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Synthesis and Structural Analysis of the Active Enantiomer of Famoxadone, a Potent Inhibitor of Cytochrome bc_1

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Abstract—Famoxadone is a newly commercialized fungicide and potent Q_o-site inhibitor of cytochrome bc_1. The *S*-(−)-enantiomer of famoxadone (the active component) was synthesized by two routes and was analyzed computationally and by X-ray crystallography. The molecule displays an extended conformation with flexibility in the structure imparted by the two terminal phenyl groups. In the crystal lattice, intermolecular hydrogen bonds occur between the NH and the oxygen atoms of the heterocycle.

Inhibition of the function of mitochondrial bc_1 has become a significant area for the discovery of fungicides useful in controlling crop diseases. Recently, five Q_o-site inhibitors of cytochrome bc_1 function have been commercialized for this purpose: azoxystrobin,[1] kresoxim-methyl,[2] metominostrobin,[3] trifloxystrobin,[4] and famoxadone[5a–e] (Fig. 1). On the basis of their origin and the methoxyacrylate-like head groups, the first four of the above-mentioned fungicides may be considered as analogues of the Q_o-site inhibitor strobilurin A.[6] Strobilurin A is a natural product found in some microorganisms.[7] In an entirely different mode of fungicide discovery, famoxadone (Famoxate®, DPX-JE874, or 5-methyl-5-(4-phenoxyphenyl)-3-phenylamino-2,4-oxazolidinedione) was optimized as a fungicide for crop protection from an initial greenhouse-active molecule[5b–e] that was until then primarily of academic interest as a novel synthetic heterocycle.[8a–c] Famoxadone differs considerably in chemical structure from strobilurin A and its commercialized mimics, and thus it presents an original toxiphore for the inhibition of cytochrome bc_1. In addition, isolates of *Saccharomyces cerevisiae* having single amino acid alterations in the Q_o center within their apocytochrome b were found to confer log scale differences in binding potencies in comparison to famoxadone among the other inhibitors examined: myxothiazol, azoxystrobin, and kresoxim-methyl.[5c,d] X-ray crystallographic studies on cytochrome bc_1 complexed with inhibitors[9a–c] are exciting new developments in the field of bioenergetics and in their utility for structure-based design. Famoxadone is racemic and the *S*-(−)-enantiomer was shown to be the active component for the inhibition of cytochrome bc_1 function and in controlling fungal diseases in crop plants.[5c] In this work we describe the synthesis and structural analyses of the *S*-(−)-enantiomer of famoxadone.

Chemistry

The racemic precursor to the more fungicidally-active *S*-(−)-enantiomer of famoxadone was the substituted atrolactic acid **1**. This could be prepared by a number of methods, including the two Grignard strategies outlined in Figure 2. The preparation of the enantiomerically-pure hydroxyester intermediates was accomplished by two chemical methods (Fig. 3). In Method A, acid **1** was converted to the corresponding acyl imidazole and coupled with excess dimethyl tartrate. The mixture of diastereomeric monoacyl tartrates, containing some diacylated material, was subjected to chromatography on silica gel to isolate the desired diasteromer as the faster-eluting monoacyl compound **2**. The corresponding methyl ester **3** was obtained by base-catalyzed transesterification in methanol, the product being separated from the dimethyl tartrate coproduct by partitioning between ether and water. By NMR analysis using a chiral shift reagent, this material was judged to be of >95% enantiomeric purity. The heterocyclic ring was then constructed by DMAP-catalyzed coupling of **3** with

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1,1′-carbonyldimidazole to form the intermediate imidazole carboxylate followed by acid-catalyzed condensation with phenylhydrazine. The crude product was purified by chromatography on silica gel and recrystallized from butyl chloride to afford the *S*-(−)-form of famoxadone, which was >95% enantiomeric excess as determined by HPLC analysis by using a chiral stationary phase and by NMR analysis using a chiral shift reagent. In Method B, the requisite *S*-(−)-enantiomer of the atrolactic ester **3** was prepared by a classical resolution of **1** with quinidine. The quinidine salt of the *R*-(+)-enantiomer crystallized preferentially, and by using 0.6 mol of quinidine, the *S*-(−)-enantiomer of **2** remained in solution. Esterification of this material with methyl iodide and Hunig's base afforded **3** which was converted to the *S*(−) enantiomer of famoxadone.

Results and Discussion

The heterocyclic ring of the *S*-(−)-enantiomer of famoxadone was anticipated to be flat and the methyl and phenyl substituents of the chiral center would remain rigid. The phenoxyl group of the *S*-(−)-enantiomer could adapt two alternative conformations. Since the phenylamino group is expected to be twisted with respect to the five remembered ring, it could be either *trans* or *cis* with respect to the phenoxyphenyl group. Thus, there are at least four general conformations for the enantiomer. A hybrid density functional theory method (B3LYP/3-21G(d) level) was utilized to investigate the conformational flexibility of the molecule.[10] The fully optimized geometry for each conformer (A–D) is shown in Figure 4. The calculated relative energies are given in Table 1. According to the theoretical calculations at the B3LYP/3-21G(d) level of theory, the *trans* conformers are favored by about 0.5 kcal/mol. It appears that there are several low energy conformers for the *S*-(−)-enantiomer of famoxadone and the preferred conformation will likely depend on the environment.

As the phenylamino nitrogen center (N1; see Fig. 5 for the numbering system) is nonplanar, there are two pos-

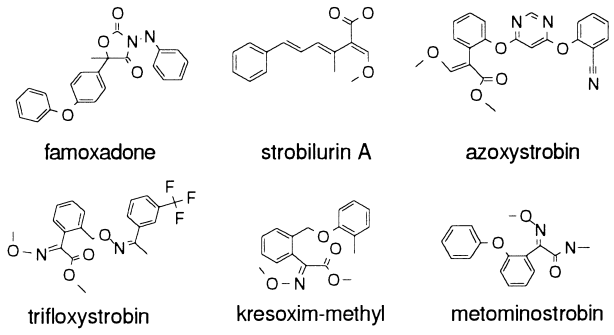

Figure 1. Q_o-site inhibitors of cytochrome bc_1 discussed in the text.

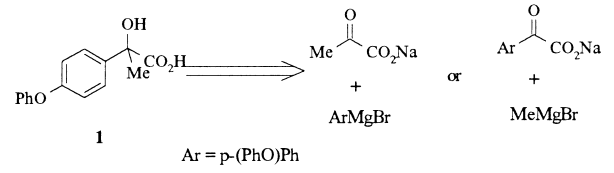

Figure 2. Preparation of atrolactic acid precursors for famoxadone.

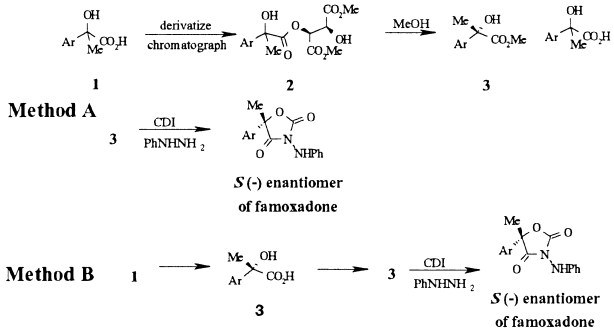

Figure 3. Preparation of chiral precursors for the *S*-(−)-enantiomer of famoxadone.

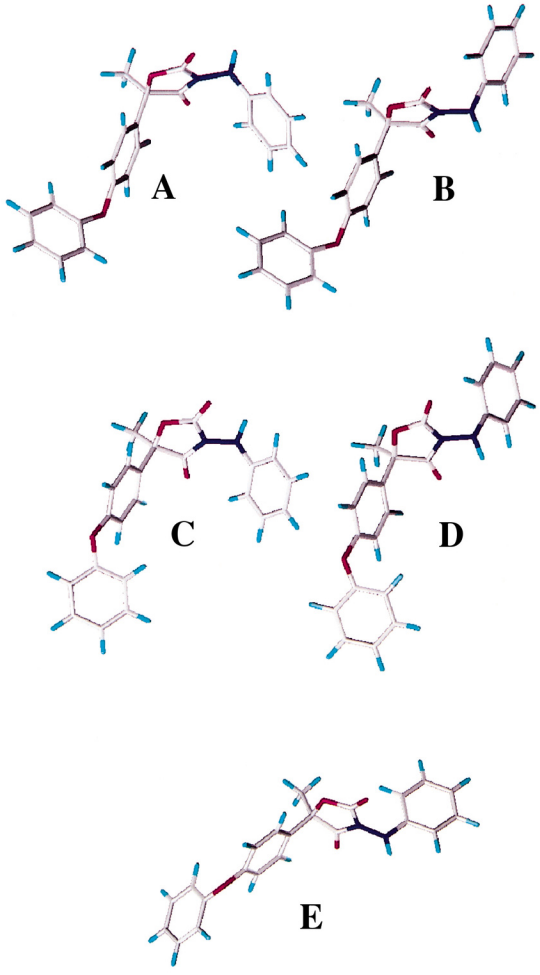

Figure 4. Five conformers of the *S*-(−)-enantiomer of famoxadone as calculated. Energy values are listed in Table 1.

Table 1. The calculated relative energies (kcal/mol) at the B3LYP/3-21G(d) and the B3LYP/6-311 + G(d,p) levels of theory

Conformer	A	B	C	D	E
B3LYP/3-21G(d)	0	−0.04	−0.45	−0.47	0.56
B3LYP/6-311 + G(d,p)	0	0.02	−0.10	−0.11	−0.37

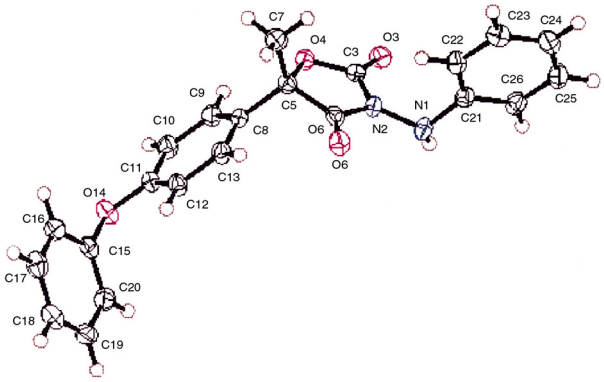

Figure 5. The conformation of the *S*-(−)-enantiomer of famoxadone as determined by X-ray crystallography. Thermal ellipsoids are drawn to the 50% probability level.

sible conformers for each conformation mentioned above depending on the relative orientation of the phenylamino lone pair. However, it is expected that the conformers effected by inversion of the N1 nitrogen center should be very close in energy. As a further check, we also examined the inverted N1 nitrogen center with respect to the conformer D. The calculated structure (E) is shown in Figure 4 and the energy value is included in Table 1. We performed additional energy calculations at a much higher level of theory (the B3LYP/6-311+G(d,p) level of theory). Again, the energy difference is quite small among the five conformers of Figure 4. Interestingly, conformer E becomes more stable than D. Undoubtedly, the biologically active conformation of the *S*-(−)-enantiomer of famoxadone will be determined by the binding pocket environment within the constraints outlined.

X-ray crystallography determined the conformation for the *S*-(−)-enantiomer famoxadone from a single crystal.[11] The asymmetric unit contains one molecule as shown in Figure 5 with thermal ellipsoids drawn to the 50% probability level. The NH group forms a hydrogen bond to an adjacent molecule with refined distances: N1 to H1 = 0.91 Å, H1 to O3 = 2.18 Å (Fig. 6). It should be noted that strobilurin A and its fungicide mimics azoxystrobin, trifloxystrobin, and kresoxim-methyl are capable of accepting a hydrogen but not in donating a hydrogen in forming hydrogen bonding interactions.

The ability to donate a hydrogen in hydrogen bonding differentiates famoxadone from the above-mentioned Q_o-site ligands. When the N1 hydrogen of famoxadone is replaced with a methyl group the IC_{50} value (35 nM for the methyl derivative of famoxadone versus 11 nM for famoxadone)[13] for the inhibition of mitochondrial electron transport (NADH to O_2) is increased by a factor of three suggesting that the NH group of famoxadone shares a hydrogen bond with cytochrome bc_1.

Conformer E, which has an inverted center at its N1 position in comparison to conformer D, is the best approximation of the X-ray structure among the calculated conformers of Figure 4. Figure 7 displays the

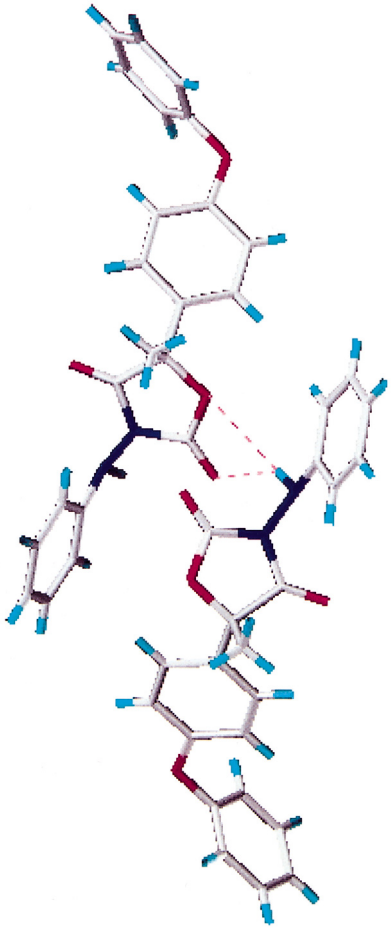

Figure 6. Hydrogen bonding of one molecule of the *S*-(−)-enantiomer of famoxadone to another in the crystal lattice. Hydrogen bonds are indicated by the dashed lines.

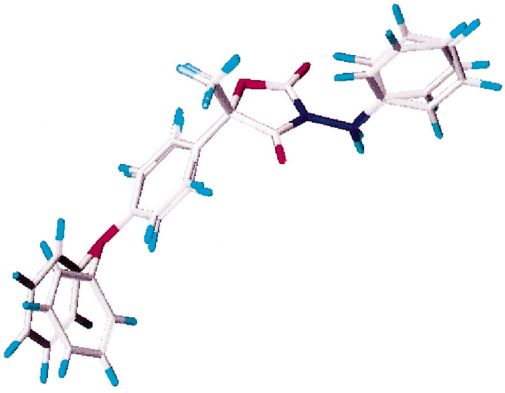

Figure 7. Superposition of the calculated (conformer E) and the X-ray crystal structures of the *S*-(−)-enantiomer of famoxadone.

superposition of the calculated conformer E and the X-ray crystal structure of the *S*-(−)-enantiomer of famoxadone. The calculated and X-ray crystal structures are nearly identical. Crystal packing forces likely cause the minor torsional differences between the two concerning the two terminal phenyl groups. Whether conformer E is the one selected by the enzyme over the other conformers of similar energies cannot be predicted.

In summary, by combining synthesis, computational chemistry, and X-ray crystallographic studies, we have elucidated the structure and the conformational flexibility of the *S*-(−)-enantiomer of famoxadone. These studies should assist in the determination of the binding mode of the molecule within the Q_o-site of cytochrome bc_1, famoxadone's biochemical target. The literature is rich with accounts of where enzyme inhibitors have been extremely valuable in understanding the function of the recipients of their poisons, and we anticipate that the inhibitor famoxadone will lead to further knowledge on the function of cytochrome bc_1. It will be of most interest to see the binding mode of the *S*-(−)-enantiomer of famoxadone within the protein and, in particular, its hydrogen bonding pattern to the apocytochrome b within cytochrome bc_1.

References and Notes

1. Beautement, K.; Clough, J. M.; de Fraine, P. J.; Godfrey, C. R. A. *Pestic. Sci.* **1991**, *31*, 499.
2. Ammermann, E.; Lorenz, G.; Schelberger, K. In *Proc 1992 Brighton Crop Prot Conf—Pests and Diseases*; British Crop Protection Council: Farnjam, UK, 1992; pp 403–410.
3. Mizutani, A.; Miki, N.; Yukioka, H.; Masuko, M. In *Mod. Fungic. Antifungal Compd., Int. Symp.,* Lyr, H.; Russell, P. E.; Sisler, H. D.; Eds; Intercept: Andover, UK, 1996; pp 93–99.
4. Hermann, D.; Fischer, W.; Knauf-Beiter, G.; Steinemann, A.; Margot, P.; Gisi, U.; Laird, D. *Phytopath.* **1998**, *88*, S37.
5. (a) Joshi, M. M.;. Sternberg, J. A. In *Proc. 1996 Brighton Crop Prot. Conf.–Pests and Diseases*; British Crop Protection Council: Farnjam, UK, 1996; pp 21–26. (b) Sternberg, J. A.; Geffken, D.; Adams, J. B. Jr.; Jordan, D. B.; Pöstages, R.; Sternberg, C. G.; Campbell, C. L.; Moberg, W. K.; Livingston, R. S. In *Synthesis and Chemistry of Agrochemicals*; Baker, D. R.; Feynes, J. G.; Basarab, G. S.; Hunt, D. A., Eds.; American Chemical Society: Washington, DC, 1998; pp 216–227. (c) Jordan, D. B.; Livingston, R. S.; Bisaha, J. J.; Duncan, K. E.; Pember, S. O.; Picollelli, M. A.; Schwartz, R. S.; Sternberg, J. A.; Tang, X. S. *Pestic. Sci.* **1999** *55*, 105. (d) Jordan, D. B.; Livingston, R. S.; Bisaha, J. J.; Duncan, K. E.; Pember, S. O.; Picollelli, M. A.; Schwartz, R. S.; Sternberg, J. A.; Tang, X. S. *Pestic. Sci.* **1999** *55*, 213. (e) Jordan, D. B.;. Kranis, K. T; Picollelli, M. A.; Schwartz, R. S.; Sternberg, J. A.; Sun, K. M. *Biochem. Soc. Trans.* **1999***27*, 577.
6. Von Jagow, G.; Link, T. A. *Methods Enzymol.* **1986**, *126*, 253.
7. Anke, T.; Oberwinkler, F.; Steglich, W.; Schramm, G. *J. Antibiot.* **1977** *30*, 806.
8. (a) Geffken, D. *Chem. Ztg.* **1979** *103*, 299. (b) Geffken, D. *Leibigs Ann. Chem.* **1982**, 211. (c) Geffken, D. *Z. Naturforsch, B: Anorg. Chem., Org. Chem.* **1987** *42*, 1202.
9. (a) Xia, D.; Yu, C.-A.; Kim, H.; Xia, J.-Z.; Kachurin, A. M.; Zhang, L.; Yu, L.; Deisenhofer, J. *Science* **1997** *277*, 60. (b) Zhang, Z.; Huang, L.; Shulmeister, V. M.; Chi, Y.-I.; Kim, K. K.; Hung, L.-W.; Crofts, A. R.; Berry, E. A.; Kim, S.-H. *Nature (London)* **1998** *392*, 677. (c) Iwata, S.; Lee, J. W.; Okada, K.; Lee, J. K.; Iwata, M.; Rasmussen, B.; Link, T. A.; Ramaswamy, S.; Jap, B. K. *Science* **1998** *281*, 64.
10. (a) Gaussian 98, Revision A.5; Gaussian, Inc.: Pittsburgh PA, 1998. (b) Becke, A. D. *J. Chem. Phys.* **1993** *98*, 5648. (c) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988** *37,* 785.
11. Crystals were grown by using a vapor diffusion method in a mixture of ethyl acetate and hexane. Data for the single-crystal, X-ray structure determination were collected with a Bruker SMART PLATFORM 1K CCD diffractometer equipped with a monochromatic MoK_α source. The SAINT Integration Package (Bruker AXS, Madison, WI) was used to obtain intensity data and orthorhombic cell parameters: $a = 5.904$ (1), $b = 16.963$ (1) and $c = 18.438$ (1) Å, volume = 1846.6 Å^3. The structure was solved by direct methods using teXsan (SIR-92) (Molecular Structure Corporation, The Woodlands, TX, 1985 and 1992) in space group $P2_12_12_1$ (No. 19) and refined using the Z program suite.[12] Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 139894.
12. Calabrese, J. C. *Z Crystal Structure Analysis Package*; E. I. DuPont de Nemours and Co., 1994.
13. IC_{50} values were determined against rat heart submitochondria by using published methods.[5c]