

## Phenolic Compounds of the roots of *Bupleurum longeradiatum*

Jae Sue Choi<sup>§</sup> and Won Sick Woo

Natural Productions Research Institute,  
Seoul National University, Seoul 110-460, Korea  
(Received July 27, 1989)

**Abstract** □ Emodin-8-O- $\beta$ -D-glucoside and narcissin were isolated from the roots of *Bupleurum longeradiatum*.

**Keywords** □ *Bupleurum longeradiatum*, Umbelliferae, emodin-8-O- $\beta$ -D-glucoside, narcissin, saikosaponin b<sub>2</sub>.

*Bupleuri Radix* is a very important crude drug in traditional Chinese medicine as an antifebrile and the enhancement of liver activity and the roots of *Bupleurum falcatum* have been used as a source of this drug in Korea. The roots of *Bupleurum longeradiatum* has, occasionally, been substituted for those of *B. falcatum* because of shortage of domestic supply, although it was reported that the pharmacologically active representative components of both plants were different.<sup>1,2)</sup>

The previous authors reported the isolation of polyacetylene,<sup>3)</sup> sterol<sup>4)</sup> and saponins<sup>5)</sup> from the roots of *B. longeradiatum*. The present paper deals with the isolation and identification of an anthraquinone glucoside and a flavonol glycoside from this plant part. Column chromatography of ethylacetate soluble fraction obtained from the methanol extract afforded two phenolic compounds (A and B) together with a known artifact saponin, saikosaponin b<sub>2</sub> which was identified by comparison of its NMR with reported data.<sup>6)</sup>

Compound A, mp 198-200°, showed characteristic positive color test (Bornträger) for hydroxyanthraquinone and showed absorption bands at 224, 273, 288 and 432 nm (log  $\epsilon$  4.47, 4.28, 4.30 and 3.91). Its IR showed the presence of a hydroxyl (3400 cm<sup>-1</sup>), two kinds of ketones (1660 and 1630 cm<sup>-1</sup>) and a glycoside bond (1000-1100 cm<sup>-1</sup>), indicating that compound A was 1,8 dihydroxyanthraquinone, one of  $\alpha$  OH groups was glycosylated.<sup>7)</sup> Its NMR showed a singlet at  $\delta$  2.40, two *meta*-coupled

doublets each of one proton with a J value of 2Hz at  $\delta$  6.98 and 7.27 and a couple of broad singlets each of one proton, at  $\delta$  7.15 and 7.47, indicating the presence of 1,6,8-trihydroxy-3-methyl system.<sup>7)</sup>

Acid hydrolysis of compound A afforded emodin, mp 254-255°, which was identified by direct comparison with authentic sample (mmp and co-TLC) and glucose as the sugar component. The attachment of the glucose moiety was established as 8-position by comparing the NMR data of the peracetate (see Experimental) with the data previously reported<sup>8)</sup> and confirmed by direct comparison with an authentic sample of emodin-8-O- $\beta$ -D-glucoside.<sup>9)</sup> This is the first report of the occurrence of anthraquinone in Umbelliferae family.<sup>10-12)</sup>

Compound B, mp 180-182°, gave characteristic flavonoid color reactions, pink with Zn + HCl and Mg + HCl, dark blue with FeCl<sub>3</sub> and a positive Molisch test and showed the presence of a hydroxyl (3300 cm<sup>-1</sup>),  $\alpha$ ,  $\beta$ -unsaturated ketone (1650 cm<sup>-1</sup>), aromatic systems (1595 and 1490 cm<sup>-1</sup>) and glycoside bond (1000-1100 cm<sup>-1</sup>) in its IR spectrum. The UV maxima at 256 and 357 nm were very similar to those reported for a number of 3-hydroxy substituted flavonols.<sup>13)</sup> It showed a bathochromic shift with AlCl<sub>3</sub> and AlCl<sub>3</sub> + HCl in band I and with NaOAc in band II which indicated the presence of free 5-hydroxy and 7-hydroxyl groups.<sup>13)</sup> A bathochromic shift of the UV with NaOEt, with an increase in intensity of band I, suggesting the presence of a free 4'-hydroxyl group.<sup>13)</sup> The <sup>1</sup>H-NMR in DMSO-d<sub>6</sub> showed a methoxy signal at  $\delta$  3.81, two *meta*-coupled doublets of one proton each at  $\delta$  6.17 (J = 1.9 Hz, H-6) and 6.40 (J = 1.9 Hz, H-8), one *ortho*-coupled doublet of one proton at  $\delta$  6.90

<sup>§</sup>Present address: Department of Nutrition and Food Science, National Fisheries University of Pusan, Pusan 608-023, Korea

( $J = 8.5$  Hz, H-5') a double-doublet of one proton at  $\delta$  7.52 ( $J = 1.8$  and 8.5 Hz, H-6'), a *meta*-coupled doublet of one proton at  $\delta$  7.84 ( $J = 1.8$  Hz, H-2') and two anomeric proton signals at  $\delta$  4.40 (broad singlet) and 5.39 (d,  $J = 6.8$  Hz). These data indicated that compound B was a 3, 5, 7, 3', 4'-oxygenated flavonoid with two sugars. The appearance of the H-2' signal at lower field than the H-6' signal suggested the presence of a 3'-methoxy-4'-hydroxy moiety in the B ring.<sup>13)</sup>

Acid hydrolysis of compound B afforded isorhamnetin, mp 305°, which was identified by direct comparison with an authentic sample<sup>14)</sup> (mmp and co-TLC) and glucose and rhamnose as the sugar components. The sugar-sugar linkage of disaccharide was deduced from its <sup>13</sup>C-NMR. All the carbon signals for rhamnose were similar to those of methyl-rhamnoside, implying a terminal sugar and the signal for primary alcohol carbon of the glucose was appeared at the low field (67.3 ppm), indicating that the disaccharide should be rutinose. Therefore, the structure of compound B was elucidated as isorhamnetin-3-O- $\beta$ -rutinoside (narcissin).

## EXPERIMENTAL METHODS

The mps were taken on a Mitamura-Riken apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Perkin-Elmer model 283 B spectrophotometer and the UV spectra were run with Gilford System 2600 spectrotometer. The NMR spectra were recorded on a Varian FT-80A.

### Plant material

The dried roots of *B. longeradiatum* were purchased from a crude drug market in Seoul and identified botanically by Prof. H.J. Chi. A voucher specimen is deposited in the herbarium of the Institute.

### Isolation of the compounds

Powdered roots (4.5kg) were extracted with MeOH. The MeOH extract (500g) was partitioned with CHCl<sub>3</sub> and then EtOAc. The EtOAc phase was concentrated *in vacuo* (26g) and subjected to silica gel column chromatography with CHCl<sub>3</sub>-MeOH (gradient) followed by Sephadex LH-20 with MeOH to yield three compounds.

Compound A (emodin-8-O- $\beta$ -D-glucoside); orange needles from MeOH, mp 198-200°, IR, UV and <sup>1</sup>H NMR: see text.

Compound B (narcissin); Crystallized from MeOH as yellowish needles, mp 180-182°, UV  $\lambda_{max}^{MeOH}$  nm(log  $\epsilon$ ): 256(4.53), 270.5(4.56), 357(4.44);

with NaOMe 270.5(4.64), 327.5(4.21), 415(4.64); with NaOAc 275.5 (4.6) 322(4.31), 375.5(4.39); with AlCl<sub>3</sub> 269 (4.58), 303(4.25), 373(4.42), 403 (4.47); with AlCl<sub>3</sub> + HCl 269(4.54), 303(4.22), 363 (4.43), 400.5(4.42), IR and <sup>1</sup>H NMR; see text.

<sup>13</sup>C NMR (DMSO-d<sub>6</sub>): 17.8(C-6'''), 56.2(OCH<sub>3</sub>), 67.3(C-6'''), 68.5(C-5'''), 70.1(C-4''), 70.5(C-3'''), 70.8(C-2'''), 72.1(C-4'''), 74.5(C-2''), 76.1(C-5''), 76.6(C-3''), 94.3(C-8), 99.2(C-6), 101.1(C-1'''), 101.7(C-1''), 104.2(C-10), 113.8(C-2''), 115.5(C-5'), 121.5(C-6'), 122.7(C-1'), 133.5(C-3), 147.2(C-3'), 149.6(C-4'), 156.7(C-2), 156.9(C-5), 161.2(C-9), 164.9(C-7), 177.5(C-4).

Saikosaponin b<sub>2</sub>; Colorless powder from MeOH, m; 217-221°, UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 245(4.35), 253 (4.41), 262(4.22); IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3400(OH), 1630 (C=C), 1000-1100 (glycoside); <sup>13</sup>C NMR (pyridine-d<sub>5</sub>)  $\delta$ : 12.9(C-24), 17.0(C-6'), 17.5(C-16), 18.6(C-25), 18.8(C-6), 22.3(C-27), 24.9(C-22), 25.3(C-29), 25.9(C-2), 32.5(C-7, 30), 32.8(C-15, 20), 35.8(C-21), 36.9(C-10), 38.7(C-1), 39.2(C-19), 41.4(C-8), 42.2(C-14), 43.7(C-4), 45.5(C-17), 48.2(C-5), 54.2 (C-9), 63.1(C-6''), 65.3(C-23), 65.6(C-28), 68.8(C-16), 71.0(C-5'), 71.8(C-2'), 72.1(C-4', 4''), 75.6 (C-2''), 78.1(C-5''), 78.2(C-3''), 82.6(C-3), 85.2 (C-3'), 105.5(C-1'), 105.9(C-1''), 126.3(C-11, 12), 133.0(C-18), 137.1(C-13).

### Acid hydrolysis of compound A

Compound A (40 mg) was refluxed with 5% H<sub>2</sub>SO<sub>4</sub> for 1 hr. After cooling, the reaction mixture was filtered to give emodin, mp 249-251°, <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 2.40(3H, s, Ar-CH<sub>3</sub>), 6.57 (1H, d,  $J = 2.5$  Hz, H-7), 7.02 (1H, brs, H-2), 7.17(1H, d,  $J = 2.5$  Hz, H-5), 7.40(1H, brs, H-4), 11.94(1H, s, OH) and 12.01(1H, s, OH).

The filtrate was neutralized with BaCO<sub>3</sub>, filtered and concentrated *in vacuo*. The residue was found to contain D-glucose by PC (pyridine-EtOAc-HOAc-H<sub>2</sub>O = 5:5:1:3) with the aid of the authentic sample.

### Acetylation of compound A

Compound A (10mg) was refluxed with Ac<sub>2</sub>/pyridine (0.4 ml each) for 2 hr. The reaction mixture was followed by the usual work-up and subjected to spectroscopy.

IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 1745, 1220 (acetate); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 2.03(6H, s, CH<sub>3</sub>CO  $\times$  2), 2.05(3H, s, CH<sub>3</sub>CO), 2.09(3H, s, CH<sub>3</sub>CO), 2.30(3H, s, Ar-CH<sub>3</sub>), 2.45(3H, s, CH<sub>3</sub>CO), 2.49(3H, s, CH<sub>3</sub>CO), around 4.20(3H, H-5' and 6'), 5.0-5.5(4H, H-1', 2', 3', 4'), 7.25(1H, brs, H-2), 7.30(1H, d,  $J = 2$

Hz, H-7), 7.75(1H, d, J = 2 Hz, H-5), 7.95(1H, brs, H-4).

#### Acid hydrolysis of compound B

A solution of compound B (100 mg) in 5% H<sub>2</sub>SO<sub>4</sub> (20 ml) was refluxed for 4 hr. The solid after cooling and separating was crystallized from MeOH to give yellowish needles of isorhamnetin, mp 305 °, <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ; 3.79(3H, s, OCH<sub>3</sub>), 6.15 (1H, d, J = 2 Hz, H-6), 6.48(1H, d, J = 2 Hz, H-8), 6.85 (1H, d, J = 8 Hz, H-5'), 7.55(1H, dd, J = 2 and 8 Hz, H-6'), 7.72(1H, d, J = 2 Hz, H-2').

The filtrate was neutralized with BaCO<sub>3</sub>, filtered and concentrated *in vacuo*. The residue was found to contain D-glucose and L-rhamnose by PC with the aid of authentic samples.

#### ACKNOWLEDGEMENT

This work was supported in part by a research grant from KOSEF.

#### LITERATURE CITED

1. Shibata, S., Kitagawa, I., Takahashi, R. and Fujimoto, H.: The chemical studies on oriental plant drugs. XIV. The constituents of *Bupleurum* spp. (1). *Yakugaku Zasshi* **86**, 1132 (1966).
2. Kimata, H., Hiyama, C., Yahara, S., Tanaka, O., Ishikawa, O. and Aiura, M.: Application of high performance liquid chromatography to the analysis of crude drugs; Separatory determination of saponins of *Bupleuri Radix*. *Chem. Pharm. Bull.* **27**, 1836 (1979).
3. Zhao, J., Guo, Y. and Meng, X.: Poisonous constituents of *Bupleurum longiadiatum*. *Shenyang Yaoxueyuan Xuebao* **2**, 301 (1985).
4. Tomitatsu, T.: Isolation of sucrose and α-spinasterol from the roots of *Bupleurum longiadiatum* Turcz. *Yakugaku Zasshi* **89**, 589

- (1969).
5. Kimata, H., Kasai, R. and Tanaka, O.: Saponins of Juk-siho and roots of *Bupleurum longiadiatum* Turcz. *Chem. Pharm. Bull.* **30**, 4373 (1982).
6. Ishii, H., Nakamura, M., Seo, S., Tori, K., Tozyo, T. and Yoshimura, Y.: Isolation, characterization and nuclear magnetic resonance spectra of new saponins from the roots of *Bupleurum falcatum* L. *Chem. Pharm. Bull.* **28**, 2367 (1980).
7. Thomson, R.H.: Naturally Occurring Quinones, Academic Press, London (1971).
8. Steglich, W. and Losel, W.: Bestimmung der Stellung von O-Substituenten bei 1,8-Dihydroxy-Anthrachinon-Derivaten mit Hilfe der NMR-Spektroskopie, *Tetrahedron* **25**, 4391 (1969).
9. Kang, S.S. and Woo, W.S.: Anthraquinones from the leaves of *Polygonum sachalinense*. *Kor. J. Pharmacogn.* **13**, 7 (1982).
10. Hegnauer, R.: Chemical Patterns and relationships of Umbelliferae. In; The biology and chemistry of the Umbelliferae (Ed. Heywood, V.H.), Academic Press, London, p. 267 (1971).
11. Carbonnier, J. and Cauwet-Marc, A.-M.: Constituants du genre *Bupleurum* L. (Umbelliferae): mise au point des connaissances actuelles. *Bull. Mus. Natn. Hist. Nat.* **1**, 213 (1979).
12. Thomson, R.H.: Naturally occurring quinones. III. Recent advances. Chapman and Hall, London (1987).
13. Mabry, T.J., Markham, K.R. and Thomas, M.B.: The systematic identification of flavonoids, Springer Verlag, N.Y. (1970).
14. Kang, S.S., Choi, J.S., Woo, W.S. and Chi, H.J.: Isorhamnetin glycosides from the leaves of *Typha latifolia*, *Kor. J. Pharmacogn.* **14**, 137 (1983).