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AN AZAPHILONE FROM *TALAROMYCES TARDIFACIENS*

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Key Work Index—*Talaromyces tardifaciens*; ascomata; azaphilone; monomethyl-(+)-mitorubrin; *Penicillium tardifaciens*.

Abstract—A new azaphilone derivative, monomethyl-(+)-mitorubrin, was isolated from the ascomata of *Talaromyces tardifaciens*. The structure was determined on the basis of spectroscopic investigations.

INTRODUCTION

Recently, several new species of *Talaromyces* were described by Udagawa *et al.* [1–5]. The colours of their ascomata on oatmeal agar are often yellow to orange or red. Although the main yellow spots observed on TLC of each dichloromethane extract of the ascomata were of different compounds, they all gave a deep red colouration with ammonia characteristic to azaphilones. In this paper, we report on the isolation and the structure determination of an azaphilone from ascomata of a new ascomycetous fungus, *T. tardifaciens* Udagawa (anamorph: *Penicillium tardifaciens* Udagawa), isolated from paddy soil from Bhaktapur, Nepal [1].

RESULTS AND DISCUSSION

The molecular formula of monomethyl-(+)-mitorubrin (**1**) was determined by high-resolution mass spectrometry as $C_{22}H_{20}O_7$. The UV spectrum of **1** (217, 255, 280, 293 and 348 nm) was essentially superimposable on that of (+)-mitorubrin (**2**) (216, 266, 292 and 346 nm), a constituent of *Hypoxylon fragiforme* Kickx [6] [(−)-mitorubrin from *Penicillium rubrum* Stoll [7]]. The ^{1}H NMR spectrum of **1** was similar to that of **2**, except for the disappearance of a chelated phenolic proton at δ 10.70 observed in **2** and the appearance of a methoxyl signal at δ3.75 in **1** (Table 1). The IR absorption region at 1715 cm^{-1} showed the presence of a non-chelated ester in **1**. The relative structure of monomethyl-(+)-mitorubrin was thus determined as **1**.

The ^{1}H and ^{13}C NMR assignment of **1** was conducted by NOE difference experiments and analyses of the ^{13}C–^{1}H COSY and COLOC spectra. The ^{1}H NMR spectra of **1**–**3** and the ^{13}C NMR spectra of **1** and **3** are compared in Tables 1 and 2, respectively.

The absolute configuration at C-7 of azaphilones so far unknown has been firmly established from the optical rotations and CD curves [8, 9]. The sign of the Cotton effect at the longest wavelength depends on the stereochemistry at the C-7 position [(+): α-methyl; (−): β-methyl]. The CD of **1** ($\Delta\varepsilon_{368}$ + 5.7) clearly showed the (*S*)-configuration at C-7. The absolute structure of monomethyl-(+)-mitorubrin was consequently concluded to be as shown in **1**.

EXPERIMENTAL

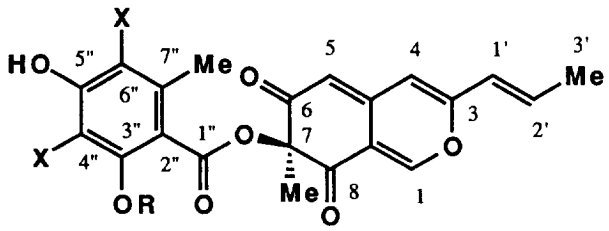
For general experimental details see ref. [10].

Isolation of monomethyl-(+)-mitorubrin (**1**). *Talaromyces tardifaciens* SUM 3017 (ATCC = 90622) was cultivated at 25° for 2 weeks in 120 petri dishes (i.d. 90 mm) containing 25 ml per dish of melted oatmeal agar. The fresh ascomata and mycelial mats, freed as far as possible from the agar substrate, were collected and extracted with

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** [11] (in acetone-d_6)

C	1	2	3*
1	8.03 (*dr s*)	8.05	7.94
4	5.56 (*d* 1.3)	6.45	5.63
5	6.44	5.59	6.12
Me-7	1.51	1.64	1.62
1′	6.24 (*dq* 15.9, 1.5)	6.22	6.01
2′	6.60 (*dq* 15.9, 6.7)	6.58	6.58
3′	1.93 (*dq* 6.7, 1.5)	1.92	1.94
OMe-3″	3.75		3.97
OH-3″		10.70	
4″	6.35 (*d* 2.0)	6.23	
OH-5″	8.64 (*br s*)	9.06	
6″	6.31 (*d* 2.0)	6.33	
Me-7″	2.38	2.61	2.49

*Compound **3** was measured in chloroform-*d*.



monomethyl-(+)-mitorubrin (1) : R = Me, X = H

(+)-mitorubrin (2) : R = H, X = H

falconensin H (3) : R = Me, X = Cl

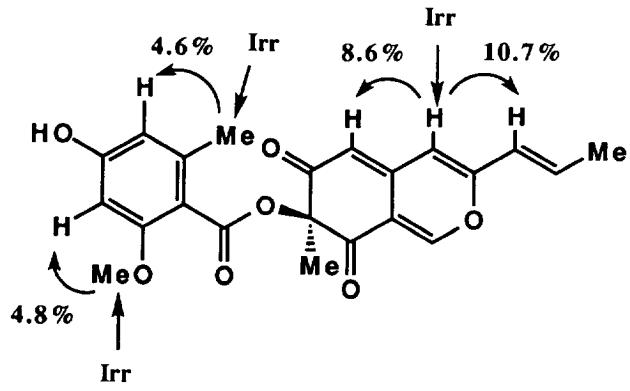


Table 2. ^{13}C NMR spectral data of compounds **1** and **3** [11]

C	1*	3†
1	154.8	153.6
3	156.6	155.6[a]
4	109.8	107.9
4a	143.6	142.8
5	109.0	108.5
6	194.0[a]	192.5[b]
7	85.9	85.6
Me-7	23.3	22.7
8	193.0[a]	191.8[b]
8a	116.4	115.1
1′	124.1	122.4
2′	135.8	135.6
3′	19.0	18.6
1″	167.3	165.2
2″	114.5	117.9
3″	161.3	153.6
OMe-3″	56.9	62.6
4″	98.6	121.0
5″	161.3	150.1[a]
6″	110.7	117.4
7″	141.4	135.0
Me-7″	20.8	17.1

*Compound **1** was measured in acetone-d_6.

†Compound **3** was measured in chloroform-*d*.

[a,b]Assignments may be interchanged.

CH_2Cl_2 at room temp. The evaporated residue (310 mg) was separated by Sephadex LH-20 into 3 fractions: CH_2Cl_2, EtOH, MeOH. The first fraction was subjected to LPLC using $CHCl_3$–EtOH (20:1) to give monomethyl-(+)-mitorubrin (**1**) (255 mg): pale yellow needles (from EtOAc, mp (dec) 226–230° (from EtOAc); $[\alpha]^{20}$ + 13.7° (MeOH; *c* 0.19); EI-MS *m/z*: 396.1220 $[M]^+$, 396.1210 for $C_{22}H_{20}O_7$, 231.0648 (231.0655 for $C_{13}H_{11}O_4$), 165.0541 (165.0551 for $C_9H_9O_3$); UV λ_{max}^{MeOH} nm (log ε): 217 (4.27), 255 (4.34), 280 (4.16), 293 (4.13), 348 (4.34), 363 (sh; 4.28); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1715 (–CO_2–), 1630 (conjugated C = O); ^{1}H NMR ($CDCl_3$): δ 1.60 (3H, *s*, Me-7), 1.94 (3H, *dd*, *J* = 7.1, 1.5 Hz, Me-3′), 2.42 (3H, *s*, Me-7″), 3.72 (3H, *s*, OMe-3″), 5.63 (1H, *d*, *J* = 1 Hz, H-4), 6.00 (1H, *dq*, *J* = 15.5, 1.5 Hz, H-1′), 6.10 (1H, *s*, H-5), 6.23 (2H, *m*, H-4″ and H-6″), 6.29 (1H, *br s*, OH-5″), 6.57 (1H, *dq*, *J* = 15.5, 7.1 Hz, H-2′), 7.93 (1H, *br s*, H-1); CD (MeOH; *c* = 4.7 × 10^{-5}) $\Delta\varepsilon^{20}$ (nm): + 4.1 (244), − 3.0 (270), − 2.7 (281), + 5.7 (368).

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