Notes

THE RELATIVE AND ABSOLUTE STEREOCHEMISTRY OF THE ANTIFUNGAL AGENT PREUSSIN

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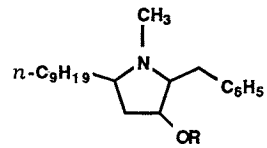
Structure **1** was reported for a novel antifungal agent, L-657,398, isolated from fermentations of *Aspergillus ochraceus*.[1] However, the relative and absolute stereochemistry of this compound were not reported. We have isolated a compound with the same structure (exclusive of stereochemistry) from fermentations of *Preussia* sp., by extraction of the mycelial cake with methanol and purification of the antibiotic by repetitive silica gel chromatography eluting with $CHCl_3$ - 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 ($[\alpha]_D^{25}$ +22.0° (*c* 1.0, $CHCl_3$)). As reported for L-657,398, the structure of preussin was determined from ^1H and ^{13}C NMR spectra and ^1H-^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 H_A to proton H_G, and from proton H_A to proton H_I.

The absolute stereochemistry was determined by using TROST's *O*-methylmandelate ester methodology.[2] The (*S*)- and (*R*)-*O*-methylmandelate esters of preussin, **3** and **4**, were synthesized and their ^1H NMR spectra compared. The chemical



1 R=H
2 R=OAc

Fig. 1. NOE's observed for preussin.

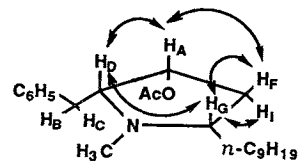
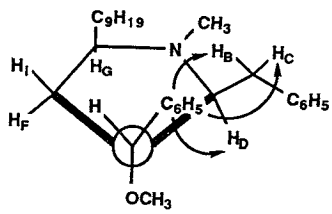


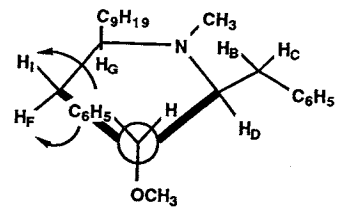
Table 1. Proton chemical shifts of the acetate (**2**) (*S*)-ester (**3**) and (*R*)-ester (**4**) in $CDCl_3$.

Proton	Chemical shift (δ)		
	2	**3**	**4**
H_B	2.95	2.71	2.91
H_C	2.87	2.57	2.82
H_D	2.47	2.30	2.53
H_I	1.34	1.45	1.08
H_F	2.36	2.40	2.19

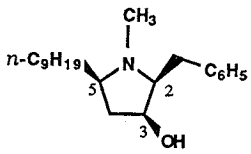
Fig. 2. Shielding interactions for the (*S*)- and (*R*)-*O*-methylmandelate esters of preussin.



3(*S*)-Preussin-(*S*)-ester (**3**)



3(*S*)-Preussin-(*R*)-ester (**4**)



Preussin (**5**)

Table 2. MIC values of preussin against various *Candida* and filamentous fungi.

Organism	MIC (μg/ml)
Candida albicans SC5314	25
C. albicans SC9721	25
C. albicans (Basilysin R) SC12,734	25
C. albicans (Aculeacin R) SCDKY53	25
C. tropicalis SC8159	25
C. tropicalis (Ampho B R) SC2963	25
C. tropicalis (Ampho B R) SC9861	6.3
C. tropicalis SC10,597	25
C. krusei (Ampho B R) SC2967	3.1
C. krusei SC2969	12.5
C. krusei SC2968	6.3
C. parakrusei SC2966	12.5
C. pseudotropicalis SC11,241	3.1
C. guilliermondii SC2996	12.5
C. stellatoidea SC2211	25
C. glabrata SC9342	25
Trichophyton menta SC2637	3.1
T. rubrum SC9199	12.5
Microsporum canis SC9327	1.6
Aspergillus fumigatus SC2100	12.5

shifts of the relevant protons of these esters and of the acetate, **2**, are shown in Table 1. As can be seen, protons H_B and H_C, and to a lesser extent proton H_D, were shifted upfield in the (*S*)-ester relative to **2**, while proton H_I, and to a lesser extent H_F, 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 (2*S*,3*S*,5*R*)-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 (CD_3COOD) 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[1], 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

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