THE STRUCTURE OF VERMISTATIN, A NEW METABOLITE FROM *PENICILLIUM VERMICULATUM*

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Separation of metabolites of *Penicillium vermiculatum* DANG present in the cultivation medium afforded three new antibiotics; vermiculine[1], vermistatin[2] and vermicillin[3]. The structure of vermiculine, a macrolide dilactone has already been solved by X-ray diffraction analysis[4]. This paper presents the structure elucidation of the second compound of this series, vermistatin.

In our previous paper[2] we reported the isolation, antibiotic and cytotoxic activity of vermistatin (**1**); it is a white crystalline compound of molecular formula $C_{18}H_{16}O_6$ (328.3), mp 213~214°C, $[\alpha]_D^{20}$ −8.5° (*c* 0.2, chloroform), with characteristic peaks in HR-MS at *m/z* 328.0950 (for $C_{18}H_{16}O_6$ calcd 328.0947, 100%), 313.0711 (for $C_{17}H_{13}O_6$ calcd 313.0712, 16%), 299.0916 (for $C_{17}H_{15}O_5$ calcd 299.0919, 17%), 269.0813 (for $C_{16}H_{13}O_4$ calcd 269.0814, 26%), 260.0689 (for $C_{14}H_{12}O_5$ calcd 260.0685, 37%), 165.0557 (for $C_9H_9O_3$ calcd 165.0552, 44%), 135.0444 (for $C_8H_7O_2$ calcd 135.0446, 48%). Its ^1H NMR spectrum displayed signals of a (1*E*)-propenyl grouping, two methoxyl groups and a further five protons at δ 6.15 to 7.42. The ^{13}C NMR spectrum provided evidence for the presence of one methyl and two methoxyl groups, seven methine and eight quaternary carbons. Correlation between carbon and hydrogen signals was provided by the two-dimensional hetero-correlated experiment[5] (Table 1). The one- and multibond ^{13}C-^1H spin-spin coupling constants jointly with the above data, suggested the molecule of **1** has to be composed of two relatively isolated moieties mutually linked through the C(3) carbon atom (Table 2). This

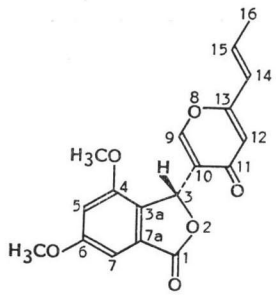

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Table 1. Chemical shifts (ppm) and H, H coupling constants (Hz) for vermistatin (**1**).

Assignment	^{13}C	^1H	$J_{H,H}$
1	170.0	—	—
3	73.6	6.45 d	0.6
3a	127.7	—	—
4	154.9	—	—
5	105.1	6.68 d	2.0
6	163.0	—	—
7	99.0	6.98 d	2.0
7a	129.3	—	—
9	153.9	7.42 d	0.6
10	123.4	—	—
11	177.2	—	—
12	112.8	6.15 dd	0.5, 0.4
13	162.1	—	—
14	123.1	6.06 dq	15.6, 1.7
15	135.9	6.60 dqd	15.6, 6.9, 0.4
16	18.5	1.92 ddd	6.9, 1.7, 0.5
C(4)-OCH_3	55.8†	3.79	—
C(6)-OCH_3	56.0†	3.88	—

† May be interchanged.

presumption accorded with the distribution of proton signals, these being determined by a two-dimensional COSY experiment optimized to long-range coupling constants[6]. Protons at 6.68 and 6.98 ppm reveal long-range coupling constants with methoxyl groups at 3.79 and 3.88 ppm, respectively. Nevertheless, no further interactions with any proton of the molecule have been found. These data, together with the absorption band at 841 cm^{-1} in the IR spectrum indicate the presence of a 1,2,3,5-tetra-substituted benzene ring; a further band at 1765 cm^{-1} (a five-membered lactone ring), the fragment at *m/z* 165 (dimethoxybenzoyl), and the ^{13}C NMR signals at δ 73.5 and 170.0 are diagnostic of

Table 2. ^{13}C-^{1}H Coupling constants (Hz) for vermistatin (**1**).

C	H									
	3	5	7	9	12	14	15	16	C(4)-OCH_3	C(6)-OCH_3
1	2.0		3.1							
3	156.4	1.7		3.3	1.3					
3a	4.6	6.0	5.9							
4	1.3	3.2	1.3						*	
5	0.9	158.9	5.3							
6	0.9	4.5	2.1							*
7		4.6	166.7							
7a	1.8		1.1							
9	6.3			195.0		0.8				
10	5.1			5.8	4.0					
11	3.7			6.0	1.5					
12					166.0	1.7	0.8			
13				8.1**	*	*	*			
14					*	*		*		
15						*	*	*		
16						*	*	*		

* Connectivities confirmed by selective INEPT experiment[11].

** Determined by 2D selective *J*-resolved experiment[12].

Table 3. Chemical shifts (ppm) for phthalide derivatives.

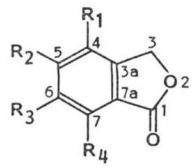
Assignment	^{13}C				^{1}H			
	2[a]	**3**[b]	**4**[b]	**4**[c]	**2**[c]	**3**[d]	**4**[d]	**4**[c]
3a	146.3	125.7	124.7	127.7	—	—	—	—
4	122.0	101.1	154.8	154.9	7.49	6.57	—	—
5	133.6	165.5	105.2	105.1	7.68	—	6.72	6.68
6	128.5	101.1	161.3	163.0	7.52	6.56	—	—
7	124.9	157.8	103.1	99.0	7.90	—	6.98	6.98
7a	125.2	103.8	127.6	129.3	—	—	—	—

[a] Ref 13.

[b] Calculated, ref 14.

[c] Measured.

[d] Calculated, ref 15.



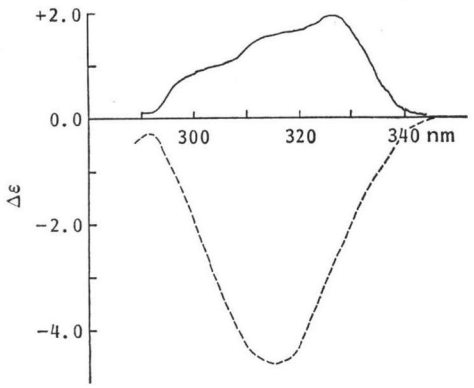
2 $R_1=R_2=R_3=R_4=H$

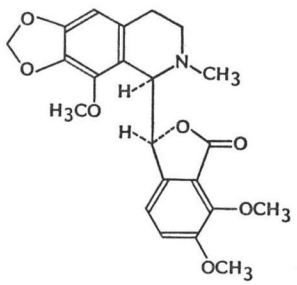
3 $R_1=R_3=H$ $R_2=R_4=OCH_3$

4 $R_1=R_3=OCH_3$ $R_2=R_4=H$

a dimethoxyphthalide grouping. Considering these findings, two *meta*-juxtaposed methoxyl groups on the phthalide ring can be in positions i) 5,7-, or ii) 4,6-. No four-band interaction between protons H(3) and H(4) was found in the spectrum and therefore, the possibility i) is not favored. Calculation involving ^{1}H and ^{13}C chemical shift data for phthalide (**2**) and consideration of the effects of methoxyl substituents (Table 3) strongly support the possibility ii), *i.e.* the presence of 4,6-dimethoxyphthalide grouping **4** in this moiety of vermistatin. The second moiety of vermistatin has to embody protons and carbons of the (1*E*)-propenyl grouping, two protons and five carbons, one of which is present as a carbonyl function (δ 177.2). Analysis of coupling constants shows that the propenyl fragment is linked to C(13), which, according to the chemical shift value (162.1 ppm) is directly attached to oxygen. Another carbon bound to

Fig. 1. CD curves of vermistatin (**1**) (——) and (−)-α-narcotin (**5**) (------) in methanol.





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oxygen is C(9) (153.9 ppm). These facts led us to propose the structure 2-(1-propenyl)-4*H*-pyran-4-one for this part of vermistatin. This proposal is in accordance with the values for 4*H*-pyranone absorption[7] found in the IR spectrum (ν(C=O) and ν(C=C), 1665 and 1649 cm^{-1}, respectively). The evaluation of $^{n}J_{H,H}$ and $^{n}J_{C,H}$ coupling constants (Tables 1 and 2) show that the phthalide and 4*H*-pyranone moieties are linked by the C(3)-C(10) bond. The UV spectrum of **1** could be considered the superposition of spectra of two individual chromophores; the phthalide (λ_{max} *ca.* 255 and 300 nm, ref 8) and the 4*H*-pyranone (λ_{max} *ca.* 270 and 310 nm ref 7). The configuration of the only center of chirality at C(3) was deduced from the circular dichroism data; the CD spectrum (Fig. 1) of **1** showed bands at λ nm (in methanol) ($\Delta\varepsilon$) 327 (+1.72), 315 (sh, +1.28) and 302 (sh, +0.80), opposite to those of (−)-α-narcotine (**5**) having the (*S*)-configuration at the phthalide center of chirality[9,10] (313 (−4.64), 253 (−5.52) nm). It follows that the configuration at C(3) of vermistatin is opposite to that of the corresponding carbon of (−)-α-narcotine (**5**) and vermistatin can be assigned the structure of 5-(4,6-dimethoxy-3*R*-phthalidyl)-2-(1*E*)-propenyl-4*H*-pyran-4-one.

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