VERMIXOCINS A AND B, TWO NOVEL METABOLITES FROM *Penicillium vermiculatum*

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Vermixocins A and B, 3-(1′-hydroxy-3′-methylbutyl)- and 3-(1′-acetoxy-3′-methylbutyl)-11-hydroxy-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[*c*,*f*][1,5]dioxocin-5-one, respectively, were isolated from the mycelium of *Penicillium vermiculatum*. Both metabolites showed cytotoxic effects on lympholeukemia cells P388.

Penicillium vermiculatum Dang. CCM F-276 (*Talaromyces flavus* var. *flavus* (Klöcker) Stolk et Samson (ATCC 26015)) produces two major metabolites depending on the carbon source: The macrolide dilactone vermiculine[1,2] and the phthalidopyranone vermistatin[3] are biosynthesized on sucrose and glucose, respectively. A new strain, specified B 5, selected from the above mentioned parental strain formed dehydroaltenusin only[4]. This compound in addition to other polyketide metabolites was isolated from the mycelium of *T. flavus* (ATCC 52201) grown on a malt extract medium[5]. Two new minor metabolites designated vermixocins A (**1**) and B (**2**) were found in the mycelium of *P. vermiculatum* cultivated under conditions optimized for vermiculin production. The structure elucidation of compounds **1** and **2** is the subject of this report.

Results and Discussion

According to the positions and intensities of UV absorption bands (Table 1), the chromophore of compound **1** is a substituted benzene ring; one of the substituents is a phenolic hydroxyl as proved by the bathochromic shift in alkaline solvent. The IR spectrum displayed strong absorption of a lactone carbonyl

Table 1. Physico-chemical properties of vermixocins A (**1**) and B (**2**).

	1	**2**
TLC (silica gel)		
Benzene	0.06	0.12
$CHCl_3$ - 2-PrOH (19 : 1)	0.26	0.45
$CHCl_3$ - MeOH (9 : 1)	0.48	0.83
$[\alpha]_D^{20}$	+21° (*c* 1, $CHCl_3$)	−54° (*c* 1, $CHCl_3$)
Molecular formula	$C_{21}H_{24}O_6$	$C_{23}H_{26}O_7$
MW	372.4	414.4
UV λ_{max}^{MeOH} nm ($\log\varepsilon$)	220 (4.09), 280 (3.63)	220 (4.28), 282 (3.70)
IR ν_{max} ($CHCl_3$) cm^{-1}	3513, 2999, 1678, 1634, 1599	3500, 2983, 1740, 1636, 1600
CD $\lambda_{extreme}^{MeCN}$ nm ($\Delta\varepsilon$)	277 (+0.325), 245 (−0.446)	276 (+0.379), 244 (−0.503)
EI-MS (160°C) *m/z* (%)	372 (45), 315 (100), 297 (24), 287 (23), 271 (22), 243 (35)	414 (62), 370 (15), 354 (100), 316 (48), 298 (75), 243 (23)

Table 2. ^{1}H NMR chemical shifts of compounds **1**~**3**.

H	**1**[a]	**1**[b]	**2**[a]	**3**[a]
1	6.81 d	6.49 d	6.87 d	6.96 d
2	7.49 d	7.31 dd	7.43 d	7.44 d
7α	5.05 m	4.67 ABq	5.09 ABq	5.11 ABq
7β	5.05 m	4.56 ABq	4.98 ABq	5.02 ABq
8	6.33 d	5.81 dq	6.37 d	6.76 d
10	6.84 d	6.79 dq	6.84 d	6.93 d
1′	5.05 dd	4.95 ddd	6.11 dd	6.11 dd
2′a	1.64 ddd	1.60 ddd	1.75 m	1.77 m
2′b	1.43 ddd	1.43 ddd	1.50 m	1.50 m
3′	1.76 m	1.81 m	1.66 m	1.64 m
4′*	0.94 d	0.93 d	0.94 d	0.95 d
5′*	0.96 d	0.94 d	0.94 d	0.95 d
C-9-CH_3	2.21 s	1.89 dd	2.23 s	2.29 s
OCH_3	3.94 s	3.78 s	4.03 s	4.02 s
C-11-$OCOCH_3$	—	—	—	2.38 s
C-1′-$OCOCH_3$	—	—	2.06 s	2.06 s

[a] $CDCl_3$.
[b] C_6D_6.
* May be interchanged.

Table 3. Coupling constants J (Hz) in ^{1}H NMR spectra of compounds **1**~**3**.

J (Hz)	**1**[a]	**1**[b]	**2**[a]	**3**[a]
1,2	8.5	8.4	8.5	8.4
2,1′	—	0.5	—	—
7α,7β	14.4	14.4	14.5	14.5
8,10	2.1	2.1	2.1	2.1
8,CH_3	—	0.7	—	—
10,CH_3	—	0.7	—	—
1′,2′a	9.0	9.1	9.2	9.1
1′,2′b	13.8	13.9	13.5	13.4
2′a,3′	5.1	5.0	5.1	5.0
2′b,3′	8.6	8.7	8.2	8.2
3′,4′	6.6	6.6	6.5	6.6
3′,5′	6.6	6.6	6.5	6.6

[a] $CDCl_3$.
[b] C_6D_6.

Table 4. ^{13}C NMR chemical shifts of vermixocins A (**1**), B (**2**) and diacetate (**3**).

C	**1**[a]	**1**[b]	**2**[a]	**3**[a]
1	117.9	117.8	117.6	118.4
2	130.9	130.9	130.7	130.7
3	136.7	137.6	134.1	134.1
4	154.1	154.8	154.5	154.4
4a	119.9	120.6	119.9	119.9
5	168.0	167.4	167.1	166.7
7	69.1	68.9	69.0	69.0
7a	125.7	126.5	125.8	127.2
8	120.5	120.9	120.7	127.8
9	134.9	134.8	135.0	134.4
10	117.7	118.0	117.7	124.8
11	147.5	148.3	147.3	145.6
11a	141.3	142.1	141.2	141.8
12a	151.7	151.9	151.5	151.1
1′	66.5	66.8	68.7	68.6
2′	47.7	48.2	45.3	45.3
3′	24.9	25.4	24.9	24.9
4′	23.2	23.7	23.1	23.1
5′	21.8	22.1	21.9	21.9
4-OCH_3	62.5	62.7	62.6	62.9
9-CH_3	20.8	20.6	20.8	20.7
11-OCO	—	—	—	169.7
11-$OCOCH_3$	—	—	—	20.6
1′-OCO	—	—	170.2	170.2
1′-$OCOCH_3$	—	—	21.2	21.2

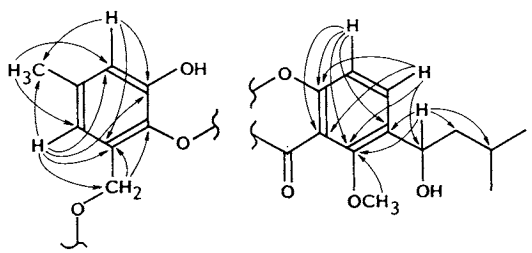
[a] $CDCl_3$.
[b] C_6D_6.

together with OH, CH and aromatic vibrations. The base peak of compound **1** at m/z 315 was generated from the molecular radical ion at m/z 372 ($C_{21}H_{24}O_6$) by the loss of a C_4H_9 fragment. The ^{1}H NMR of compound **1** dissolved in $CDCl_3$ did not afford useful information due to overlapping of several signals; sufficient resolution was achieved in C_6D_6 (Tables 2 and 3). Signals of two independent pairs of aromatic protons were observed in this spectrum: Protons of the first pair (6.49 and 7.31 ppm) were in an *ortho* position (J=8.5 Hz), those of the second (5.81 and 6.79 ppm) were *meta* arranged (J=2.1 Hz).

Both protons of the latter pair were coupled also with protons of a methyl group (1.89 ppm), which was in an *ortho* position to both protons; in addition, the proton at $\delta = 5.81$ ppm was also coupled with a pair of methylene protons (4.56 and 4.67 ppm). Further signals in the ^1H NMR spectrum of compound **1** belonged to a methoxyl group and a 1-hydroxy-3-methylbutyl moiety which was identified by a series of homonuclear decouplings. The methoxyl group has to be in the vicinity of the lactone carbonyl according to its difference in chemical shift in C_6D_6 and $CDCl_3$[6]. According to ^1H and ^{13}C NMR data (Tables 2, 3 and 4), results of a 2D-heterocorrelated experiment[7] and couplings observed in a semiselective INEPT[8] spectrum the presence of partial structures **1a** and **1b** was suggested (Fig. 1). These subunits could be linked in only two possible ways, either of which would form an eight membered lactone. Magnetization transfer through the oxymethylene protons in the INEPT experiment revealed the signal of the lactone carbonyl thus indicating the 5*H*,7*H*-dibenzo[*c*,*f*][1,5]dioxocin-5-one skeleton.

The structure of vermixocin B (**2**) differed from that of compound **1** by the presence of an acetyl group; the significant changes in chemical shifts of the protons and carbons of the side chain indicated that the hydroxyl group of this moiety was esterified. Acetylation of vermixocins A (**1**) and B (**2**) afforded the same diacetate **3**; its NMR spectra revealed changes in shifts of protons and carbons of ring C and also of H-1 and C-1 when compared with those of compound **2**. According to these data the phenolic hydroxyl group was attached to C-11. The $LiAlH_4$ reduction of all carbonyl groups in the diacetate **3** gave the diphenyl ether **4** hydroxymethylated in positions 2 and 2'. Methylation of the phenolic hydroxyl group in vermixocin A (**1**) with dimethylsulfate in aqueous KOH with simultaneous hydrolysis of the lactone group afforded the substituted phenoxybenzoic acid **5**. According to these data vermixocins A (**1**) and B (**2**) were ascribed the structures 3-(1'-hydroxy-3'-methylbutyl)- and 3-(1'-acetoxy-3'-methylbutyl)-11-hydroxy-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[*c*,*f*][1,5]dioxocin-5-one, respectively.

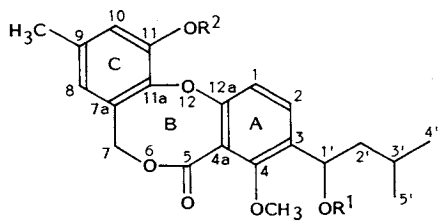
Fig. 1. ^1H-^{13}C long range connectivity pattern observed for subunits **1a** and **1b**.



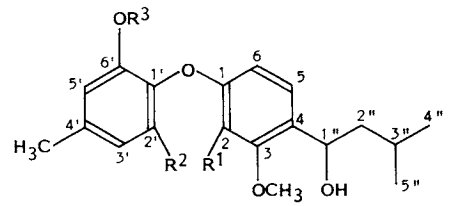
1a **1b**

Table 5. Incorporation of ^{14}C-labeled precursors into P388 cells in presence of compounds **1**~**3** (% of control).

Compound	Concentration (μg/ml)	Incorporation of		
		Thymidine	Uridine	L-Valine
1	50	110	44	96
	100	60	12	35
2	50	111	27	55
	100	110	24	54
3	50	142	48	98
	100	132	46	90



1 $R^1 = R^2 = H$
2 $R^1 = COCH_3$ $R^2 = H$
3 $R^1 = R^2 = COCH_3$



4 $R^1 = R^2 = CH_2OH$ $R^3 = H$
5 $R^1 = COOH$ $R^2 = CH_2OH$ $R^3 = CH_3$

The cytotoxic activity of the isolated compounds was studied in an *in vitro* P388 test system[9]. Vermixocins A (**1**), B (**2**) and diacetate **3** inhibited incorporation of labeled uridine and, to a less extent, L-valine (Table 5) into leukemia cells but incorporation of thymidine was enhanced. These results indicate that the isolated compounds interfere preferentially with RNA synthesis in leukemia cells.

Experimental

General

Spectral data were recorded on the following instruments: NMR, Bruker AM-300; IR, Perkin-Elmer 983; UV, Specord UV-VIS; MS, Jeol JMS 100D at 70 eV; optical rotation, Perkin-Elmer 141; CD, Jobin-Yvon Mark-III S. TLC was carried out on silica gel sheets (Silufol UV 254, Kavalier).

Isolation of Compounds 1 and 2

Concentrated liquor (25.0 g) after crystallization of vermiculine[1] was dissolved in chloroform, the solution was extracted with aqueous $NaHCO_3$, the separated organic layer was evaporated and the residue dissolved in 95% aqueous ethanol was thoroughly mixed with *n*-heptane. The lower layer after evaporation was chromatographed on silica gel in benzene-acetone (0~20%) mixture. Eluates monitored by TLC were rechromatographed on alumina in benzene-acetone (1 : 1) and purified by preparative TLC on silica gel in chloroform-2-propanol (19 : 1). Work up of bands with Rf 0.26 and 0.45 afforded amorphous compounds **1** (232 mg) and **2** (118 mg), respectively. Physico-chemical properties of isolated compounds are summarized in Table 1.

3-(1′-Acetoxy-3′-methylbutyl)-11-acetoxy-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[*c*,*f*][1,5]dioxocin-5-one (3)

Compound **1** (50 mg) dissolved in acetic anhydride (0.1 ml) with pyridine (0.1 ml) was stood for 24 hours at ambient temperature; solvents were evaporated and the residue chromatographed on silica gel in benzene to afford diacetate **3** (48 mg). For $C_{25}H_{28}O_8$ (456.5) calcd: C 65.78%, H 6.18%, found: C 65.68%, H 6.13%. UV λ_{max}^{MeOH} nm (log ε) 282 (3.73); EI-MS (direct inlet, 170°C) *m/z* (relative intensity %) 456 (36), 414 (23), 355 (30), 354 (100), 339 (6), 314 (18), 300 (23), 299 (59), 298 (17), 288 (7), 286 (6), 279 (25), 243 (11), 220 (10), 1,263 (53); NMR in Tables 2, 3 and 4.

6′-Hydroxy-4-(1″-hydroxy-3″-methylbutyl)-2,2′-bis(hydroxymethyl)-3-methoxy-4′-methyldiphenyl Ether (4)

Diacetate **3** (120 mg) was stirred with $LiAlH_4$ (45 mg) in dried diethyl ether; after 2.5 hours diluted sulfuric acid was added, the organic layer was removed, dried and the solvent was evaporated. The residue

Table 6. NMR data of compound **5** ($CDCl_3$, δ/ppm).

Position	^{13}C δ (ppm)	^1H δ (ppm)	^1H J (Hz)	Position	^{13}C δ (ppm)	^1H δ (ppm)	^1H J (Hz)
1	154.5	—	—	1″	66.4	4.95 m	
2	116.8	—	—	2″	47.1	1.64 m	
3	155.4	—	—			1.43 m	
4	136.2	—	—	3″	24.9	1.70 m	
5	129.1	7.15 d	8.5	4″	23.3	0.95 d	6.6
6	109.5	6.20 d	8.5	5″	21.9	9.93 d	6.6
1′	138.6	—	—	C-2-COOH	167.9	—	
2′	131.6	—	—	C-3-OCH_3	62.9	3.82 s	
3′	122.0	6.85 d	2.1	C-6′-OCH_3	56.0	3.67 s	
4′	134.3	—	—	C-4′-CH_3	21.4	2.28 s	
5′	113.2	6.85 d	2.1	C-2′-CH_2OH	60.8	4.41 m	
6′	151.7	—	—				

purified by preparative TLC in chloroform - methanol (9 : 1) afforded compound **4** (63 mg); Rf 0.41; for $C_{21}H_{28}O_6$ (376.4) calcd: C 67.00%, H 7.50%; found: C 66.91%, H 7.53%; EI-MS (direct inlet, 160°C): *m/z* (relative intensity %) 376 (27), 358 (15), 340 (17), 323 (46), 301 (100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 (1H, d, J=8.4 Hz, 5-H), 6.72 (1H, d, J=2.1 Hz, 3'-H), 6.70 (1H, d, J=2.1 Hz, 5'-H), 6.31 (1H, d, J=8.4 Hz, 6-H), 4.89 (1H, m, 1''-H), 4.88 (2H, s, 2-CH_2OH), 4.44 (2H, s, 2'-CH_2OH), 2.27 (3H, s, 4'-CH_3), 1.73 (1H, m, 3''-H), 1.63 (1H, m, 2a''-H), 1.44 (1H, m, 2b''-H), 0.93 (3H, d, J=6.6 Hz, 4''-H), 0.92 (3H, d, J=6.6 Hz, 5''-H).

2-Carboxy-3,6'-dimethoxy-2'-hydroxymethyl-4-(1''-hydroxy-3''-methylbutyl)-4'-methyldiphenyl Ether (**5**)

Compound **1** (100 mg) in methanolic KOH (25 ml, 1%) was mixed with dimethylsulfate (50 mg) in methanol (4 ml). The mixture was heated for 2 hours, methanol was evaporated, diluted sulfuric acid was added to pH 2.0, the suspension was extracted with chloroform - 2-propanol (3 : 1), the lower layer dried, solvents evaporated and the residue after preparative TLC on silica gel in chloroform - methanol (9 : 1) gave compound **5** (42 mg); for $C_{22}H_{28}O_7$ (404.5) calcd: C 65.33%, H 6.98%; found: C 65.25%, H 6.83%; EI-MS (direct inlet, 170°C): *m/z* (relative intensity %) 404 (31), 389 (12), 386 (25), 347 (9) 329 (100); NMR in Table 6.

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