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Effects of Compounds in Leaves of *Salix matsudana* on Arachidonic Acid Metabolism

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Apigenin 7-*O*-*β*-D-glucopyranuronide (1), luteolin 7-O-*β*-D-glucopyranuronide (2), m-hydroxybenzyl *β*-D-glucoside (3), and chrysoeriol 7-*O*-*β*-D-glucopyranuronide (4) were isolated for the first time from the leaves of *Salix matsudana*. Furthermore, the effects of compounds 1, 2 and 3 on arachidonic acid metabolism were studied. These compounds inhibited significantly the production of 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (12-HETE). In addition, the aglycon apigenin inhibited not only 12-HETE but also thromboxane B_2 (TXB_2). The effect of compound (4) on arachidonic acid metabolism is now under investigation.

Key words——*Salix matsudana*; arachidonic acid; 12-hydroxyeicosatetraenoic acid; thromboxane B_2

INTRODUCTION

The leaves of *willow* (*Salix matsudana* (Salic aceae)) have been used more than 3000 years as an herbal medicine. In the Chinese pharmacopoeia ''Ben Cao Gang Mu'', it is claimed to be an ordinary alexipharmic and antiphlogistic herb. It is sanative for suffer burn, struma, mastitis, and hypertension.[1] In a previous paper, it was reported that the extract of willow leaves improved thrombus, arteriosclerosis and hypersensitive diseases.[2]

In recent years, the incidences of thrombus, arteriosclerosis and hypersensitive diseases have continued to increase. A lot of attention has been paid to these pathological states. Blood platelets are an important factor for producing these diseases. In platelets, cyclooxygenase and lipoxygenase catalyze the initial reactions for the formation of two major arachidonic acid metabolites, thromboxane B_2 (TXB_2) and 12-hydroxy-5, 8, 10, 14- eicosatetraenoic acid (12-HETE)[3]. The cyclooxygenase mediating the production of TXA_2, an active form of TXB_2, induces platelet aggregation and the lipoxygenase reaction is also involved in atherosclerotic and allergic processes.[4] Therefore, a specific inhibitor of cyclooxygenase and lipoxygenase should be useful as a therapeutic drug for treating thrombosis and atherosclerosis. We studied the effects of 48 kinds of Chinese medicines on arachidonic acid metabolism in rabbit platelets and found leaves of *Salix matsudana* had a selective inhibitory activity against cyclooxygenase and lipoxygenase. Based on this finding, we attempted to isolate and identify the active components from the leaves of *Salix matsudana*.

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MATERIALS AND METHODS

Chemicals Sephadex LH-20 was purchased from Pharmacia Biotech Co. Unlabeled arachidonic acid was purchased from Sigma Chemical Co., [1–^{14}C]-Arachidonic acid and [5, 6, 8, 9, 11, 12, 14, 15–^{3}H] thromboxane B_2 were obtained from New England Nuclear. Silica gel 60 thin layer chromatography plastic sheets were obtained from Merck. Co.

Animals 8-week-old Wistar king male rats (250 g) were purchased from Charles River (Japan).

Preparation of rat platelets Blood drawn from cardiac aortas was collected in vessels. Platelet-rich plasma (PRP) was separated from the other blood components by centrifugation for 10 min at 220×*g* at room temperature. The PRP fraction was further centrifuged at 1500×*g* for 10 min at 4°C. The isolated platelets were washed twice with Tris/saline buffer (25 mM Tris, 130 mM NaCl, pH 7.6) containing 2 mM ethylenediaminetetraacetic acid (EDTA) and

suspended in the same buffer without EDTA or containing 1 mM $CaCl_2$.

Measurement of [1-^{14}C] arachidonic acid metabolism in rat platelets Rat platelets were preincubated with the indicated amounts of test compounds for 5 min at 37°C. Then, [1-^{14}C] arachidonic acid (0.05 ml, 0.05 μCi=1.85 KBq/tube) was added to give a final volume of 200 μl, and the mixture was incubated for 5 min at 37°C. The reaction was stopped by adding 0.5*N* formic acid (200 μl), and the products were extracted with 8 volumes of ethyl acetate. The ethyl acetate phase was evaporated under a nitrogen stream. The residue was dissolved in a small amount of ethyl acetate (40 μl), applied to precoated silica gel 60 TLC plastic sheets, and developed with $CHCl_3$-methanol-acetic acid-distilled water (90 : 8 : 1 : 0.8, v/v) together with authentic samples of 12-HETE, HHT, and thromboxane B_2. These metabolites were identified by comparison with their respective authentic sample and by GC-MS spectrometry as described previously.[5] The radioactive spots were detected by autoradiography, cut out with scissors, and counted in a liquid scintillation counter.

Plant Materials The leaves of *Salix matsudana* were collected in May on the campus of Jilin Agriculture University, (Changchun, China) and dried in the shade at room temperature.

Extract and Isolation The dried leaves of *Salix matsudana* (5 kg) were extracted with petroleum ether in a yield of 78 g which was concentrated. The residue was extracted with 95% ethanol and then concentrated under reduced pressure. The ethanol extract (655 g) was suspended in water and extracted sequentially with benzene (47 g), CH_2Cl_2 (142 g), ethyl acetate (33 g), and *n*-BuOH (141 g). The *n*-BuOH extract was suspended in MeOH and chromatographed on a sephadex LH-20 column, eluting with MeOH, and confirmed with TLC (solvent system: EtOH-butanone-formic acid-H_2O (5 : 3 : 1 : 1, v/v) to give compound 1 (212 mg) as yellow crystals, compound 2 (231 mg) as yellow crystals, and compound 3 (136 mg) as deep yellow crystals. These compounds were identified as apigenin 7-*O*-β-D-glucopyranuronide (1), luteolin 7-*O*-β-D-glucopyranuronide (2), and m-hydroxy benzyl β-D-glucoside (3) by a comparison of their spectral data with those in the literature (Chart 1).[6] In addition, another compound 4 was isolated as follows; the dried leaves of *Salix matsudana* (500 g) were extracted with 10% EtOH to give an EtOH extract in a yield of 104.9 g, which was concentrated and dried by freezing. The EtOH extract was suspended in H_2O and then extracted with *n*-BuOH. The *n*-BuOH extract was dissolved in MeOH, chromatographed on a heated sephadex LH-20 column developed with MeOH, and confirmed with TLC (solvent system: EtOH-butanone-formic acid-H_2O (5 : 3 : 1 : 1, v/v) to give compound 4 (127 mg) as yellow crystals. Compound 4 was identified as chrysoeriol 7-*O*-β-D-glucopyranuronide (4) by comparing its spectral data with those in the literature (Chart 1).[6]

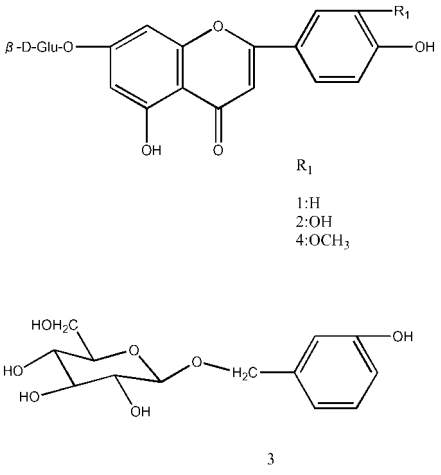

Chart 1. Structures of compounds 1–4

RESULTS AND DISCUSSION

In a previous paper, we found that the leaves of *Salix matsudana* selectivity inhibited platelet 12-lipoxygenase.[2] Compounds 1–4 were isolated from the leaves of *S. matsudana* according to the procedure shown in the isolation procedure. The present study examined the effects of compounds 1, 2 and 3 on arachidonic acid metabolism in rat platelets. As shown in Fig. 1, compounds 1, 2 and 3 significantly inhibited the production of 12-HETE (Fig. 1A, B and C). After hydrolysis, apigenin, the aglycon of apigenin 7-*O*-β-D-glucopyranoside inhibited not only 12-HETE but also TXB_2 (Fig. 1 D).

Many compounds had been isolated from willow leaves, but apigenin 7-*O*-β-D-glucopyranuronide, luteolin 7-*O*-β-D-glucopyranuronide, m-hydroxy benzyl β-D-glucoside, and chrysoeriol 7-*O*-β-D-glucopyranuronide were isolated for the first time from Salix plants.

Compounds 1, 2 and 3 inhibited the production of

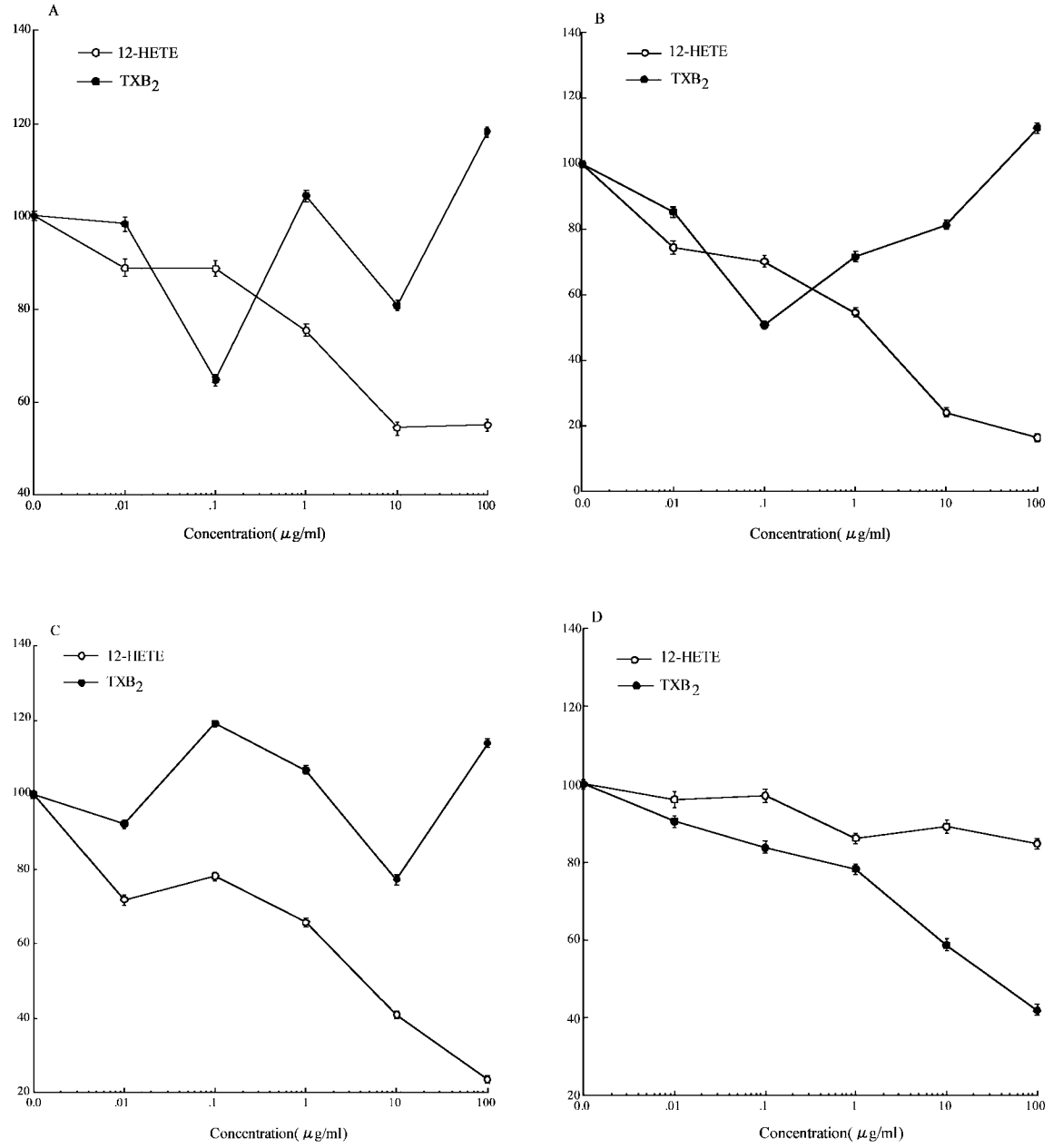

Fig. 1. Effect of Various Compounds (A, B, C and D) on Arachidonic Acid Metabolism in Rat Platelets
A: apigenin 7-*O*-*β*-D-glucopyranoside, B: luteolin 7-*O*-*β*-D-glucopyranoside, C: m-hydroxy benzyl *β*-D-glucoside, D: apigenin.

12-HETE, indicating that they maybe inhibit the activity of lipoxygenase. Therefore, they can be used to inhibit the onset of allergies and atherosclerosis. However when they inhibit the production of 12-HETE, the production of TXB_2 increases at the same time. Selective inhibition of lipooxygenase may cause activation of the cyclooxygenase pathway. The mechanisms of action of these compounds on arachidonic acid metabolism remain to be studied.

The effect of chrysoeriol 7-*O*-*β*-D-glucopyranuronide on arachidonic acid metabolism is now under investigation.

Since the incidences of thrombus and arteriosclerosis are increasing at present, new drugs to prevent and improve these diseases are urgently required. Most antithrombus drugs are chemically synthesized, and drugs from natural products are scarce. These compounds which we found in the leaves of *Salix matsudana* may be effective compounds for preventing thrombus and arteriosclerosis.

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