Antiplatelet Flavonoids from Seeds of *Psoralea corylifolia*

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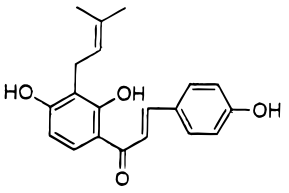
The MeOH extract of the seeds of *Psoralea corylifolia* L. was found to inhibit the aggregation of rabbit platelets induced by arachidonic acid, collagen, and platelet activating factor. Bioassay-directed fractionation led to the isolation of three flavonoids, isobavachalcone (**1**), neobavaisoflavone (**2**), and bavachin (**3**). Compounds **1** and **2** inhibited platelet aggregation.

The seeds of *P. corylifolia* L. (Leguminosae) is used to treat premature ejaculation, spermatorrhea, enuresis, backache, knee pain, and pollakiuria. Externally, it is used for vitiligo, callus, psoriasis, and alopecia.[1] Previous phytochemical studies on this drug have afforded the flavonoids isobavachalcone,[2] neobavaisoflavone,[3] and bavachin;[2] coumarins;[4] and bakuchiol.[5] In our previous studies on the development of naturally occurring antiplatelet agents, the isolation of a number of aggregation inhibitors from Chinese herbs have been reported.[6−8] In our current study, a fraction from the seeds of *Psoralea corylifolia* L. was found to inhibit platelet aggregation induced by arachidonic acid (AA). We now report here the isolation of three flavonoids from the active fraction, and the inhibition of AA-, collagen-, and platelet-activating-factor (PAF)-induced platelet aggregation by these compounds.

The MeOH extract from the seeds of *P. corylifolia* was partitioned between $CHCl_3$ and H_2O. Because the $CHCl_3$ phase exhibited strong inhibitory effects on the AA-induced platelet aggregation, it was further fractionated by chromatography on Si gel and Sephadex LH-20, with each fraction being monitored for platelet aggregation inhibitory activities. Three flavonoids, isobavachalcone (**1**), neobavaisoflavone (**2**), and bavachin (**3**), were isolated and identified by comparison of their spectral data (IR, MS, and NMR) with those previously reported.[2,3]



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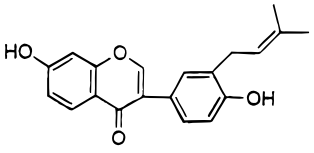

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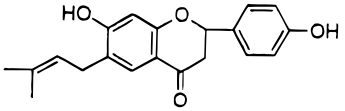

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The inhibitory effects of the isolated compounds on collagen-, AA- and PAF-induced platelet aggregation are shown in Table 1. The antiplatelet aggregation activity of compound **1** appeared to be specific as it was most effective against aggregation induced by AA, with a 50% inhibitory concentration (IC_{50}) of about 0.5 μM. On the other hand, the aggregation inhibition of compound **1** against aggregations induced by collagen (20 μg/mL) and PAF (2 nM) was only minimal, with an IC_{50} of 65.1 and 41.6 μM, respectively. Compound **2** inhibited both AA- and PAF-induced aggregation of rabbit platelets, although the degrees of inhibition were different as compared to their respective positive controls. In the case of AA inhibition, where aspirin was used as the positive control, the IC_{50} for compound **2** was 7.8 μM as compared to 32.7 μM for aspirin. In the case of PAF-induced aggregation, the inhibitory potency of compound **2** was less than that of the positive control CV-3988, as indicated by its IC_{50} of 2.5 μM vs. 1.1 μM for CV-3988. Nevertheless, the inhibitory action was concentration-dependent, as evidenced by the rightward shift of the PAF-induced concentration-aggregation curve (2−80 nM) (Figure 1).

Table 1. Effect of Flavonoids from *P. corylifoli* on the Platelet Aggregation Induced by Collagen (Col), Arachidonic Acid (AA), and Platelet Activating Factor (PAF)[a]

	IC_{50} (μM)		
compounds	col	AA	PAF
1	65.1 ± 9.1	0.5 ± 0.1	41.6 ± 6.2
2	62.4 ± 10.4	7.8 ± 2.5	2.5 ± 0.3
3	n.d.[b]	n.d.[b]	n.d.[b]
aspirin[d]	30.5 ± 5.7	32.7 ± 6.4	[c]
CV-3988[d]	[c]	[c]	1.1 ± 0.3

[a] Platelets were preincubated with various concentration of each compound or the solvent (0.5% DMSO, control) at 37 °C for 3 min, collagen (20 μg/mL), AA (100 μM), or PAF (2 nM) was added to trigger the reaction. The activity of antiplatelet aggregation (%) was calculated by the following equation: antiplatelet aggregation (%) = [1 − (platelet aggregation potency of sample/platelet aggregation potency of control)] × 100%. Then, the IC_{50} value of each compound was calculated and shown as mean ± SD (n = 3 − 6). [b] No antiaggregation activity was detectable when the test compound reached a concentration of 300 μM. [c] Not detected. [d] Positive control standard.

Several mechanisms have been suggested to be involved in the inhibition of platelet aggregation. Many flavonoids have been linked to the inhibition of cyclooxygenase in arachidonic acid metabolism.[9] The inhibition by flavonoids on platelet activation by thromboxane-dependent stimuli may be related to this effect. Further studies are required to ascertain whether this effect is responsible for the inhibition of AA-induced platelet aggregation by compounds **1** and **2**. Furthermore, the fact that compound **2** also markedly inhibited the PAF-induced platelet aggregation suggested that additional mechanisms might be involved in anti-

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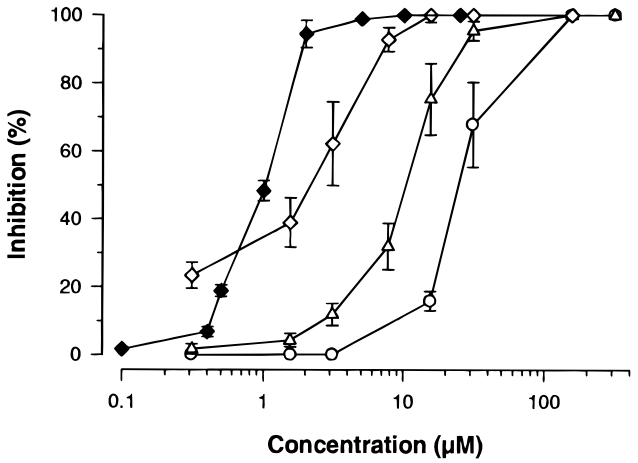

Figure 1. Concentration-dependent inhibition by neobavaisoflavone (**2**) on PAF-induced platelet aggregation. Platelets were preincubated with various concentration of neobavaisoflavone (open symbols), CV-3988 (closed symbol), or 0.5% DMSO at 37 °C for 3 min, 2 nM (◇, ◆), 5 nM (△), or 80 nM (○) of PAF was then added to trigger platelet aggregation. The percent inhibition of aggregation was calculated assuming the value of control (0.5% DMSO) to be 100% ($n = 4 - 6$).

platelet activation by compound **2**. It has been reported that some isoflavones are inhibitors of protein tyrosine kinase, which is involved in the PAF receptor-coupled activation of phospholipase C and signal transduction.[10,11] Whether this was the case in the inhibition of PAF-induced platelet aggregation by compound **2** awaits further investigations.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco Micro Melting Point Apparatus and are uncorrected. The IR spectra were recorded using a JASCO-IR-100 spectrometer. ^{1}H-NMR spectra were taken on Varian Gemini 200 FT-NMR. EIMS spectra were recorded on JEOL JMS-HX100 spectrometer. Aspirin was purchased from Sigma Chemicals. CV-3988 [(*RS*)-2-methoxy-3-(octadecylcarbamoyloxy)propyl 2-(3-thiazolio)ethyl phosphate] was purchased from Biomol Research Laboratory, Inc. (Polymouth Meeting, PA).

Plant Material. The seeds of *P. corylifolia* L. were obtained from a market in Taipei under the direction of Mr. M. T. Kao of the National Research Institute of Chinese Medicine, where voucher specimens are maintained.

Extraction and Isolation. The seeds of *P. corylifolia* (500 g) were extracted with MeOH (3 L × 3), the extract concentrated *in vacuo*, and the residue was partitioned between $CHCl_3$ and H_2O. Because the $CHCl_3$ phase was shown to inhibit AA-induced platelet aggregation, it was subsequently chromatographed on a column of Si gel (0.4 kg, 70−230 mesh) and successively eluted with CH_2Cl_2, CH_2Cl_2/Me_2CO (15:10), Me_2CO, and MeOH to yield four fractions. The active fraction (CH_2Cl_2/Me_2CO, 15:10) was chromatographed on a Si gel (0.36 kg, 230−400 mesh) column (4.5 × 40 cm) and eluted in a stepwise manner with EtOAc/*n*-hexane (15:85, 6 L), EtOAc/*n*-hexane (17:83, 3 L), EtOAc/*n*-hexane (25:75, 2 L), EtOAc/*n*-hexane (30:70, 2 L) and EtOAc/*n*-hexane (50:50, 2 L). The EtOAc/*n*-hexane (30:70) eluate was rechromatographed on a Si gel (0.1 kg, 230−400 mesh) column (2.5 × 40 cm) eluting with CH_2Cl_2/Me_2CO (40:1) and then purified by Sephadex LH-20 (MeOH) column (2.5 × 40 cm) to afford isobavachalcone (**1**) (25 mg) and bavachin (**3**) (4 mg). The EtOAc/*n*-hexane (50:50) eluate was rechromatographed on a Si gel (0.1 kg, 230−400 mesh) column (2.5 × 40 cm) eluting with CH_2Cl_2/Me_2CO (40:1) to afford neobavaisoflavone (**2**) (123 mg).

Platelet Aggregation. Platelets were obtained from fresh rabbit blood according to the washing procedures described previously.[12] Aggregation was measured by a platelet aggregation chromogenic kinetic system (Helena Laboratories, Beaumont, TX) using the turbidimetric method[13] with the absorbance of platelet suspension assigned 0% aggregation and the absorbance of platelet-free Tyrode solution assigned 100% aggregation. The final concentration of the solvent DMSO was fixed at 0.5%, which gave an absorbance equivalent to less than 10% inhibition of aggregation. Aspirin and CV-3988[14] were used as positive controls. An IC_{50} of 300 μM was considered as active.

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References and Notes

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