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Comparison of Higenamine Extraction from Unprocessed and Processed Aconite Roots

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Abstract – Higenamine is a cardiotonic constituent of Aconite root, one of the most important oriental traditional medicine. Since Aconite root contains toxic aconitine alkaloids, variously processed roots have been often used. Much works have been done with the chemical significances concerning with the toxic aconitine alkaloids during the processing periods. However, effects of processing on higenamine have not yet been previously studied. In this paper, the extract pattern and the amounts of higenamine extracted with water from unprocessed and processed Aconite roots were compared. *R*-(+)-isomer was the only higenamine enantiomer detected although racemic higenamine was reported to be separated from *Aconitum* spp. Sonication for 1 hour resulted in higher higenamine extraction (12.3 μg/g) than boiling water extraction for 3 hours (6.7 μg/g) of unprocessed Aconite root. Extraction of not only higenamine but also most of the other components of unprocessed Aconite roots were reduced with boiling in water. Similarly, reduced extraction was observed with extracts of all three processed Aconite roots (Kyung-Po-Aconite root, Dang-Po-Aconite root and Huk-Peon-Aconite root) by either sonicated extraction or boiling water extraction.

Key words – *R*-(+)-higenamine, sonication, unprocessed and processed Aconite root, HPLC-chiral column

Introduction

Aconite root, the root of *Aconitum* plant belonging to the family Ranunculaceae, is one of the most important oriental traditional medicine, used as cardiotonic, analgesic, diuretic, neuralgia, rheumatoid arthritis, remedy for paralysis and emergency (Bensky, *et al.* 1986; Tang, *et al.* 1992). The main components of Aconite root are toxic diterpenoid alkaloids including aconitine, hypaconitine and mesaconitine. Besides the diterpenoid alkaloids, it contains an isoquinoline alkaloid, higenamine, known as a cardiotonic component of this plant. Higenamine has been reported to have cardiotonic effect by stimulating cardiac β-adrenoreceptors, hypotensive effect by relaxing vascular smooth muscle, anti-thrombotic effect by inhibiting platelet aggregation and others (Kosuge, 1976; Chang, 1983; Park, *et al.* 1984; Chang, *et al.* 1986, Kim, *et al.* 1986; Yun-Choi, *et al.* 1994). Since Aconite root contains toxic aconitine alkaloids, variously processed roots have been often used in order to avoid the hazards caused by those toxicants, and the chemical significance of those processies has been extensively pursued. It has been generally accepted that several chemical transformation reactions including deacetylation, debenzoylation, oxidation *etc.* occur with the toxic aconitine alkaloids during the processing period and are responsible for the detoxification (Kitagawa, 1982). Although the effects on the contents of aconitine compounds during the processing periods have been reported by several workers (Hikino, *et al.* 1983), effects of various processing on higenamine have not been previously studied.

In the present work, the amounts of higenamine extracted from unprocessed and processed Aconite roots were compared to study the effects of processing on higenamine contained in Aconite roots.

Experimental

Plant Materials – Three types of processed Aconite roots (Kyung-Po-Aconite root, Dang-Po-Aconite root and Huk-Peon-Aconite root) were purchased from Hanyak Commercial Co., and unprocessed Aconite roots were from local crude drug market, Seoul, Korea and identified by Prof. Hyung Joon Chi, Natural Products Research Institute, Seoul National University. The voucher specimens are deposited at the

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Reagents and materials – Higenamine (racemate) was synthesized as a hydrobromide salt according to the previously reported method (Chang *et al.*, 1984). Sep-pak C_{18} (Waters Co., U.S.A.) was activated by washing with MeOH 5 ml, distilled water 5 ml and normal saline 5 ml successively before use. The reagents used were of HPLC grade (Fisher Scientific Co., U.S.A.) and filtered through a Millipore filter (0.45 μm) prior to use.

HPLC Condition – The HPLC system (Spectra Physics Co., U.S.A.) which was consisted of a Ternary Pump (SP 8800), UV Variable Wavelength Detector (SP 100), an Integrator (SP 4270) and an Injector (Rheodyne #7125), was integrated with a chiral column (4.6 × 250 mm, CHIREX 3020G-EO, Phenomenex Co., U.S.A.). The stationary phase was eluted with the mobile phase of hexane : dichloroethane : (trifluoroacetic acid / EtOH; 1/20) = 53:35:12 with the flow rate of 0.8 ml/min and the eluent was detected with UV at 284 nm (aufs:0.002).

Extraction procedure – 30 g each of powdered plant material was boiled with 240 ml of distilled water for 3 hours with reflux or sonicated in an ultrasonic cleaner (Kum Sung, Co., Korea) for 1 hour and filtered. The filtrate was washed with ethyl acetate and butanol successively and then freeze-dried.

Analysis of HPLC – The freeze-dried powder equivalent to 250 g of plant material was dissolved in 250 μl of phosphate buffered saline (PBS, pH 5.0) and applied to a Sep-pak C_{18} and washed with 5 ml of PBS (pH 5.0) and 5.0 ml of 12% MeOH/PBS and then eluted with 5 ml of 50% EtOH as shown in Scheme 1. The eluate was dried *in vacuo* and the residue was dissolved in EtOH (250 μl) and 5 μl each of the EtOH solution was injected into the HPLC system for analysis. For the quantitative analysis of higenamine contents with the standard addition method, 100 or 200 μl of higenamine (racemate) solution (HigenamineHBr 125 μg/l in 1 ml of PBS, pH 5.0) was applied to Sep-pak C_{18} together with the PBS solution of freeze-dried powder, treated in the same manner as above and injected into the HPLC system. From the increase in peak height the concentration of higenamine within the crude plant could be estimated.

Water Ext.
(equivalent to 250 μg of the crude plant)
- dissolve in 250 μl of PBS (pH 5.0)
- addition of (RS)-higenamine · HBr [125 μg/ml PBS(pH 5.0), 0, 100, 200 μl]

Sep-pak C_{18}
- wash with 5.0 ml of PBS (pH 5.0)
- wash with 5.0 ml of 12% MeOH/PBS
- elute with 5.0 ml of 50% EtOH

50% EtOH elute
- evaporate in vacuo
- dissolve in 250 μl of EtOH

HPLC analysis

Scheme 1. Pretreatment of plant extract for HPLC analysis.

Results and Discussion

The powdered crude Aconite roots (unprocessed Aconite root) and processed Aconite roots were extracted with either distilled boiling water for 3 hours once or sonicated with water for 1 hour and the higenamine extracted were analyzed with HPLC employing a chiral column (Chung, *et al*, 2000). The racemic mixture of higenamine provided two sepa-

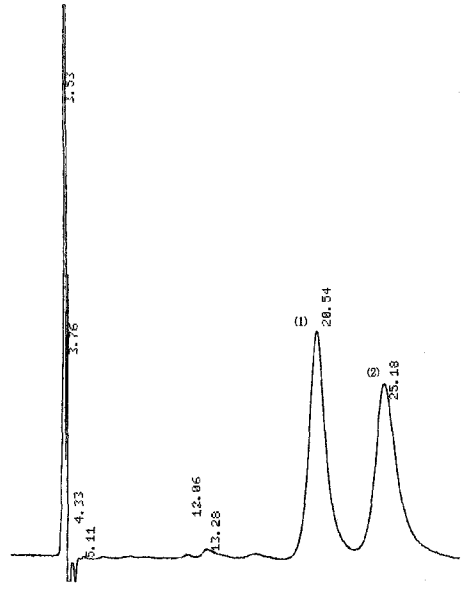

Fig. 1. HPLC chromatogram of higenamine racemic mixture Column : A 4.6259 mm chiral column (CHIREX 3020-G-EO) Mobile phase : Hexane:Dichloroethane: (TFA/EtOH;1/20) = 53:35:12. Flow rate : 0.8 ml/min Detection : 284 nm (aufs:0.002) Injection volumn : 5 l (1) : R-(+)-higenamine (2) : S-(−)-higenamine.

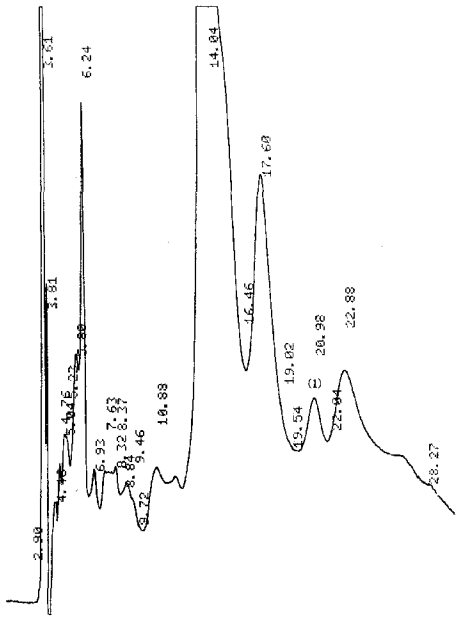

Fig. 2. HPLC chromatogram of sonicated extract of unprocessed Aconite root. The analysis was performed under the condition described in Fig. 1. (1) : R-(+)-higenamine (2) : S-(−)-higenamine.

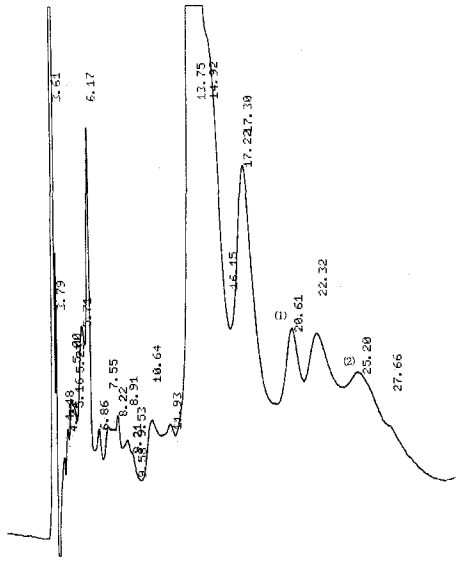

Fig. 3. HPLC chromatogram of sonicated extract of unprocessed Aconite root with the addition of standard (RS)-higenamine (40 μg/ml). The analysis was performed at the condition described in Fig. 1. (1) : R-(+)-higenamine (2) : S-(−)-higenamine.

rate peak at the retention time of 20.54 and 25.18 min as shown in Fig. 1. The enantiomers eluted with shorter retention time was identified as *R*-(+)-isomer and the other with longer retention time was identified as *S*-(−)-isomer with the aid of pure authentic samples by Chung, *et al.* (2000). As shown in the representative chromatogram of Fig. 2, a peak appeared in the region of *R*-(+)-isomer (at 20.98 min) and no peak was detected in the region of *S*-(−)-enantiomer at around 25 min as was the case with MeOH extraction of the plant material by Chung, *et al.* (2000). It was again confirmed that *R*-(+)-isomer is the only higenamine enantiomer of Aconite roots although racemic higenamine was reported to be separated from *Aconitum* spp. (Chung, *et al.*, 2000). The amount of *R*-(+)-higenamine in plants were determined by the standard addition method. Since the extracts of plants contain various other components even after careful pre-treatments, the chromatograms with plant samples usually give many disturbing peaks other than the target peaks and interferes the direct quantification of peaks. An example of a calibration graph (Fig. 5) was obtained with the chromatograms Fig. 2 (sonicated extract), Fig. 3 and 4 (sonicated extract with addition of racemic higenamine) The amount of *R*-(+)-higenamine was read

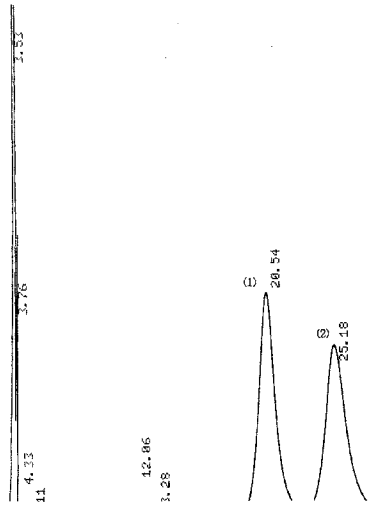

Fig. 4. HPLC chromatogram of sonicated extract of unprocessed Aconite root with the addition of standard (RS)-higenamine (80 μg/ml). The analysis was performed under the condition described in Fig. 1. (1) : R-(+)-higenamine (2) : S-(−)-higenamine.

as g in 1 ml of injected solution and this refers to μg of higenamine in 1 μg of crude plant.

The results with unprocessed Aconite roots are summerized in Table 1. Sonication resulted in higher higenamine extraction (12.3 μg/g) than boiling (3 hours) water extraction (6.7 μg/g). Actually extrac-

Table 1. Contents of higenamine in unprocessed Aconite roots

Methods of extract	Higenamine(g/g*)
Sonication	12.3
Water (boiling) extraction	6.7

*g of crude plant

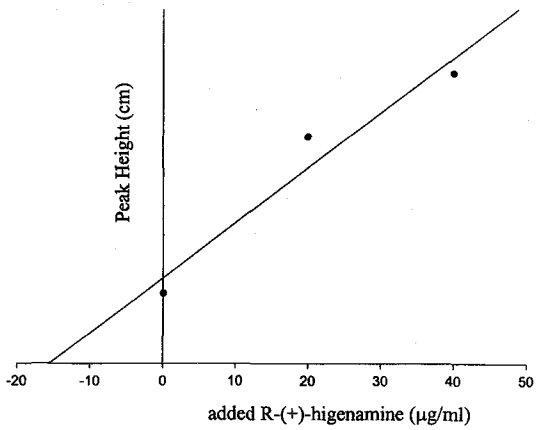

Fig. 5. Calibration curve for R-(+)-higenamine contents of sonicated extract of unprocessed Aconite root by standard addition method.

tion of not only higenamine but also most of the other components of unprocessed Aconite roots were reduced with boiling in water for 3 hours. Similar

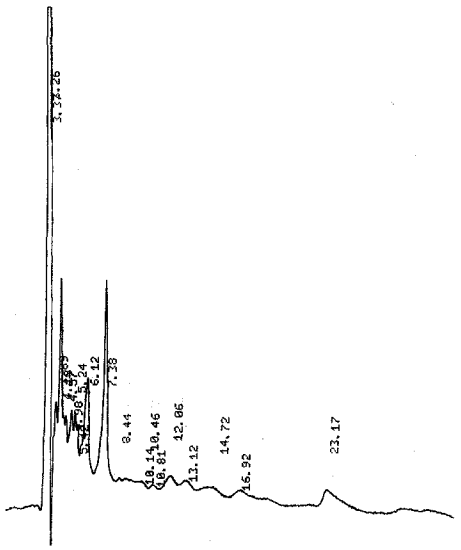

Fig. 6. HPLC chromatogram of sonicated extract of processed Aconite roots (Kyung-Po-Aconite root). The analysis was performed at the condition described in Fig. 1.

phenomend of reduced extraction were observed with extracts of all three processed Aconite roots (Kung-Po-Aconite root, Dang-Po-Aconite root and Huk-Peon-Aconite root) as shown in the representative chromatogram of Fig. 6. It is likely that the amount of solubilized starch increases with boiling Aconite root in water and so reduces the solubilities of other components of Aconite. Since most of the processing methods of Aconite roots employ high temperature and/or high pressure with water, the water extraction (either sonication or boiling) of most of the components were interfered as was the case for boiling unprocessed Aconite roots. It was noted that most of the traditional prescriptions which contain processed Aconite roots are in the form of pills (Pal-Mi-Chi-Whang-Whan, Uh-Cha-Sin-Ki-Whan and Sip-Bo-Whan, *etc.*) rather than in the form of decoction and our present results may partially explain these reasons.

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