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Analytical Studies on *Poria cocos* (I) The Quantitative Analysis of Dehydropachymic Acid

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Dehydropachymic acid contents in the crude drug, hoelen, was determined by HPLC using a Develosil P-5 (4.6×250 mm) column with CH_3CN-H_2O-AcOH (70:30:1) as a mobile phase, and monitoring absorption at 242 nm at 40°. The hoelens examined were 16 samples commercially obtained in Osaka, and 4 samples collected in Ishikawa prefecture. The dehydropachymic acid contents in the 20 hoelens were in a range of 0.013 to 0.034%. The present method of quantitative analysis of dehydropachymic acid is applicable to the determination of dehydropachymic acid contents of the Chinese medicine, Shô-hange-ka-bukuryô-tô.

Keywords——dehydropachymic acid; *Poria cocos*; hoelen; quantitative analysis; HPLC; Shô-hange-ka-bukuryô-tô

Hoelen, a sclerotium of *P. cocos*, is one of the important crude drugs in Chinese medicine. It is used for the treatment of a diuretic and for palpitation. Natori *et al.*[1] reported the isolation of several triterpenes from hoelen. They also suggested the presence in hoelen of small amounts of relating compounds having conjugated dienes. However, these compounds are difficult to separate with each other.[2] A scientific method of qualitative evaluation of hoelen is known.[3] We reported the quantitative analysis of dehydropachymic acid by HPLC.[4] Recently, we purified dehydropachymic acid by reversed phase preparative high performance liquid chromatography (HPLC) and elucidated its structure.[5] This paper deals with the quantitative determination of the acid in hoelen, by using a purified standard. This method is applicable to the quantitative analysis of dehydropachymic acid in Shô-hange-ka-bukuryô-tô.

Experimentals and Methods

Standard sample——The standard sample of dehydropachymic acid was prepared as described in our previous paper.[5] White needles, mp. 268–270°, UV λ_{EtOH}^{max} 242 nm (log ε, 4.10).

Plant materials——The hoelens used were 16 Chinese samples commercially obtained in Osaka, and 4 samples collected in Ishikawa prefecture in June 1989 and April 1990.

Preparation of sample solution——i) Hoelen: 1.0 g of powdered hoelen was extracted three times with methanol (30 ml) with sonication. The extracts were centrifuged and then filtered through a filter paper. The filtrate was evaporated under reduced pressure, the residue was dissolved in methanol (5 ml) and the solution was filtered through a 0.45 μm membrane filter (MILLEX-HV).

ii) Shô-hange-ka-bukuryô-tô extract: A mixture of hoelen (20 g), ginger (20 g) and pinellia tuber (20 g) was boiled with 600 ml of hot water until the volume of the water was reduced to about a half. Then it was filtered through a twofold gauze. The filtrate lyophilized. The prepared extract (2.0 g) was extracted with methanol (30 ml×3) with sonication. The extract was centrifuged and then filtered. The filtrate was evaporated under reduced pressure. The residue was dissolved in water (100 ml) and extracted with ether (100 ml×3). The organic layer was evaporated and the residue was dissolved in methanol (5 ml) and filtered through a 0.45 μm membrane filter.

iii) Extract of Shô-hange-ka-bukuryô-tô containing no hoelen: A mixture of ginger (20 g) and pinellia tuber (20 g) was boiled with 400 ml of hot water until the volume of the water was reduced to a half. Then the extract was treated as described above for Shô-hange-ka-bukuryô-tô extract.

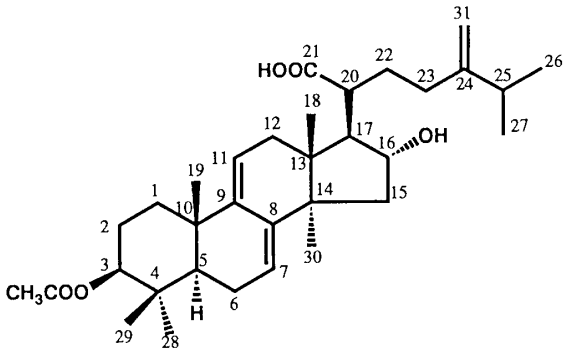

Chart 1. Structure of Dehydropachymic Acid

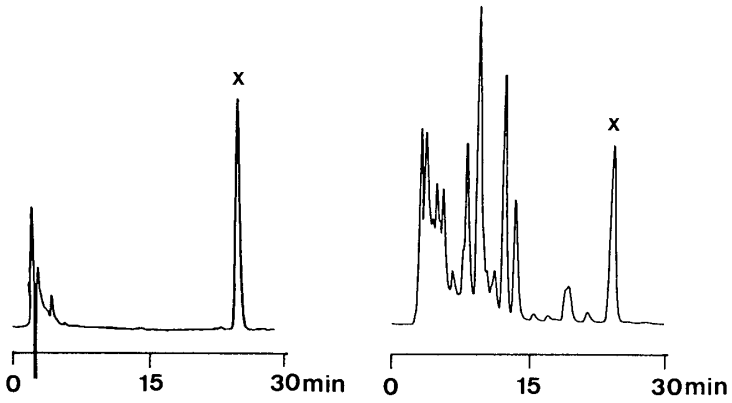

Fig. 1. HPLC Profiles of Hoelen Extract

column: Develosil ODS P-5 4.6×250 mm. mobile phase: CH_3CN-H_2O-AcOH (70:30:1). flow rate: 1.0 ml/min. detection: UV 242 nm. column temperature: 40°. X: dehydropachymic acid.

TABLE I. Contents (%) of Dehydropachymic Acid in Hoelen

Lot. 1	0.030	Lot. 2	0.026	Lot. 3	0.021
4	0.034	5	0.025	6	0.026
7	0.024	8	0.029	9	0.028
10	0.025	11	0.016	12	0.018
13	0.018	14	0.031	15	0.032
16	0.020	17	0.017	18	0.028
19	0.023	20	0.013		

Hoelen of Lot. 1–16 were commercially obtained in Osaka, and Hoelen of Lot. 17–20 were collected in Ishikawa pref. of Japan.

HPLC conditions——The HPLC system was consisted of a Model 600 pump, a U6K universal Model injector and a Model 990 photodiode alley as the detector (Waters Assoc.). The column was a Develosil ODS P-5 (4.6 mm×250 mm) and the mobile phase was a mixture of CH_3CN-H_2O-AcOH (70:30:1) The flow rate was 1.0 ml/min and the peaks were monitored at 242 nm. The column temperature was 40°.

Standard solution——The standard solution of dehydropachymic acid was prepared to contain 0.20 mg/ml (MeOH) of the acid. A good linear relationship was obtained between the acid concentrations and the detector responses (peak area) in a range of 0.01–0.20 mg/ml (r=0.999).

Results and Discussion

These solutions were analyzed by HPLC. The quantities of dehydropachymic acid in hoelen were calculated from the resulting chromatograms. The addition and recovery tests of dehydropachymic

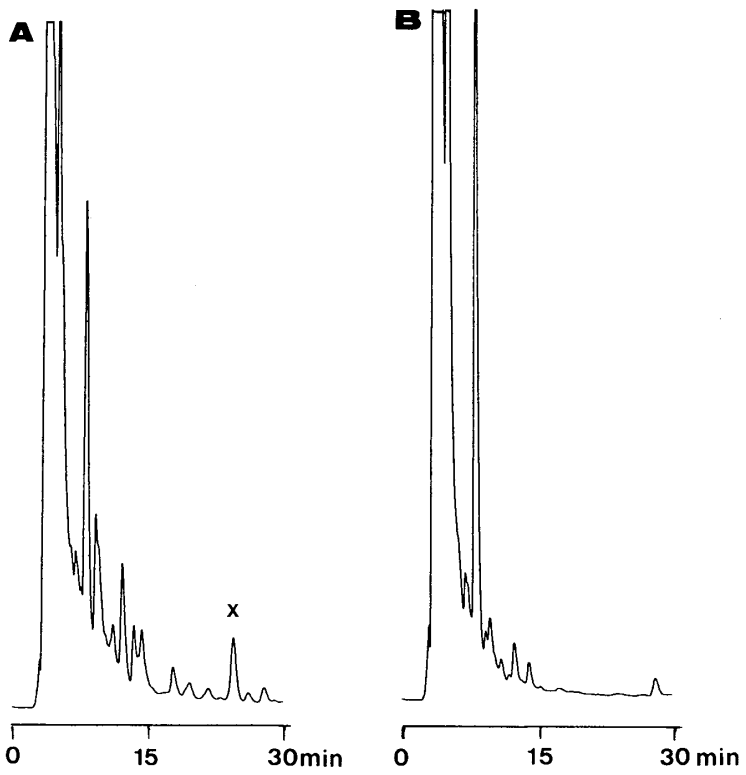

Fig. 2. HPLC Profiles of Shô-hange-ka-bukuryô-tô Extract (A) and Extract of Shô-hange-ka-bukuryô-tô Containing No Hoelen (B)

column: Develosil ODS P-5 4.6×250 mm. mobile phase: CH_3CN-H_2O-AcOH (70:30:1). flow rate: 1.0 ml/min. detection: UV 242 nm. column temperature: 40°. X: dehydropachymic acid.

acid gave reproducible results. A typical chromatogram of hoelen extract gave well resolved peaks (Fig. 1). The dehydropachymic acid contents in the 20 samples determined by HPLC varied in a range of 0.013–0.034% (Table I). A typical chromatogram of the extract of Shô-hange-ka-bukuryô-tô prepared as described above gave a good resolved peak of dehydropachymic acid (Fig. 2).

Although dehydropachymic acid is a minor component of hoelen, this method may be useful for the quality control of hoelen and Shô-hange-ka-bukuryô-tô, because the acid gives an isolated peak in the HPLC.

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References and Notes

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