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Studies on Active Substances in the Herbs Used for Oketsu ("Stagnant Blood") in Chinese Medicine. IV. On the Anticoagulative Principle in Rhei Rhizoma

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Rhei Rhizoma is an important herb in antiphlogistic, cathartic, antipyretic, anticoagulant and hemostatic prescriptions in Chinese medicine. The anticoagulative principle in the herb was isolated by a combination of partition, fractional precipitation and column chromatography on silica gel. In the isolation process, plasma recalcification time in mice was found to be useful for following the anticoagulative activity of the material. The anticoagulative principle was identified as *d*-catechin by direct comparison with an authentic sample.

Keywords—Rhei Rhizoma; stagnant blood; plasma recalcification time; anticoagulative principle; *d*-catechin

In the previous paper¹⁾ we reported the isolation of the anticoagulative principles, curcuminoids (curcumin, *p,p'*-dihydroxydicinnamoylmethane and *p*-hydroxycinnamoylferuloylmethane), in Curcumae Rhizoma, which is one of the herbs used commonly for Oketsu ("stagnant blood")²⁾ in Chinese medicine. This paper deals with the isolation and identification of the anticoagulative principle in Rhei Rhizoma (*Rheum palmatum* L.).

Rhei Rhizoma, which is a component of Daijousyatyugan (大黃蘗虫丸), Daijyoukitou (大承氣湯) and Syoujyoukitou (小承氣湯), is one of the most important herbs in Chinese medicine as a cathartic, antiphlogistic, anticoagulant, antipyretic, expellant and hemostatic.³⁾ Previous studies on this herb have been focused mostly on the chemical components, *e.g.*, anthraquinones,⁴⁻⁶⁾ dianthrones,⁷⁾ catechin⁸⁾ and rhatanin.⁹⁾ Pharmacological studies on the extract and anthraquinones of the herb to investigate the fungicidal action^{10,11)} and the hypazoturia-inducing action¹²⁾ and so on have been reported. However no study on the anticoagulative principle in the herb has been reported.

We wish to report here the isolation and identification of the anticoagulative principle in the herb. During the isolation process, plasma recalcification time¹³⁾ in mice was used for following the anticoagulative activity of the material.¹⁴⁾ Isolation was achieved by a combination of partition, fractional precipitation and silica gel column chromatography. The procedures are summarized in Chart 1.

As shown in Chart 1, ground Rhei Rhizoma was extracted with water under reflux and then the extract was partitioned between ethyl acetate and water. As the activity emerged in the ethyl acetate layer,¹⁴⁾ this fraction I was subjected to fractional precipitation with chloroform and ethyl acetate to afford the active fraction II (soluble part of active fraction I). Then this fraction was subjected to column chromatography on silica gel with chloroform-ethyl acetate-acetonitrile (v/v/v) (3:1:1) to afford three fractions, namely fractions III, IV and V. Activity was found in two fractions, fractions III and V. The thin layer chromatograms (TLC) of the fractions on silica gel are shown in Fig. 1.

As mentioned above, the activity emerged in two fractions (fractions III and V). In this

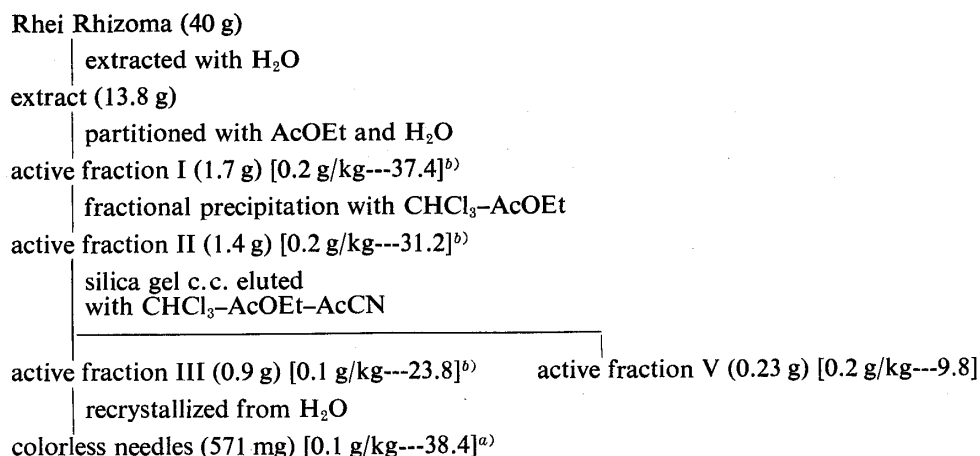


Chart 1. Isolation of the Active Principle

() indicates yields. [] indicates dose and anticoagulative activity (%). a) $p < 0.01$, b) $p < 0.05$: Significance of difference from the control group. The coagulation time of the control group was 2.40 ± 0.10 (mean \pm S.E. from 5 mice).

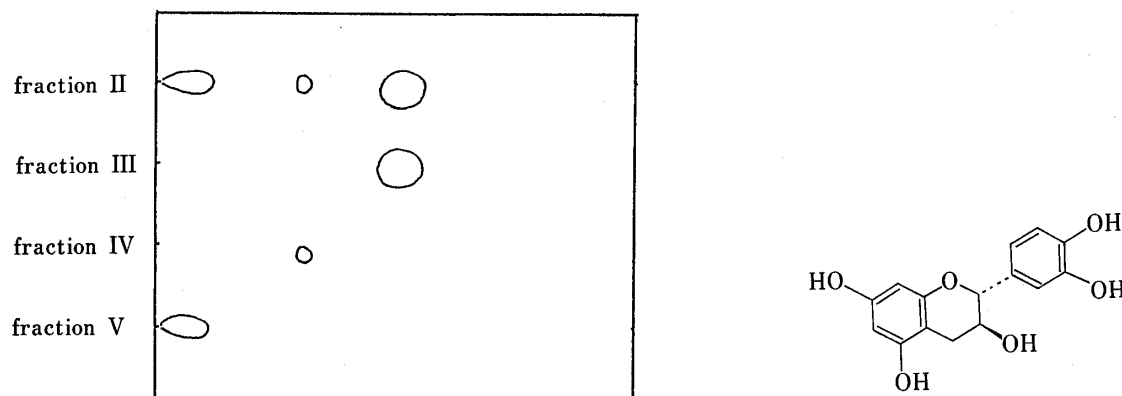


Fig. 1. Thin Layer Chromatograms of Active Fractions (Fraction II, III, IV and V)

Plate: Kieselgel H 60 (Merck Art. 5721). Solvent: CHCl₃-AcOEt-CH₃CN (3:1:1). Color reagent: I₂.

work, we will deal with fraction III, which contained the bulk of the anticoagulative activity. Work on fraction V is still in progress. Thus, the highly active fraction, fraction III, was recrystallized from water to afford colorless needles.

At higher field in the proton nuclear magnetic resonance (¹H-NMR) spectrum of the active principle, the two signals at δ 6.00 (d) and 5.86 (d) are ascribable to C₆- and C₈-H on the A-ring of a flavan skeleton and the AB₂-type signals at δ 6.76—6.88 (3H, m) are assignable to the protons of a 1,3,4-trisubstituted benzene ring. The ABX-type signals (at δ 3.01, dd, $J = 5.7$ and 15.8 Hz; C_{4e}-H, δ 2.50, dd, $J = 8.2$ and 15.8 Hz; C_{4a}-H, δ 3.98, m; C₃-H) suggested the presence of a catechin moiety in the molecule of the active principle. Thus, the active principle was characterized as *d*-catechin(*trans*-2-(3,4-dihydroxyphenyl))-3,4-dihydro-2*H*-benzopyran-3,7-triol).⁹⁾ This identification was confirmed by direct comparison with an authentic sample. The anticoagulative activity of the authentic sample was equal to that of the natural compound.

d-Catechin has been isolated from many plants, e.g., *Polygonum bistorta*,¹⁵⁾ *Chamaecyparis pisifera*,¹⁶⁾ *Eucalyptas* species (*E. gingata* and *E. sieberiana*),¹⁷⁾ *Prunus* species,¹⁸⁾ *Sanguisorba* species,¹⁹⁾ *Hypericum* species,²⁰⁾ *Ephedra helvetica*,²¹⁾ *Rheum palmatum*

L.,⁹⁾ etc. Pharmacological studies on inhibition of prostaglandin synthetase,²²⁾ mutagenicity,²³⁾ and antiviral²⁴⁾ and antibacterial²⁵⁾ activities of *d*-catechin have been reported. However, no study on the anticoagulative action of the compound has appeared. Earlier studies indicating that catechols activate the anticoagulative system²⁶⁾ and inhibit platelet aggregation²⁷⁾ support our finding that *d*-catechin inhibits blood coagulation.

It is interesting that *d*-catechin has been isolated from *Sanguisorba*¹⁹⁾ and *Hypericum*²⁰⁾ species, which are commonly used as hemostatics in Chinese medicine, because the herbs used as hemostatics in Chinese medicine appear to show not only antihemorrhagic action, but also anticoagulative action.³⁾

Experimental

¹H-NMR and carbon-13 nuclear magnetic resonance (¹³C-NMR) spectra were recorded on a JEOL FX-90 Fourier transform NMR spectrometer and are calibrated in parts per million (δ) downfield from tetramethylsilane as an internal standard. Abbreviations used: s, singlet; d, doublet; dd, double doublet; m, multiplet; t, triplet. Optical rotation was determined with a JASCO DIP-140 digital polarimeter (cell length: 100 mm). The infrared (IR) spectrum was recorded on a JASCO IRA-2 grating infrared spectrophotometer. The low-resolution mass spectrum (MS) was recorded on a JEOL JMS-D 100 instrument. Ultraviolet (UV) spectra were taken with a Shimadzu UV-360 recording spectrophotometer. Elemental analysis was done with a Perkin Elmer 240 analyzer. The melting point is uncorrected.

Assay of Anticoagulative Activity—Anticoagulative activity was followed by measuring the plasma recalcification time¹³⁾ in mice weighing 18 to 20 g. Test material homogenized in 1% methylcellulose–0.9% sodium chloride aq. was given interaperitoneally by injection. The plasma coagulation time was determined by the procedure used previously. The anticoagulative activity was calculated by means of equation 1. The statistical treatment of data was done according to the reported method.¹⁴⁾

$$\text{anticoagulative activity (\%)} = 100 \left(1 - \frac{\text{average coagulation time of treated group}}{\text{average coagulation time of control group}} \right) \quad (1)$$

Material—Rhei Rhizoma used in this study was a commercial product and was identified as *Rheum palmatum* L. by an expert.

Extraction—Ground Rhei Rhizoma (40 g) was extracted with water (40 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 (13.8 g) as a light brown powder.

Partition between Ethyl Acetate and Water—The crude extract (13.8 g) was dissolved in water (138 ml) and extracted with ethyl acetate (138 ml) four times. Concentration of the ethyl acetate layer under reduced pressure gave active fraction I (1.7 g) as a brown gum.

Silica Gel Column Chromatography—Active fraction I (1.7 g) was subjected to fractional precipitation with chloroform and ethyl acetate (1 : 1) (30 ml) to afford the soluble part (active fraction II) (1.4 g). This fraction (1.4 g) was subjected to column chromatography over silica gel (3.2 × 24 cm) with CHCl₃–AcOEt–AcCN (3 : 1 : 1) to give active fraction III (0.9 g) as a colorless gum. Fraction III (900 mg) was recrystallized from water to yield colorless needles (571 mg).

Identification of the Active Principle—The active principle has the following properties; mp 175–176 °C. MS *m/z*: 290 (M⁺), 152, 139, 123. $[\alpha]_D^{21} + 12.3^\circ$ (*c* = 0.36, acetone). *Anal.* Calcd for C₁₅H₁₄O₆: C, 62.07; H, 4.83. Found: C, 61.77; H, 4.86. ¹H-NMR (in *d*₆-acetone) δ (ppm): 7.20–8.60 (4H, br), 6.76–6.88 (3H, m), 6.00 (1H, d, *J* = 2.2 Hz), 5.86 (1H, d, *J* = 2.2 Hz), 4.55 (1H, d, *J* = 8.2 Hz), 3.98 (1H, m), 3.01 (1H, dd, *J* = 15.8 and 5.7 Hz), 2.50 (1H, dd, *J* = 8.2 and 15.8 Hz). ¹³C-NMR (in *d*₄-methanol) δ (ppm): 157.6 (s), 157.3 (s), 146.2 (s), 132.2 (d), 120.0 (d), 116.1 (d), 115.3 (d), 113.1 (d), 110.4 (s), 96.5 (d), 96.5 (d), 95.6 (s), 82.7 (d), 68.8 (d), 28.4 (t). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400, 1640, 1525, 1510, 1475, 1450, 1280, 1140, 1035 and UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log *ε*) 280 (3.71), $\lambda_{\text{max}}^{\text{MeOH}+1 \text{ N NaOH}}$ nm (log *ε*): 288 (3.75). The active principle was identified as *d*-catechin by direct comparison with an authentic sample.

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References and Notes

- 1) Part III: T. Kosuge, H. Ishida and H. Yamazaki, *Chem. Pharm. Bull.*, **33**, 1499 (1985).

- 2) D. Yakazu, *Nippon toyogakukaishi*, **25**, 1 (1969).
- 3) K. Akamatsu, "Wakanyaku," Ishiyaku Publications, Tokyo, 1966, p. 493; S. Tin, "Kanpouiyakudaiziten," Vol. II, Kodansha, Tokyo, 1982, p. 10; Kobe Chuigaku Kenkyukai, "Kanyaku No rinsyououyou," Ishiyaku Publications, Tokyo, 1969, p. 52.
- 4) A. S. Romanova and A. I. Ban'kovskii, *Med. Prom. SSSR*, **17**, 13 (1963).
- 5) S. Shibata and M. Takido, *Chem. Pharm. Bull.*, **72**, 1311 (1952).
- 6) E. Schratz and H. J. Vethacke, *Planta Med.*, **6**, 44 (1958).
- 7) J. H. Zwang, *Planta Med.*, **13**, 474 (1956); M. Miyamoto, S. Imai, M. Shinohara, S. Fujioka, M. Goto, T. Matsuoka and H. Fujimura, *Yakugaku Zasshi*, **87**, 1040 (1967).
- 8) G. Nonaka, M. Minami and I. Nishioka, *Chem. Pharm. Bull.*, **25**, 2300 (1977).
- 9) G. Nonaka, I. Nishioka and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981).
- 10) J. W. Fairbairn and M. J. R. Moss, *J. Pharm. Pharmacol.*, **22**, 584 (1970); K. Sakai, K. Yamauchi and S. Kuwano, *Planta Med.*, **37**, 370 (1979).
- 11) J. S. Agarwal, R. P. Rastogi and O. P. Srirastava, *Curr. Sci.*, **45**, 619 (1976).
- 12) T. Nagasawa, S. Shibata, H. Oura, Y. Shoyama and I. Nishioka, *Chem. Pharm. Bull.*, **28**, 1736 (1980).
- 13) I. Kanai and M. Kanai, "Rinsyoukensateiyou," Vol. VI, Kanehara Publications, Kyoto, 1973, p. 83.
- 14) Part I: T. Kosuge, H. Ishida, H. Yamazaki and M. Ishii, *Yakugaku Zasshi*, **104**, 1050 (1984).
- 15) F. Gstirner and H. Hopmann, *Arch. Pharm.*, **286**, 150 (1953).
- 16) M. Hasegawa, H. Nakamura and S. Tsuruno, *J. Japan Forestry Soc.*, **37**, 488 (1955).
- 17) W. E. Hills and A. Clarke, *Biochem. J.*, **74**, 607 (1960).
- 18) M. Hasegawa, *J. Org. Chem.*, **24**, 408 (1959); M. Hasegawa, *Shokubutsugaku Zasshi*, **82**, 458 (1969).
- 19) K. M. Dzhemukhadze and G. R. Azovtsev, *Dokl. Akad. Nauk. SSSR*, **161**, 715 (1965).
- 20) K. Akhtazhiev and G. Kitanov, *Farmatsiya*, **24**, 17 (1974); G. M. Kitanov and K. Bachev, *ibid.*, **31**, 21 (1981).
- 21) H. Friedrich and H. Wiedemeyer, *Planta Med.*, **30**, 163 (1976).
- 22) J. Bauman, G. Wurm and F. V. Bruchhausen, *Arch. Pharm.*, **313**, 330 (1980).
- 23) J. P. Brown and P. S. Dietrich, *Mutat. Res.*, **66**, 223 (1979).
- 24) A. Wacker and H. G. Eilmes, *Arzneim-Forsch*, **28**, 347 (1978).
- 25) B. Kabadi and E. R. Hammerlund, *J. Pharm. Sci.*, **52**, 1154 (1963).
- 26) G. V. Andreenko and L. V. Lyutova, *Vopr. Pitan.*, **30**, 38 (1971).
- 27) F. Jobin and F. Tremblay, *Thromb. Diath. Haemorrh.*, **22**, 468 (1969).