HYPOTENSIVE PRINCIPLES OF *PHYTOLACCA* ROOTS[1]

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The crude drug "shōriku", the roots of *Phytolacca esculenta* Van Houtte (Phytolaccaceae) indigenous to east Asia, is used as a diuretic in Oriental medicine. Another Japanese species, *P. japonica* Makino, is used as a substitution. The other species, *P. americana* Linné, native to North America and naturalized in Japan, is not used as "shōriku". Another crude drug "ikema-shōriku", originated from *Cynanchum caudatum* Maximowicz (Asclepiadaceae), is sometimes confused with "shōriku". Maeda (1) found that an extract of "shōriku" induced an increase of the excretion of urine and a decrease of blood pressure in rabbits and isolated potassium nitrate as the active principle. Although a number of chemical investigations have been carried out on "shōriku" and many compounds have been isolated (2–6), no other hypotensive principles have been recognized.

In a reexamination of the crude drug, iv injection of rats with a 50% ethanolic extract of a "shōriku" resulted in a remarkable hypotension which was not diminished through ion-exchange resin chromatography, thereby excluding the possibility that the hypotensive principle is potassium nitrate. In order to clarify the hypotensive principles of the crude drug, the present work was performed.

Fractionation of the 50% ethanol extract was carried out and was monitored by hypotensive activity in rats. The water soluble portion of the extract was adsorbed on an Amberlite IR-120 column and eluted with ammonia. When the resulting eluate was chromatographed over silica gel, it yielded γ-aminobutyric acid (GABA), a known hypotensive agent (7). However, the hypotensive potency of the extract could not be explained solely by the presence of the estimated amount of GABA (0.06%). The fraction preceding the GABA fraction on silica gel tlc, exhibited a marked hypotensive activity; this fraction was passed through an Amberlite IR-45 column. The eluate, when chromatographed over silica gel, yielded histamine (0.16%). The combined evidence demonstrates that the active principles are GABA and histamine.

The hypotensive effects of GABA (7) and histamine (8) are known; however, this is the first report of their isolation from *Phytolacca*. In particular, a rather high content of histamine in this preparation is noteworthy.

The contents of the hypotensive principles in the roots of *Phytolacca* plants and relatives were then estimated. The GABA content varied between 0.05–0.10%; no essential differences were noted among the three species of *Phytolacca* plants (table 1). On the other hand, histamine was not detected in the roots of *P. esculenta*, *P. japonica* and *C. caudatum*, with only one exception; and the roots of *P. americana* contained a large quantity of histamine (0.13–0.16%) (table 1). Thus these *Phytolacca* plants can be classified into two groups in terms of histamine content. From the histamine content of the commercially available preparations, it may be concluded that among preparations of the crude drug "shōriku" in the Japanese markets there are not only genuine

[1]Part 12 in the series on the validity of the Oriental medicines.

TABLE 1. Histamine and GABA contents of *Phytolacca* and *Cynanchum* roots.

Original plants	Habitat	Histamine content (%)[a]	GABA content (%)[a]
P. americana	Osaka, Osaka	0.16±0.01	
P. americana	Sendai, Miyagi	0.13±0.01	0.05±0.01
P. americana	Shiogama, Miyagi	0.14±0.01	
P. esculenta	Furukawa, Miyagi	—	0.06±0.01
P. esculenta	Oshima, Miyagi	—	
P. esculenta	Sakunami, Miyagi	—	0.08±0.02
P. esculenta	Sendai, Miyagi	—	0.09±0.02
P. japonica	Ikeda, Osaka	0.02±0.01	0.08±0.02
P. japonica	Kyoto, Kyoto	—	
P. japonica	Minoo, Osaka	—	0.10±0.02
C. caudatum	Sakunami, Miyagi	—	0.04±0.01
C. caudatum	Shiroishi, Miyagi	—	
"shōriku"	(Osaka)[b]	0.16±0.02	0.06±0.02
"shōriku"	(Sendai)[b]	0.02±0.01	
"shōriku"	(Sendai)[b]	0.10±0.02	
"shōriku"	(Tokyo)[b]	0.03±0.01	
"ikema-shōriku"	(Tokyo)[b]	0.06±0.02	

[a]n=3 data expressed in m±s.e.m. —: not detected.
[b]commercial preparations.

preparations from *P. esculenta* or *P. japonica* but also preparations adulterated or counterfeited by *P. americana*. Although *P. esculenta* and *P. japonica* grow wild in Japan, they are uncommon plants and are rarely cultivated for medicinal purposes. On the other hand, *P. americana* is rather commonly found in waste lands and, therefore, is now utilized as an adulterant or a counterfeit of *P. esculenta* and *P. japonica*.

One of the major differnces between *P. esculenta* and *P. japonica*, and *P. americana* is obviously the histamine content. The recorded side effects of the crude drug "shōriku" are colic, vomiting, and diarrhea (1). Therefore, it is possible that a large amount of histamine causes the side effects if contaminated with *P. americana*.

The crude drug "shōriku" is usually employed for the diuretic effect, which is apparently mediated not only by potassium nitrate but also by GABA, the diuretic action of the latter being also known (9).

The crude drug "ikema-shōriku" has now been revealed to contain a quantity of GABA comparable to "shōriku". It is also worth noting that a marketed preparation labelled "ikema-shōriku," whose original plant should be *C. caudatum*, was found by histological examinations to be a *Phytolacca* plant.

EXPERIMENTAL[2]

BLOOD PRESSURE MEASUREMENT.—Male Wistar rats weighing 300–400 g were used. A rat was anesthetized by sc injection of urethane (1.4 g/kg). A polyethylene cannula was inserted into the left common carotid artery, and the blood pressure was recorded *via* a pressure transducer on a polygraph. A test sample was dissolved in physiological saline solution; samples insoluble in saline solution were suspended in saline solution containing 2% gum arabic. The solution or suspension (1 ml/kg) was administered through a venous cannula with the aid of additional saline solution (0.1 ml).

ISOLATION OF γ-AMINOBUTYRIC ACID AND HISTAMINE.—The commercially available crude drug Radix Phytolaccae (50 g), the dried roots of a *Phytolacca* sp. (Phytolaccaceae), was extracted 5 times with hot 50% ethanol (500 ml) for 5 hr (each extraction). The extract was partitioned with water and *n*-butanol, and the water solubles (11.95 g) were passed through an Amberlite IR-120 (H^+ type, 50 ml) column. After the column

[2]Melting points were determined on a hot stage and uncorrected.

was washed with water (1000 ml), elution with 5% ammonia (1000 ml) afforded the eluate (0.80 g) which was subjected to silica gel chromatography (150 g). Elution with chloroform-methanol-17% ammonia (9:9:2) gave γ-aminobutyric acid; ir ν max (KBr) 3050 and 1558 cm^{-1}; pmr (D_2O) δ 2.07 (2H, m), 2.52 (2H, t) and 3.15 (2H, t); tlc (Rf 0.16; chloroform-ethanol-methanol-17% ammonia (2:2:1:1)). Identity with an authentic sample was confirmed by tlc, ir and pmr comparison. The substances eluted before γ-aminobutyric acid in the silica gel chromatography were collected and passed through Amberlite IR-45 (OH^- type, 10 ml). The eluate with water (200 ml) was rechromatographed on a silica gel column with the same solvent as above. When eluate was treated with diluted hydrochloric acid and concentrated, it yielded a colorless crystalline precipitate which was recrystallized from aqueous ethanol to afford histamine dihydrochloride as colorless prisms, mp 223–225°; ir ν max (KBr) 3100, 3020, 1621 and 1562 cm^{-1}; pmr (D_2O) δ 3.39 (4H, m), 7.52 (1H, s) and 8.76 (1H, s). Identity with an authentic sample was confirmed by mixed melting points, ir and pmr comparison.

DETERMINATION OF γ-AMINOBUTYRIC ACID AND HISTAMINE CONTENTS IN THE CRUDE DRUG.—Each preparation (0.5 g) was extracted 5 times with hot 50% ethanol (5 ml each) for 1 hr (each extraction). The extract was adsorbed on Amberlite IRA-400 (OH^- type, 4 ml). Elution with water (25 ml) gave the eluate A, and successive elution with 3% acetic acid (20 ml) gave the eluate B. The eluate A was adsorbed on Amberlite IRC-50 (H^+ type, 5 ml). Elution with water (25 ml) gave the eluate C, and further elution with 17% ammonia (50 ml) gave the eluate D.

The eluate B, when concentrated under reduced pressure, afforded a residue which was dissolved in water (1.0 ml). An aliquot (10 μl) of the solution was subjected to two dimentional tlc on silica gel with chloroform-ethanol-methanol-17% ammonia (2:2:1:1) as the first developing solvent and *n*-butanol-acetic acid-water (4:1:2) as the second developing solvent. γ-Aminobutyric acid (GABA) was visualized on heating with ninhydrin, and the optical density of the coloration was measured with a dual-wavelength tlc scanner (400 nm for samples and 650 nm for references). A simultaneously prepared calibration curve was used to estimate the GABA content.

Concentration of the eluate D under diminished pressure yielded a residue which was then dissolved in water (1.0 ml). An aliquot (5 μl) of the solution was subjected to chromatography on a silica gel plate with chloroform-ethanol-methanol-17% ammonia (2:2:1:1) as solvent. Histamine was visualized when heated with ninhydrin. The optical density of the coloration was determined by the same method as described above.

The results are shown in table 1.

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LITERATURE CITED

1. M. Maeda, *Tohoku Igaku Zasshi*, **5**, 85 (1922).
2. W. W. Sick, *Yakhak Hoeji*, **15**, 99 (1971); *Chem. Abstr.*, **80**, 141071n (1974).
3. W. W. Sick, *Lloydia*, **36**, 326 (1973).
4. W. W. Sick and K. S. Sik, *Yakhak Hoeji*, **17**, 152 (1973); *Chem. Abstr.*, **81**, 169658d (1974).
5. W. W. Sick and K. S. Sik, *Yakhak Hoe Chi*, **18**, 203 (1974); *Chem. Abstr.*, **82**, 152188y (1975).
6. W. W. Sick, *Yakhak Hoe Chi*, **18**, 229 (1974); *Chem. Abstr.*, **82**, 152189z (1975).
7. H. Takahashi, M. Tiba, M. Iino and T. Takayasu, *Jap. J. Physiol.*, **5**, 334 (1956).
8. D. Beleslin, *Arch. Intern. Pharmacodyn.*, **138**, 19 (1962).
9. D. Jinnai and A. Mori, *Nisshin Igaku*, **45**, 1 (1958).