Studies on Active Substances in Herbs Used for Oketsu ("Stagnant Blood") in Chinese Medicine. VI. On the Anticoagulative Principle in Paeoniae Radix

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The anticoagulative principles in Paeoniae Radix, which is one of the most important herbs and is used commonly for the treatment of female diseases in traditional oriental medicine, were investigated. In this study the measurement of plasma recalcification time in mice was found to be useful for following the anticoagulative activity of the material, and the active principles were isolated from the water extract of the herb by a combination of a partition and repeated silica gel column chromatographies.

Keywords—Paeoniae Radix; stagnant blood; anticoagulative principle; plasma recalcification time; paeoniflorin; benzoylpaeoniflorin

In the course of our study on the isolation of anticoagulative constituents in herbs used for treatment of Oketsu ("stagnant blood") in traditional Chinese medicine, we have so far reported the isolation and identification of several active principles, triolein in Persicae Semen, some curcuminoids in Curcumae Rhizoma, d-catechin in Rhei Rhizoma and paeonol in Moutan Cortex. In this paper, we will deal with the isolation and identification of anticoagulative principles in Paeoniae Radix (Paeoniae lactiflora PALL).

Paeoniae Radix is well known as one of the important herbs showing anticoagulant, anodyne and sedative action, and is used frequently treatment of female diseases in traditional oriental medicine. Previous studies on the herb have been mostly focused on the chemical components, such as tetrpenoids (e.g., paeoniflorin, benzoylpaeoniflorin, and albiflorin), tannin and paeonol. Pharmacological studies on the antiinflammatory effect of the water-soluble part of the 70% MeOH extract of the herb, and on the antiallergic and anti-platelet aggregation effects of paeoniflorin and benzoylpaeoniflorin in vitro and so on have been reported. However, no detailed study on the anticoagulative principles in the herb has appeared.

We describe here the isolation and identification of anticoagulative principles in Paeoniae Radix. During the isolation process, the anticoagulative activity of the material was determined by means of the plasma recalcification time method reported previously. Isolation of the active principles was achieved by a combination of partition and repeated column chromatographies on silica gel. The procedures are summarized in Chart 1.

The active fraction I (ethyl acetate-soluble part of the water extract) was subjected to column chromatography on silica gel with the lower layer of CHCl₃-MeOH-H₂O (7:3:1) to afford three fractions, namely fractions II₁, II₂ and II₃.

The activity emerged in all fractions. In this work, we dealt with the significantly active fractions, II₁ and II₂, which contained the bulk of the activity of fraction I. Column chromatography of active fraction II₁ on silica gel using CHCl₃-MeOH as an eluent gave two
fractions, III₁ and III₂. Significant activity was found only in fraction III₂. Final purifications of active fractions II₂ and III₂ were achieved by silica gel column chromatographies with CHCl₃–MeOH to afford two active compounds, 1 from fraction II₂ and 2 from fraction III₂. As shown in the thin layer chromatograms of each fraction in Fig. 1, silica gel column chromatography is very effective for purifying the active compounds. Anticoagulative activities of the fractions described above are shown in Chart 1. Work on other active fractions is still in progress.

Compound 1 is one of the major constituents and 2 is a minor principle in Paeoniae
Radix. Their spectral data indicated 1 to be paeoniflorin\textsuperscript{5b,10} and 2 to be 6'-O-benzoylpaeoniflorin (benzoylpaeoniflorin).\textsuperscript{11} The identifications were confirmed by direct comparisons of the spectral data (IR, NMR, UV and MS) with those of the authentic samples of paeoniflorin, obtained from Wako Co., and benzoylpaeoniflorin, prepared from 1 by the known method.\textsuperscript{12} The anticoagulative activities of authentic samples of paeoniflorin and benzoylpaeoniflorin are equal to those of 1 and 2, respectively.

Paeoniflorin and benzoylpaeoniflorin have been isolated from \textit{Paeonia lactiflora} PALL\textsuperscript{11} and \textit{Paeonia moutan} SIMS.\textsuperscript{5a} Pharmacological studies on the antiinflammatory and antiaggregatory effects of these compounds \textit{in vitro} have been reported.\textsuperscript{8,13} They were found to show an inhibitory effect on collagen-, endotoxic- and adenosine diphosphate (ADP)-induced blood platelet coagulation but not on blood aggregation \textit{in vitro}.\textsuperscript{13} Further work is necessary to elucidate in detail the mechanism of the anticoagulative effects \textit{in vivo}, in view of the reported lack of effect on blood coagulation \textit{in vitro}.

In summary, it is demonstrated that paeoniflorin and benzoylpaeoniflorin have anticoagulative activity \textit{in vivo} and might play an important role in the antiaggregatory effect of \textit{Paeoniae Radix}.

**Experimental**

Ultraviolet (UV) spectra were taken with a Shimadzu UV-360 recording spectrophotometer. Mass spectra (MS) were recorded on a JEOL JMS D-100 spectrometer. The infrared (IR) spectra were recorded on a JASCO A-2 spectrophotometer. The nuclear magnetic resonance (NMR) spectra were measured on a JEOL FX-90 Fourier-transform spectrometer (90 MHz for \textit{\textsuperscript{1}H}-NMR and 22.5 MHz for \textit{\textsuperscript{13}C}-NMR). Elemental analyses were done with a Perkin Elmer 240 analyzer. The anticoagulative activity of the material was determined by the method reported previously.\textsuperscript{3a}

**Material**—A commercial product of \textit{Paeoniae Radix} (\textit{Paeonia lactiflora} PALL) was used in this study.

**Extraction**—Ground \textit{Paeoniae Radix} (100 g) was extracted with water (500 ml) under reflux for half an hour. The mixture was centrifuged at 2500 rpm for 20 min, and the supernatant was lyophilized to give the crude extract (21.3 g).

**Partition of the H2O Extract between Water and Ethyl Acetate**—The H2O extract (21.3 g) was dissolved in water (210 ml) and extracted with ethyl acetate (210 ml) four times. Concentration of the ethyl acetate layer under reduced pressure afforded the active fraction I (1.3 g) as a brown gum.

**Silica Gel Column Chromatography of Active Fraction I**—Active fraction I (1.3 g) was subjected to column chromatography on silica gel (2.4 x 28 cm) using the lower layer of CHCl\textsubscript{3}–MeOH–H\textsubscript{2}O (7 : 3 : 1) as an eluent to afford three active fractions, namely fractions II\textsubscript{1} (230 mg), II\textsubscript{2} (475 mg) and II\textsubscript{3} (60 mg).

**Silica Gel Column Chromatography of Active Fraction II, on Silica Gel**—Active fraction II\textsubscript{1} (230 mg) was chromatographed on silica gel (0.9 x 25 cm) with CHCl\textsubscript{3}–MeOH (20 : 1) as an eluent to give the active fractions, fractions III\textsubscript{1} (94 mg) and III\textsubscript{2} (45 mg).

**Silica Gel Column Chromatography of Active Fraction II\textsubscript{2}**—Column chromatography of active fraction II\textsubscript{2} (475 mg) over silica gel (1.1 x 27 cm) using CHCl\textsubscript{3}–MeOH (10 : 1) as an eluent gave the active compound 1 (380 mg) as a white powder.

**Column Chromatography of Active Fraction III\textsubscript{1} over Silica Gel**—Active fraction III\textsubscript{1} (45 mg) was chromatographed on silica gel (0.7 x 20 cm) with CHCl\textsubscript{3}–MeOH (20 : 1) as an eluent to afford the active compound 2 (10 mg) as a white powder.

**Identification of the Active Principles, 1 and 2**—The active compounds, 1 and 2, were identified as paeoniflorin and benzoylpaeoniflorin by direct comparisons of their physical data (IR, UV, MS and NMR) with those of their authentic samples.

**References**


7) N. Nagai, Yakugaku Zasshi, 7, 288 (1887); idem, ibid., 8, 495 (1888).


