Arch Pharm Res Vol 23, No 1, 46-49, 2000

Antifungal Activity of Magnolol and Honokiol

Kyu Ho Bang[1,3], Yoon Kwan Kim[1], Byung Sun Min[2], Min Kyun Na[2], Young Ha Rhee[3], Jong Pill Lee[4], and Ki Hwan Bae[2]

[1]R & D Center, Kolon Pharmaceutical Inc., Taejon 306-220, [2]College of Pharmacy, Chungnam National University, [3]Department of Microbiology, Chungnam National University, Taejon 305-764, and [4]Natural Medicinal Products Division, Korea Food & Drug Administration, Seoul 122-704, Korea

(Received August 17, 1999)

Two neolignan compounds, magnolol (5,5'-diallyl-2,2'-dihydroxybiphenyl, **1**) and honokiol (5,5'-diallyl-2,4'-dihydroxybiphenyl, **2**), were isolated from the stem bark of *Magnolia obovata* and evaluated for antifungal activity against various human pathogenic fungi. Compound **1** and **2** showed significant inhibitory activities against *Trichophyton mentagrophytes, Microsporium gypseum, Epidermophyton floccosum, Aspergillus niger, Cryptococcus neoformans,* and *Candida albicans* with minimum inhibitory concentrations (MIC) in a range of 25-100 μg/ml. Therefore, compound **1** and **2** could be used as lead compounds for the development of novel antifungal agents.

Key words: Magnolol, Honokiol, Human pathogenic fungi, *Magnolia obovata*, Magnoliaceae

INTRODUCTION

Most synthetic antifungal drugs used to treat dermatophytosis show side effects such as toxicities against the liver and kidney as well as gastrointestinal disturbances, headaches and skin hypersensitivity (Yamaguchi, 1990; Mattew *et al.*, 1993). Since fungi are eucaryotes and thus in many ways similar to human cells, many antifungal drugs are also toxic to human cell in addition to fungal cell. There is a pressing need to find novel, safe and commercially useful antifungal agents and increasing efforts are being made to develop such compounds.

Studies on antifungal substance isolated from natural products have been reported by many researchers because natural products are known to be of less toxicities (Grayer and Harbone, 1994). We also have been focusing on the discovery of effective antifungal agents from natural products. During the course of our screening program, we found that the methanolic extract from stem bark of *Magnolia obovata* Thunberg (Magnoliaceae) show antifungal activity against *Trichophyton mentagrophytes*.

The stem bark of *M. obovata* has been used as a stomachic herb in Korea, Japan, and China (Huang, 1993). The phenolic compounds isolated from this plant were reported to exhibit potent antibacterial activities against Gram-positive bacteria, *Streptococcus mutans*, (Namba *et al.*, 1981; 1982; Bae *et al.*, 1985; Seo *et al.*, 1986) and periodontopathic microorganism such as *Polyphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Capnocytophaga gingivalis,* and *Veillonella disper* (Chang *et al.*, 1998). However, its antifungal effect against human pathogenic fungi has not been investigated yet. The antifungal activity was examined against representative human pathogenic fungi of three kinds of dermatomycosis, which were classified by Ormsby and Montgomery (1954).

In this paper, we report the isolation and structural

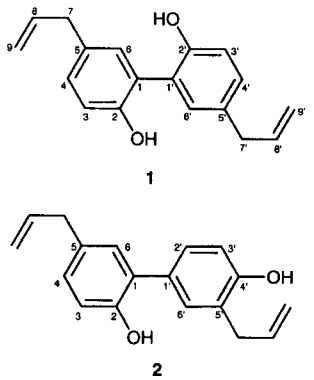

Fig. 1. Structure of magnolol (**1**) and honokiol (**2**)

Correspondence to: Ki Hwan Bae, College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
E-mail: baekh@hanbat.chungnam.ac.kr

identification of magnolol (**1**) and honokiol (**2**) from the stem bark of *M. obovata*, and evaluation of their antifungal activities.

MATERIALS AND METHODS

Instruments

Optical rotations were measured with a DIP-360 automatic polarimeter (JASCO Co.). ^{1}H and ^{13}C NMR experiments were run in $CDCl_3$ containing TMS as the internal standard, using JEOL JNA-LAA 400 WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz). Column chromatography was carried out on silica-gel (Kiesegel 60, 70-230 mesh). Thin layer chromatography (TLC) was carried out on precoated silica-gel 60 F_{254} plates, and spots were detected under UV light and by 10% H_2SO_4 followed by heating.

Plant materials

The stem bark of *M. obovata* was purchased from Tochimoto Co. (Osaka, Japan). The crude drug was identified by Prof. Bae (College of Pharmacy, Chungnam National University, Taejon, Korea). A voucher specimen is preserved at the herbarium of the College of Pharmacy, Chungnam National University.

Fungal strains

Fungal strains used in this experiment were as follows: superficial dermatomycosis, *Trichophyton mentagrophytes* KCTC 6077, *Microsporium gypseum* KCTC 1252 (from dermatophytosis of the foot), *Epidermophyton floccosum* KCTC 1246 (from dermatophytosis of the hand); deep dermatomycosis, *Cryptococcus neoformans* KCTC 7224 (from clinical specimen, Minnesota), *Aspergillus niger* KCTC 1700; Candidiasis, *Candida albicans* KCTC 1940 (from a man with bronchomycosis).

Extraction and isolation

Air dried stem bark (1.5 kg) of *M. obovata* was ground and extracted with 70% EtOH by reflux. The EtOH extract (280 g) was suspended in H_2O and extracted with EtOAc to give EtOAc soluble fraction (120 g). The EtOAc fraction was chromatographed on a silica gel column eluted with a stepwise gradient of *n*-hexane and EtOAc to give seven fractions. Repeated silica gel column chromatography of fraction 3 afforded **1** (1.6 g) and **2** (0.21 g), which were recrystalized from the EtOAc.

Magnolol (5,5'-diallyl-2,2'-dihydroxybiphenyl, 1): Colorless plates; $[\alpha]_D^{21}$ -6.6° (c=0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 3.36 (4H, d, J=6.7 Hz, H-7,7'), 5.08 (4H, m, H-9,9'), 5.53 (2H, s, OH × 2), 5.95 (2H, m, H-8,8'), 6.96 (2H, d, J=8.2 Hz, H-3,3'), 7.08 (2H, d, J=2.2 Hz, H-6,6'), 7.13 (2H, dd, J=8.2, 2.2 Hz, H-4,4'); ^{13}C-NMR ($CDCl_3$) δ 39.3 (C-7,7'), 115.8 (C-9,9'), 116.3 (C-3,3'), 123.6 (C-1,1'), 130.0 (C-4,4'), 131.2 (C-6,6'), 133.2 (C-5,5'), 137.5 (C-8,8'), 151.1 (C-2,2').

Honokiol (5,5'-diallyl-2,4'-dihydroxybiphenyl, 2): Colorless plates; $[\alpha]_D^{21}$ -4.9° (c=0.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 3.34 (2H, d, J=6.7 Hz, H-7), 3.46 (2H, d, J=6.5 Hz, H-7'), 5.10 (2H, s, OH × 2), 5.12 (4H, m, H-9,9'), 6.00 (2H, m, H-8,8'), 6.90 (1H, d, J=8.0 Hz, H-3'), 6.91 (1H, d, J=8.0 Hz, H-3), 7.02 (1H, d, J=2.2 Hz, H-6'), 7.05 (1H, dd, J=8.0, 2.2 Hz, H-4), 7.21 (1H, d, J=2.2 Hz, H-6), 7.24 (1H, dd, J=8.0, 2.2 Hz, H-2'); ^{13}C NMR ($CDCl_3$) δ 35.2 (C-7'), 39.4 (C-7), 115.6 (C-9, 9'), 116.6 (C-3), 117.0 (C-3'), 126.3 (C-1), 127.7 (C-5), 128.6 (C-2'), 128.8 (C-1'), 129.3 (C-4), 130.2 (C-6), 131.2 (C-6'), 132.2 (C-5), 136.0 (C-8), 137.8 (C-8'), 150.8 (C-2), 154.0 (C-4').

Antifungal activity test

Fungal strains used in the antifungal activity test are *C. albicans* as a representative fungus of candidiasis, *T. mentagrophytes* as that of superficial dermatomycosis and *C. neoformans* as that of deep dermatomycosis. Test compounds were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 0.2% in the reaction mixture and diluted with Sabouraud dextrose broth (pH 5.4). Six paper discs containing 25, 50, 100, 200, and 400 μg of test compounds and a control (0.2% DMSO solution) were put on fungus-inoculated plates. The cultivation was carried out at 28 for 24-48 hrs. The antifungal activity was measured by the diameter of growth inhibitory zone.

Determination of minimum inhibitory concentration (MIC)

Prior to testing, *C. albicans* and *C. neoformans* were cultured in Sabouraud dextrose agar (pH 5.6) at 28°C for 3 days, and *T. mentagrophytes*, *E. floccosum*, *M. gypseum* and *A. niger* were cultured at 28°C for 7 days. The inoculum for MIC tests was prepared by scraping fungal growth from a plate and then making a spore suspension in distilled water to give a 95% transmittance at 540 nm for *C. albicans* and *C. neoformans*, and a 90 % transmittance at 540 nm for other fungi (Moore and Jasciow, 1979).

Each compound was dissolved in 0.2% DMSO and serial two-fold dilutions were made in sterile Sabouraud dextrose broth to be 10 ml of final volumes in test tube. And then, 0.05 ml (10^5-10^6 spore suspension) of the spore suspension was inoculated into serially diluted test tubes and incubated at 28°C. They were examined daily for growth for 7 days. The MIC was determined visually

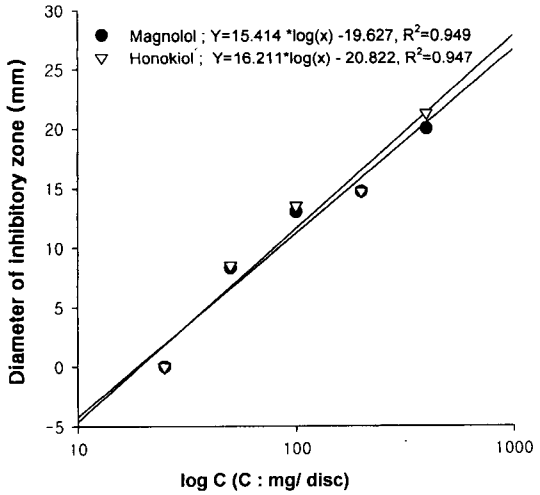

Fig. 2. Inhibitory zone *vs* added amounts of magnolol (**1**) and honokiol (**2**) per disc against *C. neoformans* KCTC 7224. According to the paper disc method, the discs (8 mm in diameter, 1.5 mm in thickness) containing various amounts of magnolol (**1**) and honokiol (**2**) were placed on Petri dishes which have been seeded with *C. neoformans* KCTC 7224. Diameter of inhibitory zones around paper discs was measured in four directions.

by judging the fungal growth in the series of test tubes.

RESULTS AND DISCUSSION

Magnolia species characteristically produce lignans, neolignans, isoquinolines and related alkaloides, and sesquiterpenes (Kijjoa *et al.*, 1989). Of these compounds, magnolol and honokiol are major compounds. We isolated the magnolol (**1**) and honokiol (**2**) from the stem bark of *M. obovata* as antifungal constituents. Two compounds were identified by comparison of the spectroscopic data (Yahara *et al.*, 1991). These compounds were already reported to show various biological activities; *i.e.*, anticholesterol activity (Kwon *et al.*, 1997), 5-lipoxygenese inhibitory activity (Fukuyama *et al.*, 1993), and antibacterial activity (Nitao *et al.*, 1991). In the previous study, we also repoted the antibacterial activity of **1** and **2** against *S. mutans* with MIC values of 6.3 μg/ml (Bae *et al.*, 1985). However, there is no publication on human pathogenic antifungal activity by this time.

In paper disk method, **1** and **2** showed antifungal activities against *T. mentagrophytes*, *C. neoformans*, and *C. albicans* (Table I). The diameter of inhibitory zone was a linear function of logarithmic concentration in a range of 25-400 μg/disc against *C. neoformans* (Fig. 2). Antifungal activity were also determined by MIC test against 6 strains of human pathogenic fungi such as *T. mentagrophytes*, *M. gypseum*, *E. floccosum*, *C. neoformans*, *A. niger*, and *C. albicans* (Table II). Compound **1** showed antifungal activity against *T. mentagrophytes*, *M. gypseum*, *E. floccosum*, *A. niger*, and *C. albicans* with MIC values from 25 to 100 μg/ml, but were practically devoid of any activity on *C. neoformans* with MIC value over 200 μg/ml (Table II). Compound **2** exhibited significant antifungal activity against all fungal strains tested with MIC values

Table I. Antifungal activities of magnolol (1) and honokiol (2) isolated from *Magnolia obovata*

Compounds	Diameter of inhibition zone (mm)[a]														
	C.albicans KCTC 1940					*T.mentagrophytes* KCTC66 07					*C.neoformans* KCTC 7224				
	25	50	100	200	400[b]	25	50	100	200	400	25	50	100	200	400
Magnolol	-[c]	-	-	12.9	14.5	-	-	9.5	12.0	16.0	-	8.3	13.0	14.7	20.0
Honokiol	-	-	13.0	14.5	18.0	-	-	12.5	16.0	19.5	-	8.5	13.5	14.7	21.3

[a] Average of three observations
[b] Added amounts of compounds per disc (μg/disc)
[c] No inhibitory activity

Table II. Minimum inhibitory concentration (MIC) of magnolol (1) and honokiol (2) against pathogenic fungi (μg/ml)

Antifungal agents	KCTC[a] 6077	KCTC[b] 1252	KCTC[c] 1246	KCTC[d] 7224	KCTC[e] 1700	KCTC[f] 1940
Magnolol	50	50	25	>200	100	25
Honikiol	25	25	25	50	50	25
Itraconazole[g]	0.13	19.5	0.03	0.13	0.3	>100
Clotrimazole[g]	4.88	1.22	0.61	2.44	1.22	0.61

[a] *T. mentagrophytes* KCTC 6077, [b] *M. gypseum* KCTC 1252, [c] *E. floccosum* KCTC 1246, [d] *C. neoformans* KCTC 7224, [e] *A. niger* KCTC 1700, [f] *C. albicans* KCTC 1940, [g] Positive control: Itraconazole-Korea Pfizer Inc., Clotrimazole-Hwail Medicines Inc.

from 25 to 50 μg/ml. Compound **1** and **2** were more active against *C. albicans* than itraconazole, an oral triazole antifungal agent, but less active than clotrimazole (Table II). The compounds **1** and **2** showed antifungal activity against *E. floccosum* and *C. albicans* with MIC value of 25 μg/ml. The antifungal effect of **2** can be evaluated to be higher than that of **1**. It can be speculated that the position of hydroxyl group on aromatic ring may influence the biological activity. Structure-activity relationship would require additional experiment.

On consideration of relatively low toxicity of natural compound and broad antifungal spectrum against all strains tested, honokiol (**2**) can be suggested as a novel antifungal lead compound to agent against pathogenic yeasts and filamentous fungi.

REFERENCES

Bae, K., Yoo, B., Lee, M. and Seo, W., Antimicrobial activities of hydroxybiphenyl derivatives (I), Antibacterial activities and HPLC determination of magnolol and honokiol. *Arch. Pharm. Res.*, 8, 85-89 (1985).

Chang, B. S., Lee, Y. M., Ku, Y., Bae, K. H. and Chung, C. P., Antimicrobial activity of magnolol and honokiol against periodontopathic microorganisms. *Planta Medica*, 64, 367-369 (1998).

Fukuyama, Y., Otoshi, Y., Miyoshi, K., Hasegawa, N., Kan, Y. and Komada, M., Structure of magnolianin, A novel triligna possessing potent 5-lipoxygenase inhibitory activity. *Tetrahedron Lett.*, 34, 1051-1054 (1993).

Grayer, R. J. and Harbone, J. B. A., Survey of antifungal compounds from higher plants. *Phytochemistry*, 37, 19-42 (1994).

Huang, K. C., *The Pharmacology of Chinese Herb's*. CRE Press, Ann Arbor, p. 173, 1993.

Kijjoa, A., Pinto, M. M. M., Tantisewie, B. and Herz, W., A biphenyl type neolignan and biphenyl ether from *Magnolia henryi*. *Phytochemistry*, 28, 1284-1286 (1989).

Kwon, B. M., Kim, M. K., Lee, S. H., Kim, J. A., Lee, I. L., Kim, Y. K. and Bok, S. H., Acyl-CoA: Cholesterol acyltransferase inhibitors from *Magnolia obovata*. *Planta Medica*, 63, 550-551 (1997).

Mattew, P. S., Omar, P. S. and Jerome, L. S., Systemic drugs in the treatment of dermatophytosis. *Int. J. Dermatology*, 32, 16-20 (1993).

Moore, G. S. and Jaciow, D. W., *Mycology for the clinical laboratory*. Reston Publishing, Virginia, pp. 262-266 (1979).

Namba, T., Tsunezuka, M., Bae, K. H. and Hattori, M., Studies on dental caries prevention by traditional Chinese medicines (part I), Screening of crude drugs for antibacterial action against *Streptococcus mutans*. *Shoyaku Zasshi*, 35, 295-302 (1981).

Namba, T., Tsunezuka, M. and Hattori, M., Dental caries by traditional Chinese medicines (part II), Potent antibacterial action of Magnoliae Cortex extracts against *Streptococcus mutans*. *Planta Medica*, 44, 100-106 (1982).

Nitao, J. K., Nair, M. G., Thorogood, D. L., Johnson, K. S. and Scriber, J. M., Bioactive neolignans from the leaves of *Magnolia virginiana*. *Phytochemistry*, 30, 2193-2195 (1991).

Ormsby, O. S. and Montgomery, M., *Disease of the skin*. Lea & Fibiger, Philadelphia, p. 1128, 1954.

Seo, W. J., Koo, S. H. and Bae, K. H., Antimicrobial activity of hydroxybiphenyl derivatives. *Arch. Pharm. Res.*, 9, 127-130 (1986).

Yahara, S., Nishiyori, T., Kohda, A., Nohara, T. and Nishioka, I., Isolation and characterization of phenolic compounds from Magnoliae Cortex produced in China. *Chem. Pharm. Bull.*, 39, 2024-2036 (1991).

Yamaguchi, H., Antifungal agents-recent trends in development and progress in research of action mechanism. *Jap. J. Clinic. Med.*, 49, 2176-2185 (1990).