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Studies on the Antihemorrhagic Substances in Herbs Classified as Hemostatics in Chinese Medicine. VIII. On the Antihemorrhagic Principle in Nelumbins Receptaculum[1)]

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The antihemorrhagic principle in Nelumbins Receptaculum, dried receptacle of *Nelumbo nucifera* GAERTNER, was isolated by a combination of partition, gel filtration through Sephadex LH-20 and column chromatography over silica gel, and identified as quercetin [2-(3,4-dihydroxy)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one].

Keywords——hemostatic; Nelumbins Receptaculum; antihemorrhagic principle; quercetin

Continuing a program of studies on the chemical elucidation of antihemorrhagic principles in herbs which are used as hemostatics in traditional Chinese medicine, we have so far examined six herbs, Sanci Ginseng Radix,[2a)] *Sanguisorba officinallis* L.,[2b)] *Hypericum erectum* THUMB.,[2c)] *Biota orientalis* L. ENDL.,[2d)] *Sophora japonica* L.[2e)] and *Cirsium japonica* DC.,[1)] and we present here our results on Nelumbins Receptaculum (蓮房).

Nelumbins Receptaculum is used as an antihemorrhagic agent especially for excess menstrual bleeding and irregular genital bleeding, and also as a remedy for dehydration caused by diarrhea in summer and for prevention of miscarriage in traditional Chinese medicine.[3)] It shortens bleeding time, and the parched preparation is more effective in animals. The unparched preparation shows antibacterial activity against *Micrococcus pyogenes aureus*.[3)]

So far there have been chemical examination of the flowers, yielding flavonoids[4)] and alkaloids,[5)] but no pharmacological study on the antihemorrhagic principle in the herb. The present paper deals with isolation of the antihemorrhagic principle in the herb and its identification. The effect of heat treatment on its biological action is discussed.

During the isolation process of the antihemorrhagic principle, Tajima's method using mice was employed for following the activity of the material, as reported previously.[1,2)] Isolation of the active principle was done by partition, gel filtration through Sephadex LH-20 and column chromatography over silica gel. The chart shows a summary of the procedure.

As shown in the chart, the unparched dry toruses of *Nelumbo nucifera* GAERT. were extracted with water. The extract was partitioned between *n*-butanol and water. The activity appeared only in the organic layer. Thus, this fraction was gel-filtered through Sephadex LH-20, using methanol as an eluent. The active fraction, thus obtained, was further chromatographed over silica gel to afford highly active fraction III. Recrystallization of this fraction from diluted ethanol gave the active principle as light yellow needles.

From the spectral data nuclear magnetic resonance (NMR), infrared (IR), mass (MS) and ultraviolet (UV), the principle was concluded to be identical with quercetin [2-(3,4-dihydroxy)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one]. This conclusion was confirmed by a direct comparison of physicochemical data with those of an authentic sample obtained from

ground toruses of *Nelumbo nucifera* GAERT. (55.5 g)

↓ extracted with H_2O under reflux

H_2O-ext (6.12 g) [1 g/kg, 2.5 min]

↓ partitioned between *n*-butanol and H_2O

active fraction I (661 mg) [0.1 g/kg, 3.2 min]

↓ gel filtration on Sephadex LH-20 with MeOH

active fraction II (225 mg) [35 mg/kg, 4.2 min]

↓ silica gel c.c. with lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)

active fraction III (11.4 mg) [2.0 mg/kg, 5.3 min]

↓ recrystallized from dil. EtOH

light yellow needles (5.5 mg) [1.5 mg/kg, 5.4 min]

( ) indicates yield. [ ] indicates dose and activity (shortening of bleeding time). Silica gel c.c., silica gel column chromatography.

Chart 1. Isolation of the Active Principle

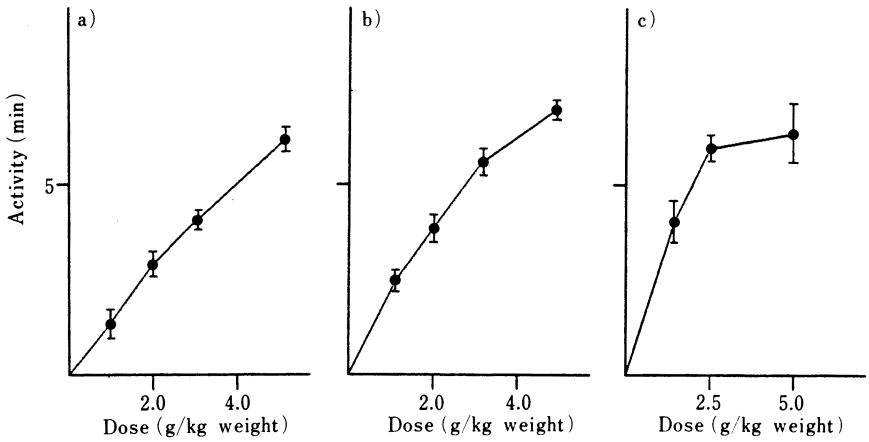

Fig. 1. Dose–Response Relationships for Antihemorrhagic Activity of Water Extracts of Parched and Unparched Herb, and Quercetin

Each point represents the mean of the antihemorrhagic activity in five different experiments with S.E. a) Water extract of unheated herb. ED_{50} = 2.05 g/kg; LD_{50} = 2500 mg/kg; (2232—2800 mg/kg). b) Water extract of parched herb. ED_{50} = 1.25 g/kg; LD_{50} = 3550 mg/kg; (3034—4154 mg/kg). c) Quercetin. ED_{50} = 1.0 mg/kg.

Wako Co. The antihemorrhagic activity of the authentic sample was equal to that of the natural product.

Quercetin is a component of cortexes, leaves and flowers of many plants, such as *Abies magnifica*,[6] *Euphoria longana*,[7] *Frugaria vesca*,[8] *Astibe thunbergii* MAG,[9] and *Sophora japonica* L.[10] It was reported that quercetin is present in the torus of *Nelumbium speciosum* by thin layer chromatography (TLC) examination.

As reported previously, a pharmacological study showed that this principle is responsible for the antihemorrhagic activity of *Sophora japonica* L.[2e] As noted above, the hemostatic activity of Nelumbins Receptaculum is also accounted for by this compound.

As shown in the figure, the antihemorrhagic activity of water extract of the parched herb is about twice that of the unparched herb in terms of ED_{50} and the LD_{50} of the water extract is apparently decreased by parching. The antihemorrhagic activities of the organic fractions obtained from the corresponding water extracts are almost equal. Analysis of the water extracts and organic fractions showed that they contain almost equal weights of quercetin. Thus, it is concluded that the water extract of the unparched herb contains material which depresses the antihemorrhagic action of quercetin. We are attempting to isolate this substance(s). The above results confirm the report that heat treatment of the herb promotes its hemostatic activity.[3]

In summary, it has been shown that the antihemorrhagic activity of Nelumbins Receptaculum is accounted for by quercetin and there is very little quantitative change of the active principle on heating of the herb under the condition described in this study. Heat treatment of the herb did decrease LD_{50} of the water extract, while its antihemorrhagic activity increased. Therefore, it is concluded that the herb should be parched before use, when it is to be used in the treatment of bleeding as an antihemorrhagic agent.

Experimental

Material——The herb examined in this study was a commercial product available in China, and was identified as *Nelumbo nucifera* GAERT. by an expert.

Extraction——Ground toruses of *Nelumbo nucifera* GAERT. (55.45 g) were extracted with 550 ml of H_2O under reflux for 0.5 h. The mixture was centrifuged at 2500 rpm for 20 min and the supernatant was lyophilized to give a brown power (6.12 g). From 55.45 g of parched herb prepared by heating 60 g of the herb on a hot plate at 200 °C for 30 min, 6.05 g of H_2O extract was obtained by following the above procedure.

Partition between *n*-Butanol and H_2O——The water extract (6.12 g) was dissolved in 150 ml of H_2O and extracted with 150 ml of *n*-butanol three times. The combined organic layer was concentrated under reduced pressure to afford 661 mg of active fraction I, as a brown gum.

Gel Filtration of Active Fraction I on Sephadex LH-20——The active fraction I (661 mg) was dissolved in 20 ml of MeOH and subjected to gel filtration through Sephadex LH-20 (3.05 × 37.0 cm), eluted with MeOH. The active fraction II (225 mg) was obtained as a yellow-brown gum.

Silica Gel Column Chromatography of Active Fraction II——This fraction II (225 mg) was chromatographed over silica gel (1.5 × 26.0 cm) to yield 11.4 mg of active fraction III, using the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent. Recrystallization of this material from diluted EtOH gave the active principle (5.5 mg) as light yellow needles. By means of the procedure described above, 5.6 mg of quercetin was also isolated from the water extract (6.05 g) of the parched herb.

Identification of the Active Principle——The active compound (mp 312—313 °C (dec.) (lit. 314 °C (dec.))[11] was identified as quercetin by direct comparison of the physicochemical data (mp, IR, UV, MS and NMR) with those of an authentic sample.

References and Notes

1) Part VII: H. Ishida, T. Umino, K. Tsuji and T. Kosuge, *Chem. Pharm. Bull.*, **35**, 861 (1987).
2) *a*) T. Kosuge, M. Yokota and A. Ochiai, *Yakugaku Zasshi*, **101**, 629 (1981); *b*) T. Kosuge, H. Ishida, M. Yokota and M. Yoshida, *Chem. Pharm. Bull.*, **32**, 4478 (1984); *c*) T. Kosuge, H. Ishida and T. Satoh, *ibid.*, **33**, 202 (1985); *d*) *Idem, ibid.*, **33**, 206 (1985); *e*) H. Ishida, T. Umino, K. Tsuji and T. Kosuge, *ibid.*, **35**, 857 (1987).
3) "Kanyaku No Rinsyoouyou," Ishiyaku Publications, Tokyo 1979, p. 250; K. Akamatsu, "Wakanyaku," Ishiyaku Publications, Tokyo, 1966, p. 468.
4) A. G. R. Nair, S. Ramakrishnan and S. Sankara, *Current Sci.* (India), **35**, 176 (1966); W. Rahman, M. Ilyas and A. W. Khan, *Naturwissenshaften*, **49**, 327 (1962).
5) K. Berhauer, *Helv. Chim. Acta*, **46**, 1783 (1963).
6) E. S. Becker and E. F. Kurth, *Tappi*, **41**, 380 (1958).
7) T. Tsukamoto, T. Tominaga and J. Takahashi, *Yakugaku Zasshi*, **69**, 40 (1949).
8) K. Herrmann, *Pharm. Zentralhalle*, **88**, 374 (1949).
9) H. Shimada, T. Sawada and S. Fukuda, *Yakugaku Zasshi*, **72**, 578 (1952).
10) K. Imai and K. Furuya, *Yakugaku Zasshi*, **72**, 1560 (1952).
11) M. Windholz, "The Merck Index," Merck & Co., Inc., Rahway, N.Y., 1983, p. 116.