Inhibitors of Prostaglandin Biosynthesis from Mucuna birdwoodiana

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Four phenolic compounds were isolated from the stalks of Mucuna birdwoodiana TUTCHER, a Chinese medicinal drug (Japanese name “鰈血液”) used to treat so-called stagnant blood. Of the four compounds, 2,6-dimethoxyphenol and N-(trans-feruloyl)tyramine inhibited prostaglandin biosynthesis, and the former showed a potent inhibitory effect on in vitro platelet aggregation.

Keywords—Mucuna birdwoodiana; Leguminosae; stagnant blood; 2,6-dimethoxyphenol; phenolic amide; N-(trans-feruloyl)tyramine; prostaglandin biosynthesis inhibitor; platelet aggregation inhibitor

In the course of our studies to find inhibitors of prostaglandin (PG) biosynthesis from medicinal plants used as crude drugs in traditional medicine, extracts of several plants used to treat so-called stagnant blood were found to inhibit PG biosynthesis. It has been recognized that PGs have very important roles in the regulation of blood flow. Therefore, the isolated inhibitors may be active principles of the medicinal plants. Previously, we reported the isolation of inhibitors of PG biosynthesis from Dalbergia odorifera (Japanese name “降真香”) and Allium chinense (Japanese name “蔔白”), which have also been used to treat stagnant blood. In this paper, we report on the inhibitors of PG biosynthesis isolated from the stalks of Mucuna birdwoodiana TUTCHER (Japanese name “鰈血液”). Its hot aqueous extract inhibited the PG biosynthesizing enzyme system (prostaglandin synthetase (PG-ase)) by 84% at a concentration of 0.75 mg/ml. This Chinese medicinal drug has been recognized as being effective to promote blood circulation or to relieve stasis, and is widely used to treat pain or numbness of the wrist, knees or other joints and irregular menstruation in present Chinese medicine. However, no study on the biologically active components of this drug has been reported.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO DS-701G spectrometer. Mass spectra (MS) were taken on a JEOL JMS-DX-300 spectrometer equipped with a JEOL JMA-200 computer. Proton and carbon-13 nuclear magnetic resonance (1H- and 13C-NMR) spectra were measured on a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard.

Assay of PG-ase—The previously reported radioisotope method was used.

Isolation of Active Principles from Mucuna birdwoodiana—Dried stalks (4.7 kg) purchased at a Hong-Kong market were successively extracted with hexane, chloroform and methanol. The inhibition of PG biosynthesis by these extracts at a concentration of 0.5 mg/ml amounted to 93, 97 and 68%, respectively. The methanol extract was further partitioned with chloroform and 80% aqueous methanol, and the chloroform-soluble portion was combined with the chloroform extract. The combined chloroform extract (15.9 g) was applied to an oxalate-treated silica gel column and eluted with chloroform and methanol to give three active fractions, which were separated and purified by chromatography on silica gel, Sephadex LH-20 and Lobar RP-8, followed by recrystallization, to afford four phenolic compounds.

2,6-Dimethoxyphenol (1) was obtained as a colorless oil (12.9 mg) and gave the following spectral data. MS m/z (rel. int. %): 154 (M+, 100), 137 (88), 109 (23), 81 (11), 63 (17).1H-NMR (CDCl3) δ: 6.39–6.90 (3H, AB2 type coupling). 13C-NMR (CDCl3) δ: 56.1 (q, OMe), 104.7 (d, C-3 and C-5), 118.8 (d, C-4), 134.6 (s, C-1), 147.0 (s, C-
Compound 1 was finally identified by direct comparison with an authentic sample purchased from Aldrich Co. (Milwaukee).

Syringic acid (2) and vanillic acid (3) were obtained as colorless powders (5.0 and 2.2 mg, respectively) and gave the following spectral data. 2: MS \( m/z \) (rel. int. %): 198 (M⁺, 100), 183 (32), 127 (17), 109 (14), 69 (19), 55 (16). ¹H-NMR (methanol-\( d_4 \)) \( \delta \): 3.87 (6H, s), 7.33 (2H, s). 3: MS \( m/z \) (rel. int. %): 168 (M⁺, 100), 153 (67), 125 (19), 106 (17), 97 (25), 78 (34), 52 (17), 51 (20). ¹H-NMR (acetone-\( d_6 \)) \( \delta \): 6.93 (1H, d, \( J=8.4 \) Hz), 7.57 (1H, d, \( J=1.0 \) Hz), 7.60 (1H, dd, \( J=8.4, 1.0 \) Hz). Compounds 2 and 3 were finally identified by direct comparison with authentic samples purchased from Tokyo-Kasei Co. (Tokyo) and Aldrich Co. (Milwaukee), respectively.

\( N\)-(trans-Feruloyl)tyramine (4) was obtained as colorless plates (mp 142—143 °C) from CHCl₃ and acetone and gave the following spectral data. IR \( v \) cm⁻¹: 1650, 1580, 1510, 1445, 1360, 1275, 1030, 980. High-resolution MS: \( C_{18}H_{19}N_0_4 \) (M⁺ \( m/z \): 313.1313, Calcd: 313.1313). MS \( m/z \) (rel. int. %): 313 (M⁺, 16), 194 (33), 193 (55), 192 (52), 177 (100), 145 (24), 120 (31), 85 (16), 83 (25). ¹H-NMR (acetone-\( d_6 \)) \( \delta \): 2.74 (2H, m), 3.49 (2H, m), 6.48 (1H, d, \( J=15.5 \) Hz), 6.75 (2H, d, \( J=8.6 \) Hz), 6.82 (1H, d, \( J=7.0 \) Hz), 7.02 (1H, d, \( J=2.5 \) Hz), 7.09 (2H, d, \( J=8.6 \) Hz), 7.14 (1H, dd, \( J=2.5, 7.0 \) Hz), 7.44 (1H, d, \( J=15.5 \) Hz), 8.03 (2H, s), 8.13 (1H, m). Compound 4 was finally identified by direct comparison with an authentic sample kindly supplied by Prof. S. Sakamura.

**Assay of Platelet Aggregation** —— The assay was carried out as described in a previous paper.³¹

**Results and Discussion**

The stalks of *M. birdwoodiana* were successively extracted with hexane, chloroform and methanol. The hexane extract was found to contain fatty acids. Since the chloroform extract inhibited PG-ase by more than 90% at a concentration of 0.5 mg/ml, the chloroform-soluble portion of the methanol extract was combined with the chloroform extract and the inhibitory principles were separated from the combined chloroform extract.

The combined chloroform extract was separated first by oxalate-impregnated silica gel column chromatography to give three active fractions not containing fatty acids. The less polar fraction than fatty acids was further separated and purified with repeated column chromatography, guided by testing of the inhibitory effect, to give 2,6-dimethoxyphenol (1) as an active principle. On the other hand, the inhibitory effect of the more polar fraction than fatty acids was widely dispersed in the course of separation. Finally, we obtained syringic acid (2) and vanillic acid (3) from active fractions derived from the more polar fraction, but they did not inhibit PG-ase. The most polar fractions among the three active fractions was also separated by repeated column chromatography and purified by recrystallization to afford \( N\)-(trans-feruloyl)tyramine (4), as an active principle.

The 50% inhibitory concentration (IC₅₀) values of 1 and 4 were 12 and 210 μM, respectively. The process of PG biosynthesis as used in our studies involves three different reactions, cyclooxygenase, hydroperoxidase and PGE₂ synthetase. Ohki *et al.* showed that the hydroperoxidase reaction was stimulated by a variety of compounds, such as tryptophan, epinephrine, phenol and hydroquinone (tryptophan-like cofactors).³⁵ Furthermore, Baumann *et al.*⁷ and Tseng *et al.*⁸ reported that phenolic acids such as vanillic acid (3) and coumaric

![Chemical structures](image-url)
acids also acted as tryptophan-like cofactors. The previous observations and the results obtained in this study indicate that the Chinese medicinal drug “鶏血藤” contains inhibitors of PG biosynthesis (1 and 4) together with activators (2 and 3). N-(trans-Feruloyl)tyramine (4) has been isolated from various plants such as Solanum melongena,9) Capsicum annuum,10) Tinospora tuberculata11) and Ipomoea aquatica.8) Recently Okuyama et al. isolated 4 from Allium chinense as an inhibitor of human platelet aggregation.12) Structure–activity relationships for the inhibition of PG biosynthesis by N-cinnamoyl-β-phenethylamine derivatives, including 4, have been reported.7)

Next, we assayed 1 for inhibitory effect on rabbit platelet aggregation. Adenosine (3.75 μM) and aspirin (11 and 111 μM) were used as positive controls for inhibition of platelet aggregation induced by adenosine diphosphate (ADP), arachidonic acid and collagen, respectively. The concentrations of 1 which gave the same degree of inhibitory effect as the positive controls were 0.45 μM in arachidonic acid-induced platelet aggregation and 32 μM in collagen-induced platelet aggregation. However, 1 did not show any effect on ADP-induced platelet aggregation even at a concentration of 1 mM. Among natural compounds isolated hitherto in our studies, 1 is the most potent inhibitor of platelet aggregation.

One of the contributing factors to blood stasis seems to be excessive platelet aggregation. Therefore, the PG biosynthesis inhibitors isolated from M. birdwoodiana may well be active principles in the therapeutic effect of the crude drug.

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References and Notes


4) The plant material was identified by Ms. Lin Mao of the Institute of Chinese Materia Medica during her stay in our laboratory. Further confirmation will be carried out at the same institute.


