Two new furanone glycosides, malfilamentosides A and B, from *Malbranchea filamentosa*

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Summary

In the course of searching for new antifungal substances in *Malbranchea* spp., two new furanone glycosides, malfilamentosides A (1) and B (2), were isolated from *Malbranchea filamentosa* along with a furanone derivative, 4-benzyl-3-phenyl-5H-furan-2-one (3) a dioxopiperazine derivative, amauromine, and an anthraquinone, erythroglaucine. The structures of 1 and 2 were established by spectroscopic and chemical investigation. Malfilamentosides A (1) and B (2) are the first examples of the glycosides of the furanone derivative from the fungal sources.

Key words : *Malbranchea filamentosa*, glycoside, malfilamentoside A, malfilamentoside B, 4-benzyl-3-phenyl-5H-furan-2-one, 6-O-methyl-N-acetyl-D-glucosamine

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Introduction

The causative fungi of severe mycoses, *e.g.*, coccidioidomycosis caused by *Coccidioides immitis* RIXFORD & GILCHRIST, paracoccidioidomycosis by *Paracoccidioides brasiliensis* (SPLENDORE) ALMEIDA, blastomycosis by *Blastomyces dermatitidis* GILCHRIST & STOKES (teleomorph: *Ajellomyces dermatitidis* McDONOUGH & LEWIS), and histoplasmosis by *Histoplasma capsulatum* DAHLING [teleomorph: *Ajellomyces capsulatus* (KWON-CHANG) MCGINNIS & KATZ], are all dimorphic hyphomycetes, of which the teleomorph belong to the family Onygenaceae, in the order Onygenales¹⁾. Fungi of the genus *Malbranchea* also belong to the Onygenaceae and are taxonomically close to the above mentioned pathogenic fungi. This fact prompted us to investigate the metabolites of *Malbranchea* fungi. Fifteen species of *Malbranchea* were cultivated on rice at 25 °C for 21 days and extracted with chloroform-methanol (1:1). The evaporated extracts were each subjected to an antifungal test against two pathogenic yeasts, *Candida albicans* (ROBIN) BERKHOUT IFM40009 and *Cryptococcus neoformans* (SANFELICE) VUILLEMIN ATCC 90112, which causes one of the deep-seated mycosis, cryptococcosis, and two pathogenic filamentous fungi, *Aspergillus fumigatus* FRESENIUS IFM 41243 and *Aspergillus niger* VAN TIEGHEM IFM41398. Three species of *Malbranchea* showed antifungal activity against some

fungi tested: *M. flava* SIGLER & CARMICHAEL IFM41299 showed antifungal activity against *C. albicans* and *A. niger*, and *M. flocciformis* SIGLER & CARMICHAEL IFM41298 showed activity against *C. albicans*, whereas *M. filamentosa* SIGLER & CARMICHAEL IFM41300 was active against *C. neoformans*.

From the constitutional investigation of *M. filamentosa* IFM41300, a furanone derivative, 4-benzyl-3-phenyl-5*H*-furan-2-one (3)²⁾, which had a moderate vasodilatory activity, was isolated along with an anthraquinone, erythroglaucone. Further analysis of the metabolites of the same fungus led to the isolation of two new furanone glycosides designated as malfilamentosides A (1) and B (2) along with a known dioxopiperazine, amauromine, originally isolated from *Amauroascus* sp.³⁾.

Compounds 1 and 2 are structurally related to 3, but they had no vasodilatory activity as well as antifungal one. This paper deals with the structural determination of 1 and 2.

Experimental

General Experimental Procedures Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 spectrometer. EI and CI mass spectra were taken with a JEOL JMS-600W spectrometer, whereas ESI-TOF-MS was taken with a Bruker microTOF spectro-meter. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ¹H and ¹³C NMR spectra were recorded on a JEOL JNM Lambda-500 spectrometer at 500.00 MHz and 125.43 MHz, respectively, using tetramethylsilane as an internal standard. Coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, quartet = q, multiplet = m, and broad = br. *J*-Values are in Hz. CD curves were determined on a JASCO J-720 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (10 × 200 mm) packed with silica gel CQ-3 (30-50 μm; Wako). HPLC was performed on a Senshu SSC-3160 pump with the flow rate of 4 mL/min using a Inersil HPLC OPS-P (10 i.d. × 250 mm) prepacked column or using an ODS prepacked column, equipped with a Shimamura YRU-883 RI-UV monitor. TLC was conducted on pre-coated Kieselgel 60 F₂₅₄ plates (Art. 5715; Merck).

Antifungal Assay by Paper Disc Method The antifungal assay was performed by the paper disc method against *C. albicans* ATCC 90028, *C. neoformans* ATCC 90112, *A. fumigatus* IFM 41362, and *A. niger* IFM 41398, as test organisms. All *Malbranchea* species were cultured at 25 °C for 21 days in a test-tube containing 10 g of moist rice. The cultivated rice was extracted with CH₂Cl₂-MeOH (1:1) and the organic layer was evaporated *in vacuo*. The extracts were applied to the paper discs (8 mm diameter) as 2.5 mg/disc and placed on the assay plates. Fungi were cultivated on potato-dextrose agar (PDA) at 25 °C. After 48-72 hour incubation, zones of inhibition (mm in diameter) were recorded. Three species of *Malbranchea* showed antifungal activity against following fungi tested: *M. flava* IFM41299 showed 10 mm and 17 mm of inhibition circle against *C. albicans* and *A. niger*, respectively. *M. flocciformis* IFM41298 showed 10 mm of inhibition circle against *C. albicans*. *M. filamentosa* IFM41300 showed 14 mm of inhibition circle against *C. neoformans*. Other strains showed no inhibition.

Isolation of Malfilamentosides A (1) and B (2) from *Malbranchea filamentosa* *M. filamentosa*,

strain IFM 41300, was cultivated on moistured rice (750 g) using 5 Roux flasks at 25 °C for 21 days. The cultivated rice was extracted with acetone and the solvent was evaporated *in vacuo*. The resulting extract was suspended in H₂O and extracted with AcOEt, after which the organic layer was evaporated *in vacuo* to obtain an AcOEt extract (10 g). This extract was chromatographed on silica gel with *n*-hexane, benzene, and CH₂Cl₂, in turn. Amauromine (235 mg) was precipitated from the hexane eluate, when the extract was concentrated. The benzene eluate was chromatographed on silica gel with CH₂Cl₂-EtOH followed by purification of HPLC on silica gel [benzene-EtOH (70:1)] to give erythroglaucine (43 mg) and 4-benzyl-3-phenyl-5*H*-furan-2-one (3) (10 mg). The CH₂Cl₂ eluate was purified by LPLC on silica gel with CH₂Cl₂-EtOH (10:1) followed by repeated purification by HPLC on silica gel using CH₂Cl₂-EtOH (10:1) and CH₂Cl₂-acetone (1:2) and on ODS using 70 % MeOH, to give malfilamentosides A (1) (9 mg) and B (2) (243 mg).

Malfilamentoside A (1): Colorless needles (from MeOH): mp 188 °C: [α]_D²⁰ +206.4° (*c* 1.00, MeOH): EI-MS *m/z* (%) 483 (M⁺, 18), 405 [(M-H₂O)⁺, 27], 248 (100): CI-MS *m/z* (isobutane) 484.1955 [(M+H)⁺, 484.1971 for C₂₆H₃₀NO₈, 100]: UV (MeOH) $\lambda_{\max}$ (log ϵ) 256 (3.98) nm: IR (KBr) $\nu_{\max}$ 3400 (-OH), 1780 (-CO₂-), 1610 (-CONH-), 1520 cm⁻¹. CD Δ (nm) +9.8 (243). The ¹H and ¹³C NMR data were summarized in Table 1.

Malfilamentoside B (2): Colorless amorphous powder: mp 113 °C (from MeOH): [α]_D²⁰ +24° (*c* 1.0, MeOH): EI-MS *m/z* (%) 264 (100): CI-MS *m/z* (isobutane) 265 (100): ESI-TOF-MS *m/z* 500.1929 [(M+H)⁺, 500.1915 for C₂₆H₃₀NO₉]: UV (MeOH) $\lambda_{\max}$ (log ϵ) 259 (3.88) nm: IR (KBr) $\nu_{\max}$ 3400 (-OH), 1770 (-CO₂-), 1650 (-CONH-), 1530. CD Δ (nm) -7.3 (260). The ¹H and ¹³C NMR data were summarized in Table 1.

Acid Hydrolysis of Malfilamentoside A (1) and Malfilamentoside B (2) Each of malfilamentoside A (1) (20 mg) or malfilamentoside B (2) (20 mg) was suspended in 4 M HCl (10 mL) and the suspension was stirred at 100 °C for 3 h. After cooling, each reaction mixture was extracted with AcOEt. The aqueous layer was chromatographed on ODS column to give 6-*O*-methyl-D-glucosamine hydrochloride (6 mg from 1; 5 mg from 2).

6-*O*-Methylglucosamine hydrochloride: Colorless powder: [α]_D²⁰ +65.3° (*c* 1.00, H₂O): positive ESI-MS *m/z* (%) 194 [(M-Cl)⁺, 77], 176 [(M-Cl-H₂O)⁺, 100]: ¹H NMR (CD₃OD): α -anomer: 3.05 (1H, dd, *J* = 9.8, 3.4 Hz, 2-H), 3.38 (1H, t, *J* = 9.8 Hz, 4-H), 3.40 (3H, s, 6-*O*-Me), 3.81 (1H, t, *J* = 9.8 Hz, 3-H), 3.65 (2H, m, 6-H₂), 3.91 (1H, m, 5-H), 5.30 (1H, d, *J* = 3.4 Hz, 1-H), β -anomer: 2.78 (1H, dd, *J* = 9.4, 8.2 Hz, 2-H), 3.71 (1H, m, 4-H), 3.40 (3H, s, 6-*O*-Me), 3.50 (1H, t, *J* = 9.4 Hz, 3-H), 3.60 (2H, m, 6-H₂), 3.43 (1H, m, 5-H), 4.74 (1H, d, *J* = 8.2 Hz, 1-H): ¹³C NMR (CD₃OD): α -anomer: 56.2 (2-C), 59.6 (6-*O*-Me), 71.4 (3-C), 71.9 (4-C), 72.1 (5-C), 72.7 (6-C), 90.8 (1-C): β -anomer: 58.7 (2-C), 59.5 (6-*O*-Me), 72.0 (6-C), 72.8 (4-C), 74.1 (3-C), 77.1 (5-C), 94.7 (1-C)

Results and discussion

The molecular formulae of malfilamentosides A (1) and B (2) were confirmed as C₂₆H₂₉NO₈ and C₂₆H₂₉NO₉, respectively, by the high resolution CI or ESI-TOF mass spectrometry. The ¹H and ¹³C NMR spectra of 1 (Table 1) were similar to those of 4-benzyl-3-phenyl-5*H*-furan-2-one (3), which formed as a co-occurring compound in the fungus of origin,²⁾ except for the presence of sugar moiety

Table 1 ^1H and ^{13}C NMR Chemical Shifts for 1-3 in CDCl_3

Carbon No.	1		2		2 in CD_3OD	3	
	C	H (J in Hz)	C	H (J in Hz)	H (J in Hz)	C	H
2	169.9		169.8			173.3	
3	136.0		140.4			127.6	
4	155.7		156.8			159.7	
5	100.8	5.76 s	101.7	6.29 s	6.29 s	71.1	4.69 s
6	32.6	3.82 d (15.6)	70.0	5.95 d (4.9)	5.94 s	34.0	3.96 s
		4.05 d (15.6)					
6-OH				3.85 br			
1'	131.1		130.7			136.1	
2' & 6'	128.5	7.14 m	127.6	7.45 m	7.34-7.48 m	128.5	7.10 m
3' & 5'	128.9	7.44 m	129.0	7.32 m	7.34-7.48 m	129.2	7.34 m
4'	127.5	7.28 m	129.5	7.32 m	7.34-7.48 m	127.4	7.28 m
1''	128.6		128.4			129.7	
2'' & 6''	129.3	7.34 m	128.9	7.40 m	7.34-7.48 m	128.9	7.53 m
3'' & 5''	128.8	7.52 m	128.5	7.32 m	7.34-7.48 m	128.7	7.47 m
4''	129.5	7.44 m	128.8	7.32 m	7.34-7.48 m	128.8	7.40 m
1'''	98.4	5.03 d (3.4)	100.0	5.25 d (3.4)	5.19 d (3.5)		
2'''	53.3	4.08 m	53.8	3.91 ddd (10.7, 7.6, 3.4)	3.90 m		
2'''-NH		5.85 d (8.6)		5.79 d (7.6)			
-COCH ₃	23.2	1.93 s	23.2	1.49 s	1.71 s		
-COCH ₃	171.5		172.6				
3'''	72.5	3.64 brd (9.8)	73.2	3.45 m	3.69 m		
4'''	71.7	3.64 brd (9.8)	71.8	3.54 brt (10.1)	3.69 m		
5'''	71.2	3.98 m	70.8	3.97 ddd (10.1, 4.6, 3.4)	3.90 m		
6'''	71.8	3.65 dd (10.7, 3.4)	71.7	3.67 dd (10.7, 3.4)	3.39 m		
		3.75 dd (10.7, 4.3)		3.75 dd (10.7, 4.6)			
6'''-O-Me	59.5	3.41 s	59.3	3.41 s	3.43 s		

[the ^1H NMR signals observed at 5.03 (1H, d), 4.09 (1H, m), 3.65 (1H, dd), 3.75 (1H, dd), 3.98 (1H, m), and 3.63 (2H, br d) coupled with the ^{13}C NMR signals at 98.4, 53.3, 72.5, 71.8, 71.2, and 71.7, respectively], an acetyl group [the ^1H NMR signal at 1.93 (3H, s) and the ^{13}C NMR signals at 23.2 and 171.5], and a methoxy group [the ^1H NMR signal at 3.41 (3H, s) coupled with the ^{13}C NMR signals at 59.5]. Another difference between 1 and 3 was the appearance of the methine proton observed at 5.76 (1H, s) coupled with the ^{13}C NMR signals at 100.8 in 1 instead of the disappearance of the methylene protons observed at 4.69 (2H, s) coupled with the ^{13}C -NMR signals at d 71.1 in 3. This result and the molecular formula of 1 suggested that malfilamentoside A (1) may be an acetylated and methylated monoglycoside of a furanone derivative with a hydroxyl group at C-5 of 3.

Acid hydrolysis of 1 by 4 M HCl gave 6-*O*-methyl-D-glucosamine hydrochloride⁴. The HMBC spectrum of 1 showed the long range couplings (Fig. 2) from the acetyl carbonyl carbon (171.5) to the NH proton [5.85 (d)] and the acetyl methyl protons (1.93) and those from the methoxy protons (3.41) to the C-6''' carbon (71.7) and from the 6'''-methylene protons [3.63 (br d)] to the methoxy carbon (59.5), which confirmed the positions of the acetyl and methoxy groups in 1. The $^3J_{\text{H,H}}$ value (3.4 Hz) of the anomer proton at C-1''' concluded that the glycoside linkage is β . From the above results and the detailed analysis of the HMBC spectrum (Fig. 2), the structure of malfilamentoside A

Fig. 1 Structures of furanone derivatives

Fig. 2 HMBC Correlations in Malfilamentosides A (1) and B (2)

(1) was confirmed as shown in Fig. 1.

The NMR spectra of malfilamentoside B (2) (Table 1) were similar to those of malfilamentoside A (1), except the appearance of 6-H observed at 5.95 (1H, d) coupled with the ^{13}C NMR signals at 70.0 in 2 instead of the methylene protons at C-6 [3.82 (1H, d) and 4.05 (1H, d)] coupled with the carbon at 32.6 in 1. This result and the molecular formula of 2 indicated that malfilamentoside B (2) is the hydroxyl derivative of malfilamentoside A (1) at C-6. The structure of malfilamentoside B (2) was confirmed as shown in Fig. 1 by acid hydrolysis experiment and HMBC correlations (Fig. 2) similarly to the case of 1. Works to clarify the stereochemistry of the genin part of 1 and 2 are now in progress.

Malfilamentosides A (1) and B (2) are first examples, in our knowledge, of the glycosides of the

furanone derivative from the fungal sources. The furanone derivative 3 has been isolated previously from *M. filamentosa*²⁾ and also recently isolated as gymnoascolide A from the Australian soil fungus *Gymnoascus reessii* BARANETZKI⁵⁾. Moreover, the furanone derivative, microperfurane (4)⁶⁾, which corresponds to the aglycon part of 1, was isolated from the ascomycete *Anixiella microperptusa* HORIE & UDAGAWA, whereas the furanone derivatives, gymnoascolides B (5) and C (6)⁶⁾ were also isolated from *G. reessii*. Since malfilamentosides A (1) and B (2) had no antifungal activity, further investigation of the antifungal compounds in the extract of *M. filamentosa* are now in progress.

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マルブランキア・フィラメントサから得られる 2 種の新規フラノン誘導体

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深在性真菌症に対する新規抗真菌剤開発の一環として, 重篤輸入真菌症の原因菌と同科のマルブランキア属菌 15 菌株について抗真菌活性を指標に検索した結果, マルブランキア・フィラメントサほか 2 種に抗真菌活性が認められた。今回は, マルブランキア・フィラメントサに着目し, その成分として 2 種の新規フラノン配糖体マルブランキオシド A (1), マルブランキオシド B (2) を単離することができた。化合物 1 および 2 の構造は各種スペクトルデータの解析および化学反応の利用により決定した。化合物 1 および 2 は菌類より得られたグルコサミンを糖部とするフラノン配糖体の例として珍しいものである。

キーワード: マルブランキア・フィラメントサ, 配糖体, マルブランキオシド A, マルブランキオシド B, 4-ベンジル-3-フェニル-5H-6-フラン-2-オン, O-メチル-N-アセチル-D-グルコサミン