THE RELATIVE AND ABSOLUTE STEREOCHEMISTRY OF THE ANTIFUNGAL AGENT PREUSSIN

Janice H. Johnson, D. W. Phillipson and Alicia D. Kahle

The Squibb Institute for Medical Research, P. O. Box 4000, Princeton, New Jersey 08543-4000, U.S.A.

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Structure 1 was reported for a novel antifungal agent, L-657,398, isolated from fermentations of Aspergillus ochraceus. However, the relative and absolute stereochemistry of this compound were not reported. We have isolated a compound with the same structure (exclusive of stereochemistry) from fermentations of Preussia sp., by extraction of the mycelial cake with methanol and purification of the antibiotic by repetitive silica gel chromatography eluting with CHCl₃-MeOH (98:2) and toluene-MeOH (95:5). Two liters of whole broth (360 g of wet mycelial cake) yielded 62 mg of the compound, which we call preussin, as a yellow oil (\([\alpha]_D^2 +22.0^\circ \) (c 1.0, CHCℓ₃)). As reported for L-657,398, the structure of preussin was determined from XH and ¹³C NMR spectra and XH-¹H connectivity experiments on the natural product, 1, and the monoacylated derivative, 2. The relative stereochemistry was then determined from a series of nuclear Overhauser effect (NOE) experiments that showed contiguity as indicated by the arrows in Fig. 1. In addition, a small NOE was observed from the methyl group of the acetate to both benzylic protons, from proton H₆ to proton H₅, and from proton H₆ to proton H₅.

The absolute stereochemistry was determined by using Trost's O-methylmandelate ester methodology. The (S)- and (R)-O-methylmandelate esters of preussin, 3 and 4, were synthesized and their ¹H NMR spectra compared. The chemical

![Fig. 1. NOE's observed for preussin.](image)

Table 1. Proton chemical shifts of the acetate (2) (S)-ester (3) and (R)-ester (4) in CDCl₃.

<table>
<thead>
<tr>
<th>Proton</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₆</td>
<td>2.95</td>
<td>2.71</td>
<td>2.91</td>
</tr>
<tr>
<td>H₅</td>
<td>2.87</td>
<td>2.57</td>
<td>2.82</td>
</tr>
<tr>
<td>H₄</td>
<td>2.47</td>
<td>2.30</td>
<td>2.53</td>
</tr>
<tr>
<td>H₃</td>
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<td>1.45</td>
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</tr>
<tr>
<td>H₂</td>
<td>2.36</td>
<td>2.40</td>
<td>2.19</td>
</tr>
</tbody>
</table>

![Fig. 2. Shielding interactions for the (S)- and (R)-O-methylmandelate esters of preussin.](image)

3(S)-Preussin-(S)-ester (3) 3(S)-Preussin-(R)-ester (4)
shifts of the relevant protons of these esters and of the acetate, 2, are shown in Table 1. As can be seen, protons H₉ and H₁₀, and to a lesser extent proton H₁, were shifted upfield in the (S)-ester relative to 2, while proton H₂, and to a lesser extent H₆, were shifted upfield in the (R)-ester relative to 2. These results are consistent with the mandelate phenyl group shielding the eclipsed protons (see Fig. 2) if the natural product possesses the (S), but not the (R), configuration at carbon 3. Thus, preussin is (2S,3S,5R)-1-methyl-5-nonyl-2(phenylmethyl)-3-pyrrolidinol, 5. Comparison of the proton and carbon chemical shifts reported for L-657,398 with the values obtained for preussin (CD₃COOD) suggests that these two compounds have the same relative stereochemistry. (However, the N-methyl carbon reported at δ 33.8 for L-657,398 is found at 38.8 in preussin; this disparity may be due to a typographical error.)

As reported for L-657,398, preussin shows antifungal activity against both filamentous fungi and yeasts. MIC values vs. several of these microorganisms are listed in Table 2.

Acknowledgments

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References
