Journal of Chromatography, 477 (1989) 458–462
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 576

Note

Separation and determination of 8β-hydroxyasterolid and perlolyrine in *Codonopsis pilosula* by reversed-phase high-performance liquid chromatography

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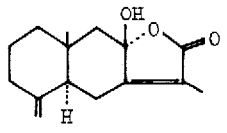
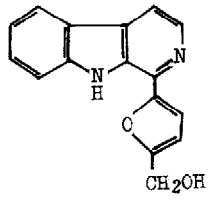
(First received February 6th, 1989; revised manuscript received April 17th, 1989)

Codonopsis pilosula (Franch.) Nannf., known as "Dangshen", is a traditional tonic medicine in China and is widely used in place of *Panax gingseng* in the treatment of shortness of breath accompanied by palpitation, lassitude and physical weakness.

There have been many reports on the investigation of the chemical ingredients and various kinds of compounds have been isolated from this herbal medicine. However, few methods for the determination of active compounds have been reported.



A. 8β-hydroxyasterolid



B. perlolyrine

This paper deals with a reversed-phase high-performance liquid chromatographic (RP-HPLC) method for separation and determination of 8β-hydroxyasterolid (A) and perlolyrine (B) respectively. 8β-Hydroxyasterolid is the first sesquiterpenoid lactone isolated from *C. pilosula*, and perlolyrine, as we reported before[1], is the first alkaloid isolated from this plant. It is very interesting that perlolyrine exists both in *C. pilosula* and in *Panax gingseng*, since the former has long been used as a substitute for the latter and similar ingredients have not yet been found in both plants. 8β-Hydroxyasterolid shows antiinflammatory properties[2] and perlolyrine belongs to the β-carboline alkaloids, which produce activity toward the benzodiazepine and γ-aminobutyric acid (GABA) receptors[3]. Due to these reasons, a method for determining their contents in different species of Codonopsis was needed. Because of the great difference in their contents in specimens, different HPLC conditions for compounds A and B have been designed.

0021-9673/89/$03.50

EXPERIMENTAL

Materials

8β-Hydroxyasterolid was isolated from *C. pilosula* (cultivated in Lucheng, Shanxi province, China) using a silica gel column (120 cm × 5 cm I.D.) eluted under low pressure with light petroleum (b.p. 30–60°C)–ethyl acetate (6:1) and then recrystallized from chloroform. The purified lactone comprised colourless platelet crystals, m.p. 168–169°C; $[\alpha]_D^{25} = +105.34°$ (*c* 0.83, $CHCl_3$). Its IR spectrum showed an hydroxyl group (3390 cm^{-1}), an α,β-unsaturated lactone group (1750, 1705 cm^{-1}) and a methene group (3090, 1650, 900 cm^{-1}). The mass spectrum showed a $(M)^+$ peak at *m/e* 248 and fragments at 230, 220, 215, 147, 44 (base peak). The UV spectrum had a maximum absorption at 224 nm in methanol. These data and the ^1H NMR spectrum were identical with those reported previously[4].

Perlolyrine was synthesized from 2-hydromethylfurfural and tryptophan according to the literature[5] and used as a reference substance for HPLC. It comprised yellow needle crystals, m.p. 183°C (subliming at 150°C) and its UV, mass and ^1H NMR spectra as well as the HPLC retention time were identical with those of the natural product.

Methanol was of analytical grade. Silica gel was obtained from Qingdao Oceanic Chemical Co.

The Codonopsis plant materials were collected from Shanxi, Sichuan provinces, etc. and identified by associate Professor Yao Damu and Professor Zhao Dawen of our Institute. The sample of Panax gingseng was provided by associate Professor Yan Kedong of the same Institute.

Apparatus

The equipment comprised a M 6000A pump, an U6K injector (Waters Assoc., Milford, MA, U.S.A.) and a SPD-1 UV–VIS detector and a Chromatopac C-R1B data processor (Shimadzu, Kyoto, Japan).

HPLC conditions

A μBondapak C_{18} column (300 mm × 4 mm), 10-μm particles, was employed. The mobile phases were methanol–water, (60:40, v/v) for compound A, (58:42) for B. The flow-rate and column temperature were 1.0 ml/min and 25°C, respectively. The detector was operated at 220 nm for A and 290 nm for B with a sensitivity of 0.08 a.u.f.s. Chart speed 2.5 mm/min.

Sample preparation

Compound A. Methanol (50 ml) was added to 2 g of powdered plant material in a stoppered flask and placed in an ultrosonic bath for 30 min. The extract was filtered and the filtrate was evaporated to dryness on a rotatory evaporator under reduced pressure. The residue was dissolved in 20 ml of water and extracted with chloroform (20 ml × 3). The extract was evaporated to dryness. The residue was dissolved in exactly 2.0 ml of methanol and filtered with a Millipore FH membrane. A 5.0-μl volume of the filtrate was injected for HPLC.

Compound B. Methanol (60 ml) was added to 6 g of powdered plant material and placed in an ultrasonic bath for 30 min. The extract was filtered and the filtrate

was evaporated to dryness. The residue was dissolved in 30 ml of 5% HCl. After elimination of lipids with light petroleum (20 ml × 2), the acidic aqueous layer was neutralized with NH_4OH and extracted with diethyl ether (20 ml × 4). The ether layers were collected, washed with a small amount of water and evaporated to dryness. The residue was dissolved in a small amount of methanol and spotted on a silica G plate (0.5 mm, 10 cm × 10 cm, prepared manually) and then developed by chloroform–methanol (9:1). A bright blue-green fluorescent band appeared on the plate. It was eluted with chloroform–methanol (1:1) and the eluate was allowed to evaporate to dryness. The residue was dissolved in precisely 0.5 ml of methanol, filtered with a Millipore FH membrane and 20.0 μl of the filtrate were subjected to HPLC.

The large amount of sugar in *C. pilosula* is the main interference in the HPLC of 8β-hydroxyasterolid. Therefore, elimination of sugar is an inevitable step. A great deal of lipid, besides sugar, also interferes with the detection of perlolyrine. These components were removed from the samples employed in the estimation of perlolyrine, which were concentrated appropriately by thin-layer chromatography (TLC) prior to HPLC. This treatment also protects the chromatographic column, thus prolonging its useful life.

Calibration graphs were constructed from the results of each of five consecutive injections. Stock solutions were prepared by dissolving 1.60 mg of compound A and 1.04 mg of B separately in 2 ml of methanol. The reference standard solutions were obtained by diluting in methanol (0.08 μg/μl for A and 0.013 μg/μl for B) and processed as described above.

RESULTS AND DISCUSSION

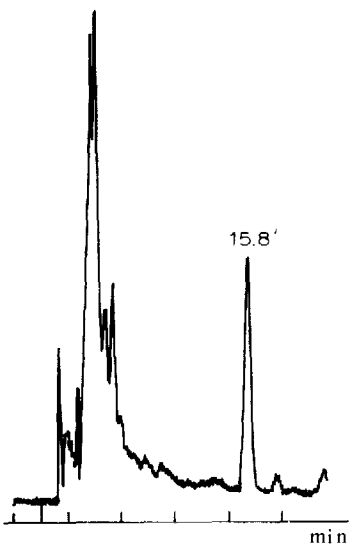
Figs. 1 and 2 show chromatograms of compound A and B in a sample. Satisfactory results were obtained.

The calibration graphs for A and B showed good linearity in the ranges of 160–800 and 26–78 ng respectively.

The recoveries of A and B were 101.6 and 101.0% respectively.

The contents of 8β-hydroxyasterolid in 10 samples, 6 cultivated and 4 wild, are presented in Table I. Rather significant variations both in quality and quantity were observed between cultivated and wild samples. For wild samples, the HPLC peak of the lactone was not found in *C. subglobosa* and in *C. tangshen*. In *C. pilosula* var. *modesta* collected in Songpan, Sichuan, the trace amount of the lactone was too low to be detected. In a sample of same species collected in Lanping, Sichuan, however, the content of the lactone was the highest among all of the samples concerned. Among cultivated samples, some variations were also observed.

The contents of perlolyrine in 11 samples, 10 of Codonopsis as above and 1 of Panax gingseng, are also shown in Table I. Rather significant variations were observed. The HPLC peak of perlolyrine was not found for *C. pilosula* var. *modesta* Nannf. (growing wild in Lanping, Sichuan) and for *C. subglobosa*. Trace amounts of the alkaloid were detected in *C. pilosula* cultivated in Gansu, Inner Mongolia and Shanxi provinces. Significant variation was also observed between the samples of the same species from different sources, *i.e.*, in two samples of *C. pilosula* var. *modesta* Nannf., growing wild in Lanping and Songpan respectively, not far away from each other geographically.



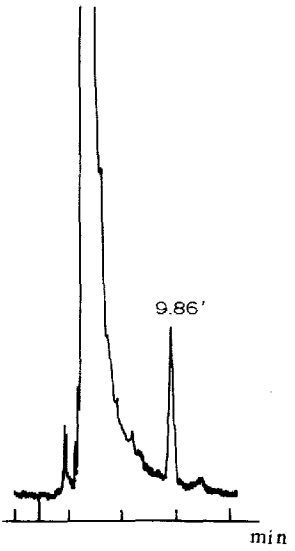


Fig. 1. HPLC trace of 8β-hydroxyasterolid.

Fig. 2. HPLC trace of perlolyrine.

The content of perlolyrine in Panax gingseng is higher than that in all Codonopsis plants concerned except *C. tangshen*.

The contents of 8β-hydroxyasterolid and perlolyrine in all the samples showed similar variations. These would be useful for quality control of the medicine.

TABLE I

CONTENTS OF 8β-HYDROXYASTEROLID AND PERLOLYRINE

No.	*Source*	*Plant*	*Content of 8β-hydroxyasterolid (‰)*	*Content of perlolyrine (‰)*
1	Lucheng, Shanxi		0.052	Trace
2	Lucheng, Shanxi		0.054	0.00020
3	Lucheng, Shanxi	*Codonopsis pilosula*	0.053	0.00015
4	Lucheng, Shanxi	(Franch.) Nannf.	0.069	0.00050
5	Gansu		0.048	Trace
6	Inner Mongolia		0.013	Trace
7	Songpan, Sichuan	*Codonopsis pilosula*	Trace	0.00013
8	Lanping, Sichuan	var. *modesta* Nannf.	0.093	–
9	Ganzi, Sichuan	*Codonopsis subglobosa*	–	–
10	Wuxi, Sichuan	*Codonopsis tangshen* Olive.	–	0.0019
11	Jilin	*Panax gingseng*		0.0010

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