Pinusolidic Acid: A Platelet-Activating Factor Inhibitor from *Biota orientalis*

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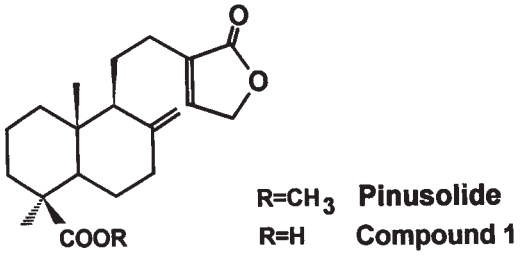
Abstract: **The water extract of *Biota orientalis* showed a potent inhibitory effect on platelet activating factor (PAF) binding to rabbit platelets using [^3H]PAF as a ligand in our previous screening studies for Korean medicinal antagonists by the activitiy-guided purification studies. Another active compound, compound 1 (IC_{50} = 2.3 × 10^{-5} M, 7.48 ± 2.11 μg/ml, n = 4) was isolated from the title plant. The chemical structure of compound 1 was elucidated as pinusolidic acid by chemical and spectrometric analyses**

In order to find new platelet activating factor (PAF) antagonists, the PAF receptor binding inhibitory effects of the water extracts of Korean medicinal plants were screened (1–3). Among the plants screened, *Biota orientalis* (L.) Endl. (Cupressaceae) showed a strong activity. In our previous study, pinusolide (IC_{50} = 2.5 × 10^{-7} M, 0.087 ± 0.009 μg/ml, n = 4) and cedrol (IC_{50} = 1.3 × 10^{-5} M, 2.9 ± 0.79 μg/ml, n = 3) were isolated from the hexane extract of this plant as new PAF antagonists by activity-guided isolation (4). The aim of this study is to find other active compounds from the plant, especially, the structural analogue of pinusolide. The isolation of the structural analogues of pinusolide will be useful in the elucidation of structure-activity relationships. Pinusolidic acid, pinusolide hydrolysate, had been isolated from the seed of *Bitoa orientalis* (5). However, no biological activity of pinusolidic acid has been reported in the literature. This paper describes the isolation, identification, and the PAF-receptor binding inhibitory activity of compound **1**, pinusolidic acid, from the leaves and branches of *Biota orientalis*.



To isolate pinusolidic acid, the acidic fraction was obtained from the $CHCl_3$ extract of the title plant. The acidic fraction afforded 5 fractions on silica gel column chromatography. Each fraction was tested for the PAF inhibition activity (data not shown). Only fraction IV showed a strong inhibitory activity (IC_{50} = 53.5 ± 2.2 μg/ml, n = 3). By subsequent column chromatographic purification of fraction IV, compound **1** was obtained in crystalline form and it could be detected by a characteristic false-positive Dragendorff's reaction. Furthermore, compound **1** was confirmed as genuine since it was detected in the total $CHCl_3$ extract on the TLC plate by the co-TLC method. And the acidified pinusolide did not yield compound **1** under the same conditions as the acidic fraction which was obtained from the $CHCl_3$ extract of the title plant. These facts mean that compound **1** is not an artefact but a genuine compound.

Compound **1** showed a PAF receptor binding inhibitory activity. The IC_{50} value of compound **1** was 7.48 ± 2.11 μg/ml (22.5 ± 0.64 μM). Thus, compound **1** was found to be a 100-fold weaker inhibitor than pinusolide. This result suggests that the C-19-methyl ester group contributes to the PAF inhibitory activity. The methyl ester of **1** was identical with pinusolide, by co-TLC and ^{1}H-NMR spectra. Thus compound **1** was identified as pinusolidic acid which had been isolated from the seed of *Biota orientalis* (5).

Materials and Methods

The crude drugs were collected in March 1993 from the garden of Seoul National University and identified as *Biota orientalis* by Prof. Hyung Joon Chi, the Botanist, Natural Products Research Institute, Seoul National University, Korea. The voucher specimens have been deposited in the herbarium of the Institute (*B. orientalis*, No. 940342).

Binding of [^3H]PAF to rabbit platelets was carried out according to the method of Valone (6) with some modifications (4). Briefly, washed rabbit platelets were prepared and incubated for 1 h at room temperature with a specific radioligand at a final concentration of 0.6 nM in tris-Tyrode buffer containing 0.25 % BSA. Free ligands were separated by suction on a Whatman GF/C glass-fiber filter and the radioactivity was counted on a liquid scintillation counter. Specific binding was defined as the difference in radioactivity between the total binding and the nonspecific binding determined in the presence of a 500-fold excess of unlabeled PAF. In a set of experiments, [^3H]PAF (0.6 nM) was incubated with different concentrations of compound **1** and the effect of the compound on the specific binding expressed as percentage inhibition of the control. The IC_{50} value was defined as the final concentration of the inhibitor required to block 50 % of the specific [^3H]PAF binding to rabbit platelet receptors.

Samples were dissolved in dimethyl sulfoxide (DMSO) and diluted with saline (final concentration of DMSO, 0.2 %). Control test was carried out with 0.2 % DMSO in saline solution instead of sample solution. Preliminary tests confirmed that 0.2 % DMSO does not interfere with the receptor binding studies.

Dried leaves and stems (1 kg) of *B. orientalis* (L.) Endl. were refluxed with hexane, the residue was dried absolutely, and subsequently refluxed with chloroform. The chloroform extract (60 g) was dried and dissolved in diethyl ether (3 l). And the ether solution was treated with 1 N NaOH solution (3 l × 3). The basic layer was acidified with 1 N HCl (to pH 6) and then extracted with diethyl ether (3 l × 3). The ether fraction was dried absolutely in vacuo. The extract (1.5 g) thus obtained was loaded onto a silica gel column (45 g, 2 ×

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30 cm) and eluted with hexane-EtOAc (10 : 1) affording fraction I (0.1 g), II (0.3 g), III (0.2 g), IV (0.5 g), and V (0.3 g), respectively. Each fraction was tested for PAF inhibition activity. Of the fractions I – V, Fr. IV (0.5 g), the strong Dragendorff's reagent positive and significantly active fraction, was rechromatographed on a silica gel (20 g, 1.6 × 20 cm) with hexane-EtOAc (3 : 1) to give crystalline, compound **1** (35 mg), which was subsequently tested for the PAF inhibition activity.

Compound **1**: $C_{20}H_{28}O_2$, colourless needles from hexane/EtOAc mixture, R_f = 0.1, hexane-EtOAc (3 : 1), false positive on the Dragendorff's reaction. m.p. 132 – 133 °C, $[\alpha]_D^{23}$: + 54.5° ($CHCl_3$, *c* 0.1).

Compound **1** (3 mg) dissolved in 50 µl of Et_2O was treated with a diethyl ether solution of excess diazomethane for 2 h. Removal of the solvent and subsequent crystallization gave the methylate (2 mg): colourless needles from EtOAc, m.p. 80 – 82 °C, $C_{21}H_{34}O_4$, R_f = 0.3, hexane-EtOAc (3 : 1), false positive in Dragendorff's reaction. Pinusolide was treated with the same procedure as the acidic $CHCl_3$ fraction which was obtained from the $CHCl_3$ extract of *B. orientalis*. Pinusolide (2 mg) dissolved in 50 µl diethyl ether was treated with 1 N NaOH solution (50 µl × 3). The basic layer was acidified with 1 N HCl (to pH 6) and then extracted with diethyl ether (50 µl × 3). The ether fraction was concentrated under nitrogen and applied on the TLC plate to determine whether pinusolidic acid was detected or not.

Acknowledgements

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Phenolic Diarylheptenones from *Alnus rubra* Bark

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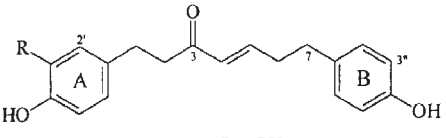
Abstract: **The novel diarylheptenone 1-(3′,4′-dihydroxyphenyl)-7-(4″-hydroxyphenyl)-4-hepten-3-one and 1,7-bis(*p*-hydroxyphenyl)-4-hepten-3-one were isolated from *Alnus rubra* bark and their structures elucidated by spectrometric techniques.**

Alnus rubra Bong. (Betulaceae) is a commonly occurring deciduous tree of the river valleys and moist coastal areas of the Pacific Northwest. Called the red alder tree, this species gets its name from the reddish-orange color which quickly develops on freshly exposed wood or bark. Indigenous peoples have used the bark of this tree for both coloring materials and medicines (1 – 3). Medicinal uses of the bark have been varied and include purgatives, general tonics, and teas for the treatment of digestive and respiratory problems. A bark tea has also been used to relieve heart pain. Previous chemical investigations have led to the isolation of triterpenoids (4, 5), the diarylheptanoid xyloside oregonin (6), and a procyanidin polymer (7). Oregonin and its aglycone were recently shown to be associated with the antibiotic activity (8) exhibited by a red alder bark methanol extract (9). In the present investigation of non-glycosidic diarylheptanoids of red alder bark, we report on the isolation and structural elucidation of the new compound 1-(3′,4′-dihydroxyphenyl)-7-(4″-hydroxyphenyl)-4-hepten-3-one (**1**) and the known compound 1,7-bis(*p*-hydroxyphenyl)-4-hepten-3-one (**2**). Compound **2** has not been previously preorted in red alder. The isolation of these compounds from red alder bark has increased the known diversity of diarylheptanoid structures in this species which is finding renewed use as an indigenous medicine.



1 R = OH

2 R = H

Materials and Methods

Extraction and isolation: *Alnus rubra* bark was collected in July 1993 from McDonald State Forest, Corvallis, Oregon. A voucher specimen is deposited at the Oregon State University Herbarium (# 139987). The freeze-dried powder (200 g) of an Me_2CO-H_2O (7 : 3) extract of fresh bark was chromatographed over two Sephadex LH-20 parallel columns (10 × 60 cm each)

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