Phytochemistry, Vol 27, No 5, pp 1517–1519, 1988
Printed in Great Britain

0031–9422/88 $3.00 + 0.00
Pergamon Press plc

SORDARIOL AND RELATED COMPOUNDS, HEXAKETIDES IN THE FUNGUS *SORDARIA MACROSPORA*

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(*Received* 24 *June* 1987)

Key Word Index—*Sordaria macrospora*, Ascomycete; hexaketides, sordariol, pyriculol.

Abstract—Extracts of the fungus *Sordaria macrospora* afforded three new hexaketides: sordariol (2-hydroxy-6-(3,4-dihydroxypent-1-enyl)-benzyl alcohol] and two related cyclization products. The structures were elucidated by spectroscopic methods.

INTRODUCTION

In a previous study [1], we used *Sordaria macrospora*, as an experimental model to study the physiology of fruiting and the fruiting active substances of fungal origin. The genetics and biology of this fungus are well documented (see, for example, [2]), but little is known of its biochemistry.

This paper reports the isolation and structural elucidation, from the medium and the mycelium, of three new hexaketides which are chemically related to the heptaketide pyriculol family, first identified in *Pyricularia oryzae* [3, 4].

The main substance is named sordariol and its chemical structure has been established as **1** by spectral studies.

RESULTS AND DISCUSSION

Analysis of both medium and mycelium extracts by TLC on silica gel with apolar solvents showed the presence of several compounds, which all displayed colours with bisdiazotized benzidine. Among them, three compounds have been isolated and their chemical structure elucidated.

The main compound, named sordariol, gave an intense red colour with the diazo reagent. Its UV spectrum was that of a styrene type molecule in which the aromatic ring is coplanar with a double-bond

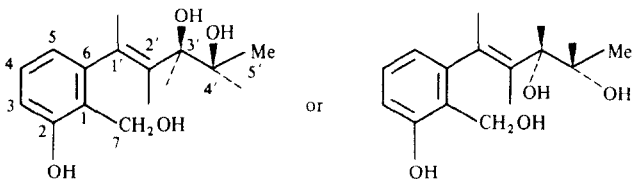
The molecular ion was not observed in EIMS but in DCIMS (NH_3) an $[M+NH_4]^+$ ion (m/z 242) was obtained. The EIMS of the TMSi derivative, showed the presence of four silyl groups ^{1}H NMR studies (Table 1) showed the presence of a side chain, attached by a double bond to an *o*-trisubstituted aromatic ring; the proposed structure of this side chain was deduced from irradiation experiments. The chemical shift of H-3′ and H-4′ indicated they must be linked to C atoms attached to oxygen as for a diol group. This was shown with TMSi and acetonide (**3**) derivatives. The other substituents of the aromatic ring had to be a hydroxymethyl group (δ4.78, *s*) and then, to explain the last TMSi group, a hydroxyl group located *ortho* to the hydroxy methylene (formation of the diacetonide **4**).

The respective positions of both these substituents were deduced: (1) from the absence of a long range coupling between H-7 and H-3, (2). from ^{13}C NMR (Table 2) and (3) from comparison of the ^{1}H NMR spectra of sordariol with those of 2-hydroxy-6-(3,4-dihydroxy-hepta-1,5-dienyl)-benzyl alcohol (**5b**) isolated from *P. oryzae* [4] and its 2-*O*-methyl derivative (**5c**), isolated from *Aspergillus variecolor* [5]. The configuration of the diol group in 3′,4′ as *erythro*, was deduced from a positive NOE between Me-5′ and H-3′ and the absence of a long range coupling between the same protons in the ^{1}H NMR spectrum of the acetonide derivative (**3**) (Table 1) [6].

UV examination of the TLC of the medium and mycelium extracts of *S. macrospora* showed that sordariol was the major component However, the use of bisdiazotized benzidine, established the presence of several other substances. Among them, we have identified two compounds (**2a** and **2b**) that possessed the same UV absorption maxima [(MeOH) 205, 268, 278 and 310 (sh) nm], gave the same intense orange reaction with the diazo reagent and the same MS for their TMSi derivatives, although they can be separated by TLC, HPLC and GC(TMSi).

^{1}H NMR (Table 1) established that compounds **2a** and **2b** are formed from sordariol by a ring closure forming an ether linkage between CH_2-7 and C-1′ to give an isobenzofuranyl group. Two similar compounds have been isolated from *P oryzae* [4] but their stereochemistry has not been established. The authors suggested that these compounds could be stereoisomers at the 1′ position. This seems obvious for **2a** and **2b** because of their easy chromatographic separation. Their configuration is under investigation. The co-occurrence of these compounds suggested that they could be isolation artefacts. However, the extraction was achieved directly from the culture medium either with EtOAc or with BuOH and we have observed their presence in the crude medium both by TLC and HPLC. Moreover, these compounds were never obtained from sordariol, by TLC on silica gel. Therefore, we think that if sordariol gives these compounds, it may be transformed by the fungus itself.

Several other substances have been isolated from this fungus, their structures are under investigation. In *P. oryzae*, pyriculol (**5a**) has been shown to be phytotoxic and to be responsible for the disease caused by the fungus



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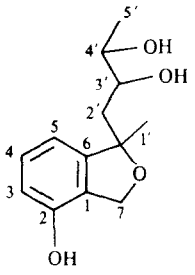
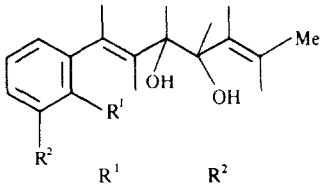
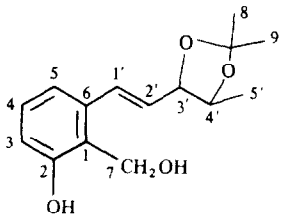


2a and 2b



	R^1	R^2	
5a	CHO	OH	Pyriculol
5b	CH_2OH	OH	
5c	CH_2OH	OMe	



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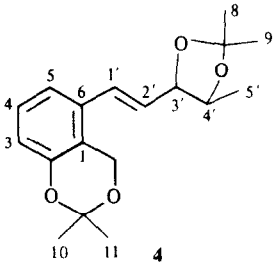

4

on rice By contrast, compound **5b**, in which the aldehyde group is reduced, was non-phytotoxic [4]

We have shown that sordariol is not phytotoxic towards rice roots (concentrations up to 750 ppm do not restrict the growth) or rice shoots. However, browning (but no necrosis) is induced on the leaves of certain varieties, resistant to the *P. oryzae* disease

EXPERIMENTAL

^1H and ^{13}C NMR, CAMECA, 350 MHz, TMS as int reference

Fungi Wild strain of *Sordaria macrospora* (Auersw) was obtained from D. Zickler and G Leblon's collection, Laboratoire des Interactions Moléculaires Génomiques, Université Paris-Sud, F-91405 Orsay

Production, extraction and purification of the metabolites S *macrospora* was maintained and grown for metabolite production on the same medium containing (g/l): glucose (10), yeast extract (3), KH_2PO_4(0 5), K_2HPO_4(0 6), $MgSO_4$ (0 5), biotin and thiamine (25 μg/l each) and trace elements (Zn, Fe, Cu, Mn, Mo), under alternating light and dark (12 hr/12 hr), at 22°

After incubation, the mycelium was separated from the broth by filtration through glass-wool and both separately extracted The broth was poured in a funnel and extracted (× 3) with *n*-BuOH (0 5 vol) The mycelium was washed with distilled H_2O and extracted with boiling 80% MeOH The crude extracts were concentrated and the dry residues taken-up in the minimum vol of MeOH. Purifications were achieved by using Chromatotron and TLC, on silica gels with the solvents Et_2O–Me_2CO (9 1) and hexane–EtOAc–MeOH (6 4 1).

Sordariol [*2-hydroxy-6-(3,4-dihydroxy-pent-1-enyl)-benzyl-alcohol*] (**1**) Amorphous powder, UV λ_{max}^{MeOH} nm (log ε) 217 (22 000), 252 (8600) and 295 (2000), EIMS *m/z* (rel int) $[M]^+$ absent, 162 (80), 161 (72), 133 (100), DCIMS (NH_3) *m/z* (rel int) 242 $[M+NH_4]^+$ (16), 224 (66), 206 (13), 189 (100), 171 (25), 162 (29), 161 (13), 133 (39), EIMS of the TMSi derivative, *m/z* (rel int) 497 $[M-15]^+$ ($C_{23}H_{45}O_4Si_4$) (0 3), 422 $[M-TMSiOH]^+$ ($C_{21}H_{38}O_3Si_3$) (0.5), 407 (2), 395 (17), 332 $[M-2\times TMSiOH]^+$ (5), 306 (20), 243 (5), 203 (100), 191 (39), 147 (24), 117 (52), 103 (11) 73 (89)

2a *and* **2b** [*1-(2,3-dihydroxybutyl)-1,3,-dihydro-isobenzofuran-4-ols*] Compound **2a** yielded white needles and **2b** an amorphous powder Both have the same UV λ_{max}^{MeOH} nm 222 (sh), 268, 278; $\lambda_{max}^{MeOH+OH^-}$ nm 215, 260, 305 EIMS of the TMSi derivatives (**2a**) *m/z* (rel int) 350 $[M-TMSiOH]^+$ ($C_{18}H_{30}O_3Si_2$) (0 3), 335 (0 3), 323 (0 6), 306 (1), 233 (33), 220 (12), 207 (100), 147 (5), 117 (26), 73 (29) (**2b**) *m/z* (rel int) 425 $[M-15]^+$ ($C_{20}H_{37}O_4Si_3$), 395 (1), 350 $[M-TMSiOH]^+$ ($C_{18}H_{30}O_3Si_2$) (2), 335 (1), 306 (1), 323 (M − 117) (1), 322 (1), 321 (1), 233 (40), 220 (10), 207 (100), 117 (17)

Acetonides **3** *and* **4** To 5 mg of sordariol were added Me_2CO (0 5 ml) and methylchoroformate (0 01 ml) and the mixture left 24 hr at room temp Purification by TLC (Et_2O–Me_2CO, 9 1) gave a monoacetonide (**3**) and a diacetonide (**4**) Compound **3** EIMS *m/z* (rel int) 264 $[M]^+$ (3), 246 $[M-H_2O]^+$ (1), 220 (20), 202 (25), 189 (24), 187 (19), 173 (23), 162 (44), 159 (19), 158 (20), 144 (58), 133 (28), 132 (32), 131 (24), 116 (32), 115 (34), 58 (57), 43 (100)

Table 1. ^{1}H NMR chemical shifts of compounds **1**, **2a**, **2b**, **3** and **4***

H	1	2a	2b	3	4
3	6 72 *dd* (8, 1 3)	6.66 *t* (8)	6 66 *d* (8)	6 77 *dd* (8, 1.3)	6 70 *dd* (8, 1 3)
4	7 07 *t* (8, 8)	7.10 *t* (8, 8)	7 11 *t* (8, 8)	7 09 *t* (8, 8)	7.10–7 20 *m*
5	7 00 *dd* (8, 1 3)	6.66 *t* (8)	6 74 *d* (8)	7 00 *dd* (8, 1 3)	7.10–7 20 *m*
1′	7 0 *brd* (15)	5 41 *m*	5 37 *m*	6 98 *brd* (16)	6.60 *brd* (16)
2′a	6 17 *dd* (15, 6 5)	1.83 *m*	1 77 *ddd* (14, 8, 8)	6 07 *dd* (16, 7 5)	6 15 *dd* (16, 7 5)
2′b	—	—	2 06 *ddd* (14, 3.5, 3 5)	—	—
3′	4 07 *ddd* (6.5, 6.5, 1 3)	3.71 *m*	3.70 *m*	4 71 *ddd*† (7.5, 6 5, 1 3)	4.70 *ddd* (7.5, 6.5, 1 3)
4′	3 77 *dt* (6 5, 6 5)	3.60 *quint* (6 5)	3 62 *m*	4 40 *quint* (6 5)	4.40 *quint* (6 5)
5′	1 20 *d* (6 5)	1 17 *d* (6 5)	1.19 *d* (6 5)	1 14 *d*† (6 5)	1 14 *d* (6 5)
7a	4 78 *s*	4.95 *brd* (12)	4 97 *brd*	4.91 *s*	4.91 *s*
7b	—	5 05 *dd* (12, 3)	5 09 *dd* (12, 3)	—	—
8	—	—	—	1.33 *s*	1 33 *s*
9	—	—	—	1.45 *s*	1.46 *s*
10	—	—	—	—	1.49 *s*
11	—	—	—	—	1 49 *s*

*1, **2a** and **2b** in MeOD, **3** and **4** in $(CD_3)_2CO$, coupling constant (J in Hz) are given in parenthesis
†Positive NOE, long range coupling absent

Table 2 ^{13}C NMR chemical shifts of carbon of compound **1** (MeOD)

C	δ	C	δ
1	125 3	1′	129 7
2	157 3	2′	132.5
3	115.4	3′	78.0
4	129 7	4′	71.7
5	130 6	5′	18.9
6	139 6		
7	56 6		

Compound **4**. EIMS *m/z* (rel. int.): 304 $[M]^+$ (7), 260 (9), 246 (6), 229 (6), 202 (56), 187 (26), 184 (5), 173 (33), 171 (39), 159 (44), 158 (31), 145 (82), 144 (41), 132 (39), 131 (24), 115 (70), 43 (100)

Acknowledgements—We thank Dr M H. Lebrun, Laboratoire de Cryptogamie, Université Paris-Sud, Centre d'Orsay, for the toxicity assays, H Waton, CNRS. Solaize, for the NMR data and C Perrin and F Gobert, (Rhône-Poulenc) for NOE experiments

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