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A BIPHENYL PHYTOALEXIN FROM *CERCIDIPHYLLUM JAPONICUM* *

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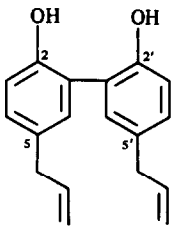
Key Word Index—*Cercidiphyllum japonicum*; Cercidiphyllaceae; katsura tree; phytoalexin; magnolol; 5,5′-diallyl-2,2′-dihydroxybiphenyl.

Abstract—A biphenyl derivative has been isolated as a phytoalexin from the fungus-inoculated twig cortical tissue of *Cercidiphyllum japonicum*. The biphenyl was shown to be magnolol on the basis of spectral evidence and direct comparison with an authentic sample.

INTRODUCTION

We have previously reported the isolation of several phytoalexins from moraceous trees [1, 2]. Screening tests for antifungal compounds from the fungus-inoculated tissues of other arboreal plants revealed that the twig cortical tissue of the katsura tree (*Cercidiphyllum japonicum*) produced a single antifungal compound (**1**), which was absent from water-treated control tissue. We report herein the isolation and characterization of this compound.



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RESULTS AND DISCUSSION

Bioassay-directed fractionation of the acetone extracts from the twig cortical tissue, which had been inoculated with *Fusarium solani* f. sp. *mori*, resulted in the isolation of the antifungal compound (**1**), which inhibited completely the conidial germination of *Bipolaris leersiae* at a concentration of 5×10^{-5} M. The chemical and spectral data of **1** suggested it to be 5,5′-diallyl-2,2′-dihydroxybiphenyl, magnolol. Direct comparison (mmp, IR and ^{1}H NMR spectra) of **1** with those of magnolol confirmed its identity. This is the first report of magnolol as a phytoalexin although it has been reported as a constituent of *Magnolia* species [3, 4] and Taiwan sassafras [5]. To our knowledge, magnolol is the second biphenyl phytoalexin. The first one, aucuparin (4-hydroxy-3,5-dimethoxybiphenyl), was reported recently from two rosaceous plants *Eriobotrya japonica* [6] and *Malus pumila* [7].

EXPERIMENTAL

TLC bioassay. Developed silica gel sheet (Merck, Kieselgel 60 F_{254}; ether) was air-dried, sprayed with a dense conidial suspension of *Bipolaris leersiae* in a potato–glucose medium, and incubated in a moist box at 25° for 2 days [8]. A fungitoxic area appeared white against a dark gray background.

Induction and isolation of magnolol (**1**). Twigs of *Cercidiphyllum japonicum* collected from Tomakomai Experiment Forest of Hokkaido University in September were stripped of epidermis with a razor-blade. The exposed cortical tissue was inoculated with a conidial suspension of *Fusarium solani* f. sp. *mori* and incubated in a moist chamber at 25° for 6 days. The browned tissue separated from the xylem was freeze-dried (523 g) and extracted with Me_2CO to afford the extracts (26.6 g) which showed a single antifungal spot (R_f 0.51) on TLC bioassay. The extracts were chromatographed on a silica gel column using CH_2Cl_2 with increasing amounts of MeOH. The combined active fractions (1.1 g) eluted with CH_2Cl_2–MeOH (100:2) were further separated on a Sephadex LH-20 column (Me_2CO) and then on a silica gel column (CH_2Cl_2) to afford a crystalline residue, which on recrystallization from C_6H_6–hexane gave **1** (226 mg) ; mp 100–101° (lit. 102°) [4], $C_{18}H_{18}O_2$ (found: *m/z* 266.1305); ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ3.36 (4H, *br d*, J = 6.7 Hz, 2 × $\underline{CH_2}CH{=}CH_2$), 5.01 [2H, *dddd*, J = 10.1, 2.1, 1.2, 1.2 Hz, 2 × $CH_2CH{=}\underline{CH_2}$ (*cis*)], 5.09 [2H, *dddd*, J = 17.1, 2.1, 1.5, 1.5 Hz, 2 × $CH_2CH{=}\underline{CH_2}$ (*trans*)], 5.99 (2H, *dddd*, J = 17.1, 10.1, 6.7, 6.7 Hz, 2 × $CH_2\underline{CH}{=}CH_2$), 6.92 (2H, *d*, J = 8.2 Hz, H-3, H-3′), 7.07 (2H, *dd*, J = 8.2, 2.1 Hz, H-4, H-4′), 7.11 (2H, *d*, J = 2.1 Hz, H-6, H-6′), 8.24 (2H, OH); ^{13}C NMR [25.15 MHz, $(CD_3)_2CO$]: *s* at δ127.0, 132.7, 153.0, *d* at δ117.4, 129.6, 132.4, 139.0, *t* at δ40.0, 115.5.

* Part 4 in the series "Studies on Stress Metabolites". For part 3 see Takasugi, M., Kawashima, S., Monde, K., Katsui, N., Masamune, T. and Shirata, A., *Phytochemistry* (in press). Paper No. 6284

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