	0.52
2	Saiko-I	(Japan)	Commercial	0.52	0.63
3	Saiko-II	(Japan)	Commercial	0.24	0.25
4	Pei-Chaihu	(China)	Commercial	0.51	0.54
5	Zuzhuye-Chaihu, *B. marginatum* var. *stenophyllum*	(China)	Wild	0.54	0.55
6	Zhuye-Chaihu, *B. marginatum*	(China)	Wild	1.15	—
7	Lijiang-Chaihu, *B. rockii*	(China)	Wild	1.18	1.12
8	Siho-I	(Korea)	Commercial	0.60	0.45
9	Juk-Siho	(Korea)	Commercial	0.11	0.08
10	Siho-II	(Korea)	Commercial	0.58	0.62

—: trace.



	R_1	R_2	R_3
21:	–Fuc^{3}–Glc	–α-OH	$-CH_2OH$
23:	–Fuc^{3}–Glc^{2}–Ac	–α-OH	$-CH_2OH$
25:	–Fuc^{3}–Glc^{3}–Ac	–α-OH	$-CH_2OH$
26:	–Fuc^{3}–Glc^{6}–Ac	–α-OH	$-CH_2OH$

Fuc: β-D-fucopyranosyl
Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl
Xyl: β-D-xylopyranosyl
Ac: acetyl

	R_1	R_2	R_3
12:	–Fuc^{3}–Glc	–β-OH	$-CH_2OH$
13:	–Fuc^{3}–Glc	–α-OH	$-CH_2OH$
14:	–Glc^{4}(–Rha, ^{6}–Glc)	–β-OH	$-CH_3$
15:	–Fuc^{3}–Glc^{2}–Xyl	–β-OH	$-CH_2OH$
16:	–Glc^{4}(–Rha, ^{6}–Glc)	–β-OH	$-CH_2OH$
17:	–Fuc^{3}–Glc^{3}–Ac	–α-OH	$-CH_2OH$
19:	–Fuc^{3}–Glc^{6}–Ac	–β-OH	$-CH_2OH$
24:	–Fuc^{3}–Glc^{2}–Ac	–β-OH	$-CH_2OH$
27:	–Fuc^{3}–Glc^{3}–Ac	–β-OH	$-CH_2OH$

Chart 2

separated by repeated column chromatography on silica gel, and by reverse-phase column chromatography on RP-8 (see Fig. 3), affording **1**—**3**, **6**—**9**, **14**, **19**, **21**—**27**.

2″-*O*-Acetylsaikosaponin-a (**22**): A white powder, $[\alpha]_D^{18}$ +41.7° (c=2.1, MeOH). *Anal.* Calcd for $C_{44}H_{70}O_{14} \cdot 7/2H_2O$: C, 59.64; H, 8.76. Found: C, 59.60; H, 8.83. **23**: A white powder, $[\alpha]_D^{18}$ −8.8° (c=1.4, MeOH). **24**: A white powder, $[\alpha]_D^{18}$ 0° (c=1.0, MeOH). **25**: A white powder, $[\alpha]_D^{18}$ −4.6° (c=1.5, MeOH). **26**: A white powder, $[\alpha]_D^{18}$ −13.7° (c=1.6, MeOH). **27**: A white powder, $[\alpha]_D^{18}$ 0° (c=1.9, MeOH).

Deacetylation of 22——A solution of **22** (50 mg) in 2% KOH–MeOH (1.5 ml) was stirred at room temperature for 30 min. The reaction mixture was neutralized with Dowex 50W and concentrated to dryness. This reaction mixture was subjected to column chromatography on RP-8 (75% aq. MeOH) to give **1** (22 mg).

Quantitative Analysis of Bupleuri Radix——See previous paper.[2)]

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