Chimia 57 (2003) 675–679

ISSN 0009–4293

Fungicidal *meta*-Substituted Strobilurin Analogs

Michael P. Walker*

Abstract: Strobilurin analogs in which the side chain is in a *meta*-relationship to the pharmacophore are active fungicides provided an *ortho*-group is also present. Syntheses, fungicidal screening results and structure–activity relationships for these compounds are described. A new pharmacophore synthesis from aniline precursors is also detailed.

Keywords: Crop protection · Cytochrome bc_1 complex · Fungicides · Mitochondrial respiration inhibitors · Strobilurin analogs

Introduction

The isolation and structural determination of the fungicidal natural product Strobilurin A (**1**) from the basidiomycete fungus *Strobilurus tenacellus* was the starting point for fruitful research programs at many companies, which have subsequently led to commercialization of several successful agricultural fungicides [1]. The site of action of this compound was shown to be inhibition of mitochondrial respiration at the cytochrome bc_1 complex [2]. The unique and simple β-methoxyacrylate containing structure proved to be readily amenable to modification with retention of activity. The natural product was unfortunately very photolabile and therefore not very effective in testing on whole plants in direct sunlight. A solution to the photostability problem was soon arrived at as scientists from both ICI (now Syngenta) and BASF simply replaced the double bond *proximal* to the β-methoxyacrylate with an aromatic ring as in the fungicidally active stilbene **2** (Fig. 1). Similar exchange of the *distal* double bond with an aromatic ring gave the biphenyl **3**, also an effective fungicide. Replacement of both double bonds led to a third class of biologically active ring constrained analogs represented by the naphthalene **4**.

From these promising beginnings scientists at BASF and ICI turned their attention to optimizing activity mainly on structure **2**. The stilbene unit (or side chain) was still not photostable enough to be part of a commercial fungicide so both companies followed an optimization program designed to replace the styrene with other lipophilic groups. After the publication of the first patents many other companies joined the search for novel side chains. A wide variety of groups proved capable of standing in for the stilbene as exemplified by the structures in Fig. 2. Most important among these side chain modifications are aryl ethers (present in **5** and **7**), aralkyl ethers (in **6** and **9**) and oximes (exemplified by **8** and **10**). A second objective of the optimization work was to replace the putative pharmacophoric β-methoxyacrylate group present in the natural product with other groups. ICI, BASF and Ciba quickly found that an oxime ester was an effective replacement (in **6** and **8**). A variety of other replacements has also been uncovered, notably the carbamate (present in **9**) and the oxime amide (*i.e.* **7**). At Du Pont we have found that certain heterocycles such as the triazolinone present in structure **10** serve as cyclic pharmacophores [3].

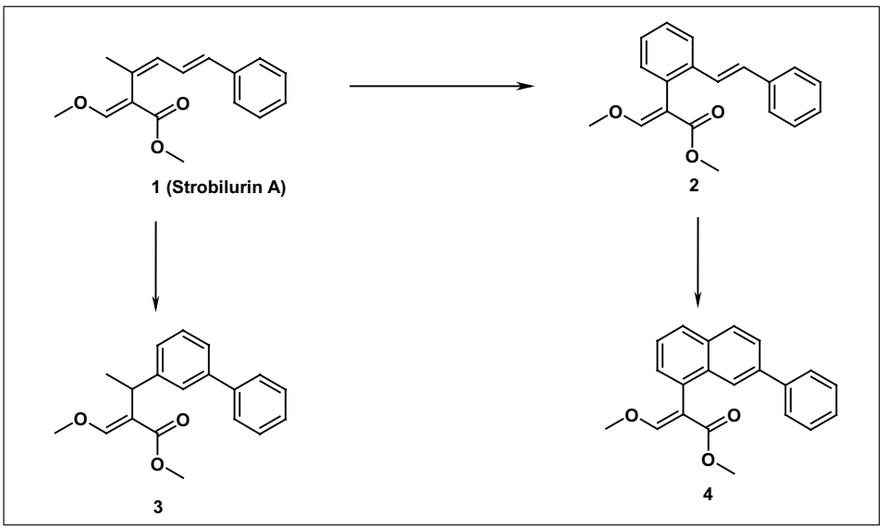


Fig. 1. Strobilurin A double bond replacement strategy pursued by ICI and BASF

*Deceased December 7, 2002

Correspondence: Dr. Thomas M. Stevenson
Du Pont Crop Protection
Stine-Haskell Research Center
P.O. Box 30
Newark, DE 19714, USA
Tel.: +1 302 366 5744
Fax: +1 302 366 5738
E-Mail: Thomas.M.Stevenson@usa.dupont.com

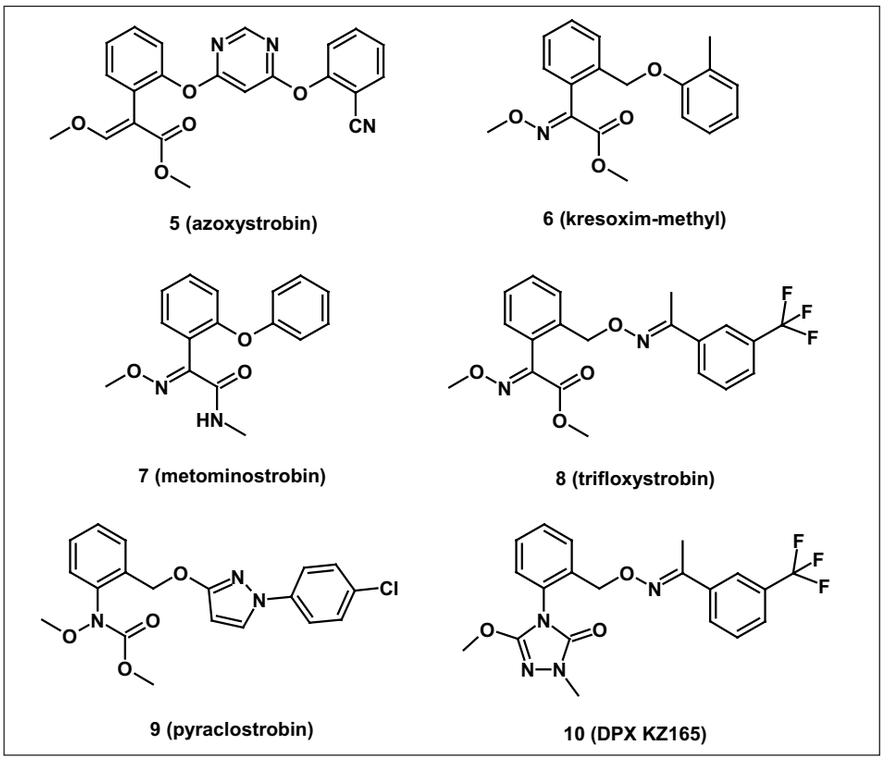
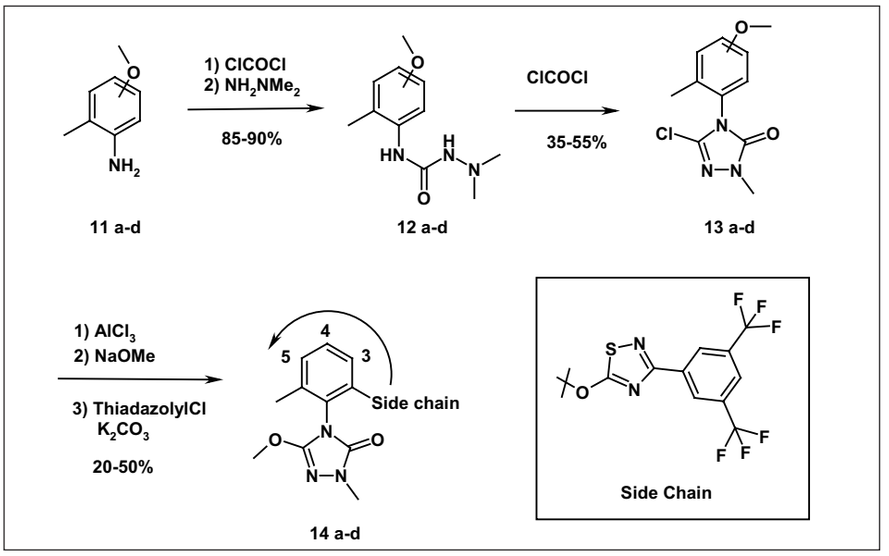


Fig. 2. Examples of various strobilurin pharmacophores and side chains



Scheme 1. Synthesis of four side chain positional isomers

Table 1. Comparative activities of the four positional isomers

Side Chain Position	2 (**14a**)	3 (**14b**)	4 (**14c**)	5 (**14d**)
Fungicidal Activity[a]	++++	++	—	—
Mitochondrial Inhibition ppb[b]	11	55	> 5000	> 5000

[a] Fungicidal activity was measured on the following pathogens: *Erysiphe graminis* (wheat powdery mildew), *Puccinia recondita* (wheat leaf rust) and *Pseudocercosporella herpotrichoides* (wheat foot rot). The following rating system is used in all of the tables: + denotes activity at 200 ppm, ++ denotes broad spectrum activity at 200 ppm, +++ denotes broad spectrum activity at 40 ppm and ++++ denotes broad spectrum activity at 10 ppm or below. See [6] for details of the assays.
[b] Measured with beef heart mitochondria by Mr. Rand Schwartz and represents the concentration in ppb required to inhibit 50% of the mitochondrial function.

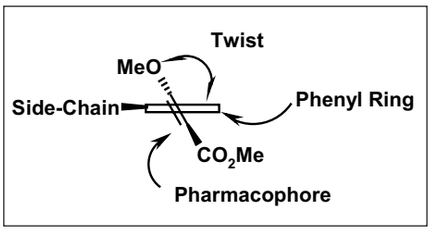


Fig. 3. Side-chain enforces a twist in strobilurin analogs

Side Chain Orientation

In our attempts to optimize the activity of strobilurin analogs derived from our triazolinone pharmacophore, we studied the effects of side chain position with respect to the pharmacophore. Previous work at ICI had established a clear preference for the point of attachment of the styrene side chain to be *ortho* to the β-methoxyacrylate for good activity [4]. Compounds with styrene groups *meta* or *para* to the pharmacophore were essentially inactive. Despite this precedent we were convinced that at least part of this preference for *ortho*-substitution could be attributed to the contribution of the side chain in twisting the pharmacophore out of plane (Fig. 3). From X-ray crystallographic measurements the twist between the pharmacophore and the aromatic ring was reported by ICI to be 86° [4]. We believed that if we synthesized strobilurin analogs with a methyl group *ortho* to the triazolinone (thereby imparting a twist to the pharmacophore) that the side chain might also be successfully attached to other positions on the ring. The myriad of different side chains that had been patented in strobilurins was also compelling evidence that a large lipophilic binding site was present in the cytochrome bc_1 complex that might accommodate substitution at other positions.

Triazolinone Pharmacophores

We chose to begin our investigation of side chain position with heterocyclic ethers derived from 3-aryl-1,2,4-thiadiazoles. Compounds bearing this side chain have extremely high levels of fungicidal activity and starting materials to make all four of the potential targets are readily available [5]. As shown in Scheme 1 synthesis began with *o*-toluidines **11a–d** with a methoxy group in each of the four possible positions.

The triazolinone intermediates **13a–d** were built up by isocyanate formation, followed by reaction with 1,1-dimethylhydrazine and ring formation with triphosgene [3]. The phenols were liberated by reaction with aluminum chloride. Displacement of the chlorides with sodium methoxide gave the four isomeric precursors to the target structures. Reaction of each isomer with commercially available 5-chloro-3-(3,5-bistrifluoromethylphenyl)-1,2,4-thiadiazole (Maybridge) in the presence of potassium carbonate gave the desired strobilurin analogs **14a–d**.

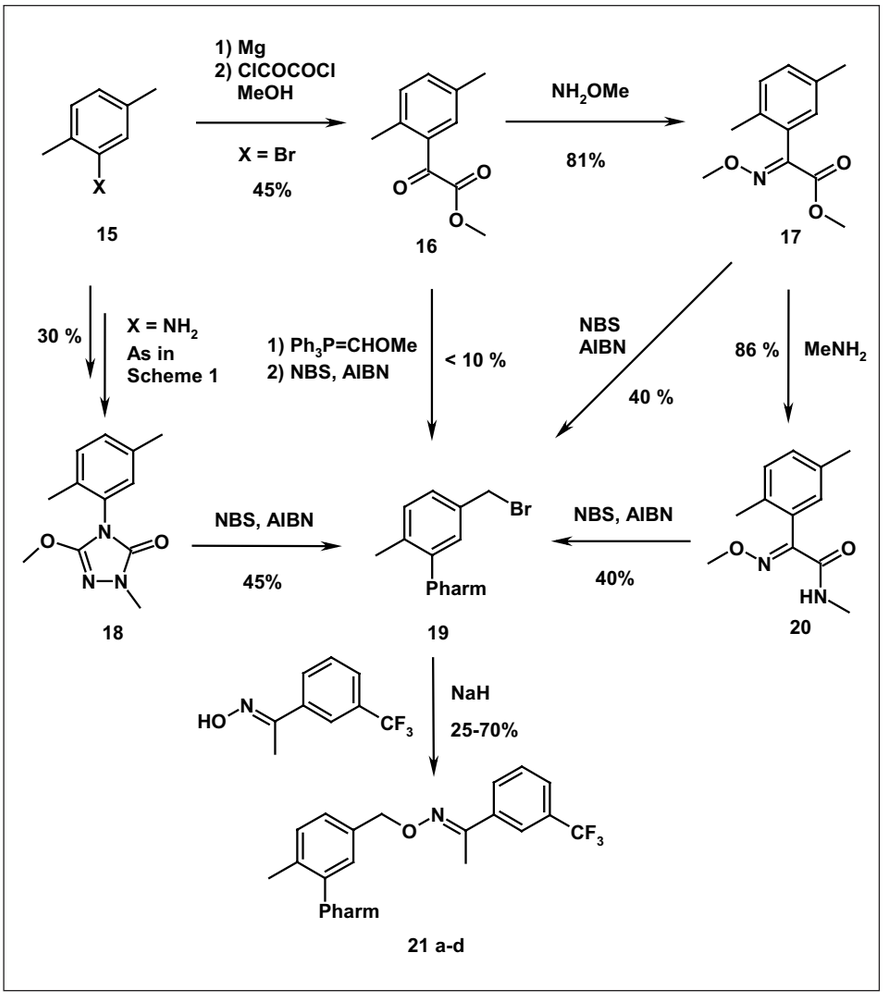
The results, from fungicidal and mitochondrial respiration testing for the four isomers, are shown in Table 1. As expected and in line with previously known structure activity relationships, the highest activity was observed with the side chain in the 2-position. Compounds with side chains in the 4- and 5-positions were virtually inactive in both fungicidal and mitochondrial testing. Gratifyingly, when the side chain was placed in the 3-position high activity was also observed. This showed that the twist imparted by the methyl group was indeed sufficient to allow binding of the 3-substituted product to the cytochrome bc_1 complex. As a control we also synthesized and tested the analogous 3-substituted compound lacking the *ortho*-methyl group and found it to be essentially inactive in the assays. [6]

Traditional Pharmacophores

We next set out to discover if the positive effect of an *ortho*-methyl group would extend to other known strobilurin pharmacophores. Our synthetic approach was based on an α-ketoester intermediate that would allow us to make oxime ester, oxime amide and methoxy acrylate pharmacophores from a common synthetic intermediate (Scheme 2). We investigated several reagents for the introduction of this function and settled on the use of oxalyl chloride. We formed the Grignard reagent derived from 2-bromo-*p*-xylene and added it to a –70 °C solution of oxalyl chloride in THF. Quenching with methanol gave the desired phenyl glyoxylate **16** in moderate yield (the main byproduct was the corresponding benzil). This could be converted, for example, to the oxime ester **17** by reaction with methoxylamine and on to the oxime amide **20** with methylamine. This approach afforded the desired intermediates from an inexpensive starting material, but differentiation of the two methyl groups turned out to be more problematic than expected as the radical bromination proved to be only moderately selective (less than 2:1 in favor of the desired product). However, separation was not difficult and the isolated benzylic bromides **19a–c** could be converted to the corresponding oxime final products without difficulty. Similar chemistry produced the methoxy acrylate **21d**, but the bromination and oxime formation proceeded in much lower overall yield. The triazolinone precursor **18** was made from 2,5-dimethylaniline by the route shown in Scheme 1. As expected, good levels of activity were observed for the products as seen in Table 2, confirming the ability of the methyl group to impart a twist to the other strobilurin pharmacophores.

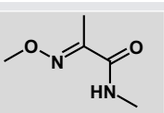
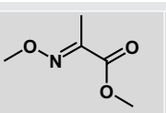
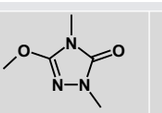
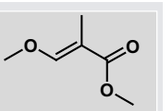
Side Chain Optimization

The highest levels of activity were seen for the oxime amide and oxime ester pharmacophores and we wanted a more robust method for synthesis of intermediates to pursue an analog program to explore structure–activity relationships on the oxime sidechain. Since selective functionalization of the 5-methyl group was our main synthetic obstacle, we searched for potential starting materials that already were appropriately functionalized at the 5-position. Commercially available 3-amino-4 methylbenzyl alcohol (**22**) was an appealing starting material, but its use required the devel-

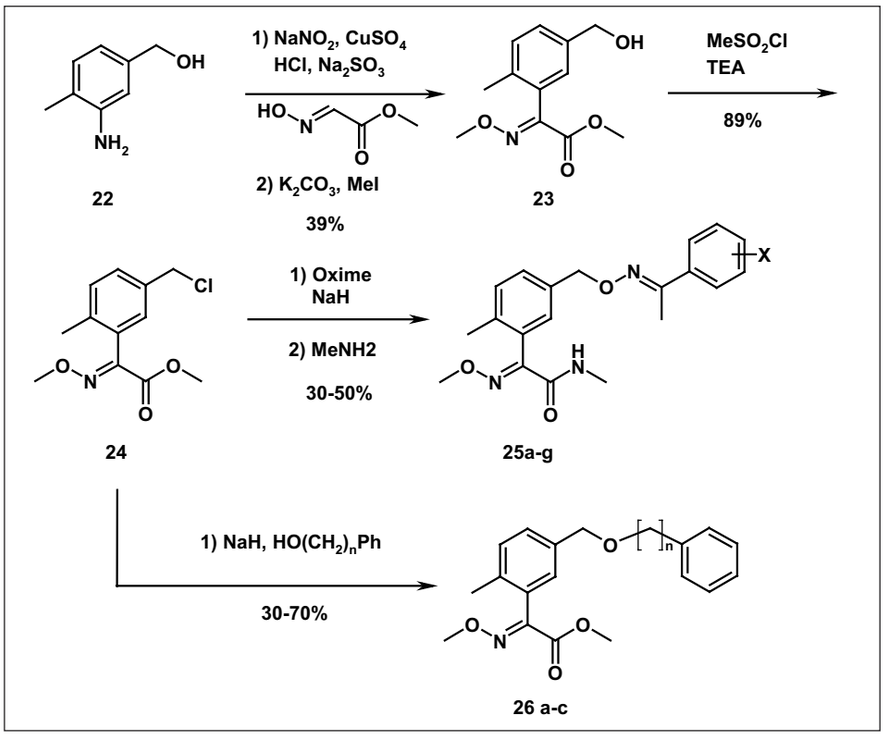


Scheme 2. Synthesis of various pharmacophores with the oxime side chain at position 3

Table 2. Comparative activity of various pharmacophores with the oxime side chain

Strobilurin Pharmacophore	 (**21a**)	 (**21b**)	 (**21c**)	 (**21d**)
Fungicidal Activity	++++	+++	+++	++

opment of an effective method for converting the amino group to the oxime ester or amide (Scheme 3). Aldoximes are known to react with diazonium salts in the presence of copper salts to form aromatic ketoximes [7]. If the oxime of methylglyoxylate could participate in such a coupling, a direct synthesis of the oxime ester pharmacophore might be possible. Indeed, when the diazonium coupling reaction was carried out in the presence of copper sulfate and sodium sulphite the desired ester oxime was isolated in good yield [8]. Methylation of the oxime with methyl iodide or dimethylsulfate in the presence of potassium carbonate produced the desired (*E*)-isomer of the oxime ester **23** in high selectivity and good yield. Conversion of the alcohol to the chloride **24** could be carried out by treatment with methanesulfonyl chloride. Reaction of the benzylic chloride with various oximes gave the target molecules, which could be converted with methylamine to the amide oximes **25a**–**c**. It was also possible to make ethers **26a**–**g** of various chain lengths from **24**, which allowed us to gauge the effect of chain length on activity (n = 0, 1, and 2). The most active materials were those with n = 0 (Scheme 3).



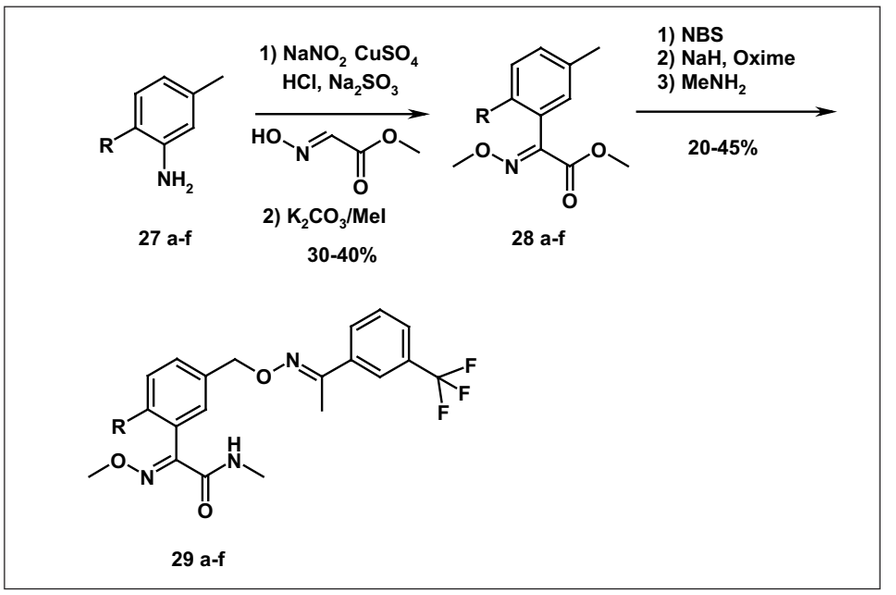
Scheme 3. Novel synthesis of traditional pharmacophores *via* diazotization

Table 3. Relative activities of various oxime substituents

Subst.	3,5-CF_3 (**25a**)	3,5-DiCl (**25b**)	3-$SiMe_3$ (**25c**)	3-OCF_3 (**25d**)	3-Cl (**25e**)	3-Me (**25f**)	4-Me (**25g**)
Fungicidal Activity	++++	+++	+++	++++	+++	++	++

Alternative *ortho*-Groups

It was also apparent that methyl groups were not the only groups that might be able to cause a twist in strobilurin pharmacophores. We found it was straightforward to synthesize compounds with a variety of halogens as well as a methoxy group *ortho* to the pharmacophore with the oxime side chain (Scheme 4). Beginning with the requisite anilines **27a**–**f** with a 3-methyl and the various *ortho*-substituents, we were able to use the diazotization route to install the pharmacophore in moderate yield. Radical bromination of the methyl group gave the requisite benzylic bromides. Reaction with the 3-trifluoromethylacetophenone oxime, and amidation completed the sequence to give **29a**–**f**. As shown in Table 4, the methyl substituent had the highest levels of fungicidal activity.



Scheme 4. Synthesis of various *ortho*-substituents

Table 4. Relative fungicidal activities of various *ortho*-groups

R	Me (**21a**)	F (**29a**)	Cl (**29b**)	Br (**29c**)	I (**29d**)	OMe (**29e**)	H (**29f**)
Fung. Activity	++++	++	++	+	++	+	-

Conclusions

Strobilurin analogs with *meta*-sidechains are quite active against a variety of commercially important plant pathogens. Highest levels of activity were found for diseases of cereal crops such as wheat foot rot, wheat leaf rust, wheat glume blotch and especially, wheat powdery mildew. Efficacy for the optimal compounds such as **21a** was displayed at 2 ppm and below for various powdery mildews. Good activity was also displayed on other important pathogens such as apple scab, rice blast and grape downy mildew.

We were able to show that *meta*-substituted strobilurins can have interesting levels of fungicidal activity provided that a substituent *ortho* to the pharmacophore was also present. This work contradicted previous findings that the sidechain needed to be in the *ortho*-position relative to the pharmacophore. We surmise that the *ortho*-group enforces a twist on the pharmacophore leading to a conformation suitable for binding to the cytochrome bc_1 complex, similar to the effect of the traditional *ortho*-side chain.

Acknowledgements

We would like to thank all the members of the plant disease control group at Du Pont for providing the biological evaluations presented here. Mitochondrial respiration assays were carried out by Mr. Rand Schwartz and Dr. Douglas Jordan. Analytical measurements were obtained by Mr. James Doughty, Mr. John Groce and Ms. Gina Blankenship. We also acknowledge helpful discussions with Dr. David A. Clark and Dr. Richard J. Brown. We would also like to thank Dr. Darius J. Robinson and Ms. Berlin Stokes (who synthesized the original thiadiazolyl triazolinone **(14a)**, which served as the comparison point for our original investigation). We would also like to thank Dr. James V. Hay and Dr. William K. Moberg for their enthusiastic support of this project.

Received: September 15, 2003

[1] For a comprehensive review of the evolution of strobilurins see: H. Sauter, W. Steglich, T. Anke, *Angew. Chem. Int. Ed.* **1999,** *38,* 1328.
[2] W.F. Becker, G. von Jagow, T. Anke, W. Steglich, FEBS Lett. **1981,** *132,* 329.
[3] R.J. Brown, B. Ashworth, J.E. Drumm, D.A. Frasier, M.A. Hanagan, C. Happersett, G.E. Koether, D.J. Robinson, K-M. Sun, P. Wojtkowski, in 'Synthesis and Chemistry of Agrochemicals VI', Eds. D.R. Baker, J.G. Feynes, G.P. Lahm, T.P. Selby, T.M. Stevenson, American Chemical Society, Washington D.C., pp. 327–341.
[4] K. Beautement, J.M. Clough, P.J. de Fraine, C.R.A. Godfrey, *Pestic. Sci.* **1991,** *31,* 499.
[5] R.J. Brown, D.M.T. Chan, M.H. Howard, D.J. Daniel, D.A. Clark, T.P. Selby (Du Pont), World Patent Application WO 97/00612, **1997.**
[6] Experimental details, physical properties and fungicidal activity for compounds in this paper are described in: M.P. Walker (Du Pont), World Patent Application WO 99/28305, **1999.**
[7] W.F. Beech, *J. Chem. Soc.* **1954,** 1297.
[8] A similar transformation was subsequently reported by Novartis: R. Waditschatka (Novartis), World Patent Application WO 00/63162, **2000.**