Biological Activity of a Heptaketide Metabolite from *Pleiochaeta setosa*

Boniface OKEKE, Françoise SEIGLE-MURANDI,* and Régine STEIMAN

Groupe pour l'Étude du Devenir des Xénobiotiques dans l'Environnement (GEDEX), Laboratoire de Botanique, Cryptogamie, Biologie Cellulaire et Génétique, UFR de Pharmacie, Université Joseph Fourier, 38243 Meylan, France

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An uncommon heptaketide metabolite, setosol (2,8-dimethyl-4-methoxy-6,10,11-trihydroxy-benzo-oxaonin), was isolated from a liquid culture filtrate of the fungus *Pleiochaeta setosa*. The biological activity of the molecule was studied by using 12 microbial strains consisting of three bacteria, three yeasts and six fungi. The level of activity was compared with those of known antibiotics and antifungal agents. The metabolite exhibited antifungal and antibiotic activity against *Drechslera oryzae*, *Gerlachia oryzae*, *Pyricularia oryzae*, *Cryptococcus neoformans*, and *Staphylococcus aureus*. The acetylated derivative of setosol did not inhibit the growth of any of the target pathogens. Phytoxicity studies on whole lupine leaves show that setosol is implicated in the pathogenesis of the brown spot disease of lupines since artificial inoculation of the leaves with the metabolite provoked lesions similar to the characteristic brown spots and lesions on lupine leaves infected by the fungus.

A heptaketide antifungal antibiotic metabolite named setosol (Fig. 1) was isolated from a phytopathogenic fungus of agronomic importance. This pathogen, *Pleiochaeta setosa* (Kirchn.) Hughes is the causal agent of the brown spot disease of *Lupinus albus* L. which is increasingly becoming an important horticultural crop in many countries. It has been isolated and identified in many parts of the world.1-3)

This work is a continuation of interest in the search for metabolites of agricultural and medical importance produced by selected strains of fungi.4,5) The aspects of production, isolation, and structural elucidation of the metabolite have been presented in an earlier work.6) The structural analysis was based on $^1$H- and $^{13}$C-NMR and MS data for both the natural product and the acetylated compound. These results showed the molecule to be a 2,8-dimethyl-4-methoxy-6,10,11-trihydroxy-benzo-oxaonin. In the present work, the biological activities of setosol (C$_{13}$H$_{14}$O) are presented as well as those of four known reference antibiotics and two fungicides. The acetylated derivative of setosol was also tested for its antibiotic and antifungal activities. In order to provide further understanding of the pathogenesis of the brown spot disease caused by *P. setosa* attack of lupine plants, the implication of setosol in the development of the disease was studied by artificials, inoculating different concentrations of setosol into hybrid variants of lupine plants, especially into the leaves. This study was carried out under controlled conditions.

**Materials and Methods**

Fungal material. *P. setosa* was isolated from volcanic rock in the Canary Islands. It was grown for identification on a solid malt extract agar (MEA) medium (1.5%). The identity was further confirmed in a greenhouse by artificial re-inoculation into lupine plants. The fungus which was grown on MEA was inoculated into hybrid white lupine plants, resulting in the typical brown lesion of the brown spot disease being provoked on the leaves.

Isolation and identification of the metabolite. The procedure for isolating and identifying the pure active fungal metabolite has already been described.4) The metabolite was isolated by stepwise centrifugal TLC with chromatotron apparatus (Harrison Research, U.S.A.). The structure was established by a detailed spectral analysis comprising COSY, 2D $^1$H-$^{13}$C direct chemical shift correlation (XHICORR), 2D $^1$H-$^{13}$C correlating via long range coupling (COLOC), heteronuclear gated decoupling (GATE-DEC) and single frequency heteronuclear gated decoupling (SFDEC) NMR techniques. The other methods used were UV, IR, and MS analyses.

Chemicals. Fosetyl-Al (Aliette), Benomyl (Benlate), clotrimazole, econazole, miconazole, ampicillin, kanamycin, streptomycin, and thiophenicol (Tables I-III) were of commercial grade purity and were obtained directly from the manufacturers.

**Bioactivity tests.** The target pathogens consisted of six phytopathogens of agronomic importance, three yeasts and three bacterial strains of medical importance (Tables I, II, and III). These organisms were isolated from various sources, the phytopathogens and yeasts being stored on MEA while a trypto-casein-soja medium was used for the bacterial strains. The storage temperature was 4°C for each. Subcultures of the phytopathogens intended for further experiments were kept at 24°C, while those of the bacterial and yeast strains were kept at 37°C. The paper disk diffusion method was used for the antimicrobial activity tests.9) The minimum inhibitory concentration (MIC) was observed in each test. Serial dilutions (500 to 0.05 mg/ml) of natural setosol, its acetylated derivative and other test chemicals were prepared in methanol. The least concentration at which the pathogens did not exhibit any growth is taken as the minimum fungicidal or bactericidal concentration. The diameter (mm) of each inhibition zone was measured, using methanol alone as a control which
did not inhibit the growth of any of the target pathogens. Dry sterile paper disks (6 mm) impregnated separately with 100 ml of each test chemical were deposited on the respective culture medium (MEA for the phytopathogens and yeasts, and tryptose- soya for the bacteria) containing solidified suspension (5 ml) of each target strain.

**Phytopathogenicity and phytotoxicity test.** Both a crude extract and the purified metabolite of *P. setosa* were tested on four clean and healthy hybrid variants of lupine plants (six months old). The hybrid plants consisted of white, blue/white, pink/white, and red lupines and were supplied by Paquet Jardin, France. The plants were cultivated in pots (7.5 x 7.5 x 10 cm) containing a mixture of brown (35%) and very light brown (65%) soil. The plants were acclimatised and left in a controlled plant room maintained at 30°C, 100% relative humidity created by micropulverization of water, and 4930 W light intensity. The light was supplied by 38 fluorescent tubes (Sylvania, Germany) of 85 W each. The plants were inoculated with different concentrations (20 to 500 mg/ml) of setosol and the crude extract, the solutions being prepared in a mixture of water and methanol (90/10, v/v). This mixture was used in order to ensure complete dissolution of the substances which were not readily soluble in water. This water/methanol mixture was also injected (20 ml) into each lupine variant as a control. Only a slight necrotic effect was provoked on the leaves at the point of contact due to the wound inflicted by the microsyringe used for introducing the extracts. The immediate reaction of the leaves after injection was noted, and subsequent observations were made after 30 min, and 24 and 72 h. The experiments were replicated four times.

### Results and Discussion

The results of the fungitoxic and antibiotic assays of natural setosol, its acetylated derivative and the other antifungal and antibiotic agents used for comparison are shown in Tables I–III. Selection of the different chemicals used for the control experiments was based on their wide trials and applications in agriculture and medicine. Table I shows MIC values for natural setosol, benomyl (benzimidazole) and fosethyl-Al or aluminium phosphite against the six phytopathogens. Setosol inhibited the mycelial growth of four of the six target pathogens. *Pyricularia oryzae*, the causal pathogen of the widely known rice blast disease, was the most sensitive to the metabolite. Benomyl and fosethyl-Al were active against the six pathogens. *Aspergillus oryzae*, *Colletotrichum musae*, *Fusarium oxysporum*, and *Gerlachia oryzae* were most sensitive to benomyl. Although fosethyl-Al inhibited the growth of all the plant pathogens, the MIC values were relatively high. This may not be surprising since fosethyl-Al is acknowledged to be most effective against Oomycetes, and comparable levels of activity on an agar–agar medium have been noted by other authors. This was attributed to the indirect mode of action of fosethyl-Al. Microscopic examination showed that none of the tested chemicals provoked any morphological modification of the organs in the phytopathogens.

The results of the growth inhibition activity of setosol and its acetylated derivative are also compared with those of clortimazole, econazole, and miconazole in Table II. The three antifungal agents, which are widely used antimicrobial drugs, were more active than natural setosol, which inhibited the growth of only *Cryptococcus neoformans* at a relatively high concentration. Clortimazole, econazole, and miconazole were active against *Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans* at the same concentration of 50 μg/ml but differences of 5 to 55% were observed in the diameter of the zones of inhibition (Table II). They were more active than natural setosol, which only showed inhibition activity against *C. neoformans*. Among the bacterial strains, setosol only inhibited the growth of *S. aureus* (Table III). This activity was lower than that of ampicillin, kanamycin, streptomycin, and thiamphenicol, since it was active at a comparatively higher concentration. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were inhibited by kanamycin and streptomycin at the same MIC level (50 μg/ml), but there was a difference in the diameter of the zones of inhibition (Table III). The acetylated derivative of setosol did not manifest any activity against any of the pathogens even at 500 μg/ml.

Both pure setosol and the unpurified crude extract were

### Table II. Antifungal Activity of the Natural Compound (1) and Its Acetylated Derivative (2) Compared with that of Three Fungicidal Agents against the Growth of Three Yeast Strains

(Ca, *Candida albicans*; Crn, *Cryptococcus neoformans*; Ct(R2), *Candida tropicalis* resistant to polyenes).

<table>
<thead>
<tr>
<th>Target yeast strains</th>
<th>MIC* (μg/ml)</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Crn</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Clortimazole</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Econazole</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Miconazole</td>
<td>50</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table III. Antibiotic Activity of the Natural Compound (1) and Its Acetylated Derivative (2) Compared with that of Four known Antibiotics against the Growth of Three Bacterial Strains

(Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Sa(s), *Staphylococcus aureus* sensitive to penicillin).

<table>
<thead>
<tr>
<th>Target bacterial strain</th>
<th>MIC* (μg/ml)</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ec</td>
<td>Pa</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
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<td>2</td>
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<td>Kanamycin</td>
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<td>Streptomycin</td>
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<td>16</td>
</tr>
<tr>
<td>Thiamphenicol</td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
</table>

* Same MIC value for the three strains.
phytotoxic to the four variants of lupine plants used in this experiment. They produced similar brown necrotic lesions on the leaves, the phytotoxicity increasing with increasing concentration of each extract. At a concentration of 50 \(\mu\)g/ml, leaf wilting preceded necrosis and started 30 min after injection. The brown necrotic effect of the metabolite on the leaves, although larger due to spreading, was similar to the effect observed on leaves infected with the fungus. The similarity of the observed reaction to the typical brown lesions caused by attack from \textit{P. setosa} in nature suggests that the metabolite is wholly or largely responsible for the phytopathogenic effect of the fungus. The genus \textit{Pleiochaeta} contains only the species \textit{P. setosa}, and this is the first report of a bioactive compound produced by the genus.

The antifungal antibiotic activity of setosol, an uncommon heptaketide metabolite produced by \textit{P. setosa} was investigated. The activity of the metabolite may be said to be limited when compared with the other antibiotics and fungicides tested. Setosol contains three hydroxyl (OH) groups at the 6, 10, and 11 positions of the aromatic carbon atoms. These OH groups are most probably responsible for its weak activity, since they are known to render the molecule unstable. It is rapidly oxidized either in a solution or when deposited on silica gel TLC plates in an ambient laboratory atmosphere, giving a deep yellow spot which is visible to the naked eye.

Acetylation of natural setosol to the triacetate derivative resulted in the introduction of three acetyl (CH\(_3\)CO) groups in place of the OH groups. This replacement rendered the molecule more stable, but inactive. Hence, the acetylated derivative did not inhibit the growth of any of the target pathogens even at very high concentrations. Other physico-chemical properties of setosol as well as its desmethyl congener (desmethyl/setosol) have been described earlier (Kaoudji \textit{et al.}, submitted). Since the metabolite does not possess a chiral carbon atom, it is proposed that it may be possible to synthesize it in the laboratory with little difficulty. The introduction of chloride or bromide atoms in place of the OH units is likely to enhance the activity of the molecule. This may be achieved either by direct laboratory synthesis or by making appropriate changes in the composition of the culture medium used for the growth of \textit{P. setosa}. Setosol presents a rare and interesting heptaketide structure that deserves further investigations.

References