Chemical Evaluation of Bupleurum Species Collected in Yunnan, China

JING-KAI DING, a HIROKO FUJINO (née KIMATA), b RYOJI KASAI, b NAOKO FUJIMOTO, b OSAMU TANAKA, a, b JUN ZHOU, a HIROMICHI MATSUURA c and TOHRU FUWA c

Kunming Institute of Botany, Academia Sinica, a Kunming, Yunnan, China, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, b Kasumi, Minami-ku, Hiroshima 734, Japan and Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd. f Shimokotachi 1624, Koda-cho, Takata-gun, Hiroshima 729-64, Japan

(Received August 24, 1985)

Saponins were isolated from roots of Bupleurum marginatum (Zhuye-Chaihu), B. marginatum var. stenophyllum (Zezhuuye-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: Zezhuuye-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-Saiko3) 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)
roots of *B. marginatum* Wall. *ex DC. var. stenophyllum* (Wolff) Shan et Y. Li

\[
\begin{align*}
\text{Et}_2\text{O layer} & \quad \text{aq. layer} \\
\text{BuOH ext. [14 g]} & \quad \text{column chromatography on silica gel} \\
(\text{CHCl}_3-\text{MeOH}-\text{H}_2\text{O} 90:10:5:75:25:1.5, \text{homogeneous}) \\
\end{align*}
\]

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% MeOH; [M80], 80% MeOH; [M75], 75% MeOH; [M70], 70% MeOH; [M65], 65% MeOH; [M60], 60% MeOH; [M55], 55% MeOH.

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. *stenophyllum* 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 (\(^{13}\text{C-NMR}\)) 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)_3Ac], 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
roots of *B. marginatum* WALL. *ex* DC.

\[
\begin{align*}
\text{[51.0 g]} & \quad \text{extd. with MeOH at room temperature} \\
\text{MeOH ext. [73 g]} & \quad 37 \text{ g} \\
\text{DIAION HP-20 column chromatography} & \\
\hline
\text{H}_{2}\text{O eluate [21.8 g]} & \quad 50\% \text{aq. MeOH} & \quad 75\% \text{aq. MeOH} & \quad \text{MeOH eluate [5.6 g]} & \quad \text{acetone eluate [1.6 g]} \\
\text{DIAION HP-20 column chromatography} & \\
\hline
\text{50\% aq. MeOH eluate [1.7 g]} & \quad 65\% \text{aq. MeOH eluate [4.8 g]} & \quad \text{MeOH eluate} \\
\text{Fr. 1} & \quad \text{Fr. 2} & \quad \text{Fr. 3} & \quad \text{Fr. 4} & \quad \text{Fr. 5} & \quad \text{Fr. 6} & \quad \text{Fr. 7} & \quad \text{Fr. 8} & \quad \text{Fr. 9} & \quad \text{Fr. 10} \\
\text{R [M70]} & \quad \text{R [M70]} & \quad \text{R [M70]} & \quad \text{R [M75]} & \quad \text{R [M75]} & \quad \text{R [M80]} & \quad \text{R [M75]} & \quad \text{R [M75]} & \quad \text{S [E-2]} & \\
\text{S [E-2]} & \quad \text{S [E-3]} & \quad \text{S [E-3]} & \quad \text{S [E-4]} & \quad \text{S [E-4]} & \quad \text{S [E-2]} & \quad \text{S [E-3]} & \quad \text{S [E-4]} & & \\
(0.026) & \quad (0.07) & \quad (0.18) & \quad (0.025) & \quad (0.067) & \quad (0.007) & \quad (0.01) & \quad (0.01) & & \\
\text{(0.013)} &
\end{align*}
\]

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

R: column chromatography on LiChrorep RP-8.
S: column chromatography on silica gel.
[M70], 70\% aq. MeOH; [M75], 75\% aq. MeOH; [M80], 80\% aq. MeOH.
[E-1], EtOAc-EtOH-\text{H}_{2}\text{O 8:2:1}; [E-2], EtOAc-EtOH-\text{H}_{2}\text{O 10:2:1}; [E-3], EtOAc-
EtOH-\text{H}_{2}\text{O 12:2:1}; [E-4], EtOAc-EtOH-\text{H}_{2}\text{O 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 \(^{13}\text{C}\)-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 \(^{13}\text{C}\)-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 \(^{13}\text{C}\)-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 \(\beta\)-d-fucopyranoside linked with the 3\(\beta\)-hydroxy group of an oleane-type triterpene. Based on this evidence, 20 can be formulated as 3-O-\(\beta\)-d-fucopyranosylsaikogenin F (=deshydroxy-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—
roots of *B. rockii* **WOLFF**

\[540 \text{ g}\]

\[
\text{exted. with MeOH at room temperature}
\]

\[
\text{MeOH ext. [129 g]}
\]

\[
65 \text{ g}
\]

DIAION HP-20 column chromatography

\[
\text{H}_2\text{O eluate [41.4 g]}
\]

\[
\text{50% aq. MeOH eluate [3.8 g]}
\]

\[
\text{80% aq. MeOH eluate [11.7 g]}
\]

\[
\text{MeOH eluate [3.0 g]}
\]

\[
\text{acetone eluate [1.9 g]}
\]

\[
4 \text{ g}
\]

R[M70]

Fr. 1 Fr. 2 Fr. 3 Fr. 4 Fr. 5 Fr. 6 Fr. 7 Fr. 8 Fr. 9

\[
\text{S[E-1]} \quad \text{S[E-3]} \quad \text{S[E-3]} \quad \text{S[E-3]} \quad \text{S[E-3]} \quad \text{S[E-4]} \quad \text{S[E-4]} \quad \text{S[E-2]} \quad \text{S[E-5]}
\]

\[
2 \quad 14 \quad 19 \quad 7 \quad 24 \quad 22 \quad 28 \quad (0.08)
\]

\[
(0.09) \quad (0.13) \quad (0.16) \quad (0.02) \quad (0.13) \quad (0.03) \quad (0.08)
\]

\[
9 \quad 23 \quad 1 \quad (0.02)
\]

\[
26 \quad 3 \quad (0.02) \quad (0.09)
\]

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$_2$O 9:2:1; [E-2], EtOAc-EtOH-H$_2$O 10:2:1; [E-3], EtOAc-EtOH-H$_2$O 12:2:1; [E-4], EtOAc-EtOH-H$_2$O 13:2:1; [E-5], EtOAc-EtOH-H$_2$O 14:2:1; [E-6], EtOAc-EtOH-H$_2$O 18:2:1; [E-7], EtOAc-EtOH-H$_2$O 20:2:1.

Solvents are all homogeneous.

\(\therefore\): 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$_2$O (8:2:1).

Detection: 10% H$_2$SO$_4$, heat.

---

**Table 1. Bupleurum spp. Distributed in Yunnan Province**

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bupleurum marginatum</em> WALL. ex DC.</td>
<td>(竹叶柴胡)<em>a</em></td>
</tr>
<tr>
<td><em>B. marginatum</em> WALL. ex DC. var. <em>stenophyllum</em> (WOLFF) SHAN et Y.LI (窄竹叶柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td><em>B. yunnanense</em> FRANCH. (雲南柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td><em>B. candelaei</em> WALL. ex DC. (川滇柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td><em>B. candelaei</em> WALL. ex DC. var. <em>atropurpureum</em> C. Y. WU (紫紅川滇柴胡)</td>
<td></td>
</tr>
<tr>
<td>*B. tenuif. BUCH.-HAM. ex D. DON (小柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td>*B. tenuif. BUCH.-HAM. ex D. DON var. <em>humble</em> FRANCH. (矮小柴胡)</td>
<td></td>
</tr>
<tr>
<td><em>B. longicaule</em> WALL. ex DC. var. <em>franchetii</em> de BOISS. (空心柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td><em>B. longicaule</em> WALL. ex DC. var. <em>amplicaula</em> C. Y. WU (有茎柴胡)</td>
<td></td>
</tr>
<tr>
<td><em>B. commelnoideum</em> de BOISS. (紫花楊枝柴胡)<em>a</em></td>
<td></td>
</tr>
<tr>
<td><em>B. rockii</em> WALL. (丽江柴胡)</td>
<td></td>
</tr>
<tr>
<td><em>B. dalhousieanum</em> (CLARKE) K.-POL. (枯枝柴胡)</td>
<td></td>
</tr>
<tr>
<td><em>B. petiolulatum</em> FRANCH. (有柄柴胡)</td>
<td></td>
</tr>
</tbody>
</table>

\(a\): Used as medicinal plants in Yunnan Province, China.
3, 6—9 and a new saponin (22). The presence of an acetoxy 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)3Ac], 421 [(Glc)(TMSi)2Ac] and 361 (421 - AcOH), indicating the location of the acetoxy 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. 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$_5$D$_5$N

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1'</td>
<td>105.9 (a)</td>
<td>106.6</td>
<td>105.9 (a)</td>
<td>104.9 (a)</td>
<td>105.7 (a)</td>
<td>106.1 (a)</td>
<td>105.8</td>
<td>105.9 (a)</td>
<td>106.1</td>
<td>106.8 (a)</td>
<td>106.3</td>
<td>106.0</td>
</tr>
<tr>
<td>C-2'</td>
<td>71.4</td>
<td>75.0</td>
<td>71.4</td>
<td>71.2 (b)</td>
<td>75.0</td>
<td>71.6</td>
<td>71.4</td>
<td>71.4</td>
<td>71.5</td>
<td>71.5</td>
<td>72.4</td>
<td>71.4</td>
</tr>
<tr>
<td>C-3'</td>
<td>85.0</td>
<td>76.7</td>
<td>85.0</td>
<td>85.7</td>
<td>76.7</td>
<td>84.9</td>
<td>85.1</td>
<td>85.2</td>
<td>84.8</td>
<td>85.1</td>
<td>75.5</td>
<td>84.2</td>
</tr>
<tr>
<td>C-4'</td>
<td>72.0</td>
<td>79.5</td>
<td>72.0</td>
<td>71.6 (b)</td>
<td>79.7</td>
<td>72.1</td>
<td>71.4</td>
<td>71.4</td>
<td>72.1</td>
<td>71.9</td>
<td>73.0</td>
<td>71.4</td>
</tr>
<tr>
<td>C-5'</td>
<td>71.0</td>
<td>75.4</td>
<td>70.9</td>
<td>70.8 (b)</td>
<td>75.4</td>
<td>71.0</td>
<td>70.7</td>
<td>70.8</td>
<td>70.9</td>
<td>71.0</td>
<td>71.3</td>
<td>70.6</td>
</tr>
<tr>
<td>C-6'</td>
<td>17.3</td>
<td>69.0</td>
<td>17.3</td>
<td>17.2</td>
<td>68.7</td>
<td>17.2</td>
<td>17.3</td>
<td>17.4</td>
<td>17.2</td>
<td>17.3</td>
<td>17.5</td>
<td>17.2</td>
</tr>
<tr>
<td>C-1''</td>
<td>106.3 (a)</td>
<td>104.9</td>
<td>106.3 (a)</td>
<td>104.3 (a)</td>
<td>105.0 (a)</td>
<td>106.2 (a)</td>
<td>105.8</td>
<td>106.0 (a)</td>
<td>106.1</td>
<td>106.6 (a)</td>
<td>103.4</td>
<td></td>
</tr>
<tr>
<td>C-2''</td>
<td>75.6</td>
<td>74.6</td>
<td>75.6</td>
<td>86.7</td>
<td>74.6</td>
<td>73.5</td>
<td>75.2</td>
<td>75.1</td>
<td>73.5</td>
<td>75.8</td>
<td>76.0</td>
<td></td>
</tr>
<tr>
<td>C-3''</td>
<td>78.3</td>
<td>78.2</td>
<td>78.6</td>
<td>77.6 (b)</td>
<td>78.2</td>
<td>79.2</td>
<td>77.9</td>
<td>77.9</td>
<td>79.1</td>
<td>78.4</td>
<td>75.4</td>
<td></td>
</tr>
<tr>
<td>C-4''</td>
<td>71.7</td>
<td>71.4</td>
<td>71.7</td>
<td>70.8 (b)</td>
<td>71.4</td>
<td>69.2</td>
<td>71.8</td>
<td>71.9</td>
<td>69.2</td>
<td>72.1</td>
<td>71.7</td>
<td></td>
</tr>
<tr>
<td>C-5''</td>
<td>78.3</td>
<td>78.2</td>
<td>78.2</td>
<td>78.1 (a)</td>
<td>78.3</td>
<td>78.5</td>
<td>75.2</td>
<td>75.3</td>
<td>78.4</td>
<td>78.7</td>
<td>78.5</td>
<td></td>
</tr>
<tr>
<td>C-6''</td>
<td>62.6</td>
<td>62.4</td>
<td>62.6</td>
<td>62.1</td>
<td>62.5</td>
<td>62.0</td>
<td>64.6</td>
<td>64.6</td>
<td>62.0</td>
<td>62.7</td>
<td>62.3</td>
<td></td>
</tr>
</tbody>
</table>

$^{13}$C-NMR spectra were observed at 22.5°C. C-1' -- C-6'; 1, 3, 4, 6--9, 18, 20, 22, β-D-fucopyranosyl; 2, 5, inner β-D-glucopyranosyl. C-1'' -- C-6''; 1, 3, 4, 6--9, 18, 20, 22, β-D-glucopyranosyl; 2, 5, terminal β-D-glucopyranosyl. C-1''' -- C-6''' d-α-l-rhamnopyranosyl, C-1''' -- C-5''' β-D-xylpyranosyl. a-d Assignments may be reversed in each column.

Into the 10-type diene saponins have been reported. 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. Zhuuye-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 (1H-NMR at 99.55 MHz and $^{13}$C-NMR at 25.00 MHz) and JEOL GX-270 (1H-NMR at 270 MHz and $^{13}$C-NMR at 67.80 MHz) spectrometers in C$_5$D$_5$N and chemical shifts are given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Trimethylsilylation for MS: see previous paper.5

Identification of the Known Saponins—Each saponin isolated in the present study was identified by comparison of the 1H- and $^{13}$C-NMR spectra, MS of the trimethylsilyl esters and optical rotation with those of an authentic sample.
Hydrolysis of saponin and identification of the resulting monosaccharides: see previous paper. 4)

Separation of Saponins of B. marginatum WALL ex DC. var. stenophyllum (WOLF) SHAN et Y. LI—The roots (250 g) collected at Qiaoja, 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 16.5%) in H₂O was washed with Et₂O and then extracted with 1-BuOH saturated with H₂O. 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 CHCl₃-MeOH-H₂O (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-Geô ODS-120A (see Fig. 1), affording 1—9, 12—17.

3°-O-Acetylsalikosaponin-a (9): A white powder, [α]D₂⁰ +63.7° (c=2.0, MeOH). Anal. Caled for C₃₄H₅₀O₁₄: 2H₂O: C, 61.52; H, 8.68. Found: C, 61.22; H, 8.42. 14: A white powder, [α]D₂⁰ +33.0° (c=0.94, 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. 13C-NMR Chemical Shifts of Sugar Moieties (Artificial Saponnins) in C3D2N

<table>
<thead>
<tr>
<th></th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>21</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1'</td>
<td>105.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.6</td>
<td>105.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.0</td>
<td>105.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.3</td>
<td>105.9</td>
<td>106.0</td>
<td>105.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105.9</td>
<td></td>
</tr>
<tr>
<td>C-2'</td>
<td>71.7</td>
<td>71.4</td>
<td>75.1</td>
<td>71.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.0</td>
<td>71.5</td>
<td>71.4</td>
<td>71.4</td>
<td>71.7</td>
<td>71.5</td>
<td>71.5</td>
<td>71.4</td>
<td>71.5</td>
<td></td>
</tr>
<tr>
<td>C-3'</td>
<td>85.1</td>
<td>84.9</td>
<td>76.7</td>
<td>85.8</td>
<td>76.7</td>
<td>84.9</td>
<td>85.2</td>
<td>85.0</td>
<td>84.2</td>
<td>84.2</td>
<td>84.8</td>
<td>85.2</td>
<td>84.8</td>
<td></td>
</tr>
<tr>
<td>C-4'</td>
<td>71.7</td>
<td>72.0</td>
<td>79.8</td>
<td>71.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.7</td>
<td>72.1</td>
<td>71.4</td>
<td>72.0</td>
<td>71.7</td>
<td>71.5</td>
<td>72.0</td>
<td>71.4</td>
<td>71.9</td>
<td></td>
</tr>
<tr>
<td>C-5'</td>
<td>70.9</td>
<td>70.8</td>
<td>75.4</td>
<td>70.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.4</td>
<td>71.0</td>
<td>70.9</td>
<td>70.9</td>
<td>70.6</td>
<td>70.5</td>
<td>70.9</td>
<td>70.9</td>
<td>70.8</td>
<td></td>
</tr>
<tr>
<td>C-6'</td>
<td>17.1</td>
<td>17.2</td>
<td>68.5</td>
<td>17.3</td>
<td>68.7</td>
<td>17.3</td>
<td>17.3</td>
<td>17.2</td>
<td>17.2</td>
<td>17.2</td>
<td>17.2</td>
<td>17.2</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>C-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>106.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.1</td>
<td>104.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.0</td>
<td>106.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.4</td>
<td>103.3</td>
<td>106.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.6</td>
<td>75.6</td>
<td>74.7</td>
<td>86.8</td>
<td>74.7</td>
<td>73.6</td>
<td>75.2</td>
<td>75.6</td>
<td>76.1</td>
<td>75.9</td>
<td>73.4</td>
<td>75.1</td>
<td>73.4</td>
<td></td>
</tr>
<tr>
<td>C-3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.2</td>
<td>87.2</td>
<td>87.2</td>
<td>77.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.2</td>
<td>79.2</td>
<td>78.0</td>
<td>78.5</td>
<td>75.4</td>
<td>75.3</td>
<td>79.1</td>
<td>77.9</td>
<td>79.0</td>
<td></td>
</tr>
<tr>
<td>C-4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.0</td>
<td>72.0</td>
<td>71.3</td>
<td>70.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.4</td>
<td>69.2</td>
<td>71.9</td>
<td>71.7</td>
<td>71.7</td>
<td>71.5</td>
<td>69.2</td>
<td>71.9</td>
<td>69.1</td>
<td></td>
</tr>
<tr>
<td>C-5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.6</td>
<td>78.5</td>
<td>78.2</td>
<td>78.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.3</td>
<td>78.4</td>
<td>75.3</td>
<td>78.2</td>
<td>78.5</td>
<td>78.3</td>
<td>78.3</td>
<td>75.3</td>
<td>78.2</td>
<td></td>
</tr>
<tr>
<td>C-6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.6</td>
<td>62.5</td>
<td>62.5</td>
<td>62.1</td>
<td>62.5</td>
<td>62.0</td>
<td>64.7</td>
<td>62.5</td>
<td>62.3</td>
<td>62.2</td>
<td>61.9</td>
<td>64.6</td>
<td>61.9</td>
<td></td>
</tr>
<tr>
<td>C-1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.8</td>
<td>107.8</td>
<td>102.9</td>
<td>72.4</td>
<td>75.9</td>
<td>72.5</td>
<td>72.4</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5</td>
<td>73.7</td>
<td>70.4</td>
<td>73.7</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>C-2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.4</td>
<td>75.9</td>
<td>72.5</td>
<td>72.4</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5</td>
<td>73.7</td>
<td>70.4</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>C-3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.4</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5</td>
<td>72.4</td>
<td>77.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.5</td>
<td>73.7</td>
<td>70.4</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>C-4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.7</td>
<td>70.4</td>
<td>73.7</td>
<td>73.7</td>
<td>70.4</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>C-5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.5</td>
<td>67.5</td>
<td>70.5</td>
<td>70.5</td>
<td>67.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td>70.5</td>
<td></td>
</tr>
<tr>
<td>C-6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.2</td>
<td>18.4</td>
<td>18.4</td>
<td></td>
</tr>
</tbody>
</table>

COO<sub>CH</sub><sub>3</sub> ........................................ 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8 170.8


13C-NMR spectra were observed at 22.5 °C. C-1'—6'; 12, 13, 15, 17, 19, 21, 23—27, β-D-frucopyranosyl; 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-xylpyranosyl. a—d) Assignments may be reversed in each column.

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-Fucopyranosylaikogenin F (20): A white powder, [α]<sub>D</sub><sup>18</sup> +68.3° (c=1.2, MeOH). Anal. Caled for C<sub>36</sub>H<sub>38</sub>O<sub>16</sub>; 5/2H<sub>2</sub>O: C, 65.13; H, 9.56. Found: C, 65.02; H, 9.25. 19: A white powder, [α]<sub>D</sub><sup>18</sup> 0° (c=3.0, MeOH).

Separation of Saponnins of B. rockii VOLFF—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<sub>2</sub>O, 50%, 80% eq. MeOH, MeOH and finally acetone), affording a crude saponnin fraction from the eluate with 80% eq. MeOH (yield 4.3%). This fraction was
Table IV. Contents of Saikosaponin-a (I) and -d (3) (by HPLC Analysis*)

<table>
<thead>
<tr>
<th></th>
<th>Bupleuri Radix</th>
<th>1 (%)</th>
<th>3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mishima-Saiko, B. falcatum (Japan)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Saiko-I (Japan)</td>
<td>0.38</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>Saiko-II (Japan)</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>4</td>
<td>Pei-Chaihu (China)</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>Zuzhuye-Chaihu, B. marginatum var. stenophyllum (China)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Zhuye-Chaihu, B. marginatum (China)</td>
<td>0.51</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>Lijiang-Chaihu, B. rokitii (China)</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Siho-I (Korea)</td>
<td>1.18</td>
<td>1.12</td>
</tr>
<tr>
<td>9</td>
<td>Juk-Siho (Korea)</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>10</td>
<td>Siho-II (Korea)</td>
<td>0.11</td>
<td>0.08</td>
</tr>
</tbody>
</table>

--- trace.

---

R₁-O

** type 10 **

R₁-O

** type 11 **

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

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, [α]_D^28 +41.7° (c = 2.1, MeOH). Anal. Caled for C_{44}H_{30}O_{14}: 7/2H_2O: C, 59.64; H, 8.76. Found: C, 59.60; H, 8.83. 23: A white powder, [α]_D^28 +8.8° (c = 1.4, MeOH). 24: A white powder, [α]_D^28 0° (c = 1.0, MeOH). 25: A white powder, [α]_D^28 -4.6° (c = 1.5, MeOH). 26: A white powder, [α]_D^28 -13.7° (c = 1.6, MeOH). 27: A white powder, [α]_D^28 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).

Acknowledgement — Our thanks are due to Emeritus Professor Chen-Yie Wu, Former Director of Kunming Institute of Botany, Academia Sinica, for his valuable advice. This study was financially supported by a Grant-in-Aid for Encouragement of Young Scientists by the Ministry of Education, Science and Culture to H. Fujino (née Kimata) (No. 59771645 in 1984). J.-K. Ding is grateful to Wako Pharmaceutical Co., Ltd. for financial support during his study at Hiroshima University.

References