Platelet Aggregation Inhibitors from *Populus sieboldii* MIQUEL

Kiyomi KAGAWA, Katsuya TOKURA,* Kiyohisa UCHIDA, Hisato KAKUSHI, and Tsutomu SHIKE

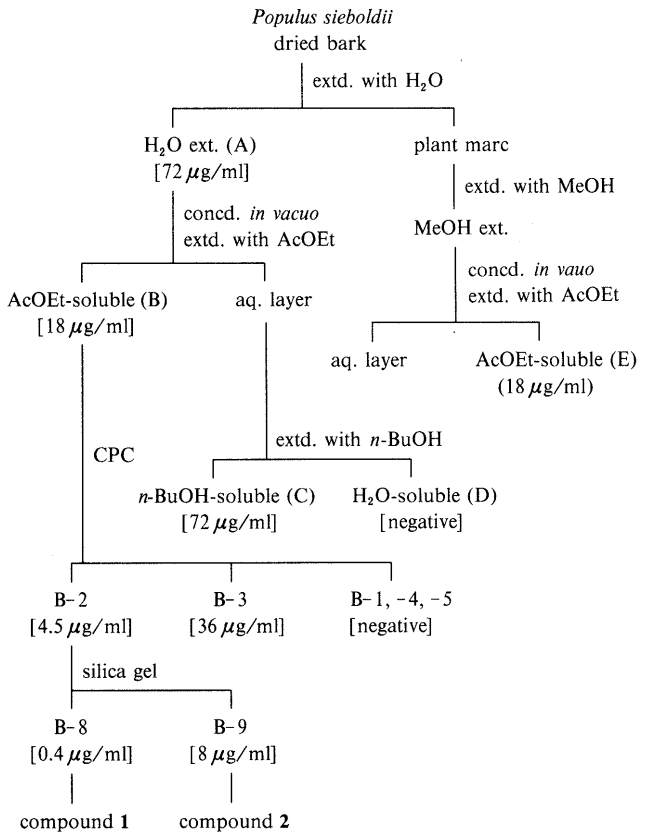
Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan. *Received January 13, 1992*

The water extract of *Populus sieboldii* MIQUEL (Salicaceae) inhibited arachidonic acid-induced platelet aggregation. Pyrocatechol and salicyl alcohol were isolated as active constituents. Pyrocatechol showed an inhibitory effect on platelet aggregation induced by arachidonic acid with IC_{100} value of 4 μM, which was 25 times more potent than aspirin.

Keywords *Populus sieboldii*; Salicaceae; pyrocatechol; salicyl alcohol; platelet aggregation inhibitor

The bark, leaves, roots and flowers of *Populus* plants have been used in traditional Chinese medicine against rheumatism, hypertension, pain, and dropsy, but no medicinal uses of *Populus sieboldii* MIQUEL (Salicaceae) have been described. In screening for platelet aggregation inhibitors from plant sources, we found that the aqueous extract of *Populus sieboldii* could inhibit platelet aggregation induced by arachidonic acid (AA). Separation and identification of the active components and the inhibition of platelet aggregation by these components are reported in this paper.

Inhibition of AA- or collagen-induced platelet aggregation was tested using rabbit platelet rich plasma. The water extract (fraction A) inhibited both AA- and collagen-induced platelet aggregation. Fraction A was fractionated as shown in Fig. 1 and each fraction obtained was tested for its inhibitory effect on AA-induced platelet aggregation.



[ ] indicates inhibitory activity (IC_{100} values) of each fraction on platelet aggregation induced by arachidonic acid.

Fig. 1. Fractionation of Water Extract of *Populus sieboldii*

The activity was expressed by concentrations causing 100% inhibition of aggregation (IC_{100} values). The active fraction B-2 was further separated to give active crystalline compounds **1** (mp 104—105 °C) and **2** (mp 82—84 °C). Infrared (IR) spectra and proton nuclear magnetic resonance (^{1}H-NMR) spectra suggested that **1** and **2** were pyrocatechol and salicyl alcohol, respectively. This was confirmed by direct comparison (mixed melting points, thin-layer chromatography, and IR and ^{1}H-NMR spectra) with commercially available samples. Compounds **1** and **2** have been isolated from *Populus* plants along with various phenolic glycosides.

The inhibitory effects of phenolic compounds **1**, **2**, eugenol (**3**), and guaiacol (**4**) on AA-induced platelet aggregation were compared with that of aspirin (**5**) (Table I). IC_{100} value of **1** was one 25th that of **5**. **1**, **2**, and **5** did not inhibit the platelet aggregation induced by U-46619 which was assumed to be a thromboxane (TX) A_2 agonist. The inhibitory effects of catechol derivatives on AA-induced platelet aggregation were studied from a mechanistic viewpoint in a recent study.[1)]

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus. IR spectra were recorded on a Hitachi 260-10 infrared spectrophotometer. ^{1}H-NMR spectra were observed on a Varian EM 390 spectrometer using tetramethylsilane (TMS) as an internal standard. Centrifugal partition chromatography (CPC) was carried out with a model CPC-LLN instrument (Sanki Engineering Co.). High performance liquid chromatography (HPLC) was performed using an Altex 110A HPLC pump equipped with a ultraviolet (UV) detector UVILOG-5III (Oyo Bunko Kiki Co.), with monitoring of the UV absorption at 254 nm.

Examination of Platelet Aggregation Platelet aggregation was tested by Born's method[2)] using an Auto-ram61 type aggregometer (Rika-Denki Co., Ltd. Tokyo) as reported earlier.[3)]

Extraction and Isolation The dried bark (700 g) of *Populus sieboldii* (collected in Kohga, Shiga Prefecture) was extracted twice with hot water and then with MeOH. One tenth of the water extract was lyophilized to give fraction A. The residual water extract was partitioned with AcOEt and *n*-BuOH successively to give fractions B (19.42 g) and C

TABLE I. Inhibitory Effect of Phenolic Compounds on AA-Induced Platelet Aggregation

Compound	Inhibition of platelet aggregation
	IC_{100} (μM)
1	4
2	200
3	1
4	4
5	100

(23.68 g). Fraction B (500 mg) was separated by CPC using the upper layer of the solvent system $CHCl_3$–MeOH–H_2O (5 : 6 : 5) as the stationary phase and the lower layer as the mobile phase to give the active fractions B-2 and B-3. Fraction B-2 (100 mg) was chromatographed on Silica gel 60 (Merck, 70—230 mesh) using a mixture of $CHCl_3$ and MeOH (4 : 1) as a developing solvent. The more polar fraction B-7 (70 mg) was separated by HPLC on a Develosil ODS column (Nomura Chemicals Co., 10—20 μm, 20 mm i.d. × 250 mm) using 50% MeOH as a developing solvent and afforded three fractions (17, 38, and 13 mg, respectively), but none of them showed activity. The less polar fraction B-6 (50 mg) was chromatographed on Silica gel 60 (Merck, 230—400 mesh) using a mixture of benzene and AcOEt (3 : 1) to obtain active fractions B-8 (21 mg) and B-9 (19 mg). Fractions B-8 and B-9 were recrystallized from benzene to afford **1** (18 mg), colorless prisms, and **2** (16 mg), colorless plates, respectively. The plant marc after water extraction was extracted with MeOH. The MeOH extract was evaporated *in vacuo* and partitioned between AcOEt and H_2O to obtain an AcOEt-soluble fraction (E, 18.63 g). Fractions E (500 mg), B-3 (112 mg), and C (1000 mg) were separated by column chromatography on Silica gel 60 (Merck, 230—400 mesh), as done to separate fraction B-6, and gave **1** (8.8, 0.8, and 2.5 mg, respectively) and **2** (6.2, trace, and 2.3 mg, respectively).

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