Some monoamine oxidase inhibitory components from *Mycelia Sterilia* derived from an Ascomycete, *Gelasinospora pseudoreticulata*

Haruhiro FUJIMOTO*, Hiromi OKUYAMA*, Yumiko MOTOHASHI*,
Eiji YOSHIDA*, and Mikio YAMAZAKI*

藤本治宏*, 奥山浩美*, 本橋弓子*, 吉田栄治*, 山崎幹夫*: 子囊菌 *Gelasinospora pseudoreticulata* 由来 *Mycelia Sterilia* からのモノアミン酸化酵素阻害活性成分

Summary

Three new components, tentatively named GP-A, -B, and -C, with monoamine oxidase (MAO) inhibitory activity and a known anthraquinone chrysophanol were isolated from *Mycelia Sterilia* derived from an Ascomycete, *Gelasinospora pseudoreticulata*. GP-A and -B, the main MAO inhibitory principles of this fungus, were deduced to be 5-hydroxy-7-methoxy-4,9-dioxonaphtho[2,3-*c*]furan and 1,3-dihydro-5-hydroxy-7-methoxy-4,9-dioxonaphtho[2,3-*c*]furan, respectively. The IC₅₀ values of GP-A, -B, and -C were evaluated against the MAO from mouse liver.

Key words: *Mycelia Sterilia* derived from *Gelasinospora pseudoreticulata*; Fungus, 菌類; MAO inhibitory activity, モノアミン酸化酵素阻害活性; 4,9-Dioxonaphtho[2,3-*c*]furan, 4,9-ジオキソナフト[2,3-*c*]フラン; Fungal metabolite, 菌類代謝産物

In our project to search the monoamine oxidase (MAO) inhibitory metabolites, some effective components from Ascomycetes, *Emericella navahoensis*^{1,2)} and *Talaromyces luteus*,³⁾ have so far been isolated. Now from the ethyl acetate extract of cultivated mycelia of *Mycelia Sterilia* derived from *Gelasinospora pseudoreticulata* Matsushima IFM4635, two new 4,9-dioxonaphtho[2,3-*c*]furans which were tentatively named GP-A (**1**) and -B (**2**) have been isolated as the main MAO inhibitory principles of this fungus. Two metabolites tentatively named GP-C (**3**) and -D (**4**) have also been isolated. This report deals with the isolation, structure elucidation, and MAO inhibitory activity of these fungal metabolites.

Materials and Methods

The MAO inhibitory activity of samples was evaluated with the modified Kraml's method described in our previous report^{1),2)} using the MAO from liver of mouse (ddY, male, 5 weeks) purchased from Nippon SLC Co. (Hamamatsu, Japan). Column chromatographic adsorbents and chemical reagents were of the purest grade commercially available. Thin layer chromatography (TLC) was carried out with silica gel plates (Merck Kieselgel 60F₂₅₄). The infrared (IR) spectra were recorded with a Hitachi IR 260-10, the ultraviolet (UV) spectra with a Hitachi U-3200, the high-resolution electron impact mass (HREI-MS) spectra

* Faculty of Pharmaceutical Sciences, Chiba University (1-33, Yayoi-cho, Inage-ku, Chiba 263)
千葉大学薬学部 (〒263 千葉市稲毛区弥生町 1-33)

with a JEOL JMS-HX110, and the ^1H - and ^{13}C -nuclear magnetic resonance (NMR) spectra expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard with a JEOL JNM-A500 at 500 and 125.65 MHz, respectively.

Isolation of GP-A (1), -B (2), -C (3), and -D (4) Mycelia Sterilia derived from *G. pseudoreticulata* IFM4635⁴⁾ was cultivated on sterilized rice⁵⁾ (180 g/flask $\times$ 167) at 25°C for 22 days. During the cultivation, the moldy rice in 107 flasks gradually became light-brown and the remainder gradually became dark-brown. The light-brown moldy rice was extracted with ethyl acetate (AcOEt) (32.1 l) with shaking at room temperature for 5 hours two times to give an extract (194 g), which was defatted with *n*-hexane-methanol (MeOH) (3: 1, v/v) (1.25 l). The defatted extract (87.5 g) was partitioned with AcOEt-H₂O (1: 1) (640 ml) into the AcOEt and the aqueous layers, which gave after evaporation the AcOEt (54.4 g) and the aqueous fractions (27.0 g), respectively. The AcOEt fraction was subjected to silica gel column chromatography to give fractions I-IV. Among them, fractions II and III were obtained as the former and the latter eluates with *n*-hexane-AcOEt (2: 1), respectively. Fraction II (662 mg) was further chromatographed repeatedly on a silica gel column to give fractions IIa-IId. Fraction IIb eluted with C₆H₆ (28 mg) was recrystallized with ethanol (EtOH) to afford **1** (18 mg). Fraction IIc eluted with C₆H₆-acetone (40: 1) (532 mg) was further subjected to chromatography on a silica gel column with CHCl₃ and then on a Sephadex LH-20 column with CHCl₃-MeOH (1: 1) to give a fraction, which was treated with CHCl₃-MeOH to afford **2** (3 mg). Fraction III (328 mg) was further chromatographed on a silica gel column with C₆H₆-acetone (40: 1), on a silica gel column with CHCl₃-AcOEt (50: 1), and successively on a Sephadex LH-20 column with CHCl₃-MeOH (1: 1) to give a fraction, which was recrystallized with CHCl₃-MeOH to afford **3** (5 mg). The dark-brown moldy rice was extracted with AcOEt (18.0 l) in the same way as for extraction of the light-brown moldy rice to give an extract (57 g), which was defatted with *n*-hexane-MeOH (3: 1) (0.36 l). The defatted extract (27.3 g) was partitioned with AcOEt-H₂O (1: 1) (132 ml) into the AcOEt and the aqueous layers, which gave after evaporation the AcOEt (25.1 g) and the aqueous fractions (2.4 g), respectively. The AcOEt fraction was chromatographed on a silica gel column with *n*-hexane-AcOEt (2: 1) repeatedly, and successively on a silica gel column with CHCl₃ to give a fraction, which was treated with CHCl₃ to afford **4** (14 mg).

GP-A (**1**): yellow needles, mp 198.0–200.0°C (from EtOH). HREI-MS m/z Calcd. for C₁₃H₈O₅ (M⁺) 244.0372. Found: 244.0364. UV_{max}^{MeOH} nm (log ϵ): 220 (4.19), 254 (sh 4.16), 260 (4.16), 284 (sh 3.94), 389 (3.85). IR_{max}^{KBr} cm⁻¹: 3140, 3100 (O-H), 1670, 1625 (C=O), 1580, 1545 (C=C), 1440, 1390, 1360, 1315, 1290, 1260, 1220, 1130 (C-O), 970, 870, 750, 690 (C-H).

GP-B (**2**): orange powder, mp 187.0–188.5°C (from CHCl₃-MeOH). HREI-MS m/z Calcd. for C₁₃H₁₀O₅ (M⁺) 246.0528. Found: 246.0526. UV_{max}^{MeOH} nm (log ϵ): 218 (4.50), 264 (4.00), 308 (3.70), 438 (3.50). IR_{max}^{KBr} cm⁻¹: 3450, 3075 (O-H), 1650, 1610 (C=O), 1570 (C=C), 1435, 1390, 1310, 1280, 1220, 1200, 1160 (C-O), 1020, 960, 890, 800, 760 (C-H).

GP-C (**3**): orange powder, mp 217.0–222.0°C (from CHCl₃-MeOH). HREI-MS m/z Calcd. for C₂₆H₂₆O₉ (M⁺) 476.1123. Found: 476.1115. UV_{max}^{MeOH} nm (log ϵ): 218 (4.50), 246

(4.40), 266 (4.30), 287 (sh 4.10), 355 (3.80), 428 (3.60). IR_{max}^{KBr} cm⁻¹: 3425 (O-H), 1660, 1610 (C=O), 1570 (C=C), 1480, 1435, 1380, 1300, 1260, 1200, 1150 (C-O), 1020, 750 (C-H).

GP-D (**4**): yellow needles, mp 181.0–185.0°C (from CHCl₃). HREI-MS *m/z* Calcd. for C₁₅H₁₀O₄ (M⁺) 254.0580. Found: 254.0605. This compound was identical with authentic chrysophanol in terms of mixed mp and TLC (solvent: C₆H₆, *n*-hexane–AcOEt (5: 1), and CHCl₃).

Results and Discussion

The AcOEt extract of the light-brown colored mycelia inhibited by 66% MAO from mouse liver at 5.0×10^{-4} g/ml. The extract was defatted and then partitioned into AcOEt and aqueous fractions. The AcOEt fraction inhibited by 44% MAO at 1.0×10^{-4} g/ml, but the aqueous fraction inhibited it by only 8% at the same concentration. Repeated chromatography of the AcOEt fraction afforded GP-A (**1**), -B (**2**), and -C (**3**) as the effective components.

GP-A (**1**) was obtained as yellow needles, C₁₅H₈O₅. The UV and IR spectra of **1** suggested the presence of carbonyl groups conjugated with C=C bonds. This compound was suggested to be a phenolic component because it was positive to the FeCl₃ reaction and soluble in 0.1 N NaOH but not in 0.1 N NaHCO₃. These and the ¹H- and ¹³C-NMR spectra of **1** indicated that **1** is a new compound having one methoxyl, four aromatic methines, six aromatic quaternary carbons, two carbonyls, one phenolic hydroxyl, and one ethereal oxygen. The NMR spectral data including spin-decoupling ¹H-NMR and two-dimensional ¹H-¹H (¹H-¹H COSY) and ¹³C-¹H shift correlation (¹³C-¹H COSY) NMR spectra of **1** in CDCl₃ (see Table 1) suggested that **1** might be composed of four partial structures A-D as shown in Fig.

Table 1. ¹H-NMR and ¹³C-NMR Data for GP-A (**1**) and -B (**2**), δ (ppm) from TMS in CDCl₃

Position	1		2	
	¹ H-NMR	¹³ C-NMR	¹ H-NMR	¹³ C-NMR
1	8.19 (br s)* ¹	145.75 (d)* ²	5.13 (2H, m)	73.56 (t)* ⁴
3	8.20 (br s)* ¹	146.21 (d)* ²	5.13 (2H, m)	73.84 (t)* ⁴
3a		122.86 (s)* ³		147.30 (s)
4		183.84 (s)		184.83 (s)
4a		112.16 (s)		110.11 (s)
5		166.15 (s)		164.76 (s)
5-OH	12.90 (s)		12.06 (s)	
6	6.70 (br d, 2.4)	106.60 (d)	6.64 (d, 2.6)	106.07 (d)
7		166.32 (s)		166.24 (s)
7-OCH ₃	3.94 (3H, s)	56.07 (q)	3.92 (3H, s)	56.13 (q)
8	7.37 (br d, 2.4)	108.14 (d)	7.20 (d, 2.6)	109.01 (d)
8a		137.25 (s)		134.67 (s)
9		178.53 (s)		180.45 (s)
9a		122.80 (s)* ³		146.71 (s)

s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. coupling constant: expressed in Hz.

*¹, *², *³, *⁴: interchangeable.

Fig. 1. Partial structures, A-D, for GP-A (**1**)

1. Among them A and B were constructed with the aid of significant cross peaks due to H-C long-range coupling with J_2 and/or J_3 in the ^1H -detected multiple-bond heteronuclear multiple quantum coherence (HMBC) NMR spectrum. Namely, A was constructed on the basis of the presence of significant cross peaks of H_3 (at δ 3.94)/C(166.32), H(6.70)/C(108.14, 112.16, 166.15, 166.32), H(7.37)/C(112.16), and OH(12.90)/C(106.60, 112.16, 166.15) in the HMBC spectrum, and B was also constructed on the basis of the presence of significant cross peaks of H(8.19)/C(122.80, 122.86, 146.21) and H(8.20)/C(122.80, 122.86, 145.75) in the HMBC spectrum (see Fig. 1). In consideration of the chemical shift of each proton and carbon in A-D, the four partial structures A-D were connected together into a whole structure, 5-hydroxy-7-methoxy-4,9-dioxonaphtho[2,3-*c*]furan (**1**) (see Fig. 2). The signal of phenolic OH at position 5, which is hydrogen-bonded with the C=O at position 4, appears at δ 12.90 in the ^1H -NMR spectrum, supporting also the proposed structure of GP-A (**1**).

GP-B (**2**) was obtained as orange powder, $\text{C}_{13}\text{H}_{10}\text{O}_5$. The UV and IR spectra of **2** suggested the presence of carbonyls conjugated with C=C bonds in **2**. This compound was also suggested to be a phenolic component because of being positive to FeCl_3 and soluble in 0.1 N NaOH but insoluble in 0.1 N NaHCO_3 . Comparison of the ^1H - and ^{13}C -NMR spectra of **2** with those of **1** indicated that all signals were quite similar to the corresponding signals of **1** except that the signals of methylene H_{2-1} (2H, δ 5.13) and -3 (2H, 5.13), methylene C-1 (73.56) and -3 (73.84), and aromatic C-3a (147.30) and -9a (146.71) newly appeared instead of disappearing of the signals of aromatic H-1 (8.19) and -3 (8.20), aromatic C-1 (145.75) and -3 (146.21), and aromatic C-3a (122.86) and -9a (122.80) (see Table 1). This fact suggested that **2** is the 1,3-dihydro derivative of **1**. Accordingly, the structure of GP-B was deduced to be 1,3-dihydro-5-hydroxy-7-methoxy-4,9-dioxonaphtho[2,3-*c*]furan (**2**), as shown in Fig. 2.

GP-C (**3**) was obtained as orange powder, $\text{C}_{26}\text{H}_{20}\text{O}_9$. The UV and IR spectra of **3** sug-

Fig. 2. Structures of GP-A (1), -B (2), and -D (4)

gested the presence of carbonyl groups conjugated with C=C bonds in **3**. This compound is also a phenolic component because of being positive to the FeCl₃ reaction and soluble in 0.1 N NaOH but not in 0.1 N NaHCO₃. Comparison of the ¹H- and ¹³C-NMR spectra of **3** with those of **2** indicated that **3** might be a dimer of **2** (the ¹H- and ¹³C-NMR spectral data of **3** are not shown here).

The AcOEt extract of the dark-brown colored mycelia inhibited by 40% the MAO at 5.0×10^{-4} g/ml. The AcOEt extract was defatted and then partitioned with AcOEt-H₂O into AcOEt and aqueous fractions. The AcOEt fraction inhibited by 27% the MAO at 1.0×10^{-4} g/ml, but the aqueous fraction did not at 1.0×10^{-4} g/ml. Repeated chromatography of the AcOEt fraction afforded GP-D (**4**), yellow needles, as the pigmental component. The physicochemical and spectral data of **4** including the UV, IR, ¹H- and ¹³C-NMR spectra suggested that **4** was to be chrysophanol (see Fig. 2), which has so far been isolated from *Penicillium islandicum* and many other fungi,^{6),7)} and identified with authentic specimen.

The IC₅₀ values of 4,9-dioxonaphtho[2,3-c]furans **1**, **2**, and **3** against the MAO from mouse liver were calculated to be 2.7×10^{-6} , 2.0×10^{-6} , and $>2.1 \times 10^{-5}$ M, respectively. However, the anthraquinone **4** inhibited only by 2.4% the MAO even at 3.9×10^{-4} M. To the best of our knowledge, this is the first isolation of 4,9-dioxonaphtho[2,3-c]furan derivative with MAO inhibitory activity from natural resources.

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