New Hexaketides Related to Sordariol in *Sordaria macrospora*

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Broths extracts of the fungus *Sordaria macrospora* afforded new hexaketides related to sordariol: sordarial, the corresponding aldehyde, heptacyclosordariolone and cyclosordariolone, two cyclic compounds. The structure of these substances has been established by spectrometric methods.

Introduction

As a part of our programme to explore the chemistry of the Ascomycete *Sordaria macrospora,* we have first investigated the compounds present in the culture filtrates of the wild strain and of several mutant strains altered in their pigmentation.

S. macrospora seems remarkable for its production of two types of secondary metabolites derivated from the polyketide pathway.

Indeed, in a previous investigation [1], we have identified, in the culture broth of the fungus, three new hexaketides: *trans*-sordariol (**1**) and the two isobenzofuranyl derivatives **7a** and **7b**. Subsequently [2], we have demonstrated that melanin in this fungus was biosynthesized *via* the 5,8-dihydroxynaphthalene pathway, by isolation and identification of several pentaketides precursors.

We now have thoroughly reinvestigated the metabolites of the culture media of the wild strain at several stages of its developmental cycle and those excreted by two mutant strains.

We describe here the isolation and the chemical identification of three new natural substances related to sordariol and of two of their derivatives.

Results and Discussion

From the EtOAc extracts of the culture media, we have isolated five substances which all, except **5**, show the same *ortho* trisubstituted aromatic cycle as sordariol (**1**).

Compound **2** for which we propose the name sordarial, is the corresponding aldehyde of **1**: their ^{1}H NMR (Table I) mainly differ at the level of H-7 (a

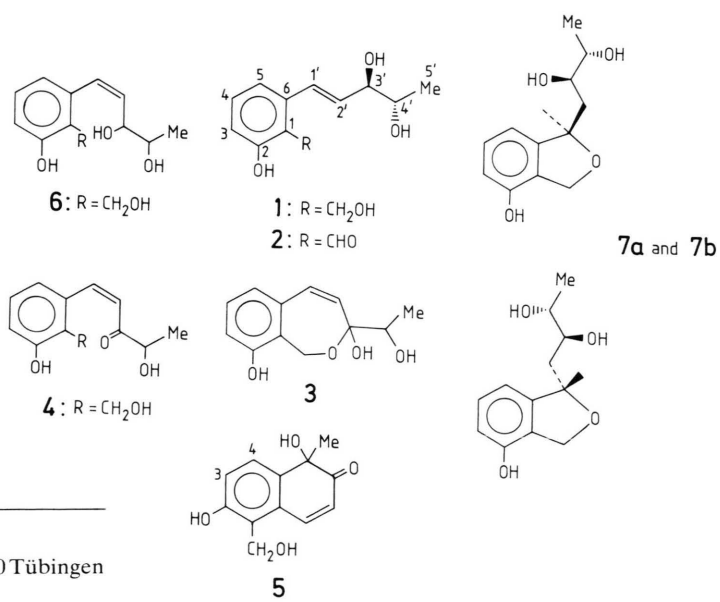

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Table I. ^{1}H NMR chemical shifts of compounds **1** to **6** (MeOD).

H	**1**	**2**	**3a**	**3b**	**4**	**5**	**6**
3	7.00 brd (8.0)	6.84 brd (8.2)	6.81 brd (7.5)	6.81 brd (7.5)	6.83 brd (7.3)	6.91 d (8.3)	6.74 brd (7.7)
4	7.07 t (8.0)	7.50 dd (8.2, 7.7)	7.07 t (7.8)	7.07 t (7.8)	7.09 t (7.8)	7.48 d (8.3)	7.08 t (8.0)
5	6.72 dd (8, 1.3)	7.04 d (7.7)	6.72 brd (8.1)	6.72 brd (8.1)	6.74 brd (7.5)		6.78 brd (7.3)
7a	4.78 s	10.4 s	5.14 d (13.7)	5.12 d (13.7)	5.08 d (13.6)	4.93 d (11.8)	4.72 d (11.6)
7b			4.52 d (13.7)	4.52 d (13.7)	4.54 d (13.6)	4.84 d (11.8)	4.64 d (11.6)
1′	7.00 brd (15.0)	7.26 brd (15.8)	6.65 d (12.6)	6.62 d (12.6)	6.69 d (12.6)	7.98 d (10.5)	6.84 brd (11.4)
2′	6.17 dd (15.0, 6.5)	6.26 dd (15.8, 6.2)	5.92 d (12.6)	5.90 d (12.6)	5.87 d (12.6)	6.15 d (10.2)	5.80 dd (11.4, 9.7)
3′	4.07 ddd (6.5, 6.5, 1.3)	4.10 ddd (6.2, 5.2, 1.5)					4.07 ddd (9.7, 5.1, 1)
4′	3.77 dq (6.5, 6.4)	3.76 dq (5.2, 6.4)	3.65 m	3.65 m	4.07 q (6.5)		3.66 dq (6.5, 5.1)
5′	1.20 d (6.4)	1.22 d (6.4)	1.12 d (6.5)	1.0 d (6.5)	0.99 d (6.5)	1.46 s	1.10 d (6.5)

Coupling constant (J in Hz) are given in parenthesis. In compounds **1**, **2**, **3a**, **3b**, **4**, and **6**, H-3 and H-5 signals could be reversed.

singlet at 10.4 ppm, 1H in **2**, instead of a singlet at 4.78 ppm, 2H in **1**). Therefore, both compounds must have identical stereochemistry at both the double bond (*trans* J = 15 and 15.8 Hz) and the diol function. The *erythro*-configuration of the latter is well established by the presence of a W long-range coupling between CH_3-5′ and H-3′ and the absence of any NOE's in the ^{1}H NMR spectra of the acetonides of **1**, **7a**, and **7b** (Table II). Sordarial is accumulated in the albino-mutant strains Bl_2 and Bl_5 media, however, it is only detected in trace amounts at the beginning of the developmental stage in the wild strain medium (Table III). The aldehyde group must be quickly reduced to the corresponding alcohol (sordariol). A comparison could be made between the couple sordarial–sordariol and the vinylogous couple: pyriculol and its reduction product. The latter compounds possess also *erythro*-configuration and have been isolated from the culture medium of *Pyricularia oryzae* [3, 4]. In the same way as sordarial, pyriculol also shows a very transient existence [5].

Compound **3** appears quantitatively important at the beginning of the developmental stage, in all media studied. It shows UV maxima at 254 and 294 nm and gives an intense red-violet colour with diazo reagent. This compound has been isolated and purified with difficulty, because of its instability: it shows a tendency to give several products (mainly one) with the same absorbance and colouring reaction, distinguished by HPLC and TLC. This transformation occurs even during ^{1}H and ^{13}C NMR recordings whose data only allow us to conclude that the product mainly exists as a mixture of two diastereoisomers with the heptacyclic structure **3**. Indeed, the ^{1}H NMR recording is the sum of two almost identical spectra partly overlapped in the aromatic field, but separated elsewhere. An endocyclic double bond (J = 12.6 Hz) can be recognized. Finally the spectra show great analogies with that of compound **1** but the lack of the H-3′ and the two signals given by H-7, as in the spectra of the cyclic compounds **7a**, **7b** (Ref. [1] and Table II) and **5** and also of the *cis*-sordariol (**6**), make them different. The remaining H has to be

Table II. ^{1}H NMR chemical shifts of acetonides of **1**, **7a**, and **7b** ($CDCl_3$).

H	**1**	**7a**	**7b**
3	6.77 dd (7.7, 1.3)	6.77 d (7.5)	6.83 d (7.4)
4	7.09 t (8)	7.17 t (7.7)	7.16 t (7.7)
5	7.00 dd (7.5, 1.3)	6.66 d (8)	6.67 d (7.9)
7a	4.91 s	5.15 dd (12, 2.7)	5.15 dd (12.1, 2.7)
7b	–	5.08 brd (12)	5.07 brd (12.1)
1′	6.98 brd (16)	5.43 brd (10)	5.36 m
2′a	6.07 dd (16, 7.5)	1.99 ddd (14, 10, 2.2)	2.10 dt (13, 7)
2′b	–	1.65 ddd (14, 3, 2)	1.92 dt (13.7, 5.8)
3′	4.71 ddd* (7.5, 6.5, 1.3)	4.45 m*	4.28 m*
4′	4.40 quint. (6.5)	4.33 quint. (6.3)	4.28 m
5′	1.14 d* (6.5)	1.16 d* (6.3)	1.20 d* (6)
Me(a)	1.45 s	1.51 s	1.47 s
Me(b)	1.33 s	1.39 s	1.33 s

Coupling constant (in Hz) are given in parenthesis.
* W long-range coupling, absence of NOE.
H-3 and H-5 signals could be reversed.

H-4′, since its irradiation only affects the CH_3-5′ coupling.

In the ^{13}C NMR spectrum, two peaks (δ = 103.2 and 102.4 ppm) characterize a *semi*-aldehydic quaternary C.

GC/MS coupling of the TMSi derivatives shows the presence of 3 TMSi groups (M^+ at m/z = 438) and a m/z = 117 ion corresponding to the CH_3–CH–OTMSi terminal group.

Compound **4** is the open tautomer of **3**. This compound shows the same UV spectrum as **3**, but differs by the yellow colour reaction with diazo reagent and a more lipophilic character (Table IV). Their ^{1}H NMR spectra are almost identical, however, the spectrum of **4** shows no doubling and a deshielded H-4′.

That could be explained by the presence of a CO group at C-3′. The MS (FAB m/z MH^+ = 223 and HPLC/MS coupling MH^+ = 223 and $M-NH_4^+$ = 240), and the presence of a CO peak in the IR spectrum confirm this structure.

4 likely is a work-up product because it is not observed by TLC or HPLC of the crude extract. In the course of the purification set **3**(**a** or **b**) must be opened.

We propose to name the open product **4**, sordariolone, and the cyclic tautomer **3**, heptacyclosordariolone. One of the diastereoisomers of the latter represents the natural form of this tautomerie.

Compound **5** shows an UV spectrum with maxima at 258 and 310 nm, and a shoulder at 382 nm explaining its yellow-green colour. The MS of the TMSi

Table III. Occurrence of hexaketides **1** to **7b** in the culture media of:

	the wild strain			the albino-mutant strains
	a	b	c	a
1	+++	++++	++	++++
2	( )	no	no	++++
3	++++	no	no	++++
5	++	+++	+	+++
6	no	no	++	no
7a	no	++	+++	++
7b	no	+	++	+

a) At the beginning (day 5) and b) the end (day 10) of the developmental cycle c) in old cultures medium. Production of the compound increase from ( ) trace to ++++.

Table IV. TLC and HPLC data of compounds **1** to **7b**.

	TLC[a] $Rf \times 100$	HPLC Rt_{min}		Colour with diazo reagent[c]
		1[b]	2[b]	
1	32	14.2	7.0	red
2	58	27.0	14.2	brown
3	61	19.6	–	red-violet
4	76	–	26.0	yellow
5	48	15.2	7.4	light brown
6	33	14.2		red
7a	45	16.2		yellow
7b	48	17.2		yellow

[a] Silica gel, hexane–EtOAc–MEOH 6:4:1.
[b] Reverse phase C_{18} column: 1) B in A, from 8% to 45% at 1 ml·min^{-1}, in 35 min.; 2) from 15% of B in A to 100% of B at 1 ml·min^{-1} in 25 min; A= H_2O–HOAc 100:2; B = ACN–H_2O–HOAc 75:25:2.
[c] *bis*-Diazotized benzidine.

Table V. ^{13}C NMR chemical shifts of carbon of compound **5** (MeOD).

C		C	
1	126.4	1′	143.3
2	156.6	2′	127.3*
3	118.4	3′	206.8
4	124.4*	4′	77.6
5	139	5′	32.3
6	129.9		
7	55.1		

* Signals may be interchanged.

derivative shows the presence of three TMSi groups of a molecule with M^+ at $m/z = 220$.

Comparative analysis of ^1H NMR data relative to **1** and **5** (Table I) shows the following differences for **5**: (i) a tetrasubstituted ring as showed by two *ortho* aromatic H at 7.48 and 6.91 ppm ($J = 8.2$ Hz), (ii) a cyclohexene bond indicated by two one H doublets at 7.98 and 7.4 ppm ($J = 10.5$ Hz), (iii) absence of H-3′, (iv) absence of H-4′ indicated by a CH_3-5′ singlet at 1.46 ppm.

From these results and from the ^{13}C NMR data (see Table V), structure **5** is attributed to this new compound that we name cyclosordariolone.

This compound accumulates in both wild and mutant strains with a delayed production, by comparison with **3**. More stable it persists a longer time in the medium.

Finally, *cis*-sordariol (**6**) has been isolated in very old culture media from both wild and mutant strains. In the ^1H NMR spectrum, *cis*- and *trans*-isomers differ, besides of their double bond coupling: $J_{trans} = 15$ Hz, $J_{cis} = 11.4$ Hz, by the signal of the CH_2OH-7 protons: *trans:* singlet, *cis:* doublet.

Cis-sordariol could be easily obtained by UV irradiation of *trans*-sordariol, therefore we think that it could be an artefact.

Among all the biochemically related structures elucidated in this study, we think that the more oxidized ones (**2**, **3**) are the first synthesized. Correlatively to the developmental cycle, their stabilization is effected, by reduction and by ring cyclization.

Experimental

Fungi

Wild strain and apigmented mutants Bl_2 et Bl_5 of *Sordaria macrospora* (Auersw.) were obtained from G. LEBLON's collection, Laboratoire des Interactions Genomiques, Université Paris-Sud, F-91405 Orsay and maintained and grown as described in [1].

Extraction and purification of metabolites

After incubation, the mycelium was filtered and the broth extracted ($3 \times ½$ vol.) with EtOAc. The crude extracts were concentrated and the dry residues taken-up in the minimum solvent (EtOAc or MeOH). Purification was achieved by using at first, either Ac-polyamide columns with a hexane–EtOAc–MeOH gradient as solvent, or CCTLC with hexane–EtOAc 6:4 and hexane–EtOAc–MeOH 6:4:1 as solvents, then HPLC (C_{18} preparative column with an ACN–H_2O gradient). Analyses were performed by TLC and HPLC (see Table IV).

Physicochemical properties of the compounds

*trans-Sordariol (**1**).* Data about this substance were given in [1] except $[\alpha]_D^{20\,°C} = +10.4°$ ($c = 1.25$ in MeOH).

*trans-Sordarial (**2**).* (2-Hydroxy-6-(3,4-dihydroxy-pent-1-enyl)-benzaldehyde). Yellow powder. UV λ_{max}^{MeOH} nm (ε): 230 (4600), 277 (6300), 351 (3100); UV $\lambda_{max}^{MeOH} + OH^-$ nm: 292 (sh), 395. $[\alpha]_D^{20°C} = +18.2°$ ($c = 2.03$ in MeOH); EI/MS of the TMSi derivative, m/z (rel. int.): 438 (M^+; 0.1), 423 (M-15; 0.2), 394 (9.4), 348 (M–TMSiOH; 2.5), 332 (5), 321 (13), 306 (8), 292 (4.9), 219 (7.3), 203 (9.5), 191 (28), 133 (3), 117 (77), 75 (10), 73 (100).

*Heptacyclosordariolone (**3**).* (3,9-Dihydroxy-9-(1-hydroxyethyl)-2,9 H-benzo-[c]-oxepin). Amorphous powder. Red-violet with *bis*-diazotized benzidine. UV λ_{max}^{MeOH} nm 252, 260 (sh), 300. EI/MS of the TMSi derivatives, m/z (rel. int.): 438 (M^+; 0.4), 423 (M-15; 0.6), 348 (M–TMSiOH; 4), 335 (18), 321 (M–CH_3–CH–OTMSi; 18), 305 (4), 232 (16), 217 (4), 203 (6), 189 (18), 147 (10), 117 (100), 75 (14), 73 (100).

*Sordariolone (**4**).* (1-(-3-Hydroxy-2-hydroxymethyl phenyl)-4-hydroxy-pent-1-en-3-one). Amorphous powder. Yellow with *bis*-diazotized benzidine. UV λ_{max}^{MeOH} nm: 252, 260, 300.

*Cyclosordariolone (**5**).* (1,6-Dihydroxy 5-hydroxymethyl 1-methyl naphthalen-2-one). Yellow-green powder; UV λ_{max}^{MeOH} nm (ε): 208 (16,400), 258 (12,000), 310 (8200), 382 sh (2800); UV $\lambda_{max}^{MeOH} + OH^-$ nm: 240 sh, 280, 328, 420. $[\alpha]_D^{20\,°C} = -77.8°$ ($c = 0.37$ in MeOH). EI/MS of the TMSi derivative: m/z

(rel. int.) 436 (M^+; 5), 421 (M-15; 8), 406 (9), 393 (5.5), 346 (M−TMSiOH; 3), 333 (41), 331 (9), 319 (100), 318 (18), 303 (6), 259 (6), 230 (6), 147 (9), 75 (12), 73 (96).

*cis-Sordariol (**6**).* From the crude extract of the culture medium, separation from the *trans* compound was achieved by TLC. UV and MS data are the same as those obtained with **1**. UV irradiation (254 nm) of **1** in MeOH affords a complete change to **6** after 10 min. After a longer time, degradation occurs.

*Acetonides of **1**, **7a** and **7b**.* To a few mg of **1**, **7a** or **7b** were added Me_2CO (0.5 ml) and methylchloroformate (0.01 ml) and the mixture left 24 h at room temperature. Purification by TLC (hexane−EtOAc 6:4) gave the corresponding acetonides.

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