Mitorubrin Derivatives on Ascomata of Some *Talaromyces* Species of Ascomycetous Fungi

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Two groups, producers of (+)- and (−)-mitorubrin derivatives, coexist in the series *Lutei* of the genus *Talaromyces*. The optical rotations of mitorubrins from *T. emodensis*, *T. hachijoensis*, and *T. wortmannii* var. *sublevisporus*, which produced mitorubrinol acetate (**5**), were all positive, whereas those from *T. austrocalifornicus* and *T. convolutus*, which produced mitorubrinal (**3**) and mitorubrinic acid (**4**), were all negative.

Frisvad et al.[1] reported profiles of secondary metabolites for 25 species of the genus *Talaromyces* (Eurotiales, Ascomycota) to provide a means of simple differentiation of the taxa. They reported that some of the yellow pigments produced by species of *Talaromyces* were anthraquinone derivatives, mitorubrin derivatives, and/or phenalenone derivatives. During our research for yellow pigments on ascomata of fungi placed in the section *Talaromyces* of the genus *Talaromyces*, we reported the isolation of an azaphilone, (+)-monomethylmitorubrin,[2] from *T. tardifaciens* Udagawa.[3] We report herein yellow pigments on ascomata of the following species belonging to the series *Lutei* in section *Talaromyces*:[4] *T. austrocalifornicus* Yaguchi et Udagawa,[5] *T. convolutus* Udagawa,[3] *T. emodensis* Udagawa,[3] *T. hachijoensis* Yaguchi, Someya et Udagawa,[3] and *T. wortmannii* C. R. Benjamin var. *sublevisporus* Yaguchi et Udagawa.[6]

The above five isolates of *Talaromyces* were grown on oatmeal agar with wheat germ at 25 °C for 2 weeks. Abundant yellow ripe ascomata produced over the entire surface of the agar medium were collected and sequentially extracted with CH_2Cl_2 and acetone to give the corresponding extracts.

Chromatography of the CH_2Cl_2 extract from the ascomata of *T. convolutus* gave an unknown azaphilone (**1**), along with mitorubrin derivatives, mitorubrin (**2**),[7] mitorubrinol (**3**),[7] and mitorubrinic acid (**4**),[8] and pre-anthraquinone derivatives, anhydroflavomannin-9,10-quinone-6,6′-di-*O*-methyl ether and flavomannin-6,6′-di-*O*-methyl ether (FDM).[9] The molecular formula of **1** was determined by HRMS as $C_{21}H_{16}O_8$. The UV spectrum of **1** was essentially superimposable on that of mitorubrin (**2**). The ^{1}H NMR spectrum of **1** was similar to that of **2**, except for a side chain at C-3: 6.56 (1H, dd), 7.30 (1H, d), 9.66 (1H, d). Oxidation of mitorubrinol (**3**) with MnO_2 gave **1**. The structure of compound **1**, which was named mitorubrinal, was thus determined to be **1**. The stereochemistry at C-7 in **1** was determined to be *R* from the optical rotation ($[\alpha]_D$ −541°).[10]

Yellow pigments on the ascomata produced by *T. austrocalifornicus* were also identified as mitorubrin derivatives **1**−**4**, and FDM.[9] These mitorubrin derivatives isolated from *T. convolutus* and *T. austrocalifornicus* all showed negative optical rotations (Table 1).

On the other hand, mitorubrin (**2**), mitorubrinol (**3**), and mitorubrinol acetate (**5**) were isolated as yellow pigments on the ascomata of *T. emodensis*, *T. hachijoensis*, and *T. wortmannii* var. *sublevisporus*, along with duclauxin[11] from the former fungus, FDM from the middle, and flavomanin[12] from the latter. The optical rotation of mitorubrin derivatives from the three above fungi were all positive[13] (Table 1).

It was clear that the main yellow pigments on ascomata of the above five species classified into series *Lutei* of the genus *Talaromyces* were mitorubrin derivatives and either pre-anthraquinone or duclauxin. Mitorubrin derivatives from one group that produced mitorubrinic acid (**4**) had the 7*R* configuration, because the compounds all gave negative optical rotations. In contrast, mitorubrins from the other group that produced mitorubrinol acetate (**5**) were 7*S* forms because of positive optical rotations[10] (Scheme 1). It is chemotaxonomically interesting that a group of producers of (7*S*)-mitorubrin derivatives and that of (7*R*)-mitorubrin derivatives coexist in the same series of *Talaromyces*.

Frisvad et al.[1] showed that *T. udagawae* and *T. wortmannii* produced both **4** and **5**, but their optical rotations were not measured. We could not find both **4** and **5** in ascomata of any single fungus.

Experimental Section

General Experimental Procedures. For general experimental details see Nozawa et al.[2] Optical rotations were measured on a JASCO DIP-181 spectrometer in dioxane.

Organism, Culture Conditions, and Extraction. The following fungi were used for this study: *T. austrocalifornicus* PF 1117,[5] *T. convolutus* SUM 3018,[3] *T. emodensis* SUM 3025,[3] *T. hachijoensis* PF 1174,[3] and *T. wortmannii* var. *sublevisporus* PF 1130.[6] Each fungus was cultivated at 25 °C for 2 weeks on 200 Petri dishes (i.d. 90 mm) containing 25 mL per dish of melted oatmeal agar with wheat germ (ground oatmeal 15 g; ground wheat germ 15 g; agar 20 g; tap water 1000 mL). The fresh ascomata and mycelial mat, freed as far as possible from the agar substrate, were collected and extracted with CH_2Cl_2 and then Me_2CO. Solvents were evaporated to obtain the CH_2Cl_2 and Me_2CO extracts.

Isolation of Yellow Pigments from *T. convolutus*. The CH_2Cl_2 extract (1.1 g) from *T. convolutus* SUM 3018 was subjected to column chromatography on Si gel yielding $CHCl_3$, $CHCl_3$−Me_2CO (10:1), and Me_2CO fractions. The $CHCl_3$−Me_2CO (10:1) fraction was separated by LPLC eluting

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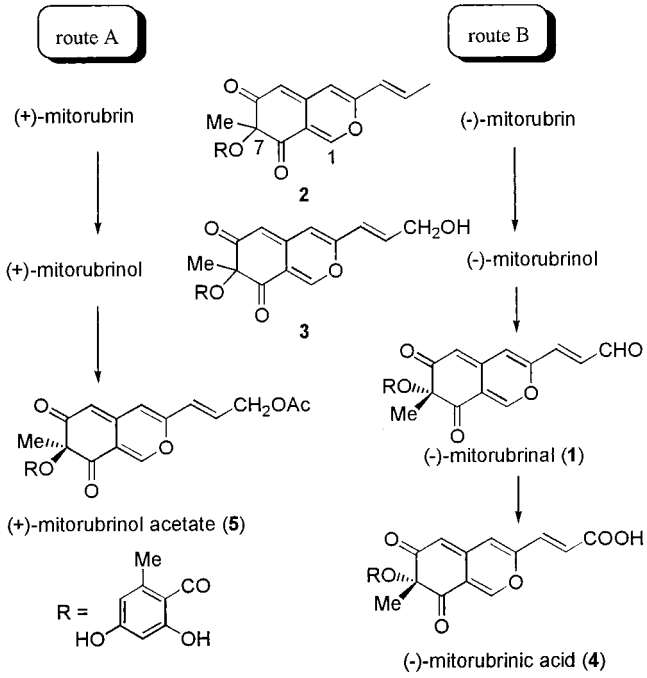
Table 1. Optical Rotations of Mitorubrin Derivatives from *Talaromyces* spp.

fungus	1	2	3	4	5
T. austrocalifornicus	−360	−428	−349	−426	ND
T. convolutus	−541	−435	−455	−581	ND
T. emodensis	ND[b]	+388	+484	ND	+487
T. hachijoensis	ND	+337	trace[a]	ND	+412
T. wortmannii var. sublevisporus	ND	+385	+450	ND	+485

[a] Detected on TLC. [b] ND none detected. Mitorubrin (**2**); mitorubrinol (**3**); mitorubrinal (**1**); mitorubrinic acid (**4**); mitorubrinol acetate (**5**).

Scheme 1. Biosynthetic Pathway of Mitorubrin Derivatives in the Series Lutei of the Genus *Talaromyces*



with C_6H_6−Me_2CO (10:1) to give mitorubrin (**2**) (189 mg), yellow prisms (from $CHCl_3$); $[\alpha]_D^{20}$ −422° (*c* 0.3) (lit. −405°),[7] and with C_6H_6−Me_2CO (5:1), followed by repeated purification on LPLC using the same solvent system to give first anhydroflavomannin-9,10-quinone-6,6′-di-*O*-methyl ether (33 mg) and then mitorubrinal (**1**) (64 mg). The Me_2CO fraction was also subjected to LPLC eluting with $CHCl_3$−EtOH (5:1) to give mitorubrinic acid (**4**) (67 mg), yellow needles (from $CHCl_3$); $[\alpha]_D^{20}$ −581° (*c* 0.07) (lit. −450°),[8] FDM (222 mg), and (−)-mitorubrinol (**3**) (111 mg), yellow needles (from hexane−EtOAc), $[\alpha]_D^{20}$ −455° (*c* 0.2) (lit. −375°),[7] in that order.

(−)-Mitorubrinal (1): yellow needles (hexane−EtOAc); mp (dec) 188.5−191 °C; $[\alpha]^{20}_D$ −541°(*c* 0.16); HREIMS *m*/*z*: 396.0848 (M^+) (calcd for $C_{21}H_{16}O_8$, 396.0845), 231.0648 (calcd for $C_{13}H_{11}O_4$, 231.0655), 165.0541 (calcd for $C_9H_9O_3$, 165.0551); UV(MeOH) λ_{max} (log ϵ) 217 (4.27), 255 (4.34), 280 (4.16), 293 (4.13), 348 (4.34), 363 (sh) (4.28) nm; IR(KBr) ν_{max} 3350 (OH), 1715 (−COO−), 1630 (conjugated C=O) cm^{-1}; ^1H NMR (Me_2-CO-d_6, 400 MHz) δ 1.61 (3H, s, 7-Me), 2.49 (3H, s, 7″-Me), 5.67 (1H, d, J = 1 Hz, 5-H), 6.10 (1H, d, J = 2.3 Hz, 4″-H), 6.23 (1H, d, 2.3 Hz, 6″-H), 6.56 (1H, dd, J = 15.6, 7.6 Hz, 2′-H), 6.98 (1H, br s, 4-H), 7.30 (1H, d, J = 15.6 Hz, 1′-H), 8.04(1H, s, 1-H), 9.16 (1H, br s, OH), 9.66 (1H, J = 7.6 Hz, 3′-H), 10.63 (1H, s, OH); ^{13}C NMR (Me_2CO-d_6, 100 MHz) δ 22.4 (Me-7), 24.9 (Me-7″), 86.6 (C-7), 101.6 (C-4″), 104.7 (C-2″), 110.7 (C-5), 112.6 (C-6″), 115.6 (C-8a), 117.9 (C-4), 132.6 (C-2′), 140.8 (C-1′), 142.3 (C-4a), 144.8 (C-7″), 153.9 (C-3), 155.0 (C-1), 163.9 (C-3″ or 5″), 166.2 (C-5″ or 3″), 170.6 (C-1″), 192.3 (C-6 or 8), 192.5 (C-8 or 6), 193.2 (C-3′).[14]

Oxidation of (−)-Mitorubrinol (3). A mixture of (−)-mitorubrinol (10 mg) and MnO_2 (100 mg) in Me_2CO (5 mL) was stirred at room temperature for 30 min. After removal of MnO_2, the filtrate was evaporated and the residue separated by LPLC ($CHCl_3$−Me_2CO, 10:1) to give (−)-mitorubrinal (**1**) 5 mg.

Isolation of Yellow Pigments from *T. wortmannii* var. *sublevisporus*. The CH_2Cl_2 extract (0.97 g) from *T. wortmannii* var. *sublevisporus* PF 1130 was chromatographed on Si gel yielding $CHCl_3$, $CHCl_3$−Me_2CO (10:1), and Me_2CO fractions. The $CHCl_3$−Me_2CO (10:1) fraction was subjected to LPLC eluting with C_6H_6−Me_2CO (10:1) to give (+)-mitorubrin (**2**) (23 mg), yellow needles (from $CHCl_3$); $[\alpha]_D^{20}$ +385° (*c* 0.5) (lit. +586°)[13] and skyrin (14 mg), and with C_6H_6−Me_2CO (6:1) followed by LPLC using the solvent system of $CHCl_3$−Me_2CO (20:1) to give (+)-mitorubrinol acetate (**5**) (65 mg), yellow plates (from EtOH); $[\alpha]_D^{20}$ +485° (*c* 0.5) (lit. +525°).[13] The Me_2CO fraction was also subjected to LPLC ($CHCl_3$−Me_2CO, 5:1) to give (+)-mitorubrinol (**3**) (5 mg), yellow needles (from $CHCl_3$); $[\alpha]_D^{20}$ +450° (*c* 0.5) (lit. +548°).[13] The Me_2CO extract (0.4 g) was washed with $CHCl_3$, and then the residue was recrystallized from Me_2CO to give microcrystals of flavomannin (12 mg).

Isolation of Yellow Pigments from other *Talaromyces* spp. The CH_2Cl_2 extract (565 mg) from *T. austrocalifornicus* PF1117 was purified by the same method as that of *T. convolutus* to give **2** (6 mg); $[\alpha]^{20}_D$ −428° (*c* 0.06), **3** (2 mg); $[\alpha]^{20}_D$ −349° (*c* 0.05), **1** (11 mg); $[\alpha]^{20}_D$ −360° (*c* 0.03), **4** (17 mg); $[\alpha]^{20}_D$ −426° (*c* 0.23), and FDM (102 mg). The CH_2Cl_2 extracts (420 mg and 300 mg, respectively) from *T. emodensis* SUM3025 and *T. hachijoensis* PF1174 were also separated in the same manner as that of *T. wortmannii* var. *sublevisporus* to give **2** (11 mg); $[\alpha]^{20}_D$ +388° (*c* 0.07), **3** (2 mg); $[\alpha]^{20}_D$ +484° (*c* 0.03), **5** (27 mg); $[\alpha]^{20}_D$ +487° (*c* 0.5), duclauxin (126 mg), and **2** (27 mg); $[\alpha]^{20}_D$ +337° (*c* 0.17), **3** (trace), **5** (80 mg) +337° (*c* 0.47), and FDM (9 mg), respectively.

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