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VERMICULINOL AND VERMICULIDIOL, MACRODIOLIDES FROM THE FUNGUS *PENICILLIUM VERMICULATUM*

MARCEL MASSIAS, LUCIE MOLHO, SYLVIE REBUFFAT, MICHELE CESARIO,* JEAN GUILHEN,* CLAUDINE PASCARD* and BERNARD BODO

Laboratoire de Chimie, UA CNRS 401, Muséum national d'Histoire naturelle, 63, rue Buffon, 75231 Paris Cedex 05, France;
* Institut de Chimie des Substances naturelles, CNRS, 91190 Gif sur Yvette, France

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Key Word Index-Penicillium uermiculatum; fungi; vermiculinol; vermiculidiol; macrodiolides; NMR, X-ray.

Abstract-Two macrodiolides, vermiculinol and vermiculidiol have been isolated from a culture of the fungus ***Penicillium uermiculatum*** and their structures elucidated from a spectroscopic study including IR, MS, **NMR** and X-ray crystallography and chemical transformations.

INTRODUCTION

As part of a program investigating fungi with antagonistic properties as source of antifungal products, an isolate of ***Penicillium vermiculatum,*** collected in Africa, was studied. From the biologically active crude extract we have isolated dehydroaltenusin, vermistatin, vermiculine **(1)** and two macrodiolides related to vermiculine: vermiculidiol (2) and vermiculinol (3). In this paper we describe the isolation and structural elucidation of these two new metabolites. Previous works on an European isolate of P. ***vermiculatum*** reported vermiculine [1–3] and vermistatin [4]. This fungus had been shown to inhibit growth of other fungi including ***Penicillium*** spp. [5]. The antifungal activity against ***P. crustosum*** that we observed with the ethyl acetate crude extract was mainly attributed to dehydroaltenusin.

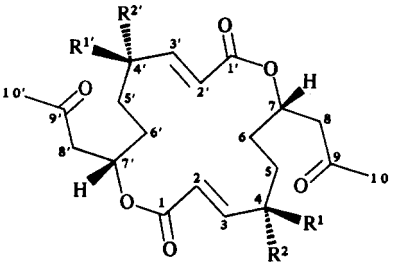
RESULTS AND DISCUSSION

Penicillium vermiculatum was grown on a Czapek-Dox medium at 27" for 30 days. The culture filtrate was extracted several times with ethyl acetate. From the concentrated extract dissolved in methanol, vermiculine was separated by crystallization. The remaining residue was chromatographed over a silica gel column and each of the eight collected fractions was further purified by TLC.

Fraction I from the column gave mainly dehydroaltenusin, characterized by its mass and ^{1}H NMR spectra, which were in agreement with the data published by Coombe ***et al.*** who isolated it from ***Alternaria*** sp. [6]. The purified dehydroaltenusin induced inhibition of ***P. crustosum*** development, when tested (disk method). Fraction II contained a substance identified as vermistatin as its mass, IR and ^{1}H NMR spectra were identical to those recently reported by Fuska et ***al.*** [4].

Vermiculine **(1)** was mostly obtained by crystallization, but also from fraction III of the column. Its structure was assigned on the basis of MS, IR and ^{1}H NMR data (Table 1). TLC purification of fraction III gave vermiculidiol(2) as a crystalline compound (mp 173-174"). The CI mass spectrum showed the pseudomolecular ion $[M + H]^+$ at m/z 397 in agreement with the molecular formula $C_{20}H_{28}O_8$. The ^{13}C NMR spectrum showed only 10 carbon atoms, so that vermiculidiol must be C-2 symmetrical (Table 2). The spectrum showed one ketone group (6 205.8), one carbonyl carbon atom (6 164.8) from an ester conjugated to a double bond and two tertiary carbons bearing an oxygen atom. The spectrum differed from that of vermiculine only by the replacement of a ketone group by a secondary alcohol group (6 68.6). The IR spectrum showed absorptions of alcohol (ν = 3567 cm-') and carbonyl (ν = 1708 and 1703 cm^{-1}). Acetylation of 2 gave a diacetate 4 ($C_{24}H_{32}O_{10}$).

The ^{1}H NMR spectrum of 2 (Table 1), assigned by using 2D COSY, showed one isolated methyl group linked to a carbonyl and a spin system involving 10 protons: the two ethylenic protons (6 5.88 and 6.79) were in the ***E*** configuration as indicated by their mutual coupling constant (J = 15.6 Hz). The ethylenic proton at δ 6.79 was vicinal to a methine proton (6 4.08) which was geminal to a hydroxyl group and deshielded at 65.22 in the acetate 4. This methine proton was linked to a methylene adjacent to a methylene itself linked to a methine (65.37) bearing an oxygen atom and linked to the methylene (62.71 and 2.88). The results allowed the spin system to be –CH=CH–CH(OH)–CH_2–CH_2–CH(OR)–CH_2–. Thus all the data agreed with structure **2** for vermiculidiol.



1 R^1, R^2 = =O, $R^{1'}$, $R^{2'}$ = =O
2 R^1 = $R^{1'}$ = H, R^2 = $R^{2'}$ = OH
3 R^1, R^2 = =O, $R^{1'}$ = H, $R^{2'}$ = OH

Table 1. ^{1}H NMR data (**1**: $CDCl_3$, 80 MHz; 2 and 3: CD_3OD, 250 MHz)

	1 δ (ppm) mult. J (Hz)	2 δ (ppm) mult. J (Hz)	3 δ (ppm) mult. J (Hz)
2	6.44 (*d* 15.8)		6.57 (*d* 15.8)
3	6.97 (*d* 15.8)		7.02 (*d* 15.8)
5	2.00–2.70		2.05 and 2.60 (*m*)
6	2.00–2.70		2.10–2.35
7	5.40 (m)		5.34 (m)
8a	2.87 (dd 17.7-6.7)		2.85 (dd 17.0-6.3)
8b	2.65 (*dd* 17.7. 6.6)		2.60 (*dd* 17.0-6.7)
10	2.15 (*s*)		*2.18 (s)
2'		5.88 (dd 15.8–1.1)	5.91 (*dd 15.7-2.1*)
3'		*6.79* (dd 15.8–6.5)	6.98 (dd 15.7–3.1)
4'		4.08 (dddd 10.3-6.5 m-3.2-1.1)	4.42 (*m*)
5'		1.54–1.92 (m)	1.91 (*m*)
6'		1.54–1.92 (m)	1.80 (*m*)
7'		*5.37 (m)*	*5.54 (m)*
X'a		*2.88* (dd 17.0-8.4)	2.99 (dd 17.4-7.8)
8'b		2.71 (dd 17.0–5.1)	2.69 (*dd* 17.4–5.6)
10		2.15 (s)	* 2.15 (*s*)

*May be reversed.

Table 2. ^{13}C NMR data (DMSO-d,; 65.1 MHz)

C	1 δ (ppm) mult.	2 δ (ppm) mult.
1	164.2 *s*	164.8 *s*
2	129.9 *d*	120.6 *d*
3	139.6 *d*	150.7 *d*
4	199.9 *s*	*68.6 *d*
5	36.9 t	30.6 *t*
6	29.2 *t*	26.7 *t*
7	70.7 *d*	*69.3 *d*
8	45.9 *t*	45.1 *t*
9	205.2 *s*	205.8 s
10	30.1 *q*	30.1 *q*

*May be reversed

Purification by TLC of fraction VI gave vermiculinol (3) which was crystallized from methanol. The pseudomolecular ion $[M+H]^+$ appeared at m/z 395 in its CI mass spectrum leading to the molecular formula $C_{20}H_{26}O_8$. Its IR spectrum showed absorption of hydroxyl group (ν 3539 cm^{-1}). Acetylation of 3 gave a monoacetate $C_{22}H_{28}O_9$. The ^{1}H NMR spectrum of vermiculinol3, more complex than those of 1 and 2, showed the spin systems of 1 and 2 (Table 1). The results were indicative of the asymmetric structure 3. Oxidation of 3 with Jones reagent gave vermiculine, which was in agreement with the proposed structure.

Confirmation of structures was provided from Jones oxidation of 2 and 3 which both led to 1. The structure of this antibiotic had been clearly elucidated by an X-ray analysis [3], showing unambiguously that vermiculine adopts a 16-membered macrocyclic ring geometry. The absolute configuration 7*S*, 7'S of the asymmetric centres C(7), C(7') of vermiculine was deduced from synthesis [7] and correlations with the structure of azalomycine B, macrodiolide with a 16-membered dilactone ring [8]. Since the vermiculine obtained from 2 or 3 had the same rotatory power as the natural one ($\alpha_D - 15°$), compounds 2 and 3 must have the same absolute S configuration at c-7.

In order to determine the relative configuration of the two other asymmetric carbon atoms C(4) and C(4') of vermiculidiol 2 a crystal was submitted to an X-ray analysis (Table 3).

The 16-membered macrocyclic ring has a two-fold crystallographic axis, perpendicular to the best molecular least-squares plane. The two lateral chains, branched on C(7) and C(7') and the hydroxyl groups are situated on the same side with regard to the mean-plane of the macrocyclic ring. Taking into account the configuration (S) of the carbon atoms (7) and (7'), The configuration (S) is assigned to asymmetric carbon atoms (4) and (4') of 2.

EXPERIMENTAL

Penicillium vermiculatum Dang., collected in the soil at Yaounde (Cameroon) was cultivated on a Czapek-Dox medium for 30 days at 27° in a 141 fermentator, with aeration and stirring. The culture filtrate was extracted several times with EtOAc, the extracts combined and the solvent evapd. The residue was dissolved in MeOH and vermiculine crystallized by cooling. The residue obtained after evapn of MeOH was chromatographed over a silica gel column with EtOAc–toluene–HCO_2H (10:5: 1). Eight fractions were collected; the main component of each fraction was further purified by TLC on silica gel plates eluted with $CHCl_3$–MeOH: (47: 3). When analysed by TLC (silica gel; EtOAc–toluene–HCO_2H:10: 5: 1) the following R, were measured: dehydroaltenusin (0.62), vermistatin (0.42). vermiculine (0.20), vermiculinol (0.08) and vermiculidiol (0.02).

Vermiculine (1). $C_{20}H_{24}O_8$, mp 160–162° (MeOH), (lit. 175–177° dec. [1]); $\alpha_D^{24} - 15°$ (MeOH; *c* 0.1); IR ν^{KBr} cm^{-1}: 2934,

1730, 1690, 1637,1297, 1169, 1029, 980, 908, 721; MS (EI, 70 eV, 200") m/z (%): 392 (2, $M^{+\cdot}$), 197 (81), 180 (24), 179 (25), 178 (29), 137 (45), 109 (42), 99 (100), 72 (87).

Vermiculidiol (2). $C_{20}H_{28}O_8$; mp 173-174" (MeOH); α_D^{24} + 34" (MeOH; *c* 0.2); IR ν^{KBr} cm^{-1}: 3567, 2950, 1708, 1703, 1642, 1390, 1355, 1308, 1281, 1217, 1183, 1151, 1029, 1008, 986, 886, 727; MS (EI, 70 eV, 200") m/z (%): 396 (0.2, $M^{+\cdot}$), 339 (1), 295 (2.5), 263 (2), 199 (32),181 (87),180 (95),163 (28), 153 (37),141 (27),139 (80), 138 (44), 123 (37), 122 (35), 97 (48), 43 (100).

Vermiculinol (3). $C_{20}H_{28}O_8$, mp 134–135° (MeOH); α_D^{24} + 6 (MeOH; *c* 0.2); IR ν^{KBr} cm^{-1}: 3539, 2927, 1724, 1700, 1644, 1382, 1299, 1159, 1033, 980, 917, 864, 718, 611; MS (EI, 70 eV, 200°) *m/z* (%): 394 (0.02, $M^{+\cdot}$), 199 (2), 197 (6), 181 (14),180 (6),179 (16), 153 (21), 138 (23), 136 (24), 123 (30), 122 (32), 107 (26), 99 (95), 97 (90), 83 (61), 81 (74), 69 (30), 67 (46), 55 (63), 43 (100); CIMS (NH,) *m/z* (%): 412 $[M+NH_4]^+$, 395 [M+H]+.

Table 3. Fractional atomic coordinates ($\times 10^4$) for non hydrogen atoms for compound $C_{20}H_{28}O_8$

	X	Y	Z	U
0	10887 (1)	7970 (3)	5054 (4)	32 (2)
C 1	10563 (1)	7743 (4)	3113 (6)	32 (2)
0 1	10718 (1)	7959 (4)	1033 (4)	49 (2)
c 2	9997 (1)	7182 (4)	3730 (6)	34 (2)
c 3	9790 (1)	7267 (4)	5969 (7)	32 (2)
c 4	9205 (1)	6735 (4)	6596 (7)	33 (2)
0 4	9201 (1)	5722 (3)	8802 (5)	49 (2)
c 5	8849 (1)	8369 (4)	7005 (8)	37 (3)
C 6	8659 (1)	9195 (4)	4597 (8)	38 (3)
c 7	8574 (1)	11176 (4)	4684 (7)	31 (2)
C 8	8208 (1)	11815 (5)	6738 (7)	35 (3)
c 9	8007 (1)	13706 (5)	6495 (8)	38 (3)
0 9	8041 (1)	14505 (4)	4575 (6)	57 (2)
c 10	7718 (2)	14560 (7)	8723 (10)	54 (4)

E.s.d's are given in parentheses

$U_{eq} = 1/3 \ \Sigma_i \Sigma_j \ U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$.

Diacetyl-vermiculidiol (4). $C_{24}H_{32}O_{10}$; mp 135" (MeOH); IR ν^{KBr} cm^{-1}: 2954, 1741, 1714, 1647, 1375, 1289, 1240, 1178, 1025, 975, 886, 727; MS (CI, NH,) m/z (%): 498 ($[M+NH_4]^+$, 100), 481 ($[M+H]^+$, 5); ^1H NMR (CDCI,, 500 MHz) δ(ppm): 6.79 (1H, *dd*, $J = 15.8$ and 6.5 Hz, H-3), 5.95 (1H, *dd*, $J = 15.8$ and 1.0 Hz, H-2). 5.37 (1H, *m*, H-7), 5.22 (1H, m, H-4), 2.84 (1H, *dd*, $J = 16.6$ and 6.9 Hz, H-8a), 2.60 (1H, *dd*, J = 16.6 and 6.6 Hz, H-8b), 2.08 (3H, *s*, OAc), 2.17 (3H, *s*, Me-CO), 1.60-1.86 (4H, massif, CH_2-5 and CH_2-6).

Vermistatin (5). $C_{18}H_{16}O_6$; mp 210-212" (MeOH), (lit. 213–214°, [4]); IR ν^{KBr} cm^{-1}: 1773, 1664, 1612, 1505, 1460, 1418, 1363, 1327, 1294, 1156, 1106, 1044, 977; MS (EI, 70 eV, 200°) *m/z* (%): 328 (100, M^+), 314 (13), 285 (33), 269 (40), 260 (43), 231 (22), 165 (30), 93 (9), 69 (16); ^1H NMR ($CDCl_3$, 250 MHz) δ(ppm): 7.43 (1H, *s*, H-2'), 6.98 (1H, *d*, $J = 1.9$ Hz, H-7), 6.68 (1H, *d*, $J = 1.9$ Hz, H-5), 6.60 (1H, *dq*, $J = 15.6$ and 6.9 Hz, H-8'), 6.46 (1H, s, H-3), 6.16 (1H, *s*, H-5'), 6.07 (1H, *dq*, $J = 15.6$ and 1.7 Hz, H-7'), 3.88 (3H, s, OMe), 3.79 (3H, s, OMe), 1.92 (3H, *dd*, $J = 6.9$ and 1.7 Hz. Me-9').

Dehydroaltenusin (6). $C_{15}H_{12}O_6$; mp 186187" (MeOH), (lit. 190–193° [6]); IR ν^{KBr} cm^{-1}: 3382, 1671, 1642, 1623, 1394, 1297, 1263, 1227, 1195, 1162, 1077; MS (EI, 70 eV, 200") m/z (%): 288 (32, M^+), 272 (IS), 260 (S), 246 (100), 244 (55), 232 (7), 229 (27), 227 (30), 215 (25), 213 (20), 197 (27), 99 (45); ^1H NMR δ(ppm): 6.73 (1H, *d*, $J = 2.3$ Hz), 6.69 (1H, *s*), 6.63 (1H, *d*, $J = 2.3$ Hz), 6.28 (1H, *s*), 3.91 (3H, s, OMe), 1.73 (3H, *s*, Me), 10.40 and 6.40 (20H).

Crystal *data*. $C_{20}H_{28}O_8$, orthorhombic, space group $P2_12_12_1$, $a = 21.116(8)$. $b = 7.560(4)$. $c = 5.493(3)$ Å; $V \sim 1001$ Å^3, $d_c = 1.31$; $Z = 2$, MoKα radiation, $l = 0.7107$ Å.

The experimental data were collected with a Philips-4-circle diffractometer using graphite monochromated MoKα radiation. From the 1097 measured independent reflections, 965 were significant [$I > 3\sigma(I)$]. The reflections were corrected for Lorentz and polarisation effects. No absorption correction was applied.

The structure was solved by direct methods [9]. The atomic coordinates and anisotropic thermal parameters were refined by least-squares refinement [10] to discrepancy factors of R= 5.92% and $R_w = 6.18\%$. The function minimized in the refinement was $\Sigma W(|F_o| - |F_{ct}|)^2$ with a weighting scheme $W = [\sigma^2(F_o) + 0.0016(F_o)^2]^{-1}$. All hydrogen atoms were found on difference Fourier syntheses and introduced in the calculations

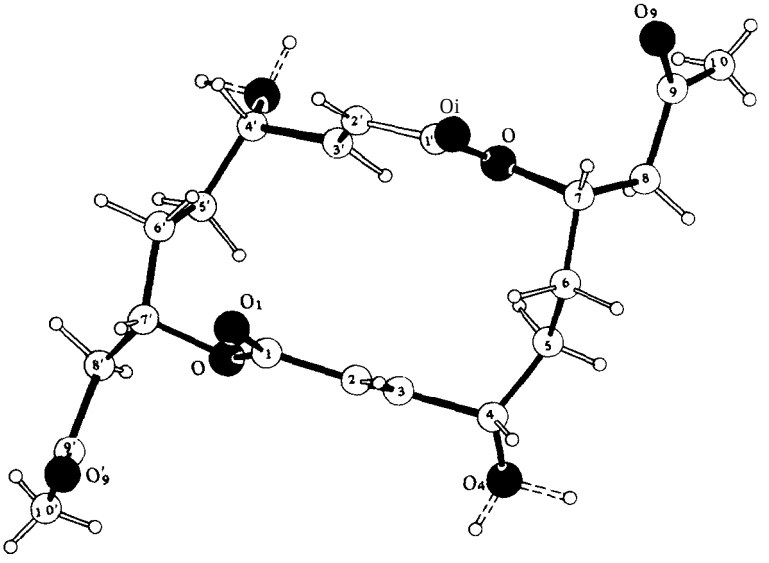


Fig. 1. Perspective view of vermiculidiol (2) showing the crystallographic numbering scheme.

with the equivalent isotropic thermal factor of the bonded atom. Their atomic coordinates were refined. A disorder is observed on the H atom of the hydroxyl group located atC(4). The occupancy factors are (2/3,1/3). No peak >0.3 e $Å^{-3}$ are observed on the final difference syntheses.

Final atomic coordinates are given in Table 3. The anisotropic thermal parameters, bond distances and angles, for this work, are available on request to the Director of the Cambridge Crystallographic Data Centre University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. The list of observed and calculated structure factors is available from the authors at the Institut de Chimie des Substances Naturelles. Figure 1 gives the perspective view of compound 2 with the numbering scheme.

Biological activity. Antimicrobial and antifungal activities were observed by using the agar impregnated disk diffusion method. ***Staphylococcus aureus (209*** P) (6 mm disk impregnated with 500 μg of substance were used), inhibition zone (mm): **1** (15 mm), 2 (0 mm), 3 (11 mm) and 6 (12 mm). *Penicillium crustosum (9* mm disk impregnated with 300 μg ofsubstance were used), inhibition zone (mm): 1 (0 mm) and 6 (15 mm).

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