Inhibition of Cyclooxygenase-2 Expression by Diarylheptanoids from the Bark of *Ahnalia hirsuta* var. *sibirica*

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Two known diarylheptanoids, oregonin (1), (5S)-1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-one-5-O-β-O-xylopyranoside and hirsutanonol (2), (5S)-1,7-bis-(3,4-dihydroxyphenoxy)-5-hydroxyheptane-3-one isolated from the bark of *Ahnalia hirsuta* var. *sibirica*, showed significant inhibitory effects on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cyclooxygenase-2 (COX-2) expression in immortalized human breast epithelial MCF10A cells.

Key words *Ahnalia hirsuta* var. *sibirica*; cyclooxygenase-2 inhibitor; diarylheptanoid

*Alnus hirsuta* var. *sibirica*, an indigenous *Alnus* species found in Korea, is a deciduous broad-leaved tree growing in the damp areas of mountain valleys. The bark of this plant has been used in Korea as a traditional medicine for antipyretic and as a health tea for alcoholism.1 Several phenolic compounds such as stilbene, flavonoid, phenylpropanoid, tannin and diarylheptanoid have been isolated from the *Alnus* species.2–5 Recently, we have isolated a diarylheptanoid named oregonin (1) from *A. hirsuta* which exhibited inhibitory effects on B16-F10 mouse melanoma cell line.6 Much attention has been focused on the role of prostaglandins in the pathogenesis of cancer because cyclooxygenase-2 (COX-2), an inducible enzyme responsible for prostaglandin biosynthesis, is accumulated at high levels in malignant or transformed cells compared with that in normal cells.7 In order to evaluate the additional antitumorigenic potential of diarylheptanoids from *A. hirsuta* var. *sibirica*, we have studied the inhibitory effects of I and its aglycone hirsutanonol (2) on the TPA-induced expression of COX-2 protein in immortalized human mammary epithelial MCF-10A cells.

MATERIALS AND METHODS

Materials Oregolin (1) and hirsutanonol (2) were isolated from the 80% acetone extract of the fresh bark of *Ahnalia hirsuta* var. *sibirica* (Chart 1). Fresh leaves (3 kg) were extracted with 80%aq. Me₃CO at room temperature 3 times to give 400 g of extract on removal of the solvent in vacuo. The extract was suspended in distilled water and the aqueous solution was filtered. The filtrate was concentrated and then applied to a column of Sephadex LH-20. Elution with H₂O containing increasing proportions of MeOH afforded 3 fractions, fr. I (70 g), II (50 g) and III (40 g). Repeated column chromatography of fr. II over MCI-gel CHP-20P and YMC ODS-gel with an H₂O–MeOH gradient yield 1.2 g of I. Repeated column chromatography of fr. III over YMC ODS-gel with 60% MeOH and Sephadex LH-20 with EtOH yielded 0.8 g of 2.

![Diagram of structures of compounds 1 and 2](image)

1: R = D-xylene
2: R = H

Chart 1. Structures of 1 and 2

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RESULTS AND DISCUSSION

In the present work, both 1 and 2 were isolated from the acetone extract of the fresh bark of *Ahnalia hirsuta* var. *sibirica* and identified by comparison with previously reported physico-chemical and spectra data (IR, MS, 1H- and 13C-NMR).5,8–9 To determine the inhibitory effects of these diarylheptanoids on TPA-induced expression of COX-2 protein in immortalized human mammary epithelial MCF-10A cells, cells were co-treated for 4 h with TPA and indicated concentrations of 1 and 2. As shown in Fig. 1, TPA induced COX-2 in human mammary epithelial cells and co-treatment with 1 reduced the TPA-induced expression of COX-2 dose-dependently. The aglycone, 2 showed more potent COX-2 inhibitory activity than 1 and the maximal inhibitory effect was

![Western Blot Analysis of COX-2 Expression in MCF-10A Cells](image)

Lane 1, DMSO control; lane 2, treated with TPA (60 µg) alone; lane 3, treated with I (12.5 µg) and TPA; lane 4, treated with 1 (25 µg) and TPA; lane 5, treated with I (50 µg) and TPA; lane 6, treated with I (100 µg) and TPA.

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observed at 50—100 μM (Fig. 2). These results indicate that 1 and 2 are potential chemopreventive agents and A. hirsuta var. sibirica is a rich source of these diarylheptanoids. There appears to be a common structural requirement in curcuminoids (curcumin and yakuchinone A), which have a diarylheptanoid moiety with lipophilic carbonyl functional groups in exhibiting anti-inflammatory and cancer chemopreventive activities.\textsuperscript{10,11} It is noteworthy that 1 and 2 are diarylheptanoid glycoside and its aglycone, respectively, with a highly hydrophilic property (Chart 1), but still possess potential COX-2 inhibitory activity.

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**REFERENCES**