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LIGNANS WITH PLATELET ACTIVATING FACTOR ANTAGONIST ACTIVITY FROM *MAGNOLIA BIONDII*

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Key Word Index—*Magnolia biondii*; Magnoliaceae; xinyi; flower buds; lignans; PAF receptor antagonist; [^{3}H]PAF receptor binding.

Abstract—Six active lignans were isolated from the flower buds of *Magnolia biondii*. They are liroresinol-B dimethyl ether, magnolin, pinoresinol dimethyl ether, fargesin, demethoxyaschantin and aschantin. All six lignans show antagonistic activities against platelet activating factor in the [^{3}H]PAF receptor binding assay.

INTRODUCTION

Platelet activating factor (PAF), identified recently as 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine, is a potent phospholipid mediator that may be involved in various inflammatory, respiratory and cardiovascular disorders [1–3]. It is synthesized in a myriad of stimulated cells and affects a variety of cells and tissues. In an attempt to regulate this new phospholipid mediator, we established a PAF receptor binding assay [4, 5] using isolated rabbit platelet membranes and [^{3}H]PAF to search for PAF receptor antagonists.

The Chinese have a long tradition of using herbs as *materia medica*. The crude extracts contain many components, both organic and inorganic. Fortunately, many enzyme or receptor assays can detect the presence of a minute amount of an active substance in a crude mixture, similar to the discovery of antibiotics in fermentation broths. Approximately 200 crude extracts of Chinese herbal preparations used for the treatment of inflammation, cardiovascular and pulmonary diseases have been tested in the PAF receptor binding assay. Kadsurenone, a potent and promising PAF receptor antagonist, was discovered and isolated from a Chinese herbal preparation of *Piper futokadsura* [6, 7]. It is a specific and competitive inhibitor of specific [^{3}H]PAF binding to rabbit platelet membranes with an equilibrium dissociation constant (K_B) of 9×10^{-8} M [8, 9].

In this report, several lignans isolated from xinyi were found to have PAF receptor antagonistic activities. Xinyi, the flower buds of *Magnolia biondii*, has been widely used in Chinese medicinal preparations for the treatment of nasal empyema and headache. Methylene chloride extracts of *M. biondii* were found to block the binding of [^{3}H]PAF to rabbit platelet membranes. The chemical structures of six active components have been determined; they are known lignans. Pinoresinol dimethyl ether (**1**), magnolin (**2**), liroresinol-B dimethyl ether (**3**) and fargesin (**4**) have been isolated from *M. fargensii* [10]. Demethoxyaschantin (**5**) has been isolated from *M. stellata* [11]. This is the first report of the existence of aschantin (**6**) in *Magnolia* spp. and this is the first report that these compounds have PAF receptor antagonistic activities.

RESULTS AND DISCUSSION

The methylene chloride extract of *M. biondii* was subjected to silica flash CC eluted with a stepwise solvent gradient of ethyl acetate in hexane. The fractions collected were tested in the [^{3}H]PAF receptor binding assay. Biologically active fractions were further fractionated by prep. TLC and HPLC. Six active components were isolated and their chemical structures were identified. These are the known lignans pinoresinol dimethyl ether (**1**), magnolin (**2**), liroresinol-B dimethyl ether (**3**), fargesin (**4**), demethoxyaschantin (**5**) and aschantin (**6**). The spectroscopic data indicated that all these compounds have a 3,7-dioxabicyclo-(3,3,0)-octane skeleton.

The six identified lignans were tested in the inhibition of the binding of [^{3}H]PAF (0.3 nM) to isolated rabbit platelet membranes (30 μg membrane protein/ml). Figure 1 shows the inhibition of [^{3}H]PAF binding to the receptor preparations by **4**, **5**, **6** (Fig. 1A), **3**, **2** and **1** (Fig. 1B). For comparison, the displacement curve for kadsurenone is also shown in Fig. 1. The ED_{50}s, the inhibitor concentration required to displace 50% of the specific [^{3}H]PAF binding, are listed in Table 1. **4** and **1** show roughly identical potency with ED_{50}s of 1.3 and 1.7 μM, respectively, which are *ca* 10 times less potent than kadsurenone. The other four lignans are less potent following the order **5** > **2** > **3** > **6**. **6** with a 3,4,5-trimethoxyphenyl group is the least potent with an ED_{50} of 10 μM. Unlike those in the series of L-652,731 [12], **6** with a 3,4,5-trimethoxyphenyl group is less potent than **5** with a 3,4-dimethoxyphenyl group. Also, **1** with a 3,4-dimethoxyphenyl group on both sides is more potent than **2** which has a 3,4-dimethoxyphenyl group on one side and a 3,4,5-trimethoxyphenyl group on the other side and **3** which has a 3,4,5-trimethoxyphenyl group on both sides of the 3,7-dioxabicyclo-(3,3,0)-octane backbone. Also the *trans* stereoisomer, **4**, clearly is more potent than the *cis* stereoisomer, **5**.

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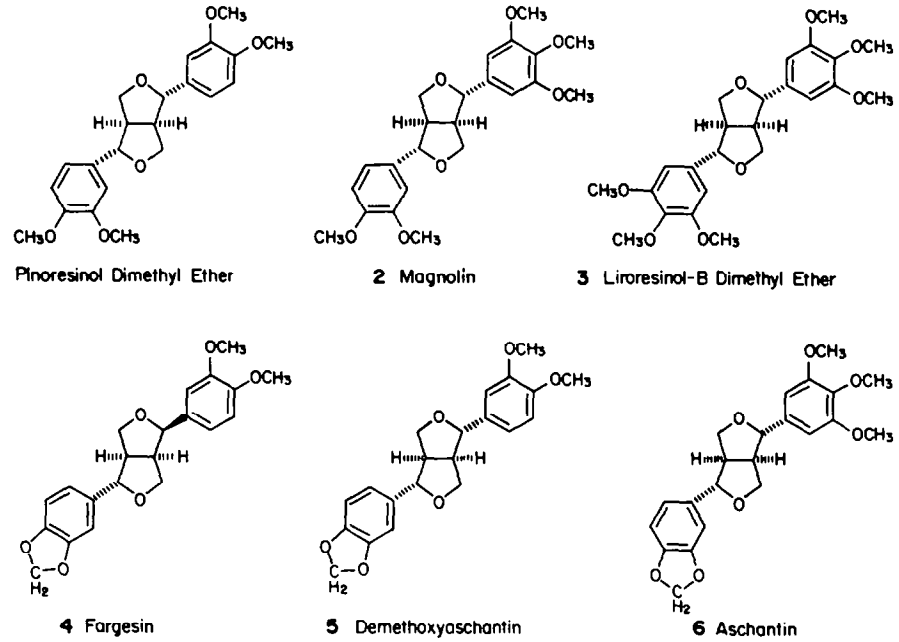

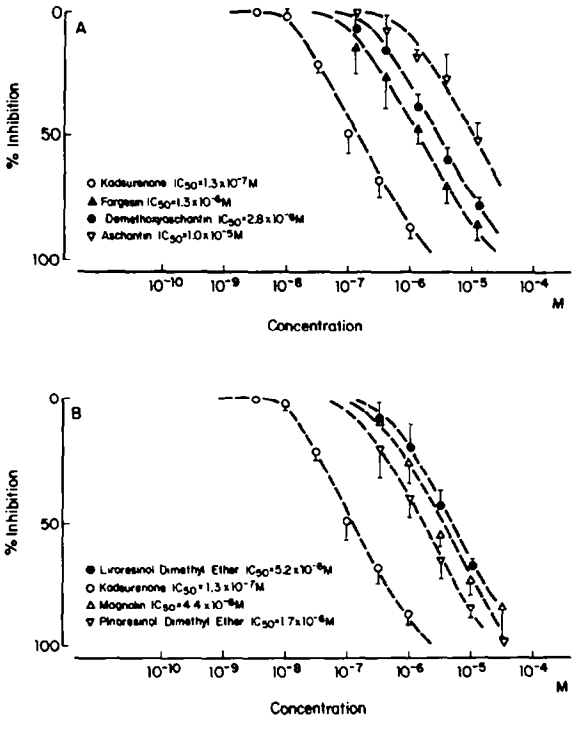

Fig. 1. Inhibition of the [^{3}H]PAF specific binding to rabbit platelet membranes. Membrane protein (30 μg) was added to an incubation mixture (1 ml) containing 0.3 nM [^{3}H]PAF and a known amount of inhibitor in a medium of 10 mM $MgCl_2$, 10 mM Tris, 0.25% BSA at pH 7. The data points are the mean of three independent experiments. In each experiment, triplicate samples were prepared. Error bar is the standard deviation. In A: kadsurenone (O——O); fargesin (▲——▲); demethoxyaschantin (●——●); aschantin (▽——▽). In B: kadsurenone (O——O); pinoresinol dimethyl ether (▽——▽); magnolin (△——△); liroresinol-B dimethyl ether (●——●).

Table 1. ED_{50} values for kadsurenone and the six isolated lignans from *Magnolia biondii* to inhibit the [^{3}H]PAF specific binding to rabbit platelet membranes

Compound	ED_{50} (μM)
Kadsurenone	0.14
Pinoresinol dimethyl ether (1)	1.7
Magnolin (2)	4.4
Liroresinol-B dimethyl ether (3)	5.2
Fargesin (4)	1.3
Demethoxyaschantin (5)	2.8
Aschantin (6)	10.0

Several lignans have also been isolated from *M. kobus* seeds and *M. fagensii* [10, 13]. Bornylmagnolol [13] was reported to have anti-allergic activity. 8,9-Dihydroxy-dihydrohonokiol and 8,9-dihydroxydihydromagnolol, extracted from the dried bark of *M. officinalis*, have been used for the treatment of contact dermatitis [14]. A neolignan named magnosalicin was also isolated as a biologically active compound from the buds of *M. salicifolia* which is used for the treatment of nasal allergy [15]. Since several reported PAF receptor antagonists including kadsurenone and L-652,731 are either lignan or lignan-like compounds, it will be interesting to see whether these lignans isolated from similar species will have the PAF receptor antagonistic activities.

EXPERIMENTAL

Isolation of lignans. Material used for the present study was purchased from Beijing, People's Republic of China, and was identified as flower bud of *M. biondii* Pump. CH_2Cl_2 extract was supplied by the Pharmaceutical School, Beijing Medical University. The CH_2Cl_2 extract (1 g) was chromatographed on silica gel. Flash column CC, collected the PAF active fractions

which were further purified by prep. TLC (silica gel 60 F254, 20 × 20 cm) developed with EtOAc–Hexane (1:3) and HPLC on Partisil M9, eluted with EtOAc–hexane (3:7), then concentrated under N_2. Six main PAF components were obtained: compound **1**, 9 mg; **2**, 13 mg; **3**, 3 mg, **4**, 4.8 mg; **5**, 4 mg and **6**, 9.5 mg. Spectroscopic data (HRMS, ^{1}H NMR in $CDCl_3$ at 400 MHz, UV in MeOH and $[\alpha]_D$ in $CHCl_3$) were found to be consistent with those reported for pinoresinol dimethyl ether (**1**), magnolin (**2**), liroresinol-B dimethyl ether (**3**) and fargesin (**4**) from *M. fargesii* [10], with demethoxyaschantin (**5**) from *M. stellata* [11] and with aschantin (**6**) from *Congo inbebs* [16, 17].

Inhibition of [^{3}H]*PAF binding to isolated rabbit platelet membranes.* Synthetic ^{3}H-labelled PAF, 1-*O*-[1′,2′-^{3}H] alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine, was purchased from New England Nuclear (Boston, MA) with a sp. act. of 45 Ci/mmol. Unlabelled PAF (1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-phosphorylcholine) was obtained from BACHEM Fine Chemicals (Torrance, CA). Rabbit platelet membranes were prepared following the procedure described in refs [12, 18] in the presence of 5 mM $MgCl_2$, 2 mM EDTA and 10 mM Tris pH 7 without NaCl. The membrane protein concn was determined with the Lowry method using BSA as standard. The binding of [^{3}H]PAF to rabbit platelet membranes was carried out in a 1 ml reaction mixture containing 30 μg membrane proteins, 0.3 nM [^{3}H]PAF and a known concn of inhibitor in a medium containing 10 mM $MgCl_2$, 10 mM Tris and 0.25% BSA pH 7. The reaction mixture was incubated at 0° for 2 hr. The free and bound [^{3}H]PAF was separated using the filtration technique described in ref. [4]. The difference between the total amount of [^{3}H]PAF bound in the absence and in the presence of unlabelled PAF (× 1000 excess) was defined as specific binding of [^{3}H]PAF. The % inhibition of PAF receptor binding in the presence of a known amount of inhibitors was expressed as:

$$\% \text{ inhibition} = \frac{\text{total binding} - \text{total binding with inhibitor}}{\text{specific binding}} \times 100\%.$$

The detailed procedure for the *in vitro* assay of the PAF receptor binding has been published elsewhere [4, 5].

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