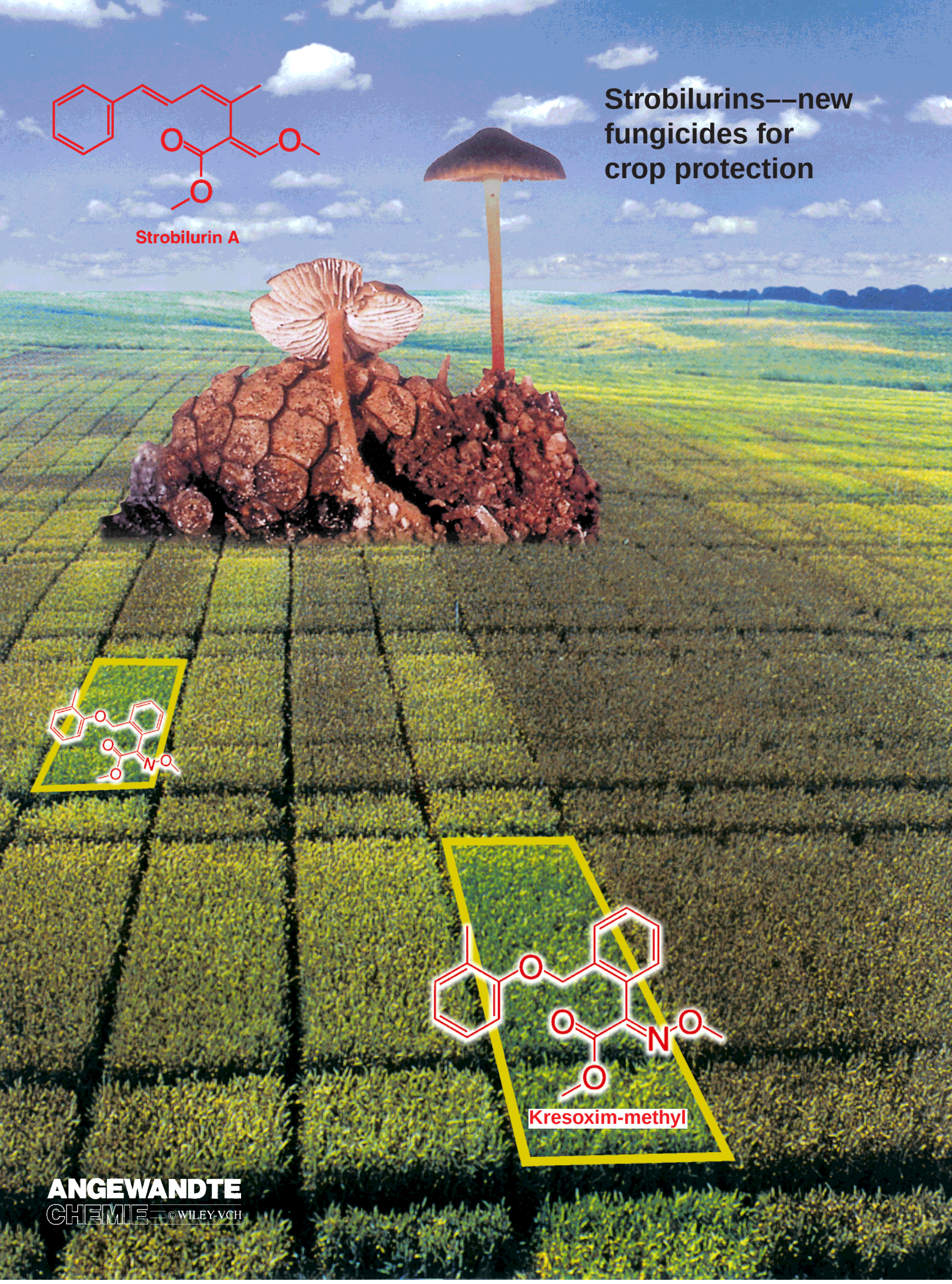

Strobilurins––new fungicides for crop protection
Strobilurin A
Kresoxim-methyl
ANGEWANDTE CHEMIE
©WILEY-VCH

Strobilurins: Evolution of a New Class of Active Substances

Hubert Sauter,* Wolfgang Steglich, and Timm Anke

Dedicated to Professor Hans-Jürgen Quadbeck-Seeger on the occasion of his 60th birthday

Nature provides active substances, as the result of special biosynthetic pathways, with the most diverse biological activities. Many of these substances perform functions that are important to the survival of the organism in which they are formed. For example, certain fungi produce antifungal strobilurins and oudemansins, agents to which the producers themselves are not sensitive, but which ward off other fungal species that grow on the same substrates. The period that followed the early breakthroughs in the 1970s—the discovery of the natural strobilurins and oudemansins, the identification of the β-methoxyacrylate group as their common structural element, and the elucidation of their mode of action—saw the focus of research activities shift away from the universities, ushering in an era of intense competition between rival industrial fungicide research teams. Their goal was, and remains, the development of fungicides with practical applications in agricultural crop protection. This review illustrates methodological approaches in the optimization of lead structures and the importance of understanding structure–activity relationships. The variation of natural product lead structures and the selection of suitable candidates for development led to an across-the-board evolution of the strobilurins as a new class of active substance. Several commercial products resulting from this effort are now already on the market, and more are expected to follow: a most intriguing success story.

Keywords: active substance research · fungicides · natural products · strobilurins · structure–activity relationships

1. Introduction

Since time immemorial, fungi have played an important part in the history of mankind, for example in the production of foodstuffs such as cheese, bread dough, and soy sauce or in alcoholic fermentation processes. In some Asian and American cultures fungi have been used by cults as the source of hallucinogens. Also when it comes to delicacies, the fruiting bodies of fungi (to the layman the actual, that is, visible, manifestations of fungi) have been valued since olden times, none more so than the truffles prized by gourmets. However, the importance of fungi as medicines was traditionally rather low compared with that of plants. This situation remained basically unchanged until the groundbreaking discovery of penicillin by Alexander Fleming in 1928 and its development as the first highly active antibiotic obtained from a mold. Since then the importance of fungi as sources of antibiotics and other pharmacologically active natural products has become ever more evident.[1]

[*] Dr. H. Sauter
BASF Aktiengesellschaft Hauptlaboratorium
Forschung Fungizide
D-67056 Ludwigshafen (Germany)
Fax: (+49) 621-60-21156
E-mail: hubert.sauter@basf-ag.de
Prof. Dr. W. Steglich
Institut für Organische Chemie der Universität
Butenandt Strasse 5–13/F, D-81377 München (Germany)
Prof. Dr. T. Anke
Fachbereich Biologie, Lehrbereich Biotechnologie
Paul-Ehrlich-Strasse, Gebäude 23
D-67663 Kaiserslautern (Germany)

2. The Natural Strobilurins and Oudemansins

2.1. Background

Strobilurins were first identified within the framework of a program begun in late 1976 aimed at discovering new antibiotic agents from basidiomycetes. This class of higher fungi, which includes many familiar species such as fly agaric and edible boletus, was in those days spurned by natural product researchers. The often sluggish growth of mycelial cultures of basidiomycetes meant they were regarded as being more difficult to handle than the extremely active substance-rich streptomycetes, which are classified as bacteria. Nevertheless, studies of the pigments and toxins of the fruiting bodies[2] promised an original and diverse secondary metabolism in mycelial cultures too. In their work on the formation of polyacetylenes in basidiomycetes, Jones and Thaller had drawn parallels between the secondary metabolism of basidiomycetes and that of higher plants, notably *Apiaceae* and

  1433-7851/99/3810-1329 $ 17.50+.50/0

Asteraceae.[3] This followed from the pioneering work by the groups of Anchel, Kavanagh, and Hervey,[4] who in the 1940s and 1950s had screened hundreds of extracts of fruiting bodies, and in some cases of corresponding fungal mycelial cultures too, for the formation of antibiotics. A pivotal discovery to emerge from their studies was pleuromutilin, a new antibacterial antibiotic with an interesting site of action,[4b] which was launched some years later by Sandoz as the semisynthetic derivative tiamulin for use as a veterinary medicine.[5] All of this, plus the congenial research environment offered by the Sonderforschungsbereich in Tübingen headed by Zähner "Chemical Biology of Microorganisms", was the spur for one of us (T.A.) to commence work on basidiomycetes. Among the first antibiotics discovered were strobilurins A and B, obtained from fermentations of *Strobilurus tenacellus* (a fungus that grows on pinecones).[6] Up till then no natural products had been isolated from this fungus. Our first publication in 1977[6] reported the physical and chemical data of strobilurins A and B as well as their powerful antibiotic activity against a range of fungal species. The high cytotoxic activity towards Ehrlich ascites tumor cells led Douros of the National Cancer Institute (USA) to ask us later the same year to provide samples of the strobilurins to screen for antitumor activity. The first 117 mg of strobilurin A were dispatched in 1978. Soon afterwards the exciting news came that strobilurin A, although exhibiting only very weak antitumor activity, showed no acute toxicity in the tumor-bearing mice.

The structure elucidation by the research group of one of us (W.S.) showed the strobilurins to have an unusually simple structure and apparently represent a new class of antifungal antibiotics whose abundance in nature was not confined to the genus *Strobilurus*.[7] Strobilurin A has also been isolated from *Cyphellopsis* cultures and from two *Mycena* species. This compound was originally thought to have a side chain with an all-*trans* 9*E* configuration, but this was later (1984) revised to 9*Z*.[8] Strobilurin A was found to show great similarity to mucidin, an antifungal antibiotic published in 1969[9] and patented in 1970[10] without a proposed structure; they had the same empirical formula, the same UV spectrum, and equal activity. However, the optical activity reported for mucidin ($+33^\circ$ ($c = 10$) at 546 nm) completely ruled out a common identity with the strobilurin A. Mucidin, which was isolated from *Oudemansiella mucida* (a fungus that grows on the trunks of beech trees), found use in Czechoslovakia as an antifungal ointment (Mucidermin "Spofa"). To be more certain that strobilurin A and mucidin were different compounds, samples of *O. mucida* were collected as starter material for mycelial cultures, which were then investigated for the formation of antifungal antibiotics. To our surprise we did not detect mucidin in these cultures, but instead a novel natural product, the crystalline, optically active oudemansin A, and the oily strobilurin A.[11] Like strobilurins A and B, oudemansin A also exhibited powerful antifungal activity. The quest to establish which compound corresponded to "mucidin" proved to be highly protracted. Czech patents and publications yielded contradictory information. One patent,[12] citing previous patents of the same origin,[10] correctly described the structure as (9*Z*)-strobilurin A. No reference was made in this work to the optical rotation

Hubert Sauter is Vice President, Research at the Main Laboratory, Fungicide Research at BASF AG. He started studying chemistry in 1964 at Freiburg im Breisgau (Germany) and additionally studied philosophy and psychology in 1965/1966. After gaining his PhD in 1973 under the guidance of H. Prinzbach, he worked as a postdoc at Harvard University (Cambridge, Massachusetts) with R. B. Woodward for one year and joined BASF in 1976. Since then he has been involved in the area of fungicide research.


H. Sauter


W. Steglich


T. Anke

Wolfgang Steglich, Professor and Director at the Institute for Organic Chemistry at the Universität München, started studying chemistry in 1951 and received his PhD with F. Weygand in 1960. Later he carried out research with D. H. R. Barton in London and then he was Professor in Berlin, Bonn, and München. His interests lie in the field of natural product chemistry including structure determination and the development of new synthetic methods.

Timm Anke, who is a biochemist and microbiologist, is Professor of Biotechnology at the Universität Kaiserslautern. In 1964 he started studying biochemistry in Tübingen and gained his PhD in 1973 under the supervision of H. Dieckmann. After postdoctoral work with F. Lipman in New York, he completed his Habilitation in Tübingen, and in 1981 he was appointed as a Professor in Kaiserslautern. His work is predominantly involved with the discovery of biologically active natural products derived from basidiomycetes and the elucidation of their action mechanisms.

previously reported for mucidin.[10] Then in 1981 with reference to the earlier patents,[10, 12] among them also the patent stating the correct *9Z* configuration, Sedmera et al. confusingly reported the identity of mucidin as that of (9*E*)-strobilurin A.[13] The optical rotation reported in the Czech patent[10b] was referred to as a "printing error". Final clarification was ultimately furnished in a 1986 paper,[14] in which a direct spectroscopic comparison established the structures of (9*Z*)-strobilurin A and mucidin to be one and the same.

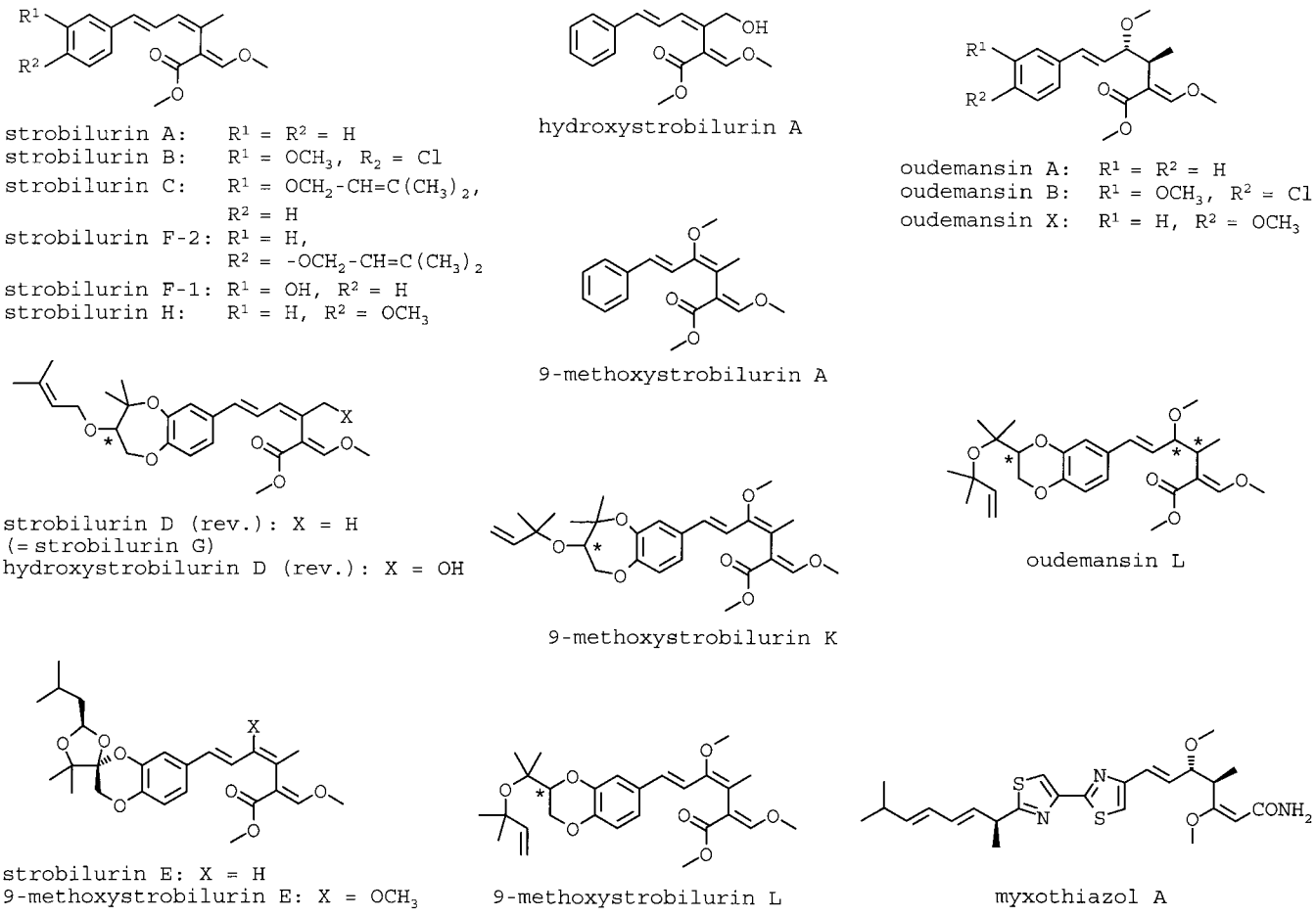
2.2. Abundance in Fungi

Fungi that produce strobilurins and oudemansins are found all over the world in all climate zones.[17b] With one exception (*Bolinea lutea*, an ascomycete) all such species are basidiomycetes.[15] Some of the structures (Scheme 1) are extremely complex, as in the case of strobilurin E, which is characterized by high antifungal activity and very high cytostatic activity.[16] The structures of the 9-methoxystrobilurins K and L, strobilurin D, and hydroxystrobilurin D have since been revised.[17] The antibacterial activity of 9-methoxystrobilurin L published by the company Xenova has, like the proposed structure, since been found to be erroneous.[18]

2.3. Function of the Natural Products for the Producers

In the search for new active substances, in the group of one of us (T.A.) basidiomycetes are normally grown on complex, highly nutrient rich media to ensure good mycelial growth and high production of secondary metabolites. However, because these conditions in no way correspond to those of their natural substrates, such as soil, leaves, or (most commonly) wood, certain strobilurin producers were also grown on their natural, presterilized substrates and then investigated for the formation of antibiotics. Formation of both oudemansin A and strobilurins was observed in a *Pterula* species, a tropical *Favolaschia* species, and in *Mycena tintinnabulum*.[19] In *Mycena tintinnabulum* strobilurin D was detected both in the fruiting bodies (caps) and in the mycelially colonized substrate (oak wood) in nature. The concentrations determined in the mycelially colonized oak wood were sufficient to inhibit the growth of other fungal species. This finding and the worldwide distribution of strobilurins and oudemansins leads us to conclude that the production of these compounds affords an advantage in terms of survival over other parasitic fungal species or fungi colonizing the same substrate.

But how do the fungi that produce strobilurins or oudemansins protect themselves against their own antifungal toxins, which are often produced in considerable quantities? We investigated this matter in collaboration with the group of



Scheme 1. The natural strobilurins and oudemansins[17b, 66] and myxothiazole A.[21a] The sterocenters whose configurations are unknown are marked with an asterisk.

Brand and von Jagow, and found that in *Strobilurus tenacellus* the site of action in the cytochrome b protein (see Section 2.4) is modified such that the binding of strobilurins and oudemansins becomes much more difficult. This is essentially due to the substitution of a small amino acid (alanine or threonine) in position 127 by a larger isoleucine residue. This resistance-inducing exchange occurs in a region that is highly preserved in the species sensitive to strobilurin-type toxins. In *S. tenacellus* an additional small amino acid in position 254 is replaced by glutamine, with the asparagine in position 261 being replaced by aspartic acid. All these amino acid residues are located in the binding envelope of the reduced coenzyme Q.[20]

2.4. Mode of Action

Studies on the mode of action of strobilurins and oudemansins revealed very early on that these compounds inhibit the respiration of fungi. Investigations with Ehrlich ascites carcinoma cell cultures showed that the site of action lay exclusively in the respiratory chain (Figure 1). Other effects

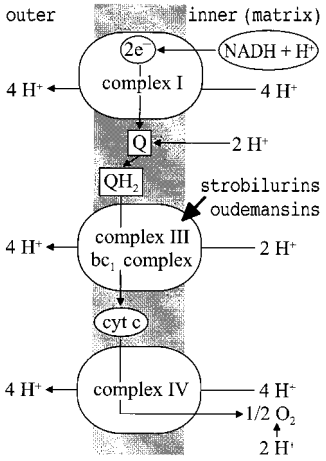


Figure 1. The site of action of strobilurins and oudemansins in the mitochondrial respiratory chain.

such as the complete inhibition of protein, RNA, and DNA synthesis were attributed by one of us (T.A.) to the resulting intracellular deficiency in ATP. The oxygen uptake and ATP synthesis of rat liver mitochondria were inhibited with both α-ketoglutarate and succinate as substrates; hence the site of action had to be in either complex III or IV of the respiratory chain.[11] A fortunate turn of events led us to find in von Jagow a collaboration partner of the utmost competence, who succeeded in localizing fairly exactly the site of action of the strobilurins and oudemansins in the mitochondrial bc_1 complex. In our first joint publication we reported that strobilurins A and B, oudemansin A, and myxothiazole, a compound isolated by the groups of Reichenbach and Höfle at the Gesellschaft für Biotechnologische Forschung (Society for Biotechnological Research) in Braunschweig,[21a] all bind at the same site of action.[21b] A common structural element of these compounds that is clearly essential to their mode of action is an (*E*)-β-methoxyacrylate subunit. Strobilurins, oudemansins, and myxothiazole bind reversibly to the ubihydroquinone oxidation (Qp) center of the bc_1 complex, a finding that has helped further our understanding of the structure and function of this part of the respiratory chain.[22] Antimycin, an antifungal antibiotic commonly found in streptomycetes that is toxic to mammals, binds to the other center of the bc_1 complex, the Qn center. The crystal structure of the bc_1 complex from bovine heart tissue was recently solved by the Deisenhofer group, and the binding sites for myxothiazole and antimycin A were identified.[23]

The first patent application for strobilurin A was submitted in collaboration with Hoechst[26] and described the synthesis of strobilurin A, which seemed like it might find use as an antimycotic agent in human medicine. However, the compound compared poorly with other antimycotics on the market, and this patent was later relinquished. Several total syntheses of the natural strobilurins and oudemansins have been described and discussed in review articles.[17b, 24]

3. Lead Structure Optimization in Rival Industrial Research Programs

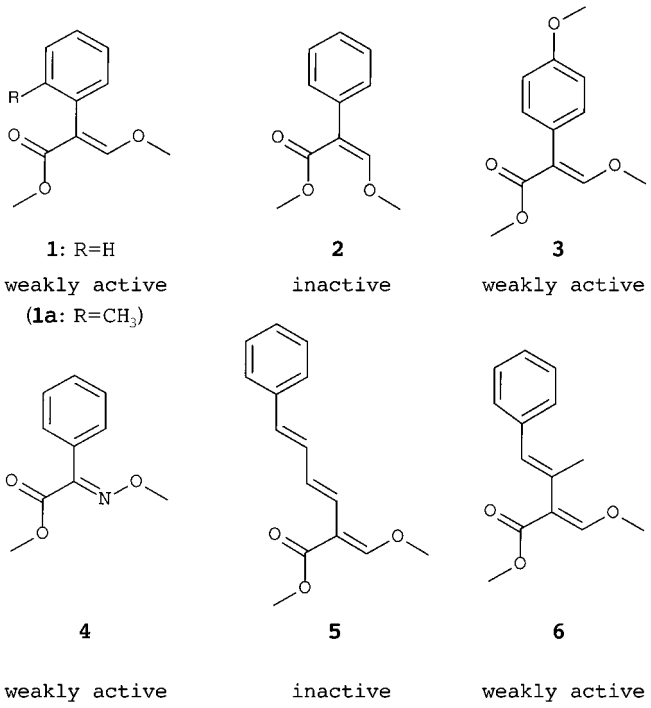
3.1. First Structural Variants

With their rare combination of favorable molecular and biological properties, the two natural products strobilurin A and oudemansin A were immediately seized upon as candidates for model compounds on which to base further synthetic modifications.[24] A clear attraction was the simplicity of their structures, which is a rarity in bioactive natural products. Moreover, the presence of the β-methoxyacrylate group in both natural products gave us a clue that these were not merely solitary active substances in isolation, but were instead part of a group of active compounds. Secondly, their antifungal activity in laboratory tests proved to be attractively high, which together with their "novel" mode of action—inhibition of respiration combined with low toxicity towards mammalian cells—therefore gave us a powerful boost.

Consequently, syntheses of molecular variants of strobilurin A were underway as early as 1977 in the research group of one of us (W.S.). The goal was easily accessible analogues with antibiotic activity that was similar or enhanced as far as possible.[25]

The β-methoxyacrylate group, whose heteroatoms are capable of intermolecular, polar interactions, was regarded as essential (i.e., as the pharmacophore) and was initially modified merely for the usual comparison purposes. The other regions of the molecule were judged to hold greater promise for structural variation. Such considerations gave rise not only to simple phenyl derivatives such as **1**–**3**, but also to norstrobilurin A (**5**) and the "shortened" analogue **6** (Scheme 2).[24, 25]

Although such compounds showed a lower level of activity than the naturally occurring model compound in laboratory tests, some did show detectable, albeit weak, activity against *Penicillium notatum* as a representative fungal species both in



Scheme 2. Structure and activity of simple strobilurin analogues. Activities were determined on the basis of the inhibition of respiration of and the agar diffusion test with *Penicillium notatum*.[24, 25]

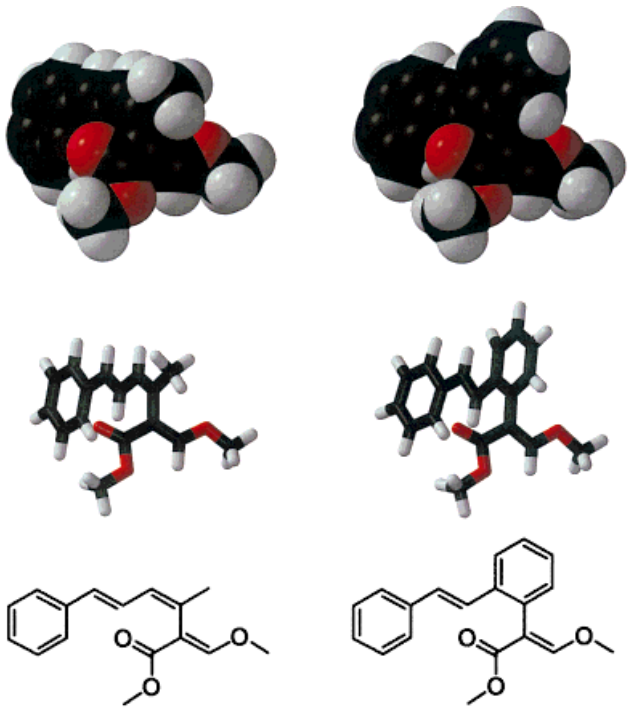


Figure 2. Computer-generated space-filling and stick models of strobilurin A (left) and the enol ether stilbene **7a** (right). Carbon: black, oxygen: red, hydrogen: white. (We are grateful to Dr. W. Altenhofen, BASF, for the calculations and the computer graphics.)

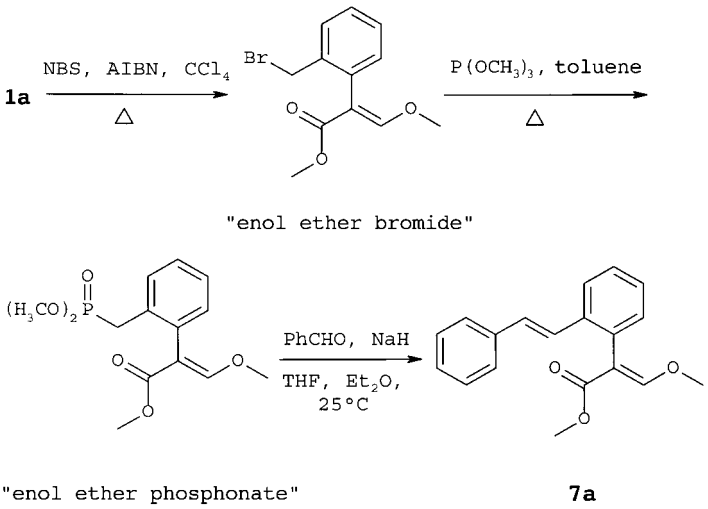
testing for inhibition of respiration and in the agar diffusion test.

From these results we were able to deduce some initial important structure–activity relationships.[24, 25] The *E* configuration of the pharmacophore as in **1** is essential, as the *Z* isomer **2** was inactive. Substituents in the aryl ring such as in **3** appeared to be "allowed"; even a C/N exchange in the enol ether group, which provided the oxime ether **4**, did not result in any appreciable reduction in activity. A loss of activity was observed for the more linear (9*E*)-norstrobilurin A (**5**) and in the similarly shaped variant **6**; lacking in both compounds is the characteristic conformation of strobilurin A and oudemansin A, in which twisting of the central single bond results in an almost right-angled orientation of the β-methoxyacrylate pharmacophore relative to the phenyl-substituted side chain (Figure 2). In the variants **1**–**4** the side chain is missing completely.

In parallel to these variations of the lead structure, collaborations were sought with industrial partners with the aim of probing the usefulness of this class of active substances for the optimization of structure and activity under the viewpoint of potential applications as agricultural fungicides or clinical antimycotics. The response from industry to our overtures was, owing to other more pressing priorities, for the most part low-key. In 1982 Zeneca (then still part of ICI) requested a sample of oudemansin, which they obtained from one of us (T.A.). Interest shown by BASF in 1983 within the framework of a publicly funded joint project[27] in collaboration with several university research groups led to a sample of strobilurin A being provided for in vivo greenhouse testing on plants infected with fungal pathogens. The test results were disappointing: The activity of strobilurin A was surprisingly weak, being barely perceptible against only two out of six phytopathogenic fungal species at high application concentrations.[28] This was contrary to expectations after the very good results in agar diffusion tests in the laboratory, and the question was posed as to what might be the possible causes.

Together with Schirmer, who at the time was responsible at BASF crop-protection research for collaboration with university research teams, we arrived at the hypothesis that the triene system of strobilurin A might have been the cause of a rapid photolytic and/or oxidative breakdown of the natural product, leading to a rapid loss of activity under the conditions of the greenhouse tests. The apparent plausibility of this hypothesis led us to dispense for the time being with verification through analytical experimentation. A different approach appeared more attractive: The idea we came up with was to stabilize the triene system with an aromatic bridge and, in the hope of improved in vivo activity, to synthesize and carry out tests on the "enol ether stilbene" **7a**. The synthesis was swiftly accomplished in the Steglich research group (Scheme 3).[29a]

As it turned out, we hit the bull's-eye with **7a**. Not only did it exhibit an activity against *P. notatum* in the inhibition of respiration test that was at least ten times higher than that of strobilurin A,[29b,c] its potency and spectrum of activity in in vivo greenhouse tests in plants infected with fungal pathogens fulfilled our ambitious expectations. Good (< 15 % infection) to very good activities (< 5 % infection) at application concentrations of 63 ppm, and in some cases even at 16 ppm, were recorded for the following fungal diseases: powdery mildew of wheat (*Erysiphe graminis*), wheat brown rust (*Puccinia recondita*), rice blast disease (*Pyricularia*



Scheme 3. Synthesis of the enol ether stilbene **7a**.[24, 29a] AIBN = 2,2′-azobisisobutyronitrile, NBS = *N*-bromosuccinimide.

oryzae), barley net blotch (*Drechslera teres*), grapevine downy mildew (*Plasmopara viticola*), and late blight (*Phytophthora infestans*).[30]

The bridging by the central arene subunit in the enol ether stilbene **7a** did not result in a reduction of affinity for the mitochondrial target, it was if anything even increased, and clearly bestowed on the molecule—as hoped—the required stability. Thus, compared with strobilurin A, **7a** represented a modified, more potent, and overall more promising lead structure.

3.2. A Further Generation of Strobilurin Variants

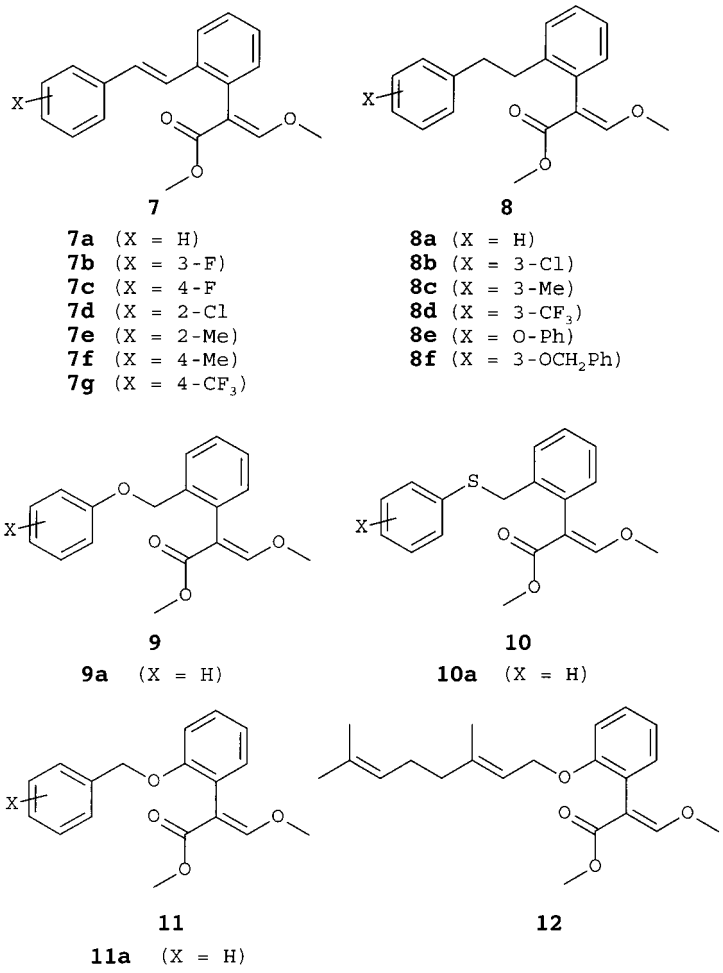
Guided by this result, and with BASF broadening their involvement in the project, we turned our attention to the synthesis of some further variants of **7**, for example **8**–**12** (Scheme 4). In laboratory tests these compounds all showed activities comparable to that of strobilurin A, and exceeded this distinctly in the greenhouse tests. Some of the new derivatives exhibited even higher activity than the enol ether stilbene **7a**, depending on the test fungus.[30]

In the design of strobilurin variants a conceptual subdivision of the enol ether stilbene lead structure into three molecular regions proved useful: side chain, arene bridge, and pharmacophore (Scheme 5). Through variation of the side chain we succeeded in obtaining compounds of type **7**,[31] **8**,[32] **9** and **10**,[33] and **11**,[34] each the subject of separate patent applications.

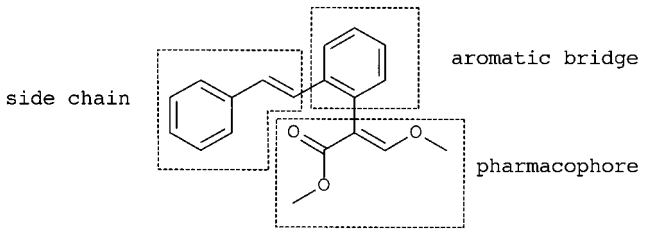
3.3. Initial Field Trials at BASF

The next step was the biological evaluation of preselected candidates under outdoor conditions. In the course of optimization of a lead structure the transition from greenhouse tests to field trials demands particularly close attention. Three aspects are of prime importance here:

- The quantities of active substance required, and hence the outlay on their synthesis, rise dramatically. For in vitro testing and other laboratory tests a few milligrams are normally sufficient. Even for an extensive series of dilutions in the greenhouse tests the amount of active material required is only about 100 mg in total. Thousands of individual structural variants can be tested per year as a matter of routine without undue difficulty. In contrast, with application of 100–1000 g ha^{-1}, preliminary field trials—normally in small plots of one square meter—require between 1 and 10 g per indication depending on the plant species, fungal pathogen, and number of treatments, that is, up to 100 g of each active substance must be supplied. Additional amounts of active substance are also required for the development of formulations suitable for application under outdoor conditions, for example as emulsion concentrates, suspension concentrates, or water-dispersible powders or granules.



Scheme 4. Strobilurin variants based on the enol ether stilbene **7a**.[24, 31–34]



Scheme 5. Structurally modified regions of the enol ether stilbene lead structure.

● For these and other practical reasons the number of compounds that can be tested under outdoor conditions is restricted to barely more than one hundred per year. This means that compounds that fail to make it to the field trial stage are essentially dropped as individual candidates in the optimization process.

● Years of experience have shown the model tests in the greenhouse to be of only limited value as a predictor of the all-important activities in field trials under real-life conditions. Clearly discrepancies[35a] are possible in the sense of false positive and false negative greenhouse results,[35b] particularly when an attempt is made to assess the order of potency within individual families of active compounds.[35c, 36] This special situation is evident from the values shown in the bottom line of Table 1. Here, the rank correlation coefficients[39] between the field trial and greenhouse test results range from usable values (0.74 for powdery mildew of wheat, 0.6 for late blight) to values of little prognostic usefulness (for example -0.3 for net blotch disease).

In this respect the selection of the "right" candidates for the field trials, which due to seasonal growing cycles are only possible at certain times of the year,[37] becomes the critical rate-determining step, and hence also the decisive step for the overall success[36] of the lead structure optimization process (see Figure 4, Section 4.4).

Of the roughly 100 strobilurin derivatives synthesized up to December 1985 at BASF, a first batch of 15 compounds was selected for small plot trials in the 1986 season and tested in various indications according to the results obtained in the earlier greenhouse tests. Table 1 summarizes these compounds and the results obtained.[38]

In field trials against powdery mildew of wheat a clear disparity is evident between the good results with the four dihydrostilbenes **8** and the lack of activity of the stilbenes **7**. This seems plausible in view of the known photolability of stilbenes, which can undergo $E \rightarrow Z$ isomerization and subsequent facile photocyclization to dihydrophenanthrenes followed by further reactions such as oxidative aromatization.[40] Overall the rank correlation with the greenhouse test results of the family of compounds tested is good ($r_s = 0.74$), though the inferiority of the stilbenes **7** in the greenhouse tests is not so pronounced as under the more severe photochemical conditions in the field trials. Particularly noteworthy was the activity of **8d** against powdery mildew of wheat in field trials. The activity of **8d** at maximum infection pressure (100% infection in the untreated plots), albeit at higher rates of application (1000 g), exceeded that of the standard reference substance, Corbel—a most encouraging result.

In tests against net blotch disease even the three stilbenes **7** tested here proved to have some activity at a lower infection pressure (35%). By contrast, the sole dihydrostilbene tested, **8d**, again showed distinctly higher activity, exceeding—albeit at double the rate of application—the level of the standard Desmel.

In tests against glume blotch on wheat **8d**, the front runner against powdery mildew of wheat, again showed high activity, this time, however, closely followed by stilbene **7e**. The tests against wheat brown rust were not especially fruitful, owing to the particularly low infection pressure (18%) and the relatively weak activity of the five dihydrostilbenes tested. In tests against late blight at a high infection pressure, the dihydrostilbene **8a** proved to be the best, although two of the stilbenes also showed measurable activity. In the case of activity against apple scab stilbene **7a** was in first place and on the level of the standard. In summary, from a structure–activity viewpoint the photolabile stilbenes were distinctly inferior to the dihydrostilbenes under outdoor conditions. The former did, however, show some activity in tests in which the interval between the application of the active substance and the evaluation of the test was relatively brief (applications against glume blotch, late blight, and apple scab), that is 8 days compared with about 30 days in the other tests. For the structure types **9** and **10** only a single example of each underwent field trials, hence a full, experimentally-based assessment of these structure types was not possible at that time.

The first results from these small-plot trials under outdoor conditions, which were of decisive importance to the assessment of the overall structure type, were obtained in May 1986. Even at this early stage some results were highly encouraging, for example the first indication that the activities of **8a**, **8b**, and **8d** were very good and of the same order as that of the standard reference material Corbel. In addition, initial results from tests against net blotch disease in barley, for example after treatment with **8b**, gave rise to high expectations and much euphoria. Not only had our good fortune seemingly seen us through the birth of this new class of fungicidal active substances, we were able to look ahead to the further development of the baby full of hope and confidence.

In the midst of our elation at this breakthrough we came face to face with a most unpleasant surprise. At the end of April 1986 a comprehensive patent application had been published by ICI (now Zeneca),[41] which was brought to our attention a short time later. The first priority date was October 19, 1984.[42] The basic formula **I** of what was an extremely broad and general claim is shown in Scheme 6. R^1 and R^2 represent a wide variety of substituents ranging from hydrogen to "optionally substituted" alkyl, aryl, cycloalkyl-alkyl, and other groups.[43] Even richer in variants were the definitions for X, Y, and Z. On the other hand, the actual core element of these structures was defined in subclaim 7[41] (formula **XII** in Scheme 6). Additional subclaims were submitted for structures of type **13**. As individual compounds "our" enol ether stilbene **7a** and the hydrogenated variant **8a** that had already attracted attention in our field trials, had been the subjects of specific claims. What was striking were the parallels with our ideas and concepts that lay at the heart of this application. All the BASF applications for the enol ether strobilurins[31–34] were hit by this patent—a disaster that set us back all the more at the time, as our field trial results had led us to the brink of verifying the great potential of the strobilurins, not to mention their potential practical applications.

Back in the laboratory we had some hard questions to ask ourselves. How could such a thing had happened? Had we been caught napping? Had indiscretions, espionage even, played a part? The answer was soon in coming and was plain to see: All this had taken place in the context of a fair contest between rival research groups.

Table 1. Test results and order of activity of strobilurins against fungal diseases in 1986 field trials[38] and greenhouse tests.[30]

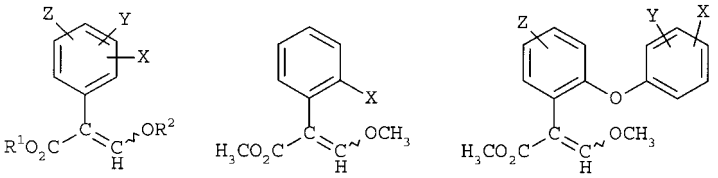
Compound	Powdery mildew of wheat[a]			Net blotch[f]			Wheat leaf spot[i]		
	infection[b] [%]	R_F[c]	R_G[d,e]	infection[g] [%]	R_F[c]	R_G[h]	infection[j] [%]	R_F[c]	R_G[k]
7a				28	5	2	30	6	1
7b	100	7	9	25	4	3			
7c	100	7	7						
7d				22	3	1			
7e	100	7	3				10	2	4.5
7f	100	7	8						
7g	100	7	6				61	7	8
8a	22	2.5	1				18	5	4.5
8b	22	2.5	5	4	1	4	13	4	4.5
8c	31	4	4				12	3	4.5
8d	15	1	2				8	1	4.5
8e							68	8	4.5
8f									
9a									
10a				18	2	5			
untreated	100			35			71		
standard	18			5			5		
r_s[u]		0.74			− 0.30			0.23	

Compound	Wheat brown rust[e]			Late blight[o]			Apple scab[r]		
	infection[m] [%]	R_F[c]	R_G[n]	infection[p] [%]	R_F[c]	R_G[q]	infection[s] [%]	R_F[c]	R_G[t]
7a				68	3	1	5	1	3
7b									
7c									
7d									
7e									
7f				61	2	2			
7g									
8a	18	5	4	33	1	3			
8b	10	1.5	2						
8c	10	1.5	4						
8d	12	3	4						
8e				84	5	5	8	2	2
8f				77	4	4	15	3	1
9a	15	4	1						
10a									
untreated	18			84			77		
standard	9			14			6		
r_s[u]		0.13			0.6			− 1.0	

[a] *Erysiphe graminis f. sp. tritici.* [b] Small plot trials in winter wheat; spontaneous infection; treatment after 2.5 % preinfection, in each case with 750 g ha^{-1} of active substance; evaluation 31 days after treatment; standard reference substance: Corbel, 750 g ha^{-1}. [c] R_F = ranking in field trials according to the definition in ref. [39] within the tested family of compounds (vertical columns). [d] R_G = ranking in greenhouse tests, in each case determined from a number of tests after treatment with various spray concentrations. [e] Determination of R_G based on greenhouse tests with spray concentrations of 16, 8, 4, 2, and 1 ppm. The ranking changes in the corresponding curative tests in the greenhouse; as might be expected, the results of these tests give a better rank correlation with the results of the (in some cases curative) field trials: $r_s = 0.87$. [f] *Pyrenophora teres f. sp. hordei* (barley net blotch). [g] Small-plot trial in winter barley; infection carried out twice 5 and 2 days before treatment with 1000 g ha^{-1} of active substance; evaluation 36 days after treatment; standard: Desmel 500 g ha^{-1}. [h] Determined from protective greenhouse tests after treatment with spray concentrations of 250, 125, 63, and 31 ppm. [i] *Leptosphaeria nodorum* (glume blotch) on wheat. [j] Small-plot trial in winter wheat. Artificial infection 3 days after treatment with 1000 g ha^{-1} of active substance; standard: Desmel 250 g ha^{-1}. [k] Determined from protective greenhouse tests after treatment with spray concentrations of 125, 63, and 31 ppm. [l] *Puccinia recondita* (wheat brown rust). [m] Small-plot trial in winter wheat; spontaneous infection; treatment without preinfection with 750 g ha^{-1} of active substance; evaluation 32 days after treatment; standard: Impact 125 g ha^{-1}. [n] Determined from curative greenhouse tests after treatment with spray concentrations of 250, 63, and 16 ppm. [o] *Phytophthora infestans* [p] Small-plot trial in potatoes; spontaneous infection; treatment carried out three times at weekly intervals with 1000 g ha^{-1} of active substance without preinfection; evaluation 30 days after last treatment; standard: Polyram-Combi (80 %) 500 g ha^{-1}. [q] Determined from protective greenhouse tests in tomato plants after treatment with spray concentrations of 63, 31, and 16 ppm. [r] *Venturia inaequalis.* [s] Tests an apple trees, strain Golden Delicious; spontaneous infection; treatment carried out 18 time at weekly intervals with 210 g ha^{-1} of active substance without preinfection; evaluation 7 days after last treatment; only the infection on the leaves present at the start of treatment was evaluated; standard: Baycor 140 g ha^{-1}. [t] Determined from curative greenhouse tests on apple seedlings after treatment with spray concentrations of 75 and 31 ppm. [u] r_s is the Spearman rank correlation coefficient.[39] Only the rankings of rows R_G and R_F are correlated against one another.

3.4. The Route of the Zeneca Research Team to Azoxystrobin[44, 45]

At the beginning of the 1980s Zeneca had embarked on a systematic and intensified research program looking into natural products as possible lead structures for fungicides. In the course of these activities Baldwin, a biochemist working in the Zeneca fungicides team, had become aware of the work already described above[21b] on the common structural elements and common mode of action of oudemansin, strobilurins A and B, and myxothiazole. Zeneca subsequently asked one of us (T.A.) to provide them with a sample of oudeman-



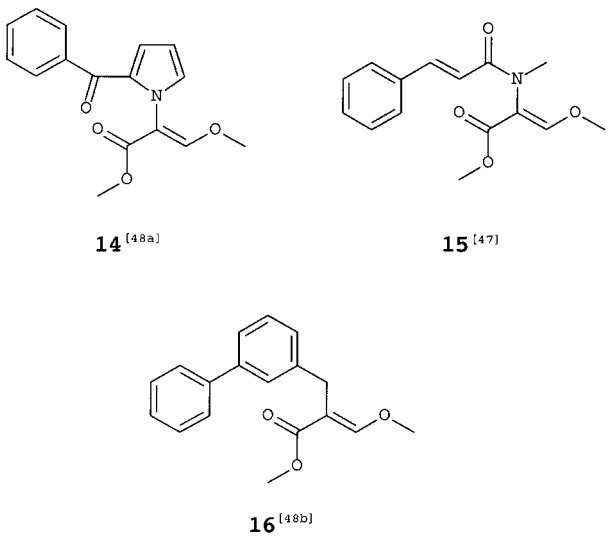
I XII 13

Scheme 6. Formulae from the claims in the ICI patent application for enol ether strobilurins[41]. See text for details.

sin A for greenhouse testing, and in June 1982 had been sent 4 mg of the crystalline substance. Zeneca's greenhouse test results[46] against eight different pathogens were highly impressive. Unlike strobilurin A, oudemansin A is sufficiently stable under greenhouse conditions and consequently shows very high fungicidal activities and a strikingly broad spectrum of activity even at the low application concentration of 33 ppm. This activity proved reproducible at Zeneca, and, spurred on by their impressive test results, the Zeneca team headed by Clough swiftly took to the topic of strobilurins—then still classified under the banner of β-methoxyacrylates—on a wide front. Their endeavors led among other things to the design and realization of stereoselective syntheses for both (9*E*)- and (9*Z*)-strobilurin A,[45e] thereby providing independent confirmation once again of the configuration we had correctly assigned to the natural product strobilurin A. Subsequent analytic investigations revealed the inadequate activity of the synthetic (9*Z*)-strobilurin A in the greenhouse tests to be due, in addition to its relatively high volatility, to its extremely high photolability.[45a, b]

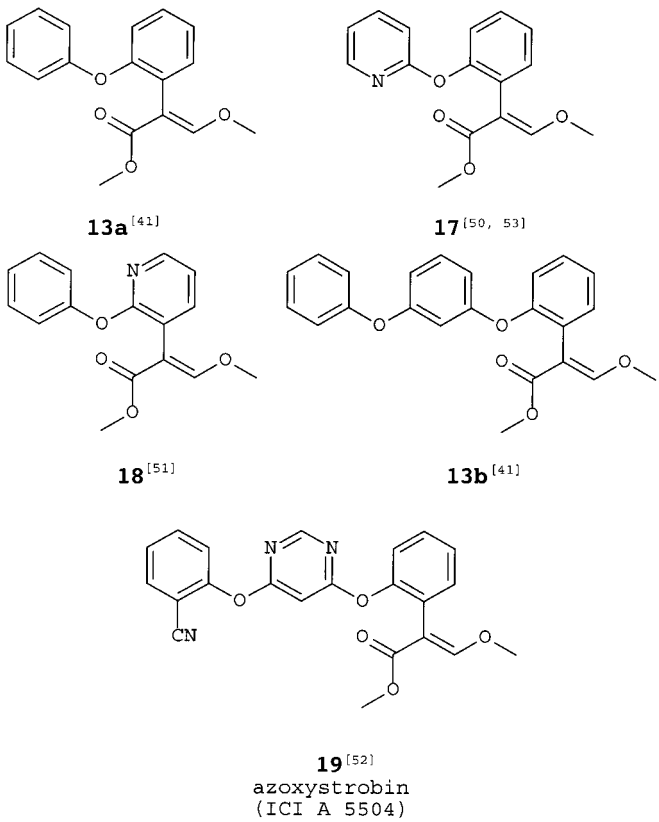
Considerations similar to ours likewise soon led the Zeneca team to the enol ether stilbene **7a**. In parallel to this, however, a range of other active, easily accessible, stabilized variants had also been synthesized, for example **14**–**16** (Scheme 7).

In the progress made at Zeneca a decisive role was played by the diphenyl ether **13a** (Scheme 8). Its photostability was even higher than that of the enol ether stilbene **7a**, it showed high activity in greenhouse and field trials, and it exhibited a broad spectrum of activity.[45e] It also possessed a molecular property that was a specific focus of Zeneca's biological screening program: the systemic transport of the active substance through the xylem and occasionally the phloem of the treated plants. This means that once applied, instead of being merely confined to the sprayed surface of the plant, a systemic active substance will penetrate the plant, thus allowing it to undergo transportation in solubilized form.[49] Systemic transport of a fungicide ultimately means that its activity will not just remain localized, but, as a consequence of the distribution process, can extend to the unsprayed parts of the plant too. Zeneca concluded that although the systemic distribution of the diphenyl ether **13a** was the reason for the good performance as a fungicide in field trials, it was also responsible for the slight phytotoxic damage sustained by some sensitive plant species. Further optimization work was consequently pursued with the aim of keeping the lipophilicity of the new structural variants sufficiently low to permit systemic transport,[49c] but without any accompanying phytotoxicity. The introduction of heteroatoms as in compounds **17** and **18** proved advantageous in this respect. Compound **13b** represented a further step forward from another viewpoint: Its activity in greenhouse tests was superior to that of **13a**, pointing to more efficient docking at the mitochondrial bc_1 complex. However, the additional phenoxy substituents enhanced the lipophilicity of the compound to such a degree it was no longer able to undergo systemic transportation.[49c, 54] With the introduction of hydrophilic structural elements into



14[48a] **15**[47] **16**[48b]

Scheme 7. Stabilized, active strobilurin variants from the early phase of the lead structure optimization at Zeneca.[45e]



13a[41] **17**[50, 53] **18**[51] **13b**[41] **19**[52]
azoxystrobin
(ICI A 5504)

Scheme 8. Strobilurin analogues with variations in the side chain and aromatic bridge on the Zeneca route to azoxystrobin.[45e]

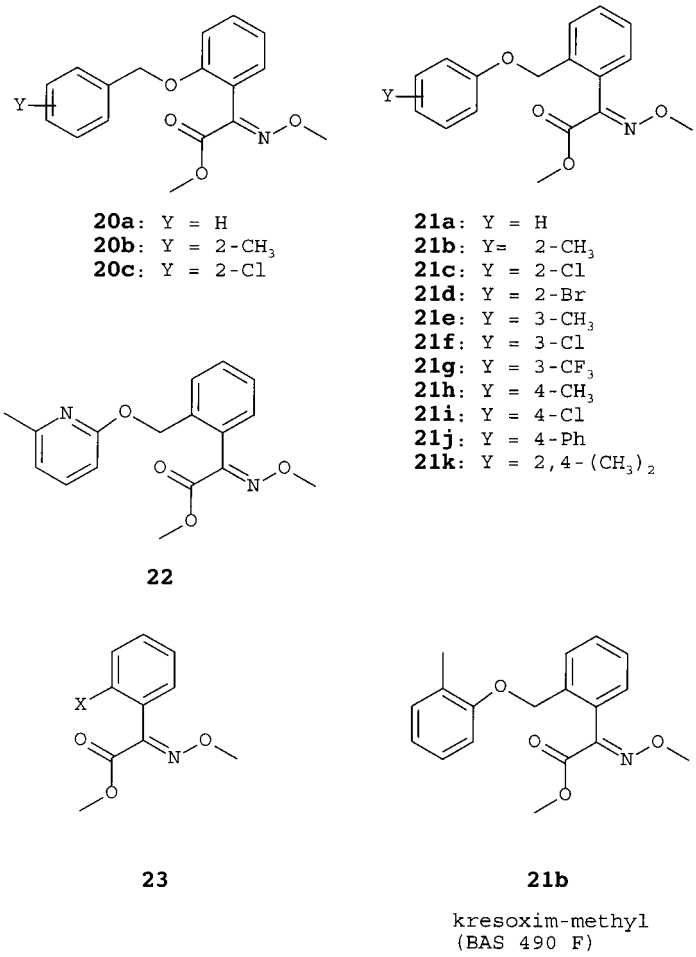
the lipophilic side chain of **13b**, the lead structure optimization finally bore fruit in the form of compound **19**. This compound, initially developed under the code number ICI A 5504, possessed the desired physicochemical and biological properties, and was subsequently christened with the common name azoxystrobin before being launched onto the German market in February 1996 under the trade name Amistar.[45e] Details of its biological action profile in comparison with that of other strobilurins are given in Section 5.3.

3.5. The Second Phase of the Lead Structure Optimization at BASF: The Route to Kresoxim-methyl[28]

With the submission of the enol ether basic application, and especially after its publication in April 1986,[41] the Zeneca team was in a relatively comfortable position from a patent point of view and could turn its attention to the optimization of the side chain while retaining the enol ether pharmacophore. In contrast, the BASF fungicide team was faced with a very different situation in May 1986. The claims published by Zeneca appeared watertight, the only real gaps that were immediately perceptible being in the arene bridge. There was a very real danger[55] that all our efforts to date had been in vain, for us at least. Nevertheless, the recent, immensely promising BASF field trial results (see Table 1) left us no alternative: A way out had to be found. The biological potential of the new class of active substances seemed too tempting, as did the wide latitude in the variation of the arene bridge and side chain discernible inter alia from the Zeneca patent claims.[41, 47] The BASF team consequently elected to go for the sole realistic loophole that still remained and to modify the pharmacophore as a way of getting around the tight confines of the Zeneca patent claims.[56]

Variation of the pharmacophore in a class of active substance always carries a very high risk of a drastic loss of activity. This assumes that the pharmacophore is, by definition, the common functional group which through intermolecular interactions gives the class of active substances a decisive energetic boost for docking at the target. In practice this nearly always means the involvement of heteroatoms with their possibilities for polar interactions and unique vectorial–spatial arrangements relative to the rest of the active substance molecule. The enol ether pharmacophore contains three such heteroatoms, each of them oxygen. Nothing was known at the time about their function in the binding process at the bc_1 complex.

In the BASF team Wenderoth and Rentzea had already embarked on the task of substituting the enol ether group of the pharmacophore by an oxime ether unit. From a pharmacophore analysis viewpoint all three original heteroatoms were still retained, as were, in all probability, their vectorial orientation *possibilities*; this was evident even without the benefit of computer models. The sole requirement was preservation of the *E* configuration of the double bond, which was known to be essential (see Scheme 2, **1** versus **2**). Only the sp^2-hybridized nitrogen atom was new. The first representatives (**20a** and **21a**) of structure types **20** and **21** (Scheme 9) had already been synthesized and subjected to greenhouse tests. From the good results obtained in vitro and with initial greenhouse tests we were confident of the high biological potential of these oxime ether structures. In response to the situation with our known competitors at Zeneca, a corresponding patent application[57] was very



Scheme 9. The first strobilurins (**20**–**22**) with an oxime ether pharmacophore of general structure **23** synthesized at BASF on the way to kresoxim-methyl.

quickly submitted in July 1986—a stroke of luck for us, as we later discovered. What we did not know at the time, but must have feared all too well, was that the Zeneca group had already been pursuing the same idea and had themselves been in the process of submitting a patent application for the oxime ethers.[58] We then had to get through the usual nerve-wracking 18-month waiting period following the submission of our patent.[42] Finally in January 1988 it emerged that this time we had got there first with our submission, by a mere two days—it looked like round two of the contest may well have gone decidedly in our favor.

In the meantime further progress had been made at BASF with the optimization of the oxime ethers. For the 1988 field-trials season two derivatives of **20** (**20b** and **20c**) had been penciled in for small-plot trials on the basis of their good activity against powdery mildew of wheat in the greenhouse tests. This activity was confirmed, and was comparable to that

of the standard Corbel and thus approached the level required for a viable competing product.

A decisive factor in our continuing efforts towards optimization of the lead structure was that from June 1987 onwards a biochemical test was available at BASF[59] which compared with the established greenhouse tests, or the field trials for that matter, was able to provide much more direct answers to the question of how effectively the strobilurin variants bind to the mitochondrial bc_1 complex. This test utilized submitochondrial preparations of yeast cells as model systems for the respiratory chain of fungi. The end result quantifies the inhibitory activity of the test substance towards the bc_1 complex in the form of an I_{50} value, which is the concentration of the active substance necessary to achieve 50% inhibition: the lower the I_{50} value, the more active the compound.[60]

With the aid of this test, we were soon able to deduce some very clear structure–activity relationships (see also Section 4.1). A series of comparisons between pairs of enol ethers and oxime ethers with identical side chains—the arene bridge was a phenylene moiety in all cases—showed the two pharmacophore types to have equal affinities on the target level, giving rise to no appreciable differences in activity. The two pharmacophores thus bind equally strongly—a pivotal finding. Something else that emerged quite clearly, again from a series of pairwise comparisons, was that derivatives of type **21** were on average about ten times more active than the corresponding regioisomers **20**. This result could not have been deduced so clearly, if at all, from greenhouse tests in which the striking differences were blurred by the effects of various factors arising from the more complex study designs, thus being hidden so to speak by background noise.[61]

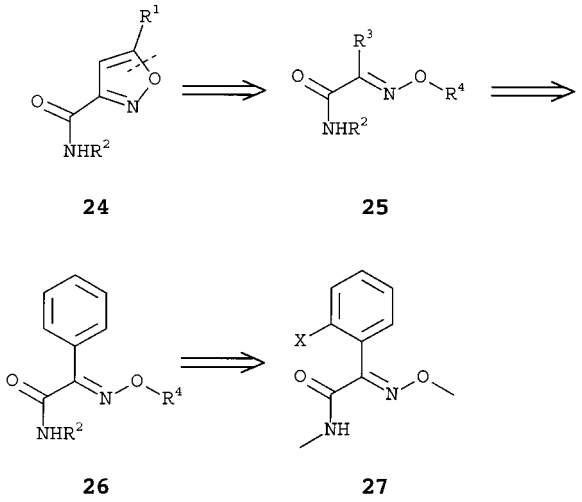
These results were of crucial importance to our future studies. First and foremost, they swung things in favor of the oxime ethers **21** with a phenoxymethyl side chain for development within our synthetic program. Secondly, they led us to focus on variants of the more active structure type **21** in the selection of the candidates for field trials during the 1989 season. Ten variants (**21b**–**21k**) plus the heterocyclic derivative **22** were subjected to field trials. Expectations were high, as remarkable results had already been obtained in the 1988 field-trials season with two derivatives of type **20** that had shown lower activity on the target level. These expectations were borne out in full: All variants of type **21** proved active to a lesser or greater degree in the field trials, particularly towards cereal mildew, but also towards other cereal infections such as wheat leaf spot and leaf blotch. Comparisons with **20b** and **20c**, the two compounds of the previous generation with a benzyloxy side chain, demonstrated clearly the superiority of the new type **21** over **20** under outdoor conditions. Furthermore, in the tests against powdery mildew of wheat the activity of the oxime ethers **21** was clearly greater than that of some of the enol ethers employed for comparison purposes.

Of the outstandingly effective active substances of type **21**, it was **21b** (later named kresoxim-methyl) that for economic and other reasons was selected as the candidate for development. This compound represented an optimal combination of high fungicidal performance and a favorable outlook for a cost-effective commercial synthesis, the sole synthon required for the construction of the side chain being the cheap bulk chemical *o*-cresol.[62]

Compound **21b**, initially developed under the code number BAS 490 F, was later given the common name kresoxim-methyl and, again in competition with Zeneca, was subsequently registered by the German authorities and launched onto the market in February 1996 a few days before azoxystrobin. Details of its biological properties and resultant market positioning are presented in Section 5.3.

3.6. The Entry of the Shionogi Team into Strobilurin Chemistry[63]

Rather by chance, and from another direction and different route entirely, fungicide researchers at Shionogi focused on the strobilurins. In their case it was not the natural products strobilurin A and oudemansin A with their extremely promising biological activities that had been the starting point, but a totally different, "in-house" class of compounds, the carbamoylisoxazoles **24** (Scheme 10).[63] These compounds



Scheme 10. Steps in Shionogi's sequence of thoughts for the synthesis of oxime ether amides **27**.[63]

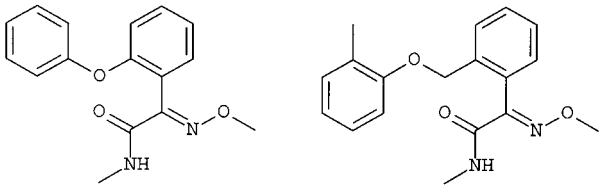
had shown fungicidal activity against rice diseases, but there was no apparent connection with strobilurins. Compounds of type **24** lack two of the properties typical of strobilurins: their broad fungicidal spectrum of activity and their characteristic mode of action in the inhibition of respiration. In the variation of structures of type **24**, trains of thought set in motion largely by purely chemical structural considerations and the efficient deployment of in-house synthetic know-how led to new structures and biological testing in the Shionogi team, culminating in the oxime ether amides of type **26** by the route outlined in Scheme 10.[63] The structure of these compounds already resembled that of the oxime ether strobilurins very closely (see, for example, **4**, **20**–**23**), hence it is no surprise that systematic variation by the Shionogi team ultimately led to compounds of type **27** and to the recognition of their biological potential and the connection with the BASF

and Zeneca strobilurins. In the knowledge[63b] of the oxime ether patents published in early 1988[57, 58] and of the other strobilurin patents published at that time, the Shionogi group submitted their own patent application,[64] based in essence on the general structure **27**. With **27** a new type of pharmacophore, in the shape of the oxime ether methyl amide, had been discovered that was isosteric with the oxime ether methyl esters.

In the course of their own studies on lead structure optimization, the BASF team had, in modifying the oxime ether pharmacophore, likewise turned their attention to the corresponding amides. However, they arrived on the scene late, as the optimization of the side chain in the development of BAS 490 F had been the top priority.

As the first and initially solitary variant of type **27**, compound **27b**, with the side chain that in the case of **21b** had proved optimal in the oxime ether series, was specifically selected and synthesized in late 1989 by BASF. The greenhouse test results were good, but not outstanding, and in the yeast test the I_{50} value of **27b** was more than ten times lower than that of **21b**. Because of this, and in the face of more attractive alternatives, it seemed unnecessary at the time to attach particular priority to this type of compound—a colossal error. It was only after further derivatives with better activities had come to light, though before the publication of the Shionogi claim,[64] that a corresponding patent application was filed by BASF in late 1990.[65] This led once more to patent interferences in the strobilurin field.

The efforts of the Shionogi team ultimately led to a development product with the code number SSF 126[66] (Scheme 11). This compound, subsequently received the common name metominostrobin, was launched onto the Japanese market in 1998 under the trade name oribright as a fungicide active against rice diseases.



27a **27b**

metominostrobin
SSF 126

Scheme 11. The Shionogi strobilurin **27a** and the first variant synthesized at BASF with an oxime ether amide pharmacophore (**27b**).

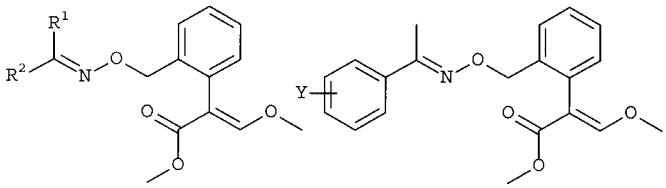
3.7. The Route of Novartis to Their Own Strobilurins

Four completely different aspects of this route merit particular attention:

- In the course of studies aimed at the identification of new, biologically active natural products, scientists at Ciba-Geigy had already isolated strobilurins in the 1980s and identified their antifungal activity,[66] but at the time had elected not to follow them up any further.

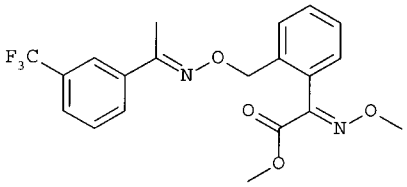
- Quite independently, and clearly soon after the publication of the Zeneca basic application on the enol ethers,[41] a fungicide synthesis group at Maag, at the time still a subsidiary of Hoffmann-La Roche, had discovered a gap in the Zeneca claims, and after charging through this gap with their own program of research, had submitted their own application at the turn of 1988/1989.[67b] Company acquisitions then saw the Maag synthesis group along with the patents they held transferred first in 1990 to Ciba-Geigy and then, following the merger with Sandoz in 1996, to Novartis: a happy homecoming of the strobilurins to the former Ciba-Geigy?[68]

- The core of the Maag application[67b] is illustrated by structures **28**–**28b** (Scheme 12), and comprises the introduction of a new side chain into the enol ether—classic "me too"



28 **28a**

28b: $R^1 = CH_3$, R^2 = heteroaryl



trifloxystrobin
CGA 279202

Scheme 12. Strobilurins with the new oxime ether side chain. Structures **28**–**28b** illustrate the core of the two conflicting patent applications submitted by Zeneca[67a] and Maag [67b] only five weeks apart.

chemistry.[69] Although it is disputable whether the side chain in **28** sidesteps the term "optionally substituted alkyl" used in the Zeneca basic application[41] and is therefore safe from legal challenge, it is undeniably a substituted methyl group. What is most striking about the strobilurins with an oxime ether side chain is their somewhat higher biological activity,[70] which, particularly in the case of derivatives of type **28a**, compares well even with the earlier, previously optimized phenoxy and phenoxymethyl side chains (for example **27a, b**, Scheme 11).

- From a patent point of view, the period that followed saw a curious state of confusion develop around this oxime ether side chain. The Zeneca group had in the meanwhile also identified the high potential of this side chain, and had submitted a corresponding patent application[67a]—based on the enol ether pharmacophore—some five weeks *ahead* of the Maag team. Further competitors joined in this almost grotesque competition, all completely in the dark as to the timing of the rival applications.[42] All of a sudden the whole world seemed to have discovered the oxime ether side chain.

Within barely more two years eight patent applications had appeared from five different companies (Table 2).[67] What was clear was that the development of the new pharmacophore variants had, with the usual time lag, in each case led to the submission of corresponding patent applications for the oxime ether side chain. An important point for Novartis was that the Maag team had managed to submit their application[67e] for the combination of an oxime ether ester pharmacophore and an oxime ether side chain three weeks ahead of BASF[67f] and eleven weeks ahead of Zeneca.[67g] The question of the Novartis compounds being dependent on the first, in the general claims very broadly defined, Zeneca patent on the strobilurins with an oxime ether pharmacophore[58] was settled in favor of Novartis, as the Zeneca patent[58] had recently been revoked by the Board of Appeal of the European Patent Office. An active substance from this group that is in the advanced stages of development at Novartis under the code number CGA 279202[71] has been given the name trifloxystrobin (Scheme 12) and is currently in the process of registration in the USA.

Table 2. The first patents for strobilurin with an oxime ether side chain (see structures **28**–**28b** in Scheme 12).

First priority date	Patent holder (applicant)	Pharmacophore	Ref.
Nov. 21, 1988	Zeneca (ICI)	enol ether[a]	[67a]
Dec. 29, 1988	Novartis (Maag)	enol ether	[67b]
Aug. 22, 1989	Nihon Nohyaku	enol ether	[67c]
Nov. 2, 1989	Ube	enol ether	[67d]
June 5, 1990	Novartis (Maag)	oxime ether ester[b]	
		thioenol ether[c]	[67e]
June 27, 1990	BASF	oxime ether ester[d]	
		oxime ether amide[e]	[67f]
Aug. 22, 1990	Zeneca (ICI)	oxime ether ester	[67g]
Jan. 30, 1991	Zeneca (ICI)	oxime ether amide	[67h]

[a] See structure **XII** in Scheme 6. [b] See structure **23** in Scheme 9. [c] The pharmacophore has the *E*-configured formula $-C(COOCH_3)=CHSCH_3$. [d] At the priority date the Zeneca enol ether application[67a] had already been published (date of publication May 1990). The claims for the enol ether initially included in the BASF application were for the most part withdrawn during the patent examination process. [e] See structure **27** in Scheme 10.

4. Structure–Activity Relationships as a Driving Force in the Lead Structure Optimization

4.1. Molecular Characteristics and Activity Variables

The activity of a fungicide under real-life conditions in a complex system of the fungal pathogen, the plant, and the environment is determined by a multitude of quite different influencing factors.[72] In the optimization of the lead structure it is useful to separate the complex of such influencing factors and the associated molecular properties in two parts.[73] A decisive factor on the one hand is the affinity of the respective active substance for its molecular target in the fungus. In the case of the strobilurins this is primarily determined by the binding affinity of the substance for the bc_1 complex of the respiratory chain. On the other hand, just as important for the in vivo activity under real-life conditions is how much of the active substance actually succeeds in reaching its target. Of critical importance here is the biokinetic behavior of the active substance, that is, the particular characteristics of the molecule that govern its absorption, transportation, breakdown, and, where appropriate, excretion.

Changes to the lead structure affect not only the target affinity of the active substance, but also, in a variety of ways, the biokinetic properties of the molecule. This ultimately influences the activity towards fungal pathogens in vivo, and has consequences for both the selectivity towards nontarget organisms (for example plants and beneficial insects) and for the environmental behavior (for example degradation and translocation in the soil and volatilization).

For a rationally conceived lead structure optimization it is important to consciously separate all these influencing factors, and wherever possible to experimentally establish at an early stage the associated molecular characteristics and their effects on target activity and the individual biokinetic parameters, and to exploit them in the optimization of the active substance structures with respect to their activity and environmental tolerability. Of particular importance for structure–activity analyses are the following, easily measurable molecular properties:

- The melting point to assess the rate of dispersion and bioavailability.
- The vapor pressure as a measure of the volatilization profile and quasisystemic mobility (see below) on the surface of the leaf.
- Lipophilicity and water solubility to determine the absorption and transportation behavior.
- The photostability to establish the lifetime of the active substance, especially under outdoor conditions.

With regard to the observable biological effects the following individual and complex parameters are of particular interest:[74]

- The activity on the level of the molecular target. For the strobilurins this means the binding constant for the bc_1 complex or—a more easily measurable option—the active substance concentration necessary for 50% inhibition of an appropriate enzyme preparation (the I_{50} value, for example from the test on yeast mitochondria used as a model system;[60] see Section 3.5).
- The activity on the cellular level of the target organisms, for example the inhibition of respiration or of the growth of fungal cultures on artificial nutrient media in the laboratory.
- The rate of metabolic degradation (half-life) of the active substance in plant or fungal cell culture models or in whole plants.
- The proportions of the active substance on the surface and in the interior of the treated leaf and in untreated leaves of the plant after application onto a particular leaf as a function of time.
- The activity in greenhouse tests on plants infected with fungal pathogens.
- The fungicidal activity under outdoor conditions.
- The effects on aquatic and terrestrial nontarget organisms in laboratory tests.

- Degradation rates and translocation behavior in the soil (laboratory model).

This complex interplay between a multitude of variables means that particular caution must be exercised in the interpretation of test results. Structure–activity trends can often be discerned or guessed at only with great difficulty. For practical reasons it is hardly feasible to investigate all new test substances[75] from every possible angle. At BASF only the yeast mitochondria test and the greenhouse tests are carried out routinely for all strobilurins. Of paramount importance therefore is the reasoned and systematic selection of candidate active substances for screening in additional, more elaborate test models.

Several examples based on different molecular variables have been published of structure–activity relationships for strobilurins in some of the tests mentioned.[28, 45a–c, 76]

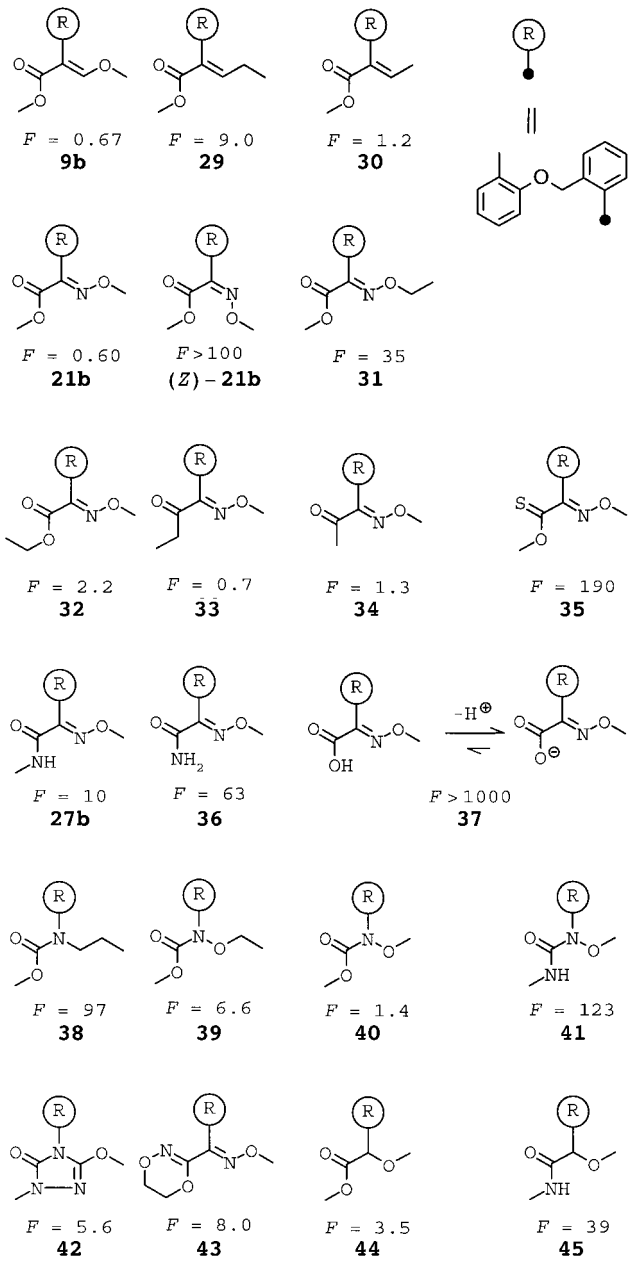
4.2. Variation of the Pharmacophore

In the identification of new pharmacophore variants the yeast mitochondria test has proved to be an extremely useful tool. To ensure the individual results were comparable, all test series were screened using a reference standard, the enol ether stilbene **7a**, against which the I_{50} value obtained for a test substance was referred:

$$F = \frac{I_{50}\ (\text{test substance})}{I_{50}\ (\mathbf{7a})}$$

By definition, $F = 1$ for **7a**, and hence the smaller the F value, the higher the activity. Scheme 13 shows a small selection from the wealth of conceivable pharmacophore variants and should give some impression of the structure–activity relationships which are discussed here with respect to just a few, though important, aspects.[77]

- What is most astonishing is the latitude with which the structures can be varied without incurring any fundamental loss of target activity. On a similar level of activity as the "classical" enol ether **9b** and oxime ether **21b** are the crotonic ester **30**,[78] the ketones **33** and **34**,[79] and the *N*-methoxycarbamate **40**.[80a] The variants **27b**,[64, 65] **41**,[80] **42**,[81] and **43**[82] as well as the mandelic acid derivatives **44** and **45**[83] also show remarkable activity.
- A drastic loss of activity is observed to a lesser or greater degree when the bulk of the substituents on either side of the periphery of the pharmacophore is increased (cf. **31/32** versus **21b**). The optimal size is clearly shown by truncated variants such as **30** (cf. **29**) and **40** (cf. **39**). The *E* configuration of the central double bond cannot be changed without suffering a catastrophic loss of activity, as shown by a comparison of (*E*)-**21b** and (*Z*)-**21b**.
- As is the case with the side chain variants (see Section 4.3, Figure 3), the activity of the pharmacophore variants is distinctly dependent on the lipophilicity of the molecule. Up to an optimum of $\lg P_{ow} > 4$, the activity increases with increasing lipophilicity, as exemplified by the isosteric ester/amide pairs **21b/27b**, **40/41**, and **44/45**. In each of these cases the ester is 0.7 $\lg P_{ow}$ units more lipophilic and shows correspondingly higher activity. In an assessment of the activity of the pharmacophore variants, the lipophilicity of the entire molecule must therefore be taken into account.



Scheme 13. Strobilurins with the kresoxim-methyl side chain and variations on the pharmacophore. Their relative activities on the target level are given below the formula; F is inversely proportional to the activity (see text).

- The only essential heteroatom in the pharmacophore is the carbonyl oxygen atom, or an equivalent which also functions as a hydrogen-bond acceptor at the target. This is illustrated particularly clearly by the distinct loss of activity observed on going from C=O to C=S (cf. **21b** and **35**) and C=CH_2.[77]
- The question of whether the C=O bond points to the "northwest" (*s-trans* conformation) relative to the second double bond or to the "south" (*s-cis* conformation) is settled unequivocally in favor of the *s-trans* conformation, as evidenced by the high activity shown by the cyclic, fixed-geometry variant **42**.[81a]

With regard to the biophysical and biokinetic properties of the individual pharmacophores, some important trends can likewise be discerned, that have consequences for the respective biological activities.

- When the remainder of the molecule is kept constant (®, see Scheme 13), the lipophilicity of the pharmacophore variants increases in the following order: cyclic variants (such as **42**, $\lg P_{ow} = 2.3$) < oxime ether amide (**27b**, $\lg P_{ow} = 2.8$) < oxime ether ester (**21b**, $\lg P_{ow} = 3.5$) < *N*-methoxycarbamate (**40**, $\lg P_{ow} = 3.7$) ≈ enol ether (**9b**, $\lg P_{ow} = 3.7$) < methyl ketone (**34**, $\lg P_{ow} = 4.0$) < crotonic ester (**30**, $\lg P_{ow} = 4.4$). The overall lipophilicity of the molecule not only influences the activity on the mitochondrial target level, but also has a considerable influence on absorption and transportation; this had to be taken into consideration in the design of new variants. Thus, in order to be in a lipophilicity area where systemic transport in plants is possible ($\lg P_{ow} < 4$[49]), the lipophilic crotonic esters require side chains that are more hydrophilic than the intrinsically more hydrophilic oxime ether amides.
- The vapor pressure of the compounds is also determined to a considerable degree by the pharmacophore type. For example, in the case of the strongly polar oxime ether amides the vapor pressure is more than an order of magnitude lower than that of the corresponding oxime ether esters (**21b**: 2.3×10^{-8} hPa; **27b**: 7.5×10^{-10} hPa).[28b] Because the vapor pressure correlates with the quasisystemic mobility of the active substance on the surface of the leaf,[28b] it has a decisive influence on the distribution of strobilurins *on* the plants requiring protection. Consequently, a key practical consideration—particularly after protective application of a fungicide[84d]—is to achieve a protective film that is as even as possible. Furthermore, for a larger number of strobilurins a correlation has also been demonstrated between vapor pressure and activity against powdery mildew of wheat[28b] in more curatively oriented[84d] field trials. Since powdery mildew of wheat is a fungal pathogen that grows largely *on* the surface of the leaf, this is understandable. Also understandable is the failure to observe—at least in curatively oriented studies—any corresponding correlation in the case of wheat brown rust, which prefers to grow *inside* the leaf.
- Likewise, for the rates of degradation of strobilurins in the soil and in plant and fungal cell cultures some general trends emerge in relation to the pharmacophore. The degradation rates tend to increase in the following order: cyclic variant (type **42**) ≤ oxime ether amide ≪ enol ether ≤ methoxycarbamate ≤ crotonic ester ≪ oxime ether ester. A consequence of this is that oxime ether amides that show extreme metabolic stability are characterized by a long duration of action under outdoor conditions. However, this is accompanied by a correspondingly high persistence in the soil—the reverse side of the coin. This long duration of action in the treated crops is ideal for systemic transport. Conversely, although the rapid metabolization of the oxime ether ester to the practically inactive carboxylic acid (cf. **37**, Scheme 13) prevents any marked systemic effects, it guarantees a particularly rapid degradation in the soil that is highly desirable, with half-lives ranging from hours to a few days.[84a]

4.3. Variation of the Side Chain and the Arene Bridge

On the level of the target, clear, quantitative structure–activity relationships have been established in accordance with a bilinear equation (Figure 3) for a series of oxime ethers of type **21**.[28a, 85] Similar correlations have been deduced for

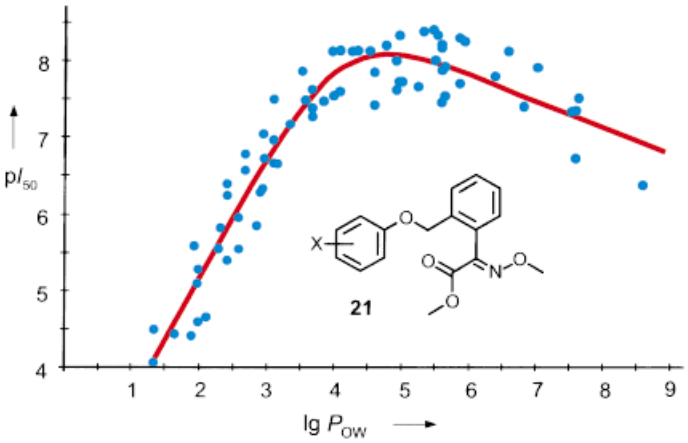


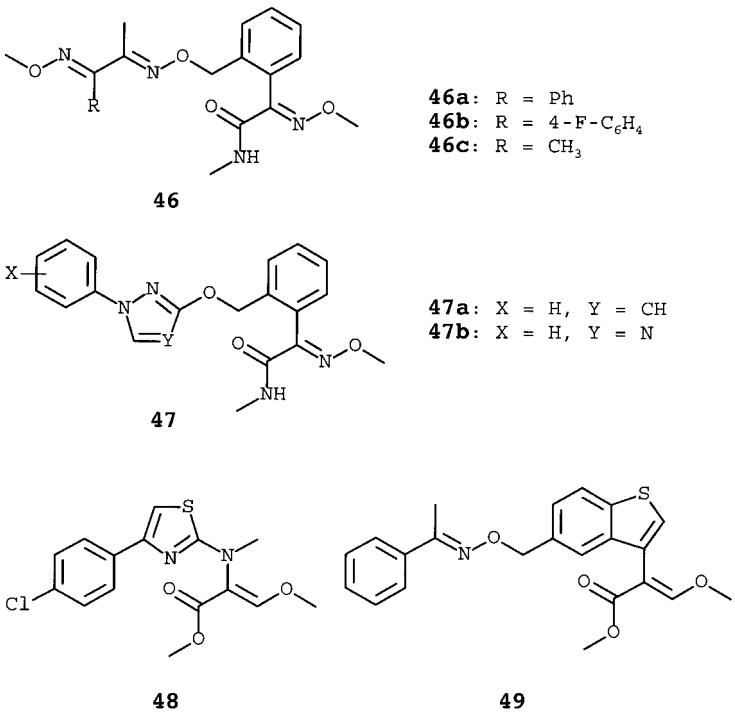
Figure 3. Target-level activity of a series of strobilurin oxime ethers **21** as a function of the lipophilicity of the substituents X. pI_{50} is the negative logarithm of the I_{50} value from the yeast mitochondria test. Each point represents a variant of structure **21**.[28]

enol ethers of type **9**, oxime ether amides, and crotonic esters and methoxycarbamates.[86] Thus, it is clearly the overall lipophilicity of the molecule—modified by the substituents X in the side chain—that is the critical influencing factor. Significant "underperformers" with respect to the curve shown in Figure 3 arise if, on account of certain substituents or substitution patterns X, the steric bulk of the side chain no longer permits optimal docking at the target.[28a]

The influence of the arene bridge and the side chain on the biokinetic properties is illustrated, for example, by the route outlined in Section 3.4 followed by the Zeneca team in the optimization of the diphenyl ether **13a** to azoxystrobin.[45a, e] A key element of their approach was the introduction of heteroatoms to specifically lower the lipophilicity of the molecule enough to allow systemic transportation, while keeping the attendant reduction in activity on the target level as low as possible.

A similar role is played by the heteroatoms in the oxime ether side chains already mentioned in Section 3.7, for example in compounds of type **28a**. Here, the side chain is two atoms longer than the phenoxymethyl side chains in **9**, without an accompanying drastic increase in lipophilicity:[87] $\lg P_{ow}$ is 3.2 for **9a** and is only slightly higher at 3.6 in the case of **28a** (with Y = H). The extension of this principle to strobilurins with "double oxime ether" side chains such as in **46** led to derivatives with comparatively low lipophilicity ($\lg P_{ow} = 3.2$ for **46a**) and very good activities in field trials.[88, 89] Even more hydrophilic side chains are present in **47a**[90] ($\lg P_{ow} = 2.6$) and **47b**[90] ($\lg P_{ow} = 1.5$).

The diverse range of variants that is possible without any fundamental loss of activity is illustrated by **48**[91] and **49**,[92] chosen more or less arbitrarily from the wealth of derivatives so far known (Scheme 14).



Scheme 14. Examples of strobilurins with variations of the side chain and aromatic bridge.

4.4. Variation of the Lead Structure and Selection of Candidates

In the evolution of a new class of active substance—as here with the strobilurins—an analogy can be drawn between the variations on the lead structure and the mutations that occur in the course of evolution in nature. The selection of individual compounds as candidates for the development of one or a handful of individual active substances to the point of marketability thus parallels the "survival of the fittest" (Figure 4). In the variation of the lead structure as well as in the selection of development candidates, optimal compromises must be found with regard to all the properties of the active substance. In both cases data obtained from studies of structure–activity relationships represent an important element of feedback control.

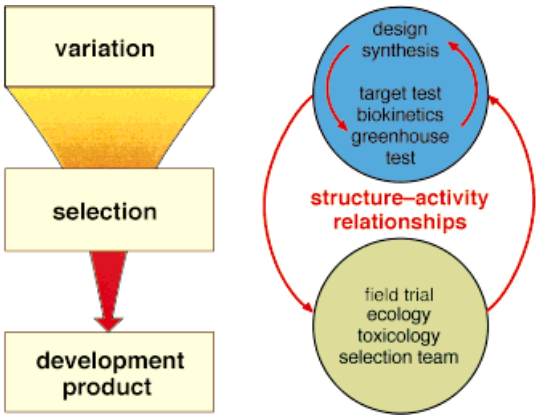


Figure 4. Schematic representation of the evolution process in the course of a lead structure optimization.

Here it is necessary to analyze and, particularly with respect to the net effects under real-life conditions, develop an understanding of the impact that structural variations within the various regions of the molecule have on the individual observables at various levels (e.g. target level, biokinetics). This is primarily a challenge for the chemist planning his synthetic program. No less critical, however, is the consideration of such structure–activity relationships in the subsequent selection process. Here, as before, the complex network of interrelationships means that it is often less a question of clearly derivable, conclusive inferences, but of provisional decisions based on "soft" data or mere estimates, or even outright speculation. Empirical social research has shown that in such situations the assessments and decisions of a team are fundamentally superior to those of even the most apt individual.[93] Therefore, in this selection process during the optimization of the lead structure, it makes sense to exploit this "team advantage"[93] to the full through the assembly of interdisciplinary teams.

5. Biological Properties of the Strobilurins

5.1. Importance of the Mode of Action

For the biological action profile of strobilurins, their mode of action (the inhibition of respiration) is of decisive importance from a number of perspectives. In all eukaryotes the bc_1 complex forms part of the mitochondrial respiratory chain. This poses the question as to the extent to which the structure of the bc_1 complexes of different species affect the binding affinities of individual strobilurin variants and hence result in selective toxicity on the target level. The submolecular intricacies of this question can be expected from knowledge of bc_1 complexes through crystal structure determinations at increasingly higher resolution.[23, 94] The relevant amino acid sequences known so far for various species show a high degree of preservation in the region of the binding envelope.[20, 95] With submitochondrial enzyme preparations of various species (fungi, housefly, rat, and maize), tests carried out on 14 strobilurins plus myxothiazole showed that *no* appreciable contributions to species selectivity can be expected at the target level.[28, 60] On the other hand, the natural abundance of fungi species resistant to bc_1 inhibitors clearly derives from mutations of the bc_1 complex that prevent the docking of strobilurins in the binding envelope.[20]

Consequently, the appearance of resistant plant pathogens in which similar mutations are present cannot be ruled out. The question of whether, and to what extent, this will be of importance in the development of serious resistance towards strobilurins under the selection pressure of continued strobilurin treatments in agricultural practice will only be answered through experience in the field over the coming years.[96]

5.2. Physiological Consequences of the Mode of Action

On the biochemical level the mode of action for the inhibition of respiration results in the arrest of ATP forma-

tion, which is coupled to the respiratory chain. The consequence on the physiological level is a particularly pronounced inhibition of growth processes that are strongly energy-, and hence, respiration-dependent. These include not only the growth of tumor cell lines, but in particular the germination of fungal spores. The strobilurin BAS 490 F has been shown in several fungal species to completely inhibit spore germination at considerably lower active substance concentrations (factors of between 10 and 10000) than are required for 50% inhibition of mycelial growth in the same species.[28b] Since among eukaryotes spore germination occurs almost exclusively in fungi,[97] this accounts on the physiological level for the selectivity that is so important for practical applications.

5.3. Action Profiles and Practical Applications of Strobilurins

Because of the high vulnerability of the fungal spore germination to strobilurins, these compounds generally show a remarkably broad action potential against virtually all phytopathogens, particularly when applied early as a protective measure. Nevertheless, the maximum activities achievable in practical use as an agricultural fungicide and the associated ecological profile will depend to a considerable degree on the biophysical and biokinetic characteristics of the active substance in question.

- Kresoxim-methyl stands out in this respect on account of two properties. The still measurable vapor pressure (2.3×10^{-8} hPa) and relatively high lipophilicity ($\lg P_{ow} = 3.5$) of the active substance allow distribution processes between the lipophilic waxy cuticle of the plant and the gas-phase "boundary layers" immediately above. Although the biokinetic properties of kresoxim-methyl prevent it from undergoing systemic transport *within* the plant (see Section 4.2), diffusion into the gas phase is sufficient for migration and distribution processes to take place *on* the surface of the plant, thereby imbuing it with quasi-systemic mobility.[28b] The second outstanding property of kresoxim-methyl is its rapid metabolic breakdown in nontarget organisms to the virtually inactive carboxylic acid. Thus, the inability to undergo systemic transportation within the plant is offset by high selectivity and a favorable ecological profile.[84]

In its practical applications kresoxim-methyl is characterized—particularly if applied protectively—by a broad spectrum of activity against a wide range of phytopathogens.[98] Peak activities—even for curative application after an infection—are achieved against cereal mildew, which grows on the surface of the leaf, and against apple scab at application rates of below 125 g ha^{-1}. Its key practical advantage is the increase in yields achievable particularly in cereal cultivation, which are distinctly higher than might be expected on the basis of its fungicidal effects alone.[99]

Kresoxim-methyl finds use in commercial products such as Discus (marketed as Stroby in Japan) as the sole active ingredient particularly in fruit production, in Brio and Mentor as a mixture with the BASF amine fungicide fenpropimorph for the control of cereal mildew, and in a three-component mixture with Fenpropimorph and the BASF triazole fungicide epoxiconazole in the broad-spectrum cereal fungicide Juwel Top. The use of kresoxim-methyl in mixtures serves both to expand the activity profile of the product and to help prevent the potential development of resistance.

- Azoxystrobin possesses distinctly different biophysical and biokinetic properties and a different, to a certain degree complementary, spectrum of activity.[45e, 100] Its vapor pressure is considerably lower (1.1×10^{-12} hPa at 25 °C), and consequently its activity, against cereal mildew, is only moderate. A slower rate of metabolic breakdown in the plant and the relatively low lipophilicity ($\lg P_{ow} = 2.5$) allows systemic transport in the treated plants. Marketed under trade names such as Amistar, Bankit, and Heritage, azoxystrobin is used to combat a wide range of phytopathogens in numerous agricultural crops.
- Metominostrobin possesses an even lower lipophilicity ($\lg P_{ow} = 2.3$) than azoxystrobin, and is about 20 times more soluble in water.[20e, 101] Its oxime ether amide group endows the pharmacophore with particularly high metabolic stability. After application onto the water surface of rice paddies or onto the soil of seedling boxes it is absorbed through the roots into the rice plants, where, following systemic transportation, it is able to fight off fungal pathogens from within.[101] It is used in Japan under the trade name Oribright particularly against blast disease *Pyricularia oryzae* and other diseases of rice.
- Like kresoxim-methyl, CGA 27 92 02 (trifloxystrobin) has an oxime ether ester pharmacophore. Compared with kresoxim-methyl it has higher lipophilicity ($\lg P_{ow} = 4.5$), a similar vapor pressure (3.4×10^{-8} hPa), and similar transport and distribution properties. It is used under the trade name FLINT against a range of fungal diseases in various agricultural crops.[71]

6. Industrial Strobilurin Syntheses

A summarizing account describes the known processes for the construction of enol ether, oxime ether, and oxime ether amide pharmacophores as well as palladium-catalyzed couplings of entire pharmacophores to the bridging arenes *ortho* to preexisting side chains.[45e] Key steps in an elegant synthesis of kresoxim-methyl are the irreversible lactone ring opening of phthalide with cresolate under alkaline conditions and the Pinner reaction of an acyl cyanide during the subsequent construction of the pharmacophore.[28a, 102] In an interesting synthetic variant yielding compounds of the trifloxystrobin type, the coupling of the side chain and pharmacophore is achieved simultaneously with the construction of the arene bridge by means of a Diels–Alder reaction.[103]

7. Economic Importance, Summary, and Outlook

What is already quite clear is that the strobilurin class of active substances represents one of the most significant product innovations in crop protection. A consequence of this is that virtually all the important crop-protection companies are now engaged in research in this field. Over 500 international patent applications have been published to date

from more than 20 companies and research institutes (Figure 5 and reference [45e]). Worldwide, industrial chemists have probably synthesized, as a conservative estimate, well over 30000 strobilurin analogues so far.

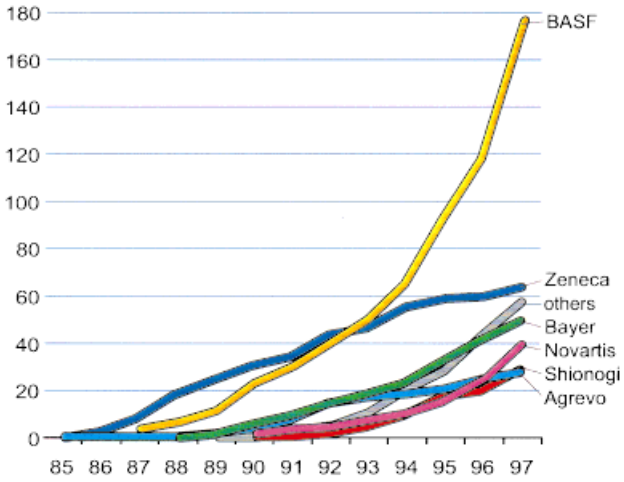


Figure 5. Number of published international strobilurin patent applications from 1986 to 1997.

The mode of action, which is novel for a fungicide, is based on inhibition of the cellular respiration of the fungal pathogen and thus a valuable asset in the management of resistance to established fungicides. It is also the reason for the particularly high vulnerability of the strongly respiration dependent spore germination of the fungus towards these active substances; this helps account for the selectivity of their action against fungi as the target organisms, a decisive factor for the generally very good environmental tolerability of strobilurins.

With their broad spectrum of activity, long duration of action, high activity at low rates of application, and outstanding environmental tolerability, the strobilurins have set new standards in the control of fungal diseases, thereby making a key contribution to integrated crop-protection strategies. Agricultural enterprises have taken this innovation on board with enthusiasm.[104] Estimates of its future market potential are correspondingly high. By 2006—that is, ten years after the market launch of the first two strobilurins kresoxim-methyl and azoxystrobin—annual sales worldwide may well total over two billion German marks, if we include all the future products expected to arise from the international competition. This figure represents approximately a 20% share of the world fungicide market. Thus, provided there is no unexpected counterproductive development of resistance, the strobilurins might in the near future assume the number one position in the market, which is currently still held by the triazoles as the largest class of fungicidal active substances.

The story of strobilurin research is in many respects also a tale of applied chemistry. It describes the evolution of a new class of active substance, and is both exciting and stimulating. It is full of impulses and results, as the researcher might wish, but it is also packed with wrong turns, false dawns, disappointments, and questions that remain unanswered. These challenges as well as the provision of natural product lead structures of the strobilurin type should be regarded as a gift of nature.

In the spirit of the concluding words of this review, nature deserves our sincere gratitude. Our special thanks must, however, be reserved above all for those scientifically inspired colleagues and co-workers of our research groups mentioned in the text as well as in the notes and literature citations.

Received: October 16, 1998 [A308IE]
German Edition: *Angew. Chem.* **1999**, *111*, 1416 – 1438

[1] *Fungal Biotechnology* (Ed.: T. Anke), Chapman and Hall, Weinheim, **1997**.
[2] Reviews on pigments: a) M. Gill, *Prod. Rep.* **1994**, *11*, 67 – 90; b) M. Gill, W. Steglich in *Progress in the Chemistry of Organic Natural Products*, *Vol. 51* (Ed.: L. Zechmeister), Springer, Wien, **1987**, pp. 1 – 317; c) toxins: A. Bresinsky, H. Besl, *Giftpilze*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, **1985**.
[3] E. R. H. Jones, V. Thaller in *Handbook of Microbiology*, *Vol. 5* (Eds.: A. I. Laskin, H. A. Lechevalier), 2nd ed., CRC Press, Boca Raton, **1984**, pp. 83 – 99.
[4] a) M. Anchel, A. Harvey, W. J. Robbins, *Proc. Natl. Acad. Sci. USA* **1950**, *36*, 300 – 305; b) F. Kavanagh, A. Hervey, W. J. Robbins, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 570 – 574.
[5] H. Egger, H. Reinshagen, *J. Antibiot.* **1976**, *29*, 915 – 922; H. Egger, H. Reinshagen, *J. Antibiot.* **1976**, *29*, 923 – 927.
[6] T. Anke, F. Oberwinkler, W. Steglich, G. Schramm, *J. Antibiot.* **1977**, *30*, 806 – 810.
[7] G. Schramm, W. Steglich, T. Anke, F. Oberwinkler, *Chem. Ber.* **1978**, *111*, 2779 – 2784.
[8] T. Anke, G. Schramm, B. Schwalge, B. Steffan, W. Steglich, *Liebigs Ann. Chem.* **1984**, 1616 – 1625.
[9] V. Musilek, J. Cerna, V. Sasek, M. Semerzieva, M. Vondracek, *Folia Microbiol.* **1969**, *14*, 377 – 387.
[10] a) V. Musilek, CS-B 136492, **1970** (priority date December 17, 1965); b) M. Vondracek, J. Capkova, J. Slechta, A. Benda, V. Musilek, J. Cudlin, CS-B 136495, **1970** (priority date January 18, 1967).
[11] T. Anke, H.-J. Hecht, G. Schramm, W. Steglich, *J. Antibiot.* **1979**, *32*, 1112 – 1117. Respiration-inhibitory activity has also been reported for mucidin: J. Subik, M. Behun, V. Musilek, *Biochem. Biophys. Res. Commun.* **1974**, *57*, 17 – 22.
[12] M. Vondracek, J. Capkova, K. Culik, CS-B 180775, **1979** (priority date August 29, 1974).
[13] P. Sedmera, V. Musilek, F. Nerud, *J. Antibiot.* **1981**, *34*, 1069.
[14] G. von Jagow, G. W. Gribble, B. L. Trumpower, *Biochemistry* **1986**, *25*, 775 – 780.
[15] T. Anke, *Can. J. Bot.* **1995**, *73* (Suppl. 1), 940 – 945.
[16] W. Weber, T. Anke, B. Steffan, W. Steglich, *J. Antibiot.* **1990**, *43*, 207 – 212.
[17] a) G. M. Nicholas, J. W. Blunt, A. L. J. Cole, M. H. G. Munro, *Tetrahedron Lett.* **1997**, *38*, 7465 – 7468; b) "Strobilurins and Oudemansins": T. Anke, W. Steglich in *Drug Discovery from Nature* (Eds.: S. Grabley, R. Thiericke), Springer, Heidelberg, **1999**, in press.
[18] K. A. Wood, D. A. Kau, S. K. Wrigley, R. Beneyto, D. V. Renno, A. M. Ainsworth, J. Penn, D. Hill, J. Killacky, P. Depledge, *J. Nat. Prod.* **1996**, *59*, 646 – 649.
[19] M. Engler, T. Anke, O. Sterner, *Z. Naturforsch. C* **1998**, *53*, 318 – 324.
[20] P. Kraiczy, U. Haase, S. Gencic, S. Flindt, T. Anke, U. Brandt, G. von Jagow, *Eur. J. Biochem.* **1996**, *235*, 54 – 63.
[21] a) W. Trowitsch, G. Reifenstahl, V. Wray, K. Gerth, *J. Antibiot.* **1980**, *33*, 1480—1490; b) W. F. Becker, G. von Jagow, T. Anke, W. Steglich, *FEBS Lett.* **1981**, *132*, 329 – 333.
[22] U. Brandt, U. Haase, H. Schägger, G. von Jagow in *Wege zu neuen Produkten und Verfahren der Biotechnologie*, *DECHEMA Monographien, Vol. 129* (Eds.: T. Anke, U. Onken), VCH, Weinheim, **1993**, pp. 27 – 38.
[23] D. Xia, C.-A. Yu, H. Kim, J.-Z. Xia, A. M. Kachurin, L. Zhang, L. Yu, J. Deisenhofer, *Science* **1997**, *277*, 60 – 66.

[24] "β-Methoxyacrylate Antibiotics: From Biological Activity to Synthetic Analogues": T. Anke, W. Steglich in *Biologically Active Molecules* (Ed.: U. P. Schlunegger), Springer, Berlin, **1989**, pp. 9–25.
[25] G. Schramm, Dissertation, Universität Bonn, **1980**.
[26] W. Steglich, G. Schramm, T. Anke, F. Oberwinkler (Hoechst AG), EP-A 044448, **1980** (priority date July 4, 1980).
[27] We are indebted to the Federal Ministry for Research and Technology (now the BMBF) for their support of the project *Constituents of Microorganisms and Plants as New Active Substances or Models for Active Substances in Crop Protection* between 1983 and 1992.
[28] a) "Strobilurins – From Natural Products to a New Class of Fungicides": H. Sauter, E. Ammermann, F. Röhl in *Crop Protection Agents from Nature: Natural Products and Analogues* (Ed.: L. G. Copping), The Royal Society of Chemistry, Cambridge, **1996**; b) "Mitochondrial respiration as a target for antifungals: lessons from research on strobilurins": H. Sauter, E. Ammermann, R. Benoit, S. Brand, R. E. Gold, W. Grammenos, H. Köhle, G. Lorenz, B. Müller, F. Röhl, U. Schirmer, J. B. Speakman, B. Wenderoth, H. Wingert in *Antifungal Agents, Discovery and Mode of Action* (Eds.: G. K. Dixon, L. G. Copping, D. W. Hollomon), BIOS Scientific Publishers, Oxford, **1995**, pp. 173–191.
[29] a) B. A. M. Schwalge, Dissertation, Universität Bonn, **1986**; b) S. Zapf, Dissertation, Universität Kaiserslautern, **1994**; c) M. Engler, Dissertation, Universität Kaiserslautern, **1997**.
[30] We would like to thank E. Ammermann for the greenhouse test results and for helpful discussions in this context.
[31] U. Schirmer, S. Karbach, E. H. Pommer, E. Ammermann, W. Steglich, B. A. M. Schwalge, T. Anke (BASF AG), EP-A 203606, **1985** (priority date May 30, 1985).
[32] U. Schirmer, S. Karbach, E. H. Pommer, E. Ammermann, W. Steglich, B. A. M. Schwalge, T. Anke (BASF AG), EP-A 229974, **1985** (priority date December 20, 1985).
[33] U. Schirmer, S. Karbach, E. H. Pommer, E. Ammermann, W. Steglich, B. A. M. Schwalge, T. Anke (BASF AG), EP-A 226917, **1985** (priority date December 20, 1985).
[34] U. Schirmer, S. Karbach, E. H. Pommer, E. Ammermann, W. Steglich, B. A. M. Schwalge, T. Anke (BASF AG), EP-A 203608, **1985** (priority date May 30, 1985).
[35] a) "Factors Involved in Apparent Discrepancies of Fungicide Performance": R. E. Gold, A. Akers, H. Sauter in *Comparing Glasshouse and Field Pesticide Performance II* (Eds.: H. G. Hewitt, J. Caseley, L. G. Copping, B. T. Grayson, D. Tyson), British Crop Protection Monograph 49, **1994**, pp. 47—56. b) For both phenomena the different time schedules of the tests appears to be critical. Whereas in the greenhouse test the interval between application of the active substance and evaluation of the test was generally only 8 days in the preliminary tests, in field trials it can be up to 30 days or longer depending on the indication. False positive results for individual test substances in the greenhouse tests can thus be easily explained in terms of a relatively rapid disappearance of the active substance through various breakdown pathways (e.g. photolysis, metabolism). More difficult to rationalize are false negative results. A key factor here could be the relatively slow "outflow" (i.e. the increase in the available concentration) of the active substance into discrete molecules that is necessary for any activity, particularly in cases where under greenhouse conditions compounds with higher melting points had been deposited onto the test plants in microcrystalline form. On the other hand, a slow, but continuous outflow under outdoor conditions can easily result in particularly good activity at the end of a trial. A further factor that might result in field trials yielding comparatively better results than the greenhouse tests could be a particularly slow "disappearance" as a result of high biostability of individual substances. c) See Table 1 for examples of rank correlations of greenhouse results against corresponding field trial results.
[36] This seemingly very serious difficulty faced by fungicide researchers increasingly became an issue during the lead structure optimization with the strobilurins, and led to an extensive reevaluation at BASF of the selection procedures adopted in going from greenhouse tests to field trials. H. Sauter is grateful to his many collaborators in this project for numerous interdisciplinary discussions on this matter, in particular the head of biological fungicide research at BASF, G. Lorenz, for her helpful support and insightful contributions.
[37] Taking advantage of the different seasonal cycles of the northern and southern hemispheres makes it possible to stage two field trial campaigns each year spaced at distinct intervals.
[38] We are grateful to E. H. Pommer for the field trial results.
[39] S. Siegel, *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill, New York, **1956**, pp. 202–213. With regard to Table 1, the Spearman rank correlation coefficients r_s are calculated according to the following equation:

$$r_s = 1 - \frac{6 \sum (R_F - R_G)^2}{n (n^2 - 1)}$$

For the definition of R_F and R_G, see Table 1; n is the number of compounds whose test results correlate with one another. In cases with equal rankings within columns, r_s was corrected in accordance with equation 9.4 on p. 207 of the book cited above.
[40] a) H. Meier, *Methoden Org. Chem.* (*Houben-Weyl*) *4th ed. 1952–*, Vol. 4/5a Photochemie I, **1975**, pp. 189–204, pp. 254, pp. 511–519. b) Experiments have shown that some enol ether stilbenes, instead of undergoing a simple photochemical cyclization of the 1,3,5-hexatriene system to form dihydrophenanthrenes, react preferentially by a different pathway involving the enol ether double bond of the pharmacophore (see refs. [45a, b]).
[41] M. J. Bushell, K. Beautement, J. M. Clough, P. DeFraine, V. M. Anthony, C. R. A. Godfrey (ICI), EP-A 178826, **1984** (priority dates October 19, 1984, December 20, 1984, date of publication April 23, 1986).
[42] For a European patent application, there is an 18-month time lag between submission (first priority date) and publication. Following the priority date of his own application the submitter must therefore wait eighteen months before he can be certain there are no competing patents in the pipeline ahead of him.
[43] A colleague jokingly remarked that the additional "optionally substituted" may well have been forgotten in the case of hydrogen.[41]
[44] From this point onwards reference is made in the text only to Zeneca, even though "ICI" may be technically more correct in the chronological sequence of events. b) In 1992/1993 ICI was split into two separate companies. The chemicals business continue to trade under the name ICI, and the life sciences enterprises were spun off and have thereafter operated under the name Zeneca.
[45] The Zeneca team has published a number of excellent reviews describing their work on the strobilurins. Reference [45e] also gives an overall account of the historical course of strobilurin research. a) "Fungicidal β-methoxyacrylates: From natural products to novel synthetic agricultural fungicides": K. Beautement, J. M. Clough, P. J. de Fraine, C. R. A. Godfrey, *Pestic. Sci.* **1991**, *31*, 499–519. b) "Fungicidal β-methoxyacrylates: From natural products to novel synthetic agricultural fungicides": J. M. Clough, P. J. de Fraine, T. E. M. Fraser, C. R. A. Godfrey, *ACS Symp. Ser.* **1992**, *504*, 372–383; c) "Role of Natural Products in Pesticide Discovery. The β-Methoxyacrylates": J. M. Clough, A. D. Evans, P. J. de Fraine, T. E. M. Fraser, C. R. A. Godfrey, D. Youle, *ACS Symp. Ser.* **1994**, *551*, 37–53; d) "Fungicidal β-Methoxyacrylates. N-Linked Pyrroles": K. Beautement, J. M. Clough, P. J. de Fraine, C. R. A. Godfrey, *ACS Symp. Ser.* **1995**, *584*, 326–342; e) "The Strobilurin Fungicides": J. M. Clough, C. R. A. Godfrey in *Fungicidal Activity* (Eds.: D. H. Hutson, J. Miyamoto), Wiley, Chichester, **1998**, pp. 109–148.
[46] One of us (T. A.) received an ICI Plant Protection Division report (*Evaluation of Oudemansin for antifungal and anti-bacterial activity, January 1984)* from the German subsidiary of ICI.
[47] J. M. Clough, I. T. Kay (ICI), EP-A 178808, **1984**, (priority dates October 18, 1984, May 23, 1985).
[48] a) P. DeFraine, B. K. Snell, J. M. Clough, V. M. Anthony, K. Beautement (ICI), EP-A 206523, **1985** (priority dates August 18, 1985, April 17, 1986); b) J. M. Clough, C. R. A. Godfrey (ICI), EP-A 212859, **1985** (priority date August 22, 1985).
[49] a) The *transport* of dissolved substances in higher plants essentially takes place within two separate transport systems. In the normally fast-flowing transpiration stream of the xylem (a system of vascular

bundles extending from the root to the tips of the leaves) minerals absorbed from the roots area are transported in an upwards direction (acropetally) towards the tips of the leaves. Transport in the other direction (basipetally) is possible only within the phloem system of the plant (sieve tubes). This is essential for example for the transport of assimilates from the place of their biosynthesis in the leaves in a downwards direction towards the leaf sheath and stem; see H. Mohr, P. Schopfer, *Pflanzenphysiologie*, Springer, Berlin, **1992**, pp. 487 – 517. b) For the systemic distribution of xenobiotics (e.g. fungicides) after application onto the leaf, the first step is generally *penetration* from the leaf surface through the waxy layer of the cuticle into the interior of the leaf, and thence into the aqueous phases of the xylem and phloem. This biophysical process corresponds in essence to a distribution process between three phases: an external aqueous or gaseous phase, the lipophilic cuticle, and the internal aqueous sap of the plant. For such processes there is consequently an optimal range for the lipid – water partition coefficients of the active substance in question, which corresponds approximately to their octanol – water partition coefficients (P_{ow}). For fungicides applied through the leaves, $\lg P_{ow}$ values of about 2 – 4 can be regarded as optimal; see "Principles of uptake and systemic transport of fungicides within the plant": F. Jacob, S. Neumann in *Modern Selective Fungicides* (Ed.: H. Lyr), Longman Scientific & Technical, Essex, **1987**, pp. 13 – 30. c) A prerequisite for efficient transport of xenobiotics in the xylem of the plant is adequate solubility in water, which should generally be greater than 10 mg L^{-1} (correlating with $\lg P_{ow}$ values below 4). However, for individual active substances with extremely high activities, lower aqueous solubilities can sometimes be sufficient for the macroscopic observation of systemic effects, even though the amount of active substance undergoing transportation may be very low. d) For efficient basipetal transport of xenobiotics in the phloem, the water solubility of the active substance must, in comparison, be distinctly higher still. Since this is a process with a low rate of transportation, the substance must remain present in the phloem for a sufficiently long time. This will be the case if an active substance itself has particularly high water solubility (preferably greater than 10 g L^{-1}, correlating with $\lg P_{ow}$ values below 0) and/or is acidic (preferably with a pK_a below 5) and is therefore present mainly as the anion in the slightly alkaline environment of the phloem (pH 7.4 – 8.7); see ref. [49b] and D. A. Kleier, *Pestic. Sci.* **1994**, *42*, 1 – 11. e) The somewhat opposing physical prerequisites on the one hand for penetration of the cuticle and on the other for transport in the aqueous systems of the plant means that highly lipophilic active substances ($\lg P_{ow} > 4$) will stand virtually no chance of systemic transportation. Conversely, active substances with very low lipophilicity ($\lg P_{ow} < 0$) are no longer easily able to penetrate the cuticle, and will require other routes of entry into the interior of the plant and/or special additives to the formulation; see P. J. G. Stevens, E. A. Baker, N. H. Anderson, *Pestic. Sci.* **1988**, *24*, 31 – 53; E. A. Baker, A. L. Hayes, R. C. Butler, *Pestic. Sci.* **1992**, *34*, 167 – 182. f) Systemic transport does, of course, mean that the active substance must have adequately high metabolic stability in the plant system to ensure its lifetime under these conditions is sufficiently long.

[50] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey (ICI), EP-A 242070, **1986** (priority dates April 17, 1986, January 22, 1987).

[51] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey, P. J. Crowley, K. Anderton (ICI), EP-A 243012, **1986** (priority dates April 17, 1986, Januray 26, 1987).

[52] C. R. A. Godfrey, I. Streeting, R. Cheetham (ICI), EP-A 382375, **1989** (priority date February 10, 1989).

[53] V. M. Anthony, J. M. Clough, P. DeFraine, C. R. A. Godfrey, I. Ferguson, P. Crowley, M. G. Hutchings (Zeneca), EP-A 242081, **1986** (priority dates April 17, 1986, December 23, 1986).

[54] The value of $\lg P_{ow}$ is 3.3 for **13a**,[45b] 5.1 for **13b**,[45c] and 2.5 for **19**.[45e]

[55] However, as one knows, there where an emergency emerges, a source of rescue emerges as well (adapted from Friedrich Hölderlin, Patmos).

[56] "Now it is no longer done with grumbling and cursing. (…) A despairing illness wants a daring medicine." (translated from Friedrich Schiller in *Die Verschwörung des Fiesco zu Genua*, act 4, scene 6).

[57] B. Wenderoth, C. Rentzea, E. Ammermann, E. H. Pommer, W. Steglich, T. Anke (BASF AG), EP-A 253213, **1986** (priority date July 16, 1986).

[58] V. M. Anthony, J. M. Clough, C. R. A. Godfrey, T. E. Wiggins (ICI), EP-A 254426, **1986** (priority date July 18, 1986).

[59] H. Sauter is indebted to F. Röhl both for the establishment and automation of this test, the reliable and expert testing of thousands of strobilurin variants, and the swift dispatch of the test results and their assessments.

[60] For details of the test system, see a) F. Röhl, H. Sauter, *Biochem. Soc. Trans.* **1994**, *22*, 63S; b) ref. [28].

[61] For the fundamental importance of biochemical test models in the discovery and optimization of lead structures, see for example J. Dancer, A. Schulz, T. Peine, K. Wright, *Pestic. Outlook* **1998**, *9*(3), 36 – 41, and references therein

[62] For the synthesis see Section 6.

[63] a) "Phenoxyphenyl alkoxyiminoacetamides. New broad spectrum fungicides": Y. Hayase, T. Kataoka, M. Masuko, M. Niikawa, M. Ichinari, H. Takenaka, T. Takahashi, Y. Hayashi, R. Takeda, *ACS Symp. Ser.* **1995**, *584*, 343 – 353; b) M. Masuko, *Annu. Rep. Shionogi Res. Lab.* **1997**, *47*, 33 – 57.

[64] Y. Hayase, T. Kataoka, H. Takenaka, M. Ichinari, M. Masuko, T. Takahashi, N. Tanimoto (Shionogi), EP-A 398692, **1989** (priority dates May 17, 1989, December 29, 1989).

[65] S. Brand, E. Ammermann, G. Lorenz, H. Sauter, K. Oberdorf, U. Kardorff, C. Künast (BASF AG), EP-A 477631, **1990** (priority date September 22, 1990).

[66] a) A. Fredenhagen, A. Kühn, H. H. Peter, V. Cuomo, U. Giuliano, *J. Antibiot.* **1990**, *43*, 655 – 660; b) A. Fredenhagen, P. Hug, H. H. Peter, *J. Antibiot.* **1990**, *43*, 661 – 667.

[67] a) P. J. de Fraine, A. Martin (Zeneca), EP-A 370629, **1988** (priority dates November 21, 1988, March 9, 1989); b) H. P. Isenring, S. Trah, B. Weiss (Hoffmann-La Roche), WO-A 90/07493, **1988** (priority date December 29, 1988); c) K. Tsubata, N. Nijno, E. Endo, Y. Yamamoto, H. Kanno (Nihon Nohyaku), EP-A 414153, **1989** (priority date August 22, 1989); d) M. Watanabe, T. Tanaka, H. Kobayashi, S. Yokoyama (Ube), EP-A 426460, **1989** (priority dates November 2, 1989, February 27, 1990); e) H. P. Isenring, B. Weiss (Ciba-Geigy), EP-A 460575, **1990** (priority dates June 5, 1990, April 23, 1991); f) S. Brand, U. Kardorff, R. Kirstgen, B. Müller, K. Oberdorf, H. Sauter, G. Lorenz, E. Ammermann, C. Künast, A. Harreus (BASF AG), EP-A 463488, **1990** (priority date June 27, 1990); g) J. M. Clough, C. R. A. Godfrey, P. J. de Fraine (Zeneca), EP-A 472300, **1990** (priority date August 22, 1990); h) J. M. Clough, C. R. A. Godfrey, P. J. de Fraine, I. R. Matthews (Zeneca), WO-A 92/13830, **1991** (priority dates January 30, 1991, August 14, 1991).

[68] BASF had also pursued a discreet interest in the acquisition of Maag, to no avail. At the forefront, however, of the interest shown by BASF was not the strobilurins, but the already exisiting fungicide business of Maag (e.g. Corbel).

[69] This term is unfortunately still used too often in a pejorative sense. This is usually unjustified since the art of finding a usable gap in a tightly formulated patent claim demands rare creativity, and secondly because such "me too" variants could always lead to unusual and unexpected increases in activity, as was the case here (see text).

[70] Compare with ref. [20e] and P. J. de Fraine, J. M. Clough, *Pestic. Sci.* **1995**, *44*, 77 – 79.

[71] a) "CGA279202. A new Plant Health Product for Protection Against Diseases of Agronomic Crops, Vegetables, Tree Crops and Turf": *Novartis Technical Bulletin* **1998**; b) "CGA279202: A new broad-spectrum strobilurin fungicide": P. Margot, F. Huggenberger, J. Amrein, B. Weiss, *Abstr. Pap. Brighton Crop Prot. Conf. Pests and Diseases* (Farnham, UK) **1998**, pp. 275 – 382.

[72] For an excellent introduction to this subject, see L. G. Copping, H. G. Hewitt, *Chemistry and Mode of Action of Crop Protection Agents*, The Royal Society of Chemistry, Cambridge, **1998**.

[73] See for example "Optimization of Physicochemical and Biophysical Properties of Pesticides": I. J. Graham-Bryce, *ACS Symp. Ser.* **1994**, *225*, 185 – 207.

[74] H. Sauter is grateful to H. Köhle for numerous discussions and ideas in this context.

[75] Over 15000 strobilurin variants have been synthesized at BASF to date.
[76] a) H. Köhle, R. E. Gold, E. Ammermann, H. Sauter, *Biochem. Soc. Trans.* **1994**, *22*, 65S.
[77] A more detailed discussion is given in ref. [28a].
[78] B. Wenderoth, H. Sauter, E. Ammermann, E. H. Pommer (BASF), EP-A 280185, **1987** (priority date February 20, 1987).
[79] R. Benoit, H. Sauter, R. Kirstgen, G. Lorenz, E. Ammermann (BASF), EP-A 498188, **1991** (priority date February 7, 1991).
[80] a) B. Müller, H. Sauter, F. Röhl, R. Dötzer, G. Lorenz, E. Ammermann (BASF), WO-A 93/15046, **1992** (priority dates January 29, 1992, June 26, 1992, October 5, 1992). b) Here there was further competition between rival patents. Ishihara's more weakly active *N*-alkylcarbamates were submitted first: T. Komyoji, I. Shigehava, N. Matsuo, H. Shimoharada, T. Ohshima, T. Akagi, S. Mitani (Ishihara), EP-A 498 396, **1991** (priority dates February 7, 1991, October 23, 1991). This was followed by our application.[80a] Finally, Nihon Nohyaku also submitted an application for the *N*-methoxycarbamates: c) H. Ohnishi, S. Tajma, Y. Yamamoto, H. Kanno (Nihon Nohyaku), EP-A 619 301, **1993** (priority date April 4, 1993).
[81] a) R. J. Brown, K.-M. Sun, D. A. Frasier (DuPont), WO-A 95/14009, **1993** (priority date November 19, 1993). b) Shortly after the publication of this application in May 1995 Novartis had submitted patent applications for strobilurins with similar cyclic pharmacophores and specific side chains: H. Ziegler, R. Zurflüh, A. C. O'Sullivan (Novartis), WO-A 97/02255, **1995** (priority dates July 4, 1995, May 24, 1996).
[82] B. W. Krüger, L. Assmann, H. Gayer, P. Gerdes, U. Heinemann, D. Kuhnt, U. Philipp, T. Seitz, J. Stetter, R. Tiemann, H. W. Dehne, S. Dutzmann, G. Häussler (Bayer AG), WO-A 95/04728, **1993** (priority dates August 11, 1993, March 10, 1994).
[83] a) T. Ohtsuka, T. Murashi, S. Suzuki, M. Masuko, H. Takenaka (Shionogi), WO-A 95/27693, **1994** (priority dates April 6, 1994, July 7, 1994, November 4, 1994, January 27, 1995); b) K. Oberdorf, H. Sauter, H. König, A. Harreus, B. Müller, R. Kirstgen, W. Grammenos, H. Bayer, F. Röhl, G. Lorenz, E. Ammermann, V. Harries (BASF AG), WO-A 96/07633, **1994** (priority date September 10, 1994).
[84] a) "The synthetic strobilurin BAS490F: profile of a modern fungicide": R. E. Gold, E. Ammermann, H. Köhle, G. M. E. Leinhos, G. Lorenz, J. B. Speakman, M. Stark-Urnau, H. Sauter in *Modern Fungicides and Antifungal Compounds* (Eds.: H Lyr, P. E. Russell, H. D. Sisler), Intercept, Andover, **1996**, pp. 79–92; b) R. E. Gold, G. M. E. Leinhos, *Pestic. Sci.* **1995**, *43*, 250–253. c) *Kresoxim-methyl*, active substance brochure of BASF, Ludwigshafen, **1993**. d) The terms "protective" and "curative" refer to the timing of the fungicide treatment, that is, whether it is before or after a fungal infection.
[85] H. Sauter is indebted to H. Kubinyi for these and other QSAR calculations and for numerous other informative discussions.
[86] W. Grammenos, H. Kubinyi, H. Sauter, unpublished results.
[87] Furthermore, extending the bridging subunit linking the two aromatic rings of the phenoxy side chain in **13a** to the phenoxymethyl side chain of **9a** increases the value of $\lg P_{ow}$ only by about 0.3.
[88] Here too there was—at least in hindsight—a fascinating patent competition: a) H. Ziegler, S. Trah, S. Farooq, R. Zurflüh (Ciba-Geigy), WO-A 95/18789, **1994** (priority dates January 5, 1994, July 1, 1994); b) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21153, **1994** (priority dates February 4, 1994, June 17, 1994); c) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21154, **1994** (Prioriotät: 4.2.1994, 17.6.1994); d) H. Bayer, H. Sauter, R. Müller, W. Grammenos, A. Harreus, R. Kirstgen, F. Röhl, E. Ammermann, G. Lorenz (BASF AG), WO-A 95/21156, **1994** (priority dates February 4, 1994, June 17, 1994). e) See also ref. [81b], in which Novartis claims the special "double oxime ether" side chain with a "new" cyclic pharmacophore.
[89] Despite ceding priority, BASF submitted its own patent applications for active substance mixtures with some of these strobilurins with a "double oxime ether" side chain, for example B. Schwalge, R. Müller, H. Bayer, H. Sauter, R. Saur, K. Schelberger, E. Ammermann, G. Lorenz, S. Strathmann (BASF AG), WO-A 97/06678, **1995** (priority date August 17, 1995).
[90] R. Kirstgen, W. Grammenos, H. Bayer, R. Doetzer, H. Koenig, K. Oberdorf, H. Sauter, H. Wingert, G. Lorenz, E. Ammermann, V. Harries (BASF AG), WO-A 94/19331, **1993** (priority date February 23, 1993).
[91] G. Kleefeld, A. Klausener, W. Krämer, W. Brandes, S. Dutzmann, G. Häussler (Bayer AG), EP-A 331966, **1988** (priority date March 5, 1988).
[92] S. Trah, F. Gantz (Maag AG), EP-A 433233, **1989** (priority date December 14, 1989).
[93] a) P. R. Hofstätter, *Gruppendynamik*, Rowohlt, Hamburg, **1957**, pp. 27–63; b) "Teamarbeit und Einzelarbeit aus Sicht der Arbeitspsychologie": B. Biäsch, *Ind. Org.* **1962**, *31*, 139–142.
[94] a) S. Iwata, J. W. Lee, K. Okada, J. K. Lee, M. Iwata, B. Rasmussen, T. A. Link, S. Ramaswamy, B. K. Jap, *Science* **1998**, *281*, 64–71; b) Z. Zhang, L. Huang, V. M. Shulmeister, Y.-I. Chi, K. K. Kim, L.-W. Hung, A. R. Crofts, E. A. Berry, S.-H. Kim, *Nature* **1998**, *392*, 677–684.
[95] a) M. Degli Eposti, S. de Vries, M. Crimi, A. X. Gnelli, T. Patarnello, A. Meyer, *Biochim. Biophys. Acta* **1993**, *1143*, 243–271; b) U. Brandt, B. L. Trumpower, *Crit. Rev. Biochem.* **1994**, *29*, 165–197.
[96] a) "Fungicide Resistance": S. J. Kendall, D. W. Hollomon in *Fungicidal Activity* (Eds.: D. Hutson, J. Miyamoto), Wiley, Chichester, **1998**, pp. 7–108. b) Modern strategies for preventive resistance management include the use of mixtures and alternating treatments with fungicides of other active substance classes as well as pan-industrial cooperation and the agreement of application guidelines in FRAC (Fungicide Resistance Action Committee); compare with ref. [72], p. 111.
[97] Besides fungi, only algae and mosses depend on spore germination.
[98] "BAS490F – a broad-spectrum fungicide with a new mode of action": E. Ammermann, G. Lorenz, K. Schelberger, B. Wenderoth, H. Sauter, C. Rentzea, *Abstr. Pap. Brighton Crop Prot. Conf. Pests and Diseases, Vol. 1* (Farnham, UK) **1992**, pp. 403–410.
[99] a) H. Köhle, K. Grossmann, G. Retzlaff, A. Akers, *Gesunde Pflanz.* **1997**, *49*(8), 1–5; b) R. Saur, B. H. Menck, G. Prigge, *Gesunde Pflanz.* **1997**, *49*(5), 151–158.
[100] "ICIA5504: a novel, broad spectrum, systemic β-methoxyacrylate fungicide": J. R. Godwin, V. M. Anthony, J. M. Clough, C. R. A. Godfrey, *Abstr. Pap. Brighton Crop Prot. Conf. Pests and* Diseases, *Vol. 1* (Farnham, UK) **1992**, pp. 435–442.
[101] a) M. Masuko, M. Niikawa, T. Kataoka, M. Ichinari, H. Takenaka, Y. Hayase, Y. Hayashi, R. Takeda, *Abstr. Pap. 6th Int. Congr. Plant Pathology* (Montreal) **1993**, p. 91; b) M. Masuko, M. Niikawa, T. Kataoka, M. Ichinari, H. Takenaka, Y. Hayase, Y, Hayashi, R. Takeda, poster presented at the 6th International Congress of Plant Pathology, Montreal, Canada **1993**.
[102] H. Wingert, B. Wolf, B. Benoit, H. Sauter, M. Hepp, W. Grammenos, T. Kükenhöhner (BASF AG), EP-A 493711, **1990**, (priority date December 31, 1990).
[103] O. Hueter, A. Pfiffner, H. Szcepanski, M. Zeller, *Abstr. Pap. 9th Int. Congr. Pest. Chem.* (London) **1998**, pp. 1A-008.
[104] a) "Strobilurin in der Wintergerste verbessern die Qualität": K. Schlüter, *Landpost* (Stuttgart) **1997**, June 7, p. 34; b) "Fungizid-Nachlese 1997 – Das Jahr der Strobis": J. Wörle, *dlz agrarmagazin* (München) **1997**, issue 11, p. 26; c) "Strobilurin: Erfahrungen aus 1997 nutzen": J. Frahm, *top agrar* **1998**, issue 1, p. 60; d) "Fungizid-Einsatz im Getreide 1998 – Die Karten werden neu gemischt": H. Hanhart, J. Frahm, *top agrar* **1998**, issue 1, p. 64.