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NEW VERMISTATIN DERIVATIVES ISOLATED FROM *PENICILLIUM SIMPLICISSIMUM*

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Abstract – Four new vermistatin derivatives, dihydrovermistatin (**1**), acetoxy-dihydrovermistatin (**2**), hydroxydihydrovermistatin (**3**), and penisimplicissin (**4**) were isolated along with vermistatin (**5**), penicillide, and funicone from the extract of *Penicillium simplicissimum* IFM 53375. The structures of **1** – **4** were determined by spectroscopic and chemical methods.

INTRODUCTION

During our search for new antifungal compounds against pathogenic filamentous fungi, *Aspergillus fumigatus* and *A. niger*, and/or pathogenic yeasts, *Candida albicans* and *Cryptococcus neoformans* from fungal sourses,[1] we found that the extract of *Penicillium simplicissimu*m IFM 53375 showed characteristic antifungal activity against *A. fumigatus*. Recently new meroterpenoids, penisimplicins A and B, were isolated from the above extract.[2] Further fractionation of the extract led to the isolation of four new vermistatin derivatives designed as dihydrovermistatin (**1**), acetoxydihydrovermistatin (**2**), hydroxydihydrovermistatin (**3**), and penisimplicissin (**4**) along with penicillide[3], vermistatin (**5**)[4], and funicone.[5] In this paper, we report the structural determination of four new vermistatin derivatives (**1** – **4**).

RESULTS AND DISCUSSION

The molecular formulae of **1**- **4** were determined by HREI-MS spectrum as $C_{18}H_{18}O_6$, $C_{20}H_{20}O_8$, $C_{18}H_{18}O_7$ and $C_{16}H_{14}O_6$, respectively. The UV spectra of **1**-**4** were almost superimposable with those of vermistatin (**5**).[4] The ^{1}H-NMR spectra of **1**-**4** (Table 1) showed commonly two methoxy signals, a methine proton bearing a hydroxyl group, two aromatic or olefinic protons, and a pair of *meta*-coupled

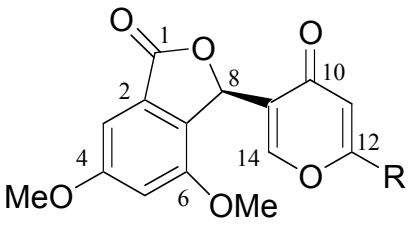
doublet aromatic protons as same as **5**, except for the side chain. The above results indicated that **1**-**4** were the derivative of vermistatin (**5**).

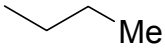
Table 1. ^{1}H- and ^{13}C-NMR spectral data for **1**- **5** in $CDCl_3$ (δ in ppm)

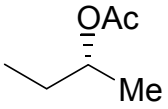
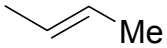
No	**1**		**2**		**3**		**4**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	170.0		170.0		170.2		170.0		170.0	
2	129.3		129.3		129.4		129.3		129.3	
3	99.0	6.98 d (1.9)	99.0	6.98 d (2.0)	99.0	6.97 d (2.0)	99.0	6.98 d (1.8)	99.0	6.99 d
4	163.1		163.1		163.1		163.1		163.1	
5	105.1	6.69 d (1.9)	105.2	6.69 d (2.0)	105.0	6.66 d (2.0)	105.1	6.68 d (1.8)	105.1	6.68 d
6	154.9		154.9		154.8		154.8		154.7	
7	127.7		127.5		127.5		127.7		127.6	
8	73.5	6.46 s	73.4	6.44 (s)	74.2	6.32 s	73.5	6.45 s	73.5	6.46 s
9	123.4		123.7		122.8		123.4		123.6	
10	177.1		176.7		177.3		176.9		177.0	
11	114.4	6.20 s	116.3	6.22 (s)	116.0	6.21 s	115.1	6.19 s	112.9	6.16 s
12	169.4		165.1		167.2		165.9		162.1	
14	154.5	7.44 s	154.6	7.45 (s)	155.3	7.64 s	154.4	7.42 s	153.8	7.43 s
15	35.3	2.48 t (7.6)	39.9	2.70 dd (14.6,5.2)	43.3	2.57 dd (14.6,8.2)	19.7	2.26 s	123.1	6.07 d
				2.77 dd (14.6,7.3)		2.64 dd (14.6,4.0)				
16	19.9	1.67 qt (7.4,7.6)	67.4	5.19 qdd (6.4,7.3,5.2)	65.3	4.18 qdd (6.4,8.2,4.0)			135.9	6.60 dq
17	13.4	0.98 t (7.4)	19.8	1.30 d (6.4)	23.4	1.28 d (6.4)			18.5	1.92 d
4-OCH_3	56.0	3.88 s	56.0	3.88 s	56.0	3.87 s	56.0	3.88 s	56.0	3.88 s
6-OCH_3	55.8	3.79 s	55.8	3.79 s	55.8	3.78 s	55.8	3.79 s	55.8	3.79 s
$COCH_3$			21.1	2.02 s						
$\underline{C}OCH_3$			170.2							

The side chain of **1**, **2**, **3**, and **4** were confirmed as propyl, 2-acetyloxypropyl, 2-hydroxypropyl, and methyl groups, respectively, from the analysis of ^{1}H-NMR spectra. The ^{1}H- and ^{13}C-NMR spectral assignments of **1** - **4** (Table 1) were determined from the detailed analyses of the ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. Hydrogenation of vermistatin (**5**) using H_2 gas under the presence of 5% Pd-C led to dihydrovermistatin (**1**), whereas acetylation of hydroxydihydrovermistatin (**3**) gave acetoxydihydro-vermistatin (**2**). From the CD cspectra of **1-4** compared with that of vermistatin (**5**), the stereochemistry at C-8 in **1** - **4** was confirmed as the same as that of vermistatin (**5**).

In order to confirm the absolute configuration of the secondary alcohol in the side chain of **2** and **3**, the advanced Mosher's method[6] was applied to **3**. The (*R*)- and (*S*)-α-methoxy-α-trifluoromethyl-phenylacetic acid (MTPA) esters of **3** were synthesized and the values of the chemical shift differences between the (*S*)- and (*R*)-MTPA esters [$\Delta\delta = \delta_S$-δ_R in Hz (500 MHz)] were calculated. From the results (Figure 1), the secondary hydroxyl group in **3** was assigned as *R*-configuration. Therefore, the absolute configuration of the acetoxyl group in **2** was determined as *R*-configuration. The absolute structures of four vermistatin derivatives were consequently confirmed as depicted in **1** - **4**.



1 : R =  **4** : R = Me

2 : R =  **5** : R = 

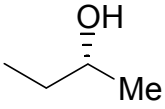
3 : R = 

Chart 1.

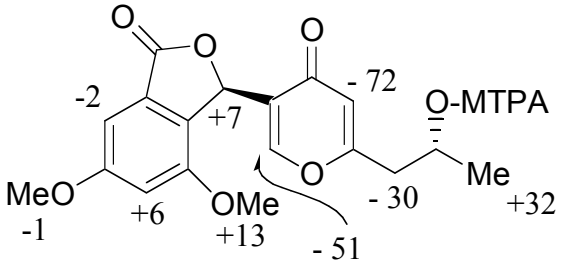


Figure 1. Differences of chemical shifts (Δδ in Hz) between the (*S*)- and (*R*)-MTPA esters of **3**

Vermistatin (**5**) was originally isolated as the cytotoxic compound for tumor cells from *Penicillium vermiculatum*,[7] which is the synonym for an anamorph of *Talaromyces flavus*.[8] Vermistatin (**5**) was also isolated as fijiensin, phytotoxic on various banana cultivars, from *Mycosphaerella fijiensis*, the causal agent of Black Sigatoka disease of bananas.[9] Recently Arai *et al.* reported[8] the isolation of vermistatin (**5**) along with funicone and its derivatives from *T. flavus*. On the taxonomy, the teleomorphic states of *Penicillium* are broadly divided into two genera, *Talaromyces* and *Eupenicillium*. The place of *P. simplicissimum*, which is a member of the group including *Eupenicillium*, is phylogenetically different from that of *P. vermiculatum* in the genus *Penicillium*. From this point of view, it is taxonomically interesting that *P. simplicissimum* produced vermistatin (**5**) and its derivatives (**1**-**4**) along with funicone. Since **1** – **5** showed almost no antifungal activity, further investigation for the antifungal substances in *P. simplicissimum* will be attempted.

EXPERIMENTAL

General Experimental Procedures. General experimental procedures were described in the previous paper.[1]

Isolation of the metabolite from *P. simplicissimum*. *P. simplicissimum* IFM 53375 was cultured at 25 °C for 21 days in 16 Roux flasks containing 150 g of moist rice in each flask. The fermented rice (2.5 kg) was extracted with CH_2Cl_2-MeOH (1:1) (8 L) at rt for 16 h and the organic layer was evaporated *in vacuo*. The resultant extract (56 g) was suspended in H_2O and extracted with EtOAc, and then the organic layer was evaporated *in vacuo*, respectively. The EtOAc extract (40 g), which showed the strong antifungal activity against *A. fumigatus*, was separated by column chromatography on silica gel (600 g) into six fractions: CH_2Cl_2 (50:1) (8 g), CH_2Cl_2-EtOH (20:1) (14 g), CH_2Cl_2-EtOH (10:1) (4 g), CH_2Cl_2-EtOH (5:1) (4 g), CH_2Cl_2-EtOH (1:1) (5 g), and EtOH (1.5 g). The 2nd fraction [CH_2Cl_2-EtOH (20:1)] was purified by LPLC on a silica gel column using benzene-acetone (15:1) followed by the further

purification of HPLC on silica gel column [CH_2Cl_2-acetone (15:1)] to give penicillide[3] (35 mg), dihydrovermistatin (**1**) (20mg), vermistatin[4] (**5**) (1.0 g), funicone[5] (8 mg), penisimplicissin (**4**) (15 mg), and acetoxydihydrovermistatin (**2**) (20 mg). The 3rd fraction [CH_2Cl_2-EtOH (10:1)] was also purified with LPLC on a silica gel column using CH_2Cl_2-EtOH (20:1), followed by the purification with HPLC on a silica gel column using CH_2Cl_2-acetone (2:1) to give hydroxydihydrovermistatin (**3**) (32 mg). Penicillide, funicone, and **5** were identified by the comparison with the published data.[3-5]

Dihydrovermistatin (**1**) : Colorless needles (from MeOH); mp 143-145°C; $[\alpha]_D^{19}$ -108° (*c* = 0.10, $CHCl_3$); IR (KBr) ν_{max}: 1770(CO_2), 1665 (CO) cm^{-1}; UV (MeOH) λ_{max} (log ε): 210 (4.6), 245 (4.1), 303 (3.6); EI-MS *m/z*: 330.1094 (M^+, 330.1103 for $C_{18}H_{18}O_6$, 90), 287 (17), 217 (100); CD($\Delta\varepsilon$): 210 (+13.9), 222 (-16.4), 250 (+2.2), 303 (-1.0) nm. The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Acetoxydihydrovermistatin (**2**) : White amorphous powder; $[\alpha]_D^{19}$ -80° (*c* = 0.20, $CHCl_3$); IR (KBr) ν_{max}: 1770(CO_2), 1660 (CO) cm^{-1}; UV (MeOH) λ_{max} (log ε): 210 (4.7), 246 (4.2), 305 (3.7); EI-MS *m/z*: 388.1160 (M^+, 388.1158 for $C_{20}H_{20}O_8$, 63), 328 (100), 217 (33); CD($\Delta\varepsilon$): 212 (+18.6), 222 (-42.5), 250 (+3.6), 303 (-3.8) nm. The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Hydroxydihydrovermistatin (**3**) : Colorless needles (from MeOH); mp 184-185°C; $[\alpha]_D^{19}$ -85° (*c* = 0.13, $CHCl_3$); IR (KBr)ν_{max}: 3380(OH), 1770(CO_2), 1660 (CO) cm^{-1}; UV (MeOH) λ_{max} (log ε): 210 (4.6), 248 (4.2), 306 (3.6) nm; EI-MS *m/z*: 346.1064 (M^+, 346.1053 for $C_{18}H_{18}O_7$, 100), 287 (23), 217 (42); CD($\Delta\varepsilon$): 210 (+21.1), 222 (-22.6), 249 (+3.16), 301 (-0.8), 325 (+0.5) nm. The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Penisimplicissin (**4**) : Colorless needles (from acetone); mp 185-186°C; $[\alpha]_D^{19}$ -119° (*c* = 0.10 , $CHCl_3$); IR (KBr) ν_{max}: 1770(CO_2), 1665 (CO) cm^{-1}; UV (MeOH) λ_{max} (log ε): 210 (4.6), 247 (4.1), 305 (3.5); EI-MS *m/z*: 302.0793 (M^+, 302.0790 for $C_{16}H_{14}O_6$, 100), 287 (M-Me, 10), 259 (46), 217 (38); CD($\Delta\varepsilon$): 210 (+15.4), 222 (-21.2), 250 (+2.4), 303 (-1.5) nm. The assignments of ^{1}H- and ^{13}C-NMR signals are summarized in Table 1.

Synthesis of (S)- and (R)-MTPA esters of hydroxydihydrovermistatin (3).

Dicyclohexylcarbodiimide (16 mg), 4-dimethylaminopyridine (6 mg), and (*S*)- or (*R*)-MTPA (16 mg) were added to a solution of hydroxydihydrovermistatin (**3**) (3.5 mg) in CH_2Cl_2 (1 mL). The reaction mixture was kept at 40°C for 1.5 h, and then washed with 0.5M HCl, saturated $NaHCO_3$, and water successively, and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by HPLC (silica gel) (CH_2Cl_2-acetone 15:1) to afford the (*R*)- or (*S*)-MTPA ester of **3** [1.1 mg for (*S*), 2.6mg for (*R*)].

(*S*)-MTPA ester of **3**: White amorphous powder. ^{1}H-NMR ($CDCl_3$, 270 MHz; except for the phenyl signals): δ 7.30 (1H, s, H-14), 6.99 (1H, d, *J*= 1.8 Hz, H-3), 6.69 (1H, d, *J*= 1.8 Hz, H-5), 6.47 (1H, s,

H-8), 6.06 (1H, s, H-11), 5.50 (1H, qt, *J*= 6.4, 6.4 Hz, H-16), 3.89 (3H, s, 4-OCH_3), 3.75 (3H, s, 6-OCH_3), 2.76 (2H, d, *J*= 6.4 Hz, H-15), 1.44 (3H, d, *J*= 6.4 Hz, H-17).

(*R*)-MTPA ester of **3**: White amorphous powder. ^{1}H-NMR ($CDCl_3$, 270 MHz; except for the phenyl signals): δ 7.39 (1H, s, H-14), 6.99 (1H, d, *J*= 1.8 Hz, H-3), 6.67 (1H, d, *J*= 1.8 Hz, H-5), 6.45 (1H, s, H-8), 6.19 (1H, s, H-11), 5.46 (1H, qt, *J*= 6.4, 6.4 Hz, H-16), 3.89 (3H, s, 4-OCH_3), 3.72 (3H, s, 6-OCH_3), 2.81 (2H, d, *J*= 6.4 Hz, H-15), 1.37 (3H, d, *J*= 6.4 Hz, H-17).

Acetylation of hydroxydihydrovermistatin (3). A mixture of **3** (5 mg), acetic anhydride (0.6 mL), and pyridine (0.1 mL) was stirred at rt for 16 h. Work-up in the usual manner provided a viscous oil, which was purified by HPLC on silica gel with CH_2Cl_2-acetone (10:1) to give an acetate (4 mg). This acetate was identical to acetoxydihydrovermistatin (**2**) based on the spectroscopic data including the optical rotation and CD curve.

Hydrogenation of vermistatin (5). 5% Pd-C (20 mg) was suspended in a solution of vermistatin (**5**) (20 mg) in MeOH (4 mL) and the mixture stirred at rt in a hydrogen atmosphere for 10 min. After removal of the catalysts, the solvent was evaporated in vacuo. The residue was purified by HPLC on silica gel with CH_2Cl_2-acetone (15:1) to give a dihydro derivative (16 mg). This compound was identical to dihydrovermistatin (**1**) based on the spectroscopic data including the optical rotation and CD curve.

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