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4-Benzyl-3-phenyl-5*H*-furan-2-one, a vasodilator isolated from *Malbranchea filamentosa* IFM 41300

Tomoo Hosoe [a,*], Toru Iizuka [a], Shin-ichirou Komai [a], Daigo Wakana [a], Takeshi Itabashi [a], Koohei Nozawa [a], Kazutaka Fukushima [b], Ken-ichi Kawai [a]

[a] *Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*
[b] *Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8673, Japan*

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Abstract

Screening of *Malbranchea filamentosa* IFM 41300 for bioactive compounds led to the identification of 4-benzyl-3-phenyl-5*H*-furan-2-one (**1**) as a vasodilator and erythroglaucin (**2**). The structure of **1** was established on the basis of spectroscopic and chemical investigations. Compound **1** inhibited Ca^{2+}-induced vasocintraction in aortic rings pretreated with high K^+ (60 mM) or norepinephrine. Finally, compound **1** did not exhibit activity against human pathogenic microorganisms.

Keywords: 4-Benzyl-3-phenyl-5*H*-furan-2-one; Vasodilator; *Malbranchea filamentosa* fungus; Chemical structure

1. Introduction

In our search for new bioactive compounds, we isolated a vasodilator from a CH_2Cl_2/MeOH (1:1) extract of the fungus *Malbranchea filamentosa* Sigler and Carmichael IFM 41300 (isolated from an Argentine soil sample): 4-benzyl-3-phenyl-5*H*-furan-2-one (**1**), a new furanone derivative (Fig. 1); and along with erythroglaucin (**2**), which has been previously reported (Braun, 1981; Chandrasenan et al., 1960; Siliva et al., 1979; Bruno and Paul, 1988). In this paper, we describe the isolation, structural determination, and biological activity of **1**.

2. Results and discussion

The molecular formula of **1** was determined to be $C_{17}H_{14}O_2$ by high-resolution electron-impact ionization mass spectrometry (HR-EIMS). The ^1H NMR spectrum of **1** showed 14 protons, which were assigned to 10 aromatic protons (δ 7.10–7.53) and two sets of methylene protons, [δ 3.96 (2H, s) and 4.69 (2H, s)]. The ^{13}C NMR spectrum of **1** showed 17 carbons,which we assigned to 2 sp^3 methylene carbons, one of which was bearing an oxygen function (δ 71.1), and 15 sp^2 carbons, including one carbonyl carbon (δ 173.3). The ^1H–^1H COSY spectrum established that two sets of 1-disubstituted aromatic rings were present, allowing assignment of 10 sp^2 methines [δ 7.10 (2H, 2′-H), 7.34 (2H, 3′-H), 7.28 (1H, 4′-H) and δ 7.53 (2H, 2″-H), 7.47 (2H, 3″-H), 7.40 (1H, 4″-H)]. The infrared (IR) absorption at 1750 cm^{-1} (strong), the ultraviolet (UV) absorption at 254 nm, and the HMBC correlations (Fig. 2), which were observed from 5-C (δ 4.69) to C-2 (δ 173.3), C-3 (δ 127.6), C-4 (δ 159.7) and C-6 (δ 34.0), indicated the existence of an α,β-unsaturated γ-lactone ring. HMBC correlations were also observed for 6-H_2 (δ 3.96) to C-3 (δ 127.6), C-4 (δ 159.7), C-5 (δ 71.1), C-1′ (δ 136.1) and C-2′ (δ 128.5), which indicated a benzyl group attached at C-4. Based on these results, compound **1** was found to be 4-benzyl-3-phenyl-5*H*-furan-2-one (Fig. 1).

The vasodilatory effects of **1** were examined in high k^+ (60 mM)-treated isolated rat thoracic aorta. As shown in

* Corresponding author. Tel.: +81 3 5498 5790; fax: +81 3 5498 5788.
E-mail address: hosoe@hoshi.ac.jp (T. Hosoe).

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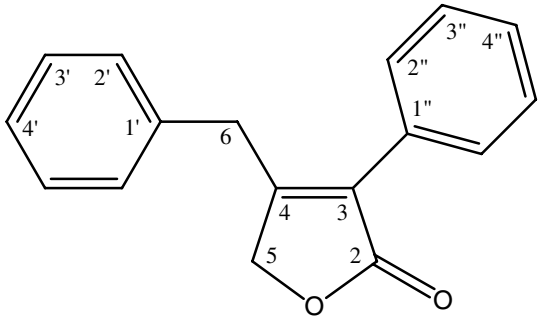

Fig. 1. Chemical structure of compound **1**.

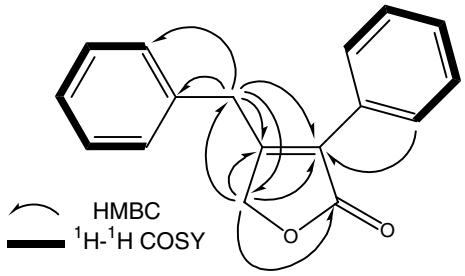

Fig. 2. ^{1}H–^{1}H COSY and the important HMBC correlations of compound **1**.

Fig. 3, **1** (10^{-6}–10^{-7} M) caused a dose-dependent inhibition of the Ca^{2+}-induced contraction in a Ca^{2+}-free, high K^+ solution. We also examined the ability of **1** (10^{-5}–10^{-6} M) to inhibit Ca^{2+}-induced vasocontraction in aorta that had been pretreated with Ca^{2+}-free medium containing 10^{-6} M norepinephrine (NE) and 10^{-6} M nicardipine. Fig. 4 shows that **1** was less potent than when the contractions were induced in the presence of high K^+.

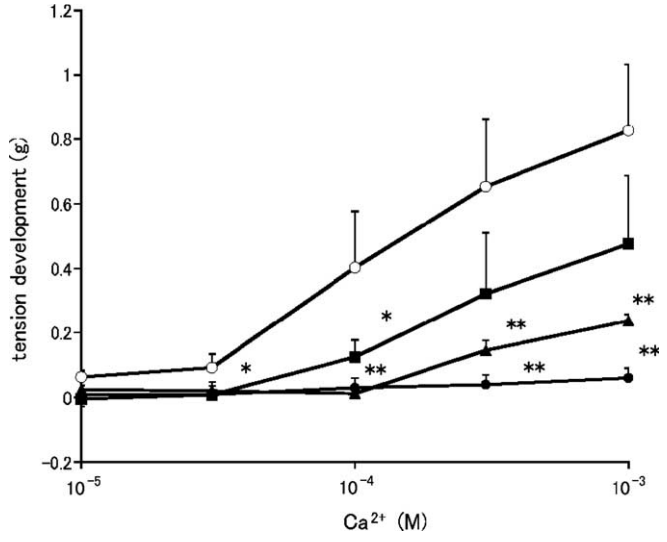

Fig. 3. Dose–response of compounds on Ca^{2+}-induced contraction of aortic rings pretreated with high K^+. Aortic rings were preincubated for 1 h in Ca^{2+}-free medium containing high (60 mM) K^+. Saline (control) (○), 10^{-6} M compound **1** (▲), 10^{-7} compound **1** (■), or 10^{-8} M nicardipine (●) were then added, and contraction was induced by the addition of Ca^{2+}. Values represent the means ± SE of three determinations: $*P < 0.05$; $**P < 0.01$ vs. control.

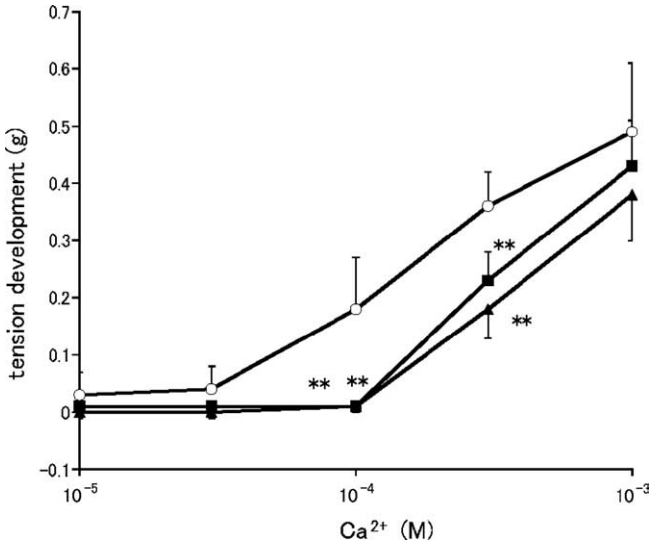

Fig. 4. Dose–response of compounds on Ca^{2+}-induced contraction of aortic rings pretreated with NE nicardipine. Aortic rings were preincubated for 1 h in Ca^{2+}-free medium containing NE (10^{-6} M) and nicardipine (10^{-6} M). Saline (control) (○) 10^{-5} M compound **1** (▲), or 10^{-6} M compound **1** (■) were then added, and contraction was induced by the addition of Ca^{2+}. Values represent the means ± SE of three determinations: $**P < 0.01$ vs. control.

In many cases, vasoconstrictor-induced contraction is largely mediated by Ca^{2+} influx so that inhibitors of Ca^{2+} influx cause vasodilation. Indeed, nicardipine, a voltage-dependent Ca^{2+} channel blocker, potently inhibited high K^+-induced vasocontraction. In contrast, **1** showed only moderate dose-dependent effects on high K^+-induced vasocontraction. These results demonstrate that **1** relaxes high K^+-induced vasocontraction and indicate that the vasodilatory effect of **1** may be due to the inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. In addition, **1** weakly inhibited the contractions induced by NE in the presence of nicardipine, which suggests that other mechanisms may be partially involved in its vasodilatory activity.

We further found that compound **1** at a concentration of 100 μg/disc showed no antimicrobial activity against *Aspergillus fumigatus* Fresenius IFM 41243, *Aspergillus niger* Van Tiehem IFM 41398, *Candida albicans* (Robin) Berkhout IFM 40009, *Cryptococcus neoformans* (Sanfelice) Vullemin ATCC 90112, *Bacillus subtilis* ATCC 6633, or *Escheria coli* strain B.

In summary, we identified **1** as a new vasodilator.

3. Experimental

3.1. General

HR-EIMS and CIMS were taken with a JEOL JMS-MS600W spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrometer and a JASCO FT/IR-200 spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Lambda-500 (^{1}H, 500.00 MHz;

^{13}C, 125.25 MHz) spectrometer with tetramethylsilane as an internal standard. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). Low-pressure liquid chromatography was performed using an FMI Lab Pump (model RP-SY) and a glass column (200 × 10 mm) packed with Silica gel CQ-3 (30–50 μm; Wako). Compounds separated by TLC were visualized by illumination with UV light at 254 nm and/or by spraying with 5% H_2SO_4 and then healing.

3.2. Fermentation, extraction, and isolation

M. filamentosa IFM 41300 was cultivated at 25 °C for 21 days in five Roux flasks, each containing 150 g of moist rice. The cultivated rice was extracted with 1:1 CH_2Cl_2/MeOH, and the organic layer was evaporated in vacuo. The resulting extract (21 g) was suspended in water and extracted with EtOAc, after which the organic layer was evaporated in vacuo to obtain the EtOAc extract (10 g).

The EtOAc extract was separated by column chromatography over silica gel (200 g) into six fractions: 50:1 CH_2Cl_2/EtOH (233 mg), 20:1 CH_2Cl_2/EtOH (304 mg), 10:1 CH_2Cl_2/EtOH (5.71 g), 5:1 CH_2Cl_2/EtOH (2.83 g), 1:1 CH_2Cl_2/EtOH (505 mg), and EtOH (138 mg). The second fraction (20:1 CH_2Cl_2/EtOH) was separated by low-pressure liquid chromatography on silica gel with 35:1 C_6H_6/EtOAc (35:1) to give **1** (28.1 mg). Erythroglaucin (**2**, 60.6 mg) was obtained from the first fraction (50:1 CH_2Cl_2/EtOH) as a precipitate. Compound **2** was identified by comparison with published data (Bruno and Paul, 1988).

3.3. 4-Benzyl-3-phenyl-5H-furan-2-one (*1*)

Colorless, viscous oil. EIMS *m/z*, 250[M^+] (25), 205 (52), 115 (97), 91 (100); CIMS *m/z*, 251 [M + H]$^+$; HR-EIMS, found: 250.0996 (M^+), calculated: 250.0994 (for $C_{17}H_{14}O_2$); UV(MeOH) λ_{max} nm (log ε): 249 (3.95), 208 (4.21); IR ν_{max} cm^{-1}: 3030, 2920, 1750, 1500, 1450, 1110, 1040; ^1H NMR spectral data (500 MHz, $CDC1_3$): δ 3.96 (2H, *s*, 6-H), 4.69 (2H, *s*, 5-H), 7.10 (2H, *m*, 2′-H), 7.28 (1H, *m*, 4′-H),7.34 (2H, *m*, 3′-H), 7.40 (1H, *m*, 4″-H), 7.47 (2H, *m*, 3″-H), 7.53 (2H, *m*, 2″-H); ^{13}C NMR spectral data (125.43 MHz, $CDC1_3$): δ 34.0 (*t*, C-6), 71.1 (*t*, C-5), 127.4 (*d*, C-4′), 127.6 (*s*, C-3), 128.5 (*d*, C-2′), 128.7 (*d*, C-3″), 128.8 (*d*, C-4″), 128.9 (*d*, C-2″), 129.2 (*d*, C-3′), 129.7 (*s*, C-1″), 136.1 (*s*, C-1′), 159.7 (*s*, C-4), 173.3 (*s*, C-2).

3.4. Assay of vasorelaxation

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science. Sports Culture, and Technology of Japan.

3.4.1. Preparation of rat aortic rings

Preparation of aortic rings and measurement of tension were performed as previously described (Nagai et al., 1996). Male Wistar rats weighting 230–260 g were sacrificed by decapitation. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS; 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut in ring preparations 3 mm in length. The tissue was placed in a well oxygenated (95% O_2, 5% CO_2) bath containing 10 ml KHS solution at 37 °C with one end connected to a tissue holder and the other to a force–displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

3.4.2. Experimental protocol

For examination of Ca^{2+}-induced contraction in depolarized muscle, the aortic rings were exposed to Ca^{2+}-free KHS containing 0.01 mM ethyleneglycol-bis-(β-aminoethyl ether)-tetraacetic acid (EGTA) and were depolarized with isotonic K^+ (60 mM). The aortic rings were exposed to 10^{-7} or 10^{-6} M of **1** for 1 h, after which Ca^{2+} (10^{-5}–10^{-3} M) was cumulatively applied to the depolarized aorta in Ca^{2+}-free KHS. Nicardipine (10^{-7} M) was added as a positive control to the organ bath in the same manner as for **1**. For examination of Ca^{2+}-induced contraction in the presence of NE, the aortic rings were exposed to 10^{-6} or 10^{-5} M of **1** in Ca^{2+}-free KHS containing 0.01 mM EGTA for 1 h, followed by the addition of 10^{-6} M nicardipine and 10^{-6} M NE. Next, Ca^{2+} (10^{-5}–10^{-3} M) was added cumulatively to the bath. Compound **1** was dissolved in DMSO and diluted with saline. The final concentration of DMSO in the organ bath was less than 0.1%, which did not affect contraction or relaxation. All other drugs were dissolved in saline.

3.4.3. Statistical analysis

The statistical significance of differences between mean values was examined by a Dunnett-type Bonferroni's multiple *t*-test. *P* values of less than 5% were considered significant.

3.5. Antibacterial and antifungal activities of *1*

Antibacterial and antifungal activities were semi-quantitatively determined by the paper disc-agar diffusion method using 8-mm discs loaded with 5 or 100 μg of compound as described previously (Hosoe et al., 2004). The test organisms used were *A. fumigatus* IFM 41243, *A. niger* IFM 41398, *C. albicans* IFM 40009, *C. neoformans* ATCC 90112, *B. subtilis* ATCC 6633, and *E. coli* strain B.

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