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Isolation and Identification of Inhibitory Compounds on TNF-α Production from *Magnolia fargesii*

Sook-Hee Chae, Pyoung-Su Kim, Jae-Youl Cho, Ji-Soo Park, Jae-Ho Lee, Eun-Sook Yoo, Kyong-Up Baik, Jong-Soo Lee, Myung-Hwan Park

Research and Development Center, Daewoong Pharmaceutical Co., Sungnam 462-120, Korea

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Three TNFα-inhibitory lignans were isolated from the flower buds of *Magnolia fargesii* through bioassay-guided isolation. They were identified as eudesmin, magnolin and lirioresinol-B dimethylether on the basis of their spectroscopic data. All three lignans showed inhibitory effects on TNF-α production in LPS-stimulated murine macrophage cell line, RAW264.7 and eudesmin showed the strongest activity (IC_{50}=51 μM).

Key words : *Magnolia fargesii*, bioassay-guided isolation, flower buds, lignans, TNF-α, murine macrophage cell line

INTRODUCTION

Plants are a valuable source of a vast array of bioactive lead structures from which potentially more potent and less toxic drugs may be synthesized. In most instances, these natural products belong to a rather broad metabolic group, collectively referred to as secondary metabolites. In contrast to the ubiquitous distribution of primary metabolism, plants synthesize individual compounds through their own peculiar secondary metabolism. Accordingly, biologically active compounds are contained to certain plant species.

In our previous screening of 118 medicinal plant extracts for their inhibitory effects on TNF-α production in LPS-stimulated murine macrophage cell line, the extract of *Magnolia fargesii* (flower bud) showed 60.5% inhibition at the final concentration of 100 μg/ml.

The flower buds of *Magnolia fargesii* have been used as the source of a Chinese crude drug, "shin-i" and have been used for the treatment of various kinds of nasal diseases and headache (Miyazawa *et al.*, 1992).

To isolate the active principle from flower buds of *Magnolia fargesii*, the crude extract was subjected to sequential solvent extraction, preparative TLC and HPLC through activity-guided fractionation scheme (Fig. 1).

MATERIALS AND METHODS

Materials

Correspondence to: Sook-Hee Chae, Research and Development Center, Daewoong Pharmaceutical Co., Sungnam 462-120, Korea

The flower buds of *Magnolia fargesii* were purchased from Korean local herb market and identified taxonomically. A voucher specimen was deposited in our laboratory. Precoated TLC plates (precoated silicagel 60 F_{254}) and Lichrosorb RP8 column were purchased from Merck (Germany). RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco (USA). LPS (*E. coli* 055:B5) was obtained from Sigma (USA)

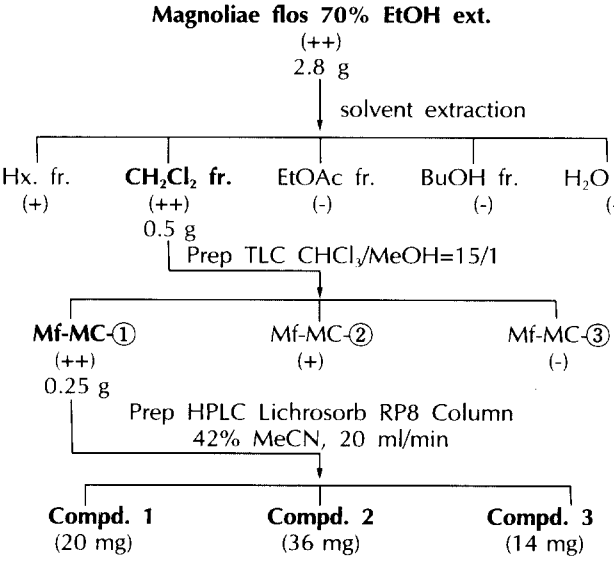

Fig. 1. Procedures of assay-guided isolation of active compounds from Magnoliae flos. The inhibitory potency of each sample was evaluated as % of inhibition compared with the control and is indicated as one of the three grades, more than 50% (++), 20% to 50% (+) and less than 20% (-) of inhibition.

and TNF-α enzyme-linked immunosorbent assay (ELISA) kit was obtained from Amersham (UK).

Extraction and Isolation

Air-dried flower buds of *Magnolia fargesii* were pulverized using milling machine and extracted at 95°C with 70% aq. EtOH (×3). The extract was filtered and the filtrate was concentrated *in vacuo* and lyophilized. The freeze dried extract was resuspended in distilled water and extracted with hexane, methylene dichloride, ethylacetate and n-butanol sequentially. Among these solvent fractions, the methylene dichloride extract showed the strongest activity and was subjected to preparative TLC (Kieselgel 60 F_{254}, 1 mm) in $CHCl_3$/MeOH (15/1) solvent system to give 3 subfractions (Mf-MC-①~③). Preparative HPLC (Rainin, USA) of the bioactive subfraction (Mf-MC-①) on Lichrosorb RP-8 (7 μm, 250×25 mm), eluted with 42% MeCN (flow=20 ml/min) resulted in the isolation of 3 pure compounds (1, 20 mg; 2, 36 mg; 3, 14 mg). Fig. 1 shows the isolation scheme of the active principle from *Magnolia fargesii*.

Structure elucidation

For the structure elucidation of compound 1~3, ^1H and ^{13}C-NMR and heteronuclear multiple quantum correlation (HMQC) spectra were recorded in $CDCl_3$ on Bruker AMX300 instrument operating at 300 MHz for ^1H and 75 MHz for ^{13}C-NMR. Chemical shifts are reported in ppm using tetramethylsilane (TMS) as the internal standard (0 ppm).

TNF-α inhibitory activity assay

Each subfraction and compound was dissolved in RPMI 1640 medium containing 0.89% polypropylene glycol, 0.1% ethanol, 0.01% dimethyl sulfoxide as a final concentration and used as test samples. RAW 264.7 cells (1×10^6 cells/ml) were seeded into a 24-well plate and preincubated in 2 ml of RPMI 1640 medium supplemented with 5% FBS, 100 units/ml of penicillin, and 100 μg/ml of streptomycin at 37°C in a humidified atmosphere containing 5% CO_2 for 18 h. After preincubation, the medium was removed. The cells were then treated with 450 μl of LPS (1 μg/ml; dissolved in RPMI 1640 medium) and 50 μl of test sample. Control group was treated with LPS only, and blank group with RPMI 1640 medium instead of LPS and test sample. After 6 h incubation at the same condition of preincubation, the culture medium was centrifuged at 12,000 rpm for 3 min. The supernate was subjected to an ELISA to determine the TNF-α amount, using Amersham TNF-α ELISA kit, following the manufacturer's protocol. TNF-α inhibitory activity was calculated as follows:

$$\text{inhibition (\%)} = \left(1 - \frac{\text{sample } A_{450}\text{-blank } A_{450}}{\text{control } A_{450}\text{-blank } A_{450}}\right) \times 100$$

RESULTS AND DISCUSSION

TNF-α is a proinflammatory cytokine which plays a major role in various kinds of inflammatory diseases and has been an attractive target for anti-inflammatory therapy (Beutler, 1992).

To isolate the TNF-α inhibitory components from the flower buds of *Magnolia fargesii*, the alcoholic crude extract was subjected to sequential extraction and preparative TLC and HPLC through bioassay-guided isolation scheme (Fig. 1). Three active compounds

Table I. ^1H-NMR spectral data (300 MHz, $CDCl_3$) for compounds 1-3

H	compound 1	compound 2	compound 3
1	3.13m	3.12m	3.12m
2	4.77d	4.77dd	4.77d
4ax	3.66m	3.94dd	3.93-3.97dd
4eq	4.25-4.30m	4.29m	4.30-4.35m
5	3.13m	3.12m	3.12m
6	4.77d	4.77dd	4.77d
8ax	3.66m	3.94dd	3.93-3.97dd
8eq	4.25-4.30m	4.79m	4.30-4.35m
Ar	6.84-6.93m	6.59s	6.59s
		ca.6.88m	
OMe	3.89s	3.85s	3.85s
	3.93s	3.89s	3.91s
		3.91s	

δ in ppm

Table II. ^{13}C-NMR spectral data (75 MHz, $CDCl_3$) for compounds 1-3

C	compound 1	compound 2	compound 3
1	54.5	54.5	54.7
5	54.5	54.8	54.7
2	86.1	86.1	86.3
6	86.1	86.4	86.3
4	72.1	72.1	72.3
8	72.1	72.3	72.3
1'	133.9	133.8	137.1
2'	109.5	109.5	103.1
3'	149.5	149.0	153.8
4'	149.0	149.5	137.8
5'	111.4	111.4	153.8
6'	118.6	118.6	103.1
1"	133.9	137.2	137.1
2"	109.5	103.1	103.1
3"	149.5	153.8	153.8
4"	149.0	137.8	137.8
5"	111.4	153.8	153.8
6"	118.6	103.1	103.1
OMe	56.3(4)	56.3(2)	56.9(4)
		56.5(2)	61.2(2)
		61.2(1)	

Signal assignments were confirmed by HMQC spectroscopy.

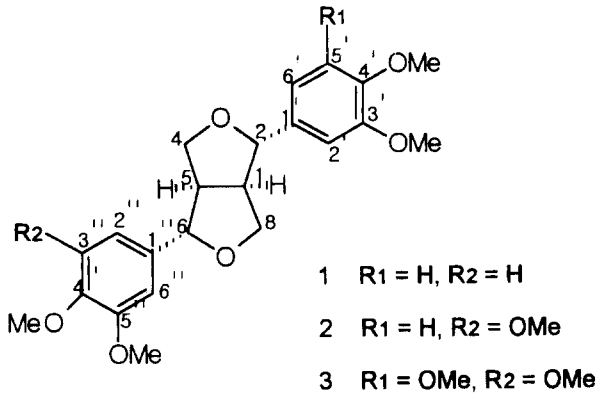

Fig. 2. Structures of three isolated lignans from Magnoliae flos.

were isolated as oils and their chemical structures were identified by NMR data (Table I, II). They were known lignans, namely eudesmin (1), $C_{22}H_{26}O_6$, magnolin (2), $C_{23}H_{28}O_7$, and lirioresinol-B dimethylether (3), $C_{24}H_{30}O_8$ (Fig. 2). The spectroscopic data indicated that all these compounds have a 2,6-diaryl-3, 7-dioxabicyclooctane skeleton. Two aryl group of these 3 compounds are interpreted to be in diequatorial position by NMR data (Table I, II), for endo aryl group bonded at C-2 at 3,7-dioxabicyclooctane system is held very close to the endo hydrogen atom (C 8-H) on the opposite ring, which causes a high field shift by the anisotropic effect of the aryl group (Lindsat *et al.*, 1968; Pelter *et al.*, 1977).

Lignans are widely distributed in terrestrial plants and exhibit variety of biological activities, e.g. antitumor, antimitotic and antiviral (MacRae and Towers, 1984). A variety of bioactive lignans with the same carbon skeleton of eudesmin such as fargesin, aschantin, pinoresinol etc. have been isolated from *Magnolia* spp. and shown to have anti-PAF activity (Pan *et al.*, 1987).

The inhibitory potency of three isolated compounds on TNF-α production was evaluated (Table III). The most potent compound was eudesmin and its IC_{50} was 51 μM. This is the first report that these compounds have anti-TNFα activities. A further research on biological mechanism of this carbon skeleton are currently under investigation and this will be able to clarify the structure-activity relationship in these lignan compounds.

Table III. Inhibitory effects of three isolated lignans from *Magnolia fargesii*

sample	R_1, R_2	Conc. (μg/ml)	% inhibition
Mf-MC-①	-	25	53
Compd.1[a]	R_1=H, R_2=H	12.5	40
Compd.2	R_1=OMe, R_2=H	12.5	23
Compd.3	R_1=OMe, R_2=OMe	12.5	26

[a]IC_{50} value: 19.8 μg/ml (51 μM)

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