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New Methods for Identification of Alismatis Rhizoma by Means of Electrophoresis, Paper Partition Chromatography, and Thin-Layer Chromatography

MASASHI TOMODA,* NORIKO SHIMIZU, and KEIKO KATOH

Kyoritsu College of Pharmacy, Shibakōen, Minato-ku, Tokyo 105, Japan

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Procedures for the identification of decoction of Alismatis Rhizoma (rhizome of *Alisma orientale* JUZEPCZUK or related species) by cellulose acetate membrane electrophoresis, paper partition chromatography, and thin-layer chromatography were developed. These methods are due to the presence of a characteristic new component.

Keywords——identification; Alismatis Rhizoma; *Alisma orientale*; electrophoresis; paper chromatography; thin-layer chromatography; lactose hexaphosphate

Alismatis Rhizoma, the rhizome of *Alisma orientale* JUZEPCZUK or related species (Alismataceae), has been used as an important component of many oriental pharmaceutical preparations. However, no effective method for its identification in decoctions and preparations is yet available.

In recent years, we have proposed new methods for identification of Zizyphi Fructus and Angelicae Radix by means of electrophoresis with color and precipitation reactions.[1,2] In this paper, we report the development of several methods for identification of Alismatis Rhizoma by utilizing precipitation procedures, electrophoresis, paper partition chromatography (PPC), and thin-layer chromatography (TLC).

Experimental

Materials——The fresh material was obtained from plants cultivated in Saitama prefecture. Chinese crude drugs were purchased from Nakai-Kōshindo Co. in Kobe. Various oriental pharmaceutical preparations were prepared in our laboratory.

Precipitation Procedures——The crude drug (1 g) was sliced and extracted with water (10 ml) in a boiling water-bath for 10 min. After filtration, 5% cetyltrimethyl ammonium bromide (0.6 ml) was added to the filtrate, then the mixture was centrifuged (3500 rpm, 10 min). The precipitate was dissolved in 0.5 M sodium chloride (1 ml) and the solution was centrifuged, if necessary. The resulting solution was added to four volumes of ethanol. After centrifugation, the precipitate was mixed well with 80% ethanol (4 ml), then centrifuged again. This treatment with 80% ethanol was repeated three times. The final precipitate was dried *in vacuo*, then dissolved in water (0.08 ml). This solution was used as the sample for electrophoresis (1 μl), PPC (1 μl), and TLC (0.2 μl).

Electrophoresis——Electrophoresis was carried out with Separax (Fuji Film Co., 6 × 21 cm long) using buffer A, 0.08 M pyridine–0.04 M acetic acid (pH 5.4), at 420 V for 30 min. The inside of the apparatus was cooled with dry ice. The sample was applied in a line at a distance of 7 cm from the cathode, and was visualized with 0.5% toluidine blue in 3% acetic acid. After being dipped for 5 min, the membrane was washed with 1% acetic acid.

PPC——The sample was subjected to PPC by the ascending method using Tōyō-Roshi No. 51 and solvent A, methanol–formic acid–water (10 : 2 : 1). It was visualized with the toluidine blue reagent as described above, and with the Hanes–Isherwood reagent.[3]

TLC——This was carried out by the ascending method with Avicel SF cellulose (Funakoshi Co.) using the same solvent A as in PPC. The Hanes–Isherwood reagent was used for detection.

Results and Discussion

Color Reaction of the Samples Obtained by the Precipitation Procedures

Alismatis Rhizoma and thirty-seven other crude drugs used in related pharmaceutical preparations were treated by the precipitation procedures described above. When the final samples were subjected to cellulose acetate electrophoresis, detection with the toluidine blue reagent showed that nine samples in addition to Alismatis Rhizoma were positive, while twenty-eight samples were negative. All of the positive samples gave reddish-violet spots. The results are listed in Table I.

Results of Electrophoresis

The electrophoretic patterns of the color reaction-positive crude drugs are shown in Fig.

TABLE I. Results of Detection of the Samples with Toluidine Blue after Electrophoresis

Crude drug	Result	Crude drug	Result
Atractylodis Rhizoma	−	Moutan Cortex	−
Atractylodis Lanceae Rhizoma	−	Paeoniae Radix	−
Artemisiae Capillaris Herba	+	Nelumbi Fructus	+
Plantaginis Semen	−	Akebiae Caulis	−
Rehmanniae Radix	−	Aconiti Tuber	−
Scutellariae Radix	−	Cinnamomi Cortex	−
Gardeniae Fructus	+	Magnoliae Cortex	−
Gentianae Scabrae Radix	+	Achyranthis Radix	+
Angelicae Radix	+	Gastrodiae Tuber	−
Bupleuri Radix	−	Zingiberis Rhizoma	−
Cnidii Rhizoma	−	Pinelliae Tuber	−
Ginseng Radix	−	Maltum	−
Corni Fructus	+	Dioscoreae Rhizoma	+
Zizyphi Fructus	+	Alismatis Rhizoma	+
Aurantii Nobilis Pericarpium	−	Hoelen	−
Phellodendri Cortex	−	Polyporus	−
Astragali Radix	−	Aspergillus	−
Glycyrrhizae Radix	−	Gelatinum	−
Crataegi Fructus	−	Talcum	−

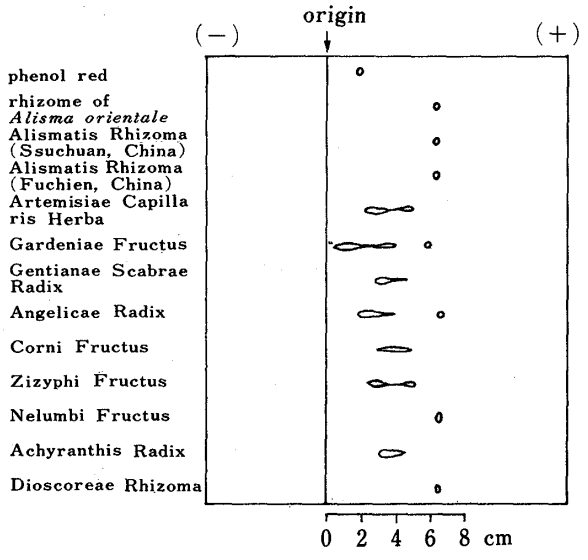

Fig. 1. Cellulose Acetate Membrane Electrophoresis with Pyridine-Acetic Acid Buffer (pH 5.4)

1. Alismatis Rhizoma gave a single clear spot having a relative migration value of 3.50 with respect to standard phenol red.

Results of PPC and TLC

Alismatis Rhizoma was the only sample which gave a single spot having *Rf* values of 0.46 in PPC and 0.56 in cellulose TLC. Any crude drug of the others tested gave no spot in PPC and TLC. For the purpose of detecting the spots in PPC, the toluidine blue reagent was superior to the Hanes–Isherwood reagent in sensitivity. However, the toluidine blue reagent was not suitable for TLC because of the required dipping process. The Hanes–Isherwood reagent resulted in sensitive detection of the spot from Alismatis Rhizoma in TLC.

Identification of the Crude Drug in Pharmaceutical Preparations

Thirteen kinds of oriental pharmaceutical preparations (Irei-to 胃苓湯, Inchingorei-san 茵蔯五苓散, Keihi-to 啓脾湯, Goshajinki-gan 牛車腎気丸, Gorei-san 五苓散, Sairei-to 柴苓湯, Shirei-to 四苓湯, Chorei-to 猪苓湯, Toukishakuyaku-san 当帰芍薬散, Hachimijiougan 八味地黄丸, Hangebyakujutsutenma-to 半夏白朮天麻湯, Ryutanshakan-to 竜胆瀉肝湯, Rokumi-gan 六味丸)[4] gave the characteristic spot of Alismatis Rhizoma on cellulose acetate membrane electrophoresis. In these cases, a portion of each preparation containing the extract from 1 g of Alismatis Rhizoma was taken through the precipitation procedures described above. As shown in Fig. 1, several crude drugs gave spots having migration values similar to that of Alismatis Rhizoma. PPC or TLC are simpler and more specific methods than electrophoresis, but electrophoresis was nevertheless the most sensitive method of the three. All thirteen pharmaceutical preparations tested gave the spot derived from Alismatis Rhizoma in PPC and TLC.

A New Characteristic Color-Reactive Substance in Alismatis Rhizoma

Both the precipitation reaction with cetyltrimethyl ammonium bromide[5] and the color reaction with toluidine blue[6] usually depend on various acidic polysaccharides in crude drugs.[1,2] However, we did not find any acidic polysaccharide in the water extract from Alismatis Rhizoma. Based on the result of reaction with the Hanes–Isherwood reagent, we concluded that the compound responsible for the characteristic reactions of Alismatis Rhizoma was a kind of phosphate. We isolated this substance from the crude drug, and identified it as a new natural product, lactose hexaphosphate. Details of the structural elucidation of this substance will be reported in a separate paper.

References and Notes

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