ANOTHER ANTIBIOTIC FROM THE BASIDIOMYCETE *Oudemansiella mucida*

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The structure *III* was assigned on the basis of spectroscopic measurements to an another antifungal antibiotic isolated from the mycelial culture of the fungus *Oudemansiella mucida*.

Two related compounds have been so far detected in the cultures of basidiomycete *Oudemansiella mucida*: mucidin (*I*) (refs[1-3], Mucidermin Spofa) and oudemansin[4] (*II*). Mucidin was found[5] to be identical to strobilurin A (ref.[6]). Its biosynthesis is connected to shikimate and acetato-malonate pathways[7]. Both compounds were also synthetized[8-10]. Another compound with antifungal activity was isolated in the course of work with the aforementioned fungus[11]. This paper is devoted to its structure elucidation.

New compound is colorless and optically inactive. It has elemental composition $C_{17}H_{20}O_4$ (high resolution mass spectroscopy and elemental analysis) *i.e.* it contains the increment CH_2O more than mucidin. This difference is observed not only for the molecular ions in the mass spectra but also for their daughter ions, including the base peak, *m/z* 151 (Fig. 1). However, the composition of the *m/z* 75 ion ($C_3H_7O_2$) remained unchanged. This ion suggests the presence of β-methoxyacrylate moiety in the molecule[6]. This conclusion is further supported by signals of corresponding protons (7·423 s, 3·730 s and 3·832 s) in the ^{1}H NMR spectrum (Table I). Comparison of corresponding vicinal proton–proton coupling constants (Table II) shows that both *I* and *III* have the same (*all-trans*) relative configuration of the double bonds. Also their UV/VIS spectra are very similar. New metabolite has one aromatic proton less and contains an additional methoxy group. This group gives rise to a singlet at 3·79 ppm that indicates its attachement to a sp^2-type carbon. Remaining four aromatic protons form an AA'BB' system (6·827 and 7·285 ppm, $J_{AB} + J_{AB'} = 9$ Hz), typical for a *para*-disubstituted aromatic ring. This conclusion is supported by ^{13}C NMR spectrum (Table I). The changes in ^{13}C chemical shifts are consistent with a known substituent effect of the methoxyl group[12]: besides

TABLE I

Comparison of ^1H and ^{13}C chemical shifts of compounds *I* and *III*

Atom	^1H NMR[a]		^{13}C NMR[b]		
	I	*III*	*I*[c]	*III*	Δ[d]
1	—	—	167·3 s	167·9 s	0·6
2	—	—	110·3 s	110·0 s	— 0·7
3	—	—	131·0 s	130·7 s	— 0·3
4	6·230 ddq	6·227 ddq	129·4 d	129·9 d	0·5
5	6·638 dd	6·515 dd	126·2 d	124·6 d	— 1·6
6	6·487 dd	6·384 dd	130·7 d	130·7 d	0·0
7	7·432 s	7·423 s	158·5 d	158·7 d	0·2
8	1·970 d	1·960 d	23·3 q	23·5 q	0·2
9	3·730 s	3·730 s	51·1 q	51·5 q	0·4
10	3·840 s	3·832 s	61·4 q	61·9 q	0·5
11	—	—	137·4 s	129·9 s	— 7·5
12, 16	7·130	6·827[e]	125·9 d[f]	127·5 d[f]	1·6
13, 15	to	7·285[e]	128·1 d[f]	114·0 d[f]	—14·1
14	7·380 mt	—	126·8 d	158·9 s	32·1
17	—	3·790 s	—	55·2 q	—

[a] C^2HCl_3, 200 MHz, chemical shift in δ-scale followed by the abbreviation of signal multiplicity; [b] C^2HCl_3, 15·036 MHz, chemical shift given in δ-scale, multiplicity from the offresonance experiment; [c] ref.[7]; [d] difference $\delta_{III} - \delta_I$ (ppm); [e] AA'BB'system; [f] 2C.

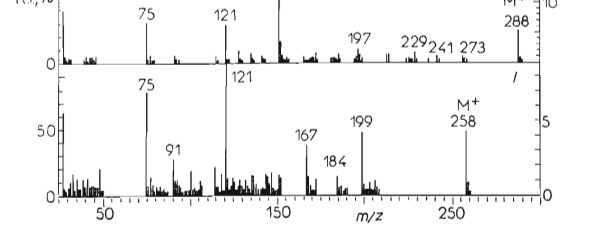
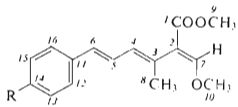


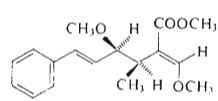
FIG. 1

Mass spectra of compounds *I* and *III*

marked downfield shift of the substituted atom, shifts in the same direction are observed in *ortho-* and *para-* positions; the effect in the side chain decreases with increasing distance from the site of substitution. Therefore, the structure *III* is assigned to the investigated compound. Its biogenesis is very likely similar to that of *I* since the additional oxidations are not uncommon with the shikimate pathway metabolites.



I, R = H
III, R = OCH_3



II

Data on biological activity (Table III) were obtained by a linear regression of the relationship $d = d_0 + k \cdot \ln c$ between the diameter of the inhibition zone d and the concentration of tested substance c. The new compound inhibited the growth of the same test organisms as *I* but it was less effective; its activity increased with increasing concentration more rapidly (*Fusarium oxysporum* being an exception). It seems to be more toxic in peroral application to mouse (LD_{50} 422·5 and 825 mg/kg for *III* and *I*, respectively).

EXPERIMENTAL

Oudemansiella mucida was cultivated as described earlier[1-3]. UV spectra were measured in methanol on a Unicam SP-700 instrument. Infrared spectra were measured in KBr pellets on a Unicam SP-200 G spectrophotometer. Mass spectra were recorded using Varian MAT 311 instrument (70 eV, ionizing current 1 mA, direct inlet temperature 150°C, ion source temperature 200°C). High resolution measurements (±5 ppm) were performed using the "peak-matching"

TABLE II

^{1}H NMR coupling constants (Hz, at 200 MHz) of compounds *I* and *III*

Compound	Coupled protons			
	4·5	4·6	4·8	5·6
I	10·0	1·0	1·3	15·4
III	8·0	2·4	1·4	15·4

technique with perfluorokerosene standard. ^{1}H NMR spectra were measured on a Varian XL-200 spectrometer (200 MHz, FT mode, deuteriochloroform, tetramethylsilane, 25 °C), ^{13}C NMR spectra on a Jeol FX-60 instrument (15·036 MHz, FT mode, deuteriochloroform, tetramethylsilane, 25 °C). Chemical shifts were calculated (± 0·001 and ± 0·03 ppm for ^{1}H and ^{13}C, respectively) from the digitally obtained address differences. They are in the δ-scale. Biological activity of *I* and *III* was determined by an agar diffusion test. The results were evaluated by the MLTR program[13]. Acute toxicity to mouse (Velaz, average weight 20 g) was tested by peroral application of olive oil solution. The results were evaluated by probit method[14].

Isolation

Mother liquors (1 800 g) after crystallization of mucidin were evaporated. The chromatographic treatment of the residue on aluminum oxide (compound–adsorbent 1 : 10) in the system light petroleum–ether 9 : 1 was stopped after the elution of mucidin. The adsorbent was extruded from the column, sliced off and the portion containing compound exhibiting dark fluorescence in the UV light was extracted by chloroform. Light petroleum was added to the yellow oil remaining after the solvent evaporation and the mixture was allowed to stand 12 h at +3°C. Compound *III* (196 g) was filtered off, washed with light petroleum and dried *in vacuo*. Pure compound obtained by crystallization from light petroleum melted at 70—71°C.

1-Methoxy-2-methoxycarbonyl-3-methyl-6-(4′-methoxyphenyl)hexa-(1*E*, 3*E*, 5*E*)-triene (*III*)

For $C_{17}H_{20}O_4$ (288·3) was calculated 70·81% C, 6·99% H, found: 71·0% C, 7·11% H. UV spectrum (methanol, λ_{max}, ε): 222 (21 400), 227 (21 200), 299 (29 400). IR spectrum (KBr): 665, 743, 768, 795, 814, 832, 859, 884, 918, 972, 1 001, 1 031, 1 073, 1 120, 1 148, 1 185, 1 195, 1 240, 1 255,

TABLE III

Comparison of biological activity of compounds *I* and *III*

Test organism[a]	Compound	d_0 mm	k[b]	s.e.c.[c]	r[d]	F[e]
Candida pseudotropicalis	*I*	28·0	3·605 ± 0·166	0·608	0·995	473·5
	III	13·7	4·687 ± 0·171	0·627	0·997	752·2
Saccharomyces cerevisiae	*I*	42·5	3·760 ± 0·450	1·653	0·966	69·7
	III	35·9	4·841 ± 0·280	1·027	0·992	299·0
Torula utilis	*I*	26·8	3·605 ± 0·295	1·081	0·984	149·8
	III	13·3	4·352 ± 0·113	0·415	0·998	1 605·5
Fusarium oxysporum	*I*	29·5	4·945 ± 0·996	3·654	0·912	24·7
	III	24·1	3·812 ± 0·419	1·538	0·971	82·7

[a] Agar diffusion test, regression equation $d = d_0 + k \cdot \ln c$; [b] regression coefficient and its error; [c] standard error of the estimate; [d] multiple correlation coefficient; [e] F-value.

1 303, 1 319, 1 339, 1 370, 1 396, 1 438, 1 447, 1 471, 1 508, 1 519, 1 579, 1 610, 1 630, 1 647, 1 714, 1 831, 1 845, 2 880, 2 910, 2 945, 2 965, 3 030, 3 070 cm^{-1}. Mass spectrum *m/z* (% of relative intensity, elemental composition): 288 (23, $C_{17}H_{20}O_4$, M^+), 273 (0·3, $C_{16}H_{17}O_4$), 256 (3, $C_{16}H_{16}O_3$), 241 (5, $C_{15}H_{13}O_3$), 229 (6, $C_{15}H_{17}O_2$), 214 (6, $C_{14}H_{14}O_2$), 213 (6, $C_{14}H_{13}O_2$), 197 (10, $C_{14}H_{13}O$), 185 (7, $C_{13}H_{13}O$), 151 (100, $C_9H_{11}O_2$), 141 (5, $C_{11}H_9$), 135 (7, $C_8H_7O_2$), 128 (9, $C_{10}H_8$), 121 (28, C_8H_9O), 115 (5, C_9H_7), 91 (5, C_7H_7), 75 (30, $C_3H_7O_2$).

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