New Hexaketides Related to Sordariol in *Sordaria macrospora*

Marie Louise Bouillant, Jacques Bernilion, Jean Favre-Bonvin, and Nadia Salin

Laboratoire de Mycochimie (UA CNRS 1127) I.C.B.M.C., Université Claude Bernard (Lyon I), F-69622 Villeurbanne Cedex, France


*Sordaria macrospora*, Ascomycete, New Hexaketides, Sordarial, Sordariol

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 

\[ \text{H NMR (Table I) mainly differ at the level of H-7 (a} \]

Reprint requests to Dr. M. L. Bouillant.

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

<table>
<thead>
<tr>
<th>H</th>
<th>1</th>
<th>2</th>
<th>3a</th>
<th>3b</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>3</td>
<td>7.00 brd</td>
<td>6.84 brd</td>
<td>6.81 brd</td>
<td>6.81 brd</td>
<td>6.83 brd</td>
<td>6.91 d</td>
<td>6.74 brd</td>
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<td>(8.3)</td>
<td>(7.7)</td>
</tr>
<tr>
<td>4</td>
<td>7.07 t</td>
<td>7.50 dd</td>
<td>7.07 t</td>
<td>7.07 t</td>
<td>7.09 t</td>
<td>7.48 d</td>
<td>7.08 t</td>
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<td>(8.3)</td>
<td>(8.0)</td>
</tr>
<tr>
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<tr>
<td></td>
<td>(8.1, 1.3)</td>
<td>(7.7)</td>
<td>(8.1)</td>
<td>(8.1)</td>
<td>(7.5)</td>
<td>(7.3)</td>
<td>(7.3)</td>
</tr>
<tr>
<td>7a</td>
<td>4.78 s</td>
<td>10.4 s</td>
<td>5.14 d</td>
<td>5.12 d</td>
<td>5.08 d</td>
<td>4.93 d</td>
<td>4.72 d</td>
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<td>(13.7)</td>
<td>(13.6)</td>
<td>(11.8)</td>
<td>(11.6)</td>
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<tr>
<td>7b</td>
<td>4.52 d</td>
<td>4.52 d</td>
<td>4.54 d</td>
<td>4.84 d</td>
<td>4.84 d</td>
<td>4.64 d</td>
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<td>(13.7)</td>
<td>(13.6)</td>
<td>(11.8)</td>
<td>(11.6)</td>
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<tr>
<td>1'</td>
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<td>(12.6)</td>
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<td>(12.6)</td>
<td>(10.5)</td>
<td>(11.4)</td>
</tr>
<tr>
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<td>(15.8, 6.2)</td>
<td>(12.6)</td>
<td>(12.6)</td>
<td>(12.6)</td>
<td>(10.2)</td>
<td>(11.4, 9.7)</td>
</tr>
<tr>
<td>3'</td>
<td>4.07 ddd</td>
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<td>3.65 m</td>
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<td>3.66 dq</td>
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<td>(6.2, 5.2, 1.5)</td>
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<td>(6.5)</td>
<td>(6.5, 5.1)</td>
<td></td>
</tr>
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<td>3.77 dq</td>
<td>3.76 dq</td>
<td>3.65 m</td>
<td>3.65 m</td>
<td>4.07 q</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.5, 6.4)</td>
<td>(5.2, 6.4)</td>
<td></td>
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<td>(6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
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<td>1.22 d</td>
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<td>0.99 d</td>
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<td>(6.4)</td>
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<td>(6.5)</td>
<td>(6.5)</td>
<td>(6.5)</td>
<td>(6.5)</td>
</tr>
</tbody>
</table>

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 ($\text{trans } J = 15$ 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 B1 and B5 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 $^1$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. 'H NMR chemical shifts of acetonides of 1, 7a, and 7b (CDCl₃).

<table>
<thead>
<tr>
<th>H</th>
<th>1</th>
<th>7a</th>
<th>7b</th>
</tr>
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<tbody>
<tr>
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<td>(7.7)</td>
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<td>(7.5, 1.3)</td>
<td>(8)</td>
<td>(7.9)</td>
</tr>
<tr>
<td>7a</td>
<td>4.91 s</td>
<td>5.15 dd</td>
<td>5.15 dd</td>
</tr>
<tr>
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<td></td>
<td>(12, 2.7)</td>
<td>(12.1, 2.7)</td>
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<td>5.08 brd</td>
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<td>(12.1)</td>
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<td>(16)</td>
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<td></td>
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<td>2'a</td>
<td>6.07 dd</td>
<td>1.99 ddd</td>
<td>2.10 dt</td>
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<td></td>
<td>(16, 7.5)</td>
<td>(14, 10, 2.2)</td>
<td>(13, 7)</td>
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<td>2'b</td>
<td></td>
<td>1.65 ddd</td>
<td>1.92 dt</td>
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<td>(14, 3, 2)</td>
<td>(13.7, 5.8)</td>
</tr>
<tr>
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<td>4.45 m*</td>
<td>4.28 m*</td>
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<tr>
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<tr>
<td>4'</td>
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<td>4.33 quint.</td>
<td>4.28 m</td>
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<td>(6.3)</td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>1.14 d*</td>
<td>1.16 d*</td>
<td>1.20 d*</td>
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<td>(6)</td>
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<tr>
<td>Me(b)</td>
<td>1.33 s</td>
<td>1.39 s</td>
<td>1.33 s</td>
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Coupling constant (in Hz) are given in parenthesis.

* W long-range coupling, absence of NOE.

H-3 and H-5 signals could be reversed.

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

<table>
<thead>
<tr>
<th>the wild strain</th>
<th>the albino-mutant strains</th>
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<tr>
<td>a</td>
<td>b</td>
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<tr>
<td>1</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>( )</td>
</tr>
<tr>
<td>3</td>
<td>++++</td>
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<td>5</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>no</td>
</tr>
<tr>
<td>7a</td>
<td>no</td>
</tr>
<tr>
<td>7b</td>
<td>no</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Colour with diazo reagent</th>
<th>TLC²</th>
<th>HPLC ² ³</th>
<th>HPLC ³ ²</th>
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<tbody>
<tr>
<td>1</td>
<td>red</td>
<td>32</td>
<td>14.2</td>
</tr>
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<td>2</td>
<td>brown</td>
<td>58</td>
<td>27.0</td>
</tr>
<tr>
<td>3</td>
<td>red-violet</td>
<td>61</td>
<td>19.6</td>
</tr>
<tr>
<td>4</td>
<td>yellow</td>
<td>76</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>light brown</td>
<td>48</td>
<td>15.2</td>
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<tr>
<td>6</td>
<td>red</td>
<td>33</td>
<td>14.2</td>
</tr>
<tr>
<td>7a</td>
<td>yellow</td>
<td>45</td>
<td>16.2</td>
</tr>
<tr>
<td>7b</td>
<td>yellow</td>
<td>48</td>
<td>17.2</td>
</tr>
</tbody>
</table>

³ Reverse phase C₁₈ 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 = H₂O–HOAc 100:2; B = ACN–H₂O–HOAc 75:25:2.

²⁄³ bis-Diazotized benzidine.
Table V. 13 C NMR chemical shifts of carbon of compound 5 (MeOD).

<table>
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<th>C</th>
<th>C'</th>
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<tr>
<td>1</td>
<td>126.4</td>
<td>1' 143.3</td>
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<tr>
<td>2</td>
<td>156.6</td>
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<tr>
<td>3</td>
<td>118.4</td>
<td>3' 206.8</td>
</tr>
<tr>
<td>4</td>
<td>124.4*</td>
<td>4' 77.6</td>
</tr>
<tr>
<td>5</td>
<td>139</td>
<td>5' 32.3</td>
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<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>55.1</td>
<td></td>
</tr>
</tbody>
</table>

* 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 CH3-5' singlet at 1.46 ppm.

From these results and from the 13C 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, Jcis = 11.4 Hz, by the signal of the CH2OH-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 biochemicaly 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 Bl2 et Bl5 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 x 1/2 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 (C18 preparative column with an ACN–H2O 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 [α]D20°C = +10.4° (c = 1.25 in MeOH).

trans-Sordarial (2). (2-Hydroxy-6-(3,4-dihydroxy-pent-1-enyl)-benzaldehyde). Yellow powder. UV λmaxOH nm (ε): 230 (4600), 277 (6300), 351 (3100); UV λmax + OH⁻ nm: 292 (sh), 395. [α]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).

Heptacycllosordariolone (3). (3,9-Dihydroxy-9-(1-hydroxyethyl)-2,9 H-benzo-[c]-oxepin). Amorphous powder. Red-violet with bis-diazotized benzidine. UV λmaxOH 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-CH3-CH-OTMSi; 18), 305 (4), 232 (16), 217 (4), 203 (6), 189 (18), 147 (10), 117 (100), 75 (14), 73 (100).


Cyclosordariolone (5). (1,6-Dihydroxy 5-hydroxymethyl 1-methyl naphthalen-2-one). Yellow-green powder; UV λmaxOH nm (ε): 208 (16,400), 258 (12,000), 310 (8200), 382 (2800); UV λmax + OH⁻ nm: 240 sh, 280, 328, 420. [α]D 20°C = -77.8° (c = 0.37 in MeOH). EI/MS of the TMSi derivative: m/z...
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 Me₂CO (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.

Acknowledgements
We thank H. Waton, CNRS, Solaize, for the NMR data and D. Davoust (Université de Rouen) for NOE experiments.