Platelet Anti-Aggregation of *Paeony* Root

Sam Sik Kang, Ju Sun Kim, Eun Mi Kim and Hye Sook Yun-Choi

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
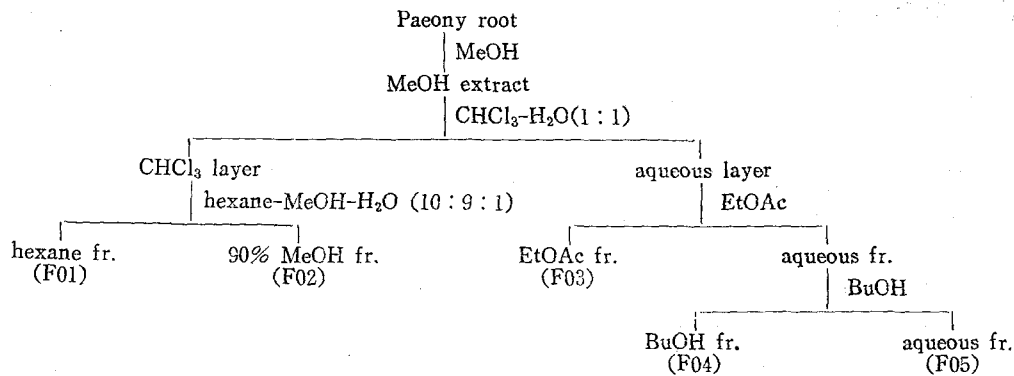
Abstract—Paeony root was extracted and fractionated for the identification of the platelet anti-aggregatory components. And gallic acid methylester was isolated as the major component from the active sub-fraction F302.

Keywords—Paeony root • platelet anti-aggregation • gallic acid methylester

Platelet aggregation is a crucial factor in the pathogenesis of ischemic diseases. Since platelets readily aggregate in response to a variety of endogenous substances and secrete various substances that cause further aggregation, they can initiate thrombus formation and precipitate thromboembolism leading to ischemic diseases. In addition, substances secreted from platelets can mediate many other biologic reactions and may also be involved in atherogenesis and other pathologic processes.[1] Hence, drugs that inhibit platelet aggregation or secretion could have wide biological implications. In an attempt to search for inhibitors of blood platelet aggregation, extracts of various plant materials which have folkloric reputation for the the treatment of symptoms related to thromboembolic disorders were evaluated.[2,3] Paeony root was one of the plant materials that showed strong inhibitory activities against ADP-, arachidonic acid(AA)-, or collagen induced platelet aggregation. Thus, the work was proceeded to the activity guided treatments and fractionations of the extract of paeony root for the identification of the platelet anti-aggregatory components.

Paeony root has been considered as one of the most important crude drugs used in traditional oriental medicine and has been employed as a circulatory tonic in care of weakness, night sweats, lumbar pain, etc.[4] Roots of various *Paeonia* sp. (Ranunculaceae) including *P. albiflora*, *P. lactiflora*, and *P. officinale* have been utilized as Paeony root and several monoterpenoid glucoside components including paeoniflorin, albiflorin, galloylpaeoniflorin have been isolated from this crude drug.[5~7] For the purpose of the identification of platelet anti-aggregatory component, Paeony root was extracted and fractionated into five solvent fractions as shown in Scheme 1(F01~F05). And each fraction was applied to the platelet aggregation systems. Those samples with low solubility or high turbidity were tested with the smear method and the most commonly used turbidimetric method was used as long as sample was solubilized in either 10% EtOH or PPP and the results are summerized in Table I. EtOAc soluble fr. (F03) showed strong inhibition (showing less than 10% aggregation) at the conc of 1 mg/ml against collagen or AA induced aggregation and at 3mg/ml against ADP induced aggregation. F02 (90% MeOH fr.) was also highly inhibitory, however less than F03, showing less than 10% aggregation at the conc of 5 mg/ml. Milder inhibitory effects were observed with BuOH soluble fr. (F04) comparing with F02 or F03 against all the three aggregating agents tested. Hexane soluble fr. (F01) and H_2O fr. (F05) did

```
                        Paeony root
                          | MeOH
                     MeOH extract
                          | CHCl₃-H₂O(1 : 1)
        ┌─────────────────────────────────────┐
    CHCl₃ layer                          aqueous layer
        | hexane-MeOH-H₂O (10 : 9 : 1)        | EtOAc
    ┌───────────────┐               ┌──────────────────┐
  hexane fr.      90% MeOH fr.    EtOAc fr.         aqueous fr.
   (F01)            (F02)          (F03)              | BuOH
                                            ┌──────────────────┐
                                          BuOH fr.        aqueous fr.
                                           (F04)             (F05)
```

Scheme 1. Extraction and fractionation of Paeony root.

Table I. Degrees of platelet aggregation with F01~F05

Fr. \ Aggregating agents / Sample Concn.(mg/ml)	ADP			Collagen			AA		
	5	3	1	5	3	1	5	3	1
F01	₩*			₩*			₩*		
F02	—*	+*	+	—*	±*	+	—*	±*	±
F03		—	₩			—			—
F04	±*	+	₩		—	₩	±*	±	₩
F05	₩*	₩		+*	₩		₩*	₩	

*Tested with smear method.

The degrees of aggregation were judged as the followings

	Smear method	Turbidimetric method(% aggregation)
₩	full aggregation	>80
+	intermediate aggregation	30~80
±	slight aggregation	10~30
—	no aggregation	<10

not inhibit the platelet aggregation in the system presently adopted. F03, the most active fr., was applied to a column chromatography and again fractionated into five sub-fractions(F301~F305). The inhibitory activities were tested against collagen-induced aggregation yielding F302 the most potent sub-fraction. Platelet aggregation was fully inhibited (—, no aggregation) at the conc of 0.5 mg/ml, 88% inhibited (+, slight aggregation) at the conc of 0.25 mg/ml, and 36% inhibited (intermediate aggregation) at the conc of 0.1 mg/ml against collagen-induced aggregations. Gallic acid methylester was isolated as the major component from the active sub-

fraction F302 and the works are presently being undertaken.

Experimental

General experimental procedures—Melting point was determined on a Mitamura-Riken apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. ¹H-NMR spectra were obtained on a Varian FT-80A (80 MHz) using TMS as an internal standard. MS spectra were determined on a Hewlett-Packard 5985B GC/MS System equipped with a direct inlet system. Platelet

aggregation (turbidimetric method) were measured with a Chrono-Log Whole Blood Lumi-Aggregation System.

Plant material and reagents—Parony root was purchased from a local crude drug market and identified by Prof. Hyung Joon Chi of Natural Products Research Institute, Seoul National University. Voucher specimens were deposited at the Institute. ADP (adenosine 5'-diphosphate dicyclohexyl ammonium salt), AA (Arachidonic acid sodium salt) and collagen (Platelet aggregation reagent) were purchased from Sigma Chemical Company.

Extraction and Fractionation—The dried chopped Paeony root (3 kg) was extracted three times with refluxing MeOH for 4hr and the resulting MeOH extract was fractionated as described in Scheme. 1. The MeOH residue (365 g) was partitioned between $CHCl_3$ and H_2O(1 : 1). The $CHCl_3$ layer was evaporated and the residue partitioned between hexane and 10% H_2O in MeOH (1 : 1) to afford the hexane soluble fr. (F01, 20.9 g) and the aqueous MeOH fr. (F02, 13.5 g). The aqueous layer after removing the $CHCl_3$ solubles was successively partitioned with EtOAc and then BuOH. Reduced pressure evaporation of each layer afforded EtOAc fr. (F03, 55.8g), BuOH fr. (F04, 65.7 g) and H_2O fr. (F05, 200 g). Fifty g of EtOAc fr. (F03) was applied to a silica gel (No. 9385) column and then eluted with 5% $CHCl_3$-MeOH and obtained 5 sub-fractions (F301~F305).

Separation of gallic acid methylester[8]—An active sub-fraction, F302, was applied to a SiO_2 column, eluted with hexane-EtOAc (8 : 5) and obtained gallic acid methylester as pale-yellow stout crystal after recrystallization from acetone. mp 196~198°; IR ν_{max}^{KBr} 3360, 1695, 1620, 1536, 1440, 1374 cm^{-1}; UV λ_{max}(log ε) (MeOH) 276(4.09) nm; ^1H-NMR (DMSO-d$_6$): δ 3.74(3H, s, COOCH$_3$), 6.93(2H, s, H-2,6); MS m/z(rel. int., %) 184(M$^+$, 59.3), 153(100),

125(20.7), 107(4.5), 79(8.5).

Preparation of platelet rich plasma (PRP)—Blood was drawn from hearts of $CHCl_3$-anesthetized Sprague-Dawley rats (males, 220±30 g) into a plastic syringe containing 1/10 vol. of 2.2% trisodium citrate solution. The citrated blood was centrifuged (200×g) for 10 min at room temperature to give supernatant platelet rich plasma (PRP). The remaining blood was centrifuged at 2000×g for 30 min and the resulting platelet poor plasma (PPP) was used for the preparation of sample solutions.

Modified smear method[2]—The test sample solution (20 μl prepared in either saline-EtOH or PPP) was added to 160 μl of PRP. The mixture was incubated for 2 min at 37° and 20 μl of either one of the solutions of ADP, AA or collagen was added, to give the final concentrations of $7.5×10^{-7}$ g/ml, $6×10^{-5}$ g/ml and $1×10^{-5}$ g/ml respectively. After vigorous agitation for 10 sec, and incubation at 37° for 4 min,, thin smears were prepared on glass slides and quickly dried in the air. The smears were stained with a Wright-Giemsa stain, washed and dried. The stained smears were subjected to an examination under an ordinary light microscope using an oil immersion objective lens (1000×). The degrees of aggregation of platelets were judged as described by Yun-Choi *et al.*[2]

Turbidimetric method[9]—The turbidimetric measurements of platelet aggregation were performed by the method of Born *et al.*[9,10] with a Chrono-Log whole blood platelet aggregometer. The lower and upper limits were set with PRP and with PPP. After 2 min of pre-incubation of PRP (450 μl) with 50 μl of sample or vehicle (saline-EtOH or PPP) at 37° with stirring at 1000 rpm, 4~6.5 μl of either one of the solutions of ADP, collagen or AA was added, to give the final conc. of $3.4×10^{-6}$ g/ml, $1×10^{-5}$ g/ml and $6.5×10^{-5}$ g/ml respectively, to induce platelet aggregation. The degree of aggregation

of each sample was judged from the initial maximum aggregation.

Literature Cited

1. Philip, R.B.: *Meth. Find. Exptl. Pharmacol.* 1, 197 (1979).

2. Yun-Choi, H.S., Kim, S.O., Kim, J.H., Lee,

3. Yun-Choi, H.S., Kim, J.H. and Lee, J.R.: *Kor. J. Pharmacogn.* 17, 19 (1986).

4. Perry, L.M.: "Medicinal Plants of East and Southeast Asia," MIT Press, Cambridge, p.336 (1980).

5. Shibata, S., Aimi, N. and Watanabe, M.: *Tetrahedron Lett.* 1991 (1964).

6. Kaneda, M., Iitaka, Y. and Shibata, S.: *Tetrahedron* 28, 4309 (1972).

7. Kang, S.S., Shin, K.H. and Chi, H.-J.: *Arch. Pharm. Res.* 14, 52 (1991).

8. Kim, Y.H.: *Kor. J. Pharmacogn.* 22, 22 (1991).

9. Born, G.V.R. and Cross, M.I.: *J. Physiol.* 168, 178 (1961).

10. Born, G.V.R.: *Nature* 194, 927 (1962).

J.R. and Cho, H.-I.: *J. Nat. Prod.* 48, 363 (1983).