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# Salts of *Myo*-Inositol Hexaphosphate in Alismatis Rhizoma and Angelicae Radix as an Indicator for Identification*

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In previous reports, we proposed several methods for identification of Alismatis Rhizoma and Angelicae Radix. These methods included electrophoresis, PPC, and TLC. Electrophoresis is particularly useful for identification owing to the presence of a characteristic component.

In this paper, we report the characterization of this component and a revision of the methods for identification of these crude drugs.

The crude drugs were extracted with hot water. After filtration, the extract was treated with cetyltrimethylammonium bromide. The resulting precipitate was dissolved in sodium chloride solution and this solution was poured into ethanol. The precipitate obtained was dissolved in water, and applied successively to columns of Toyopearl HW 60 and Sephadex G-25 in the case of Alismatis Rhizoma. For the isolation from Angelicae Radix, column chromatography was carried out with Sephadex G-50 and G-25 successively.

The characteristic substances (Al and An) obtained from Alismatis Rhizoma and Angelicae Radix, respectively, gave a single spot on electrophoresis, PPC and TLC. The two substances showed similar mobilities to each other in acidic buffer and mobile phase.

The substances were hydrolyzed with 2 N hydrochloric acid at 125°C for 20 h. The sole organic product was purified on a column of Sephadex G-25. The $^{13}$C-NMR spectrum of the purified product showed four signals at $\delta$ 73.765, 74.791, 75.033 and 76.949, and their integral ratio was *ca.* 2 : 1 : 2 : 1. By comparison of the $^{13}$C-NMR spectrum with that of an authentic sample, the product was identified as *myo*-inositol.

The purified product was acetylated and analyzed by GLC. *Myo*-inositol hexaacetate was identified by GLC, and it was clearly distinguishable from hexaacetates of *epi*-and *scyllo*-inositols. The substances Al and An also produced *myo*-inositol on treatement with an alkaline phosphatase, and the identity of the product was confirmed by GLC of its acetate.

The $^{13}$C-NMR spectra of the substances Al and An each showed four signals at $\delta$ 76.002, 77.917, 78.754 and 80.157, and $\delta$ 75.894, 77.890, 78.565 and 79.995, respectively. Their integral ratio was *ca.* 2 : 1 : 2 : 1 in both cases. The three-sodium salt of *myo*-inositol hexaphosphate (phytic acid) prepared from phytin gave a $^{13}$C-NMR spectrum having four signals at $\delta$ 75.840, 77.890, 78.538 and 79.995 in the integral ratio of *ca.* 2 : 1 :

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2 : 1.

Based on the accumulated evidence described above, it can be concluded that substances Al and An are both sodium salts of *myo*-inositol haxaphosphate. They are partly combined with magnesium, but the kind and the content of cations are not important from the viewpoint of the identification of crude drugs. No calcium was found in the substances Al and An.

After studies on one hundred and three kinds of crude drugs, which are generally used in oriental pharmaceutical preparations, by means of spot test, electrophoresis and GLC of the acetates of acid hydrolyzates, twenty-three kinds of samples were found to be *myo*-inositol phosphate-containing crude drugs.

In order to find which crude drugs contain phytic acid salts in abundance, the hot water extracts of these twenty-three crude drugs were subjected to precipitation procedures under the same conditions as in a previous report, except that the final dried precipitates, obtained from one gram of each sample, were dissolved in 10 ml of water. Each of the resulting solutions (0.5 $\mu$l) was used as a sample for electrophoresis. Only four crude drugs, Alismatis Rhizoma, Angelicae Radix, Nelumbi Fructus, and Saposhnikoviae Radix, gave clear positive spots with toluidine blue under these conditions. Alismatis Rhizoma and Nelumbi Fructus gave a single spot of *myo*-inositol hexaphosphate. On the other hand, Saposhnikoviae Radix showed additional tailing in the electrophoretic pattern, probably due to pectic substances, as also did Angelicae Radix.