Merodrimanes and Other Constituents from *Talaromyces thailandiasis*

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Received October 20, 2006

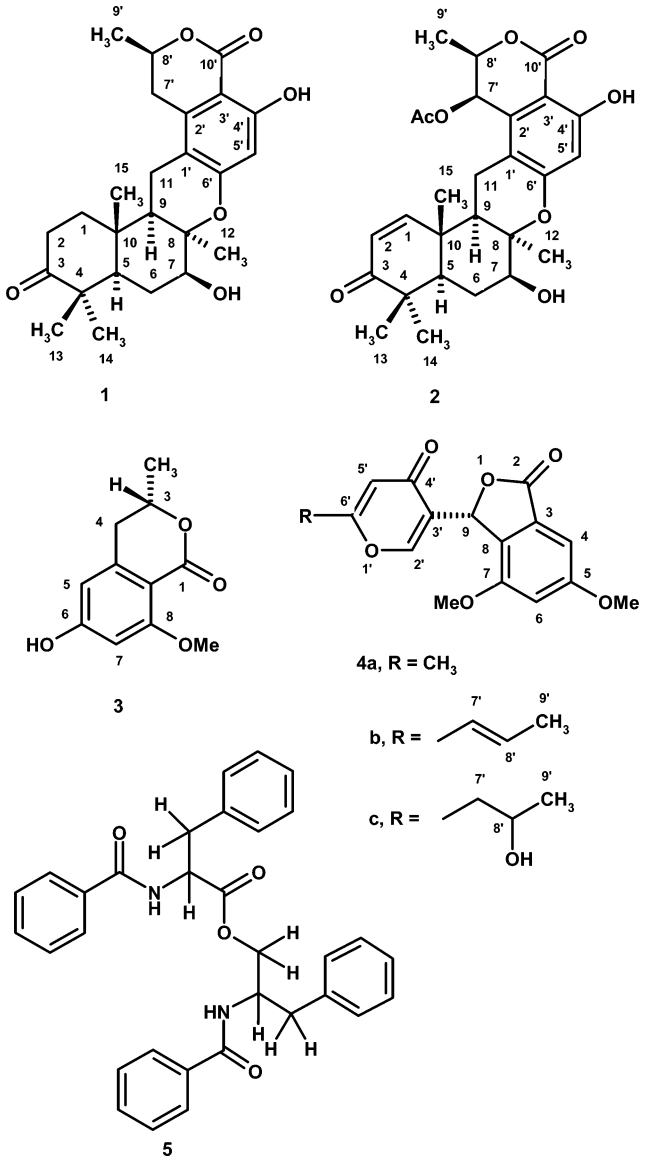
Chemical study of a previously undescribed fungus, *Talaromyces thailandiasis*, furnished the two new merodrimanes thailandolides A (**1**) and B (**2**), an *O*-methylated derivative (**3**) of the aromatic fragment incorporated in thailandolide B, and three known closely related 1(3*H*)-isobenzofuran derivatives, penisimplicissin (**4a**), vermistatin (**4b**), and hydroxydihydrovermistatin (**4c**). Structures were established by spectroscopic measurements and confirmed by X-ray analyses of compounds **1** and **4b**. The unusual peptide analogue *N*-benzoylphenylalanyl-*N*-benzoylphenyl alaninate (**5**) isolated earlier from a higher plant was also found.

Since the spiroketals talaromycins A−F from the ascomycetous fungus *Talaromyces stipitatus* were described more than 20 years ago,[1−3] a spate of articles dealing with secondary metabolites from other members of the genus have appeared, the most recent one describing the isolation of wortmannilactones A−D from *Talaromyces wortmannii*.[4] Almost all secondary metabolites isolated from *Talaramyces* species so far appear to be of polyketide origin, the exception being wortmannin, also from *T. wortmannii*,[5] which appears to be a partially oxidized steroid.

We have recently described two new oxyphenalenone dimers, bacillisporins D and E, from *Talaromyces bacillisporus* in addition to the previously known analogues duclauxin and bacillisporins A−C.[6] We now report the isolation of two new merodrimanes (**1** and **2**) from a previously undescribed fungus, *Talaromyces thailandiasis* Manoch and Dethoup,[7] as the first terpenoids found in this genus of *Penicillium* teleomorphs. Other metabolites produced by this fungus were the methyl ether **3** of the ketide half of **1**, three closely related known γ-pyrones, **4a**−**c**, incorporating a 1(3*H*)-isobenzofuranone, *N*-benzoylphenylalanyl-*N*-benzoylphenylalaninate (**5**) previously reported by one of us from *Croton hieronymi*,[8] and 2-glycerylpalmitate.

That compound **1**, which we have named thailandolide A, was a 3-oxo-7β-hydroxydrimane linked through a tertiary oxygen to an aromatic moiety incorporating a lactone function in the manner characteristic of merodrimanes known from fungi of the genus *Stachybotrys*,[9] but with the lactone function closed to C-8 of the drimane portion as in the kampanols from *Stachybotrys kampalensis*,[10] was deduced from the ^{1}H and ^{13}C NMR spectra, listed in Table 1. The probable stereochemistries of C-8 and C-8′ and the location of the phenolic hydroxyl group on C-4′ of the aromatic ring deduced from chemical shifts and coupling constants in Table 1 as well as COSY and NOESY data were confirmed by an X-ray analysis. The ORTEP diagram of **1** (Figure 1) led to the relative configuration shown in formula **1**.

^{1}H and ^{13}C NMR spectroscopic data of compound **2** (thailandolide B) demonstrated that it differed from **1** in being the corresponding enone that carried an extra β-oriented acetoxy group on C-7′ of the aromatic ring. An additional compound present in



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10.1021/np0680578 CCC: $37.00
Published on Web 05/31/2007

Table 1. NMR Data of Compounds **1** and **2** in $CDCl_3$ (^1H 300 MHz, ^{13}C 75 MHz)

	1		**2**	
position	δ_H (J in Hz)	δ_C (DEPT)	δ_H	δ_C
1α	2.01c	32.1t	7.16d (10.3)	156.2d
1β	1.7c			
2α	2.49c	33.6t	6.00d (10.3)	127.5d
2β	2.65td (11, 4.8)			
3		207.3s		203.7s
4		46.9s		44.6s
5	1.95dd (14,3)	43.6d	2.18dd (14, 4.6)	42.4d
6α	1.59	28.2t	2.2−2.3c	26.5t
6β	2.18ddd (14, 7.5, 3)		1.85ddd (14,14,2.4)	
7	4.03t (7.5)	73.0d	4.18dd (8.6, 2)	71.8d
8		78.3s		79.7s
9	2.05dd (12,6.2)	40.8d	2.27dd (14, 5)	41.8d
10		35.7s		38.4s
11α	2.5c	19.5t	2.62dd (15, 14)	21.5t
11β	2.5c		2.96dd (15, 5)	
12[a]	1.37s	23.1q	1.27s	21.4q
13[a]	1.11s	20.0q	1.11s	21.3q
14[a]	1.12s	28.6q	1.12s	27.6q
15[a]	1.05s	22.8q	1.36s	27.4q
1′		102.2s		102.2s
2′		139.1s		135.8s
3′		109.9s		112.3s
4′		162.3s		162.2s
5′	6.35s	103.1d	6.50s	106.3d
6		158.6s		159.6s
7′α	2.86dd (16.5, 3.5)	31.7t	6.17d (1.7)	64.1d
7β	2.72dd (16.5, 12)			
8′	4.65ddq (12, 3.5, 6.3)	74.7d	4.73qd (6.6, 1.7)	76.0d
9′	1.56d (6.3)	21.0q	1.49d (6.6)	16.4q
10		170.0s		168.8s
7-OH	3.11brs			
4′-OH	11.09s		11.06s	
Ac			2.17s[a]	20.7q 170.6s

[a] Intensity three protons.

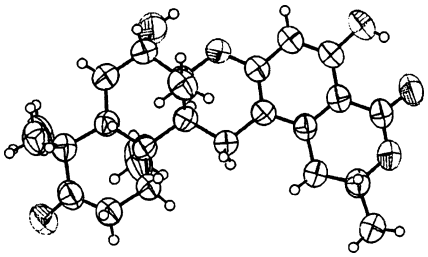

Figure 1. ORTEP view of compound **1**.

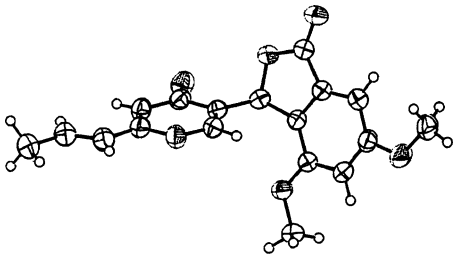

Figure 2. ORTEP view of compound **4b**.

the extract was **3**, an *O*-methylated derivative of the aromatic fragment incorporated in **2**. The ring conformation of **3** is such that the methyl group is quasiequatorial, as can be gleaned from the H-3/H-4a,b coupling constants and conforms to the stereochemistry of the C-8′-methyl group in **1**. The location of the hydroxyl group on C-6 and the methoxy group on C-8 is shown by the presence of hydrogen bonding to the carbonyl, as evidenced by a broad −OH signal at δ 10.29, the COSY spectrum, which exhibited cross-peaks between H-5 and H-1′a,b and between H-3 and the OMe group, and the HMBC spectrum with cross-peaks between H-5 and CH_2-1′, C-3, C-1, and C-0, between H-3 and C-1, C-5, and CO, and between −OMe and CO.

Three further constituents of the extract were three 1(3*H*)-isobenzofuranone derivatives, **4a**−**c**. Compound **4b** was identical with vermistatin, which has been isolated previously from cultures of *Penicillium verticulatum*,[11,12] *verruculosum*,[13] and *simplicissimum*[14] as well as from fungal cultures related to *Talaromyces flavus*,[15−17] while compounds **4a** and **4c** were identical with penisimplicissin and a hydrated analogue of vermistatin, both recently reported from *Pencillium simplicissimum*.[14] The structure of **4b** was confirmed by an X-ray analysis (Figure 2).

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded at ambient temperature in $CDCl_3$ on a Bruker AMC instrument operating at 300.13 and 75.47 MHz, respectively, or on a Bruker DRX instrument operating at 500 and 125 MHz, respectively. EI mass spectra were measured on a Hitachi Perkin-Elmer RMV-GM instrument. HR mass spectra were measured on a Kratos Concept II 2 sector mass spectrometer. The accelerating voltage was 8 kV. Melting points were recorded on a Bock monoscope and are uncorrected. Optical rotations were determined on a Polax-2L instrument. Si gel for chromatography was silica gel 60 (0.2−0.5 mm Merck) for analytical work and for preparative TLC Merck silica gel 60 GF 254.

Fungal Material. *T. thailandiasis* was isolated by L.M. from a soil sample collected in Trat Province, Southern Thailand, in August 2003. The strain, accession number KPFC 3399, is on deposit in the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University.

Fermentation, Extraction, and Isolation. Fifty 1 L Erlenmeyer flasks, each containing 200 g of rice and 100 mL of H_2O, were inoculated with 10 mycelium plugs from the *T. thailandiasis* culture and incubated at 28 °C for 30 days. At the end of this period and after the subsequent addition of 400 mL of ethyl acetate to each flask the

mixture was allowed to stand for 3 days and filtered using filter paper. Concentration of the filtrate at reduced pressure to a volume of 3 L, addition of anhydrous sodium sulfate, filtration, and evaporation at reduced pressure furnished 79.2 g of dark brown viscous ethyl acetate extract, which was extracted with $CHCl_3$ (3 × 500 mL). Combination of the $CHCl_3$ extracts followed by evaporation at reduced pressure afforded 51.4 g of viscous $CHCl_3$ extract. A portion (40 g) was applied to a Si gel column (200 g) and eluted with $CHCl_3$−petroleum ether and $CHCl_3$−acetone mixtures, 300 mL fractions being eluted as follows: Fractions 1−202 ($CHCl_3$−petroleum ether, 1:1), 203−265 ($CHCl_3$−petroleum ether, 7:3), 266−285 ($CHCl_3$−petroleum ether, 9:1), 286−316 ($CHCl_3$−acetone, 9:1), 317−343 ($CHCl_3$−acetone, 4:1), and 344−368 ($CHCl_3$−acetone, 7:3).

Fractions 65−75 (345 mg) were combined and recrystallized from a mixture of $CHCl_3$−petroleum ether to give 200 mg of **5** as colorless crystals: mp 214−215°, lit. mp 212.5−213[8] $[\alpha]_D^{24}$ +263 ($CHCl_3$, *c* 0.38 g/100 mL); FABMS *m*/*z* 507 $(M + H)^+$; ^1H and ^{13}C NMR spectra identical with those reported earlier.[8]

Fractions 77−90 and 93−95 were combined (179 mg). Recrystallization from $CHCl_3$−petroleum ether afforded **4b** (vermistatin, 59 mg), identified by HRMS, ^1H and ^{13}C NMR,[14] and an X-ray crystallographic analysis (Figure 2). Combination of fractions 91 and 92 followed by TLC (Si gel, $CHCl_3$−MeOH−HCO_2H, 95:5:1) furnished 11.3 mg of **2** and an additional 20 mg of **4b**. Combination of fractions 97−107 (244 mg) followed by recrystallization from $CHCl_3$−petroleum ether afforded **1** (200 mg). Combination of fractions 111−130 (570 mg) and recrystallization from $CHCl_3$−petroleum ether gave a yellow solid (45 mg), which was purified by TLC (Si gel, $CHCl_3$−Me_2O−HCO_2H, 95:5:1) to give 15.4 mg of **4a** (penisimplicissin, 15.4 mg) identified by MS and ^1H and ^{13}C NMR spectrometry.[14] Fractions 145−159 (121 mg) were combined and purified by TLC (Si gel, $CHCl_3$−Me_2O−EtOAc−HCO_2H, 85:10:5:1) to give 11.7 mg of 2-glycerylpalmitate identified by HRMS and ^1H and ^{13}C NMR spectrometry.

Fractions 221−280 of the initial chromatogram were combined and chromatographed over Si gel (10 g) using $CHCl_3$−petroleum ether, 100 mL fractions being collected as follows: fractions 1−50 ($CHCl_3$−petroleum ether, 1:1), 51−74 ($CHCl_3$−petroleum ether, 7:3), 75−80 ($CHCl_3$−petroleum ether, 9:1). Fractions 28−32 (127 mg) were combined and purified by TLC (Si gel, $CHCl_3$−Me_2O−HCO_2H, 4:1:0.1) to give 37 mg of **3**. Fractions 286−313 (2.2 g) were combined and rechromatographed over Si gel (12 g), using $CHCl_3$−petroleum ether mixtures and 100 mL subfractions as follows: subfractions 1−25 ($CHCl_3$−petroleum ether, 7:3) and subfractions 26−50 ($CHCl_3$−petroleum ether, 9:1). Subfractions 16−24 were combined (220 mg) and purified by TLC (Si gel, $CHCl_3$−Me_2O−HCO_2H, 4:1:0.1) to give 46 mg of **4c** identified by MS and ^1H and ^{13}C NMR spectrometry.[14]

Thailandolide A (1): white, crystalline solid; mp 277−278 °C; $[\alpha]_D^{24}$ −256 ($CHCl_3$, *c* 0.039 g/100 mL); ^1H and ^{13}C NMR, see Table 1; + FABHRMS *m*/*z* 429.22778 $(M + H)^+$ (calcd for $C_{25}H_{33}O_6$, 429.22771); ^1H and ^{13}C NMR spectra in Table 1.

Thailandolide B (2): white, crystalline solid; mp 274−275 °C; $[\alpha]_D^{24}$ +134 ($CHCl_3$, *c* 0.14 g/100 mL); ^1H and ^{13}C NMR, see Table 1; + FABHRMS *m*/*z* 485.21748 $(M + H)^+$ (calcd for $C_{27}H_{33}O_8$, 485.21754).

3-Methyl-6-hydroxy-8-methoxy-3,4-dihydroisocoumarin (3): gum; $[\alpha]_D^{27}$ −46.5 ($CHCl_3$, *c* 0.043 g/100 mL); ^{13}C NMR ($CDCl_3$) δ 162.9 (C, C-8), 162.8 (C, C-1), 161.3 (C, C-6), 144.0 (C, C-4a), 106.2 (CH, C-5), 104.6 (C, C-8a), 98.4 (CH, C-7), 72.9 (CH, C-3), 35.5 (CH_2, C-4), 20.4 (CH_3); ^1H NMR 10.52 (brs, OH), 6.37 (s, H-7), 6.26 (s, H-5), 4.41 (m, H-3), 3.74 (s, 3p, OMe), 2.86 (dd, 16, 3.1, H-4) 2.27 (dd, 16, 10.9, H-4b), 1.32 (3p, d, 6.2, 3-Me), + FABHRMS *m*/*z* 209.08148 $(M + H)^+$ (calcd for $C_{11}H_{13}O_4$, 209.08138).

X-ray Crystal Structure of 1. Suitable crystals were obtained by slow evaporation of a solution in chloroform−petroleum ether and were orthorhombic, space group $P2_12_12_1$, cell volume $V = 2271.2(10)$ Å, $a = 11.0763(3)$ Å, $b = 12.831(3)$ Å, $c = 15.981(4)$ Å (uncertainties in parentheses). There were four molecules per unit cell, calculated density 1.253 g/cm^3. Diffraction data were collected at 293 K with a Stoe IPDS plate equipped with Mo Kα radiation ($d = 0.71073$ Å; 3066 independent reflections were measured, of which 2057 were observed ($I > 2\sigma(I)$). The structure was solved by direct methods using SHELXS-97[18] and refined with SHELXL-97.[19] Hydrogen atoms bonded to C-5, C-7, C-9, C-5′, and C-8′ were refined freely with isotropic radiation parameters. The rest of the hydrogen atoms were positioned with idealized geometry and refined attached to their parent C or D atoms. The refinement converged to R ($I > 2\sigma(I)$) = 8.29% and wR^2 ($I > 2\sigma(I)$) = 23.25%. An ORTEP view is shown in Figure 1. Full details of the data collection, refinement, and tables of atomic coordinates, bond lengths and angles, and torsion angles have been deposited with the Cambridge Crystallographic Data Centre.[20]

X-ray Crystal Structure of 4b. Suitable crystals were obtained by slow evaporation of a solution in chloroform−petroleum ether and were triclinic, space group *P*1, cell volume $V = 769.1(4)$ Å, $a = 4.5194(12)$ Å, $b = 8.666(2)$ Å, $c = 19.755(6)$ Å, $\alpha = 86.73(3)°$, $\beta = 86,13(3)°$, and $\gamma = 86.13(3)°$ (uncertainties in parentheses). There were two molecules per unit cell, calculated density 1.418 g/cm^3. Diffraction data were collected as described in the previous paragraph. A total of 3450 independent reflections were measured, of which 2689 were observed ($I > 2\sigma(I)$). The structure was solved as described in the previous paragraph. Carbon and oxygen atoms were refined freely with isotropic displacement parameters. The rest of the hydrogen atoms were positioned with idealized geometry and refined attached to their parent C or D atoms. The refinement converged to R ($I > 2\sigma(I)$) = 5.51% and wR^2 ($I > 2\sigma(I)$) = 14.62%. An ORTEP view is shown in Figure 1. Full details of the data collection and refinement and tables of atomic coordinates, bond lengths and angles, and torsion angles have been deposited with the Cambridge Crystallographic Data Centre.[20]

Acknowledgment. Work in Portugal was supported by Fundação para a Ciência e Tecnologia of Portugal (Unidade de I & D 226/96), POCT (QAIII), FEDER, and CIIMAR Pluriannual. T.D. thanks the Thailand Research Fund (TRF) for a fellowship under the RGJ-PhD program.

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(20) Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge C82 IE2, UK (fax: +44-(0)223-336033 or e-mail: deposit@ccdc.cam.ac.uk). Deposition numbers are CCDC 641403 and 641484.

NP0680578