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# Inhibition of Mast Cell Histamine Release by 2,6-Dimethoxy-*p*-benzoquinone Isolated from *Berchemia racemosa*

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The MeOH extract of *Berchemia racemosa* has an inhibitory activity on histamine release from rat mast cells. From the active fraction of the extract, 2,6-dimethoxy-*p*-benzoquinone was isolated. It shows strong inhibition of histamine release from rat mast cells induced by compound 48/80 and by concanavalin A, with $IC_{50}$ values of 6 and 8 $\mu$M, respectively.

**Keywords**——*Berchemia racemosa*; Rhamnaceae; benzoquinone; 2,6-dimethoxy-*p*-benzoquinone; mast cell; histamine release inhibitor

As a continuation of our studies to search for pharmacologically active constituents in natural sources, we investigated the active constituents of an indigenous crude drug, *Berchemia racemosa* SIEB. *et* ZUCC. (Rhamnaceae) by means of a previously described bioassay system for inhibition of histamine release from rat mast cells.[1,2]

The stems of *Berchemia racemosa* are locally used for the treatment of gall stone, liver diseases, neuralgia and stomach cramp, and the related plant, *B. floribunda*, has been used in traditional Chinese medicine as an antipyretic, a diuretic and for the treatment of rheumatism and lumbago.[3]

In our bioassay, the MeOH extract of the plant showed activity to inhibit the histamine release from rat mast cells induced by compound 48/80 and by concanavalin A. Therefore, the stems of *Berchemia racemosa* were extracted with MeOH and the extract was further fractionated by using several solvents, with monitoring of the bioactivity of each fraction. From the most active ethyl acetate-soluble fraction, a crystalline compound **1** was isolated (Fig. 1).

Compound **1** (mp 179—182 °C) has characteristic ultraviolet (UV) absorptions at 285 nm (log $\varepsilon$: 4.08) and 375 nm (log $\varepsilon$: 2.48), and gave a simple proton nuclear magnetic resonance ($^1$H-NMR) spectrum (in $CDCl_3$, $\delta$ 3.83, s, 6H and 5.86, s, 2H) and carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum (in $CDCl_3$, $\delta$ 186.2, s, 176.0, s, 156.8, s, 107.1, d, and 56.3, q).

From these data, **1** is strongly suggested to be 2,6-dimethoxy-*p*-benzoquinone, and this was confirmed by direct comparison (thin layer chromatography (TLC) behavior, infrared (IR), UV, $^1$H-NMR, $^{13}$C-NMR, mass spectrum (MS) and mixed melting point) with an authentic sample.[4]

The inhibitory activity of **1** on histamine release from rat mast cells was measured at various concentrations (Fig. 2). The results showed that compound **1** had strong inhibitory activity on histamine release caused by two different agents, and the activities were clearly dose-dependent. Among many compounds tested at various times in our laboratory, **1** appeared to be one of the most effective. The $IC_{50}$'s of **1** were 8 $\mu$M for concanavalin A, and

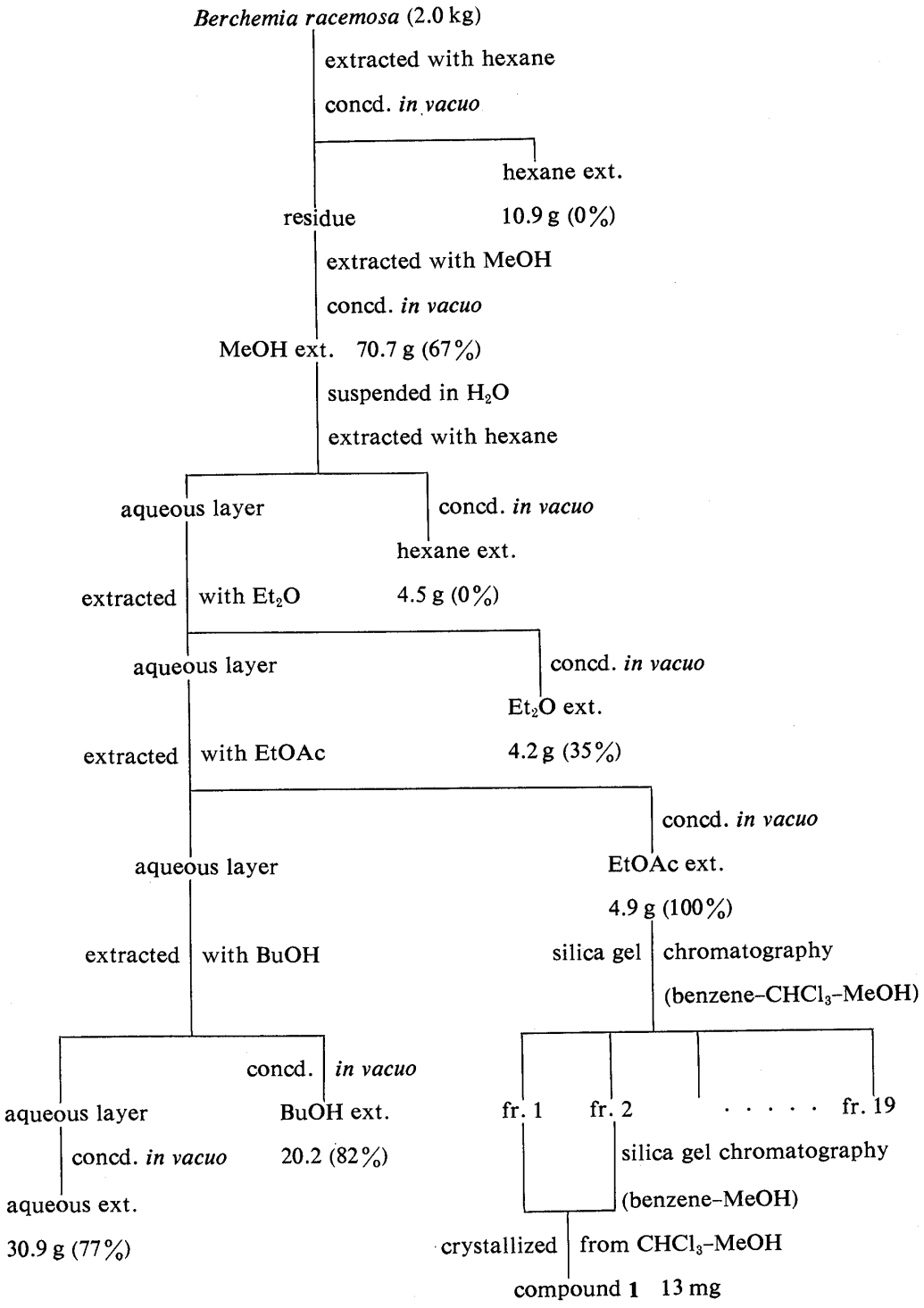


Fig. 1. Fractionation of Biologically Active Constituents of *Berchemia racemosa*

Values in parentheses indicate the inhibitory activities of each fraction on histamine release from rat mast cells at the concentration of 0.1 mg of extract in 1 ml of assay medium with compound 48/80. For compound **1**, see Fig. 2.

6 $\mu$M for compound 48/80, which are comparable to those of baicalin (3 and 4 $\mu$M, respectively). The flavonoid glucuronide, baicalin has the most potent antihistamine release activity so far found among natural products. Recently, fairly strong activities were reported for several flavonoids and biflavonoids, using compound 48/80 and ionophore A23187 as releasers. However, as far as the $IC_{50}$ values with compound 48/80 are concerned, all of the

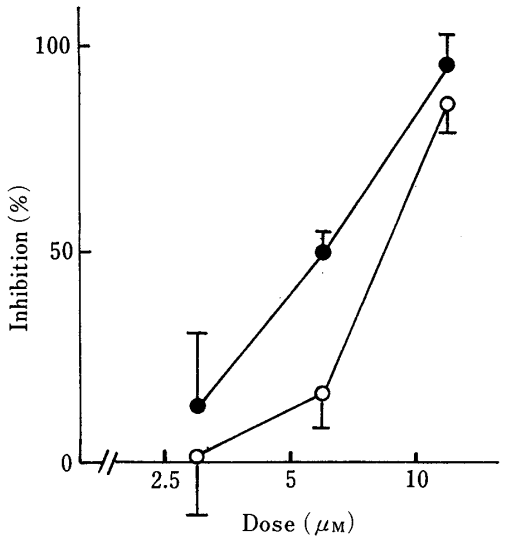


Fig. 2. Effect of 2,6-Dimethoxy-*p*-benzoquinone (**1**) on the Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 (●) and by Concanavalin A (○)

Values are means of six and eight duplicated experiments with compound 48/80 and concanavalin A, respectively.

tested compounds were less active than **1**, the most active of them being luteorin ($IC_{50}$: 10 μM).[5]

While **1** is known to occur in a wide variety of plants *i.e.* genera *Adonis*,[6] *Euricoma*,[7] *Acer*,[4] *Betula*,[8] *Marsdenia*,[9] *Fraxinus*,[10] *Xanthoxylum*,[11] and fungus, *Polyporus fumosus*,[12] this is the first report of its isolation from a plant belonging to Rhamnaceae.

With respect to other biological activities of **1**, it was reported that **1** showed weak inhibition of the growth of gram-positive bacteria, *Staphyloccocus aureus* and *Escherichia coli* (minimum effective concentrations: 62.5 and 1000 μM, respectively),[13] and of the fungus *Trichophyton interdigitale* (minimum inhibition concentration: 300 μM).[8] Antitumor activity of **1** was also observed against Ehrlich ascites carcinoma.[13] Furthermore, **1** inhibited adenosine 3′,5′-cyclic monophosphate (c-AMP) phosphodiesterase activity ($IC_{50}$ = 150 μM).[14] Oral administration of **1** to intact rats at a dose of 50 mg/kg inhibited the tissue granulation induced by the subcutaneous implantation of a cotton pellet.[4] Inhibition by **1** of platelet aggregation induced by arachidonic acid and collagen was reported at concentrations of 145 and 49 μM ($IC_{50}$ values), respectively.[10]

Our results on the inhibition of histamine release from mast cells may be related to some of these biological activities. The mechanism of inhibition of histamine release of **1** may involve the electron transfer system in the mast cells.

Compound **1** was also isolated from the BuOH fraction, and the investigation of other biologically active substances from this plant is in progress.

## Experimental

The melting point is uncorrected. $^1$H-NMR and $^{13}$C-NMR spectra were obtained on a JEOL FX-100 instrument at 100 MHz and 25 MHz, respectively.

**Plant Material**——*Berchemia racemosa* SIEB. *et* ZUCC. was collected in the vicinity of Taishaku-kyo, Hiroshima Prefecture, Japan. A specimen was deposited at The Herbarium of the Experimental Station of Medicinal Plants, Hiroshima University School of Medicine.

**Bioassay**——The inhibiting effect of crude extract of the plants, each fraction or the isolated compound on histamine release from rat peritoneal mast cells was assayed by the high performance liquid chromatography (HPLC)-fluorometry method previously reported.[1]

Cells were preincubated with **1** for 10 min at 37 °C. Secretion was induced by 2.5 μg/ml compound 48/80 or 10 μg/ml concanavalin A with 10 μg/ml phosphatidylserine. Percent inhibition was calculated as 100 − (released histamine with sample and releaser − blank)/(released histamine with releaser − blank) × 100, where the blank value is released histamine with the mast cell preparation only. Basal activities of releasers compound 48/80 and concanavalin A, are in the range of 40—70%, and 30—40%, respectively, of total histamine (10—40 pg/cell) secreted in the presence of 0.05% Triton X-100. The inhibitory activity of the positive control (1 mM hydrocortisone) is 50—70% with

compound 48/80, and 60—80% with concanavalin A.

**Extraction and Separation of the Constituent of *Berchemia racemosa*——**Dried stems of the plants (2.0 kg) were crushed and extracted with hexane and MeOH successively. The biologically active MeOH extract was suspended in water and extracted with hexane, $Et_2O$, EtOAc, BuOH and water successively. The most biologically active EtOAc extract was chromatographed on silica gel. Elution with benzene–$CHCl_3$ and $CHCl_3$–MeOH gave 19 fractions. From the first two fractions, active compound **1** was obtained, and it was purified by silica gel chromatography with benzene–MeOH, then crystallized from $CHCl_3$–MeOH to afford yellow needles (13 mg).

**Compound 1 (2,6-Dimethoxy-*p*-benzoquinone)——**Yellow needles from $CHCl_3$–MeOH, mp 179—182 °C.[15] MS *m/z*: 168 ($M^+$), 80, 69, 53, 41, 15. UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 285 (4.08), 375 (2.48). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1692, 1640, 1622, 1592, 1323, 1260. $^1$H-NMR ($CDCl_3$) see the text; identical with reported values[4] and with the spectrum of an authentic sample. $^{13}$C-NMR data are given in the text (identical with those of the authentic sample).

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## References and Notes

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15) Significantly different from the reported value,[4] however, no decrease of melting point was observed on mixed melting point measurement with an authentic sample, and the curious melting behavior of **1** was mentioned in the literature.[11]