Note

Isolation and Identification of Tentoxin from *Alternaria porri* (Ellis) Ciferri†

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Received March 22, 1990

In the course of our investigation on the bioactive products of *Alternaria porri* (Ellis) Ciferri, the causal fungus of black spot disease in the stone-leek (Japanese name: negi) and onion, we have reported hitherto three reduced anthraquinone derivatives, altersolanol A and B and dactylariol,1* which inhibited the elongation of the root in seeds of lettuce and stone-leek. Altersolanol A also showed antimicrobial activities against some Gram-positive and negative bacteria,1* and altersolanol B showed high cytotoxicity to Hela2) and Ehrlish ascites carcinoma.3) Moreover, we have isolated anthraquinone derivative dimers named alterporriol A,4) B5) and C,6) using onion decoction medium, and determined their chemical structures. However, they have been detected by an HPLC analysis not only from *Alternaria porri* but also from *A. solani* when using potato-sucrose medium.7) In contrast, they were not detected from *A. solani* when using onion decoction medium.7) These results imply that *A. porri* and *A. solani* are closely related, although they are classified into different species of the *Alternaria* genus. In our experiments, alterporriol A, B and C were not detected from *A. mali* and *A. japonica*, using several kinds of artificial and natural media, by HPLC analysis.8) These results suggest that *A. porri* and *A. solani* are characteristic among the *Alternaria* species, and that they are slightly different from each other from the chemotaxonomical point of view. This communication describes the isolation and identification of another bioactive compound, tentoxin (Fig. 1), from *Alternaria porri*.

The culture conditions, using stone-leek decoction, were the same as those previously reported.9) After fermenting for thirty days, the active substances were extracted with ethyl acetate from the culture liquid. After concentrating, the extract was subjected to preparative TLC (Merck Kieselgel 60PF254) with a solvent system of chloroform-acetone-formic acid (200:100:1). Repeated preparative TLC (Rf: 0.37), which was followed by recrystallization from benzene, gave colorless needles of mp 173–174°C (lit. 172–173°C), in a yield of 17.2 mg from 25.6 l of culture medium. The SIMS mass spectrum showed a peak at m/z 415 (MH+). Its molecular formula was determined to be C22H30N4O4 (M+; found, 414.2259; calcld., 414.2266) by EI-high-resolution mass spectral data. The IR spectrum showed the presence of an amide group (1670, 1630 cm⁻¹), and a monosubstituted phenyl group (1520, 760, 700 cm⁻¹). The ¹H-NMR spectrum of (400 MHz) in CDCl₃ exhibited the presence of two imino protons (δ 8.10, 7.20), an olefinic proton (δ 7.75), five aromatic protons (δ 7.41, 2.81) and three C-methyl protons (δ 1.54, 0.63, 0.51). The results from the ¹³C-NMR spectrum are given in Table I, showing that twenty two signals could be found in C₂₂H₃₀N₄O₄. Based on these spectral data, the compound

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**Table I.** ¹³C NMR Spectral Data of Tentoxin (INEPT method) (100 MHz, (CD₃)₂CO with TMS)

<table>
<thead>
<tr>
<th>Signal</th>
<th>¹³C (ppm)</th>
<th>Assignment</th>
</tr>
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<tbody>
<tr>
<td>C=O</td>
<td>172.44</td>
<td>H=O</td>
</tr>
<tr>
<td></td>
<td>172.00</td>
<td>CH=O</td>
</tr>
<tr>
<td></td>
<td>170.35</td>
<td>CH=O</td>
</tr>
<tr>
<td></td>
<td>164.66</td>
<td>N–CH₂</td>
</tr>
<tr>
<td></td>
<td>133.66</td>
<td>N–CH₃</td>
</tr>
<tr>
<td>Aromatic</td>
<td>131.30</td>
<td>C=–C</td>
</tr>
<tr>
<td>Aromatic</td>
<td>131.04</td>
<td>C=–CH₂</td>
</tr>
<tr>
<td>Aromatic</td>
<td>130.41</td>
<td>C=–CH₃</td>
</tr>
<tr>
<td>Aromatic</td>
<td>130.06</td>
<td>C=–CH₂</td>
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<tr>
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<td>C=–CH₃</td>
</tr>
<tr>
<td>Aromatic</td>
<td>129.18</td>
<td>C=–CH₂</td>
</tr>
<tr>
<td>Quat.-C</td>
<td>127.52</td>
<td>C=–C</td>
</tr>
</tbody>
</table>

* Values recorded in CD₃OD.
was presumed to be tentoxin, a phytotoxin of another *Alternaria* genus. This presumption was proved to be correct by comparing the IR, NMR and mass spectra with those of an authentic sample previously reported.

Tentoxin is known as a nonspecific toxin produced by some *Alternaria* species, i.e., *A. tenuis*, *A. mali*, *A. citri*, *A. alternata* and *A. longipes*, which produces several chlorosis in the cotyledons of many dicotyledous plant species. From the chemotaxonomical point of view, it is of interest that tentoxin was proved to be a metabolite of *Alternaria porri*.

**Experimental**

The strain of *Alternaria porri* used in this experiment was purchased from IFO (Institute for Fermentation, Osaka), strain no. 9762. 1H- and 13C-NMR spectra were recorded with a JEOL GX-400 spectrometer, using TMS as an internal standard. Mass spectra (MS) were measured on a Hitachi M-80B mass spectrometer, and IR spectra were recorded on a Shimadzu FTIR-4200 infrared spectrometer.

**Physicochemical properties of tentoxin.**

Colorless needles, mp 173–174°C (uncorr.), [α] D 10 – 117° (MeOH, c 0.3). IR ν max (KBr): 3350 (NH), 2950, 1450 (CH3, CH2), 1670, 1630 (CONH), 1520, 760, 700 (phenyl). MS (LC/API) (H2O:MeOH = 1:4) m/z: 415 (MH+), 397 (MH+ – H2O), 379 (MH+ – 2H2O), 358 (MH+ – A), 330 (MH+ – B), 302 (MH+ – C), 273 (MH+ – B – A), 217 (MH+ – C – B), 199 (MH+ – D – A), 143 (MH+ – D – C). MS (EI): found, 414.2259 (M+); C22H30N4O4 requires 414.2266. NMR δ (CDCl3): 8.10 (1H, broad s), 7.75 (1H, s), 7.41 (5H, s), 7.20 (1H, broad d, J = 7.9 Hz), 5.01 (1H, dd, J = 10.2 Hz), 4.30 (1H, q), 4.13 (1H, q, J = 7.9 Hz), 3.50 (1H, d, J = 15.3 Hz), 3.19 (3H, s), 2.81 (3H, s), 2.04 (1H, m), 1.54 (3H, d, J = 6.5 Hz), 1.26 (2H, m), 0.63 (3H, d, J = 5.0 Hz).

**References**

8. R. Suemitsu and K. Horiuchi, not published.