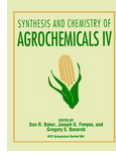
SYNTHESIS AND CHEMISTRY OF
AGROCHEMICALS IV
Donald R. Baker, Joseph G. Fenyes, and Gregory S. Basarab
ACS Symposium Series 1994

Synthesis and Chemistry of Agrochemicals IV

In Synthesis and Chemistry of Agrochemicals IV; Baker, D., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1995.

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Synthesis and Chemistry of Agrochemicals IV

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ACS Symposium Series

M. Joan Comstock, *Series Editor*

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Foreword

THE ACS SYMPOSIUM SERIES was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of this series is to publish comprehensive books developed from symposia, which are usually "snapshots in time" of the current research being done on a topic, plus some review material on the topic. For this reason, it is necessary that the papers be published as quickly as possible.

Before a symposium-based book is put under contract, the proposed table of contents is reviewed for appropriateness to the topic and for comprehensiveness of the collection. Some papers are excluded at this point, and others are added to round out the scope of the volume. In addition, a draft of each paper is peer-reviewed prior to final acceptance or rejection. This anonymous review process is supervised by the organizer(s) of the symposium, who become the editor(s) of the book. The authors then revise their papers according to the recommendations of both the reviewers and the editors, prepare camera-ready copy, and submit the final papers to the editors, who check that all necessary revisions have been made.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

M. Joan Comstock
Series Editor

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Preface

RACHEL CARSON, 32 years ago in her book *Silent Spring*, discussed the dangers of certain chemicals used in industry, agriculture, and health care. Carson implicated DDT in particular for its persistence in the environment and its deleterious effects on off-target organisms. During and after the second World War, DDT was even used for treating humans for lice and related conditions. To a large extent, we can credit Rachel Carson and the publication of *Silent Spring* for many changes in public, industrial, and academic policy. In the book's last chapter, entitled "The Other Road," Carson pointed out alternatives to synthetic agrochemicals, such as the use of natural products and the development of male sterilization methods for insect control, and she encouraged the use of safer agrochemicals. During the following decades we witnessed the banning of DDT and many other polychlorinated hydrocarbons, including cyclodienes such as Aldrin and related insecticides.

Improvement in the accuracy of analytical instruments and the development of newer and more sophisticated methods for toxicological testing have aided the progression toward newer and safer chemicals for agricultural and industrial uses. The overview chapter in this volume entitled "Bioassays in the Discovery Process of Agrochemicals" describes various methods used to evaluate biological activity. However, beyond activity, an agrochemical must show favorable properties relating to mammalian and fish toxicity, mutagenicity, teratogenicity, etc., for submission to government regulatory agencies for registration. As a result of the strict standards imposed by the industry on its research, we now have herbicides (sulfonylureas and imidazolinones) with application rates of grams rather than pounds per acre. We have safer, less persistent agrochemicals in place of highly chlorinated fungicides and insecticides. We continue to strive quite successfully to find increasingly safer chemicals for agrochemical uses.

As with the previous three volumes, our goal is to inform the reader of the current trends in research for safe, efficient, biologically active chemicals. The organization of this book is similar to that of the preceding three volumes. After the overview chapter and a second chapter reviewing bioisosterism in agrochemicals, a section of chapters describes the discovery of new plant control agents. The following section deals with control of insects and acarids. The final section covers the control of fungal diseases.

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We express our appreciation to the authors who shared the results of their interesting work with us during the symposia. Special thanks go to those who spent many extra hours preparing the chapters for publication in this volume. We hope that the readers—be they chemists, microbiologists, entomologists, plant physiologists, or medicinal chemists—will find the chapters interesting, useful and, above all, stimulating.

Last, but not least, we also thank our employers, Buckman Laboratories International, Inc., DuPont, and Zeneca Ag Products; without their generous support of the symposia, this volume and the previous three volumes could not have been published.

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Chapter 1

Bioassays in the Discovery Process of Agrochemicals

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Agrochemical discovery relies on a variety of assays to reflect the attributes of a chemical that are ultimately useful to the farmer. Among these attributes are spectrum of pest control, use rates, mode-of-action, phytotoxicity to crops, and safety to workers and to the environment. Their characterization is quite complex and no one set of protocols will guarantee progress towards compounds of commercial potential. Furthermore, the course to commercialization grows more arduous due to the increase in regulation driving up development costs and to the modernization and sophistication of laboratory methods driving up research costs. Described herein are evolving trends that we envision for the design of assays key to the discovery of newer and more effective crop protection chemicals. We have divided discovery assays into five types: enzyme/receptor, biochemical pathway, cellular, greenhouse and field assays and suggest that all of the types will be integrated for the successful identification of novel chemistry to protect crops from weeds, insects and fungi.

An irony of modern life is that those commodities that are truly most precious are those that we do not pay for dearly. We pay tremendous prices for diamonds which contribute little to day-to-day livelihood. Conversely, we pay relatively little for our food, expecting abundant supplies at the local grocery store. Even when famine sweeps across countries or regions, the cause is usually rooted in politics rather than actual supply or failures by worldwide farming. Today the world is able to feed its population; some regions are perhaps more successful than others, but overall food is cheap and plentiful. It would be hard to imagine this world otherwise and it offers us some measure of hope that few of the world conflicts deal with access to the necessities of life such as food, water and shelter.

At this point in time, the farmer efficiently feeds the world having met the challenge of population growth; the growth in productivy of United States agriculture, for

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example, has outstripped that of the non-farm private sector by nearly four times since World War II (*1*). The reasons for the productivity increases are many, including automation and mechanization, pest control with agrochemicals, the use of superior hybrid crops and the adoption of alternative farming practices. In this book we divided agrochemical discovery into three areas: the control of weeds and plant growth; the control of insects, acarids and nematodes; and the control of fungi. This is the fourth in a series of ACS Books dedicated to the synthesis and chemistry of agrochemicals, the previous being published in 1989, 1991 and 1992 (*2-4*).

Ongoing Concerns and Prospects

The overview of the last book in this series raised a number of issues concerning the safety of the food supply and the health of the environment due to agrochemical use (*5*). These issues remain today and the pace of the effort to bring agrochemicals with higher safety margins to the farmer, the environment and the consumer has accelerated as lower use rate, more pest-specific agrochemicals have entered the marketplace. Though this pace may not seem fast enough for many of the general public, the evaluation and selection of analogues for development include toxicology and environmental fate determinations much earlier during the discovery process than in the past. Government regulatory agencies are trying to use risk-benefit assessments for setting pesticide residue allowances in food (*6*), but pressures to limit pesticide use continues unabated and public fears persist (*7*). In the United States, the courts have mandated strict interpretation of the Delaney Clause, a piece of legislation that prohibits any level of a food addititive found to induce cancer in animals (*8*). The chemical industry and others involved with food production have a duty to address public fears as well as to use technological advances to avoid the potential for environmental abuse and adverse food contamination.

As documentation of resistance continues, the efficacy of modern agrochemicals becomes clouded particularly since many have single-site modes-of-action leaving themselves more susceptible to resistance development (*9*). The registration process dictated by the European Economic Community (EEC) now requires data about resistance potential for re-registration of older agrochemicals and introduction of new agrochemicals (*10*). Resistance potential may be difficult to assess, but the efficacy of new agrochemicals will abate more quickly if they share modes-of-action with older ones that have encountered field resistance. As a result (and for other reasons), there remain coordinated efforts to discover agrochemicals through biochemical means (*11,12*). Though the agrochemical industry has yet to discover a commercially viable, new mode-of-action pesticide directed by design to bind to a protein target, the integration of protein binding assays into the discovery and optimization process continues unabated. Within this volume, the chapters by Finn *et al.*, Markley *et al.*, and Cloudsdale *et al.* correlate enzyme inhibition data with greenhouse data in efforts to better understand structure-activity-relationships (SAR).

Assaying for Agrochemical Utility

The discovery process begins with reliable and efficient assays that predict the ability of a chemical to protect a field crop. This discovery process entails not only

identification of new classes of chemistry and new modes-of-action, but also optimization of the characteristics necessary for commercialization through the iterative process of synthesis and biological evaluation. With unlimited resources, evaluation of all natural or synthesized chemicals would be carried out in the field, in the greenhouse and *in vitro*, somewhat simultaneously, affording the maximum amount of data at the earliest possible time to drive further research. Practicality dictates that less rigorous, more efficient protocols be developed, historically in the form of greenhouse assays in which a variety of crops and/or pests are exposed to a given chemical at a variety of rates and conditions. One of the advantages of agrochemical over pharmaceutical primary assays is that exposure of the ultimate host to a given chemical is routine and acceptable. We will test an antifungal compound on a plant in the greenhouse long before we will test one on a human subject. Since most, if not all agrochemicals operate by binding protein targets to disrupt cellular physiology, the development of *in vitro* assays has become increasingly important. Among the *in vitro* assays that we envision are cellular assays (growth assays for fungi or tissue culture assays for plants and insects), biochemical pathway assays and enzyme or receptor assays (see Table I).

Table I. Attributes for the Types of Agrochemical Assays

TYPES OF ASSAYS	TRACK RECORD FOR PREDICTING UTILITY	CONSTRAINTS OF ASSAY ON EFFICACY	# OF BIOCHEMICAL TARGETS	THROUGH PUT	SPACE AND FACILITY NEEDS	AMOUNT OF COMPOUND NEEDED
FIELD	V. GOOD	V. HIGH	MANY	V. LOW	V. HIGH	V. HIGH
GREENHOUSE	GOOD	HIGH	MANY	MEDIUM	HIGH	HIGH
CELLULAR	FAIR	MEDIUM	MEDIUM	HIGH	LOW	LOW
BIOCHEMICAL PATHWAY	POOR	LOW	FEW	HIGH	LOW	LOW
ENZYME OR RECEPTOR	POOR	LOW	V. FEW	V. HIGH	LOW	LOW

As mentioned, greenhouse assays have historically driven the agrochemical discovery process and will continue to do so in the future. However, the utility of other assays will expand as we better understand the relationships between a chemical, its biochemical target and its efficacy. A compound must overcome more and more hurdles to show activity as testing proceeds from enzyme or receptor assays progressively to cellular assays and ultimately to field assays. We have termed this Constraints of As-

say on Efficacy in Table I. A living cell may prevent penetration of a compound effective in a protein binding assay or may metabolize the compound precluding expression of its activity in cellular assays. A compound active in cellular assays may not translocate appropriately through a plant or may undergo metabolism by the plant precluding the expression of greenhouse activity. A greenhouse active compound may not survive the intense exposure to sunlight or rain in the field again precluding the expression of activity. Correlation with practical utility for the farmer improves proceeding up the levels of assays and, hence, the track record is better (see Table I). Though the precise identity of the biochemical targets may not be apparent while proceeding from protein binding to field assays, the physiological effects of many more biochemical targets are being investigated. On the other hand, progressing down the levels of assays in Table I will more likely afford activity in a given class of chemistry since physical and chemical attributes are less constraining. The throughput is higher (more compounds assayed in a shorter time span) progressing down Table I and the physical space requirement is lower as is the amount of chemical necessary.

Higher throughput, lower chemical input assays grow in importance as ever increasing numbers and ever decreasing amounts of compounds such as natural products become available. Advances in extraction and analytical technology have fostered the isolation of even sub-milligram amounts of material (*13*), less than what greenhouse assays typically require thereby precluding the testing of many natural products. Ironically, the pharmaceutical industry has relied more on natural products than the agrochemical industry even though the role of many natural products is to enhance an organism's defensive and competitive situation (*14*) and there should be many more natural products (and natural product leads) with insecticidal (*15*), herbicidal (*16*) or fungicidal (*17*) utility than with medicinal utility. Cyclosporin, isolated from a bacterium found in a Norwegian soil sample, indirectly turns off a signal for the production of T-cells during the mammalian immune response (*18*) and has a function that has little to do with its role in nature. However, it is not hard to imagine that a plant would produce defensive chemicals against insect or fungal pests or that a fungus may produce a toxin to outcompete other fungi or to help invade a plant. By one estimation, less than one percent of the plant kingdom has been brought into some form of cultivation (*19*), much less fractionated to look for chemicals of agrochemical utility. The percentages of characterized species are probably far less for the animal and microbial kingdoms and further exploration into natural products for agricultural utilities ought to become less constrained by sample size.

Similarly, combinatorial methods generate huge libraries of only small quantities of chemicals by covalently combining a variety of building block units in either a directed or random fashion. The methods may rely on biological techniques in which perhaps randomly synthesized oligonucleotides are spliced onto a phage or plasmid vector for cellular expression of peptide fragments onto a cell surface protein, phage particle or plasmid (*20*). To identify a potential lead, a variety of methods to identify the active peptide must follow an assay for a desired activity (*i.e.*, binding affinity with a protein of agrochemical interest). Alternatively, automated techniques exist to synthesize peptide libraries on solid supports either with a knowledge of sequence up front or in random fashion requiring an efficient scheme to identify active principles. Clearly the challenges remain to mesh an assay of agrochemical importance with the combinatorial products

and to then design a non-peptide lead from the identified peptide sequence. Synthetic chemists have also sought imaginative non-peptidic molecular diversity (*21*); again, efficiently meshing the chemistry with agriculturally relevant assays will certainly be investigated over the next few years. The net result is that these methods produce numbers of chemicals beyond the capabilities of greenhouse assays and therefore, the development of appropriate assays becomes all important to exploit the technology. The trends in the development of the various levels of assays and their relationship to chemistry will be the focus of the remainder of this chapter.

Enzyme and Receptor Assays

Besides the pressures of compound numbers and quantities, another obvious reason for the incorporation of biochemical assays into discovery efforts is simply that agrochemicals operate, generally, by the interaction of a small molecule with a protein. The protein may be an enzyme or a non-catalytic receptor site, crucial in some manner to the well-being of the organism. There would be a measure of elegance in purposefully designing a compound that would both effectively shut down a biochemical process and afford, as a result, greenhouse activity. Furthermore targeting biochemical sites offers potential avenues around problems of resistance. On one level, chemical inhibition of a novel target site precludes cross-resistance to existing agrochemicals. On another level, designing a small molecule that binds to a protein active site could decrease the likelihood of resistance development by mutation of the protein (*22*). Though such mutations are not the only pathways to resistance, they clearly are a major source.

Target-Site Validation. Earnest evaluation of target sites should precede establishment of protein binding assays with particular attention being paid to validation of target sites, that is rationalization that binding to a given receptor or enzyme will lead to a desired physiological response. Ideally, chemical validation of a given target site would create a commercial agrochemical with a novel mode-of-action. If chemical validation is untenable, then genetic techniques become necessary, either through mutant variants lacking individual proteins, or through gene disruption and anti-sense constructs to vary the expression level of a given protein. Approaches to validation will be outlined in the following paragraphs.

Chemical Validation. Protein targets that already benefit from chemical validation through empirical discovery offer the clearest choice for inhibitor design. Information on how a pesticide class binds to its target site can inspire the design of improved analogues. Greenhouse SAR do not often reveal the reasons for the up and down swings of activity which may be due to differential inherent binding affinity with proteins. However, SAR variations also may result from dissimilar physical-chemical properties of the chemistry and, consequently, to disparate propensities to overcome the hurdles of penetration into the plant, translocation to the site-of-action, metabolism, degradation by UV radiation, *etc.* Kinetic experiments can often suggest the mode of binding of chemicals to protein targets distinguishing among competitive binding relative to a substrate or other ligand, uncompetitive binding to some alternate protein complex form or noncompetitive binding to an allosteric protein site. Kinetic experiments show that sulfonylurea, imidazolinone and sulfonanilide herbicides operate by in-

hibition of acetolactate synthase (ALS) (and, as a result, of branched chain amino acid biosynthesis) at an allosteric vestigial quinone binding site (*23*), suggesting that the design of active site inhibitors could offer a new class of herbicides that would not show cross-resistance (Abell, L. M.; Kerschen, J. A. *Pestic. Sci.*, in press).

Alternatively, target sites for inhibitor design often emerge from the knowledge of pathway susceptibility to agrochemicals. Due to the commercial payoff of ALS inhibitors, researchers have investigated other steps in the biosynthetic route to branched chain amino acids as targets for pesticide design (*25*). This approach suffers from the fact that not all steps in a pathway are equally important to the viability of an organism. Aulabaugh *et al.* demonstrated that greater than 90% *in vivo* inhibition of ketol acid reductoisomerase (KARI), the step subsequent to ALS in the branch chain amino acid pathway, does not significantly induce phytotoxicity, suggesting that the plant has more than enough of this enzyme to maintain the flux through the biosynthetic pathway (*24*). Thus, the substrate turnover inside the cell may vary from step to step. Alternatively, the buildup of one intermediate over another may contribute more to toxicity than depletion of the final product or else a given intermediate of a pathway may have a secondary physiological or metabolic role.

Genetic Validation. Recently there has been work in genetic techniques to attenuate but not eliminate the metabolic activity of protein targets to better understand their role in cellular processes and to offer a means for validation. It is well understood that generation of mutant organisms deficient in an essential metabolic activity may retard normal growth (*23*). However, doing so may not be enough to identify ideal target sites where partial binding *in vivo* is enough to see a lethal or growth retarding effect. The KARI example above showcases a less than ideal target site, though no one has yet reported the genetic attenuation of KARI in a plant. The techniques for partial elimination of protein activity include site-directed mutagenesis in which key amino acid residues are modified to afford a protein of decreased viability (*26*), gene disruption in which the gene promoter region is clipped to decrease (but not eliminate) protein expression (*27*) and anti-sense RNA expression in which a gene is inserted into an organism to produce segments of opposite stranded messenger RNA to complement the normal sense messenger RNA and thereby attenuate protein expression (*12*). Höfgen *et al.* recently showed that attenuation of tobacco glutamate 1-semialdehyde aminotransferase (GSA-AT) in the chlorophyll biosynthetic pathway elicited a variety of chlorophyll deficient phenotypes (*28*).

Beyond serving to elucidate cellular physiology, validation experiments instill confidence in those who design assays to screen compound inventories or libraries. Those who synthesize compounds can either pursue classical SAR follow-up of empirically discovered leads design chemistry from mechanistic or structural information about the target site.

Structure-based Design. The last few years in particular have seen an explosion in the use of protein structural information for inhibitor design, mostly for targets of medicinal interest. The Protein Data Bank currently lists full atomic coordinates for nearly 2500 proteins, up from 230 ten years ago (*29*). This growth is due to contributions from three areas of technology: molecular biology to provide ample quantities of protein for study, nuclear magnetic resonance and protein crystallography to solve pro-

tein structure and computer technology, again to solve protein structure but also to enhance visualization and manipulation of protein models (*30*). Improving binding affinities of small molecules to proteins has seen greater progress than the quite difficult task of designing inhibitors from protein models without a bound ligand (substrate or inhibitor). A variety of software programs offers structural insight into binding processes including graphics based programs that aid molecular calculations, inhibitor docking, and conformational and similarity searching (*30,31*). There are now attempts to assemble random or semi-random *de novo* structures computationally in protein pockets generating a library of structures that may not be intuitive to a chemist (*31*). To date, the literature has reported the solutions of the structures of five proteins that are closely related to validated pesticide targets: enol pyruvate shikimate phosphate synthase (*32*), photosystem II (*33*), glutamine synthetase (*34*), nicotinic acetylcholine receptor (*35*) and acetylcholine esterase (*36*). The structure-based approach requires easily purified, preferably crystallizable proteins, excluding most membrane soluble proteins. Homology modeling can circumvent this limitation if the amino acid sequence of a protein shares sufficient similarity to that of proteins with determined structures. Morris and Richards used the X-ray crystal structure of cytochrome $P\text{-}450_{cam}$, the enzyme from the camphor utilizing bacterium *Pseudomonas putida,* to construct a three dimensional model of substrate bound cytochrome $P\text{-}450_{14DM}$ (the sterol C-14 demethylase target-site of triazole fungicides) using the known amino acid sequence from yeast (*37*). Structure-based design is clearly in its infancy even though entire companies devoted to discovery via this concept have emerged (*31,38*).

Biochemical Pathway Assays

As mentioned, activity of a pesticide via binding to one protein of a biochemical pathway suggests investigation of the entire biochemical pathway. Such studies apply mainly to herbicide and fungicide discovery since most insecticides operate on neuroreceptors or hormonal binding sites, the notable exceptions being insect growth regulants that block N-acetylglucosamine incorporation into chitin (*39*) and mitochondrial respiration inhibitors (*40*). There is an economy of design in fashioning assays that implicate a number of different protein targets since the ideal enzyme to inhibit in a pathway is often unknown. Experiments can be constructed to measure the contribution of one enzyme to pathway flux by chemical inhibition and analysis for incorporation of a radioactive starting substrate into the end product. Flux experiments require a well-characterized enzyme with inhibitors of known inhibition constant (K_i); interest in the enzyme usually comes from empirical discovery of an inhibitor (herbicide or fungicide). There are 21 or more enzymes involved in lipid biosynthesis and acetyl-CoA carboxylase (ACCase), the target site of cyclohexanedione and aryloxyphenoxypropionate herbicides, controls 58 and 52 % of the flux through the pathway in barley and maize, respectively (*41*). Hence, it follows that ACCase is the rate-limiting step in lipid biosynthesis and the remaining 20 steps control 42 and 38% of the flux. Similar flux investigations of pathways of agrochemical significance have been carried out on carotenoid biosynthesis (*42*), aromatic amino acid biosynthesis (*43*), and photosynthesis (*44*).

Whether the results of these investigations prescribe exploration into other steps of the pathways is a matter of interpretation, but using radiolabeled metabolites to

examine pathway flux can identify inhibition of any of the pathway steps. Bisaha *et al.* discuss a sterol biosynthesis assay relevant to commercial triazole and morpholine fungicides in which fungal organisms are grown in the presence of radiolabeled acetate and a test chemical; fractionated cells are saponified and the sterol composition of the hexane soluble extract analyzed by high performance liquid chromatography, HPLC (*45*). In the absence of inhibition, a characteristic peak for the end product sterol (*i.e.*, ergosterol) prevails. When inhibition causes build-up of any of the pathway sterols in HPLC tracings, correlation to retention time standards for the sterols starting from squalene identifies a mode-of-action. This is perhaps especially valuable for setting priorities as chemistry with a new mode-of-action has greater importance than that with an established one due to the potential for cross-resistance. These studies may require radiolabeled probes, but they offer automated, highly sensitive assays for a number of biochemical steps within a pathway. Barry *et al.* described HPLC methods to study carotenoid biosynthesis without the use of radiolabeled precursors (*46*).

Inhibition of respiration via blockage of mitochondrial electron transport and oxidative phosphorylation is also of interest as a multiple step pathway having relevance to insects, fungi and plants (and on the downside, to mammals). Clearly, specificity of action would be worthwhile and assays for mitochondrial electron transport for each of the pest classes demonstrate substantial utility. The assays measure either the rates of oxygen consumption or conversion of NADH to NAD spectrophotometrically; addition of various known inhibitors of the various pathway complexes can identify the site of action of a given xenobiotic (*47,48*).

Cellular Assays

Cellular or culture assays help delineate the causes of discrepancies between enzyme or receptor assays and greenhouse assays, while maintaining strictly controlled, reproducible conditions and offering fewer barriers to chemical penetration. Automated microplate fungal growth assays can serve as an initial screening method for large numbers of compounds at multiple dilutions for the commercially important pathogens that can be grown in liquid-culture. Indeed, natural product chemists and pharmaceutical firms utilize growth assays as the primary means of identifying activity. Growth cultures deficient in key nutrients can operate as a first step for determining the mode-of-action of a fungicide as restoration of growth via systematic nutrient supplementation implicates the anabolic pathway responsible for inhibition. Similarly, growth of photosynthetic algae and bacteria in culture serves as a model for plant systems. Growth assays of whole plants such as arabadopsis or lemna in microplates, though not quite cellular in nature, parallel those for fungal species in the ability to scale-up analysis. Rapidly growing plant cell cultures serve to model root and meristem growth and offer the flexibility of designing growth assays based on agronomically important crops (*49*). Finally, since most commercial insecticides attack the insect nervous system, methods to investigate the interaction of chemicals on either intact or cultured nerve cells have been developed (*50*). Electrophysiological and ligand binding assays of the nerve cells show, among other activities, operative nicotinic acetylcholinergic receptors (*51*), γ-aminobutyric acid (GABA) receptors (*52,53*) and sodium channels receptors (*54*), the binding sites of imidacloprid, cyclodiene insecticides and pyrethroids, respectively.

Greenhouse Assays

Greenhouse assays bear the burden of having to meet two contradictory objectives: first, the assays must control conditions to produce meaningful and reproducible results, and second, they must anticipate performance of chemicals in the field in which conditions are highly variable and difficult to predict (*55,56*). To a certain extent, these two objectives are separated by standardizing first tier lead discovery assays while building flexibility into (but still controlling) conditions in advanced level greenhouse assays. The first tier lead discovery assays sift through large numbers of compounds in an automated or semi-automated fashion. Advanced greenhouse assays will test compound efficacy while simulating some of the pressures due to outside climatic conditions, but will do so with fewer numbers of compounds. As a rule, as assays become more standardized allowing the study of larger and larger numbers of compounds, they will reflect the expression of activity in the field more poorly.

For the most part, greenhouse herbicide assays control temperature, soil type, moisture and water conditions, plant genome, light characteristics and spray schedules. Similarly, fungicide and insecticide assays standardize fungal isolates and their culture conditions, and insect genomes and their rearing conditions, respectively. For example in fungicide research, matching the host cultivar and the fungal isolate for maximum disease expression obviates the use of more popular crop varieties (*57*). Furthermore, weed germination, pathogen inoculation and insect infestation for each of the pest classes must be synchronous with a spray schedule and the cultivation of the test crop. This is all quite in contrast to the situation in the field where broad ranges of fungal, insect and weed sub-species exist. A number of weather-related variables such as abnormal winds or excessive rainfall resulting in plant abrasion or saturation of the soil might also be unanticipated in design of greenhouse assays. Sunlight levels and temperature gradations over the course of a field season all affect leaf waxes and cuticle development and therefore susceptibility to fungi and insects not to mention adsorption of test chemicals. Hence, establishment of greenhouse assays and field assays must consider experimental design and the concomitant techniques of data analysis quite carefully.

We anticipate that current greenhouse assays will incorporate a number of strategies to improve correlation to the field. We can expect greenhouse evaluation of a larger variety of plant, pathogen or insect genomes. Included in this will be more systematic evaluation of pesticide resistant phenotypes overcoming the logistics of moving pest isolates across international borders to prevent their spread. Hence, leveraging research by establishing collaborations among research laboratories around the world becomes more and more important.

Expansion of the role of formulation science as a tool to understand laboratory-field correlations will continue (*58*). Development organizations optimize formulations for commercialization usually finding that every chemical has a unique formulation for ideal field characteristics. Nonetheless, the testing of general formulations that enhance the efficacy of chemical classes rather than just a single chemical will improve evaluation of analogues. As the understanding of the effects of formulations improves, their utility in discovery research will grow.

Finally, we can expect closer examination of the fate of test chemicals in plants to explain the difference in data from greenhouse and *in vitro* assays, requiring perhaps

the synthesis of radiolabeled compounds to determine metabolic fate and translocation properties. Alternatively other analytical techniques (*e.g.*, gas chromatography or high performance liquid chromatography in conjunction with mass spectrometry) will develop to quantify chemicals in a plant matrix for research purposes, not just for developmental purposes. There will be an increasing role for other specialized laboratory tests (*e.g.*, photodegradation, hydrolysis half-life, soil stability, *etc.*) for aiding the understanding of greenhouse activity to the field (*59*). We can therefore expect closer collaborations between those who design analytical techniques and those who design greenhouse experiments.

Field Assays

Some of the limitations described above for greenhouse evaluation of agrochemicals hold for field evaluations in that a few field tests do not represent the total set of conditions a crop protection agent may see in commercial use. Any given field plot will use only a few plant cultivars. Often, insects and fungi (of narrow genotype) are artificially introduced into field tests in order to supply sufficient pressures for compound evaluation. Periods of drought or excessive rain can render a crop unusually susceptible to phytotoxicity to a pesticide.

Due to the vagaries of field assays and the limitations of greenhouse assays, performances of compounds of the same chemical family can vary even to the extent that there is inversion of relative efficacy. Well recognized, for example, is that compound physical properties play important roles in affecting translocation through the plant and, in turn, expression of bioactivity. The translocation properties can differ in young plants or seedlings versus large, mature field plants (*60*). Furthermore, the physiology of the important fruiting stage of plants is often impractical to reproduce in the greenhouse. Even different formulations of the same compound can perform differently in a relative sense between greenhouse and field assays. Hence, we can expect the testing of a larger number of analogues and formulations in the field as a method to sort out potential development candidates. This will require, perhaps, smaller plot size and more meticulous methods of application. The concept of transfer ratios (or transfer factors) has been introduced as the ratio of rates required for expression of activity in the greenhouse and the field (*61*). Often the development arm of agrochemical companies establishes field testing rates to determine the rates at which to market a given chemical. In addition, due to plot size limitation, application of only one or two doses of a given chemical may be possible. These testing schemes run counter to establishing the dose-response data necessary for determination of transfer ratios and correlation to greenhouse assays. Again, test design and data analysis need more attention.

Otherwise, biologists will continue to experiment with methods to mitigate the differences observed between laboratory and field conditions. One can imagine treatment of plants with a chemical in greenhouse pots before transferring them outdoors for exposure to the environment (*62*). Alternatively, exposure of plants sprayed in pots in the field to the weather for varying intervals can precede transfer indoors for inoculation with fungi or exposure to insects in more controlled environments (*63*). The imagination of scientists becomes key as they continue to manipulate the variables of test conditions in an iterative cycle between the greenhouse and field in order to close the gap between expressed efficacies.

Conclusions

As the competitive environment for the discovery of new agrochemicals continues to grow, the discovery process becomes ever more difficult and therefore expensive. Markets continue to shift, either due to development of resistance, emergence of modern farming practices in developing nations or changes in pest pressures. No research laboratory can afford to carry out all the methods described here and elsewhere to design the next agrochemical, but must rather select among the various assay options or develop new methodology for maximum efficiency. Choosing among assay options must concern itself with the goals of a particular discovery program and the aims of synthetic design. There is little sense to start up an assay without having the results of the assay direct synthesis and design in some fashion more efficiently than other possible assays. Two or more assays in conjunction may be necessary to direct design, especially if the information they give is complementary and additive. Different assays can have different goals and therefore different sets of compounds to evaluate, but they all must have a unique purpose for incorporation into a discovery program. Ultimately, the design of assays at all levels should have the goal of shortening the timeline to the discovery of safer, more targeted crop protection agents based on rational pharmacological principles.

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Chapter 2

Bioisosterism in Agrochemicals

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A difficulty which agrochemists responsible for new agrochemical exploration meet during their practical experimental process, is lead optimization and how to systematically synthesize many diversified derivatives starting from a certain lead structure. The concept of 'Bioisosterism' is one of the sophisticated optimizations found useful for designing new qualified structures that has been applied in various field of agrochemicals. This principle has proved to be useful as shown by a large numbers of successes in molecular optimization. This chapter reviews bioisosterism in agrochemicals and introduces some examples of its successful applications for practical exploration of new agrochemicals.

This chapter reviews progress in the use of bioisosterism in agrochemicals design. Bioisosterism can be a useful methodology for the exploration of new agrochemicals. The concept of bioisosterism has been well described in reviews by Thornber (*1*) and Lipinski (*2*). According to Thornber's definition, bioisosters are groups or molecules which have chemical and physical similarities producing broadly similar biological properties.

Definition

Isosterism originally meant a close similarity of molecules or ions which have the same number of atoms and valence electrons *e.g.* CO and N_2. And such a similarity was thought to explain the analogies of physical constants of molecules. Then, its definition was expanded to the molecules which have the same number of valence electrons but different number of atoms *e.g.* -O-, -NH-, and -CH_2-. However, it has been shown that even such a broader definition is too restrictive to relate biological properties to the physical and chemical properties of the molecule.

"Bioisosterism" has been introduced to describe the phenomena where molecules possessing related structure have similar or antagonistic properties. "Non-classical" isosterism is also used interchangeably with bioisosterism, particularly when molecules do not have the same number of atoms and valence electrons, but show a similarity in some important parameters to a certain extent. Since no two substituents are exactly alike, any substitution will result in changes in size, shape, electronic distribution,

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lipophilicity, p*K*a, chemical reactivity, susceptibility to metabolism, and so on. Thus, purpose of the bioisosteric approach is the total change induced by the substituent replacement will result in improved potency, selectivity, duration of action, bioavailability, and/or a reduction in toxicity. Although applications in medicinal chemistry have been explained in the foregoing reviews, no descriptions have been made as to the bioisosterism and molecular modification in agrochemicals. Examples of the "classical" and " non-classical" isosters are shown in Tables I and II, respectively. In the following section, application examples of bioisosteric replacements to agrochemicals are shown.

Table I. Classical Isosters

Atoms and groups which have the same number of valence electrons.

A Univalent

-F, -OH, $-NH_2$, $-CH_3$

B Bivalent

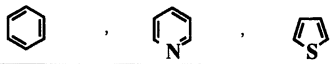
-O-, -S-, -Se-, $-CH_2-$, -NH-

C Tervalent

-CH=, -N=

D Quadrivalent

Ring equivalents



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Classical Bioisosters

Ring Equivalents. The first applications of isosterism are found in ring (or atom) equivalents as they are encountered in the pair of benzene and pyridine , or the pair, benzene and thiophene. Especially, isosteric replacement of benzenes by CF_3-substituted pyridines led to a number of useful agrochemicals. In the following example, application of ring equivalent to the herbicidal aryloxy-phenoxy- propionic acid ester is shown. Replacement of benzene with pyridine did not make any considerable enhancement in herbicidal activity. However, translocation ability in plants was remarkably improved by the replacement (*3*). Since 5-CF_3-2-pyridyloxy group is less lipophilic (π =1.12) compared with the corresponding phenoxy group (4-CF_3-phenoxy ; π =2.08), the molecule bearing the CF_3-pyridyloxy group is supposed to possess the favorable partition parameter to translocate in the plant tissue. Thus-prepared fluazifop butyl is now widely used in the world broadleaf crop market as a graminicide.

fluazifop butyl

Table II. Non-classical Isosters

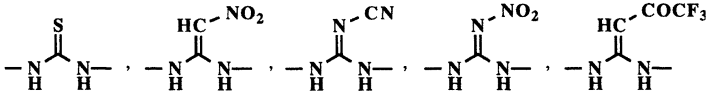
Carboxylic acid group

$-CO_2H$, $-SO_2NHR$, $-SO_3H$, $-PO(OH)_2$, $-PO(OH)NH_2$, -CONHCN,

Halogen

-F, -Cl, -Br, -I, $-CF_3$, -CN, -SCN, $-N(CN)_2$, $-C(CN)_3$

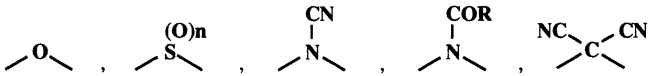
Thiourea



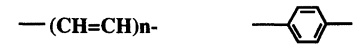
Hydroxy-group

-OH, -NHCOR, $-NHSO_2R$, $-CH_2OH$, $-NHCONH_2$, -NHCN, $-CH(CN)_2$

Ether



Conjugated double bond



Activating group

Aldehyde - Nitrile

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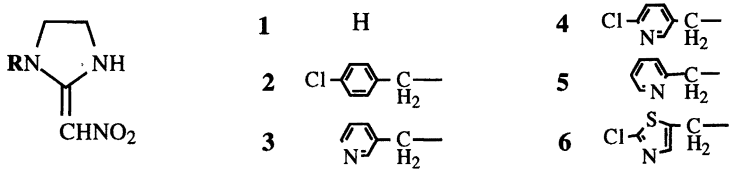
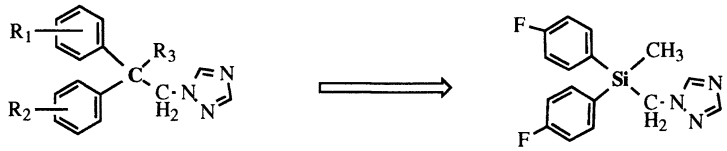
Kagabu *et al.* (*4*) found during their study on the substitution effects in the known nitromethylenyl heterocycles (*5*) that the benzylation of a nitrogen atom of imidazolidine **1** appreciably enhanced the insecticidal activity. The efficacy strongly depends upon the nature of substituents and their position on the phenyl ring. Among the compounds tested, 4-chlorobenzyl derivative **2**, was the most active. Insecticidal activity LC_{90} against green rice planthopper *Nephotettix cincticeps* was 40 ppm. Replacement of the benzene ring by a pyridine ring **3** not only increased the activity (LC_{90} : 8 ppm) by an order magnitude, but also remarkably expanded the spectrum.



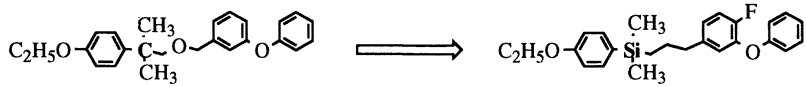
The 6-chloro-nicotinyl bioisoster **4** of proto-type **2** showed extraordinary activity (LC_{90} : 0.32 ppm) (*6*). Since the 2-pyridyl bioisoster **5** did not enhance the activity, it is suggested that the distance between nitrogen atoms in the heterocycles and "acidic" amide proton is crucial to maximum activity. 2-Chloro-5-thiazolyl derivative **6** also showed remarkably strong activity.

Quadrivalent Atoms (Organosilicon Compounds). Although both carbon and silicon are members of group IV of the periodic table, few examples of the substitution of silicon for carbon have been reported in agrochemicals. The first agrochemical commercialized which contains silicon is the triazole fungicide, flusilazole (*7*).



flusilazole

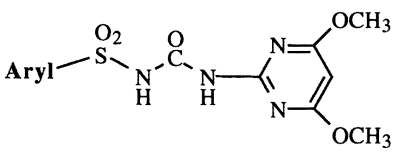
Replacement of cumyl ether group with dimethylphenylsilylmethyl group gives a silicon containing insecticide (*8*).



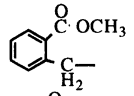
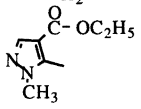
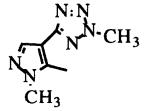
ethofenprox **silafluofen**

Non-classical Bioisosters

Bioisosters in Sulfonyl Urea Rice Herbicides. Bensulfuron-methyl is a widely-used rice herbicide in Japan. Standard rate of bensulfuron-methyl is around 50 g/ha (*9*). Nissan Chemical developed second sulfonyl urea rice herbicide pyrazosulfuron-ethyl, which has a pyrazole ring bearing carboxylate substituent at 4-position thereof in the Aryl portion and can be applied at rate of 25 g/ha (*10*). Probably influenced by pyrazosulfuron-ethyl, Dupont performed further exploration of pyrazolyl-sulfonylurea herbicides resulting in the development of azimsulfuron, which has a methyltetrazolyl moiety as a bioisoster of carboxylate group at the same position of pyrazole (*11*). Azimsulfuron shows longer persistence than pyrazosulfuron-ethyl in the paddy field and is expected to show activity against one of the noxious weeds, water chestnut, *Eleocharis kuroguwai Ohwi* by combining with bensulfuron-methyl.

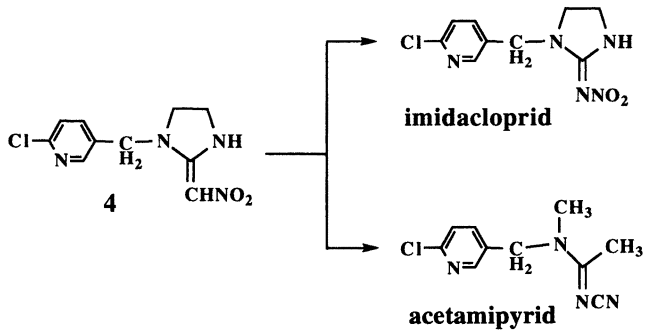


Bridge Heterocycle

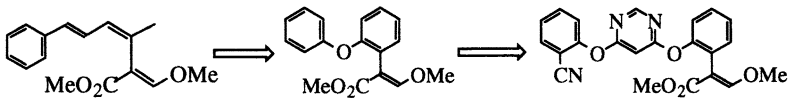
Code	Aryl
bensulfuron-methyl	
pyrazosulfuron-ethyl	
azimsulfuron	

Thiourea. As described previously, the insecticidal activity of the nitromethylene insecticide was enhanced by the introduction of 6-chloro-3-pyridylmethyl group. However, compound **5** possesses two disadvantageous problems for commercialization.

In order to overcome photochemical instability and high mammalian toxicity (*12*), isosters of the nitromethylene group, namely N-nitro guanidine (imidacloprid) and N-cyanoamidine (acetamipyrid), were prepared (*13*).

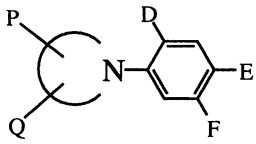


Conjugated Double Bond. In natural products, alkanedienyl moieties are frequently observed. These moieties are often photochemically unstable. It is desirable to replace these labile groups with other lipophilic isosters with higher photostability. In strobilurin A, which is a fungicidal compound derived from the mycelia of various Basidiomycete fungi and shows activity against broad spectrum of fungi, the phenylpentadienyl moiety is characteristic. The photochemical instability of the moiety is thought to be the main reason for the inactivity of this molecule *in vivo* (*14*). Replacement of the phenylpentadienyl group with a diphenyl ether **7** dramatically increased stability to light. Further optimization led to higher activity and less phytotoxicity in compound ICI A 5504. The pentadiene bridge of strobilurin A was effectively replaced by the phenoxypyrimidinyloxy group.



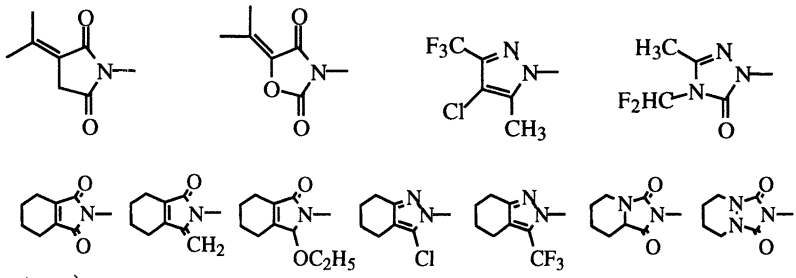
Strobilurin A **7** **ICI A 5504**

Bioisosters in Peroxidizing Herbicides. Cyclic imides and phenylpyrazoles show the identical herbicidal symptoms as diphenyl ethers when applied to weeds. Actually, these N-phenyl-heterocycle herbicides and diphenyl ethers are suggested to have the same target enzyme (protoporphyrinogen IX oxidase)(*15*). Consequently, structure-activity relationships of diphenyl ether herbicides have been utilized in drug design of N-phenyl-heterocycle herbicides. For example, in cyclic imide herbicides, a similar substituent pattern is observed for the phenyl ring. A variety of bioisosters of the cyclic imide moiety is adopted in these derivatives.

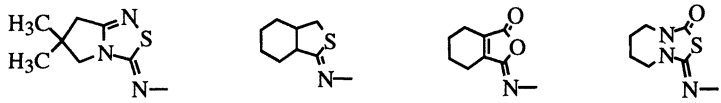


D : F, Cl
E : Cl
F : H, O-alkyl, S-alkyl

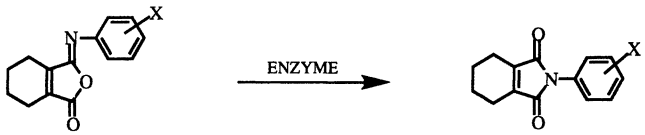
(endo type)



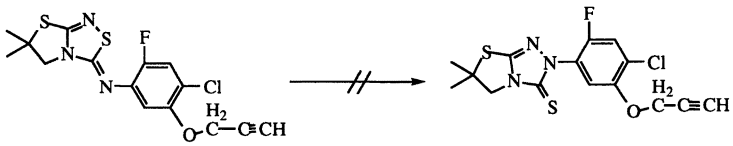
(exo type)



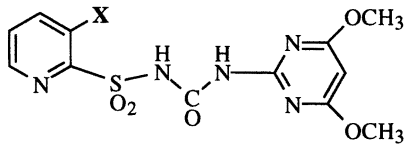
It was reported that a certain type of prodrug isoimide compounds are rearranged into parent imides under the presence of enzymes as shown in the following equation (*16*).



Further, certain thiadiazolidines were reported to be rapidly and spontaneously isomerized via unstable intermediates to the corresponding triazolidine isomers (*17*). On the other hand, a series of fused Δ^{2}-1,2,4-thiadiazolines, which show similar peroxidizing herbicidal activity, were reported not to be isomerized under several conditions to the corresponding triazolinethione, where the nitrogen locates in the ring system (18). Hagiwara *et al.* , with the intention of understanding bioisosterism among three peroxidizing herbicidal skeletons, studied those electrostatic properties by means of molecular orbital calculations and computational techniques, ending up with the proposal of a hypothetical model of active structures required for the herbicidal activity (*19*).

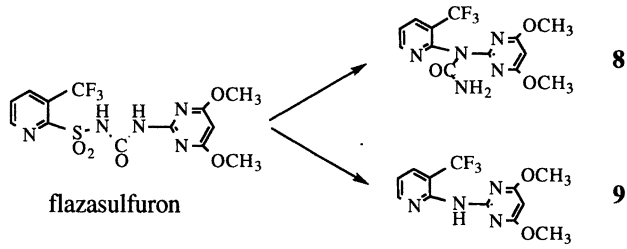


Electron-withdrawing substituent. Although sulfonyl urea herbicides have proved to be highly active, one of problems, which initial sulfonyl ureas encountered was carry-over in some recropping situations. One of the successful strategies tried to overcome this problem was the introduction of an electron-withdrawing group to 3-position of the pyridine ring of 2-pyridyl-sulfonylureas described in the following structure. Nicosulfuron (*20*) and rimsulfuron (*21*) have been commercialized for corn and flazasulfuron (*3*) for turf.

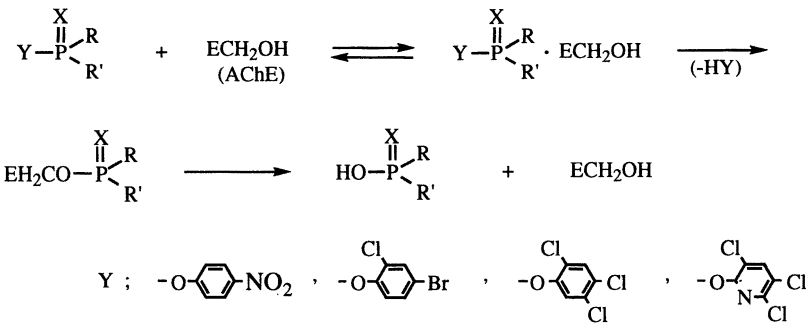


X	common name
$-CF_3$	flazasulfuron
$-CON(CH_3)_2$	nicosulfuron
$-SO_2C_2H_5$	rimsulfuron

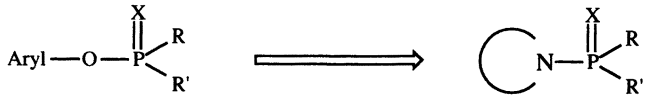
As an example flazasulfuron subjected to hydrolysis in aqueous media, decomposes due to the electron-deficient character of carbon at 2-position of pyridine surrounded by three electron-withdrawing moieties such as nuclear nitrogen, sulfonyl bridge and substituent **X** according to the chemical degradation scheme shown below to afford asymmetric 1,1-disubstituted urea **8**, which in turn is hydrolyzed to pyridyl-pyrimidinyl - amine **9**(*3*). This chemical reactivity is a new mode of degradation, not seen in conventional sulfonylurea herbicides. The half life of flazasulfuron for instance was found to be as short as less than two weeks in soil.



N-Heterocycles as the activating group of the phosphoryl bond. The organophosphorous insecticides bind to acetylcholinesterase(AChE) according to the following equation, where R and R' are generally lower alkyl, alkoxy, alkylthio, or substituted amino groups; X is oxygen or sulfur; and Y is a good leaving group. As the leaving group, aryloxy groups substituted with electron-withdrawing substituents are usually favorable.



An attempt was made to introduce N-heterocycles as the leaving group in place of aryloxy group of organo-phosphorous insecticide.

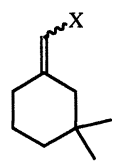


Structure activity relationship (SAR) of these N-phosphoryl-heterocycles (*22*) can be reasonably interpreted by taking into account the acidity of the hydrogen substituted at the nitrogen atom of the parent heterocycle. In Table III, the relationship between the acidity constant (p*K*a) of the parent heterocyclic ring and activities of the corresponding organophosphorous insecticides is shown. An optimum p*K*a seems to exist in the heterocycles. A plausible explanation for this behavior is as follows. When acidity is too low, activation of the phosphoryl group by the heterocycle becomes insufficient, thereby lowering the insecticidal activity. On the other hand, when the p*K*a value is too low, the heterocycle shows increased tendency as a leaving group to such an extent that the molecule itself becomes unstable. Based on the extensive studies on these derivatives, fosthiazate (heterocycle : thiazolidin-2-one) was selected for further development.

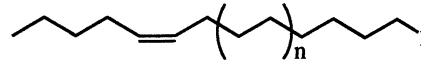
Table III. Relationship between p*K*a of Parent Heterocycles and Activity

					
p*K*a	25.0	11.8	11.6	10.5	9.6
Nematicidal Activity	–	+++	+++	–	–

Aldehyde - Nitrile. It was found in the search for stable sex pheromone analogs with favorable chemical and biological properties that replacement of the aldehyde moiety in pheromones with a nitrile group leads to bioisosters that retained biological activity and had a good potential in practical applications against lepidopteran and coleopteran insect (*23*).



X = CHO, CN



X = CHO, CN

Conclusion

Definition, utilization of bioisosterism, and examples of successful results of new agrochemicals exploration by using the concept of bioisosterism were described in this chapter. Bioisosterism has remained useful as one of the practical chemorational approaches and its position as the best possible approximation for explaining and predicting chemical and biological similarities and analogies has not been replaced by any defendable assumptions. Although there is a discussion that bioisosterism is part of the spectrum of QSAR (*24*), the simple and qualitative concept may be most useful to the agrochemists responsible for new agrochemicals exploration with respect to the point that a chemical structure of a compound, whose activity may be predicted, can be assembled by means of solely visual sense based on one's own chemorational design without any computer support. The concept of bioisosters can be extensively utilized in the future by agrochemists who put an appropriate chemorational emphasis on one of its multiple-faces, which the chemical structure originally possesses, depending upon the necessity of design for new agrochemicals exploration.

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Chapter 3

Synthesis and Herbicidal Activity of Sultamsulfonamides

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The regioselectively methylated 1-thia-2-azacyclopentane-1,1-dioxides (propanesultams) and 1-thia-2-azacyclohexane-1,1-dioxides (butanesultams) and 3-halomethylpropanesultams were synthesized, and converted into 5-phenyl-1-(sultam-N-sulfonyl-carbamoyl)pyrazolines **1** as a new ALS inhibitor. Some of these showed strong herbicidal actvity against broad-leaved weeds without phytotoxicity to wheat. In this chapter, syntheses of mono-, di- and trimethylpropanesultams, mono- and dimethyl-butanesultams and halomethylpropanesultams, and furthermore, synthetic procedure and herbicidal activity of **1** will be discussed.

Propane- and butanesultams have been utilized as building blocks in the synthesis of new pharmaceuticals, as for example in the case of anticonvulsants, a PGE_2 analog, and antihistamines-H_1 (*1-3*). Since this was not the case in the field of agro-chemicals, we looked for opportunities for their use in the synthesis of new herbicides. On the other hand, Wellinga *et. al.* reported the synthesis of herbicidally active benzenesulfonamides incorporating a pyrazoline moiety (*4*). On the basis of reported structural requirements for known inhibitors, we suspected that these compounds were ALS inhibitors. In our continuing studies directed toward new ALS inhibitors, we incorporated both the sultam (cyclic sulfonamide) and pyrazoline moieties into the same molecule, and discovered a new class of herbicidally active sultamsulfonamides **1** (Figure 1) (*5*).

We first describe the synthesis of propane- and butanesultams in detail (Figure 2). In support of structure-activity studies in the sultamsulfonamide series, we elaborated facile methods for the synthesis of mono-, di- and trimethylpropanesultams **7a-d**, mono- and dimethyl- butanesultams **11a-c**, and 3-halomethylpropanesultams **23a-d**, not obtainable by reported methods (*6-8*). Second, we describe the synthesis of 5-phenylpyrazolines **32a,b**, and furthermore, the synthesis and herbicidal activity of sultamsulfonamides **1**.

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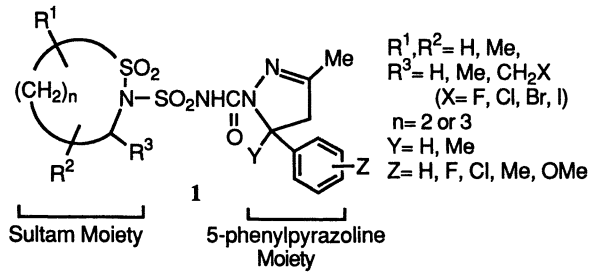


Figure 1. Sultamsulfonamides 1

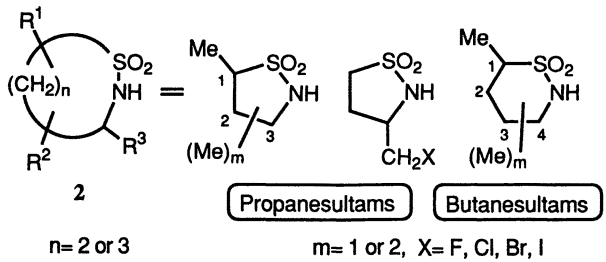


Figure 2. Synthesized Propane- and Butanesultams 2

Synthesis of Propane- and Butanesultams

Our target compounds were mono-, di- and trimethylpropanesultams, mono- and dimethylbutanesultams and halomethylpropanesultams. The introduction of the methyl group into 1-position of sultam ring was necessary for enhancing the herbicidal activity of sultamsulfonamides **1**, and 3-halomethyl derivatives were designed for improving the properties of herbicidal activity.

Mono-, di- and trimethylpropanesultams To construct the sulfonamide moiety of title compounds **7a-d**, initial Michael addition of benzyl mercaptan to the methylated α,β-unsaturated aldehydes **3a,b** was utilized since the benzylthio group introduced at this stage could subsequently be converted into a sulfonamide group by oxidative chlorination and amination. The synthesis of 1-methyl- and 1,2-dimethylpropanesultams **7a,b** is shown in Figure 3. Thus, reaction of benzyl mercaptan with α,β-unsaturated aldehydes **3a,b** afforded the Michael adducts **4a,b** in good yield. The aldehydes **4a,b** were reduced with $NaBH_4$ to afford primary alcohols **5a,b**, which were then converted into sulfonamides **6a,b** *via* chlorination of the hydroxyl group, oxidative chlorination of the benzylthio group, and amination of the chlorosulfonyl group. Cyclization of **6a** and **6b** with KOH afforded 1-methylpropanesultam **7a** and 1,2-dimethylpropanesultam **7b**, respectively.

Grignard addition of MeMgI to the Michael adducts **4a,b** (Figure 4) afforded secondary alcohols **5c,d**, which were converted into 1,3-dimethylpropanesultam **7c** and 1,2,3-trimethylpropanesultam **7d** in the same method as mentioned above.

Mono- and dimethylbutanesultams The synthesis of 1-methyl-, 1,2-dimethyl- and 1,4-dimethylbutanesultams **11a-c** is shown in Figure 5. Butanesultams **8a-c** were first protected as their methoxymethyl (MOM) derivatives and the resultant compounds **9a-c** were lithiated with n-BuLi, reacted with MeI at -25°C, and the 1-methylsultam derivatives **10a-c**, then deprotected with HCl under reflux, affording 1-methylbutanesultam **11a**, 1,2-dimethylbutanesultam **11b** and 1,4-dimethylbutanesultam **11c** in moderate yields.

Halomethylpropanesultams Firstly, we investigated the synthesis of 3-methoxycarbonylpropanesultam **15** as the key intermediate in the synthesis of 3-halomethylpropanesultams (Figure 6). As the precursor of **15**, we attempted the synthesis of amino acid derivative **14**. However, when we attempted the oxidative chlorination of thiol **13** with gaseous chlorine, explosive decomposition occurred. We thought it occurred as a result of the generation of N-chloro compound like chloramine, which derived from the insufficient deactivation of amino group on **13.** As this synthetic route to **15** was so dangerous, we investigated another route as shown in Figure 7. The aldehyde **17**, obtained by Michael addition of benzyl mercaptan to acrolein, was converted into hydroxy acid derivative **18** in four steps. Mesylation of hydroxy group, treatment with n-$Bu_4N^+Br^-$, oxidative chlorination of the benzylthio group and amination were subsequently carried out to yield bromo acid derivative **19**. Cyclization of **19** with K_2CO_3 afforded 3-methoxycarbonylpropanesultam **15**.

Conversion of the methoxycarbonyl group of **15** into the halomethyl group is shown in Figure 8. Thus, the 3-methoxycarbonyl derivative **15** was protected with a benzyloxymethyl group (BOM) in place of MOM to increase the lipophilicity, and then reacted with $LiAlH_4$ and mesylated. Compound **21** was reacted with n-$Bu_4N^+X^-$ (X= F, Cl, Br, I) to give the 3-halomethyl derivatives **22a-d**, which were deprotected with HCl to afford the 3-halomethylpropanesultams **23a-d**. Nucleophilic displacements with n-$Bu_4N^+Cl^-$ and n-$Bu_4N^+Br^-$ easily proceeded in 90% and 89% yields respectively, while the fluoride and iodide were formed in moderate yields.

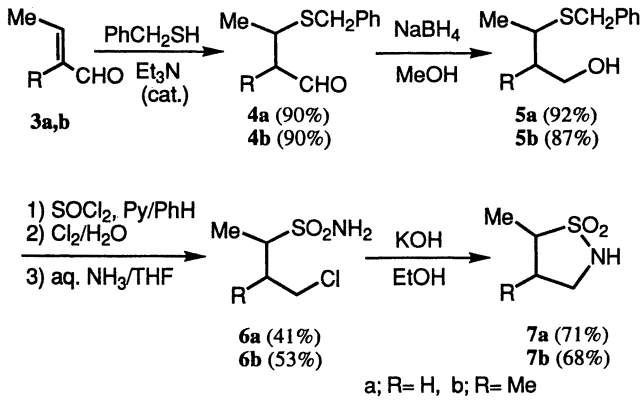


Figure 3. Synthesis of 1-Methyl- and 1,2-Dimethylpropanesultams 7a,b

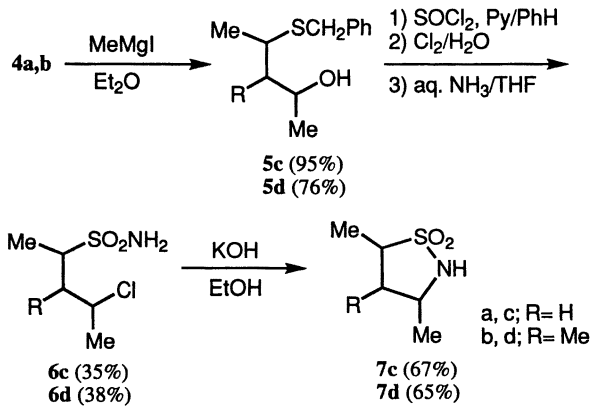
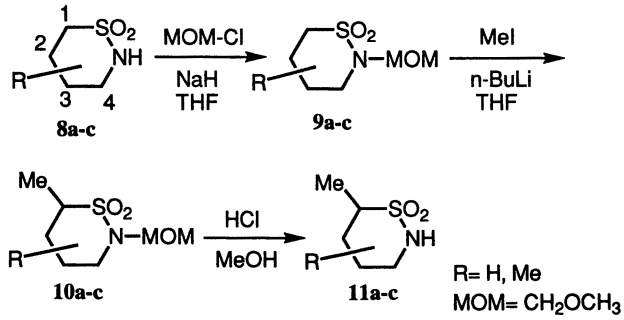


Figure 4. Synthesis of 1,3-Dimethyl- and 1,2,3-Trimethylpropanesultams 7c,d



R	Methylation		Deprotection	
	Yield (%) of **10a-c**		Yield (%) of **11a-c**	
H	**10a**	68	**11a**	80
2-Me	**10b**	56	**11b**	68
4-Me	**10c**	55	**11c**	63

Figure 5. Synthesis of 1-Methyl-, 1,2-Dimethyl- and 1,4-Dimethylbutanesultams 11a-c

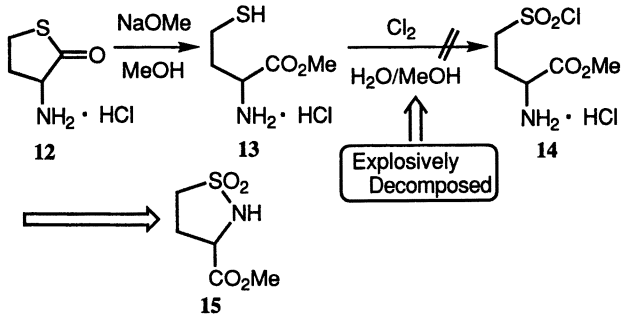


Figure 6. Attempted Synthetic Route to 3-Methoxycarbonylpropanesultam 15 with Amino Acid Derivative 14

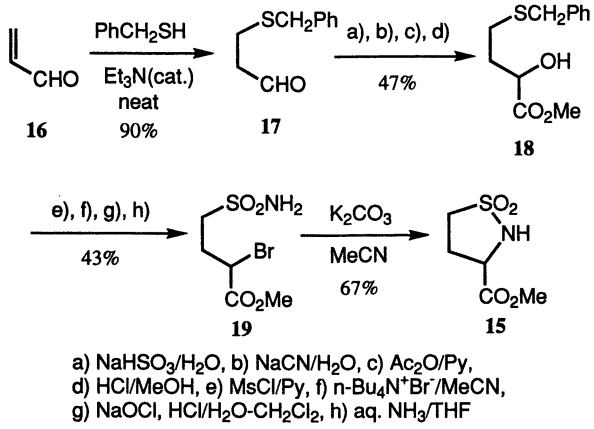
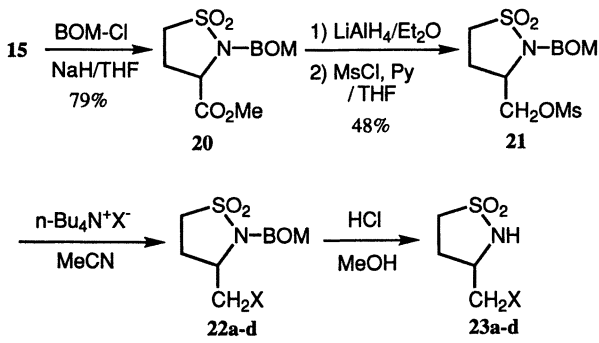


Figure 7. Synthesis of 3-Methoxycarbonylpropanesultam 15



X	Halogenation Yield (%) of **22a-d**		Deprotection Yield (%) of **23a-d**	
F	**22a**	40	**23a**	60
Cl	**22b**	90	**23b**	66
Br	**22c**	89	**23c**	75
I	**22d**	60	**23d**	76

Figure 8. Synthesis of 3-Halomethylpropanesultams 23a-d

Synthesis of 5-Phenylpyrazolines

5-Phenylpyrazolines **32a** are easily obtained by the reaction of benzylideneacetones **31a,b** with hydrazine hydrate. We thus studied several convienient methods for the synthesis of benzylideneacetones **31a,b** (Figure 9).

In Route 1, Wittig reaction of benzaldehydes **24** with phosphoranylidene-2-propanone was carried out to obtain β-unsubstituted **31a** in good yields. To introduce methyl group into β-position of **31a**, we used three methods, Routes 2, 3 and 4. That is, acylation of exo-methylene group on α-methylstyrene **25** with acetic anhydride afforded **31b** (**Z**= H) in 45 % yield (Route 2) (*9*). In Route 3, used when **Z** was an elerctron-withdrawing group (EWG), acetophenones **26** were condensed with acetone imine **27** to afford hydroxy imines **28**, which were then dehydrated and hydrolyzed to afford **31b** (*10*). On the other hand, when **Z** was an electron-donating group (EDG), Acetophenones **26** were converted by Reformatsky reaction into hydroxy esters **29**, which were then dehydrated and reacted with MeLi to afford **31b** (Route 4) (*11*).

Synthesis of Sultamsulfonamides

Three methods were utilized to synthesize sultamsulfonamides **1** (Figure 10). In Route 1, propane- and butanesultams **2** were converted into phenyl N-sultamsulfonylcarbamates **33** and then reacted with 5-phenylpyrazolines **32a,b** to obtain sultamsulfonamides **1**. In Route 2, sultamsulfonyl isocyanates **34** were reacted with **32a,b**. And in Route 3, propane- and butanesultams **2** were condensed with pyrazoline derivatives **35**, which derived from **32a,b** and chlorosulfonyl isocyanate.

Herbicidal Activity

We discuss some structure-activity relationship of sultamsulfonamides **1**. Post-emergence greenhouse activity at 160 g/ha for two sets of analogs is given in Tables I and II. Visual assessments were made at 14 days after application using a rating scale of 0 (no injury) to 9 (complete kill).

Table I shows the influence of the methyl group on propanesultam ring on herbicidal activity. 1-Methylpropanesultam derivatives **36,40** showed strong herbicidal activity against broad-leaved weeds (black nightshade, hairy galinsoga, Indian field cress) without phytotoxicity to wheat. When a methyl group was introduced into the 3-position on sultam ring, the phytotoxicity to wheat seriously increased (Compound **37,39**). And the introduction of a methyl group into 2-position conciderably reduced herbicidal activity (Compound **38**). Table I also shows that a methyl group in the 5-position of the pyrazoline ring (Compound **40**) did not influence on herbicidal activity against broad-leaved weeds.

Table II shows the influence of the substituent **Z** on the phenyl group of pyrazoline ring on herbicidal activity. The order of herbicidal activity derived from substitution position of **Z** was :

$$o\text{-position} = m\text{-position} >> p\text{-position}$$

When *m*-position of phenyl group was substituted with a chlorine atom (EWG), or methoxy group (EDG), the herbicidal activity slightly decreased, as compared with unsubstituted compound.

The sultamsulfonylureas, which were bonded to 2-amino group of 4,6-dimethoxypyrimidine or 4-methoxy-6-methyltriazine in place of 5-phenylpyrazolines **32a,b**, killed both broad-leaved weeds and gramineous weeds, and its phytotoxicity to wheat was unacceptable (*12-13*). Sultamsulfonamides **1** on the other hand exhibited selectivity for gramineous weeds and showed no phytotoxicity to wheat.

In Synthesis and Chemistry of Agrochemicals IV; Baker, D., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1995.

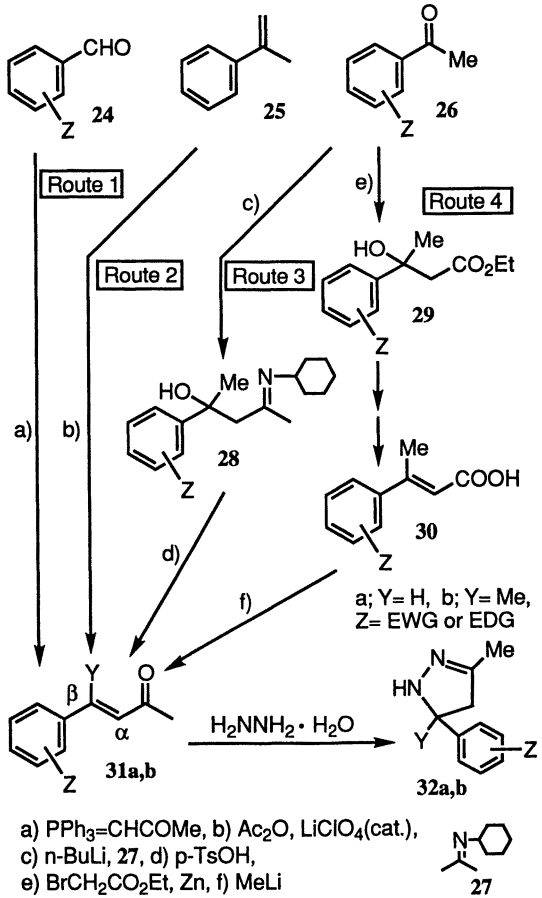


Figure 9. Synthesis of 5-Phenylpyrazolines 32a,b

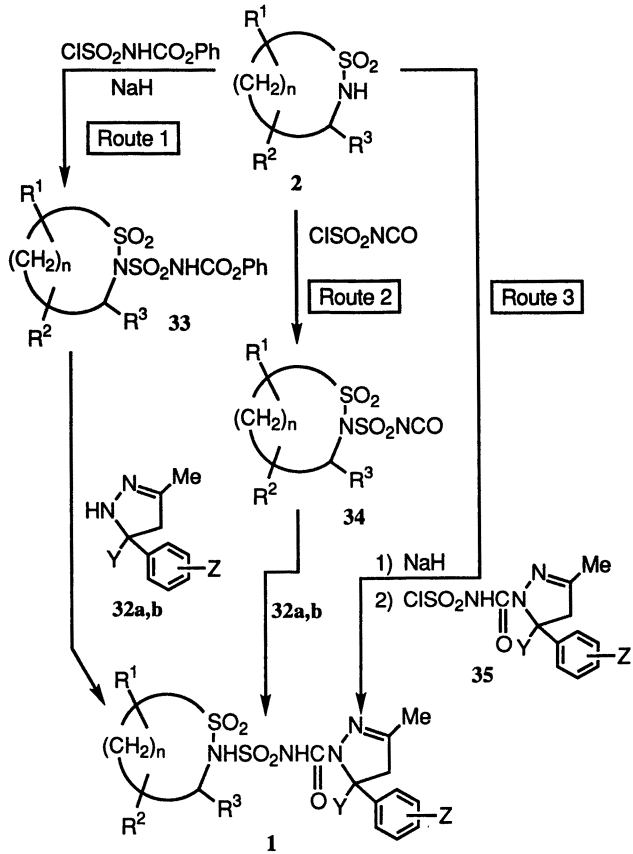
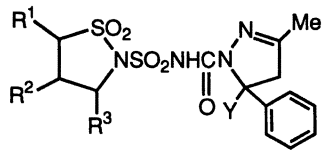


Figure 10. Synthesis of Sultamsulfonamides 1

Table I. Influence of the Methyl Group on Herbicidal Activity

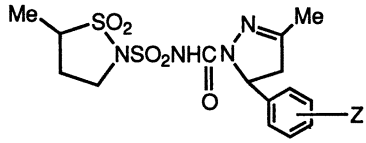


Postemergence Rating at 160 g/ha (Greenhouse, 14DAT)

Compound	R^1	R^2	R^3	Y	SOLNI	GASCI	RORAT	TRZAX
36	Me	H	H	H	9	9	9	0
37	H	H	Me	H	9	9	7	5
38	Me	Me	H	H	4	4	9	0
39	Me	H	Me	H	9	9	6	2
40	Me	H	H	Me	9	9	9	0

0= No Injury, 9= Completely Killed
SOLNI: black nightshade, GASCI: hairy galinsoga
RORAT: Indian field cress, TRZAX: wheat

Table II. Influence of the Substituent Z on Herbicidal Activity



Postemergence Rating at 160 g/ha (Greenhouse, 14DAT)

Compound	Z	SOLNI	GASCI	RORAT	TRZAX
41	*m*-H	9	9	9	0
42	*m*-Cl	8	8	9	0
43	*m*-OMe	7	9	8	0

0= No Injury, 9= Completely Killed

And as concerns the 3-halomethylpropanesultam derivatives, they showed very weak herbicidal activity.

Conclusion

Synthesis of the regioselectively methylated propanesultams and butanesultams was achieved, using Michael addition of benzylmercaptan to α,β-unsaturated aldehydes and the methylation of 1-position on butanesultams *via* lithiation as the key steps, respectively. We also applied the Michael adducts to synthesize 3-methoxycarbonyl-propanesultam, and which was converted into 3-halomethylpropanesultam *via* mild halogenation with n-$Bu_4N^+X^-$. These propanesultams and butanesultams have been converted into sultamsulfonamides **1** . And some of them showed strong herbicidal activity against broad-leaved weeds without phytotoxicity to wheat, furthermore, **1** showed *in vitro* inhibitory activity against ALS.

Acknowledgment

We wish to express our thanks to Dr. Mamoru Hayashi, General Manager of Central Research Institute, Nissan Chemical Industries Ltd., Dr. Gozyo Sakata, Manager of New Business Planning Dept., Nissan Chemical Industries Ltd., and Dr. Kazutaka Arai, General Manager of Agricultural Chemicals Research Dept., Central Research Institute, Nissan Chemical Industries Ltd., for their continuing guidance and encouragement.

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Chapter 4

Herbicidal Sulfonylamides

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A large number of disparte chemical classes have been shown to inhibit the enzyme acetolactate synthase. The herbicidal activity resulting from the inhibition of this enzyme has resulted in the introduction of novel compounds with low use rates and excellent toxicological and environmental profiles. The design and synthesis of a series of sulfonylamides that inhibit this enzyme are described. Structure activity and herbicidal properties of these compounds are discussed.

The low use rates and excellent toxicological and environmental properties of herbicides that act by the inhibition of acetolactate synthase (ALS), such as the sulfonylureas of DuPont and the imidazolinones of American Cyanamid, led numerous companies to conduct synthetic efforts on this mode-of-action. The more recent introductions of flumetsulam by Dow-Elanco and pyrithiobac by Kumiai are prime examples of this follow up. At the outset of this project, only the sulfonylureas and the imidazolinones were known. The literature at the time suggested that these compounds inhibited binding of the second molecule of either pyruvate or α-ketobutyrate to the enzyme *(1)*.

Studying the mechanism of the enzyme-catalyzed reaction, we hypothesized that the imidazolinones were reaction intermediate analogs resembling the adduct between hydroxyethylthiamine and a second molecule of pyruvate or α-ketobutyrate. We postulated that some of the imidazolinone functionality might mimic the highly branched carbon chain of the adduct. Since one essential aspect of the herbicides appeared to be an ionizable functional group that could mimic a carboxylate, the diol **1**, in which the carboxylate has been replaced by a sulfonyl amide, was chosen as a target compound (Figure 1). Whilst this biochemical hypothesis is now known to be incorrect *(2)*, it nevertheless led to a fruitful avenue of research.

Synthesis and SAR

The preparation of **1** was straightforward (Figure 2), but the target compound was completely devoid of activity. However, its precursor **2** was weakly active both in the greenhouse, displaying typical ALS symptomology at high rates, and on the enzyme (I_{50} = 60μM), and this encouraged us to pursue this chemistry. Analogs having free

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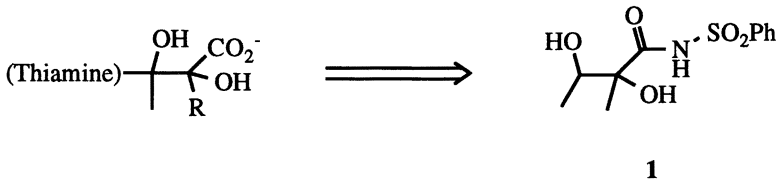
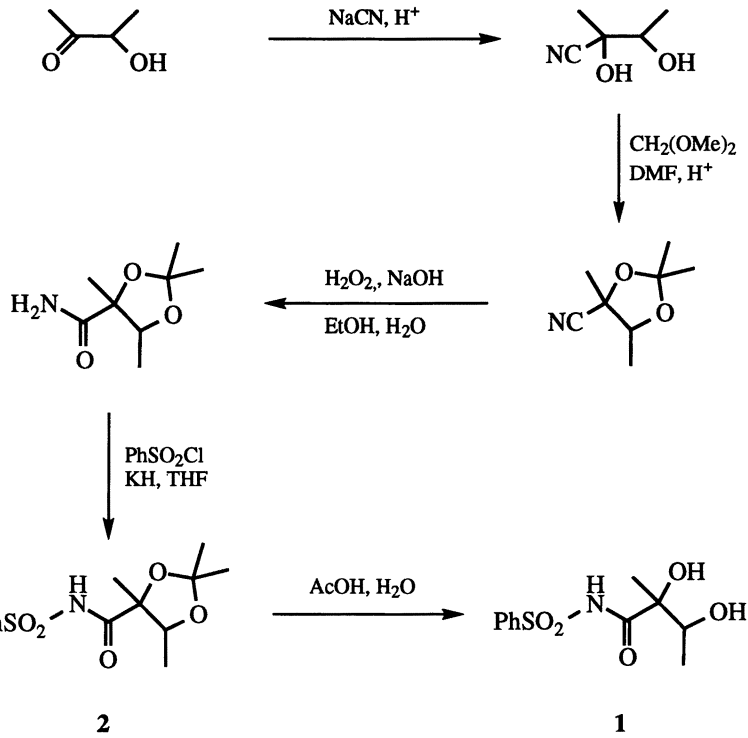


1

Figure 1

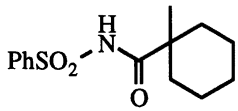


2 **1**

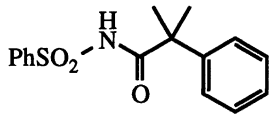
Figure 2

hydroxyl groups as illustrated by **1** and less substituted patterns as shown in Figure 3 were inactive; however, the substituted carbocycle **3** was active. The gem-dimethylphenylacetic acid derivative **4** was still more active showing an I_{50} of 1μM in our enzyme assay. Chlorsulfuron, which was chosen as a standard for this work, gave an I_{50} of 10nM. Based on the excellent intrinsic activity seen for **4** in the *in vitro* assay, it was decided to investigate the structure-activity relationships of its analogs.

Active

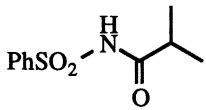


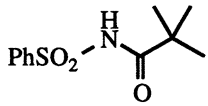
3

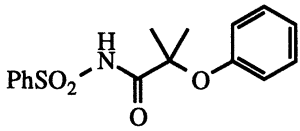


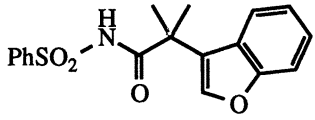
4

Inactive









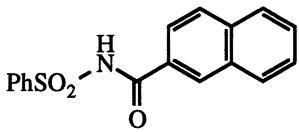
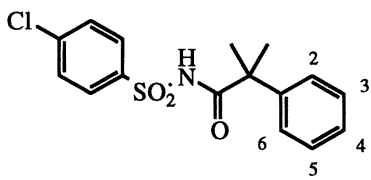


Figure 3

The general synthetic strategy for all of these compounds was to prepare the amides by hydrolysis of the appropriate nitrile and react them in the presence of excess base with a sulfonyl chloride. KH consistently gave better yields than NaH and was used routinely as the base. It was initially found that, in contrast to the sulfonylureas, the sulfonylamides containing a p-chlorosulfonyl moiety gave superior binding activity in the enzyme assay together with the highest greenhouse activity. Therefore we concentrated our studies on structure activity relationships for the aromatic ring and the bridging carbon of the carboxamide moiety.

With the phenylacetamide derivatives it was rapidly found that the presence of an ortho-halo substitutent significantly improved activity (compound **5**, Table I). The dihalo substitution pattern gave the best activity for this particular structure type with both the 2,4-dichloro **6** and 2,4-difluoro **9** patterns producing compounds equal in activity to chlorsulfuron in the enzyme assay. Of particular interest was the deleterious effect of the 2,6-disubstitution pattern. Comparing the values for the 2,4-, 2,5- and 2,6-dihalo compounds **6-11** indicates that one side of the phenyl ring is severely compressed. Even substitution of hydrogen by fluorine at positions 5 or 6 lowers activity significantly, with the greatest effect obvious at the 6-position.

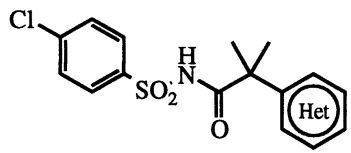
Table I. Effect of phenyl substitution pattern on enzyme activity



Cmpd	Substituent Pattern	I_{50}(nM)
	H	250
5	2-Cl	12
	2-OMe	350
	4-F	90
	4-Cl	200
	4-NO_2	360
	4-CF_3	1,600
6	2,4-diCl	16
7	2,5-diCl	920
8	2,6-diCl	30,000
9	2,4-diF	10
	2,3-diF	93
10	2,5-diF	130
11	2,6-diF	1,000
	3,4-diF	500
	chlorsulfuron	10

Heterocyclic acetamides were also explored, especially in the light of the pyrimidine and triazine systems present in the sulfonylureas and the pyridine and quinoline systems in the imidazolinones. Only the 2- and 3-pyridylacetamides showed activity comparable to the phenyl system, with the 2-pyridylacetamide **12** showing the best greenhouse activity (Table II).

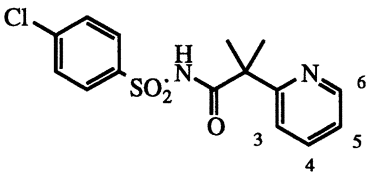
Table II. Effect of heterocyclic substitution



Cmpd	Substituent Pattern	I_{50}(nM)
12	2-pyridyl	300
13	3-pyridyl	2000
	4-pyridyl	9,000
	2-pyrimidinyl	24,000

Substitution of the 4-pyridyl and 2-pyrimidinyl derivatives failed to improve activity. Investigation of the allowed substitution pattern for the 2- and 3-pyridyl systems showed great similarities to the phenyl series. Thus for the 2-pyridyl system, introduction of a 5-chloro substitutent marginally increased activity, whereas the 3-chloro compound **14** was essentially devoid of activity suggesting that the pyridine nitrogen atom occupies the same site as the halogen in the 2-position of the phenyl series (Table III).

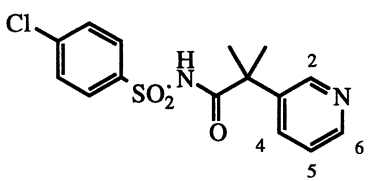
Table III. Substituted 2-pyridyl derivatives



Cmpd	Substituent Pattern	I_{50}(nM)
12	-	300
	5-Cl	100
	6-OMe	170
	6-F	750
	5-CF_3	2,500
	3-Cl, 5-CF_3	inactive
14	3-Cl	inactive

For the 3-pyridyl series, the 2-chloro compound **15** (Table IV) proved to be the most effective compound in our greenhouse tests even though it was less active on the enzyme than the corresponding phenyl derivative **5** (Table I).

Table IV. Substituted 3-pyridyl derivatives

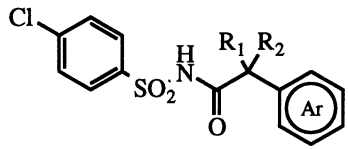


Cmpd	Substituent Pattern	I_{50}(nM)
13	-	200
15	2-Cl	50
	2,6-diCl	90
	2-Br	75
	2-F	100
	2-OMe	160
	2-Me*	130

* exists as a zwitterion

Investigation of the bridge substituent pattern revealed that only compounds containing the gem-dimethyl or cyclopropyl groups could be tolerated well by the enzyme. In the phenyl series, analogs with the gem-dimethyl and cyclopropyl substitution patterns were equal in intrinsic activity. The chloro/methyl bridge lowered activity 120-fold whilst the mono-methyl compound was inactive (Table V). In the 2-pyridyl system a number of alkyl substituents were prepared as shown in Figure 4. Increasing steric bulk with the methyl/isopropyl functionality, so prevalent in the American Cyanamid imidazolinones, severely reduced activity. Only the methyl/ethyl or methyl/monofluoromethyl compounds gave reasonable activity. Whilst the cyclopropyl derivatives showed *in vitro* activity comparable to the gem-dimethyl analogs, their greenhouse activity was much less.

Table V. Alkyl substitution pattern



Cmpd	R^1	R^2	Ar	I_{50} (nM)
	Me	Me	Phenyl	250
	- CH_2CH_2-		Phenyl	350
	Me	Cl	Phenyl	3,000
	Me	H	Phenyl	inactive
12	Me	Me	2-Pyridyl	300
	Me	Et	2-Pyridyl	400
	Me	CH_2F	2-Pyridyl	600
	Me	CHF_2	2-Pyridyl	8,000
	Me	i-Pr	2-Pyridyl	2,000
	Me	n-Pr	2-Pyridyl	1,500

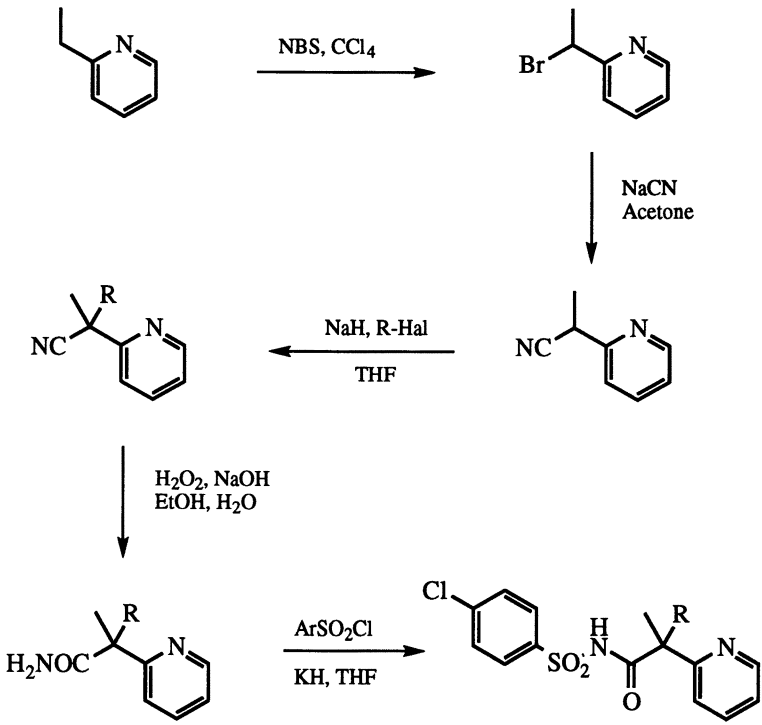
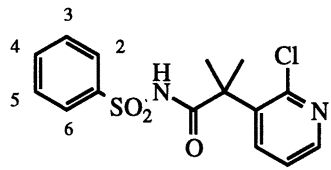


Figure 4

The phenylsulfonyl substitution pattern was most thoroughly investigated for the 2-chloro-3-pyridyl system (Table VI). In contrast to the sulfonyl ureas, where substitution in the ortho-position improved activity and substitution in the para-position decreased activity, the opposite was found for this series(*3*). Thus only the ortho-fluoro compound **16** showed improved activity over the unsubstituted analog and the ortho-methoxycarbonyl derivative **17** was very weak. The para-position could tolerate various electron withdrawing groups. 2,4-Dihalosubstituted compounds such as **18** and **19** gave the best intrinsic activity; however, their greenhouse activity was no better than that for compound **15.**

Table VI. Effect of phenylsulfonyl substituent pattern on activity



Cmpd	Substituent Pattern	I_{50}(nM)
	-	160
	2-Cl	160
16	2-F	40
	2-Br	200
17	2-CO_2Me	2,000
	3-Cl	900
15	4-Cl	50
	4-F	200
	4-Br	70
	4-I	60
	4-OMe	800
	4-NO_2	40
	4-CF_3	80
	4-Me	100
	4-Et	600
	4-iPr	1,500
	4-CO_2Me	inactive
	2,4-diCl	40
18	2,4-diF	20
19	2-F,4-Br	30
	2-Cl,4-Br	80
	3,4-diCl	30
	3-CF_3	700

Structure activity trends for this series are summarized in Figure 5. Thus for the phenylsulfonyl group, electron withdrawing groups at the 4-position were best. For the bridging carbon of the carboxamide moiety, only cyclopropyl and gem-dimethyl substitution patterns gave good activity, while for the aromatic ring, the 2-position had a strong electronic dependence with chloro-substitution optimal. Positions 3 and 4 were most variable, but positions 5 and 6 were sterically crowded and substitution invariably decreased activity.

After this work was completed, Alverado *et al* published their work on some related sulfonylamides that were also shown to inhibit ALS (*4*). This is illustrated by the general structure shown in Figure 6. In contrast to the sulfonylamides described above, the most active compounds contained the methyl/isopropyl functionality on the bridging carbon and acetyl was the prefered substitution on the nitrogen atom.

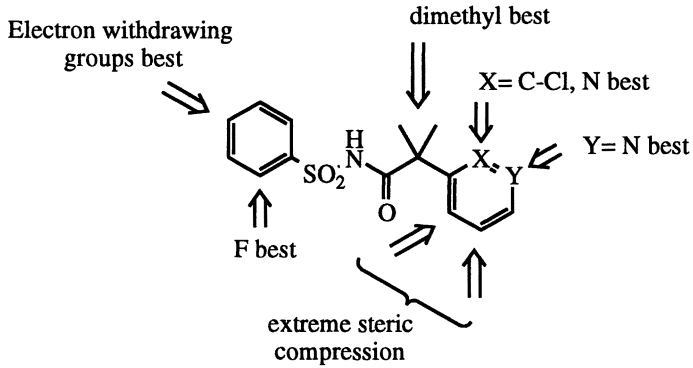


Figure 5

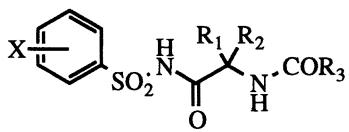


Figure 6

Herbicidal Activity

These compounds were most effective as postemergent broadleaf herbicides. However, the high intrinsic activity observed for these compounds was not carried over to the greenhouse as illustrated in Table VII. In some cases, preparation of an amine salt slightly improved activity over the parent acid, but the low use rates expected for compounds of this activity level did not materialize. Investigation of the physical properties of these compounds did not give any clues to their poor greenhouse activity. Thus compound **15** showed a similar logP to chlorosulfuron. As seen from the table, these compounds were quite weak against pigweed and sicklepod. This, together with their relatively high use rates, prevented further development of this series of compounds.

Table VII. Herbicidal activity

Cmpd	Rate Kg/ha	Postemergent Control (%) MG	CB	VL	PW	SP	WH	CN
6	0.3	80	80	90	0	0	0	0
6	0.1	30	40	70	0	0	0	0
9*	0.3	90	80	98	20	0	20	50
9*	0.1	80	80	98	20	0	20	20
12	0.3	98	98	98	45	50	0	30
12	0.1	98	60	95	40	20	0	20
12	0.3	40	50	98	30	0	0	0
12*	0.3	98	85	98	30	30	0	40
12*	0.1	95	80	98	20	20	0	20
12*	0.03	95	80	98	0	0	0	0
15	0.3	99	99	99	90	90	55	50
15	0.1	99	99	99	85	85	30	30
15	0.03	95	50	99	60	60	20	10
15*	0.3	99	98	99	95	95	60	80
15*	0.1	99	98	99	90	90	20	40
15*	0.03	95	98	90	85	80	0	20

*Isopropylamine salt

MG =morning glory, CB =cocklebur, VL =velvetleaf, PW =pigweed,SP =sicklepod, WH =wheat, CN =corn

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Chapter 5

Synthesis of Imidazolinone Herbicides with Reduced Soil Persistence

Derivatives of 5-Formyl-2-imidazolin-2-yl-nicotinic Acids

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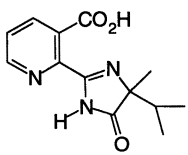
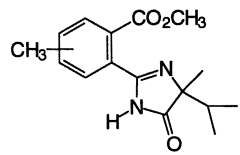
The discovery of highly active herbicides that have reduced soil persistence is of increasing importance. Our approach to this goal began by identifying a surprisingly inactive imidazolinone, 5-formyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-nicotinic acid. Acetal and oxime derivatives of this compound displayed strong herbicidal activity. These herbicides were shown to have significantly reduced soil persistence when compared to other imidazolinone herbicides. Metabolism of the acetal functionality was shown to be the mechanism responsible for the reduced soil persistence.

The evolution of a herbicide series depends on the discovery of new series members possessing different and beneficial biological properties. At American Cyanamid, significant attention has been spent on developing the imidazolinone herbicide series(*1-3*). This series includes the total vegetation control herbicide imazapyr, a wild oat herbicide imazamethabenz-methyl and two herbicides used for weed control in soybean/legumes imazethapyr and imazaquin. These herbicides have gained widespread acceptance in the marketplace. Following this success, efforts were undertaken to find novel members of this series with different biological properties. The properties desired were selectivity in crops other than soybeans / legumes and reduced soil persistence.



imazapyr



imazamethabenz-methyl

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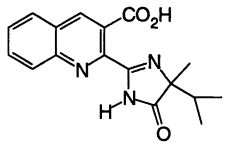
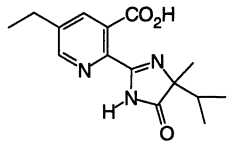


imazaquin



imazethapyr

To achieve this goal, we sought to identify appropriate functional groups to act as metabolic handles. This was accomplished through the use of a concept we call "retro-metabolic analysis" (Figure 1). The first step in this process is the discovery of an imidazolinone, containing a functional group (Z) that has weak (or no) herbicidal activity. Chemical derivatization of Z can potentially yield herbicidal compounds, incorporating a new functional group (Y-Z). The new functional group can act as a metabolic handle by the reverse chemical process (Y-Z to Z). Crop selectivity is possible if this process occurs in the desired crops. In soil, this process will result in herbicides with reduced soil persistence. Because of the abundance of hydrolytic pathways, we focused on Y and Z groups related by hydration-dehydration processes.

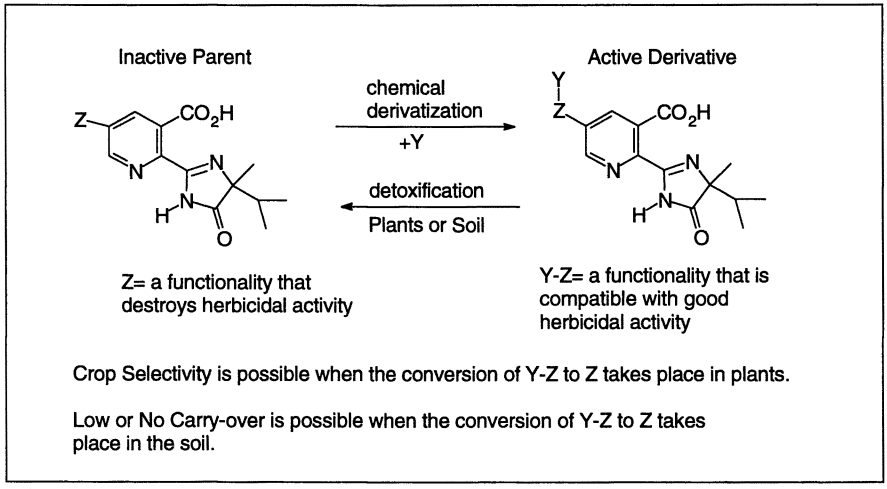


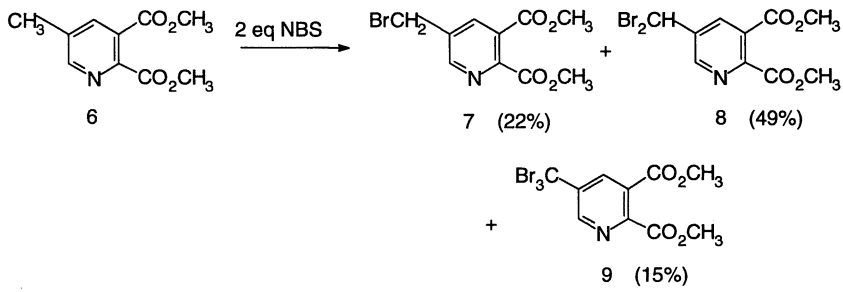
Figure 1: Rationale for Discovering Imidazolinones with Novel Metabolic Handles

While preparing a series of 5-substituted pyridine imidazolinones, we found a set of compounds that fit the above rationale. Aldehyde **1** had no preemergence herbicidal activity at the highest rate tested (1/2 kg/ha), even on sugar beets, a species normally very sensitive to imidazolinones. We were surprised by this result because a close relative to this compound, ketone **3**, displayed moderate herbicidal activity. In stark contrast, an acetal derivative **2** displayed excellent herbicidal activity both pre- and postemergence. This high level of herbicidal activity was also unexpected because a related compound **4** is only a moderate herbicide.

Synthesis

Synthesis of Acetal Derivatives:

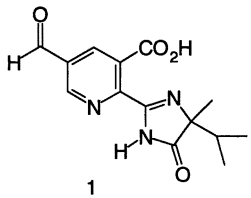
Acetal derivatives of **1** can be synthesized by several routes with the acetal functionality introduced at either the pyridine diester or the imidazolinylnicotinic acid stage. Beginning with pyridine diester **6** (*4*), NBS oxidation afforded a mixture of mono, di and tribromomethyl products (**7**, **8**, **9**). These were readily separated by liquid chromatography and the monobrominated product retreated with NBS to provide more of the desired dibromomethyl product **8**.



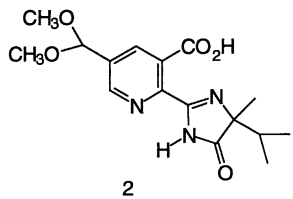
6 7 (22%) 8 (49%) 9 (15%)

The 5-dibromomethylpyridine **8** is a versatile intermediate. The silver assisted solvolysis of **8** in methanol proceeded very cleanly to afford dimethyl acetal **10** in high yield. This solvolysis reaction proved to be very general and was used to prepare cyclic acetal **11** and aldehyde **12**. This reaction can be extended to the preparation of aminals **13** and diaziridines **14**, **15**, although these yields are lower.

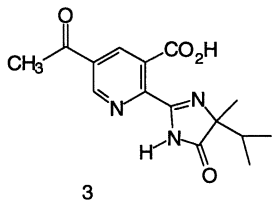
Table I contains the herbicidal data and the *in vitro* AHAS enzyme screen data for these imidazolinones. The mode of action of the imidazolinone herbicides is inhibition of the enzyme acetohydroxyacid synthase (AHAS) and data from the *in vitro* AHAS enzyme screen can sometimes explain herbicidal activity. The inhibition data is listed as a relative inhibition concentration (RIC), which is the ratio of the activity of the test compound relative to the standard **5**. Of the four compounds, the weak *in vitro* inhibition shown by the ketal **4** compound explains why that compound has only modest activity. The modest activity of the ketone **3** is not surprising because other imidazolinones with electron withdrawing groups in this position had shown similar levels of activity. What is surprising are the dramatic differences between the very active acetal **2** and the inactive aldehyde **1**. These differences are not due to differences at the enzymatic level because both the aldehyde and acetal are good AHAS inhibitors. Knowing this, we speculated that the aldehyde functionality might be acting as a metabolic handle. Upon this discovery, we initiated a program to prepare other aldehyde derivatives with the hope of finding imidazolinones with novel biological activity.



1



2



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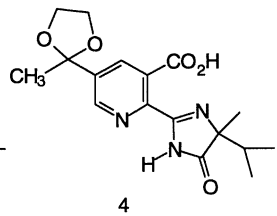
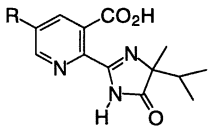
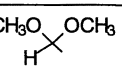
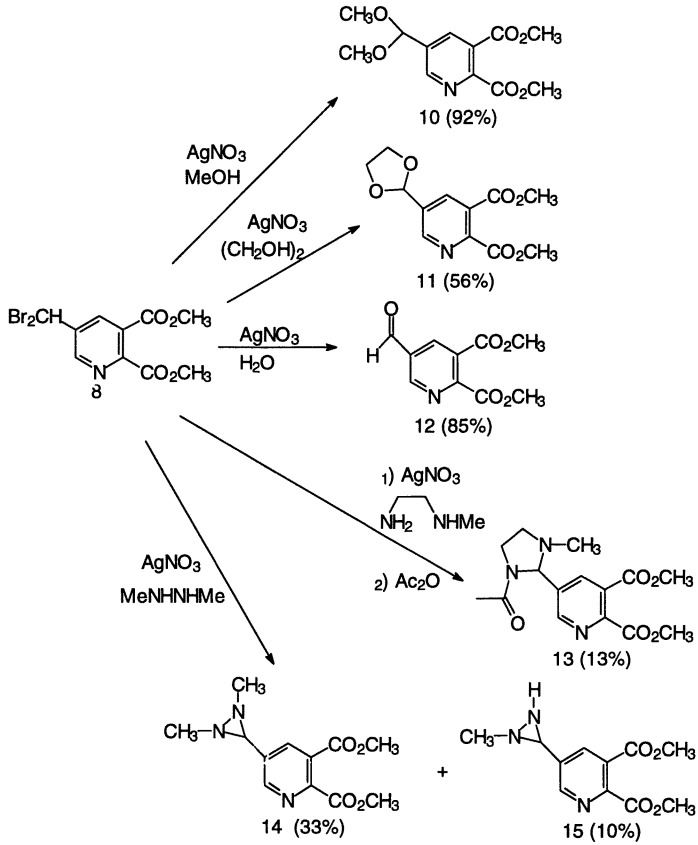


4

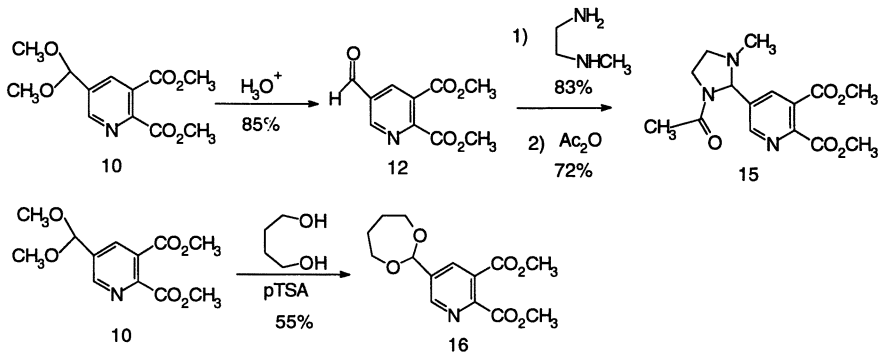
Table I: Biological Activity of Imidazolinones **1-4**



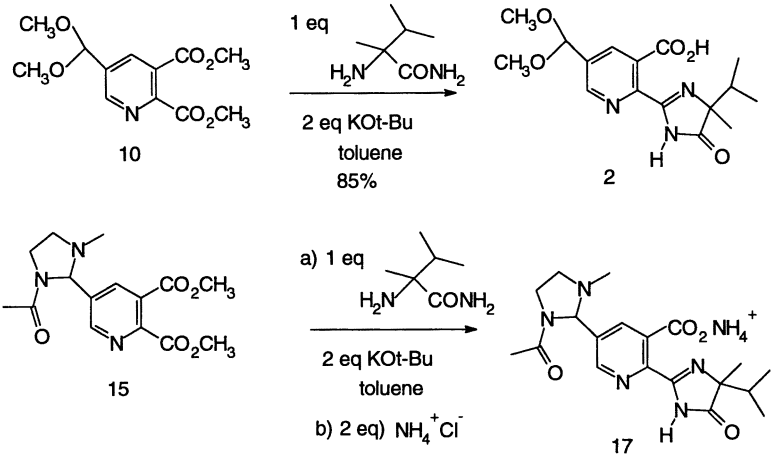
compound #	R	PRE control rate kg/ha	POST control rate kg/ha	AHAS RIC
1		**Inactive**	weak at 0.500	2.0
2		0.063	0.032	2.8
3		0.250	0.500	0.5
4		moderate at 0.500	0.500	14.7
5	H			1.0



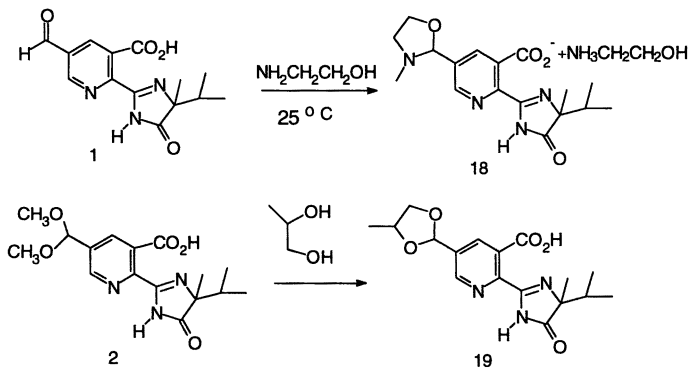
An alternate route for preparing a variety of acetals utilized hydrolysis of **10** to aldehyde **12** followed by a standard condensation reaction (*5*) with diol to form a cyclic acetal. Alternatively, an acetal exchange reaction using dimethylacetal **11** provided the desired acetal product **16** in modest yield. A transesterification side reaction with the diol was responsible for the modest yield in this reaction.



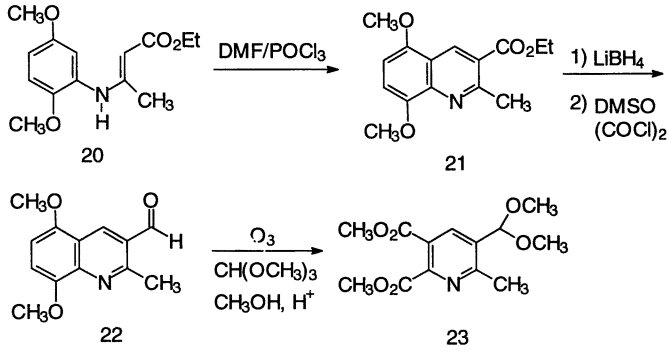
The acetal diesters were readily converted to their corresponding imidazolinone acids by treatment in toluene with one equivalent of the aminoamide and two equivalents of potassium *tert*-butoxide (*6*). This reaction proved to be very general in the acetal series and generally proceeded in very high yield. Even very acid labile derivatives such as this aminal **17** can be prepared by using a modification which uses two equivalents of ammonium chloride to neutralize the base during the workup.



The acetal functionality can also be prepared at the imidazolinyl-nicotinic acid stage using either aldehyde **1** or acetal **2**. This route is preferred for very labile acetals such as the N,O acetal **18**.

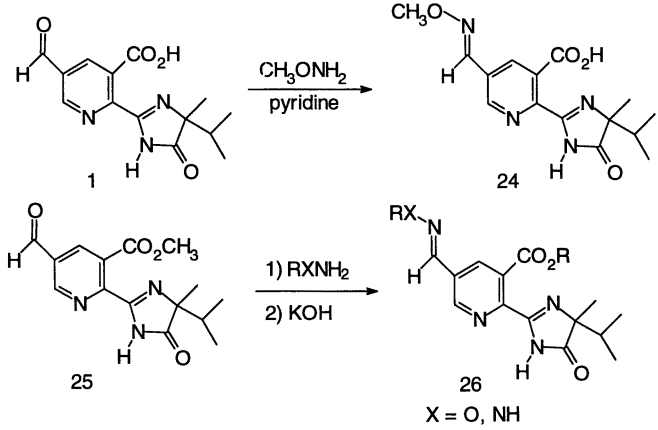


The preparation of 6-substituted derivatives required a synthetic route to 6-substituted-5-formyl-pyridine diesters. Treatment of enamine **20**, formed from dimethoxyaniline and ethyl acetoacetate, with Vilsmeier's reagent afforded quinoline **21** (*7*). The ester was reduced to an alcohol and Swern oxidation provided the aldehyde **22**. The quinoline aldehyde **22** was oxidized with ozone using methanol and trimethyl orthoformate as solvent (*8*) to yield the desired pyridine diester **23**.



Synthesis of Oxime Derivatives

A second set of aldehyde derivatives examined were oximes and hydrazones. These compounds were readily prepared by treatment of a 5-formylpyridine with an alkoxyamine or hydrazine (*9*). This reaction worked well using either a 5-formyl nicotinic acid **1** or nicotinic ester **25**.



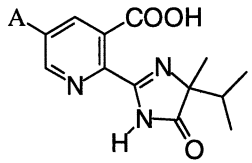
Biological Activity

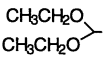
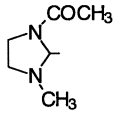
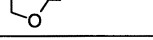
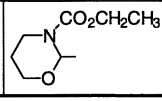
Herbicidal and enzymatic ratings for a variety of acetal derivatives are compiled in Table II. The control rate given in this table is the lowest rate required to kill seven of the ten weeds in the herbicide screen. When control of 7 of 10 weeds was not achieved at the highest rate tested, the terms moderate, moderate to weak, weak and inactive are used to indicate the level of activity. As in Table I, enzymatic data is given in relative inhibition concentration (RIC) values using compound **5** as the reference.

The most herbicidally active members are the sterically small O,O acetals. These include: the dimethyl acetal, the cyclic dioxolane , the dioxane and the

dioxepane. Larger acetals, such as the diethyl acetal and the 5,5-dimethyl-1,3-dioxane are several rates less active. Comparison of the *in vitro* AHAS data explains this result: the larger acetals are significantly weaker enzyme inhibitors. This result is consistent with the weak herbicidal and *in vitro* activity of ketal **4** (Table I). Replacing one or both of the acetal oxygens with sulfur or nitrogen reduces herbicidal activity. Of these derivatives, the oxathiolane, **34,** is the most interesting, showing good broad spectrum activity postemergence at 0.125 kg/ha.

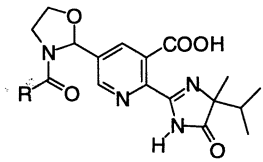
TableII: Biological Activity of Acetal Derivatives



#	A	PRE	POST	AHAS (RIC)	#	A	PRE	POST	AHAS (RIC)
2		0.063	0.032	2.8	34		0.500	0.125	0.6
27		0.500	0.250	15.9	35		moderate at 0.500	0.500	0.01
28		0.063	0.063	0.9	17		inactive at 0.500	very weak at 1.0	25.5
29		0.063	0.063	1.8	36		inactive at 0.500	weak at 1.00	1.6
30		0.063	0.063	1.0	37		inactive at 0.500	moderate at 1.00	2.0
19		0.250	0.250	1.6	38		moderate at 0.500	0.250	1.1
31		moderate at 0.500	moderate at 1.000	31.9	39		moderate at 0.500	1.00	1.3
32		inactive at 0.500	moderate at 1.00	2.0	40		moderate to weak at 0.500	moderate at 1.00	15.6
33		very weak at 0.500	moderate to weak at 0.500	1.6					

The N-acyl-oxazolidines series of compounds display a range of herbicidal activities, dependent on the acyl group. These differences are related to hydrolytic stability. Table III lists the half life at pH 2, the *in vivo* and *in vitro* activity for three N-acyl-oxazolidines. The carbamate, the most hydrolytically stable compound, is significantly more active postemergence than the other analogs. The least active compound, the amide, is also the least stable to acid hydrolysis. As a comparison, the dimethyl acetal, **2**, has a solution half life of 2 days at pH 2.

Table III: Half Life of Oxaxolidine Derivatives at pH 2.0

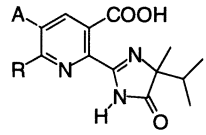


#	R	$T_{1/2}$	POST control rate	AHAS RIC
36	CH_3	5 min	weak at 1.00	1.6
37	$(CH_3)_2N$	2 hour	moderate at 1.00	2.0
38	CH_3CH_2O	stable for 4 days	0.25	1.1

Table IV summarizes the activity of acetals bearing a substituent in the six position of the pyridine ring. As a group, these compounds showed reduced herbicidal activity compared to 6-unsubstituted compounds. For example, the 6-methoxy-5-dioxolane-pyridine, **42**, is a fairly weak herbicide. The 6-methyl-analogs, however, displayed good herbicidal activity postemergence. Interestingly, although 6-methyl-5-dimethoxymethylpyridine, **41**, and 6-methyl-5-dioxolane, **43**, displayed good broad spectrum activity postemergence at 0.125 kg/ha, they were almost inactive at 0.125 kg/ha preemergence. The difference in activity preemergence versus postemergence suggests that these compounds are more rapidly degraded in the soil than 6-unsubstituted-pyridines.

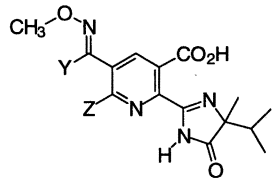
Of the different imino series we examined, the oxime series was the most active. As was expected from the SAR of the acetal series, substituent size has a tremendous effect on herbicidal activity. The oximes derived from the aldehyde were significantly more active than those derived from the 4-acetylpyridine. The data in Table V shows the dramatic difference in herbicidal activity among a series of oximes. The differences in the herbicidal activity correspond directly to the level of enzyme inhibition.

Table IV: Biological Activity of 6-Substituted Acetal Derivatives



#	A	R	PRE	POST	AHAS RIC
28		H	0.063	0.063	0.9
41		**CH_3**	moderate at 0.500	0.125	1.1
42		**CH_3O**	moderate at 0.500	moderate at 0.500	6.0
43		**CH_3**	0.500	0.125	0.7
44		**CH_3CH_2**	moderate at 0.500	1.00	-
45		**CH_3**	0.500	0.250	15.5

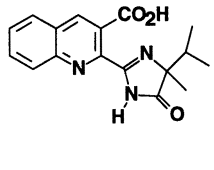
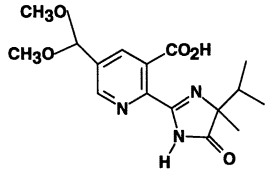
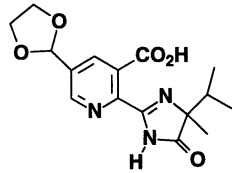
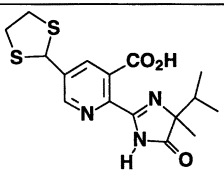
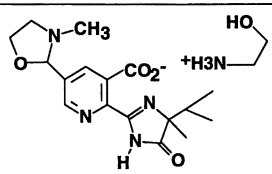
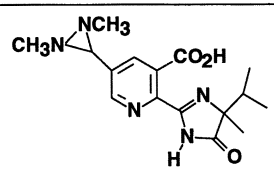
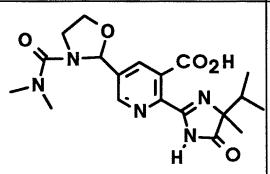
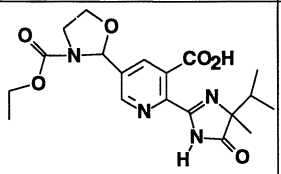
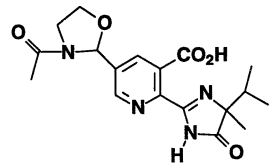
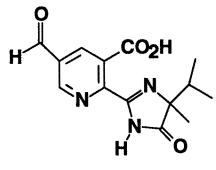
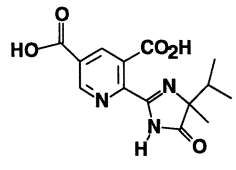
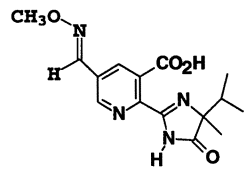
Table V: Biological Activity of Selected Oxime Derivatives



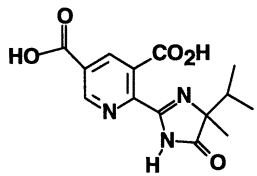
#	Y	Z	PRE	POST	AHAS RIC
24	H	H	0.032	0.024	0.5
46	H	CH_3	0.250	0.125	not tested
47	CH_3	H	0.125	0.250	2.4
48	CH_3	CH_3	moderate at 0.500	1.00	21.4

Several members of the acetal series were tested in an *in vitro* screen to measured herbicide dissipation in the soil. In this assay, a compound was applied to soil and the sample kept at temperatures and soil moistures that simulate field conditions. At intervals, portions of the soil sample were extracted with water and analyzed by HPLC. The results are summarized in Table VI by half life values, which are an indicator of herbicide bioavailablity

Table VI: Results of the *in vitro* Herbicide Dissipation Screen

 **imazaquin** $T_{1/2}$ = >60 days	 **2** $T_{1/2}$ =32 days	 **28** $T_{1/2}$ =14 days
 **33** $T_{1/2}$ = 2 days	 **18** $T_{1/2}$ = << 1 day	 **35** $T_{1/2}$ = << 1 day
 **37** $T_{1/2}$ = 20 days	 **38** $T_{1/2}$ = >> 60 days	 **36** $T_{1/2}$ => 60 days
 **1** $T_{1/2}$ = << 1 day	 **49** $T_{1/2}$ = >> 60 days	 **24** $T_{1/2}$ = 25 days

over time. Most compounds in the acetal series undergo fairly rapid dissipation. The very rapid dissipation of the thioketal compound confirms the expectation that compounds with major differences between their pre- and postemergence activities are rapidly degraded in the soil. In addition to providing half life values, the dissipation screen indicated that the major soil metabolite for every acetal and the oxime included on this study was the 3,5-pyridine dicarboxcylic acid **49**. The 5-formyl imidazolinone **1** was also found to rapidly degrade to this diacid. This indicates that an oxidative metabolism is the primary method for herbicide detoxification in the soil.



49

The results from the soil dissipation screen were confirmed by field studies. Figure 2 shows the results of an experiment where sunflowers were planted back into a field treated with imidazolinone herbicides. The imidazolinones in this test were **2** (the dimethylacetal), imazaquin and imazethapyr. Sunflowers are sensitive to all three herbicides, as shown by the high herbicidal injury observed immediately after treatment. The quick drop-off in injury ratings for plant-backs into the plot treated with **2** demonstrate that this herbicide is rapidly degraded under field conditions.

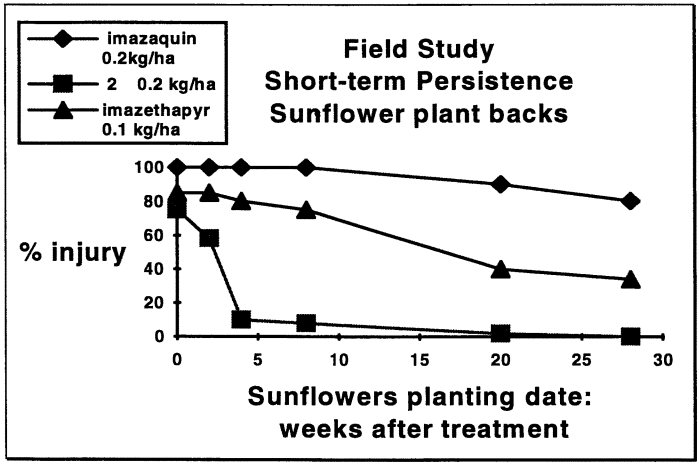


Figure 2: Sunflower Plantbacks into Fields Treated with Imidazolinone Herbicides

Figure 3 shows a similar study using sugarbeets as the plant-back crop. In this study, soil samples were taken from fields treated with imidazolinone herbicides and sugarbeets were planted into this soil and grown in the greenhouse. Plant-backs into soil treated with **2** show a quick reduction in sugarbeet injury indicating rapid degradation. It should be noted that sugarbeets are very sensitive to imidazolinones and there is a concentration of the herbicide that needs to be reached before sugarbeet growth can begin. Thus, the flat line shown by the imazaquin samples indicates that this concentration has not been reached.

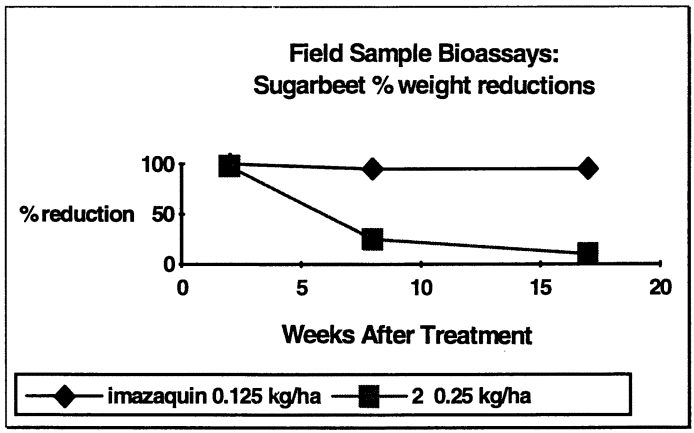


Figure 3: Sugarbeet Bioassays of Field Samples

The results of the biological studies conclusively demonstrate that acetal derivatives are rapidly degraded in the soil. There are two major pathways by which these compounds may be degraded (Figure 4). A hydrolytic pathway where the acetal is hydrolyzed to the aldehyde appears to be important for very hydrolytically labile acetal derivatives (e.g. N-acyloxazolidines). Microbial oxidation leading to diacid derivatives is the probable pathway by which soil degradation occurs for the rest of the acetal series. The oxidation pathway explains why the hydrolytically more stable cyclic acetals break down in the soil as fast or faster than acyclic acetals. The oxidation mechanism also explains why some compounds that are very active *in vitro* and hydrolytically very stable at pH 2, show such a dramatic difference in activity postemergence compared to preemergence. Similar oxidative mechanisms have been shown to occur for other 5-alkyl pyridine imidazolinones.

In summary, imidazolinone herbicides with reduced soil persistence were found by examining derivatives of an almost inactive member of the imidazolinone series. Compounds such as the dimethoxymethyl and the dioxolane are excellent herbicides with low soil persistence. They provide broad spectrum weed control but break down sufficiently fast to allow for very flexible crop rotation. Even the most sensitive crop species (such as sugar beets) can be used as a follow crop. Although no new crop selectivities were discovered, the methodology and concepts used in this project could help lead to the discovery of such compounds. The concept of examining derivatives of an inactive analog could prove to be

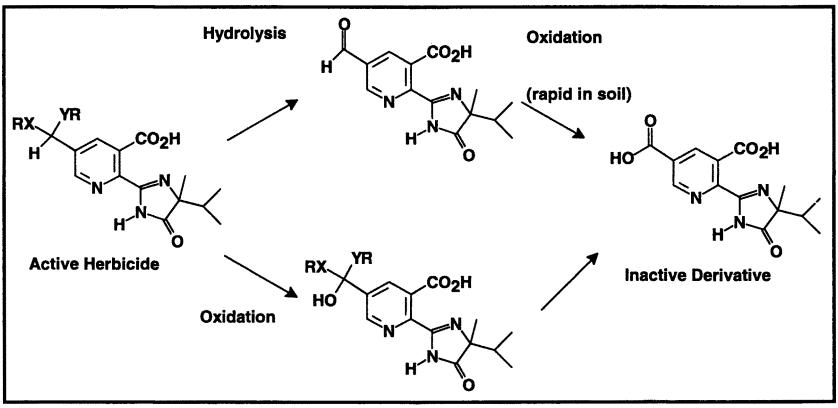


Figure 4: Different Mechanisms of Soil Degradation for Acetal Derivatives

useful method for the discovery of other herbicides with shorter residual activity and/or different crop selectivities.

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Chapter 6

Cyclic Imidate Derivatives of 5-Amino-2,6-bis(polyfluoroalkyl)pyridine-3-carboxylates

Synthesis and Herbicidal Activity

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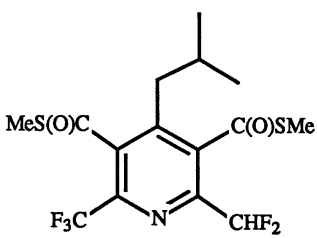
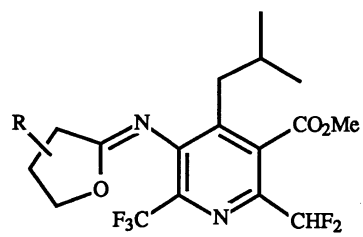
Certain 4-halobutyramide derivatives of 5-amino-2,6-bis(polyfluoro alkyl)-4-*iso*butylpyridine-3-carboxylate showed considerably higher herbicidal activity than the corresponding non-halogenated amides. Comparison of activities of several halogenated amides indicated that the unusual activity of 4-halobutyramides may be related to their ability to cyclize to a five-membered heterocyclic system. Lewis acid catalyzed cyclization of 4-halobutyramides gave the corresponding cyclic imidates, which are highly active herbicides.

The herbicidal properties of 2,6-(polyfluoroalkyl)-pyridine-3,5-dicarboxylates were first disclosed in 1985 by Lee and coworkers (*1*). Since then a large number of pyridine derivatives have been synthesized and screened for herbicidal activity (*2-8*). In general, these compounds exhibit pre-emergence and early post-emergence control of annual grasses and small-seeded broadleaf weeds. The mechanism of action has been determined to be inhibition of cell division by disruption of the formation of microtubules (*9*). The commercial potential of pyridines has been explored thoroughly over the past several years. These efforts have led to the development of a new turf herbicide Dimension (**1a**) which is a pyridine-3,5-dithiocarboxylate derivative.



1a



1b

0097–6156/95/0584–0060$12.00/0

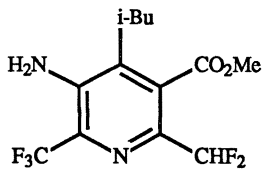
In the course of investigating various analogs of **1a**, we have discovered a new family of highly active pyridine herbicides represented by the general formula **1b** (*10, 11*). These are cyclic imidate derivatives of 5-amino-2,6-bis(polyfluoroalkyl)-4-isobutylpyridine-3-monocarboxylate. In this paper we present the unusual activity of certain halogenated amide derivatives of 5-amino-2,6-bis(polyfluoroalkyl)pyridine-3-carboxylate and the structure-activity rationalization which led to the conception and synthesis of cyclic imidates as a new family of pyridine herbicides. Also detailed herein is a brief account of the synthesis and biological activity of some heterocyclic analogs of cyclic imidates.

Pre-emergence Herbicidal Activity.

A description of the protocol of pre-emergence assay has been previously reported (*10, 11*). The assay included five narrowleaf weeds (Downy Brome, Proso Millet, Barnyard Grass, Large Crab Grass, and Green Foxtail) and six broadleaf weeds (Cocklebur, Wild Buckwheat, Morning Glory, Hemp Sesbania, Jimsonweed, and Velvetleaf). The application rates of test compounds ranged from 0.01 to 2.5 lb/acre in multiples of 2 (2.5, 1.25, 0.62 lb/acre, etc.). Herbicidal activity is expressed as narrowleaf weed GR80 (NL WGR80) and broadleaf weed GR80 (BL WGR80) which is the amount of herbicide in pounds per acre, averaged over all five narrowleaf species and all six broadleaf species, respectively, required to inhibit 80% of weed growth relative to that of the untreated control.

Discovery of Cyclic Imidates.

In the course of investigating the functional group chemistry of amino-pyridinecarboxylate **2**, a readily obtained intermediate from the corresponding pyridine-3,5-dicarboxylate (*10, 11*), several amide derivatives were prepared by acylation with aliphatic acyl halides. Upon screening for herbicidal activity, these amides proved to be generally inactive. However, an exception to the general trend was realized in the case of a 4-bromobutyramide derivative **3** which displayed unusually high narrowleaf activity (NL WGR80 = 0.04).



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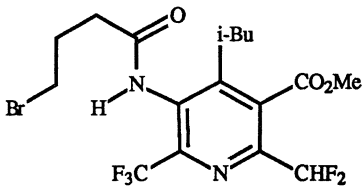

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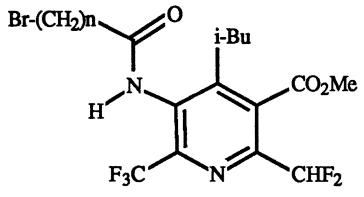
In order to determine the origin of the unusual herbicidal activity of **3**, we carried out a systematic synthesis of structurally diverse halogenated amides. Compound **2** was acylated with selected acyl halides so as to obtain halogenated amide moieties which included variations in the length of the amide chain, the degree of branching, the number and position of halogen atoms, and the nature of the halogen. The biological activity of halogenated amides follows a well-defined pattern. Some of the key structure-activity correlations are as follows:

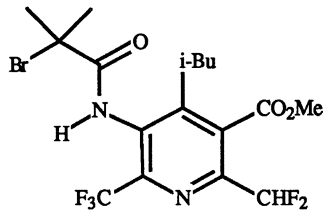
1. 4-Bromobutyryl amide **3** is substantially more active (100x) than the corresponding 5-bromovaleramide, 3-bromopropionamide and 2-bromoacetamide.

In general, butyramide represents the optimum chain length for best activity.



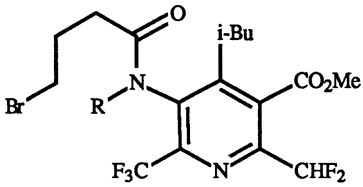
	n	NL WGR80
4	4	5.2
3	3	0.04
5	2	3.6
6	1	>10

2. The linear arrangement of the amide chain is critical for high herbicidal activity. Branched amide groups containing one halogen and four carbon atoms are much less active. For example, compound 7 had a NL WGR80 of 0.7.



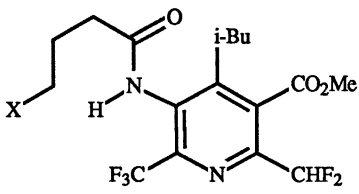
7

3. The "N-H" bond in **3** is critical for activity. Replacement of the N-H group with an N-methyl group lowers the activity by two orders of magnitude.



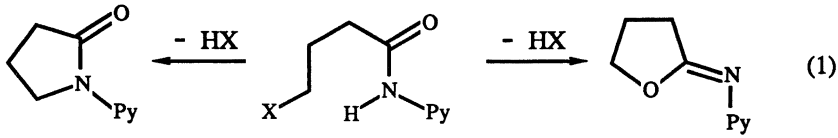
	R	NL WGR80
3	H	0.04
8	Me	3.04

4. Among the 4-iodo-, 4-bromo-, and 4-chlorobutyramides, the activity order with respect to the halogen substituent is I > Br > Cl based on average GR80 values for all narrowleaf species. It is important to note that the activity order for the halogen substituents is also the order of their ability as leaving groups in displacement reactions.



	X	NL GR 80
9	Cl	1.0
3	Br	0.19
10	I	0.11

The significant loss of activity by replacing the N-H group with N-Me, the similarity between the order of activity with respect to the halogen group and the order of reactivity in substitution reactions (I > Br > Cl), and the specific requirement of a five atom amide chain for high herbicidal activity led us to postulate an intramolecular cyclization of halogenated butyramides as a mechanism of biological activity. The cyclization of **3** with the loss of HBr is effectively an intramolecular alkylation of the amide moiety to form a five-membered ring. Since the amide group is an ambident nucleophile, the alkylation can occur either at the oxygen or at the nitrogen atom. O-alkylation leads to a cyclic imidate whereas N-alkylation leads to a pyrrolidone structure (equation 1).



In order to determine the validity of the cyclization mechanism for biological activity, we needed to synthesize both the pyrrolidone and the cyclic imidate derivatives shown in equation 1 and compare their herbicidal activities. This was accomplished by cyclization of **3** under two different conditions as shown in Figure 1. Anionic cyclization of **3** with a strong base such as sodium bis(trimethyl silyl)amide gave the pyrrolidone **11** exclusively. In contrast, reaction of **3** with silver tetrafluoroborate or ferric chloride afforded the cyclic imidate **12** as the sole cyclization product. The structures of compounds **11** and **12** were assigned based on a comparison of their ^{13}C NMR spectra with those of some model compounds reported in the literature (*12*). Further evidence for the cyclic imidate structure of **12** was derived from the X-Ray structure of the α-methyl analog as described later in this paper.

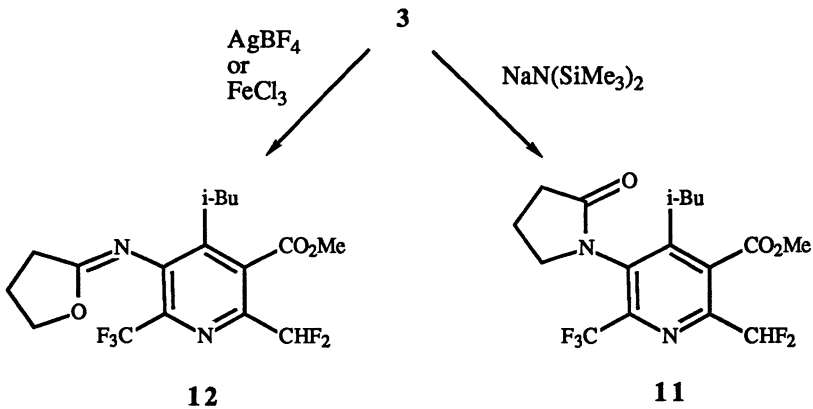


Figure 1. Cyclization of 4-bromobutyramide derivative **3**

The two modes of cyclization depicted in Figure 1 can be broadly distinguished as S_N1 and S_N2 type alkylations. Complexation of silver tetrafluoroborate with the halogen results in the development of a positive charge on the adjacent carbon and the cyclization then proceeds via a transition state with substantial amount of positive character (S_N1 type). Conversely, sodium bis(trimethylsilyl)amide

generates the anion of the amide moiety, which then cyclizes via an S_N2 transition state. The O- versus N-alkylation is readily explained by Kornblum's hypothesis (*13*) concerning the reaction of ambident nucleophiles. The hypothesis states that in reactions of ambident nucleophiles with alkyl halides, as the positive nature of the transition state increases, bond formation at the ambident anion atom having the higher electron density is preferred since it can better accommodate the positive charge. In an amide nucleophile, oxygen being more electronegative than nitrogen, predominant O-alkylation can be expected under S_N1 conditions. In an S_N2 mechanism, the preference is for bond formation at the atom less able to bear the negative charge. Consequently, amide anions are predominantly N-alkylated. Compound **12** exhibited impressive herbicidal activity against annual narrowleaf weeds (NL WGR80=0.02). The pyrrolidone **11** was at least five-fold less active (NL WGR80=0.1). Thus, the experiment added validity to the cyclization postulate and indicated that the cyclization of compound **3** to the cyclic imidate structure was most likely responsible for the high biological activity.

Synthesis of Substituted Cyclic Imidates.

The majority of the α-substituted cyclic imidates were prepared according to the general protocol depicted in Figure 2. The aminopyridine **2** was acylated with a variety of α-substituted 4-bromobutyryl chlorides **13a-i** and the resulting amides **14a-i** were cyclized with silver tetrafluoroborate to obtain the cyclic imidates **15a-i**.

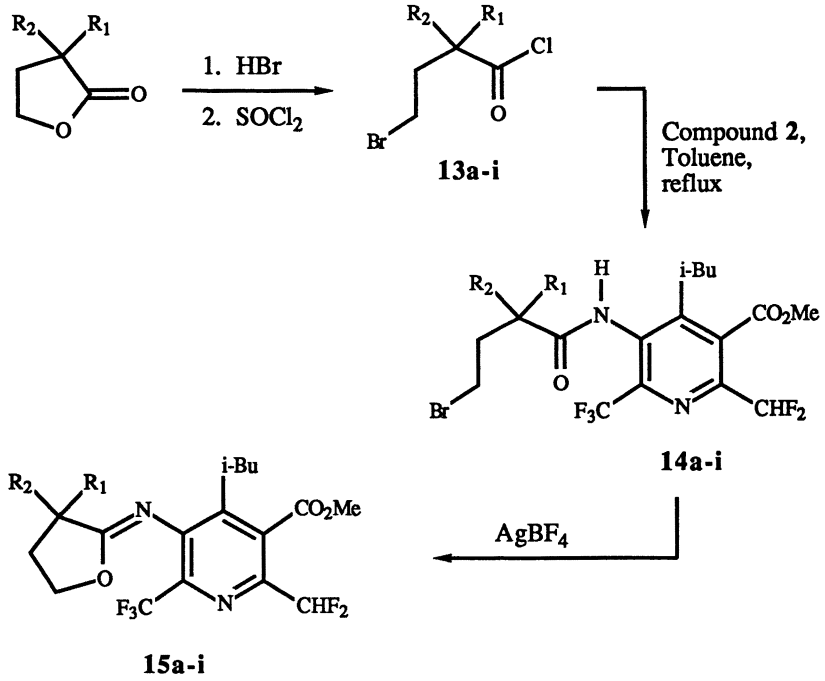
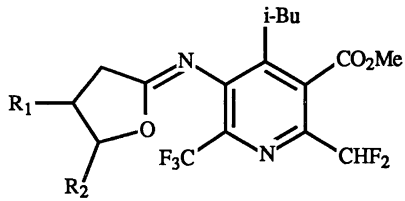


Figure 2. Synthesis of α-substituted cyclic imidates

The 4-bromobutyryl chloride precursors were obtained in a two step sequence from α-substituted butyrolactones, by first cleaving the lactone ring with HBr to give the corresponding 4-bromobutyric acids followed by treatment with thionyl chloride. The general strategy described in Figure 2 can also be employed in the preparation of β-substituted cyclic imidates. Thus, the β-methyl analog **16** and the β-fluoro analog **17** were synthesized starting from β-methyl and β-fluoro butyrolactones, respectively. Compound **17** proved to be extremely succeptible to dehydrofluorination and underwent decomposition upon chromatography on silica gel. Consequently, the herbicidal activity of **17** could not be determined.



16 $R_1 = Me, R_2 = H$

17 $R_1 = F, R_2 = H$

18 $R_1 = H, R_2 = Me$

The synthesis of the γ-methyl substituted cyclic imidate **18** is described in Figure 3. The unsaturated amide **19** was brominated and the resulting dibromo derivative was cyclized with silver tetrafluoroborate to provide the γ-(bromomethyl) cyclic imidate **20**. Reduction of the bromomethyl group to methyl was accomplished by treatment with tributyltin hydride.

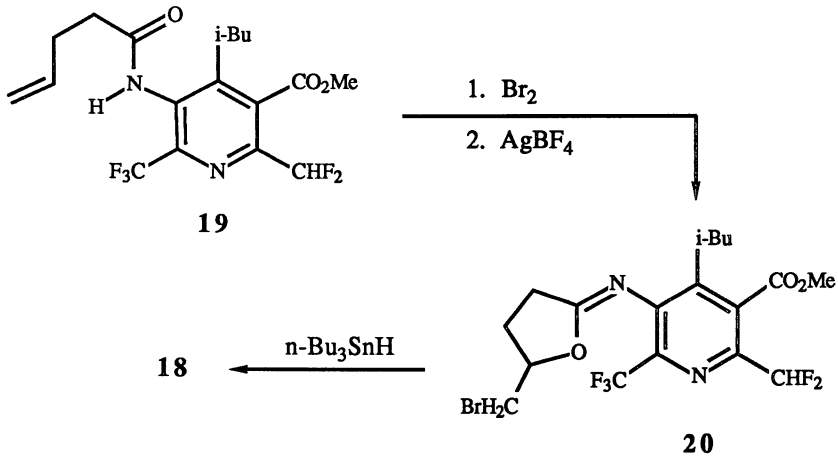


Figure 3. Synthesis of γ-methyl substituted cyclic imidate **18**

Stereochemistry of Cyclic Imidates.

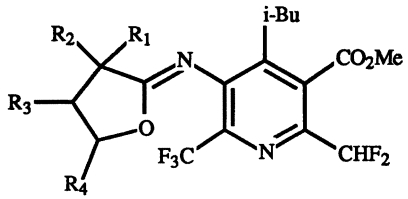
There are two possible geometric isomers for the cyclic imidate system, E and Z, based on the relative orientation of the imidate oxygen and the pyridine ring with respect to the carbon-nitrogen double bond. Cyclizations of haloamides **3** and **14a-i** with either silver tetrafluoroborate or ferric chloride generally yielded a single isomer of the cyclic imidate product. Determination of the exact stereochemistry of

12 and **15a-i** through NMR studies proved to be tenuous. The Z configuration of the cyclic imidate moiety was finally deduced by a single-crystal X-ray analysis of **15a**. The exclusive formation of Z-cyclic imidates is ascribed to their high configurational stability. This is consistent with some earlier observations on simpler imidate systems reported by Moriarity and coworkers (*14*). The high barrier to inversion in the Z-imidates was proposed to result from repulsion between the nonbonding electrons on oxygen and the electrons localized in a p-orbital on nitrogen in the transition state of inversion.

Structure-Activity Correlations of Substituted Cyclic Imidates.

The narrowleaf (NL WGR80) and broadleaf (BL WGR80) herbicidal activities of cyclic imidates **12, 15a-i, 16, 18** and **20** are listed in Table I.

Table I. Structure-Activity Correlations of Substituted Cyclic Imidates



compd	R_1	R_2	R_3	R_4	WGR 80 (lb / acre) NL	BL
12	H	H	H	H	0.02	0.14
15a	Me	H	H	H	0.02	0.24
15b	F	H	H	H	0.04	0.80
15c	Cl	H	H	H	0.06	0.21
15d	Br	H	H	H	0.20	4.60
15e	OMe	H	H	H	0.05	0.75
15f	SMe	H	H	H	0.12	2.91
15g	Me	Me	H	H	0.14	4.46
15h	Et	H	H	H	0.10	1.73
15i	Me	F	H	H	0.06	0.30
16	H	H	Me	H	0.20	1.86
18	H	H	H	Me	0.16	1.38
20	H	H	H	CH_2Br	0.48	5.77

Comparisons between the activities of individual compounds reveal the following structure-activity correlations: (1) Among the three possible methyl substituted cyclic imidates, α-methyl analog **15a** is the most active. The β- and γ-methyl derivatives **16** and **18** are approximately 8-10 fold less active toward narrowleaf weeds and 6-8 fold less active toward broadleaf weeds. (2) Among the α-alkyl substituted cyclic imidates, α-methyl analog **15a** is significantly more active than the corresponding ethyl and gem-dimethyl derivatives **15h** and **15g**, respectively. (3) Among the halogen substituted cyclic imidates, the order of herbicidal activity is **F > Cl > Br** for narrowleaf weeds and **Cl > F > Br** for broadleaf weeds. (4) The highest narrowleaf weed activity was exhibited by the unsubstituted cyclic imidate **12** and the α-methyl substituted cyclic imidate **15a**.

Heterocyclic analogs of Cyclic Imidates.

Encouraged by the high activity of **12**, we proceeded to synthesize a few other five-membered heterocyclic analogs of the cyclic imidate moiety. Herein we describe the preparation of a cyclic thioimidate **21**, a cyclic amidine **22** and a cyclic iminocarbonate **26** (Figures 4 and 5).

The 4-bromobutyryl amide **3** was converted to the corresponding imidoyl chloride by reaction with phosphorus pentachloride. Treatment of the imidoyl chloride with lithium sulfide resulted in cyclization to afford **21**. The cyclic amidine derivative **22** was prepared in one step by condensing N-methyl pyrrolidone dimethyl acetal with the aminopyridine **2** under acid catalysis. The synthesis of cyclic iminocarbonate **26** involved three sequential steps: (i) Curtius reaction of the acid chloride **23** with sodium azide in a mixture of ethylene glycol and acetone to give the hydroxyethyl carbamate **24**; (ii) Conversion of compound **24** to the corresponding chloroethyl carbamate **25** with thionyl chloride; and (iii) Cyclization with silver tetrafluoroborate.

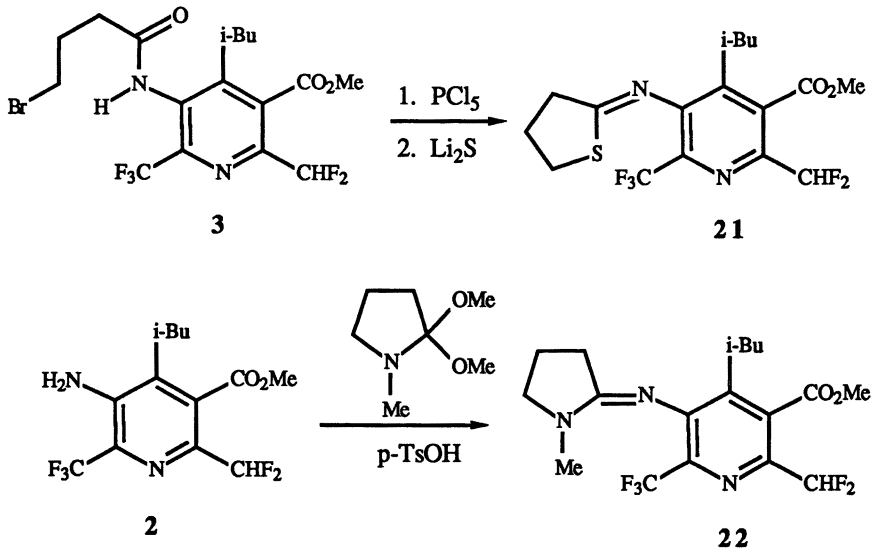


Figure 4. Synthesis of sulfur and nitrogen analogs of cyclic imidates

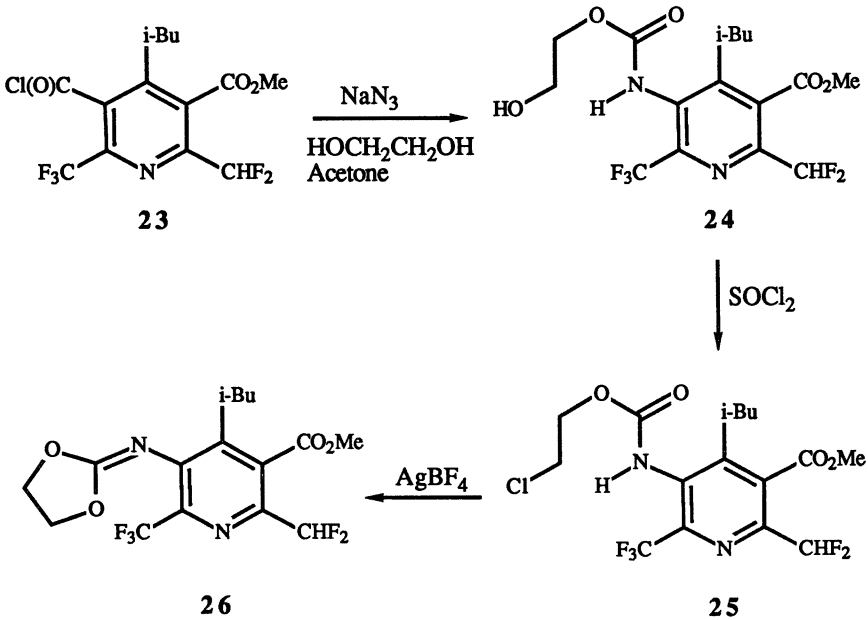
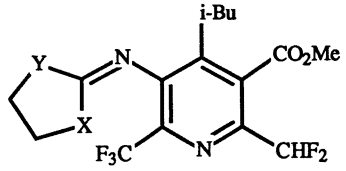


Figure 5. Synthesis of cyclic iminocarbonate **26**

Table II. Structure-Activity Correlations of Heterocyclic Analogs of Cyclic Imidates



compd	X	Y	WGR 80 (lb / acre) NL	BL
12	O	CH_2	0.02	0.14
21	S	CH_2	0.02	0.24
22	NMe	CH_2	0.06	0.96
26	O	O	0.23	0.92

The herbicidal activities of **12** and its five-membered heterocyclic analogs are compared in Table II. Although compounds **12** and **21** had comparable activity on narrowleaf weeds, **12** exhibited slightly higher broadleaf activity. By comparison, the cyclic amidine **22** and the cyclic iminocarbonate **26** were significantly less active on both narrowleaf and broadleaf weeds.

Conclusions

In summary, the unusual herbicidal activity of a 4-halobutyramide derivative **3** was postulated to be due to its ability to cyclize to a five membered heterocyclic system based on the structure-activity correlations of related halogenated amides. Cyclization of **3** using a Lewis acid such as silver tetrafluoroborate or ferric chloride gave the cyclic imidate **12**, which proved to be a highly active herbicide. The pyrrolidone derivative **13**, obtained by anionic cyclization of **3** using a strong base, was significantly less active. Among the analogs of cyclic imidate **12**, several α-substituted derivatives as well as a cyclic thioimidate also showed high levels of herbicidal activity.

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Chapter 7

Thiazolo[4,5-*b*]pyridine-3(2*H*)-acetic Acid Derivatives

Synthesis and Herbicidal Activity

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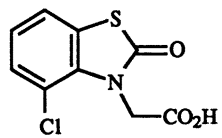
Several examples of 5-(haloalkyl) substituted thiazolo[4,5-*b*]pyridine-3(2*H*)-acetic acid derivatives were prepared as pyridine analogues of the commercial herbicide Benazolin. The heterocyclic ring system was constructed via a novel condensation reaction of certain β-ethoxyenones with methyl 4-imino-2-thioxo-3-thiazolidine acetate. Most of the compounds in this study exhibited auxin-like herbicidal symptoms and higher activity on broadleaf weeds than narrowleaf weeds.

The auxin-like herbicidal activity of 2-oxo-benzothiazole-3(2*H*)-acetic acid derivatives were first described by Brookes and Leafe in 1963 (*1*). The 4-chloro analogue in this series, Benazolin (**1**) has found commercial utility as a post-emergence herbicide. It is principally used in combination with phenoxy herbicides such as 2,4-D, MCPA, and MCPB for the selective control of certain broadleaf weeds such as chickweed and cleavers in cereal crops and oilseed crops (*2*, *3*). The herbicidal effects of **1** are similar to those exhibited by phenoxy herbicides with hormonal activity (*1*, *4*, *5*). The auxin-like activity of **1** has been further demonstrated by comparison with indole-3-acetic acid in a pea straight growth bioassay (*6*).



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The effect of replacing the benzene ring of **1** with a heterocyclic ring on herbicidal activity has not been previously reported. As part of our effort to utilize 2-(haloalkyl) substituted pyridine substructures in designing newer agrochemicals (*7*), we have prepared several thiazolo[4,5-*b*]pyridine-3(2*H*)-acetic acid derivatives

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represented by the general formula **2** (Figure 1). In this paper we describe the methods of synthesis and the structure-herbicidal activity correlations of compounds **2**.

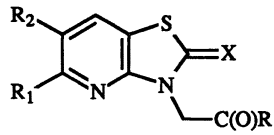

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Figure 1. General formula of compounds in the present study: R = OH, OMe, NHOMe, N(Me)OMe; R_1 = Haloalkyl; R_2 = H, Cl; X = O, S

Synthesis.

Assembly of the Heterocyclic Ring System. We envisioned the construction of thiazolo[4,5-*b*]pyridine ring system by a cyclocondensation of the appropriately substituted β-ethoxyenones **3** and the previously known methyl 4-imino-2-thioxo-3-thiazolidine acetate **4** (*8*). This condensation, in theory, can produce structure **5** as well as its regioisomer **6** (Figure 2).

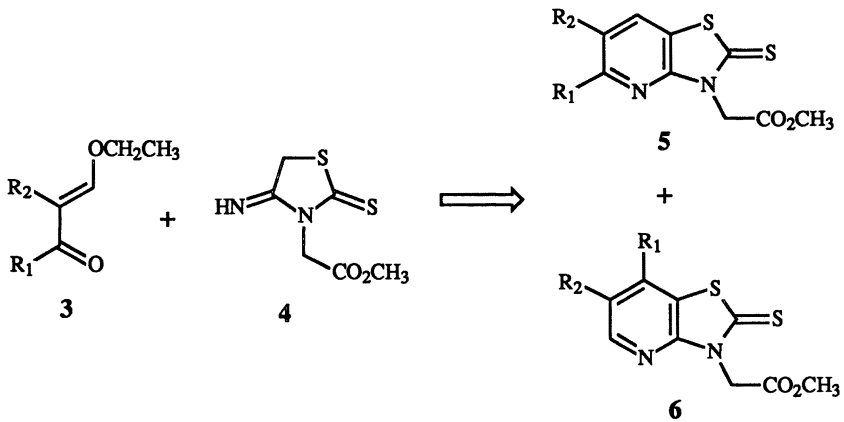


Figure 2. Proposed synthetic route for the construction of the thiazolo[4,5-*b*]pyridine ring system

The β-ethoxyenone intermediates were prepared as shown in Figure 3. Ethyl vinyl ether (**7**) was acylated with trifluoroacetic anhydride and chlorodifluoroacetic anhydride as reported in the literature (*9*) to give enones **3a** and **3b**, respectively. Chlorination of **3a** and **3b** with N-chlorosuccinimide provided the corresponding chloro derivatives **3c** and **3d**, respectively. Figure 4 describes the synthesis of methyl 4-imino-2-thioxo-3-thiazolidine acetate (**4**) and subsequent condensation with β-ethoxyenones **3a-d**. Thus, glycine methyl ester was treated with carbon disulfide and sodium hydroxide to produce the dithiocarbamate derivative. Addition

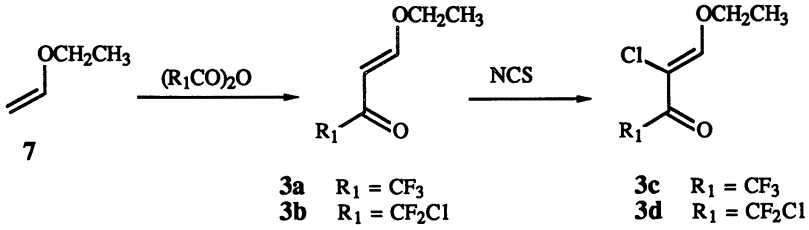


Figure 3. Synthesis of β-ethoxy enone intermediates

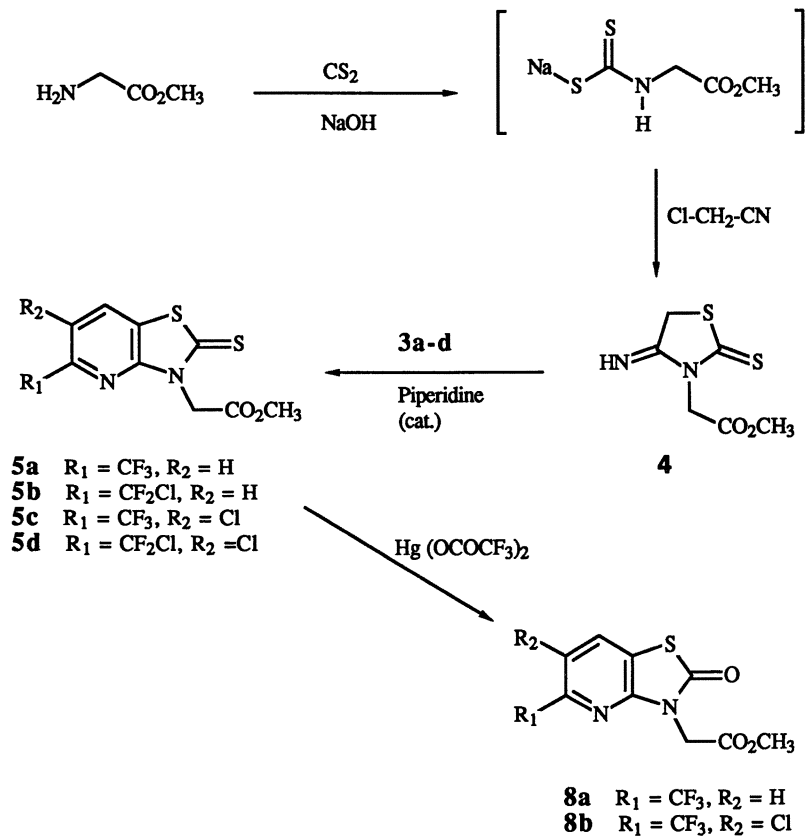


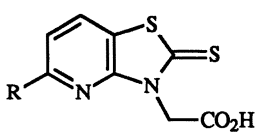
Figure 4. Synthesis of 2-thioxo and 2-oxo-thiazolo[4,5-*b*]pyridine-3(2*H*)-acetates

of chloroacetonitrile to the reaction mixture resulted in the alkylation of the dithiocarbamate derivative and concurrent cyclization to give compound **4**. The condensation of β-ethoxyenones **3a-d** with compound **4** in the presence of a catalytic amount of piperidine gave the thiazolo[4,5-*b*]pyridine derivatives **5a-d** as a single regioisomer in each case.

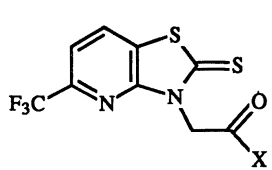
Regiochemistry of cyclization. The exact location of the haloalkyl group in **5a-d** was determined by employing Selective INADEQUATE (Incredible Natural Abundance Double Quantum Transfer Experiment) NMR (*10*), a new technique for establishing carbon-carbon connectivity. In this experiment a selective pulse is applied to one particular carbon resonance. This results in a very characteristic signal, an antiphase doublet, at the NMR frequency of any carbon or carbons directly bonded to the one which was selectively pulsed. In the ^{13}C spectra of compounds **5a-d**, the carbon bearing the haloalkyl group is readily identified due to its coupling with the fluorines. Four separate INADEQUATE spectra were obtained in each case by selectively pulsing at each of the remaining four pyridine ring-carbon frequencies individually. An antiphase doublet at the frequency of the carbon bearing the haloalkyl group was observed in only one of the four spectra indicating only one adjacent ring-carbon. Thus, the haloalkyl group must be at position 5 and not at position 7.

Desulfurization of 2-thioxo-thiazolo[4,5-*b*]pyridine-3(2*H*)-acetates. Conversion of thioesters to carboxylic esters has been traditionally carried out via desulfurization with mercuric acetate (*11*). Desulfurization of thioamides with mercuric acetate is also known, however their reactivity is less than the corresponding thioesters. Although a similar reaction of thiocarbamates has not been reported previously, a substantially lower reactivity is expected based upon the reduced nucleophilicity of the thiocarbonyl group. Indeed, compounds **5** reacted very sluggishly with mercuric acetate even in refluxing chloroform. Generally, less than 10% conversion was observed upon refluxing the reaction mixture for 24 h. This problem was overcome by utilizing a more electrophilic mercury salt, mercuric trifluoroacetate. Refluxing compounds **5** with stoichiometric amount of mercuric trifluoroacetate in methylene chloride for 6 h gave the corresponding oxo compounds **8** in good yield (Figure 4). In general, mercuric trifluoroacetate may be an effective alternative to mercuric acetate in the desulfurization of relatively electron-deficient thiocarbonyl compounds.

Derivatization of 2-thioxo-thiazolo[4,5-*b*]pyridine-3(2*H*)-acetates. The ester groups of compounds **5a-d** were shown to be amenable to further transformations. Thus, compounds **5a** and **5b** were hydrolyzed to the corresponding acids **9a** and **9b**, respectively, by treatment with sodium hydroxide (Figure 5). Alkylation of **9a** with



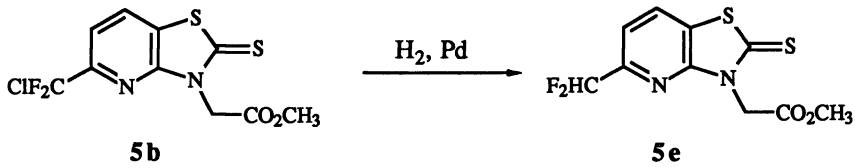
9a R = CF_3
9b R = CF_2Cl



	X
10	-OCH(Me)CO_2Et
11	-NHOMe
12	-N(Me)OMe

Figure 5. Carboxylic acid, ester and amide derivatives from compound **5a**

ethyl 2-bromopropionate in the presence of potassium carbonate gave the lactate ester **10**. Alternatively, the carboxylic acid group of **9a** was first converted to the acid chloride by reaction with thionyl chloride and the acid chloride was treated with methoxyamine or N,O-dimethylhydroxylamine to obtain amides **11** and **12**, respectively. Figure 6 describes the hydrogenolysis of the chlorodifluoromethyl group of compound **5b** to produce the 5-(difluoromethyl)-thiazolo[4,5-*b*]pyridine derivative **5e**. Finally, an α-methyl substituted thiazolo[4,5-*b*]pyridine-3(2*H*)-acetate derivative **13** was synthesized starting from DL-alanine ethyl ester (Figure 7) and utilizing a similar sequence of reactions as described in Figure 4.



5b **5e**

Figure 6. Synthesis of the 5-(difluoromethyl) derivative **5e**

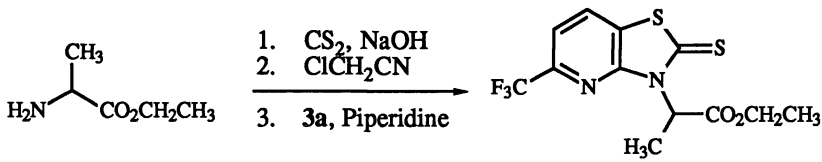


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Figure 7. α-Methyl substituted thiazolo[4,5-*b*]pyridine-3(2*H*)-acetate derivative

Determination of Herbicidal Activity.

All of the synthetic compounds described above were evaluated in pre-plant incorporated and postemergence herbicide assays. The evaluations included two crops: corn and wheat, two narrowleaf weed species: barnyard grass (*Echinochloa crus-galli*) and downy brome (*Bromus tectorum*) and four broadleaf weed species: velvetleaf (*Abutilon theophrasti*), morning glory (*Ipomoea spp.*), cleavers (*Galium aparine*) and common chickweed (*Stellaria media*). The application rates of test compounds ranged from 0.07 to 11.2 kg/Ha. The activity data are expressed as GR 20's for crops and GR 80's for weeds which is the rate of herbicide (kg/Ha) that causes 20% crop injury and 80% weed injury, respectively.

Structure-Activity Correlations.

Tables I and II provide the herbicidal activity data from the pre-plant incorporated and post-emergence assays, respectively. In general, the compounds were more active on broadleaf than narrowleaf species with the difference being most apparent in post-emergence evaluations. All of the active compounds exhibited auxin-like

herbicidal symptoms characterized by epinastic response and formative action. Shoot apex inhibition was also noted in several post-emergence evaluations.

Most compounds showed a high degree of selectivity in corn in both pre-plant incorporated and postemergence assays. Selectivity in wheat was high in post-emergence tests and moderate in pre-plant incorporated tests.

Table I. Pre-Plant-Incorporated Herbicidal Activity of Compounds 5, 8-13, and Benazolin (1) against Crop Plants and Representative Weed Species[a]

	GR20 (Kg/ha)		GR80 (Kg/ha)					
compd	corn	wheat	BG	DB	VL	MG	GA	CW
5a	>11	11.0	6.4	7.5	4.9	0.4	0.7	0.9
5b	5.6	11.0	>11	>11	4.6	2.0	0.7	0.7
5c	>11	5.6	>11	7.5	>11	0.7	0.9	0.2
5d	>11	>11	>11	>11	>11	9.0	7.5	6.4
5e	>11	5.6	11.0	6.9	4.7	2.0	0.9	3.0
8a	4.7	2.2	5.6	5.2	1.1	0.7	0.8	2.2
8b	>11	5.6	6.9	>11	9.0	0.2	0.2	1.1
9a	>11	1.1	7.5	10.0	3.0	0.2	0.7	0.8
9b	>11	0.8	11.0	0.2	0.6	0.2	7.5	5.9
10	>11	>11	>11	>11	5.9	2.6	0.9	1.9
11	>11	5.6	>11	>11	4.4	3.4	4.6	3.4
12	>11	>11	>11	>11	>11	>11	>11	>11
13	>11	>11	>11	>11	>11	>11	>11	>11
1	>11	2.0	5.0	8.6	0.8	0.9	0.5	0.1

[a] Key to the weed species in this study: BG, barnyard grass; DB, downy brome; VL, velvetleaf; MG, morning glory; GA, cleavers; CW, common chickweed.

Three general correlations of structure and activity are summarized as follows: (1) A dramatic difference in activity against weeds was observed between compound **5a** and the corresponding α-methyl analogue **13**. The former had some activity against most broadleaf weeds whereas the latter was virtually inactive indicating a large variability of activity with respect to the α-substituent.
(2) Interestingly, among the carboxylic esters in the 2-thioxo series, compound **5d** (5-CF_2Cl, 6-Cl) was significantly less active than compounds **5a** (5-CF_3, 6-H), **5b** (5-CF_2Cl, 6-H), **5c** (5-CF_3, 6-Cl) and **5e** (5-CF_2H, 6-H). Thus, the herbicidal

activity was critically dependent on the combined effect of the 5- and 6-position substituents.
(3) Amides **11** and **12** were less active than most of the corresponding acid and ester derivatives.

Further correlations can be made between structure and activity on specific weeds. Thus, in pre-plant incorporated tests, cleavers and morning glory were the most succeptible species (Table I). Although several compounds had high activity on cleavers, compound **8b** had higher combined activity on cleavers and morning glory than all other compounds including Benazolin (**1**). On the remaining broadleaf weed species, Benazolin was generally more active than the compounds in this study.

In post-emergence tests, morning glory was the most sensitive species (Table II). Again, compound **8b** was the most active, closely followed by the lactate ester **10**. Compound **8b** was also substantially more active than Benazolin. On all other species, Benazolin had higher activity than the analogues in this study.

Table II. Postemergence Herbicidal Activity of Compounds 5, 8-13 and Benazolin (1) against Crop Plants and Representative Weed Species[a]

	GR20 (Kg/ha)		GR80 (Kg/ha)					
compd	corn	wheat	BG	DB	VL	MG	GA	CW
5a	>11	11.0	>11	>11	5.6	1.1	1.1	0.3
5b	>11	11.0	>11	>11	9.0	11.0	7.5	11.0
5c	>11	>11	>11	>11	7.5	5.6	3.7	1.1
5d	>11	>11	>11	>11	>11	>11	>11	>11
5e	>11	7.5	>11	>11	6.9	1.1	7.5	>11
8a	>11	11.0	>11	>11	4.1	0.5	4.5	3.7
8b	>11	7.5	>11	>11	>11	0.2	8.2	4.1
9a	>11	>11	>11	>11	5.9	1.1	1.1	6.4
9b	>11	9.5	>11	>11	6.4	4.5	2.4	4.7
10	>11	>11	>11	>11	1.1	0.3	7.5	4.7
11	>11	>11	>11	>11	>11	>11	>11	>11
12	>11	>11	>11	>11	>11	>11	>11	>11
13	>11	>11	>11	>11	>11	>11	>11	>11
1	>11	>11	>11	>11	0.9	5.6	0.1	0.3

[a] Key to the weed species in this study: BG, barnyard grass; DB, downy brome; VL, velvetleaf; MG, morning glory; GA, cleavers; CW, common chickweed.

Conclusions

The results from this study demonstrated that replacing the benzene ring of Benazolin (**1**) with a pyridine ring did not alter the general nature and the spectrum of activity. However, the levels of activity varied significantly depending on the species. Structure-activity correlations of several compounds of the general formula **2** revealed that a combination of 2-oxo and 6-chloro substituents resulted in higher activity than **1** in certain broadleaf weeds.

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Chapter 8

Herbicidal 1-(2,4-Dihalo-5-phenoxyphenyl)-4-difluoromethyl-4,5-dihydro-3-methyl-1,2,4-triazolin-5(1*H*)-one Derivatives

Synthesis and Structure–Activity Relationships

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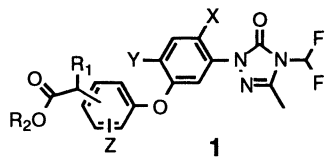
1-(2,4-Dihalo-5-phenoxyphenyl)-4-difluoromethyl-4,5-dihydro-3-methyl-1,2,4-triazolin-5(1H)-ones **1** are a new class of highly active postemergence herbicides. The mechanism of action was found to involve inhibition of the enzyme protoporphyrinogen oxidase which results in the buildup of a photodynamic toxicant, protoporphyrin IX. When compounds **1** were applied postemergence they provided excellent weed control at 7-15 grams/hectare. These herbicides were designed to act as substrate inhibitors of tetrapyrrole-handling enzymes in the chlorophyll synthesis pathway by mimicking the three ring-propionate portion of the tetrapyrrole molecule. Synthesis, biological properties, and SAR are discussed.



1

We have been interested for over a decade in the herbicidal properties of a variety of chemistries that share a common site of action, inhibition of protoporphyrinogen oxidase (Protox)(*1*). Even though several light dependent peroxidizing herbicides such as Ronstar® herbicide and several diphenyl ether herbicides such as Blazer® and Goal® brands have been in commercial use for several decades it was not till recently that their mechanism of action was elucidated (*2,3,4*).

When initial findings suggested that these herbicides acted by inhibiting an enzyme in the chlorophyll synthesis pathway, which in turn led to the accumulation of protoporphyrin IX, it was thought that the inhibited enzyme was magnesium chelatase, which is responsible for the insertion of magnesium into the tetrapyrrole ring during chlorophyll synthesis (*5,6*).

In 1987 we concluded that protoporphyrin IX buildup was a result of the ability of peroxidizing herbicides to mimic the tetrapyrrole substrate and to competitively bind at the same site as the tetrapyrrole molecule. Close examination of the molecular shape of the peroxidizing herbicides and the tetrapyrrole ring soon pointed out the strong similarities between the two molecules. To test this hypothesis we

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proposed that if the two ring molecule from a peroxidizing herbicide such as the diphenyl ethers and the aryl triazolinones (*7*) were mimicking two of the pyrrole rings of the tertrapyrrole molecule, then a three ring molecule with a propionate side chain would more closely resemble the tetrapyrrole ring substrate as shown in Figure 1(*8*).

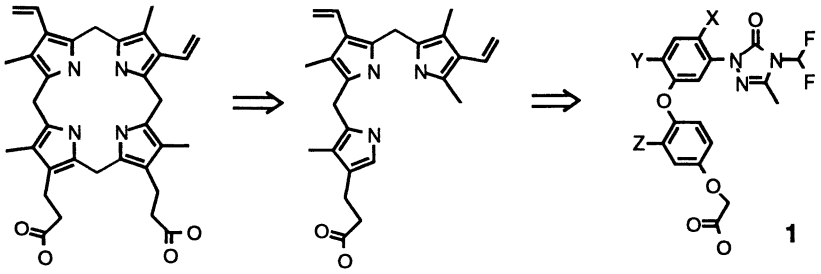


Figure 1. Phenoxyphenoxy propionates **1** as three ring mimics of tetrapyrroles.

We found that compounds with the general chemical structure **1** were among the most active peroxidizing herbicides tested in our laboratories and, as we will be discussing later, the oxypropionate moiety was essential for optimum activity. Subsequently, Matringe et al (*2,3*) and Witkowski and Halling (*4*) showed that peroxidizing herbicides inhibit protoporphyrinogen oxidase and not Mg chelatase as was initially thought. These findings led to further refinements of this model, which will be presented later.

More recently, Duke et al. proposed that peroxidizing herbicides were competitive inhibitors of Protox, requiring a bicyclic ring system and mimicking one half of the protogen molecule for a good fit into the active site (*9,10*). Our early appreciation of the structural similarities between the tetrapyrrole ring substrate and the peroxidizing herbicides allowed us to design a new class of herbicide chemistry, as well as to demonstrate that polycyclic molecules, such as compounds of general structure **1**, are inhibitors of Protox. When applied postemergence, compound **6** (Z=H, R_1,R_2=CH_3) causes rapid desiccation of sensitive weed species at application rates as low as 7.8 g/ha.

Synthesis

The synthesis of the majority of the compounds described in this work involves the derivatization of the key anilino intermediate **4**, which is prepared in good yields in two steps from the reaction of the appropriately substituted phenol **2** with a halonitrobenzene, followed by catalytic hydrogenation with PtO_2 catalyst in ethanol. The synthesis of the phenol intermediate **2** has been previously described (*7*).

The p-aminophenoxyphenyl compound **4** can be converted to the corresponding p-hydroxyphenoxyphenyl derivative **5** by the diazotization of compound **4** with $NaNO_2$ in sulfuric acid, followed by hydrolysis with $CuSO_4$ in refluxing water/xylene. Treatment of the p-hydroxyphenoxyphenyl derivative **5** with K_2CO_3 and 1-halo esters or alkyl halides resulted in good yields of the desired product **6** (Figure 2). This approach was used to obtain the para substituted phenoxyphenoxy oxypropionates, as well as other para substituted alkoxy derivatives.

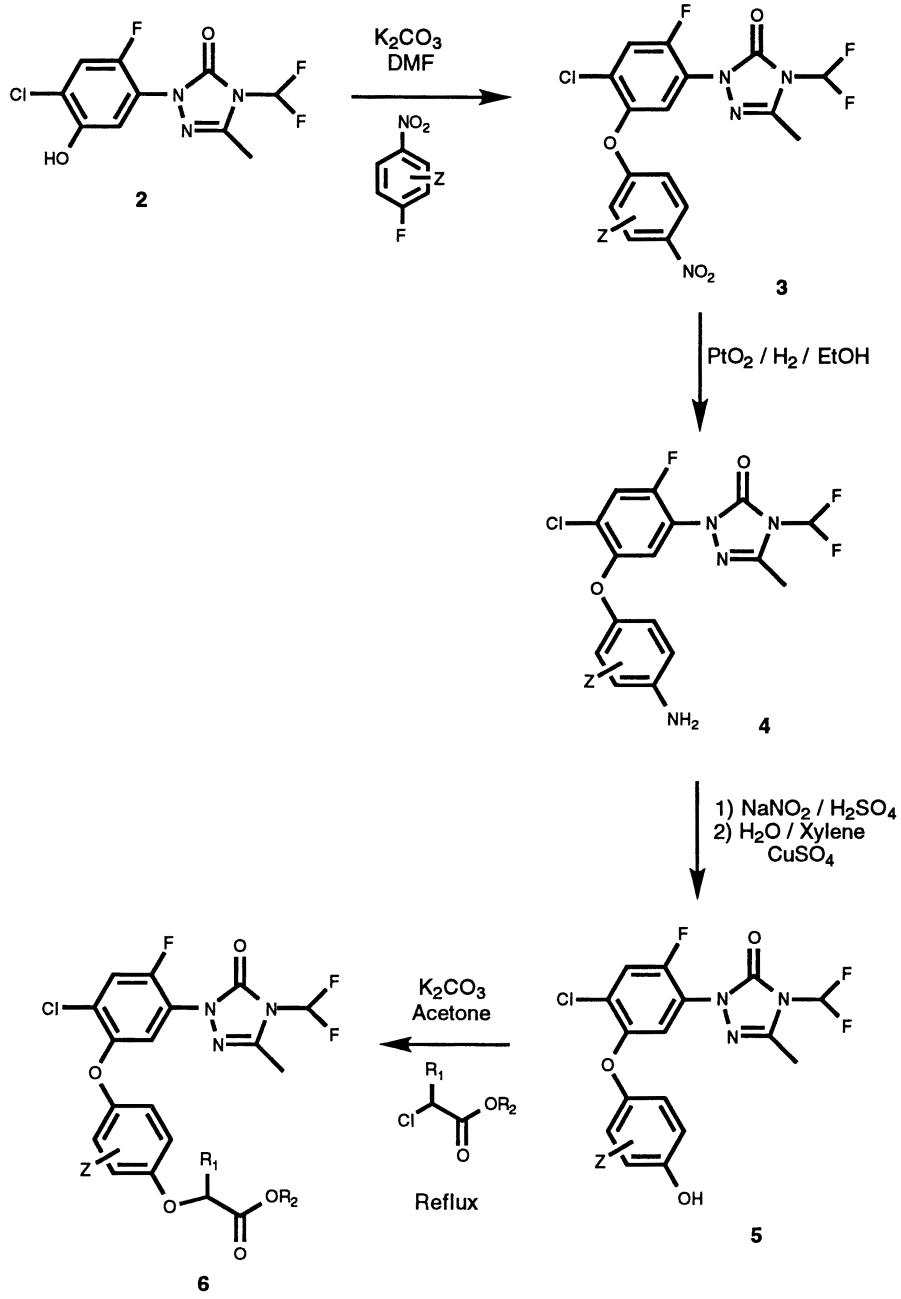


Figure 2. Synthesis of para-substituted phenoxyphenoxy derivatives.

When the substituent Z = 2-OCH_3, as in the intermediate **7**, the diazotization of the amino group followed by treatment with ethanol/toluene resulted in the meta-substituted methoxyphenoxy derivative **8**. Demethylation of compound **8** with BBr_3 gave quantitative yields of the meta-substituted phenol **9**, which in turn becomes the starting material for the synthesis of the meta-substituted oxypropionate **10** (Figure 3).

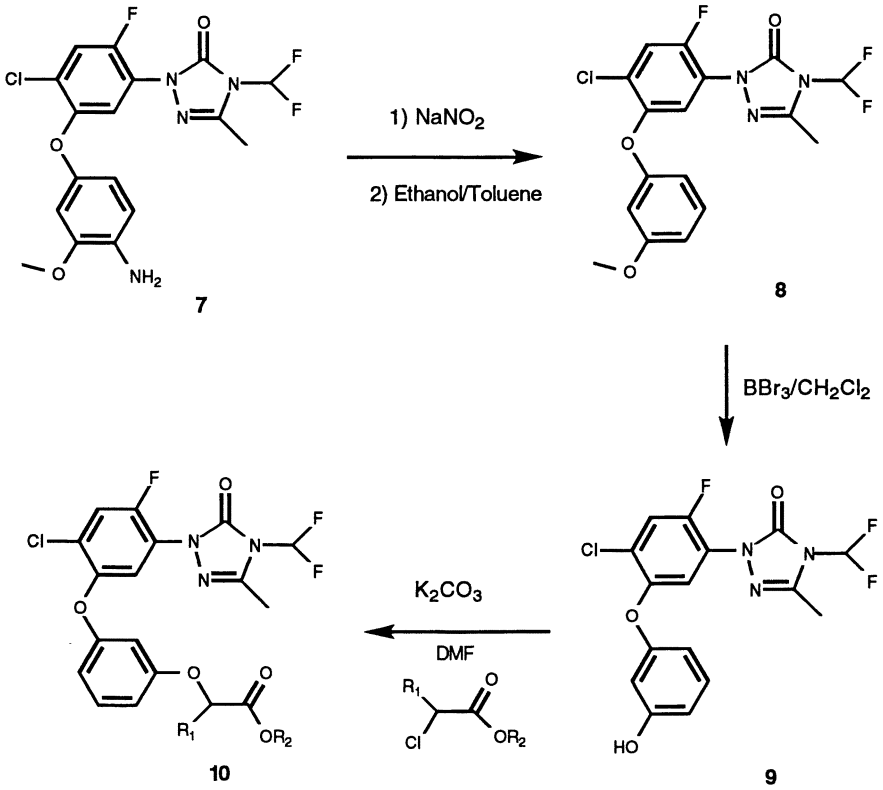


Figure 3. Synthesis of meta-substituted phenoxyphenoxy derivatives.

The 5-oxypropionate-2-pyridyloxy derivative **14** was prepared in a similar way as previously described for the synthesis of the para-substituted phenoxyphenoxy analogs **6** (Figure 2), with the exception of the step where the amino group is converted to the hydroxy group. Attempts to apply the same diazotization conditions resulted in poor yields of the phenol intermediate **13**. A better synthesis of the required hydroxy intermediate **13** involved the initial diazotization of the 5-amino-2-pyridyloxy intermediate **11** with aqueous HCl followed by the addition of tetrafluoroboric acid to give the corresponding tetrafluoroborate salt **12** in good yields. Treatment of compound **12** with potassium carbonate and trifluoroacetic acid gave the desired product **13** in good yields(*11*), which could then be further derivatized by following standard procedures as shown in Figure 4.

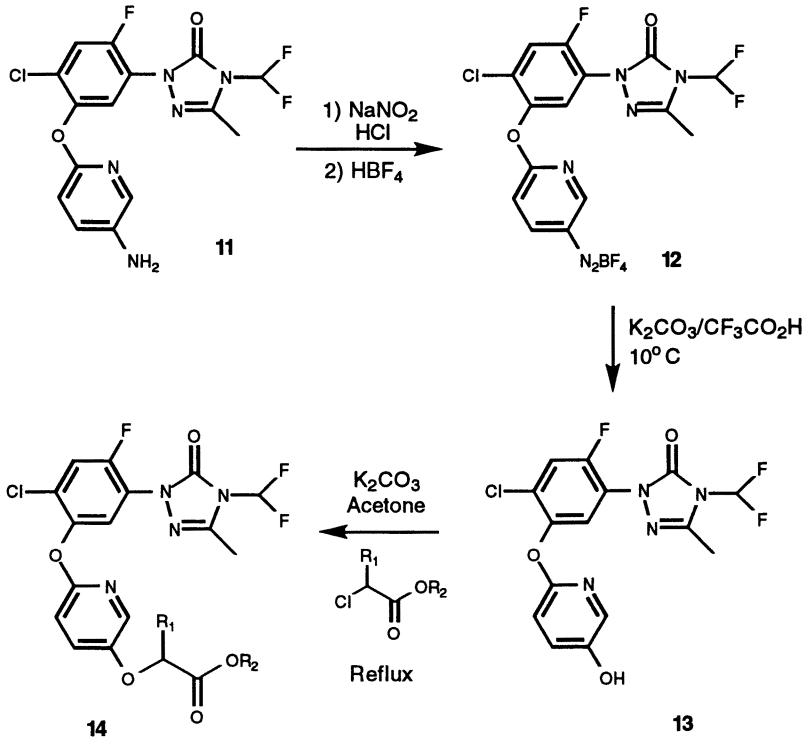


Figure 4. Synthesis of phenoxy-2-pyridyloxy -5-oxypropionates.

The synthesis of the propionate derivatives **15**, where the oxygen from the oxypropionate moity has been replaced with a methylene group, was accomplished by using the Meerwein reaction. Diazotization of the amino group of compound **4** with sodium nitrite and concentrated hydrochloric acid in acetone as a solvent followed by the addition of excess alkyl acrylate and cuprous chloride gave the desired product **15** in excellent yields (Figure 5).

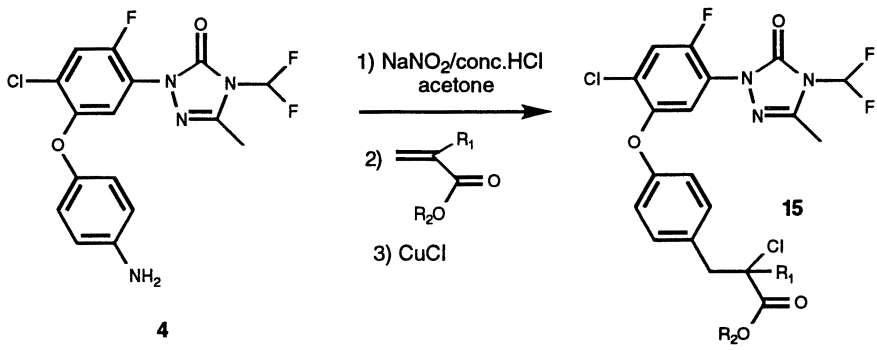


Figure 5. Synthesis of para-phenoxyphenyl-alpha-chloropropionates.

Biological Testing

The compounds described were tested preemergence and postemergence on various weeds and crops in the greenhouse The seeds of the plant test species were planted in furrows in steam-sterilized sandy loam soil contained in disposable fiber flats. A topping soil of equal portions of sand and sandy loam soil was placed uniformly on top of each flat to a depth of approximately 0.5 cm.

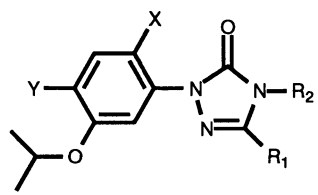
The flats were placed in a greenhouse and watered for 8-10 days, then the foliage of the emerged test plants was sprayed with a solution of the test compound in acetone-water containing up to 5 ml liter $^{-1}$ sorbitan monolaurate emulsifier/solubilizer. The concentration of the test compound in solution was varied to give a range of application rates.

Phytotoxicity data were taken as percentage control, determined by a method similar to the 0-100 rating system described previously (*12*), with 0% control of crops or weeds showing no effect relative to controls, and 100% control indicating complete crop or weed destruction. Biological data in Tables I-VI are presented as the postemergence application rate required to give 90% control as compared with untreated plants. In general, the 95% confidence interval for individual ED_{90} values in these tests is $ED_{90}/2$ to $ED_{90}x2$ (e.g., the CI for an ED_{90} of 30 g ha^{-1} is 15-60). The weeds species used in this study were morningglory, velvetleaf, johnsongrass, green foxtail, and barnyardgrass.

Structure-Activity Relationships

Aromatic substitution

Structure-activity studies were directed towards the optimization of the phenoxypropionate ring and the propionate group. We have previously discussed the structure-activity relationship of both the triazolinone ring and the aromatic ring of related herbicides **16**. The conclusion from that study was that the following chemical groups X=F, Y=Cl, R_1=CH_3 and R_2=CHF_2 were required for optimum biological activity(*7*).



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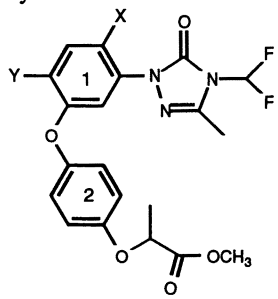
Representative examples with different X and Y groups were prepared in the phenoxyphenoxy propionate chemistry, and it was found that the same structure-activity relationship rules that applied to the molecule shown above also applied to the phenoxyphenoxy propionate chemistry. The most active compounds had X=F and Y=Cl (Table I).

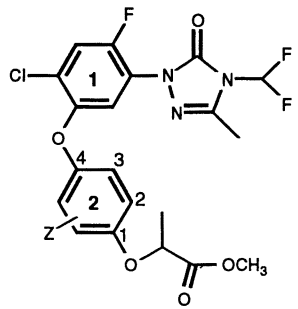
Table I. Effect of Groups at Positions 2(X) and 4(Y) of Aromatic Ring 1 on Biological Activity



X	Y	Rate required to provide 90% control of both morningglory and velvetleaf when applied postemergence (grams/ha)
F	Cl	7.8
Cl	Cl	31.3
H	Cl	1000
Cl	H	2000
H	H	2000

Halogens at position 3 of the aromatic ring 2 resulted in compounds with biological activity comparable to that of the parent compound. This is particularly true when Z=3-chlorine and 3-fluorine. Substitution at the 2 position of ring 2 resulted in a dramatic reduction of biological activity (Table II).

Table II. Effect of Position 2 and 3 of Aromatic Ring 2 on Biological Activity

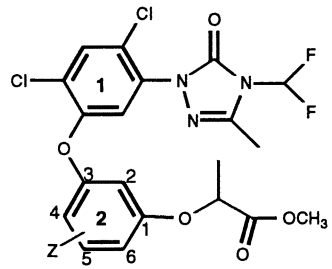


Z	Rate required to provide 90% control of morningglory, velvetleaf, johnsongrass and barnyardgrass when applied postemergence (grams/ha)
H	31.3
3-F	31.3
3-Cl	31.3
2-Cl	125

When the oxypropionate group was in the meta position, the resulting compounds were found to have comparable biological activity to that of the corresponding para- phenoxyphenoxy propionate analog. Analogs with a halogen in position 4 of ring 2 retained biological activity, while introducing halogens at both positions 4 and 6 resulted in loss of biological activity. When groups such as NO_2 and NH_2 were introduced at position 4 of ring 2, the resulting compounds were significantly less active (Table III).

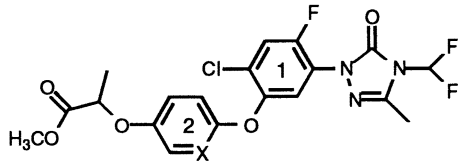
Replacing the aromatic ring 2 in compound **6** with a 2-pyridine group resulted in a somewhat less active molecule (Table IV).

Table III. Meta Substituted Phenoxyphenoxy Propionate Derivatives



Z	Rate required to provide 90% control of morningglory, velvetleaf, johnsongrass and green foxtail when applied postemergence (grams/ha)
H	62.5
4-F	62.5
4-F,6-Cl	125
4-NO_2	250
4-NH_2	>500

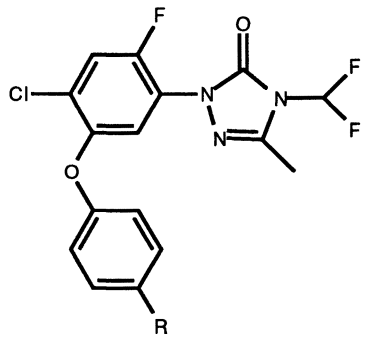
Table IV. Effect of Pyridine Ring 2 on Biological Activity



X	Rate required to provide 90% control of morningglory, velvetleaf, johnsongrass and barnyardgrass when applied postemergence (grams/ha)
CH	31.3
N	62.5

We have previously mentioned the importance of the presence of the ester side chain on phenyl ring 2 in our original design of the phenoxyphenoxy herbicides. Several other R groups were investigated and they resulted in compounds with significantly less biological activity. It is interesting to note that the compound where R=CO_2CH_3 is significantly less active than the compound where R=$OCH(CH_3)CO_2CH_3$, pointing out the need for the two atom linkage between the ester and the aromatic ring for optimum biological activity (Table V).

Table V. Effect of Phenyl Ring 2 Substituents on Biological Activity

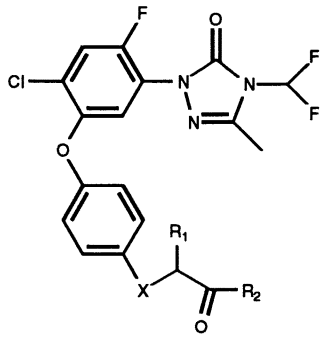


R	Rate required to provide 90% control of morningglory, velvetleaf, johnsongrass and barnyardgrass when applied postemergence (grams/ha)
$OCH(CH_3)CO_2CH_3$	31.3
OCH_3	500
CO_2CH_3	500
H	1000
NH_2	4000
NO_2	>4000

Effect of X, R_1 and R_2 groups of the ester side chain on biological activity

Several acid derivatives of the ester side chain in ring 2 were prepared, including amides, acids and their salts. The best control of both broadleaf and grass weeds was obtained with the ester derivatives, particularly when X=O, R_1=CH_3 and R_2=OCH_3. The amides and acids, though highly active, were not as effective in controlling grass weeds. It is interesting to note that replacing oxygen with a methylene group, to give R=$CH_2CH_2CO_2CH_3$, resulted in excellent broadleaf weed control with a significant decrease in grass weed control. Replacement of the oxygen atom with an NH group, X=NH, resulted in a dramatic loss of biological activity. The broadleaf weeds morningglory and velvetleaf, and the grass weeds barnyardgrass and johnsongrass, were used for Table VI.

Table VI. Effect of X, R_1 and R_2 Groups of the Ester Side Chain on Biological Activity



			Rate required to provide 90% control of weeds tested (grams/ha)	
X	R_1	R_2	Broadleaf	Grass
O	CH_3	OCH_3	7.8	31.3
O	CH_3	NH_2	15.6	62.5
O	CH_3	OH	7.8	62.5
O	H	OCH_3	7.8	62.5
CH_2	Cl	OCH_3	3.9	>125
NH	CH_3	OCH_2CH_3	4000	>4000

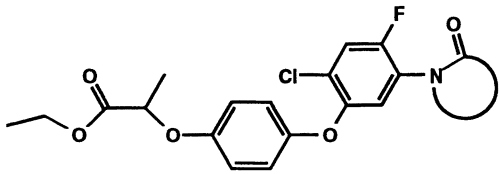
Effect of heterocyclic ring on biological activity

In addition to the triazolin-5-one, we investigated a number of other heterocyclic rings. In general the triazolin-5-one gave the best overall activity, providing good control of both broadleaf and grass weeds. The tetrahydrophtalimide and the hydantoin heterocycles required higher application rates than the triazolin-5-one for broadleaf weed control, while grass weed control for these two rings was in general poor (Table VII).

Potential of heteroaryl phenoxyphenoxy oxypropionates as rice herbicides

Several compounds in this new class of postemergence herbicides have shown promise as potential rice herbicides. Compound **17** provided excellent control of a number of weeds when applied postemergence at rates as low as 3.9 g/ha in greenhouse tests. Rice was tolerant at this rate (Table VIII).

Table VII. Effect of Heterocyclic Ring on Biological Activity



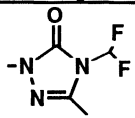
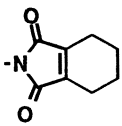
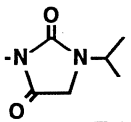
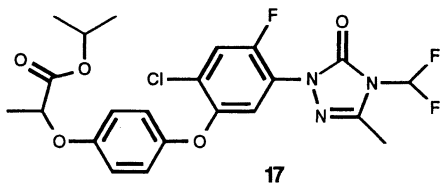
Heterocyclic ring	Rate grams a.i./ha	Greenhouse postemergence activity phytotoxicity(% control) Velvetleaf	Morningglory	Barnyard grass	Johnson grass
	7.8	100	100	40	60
	31.3	100	100	90	80
	15.6	90	95	20	20
	62.5	100	100	30	20
	62.5	100	95	40	30
	250	100	100	70	60

Table VIII. Potential of phenoxyphenoxy oxypropionates as rice herbicides



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Rate grams a.i./ha	Rice	Grenhouse postemergence activity phytotoxicity (% control) Cocklebur	Velvetleaf	Morningglory	Barnyard grass	Nightshade
3.9	5	100	100	100	20	100
7.8	5	100	100	100	40	100
15.6	10	100	100	100	50	100
31.3	20	100	100	100	90	100

Summary

The 1-(2,4-dihalo-5-phenoxyphenyl)-4-difluoromethyl-4,5-dihydro-3-methyl-1,2,4-triazolin-5(1H)-ones **1** are a new class of highly active postemergence herbicides providing excellent weed control at rates as low as 7-15 grams/hectare. Crop selectivity was observed in some cases, such as the rice selectivity obtained with compound **17**. The rational design of this novel class of herbicides was based on the assumption that peroxidizing herbicides acted as substrate inhibitors of tetrapyrrole handling enzymes in the chlorophyll synthesis pathway. This approach allowed us to discover not only a highly active class of herbicides but also an area in which chemistry deviated greatly from that of previously known peroxidizing herbicides.

Acknowledgments

The authors would like to acknowledge the contributions of Blaik P. Halling, Debra A. Witkowski and M. Joan Plummer for their work in elucidating the mechanism of action of these herbicides (*4,6*). We also would like to express our thanks to James T. Bahr and William A. Van Saun for their encouragement and advice. Finally, the authors acknowledge the support of FMC Corporation.

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Chapter 9

Alkyl 3-[2,4-Disubstituted-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)phenyl]propenoate Derivatives

Synthesis and Structure–Activity Relationships

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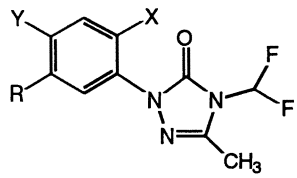
Structure-activity investigations of the five position of the aryl ring of 1-(2,4,5-trisubstitutedphenyl)-4,5-dihydro-1,2,4-triazol-5(H)-one **1** resulted in the discovery of a highly active class of postemergence herbicides with excellent control of key broadleaf weeds. The synthesis and structure-activity relationship of these compounds are discussed. The weed control and crop selectivity of F8426 are presented in detail.



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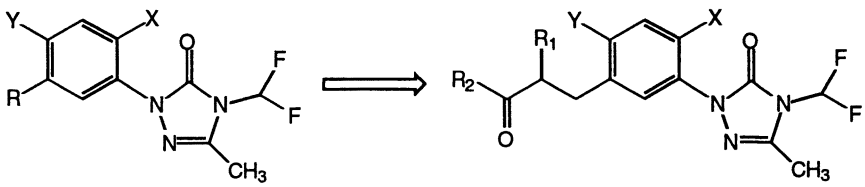
Our previous structure-activity investigations of aryltriazolinone herbicides **1**, have resulted in a number of significant discoveries, including sulfentrazone (**1**, X, Y = Cl; R = $NHSO_2CH_3$), a soybean herbicide currently in development by FMC Corporation (*1,2,3*). Recently we discovered that a 2-halopropionate ester group at the five position of the phenyl ring results in a highly active class of postemergence herbicides with good cereal tolerance (*4*).



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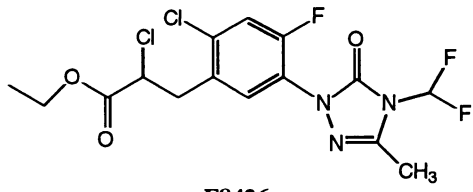
F8426, is a new experimental postemergence herbicide in development at FMC Corporation (*5*). The mechanism of action was found to involve the inhibition of the enzyme protoporphyrinogen oxidase (Protox), which results in the build-up of a photodynamic toxicant, protoporphyrin IX (*6-11*). Applied postemergence, F8426 herbicide causes rapid dessication of sensitive weed species at field rates between 4 and 35 g/ha. Soil activity of F8426 is observed at higher rates, 70 to 500 g/ha.



F8426

Synthesis

Synthesis of this class of compounds involves the use of substituted arylhydrazines as starting materials to prepare the corresponding substituted aniline triazolinone intermediates **2**, as previously reported (*12*). The Meerwein reaction was used for the synthesis of the alkyl halopropionate chain. Treatment of the aniline intermediate **2** with tert-butyl nitrite, alkyl acrylate and copper II chloride in acetonitrile gives the desired product **3** in excellent yields (*4,13*) (Figure 1).

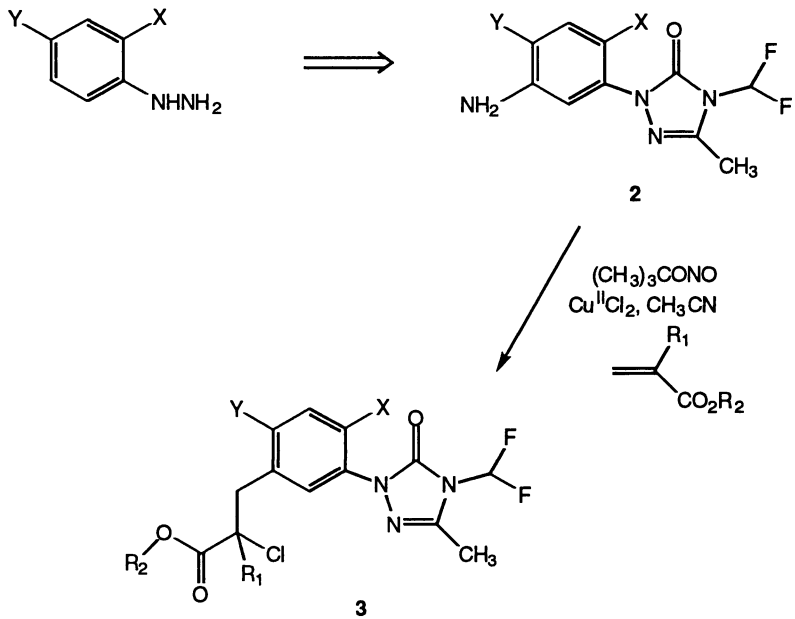


Figure 1. General synthesis of aryltriazolinones.

An alternative synthesis involves the use of 2-chloro-4-fluoro-5-nitrobenzaldehyde **4** as starting materials (*4*). The aldehyde is protected during the synthesis of the heterocycle by reacting it with 1,3-propanedithiol. The protecting group was then removed to regenerate the aldehyde **6** which was reacted with (carbethoxymethylene) triphenylphosphorane. The trans isomer **7** is obtained which can then be hydrogenated to the saturated propionate derivative **8** as shown in Figure 2.

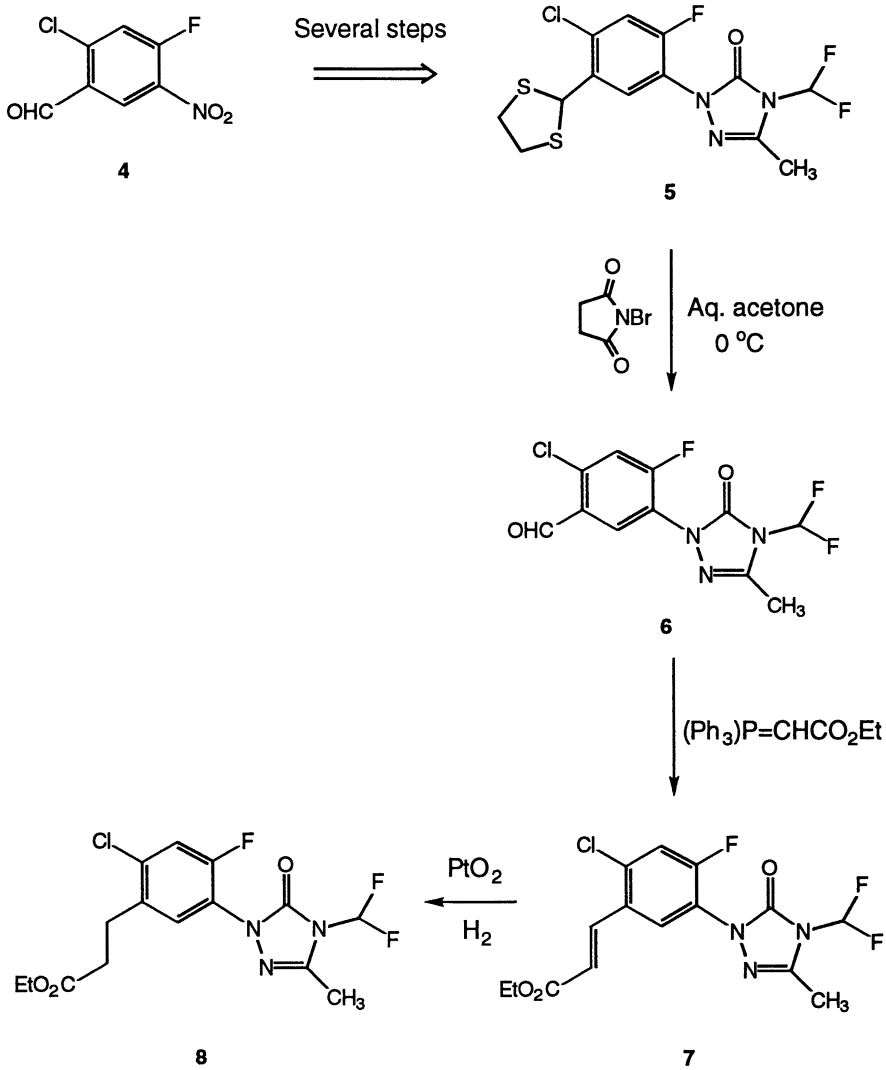


Figure 2. Alternative synthesis of aryltriazolinones.

Biological Testing

The compounds described were tested preemergence and postemergence on various weeds and crops in the greenhouse. All biological data for Tables I-VIII refer to postemergence application. The seeds of the plant test species were planted in furrows in steam-sterilized sandy loam soil contained in disposable fiber flats. A topping soil of equal portions of sand and sandy loam soil was placed uniformly on top of each flat to a depth of approximately 0.5 cm.

The flats were placed in a greenhouse and watered for 8-10 days, then the foliage of the emerged test plants was sprayed with a solution of the test compound in acetone-water containing up to 5 ml liter $^{-1}$ sorbitan monolaurate emulsifier/solubilizer. The concentration of the test compound in solution was varied to give a range of application rates.

Phytotoxicity data were taken as percentage control, determined by a method similar to the 0-100 rating system described previously (*14*) , with 0% control of crops or weeds showing no effect relative to controls, and 100% control indicating complete crop or weed destruction. The biological data in Tables I- VIII are presented as the postemergence application rate required to give 90% control as compared with untreated plants. In general, the 95% confidence interval for individual ED_{90} values in these tests is $ED_{90}/2$ to $ED_{90}x2$ (e.g., the CI for an ED_{90} of 30 g ha^{-1} is 15-60). The weeds species used in this study were morningglory and velvetleaf.

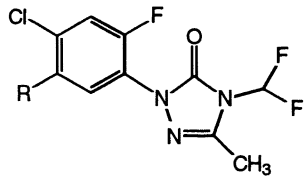
Structure-Activity Relationships

Aromatic Substitution. The pre- and postemergence activity of aryl triazolinone herbicides is greatly affected by the substituents in the aromatic portion of the molecule. We have previously discussed the effect that various chemical groups at position five of the phenyl ring have on preemergence biological activity, weed control spectrum and crop tolerance of aryl triazolinones (*12*). In that study we concluded that the substituents at position five of the phenyl ring not only influence the degree of preemergence herbicidal activity but also the weed spectrum and crop tolerance. In the present work we found that the substituents at position five of the phenyl ring also influence the degree of postemergence biological activity as well as the weed spectrum and crop tolerance. For instance, R groups such as the methoxy and the propargyloxy resulted in compounds that provided good broadleaf weed control at low application rates, but their presence also resulted in high wheat injury. When R=NH_2 or OC_6H_5, the resulting compounds were considerably less active than the parent compound, R=H. In contrast, the ethyl 2-chloropropionate group is able to provide both good weed control and wheat tolerance (Table I).

The structure-activity relationship of chemical groups at the 2 and 4 position of the phenyl ring parallels that of related aryl triazolinone herbicides (*12*). Compounds where X=F and Y=Cl provided the best postemergence biological activity, with compounds where X=F and Y=Br providing comparable activity. When X and Y were both chlorine or fluorine there was a significant reduction of activity (Table II).

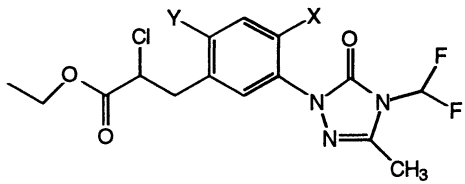
The ester group was replaced with a variety of other chemical groups, all of which resulted in a dramatic reduction in biological activity. This was particularly true when the ester group was replaced with a methanesulfonyl or a phenyl group (Table III).

Table I. Effect of R Groups at Position Five of Aromatic Ring on the Postemergence Biological Activity of Aryltriazolinones



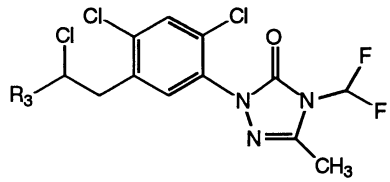
R	Rate required to provide 90% control of weeds tested (g/ha)	Rate required to provide 20% wheat injury (g/ha)
$CH_2CHClCO_2Et$	15	250
$OCH_2C{\equiv}CH$	15	8
OCH_3	100	100
H	250	250
OC_6H_5	1000	100
NH_2	2000	500

Table II. Effect of Groups X and Y of Aromatic Ring on Postemergence Biological Activity



Substituents		Rate required to provide 90% control of weeds tested
X	Y	(g/ha)
F	Cl	15
F	Br	15
Cl	Cl	30
F	F	300

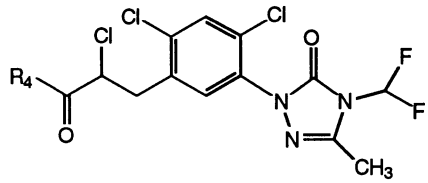
Table III. Effect of R_3 Group on Postemergence Biological Activity



R_3	Rate required to provide 90% control of weeds tested (g/ha)
CO_2Et	15
$PO(OEt)_2$	250
CN	300
SO_2CH_3	>300
C_6H_5	>300

Several carboxylic acid derivatives were prepared including a variety of esters, amides and substituted amides. The ester derivatives provided the best postemergence broadleaf weed activity and wheat tolerance. The ester derivatives were more active than the corresponding acid derivatives. The amide derivatives, though fairly active, resulted in higher wheat phytotoxicity than the esters (Table IV).

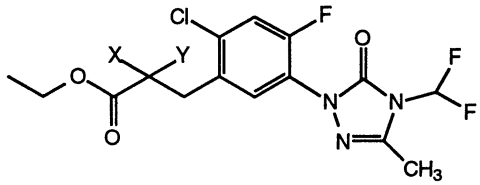
Table IV. Effect of Carboxylic Acid Derivatives on Postemergence Biological Activity



R_4	Rate required to provide 90% control of weeds tested (g/ha)	Rate required to provide 20% wheat injury (g/ha)
OCH_3	30	125
OCH_2CH_3	30	125
OH	125	60
NH_2	125	30
$NHCH_3$	125	60

Of all the chemical groups investigated at the alpha-position of the propionate group, X=H and Y=Cl gave the highest biological activity. Replacement of the alpha-chlorine with hydrogen, bromine or a methyl group resulted in a reduction of biological activity (Table V).

Table V. Effect of Substituents in the Propionate Group on Postemergence Biological Activity



Substituents		Rate required to provide 90% control of weeds tested
X	Y	(g/ha)
H	Cl	15
CH_3	Cl	30
H	Br	300
H	H	300
H	CH_3	300

F8426 Herbicidal Activity

F8426 was tested in small plot field trials in the United States, Canada, Europe, selected east Asian countries and Australia during several seasons. The United States field performance of F8426 alone at 28 to 40 days after treatment at 35 grams per hectare is shown here (Table VI). These data are from the 1990 to 1992 testing seasons for the 50 and 240 gram per liter EC formulations. The number of observations varies from as few as two for common sunflower to as many as 19 for kochia. Weed control varies from only 61% control of wild buckwheat to 98% control of field pennycress. For most species control is in the 80 to 90% range; wild buckwheat and three of the mustard species were less well controlled. For some weed species F8426 does not kill the growing point of all plants present.

We have conducted one field trial with sulfonylurea-resistant kochia. Both the EC formulation of F8426, and F8426 combined with 2,4-D ester, provided good to excellent control of this kochia population, while Harmony® Extra herbicide failed to provide either control or suppression (Table VII).

Table VI. Postemergence Weed Control of F8426 Under Field Conditions in the United States

Species	35 g/ha
kochia	87(19)
pigweed	85(7)
Russian thistle	83(9)
wild buckwheat	61(18)
common lambsquarters	88(9)
common sunflower	94(2)
velvetleaf	92(5)
shepherd's purse	97(4)
smallseed falseflax	69(3)
blue mustard	73(10)
tansy mustard	70(7)
flixweed	83(14)
bushy wallflower	91(5)
wild mustard	83(14)
field pennycress	98(5)

Table VII. F8426 Postemergence Control of Sulfonyl Urea-Resistant Kochia

	% Kochia Control (30 DAT)
Harmony® Extra herbicide 26 g/ha	25
F8426 35 g/ha	83
F8426 35 g/ha plus 2,4-D ester140 g/ha	91
2,4-D ester 280 g/ha plus Dicamba 140 g/ha	95

F8426 has similar tolerance in other cereal crops. A side-by-side field trial was conducted to compare multiple cultivars and multiple cereal crops. The injury at seven days after treatment to three-leaf stage crops ranged from 4% to 8% at the 35 gram per hectare rate. At the 70 grams per hectare rate, the injury varied from 7% to 13%. There were no significant differences among cultivars nor among the several cereal crops (Table VIII).

In Europe, F8426 provides control or strong suppression of several important weeds common in cereal crops when applied postemergence. At an application rate of 20 g AI/ha F8426 controlled *Galium aparine*, *Veronica hederfolia*, *Veronica persica*, *Capsella bursa-pastoris*, *Chenopodium album*, and *Lamium purpureum* (*5*).

Table VIII. Cereal Tolerance to Postemergence Application of F8426

Side-by-side Trial		% Necrosis at 7 DAT	
Crop/cultivar		35 g/ha	70 g/ha
Spring Wheat	Butte	7	11
	Marshall	6	10
	Vance	6	10
Winter Wheat	Cardinal	5	11
	Caldwell	6	10
	Howell	5	8
Spring Barley	Robust	7	13
	Azure	7	11
	IFS:2619D	7	11
Winter Barley	Pike	8	9
	Wysor	6	11
Winter Oats	Ogle	6	12
Winter Rye	IFS:5432	4	7

Summary

F8426 is a new postemergence broadleaf herbicide for use in cereal crops. It will offer growers a new, low-rate, herbicide for cereals. It should be a significant contributor to the management of sulfonylurea-resistant broadleaf weed populations.

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Chapter 10

Tetrahydropyridazine Derivatives

Synthesis, Reactivity, and Herbicidal Activity of Heterocycle Fused Tetrahydropyridazines

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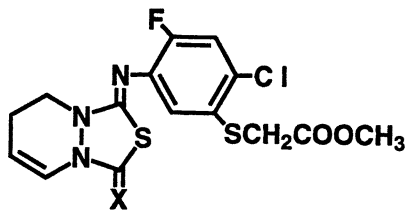
NCI-876648; methyl [[2-chloro-5-[(7,8-dihydro-3-oxo-1H, 3H-[1,3,4] thiadiazolo[3,4-a] pyridazin-1-ylidene) amino]-4-fluorophenyl] thio] acetate **1a**, and NCI-876649; methyl [[2-chloro-5-[(7,8-dihydro-3-thioxo-1H, 3H-[1,3,4] thiadiazolo[3,4-a] pyridazin-1-ylidene) amino]-4-fluorophenyl] thio] acetate **2a** are novel postemergence soybean herbicides. **1a** and **2a** belong to a new class of light dependent herbicides. The transformations of **1a** and **2a** to the corresponding triazolopyridazines **12a** and **13a** proceeded in good yield in the presence of nucleophiles, but the ring opened compounds (eg.**14a**) were obtained when the nucleophiles had a weak leaving ability. Although the triazolopyridazines were more active on weeds than the tetrahydropyridazines **1a** and **2a**, the soybean selectivity of the triazolopyridazines were inferior. It is suggested that the transformations of tetrahydropyridazines, **1a**, **2a**, and the soybean selectivity are closely related.



NCI-876 648, 1a (X=O)
NCI-876 649, 2a (X=S)

A number of herbicides have been used to protect and increase the productivity of important crops such as soybean, corn, wheat, rice, *etc.* In recent years, a need to control weed growth by using lower doses of the highly active agents has arisen from the desire to decrease both environmental impact and the cost of cultivation for farmers.

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We researched a light dependent herbicide which has proven highly effective in controlling the growth of many types of weeds at low dosage. It was difficult, however, for us to make compounds that showed excellent margins of broadleaf crop safety and high herbicidal activity in a post emergence treatment. We introduced heterocycle-fused, partially unsaturated or saturated, pyridazines into the tetrahydrophthalimide herbicide structure to improve the chemical properties , herbicidal activity, and crop selectivity. This exercise resulted in the discovery of the novel post emergence herbicides NCI-876648 **1a** (X=O) and NCI-876649 **2a** (X=S) for soybeans (*1,2*). These potent soybean herbicides are being developed by Nissan Chemical Industries Ltd. The key chemical feature of these compounds is the tetrahydro-pyridazine system which shows unique reactivity. **1a** and **2a** belong to a new class of light dependent herbicides that leads treated plants to accumulate protoporphyrin IX. They are effective in providing broadleaf control and soybean safety when applied post emergence at rates from 25 to 50g a.i./ha.

In this paper, our approach to the synthesis, the relationship of structure and activity, and the biological activity of these herbicides and the related compounds, will be discussed.

Research Approaches with THPD

During the course of our herbicidal research, we made an accidental finding (Figure 1). In an effort to synthesize hexahydropyridazine **4** (*3*), we attempted to hydrolyze hexahydropyridazine-1,2-dicarboxylate **3**, but discovered instead an unexpected compound identified as *1,4,5,6-tetrahydropyridazine* (**THPD**) which is known in the literature (*4*). It would appear that due to prolonged reaction time with exposure to air **4** was changed to THPD by oxidation. We have utilized THPD as a useful building block as well as other 1,2-dihydropyridazines.

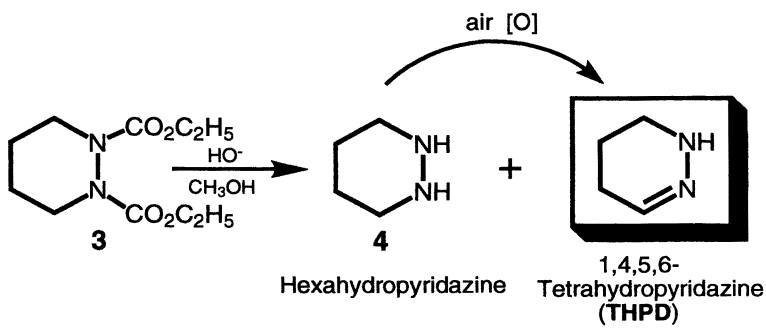

Figure 1. THPD Formation by Hydrolysis and Oxidation

We planed to introduce the heterocycle-fused pyridazines into the "A part" of the molecule that is a typical of light dependent herbicides (Figure 2). The introduction of nitrogen and sulphur atoms were intended to change the original properties such as lipophilicity, and solubility. The introduction of a partially unsaturated bond was maintained to provide a metabolic position in the molecules to give a selectivity between weeds and crops.

Figure 3 shows the new heterocycle-fused pyridazines and their relative broadleaf post emergent herbicidal activities. R represents 4-chloro-2-fluorophenyl group and X

means either oxygen and sulphur. Among these heterocycles, partially saturated thiadiazolopyridazines and partially saturated triazolopyridazines were highly effective on broadleaves when applied by either pre- or postemergence treatment. We then synthesized analogs of these compounds for activity optimization.

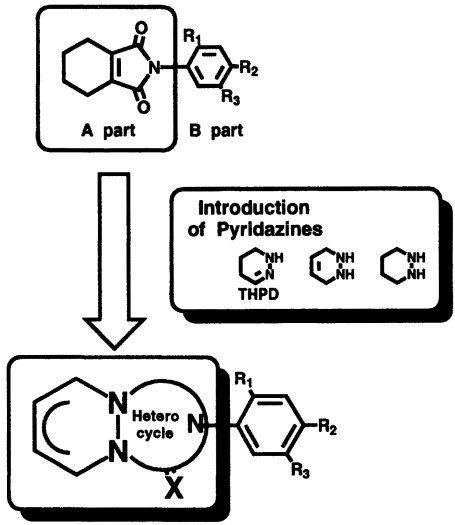

Figure 2. Our Research Approach to Light Dependent Herbicide

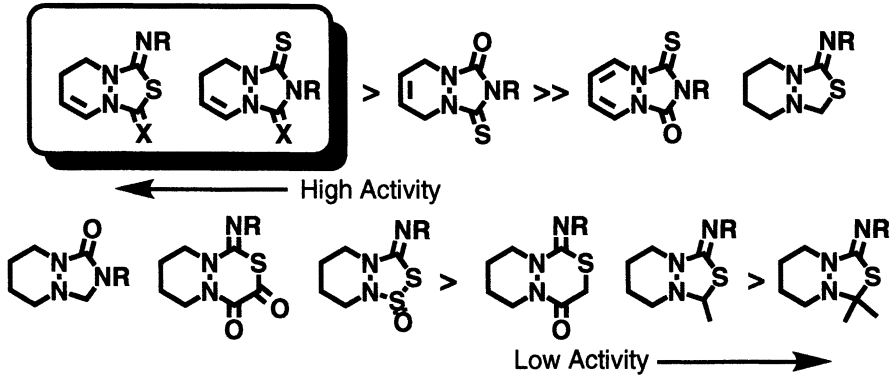

Figure 3. New Heterocycle-fused Pyridazines and Their Relative Herbicidal Activities

Chemical Results and Discussion

Facile Synthesis of THPD. For further research work, we needed a more efficient synthesis of THPD (Figure 4). A few syntheses of THPD are known in literature (*3,4*), but these were impractical because they were multistep. We have developed a facile syntheses of THPD (*5,6*). The first was *via* 2-(4-chlorobutyl)-1,3-dioxorane **5** which could be obtained by the *Rosenmund* reduction of 4-chlorobutyrylchloride (*7*). 2-(4-chlorobutyl)-1,3-dioxorane was allowed to react with hydrazine mono hydrate to yield 2-(4-hydrazinobutyl)-1,3-dioxorane **6**, which was then deprotected with dilute hydrochloric acid solution to afford THPD in 89% overall yield.

The second route was not so high yielding, a mere 21%, but 1,4-diaminobutane **7** is readily available and may be oxidized with sodium hypochloride solution to form THPD directly. We couldn't isolate the azo-compound **8**, but this reaction could possibly proceed through **8**, as **8** is unstable and quickly isomerizes to THPD under basic conditions (*4*).

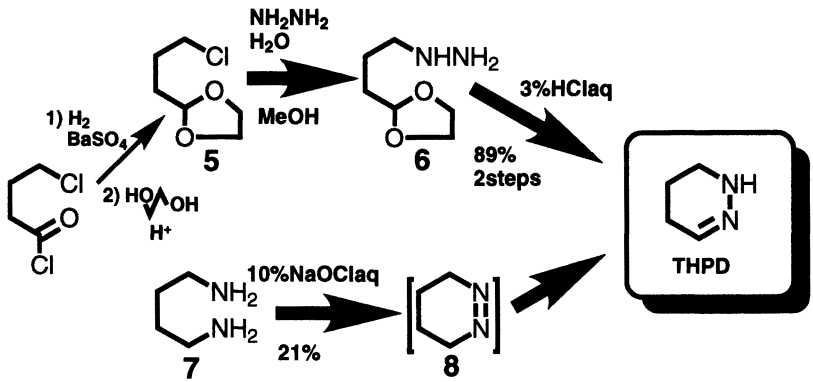

Figure 4. More Efficient Synthesis of 1,4,5,6-Tetrahydropyridazine (THPD)

New Synthesis of Thiadiazolopyridazines. The key steps of the new synthetic routes to partially saturated thiadiazolopyridazines **1**, and **2** are given in Figure 5 (*8-10*).

The first step is the reaction of THPD with isothiocyanates. THPD was dissolved in an inert solvent such as THF or benzene at room temperature and the resulting solution was treated with one equivalent of a phenylisothiocyanate which was made from the corresponding aniline (*11,12*) with thiophosgene in the usual manner (*18*). After several hours the mixture was evaporated *in vacuo*. The crude product was washed with water, then purified *via* chromatography to give the corresponding thiosemicarbazone **9** in a 63-93% yield.

The second step is the intramolecular cyclization reaction. One equivalent of **9** and two equivalents of a base such as pyridine, triethylamine, N,N-dimethylaniline, or DBU were dissolved in dichloromethane at 0 °C. To this mixture, one equivalent of thiophosgene, phosgenedimer, or phosgenetrimer was added at 0 °C, with stirring and then allowed to gradually warm to room temperature. After completion, ice water was added, and the organic layer was washed with water and brine, then dried with anhydrous sodium sulfate and given the usual work up and purification. The resulting

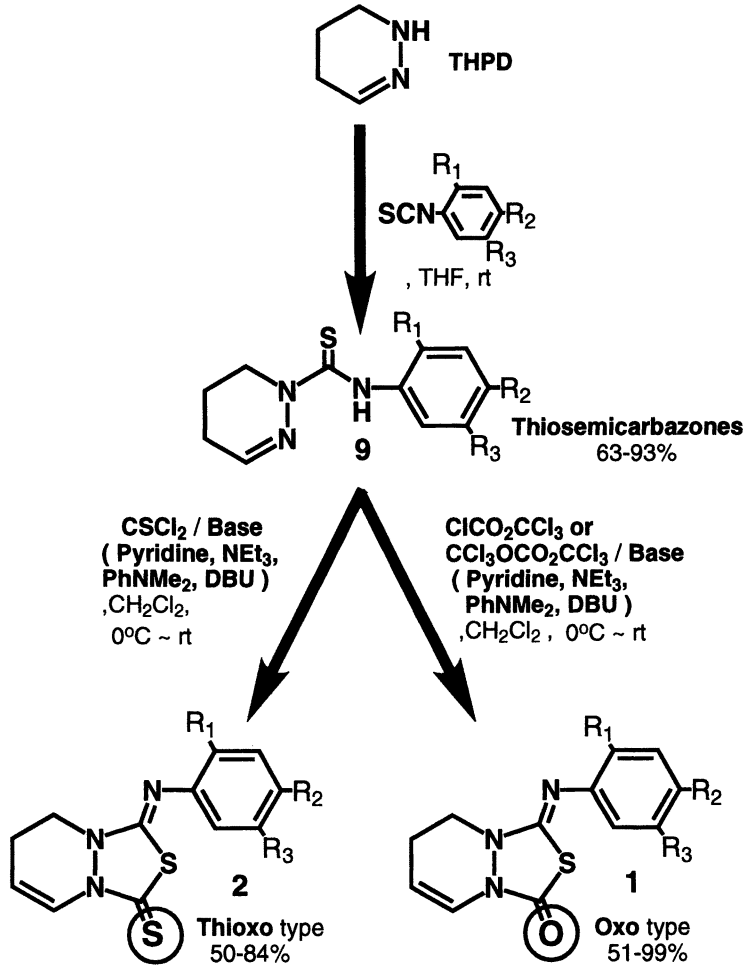

Figure 5. New Synthetic Route of Thiadiazolopyridazines by THPD

products **2** (Thioxo type) and **1** (Oxo type) were obtained in 50-84% and 51-99% yield respectively.

Figure 6 illustrates a postulated pathway of cyclization for compounds **1** and **2** (*13*). Thiosemicarbazones **9** can exist in the enole form **9a** which reacts with one equivalent of a phosgene derivative ($CXCl_2$; X=O,S) in the presence of one equivalent base to afford compound **10**. Subsequently, with one equivalent base, **10** (X=O,S) was

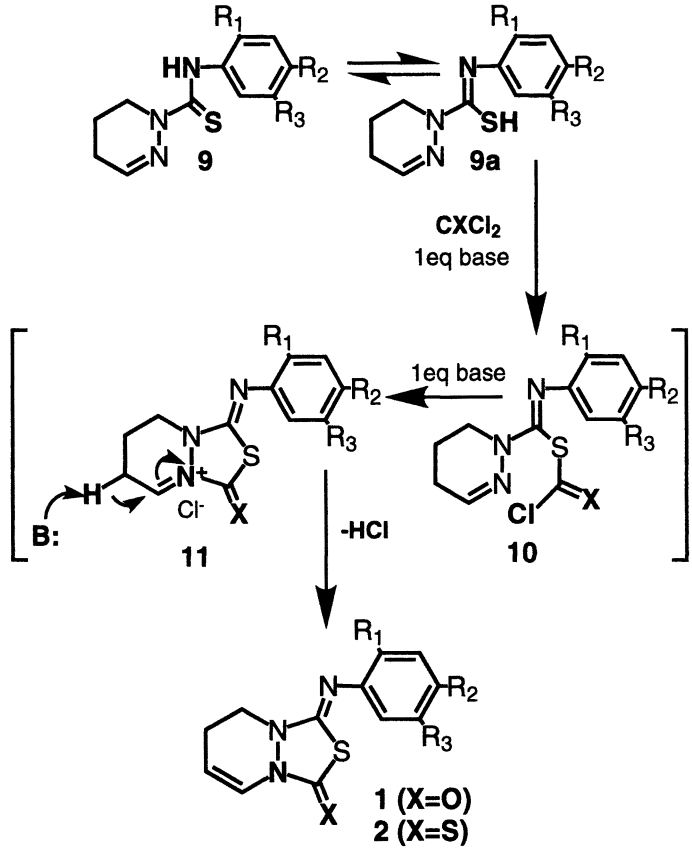

Figure 6. Postulated Pathway of Cyclization

converted to **11** (X=O,S, B: = base) by intramolecular cyclization. The cyclization and elimination reactions may take place in a concerted fashion to form **1** (X=O) and **2** (X=S).

Rearrangement of Thiadiazolopyridazines with Nucleophiles. The reactions of **1a** with nucleophiles such as alkoxides and amines are shown in Figure 7 (*14*). **1a** easily reacted with a catalytic amount of sodium methoxide to give the corresponding triazolopyridazine **12a** in over 90% yield, similarly reaction with one equivalent of dimethylamine, also produced **12a** in 82% yield. However, with one equivalent of methylamine, **1a** gave the ring opened compound **14a** in an 87% yield. Two possible explanations for the failure of methylamine to afford **12a** are: 1) The leaving ability of methylamine is lower than that of alkoxide and dimethylamine, and/or 2) The proton of

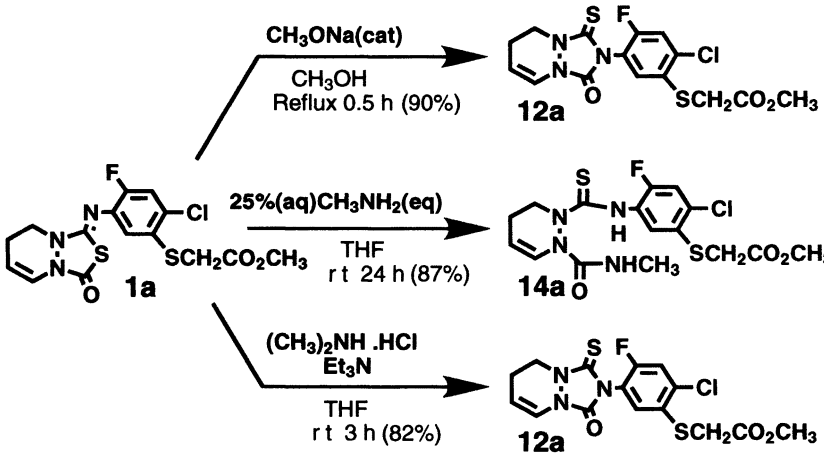

Figure 7. Rearrangement of Thiadiazolopyridazines with Nucleophiles

methylamine may reduce the positive charge at the carbonyl carbon atom through a resonance effect.

The above facts may lead to a possible pathway of ring transformations as illustrated in Figure 8 (*15*). Nucleophiles (Nu:) such as alkoxides and secondary amines which have better leaving abilities attack at a carbonyl bonded to sulphur atom in molecules **1** (X=O) and **2** (X=S). Ring opening affords **14**, and rotation followed by nitrogen as shown anion attack in **15**, produces the triazolopyridazines **12** (X=O) and **13** (X=S). Only one closely related reaction, the transformation of 1,3,4-oxadiazoline-5-one to triazolidine-2,5-dione under basic conditions, is known in literature (*16*). This transformation may be loosely categorized as a *Dimroth* rearrangement.

Preparation of Triazolopyridazines by Ring Transformation. Triazolopyridazines **12** (X=O) and **13** (X=S) may be easily obtained from thiadiazolopyridazines **1** (X=O) and **2** (X=S) by the *Dimroth* type rearrangement (ring transformation) in over 80% yield when bases such as alkoxides, sodium hydride, potassium hydroxide, secondary amines, and *etc.* are present as shown in Figure 9. This rearrangement reaction was a very useful preparation of triazolopyridazines because of the simple procedure and its high yield.

In contrast with the rearrangement reaction, an alternative for the synthesis of triazolopyridazines **19** and **12b** is shown in Figure 10 (*14*). These syntheses involved 1) the treatment of the appropriate aniline **16** with phosgendimer followed by THPD to give the semicarbazone **17** which was reacted with methylchloroformate to afford compound **18**, which was cyclized with a catalytic amount of sodium methoxide to yield triazolopyridazine **19** and 2) treatment of **16** with methylchloroformate to give carbamate **20** which was treated with thiophosgen followed by THPD to produce compound **21**, which was cyclized with a catalytic amount of sodium methoxide to give triazolopyridazine **12b**.

The reaction of **17** with phosgendimer in the presence of bases such as pyridine, triethylamine, and sodium hydride did not afford **19**.

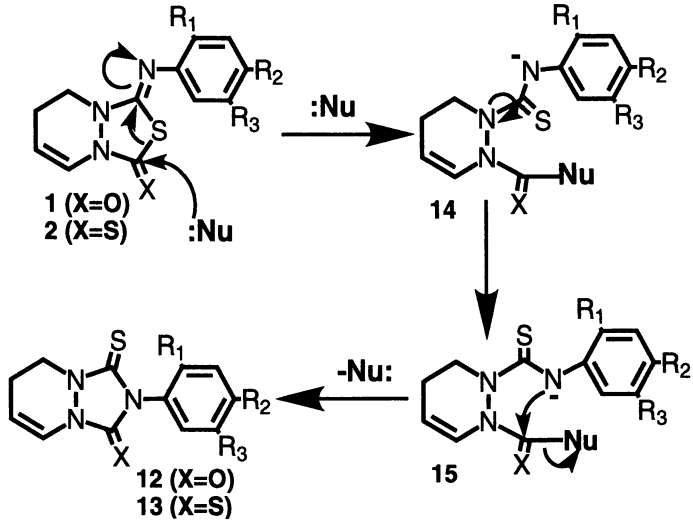

Figure 8. Possible Course of Ring Transformation

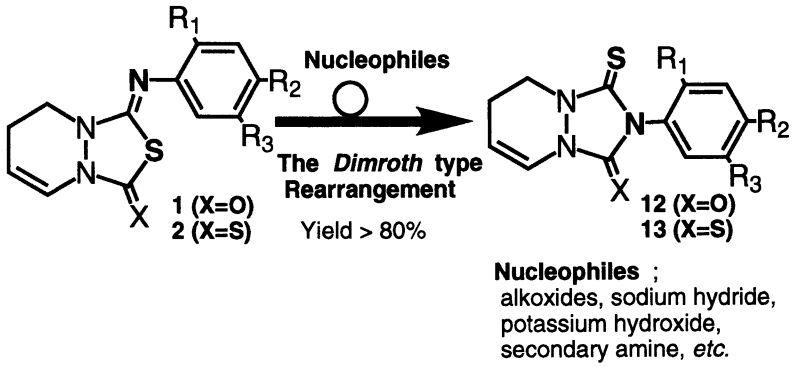

Figure 9. Synthesis of Triazolopyridazines from Thiadiazolopyridazines

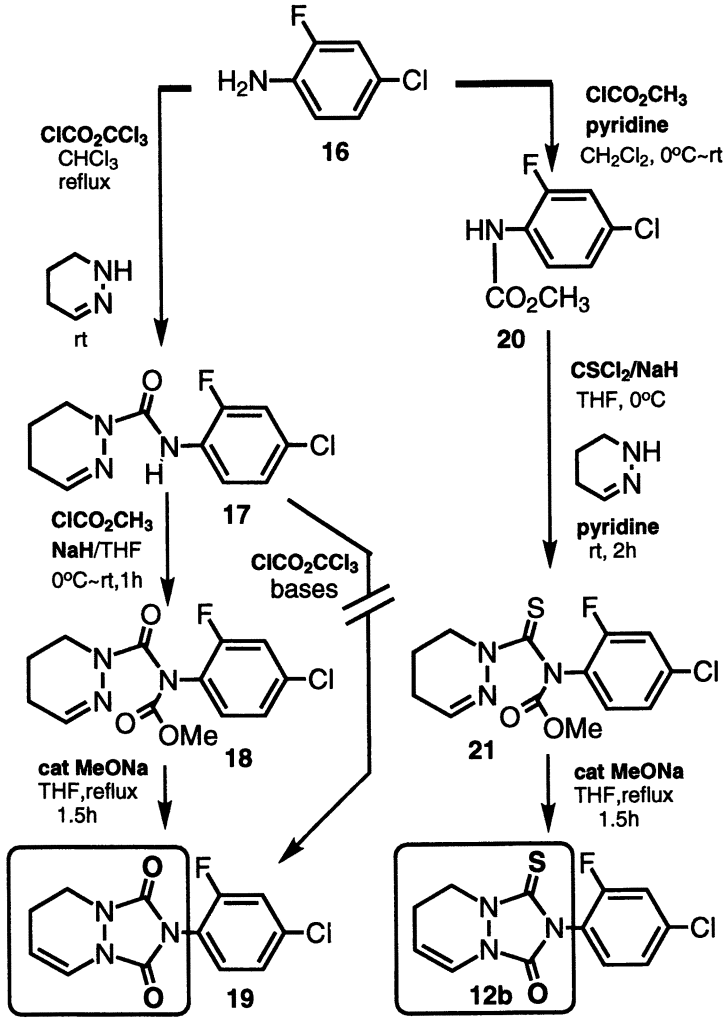

Figure 10. Alternative Synthesis of Triazolopyridazines

Reactivity of Triazolopyridazines for Alcohols. Heating triazolopyridazine **22** with a catalytic amount of sulfuric acid in primary alcohols gave alcohol adducts **23** (*17*). The effect of X-atom (**22**) on alcohol addition is shown in Figure 11. It was obvious that the reactivity of the enamides structure in **22** was effected by X-atom which was either oxygen or sulfur.

Figure 12 illustrates a possible pathway of alcohol addition to enamides **22**. Protonation of **22** gives the cationic intermediate **24** which readily reacts with the alcohol to give **23**. The herbicidal activity of **23** was lower than that of **22**.

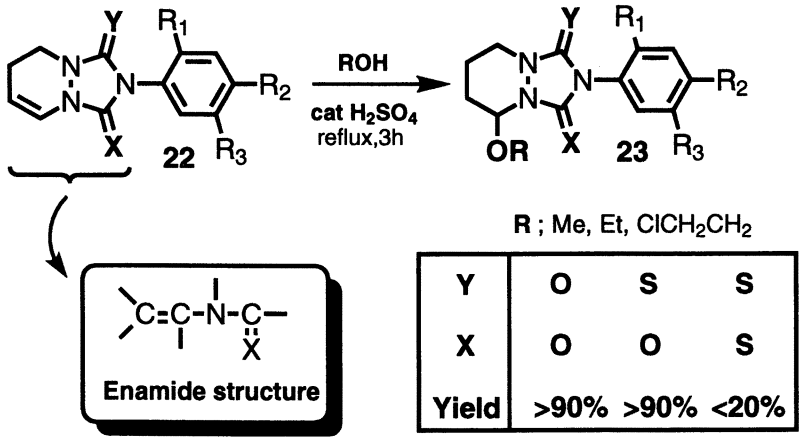

Figure 11. Alcohol Addition to Enamides

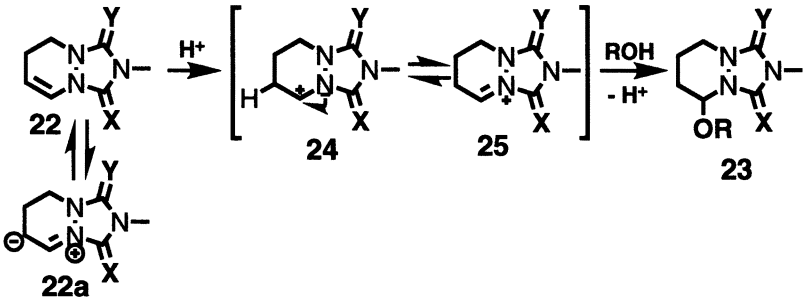

Figure 12. Possible Mechanism of Alcohol Addition to Enamides

Biological Results and Discussion

Effect of Tetrahydropyridazines on Soybean Selectivity. All the compounds described were screened for herbicidal activity on various weeds and crops both pre- and post emergence in a green house. Among these, thiadiazolopyridazines **1** and **2** and triazolopyridazines **12** and **13** were active both in the pre- and postemergence applications. They were generally more effective for broadleaf than grass control when applied post emergence. The spectrum of weed control and the crop selectivity depended on the nature of the heteroring-fused partially unsaturated pyridazines and chemical groups at the fifth position of the phenyl ring (*1*).

The effect of the tetrahydropyridazines (thiadiazolo-, and triazolopyridazines) on the post emergence herbicidal activities for broad-leaved weeds and soybean in the greenhouse at the 2-4 leaf stages is summarized in Table I. These weeds were velvetleaf (*Abutilon theophrasti*), cocklebur (*Xanthium pensylvanicum*), redroot pigweed (*Amaranthus retroflexus*), morningglory (*Ipomoea purpurea*), lambsquaters (*Chenopodium album*), and jimsonweed (*Datura stramonium*). All are important weeds in soybean fields. The application rates were from 10 to 40g a.i./ha. All compounds demonstrated excellent control of broadleaves except cocklebur, while providing good tolerance for soybean. Judging from the effectiveness against cocklebur, triazolopyridazines **12a**, **13a** were more active than thiadiazolopyridazines **1a**, **2a**. Their phytotoxity to soybean was similar. Although **12a** was most active on weeds, an increase in herbicidal activity was accompanied by an increase in soybean phytotoxity, therefore we selected **1a** and **2a** for field trials.

Field trial results in the United States of America are given in Table II (*2*). At rates of 25 to 50g a.i./ha, NCI 876 648 and 649 showed high herbicidal activity against velvetleaf (*Abutilon theophrasti*) and morningglory (*Ipomoea purpurea*) in soybean fields in comparison with the reference herbicides, acifluorfen and bentazone. Twentyone days after treatment, they provided adequate selectivity to soybean and did not produce loss in the soybean crop yield. They demonstrated especially high herbicidal activity on velvetleaf (<25g a.i./ha).

Considerations for Soybean Selectivity. We take all the above mentioned results into consideration. We propose four possibilities for selectivity to soybean demonstrated by compounds **1a** and **2a**.

The first is that both thiadiazolopyridazines (**1a**, **2a**) and triazolopyridazines (**12a**, **13a**) are active isomers in plants. The propensity to ring open the thiadiazolopyridazines in soybeans is higher than that in weeds. Nucleophiles in soybean attack the carbonyl of thiadiazolopyridazines to afford the ring opened compounds such as **14a** which is inactive for soybean, since all ring opened compounds we synthesized were less active. In contrast nucleophiles in weeds do not attack, or attack but then the triazolopyridazines form *via* the ring opened compounds thus generating another toxic compound.

The second possibility is that the herbicidally active isomer is the triazolopyridazines. The difference in selectivity between soybean and weeds depends on their ability to promote the ring transformation from thiadiazolopyridazines to triazolopyridazines.

Third, the substituent at the fifth position of the phenyl ring, the thioglycol acid group improves the selectivity (*1*), since the free acid showed weak herbicidal activity, and/or the sulphur atom could easily be oxidized to detoxify in soybean.

The fourth possibility is that the enamide structure which has reactivity for nucleophiles in plants is metabolized to give inactive compounds.

These are four possible occurrences in the selectivity emergence for soybean, however, it should also be known that more than two possibilities may occur simultaneously.

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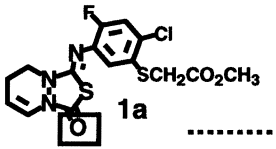
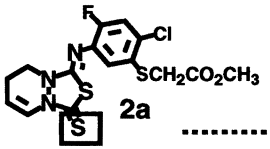
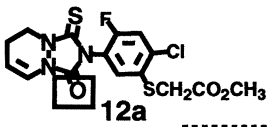
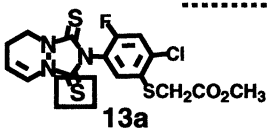
Table I. Effect of the Tetrahydropyridazine Derivatives on the Post Emergence Herbicidal Activity in a Greenhouse (2-4 leaf stage)

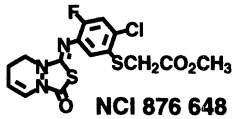
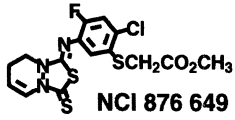
Compound	g ai/ha	ABUTH	XANPE	AMARE	IPOPU	CHEAL	DATST	Soybean
		0 (no effect) ~ 9 (completely killed) < 21DAT >						
 1a	10	9	5	9	9	8	9	0
	20	9	7	9	9	9	9	1
	40	9	8	9	9	9	9	2
 2a	10	9	4	9	9	8	9	0
	20	9	6	9	9	9	9	0
	40	9	8	9	9	9	9	1
 12a	10	9	6	9	9	8	9	1
	20	9	8	9	9	9	9	2
	40	9	9	9	9	9	9	4
 13a	10	9	4	9	9	8	9	0
	20	9	7	9	9	9	9	1
	40	9	8	9	9	9	9	2

ABUTH = Velvetleaf, XANPE = Cocklebur, AMARE = Redroot pigweed,
IPOPU = Morningglory, CHEAL = Lambsquarters, DATST = Jimsonweed

In Synthesis and Chemistry of Agrochemicals IV; Baker, D., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1995.

Table II. Field Trial Results in the U. S. A.

Post (leaf stage of weeds : 2-4)

Compound	g ai/ha	Soybean			Weed Control (21DAT)	
		2DAT	10DAT	21DAT	ABUTH	IPOPU
 NCI 876 648	25	16	12	1	98	65
	50	26	12	1	100	77
 NCI 876 649	25	5	3	0	83	83
	50	14	6	0	100	83
Acifluorfen	560	19	7	0	29	92
Bentazon	1120	7	5	0	89	83

0 = no effect, 100 = completely killed (Mean values in 3 locations)
ABUTH = Velvetleaf, IPOPU = Morningglory

Conclusions

1,4,5,6-Tetrahydropyridazine (THPD) was a useful "building block" of the heteroring fused pyridazine systems. We utilized it in our research and found a new synthesis of thiadiazolopyridazines with "THPD". The thiadiazolopyridazines easily afforded the corresponding triazolopyridazines *via* a *Dimroth* type rearrangement. Some of the thiadiazolo- and triazolopyridazines provided high herbicidal activity. It was suggested that the transformations for **1a**, **2a** and the soybean selectivity were closely related. NCI-876648 and NCI-876649 are promising post-emergence herbicides for use against broadleaves, especially velvetleaf, in soybean.

Acknowledgments

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Chapter 11

3-Aryl-4-substituted-5-(halo)alkylisoxazoles

Design, Synthesis, and Herbicidal Activity of a Unique Class of Pre- and Postemergent Herbicides

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The 3-aryl-4-substituted-5-(halo)alkyl-4-isoxazoles are a structurally unique class of herbicides. They are readily prepared by 1,3-dipolar cycloaddition reactions of benzohydroximinoyl chlorides and haloalkyl substituted acetylenic esters. Conversion of these trisubstituted isoxazolecarboxylate esters to 4-isoxazolecarboxamides affords compounds having significant preemergent and postemergent herbicidal activity against a variety of weed species. A series of related 4-aminoisoxazoles were prepared which exhibited preemergent activity. The nature of the substitutents on the aryl and the isoxazole ring determine the scope and level of activity, crop selectivity and ultimate field performance.

In the mid 1980's, we became interested in exploring the chemistry of fluorinated acetylenes as intermediates for preparation of new biologically active materials. Selective introduction of fluorine, made available by the use of new reagents, has led to the discovery of numerous fluorine containing medicinals and agrochemicals (*1*). The trifluoromethyl group in particular has appeared in a number of agrochemical products including acifluorfen, fluazifop, trifluralin and, most recently, dithiopyr (*2,3*). Acetylenic esters **1** having fluoroalkyl groups in the beta position can provide facile entry to a host of fluoroalkyl substituted carbocyclic and heterocyclic rings (Figure 1). Due to the electron deficient nature of acetylenes **1**, they react readily with nucleophiles to give either acyclic or cyclic products and with dienes or 1,3 dipoles to give carbocyclic and heterocyclic rings, respectively. Cyclocondensation reactions of **1** with bidentate nucleophiles gives heterocyclic rings having a 1-3 relation between the carbonyl or hydroxy functionality and the fluoroalkyl group, whereas cycloaddition affords hetero or carbocyclic rings having a 1-2 relation between an ester and fluoroalkyl group. The addition of **1** to hydrazines by cyclocondensation provides regioselective synthesis of 3-hydroxypyrazoles (*4,5*) which are useful intermediates for the preparation of herbicidal pyrazole phenyl ethers (*6*).

The first 3-aryl-5-fluoroalkyl-4-isoxazolecarboxylate ester **4**, which exhibited weak herbicidal activity, was prepared in the late 1970s by cycloaddition of benzohydroximinoyl chloride **2** with enamine **3** in an overall yield of 3.5% (Figure 2). The unique structure of this trisubstituted isoxazole coupled with its activity drew our attention as a possible target for investigation. An improved synthesis, however, was

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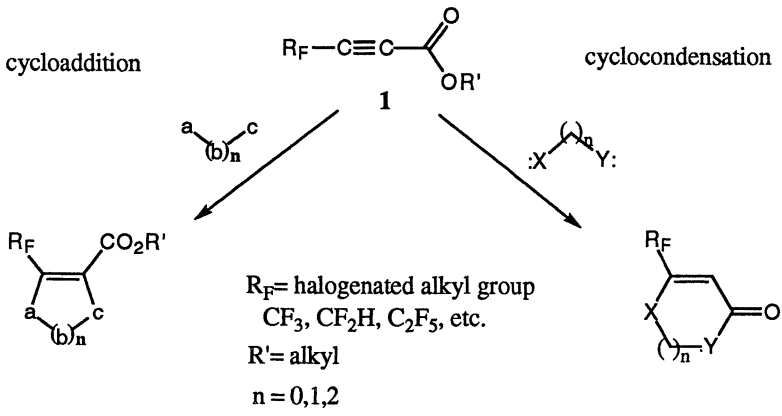


Figure 1. Synthetic Potential of Perfluoroalkylacetylenic Esters

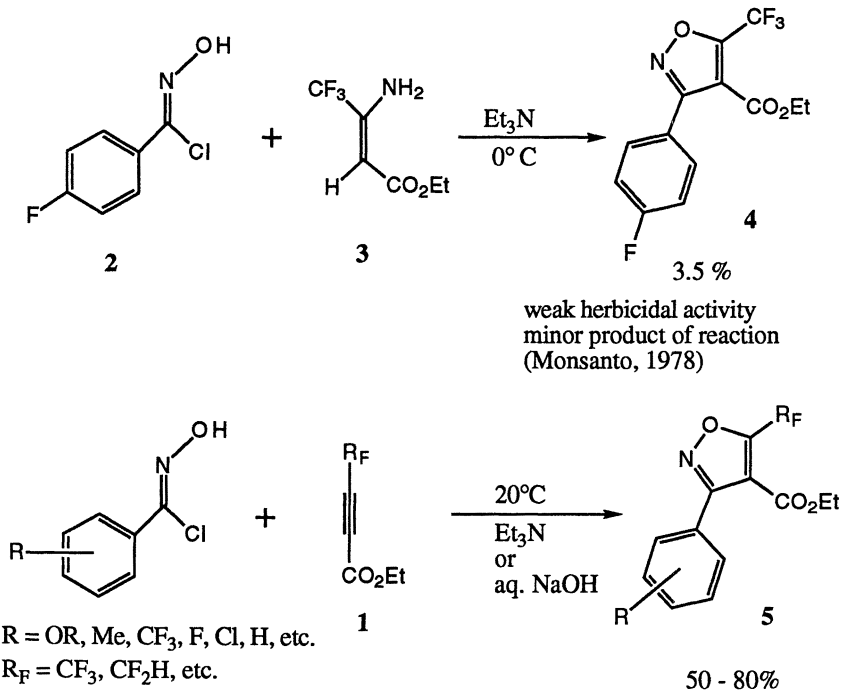


Figure 2. Nitrile Oxide Cycloadditions

required to prepare suitable quantities of material for structure-activity studies (SAR) and preparation of derivatives. A limited number of 3-aryl-5-trifluoromethyl-isxoazole carboxylates **5** had been previously prepared (*7*). We found that cycloaddition of **1** with nitrile oxides (prepared via dehydrohalogenation of the

corresponding N-hydroxyimidoyl chlorides) is general and affords direct entry to unique 3-aryl-5-haloalkyl-4-isoxazoles carboxylate esters **5** in good yields. The reaction is regioselective and can accomodate a variety of substitution patterns on the phenyl ring. Conversion of these esters to isoxazolecarboxamides provided a series of active herbicides (*8*) which is the subject of this report.

Synthesis of Trisubstituted Isoxazoles

The ability to prepare a series of 3-aryl-5-fluoroalkyl-4-isoxazolecarboxylate esters **5** depends on the availability of the fluoroalkyl substituted acetylenic esters **1** (Figure 3). Preparation of acetylene **1** ($R_F = CF_3$, R' = Et) had been reported by thermolysis of acylmethylenephosphorane **6**, however, the overall yields from available starting materials were low and the preparation of **6** was complicated by the presence of an equimolar amount of phosphonium salt (*9*). A single step procedure has been developed for preparation of **6** from a commercially available phosphonium salt which avoids the isolation of intermediate phosphoranes and greatly improves the yield by eliminating side products from transylidation reactions (*10,11*). Thermolysis of **6** is surprisingly straightforward and can be carried out on multikilogram scale to afford **1** in yields of 70-90%. As the phosphorane is heated to near its melting point, the acetylenic ester is formed and collected by distillation in a dry ice-acetone trap. This method has the advantage of removing the acetylene **1** from the reaction medium and providing it in analytically pure form.

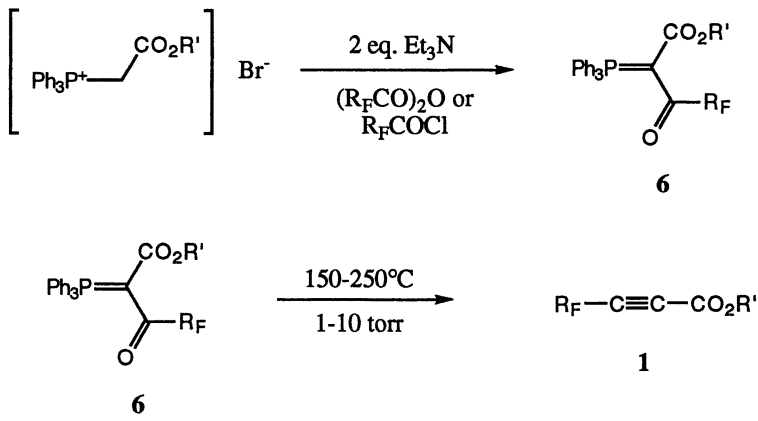


Figure 3. Preparation of Acetylenic Esters **1**

The nitrile oxides required for the cycloaddition reaction with **1** were obtained from benzohydroximinoyl chlorides by *in situ* treatment with either triethylamine or aqueous hydroxide (Figure 2). Better results were obtained with a two phase, aqueous sodium hydroxide-methylene chloride conditions rather than triethylamine. A number of side products were eliminated giving rise to overall higher yields of the desired regioisomeric product **5**.

Although some of the isoxazole esters **5** have herbicidal activity, the best candidates were prepared by derivitization of the esters to primary and secondary amides (Figure 4). The carboxylate functionality is remarkably stable to hydrolysis, due to the steric hinderance of the flanking 3-aryl and 5-fluoroalkyl substituents. As is often observed with isoxazoles, treament with strong base gave complete hydrolysis of the isoxazole rings to the corresponding benzoic acids. Fortunately the isoxazole ring is stable towards acid and under forcing conditions the carboxylic acids

7 can be obtained. Treatment with mixed acids such as acetic acid and concentrated HCl for 3 days gave good yields of **7**. The acid chlorides **8** were obtained by reaction with either oxalyl chloride or thionyl chloride and were stable enough for long term storage and, in many cases, distillation. The acid chlorides were used to prepare a series of carbonyl derivatives **9** by reaction with ammonia, primary and secondary amines, alcohols and mercaptans.

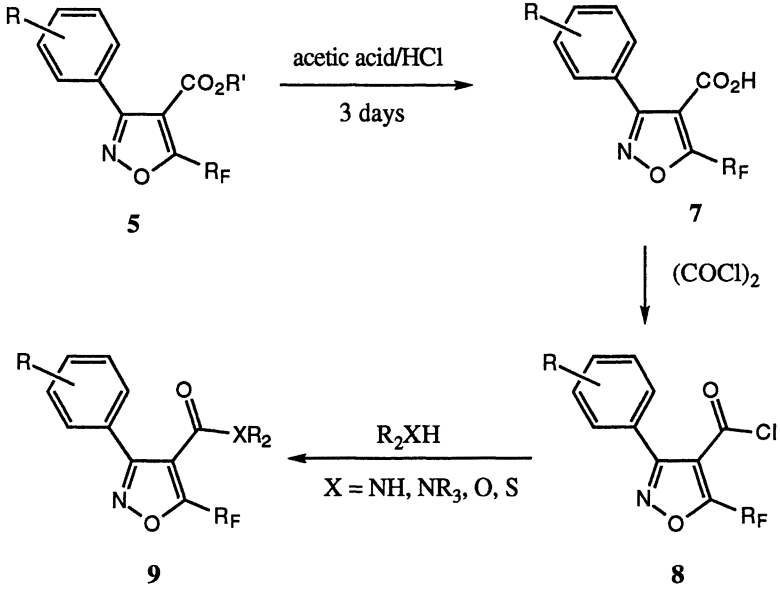


Figure 4. Synthesis of Isoxazole-4-carboxylate Derivatives

In addition to the compounds of structure **9**, it was also possible to convert isoxazole carboxylic acid **7** to 4-aminoisoxazoles **12** (Figure 5). In our initial investigations, we prepared acyl azide **11** by treatment of the acid chloride with sodium azide. These azides were heated to give an isocyanate via the Curtius rearrangement followed by hydrolysis to aminoisoxazole **12**. The crude product, however, contained a substantial amount of a bis-urea side product from reaction of the intermediate isocyanate with **12**. It was much more convenient to prepare **12** using Yamada's method (*12*) with diphenylphosphoryl azide to give carbamate **10** as an intermediate rather than the acyl azide **11**. Hydrolysis of **10** was carried out either with aqueous acid or with trimethylsilyl iodide to give aminoisoxazole **12**.

Herbicidal Properties

The 3-aryl-5-haloalkyl-4-isoxazolecarboxamides **9** were found to have an absolute requirement of light for herbicidal activity. In addition, they were shown to cause accumulation of protoporphyrin IX (PROTO) in treated cucumber cotyledon to levels known to be phytotoxic. These results indicated that they are inhibitors of protoporphyrin IX oxidase (PROTOX) (*13*), and have a mechanism of action similar to that of nitrodiphenyl ethers or N-phenylimide herbicides (*14, 15*). The aminoisoxazoles **12**, however, were found to be seed germination inhibitors and only exhibited preemergent activity. Light was not required for herbicidal activity of these compounds.

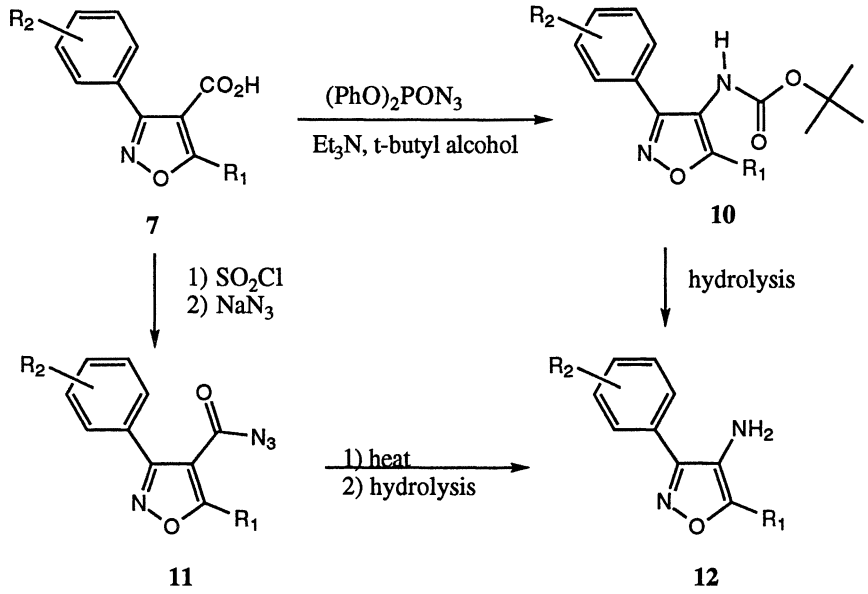
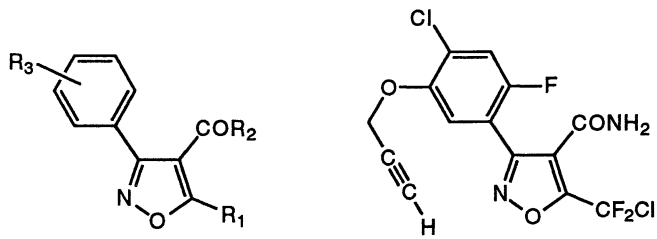


Figure 5. 3-Aryl-4-aminoisoxazoles

As might be expected in a series of compounds with multiple modes of action, structure could be optimized for either the PROTOX or seed germination inhibition activity. Compound **13,** having a carboxamide in the 4 position, is typical of the PROTOX structural types (Figure 6). It was one of the most active compounds in our tests providing 80% control of broadleaf weeds at 40 g/ha and 10 g/ha in preemergent and postemergent tests, respectively.



13

Structure-Activity Relationship
PROTOX Inhibition

R_1 - $CF_2Cl > CF_3 > CF_2H, CHMe_2 > CF_2CF_3, CCl_3 >>> CH_3, H$

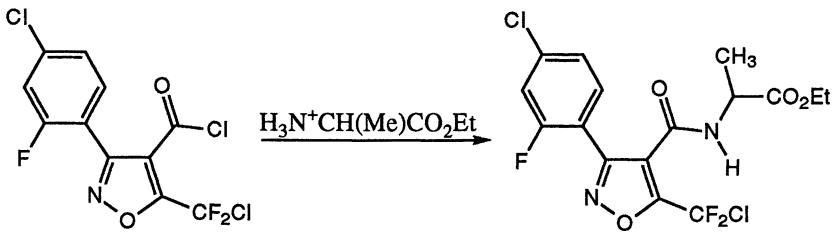
R_2 - NH_2, NHR > OEt, OMe, Cl >>> OH

R_3 - 4-halo, 2,4-dihalo, 4-CF_3 > 4-NO_2, 4-OMe >>> H, 3-subst., 2,6-dihalo

Figure 6. SAR of PROTOX Inhibitors

The effect of the R_1 group (5 position of the isoxazole ring) on PROTOX activity was pronounced in this series. Fluoroalkyl or sterically bulky groups were necessary for activity with the CF_2Cl group consistently providing the best results. The CF_3 compounds were anywhere from 2X to 100X weaker than the corresponding CF_2Cl compound on broadleaf species in preemergent tests. Difluoromethyl or isopropyl groups were slightly weaker than the CF_3 compounds. The CF_2CF_3 and CCl_3 compounds were active only at rates in excess of 5 kg/ha and most non-halogenated compounds such as methyl or H were completely inactive. Surprisingly, the CF_2H and the non-halogenated isopropyl substituted compounds were very similar in activity, being less active than CF_3 compounds, but considerably more active than the CF_2CF_3 or CCl_3 compounds. The isopropyl compounds (R_1 = $CHMe_2$) were the only examples of active PROTOX inhibitors in this series having a non-halogenated group at this position. Clearly, steric bulk as well as electron properties are important for good herbicidal activity.

In the 4 position of the isoxazole ring, the primary and secondary carboxamides provided the most active compounds. Only the small alkyl esters, such as methyl and ethyl esters, gave significant levels of herbicidal activity and they were often 10X to 50X less active than the carboxamides. The carboxylic acids and their salts were inactive. Substitution pattern on the phenyl ring also had an important role in determining PROTOX type activity. Substitution in the para or 4 position of the phenyl ring was required for activity. Halogens gave the best results, particularly for the 2,4-dihalo compounds.



14 **15**

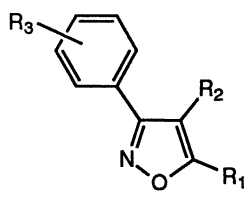
Compound **15**	Preemergent GR80 VL	BG	Postemergent GR80 VL	BG
R-enantiomer	0.055	0.20	0.063	0.16
S-enantiomer	2.7	0.41	0.55	3.1

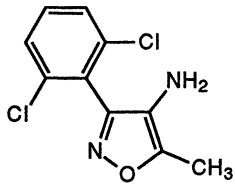
GR80 - amount of material in kg/ha required to give an 80% reduction in plant growth compared to controls. VL - velvetleaf; BG - barnyardgrass.

Figure 7. Herbicidal Activity of Chiral Isoxazoles

A number of chiral aminoester analogs of **9** were investigated for comparisons of activity (Figure 7). The secondary amides prepared by acylation with isoxazole carbonyl chloride **14** were easily obtained in enantiomerically pure form from the available (R) and (S)-aminoesters. Enantiomeric purity of the products was established as greater than 99% by HPLC with a chiral stationary phase (*16*). Even small amounts of enantiomeric impurities can make it difficult to determine the relative activities of the two antipodes. A 1% impurity of the active component in an "inactive" isomer would give rise to one hundreth the response in a bioassay. The differences in activity level between the enantiomers of **15** depended on the particular

weed species. In preemergent tests, only a 2X difference in activity was observed for barnyardgrass, whereas the difference was nearly 50X for velvetleaf.





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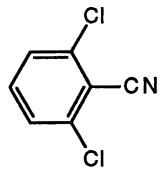

Casoron®

Structure-Activity Relationship
Seed Germination Inhibition

R_1 - CH_3 > H > CF_3

R_2 - NH_2 > CON_3 >> $NHCO_2$t-butyl, CO_2R

R_3 - 2,6-dihalo, 2-halo >>> H, other substitutions

Figure 8. SAR of Isoxazole Seed Germination Inhibitors

Aminoisoxazole **16** was the most active seed germination inhibitor having greater than 80% preemergent control of narrowleaf and broadleaf species at 1 kg/ha (Figure 8). The spectrum and level of unit activity was similar to that of 2,6-dichlorobenzonitrile or Casoron®, a known preemergent herbicide. Based on structural similarities, **16** could be a pro-herbicide of compounds similar to Casoron®. A retro-cycloaddition process would be expected to give the nitrile oxide analog of 2,6-dichlorobenzonitrile. The 5-methylisoxazoles (R_1 = Me) were somewhat more active than other 5 position analogs such as CF_3 or hydrogen. An amino group in the 4 position was critical for activity. Simple derivatives of the amines such as amides, carbamates or ureas were completely inactive. The acyl azides (R_2 = CON_3) were about 5X weaker than the amines and were the only other group to provide activity at this position. The ortho positions of the phenyl ring required a halogen atom for activity with the disubstituted 2,6-dichloro- and 2-chloro-phenyl isoxazoles being the most active. In terms of overall unit activity on either a per gram or per mole basis, the aminoisoxazoles such as **16** never approached the level of activity found in the PROTOX inhibitors such as **13**.

Summary

The 3-aryl-4-substituted-5-(halo)alkylisoxazoles are a unique structural class of herbicides. Two different types of activity were observed based on the whole plant symptoms in pre- and postemergent tests. The 3-aryl-5-haloalkyl-4-isoxazole carboxamides were highly active pre- and postemergent compounds, whereas the 4-aminoisoxazoles exhibited seed germination activity in preemergent tests.

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Chapter 12

6-Aryloxy-1*H*-benzotriazoles

Synthesis and Herbicidal Activity

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6-Aryloxy-1H-benzotriazoles represent a new and novel class of highly active pre- and postemergence herbicides related to the membrane disrupter diphenyl ether herbicides which elicit their herbicidal effects by inhibition of protoporphyrinogen oxidase. Several members of this class control a variety of broadleaf weed species at rates below 1 g/ha on postemergence application in the greenhouse. The initial discovery by PPG Industries, as well as the synthetic development and herbicidal evaluation of 6-aryloxy-1H-benzotriazoles at American Cyanamid is described.

Although diphenyl ether herbicides have been in commercial use since the early 1970's, the mechanism by which they elicit their herbicidal effects at the enzyme level has been elucidated only recently. Light-dependent membrane disrupter herbicides belonging to the diphenyl ether class act through the formation of singlet oxygen and subsequent lipid peroxidation of the plant cell membrane, resulting eventually in cell death. The site of action of these herbicides is protoporphyrinogen oxidase, the last common enzyme in the biosynthesis of heme and chlorophyll (1-4). The end result of inhibition of this enzyme is the accumulation of the potent photodynamic toxicant, Protoporphyrin IX. Much effort has been directed recently toward mechanistic studies of the porphyrin pathway, and Duke (5) has recently suggested that biorational design of inhibitors of porphyrin substrate-requiring enzymes could result in herbicides that are highly specific for chlorophyll-synthesizing organisms.

In the mid 1980's, diphenyl ether **2** (PPG-1013) was in development at PPG Industries as a herbicide for broadleaf weed control in wheat (6). One of the steps in the synthesis of this material involved the alkylation of oxime **1** as depicted in Figure 1. In the process of purifying a large development sample, an impurity arising during this alkylation step was detected. It was isolated and characterized, and shown to have structure **3**. This impurity was evaluated in the greenhouse and was found to possess herbicidal activity; at the time, this observation was unexpected on the basis of existing diphenyl ether literature. Consequently this impurity was considered a potential lead, and a synthesis program directed toward aryloxybenzheterocycles of type **4** was

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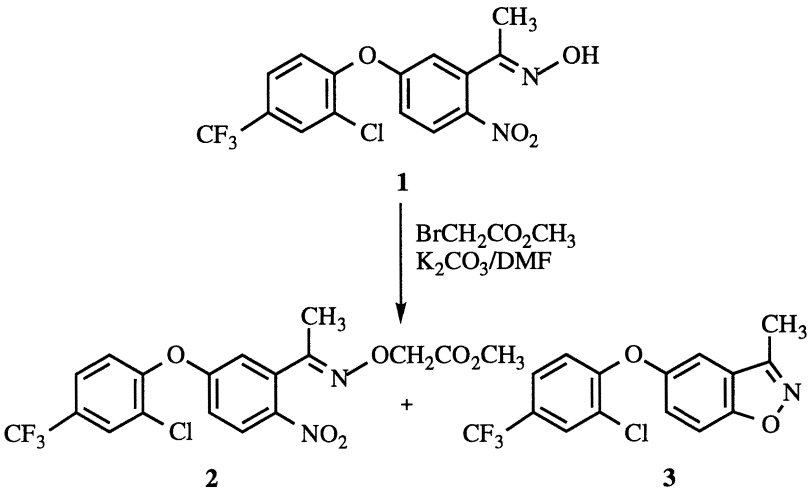


Figure 1. Origin of Bicyclic Diphenyl ether Series at PPG Industries

initiated. One of the bicyclic classes examined at PPG Industries was the 6-aryloxy-1H-benzotriazole class **5**. This chapter describes the initial work carried out at PPG Industries, and subsequent attempts at lead optimization performed at the Agricultural Research Division of American Cyanamid Co. (7). Workers at Imperial Chemical Industries have carried out similar efforts (8).

Synthesis

Non-regioselective Route to Aryloxybenzotriazoles. The initial route used to construct aryloxybenzotriazoles is described in Figure 2. The key step was non-regioselective N-alkylation of 1H-benzotriazole **6**, which gave rise to a mixture of isomers **7-9**. These were separated by chromatography, and structural assignments were made on the basis of detailed NMR studies. Greenhouse evaluation demonstrated the 6-aryloxy-1H-benzotriazoles to be the most active of the three regioisomeric series. The focus then shifted to the development of a regiospecific route to the desired 6-aryloxy-1H-benzotriazoles. One such route is shown in Figure 3. Reaction of activated aryl halide **10** with phenol **11** afforded diphenyl ether **12**. Introduction of an amine fragment *via* displacement of leaving group Z yielded diphenyl ether **13**, and subsequent nitro reduction and diazotization/cyclization afforded the desired regioisomer **14**. The main utility of this general method was in allowing for facile variation of the N_1-R group.

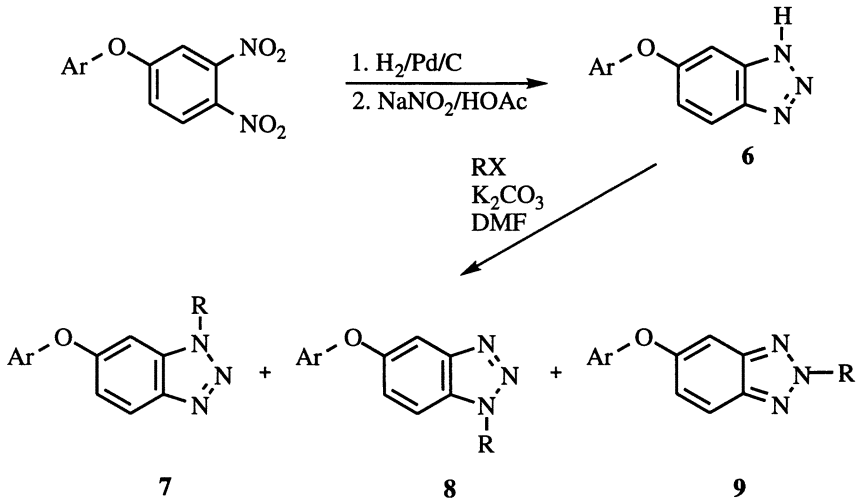


Figure 2. Non-regioselective Route to Aryloxybenzotriazoles

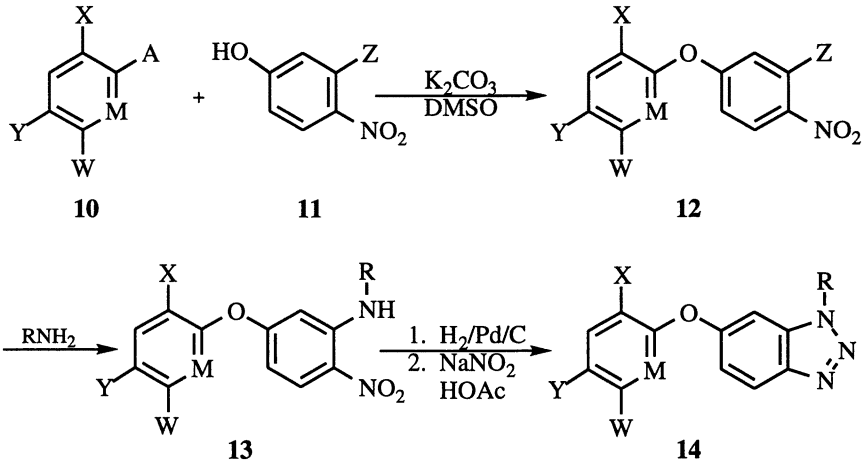


Figure 3. Regiospecific Route to 6-Aryloxy-1H-benzotriazoles

One limitation of this method is depicted in Figure 4. When the amine fragment employed in this sequence was an α-amino methyl ester or acid (**15,** R' = CH_3 or H), the product was not the expected benzotriazole **16** but the lactam **18**. This product arose by facile cyclization of the reduction product **17** prior to diazotization. This problem occurred only with α-amino methyl esters or acids; with higher homologs (i.e., β-aminoacids, etc.) lactam formation was not a problem.

One solution to this problem involved operation at the aldehyde oxidation state as shown in Figure 5. Using a protected aldehyde **19**, the amine fragment was introduced by displacement of Z affording **20**; the nitro group was then reduced, and the product diazotized and cyclized to give the benzotriazole **21**. The dimethyl acetal was then hydrolyzed to the aldehyde **22** followed by oxidation to the acid **23** and esterified to give the target **16**. Both the acid **23** and the aldehyde **22** were transformed into a variety of standard derivatives.

An alternate solution to the problem of lactam formation involved the use of a bulky ester as shown in Figure 6. By simply using the t-butyl ester **24** rather than the corresponding methyl ester, lactam formation was disfavored allowing the desired diazotization/cyclization step (**24** -> **25**) to proceed. The t-butyl ester **25** could then be cleaved to allow for subsequent transformation.

Figure 7 shows a modification of the regiospecific route to 6-aryloxy-1H-benzotriazoles which allowed for facile variation of the aryloxy group. Protection of phenol **26** followed by the usual sequence afforded benzotriazole **27**. Deprotection afforded the key hydroxybenzotriazole **28**, which was readily transformed into a variety of substituted aryloxybenzotriazoles **14**.

Two deaza analogs of the benzotriazole nucleus were investigated. The route to aryloxyindazoles is depicted in Figure 8. Protected hydroxyindazole **30** was prepared from aminoindazole **29** and coupled with activated aryl halide **10** to afford phenoxyindazole **31.** Deprotection to **32** and subsequent alkylation afforded mixtures of the desired indazole **33** along with regioisomer **34**. These products were separated by chromatography, and the structures assigned on the basis of NMR spectral data.

Aryloxyindole analogs were synthesized *via* the route depicted in Figure 9. Enamine **35** was prepared as depicted and converted to protected indole **36**. Deprotection and coupling with activated halide **10** afforded aryloxyindole **37** which upon alkylation afforded the desired indole **38.**

Biological Evaluation

At the outset of this program, a standard greenhouse protocol was established for initial evaluation of analogs. Since the analogs were more active postemergence than preemergence, they were evaluated postemergence at rates in the range 500 - 32 g/ha, and preemergence at a rate of 500 g/ha on the species listed in Table I.

Structure-Activity Relationships. The following structure-activity relationship comparisons are based on postemergence greenhouse data, since the aryloxybenzotriazoles were more effective on postemergence application. Important structural changes studied included regiochemistry of benzotriazole substitution, substitution in the aryloxy ring, and variation of the N-1 substituent. The herbicidal activity is represented as the lowest applied rate (control rate) that completely controlled the given weed species.

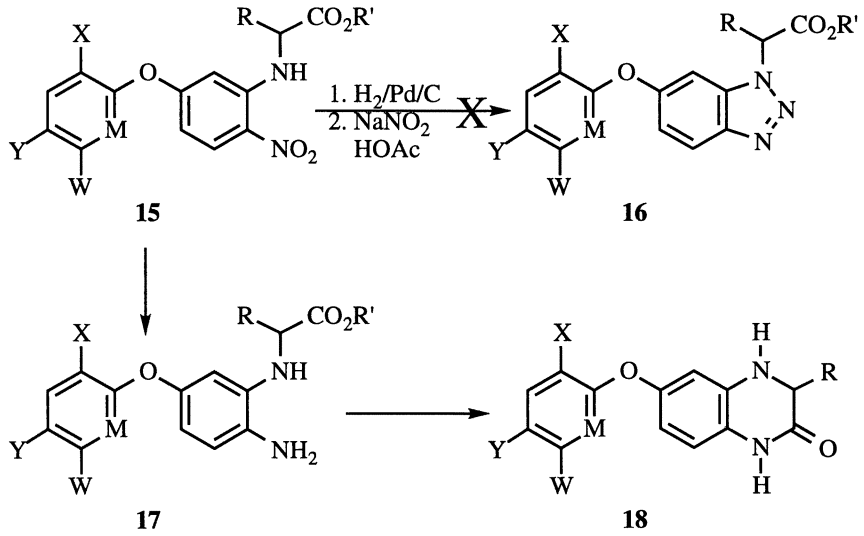


Figure 4. Lactam Formation during Attempted 6-Aryloxy-1H-benzotriazole Synthesis

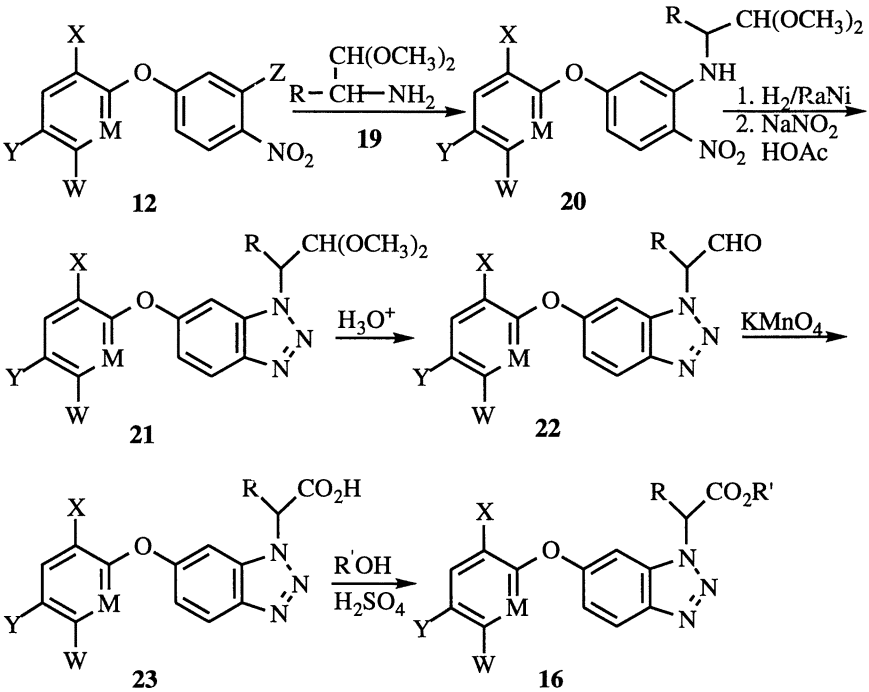


Figure 5. Aldehyde Route to 6-Aryloxy-1H-benzotriazoles

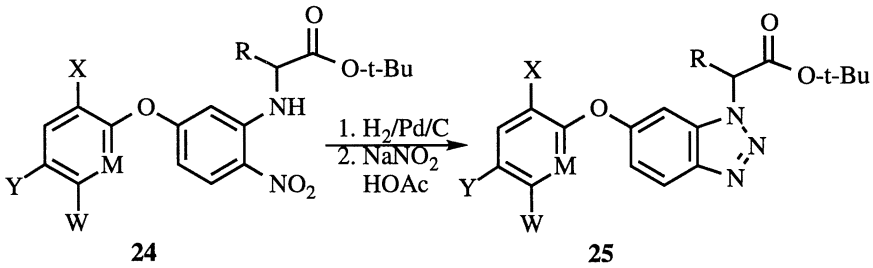


Figure 6. t-Butyl Ester Route to 6-Aryloxy-1H-benzotriazoles

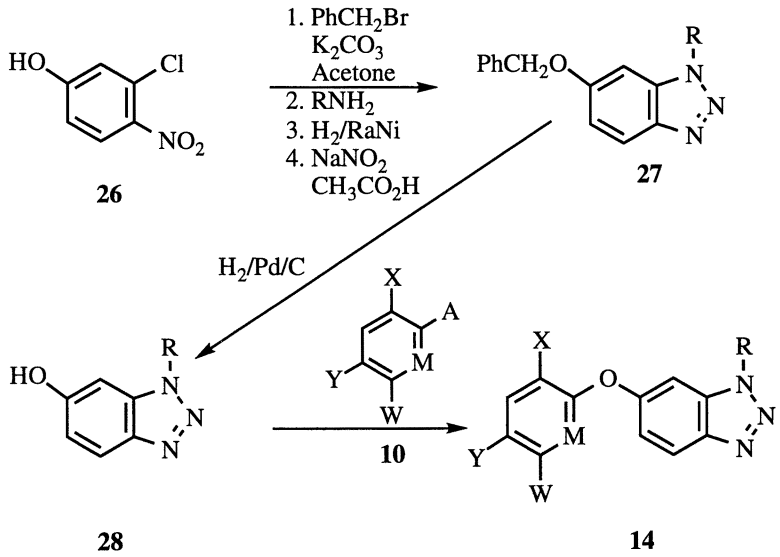


Figure 7. Variation of Regiospecific Route to 6-Aryloxy-1H-benzotriazoles

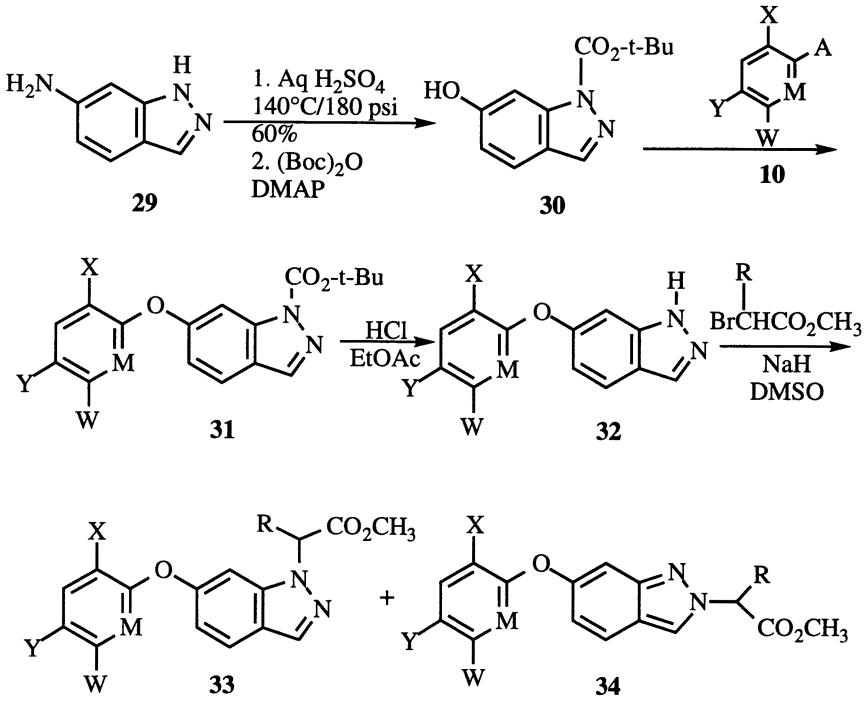


Figure 8. Synthesis of Aryloxindazoles

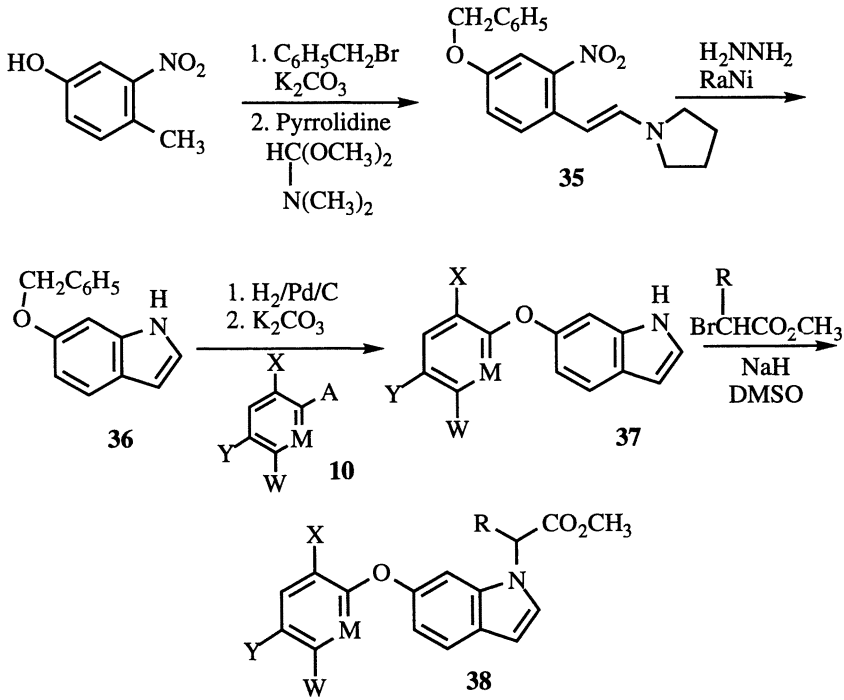


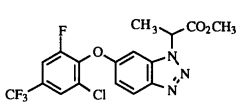
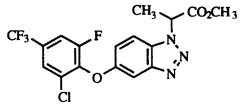
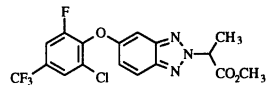
Figure 9. Synthesis of Aryloxyindoles

Table I. Species Utilized in Initial Greenhouse Evaluation

Broadleaf Weeds	Grass Weeds	Crops
Morningglory	Barnyardgrass	Corn
Pigweed	Large Crabgrass	Rice
Ragweed	Green Foxtail	Soybean
Velvetleaf	Proso Millet	Winter Wheat

Regioichemistry of Benzotriazole Substitution. Data for one set of regioisomers is shown in Table II. This is a greenhouse evaluation of postemergence activity in the rate range 500 - 32 g/ha according to the initial greenhouse protocol. Of the three regioisomeric types, the 6-aryloxy-1H-benzotriazole **39** is clearly the most active. The regioisomeric 5-aryloxy isomer **40** is almost inactive in this rate range. Curiously, the 2H-benzotriazole **41** retains some of the broadleaf activity, but lacks the grass activity of the most active regioisomer. Although three other regioisomeric aryloxybenzotriazoles are possible, they were not investigated, primarily because previous experience with other benzheterocyclic systems suggested that they would be of lesser interest.

Table II. Activity of Regioisomeric Aryoxybenzotriazoles

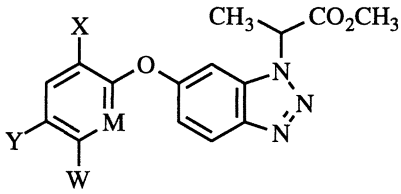
	Postemergence control rates (g/ha)		
			
Species	**39**	**40**	**41**
Pigweed	<32	250	<32
Ragweed	<32	>500	500
Velvetleaf	<32	>500	<32
Morningglory	<32	250	<32
Barnyardgrass	<32	>500	>500
Green foxtail	<32	>500	>500
Crabgrass	<32	>500	>500
Proso millet	<32	>500	>500

Aryloxy Substitution. Looking next at the effect of variation of the aryloxy substitution pattern, the best patterns in typical diphenyl ether herbicides were also the best in this series. Table III lists postemergence control rates for a set of analogs on a representative broadleaf and grass species, pigweed and barnyardgrass, respectively. As can be seen, best activity was found with 2-halo-4-trifluoromethyl- and 2,6-dihalo-4-trifluoromethyl substitution patterns.

N-Substitution. In examining the effect on activity of variation of the N1-substituent it was quickly realized that incorporation of an ester functionality into the side chain dramatically improved activity (Table IV). The simple N1-methoxycarbonylmethyl analog controlled all weeds at the lowest rate tested in the initial greenhouse protocol and was considerably more active than the other simple N1-substituted analogs listed in Table IV.

Another observation made early on was that substitution alpha to the ester with a small group generally boosted activity. Table V lists postemergence data for two analogs at rates between 8 - 0.5 g/ha. Looking first at the broadleaf weeds, the α-methyl analog controls all 10 weeds at 2 g/ha or less. Six are controlled at rates below 0.5 g/ha, the

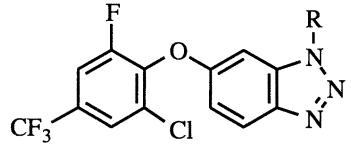
Table III. The Effects of Aryloxy Subsitution on Activity



Postemergence control rates (g/ha)

X	Y	W	M	Pigweed	Barnyardgrass
F	CF_3	H	C-Cl	<32	<32
H	CF_3	H	C-Cl	<32	125
Cl	CF_3	H	C-Cl	<32	500
H	CF_3	H	C-CN	63	63
H	CF_3	H	C-NO_2	<32	125
Cl	CF_3	H	N	<32	250
H	CF_3	H	N	63	500
Cl	Cl	H	N	<32	125
H	NO_2	H	C-CF_3	>500	>500
Cl	CF_3	H	C-NO_2	125	>500
Cl	CF_3	NO_2	C-H	>500	>500
NO_2	CF_3	H	C-NO_2	>500	>500
NO_2	CF_3	Cl	C-H	>500	>500
F	CH_3CO	H	C-H	>500	>500
C_6H_5CO	CF_3	H	C-H	>500	>500

Table IV. The Effect of N-Substituent Variation on Acivity

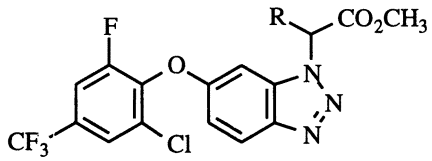


Postemergence control rates (g/ha)

Species	R = $CH_2CO_2CH_3$	H	CH_2CH_3	$CH_2C\equiv CH$	CH_2 
Pigweed	<32	125	63	<32	<32
Ragweed	<32	125	250	250	>500
Velvetleaf	<32	250	<32	250	>500
Morningglory	<32	<32	<32	<32	125
Barnyardgrass	<32	>500	>500	250	>500
Green foxtail	<32	>500	>500	63	>500
Crabgrass	<32	250	>500	500	>500
Proso millet	<32	>500	>500	250	>500

lowest rate in this evaluation. The unsubstituted analog is slightly less active. Considering the grass weeds next, control rates for both analogs are slightly higher, and again the α-methyl analog is the more active. Examination of the crop data indicates that both analogs are relatively non-selective. Although not shown here, the activity of the α,α-dimethyl analog also closely approached that of the unsubstituted compound. There was a limit to the size of the group which could be placed in the alpha position, however; phenyl substitution, for example, reduced activity considerably.

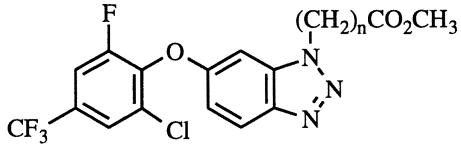
Table V. The Effect on Activity of Substitution α to the Ester



	Postemergence control rates (g/ha)	
Species	R = CH_3	R = H
Pigweed	<0.5	0.5
Common ragweed	<0.5	1.0
Wild mustard	<0.5	0.5
Prickly sida	<0.5	4.0
Velvetleaf	1.0	>8.0
Morningglory spp.	<0.5	0.5
Florida beggarweed	2.0	2.0
Devils beggartick	1.0	4.0
Hemp sesbania	<0.5	2.0
Cocklebur	2.0	2.0
Green foxtail	1.0	2.0
Crabgrass	8.0	>8.0
Yellow millet	8.0	>8.0
Barnyardgras	4.0	4.0
Tebonnet rice	8.0	8.0
Soybean	4.0	4.0
Field corn	4.0	8.0
Winter wheat	8.0	>8.0

Chain extension. The effect of extending the length of the N1-ester-containing side chain on activity is given in Table VI. Good broadleaf weed control is maintained out to n = 4, while grass activity starts to diminish at n = 2, and has markedly decreased by n = 4.

Table VI. The Effect on Activity of Chain Extension



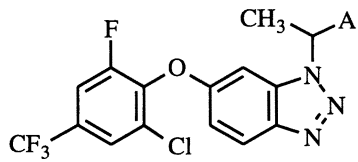
Postemergence control rates (g/ha)

Species	n=1	n=2	n=3	n=4	n=5
Pigweed	<32	<32	<32	<32	<32
Ragweed	<32	<32	<32	<32	63
Velvetleaf	<32	<32	<32	<32	250
Morningglory	<32	<32	<32	<32	<32
Barnyardgrass	<32	125	500	500	>500
Green foxtail	<32	250	125	250	>500
Crabgrass	<32	<32	125	250	500
Proso millet	<32	250	125	500	>500

Ester replacement. Using the α-methyl substituted ester as a benchmark, a number of replacements for the ester moiety were investigated. Table VII shows the effect of ester replacement with groups of the same oxidation state. Good broadleaf weed activity was maintained, but grass activity dropped off considerably upon replacement of the ester.

Similarly, replacement with functionalities at the aldehyde and alcohol oxidation states resulted in a similar decrease in control of grass weeds as depicted in Table VIII.

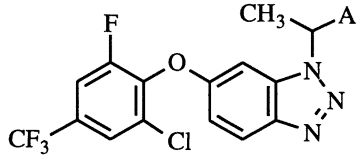
Table VII. Ester Replacements - Acid Oxidation Level



Postemergence control rates (g/ha)

Species A=	-CO_2CH_3	-COOH	-CN	-$CONH_2$	-$CONHSO_2CH_3$
Pigweed	<32	<32	<32	<32	<32
Ragweed	<32	<32	<32	<32	63
Velvetleaf	<32	<32	<32	<32	250
Morningglory	<32	<32	<32	<32	<32
Barnyardgrass	<32	63	125	125	500
Green foxtail	<32	125	63	<32	250
Crabgrass	<32	125	63	<32	250
Proso millet	<32	125	250	125	250

Table VIII. Ester Replacements - Aldehyde and Alcohol Oxidation Level



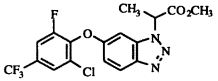
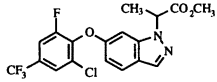
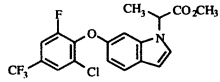
Postemergence control rates (g/ha)

Species	A= -CO_2CH_3	-$CH(OCH_3)_2$	-CH=NOH	-CHO	-CH_2OH
Pigweed	<32	<32	<32	<32	<32
Ragweed	<32	63	<32	<32	63
Velvetleaf	<32	<32	<32	<32	250
Morningglory	<32	<32	<32	<32	<32
Barnyardgrass	<32	125	500	250	250
Green foxtail	<32	63	125	63	63
Crabgrass	<32	<32	<32	<32	<32
Proso millet	<32	63	500	125	250

Benzotriazole Replacement. A comparison (Table IX) of the benzotriazole analog **39** with the two deaza systems revealed that replacement of the benzotriazole nucleus with an indazole (**42**) gave an analog which retained only the broadleaf activity of the benzotriazole, while similar replacement by indole (**43**) yielded an analog with little activity. Both of these systems have been described recently by workers at Imperial Chemical Industries (9,10).

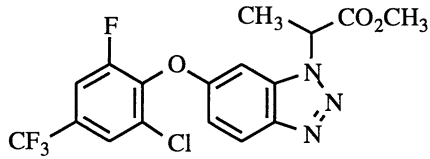
Table IX. Deaza analogs

Postemergence control rates (g/ha)

Species	 **39**	 **42**	 **43**
Pigweed	<32	<32	<32
Ragweed	<32	<32	500
Velvetleaf	<32	<32	250
Morningglory	<32	<32	500
Barnyardgrass	<32	250	>500
Green foxtail	<32	250	>500
Crabgrass	<32	250	>500
Proso millet	<32	250	>500

Preemergence Activity. Aryloxybenzotriazoles in this series also had preemergence activity. Table X lists preemergence data for the previously described α-methyl analog (**39**). Comparison of this data with data given in Table V, reveals that control rates are considerably higher preemergence than postemergence. As was true for postemergence application, preemergence rates for broadleaf weeds were lower than those for grasses. Although it is not shown here, selectivity was better preemergence than postemergence, but was still marginal.

Table X. Preemergence Activity



Preemergence control rates (g/ha)

Species	Rate
Pigweed	<32
Common ragweed	63
Velvetleaf	63
Morningglory spp.	63
Hemp sesbania	63
Cocklebur	125
Green foxtail	<32
Crabgrass	250
Yellow millet	125
Barnyardgrass	250

Conclusion

A regiospecific route to 6-aryloxy-1H-benzotriazoles was developed and utilized in the synthesis of analogs for structure-activity studies. The 6-aryloxy-1H-benzotriazoles are a new class of membrane disrupter herbicides, with activity both preemergence and postemergence on a wide variety of weeds. Postemergence activity was optimized primarily by focusing on the aryloxy substitution pattern and the substituent on N1 of the most active regioisomeric series.

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Chapter 13

Aryloxyindolin-2(3*H*)-ones

Synthesis and Herbicidal Activity

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The synthesis and structure-activity relationship of a series of regioisomeric aryloxyindolin-2(3*H*)-ones, a novel class of diphenyl ether herbicides, is described. The key step in the synthesis of these compounds is the formation of the indolin-2-one ring *via* a modified Fischer indole synthesis or a Sommelet-Hauser type rearrangement. These compounds, while exhibiting a broad spectrum of weed control, are most potent on broadleaf weeds when applied postemergence. A sub-class of these compounds, containing a spirocyclopropyl group, also exhibit unexpected selectivity in transplanted paddy rice.

Since the initial discovery of the diphenyl ether nitrofen, a number of agrochemical companies have been involved in the search for other examples of this class of membrane disrupting herbicides. The subsequent development of further generations of diphenyl ether herbicides led to the discovery of lactofen. During the 1980's, chemists at PPG Industries were actively pursuing the synthesis of lactofen analogs, whereby, during the alkylation of a phenylhydroxamic acid analog, a minor byproduct was isolated and subsequently found to have high levels of herbicidal activity. Serendipitously, they had discovered the first example of a bicyclic diphenyl ether (*1*). Immediately realizing the lack of prior art, they proposed a number of novel bicyclic diphenyl ether ring systems and began making limited forays into some of these new areas. American Cyanamid then acquired the above portion of PPG Industries' crop protection chemicals business. Subsequently, chemists at American Cyanamid Company's Agricultural Research Division began exploring some of these areas of chemistry.

One of the bicyclic diphenyl ether classes briefly examined at PPG was the aryloxyindolin-2(3*H*)-ones, **1**, where a rather limited number of compounds were prepared. In an effort to gain a better understanding of the structure-activity relationship in this class it was necessary to prepare a broader range of analogs, including modifications of the indolin-2-one substitution pattern, regiochemical variation and aryloxy group replacements. This chapter describes the initial work carried out at PPG Industries and the subsequent research performed at the Agricultural Research Division of American Cyanamid Co.

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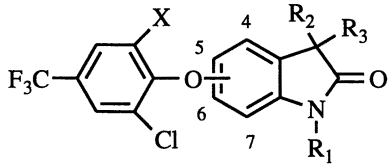


1 X=H, F

Synthesis

Preparation of Aryloxyindolin-2-ones *via* Modified Fischer Indole Synthesis. The aryloxyindolin-2-ones were initially prepared as shown in Figure 1 (*2*). Heating *para*-methoxyphenylhydrazine **2** with a variety of ketones of type **3** using a modification of the Fischer indole synthesis gave moderate yields of the 3*H*-indole (indolenine) **4.** N-alkylation to the indolenium salt **5** was followed by alkaline oxidation to give a series of 5-methoxyindolin-2-ones **6**. Acid induced cleavage of the methyl ether of **6** gave 5-hydroxyindolin-2-ones **7**, which, upon reaction with the benzotrifluorides **8**, furnished the 5-phenoxyindolin-2-ones **9**.

A limited set of analogs were prepared following the synthetic scheme outlined in Figure 1. The substitution pattern in the indolin-2-one 5-membered ring was limited to either trisubstitution (R_1, R_2, R_3 = alkyl) or no substitution (R_1, R_2, R_3 = H). Furthermore, the alkyl groups were restricted to C_1 - C_3 alkyl. Finally, only 5-phenoxy analogs were prepared starting from compound **2.**

In order to establish an effective structure-activity relationship in this class it was necessary to broaden the scope of functional groups and the substitution pattern in the indolin-2-one ring as well as vary the regiochemistry of the aryloxy group. It was reasoned that the rather harsh conditions used in the Fischer indole process would prevent the incorporation of more sensitive functionality. Accordingly, an alternative preparation of the aryloxyindolin-2-ones was warranted.

A variety of preparative methods of the indolin-2-one ring system are known. Reviews of some of the classical methods of indolin-2-one preparations (*3-5*) as well as a review highlighting the more recent literature (*6*) have appeared. It was of prime importance that the preparation of the indolin-2-one ring be carried out under conditions general enough for the inclusion of a wide variety of substituent types. Second, the ability to prepare a variety of analogs from a common intermediate was desirable. After examining a number of classical methods of indolin-2-one preparation, the Sommelet-Hauser type cyclization reported by Gassman (*7-9)* was found to be the method of choice.

Preparation of Aryloxyindolin-2-ones *via* Sommelet-Hauser type Cyclization. The aryloxyindolin-2-ones were prepared as shown in Figure 2 (*10,11*). Reaction of *para*-aminophenol **10** with the benzotrifluorides **8** gave the *para*-phenoxyanilines **12.** Similarly, *meta*-aminophenol **11** gave the *meta*-phenoxyanilines **13**. Treatment of aniline **12** with the chlorosulfonium salt of ethyl (methylthio)acetate followed by base-induced rearrangement gave an *ortho*-amino-α-(methylthio)phenylacetate, which, upon treatment with acid, cyclized to the 5-phenoxyindolin-2-ones **14**. Similar treatment of the *meta*-phenoxyanilines **13** gave a mixture of the 6- and 4-phenoxyindolin-2-ones **15** and **16** due to the existence of two possible *ortho*-rearrangements. The ratio of the 6-phenoxy/4-phenoxy isomer was 2.4/1 (X=H) and 2.6/1 (X=F). Thus, conversion of the aminophenols to the phenoxyanilines followed by the subsequent Sommelet-Hauser type rearrangement

allows for the preparation of the desired phenoxyindolin-2-one intermediates in a simple two step process.

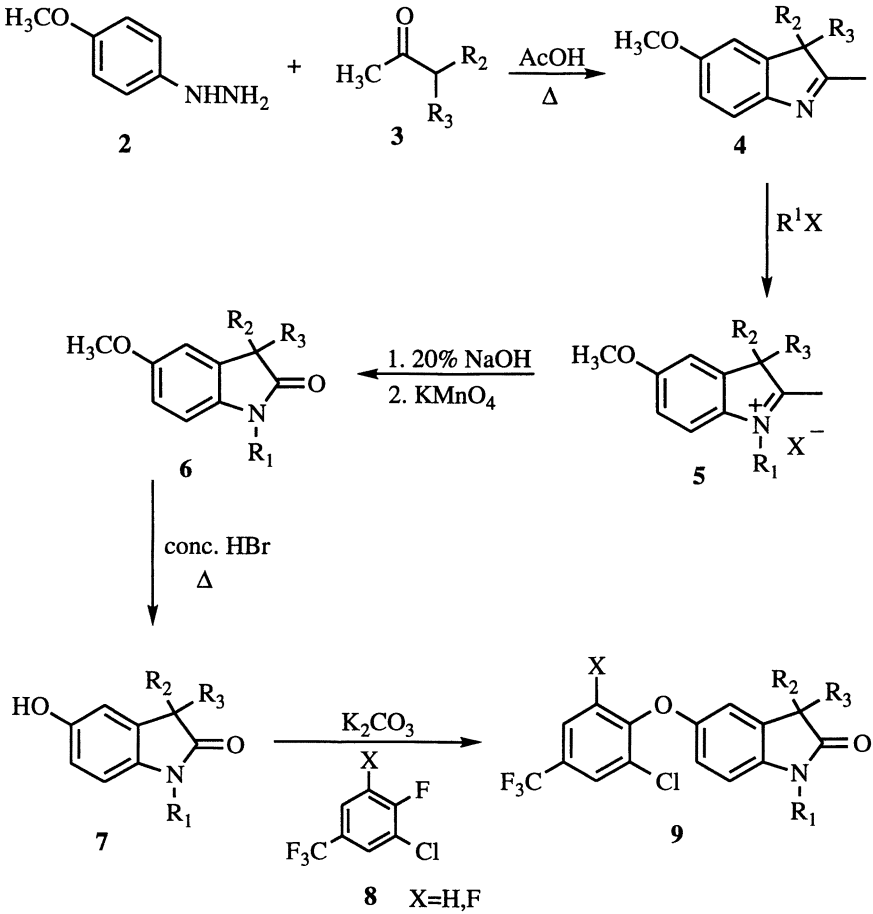


Figure 1. Preparation of 5-aryloxyindolin-2-ones from 4-methoxyphenylhydrazine *via* modified Fischer indole synthesis

Alkylation Studies. The regioselective alkylation of indolin-2-ones has been the subject of numerous studies (*6*). When the indolin-2-one five-membered ring is unsubstituted, alkylation usually leads to mixtures of C- and N-alkylated products. Often, a blocking group has been used to direct the alkylation.

It was realized that the 3-(methylthio) group of the phenoxyindolin-2-ones **14-16** might act as a potential activating/blocking group for subsequent alkylation reactions, expectations that were subsequently borne out. Treatment of phenoxyindolin-2-ones **14-16** with a variety of primary alkyl halides gave exclusive C-alkylation while lower yields and competing O-alkylation were observed in the reaction with branched alkyl halides (Figure 3) (*12*). The 3-alkyl-3-(methylthio)indolin-2-ones **17** were then

subsequently N-alkylated to give **18**. Further utility of this approach was demonstrated by the fact that reductive desulfurization, when coupled with the stepwise alkylation reactions, gave a variety of 3-, 1,3- and 1,3,3-alkylated indolin-2-ones in moderate to excellent yield (*12*).

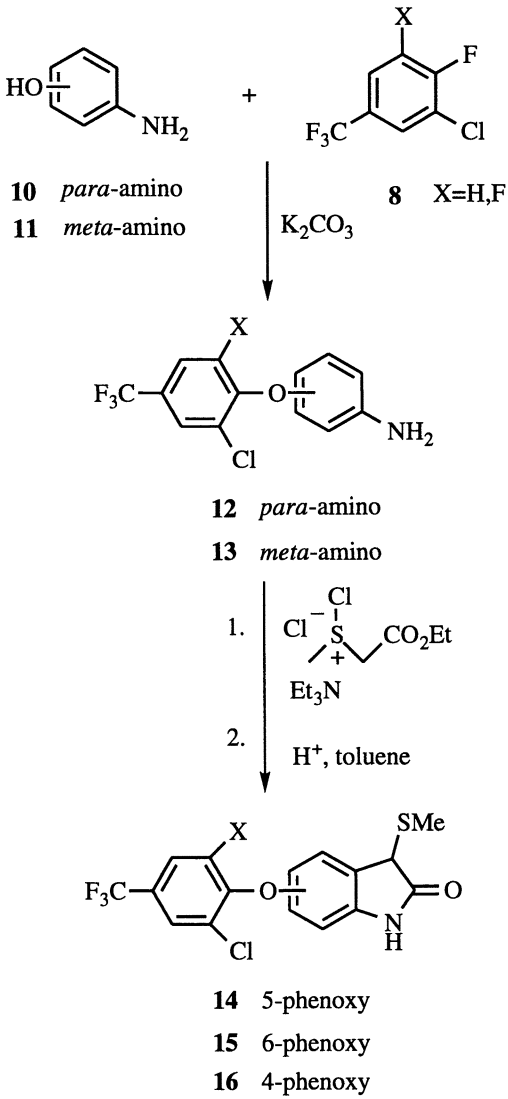


Figure 2. Preparation of regioisomeric aryloxyindolin-2-ones from aminophenols *via* Sommelet-Hauser type cyclization.

The stepwise alkylation approach has some limitations which preclude the preparation of certain compounds. Compounds containing branched alkyl groups at the indolin-2-one C-3 could be prepared in only low yield. In addition, the preparation of compounds containing C-3 spirocyclic substitution would not be straightforward using this approach.

In order to circumvent the difficulty associated with the incorporation of branched C-3 alkyl groups, an alternative approach was used. Conversion of the unsubstituted indolin-2-one **21** (obtained from the desulfurization of compound **14**) to the N-methylindolin-2-one **22** was initially carried out (Figure 4). As mentioned previously, alkylation of unsubstituted indolin-2-ones often leads to mixtures of products. A wide variety of conditions (bases, solvents and electrophiles) were examined for the N-alkylation of **21**. The observation that compound **22** could be obtained cleanly with only a specific set of reaction conditions (sodium hydride, dimethyl sulfate, toluene) was quite enigmatic; any variation led to a complex product mixture.

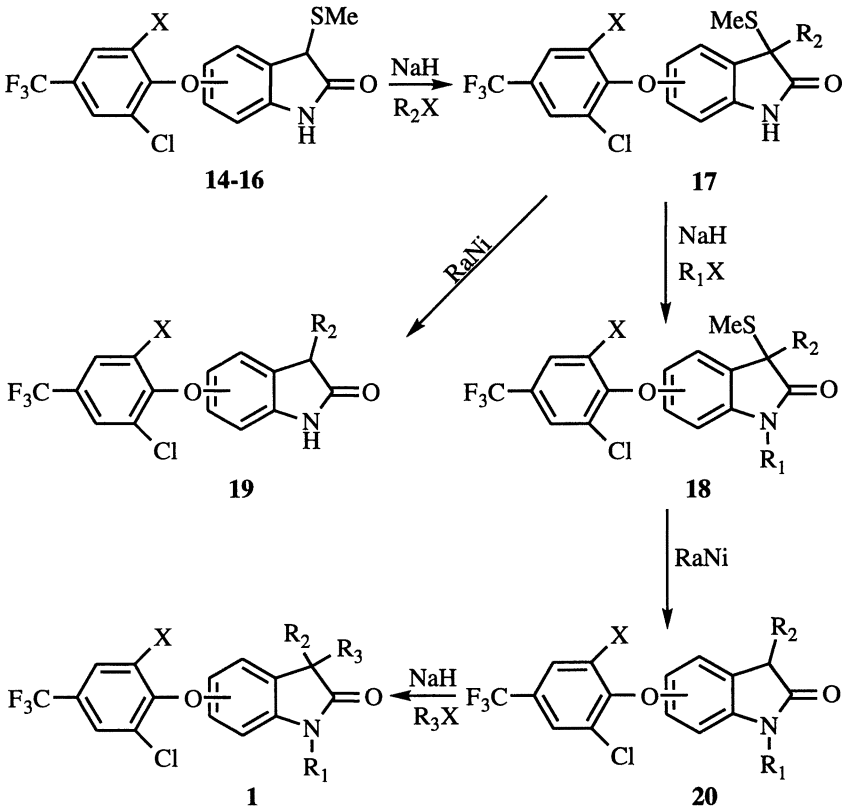
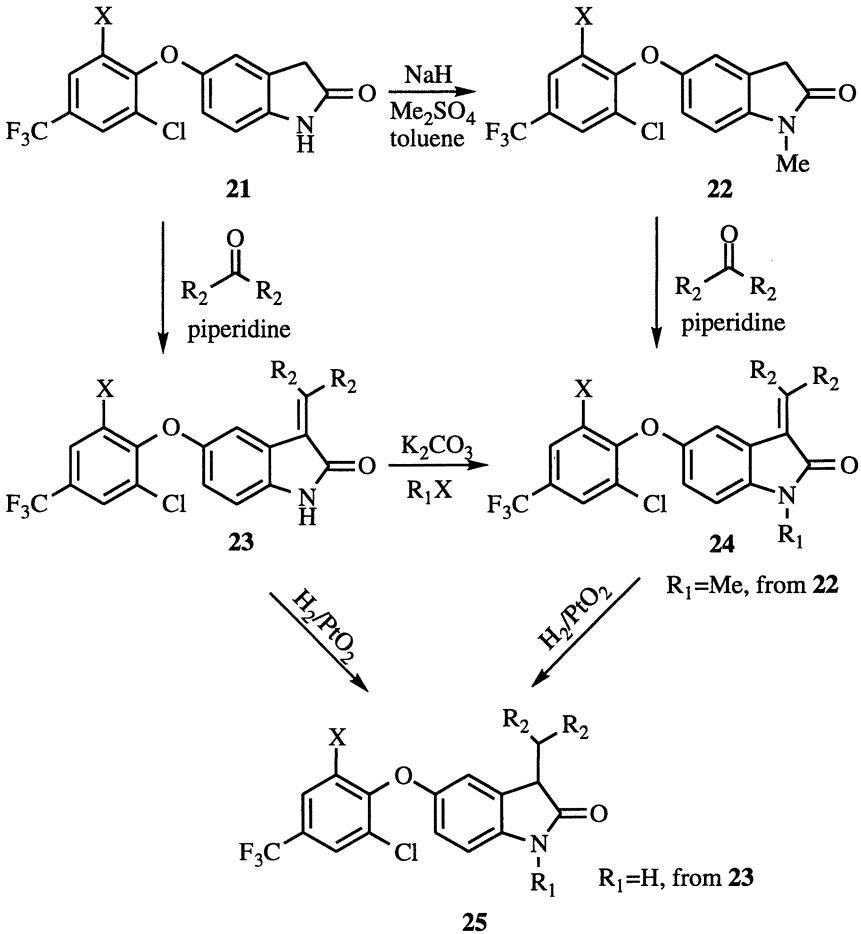


Figure 3. Regiospecific alkylation of 3-(methylthio)indolin-2-ones

Compound **22** was transformed smoothly to a series of 3-alkylidene-substituted indolin-2-ones **24** (R_1=Me) in good yield by reaction with a variety of ketones in the presence of piperidine. Both cyclic and acyclic ketones were prepared. Catalytic reduction over platinum oxide gave the desired branched C-3 alkylated indolin-2-ones

25 in excellent yield. Reversal of this sequence, i.e., initial treatment of unsubstituted compound **21** with ketones gave **23** which could then be N-alkylated in good yield to **24**, offers a general method for the preparation of N-alkyl analogs.

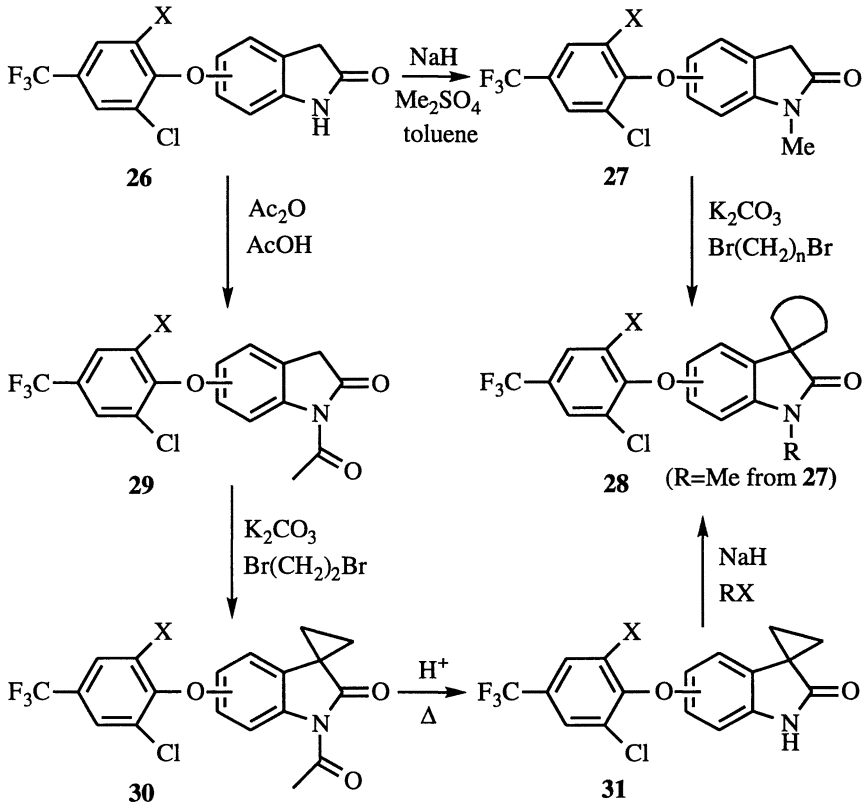


21 **22** **23** **24** **25**

Figure 4. Preparation and reduction of 3-alkylidene-substituted indolin-2-ones

The inclusion of C-3 spirocyclic substituents required another variation in the alkylation approach. Treatment of the N-methyl analog **27** with a variety of α,ω–dibromoalkanes gave a series of C-3 spirocyclic indolin-2-ones **28** of various ring sizes (Figure 5). To circumvent the restriction to N-methyl analogs, selective N-protection of compound **26** was sought. After several unsuccessful attempts, the acetyl group was found to be the protecting group of choice (*13*). Heating **26** in acetic anhydride/acetic acid allowed for the smooth transformation to **29**. C-3 spirocyclization was effected by treating compound **29** with 1,2-dibromoethane. N-

deprotection then furnished compound **31**. A variety of N-alkyl analogs were then prepared upon treatment of **31** with a number of electrophiles.



26 **27** **29** **28** **30** **31**

Figure 5. Preparation of C-3 spirocyclic indolin-2-ones

Biological Activity

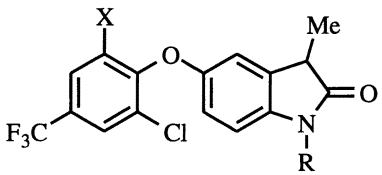
The aryloxyindolin-2-ones were tested in the greenhouse in an advanced screen on a variety of broadleaf and grass weeds, both pre- and postemergence. The broadleaf weeds included morningglory (*Ipomoea spp.*), redroot pigweed (*Amaranthus retroflexus*), common ragweed (*Ambrosia artemisiifolia*) and velvetleaf (*Abutilon theophrasti*). The grasses included barnyardgrass (*Echinochloa crus-galli*), crabgrass (*Digitaria sanguinalis*), green foxtail (*Setaria viridis*) and proso millet (*Panicum miliaceum*). The crops included soybean (*Glycine max*), corn (*Zea mays*), winter wheat (*Triticum aestivum*) and rice (*Oryza sativa*).

The observation that one of the aryloxyindolin-2-ones possessed excellent pre- and postemergent activity with safety in rice prompted the investigation of several closely related compounds for their utility as potential paddy rice herbicides. Weeds in this rice screen included barnyardgrass (*Echinochloa crus-galli*), flatstage (*Cyperus serotinus*) and arrowhead (*Sagittaria pygmaea*).

Structure-Activity Relationships. Like other diphenyl ether herbicides, the aryloxyindolin-2-ones are generally most efficacious against broadleaf weeds postemergence. Therefore, the following structure-activity relationship comparisons are based on postemergence broadleaf weed data. The most important structural changes studied include substitution in the aryloxy ring, regiochemistry of the aryloxy group and changes in the N-1 and C-3 indolin-2-one substitution pattern. The herbicidal activity is represented as the lowest applied rates that completely control the weed (in grams per hectare).

Aryloxy Substitution. Compounds containing four fluorines in the aryloxy ring (X=F) control the broadleaf weeds at a lower rate than those containing only three fluorines (X=H) (Table I).

Table I. Aryloxy Substitution vs. Activity



Postemergence control rate (g/ha)

X	R	morningglory	pigweed	ragweed	velvetleaf
F	Et	125	8*	250	63
H	Et	250	250	500	250
F	Me	16*	8*	32	8*
H	Me	125	125	125	32

* Lowest rate tested.

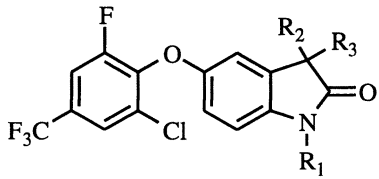
N- and C-Substitution. The herbicidal activity is highly dependent upon changes in the substitution pattern in the indolin-2-one 5-membered ring (Table II). Compounds containing N-substitution (methyl or ethyl) were considerably more active than the analogous N-H compounds. A biological effect was also noted for modifications in C-3 substitution. Changing the substitution on C-3 from unsubstituted (R_2, R_3 = H, H) to disubstituted (R_2, R_3 = SMe, Me or Me, Me) caused an increase in herbicidal activity independent of the substituent R_1.

Spirocyclic Ring Size. The effect of spirocyclic ring size on the herbicidal activity is shown in Table III. As the ring size is increased from 3- to 5- to 6-membered the herbicidal activity decreases.

N-Substitution in Spirocyclic Systems. The effect of changes in N-substitution in the 3-spirocyclopropyl-5-aryloxyindolin-2-ones on herbicidal activity is shown in Table IV. In general, the substituents that confer the greatest herbicidal

activity are smaller non-polar groups. As substituent lipophilicity increases beyond a certain point (last three entries) the herbicidal activity decreases to below that of the unsubstituted N-H compound.

Table II. N- and C-Substitution vs. Activity

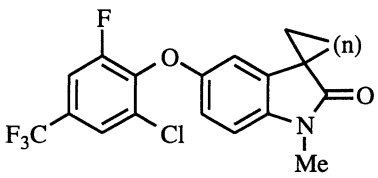


Postemergence control rate (g/ha)

R_1	R_2	R_3	morningglory	pigweed	ragweed	velvetleaf
Me	H	H	125	8*	250	63
H	H	H	>1000	-	>1000	>1000
Me	SMe	Me	16	8*	500	16
H	SMe	Me	500	32*	500	250
Et	Me	Me	32	8*	250	32
H	Me	Me	125	63	500	63

* Lowest rate tested.

Table III. Spirocyclic Ring Size vs. Activity

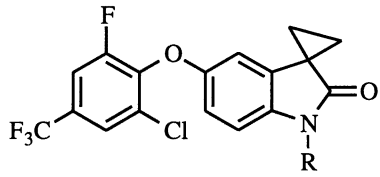


Postemergence control rate (g/ha)

n	morningglory	pigweed	ragweed	velvetleaf
1	8*	8*	16	16
3	32	32	32	32
4	32	32	125	63

* Lowest rate tested.

Table IV. N-Substitution in Spirocyclic Systems vs. Activity



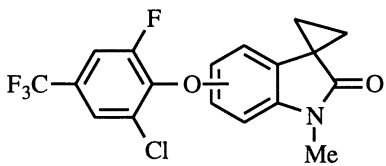
Postemergence control rate (g/ha)

R	morningglory	pigweed	ragweed	velvetleaf
Me	8*	8*	16	16
propargyl	250	63	32*	32*
Et	63	32*	>500	32*
allyl	500	32*	>500	125
n-Pr	>500	125	>500	32*
H	500	63	>500	63
CH_2SCH_3	>500	63	>500	250
i-Pr	>500	500	>500	250
$CH_2CH(CH_3)_2$	>500	>500	>500	>500

* Lowest rate tested.

Aryloxy Regiochemistry. The Sommelet-Hauser type cyclization allowed for the preparation of 4- 5- and 6-aryloxyindolin-2-ones. No examples of the 7-aryloxy regioisomers were prepared. As shown in Table V, the herbicidal activity is highly dependent upon the regiochemistry of the aryloxy group with activity decreasing in the order 5- > 6- >> 4-aryloxy.

Table V. Aryloxy Regiochemistry vs. Activity



Postemergence control rate (g/ha)

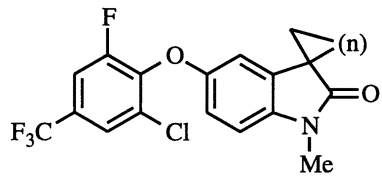
phenoxy position	morningglory	pigweed	ragweed	velvetleaf
5-	8*	8*	16	16
6-	125	63	>500	>500
4-	>500	>500	>500	>500

* Lowest rate tested.

Paddy Rice Evaluations. One of the C-3 spirocyclic aryloxyindolin-2-ones was observed to have better than expected preemergence herbicidal activity with safety in rice. A number of other spirocyclic analogs were then evaluated for their pre- and early postemergence weed control in post-transplanted rice seedlings under flooded conditions.

Spirocyclic Ring Size. The size of the spirocyclic ring has a pronounced effect on the herbicidal activity (Table VI). As the spirocyclic ring size increases from 3- to 5- to 6-membered, the preemergence herbicidal activity decreases markedly on barnyardgrass and flatstage. The herbicidal effect on arrowhead also follows this trend but is less pronounced. When applied post-transplant to rice all of the compounds were non-injurious at 1000 grams per hectare, the highest applied rate.

Table VI. Spirocyclic ring size vs. Activity in Transplanted Rice

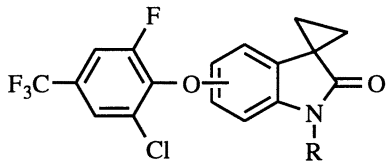


	Preemergence control rate (g/ha)			Safe at (g/ha)
n	barnyardgrass	flatstage	arrowhead	tebonnet rice
1	32	16	500	>1000
3	125	500	>1000	>1000
4	500	>1000	>1000	>1000

N-Substitution and Aryloxy Regiochemistry in Spirocyclic Systems. Changes in the substituent on nitrogen has a great effect on herbicidal activity on barnyardgrass in post-transplanted rice (Table VII). As the substituent size at N-1 increases in the 5-aryloxyindolin-2-one class there is a dramatic decrease in pre-emergence barnyardgrass activity. Note that the 6-aryloxy analog (last entry) controlled barnyardgrass at nearly the same rate as the most active 5-aryloxy analog. This was unanticipated as the results obtained on the upland species described previously showed a greater difference in herbicidal activity for the regioisomers. All of the compounds shown were safe in transplanted rice at the highest rates tested.

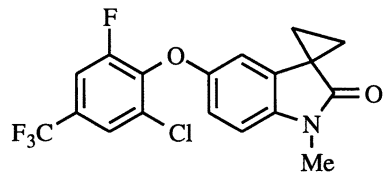
Timing of Herbicidal Application. The timing of the herbicidal application is very important to the effectiveness of barnyardgrass control in transplanted rice. Ten-day old rice seedlings were transferred into pots containing barnyardgrass seeds. After flooding the pots, the herbicide was applied at a range of rates (8-1000 grams per hectare) at various intervals (0-12 days) after transplanting and seeding. The tests were then rated three to five weeks after treatment. As shown in Table VIII, the applied herbicide maintains its effectiveness on barnyardgrass pre- and early postemergence. The herbicidal effect then decreases with increasing time after barnyardgrass seeding. Safety in the transplanted rice is maintained at all rates tested and application timings.

Table VII. N-Substitution and Aryloxy Regiochemistry vs. Activity in Transplanted Rice



		Preemergence control rate (g/ha)	Safe at (g/ha)
phenoxy position	R	barnyardgrass	tebonnet rice
5-	Me	16	>1000
5-	Et	32	>1000
5-	propargyl	63	>500
5-	allyl	250	>1000
5-	n-Pr	1000	>1000
5-	i-Pr	>1000	>1000
5-	$CH_2CH(CH_3)_2$	>1000	>1000
6-	Me	32	>1000

Table VIII. Timing of Herbicidal Application vs. Activity



Time of herbicide application (days after transplanting)	barnyardgrass control rate (g/ha)
0	32
3	32
6	63
9	250
12	500

Conclusion

The aryloxyindolin-2-ones, a novel class of membrane disrupting herbicides, have been prepared *via* two pathways. These compounds have been shown to be active both pre- and postemergence on a wide variety of weeds. The optimum substitution pattern in the indolin-2-one ring has been defined to include N-methyl and C-3 spirocyclopropyl groups. The regiochemistry was optimized with the aryloxy group at C-5. In addition, compounds containing a C-3 spirocyclopropyl group offer excellent preemergence barnyardgrass control in transplanted rice.

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Chapter 14

5-Aryloxybenzisoxazole Esters

Synthesis and Herbicidal Activity

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A series of benzisoxazole glycolate and acetate ester diphenyl ethers were prepared. The preparation of intermediate 5-hydroxybenzisoxazoleacetic acid from 4,6-dihydroxycoumarin was improved by reaction in the presence of excess hydroxylamine hydrochloride. The resultant diphenyl ether herbicides were potent total vegetation control pre- and postemergence herbicides.

The benzisoxazole diphenyl ethers are part of a third generation series of membrane disrupter herbicides. They share a common mode of action and bear a structural similarity to nitrofen and acifluorfen, the first and second generation herbicides of this class. Each succeeding generation has an increasing amount of structural complexity.

The bicyclic series of diphenyl ether herbicides was discovered by chemists at PPG Industries. They were looking for ways to improve the activity of diphenyl ether herbicides such as acifluorfen. Acifluorfen and its esters were gaining prominence at the same time as herbicides such as diclofop methyl, Figure 1. As these classes of herbicides shared a diphenyl ether backbone, the chemists at PPG Industries reasoned that the propionate group present in diclofop methyl might be appended to acifluorfen to improve its activity. The resultant combination afforded the commercial product lactofen. With this change, they began to investigate other methods of incorporating a propionate unit with the diphenyl ether nucleus. Another herbicide candidate meeting those requirements was PPG 1013. It was synthesized by alkylation of its oxime precursor with an α-bromopropionate. It was during oxime alkylation that some of the oxime anion cyclized with displacement of the nitro group to form the methyl benzisoxazole **1**. The herbicidal activity of this simple derivative started a new search for novel bicyclic diphenyl ethers. PPG Industries has successfully identified several related herbicidal bicyclic diphenyl ethers including benzotriazoles (*2*), benzoxazolones (*2*), benzisoxazoles (*3*), benzisoxazole ethers (*4*), indolinones (*5*), and tetralones and indanones (*6*).

The acquisition of a package of herbicide lead areas from PPG Industries by American Cyanamid Company allowed Cyanamid to continue to explore this area of chemistry. Thus, using acifluorfen (as well as other diphenyl ether herbicides) as a model for protoporphyrinogen oxidase inhibitors, there are several patterns which one can observe that result in good herbicidal activity, Figure 2. Our objective was to

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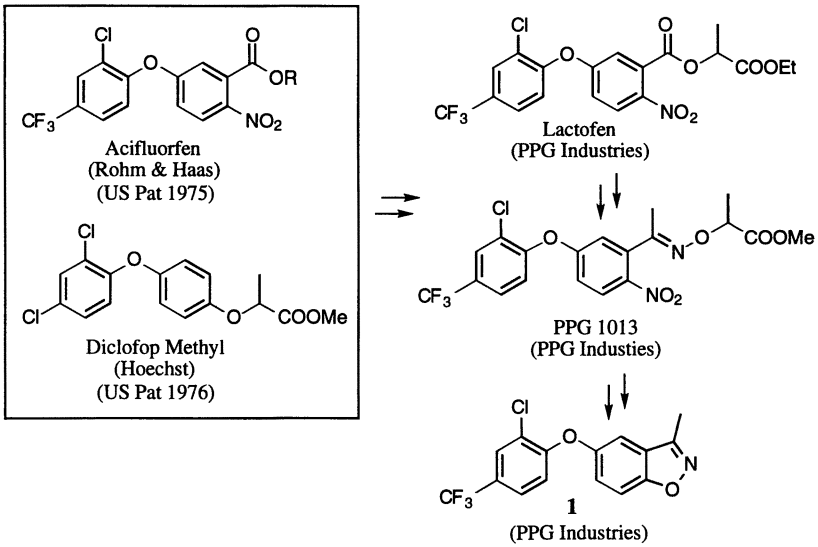
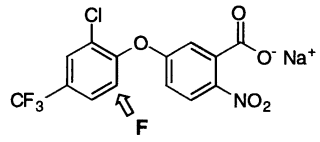


Figure 1. Discovery of Bicyclic Diphenyl Ether Herbicides



- The substitution pattern of acifluorfen is either optimal or very nearly optimal, i.e. groups in those positions result in higher activity than any other groups or arrangements
- Fluoro containing diphenyl ether is more active than des fluoro
- Maintain regiochemistry
- Nitro or halogen para to ether linkage
- Three substituents maximum
- Carboxy one of several groups that can be placed in the meta position
- Addition/replacement of a propionate substituent preferred in a meta position, but not a set distance from the remaining structure

Figure 2. Conventional Wisdom for Diphenyl Ether Substituents

prepare new bicyclic diphenyl ether herbicides that could maintain these patterns, and determine if incorporation of an acetate or propionate unit into these analogs would further improve the activity.

The route we chose to follow was to further elaborate simple bicyclic diphenyl ether herbicides by incorporating an acetate or propionate unit. This chapter will discuss the synthesis of benzisoxazole glycolate ether and acetate ester diphenyl ethers as well as their herbicidal activity. Several of the compounds reported herein have also been reported in patents (*4, 7*).

Synthetic Methods

Preparation of Ethers of 5-Aryloxy-3-hydroxybenzisoxazole. A 3-hydroxybenzisoxazole **5** can incorporate a propionate group in a manner very similar to that found in lactofen. It was prepared by coupling of 2,5-dihydroxybenzoic acid with aryl halide **2**, Figure 3. The reaction afforded a high yield with no evidence of formation of the regioisomer. The resultant acid **3** was esterified, converted to the hydroxamic acid **4**, and cyclized to the desired hydroxybenzisoxazole **5** as shown. The key mechanistic step was the reaction of the intermediate hydroxamic acid cyclic carbonate **A** (*8*). This cyclization has also been demonstrated by Kinstle and Darlage (*9*), who isolated a hydroxamic acid cyclic carbonate intermediate and treated it with triethylamine in a separate step to form a benzisoxazole. Small amounts of the benzoxazolone **6** were also formed from a competing Curtius reaction.

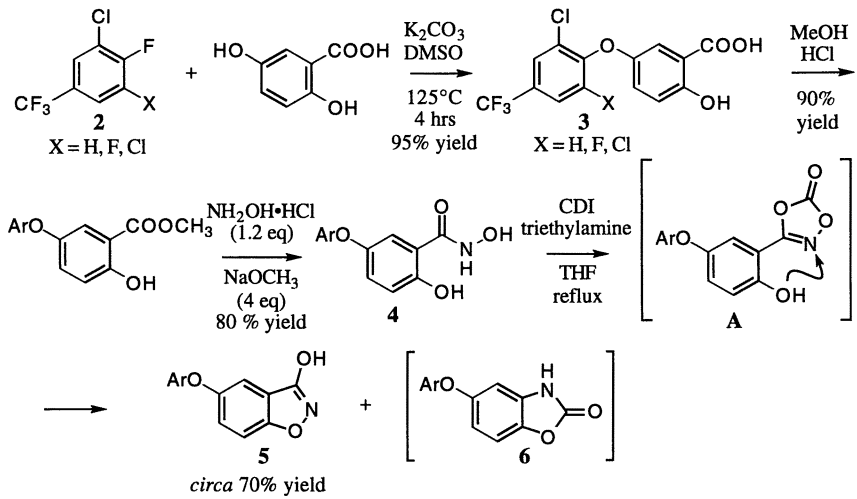


Figure 3. Preparation of 3-Hydroxybenzisoxazole Diphenyl Ethers.

Alkylation of 3-hydroxybenzisoxazole **5** with an α-bromopropionate occurs on the oxygen and nitrogen atoms, with the *O*-alkylation product **7** predominating. The *N*-alkylation product **B** reacts further resulting in cleavage of the benzisoxazole ring and recyclization to give the product **8** shown (*10*). This *N*-alkylation product has very weak herbicidal properties. **(See Figure 4.)**

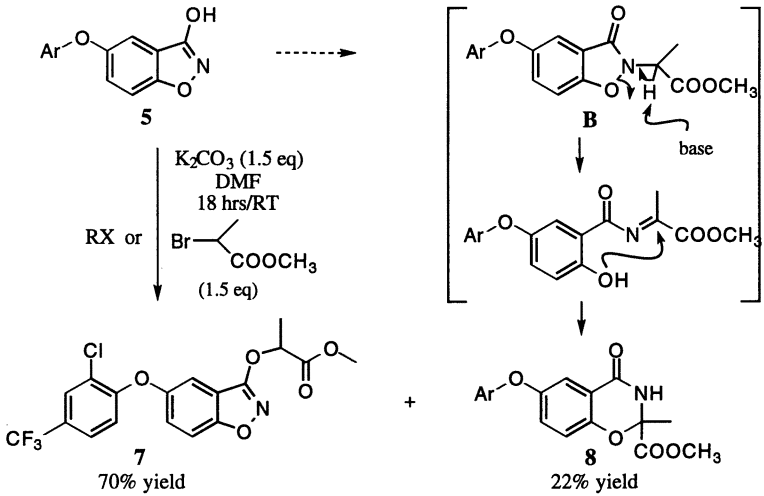


Figure 4. *N*- vs *O*-Alkylation of 3-Hydroxybenzisoxazole

Preparation of Esters of 5-Aryloxybenzisoxazole-3-acetic Acid. Our strategy in the preparation of a benzisoxazoleacetic acid diphenyl ether was to couple the known 5-hydroxybenzisoxazole-3-acetic acid methyl ester **10** with aryl halide **2**. The benzisoxazole-3-acetic acid **10** (R = H) was reported to have been prepared in good yield from 4,6-dihydroxycoumarin, **9**, by Giannella, Gualtieri, and Melchiorre (*11*) as shown in Figure 5. However, we had difficulty reproducing the literature results. In some instances, none of the desired product was formed.

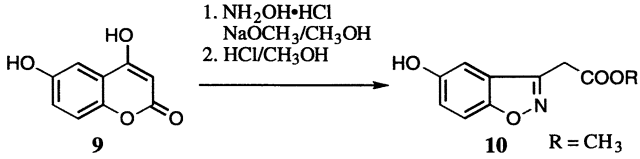


Figure 5. Preparation of 5-Hydroxybenzisoxazole-3-acetic Acid

We systematically examined the variables to determine why we were getting poor results. We reasoned that coumarin **9** must react as its keto form **D** and that the high pH conditions might reduce the ketone concentrations by forming salt **C**, thus slowing the reaction, Figure 6. We examined the effect of lowering the reaction pH by addition of acid, as additional hydroxylamine hydrochloride. Thus, an excess of hydroxylamine hydrochloride (besides the hydroxylamine normally used) was reacted with **9** to give good and consistent yields of **10**. However, the preparation of **10** was not without its own variant. Formation of the sodium salt of **10** to remove neutral by-products resulted in the salt crystallizing. However, a mixture of the sodium salt and the free acid were converted to the methyl ester **10** (R = Me) in high overall yield upon treatment with methanolic hydrogen chloride.

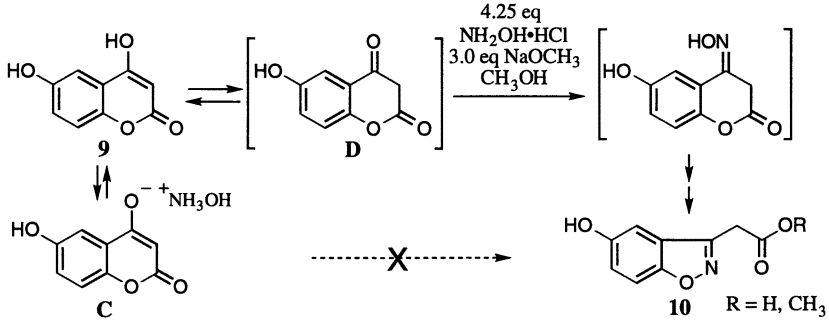


Figure 6. Formation of 5-Hydroxybenzisoxazole-3-acetic Acid.

Diphenyl Ether Formation and α–Substitution. Compound **10** was coupled with 3-chloro-4,5-difluorobenzotrifluoride, **2**, to give the desired diphenyl ether **11** (X = F) in 72% yield, Figure 7. Coupling of the less reactive chlorofluorobenzotrifluoride **2** (X = H) with ester **10** gave the diphenyl ether **11** in a moderate (35%) yield. However, when **2** was replaced with 3-bromo-4-chlorobenzotrifluoride, no coupling with **10** was observed. Heating the reaction to 100°C resulted in the consumption of **10** thus limiting analog preparation by this method.

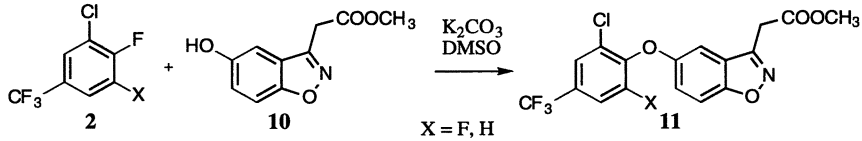


Figure 7. Formation of Diphenyl Ether

When acid **10** (R = H) was coupled with chlorofluorobenzotrifluoride **2** under more vigorous conditions, the decarboxylated product **1** resulted, Figure 8.

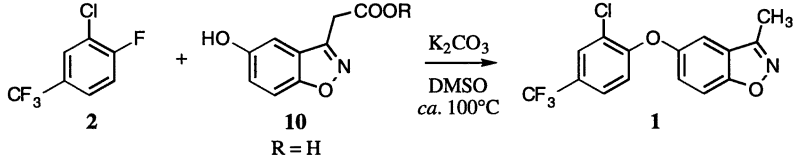


Figure 8. Formation of Diphenyl Ether with Decarboxylation.

When a more reactive benzotrifluoride such as **12** was used, the coupling reaction was accompanied by the formation of *bis*-adduct **14**, Figure 9. As we had succeeded in isolation of the desired product **13**, no effort was made to improve the ratio of product to by-product. Similar by-products could be formed upon treatment of **10** with other highly reactive aryl halides.

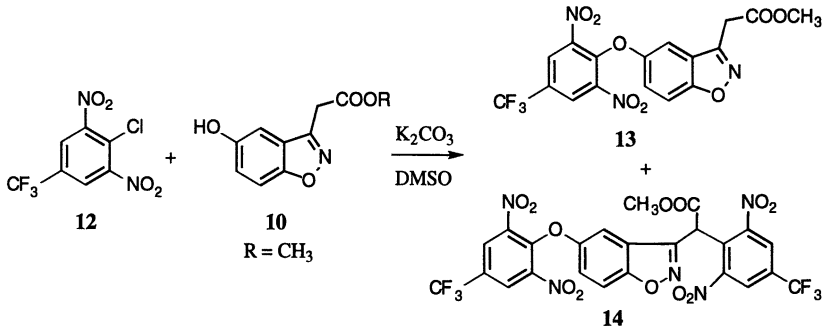


Figure 9. Benzisoxazole Arylation During Diphenyl Ether Formation.

Our next objective was to further improve the herbicidal activity by introduction of an α-methyl group. The introduction of α-substituents into benzisoxazoleacetic esters by alkylation had already been described by Ueda *et al.* (*13*). We were pleased to find that **11** could be cleanly monoalkylated with an alkyl halide, Figure 10. We did not observe any *bis*-alkylation or cleavage of the benzisoxazole ring taking place. We also prepared α-propynyl, α-allyl, and α-carbomethoxymethyl analogs **15** from the respective halides.

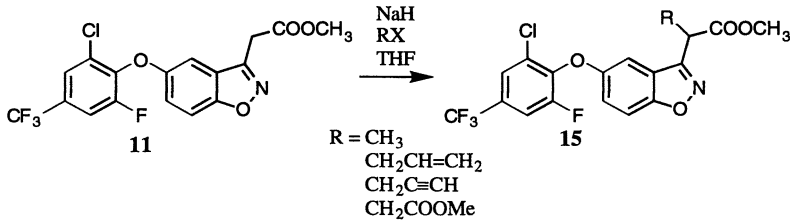


Figure 10. Benzisoxazole α-Alkylation.

We also attempted to introduce an alkylidene side chain via a Knoevenagel condensation. However, reaction of **11** with piperidine in refluxing acetone did not give the isopropylidene analog expected, but showed the competing reactivity of the benzotrifluoride ring and afforded the piperidine displacement product **16**, Figure 11.

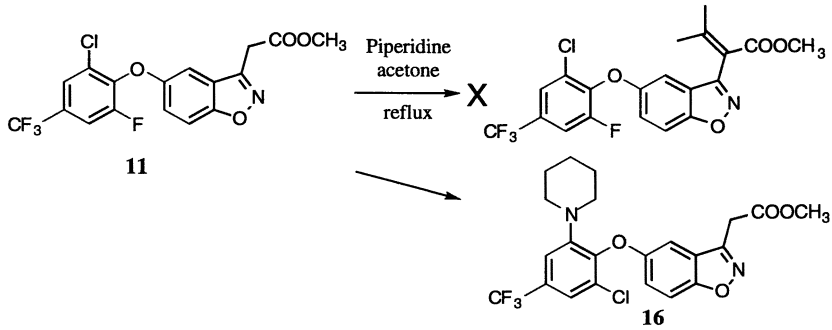


Figure 11. Piperidine Displacement of Fluorine during Attempted Knoevenagel Condensation.

Carboxymethyl Replacement. We now turned our attention to modifying the ester group. We reasoned that changing the oxidative and hydrolytic states of the ester might affect the biological rates of oxidation and hydrolysis and consequently have a positive effect on crop selectivity. Thus, reaction of **11** with ammonia in ethanol gave amide **17**, Figure 12. Although the fluoride was displaced with piperidine under the Knoevenagel conditions, no displacement was observed during amide formation. The resulting amide **17** was dehydrated to nitrile **18**.

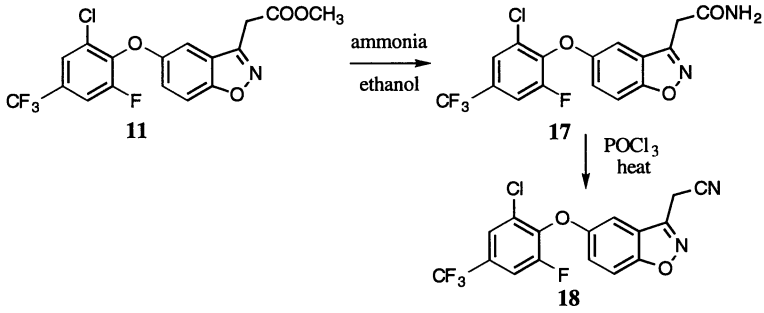


Figure 12. Formation of Amide and Nitrile From Benzisoxazole Ester.

The ester **11** was hydrolyzed to acid **19**, converted to its acid chloride; the acid chloride was used to prepare ethyl, allyl, and isopropyl esters **20**, Figure 13.

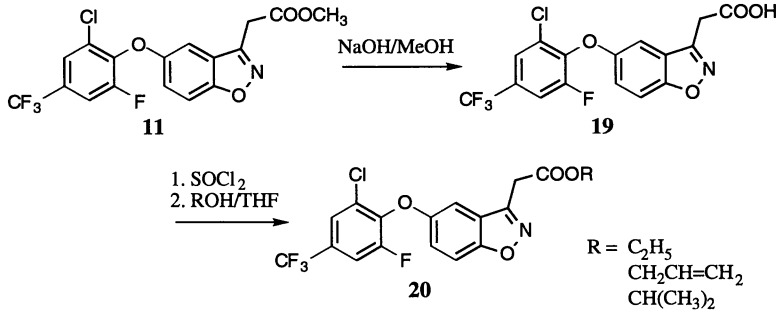


Figure 13. Formation of Esters From Benzisoxazoleacetic Acid.

The allyl bromide alkylation product was used in the preparation of a butenyl product **21**, Figure 14. This synthesis makes advantageous use of the ease with which a benzisoxazoleacetic acid can be decarboxylated, a reaction noted by Cassini, Gualtieri, and Stein (*12*).

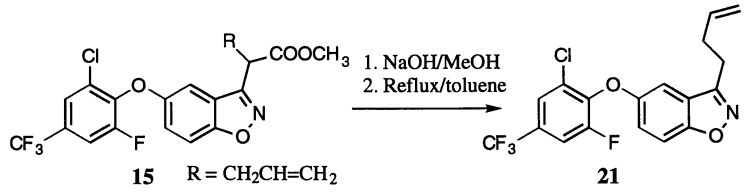


Figure 14. Preparation of Butenylbenzisoxazole via Decarboxylation.

Lithium aluminum hydride reduction of the ester group of **11** gave the hydroxyethyl benzisoxazole **22** in low yield, Figure 15. The low yield may reflect the instability of the benzisoxazole ring to lithium aluminum hydride although no ring cleaved products were isolated.

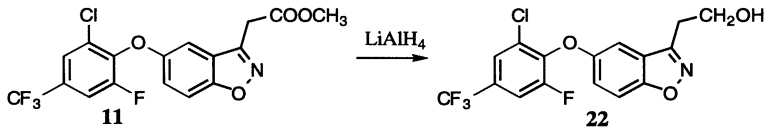


Figure 15. Lithium Aluminum Hydride Reduction of Benzisoxazole Ester

Herbicidal Activity

Mechanism of Action. The mechanism of action of the diphenyl ether herbicides is thought to be inhibition of protoporphyrinogen IX oxidase (*14, 15*), Figure 16. An unusual aspect of this mechanism is that the inhibition results in a buildup of protoporphyrin IX, the product of the oxidase enzyme. This occurs because the protoporphyrinogen diffuses from the chloroplast, where it is produced and consumed under normal conditions, and becomes oxidized to protoporphyrin IX in the cytosol. The membrane disruption that results is a consequence of protoporphyrin IX catalyzing production of singlet oxygen in the presence of light. Thus, the plant produces the toxicant itself.

An additional interesting factor in the biosynthesis is that porphyrin biosynthesis is regulated by heme. Heme, in turn, is co-produced with chlorophyll. Blockage of protoporphyrin IX biosynthesis blocks the biosynthesis of chlorophyll and heme and results in a deficiency in heme levels. The porphyrin biosynthesis is no longer feedback regulated by heme and porphyrins are overproduced, thus furthering the phytotoxic effects.

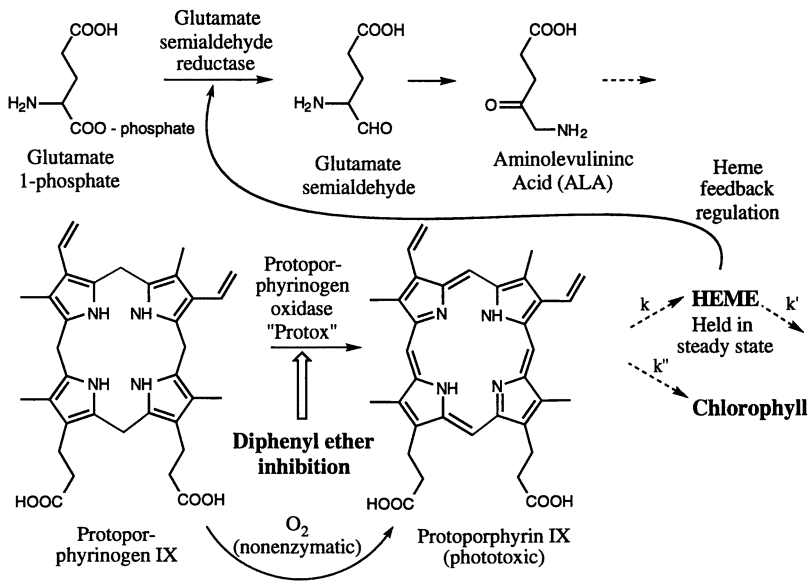
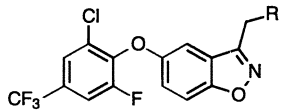


Figure 16. Mechanism of Protophorphyrinogen Oxidase Inhibition.

Structure-Activity Relationships. The structure-activity data was gathered from greenhouse application of the title compounds to a variety of weed and crop species in pre- and post-emergence tests. In general, the benzisoxazole diphenyl ether herbicides were more effective against broadleafed species, but data on grasses has been included to give a broader scale upon which to gauge the potency of a compound. The broadleafed species reported in the tables were velvetleaf (*Abutilon theophrasti*), ragweed (*Ambrosia artemisiifolia*), and morning glory (*Ipomoea spp.*), while the grasses were crabgrass (*Digitaria sanguinalis*) and green foxtail (*Setaria viridis*). The crop species soybean (*Glycine max*), corn (*Zea mays*), and winter wheat (*Triticum aestivum*) were tested, but because no crop selectivity was found, that data is not included in the tables.

The lack of success in finding crop selectivity from changes in the basic structure may be due to the rapid onset of toxicity in a post-emergence herbicide test. It is thought that the onset of symptoms occurs much faster than the combined factors required to detoxify a compound. Although the result of protoporphyrinogen oxidase inhibition is light induced membrane disruption, the herbicidal activity of this class was not limited to post-emergence applications. Table I shows comparative pre- and postemergence activity for two benzisoxazole diphenyl ether herbicides. Although the R-groups are quite different, a significant level of activity is noted for each compound in each application. The superior post-emergence potency is most evident for the ester.

Table I. Pre- Versus Postemergence Herbicide Activity of Benzisoxazole Diphenyl Ether Candidates



Control Rates in grams/hectare

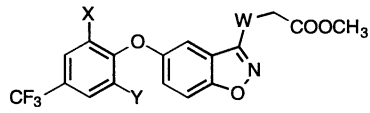
Application timing	R	Velvetleaf	Ragweed	Morning glory	Crabgrass	Green foxtail
Pre	COOMe	250	125	250	125	250
Post	COOMe	1	1	250	64	64
Pre	$CH_2CH{=}CH_2$	250	>500	250	500	125
Post	$CH_2CH{=}CH_2$	125	250	<32	250	250

(The '<' or '>' marks indicate lowest and highest rates tested, respectively.)

The preferred substitution pattern of the isolated aryloxy phenyl followed our expected results, namely that a chloro-fluoro combination performed the best (Table II). It is interesting to note that considerable activity was found for compounds in which the chloro or fluoro was replaced by other groups. However, replacing both the chlorine and fluorine with nitro resulted in a compound completely inactive at the highest rate tested.

The effect of the alkyl substituent on the herbicidal activity shows interesting differences in comparing the *C* vs *O*-alkyl series (Tables III and IV). In the ether series, the activity decreased rapidly as the ester moiety was altered and lost if the carbomethoxy was replaced with a methyl or chlorine. The allyl and propargyl ethers were also much less active than an ester. The greatest activity was found with a propionate group.

Table II. Aryl Substitution Effect on Post-emergence Herbicidal Activity

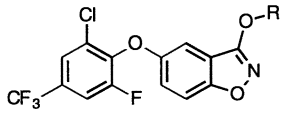


Control Rates in grams/hectare

X	Y	W	Velvetleaf	Ragweed	Morning glory	Crabgrass	Green foxtail
Cl	H	-	32	<32	<32	250	>500
Cl	F	-O-	2	4	8	<4000	<4000
Cl	F	-	32	1	1	250	64
Cl	Cl	-O-	<64	64	<64	>1000	500
NO_2	H	-	-	500	250	>500	>500
NO_2	NO_2	-	-	>>500	>>500	>>500	>>500
Cl	piperidine	-	-	32	250	>500	>500
F (perfluoro)	F (perfluoro)	-	-	<32	32	125	>500

(The '<' or '>' marks indicate lowest and highest rates tested, respectively. A '>>' indicates these was very little or no activity at that tested rate.)

Table III. Alkyl Ether Substitution Effects on Post-emergence Herbicidal Activity



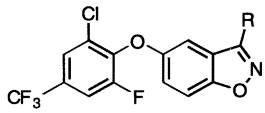
Control Rates in grams/hectare

R	Velvetleaf	Ragweed	Morning glory	Crabgrass	Green foxtail
CH_2COOMe	2	4	4	<4000	<4000
$CH(CH_3)COOMe$	0.5	1	1	125	250
CH_2COOH	<125	<125	125	125	500
$CH(CH_3)COOH$	32	250	125	500	500
$CH_2C{\equiv}CH$	500	>500	>500	>500	>>500
$CH_2CH{=}CH_2$	>>500	-	>500	>>500	>>500
$CH(CH_3)_2$	<1000	>1000	>>1000	>>1000	>>1000
$CH(Cl)CH_3$	<4000	>4000	>4000	>>4000	>>4000

(The '<' or '>' marks indicate lowest and highest rates tested, respectively. A '>>' indicates these was very little or no activity at the highest tested rate.)

In contrast, the herbicidal activity of the C-linked series was less sensitive to the changes in the ester functionality. This may indicate improved binding of the benzisoxazole or that biological oxidation or hydrolysis is a facile process and thus diminishes the expected differences. It was also noteworthy that the enhancement in activity of the propionate was much less than expected compared to the activity of the acetate, see Table 4. This result was not expected based upon the benzotriazole diphenyl ether precedents, see Condon, *et al.* (*16*), reported earlier in this symposium.

Table IV. Benzisoxazole Alkyl Substitution Effect on Post-emergence Herbicidal Activity



Control Rates in grams/hectare

R	Velvetleaf	Ragweed	Morning glory	Crabgrass	Green foxtail
CH_2COOMe	32	1	1	250	64
$CH(CH_3)COOMe$	1	32	32	125	64
CH_2COOH	32	-	<32	64	500
$CH_2COOiPr$	64	<32	<32	125	500
$CH_2COOCH2CH{=}CH_2$	32	<32	<32	64	250
CH_2COOEt	64	-	<32	<32	250
CH_2CH_2OH	64	-	125	500	>500
CH_2CN	250	-	64	500	>500
CH_2CONH_2	<32	<32	32	64	500
$CH(CH_2COOMe)COOMe$	-	125	125	>500	>500
$CH(CH_2CH{=}CH_2)COOMe$	-	<32	250	500	>500
$CH(CH_2C{\equiv}CH)COOMe$	-	32	125	125	>500
$CH(CH_2CH{=}CH_2)COOH$	64	-	250	250	>500
CH_3	64	>500	125	>500	>500
$CH_2CH_2CH{=}CH_2$	125	250	<32	250	250

(The '<' or '>' marks indicate lowest and highest rates tested, respectively.)

Conclusion. The benzisoxazole diphenyl ether herbicides follow a logical development from previous membrane disrupting diphenyl ether herbicides. A substitution pattern consistent with previous analogs was successfully incorporated which led to high levels of activity. The resulting products were most active as post-emergence broad leaf herbicides without significant crop tolerance.

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Chapter 15

Phytoene Desaturase

A Model for the Optimization of Inhibitors

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A model of the herbicide binding site of phytoene desaturase was constructed by the overlay of five structurally dissimilar inhibitors, and this was used to predict the likely levels of herbicidal activity of some simple analogues of the newly discovered inhibitor, 2-(3-trifluoromethylbenzyl)-3-ketomorpholine. The inhibitory activity of the most active analogue, *trans*-5-methyl-2-(3-trifluoromethylbenzyl) - 3-ketomorpholine, was shown to reside almost exclusively with the (2*R*),(5*S*)-form, and this stereochemical information was incorporated into the model

Inhibition of carotenoid biosynthesis has received a great deal of attention over the past fifteen to twenty years, and is now a well established mechanism of herbicide action which has been the subject of a recent review (*1*). Of the many steps involved in this pathway, the one most commonly encountered in the herbicide literature is inhibition of the desaturation of phytoene to phytofluene (*2*). Although relatively little is known about the enzyme which carries out this step (phytoene desaturase), several classes of structurally diverse inhibitors are known for which some structure-activity information is available (*3-7*). Based on the assumptions that these inhibitors bind in the same way to the same site of the enzyme, and that the optimum herbicidal compound from each series reflects the correct steric and electronic properties necessary to bind tightly to this site (in addition to possessing properties necessary for good uptake and translocation), we reasoned that if a satisfactory molecular overlay of the optimum herbicide from each class of inhibitor could be achieved, then the total union volume of this overlay might give a good indication of the overall steric and electronic requirements at the herbicide binding site.

In this paper we describe our attempts to construct a such model of the herbicide binding site of phytoene desaturase, and how we used it to predict the likely herbicidal activities of a number of analogues of the ketomorpholine **1**, a newly discovered herbicidal inhibitor of phytoene desaturase (*8*).

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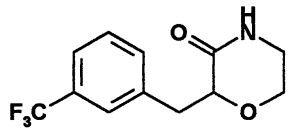

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Results and Discussion

Construction of the Model. The following compounds were chosen as the optimum examples from each of five structurally distinct classes of phytoene desaturase inhibitors: Norflurazon **2** (pyridazinones), Fluridone **3** (pyridones), Flurtamone **4** (aminofuranones), Fluorochloridone **5** (pyrrolidones) and Diflufenican **6** (nicotinamides). The model of the herbicide binding site was then constructed in four stages, as described below.

Identification of Common Structural Features. Structural features common to all, or a subset, of the compounds were required as a basis on which to carry out the molecular overlay. The structural elements selected are highlighted in Figure 1. The only feature common to all of the inhibitors is the lipophilic 3-trifluoromethylphenyl substituent (labelled #1). Compounds **2-5** each contain an amide or vinylogous amide carbonyl group (#2) which forms part of the central heterocyclic ring; compound **6** does not contain such a group, though it does possess a diaryl ether (imidate-like) oxygen atom and an exocyclic amide carbonyl oxygen atom (both #2), either of which might occupy a region of space close to the carbonyl oxygen atoms (#2) of the other inhibitors (and thus may bind to a hypothetical hydrogen bond donor or similar function in this region of the binding site). Compound **2** contains a vinylogous amide NH function (#3), which in its more stable *s*-trans form might occupy a region of space close to that occupied by the NH function of the exocyclic amide group (#3) of compound **6** (and thus form hydrogen bonds to a hypothetical hydrogen bond acceptor in this region of the binding site). Finally, the (lipophilic) *N*-methyl substituent (#4) of compound **2** might occupy the same region of space as the chlorine atom (#4) of the chloromethyl group of compound **5**.

Preliminary Overlay of Compounds 2-6. Since it is not known whether the enantiomers of either Flurtamone **4** or Fluorochloridone **5** show any differential activity, we arbitrarily chose to use the (*R*)-form of **4** and the (3*S*),(4*S*)-form of **5** in the initial construction of the model. The molecular modelling package SYBYL (available from Tripos Associates Inc., St. Louis, Missouri, USA) was used to build each of compounds **2-6** (the conformations of saturated rings were obtained from the Cambridge Crystallographic Database). These were then overlayed by matching up the common features highlighted above, allowing free rotation of all acyclic bonds.

Energy Minimization of Conformations. The conformation of each of compounds **2-6** obtained from the molecular overlay described above was minimized using the program MAXIMIN II (*9*).

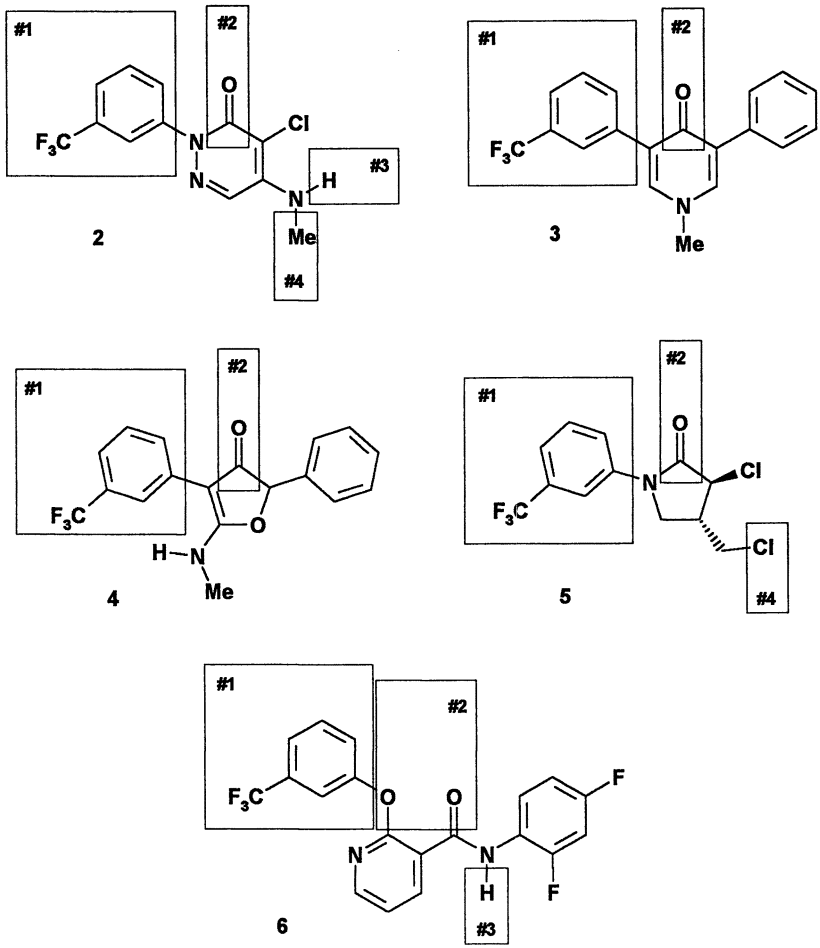


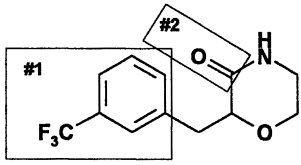
Figure 1. Common Structural Features Used in Overlay of Inhibitors

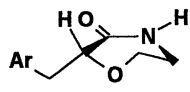
Final Overlay of Compounds 2-6. The energy minimized conformations of each of compounds **2-6** were then overlayed by matching up the common features highlighted in Figure 1. Figure 2 shows orthogonal views of the resultant overlay in which three regions can be defined; regions X (substituted phenyl ring) and Y (central heterocyclic ring), in which the steric (and electronic) requirements appear to be relatively well defined, and region Z, which is less well defined and appears to be sterically much more tolerant (the orientations of the substituents in this region cannot be described with any certainty).

Before any attempts could be made to use this model predictively, the effects of arbitrarily choosing a single enantiomer for each of compounds **4** and **5** in its construction needed to be investigated. The 2-phenyl substituent of (*R*)-Flurtamone is located entirely within the ill-defined region Z; if the (*S*)-form were also found to show activity then region Z would need to be extended, and if the (*S*)-enantiomer

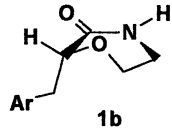
were found to be the only active form then region Z would need to be redefined. Thus, the effect of choosing a single enantiomer of compound **4** had the effect of making region Z potentially less well defined than it appears in Figure 2. For Fluorochloridone, if the (3*R*),(4*R*)-enantiomer should be found to be the active form then the entire model would need to be inverted, whereas if both enantiomers were found to possess activity then region Y would need to be expanded to accommodate the (3*R*),(4*R*)-form. These are factors which need to be taken into account when using the model.

Fitting Compound 1 into the Model. Since it was not known whether the enantiomers of compound **1** displayed differential activity, both were initially fitted into the model. Two conformations of each enantiomer were considered; conformation **1a** has the substituted benzyl group in a pseudo-equatorial orientation, whereas **1b** has it pseudo-axial [only the (2*R*)-forms are displayed in the diagrams for clarity]. The conformers were built using SYBYL, and each was fitted into the model by overlaying the 3-trifluoromethylphenyl substituent (#1) and the lactam carbonyl group (#2). A much better fit into the sterically restricted regions X and Y of the model was obtained for the (2*R*)-form of the pseudo-axial conformer **1b**. The fact that one enantiomeric form fits the model better than the other means that, as for Fluorochloridone, if the opposite enantiomer were subsequently found to be the active form, the entire model would need to be inverted.





1a

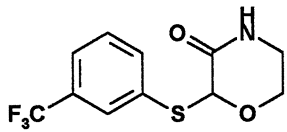


1b

Conformational analyses of molecules **1** and **7** (which is also a herbicidal inhibitor of phytoene desaturase) were performed to try to determine the energy differences between the axial and equatorial conformers, and hence whether the axial conformation is accessible to either compound (*10*). The low energy rotamers of each conformation of both compounds were generated using the conformational searching functionality available within SYBYL. The conformations thus generated were then further geometry optimized by molecular mechanics [AESOP (*11*)] and a semi-empirical molecular orbital method (AM1) using the SPARTAN program (*12*). Single point *ab initio* calculations, using the SPARTAN program with the STO-3G basis set, were also performed on the molecular mechanics optimized geometries.

There was some disagreement between the three methods used. For compound **1** the molecular mechanics and *ab initio* calculations favoured an equatorial conformation (by 2.7 and 1.4 kcal/mol, respectively) whereas the semi-empirical method favoured an axial conformation (by 0.3 kcal/mol). For molecule **7** the molecular mechanics and semi-empirical methods favoured an axial conformation (by 0.8 and 2.0 kcal/mol, respectively) while the *ab initio* calculations marginally favoured an equatorial conformation (by 0.1 kcal/mol). Thus, although

there are some discrepancies between the methods used, these calculations do indicate that an axial conformation for compound **1** is at least accessible to the molecule without it incurring an undue energy penalty, and that compound **7** is more likely to adopt an axial conformation than compound **1**. On the basis of these calculations, we believed it reasonable to use the pseudo-axial conformations of compounds **1** and **7** in this modelling work.



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The union volume of the overlay of compounds **2-6** (looking from the same perspective as in the lower diagram of Figure 2) is shown in Figure 3 (yellow); the space outside this union volume which is occupied by compound **1** following fitting conformer **1b** into the model is shown in red. Clearly, this compound fits largely inside regions X and Y of the overall volume described by compounds **2-6**. The total union volume of compounds **1-6** was used as our steric model for the herbicide binding site in all subsequent work; this we have termed the 'active volume', *i.e.* the total volume likely to be available to an inhibitor at the binding site.

Activity Predictions for Substituted Analogues of Compound 1. Methyl groups were built on to compound **1** at each of the positions shown in Figure 4; this was done without altering conformation **1b**. (Note:- the stereochemistry depicted in Figure 4 represents the relative rather than the absolute stereochemistry of these analogues.) Each compound was then fitted into the model by superimposing it on compound **1**, and the extent to which the added methyl group protruded from the active volume described by compounds **1-6** was calculated. The results are summarized in Table I. The volume of a methyl group added in this way is approximately 15 $Å^3$; thus a figure of 1-3 $Å^3$ means that the added substituent is essentially inside the active volume (and hence that the compound stands a high chance of showing activity), whereas a larger figure means that it is largely outside the active volume (and therefore the compound is less likely to show any activity).

These results suggest that compound **1D** should fit best into the model. However, the required conformation involves a potentially high energy 1,3-diaxial interaction. Attempts to calculate the difference in energy between this and other possible conformers of the molecule were unsuccessful; when this conformation was geometry optimized using either molecular mechanics or the semi-empirical method, the molecule flipped from a pseudo-chair to a pseudo-boat conformation to relieve the strain imposed by the steric clash between the substituents (*10*). Thus, these calculations suggest that compound **1D** will adopt an alternative preferred conformation in which one or both of the substituents assume an equatorial orientation. Such conformations give a very poor fit into the model, and thus it was concluded that compound **1D** stands only a low chance of being active.

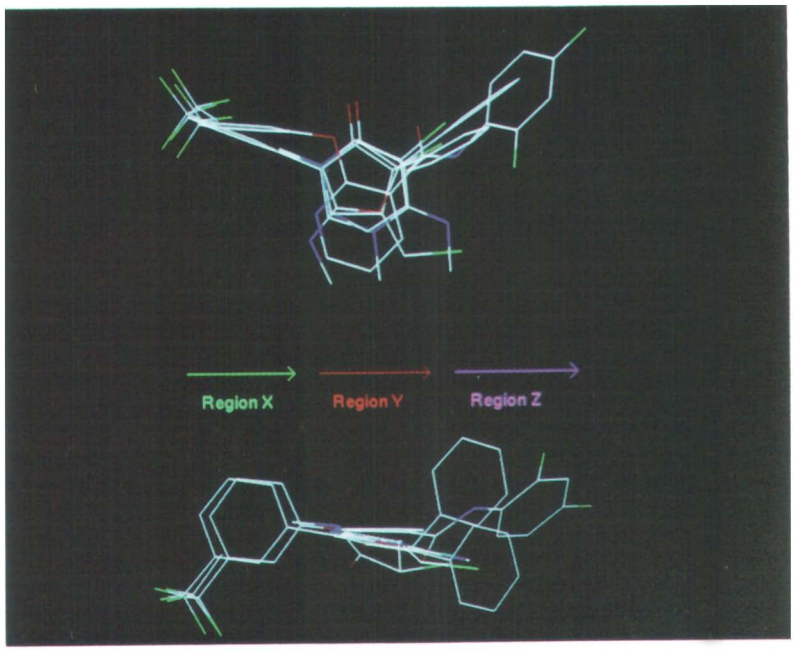


Figure 2. Orthogonal Views of the Overlay of Compounds **2-5**

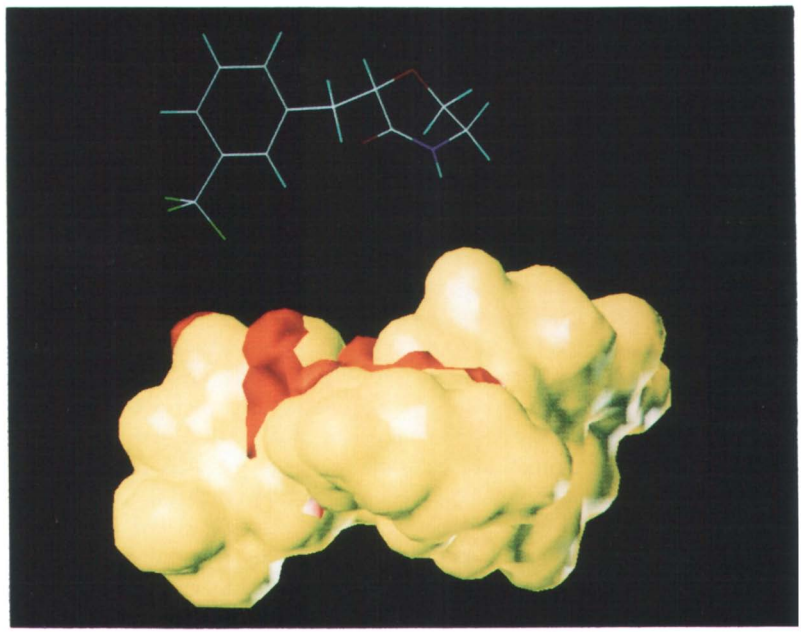


Figure 3. Union Volume Overlay of Compounds 2-5 (yellow), and Result of Adding Compound 1 (red)

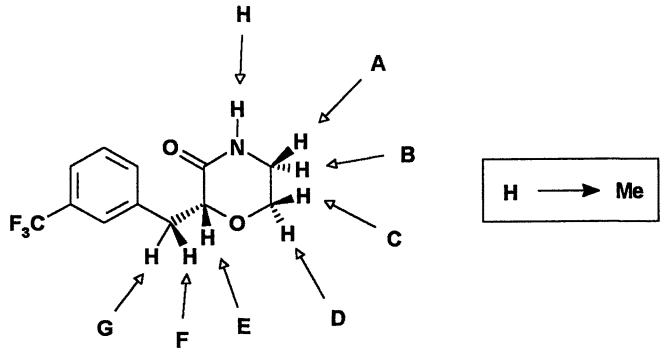


Figure 4. Sites for Addition of Methyl Groups to Compound **1**

Table I. Protrusion of Methylated Analogues **1A-1H** from Active Volume

Analogue	*Protruding Volume* (Å^3)
1A	1.9
1B	8.2
1C	8.2
1D	1.0
1E	10.5
1F	10.5
1G	6.8
1H	6.2

Compound **1A** also provides a good fit. Moreover, for this compound the added methyl substituent points towards the sterically more tolerant region Z, whereas the added substituents of the remaining analogues protrude out of the much tighter steric constraints of regions X and Y. On this basis, our model predicts that for the analogues **1A-1H**, compound **1A** is the one most likely to show herbicidal activity by inhibition of phytoene desaturase.

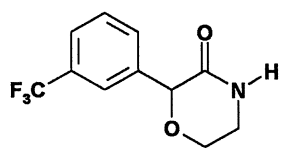
This was indeed found to be the case; when these methylated analogues were prepared (*13*), compound **1A** was the only one which showed any significant herbicidal activity - the remainder were poorly active at best (Table II). The figures quoted in Table II for the relative herbicidal activity of each of these analogues refer to the average level of pre-emergence control of a range of broad-leaved plant species and grasses at a given rate, compared to the activity of compound **1** which has been normalized to 100. Also, it should be noted that compound **1E** was not prepared; the figure given in Table II refers to the activity of the corresponding methylated analogue of compound **7**, relative to that of compound **7** (normalized to 100).

Table II. Herbicidal Activities of Analogues **A-H** of Compounds **1** and **7**

Analogue	*Relative Herbicidal Activity* (*cf* compound **1** = 100)
1A	180
1B	20
1C	0
1D	0
7E	<10 (*c.f.* compound **7** = 100)
1F	<10
1G	<10
1H	25

In order to further quantify the relative activities of compounds **1** and **1A**, the *in vitro* activity of each was determined against phytoene desaturase using the method described by Beyer and coworkers (*14*). Compound **1** has an I_{50} value of 370 μM, whereas compound **1A** has an I_{50} of 80 μM (*15*). The increased activity of compound **1A** is explained in terms of our model by the molecule occupying more of the space available to it at the herbicide binding site, and thus increasing the binding energy by additional Van der Waals interactions.

Homologues of Compound 1. The homologues **8** and **9** were built using SYBYL, and various conformations of each of these were fitted into the model. Compound **8** gave, at best, only a poor fit into regions X and Y of the active volume (protruding by at least 17 Å^3), and was found to be virtually inactive as a herbicide (it showed some weak, transient bleaching symptoms in a pre-emergence test). Compound **9** gave a very bad fit into the model (protruding by *ca* 38 Å^3), and displayed no herbicidal properties whatsoever (*13*).



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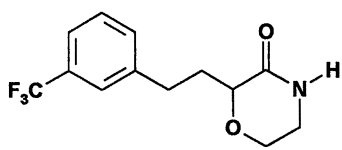

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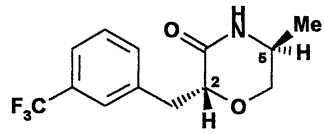
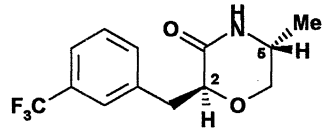
Chiral Nature of the Herbicide Binding Site. The results presented in the preceding sections referred to the relative activities of racemic analogues of compound (**1**). The increased activity of compound **1A** prompted us to investigate the relative activities of its enantiomers [which are readily available from *D*- and *L*-alaninol (*13*)]. The activity was found to reside virtually exclusively with the (2*R*),(5*S*)-form (Figure 5),

thus demonstrating a marked chiral preference for inhibitors of phytoene desaturase. The active enantiomer of compound **1A** is the one used in the modelling work, and thus our model, as described above, also reflects the correct absolute stereochemical requirements at the herbicide binding site.



(2*R*),(5*S*)-form; I_{50} = 37μM



(2*S*),(5*R*)-form; I_{50} > 500μM

Figure 5. *In Vitro* Activity of Enantiomers of Compound **1A**

Conclusions

A steric model for the herbicide binding site of phytoene desaturase has been constructed based on the assumption that a number of structurally diverse inhibitors bind in the same way at the same site on the enzyme. This model has been used to predict the likely levels of herbicidal activity for a number of analogues of the novel inhibitor, 2-(3-trifluoromethylbenzyl)-3-ketomorpholine. The chiral nature of the herbicide binding site has been demonstrated and incorporated into the model. This model may be of use in the design and optimization of new inhibitors of phytoene desaturase.

Acknowledgments

The author would like to thank J. Delaney and G. Sexton for their help in the construction of this model, R. Viner for carrying out the molecular orbital calculations and D.L. Bartlett for the phytoene desaturase *in vitro* determinations.

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Chapter 16

Aryl-Substituted Quinoxalines and Related Heteroarenes as Novel Herbicides Prepared via Palladium-Catalyzed Cross-Coupling Methods

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In follow-up to our discovery and investigation of aryl-substituted triazolo[1,2-*a*]pyrimidines as a novel class of bleaching herbicides, we have found that related aryl-substituted quinoxalines and other heteroarenes of formula **3** are also herbicidal. In some cases, high levels of broad-spectrum activity with crop selectivity were observed. Compounds of this class were found to show preemergence control of a number of key grasses and broadleaf weeds in cereals. These aryl-substituted heterocycles and their N-oxides were readily made via palladium-catalyzed cross-coupling reactions of bromoheterocyclic precursors with an arylstannane or arylboronic acid. The bleaching symptoms observed for these compounds on plants were the same as that observed for the structually related aryltriazolo[1,2-*a*]pyrimidines herbicides which we previously found to be carotenoid biosynthesis inhibitors. A summary of the synthesis and biological activity of these novel aryl-substituted nitrogen-containing heteroarenes is reported.

We previously reported that aryl-substituted triazolo[1,2-*a*]pyrimidines such as **1** and related substituted heterocycles such as triazolo[1,2-*a*]pyridine **2** represented a new family of highly active herbicides which inhibit carotenoid biosynthesis (*1*). In continuation of our effort in this area, we wish to report on the synthesis and herbicidal activity of structually related aryl-substituted quinoxalines and other heteroarenes of formula **3** (*2*). As with triazolo[1,2-*a*]pyrimidines and triazolo[1,2-*a*]pyridines (*1,3*), we observed similar structure-activity relationships for heterocycles of formula **3** and found that the same substitution was generally required for optimum activity. Compounds of formula **3** were readily prepared via palladium-catalyzed cross-couplings of bromoheterocycles of formula **4** with an arylstannane or arylboronic acid.

Chemistry

Syntheses of a number of aryl-substituted nitrogen-containing heteroarenes of formula **3** are outlined in Figures 1-8. In most cases, *meta*-trifluoromethylphenylstannane **5** was coupled with bromoheterocycles of formula **4** in the presence tetrakis-(triphenylphosphine)palladium(0) [3-5 mole %]. Boronic acid cross-couplings also worked quite well and are represented by reactions involving the arylboronic acids **6**

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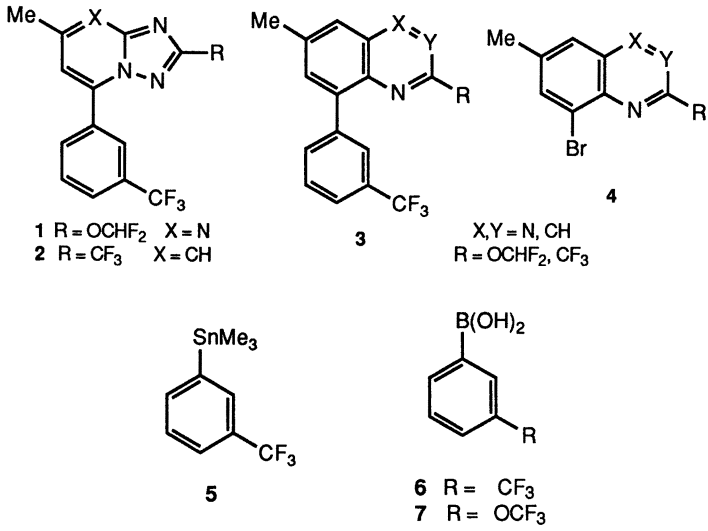


(R = CF_3) and **7** (R = OCF_3). Arylstannane **5** and the arylboronic acids **6** and **7** were readily made from the corresponding arylbromides by metal-halogen exchange and trapping with trimethyltin chloride (in the case of **5**) or with trimethyl borate (followed by acid hydrolysis in the case of **6** and **7**). We found that these arylboronic acids existed partially in the anhydride form.

Preparation of the 8-aryl-2-difluoromethoxy-6-methylquinoline **8** is shown in Figure 1. Reaction of 2-bromo-4-toluidine with cinnamoyl chloride gave the cinnamanilide **9**. Using the conditions of Colonge and Chambard (*4,5*), heating cinnamanilide **9** with three equivalents of aluminum chloride neat at 100°C gave a crude yield of bromoquinolone **10** (contaminated with some debrominated quinolone by-product). Treating this crude quinolone mixture with difluorocarbene [generated from Freon-22® (chlorodifluoromethane) and 50% aqueous sodium hydroxide in the presence of the phase transfer catalyst tetra-*n*-butylammonium bromide in dioxane] resulted in almost exclusive alkylation on oxygen to provide the bromo-difluoromethoxyquinoline **11** in 26% yield (two steps). Significant alkylation was not observed on the quinolone ring nitrogen and this was most likely due in part to steric hinderance by the bromo group at the peri-position on the quinolone **10**. Palladium-catalyzed cross-coupling of bromoquinoline **11** with arylstannane **5** in the presence of tetrakis(triphenylphosphine)palladium(0) gave the arylquinoline **8** in 71% yield.

Figure 2 illustrates the synthesis of two regioisomeric aryldifluoromethoxy-quinoxalines **12** and **13**. Catalytic reduction of 2-bromo-6-nitro-4-toluidine (**14**) (*6*) gave the corresponding phenylenediamine **15** which on condensation with aqueous glyoxylic acid at room temperature provided a ca. 2:5 mixture of the two quinoxalinone regioisomers **16** and **17** in 40% yield (two steps). X-ray crystal analysis of the major quinoxalinone, which was obtained pure by fractional crystallization, was shown to be structure **17**. Treatment of the above quinoxalinone mixture with difluorocarbene (generated from Freon-22®) afforded a regioisomeric mixture of the two bromodifluoromethoxyquinoxalines **18** and **19** which were also isolated in about the same ratio (90% yield). Again, alkylation occurred

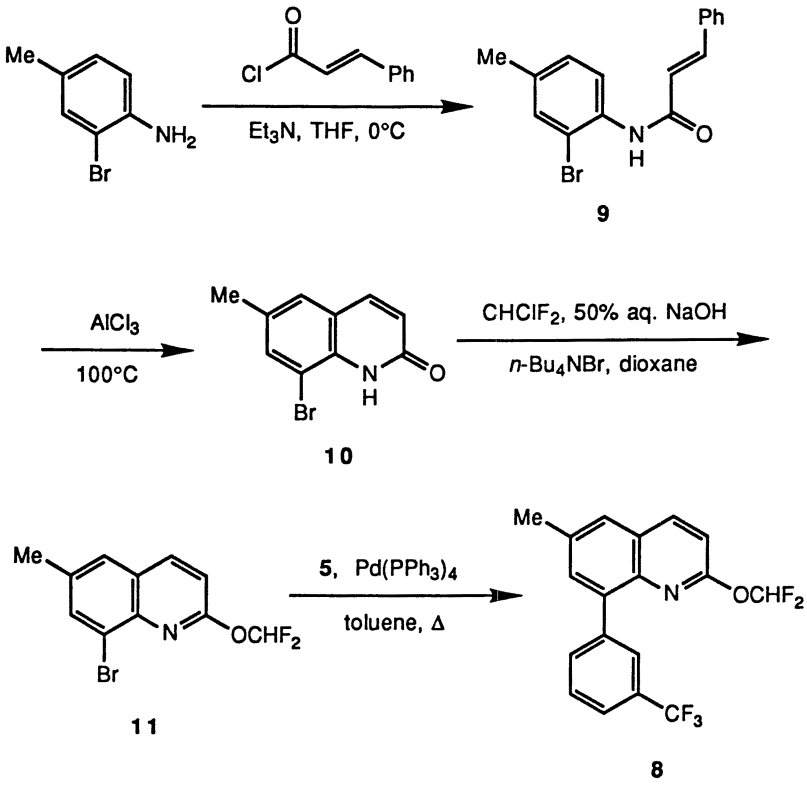


Figure 1. Synthesis of an 8-Aryl-2-difluoromethoxy-6-methylquinoline.

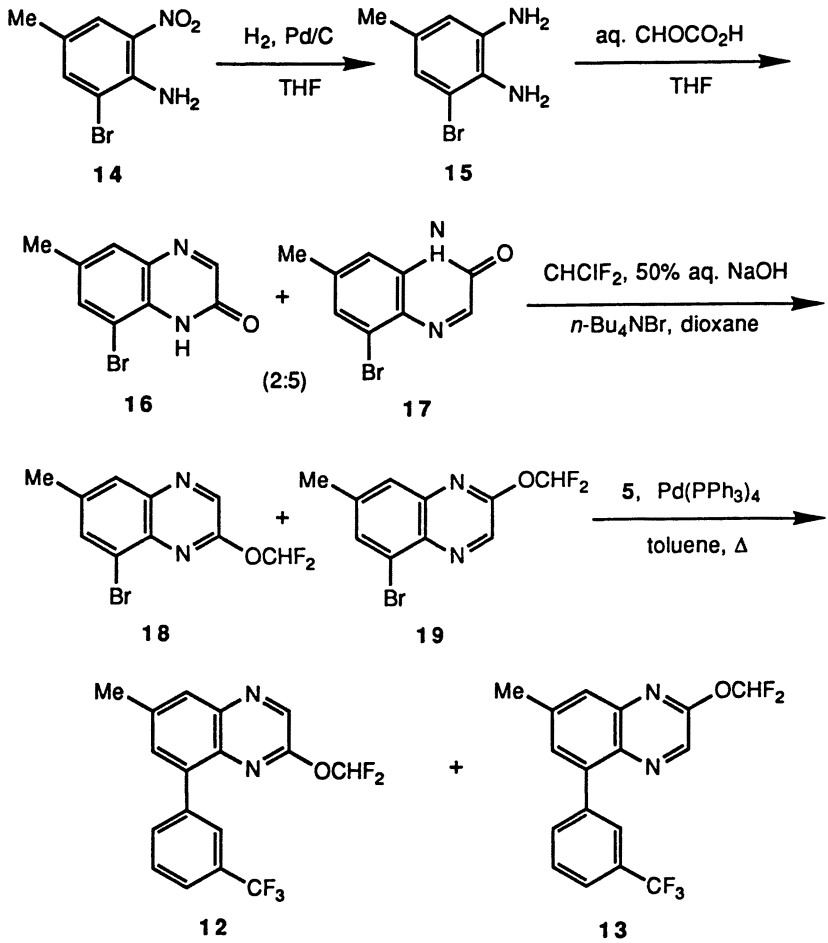


Figure 2. Synthesis of Aryldifluoromethoxyquinoxalines.

predominantly on oxygen rather than on the heterocyclic ring nitrogens. Palladium-catalyzed cross-coupling of the mixture of bromoquinoxalines **18** and **19** with arylstannane **5**, using tetrakis(triphenylphosphine)palladium(0) as catalyst afforded, in a combined yield of 55%, arylquinoxalines **12** and **13**, which were separated by flash chromatography.

A regioselective synthesis of quinoxaline **12** is summarized in Figure 3. Following a known method for the regioselective synthesis of substituted quinoxalinone-N-oxides (*7,8*), reaction of 2-bromo-6-nitro-4-toluidine (**14**) with diketene gave acetoacetanilide **20** (52% yield) which underwent ring cyclization to quinoxalinone-N-oxide **21** in aqueous sodium hydroxide/isopropanol (75% yield). Treatment of **21** with difluorocarbene (generated from Freon-22®) afforded a mixture of the difluoromethoxy-substituted bromoquinoxaline-N-oxide **22** and the corresponding deoxygenated quinoxaline **18** (isolated in yields of 43% and 30%, respectively, after flash chromatography). Cross-coupling of N-oxide **22** with arylstannane **5** in the presence of tetrakis(triphenylphosphine)palladium(0) gave arylquinoxaline-N-oxide **23** (73% yield) which was deoxygenated to **12** (53% yield) by catalytic hydrogenation.

Another synthesis of aryldifluoromethoxyquinoxalines, with trifluoromethoxy rather than trifluoromethyl substitution on the aryl ring, is shown in Figure 4. In this case, the cross-coupling step was carried out in the beginning of this reaction sequence rather than at the end. Palladium-catalyzed cross-coupling of 2-bromo-6-nitro-4-toluidine (**14**) with arylboronic acid **7** afforded the *meta*-trifluoromethoxyphenyl-substituted nitroaniline **24** in 86% yield. Catalytic hydrogenation of **24** followed by condensation with glyoxylic acid provided a mixture of quinoxalinones **25** and **26** (ca. 1:2 ratio). Reaction of this mixture with difluorocarbene (generated from Freon-22®) produced, in a combined yield of 70% from **24**, the aryldifluoromethoxyquinoxalines **27** and **28**, which were separated by flash chromatography.

The preparation of aryltrifluoromethylquinoxalines **29** and **30** is illustrated in Figure 5. In this procedure, 1,1-dibromo-3,3,3-trifluoroacetone was hydrolyzed in aqueous sodium acetate to the ketoaldehyde which was reacted *in situ* with 3-bromo-5-methylphenylenediamine **15** to afford, in 74% yield, a ca. 3:1 mixture of the bromotrifluoromethylquinoxalines **31** and **32**. Without separating these bromoquinoxalines, the mixture was cross-coupled with arylstannane **5** via palladium(0) catalysis to generate, in a combined yield of 82%, arylquinoxalines **29** and **30**, which were separated by flash chromatography.

In Figure 6, the procedure for making an 8-aryl-6-methyl-2-trifluoromethyl-quinazoline is outlined. Bromoanthranilic acid **31** (*9*) was converted to the corresponding anthranilamide **32** (21% yield) which on condensation with ethyl trifluoroacetate in sodium ethoxide/ethanol was transformed to the 8-bromo-2-trifluoromethylquinazolinone **33** (65% yield). Palladium(0)-catalyzed cross-coupling of **33** with arylboronic acid **6** gave the arylquinazolinone **34** in 41% yield. Heating **34** in phosphorous oxychloride produced the 4-chloroquinazoline **35** in 75% yield. Catalytic hydrogenolysis of the chloro group on **35** resulted in generation of the desired arylquinazoline **36** (31% yield).

The synthesis of 5-aryl-3-difluoromethoxy-7-methylbenzotriazine **37** and its N-oxide **38** is illustrated in Figure 7. By an established method (*10*), 2-bromo-6-nitro-4-toluidine (**14**) was converted to 3-amino-5-bromo-7-methylbenzotriazine-N-oxide **39** by reacting with cyanamide in a mixture of concentrated hydrochloric acid and glacial acetic acid (CAUTION: extremely exothermic reaction) followed by treatment

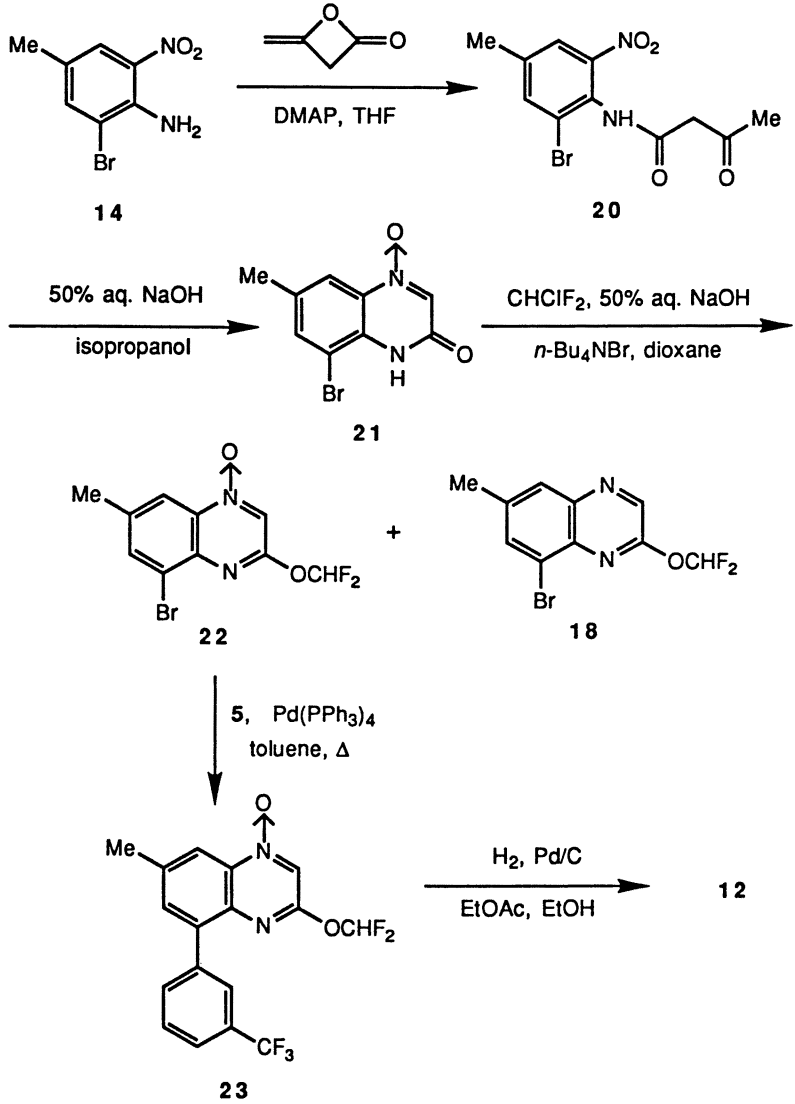


Figure 3. Regioselective Synthesis of an 8-Aryl-2-difluoromethoxy-6-methylquinoxaline.

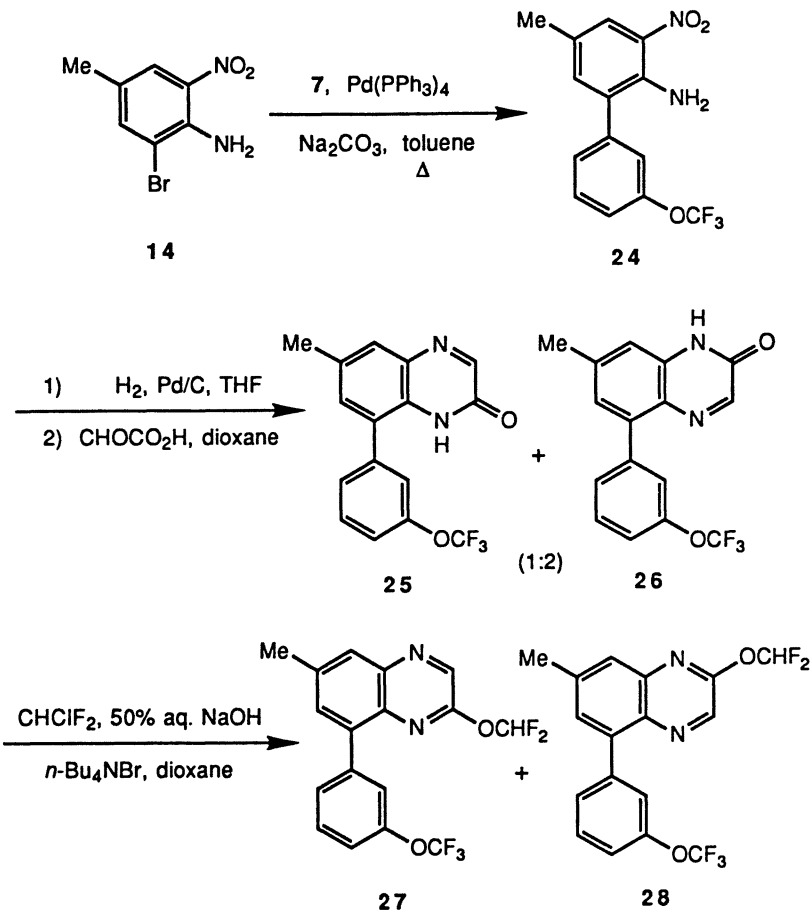


Figure 4. Alternative Synthesis of Arylquinoxalines.

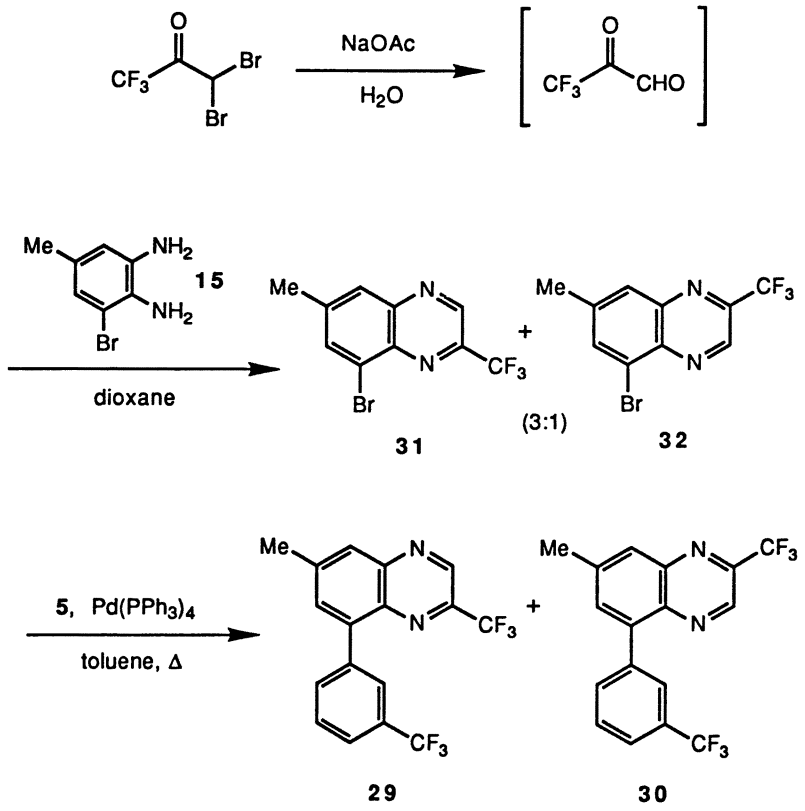


Figure 5. Synthesis of Aryltrifluoromethylquinoxalines.

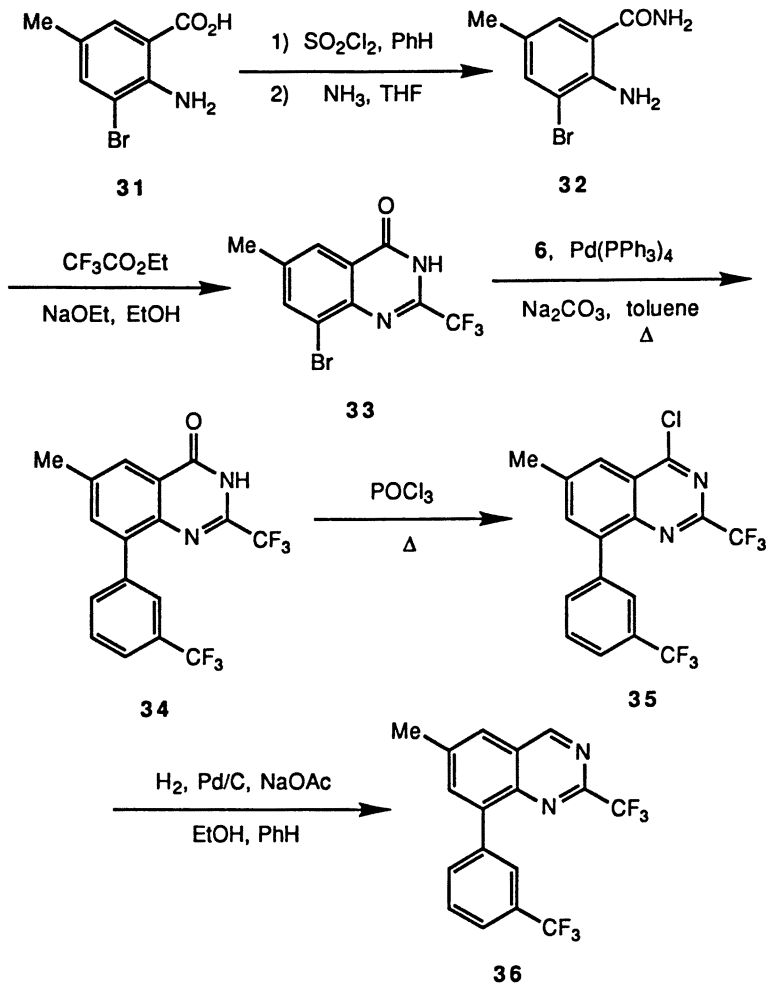


Figure 6. Synthesis of an 8-Aryl-6-methyl-2-trifluoromethylquinazoline.

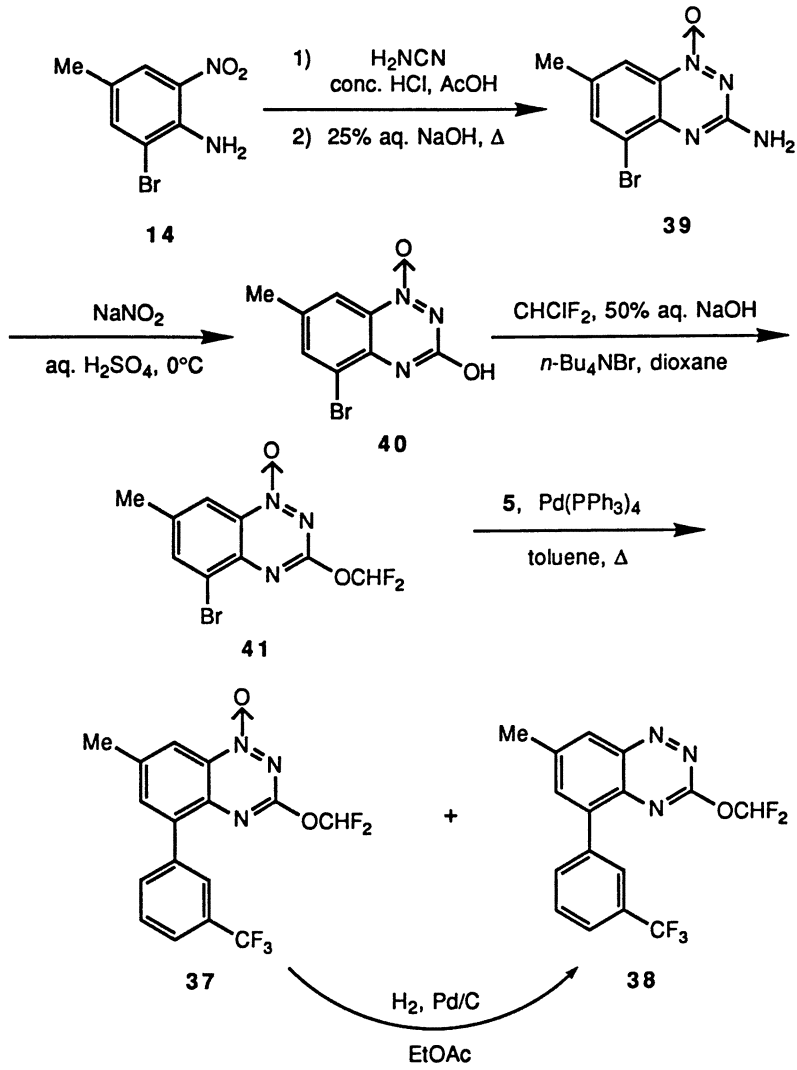


Figure 7. Synthesis of a 5-Aryl-3-difluoromethoxy-7-methylbenzotriazine.

with 25% aqueous sodium hydroxide. Diazotization of **39** with sodium nitrite in aqueous sulfuric acid gave the hydroxybenzotriazine-N-oxide **40** which on reaction with difluorocarbene (generated from Freon-22®) produced 5-bromo-3-difluoromethoxy-7-methylbenzotriazine-N-oxide **41**. Some deoxygenation occurred on palladium(0)-catalyzed cross-coupling of **41** with arylstannane **5** and a mixture of aryl-benzotriazine-N-oxide **37** and arylbenzotriazine **38** was obtained. Flash chromatography provided **37** and **38** in yields of 63% and 13%, respectively. The N-oxide **37** also underwent deoxygenation by catalytic hydrogenation to afford benzotriazine **38** in 68% yield.

In Figure 8, the preparation of 8-aryl-4-difluoromethoxy-6-methylcinnoline **42** is outlined. Unlike the other aryl-substituted heteroarenes discussed, **42** is not represented by general formula **3** and was chosen as a target to further explore the effect on activity by having the difluoromethoxy group located at a different position on the heterocycle. Treating the t-butyl phenylcarbamate **43** with two equivalents of n-butyllithium and quenching with acetaldehyde followed by oxidation with chromium trioxide gave the carbamoyl-substituted acetophenone **44** in 65% yield. Acid hydrolysis of the carbamate functionality and diazotization afforded the 4-hydroxycinnoline **45** in 62% yield. Bromination then provided **46** in 86% yield. Reacting **46** with difluorocarbene (generated from Freon-22®) gave 8-bromo-4-difluoromethoxy-6-methylcinnoline **47** which was cross-coupled with arylstannane **5** in the presence of bis(triphenylphosphine)palladium(II) chloride to afford **42** in 76% yield (two steps).

Herbicidal Results

A number of these compounds were found to show significant herbicidal activity and produced bleaching symptoms (albinism) on affected plants. They were generally more effective when applied preemergence and demonstrated broad-spectrum activity on both grasses and broadleaf weeds. Crop selectivity was also observed and the potential of this class of compounds as weed control agents in cereals was of particular interest. In Table I, preemergence herbicidal data for several aryl-substituted heteroarenes and two N-oxide derivatives are reported at 400 and 100 g/ha. Average percent control is shown for the following grasses and broadleaves: giant foxtail (*Setaria faberii*), crabgrass (*Digitaria sanguinalis*), blackgrass (*Alopecurus myosuroides*), wild oats (*Avena fatua*), lambsquarter (*Chenopodium alba*), cleavers (Galium aparine), wild buckwheat (Polygonum convolvulus), and chickweed (*Stellaria media*). Comparision of the difluoromethoxy-substituted heterocycles in Table I shows that quinoline **8** was almost inactive at the rates tested whereas quinoxaline **12** was highly active and benzotriazine **38** was moderately active. In the case of trifluoromethyl-substituted heteroarenes, quinoxaline **29** was moderately active and quinazoline **36** was very active. Therefore, the highest levels of actvity were obtained with heterocycles having two ring nitrogens (quinoxaline and quinazoline) rather than with systems containing one (quinoline) or three (benzotriazine) nitrogens. Quinoxaline **12** was more active than its N-oxide **23** but benzotriazine **38** and its N-oxide **37** were found to be comparable in efficacy.

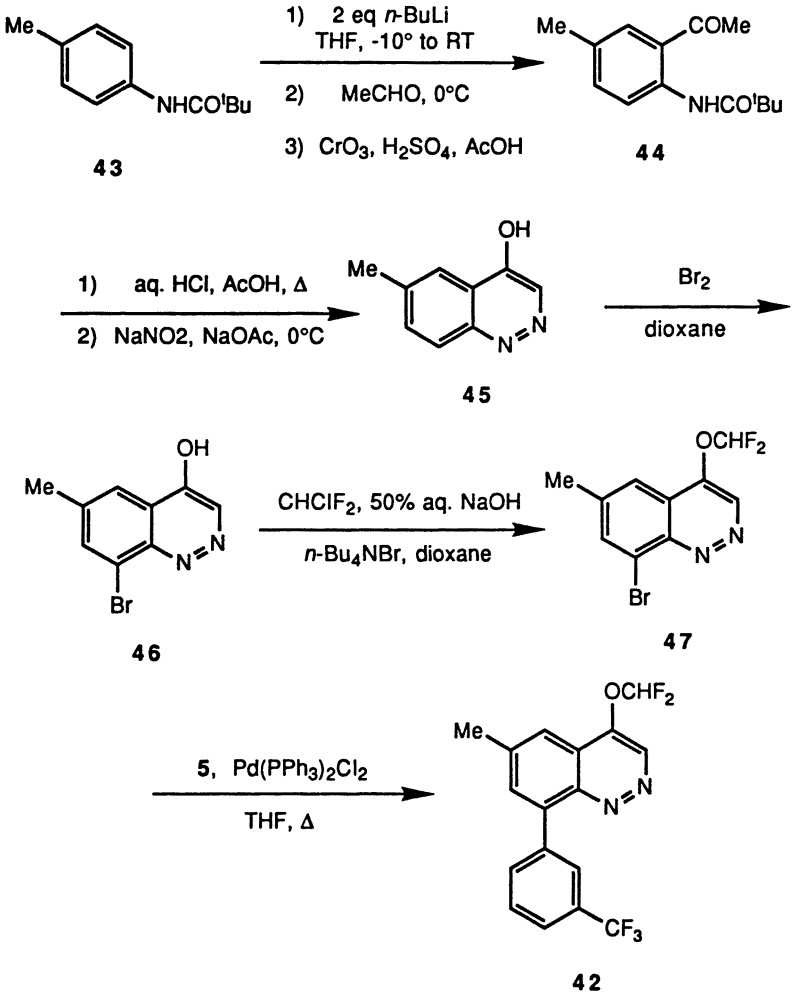
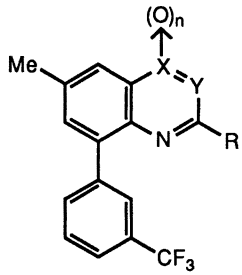


Figure 8. Synthesis of an 8-Aryl-4-difluoromethoxy-6-methylcinnoline.

Table I. Preemergence Herbicidal Activity of Aryl-Substituted Heteroarenes at 400 and 100 g/ha



No.	X	Y	R	n	Average % Control* 400 g/ha	100 g/ha
8	CH	CH	$OCHF_2$	0	5	-
12	N	CH	$OCHF_2$	0	100	96
23	N	CH	$OCHF_2$	1	96	40
29	N	CH	CF_3	0	90	61
36	CH	N	CF_3	0	93	90
37	N	N	$OCHF_2$	1	84	66
38	N	N	$OCHF_2$	0	86	56

* Grasses and Broadleaves

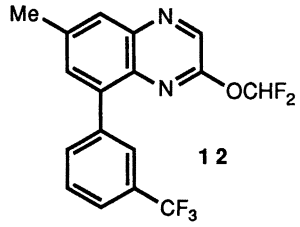
Quinoxaline **12** was found to be one of the most active analogs prepared in this area and showed broad-spectrum herbicidal activity down to 31 g/ha. Preemergence data for **12** at 62 and 31 g/ha are given in Table II. Although this compound was highly active on a number of key weeds in cereals, the safety margin to wheat and barley was found to be very narrow (as shown in Table II).

As we observed with previously studied ring systems such as the triazolo[1,2-*a*]pyrimidines (*1,3*), trifluoromethoxy substitution in place of the trifluoromethyl group at the *meta*-position of the phenyl ring also gave rise to high levels of activity. Quinoxaline **27** was found to have activity comparable to **12** and also demonstrated cereal crop tolerence.

Quinoxalines **13, 28,** and **30** showed significantly lower levels of herbicidal activity than their regioisomeric counterparts **12, 27,** and **29**, respectively. In addition, cinnoline **42** was also found to have very little actvity. These results clearly demonstrated that moving the difluoromethoxy or trifluoromethyl substitution on the heterocycle to other locations resulted in a substantial loss of efficacy.

In summary, we found that substituted quinoxalines and quinazolines of formula **3** provided the highest levels of preemergence broad-spectrum herbicidal activity with some selectivity to cereal crops being observed. The level of activity was greatly influenced by the regiochemistry of the difluoromethoxy or trifluoromethyl substitution on the heterocycle. N-oxides of some aryl-substituted heterocycles also had significant activity.

Table II. Preemergence Herbicidal Activity of Quinoxaline 12 at 62 and 31 g/ha



12

Weed/Crop	% Control 62 g/ha	31 g/ha
Giant Foxtail	100	100
Crabgrass	100	100
Blackgrass	100	95
Wild Oats	50	40
Lambsquarter	100	100
Chickweed	100	95
Wild Buckwheat	100	75
Cleavers	100	50
Spring Wheat	30	20
Winter Barley	10	10

Conclusion

In follow-up to our initial discovery and investigation of aryltriazolopyrimidines as carotenoid biosynthesis inhibiting herbicides, we have explored other heterocyclic derivatives and found novel aryl-substituted heteroarenes of formula **3** to be herbicidal as well. The most active analogs were quinoxaline and quinazoline analogs with some compounds such as **12** showing broad-spectrum activity at rates down to 31 g/ha. Compounds of this class demonstrated crop selectivity and provided preemergence control of a number of key grasses and broadleaf weeds in cereals. The bleaching symptoms observed for these compounds on plants were the same as that observed for aryltriazolo[1,2-*a*]pyrimidines which we previously reported to be inhibitors of phytoene desaturase in the carotenoid biosynthesis pathway (*1*). These aryl-substituted heteroarenes and their N-oxides were readily made via palladium-catalyzed cross-coupling reactions of bromoheterocyclic precursors with an arylstannane or arylboronic acid.

Acknowledgments

We acknowledge with gratitude all of the biologists who conducted herbicidal evaluations on these compounds. Special thanks are also due T. Neubert for technical assistance and H. M. Brown, S. K. Gee, J. V. Hay, and A. D. Wolf for technical advice given to this program. We also express our appreciation to J. C. Calabrese (Central Research and Development, Du Pont) for the X-ray crystal analysis. Finally, we thank Professor V. Snieckus for all of his very helpful advice and ideas provided on the chemistry of this program.

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Chapter 17

Novel Pyrimidine Bleaching Herbicides

Synthesis and Herbicidal Activity

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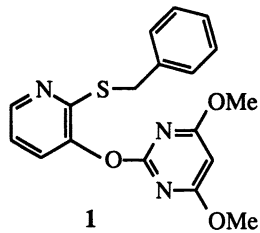
In the course of searching for new ALS (Acetolactate synthase) - inhibiting herbicides, new bleaching herbicides were found. These are pyrimidine derivatives represented by the compound **1**.



1

The compound and it's analogs exhibited bleaching symptomology, and several of the compounds showed good performance in paddy condition at 1 kg/ha. Synthetic routes to these compounds and their herbicidal activitys are described.

As part of a program searching for new ALS-inhibiting herbicides, synthesis of the molecule **3** containing sulfonamide group as an acidic part instead of carboxyl group of pyrithiobac **2** (*1,2*) was planned (Figure 1). Evaluation of the herbicidal activity revealed that the compound **3** did not have any herbicidal activity at 5 kg/ha.The compounds discussed here and represented by the formula **1** were synthesized as the intermediates to compound **3**. Evaluation of herbicidal activity of the synthetic intermediates of **3** lead to compound **1**, which exhibited bleaching symptomology. This type of bleaching substance is not reported in the literature, and the investigation of new bleaching herbicides was began by putting the molecule **1** as the lead molecule.

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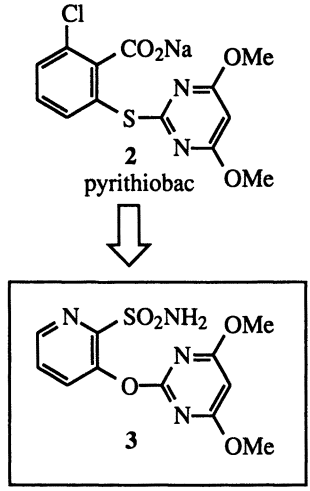


Figure1. Initial target

We would like to describe here the synthesis and the herbicidal activitys of the newly discovered pyrimidines and the initially designed molecules.

Synthesis

Synthesis of 1 and the failure to obtain 3 from 1. As shown in Figure 2, the reaction of 2-Chloro-3-pyridinol **4** with 4,6-dimethoxy-2-methanesulfonyl-pyrimidine **5** gave 3-(4,6-dimethoxypyrimidine-2-yl)-oxy-2-chloropyridine **6**. This chloride **6** was then reacted with benzylmercaptan to obtain benzylthioether **1**. Oxidation of this thioether **1** with chlorine, followed by the reaction with tert-butylamine gave the sulfonamide **7**, and deprotection of it's tert-buthyl group with trifluoroacetic acid gave the sulfonamide **8**, which was different from the desired product **3**. The oxidation step introduced an extra chlorine atom on it's pyrimidine ring. The another route to obtain **3** is described later.

Synthesis of analogs of 1. reactions leading to 14,15,16,17,18 and 19. The analogs of **1** were synthesized as shown in Figure 3. The reaction of **4** with 4,6-dimethoxy-2-methanesulfonypyrimidine **5** and 4,6-dimethyl-2-chloropyrimidine **9** gave 3-substituted pyridyl-2-chlorides **6** and **10** respectively. The reaction of **12** with 4,6-dimethoxy-2-methanesulfonypyrimidine **5** gave 6-substituted pyridyl-2-chloride **13**. The reaction of these chlorides **6,10** and **13** with thiols gave the several analogous compounds of **1**, i.e.**14,15,17,18** and **19**. The coupling of 2-Mercapto-3-pyridinol **11** with 4,6-dimethoxy-2-methanesulfonylpyrimidine **5** gave 2,3-bis(4,6-dimethoxy-pyrimidine)-substituted analog **16**.

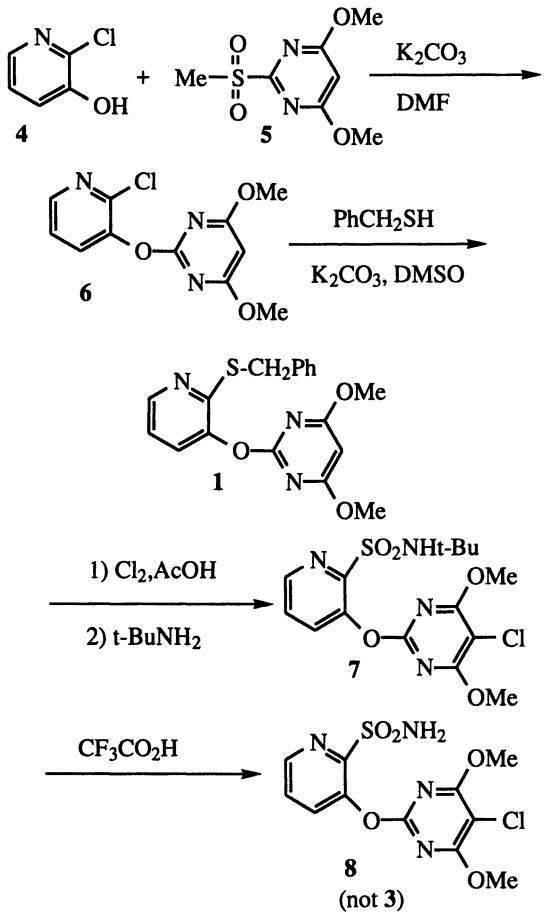


Figure2. Synthesis of **1** and the failure to obtain **3** from **1**

Synthesis of analogs of 1. reactions leading to 20,21 and 22. Oxidation of the sulfide **1** with hydrogen peroxide in the presence of sodium tangstanate gave the chromatografically separable mixture of the sulfoxide **20** and the sulfone **21**. The ether **22** was prepared by the reaction of the sulfone **21** with benzyl alcohol (Figure 4).

Synthesis of 3. The synthesis of the initially planned sulfonamide **3** was accomplished as shown in Figure 5. The reaction of 2-chloro-3-pyridinol **4** with

benzyl bromide, followed by the reaction with benzylmercaptan gave bis-benzylated product **23**. Oxidation of **23** with chlorine, followed by reaction of t-butylamine gave t-butyl 3-benzyloxy-pyridin-2-sulfonamide **24**. Hydrogenolysis of it's benzyl group gave t-butyl 3-hydroxypyridin-2-sulfonamide **25**, which was then reacted with the 4,6-dimethoxy-2-methanesulfonylpyrimidine **5** to give **26**. Deprotection of t-butyl group gave the sulfonamide **3**.

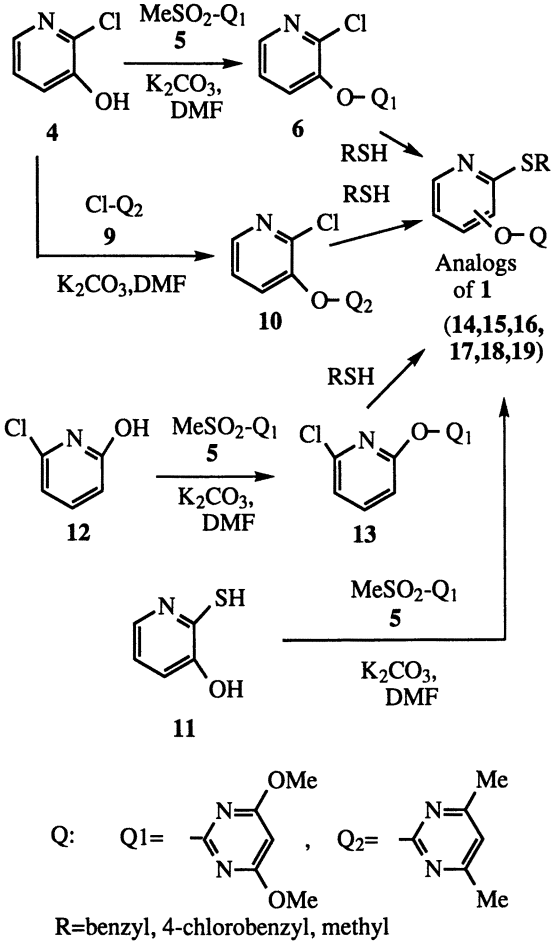


Figure 3. Synthesis of analogs of **1** (part 1)

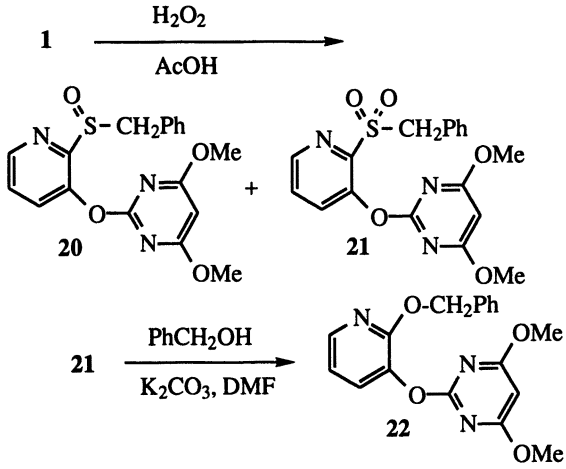


Figure4. Synthesis of analogs of 1 (part 2)

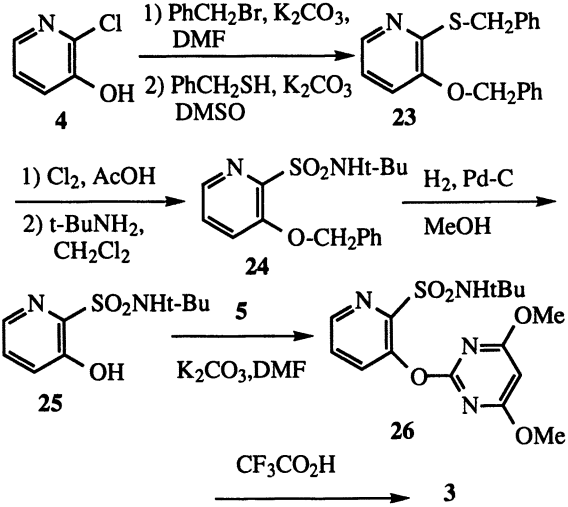
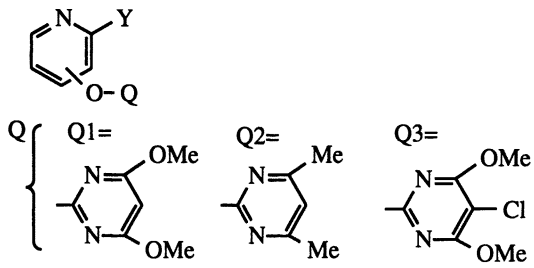


Figure5. Synthesis of 3

Table I shows the compounds synthesized by above reactions (Figure 2~5).

Table I. Lists of compounds synthesized by above reactions



Compound	Substitution pattern on pyridine ring	Y	Q
1	2,3-	benzylthio	Q1
3	2,3-	aminosulfonyl	Q1
6	2,3-	chlorine	Q1
8	2,3-	aminosulfonyl	Q3
10	2,3-	chlorine	Q2
13	2,6-	chlorine	Q1
14	2,3-	4-chlorobenzylthio	Q1
15	2,3-	methylthio	Q1
16	2,3-	4,6-dimethoxypyrimidin-2-ylthio	Q1
17	2,3-	benzylthio	Q2
18	2,6-	benzylthio	Q1
19	2,6-	4-chlorobenzylthio	Q1
20	2,3-	benzylsulfoxy	Q1
21	2,3-	benzylsulfonyl	Q1
22	2,3-	benzyloxy	Q1
26	2,3-	t-buthylaminosulfonyl	Q1

Weed species and herbicidal activity rating

The pyrimidine derivatives were tested on monocot and dicot weeds with pre- and post-emergence treatments under both paddy and upland conditions in greenhouse pot tests.

The monocot weeds included *Echinochloa oryzicola* (Ech), *Scirpus juncoides* (Sci) and *Monochoria vaginalis* (Mon) in paddy conditions, and *Echinochloa crus-galli* (Ech), *Digitaria sanguinalis* (Dig), *Setaria faberi* (Set) and *Avena fatua* (Ave) in upland conditions. The dicot weeds included *Lindernia procunbens* (Lin) in paddy conditions, and *Amaranthus viridis* (Ama), *Abutilon theophrasti* (Abu), *Xanthium strumarium L.* (Xan), *Polygonum pensylvanicum* (Pol) and *Datura stramonium* (Dat) in upland conditions.

Especially, rice(*Oryza sativa*; Ory) was tested in paddy conditions.

The herbicidal activity rating used is as follows.

0= No activity	
1= 1 to 10% injury	6= 51 to 60% injury
2= 11 to 20% injury	7= 61 to 70% injury
3= 21 to 30% injury	8= 71 to 80% injury
4= 31 to 40% injury	9= 81 to 90% injury
5= 41 to 50% injury	10= 91 to 100% injury

Herbicidal Activities

The newly discovered pyrimidines represented by the compound **1** exhibited mainly bleaching symptomology(chlorosis). Table II, III, IV and V show the herbicidal activity of them.

Paddy conditions. As shown in Table II, the compound **1** exhibited excellent herbicidal activity in paddy conditions with slight damage against rice at 1 kg/ha level. The initially designed sulfonamide **3** did not exhibit any herbicidal activity even at 5 kg/ha.The herbicidal data of the compounds listed in the Table showed us the interesting change of activity level accompanied by change of the partial structure of **1**. Conversion of it's benzylthio group into chlorine atom, methylthio, 4,6-dimethoxypyrimidin-2-ylthio and t-butylaminosulfonyl group(compound No.**6**, **15**, **16** and **26**) led almost complete loss of activity even at 5 kg/ha. Conversion of the benzylthio group into it's oxidized forms(compound No.**20**, **21**) resulted a marked decline in the activity was observed. Substitution by benzyloxy group(compound No.**22**) reduced the activity and brought the loss of selectivity to rice. On the otherhand, 4-Chlorobenzylthio-substituted one **14** offer level of activity similar to benzylthio-substituted one **1**.The activity of the compounds(**1**, **6**) bearing 4,6-dimethoxy pyrimidine was much stronger than that of 4,6-dimethylpyrimidine(Compound No.**17**, **10**).

2,6-substituted compounds(Compound No.**18**, **19**) offer levels of activity weaker than the original 2,3-substituted compounds(Compound No.**1**, **14**). Conversion of it's benzylthio group(Compound No.**18**) into chlorine atom(Compound No.**13**) led almost complete loss of activity even at 5 kg/ha as same as in the case of the

compound **6**. It is interesting that both 4-chlorobenzylthio-substituted compounds(Compound No.**14** and **19**) show good selectivity to rice compared with their benzylthio-substituted ones(Compound No.**1** and **18**).Table III explains the data of pre-emergence activity in paddy conditions. The compounds showed almost the same tendency as in post-emergence treatments.

Table II. Post-Emergence Herbicidal Activity of **1** and it's analogs (paddy condition)

Compound	rate(kg/ha)	Ech	Sci	Mon	Lin	Ory
1	5.0	10	10	10	10	7
	1.0	10	10	10	10	2
	0.6	5	8	0	2	0
3	5.0	0	0	0	0	0
6	5.0	0	0	0	0	0
8	5.0	0	0	0	0	0
10	5.0	0	0	0	0	0
13	5.0	0	0	3	0	0
	1.0	0	0	0	0	0
14	5.0	10	10	10	10	0
	1.0	10	7	10	9	0
15	5.0	3	0	0	4	2
	1.0	0	0	0	0	0
16	5.0	3	0	0	0	0
	1.0	0	0	0	0	0
17	5.0	2	-	2	7	3
	1.0	0	-	0	0	0
18	5.0	10	5	10	10	3
19	5.0	10	8	10	10	0
	1.0	4	8	8	9	0
20	5.0	3	3	5	0	0
	1.0	2	3	0	0	0
21	5.0	6	8	4	4	6
	1.0	6	3	0	0	3
22	5.0	10	-	8	4	10
	1.0	4	0	0	0	4
26	5.0	0	3	3	0	0
	1.0	0	0	0	0	0

Table III. Pre-Emergence Herbicidal Activity of **1** and it's analogs (paddy condition)

Compound	rate(kg/ha)	Ech	Sci	Mon	Lin	Ory
1	1.0	10	7	10	10	4
3	5.0	0	0	0	0	0
6	5.0	0	0	0	0	0
8	5.0	0	0	3	0	0
	1.0	0	0	0	0	0
10	5.0	0	0	3	5	0
	1.0	0	0	0	3	0
13	5.0	0	0	0	3	0
	1.0	0	0	0	0	0
14	5.0	10	9	10	10	2
	1.0	10	9	10	10	1
15	5.0	0	3	0	4	0
	1.0	0	0	0	0	0
16	5.0	3	2	3	5	0
	1.0	0	0	0	0	0
17	5.0	4	-	0	4	0
	1.0	0	-	0	0	0
18	5.0	10	7	10	10	5
	1.0	0	3	3	0	0
19	5.0	10	9	10	10	0
	1.0	7	8	10	10	0
20	5.0	9	6	5	6	7
	1.0	3	0	3	3	0
21	5.0	8	8	7	8	8
	1.0	3	0	0	4	0
22	5.0	10	9	10	6	10
	1.0	6	-	7	0	5
26	5.0	5	0	0	0	0
	1.0	0	0	0	0	0

Upland conditions. Table IV explains the data of post-emergence activity. The compounds(**1**,**14**,**18** and **19**) bearing (4-chloro)benzylthio group were active in the conditions, but, herbicidal spectrum of them were insufficient at 1kg/ha. Table V explains the data of pre-emergence activity. The compounds(**1** and **18**)

bearing benzylthio group had weak activity in the conditions, and the compounds(**14** and **19**) bearing 4-chlorobenzylthio group did not show the activity at 5kg/ha.

The above limited data suggest us there is a close relation between the unique structure of benzylthiopyridine(substituted by 4,6-dimethoxypyrimidine) with the appearance of activity and of selectivity to rice. And also, these information is thought to be useful to design new herbicides.

Table IV. Post-Emergence Herbicidal Activity of **1** and it's analogs (upland condition)

Compound	rate(kg/ha)	Ech	Dig	Set	Ave	Ama	Abu	Xan	Pol	Dat
1	1.0	6	10	10	3	9	6	4	10	10
14	5.0	10	10	10	10	10	10	4	10	10
	1.0	7	4	4	7	10	10	4	10	10
18	5.0	10	10	10	4	10	10	7	9	10
	1.0	3	5	4	0	10	10	6	-	10
19	5.0	10	10	9	10	10	10	6	10	10
	1.0	4	4	9	9	10	10	6	10	10

Table V. Pre-Emergence Herbicidal Activity of **1** and it's analogs (upland condition)

Compound	rate(kg/ha)	Ech	Dig	Set	Ave	Ama	Abu	Xan	Pol	Dat
1	1.0	8	9	10	4	10	0	3	0	0
14	5.0	0	0	0	0	0	0	0	0	0
18	5.0	9	10	10	3	10	10	0	7	-
	1.0	0	9	0	0	8	0	0	5	-
19	5.0	0	0	0	0	0	0	0	0	0

Mode of Action

The symptoms caused by treatments with these compounds on the leaves of susceptible species was mainly chlorosis. This injury is reminiscent of diflufenican-like activity and was not similar to that displayed by ALS inhibitors. It's symptoms

suggests the inhibition of biosynthetic pathway of carotenoids or chlorophyls. The study of mode of action on the molecules described here has not been done.

Conclusions

In the course of search for new ALS-inhibiting herbicides, novel herbicidal bleaching pyrimidine derivatives were found. Several pyrimidines were prepared and evaluated and showed excellent herbicidal property in paddy conditions with activity levels in the 1 to 5 kg a.i./ha range. This brief research revealed the outline of their herbicidal properties.

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Chapter 18

Oxazine Ether Herbicides

Novel Grass-Selective Compounds

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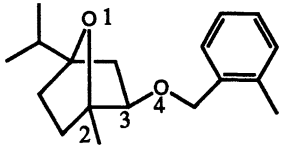
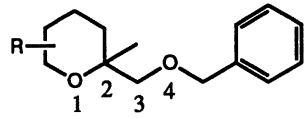
Benzylic ethers of oxazine-6-methanols are very effective grass herbicides which show safety to a number of major crops. We have found 2 different synthetic routes to these compounds. The first route is based on the reaction of nitroso-olefins with allylic alcohols. The second route relies on a novel reaction of oxime dianions with chloro-epoxides. We will describe our studies on the discovery and optimization of these syntheses and the biology of the oxazine ethers.

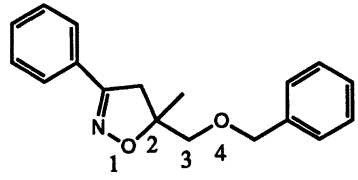
Over the past 2 decades chemists from a number of companies have explored benzylic ethers as herbicides. This area began with the discovery that monocyclic oxygen heterocycles substituted with benzylic ethers had good herbicidal activity (1). Optimization of the chemistry at Shell provided the only commercial product in the area, the bicyclic ether Cinmethylin (2). The common key structural feature of active compounds was the 1,4-relationship of the oxygen of the heterocycle and the oxygen of the benzylic ether. More recently, scientists at BASF showed that nitrogen could also be present in the heterocycle with isoxazoline ethers (3).



Cinmethylin
Shell



Bayer



BASF

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Synthesis

We wanted to explore the synthesis of 6-membered nitrogen heterocycles since work by Bayer and Shell indicated that 6-membered oxygen heterocycles were at least as active as 5-membered ones (4). As shown in figure 1 Gilchrist and others have reported that 1,2-oxazines can be readily prepared by the reaction of nitroso-alkenes with alkenes (5). This reaction works best with electron rich alkenes. The target structures for benzylic ethers of oxazines would require fairly electron rich allylic alcohols as starting materials so we felt confident that this approach would be successful.

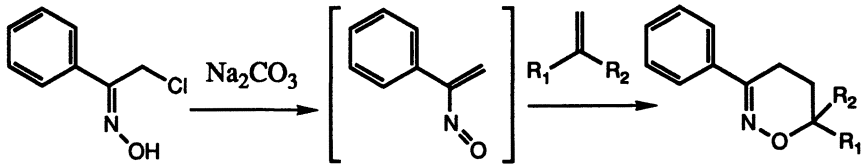
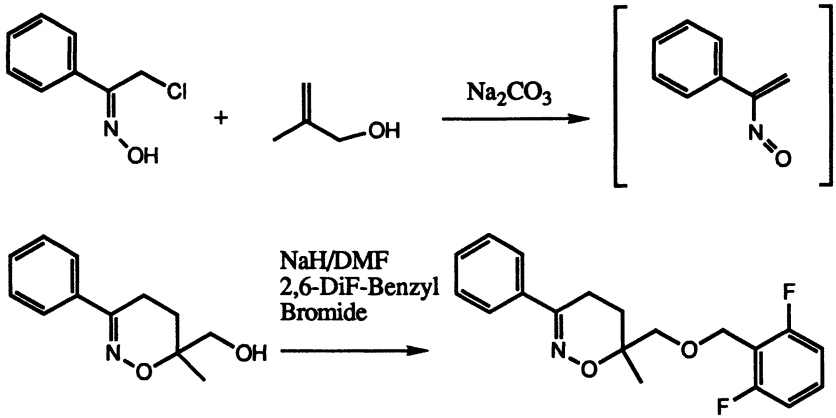


Figure 1. Nitroso-olefin route to 1,2-oxazines.

Monocyclic Oxazine Ethers. We began with the most studied nitrosoalkene system derived from the oxime of 2-chloroacetophenone. Gilchrist has reported that the optimal reaction conditions for producing nitrosoalkenes from halo-oximes is with sodium carbonate in dichloromethane(5). Use of these heterogeneous conditions ensures a slow generation of the reactive intermediate. When we treated the oxime under those conditions in the presence of methallyl alcohol we were able to isolate the desired alcohol in moderate yield (Figure 2). We were able to readily benzylate the alcohol with a variety of benzylic bromides and chlorides using sodium hydride as base. The compounds showed good herbicidal activity especially on grasses at 100 to 400 g/Ha. and we began to explore this area further.



30%

Figure 2. First synthesis of a substituted 1,2-oxazine benzylic ether.

We studied the reaction of a variety of different substituted arylnitrosoalkenes with methallyl alcohol. The commercially available arylketones were brominated with triethyl-benzylammonium bromide perbromide. Treatment of the bromides with hydroxylamine hydrochloride in

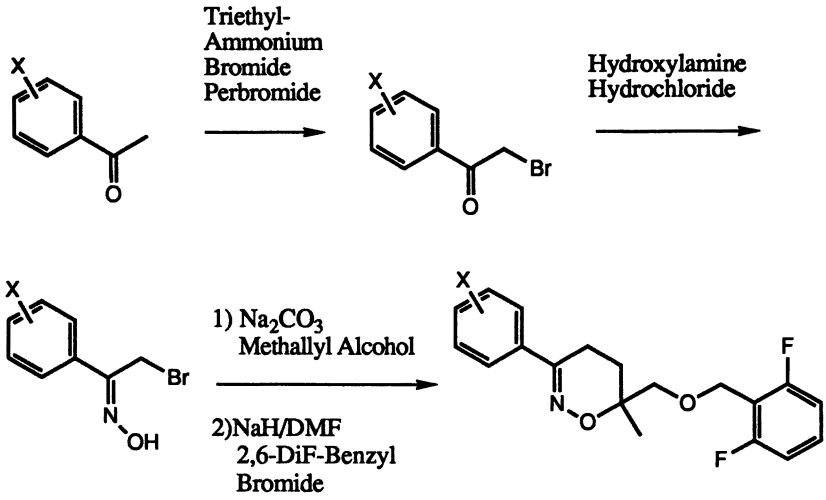


Figure 3. Synthesis of substituted 3-aryl-1,2-oxazines.

methanol led to the oximes. These were then converted as before to the desired oxazine ethers (Figure 3).

Based on our knowledge of cyclic ether herbicides we were interested in increasing bulk at the 3-position of the oxazine. Cinmethylin has an isopropyl group in the same relative position of space as our 3-position. Our initial attempt to increase bulk was with a t-butyl group through the oxime of commercially available bromopinacolone. When we treated this bromooxime with an excess of methallyl alcohol under our standard conditions the desired product was produced in greater than 90 % yield (Figure 4). This was again benzylated with a variety of benzylic halides. These compounds proved to be several times more active as herbicides than the 3-aryloxazines.

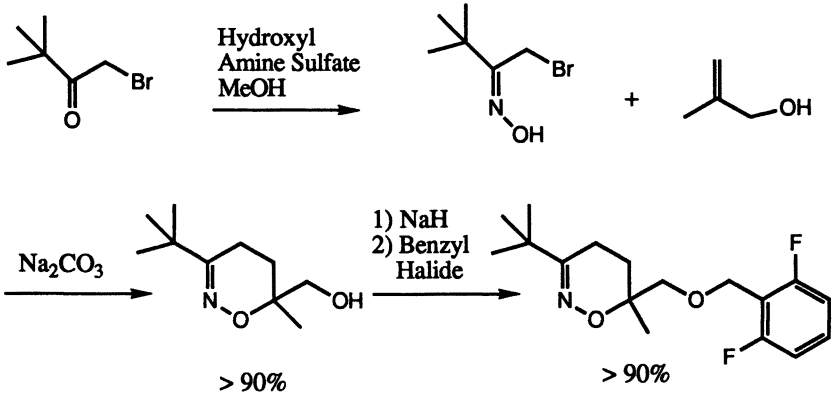
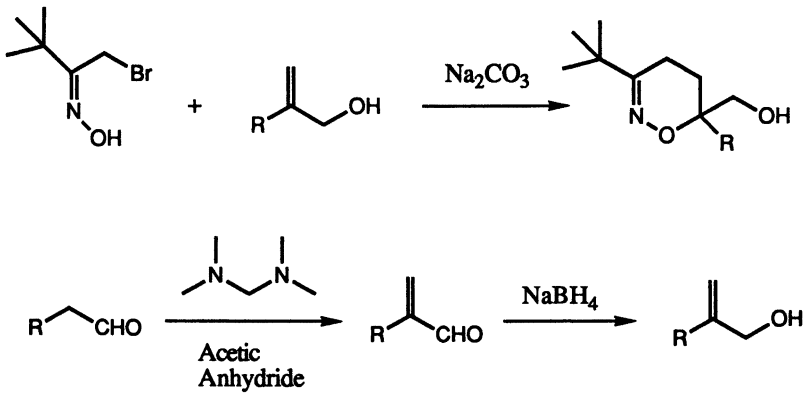


Figure 4. Synthesis of 3-t-butyl-1,2-oxazines.

After observing the high herbicidal activity of the 3-t-butyloxazines we used this system in our optimization work. Of the allylic alcohols we needed to explore the structure activity trends in the 6-position only allyl and methallyl alcohol were commercially available. Longer chain alcohols were synthesized by hydride reduction of the unsaturated aldehyde. If these aldehydes were not

available they could be made by Mannich reaction of the parent aldehyde as shown in Figure 5 (6). Similarly reactions of secondary allylic alcohols were also studied.



R = ethyl, n-propyl, i-propyl, n-butyl

Figure 5. Variation of the 6-substituent.

Ring Constrained Alcohols. Next, we turned to conformationally restricted molecules. The first class was exemplified by using cyclic allylic alcohols. Formally this restrains the alcohol in a ring. The requisite alcohols were made by borohydride reduction of the 5- and 6-membered cyclic enones. These underwent smooth cycloaddition with the t-butyl nitrosoalkene (Figure 6). An alternative conformationally restrained system in which the other 6-substituent forms the ring was also prepared. Methyl cyclohexenecarboxylate was cleanly reduced to the allylic alcohol by lithium aluminum hydride. Cycloaddition with the nitrosoolefin and benzylation completed the sequence(Figure 7). These conformationally restricted compounds showed little herbicidal activity.

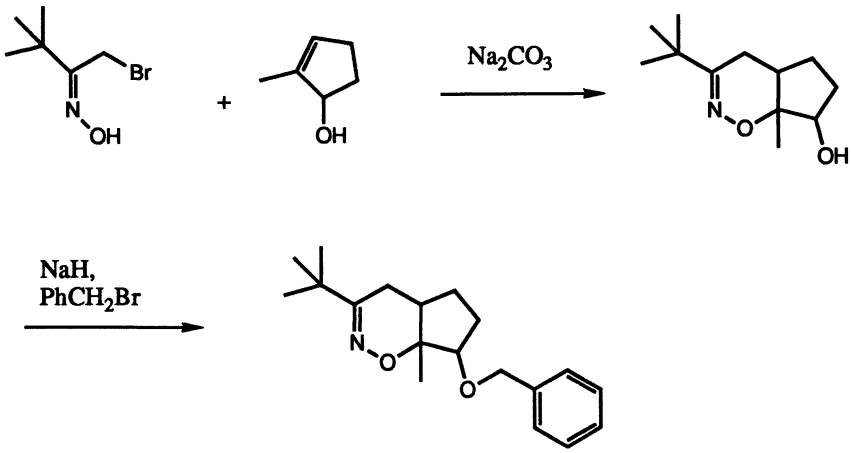


Figure 6. 1,2-Oxazines derived from cyclic allylic alcohols.

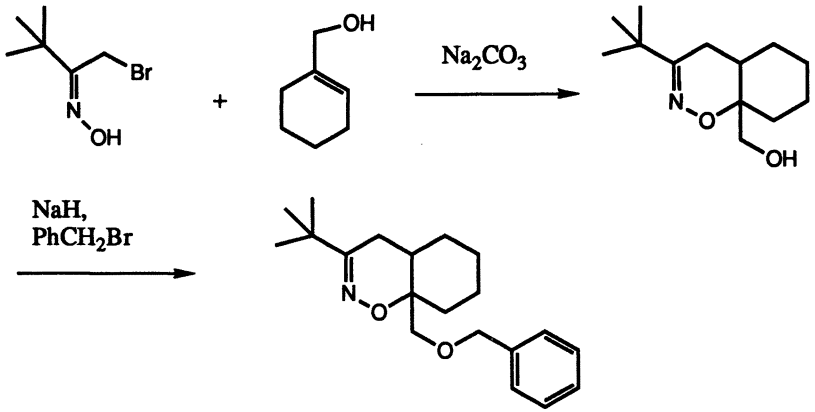


Figure 7. An alternative conformationally restrained 1,2-oxazine system.

Ring Constrained Benzylic Ethers. Compounds in which the benzylic halide is constrained in a ring were also investigated. In our initial attempts we treated our oxazine alcohol with sodium hydride and indanyl chloride. No benzylation occurred and indene was produced. In order to circumvent this elimination reaction we decided to use a preformed benzylic allylic ether as the dienophile. This could be prepared from indan-1-ol by reaction with commercially available methallyl chloride. The ether gave the desired herbicidally active product from reaction with the t-butyl nitrosoolefin (Figure 8). This technique was used to prepare a variety of different constrained benzylic groups including compounds from both antipodes of optically active indan-1-ol.

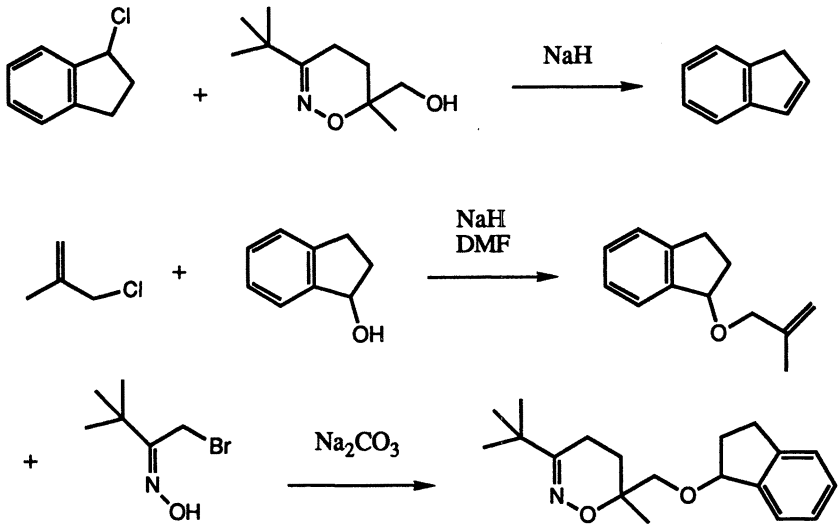


Figure 8. Synthesis of 1,2-oxazines with ring constrained benzylic ethers.

Bi- and Tricyclic Oxazine Ethers. We also explored the use of cyclic nitrosoolefins as shown in Figure 9. A constrained 3-aryl system was derived

from 2-bromotetralone oxime and methallyl alcohol. The product was produced as a separable mixture of diastereomers. A cyclic version of the 3-t-butyloxazines was produced in modest yield from the bromo oxime of commercially available 2,2-dimethylcyclopentanone. The benzyl ethers of both classes of compounds showed good herbicidal activity at 200 g/ha.

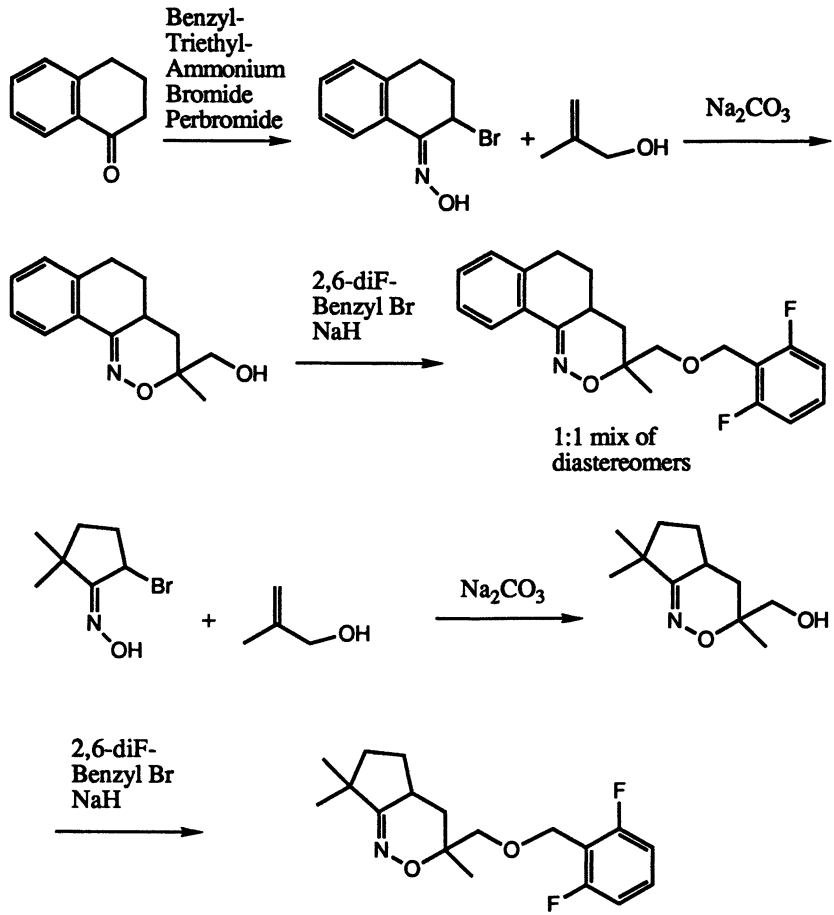


Figure 9. 1,2-Oxazines derived from cyclic nitroso-olefins.

The cycloaddition route was very successful in the cases where there is no opportunity for isomerization of the nitrosoolefin from the reactive s-cis conformation. The optimal yields are produced from compounds lacking a hydrogen alpha to the oxime. However, no cycloadduct is isolated from the reaction of 2-chlorocyclohexanone oxime with methallyl alcohol. The nitrosoolefin is apparently produced as evidenced by a transient blue color, but probably adopts the s-trans conformation (Figure 10). It appears that nitrosoolefins which can isomerize to the s-trans conformation will not react with methallyl alcohol.

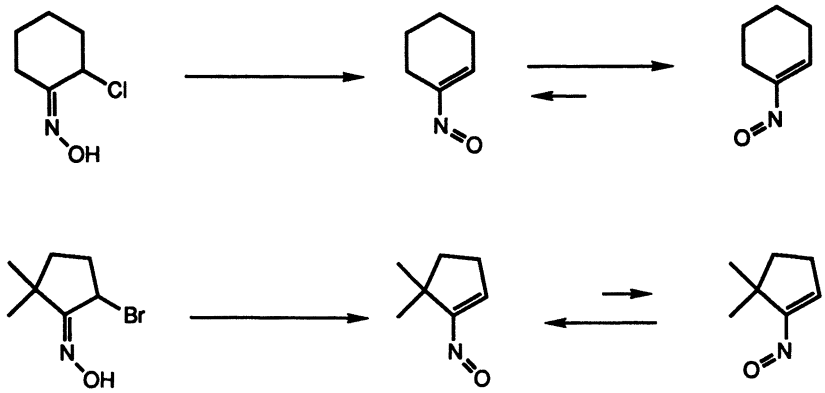


Figure 10. Possible isomerization of cyclic nitroso-olefins.

Dianion Route to Oxazines. In order to synthesize compounds which had alpha hydrogens at the 3-position of the oxazine we needed another route. In 1990 Jordanian workers reported that they could make isoxazolines through oxime dianions probably via an epoxide intermediate (7). Treatment of the acetophenone oxime dianion with phenacyl bromide gave 3,5-diphenylisoxazoline-5-methanol in good yield. Figure 11 shows our modification of this sequence with chloroacetone and cyclohexanone oxime to give the cyclic product in 70 % yield.

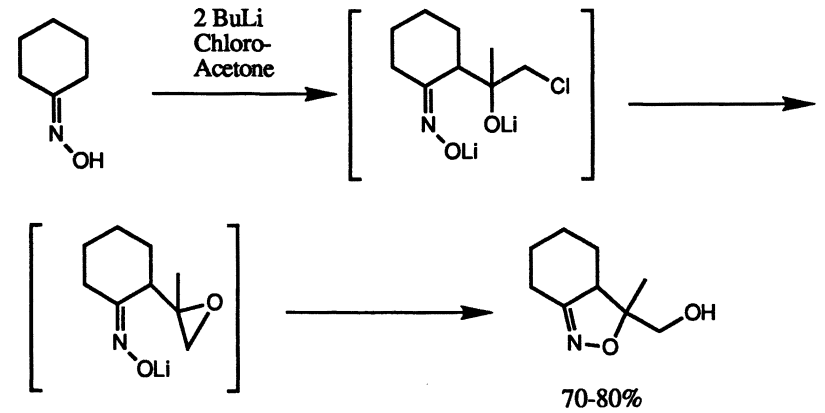


Figure 11. Synthesis of bicyclic isoxazolines via a dianion approach.

For this approach to be a viable one to oxazines we needed to make an epoxide intermediate which would be one carbon further removed from the oxime. We found that Maybridge sold 1-chloromethyl-1-methyloxirane which appeared to be a good precursor to a homologated epoxide intermediate. We treated cyclopentanone oxime with 2 equivalents of n-butyl lithium and quenched with the oxirane. On acidic or basic workup we isolated the desired oxazine in moderate yield (Figure 12). This route was used with a variety of cyclic ketones and also for 3-methyl, 3-ethyl, and 3-isopropyl oxazines.

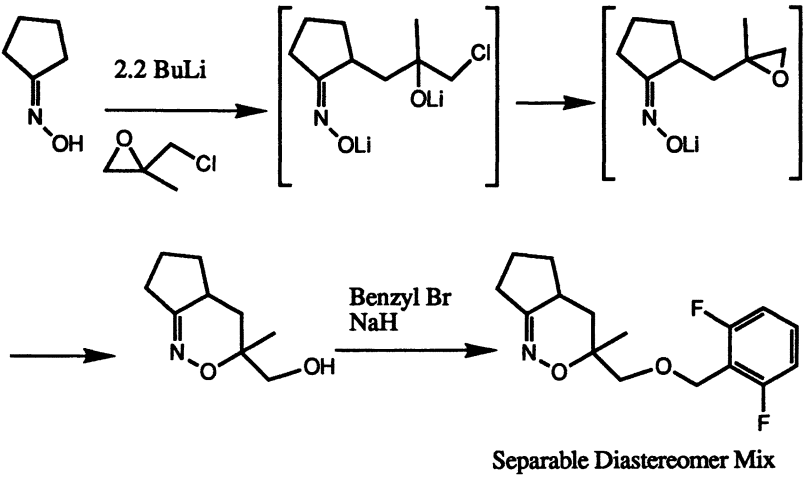


Figure 12. Synthesis of 1,2-oxazines via a dianion approach.

Biology

The oxazine ethers, represented by the structure in Figure 13, show high herbicidal activity on a wide variety of grasses and significant safety to cereals, rice, soybeans, and corn in preemergence tests (8). The activity in post-emergence testing is substantially lower. However, the activity under paddy type conditions on barnyardgrass is generally higher than the postemergence activity would predict. Results of the preemergence herbicidal screening of some representative compounds are shown in Table I.

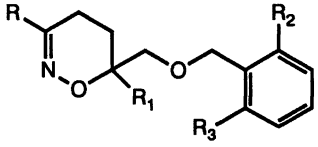


Figure 13. Generic structure of herbicidal 1,2-oxazines.

Table I: Preemergence Control of Grass Weeds at 50 g/Ha
(R_1 = Me, R_2 = R_3 = F)

R =	BKG	BYG	CBG	Wheat	Rice
t-Bu	90	100	90	0	0
i-Pr	30	90	70	0	20
Ph	20	90	60	0	0
1-(1-Me-c-Pr)	0 (90)	0 (100)	0 (100)	0 (0)	0 (10)

BKG = blackgrass, BYG = barnyardgrass, CBG = crabgrass. Numbers in parentheses were obtained at 200 g/Ha.

Structure-Activity Trends. Structure-activity trends for the 3-position emphasize the importance of bulk. However, very large groups such as adamantyl decreased herbicidal activity.

R = t-Bu > i-Pr > Ph > 1-(1-Me-Cyclopropyl) > Dimethylbenzyl > 4-F-Ph > Me > CF_3 > $COCH_3$ > Adamantyl

Substitution at the 6 position was straightforward. Any additional substitution on the ether carbon decreased activity. For the second substituent ethyl was optimal.

R_1 = Et > Me > n-Pr > i-Pr > H > n-Bu

The benzyl substituents were optimally either 2- or 2,6-substituted. The optimal substitution pattern for activity was 2, 6-difluoro. Substituents alpha to the phenyl ring decreased activity unless they were constrained in a ring.

R_2, R_3 = 2, 6-di-F > 2-F > 2-Cl,6-F > 2-H > 2, 6-di-Cl > 2-Cl > 2-Me

In conclusion, oxazine ethers are active and selective herbicides. They are readily synthesized in high yield via a hetero Diels-Alder reaction or via the reaction of oxime dianions with functionalized epoxides. The ease of synthesis, simplicity of structure, and high activity against important weeds combine to make oxazine ethers an interesting new class of herbicides.

Acknowledgments

We would like to thank all of the biologists who evaluated the compounds discussed above especially, Dave Fitzgerald who collated all of the biological data for these compounds. We would also like to thank Onorato Campopiano, Paul Liang, Jim Powell, and Jim Hay for technical advice and suggestions.

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Chapter 19

Herbicidally Active Sulfamoyl Nucleosides

Isolation and Synthesis

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The isolation of the herbicidal nucleoside 2-chloro-5'-O-sulfamoyl-adenosine **1** was reported. Its relation to other herbicidal nucleosides was described. Two new and direct synthetic routes to **1** were established and a number of derivatives were prepared. Herbicidal activity was found in analogs structurally close to **1**. An in vitro toxicological screen was applied to these compounds.

Nature produces a variety of materials and compounds which can be put to a number of uses. In the agricultural field it has long been known both in folklore and among scientists that plants, animals, and microorganisms possess pesticidal activities, which can be used for crop protection.[1] The isolation and characterisation of pure pesticidally active compounds from such living organisms resulted in a development which proved fruitful for both agricultural and scientific circles, as well as for the chemical industry.[2-4] Natural products could then be applied in a quantitative manner, and the possibility of preparing them synthetically made them amenable to large scale production. Furthermore, structural variation of pesticidally active compound led to compounds possessing better biological properties.

Since 1977, we at Ciba have been isolating pesticidally active compounds from a variety of sources, mainly plants and microorganisms. In addition we screen pure metabolites received from academic institutions, and have found a number of interesting lead compounds from both internal and external efforts. Here we describe the isolation of the herbicidal nucleoside **1** and the synthesis and biological activity of a series of analogs.

A number of herbicidally active nucleosides are known. Toyocamycin,[5] herbicidin,[6-8] arabinosyl-adenosine,[9] tubercidin,[10] herbiplanin,[11] formycin A and B,[12] 3-hydroxyuridine,[13] dehydrosinefungin,[14] F-2787,[15] 5'-deoxy-toyocamycin,[16] coaristeromycin,[16] 5'-deoxyguanidine,[16] coformycin,[16] nebularin[17] and hydantocidin[18] have been described in patents and chemical journals. In our own laboratories aristeromycin, formycin A, nebularin, and hydantocidin[19] had also been isolated and tested.

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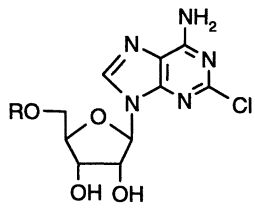
Recently we found the bacterium Streptomyces albus R 2374 (Tü-3389) to exhibit an interesting herbicidal activity.[20] A 30L fermentation batch of this bacterium was fractionated by filtration, concentration, and solvent extaction. The herbicidal activity of the fractions was assayed with Nasturtium officinale (fountain cress) and Agrostis tenuis (bentgrass). Further fractionation of the water soluble material by repeated size exclusion (sephadex-LH-20) and reversed-phase column chromatography (C-18) yielded 5'-O-sulfamoyl-2-chloro-adenosine **1** (223mg) and 2-chloro-adenosine **2** (44mg).[20] **1** had previously been isolated because of its antibacterial activity,[21] and even earlier had been prepared by synthesis.[22] We repeated the synthesis of **1** (Fig. 1) to prepare enough material for biological testing and found the compound to possess potent herbicidal activity especially on broad leaf weeds in post-emergent application (Table IV).



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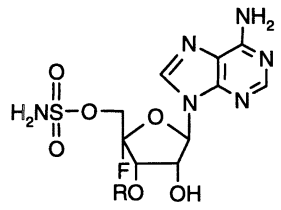
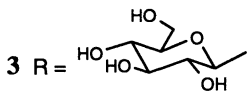
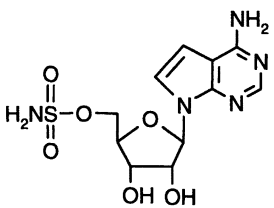
2 R = H



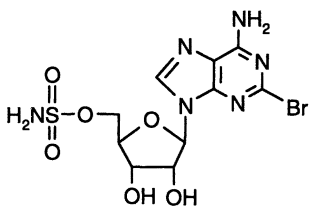


3 R =

4 R = H nucleocidin



5 5'-O-sulfamoyl-tubercidin



6

The discovery of the herbicidal activity of **1** was foreshadowed by the isolation in our laboratories some years previously of the weak herbicide 3'-(1β-glucosyl)-nucleocidin **3** from the streptomyces strain R-156. Also foreknown were the herbicidally active 5'-O-sulfamoyl-tubercidin[23] **5** and 5'-O-sulfamoyl-2-bromoadenosine[24] **6**. In fact one might assume from the description of the fermentation conditions used for the preparation of **6** that the Kureha[24] chemists were aware of the herbicidal activity of **1** itself.

The 5'-O-sulfamoyl nucleosides themselves are a class of natural products which, since the isolation of nucleocidin **4** by Lederle chemists in 1957,[25] has been

shown to exhibit a wide variety of biological activities,[26] accompanied unfortunately by a level of mammal toxicity, which prevented the commercialisation of any of the compounds. It has been suggested that the sulfamoyl group mimics both sterically and electronically a phosphate moiety,[27,28] making these molecules antimetabolites of adenosine monophosphate (AMP). These compounds have been shown to inhibit protein synthesis, more specifically aminoacyl tRNA synthase.[29] Because nucleocidin **5** and 5'-O-sulfamoyl-adenosine **7** are reported to have an oral LD_{50} in rat of between 1 and 5 mg/kg[30] the approximate oral LD_{50} of **1** was determined at the toxicological facilities of Ciba in Sisseln, Switzerland. It was found to be between 1 and 25 mg/kg. Despite this toxicity, the herbicidal activity of **1** was so encouraging, that a synthesis program was started. Of course such toxic compounds had to be handled with the neccessary care. We were furthermore attracted by the fact that no synthetic work has been described with a view towards the herbicidal activity of this interesting class of compounds, and hoped that some of the herbicidally active compounds would be substantially less toxic than **1**.

Synthesis

5'-O-sulfamoyl nucleosides have been prepared exclusively till now by sulfamoylation at 5' of 2', 3' protected nucleosides, which are most conveniently obtained by selective acetonation of the free nucleosides at 2' and 3' (Fig. 1). This methodology was used for the preparation of some of the compounds described herein. Several improvements were made to this pathway during the course of the work and a new direct approach to 5'-O-sulfamoyl-nucleosides was developed.

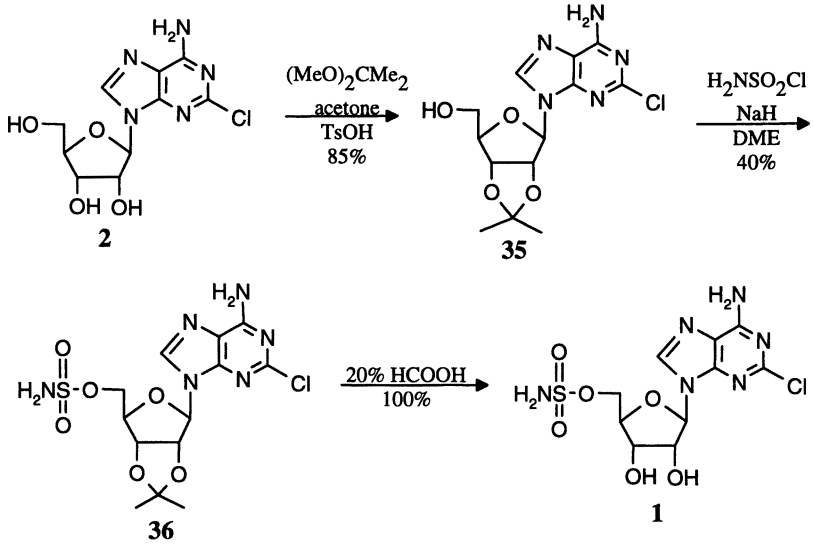


Figure 1. Literature method for the preparation of **1**.

It was clear that the sulfamoylation step would have to be performed many times in the course of this project so this reaction was examined in some detail. The base / solvent combinations which have been described for the sulfamoylation of hydroxy groups are:- NaH / DME,[31] NaH / DMF,[28] $(Bu_3Sn)_2O$ / CH_2Cl_2,[32] 4Å Sieves / CH_2Cl_2,[33] and Et_3N / CH_2Cl_2.[34] However, these conditions were either inconvenient or low yielding, so a study of this reaction was performed using the substrate shown below. It was found that the solvents affording both rapid reactions and high yields were DMF, NMP > MeCN > acetone > CH_2Cl_2. The most useful bases were $CaCO_3$, imidazole > $NaHCO_3$, 2,6-di-t-butyl-pyridine > lutidine, Et_3N, iPr_2NEt, $((Bu_3Sn)_2O$. It was interesting that the weakest bases provided the highest yields. One possible cause of yield diminution may be the known reaction of Et_3N with sulfamoyl chloride yielding the zwitterionic $HNSO_2NEt_3$.[35] This reaction and the corresponding reaction of bases of similar strength may drain sulfamoyl chloride from the sulfamoylation reaction mixture. Irrespective of the mechanism, consistently rapid and high yielding reactions of 2',3'-protected nucleosides were observed with $CaCO_3$ or imidazole in DMF or NMP.

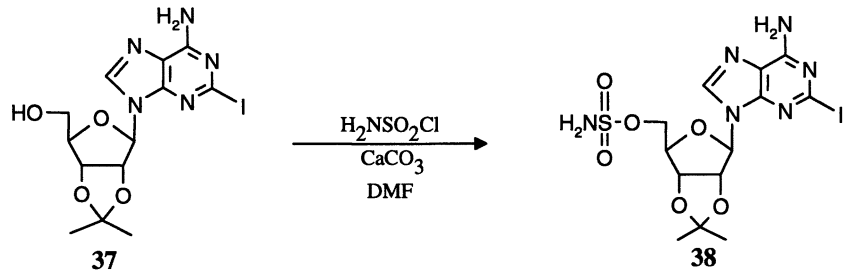
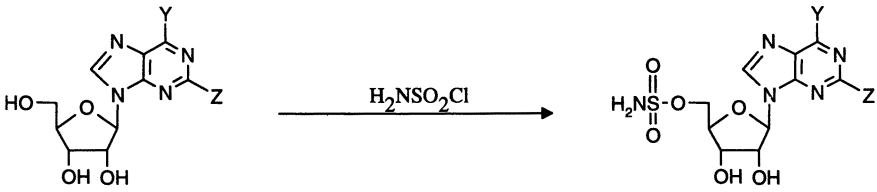


Figure 2. Optimisation of the Sulfamoylation Reaction.

In fact this reaction became so reliable that it was possible to sulfamoylate unprotected nucleosides. The 5'-O-sulfamoyl compound was the major product, but some 2'-O-sulfamoyl and 3'-O-sulfamoyl derivatives were formed as well necessitating a careful chromatography, which resulted in reduced yields. However, although this reaction is low-yielding, it is a rapid method for generating small amounts of products for initial testing, and the compounds shown below were prepared by this method. Apart from the purine nucleosides shown in table I, a number of other derivatives were prepared by the one step direct sulfamoylation of the free nucleoside again in modest yield. 5'-O-Sulfamoyl-8-bromo-adenosine was unstable yielding **46** after purification.[31] A similar reaction was observed with arabinosinyl-adenosine. Neither of these salts showed herbicidal activity.

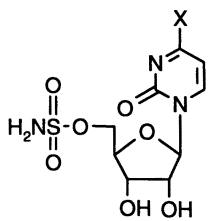
A novel and direct method of preparing sulfamoyl nucleosides was developed by condensing purine bases or other heterocycles to a ribose unit already containing the sulfamate moiety (Fig. 3). The key intermediate **47** was prepared on a Kg scale in

Table I. Direct Sulfamoylation of Unprotected Nucleosides.

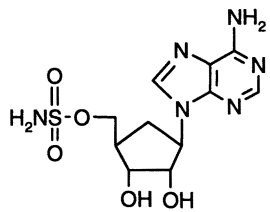


Y	Z	Solvent	Base	Compound	Yield (%)
NH_2	H	DMF	$CaCO_3$	**7**	34
NH_2	Cl	DMF	$CaCO_3$	**1**	35
NH_2	H	DMF	2,6-di-t.butyl-pyridine	**39**[a]	48
NH_2	CH_3	DMF	imidazole	**25**	15
H	NH_2	NMP	$CaCO_3$	**31**	16
OMe	H	DMF	$CaCO_3$	**11**	18
SMe	H	DMF	$CaCO_3$	**12**	15
SH	H	NMP	$CaCO_3$	**10**	5
NH_2	CN	DMF	imidazole	**24**	15
H	H	NMP	$CaCO_3$	**30**	25
OH	H	NMP	$CaCO_3$	**9**	20
	H	NMP	$CaCO_3$	**8**	36

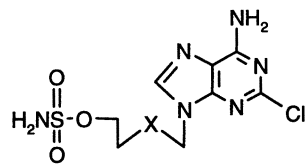
a) With $MeNHSO_2Cl$ instead of H_2NSO_2Cl



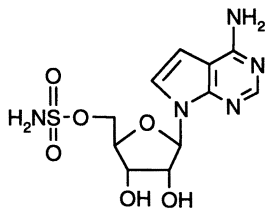
40 X = NH_2 10%
41 X = OH 11%



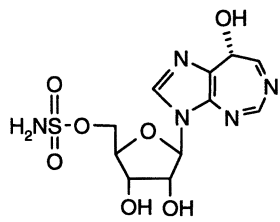
42 26%



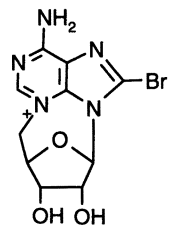
43 X = O 19%
44 X = CH_2 32%



5 16%

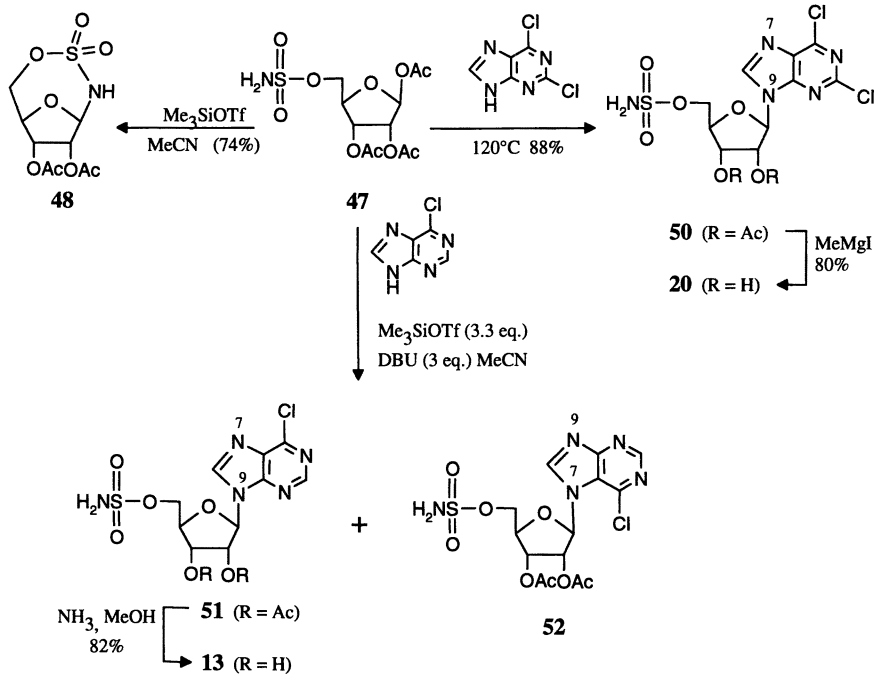


45 20%



46 15%

five steps from ribose without chromatography.[36] Using the usual Vorbrüggen conditions in conjunction with purine bases only the cyclised product **48** was obtained.[36] The crucial modification in reaction conditions, leading to the desired compounds in high yields, was the presence of a tertiary base in the reaction mixture. Drying the solvent became unneccessary, and prior silylation of the purine base could be dispensed with.[36] For low melting (<120 °C) purine bases simply melting the base together with **47** led to good yields of the product. The 2,6-diamino derivative **26** (Table IIIc) was prepared by this method.

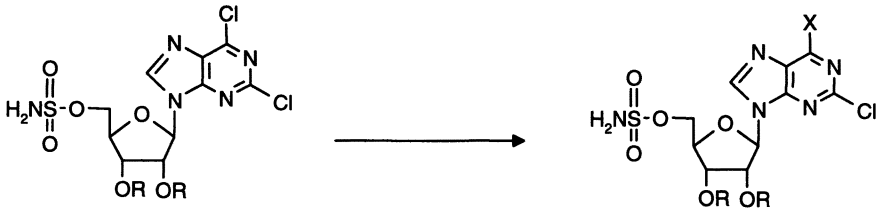


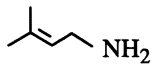
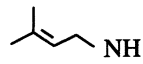
Temp. (°C)	Ratio **51**:**52**	Yield (%)
0	5:95	53
0→60	100:0	84

Figure 3. Nucleoside Formation from **47** Using Modified Conditions.

The coupled products **50**, **20**, **51**, and **13** could be further derivatised at C6, allowing a considerable diversification from a late intermediate (Table II).[36]

Table II. Derivatisation of the Sulfamoyl-Nucleosides **20** and **50**.



Starting Material	R	Conditions	Product	X	R	Yield
50	Ac	NH_3, MeOH	**1**	H_2N	H	63%
			19	MeO	H	22%
50	Ac	$MeNH_2$	**17**	MeNH	H	43%
50	Ac		**15**		H	78%
20	H	MeSNa	**18**	MeS	H	75%
50	Ac	H_2, Pd/C	**34**	H	Ac	52%
34	Ac	NH_3, MeOH	**14**	H	H	76%

Displacement of the 6-chloro group from **53**[37] (Fig. 4) led to **55**, which is a precursor to both **1** and the methoxy analog **23** (Table IIIc). The imidazole derivative **54** was deacetylated using a lipase[38] as first step in its conversion to **16** (Table IIIb). Other methods displaced the imidazole group from the purine heterocycle.

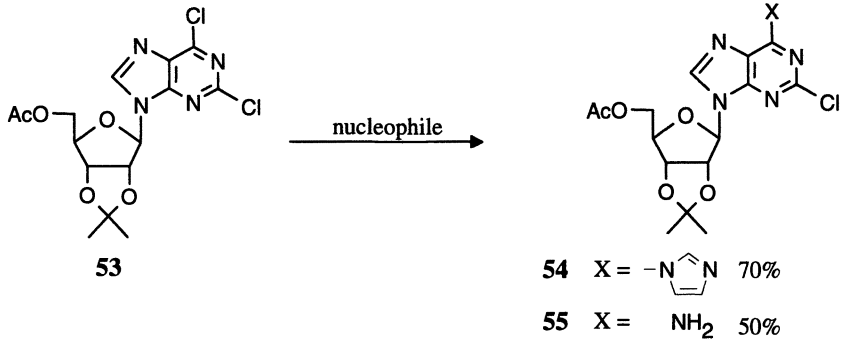


Figure 4. Preparation of **54** and **55**.

Condensation of esters onto the imidazole derivative **56** was a convenient method for introducing alkyl groups at C-2 (Figure 5).[39] The compounds **57** and **58** were then elaborated to the sulfamoyl nucleosides in a conventional manner.

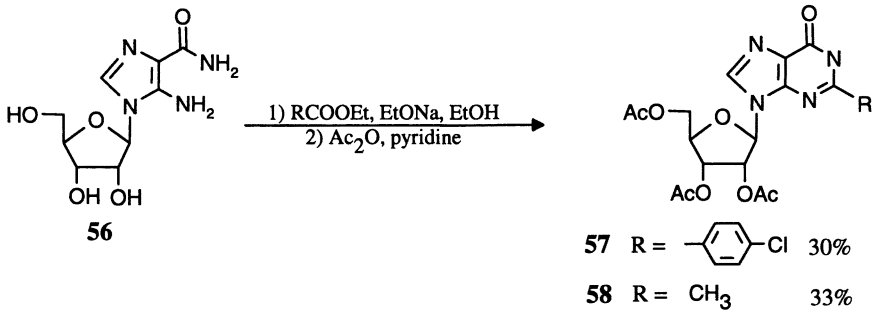


Figure 5. Preparation of 2-alkyl derivatives.

The palladium catalysed reactions shown in figure 6 allowed interesting C-2 derivatives to be prepared.[40]

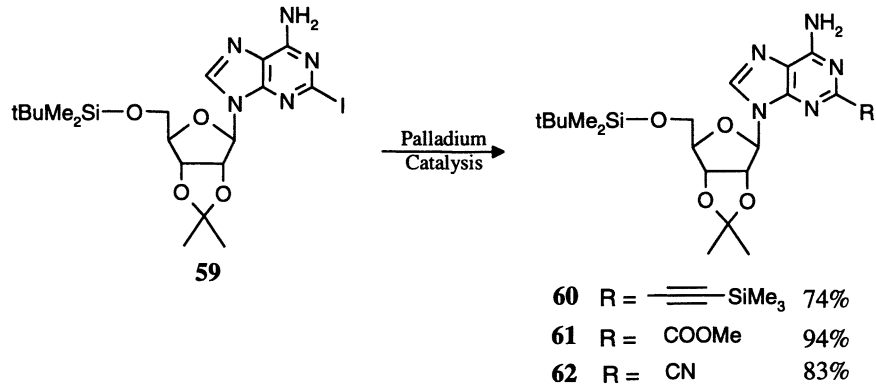


Figure 6. Palladium catalysed introduction of C-2 substituents.

Substitution of chloride in the herbicidally active late intermediate **14** was readily possible (Figure 7).

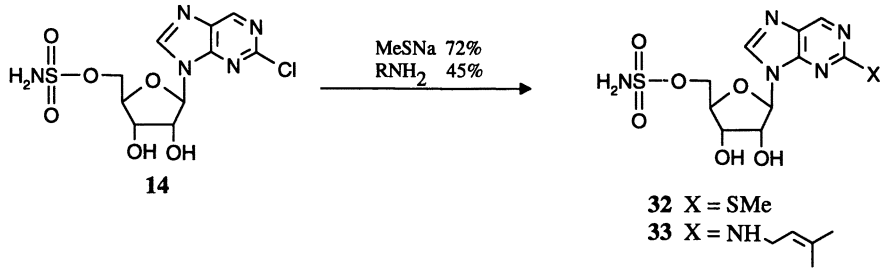


Figure 7. Preparation of C-2 substituted nebularin derivatives.

The 8-bromo-derivative **29** was prepared by selective bromination of **35** (Figure 8).

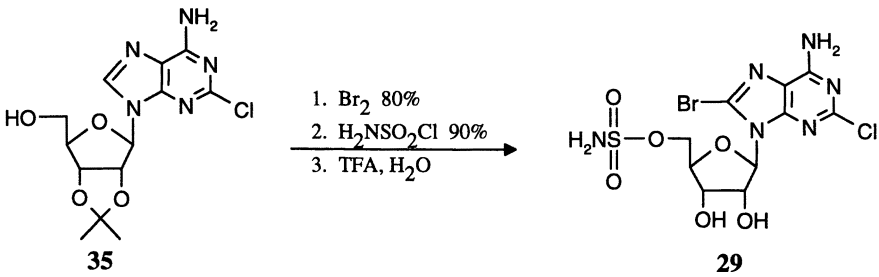


Figure 8. Preparation of **29**.

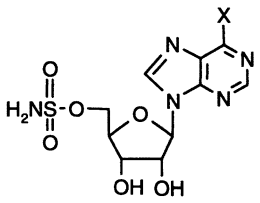
Herbicidal Activity

The compounds prepared and shown in table III were tested against a variety of crop and nuisance plants in greenhouse trials. The results of a representative group of dicot weeds are listed in table IV. Table III lists the compounds in groups of related structure. Within the groups the compounds are ordered according to their biological activity. Although none of the compounds reached the level of activity of **1**, it is possible to see a number of trends in the structure activity relationships.

Changes in the Purine Moiety. It is seen from table IIIa that compounds lacking the 2-chloro substituent were virtually inactive, with the exception of the nebularin derivative **30** (Table IIIe). As nebularin is an inhibitor of adenosine deaminase, and is itself a weak herbicide, it was thought that coformycin, which is also a weak herbicide and inhibitor of adenosine deaminase, could similarly be potentiated through sulfamoylation. However this was not the case, and **45** (Fig. 2) was devoid of herbicidal activity in our tests. Replacement of the 6-amino group in **1** (Table IIIb) led mostly to weakly active compounds, but the dimethylallyl derivative **15** and the amino-deleted compound **14** retained substantial activity. Substitution of the 2-chloro of **1** with other groups (Table IIIc) resulted in lower activity, although the Kureha compound **6**[24] was almost as active. Addition of an additional bromine atom at position 8 led to the inactive compound **29**. Replacement of the chloro group of the relatively potent derivative **14** led to a loss of activity (Table IIIe). The cytosine and uridine derivatives **40** and **41** (Table I) were inactive. Similarly, a number of benztriazole, benzimidazole, and triazole derivatives,[36] which were prepared according to fig. 3, were inactive. The Meiji Seika derivative **5**[23] was a weak herbicide.

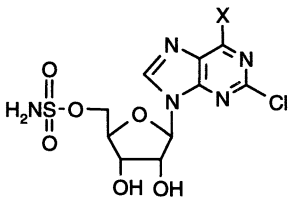
Changes in the Ribose Moiety. The methyl sulfamoyl derivative **39** (Table I) with a substituent formally added to this critical phosphate-mimetic group was inactive. No further derivatives at this moiety were prepared. The aristeromycin derivative **42** (Table I) was a weaker herbicide than aristeromycin itself. The acyclic compounds

Table III. The Structures of the Compounds from Table IV.



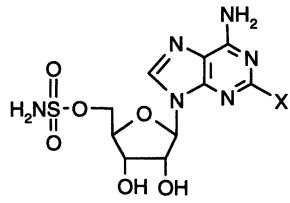
	X
30	H
7	NH_2
8	
9	OH
10	SH
11	OMe
12	SMe
13	Cl

Table IIIa



	X
1	NH_2
14	H
15	
16	
17	NHMe
18	SMe
19	OMe
20	Cl

Table IIIb



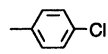
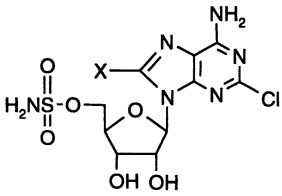
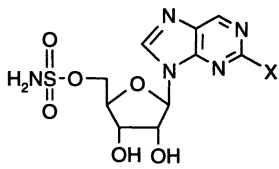
	X
1	Cl
6	Br
21	I
22	C≡CH
23	OMe
24	CN
25	CH_3
26	NH_2
7	H
27	COOMe
28	

Table IIIc



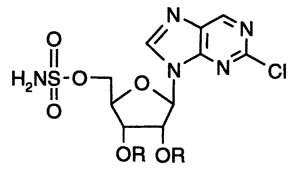
	X
1	H
29	Br

Table IIId



	X
30	H
14	Cl
31	NH_2
32	SMe
33	

Table IIIe



	X
14	H
34	Ac

Table IIIf

43 and **44** (Table I) with the ribose-mimetic chains are prepared from simple starting materials in comparison to the relatively expensive ribosides. However, they were inactive. The 2', 3'-acetonides, which were often intermediates in the synthesis of

free sulfamoyl nucleosides, were all inactive, but the 2', 3'-diacetates were often very active compounds as can be seen by comparing the herbicidal activities of **14** and **34** (Table IIIf) for example. In view of the ubiquitous nature of esterases, we suspect these esters to be prodrugs.

Table IV. Herbicidal Activity. Values are g/Ha giving 80% Effective Control on Post-Emergent Application.

	White Mustard	Chickweed	Morningglory	Speedwell	Cocklebur
	Sinapis alba	*Stellaria media*	*Ipomoea purpurea*	*Veronica persica*	*Xanthium spinosum*
1	< 60	< 60	1000	<60	<60
2	-	-	-	-	-
3	n.t.	-	2000	-	n.t.
5	303340				
6	< 60	<60	500	125	60
7	-	-	-	-	-
8	-	500	-	2000	1000
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	<60	250	500	125	125
15	60	125	2000	250	125
16	<250	500	2000	n.t.	n.t.
17	125	2000	-	2000	1000
18	500	-	-	n.t.	n.t.
19	500	-	-	-	-
20	2000	-	n.t.	n.t.	n.t.
21	<250	250	500	250	250
22	250	250	500	n.t.	n.t.
23	500	2000	2000	n.t.	n.t.
24	500	2000	-	n.t.	n.t.
25	500	-	2000	n.t.	n.t.
26	2000	2000	-	-	1000
27	-	-	n.t.	n.t.	n.t.
28	-	-	n.t.	n.t.	n.t.
29	-	-	-	-	-
30	500	2000	2000	250	125
31	283915				
32	303338				
33	305750				
34	<60	500	2000	250	125

- = >2000 g/Ha. n.t. = not tested.

Mammalian Toxicity

Because of the known toxicity of **1**, **4**, and **7**, it was clear from the beginning of the work that this issue would require careful consideration. The success of the project demanded compounds which are not only herbicidally active but also substantially less toxic than the lead compound **1**. For economic as well as ethical reasons it was impractical to screen the new sulfamoyl nucleosides for toxicity using approximate LD_{50}'s in rodents. However, because these compounds are known to inhibit protein synthesis,[29] which is an intracellular process, the toxicity of the compounds observed in rodents can be expected to be mirrored by a cytotoxicity found in cell cultures. Therefore cytotoxicity was applied as an inexpensive *in vitro* screening tool requiring minimal animal sacrifice. The cytotoxicity of several compounds was examined in a mouse fibroblast cell line,[41] and a good correlation between herbicidal activity and mammalian cytotoxicity was observed. (Table V). In fact even the weakly active or inactive herbicides **26** and **7** were also cytotoxic, giving the impression that mammalian toxicity was more common than herbicidal activity in this series.

Table V. Herbicidal Activity versus Mammalian Cytotoxicity.

Compound	Herbicidal Activity	LD_{50} ng/ml mice fibroblasts
1	strongly active	13
21	strongly active	28
14	active	45
34	active	45
30	active	46
26	weakly active	190
23	weakly active	650
17	weakly active	1080
19	weakly active	1190
20	marginally active	45000
7	inactive	121
2	inactive	10040
43	inactive	14180
29	inactive	30750
39	inactive	>100000

Conclusion

Nucleosides exhibit a variety of biological activities and many of them have found commercial use.[39] In the herbicidal field a number of nucleosides are known, usually with herbicidal activity too weak to be of practical relevance. The isolation of **1** provided a compound whose herbicidal activity was so potent that the class of nucleosides became a worthwhile object of industrial attention. The use of **1** itself as

a herbicide is difficult due to the high preparation price and prohibitive for toxicological reasons. However the herbicidal activity of **1** was so high, that it seemed worthwhile preparing a series of analogs, with **1** as lead structure. The hope was that highly active herbicides could be obtained with a simpler structure and a considerably improved toxicological profile than **1** itself.

Two new and direct synthetic routes to **1** were established and a number of analogs were prepared. An in vitro toxicological screen was applied to these compounds. However herbicidal activity was only found in compounds structurally close to **1**. More seriously, the toxicity of the compounds correlated with the herbicidal activity.

It is often the case that conclusions which at one time appear hard and fast are invalidated by results obtained later, so we will not state definitely that is impossible to prepare sulfamoyl nucleosides cheaply with potent herbicidal activity and low toxicity. However the results obtained and described here were sufficient to discourage us from further work. Should other workers take up the challenge, it is hoped they would find the methods and experiences outlined here helpful.

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Chapter 20

Aryloxy- and Pyridyloxyphenylcyclohexanedione Grass Herbicides

Synthesis and Herbicidal Activity

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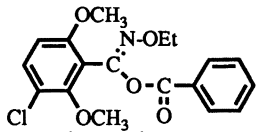
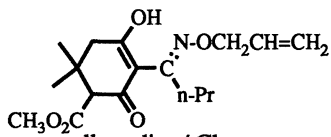
A series of aryloxy- and pyridyloxyphenylcyclohexanediones was prepared and evaluated as potential grass herbicides. In addition to the postemergent whole plant activity, ACCase enzyme inhibition as well as log K_{ow} measurements of many of the compounds are presented. By optimizing the water solubility of these compounds, it was possible to transform a series that possessed excellent enzyme activity but was devoid of whole plant activity into one that had excellent postemergent activity equal to or better than several commercial standards.

In the early 70's, Nippon Soda Co., Ltd., discovered a new class of highly active postemergent grass weed herbicides which are commonly referred to as cyclohexanediones. They (*1,2*) reported that the discovery of this unique area of herbicidal chemistry originated with the observation that several derivatives of their miticide, benzoximate, exhibited weak herbicidal activity on grass weeds.

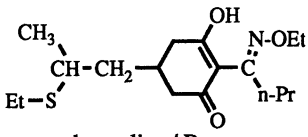


benzoximate

Their research in this area culminated in the commercialization of two products, alloxydim and sethoxydim. Sethoxydim is the active ingredient in Poast herbicide sold in the U.S. by BASF for postemergent grass weed control in soybeans. Many other companies have carried out research in this area of herbicidal chemistry and several of the cyclohexanediones which are in development or in the market place are shown below.



alloxydim / Clout



sethoxydim / Poast

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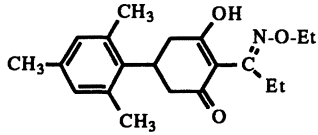
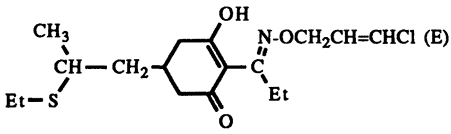
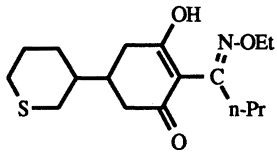


tralkoxydim / Grasp



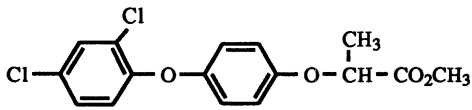
clethodim / Select



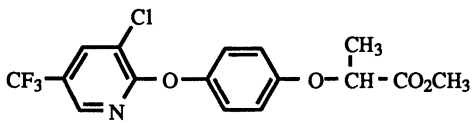
cycloxydim

Tralkoxydim is the only cyclohexanedione known to have adequate tolerance for use in a grass crop, wheat. It has been developed by ICI (*3*) for wild oats control. Most cyclohexanediones have minimal preemergent herbicidal activity.

Dow invested much effort in developing the aryloxyphenoxypropionic acid area of grass herbicides. The original discovery in this area was made by Hoechst AG and led to their development of diclofop, the active ingredient in Hoelon , used for wild oats control in cereals. Dow's research in this area resulted in the discovery of a variety of aryloxyphenoxypropionic acids including haloxyfop, the active ingredient in DowElanco's Gallant herbicide used for grass weed control in soybeans.



diclofop methyl / Hoelon

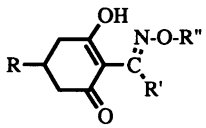


haloxyfop methyl / Gallant

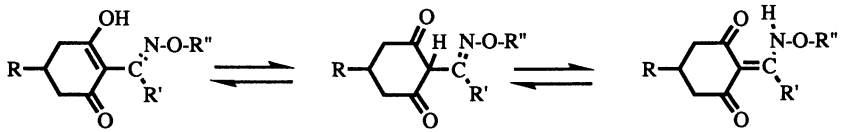
J. Secor and C. Cséke (*4*) discovered that both the cyclohexanediones and aryloxyphenoxypropionic acids inhibit the same enzyme system, acetyl CoA carboxylase (ACCase; EC 6.4.1.2). This discovery offered us a unique insight into the cyclohexanediones. Knowledge of the innate enzyme inhibition ability could possibly guide us to greater whole plant activity. This may be especially important with cyclohexanediones where metabolism in both the soil as well as plant tissue appears to play a greater role than with aryloxyphenoxypropionic acids. Based on our toxicological research, it appears that the cyclohexanedione grass herbicides are also readily metabolized in mammals.

Results and Discussion

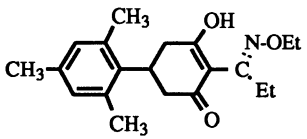
At the time the decision was made to initiate work in cyclohexanediones, many patents had been issued to various companies in this area of grass herbicides. In the generic formula shown below, it appeared that for optimal whole plant activity R' was limited to either an ethyl or an n-propyl group. R" could be Et, $-CH_2CH=CH_2$ or $-CH_2CH=CHCl$ (E) as well as substituted benzyl. The R group could be varied greatly. This is evidenced by the compounds shown on pages one and two. A variety of alkyl groups are found in alloxydim, sethoxydim and cycloxydim. In the case of alloxydim, two alkyl groups are present. Aromatic rings as in tralkoxydim are also well tolerated.



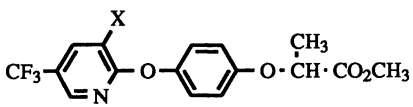
It is important to recognize that the cyclohexanediones can exist in three tautomeric forms as shown below. The cyclohexanediones are acidic molecules, sethoxydim is reported to have a pKa of 4.6 (*5*). This is very important and most likely accounts for the fact that these herbicides are systemic and phloem mobile. Aryloxyphenoxypropionic acids also have similar physical properties. Systemic herbicides offer to the grower much more consistency than contact materials, and in the case of these graminicides, much larger weeds can be controlled. Total coverage of the plant as well as stage of growth of the weed are usually less critical with systemic materials. That the ACCase enzyme inhibited by these materials is a water soluble enzyme as opposed to a membrane-bound one and resides in the meristem or growing point of the plant also requires that these xenobiotics be phloem mobile.



With the knowledge gained by reviewing the patent literature on cyclohexanediones and an understanding of aryloxyphenoxypropionic acids, it was decided to look at the two areas of chemistry in the following way. The three aryl methyl groups in tralkoxydim add greatly to its lipophilicity as does the pyridyloxy group in haloxyfop and the corresponding fluoro analog.



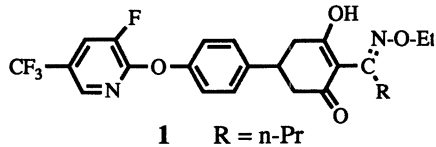
tralkoxydim



X = Cl, haloxyfop
X = F

Can the three methyl groups in tralkoxydim be replaced with the pyridyloxy group and, thereby, give us a new patentable cyclohexanedione grass herbicide?

The following two compounds, **1** and **2**, were made initially to answer this question. As shown in Table I, both compounds had good enzyme inhibition with **1** about twice as active as haloxyfop and equal in activity to tralkoxydim.



1 R = n-Pr
2 R = Et

They also exhibited good whole plant activity on a broad spectrum of grass weeds. The ethyl analog is more active than the n-propyl derivative. Replacing the 3-fluoro group in **2** with a chlorine gave **3** which is less active both enzymatically as well as on the whole plants (Table I).

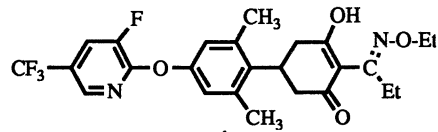
Table I. Enzymatic Inhibition and Postemergent Activity

Compound	I_{50} (μM)*	GR_{80} (ppm)**
Haloxyfop	1.21	11
Sethoxydim	-----	48
Tralkoxydim	0.760	200
1	0.656	99
2	1.12	74
3	1.14	383
4	0.286	209

*Concentration required to achieve 50% inhibition (I_{50}) of ACCase isolated from etiolated corn

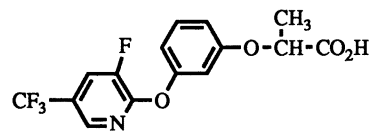
**Whole plant postemergent activity is given as an average concentration required to achieve 80% control of the following weeds: Rox orang', Barnyard grass, Giant foxtail, Fall panicum, Signal grass and Crabgrass

Are the 2,6 - dimethyl groups present in tralkoxydim important for steric reasons in addition to adding to the overall lipophilicity of the molecule? Compound **4** was made to address this issue. As shown in Table I, the material has excellent enzyme inhibition but less whole plant activity than **2**.

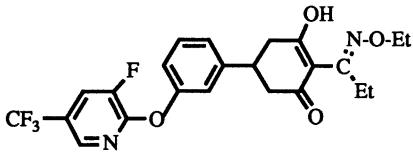


4

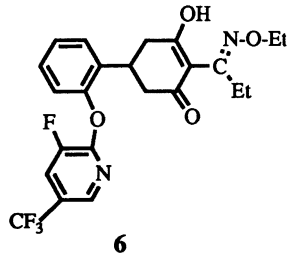
With aryloxyphenoxypropionic acids such as haloxyfop, the pyridyloxy group must be para to the phenyloxypropionic acid moiety. The meta isomer shown below is not active.



There was no reason to believe this would be the same with cyclohexanediones. In fact, with all the flexibility one has in this area, one might expect the meta isomer would be active. Both the meta and ortho isomers, **5** and **6**, were prepared.



5



6

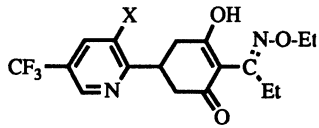
As shown in Table II, the meta orientation is actually better than the para both *in vitro* and *in vivo*. The ortho isomer is inactive.

Table II. Enzymatic Inhibition and Postemergent Activity†

Compound	I_{50} (μM)*	GR_{80} (ppm)**
2 (para)	1.12	74
5 (meta)	0.169	60
6 (ortho)	>1200	>1000
7	3.77	73
8	10.53	60

†See Table I for definition of * and **.

What happens if one removes the oxyphenyl group and attaches the pyridyl group directly to the cyclohexanedione ring? Both the 3-chloro-5-trifluoromethyl and 3-fluoro-5-trifluoromethyl pyridyl analogs were made. It is interesting to note (Table II) that even though the two materials are much less active at the enzyme level than either the meta or para oxyphenyl compounds, their whole plant activities were comparable.

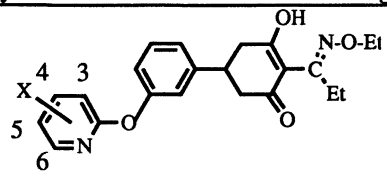


7 X = Cl
8 X = F

Does the oxyphenyl group play a part in enzyme binding? If it does, there should be compounds which have this moiety present and that have much greater whole plant activity. The minimal differences in whole plant activity with these four compounds is most unusual considering their large structural differences as well as enzyme inhibition differences. As mentioned earlier, the amount of flexibility one has in making modifications at the 5- position of cyclohexanediones is very unusual and makes it difficult to use whole plant activity as a guide to synthesis.

In addition to the 3-halo-5-trifluoromethylpyridyloxy derivatives, a variety of pyridine analogs as shown in Table III were prepared. Note that the whole plant activity for **5** given in Table III is different from that presented in earlier tables. This is due to variability from test to test and where side by side comparisons can be given, we will do so. Of this group of compounds, **5** has the highest whole plant activity with the 5-trifluoromethyl derivative, **14**, close behind.

Table III. Enzymatic Inhibition and Postemergent Activity†



Compound	X	I(μM)*	GR_{80}(ppm)**
5	3-F-5-CF_3	0.169	112
9	3-Cl-5-CF_3	0.263	186
10	3,5-diCl	0.120	1000
11	6-CF_3	0.591	864
12	4-CF_3	0.129	267
13	3-CF_3	0.417	1000
14	5-CF_3	0.123	121

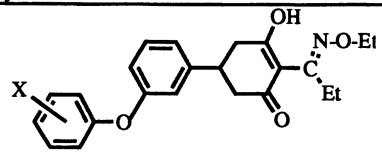
†See Table I for definition of * and **.

Since the pyridyloxyphenyl cyclohexanediones were good grass herbicides, several aryloxyphenyl derivatives were prepared as shown in Table IV. We found these materials to be excellent ACCase enzyme inhibitors, the most active of any compounds that had been tested up to that time. It was most surprising as well as disappointing to find that most of these aryloxyphenoxy cyclohexanediones had no whole plant activity. The only active one was the fluoro-cyano analog, **19**. Apparently these compounds are not getting to the site of action in the weed. Note in Table IV that the 3,4-dichlorophenyl compound, **17**, has 120 times more activity at the enzyme level than **8**, a pyridyl analog. However **17** has no whole plant activity while **8** has very respectable activity.

Why are the aryloxyphenyl cyclohexanediones inactive at the whole plant level while the pyridyloxyphenyl derivatives are active? Some of the differences which must be considered include volatility, penetration, acidity, metabolism, as well as log K_{ow} or water solubility. It does seem possible that the phenyl substituted compounds could be more readily metabolized in the plant than the pyridyl ones. If this occurred, it would be most likely via aromatic hydroxylation by mixed-function oxidases. However, two halogens on a ring as in **17** and **18** should slow down this process to such a degree to allow the compounds to get to the enzyme binding site and express whole plant activity.

If metabolism could not account for the difference, then it seemed most likely that water solubility differences could. The log K_{ow} values of a variety of our cyclohexanediones were measured and compared to haloxyfop. As shown in Table V, there is a wide range of log K_{ow}s. The compounds with the lower log K_{ow}s have the greater whole plant activity. The fluoro-cyano compound, **19**, appears to be an exception. Its whole plant activity is less than one would expect. It is possible that the cyano group is being hydrolyzed to the acid in the plant and that this compound is less active.

Table IV. Enzymatic Inhibition and Postemergent Activity†



Compound	X	I_{50}(μM)*	GR_{80}(ppm)**
15	3-CF_3	0.0939	>750
16	4-Cl	0.104	>750
17	3,4-Cl	<0.0848	>1000
18	3,5-Cl	0.138	>1000
19	2-F-4-CN	0.166	221
20	2-F-4-NO_2	0.0226	1000
21	2-F-4-CH_3CO-	0.0478	1000

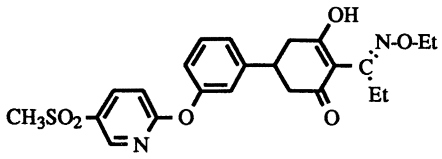
†See Table I for definition of * and **.

Table V. Log K_{ow} vs Biological Activity†

Compound	Log K_{ow}	I_{50}(μM)*	GR_{80}(ppm)**
Haloxyfop	2.83	1.21	11
8	3.64	10.53	60
19	3.76	0.166	221
5	4.63	0.169	60
Tralkoxydim	4.81	0.760	200
10	5.19	0.120	1000
17	6.32	<0.0848	>1000

†See Table I for definition of * and **.

In light of current knowledge (*6, 7*) concerning the importance of water solubility as well as acidity for systemic phloem mobile herbicides, it would seem most likely that water solubility of this type of herbicide would be very important. In order for the compounds to kill the plant, they must move from the leaf surface into the plant and then to the meristem via the phloem which has a pH of about 8.0. The amount of material in the aqueous phases of the plant would be crucial. Note that at the time the log K_{ow} measurements were made, **8** was the most water soluble cyclohexanedione we had made. Even though it possessed poor enzyme activity, it was one of the most active compounds in whole plants which we had made . The log K_{ow} measurements suggested that in order to make cyclohexanediones with whole plant activity equivalent to or greater than that of haloxyfop, it was important to synthesize compounds with greater water solubility (lower log K_{ow}s). With this in mind, the following three compounds were made.



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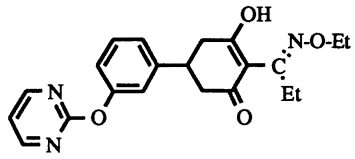

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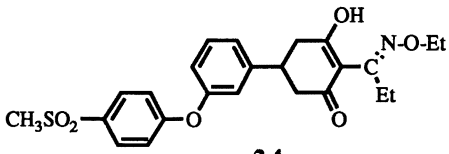


24

A methylsulfonyl group imparts the greatest water solubility to a molecule of any stable nonconjugatable moiety typically used in biological synthesis (*8*). It also seems likely that the sulfur groups in sethoxydim, clethodim and cycloxydim are oxidized in the plant to the sulfoxide and/or the sulfone and that these are active herbicides.

We were pleased to find that **22** has postemergent grass weed activity equal to that of haloxyfop (Table VI). In addition the other two compounds are also very active.

Table VI. Enzymatic Inhibition and Postemergent Activity†

Compound	Log K_{ow}	I_{50}(μM)*	GR_{80}(ppm)**
22	2.47	0.281	11
23	2.48	1.19	38
24	2.81	0.107	63
Haloxyfop	2.83	1.21	11
Tralkoxydim	4.81	0.760	200

†See Table I for definition of * and **.

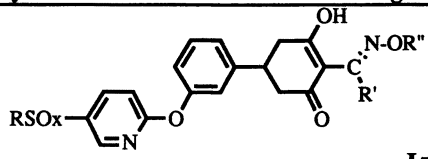
Simply replacing a chloro group in **16** (Table IV) with a methylsulfonyl group, **24**, allows one to go from an inactive compound to a very active one (note that the two compounds have the same levels of enzyme activity). It is also important to note that the log K_{ow} values of the three new compounds are in the same range as that of haloxyfop. This supports the hypothesis that the water solubility of

cyclohexanediones is very important for the expression of whole plant activity, actually more important than enzyme activity.

Compound **22** was field tested in various locations around the world. It showed postemergent grass weed activity comparable to haloxyfop. It also controlled several perennial grass weeds including Johnsongrass which is most important in soybeans grown in the southern part of the U.S. as well as in South America. It does not have the preemergent activity that haloxyfop possesses. Haloxyfop's good soil activity coupled with it's excellent postemergent activity gives the grower very consistent grass weed control in soybeans under a variety of weather conditions.

With the discovery of **22**, a series of compounds closely related to it were prepared. As shown in Table VII, the methylthio analog, **28**, has comparable activity and it was also field tested. It is interesting to note that the *trans*- chloroallyl analog **26** is extremely active *in vitro* but its whole plant activity is not impressive.

Table VII. Enzymatic Inhibition and Postemergent Activity†



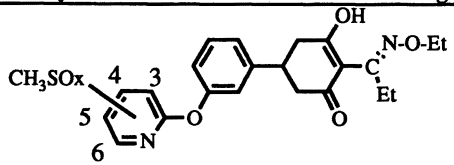
Compound	R	X	R'	R"	I_{50} (μM)*	GR_{80} (ppm)**
25	CH_3	2	n-Pr	Et	0.690	17
26	CH_3	2	Et	$CH_2CH{=}CHCl(E)$	0.0158	63
27	CH_3	2	Et	$CH_2CH{=}CH_2$	0.136	34
28	CH_3	0	Et	Et	0.321	15
29	CH_3	1	Et	Et	1.03	14
30	Et	0	Et	Et	0.204	19
31	Et	2	Et	Et	0.212	25
32	i-propyl	0	Et	Et	0.0308	78
33	i-propyl	2	Et	Et	0.206	125

†See Table I for definition of * and **.

Movement of either a methylthio or methylsulfonyl group around the pyridine ring as shown in the following four compounds resulted in somewhat less active compounds as shown in Table VIII.

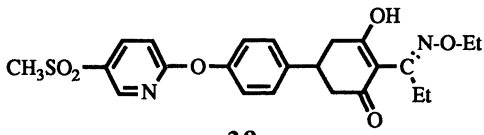
The para -pyridyloxy isomer of **22**, **38**, was prepared and shown to have quite good whole plant activity. Removal of the oxyphenyl moiety resulted in **40**, which also had good whole plant activity even though the *in vitro* activity was low. It also exhibited good wheat selectivity whilst being quite active on wild oats and blackgrass, two important grass weeds in European cereals. This was shown to be due to differential uptake between the crop and the weeds. For consistent results under varying field conditions, the herbicide must be metabolized in the crop of interest. The corresponding sulfide, **39**, showed greater whole plant activity as well as enzyme activity than the sulfone, **40**. However, it was not safe on wheat. This data is shown in Table IX.

Table VIII. Enzymatic Inhibition and Postemergent Activity†



Compound	X	Position on Ring	I_{50}(μM)*	GR_{80}(ppm)**
34	0	6	0.326	19
35	2	6	0.761	28
36	0	4	0.211	19
37	2	4	0.0872	24

†See Table I for definition of * and **.



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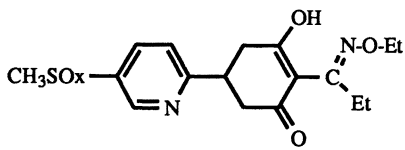
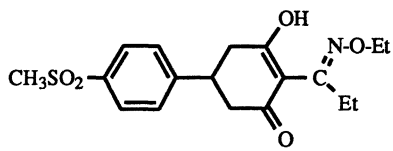


39 X = 0
40 X = 2



41

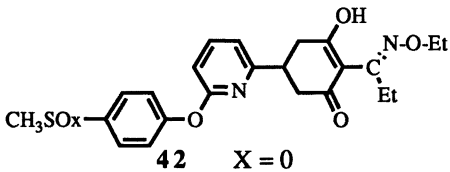
Table IX. Enzymatic Inhibition and Postemergent Activity†

Compound	I_{50}(μM)*	GR_{80}(ppm)**
38	1.68	34
39	4.01	23
40	12.34	46
41	2.87	17

†See Table I for definition of * and **.

The more simple para-methylsulfonyphenyl cyclohexanedione, **41**, exhibited excellent whole plant activity as shown in Table IX. It was not selective for wheat and is patented (*9*).

The next question addressed was whether the pyridine could be moved from the "end of the molecule" to the middle. The log K_{ow} should remain approximately the same. The following three pyridyl-cyclohexanediones were prepared. As shown in Table X, none of the compounds were as active as **22** or **28** even though their water solubilities should be comparable.



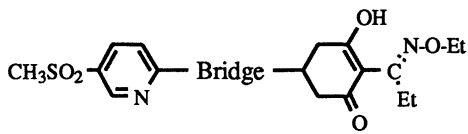
42 X = 0
43 X = 1
44 X = 2

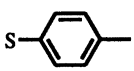
Table X. Enzymatic Inhibition and Postemergent Activity†

Compound	I_{50}(μM)*	GR_{80}(ppm)**
42	0.155	69
43	1.28	92
44	0.574	72
45	282	39
46	1780	54
47	2900	60

†See Table I for definition of * and **.

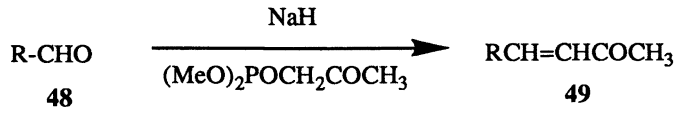
Since the oxyphenyl moiety enhanced the enzyme inhibition, several other bridging groups between pyridine and cyclohexanedione rings were considered. These included p-thiophenyl, oxymethyl, and oxyethyl groups. None of these compounds showed activity comparable to **22** (which is shown in Table VI) as shown in Table X.



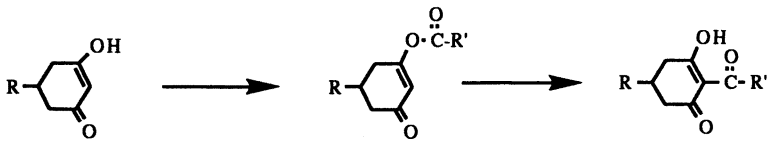
	Bridge
45	
46	OCH_2
47	OCH_2CH_2

Chemistry

A general procedure for the preparation of cyclohexanedione grass herbicides is shown in Scheme I. The method requires the preparation of an aldehyde **48** which is then converted to a vinyl ketone **49** via an Aldol condensation. The simplest way to accomplish this is to treat the aldehyde with acetone and catalytic amount of sodium hydroxide in water. This works well with the pyridyloxybenzaldehydes but not with pyridylaldehydes. For such materials we used a modification of the Wittig reaction sometimes referred to as the Horner-Emmons reaction. This involves reacting dimethyl acetylmethylphosphonate with sodium hydride and this in turn with the aldehyde.



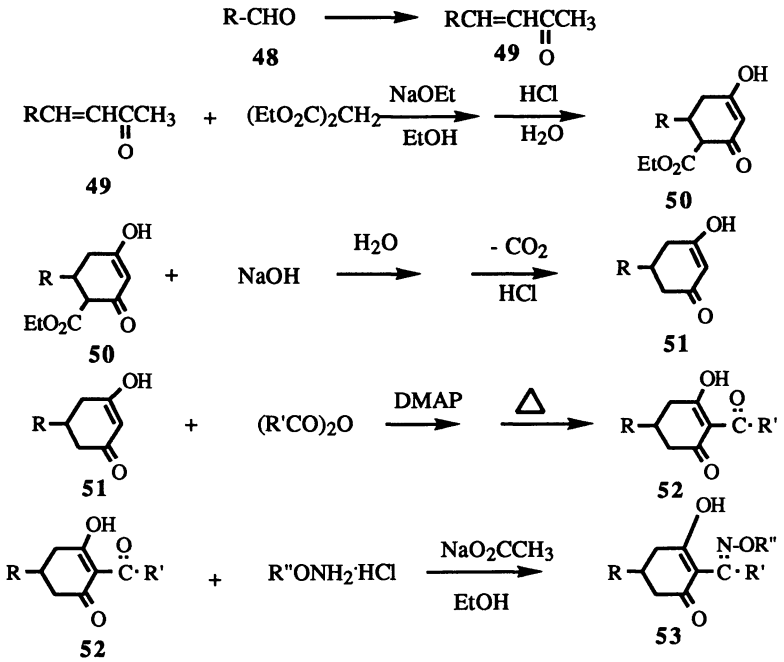
The vinyl ketones **49** are then reacted with diethyl sodiomalonate in ethanol at room temperature. The product **50** obtained upon acidification is a 5-substituted-4-carboethoxy-3-hydroxy-cyclohex-2-en-1-one. The reaction works very well and high yields of product are obtained. This ester is then saponified and decarboxylated giving the desired 5-substituted cyclohexanedione **51**. The dione is then acylated with either propionic or butyric anhydride in the presence of 4-dimethylaminopyridine (DMAP) or other bases such as pyridine. The initial product formed is the O-acylated material as shown below which in the presence of DMAP undergoes rearrangement to the trione **52** in refluxing benzene. Many different kinds of catalysts have been used over the years to affect this rearrangement especially Lewis acids such as aluminum chloride. DMAP is by far the easiest to use and results in high yields of desired trione.



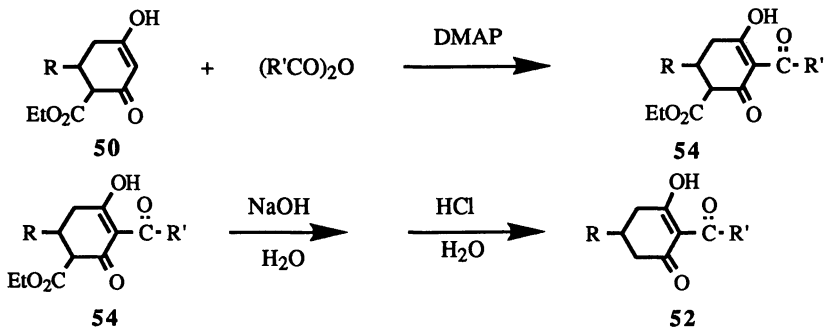
The final step in the formation of a cyclohexanedione grass herbicide is an oximination. The reaction is run by simply treating the trione **52** with the appropriate alkoxyamine hydrochloride in the presence of sodium acetate as a mild base.

As shown in Scheme II, one can carry out the acylation and rearrangement prior to the hydrolysis of the ester and decarboxylation of the acid. The rearrangement often occurs at room temperature while in the previous route, it required temperatures of 75-80°C.

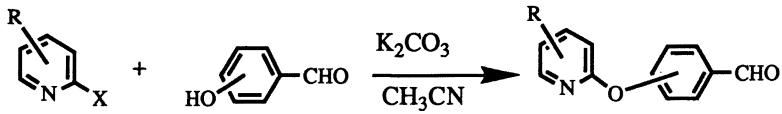
The initial aldehydes required were prepared in the following ways. Displacement of a reactive 2-pyridyl halogen with ortho, meta or para hydroxybenzaldeyde gave the desired pyridyloxybenzaldehydes in good yield.



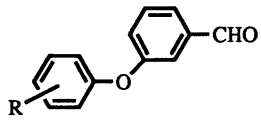
Scheme I



Scheme II



The following four phenoxybenzaldehydes were commercially available.



R = 4-Cl; 3,4 - Cl; 3,5 - Cl; 3 - CF_3

The picolinaldehydes were prepared by DIBAL-H reduction of the corresponding methyl picolinates at low temperature.

Conclusions

A unique series of cyclohexanedione grass herbicides has been prepared. These include a variety of 5-pyridyloxyphenyl-, 5-aryloxyphenyl- as well as 5-pyridyl substituted analogs. Many of the compounds exhibit good levels of whole plant activity on a variety of grass weeds when applied postemergence. The materials have been shown to inhibit acetyl-Co A carboxylase enzyme (EC 6.4.1.2) isolated from etiolated corn. In order to optimize the whole plant activity of this class of herbicides, it was found that a balance of inherent enzyme inhibition activity with the overall water solubility of the compounds is very important. The most active compound in this series is 2-(1-(ethoxyimino)propyl)-3-hydroxy-5-(3-(5-(methylsulfonyl)-2-pyridyloxy)phenyl)cyclohex-2-en-1-one. It has activity in both the greenhouse as well as the field equal to or greater than the current postemergent grass herbicides used in the marketplace including haloxyfop. In addition to annual grass weeds, it gave excellent control of several perennial weeds including Johnsongrass. A series of patents covering many of the compounds discussed in this chapter have been issued (10).

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Chapter 21

Insecticidal 1-Cyclopropyl-1,4-diarylbutenes and 1,4-Diarylbutanes

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Our investigations into pyrethroid insecticides in which various isosteric groups replaced the ester functionality have yielded a number of novel structures with insecticidal activity equal or superior to commercial standards. Some of these molecules also have miticidal activity. This chapter describes the synthesis and biological activity of compounds containing an alkene or epoxide linker group in place of the ester. We discuss spectrum and residual activity for several of these compounds. We selected one of the alkenes, 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)-2-butene, for further testing and showed it to have excellent activity against rice pests, low mammalian toxicity, and very low toxicity to fish.

The pyrethroids have been tremendously successful as commercial insecticides. This success in large part is due to their high intrinsic activity against a broad spectrum of insect pests, their low mammalian toxicity, and their generally low environmental mobility and persistence. Over the past 45 years, a great deal of research has provided definitions for the structure-activity relationships of the pyrethroids. Many potent insecticides have resulted from variations on the basic structure of these compounds.

The great majority of pyrethroids contain an ester linkage, e.g., fenvalerate **1**. In recent years, however, reports have described a number of isosteric linkages, e.g.,

- Ethers, such as ethofenprox **2** and F7704 **3** (*1*)
- Alkanes, such as MTI800 **4** and F7869 **5** (*4*)
- Oxime ethers, such as **6** (*2,3*)

Some of the non-ester bioisosteres have demonstrated desirable properties not commonly associated with pyrethroid insecticides, such as miticidal activity and low fish toxicity (*1*).

The tolerance towards variation in the linker group demonstrated thus far by the pyrethroids suggested the possibility of further modification. We proposed a series

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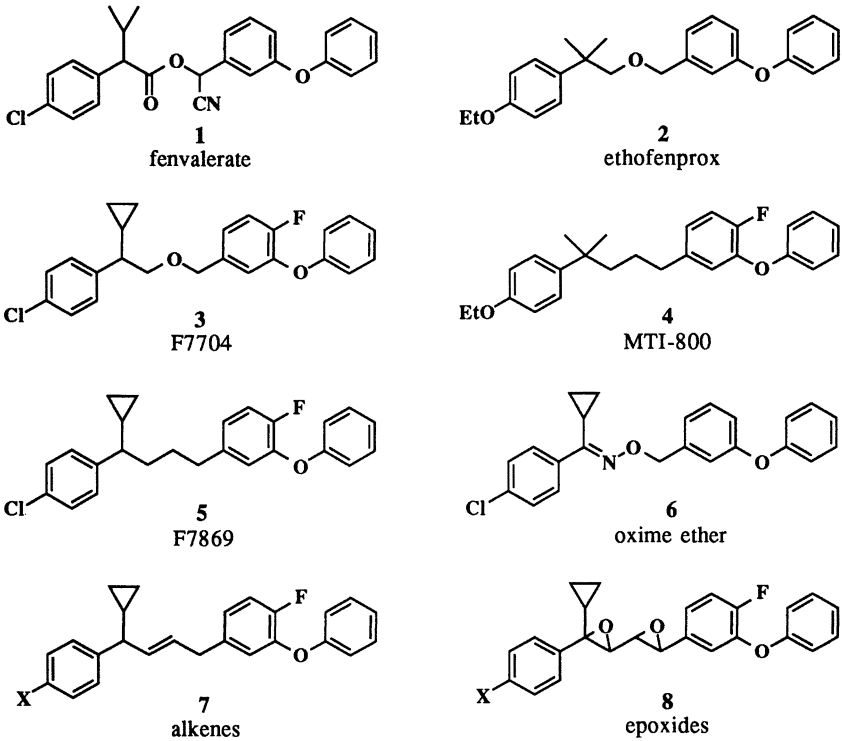


of alkenes **7** and the corresponding epoxides **8** as potential insecticides. This chapter describes the synthesis and biological activity of these molecules.

Synthesis

Synthesis of 1-Butenes and 1,2-Epoxides. Cullen et al. have previously described synthesis of the 1-butenes **9** by a Wittig addition to the appropriate aryl cyclopropyl ketone (*4*). We prepared the corresponding epoxide **10** as shown in Figure 1, by treating **9** with an excess of *m*-chloroperoxybenzoic acid at 0 °C. Yield (unoptimized) after chromatography was 34%.

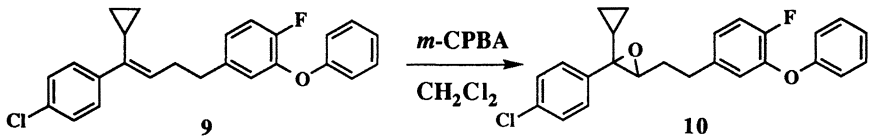


Figure 1. Synthesis of 1,2-epoxides.

Synthesis of 3-Butenes and 3,4-Epoxides. We describe the synthesis of 3-cyclopropyl-3-aryl-propionaldehyde **11** elsewhere in this volume (*5*). Treatment of this aldehyde with the phosphorane **12** in anhydrous THF yielded the 3-butene **13** as shown in Figure 2. Treatment of **13** with an excess of *m*-chloroperoxybenzoic acid at 0 °C gave the epoxide **14**. Yield after chromatography was 92%.

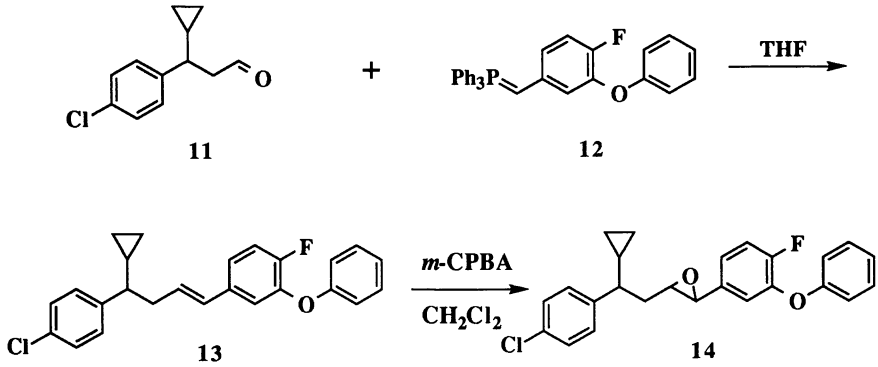


Figure 2. Synthesis of 3-butenes and 3,4-epoxides.

Synthesis of 1,3-Butadienes and 1,2-3,4-(Bis)epoxides. Cullen et al. previously described the synthesis of the 1,3-butadienes **15** (*4*). Treatment of **15** with an excess of *m*-chloroperoxybenzoic acid at 0 °C gave the (bis)epoxide **16**. After workup, we purified the crude mixture by radially-accelerated chromatography to give two fractions, each containing a mixture of diastereomeric (bis)epoxides. NMR analysis indicated a mixture of approximately 70:30 in one fraction and 30:70 in the other. Combined yield after chromatography was 22%. (See Figure 3.)

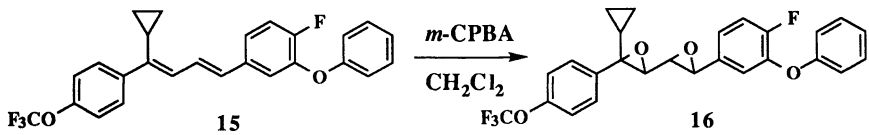


Figure 3. Synthesis of (bis)epoxides.

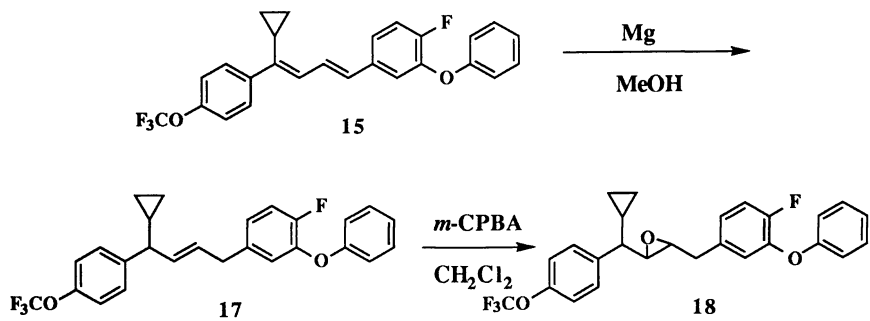


Figure 4. Synthesis of 2-butenes and 2,3-epoxides

Synthesis of 2-Butenes and 2,3-Epoxides. We prepared the 2-butene analogs **17** in 80% yield by reducing the 1,3-butadiene **15** with excess magnesium in refluxing methanol, as shown in Figure 4 (*6*). Treatment of **17** with an excess of *m*-chloroperoxybenzoic acid at 0 °C gave the epoxide **18**. Yield after chromatography was 60%.

Biological Testing

Foliar Evaluation. We screened the compounds for insecticidal and acaricidal activity against the following species: cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), Mexican bean beetle (*Epilachna varivestis*), brown planthopper (*Nilaparvata lugeus*), and twospotted spider mite (*Tetranychus urticae*).

To determine the activity against cabbage looper, tobacco budworm, and Mexican bean beetle, we sprayed the upper and lower surfaces of the leaves of pinto bean plants with test solution until run-off. Second instar larvae (ten larvae for each of two replicates for each compound) infested the plants after the foliage had dried. Appropriate dilution from a stock solution of the experimental compound in 10% acetone/water gave the test solutions.

We also determined the activity against mites on pinto bean plants. We pre-infested the bean leaves with adult mites (about 75 mites for each of two replicates for each compound), then sprayed until run-off with test solution.

To prevent escape of the insects from the test site, capped cups or other appropriate containers contained the treated plant or excised leaves. Experimental protocol required holding the tests at 25°C and 50% relative humidity for an exposure period of 48 hours. At the end of this time, we determined percent mortality and used probit analysis to determine LC_{50} values.

Testing against brown planthopper employed TN1 rice plants that were 35-50 days old. Plants trimmed to 12 inches in height and to contain four tillers were sprayed to run-off and allowed to dry. Twenty brown planthopper nymphs (2nd-3rd instar) infested each pot, which was then covered with a mylar cage. Probit analysis of mortality readings 24 hours after infestation determined the LC_{50} values.

Foliar Evaluation for Residual Activity. We determined efficacy in residual testing by spraying the test plants to run-off with aqueous dilutions of the compounds. The treated plants were held under greenhouse conditions for the appropriate time before infestation with insects. The test was then completed as described for the initial evaluations.

Toxicity Testing Against Aquatic Organisms. We determined the fish toxicity by testing against fathead minnows (*Pimephales promelas*). Fish testing required the test materials be prepared as acetone (1%)/water solutions diluted to the appropriate volume and allowed to equilibrate for five to six hours in one-quart glass containers before infestation with three minnows (25-35 mm in length). Mortality counts after 24 hours determined the PI_{50} values.

I. T. Corporation Laboratories performed testing for toxicity to water flea (*Daphnia magna*). The test protocol required two replicates of ten *Daphnia* per replicate at each concentration, with concentrations ranging from 7.6 to 9.5 x 10^{-4} mg/L. Mortality after 48 hours determined the PI_{50} values. Water-quality parameters such as temperature, pH, and dissolved oxygen remained within acceptable limits for the duration of the test.

Results and Discussion

The 1-butene isomer proved to be the least efficacious of the regioisomeric alkenes. Table I compares the foliar activity of the 1,4-diaryl-1-cyclopropylbut-1-ene to a 1,4-diaryl-1-cyclopropylbut-3-ene. Compounds **9** and **13** are nearly equivalent against cabbage looper, but the 1-butene is nearly 3X less active against Mexican bean beetle. Epoxidation of the 1-butene to give **10** increased its foliar activity, particularly against cabbage looper. Interestingly, epoxidation of the 3-butene **13** had the opposite effect. The resulting epoxide **14** had very poor insecticidal activity.

Table I. Foliar Activity of 1-Cyclopropyl-1,4-diarylbutenes and Their Epoxides

Compound		LC_{50} (ppm) vs. Cabbage Looper	Mexican Bean Beetle
9	(1-butene)	87	110
10	(1,2-epoxide)	19	78
13	(3-butene)	70	41
14	(3,4-epoxide)	300	400

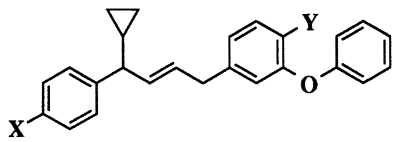
The 1,3-butadiene **15** described in Table II had very poor insecticidal activity. Epoxidation in this case gave a significant increase in insecticidal activity. During purification of the diastereomeric mixture **16** that resulted from epoxidation of the 1,3-butadiene **15**, we obtained a partial separation of diastereomers. We tested these individually, as diastereomeric mixture **16A** and **16B**. These mixtures had approximately the same activity against cabbage looper, but mixture **16A**, which eluted first during chromatography on silica gel, was at least 5X as active against Mexican bean beetle. Sample availability did not permit further separation of diastereomers or resolution of enantiomers for individual testing. However, numerous studies have documented the importance of stereochemistry for optimal pyrethroid activity for both the ester and non-ester pyrethroids, and so it is likely that resolution would lead to a further increase in insecticidal activity.

Table II. Foliar Activity of 1,4-diarylbutadienes and Their Epoxides

Compound		LC_{50} (ppm) vs. Cabbage Looper	Mexican Bean Beetle
15	(1,3-butadiene)	~200	~300
16A	((bis)epoxide mix A)	3	20
16B	((bis)epoxide mix B)	2	> 100

The 2-butenes **17, 19-22** were the most active of the regioisomeric alkenes. Table III summarizes the insecticidal activity of some of these compounds. Compounds containing a 4-fluoro-3-phenoxyphenyl moiety (**17, 20, 21**) were generally the most active, a pattern that we also observed in other classes of non-ester chemistry (*1,4,5*). The best of these compounds also exhibited modest levels of miticidal activity. Table III shows that some of the 2-butenes were comparable or superior to the standards cypermethrin and the alkane form of **21** (compound **5**).

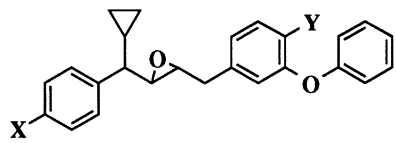
Table III. Foliar Activity of 1-Cyclopropyl-1,4-diarylbut-2-enes



Compound	X	Y	LC_{50} (ppm) vs. Cabbage Looper	Mexican Bean Beetle	Twospotted Spider Mite
17	CF_3O	F	3.4	1.2	25
19	CF_3O	H	41	3	14
20	CF_3	F	2.1	0.4	38
21	Cl	F	12	1.3	inactive
22	Cl	H	49	2.8	inactive
5 (butane)	Cl	F	1	6	225
cypermethrin			2	1	170

Epoxidation of the 2-butenes decreased their insecticidal and miticidal activity, as illustrated by the compounds shown in Table IV.

Table IV. Foliar Activity of 1-Cyclopropyl-1,4-diarylbut-2-ene Epoxides



Compound	X	Y	LC_{50} (ppm) vs. Cabbage Looper	Mexican Bean Beetle	Twospotted Spider Mite
18	CF_3O	F	15	6.8	~150
23	CF_3	F	42	7.7	~150
24	CF_3	H	~300	~100	~300

We were concerned about the stability of the epoxides and selected two for residual testing. Table V shows the 3-day and 7-day residual activity for the 2,3-epoxide **18** and the (bis)epoxide **16A**. The (bis)epoxide is approximately equivalent to cypermethrin initially and after three days, but its activity drops off significantly after 7 days.

Table V. Residual Foliar Activity of 1-Cyclopropyl-1,4-diarylbutene Epoxides

Compound	LC_{50} (ppm) vs. Cabbage Looper			Mexican Bean Beetle		
	Initial	3-Day	7-Day	Initial	3-Day	7-Day
18 (2,3-epoxide)	20	~50	>50	7	10	40
16A ((bis)epoxide)	2	3	20	5	3	20
Cypermethrin	2	5	5	1	4	3

We selected a different set of 2-butene analogs for residual testing and replaced Mexican bean beetle with tobacco budworm as one of the test species. Table VI gives the results of these tests. In this case, the commercial rice insecticide ethofenprox was included as the standard. Compound **21** showed particularly good residual activity out to seven days. On this basis, we selected it for further testing.

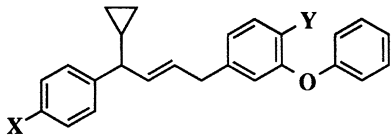
Table VI. Residual Foliar Activity of 1-Cyclopropyl-1,4-diarylbut-2-enes

Compound	LC_{50} (ppm) vs. Cabbage Looper			Tobacco Budworm		
	Initial	3-Day	7-Day	Initial	3-Day	7-Day
20	2.1	12	>16	2.4	>16	>16
21	1	1.5	5.9	2.2	5.8	18
ethofenprox	91	250	>500	52	--	--

Table VII shows the results of testing **21** and several other 2-butene analogs against brown planthopper, an important rice pest. All of these analogs had very good foliar activity against this pest when compared to the commercial standard ethofenprox.

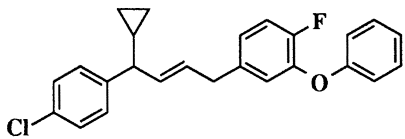
We selected compound **21** for further testing for mammalian toxicity and for toxicity to fish and aquatic invertebrates. Table VIII summarizes the results of these tests and compares the results to those obtained for the standard ethofenprox. Mammalian toxicity data for ethofenprox was taken from the product literature, and consequently no direct comparison to **21** at the highest rate used in our in-house testing was possible. The fish safety of these compounds, relative to typical ester pyrethroids, is remarkable. For example, cypermethrin has an LC_{50} against fathead minnow of only 0.001 ppm.

Table VII. Foliar Activity of 1-Cyclopropyl-1,4-diarylbut-2-enes Against Brown Planthopper



Compound	X	Y	LC_{50} (ppm) vs. Brown Planthopper
17	CF_3O	F	1
19	CF_3O	H	2
21	Cl	F	2
22	Cl	H	5
ethofenprox			31

Table VIII. Mammalian and Aquatic Organism Toxicity



Assay	2-Butene	Ethofenprox
Acute oral LC_{50}, mouse	> 500 mg/kg	> 300 mg/kg
Acute dermal LC_{50}, mouse	> 200 mg/kg	> 2100 mg/kg
Fathead minnow LC_{50}	100 ppm	38 ppm
***Daphnia magna* LC_{50}**	< 1 ppb	< 1 ppb

Conclusions.

The 1-Cyclopropyl-1,4-diarylbut-2-enes had the most promising insecticidal activity of all the compound classes investigated in this study. The best of these analogs, **21**, had initial and residual insecticidal activity comparable to cypermethrin and significantly better than ethofenprox. Compound **21** demonstrated mammalian safety in oral and dermal testing. Interestingly, **21** was approximately 3 times less toxic to fish than ethofenprox, and 10^5 times less toxic than cypermethrin! The 1-butene, 3-butene, and 1,3-butadiene isomers were all less active than the 2-butenes.

Epoxidation of the 1-butenes increased the insecticidal activity (by 4X versus cabbage looper), while epoxidation of the 3-butenes decreased it (by 4X). However, epoxidation of the 1,3-butadienes increased the cabbage looper activity by approximately 100X. Partial separation of diastereomers in the (bis)epoxide mixture showed that they differed in insecticidal activity.

Acknowledgments

The authors would like to thank Charles M. Langevine, George L. Meindl, Leslie Stratton, and James H. Strickland for their assistance in synthesizing and testing some of the compounds described in this chapter. We also acknowledge John F. Engel and Jane A. Dybas for their encouragement and advice. Finally, we acknowledge FMC Corporation for supporting this work.

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Chapter 22

Novel 1-Cyclopropyl-1-(4-substituted phenyl)-4-cyano-(4-fluoro-3-phenoxyphenyl)butane Insecticides

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4-Cyano-1-cyclopropyl-1,4-diarylbutanes are potent broad-spectrum insecticides, and, in some cases acaricides. The chemistry and biological activity of these compounds are discussed. The biological activity is compared to both relevant commercial standards and the corresponding unsubstituted compounds, the 1,4-diaryl-1-cyclopropylbutanes.

The objective of our research program was to discover an insecticide that had excellent foliar activity. Additionally, we set the criteria that our compounds possess fish and mammalian safety equivalent to ethofenprox, **1**, and MTI-800, **2**. This chapter reports on the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-substituted-3-phenoxyphenyl)butane, **3**, where the fenvalerate ester functionality, **4**, is replaced by an ethylene unit and the cyano group is retained. The structures of the compounds referred to in this chapter are in Figure 1.

Previously we reported on changes to the ester linkage of fenvalerate. One part of that program was the preparation of 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)butane, **5**. In that instance, we replaced the ester linkage of fenvalerate by an ethylene linkage and removed the cyano group (*1*, *2*). These compounds are effective foliar insecticides with good initial and residual activity against worms. They are also effective against hoppers and can be considered potential rice insecticides. Finally, compounds, such as **5** are the equivalent of ethofenprox in regard to insecticidal activity, mammalian safety, and fish toxicity (*3*, *4*).

As depicted in Figure 2, positions X, Y, and Z represent those portions of the core structure that were examined for their effects on biological activity. Previous work in this area shows the cyclopropyl group is essential for good foliar activity (*3*). It was also found that when Y is fluorine that insecticidal activity is enhanced. This finding was applied to the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-substituted-3-phenoxyphenyl)butane discussed in this work.

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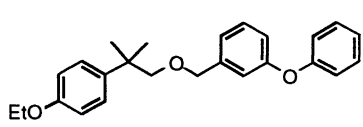
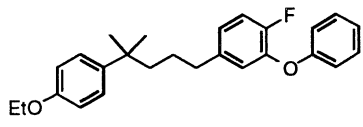
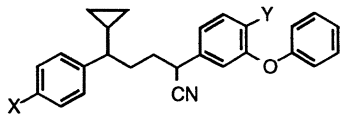


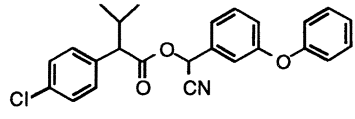
1
ethofenprox



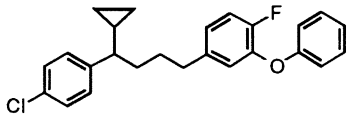
2
MTI-800



3

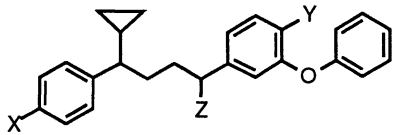


4
fenvalerate



5

Figure 1. Structures of Compounds Discussed



X= Cl, CF_3, OCF_3, OC_2H_5, H
Y= H or F
Z= CN, CH_3, or H

Figure 2. Positions for Synthesis

Synthesis

Synthesis of the 4-methyl Derivative. Figure 3. The general syntheses for the 4-methyl and 4-cyano compounds are shown in Figures 3 and 4. Synthesis details have been reported previously in greater detail and will only be outlined here. (*5*)

Treatment of 4-chlorophenyl cyclopropyl ketone with vinyl magnesium bromide gave the tertiary allylic alcohol **7** in 96% yield. Reaction of **7** with thionyl chloride gave 1-cyclopropyl-1-(4-chlorophenyl)-3-chloro-1-propene, **8**, in 90% yield. Treatment of **8** with triphenylphosphine gave 3-cyclopropyl-3-(4-chlorophenyl)-2-propenyltriphenylphosphonium salt **9**. Compound **9** is washed with toluene, removing many impurities, thereby allowing an easier purification

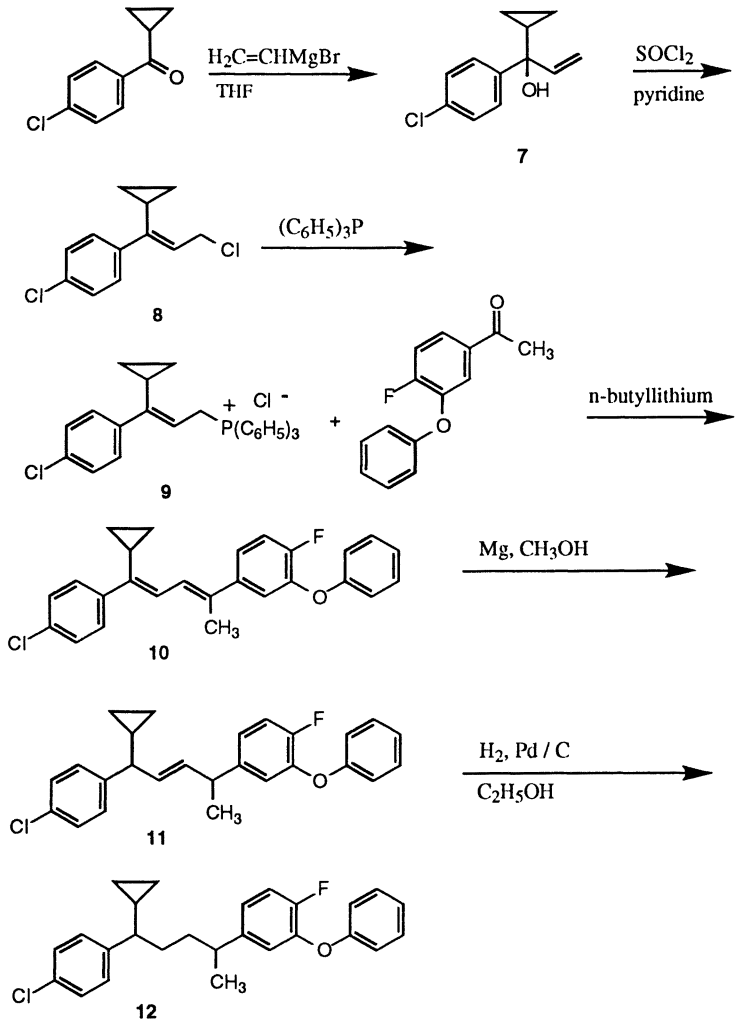


Figure 3. Synthesis Route to 1-Cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)pentane

when the Wittig reaction was performed. Compound **9** was added to a solution of n-butyllithium. Methyl (4-fluoro-3-phenoxyphenyl)ketone is added. After an aqueous work-up, the 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)-1,3-pentadiene, **10**, was purified by column chromatography. The pentadiene was stirred in magnesium and methanol. After an acidic work-up, the product, 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)-2-pentene, **11** was isolated. Catalytic reduction of **11** with palladium on charcoal gave the desired product, 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)pentane, **12**.

Synthesis of the 4-Cyano Derivative. Figure 4. The anion of trimethylsilyl–phosphonoacetate was treated with 4-chlorophenyl cyclopropyl ketone and gave methyl 3-cyclopropyl-3-(4-chlorophenyl)propenoate, **13**. The double bond of **13** was reduced using magnesium in methanol providing methyl 3-cyclopropyl-3-(4-chlorophenyl)propanoate, **14**. Compound **14** was reduced with lithium aluminum hydride in good yield to give 3-cyclopropyl-3-(4-chlorophenyl)propan-1-ol, **15**. Using pyridinium chlorochromate in methylene chloride, **15** was oxidized to 3-cyclopropyl-3-(4-chlorophenyl)-propronaldehyde, **16**, in 25% yield. Next, a mixture of **16** and 4-fluoro-3-phenoxyphenylacetonitrile in methanol was treated with a 25% sodium methoxide solution. After an aqueous work-up and purification by column chromatography, the product was 1-cyclopropyl-1-(4-chlorophenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)-3-butene, **17**. Reduction of **17** gave the desired product 1-cyclopropyl-1-(4-chlorophenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane, **18**.

Biological Testing

The compounds were screened for insecticidal and acaricidal activity against the following species: cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), Mexican bean beetle (*Epilachna varivestis*), and two-spotted spider mite (*Tetranychus urticae*).

The activity against cabbage looper (CL), tobacco budworm (TBW), and Mexican bean beetle (MBB) was determined by spraying the upper and lower surfaces of the leaves of pinto bean plants with test solution until run-off and infesting with second instar larvae (ten larvae for each of two replicates for each compound) after the foliage had dried.

The activity against mites (TSM) was determined on pinto bean plants. The bean leaves were pre-infested with adult mites (about 75 mites for each of two replicates for each compound), then sprayed until run-off with test solution.

To prevent escape of the insects from the test site, the treated plant or excised leaves were placed in capped cups or other appropriate containers. The tests were transferred to a holding room at 25°C and 50% relative humidity for an exposure period of 48 hours. At the end of this time, percent mortality was determined and LC_{50} values were determined by probit analysis.

Efficacy in residual testing was determined by spraying the test plants to run-off with aqueous dilutions of the compounds. The treated plants were held under greenhouse conditions for the appropriate period of time before infestation with insects. The test was then completed as described for the initial evaluations.

Results and Discussion

We first examined substitution at Z. The data in Table I show quite conclusively that cyano and hydrogen are preferred to methyl in foliar applications against cabbage looper and Mexican bean beetle. The comparison of cyano versus hydrogen is not as clear and so additional analogs were prepared.

Table II compares the foliar activity of the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane, and 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(3-phenoxyphenyl)butane. These compounds are not effective miticides, with the best compounds having an LC_{50} of 100 ppm. In this area, we changed substituents at three positions on the molecule and cannot quantitate the contributions of X, Y, and Z independently. Any conclusions would be qualitative. The foliar data for the 1-cyclopropyl-1-(4-X-phenyl)-4- (4-Y-3-phenoxyphenyl)butanes are included in Table II.

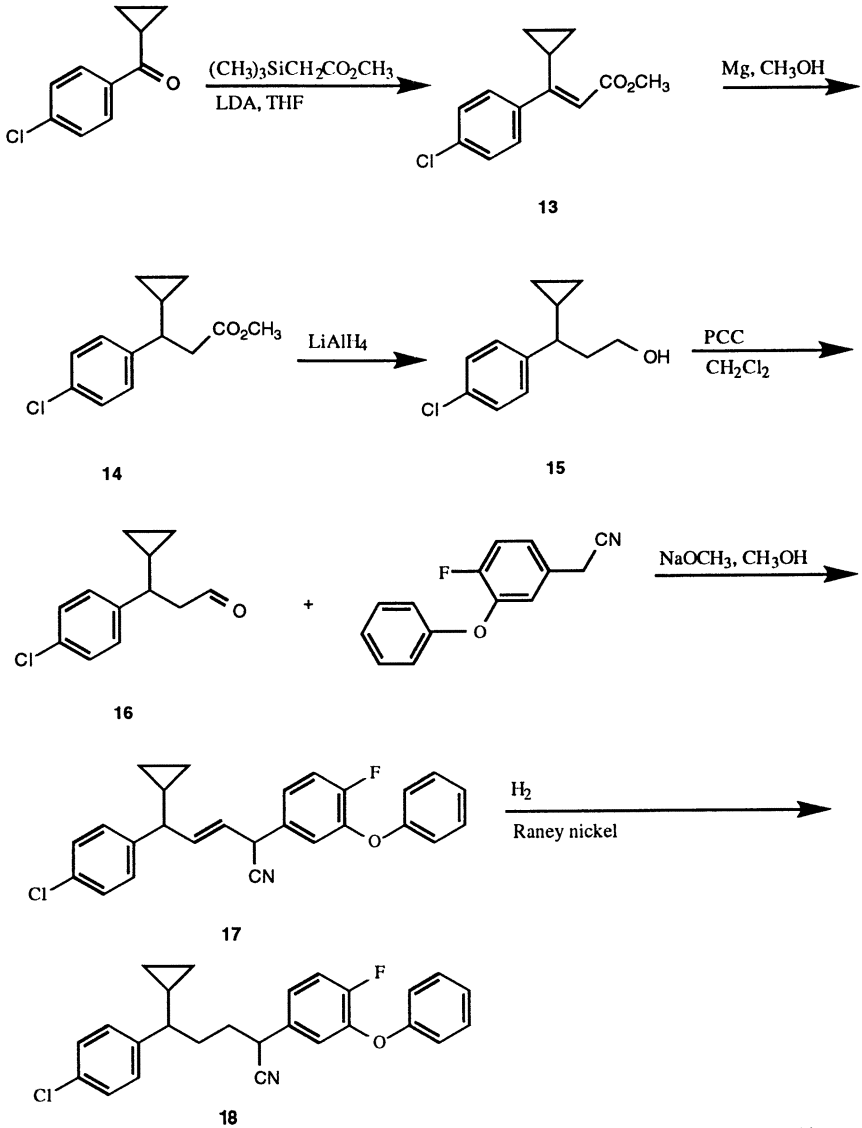
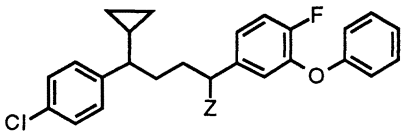


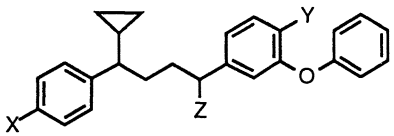
Figure 4. Synthesis of 1-Cyclopropyl-1-(4-chlorophenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane

Table I. Foliar Activity of 1-Cyclopropyl-1-(4-chlorophenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane and 1-Cyclopropyl-1-(4-chlorophenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane



	LC_{50} (PPM)	
Y	Cabbage Looper	Mexican Bean Beetle
CH_3	350	40
CN	4.6	22
H	1.0	6.0

Table II. Foliar Activity of Targets



X	Y	Z	Tobacco Budworm	Cabbage Looper	Mexican Bean Beetle	Two-spotted Spider Mite
F	F	CN	23	4.9	3.8	470
F	H	CN	61	34	21	550
Cl	F	CN	66	4.6	22	I
Cl	H	CN	69	8.1	2.2	600
Cl	F	H	8	1	6	225
Cl	H	H	15	3	17	344
CF_3	F	CN	30	5.5	22	290
CF_3	F	H	9	10	8	16
CF_3	H	H	33	13	23	123
OCF_3	F	CN	19	5	2	100
OCF_3	H	CN	46	20	2	100
OCF_3	F	H	14	5	2	13
OCF_3	H	H	-	19	5	53
OC_2H_5	F	CN	150	12	15	150
OC_2H_5	F	H	86	51	2	390
OC_2H_5	H	H	190	97	12	500
H	F	CN	85	110	18	-
H	H	CN	190	250	-	I
ethofenprox			52	91	37	-
cypermethrin			4	2	1	-

I = inactive
- = not tested

Free-Wilson Analysis

The Free-Wilson approach has great value when there is a lead molecule with many positions for substitution and the set of substituents is limited by some constraint. The major constraint for us was to identify the positions to optimize simultaneously. Furthermore, extensive foliar evaluation of many targets against a host of insect species could overwhelm the testing resources of any organization.

There are several assumptions that are used in a Free-Wilson analysis. The additivity assumption assumes that the contribution of any group on activity is dependent on position and is constant as long as that group is in that position. The method is most useful when three or more positions of a lead are subject to variation. As the complexity increases, the method becomes more valuable and efficient. In our situation, with three positions, we felt this approach was a good choice. Also, consideration of physiochemical properties is not necessary. The group contributions embody all the physico-chemical factors that play a role in determining activity. The group contribution of hydrogen is 0.0 because it is chosen to be the reference. A given substituent may increase or decrease activity when compared to hydrogen. These assumptions hold regardless of what other substituents are present in the molecule *(6-8)*.

As long as the additivity assumption holds, the Free-Wilson analysis is a powerful tool. There are situations where the method can fail. Some reasons for failure are intramolecular hydrogen bonding, chelation and special steric considerations. An examination of our compounds did not reveal any reasons for concern.

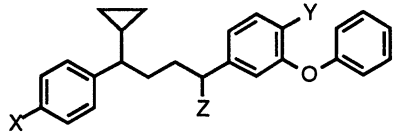
Three species were used in the analysis: tobacco budworm, cabbage looper, and Mexican bean beetle. The obviously inferior mite activity of the compounds where Z equals cyano did not warrant analysis. The foliar evaluation data in Table II is reported as an LC_{50} in part per million. In the Free-Wilson analyses, the data is manipulated as a pLC_{50}, which is the negative log of the LC_{50} divided by the molecular weight of the compound multiplied by 1000. Therefore, the group contributions to foliar activity are in log units. In the tables, the numbers in parentheses are the number of times that particular substituent occurs.

Table III shows the Free-Wilson group contributions for tobacco budworm control. This table shows quite clearly that Z equal to cyano does not increase the foliar activity against tobacco budworm. It causes more than half a log unit loss in activity. The best X groups are fluoro and trifluoromethoxy. Here these substituents increased foliar activity by half an order of magnitude. The Free-Wilson analysis shows that the cyano group is not an effective contributor to activity and we can quantitate that contribution. Given the economic importance of tobacco budworm, this strongly indicates against incorporating this particular structural modification.

Table IV shows the group contributions to cabbage looper. Again, when Z is cyano there is a negative contribution to foliar efficacy, -0.26 log units. For cabbage looper the X substituent appears to be the primary contributor to foliar activity. The chloro group increases activity by nearly an order of magnitude and the fluoro contributes 0.75 units. As in the case of the tobacco budworm, the cyano group is a negative contributor, though to a lesser extent.

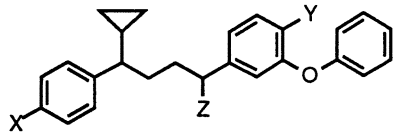
Table V provides data on the coleopteran insect, Mexican bean beetle. The results are different from the previous tables. In this case, Z, as either hydrogen or cyano, is essentially neutral. It exerts neither a positive nor negative effect on foliar activity. The one substituent that contributes to foliar activity is X as trifluoromethoxy; a positive 0.73 log units.

Table III. Free-Wilson Group Contributions for Tobacco Budworm Control



Tobacco Budworm Foliar pLC_{50} (log units) Activity of Unsubstituted Parent (theoretical) = 3.90			
Substituent	X	Y	Z
H	0.00 (3)	0.00 (8)	0.00 (8)
F	0.49 (2)	0.31 (9)	
Cl	0.35 (4)		
CF_3	0.38 (3)		
CF_3O	0.53 (3)		
C_2H_5O	-0.58 (2)		
CN			-0.53 (9)

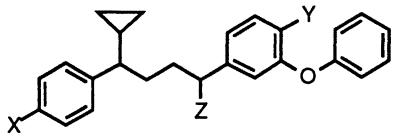
Table IV. Free-Wilson Group Contributions for Cabbage Looper Control



Cabbage Looper Foliar pLC_{50} (log units) Activity of Unsubstituted Parent (theoretical) = 3.98			
Substituent	X	Y	Z
H	0.00 (2)	0.00 (9)	0.0 0 (9)
F	0.75 (2)	0.48 (8)	
Cl	0.99 (4)		
CF_3	0.34 (3)		
CF_3O	0.56 (3)		
C_2H_5O	-0.20 (3)		
CN			-0.26 (8)

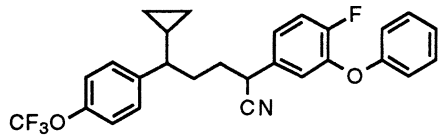
The results of an examination of the various Free-Wilson group contributions suggest that the best compound should be 1-cyclopropyl-1-(4-trifluoromethoxyphenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane. This compound was selected for residual testing, which is the next step in evaluating our interest in a particular compound. The results are reported in Table VI. The compound does have residual activity against lepidoptera and coleoptera. Yet, when this activity is compared to the best compound where Z is hydrogen, 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)butane, Table VII, it is apparent that the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)-butanes do not have the residual activity of the 1-cyclopropyl-1-(4-substituted-phenyl)-4-(4-fluoro-3-phenoxyphenyl)butanes.

Table V. Free-Wilson Group Contributions for Mexican Bean Beetle Control



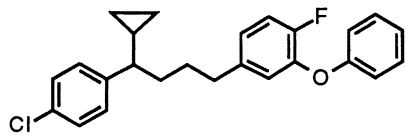
Mexican Bean Beetle Foliar pLC_{50} (log units) Activity of Unsubstituted Parent (theoretical) = 4.51			
Substituent	X	Y	Z
H	0.00 (3)	0.00 (9)	0.00 (8)
F	0.10 (2)	0.48 (9)	
Cl	0.13 (4)		
CF_3	-0.16 (3)		
CF_3O	0.73 (4)		
C_2H_5O	0.16 (3)		
CN			-0.10 (9)

Table VI. Residual Activity of 1-Cyclopropyl-1-(4-trifluoromethoxyphenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butane



	Foliar LC_{50} (ppm)		
Species	Initial	3 Day	7 Day
Cabbage Looper	5	14	13
Tobacco Budworm	19	41	60

Table VII. Residual Activity of 1-Cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)butane



	Foliar LC_{50} (ppm)		
Species	Initial	3 Day	7 Day
Cabbage Looper	1	1	5
Tobacco Budworm	2	9	17

The Free-Wilson analysis shows us that when Z is hydrogen it contributes more to foliar lepidopteran activity than Z as cyano. With the foliar data in hand and the substituent group contributions quantified, we were confident in our decision not to send the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4(4-fluoro-3-phenoxyphenyl)butanes for acute toxicity and aquatic safety testing. Instead, we selected 1-cyclopropyl-1-(4-chlorophenyl)-4-(4-fluoro-3-phenoxyphenyl)butane,**5**, for that testing and the results are reported elsewhere (3). Overall, the 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)butanes are effective foliar insecticides and possess residual activity against lepedoptera.

Conclusions

The 1-cyclopropyl-1-(4-substituted phenyl)-4-cyano-4-(4-fluoro-3-phenoxyphenyl)-butane are effective as foliar insecticides. The compounds also have residual activity against lepidoptera and coleoptera. However, the presence of the cyano group does not confer a significant increase in insecticidal activity.

Acknowledgments

The authors acknowledge the contributions of our many co-workers in this program. Charles M. Langevine and Dominic P. Suarez prepared several of the compounds; Kathleen A. Boyler, George L. Meindl, Mina Reed, and Carmela E. Barrett were responsible for the insecticide data. Finally, the authors acknowledge the support of FMC Corporation.

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Chapter 23

(−)-(4-Fluoro-3-phenoxy)methyl 2-Cyclopropyl-2-(4-trifluoromethoxyphenyl)ethyl Ether, a Highly Efficacious Nonester Pyrethroid Insecticide and Acaricide

Synthesis and Enzymatic Resolution

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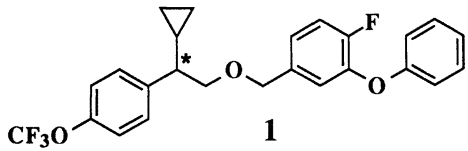
(-)-(4-Fluoro-3-phenoxy)methyl 2-cyclopropyl-2-(4-trifluoromethoxyphenyl)ethyl ether **1** is a highly efficacious insecticide/acaricide discovered in our recent work on non-ester pyrethroids. It is equivalent to bifenthrin, cyfluthrin and deltamethrin in insecticidal potency while offering significantly improved acaricidal activity relative to these commercial standards. Limited field testing supported these findings. We found an efficient enzymatic method for resolving the alcohol precursor to **1**. We also identified a one-step reaction for quantitatively converting the unwanted (+)-enantiomer of the alcohol back to the alkene from which the alcohol is prepared. This recycle step effectively permits all of the racemic alcohol to be converted into the desired (-)-enantiomer.



1
(-)-enantiomer

Our previous investigations into the structure-activity relationships of non-ester pyrethroids revealed that, in general, the ether series compounds **2** were more active insecticides and acaricides than the alkanes **3**, the 4-cyano alkanes **4**, the alkenes **5**, the epoxides **6**, and the oxime ethers **7** (*1,2,3,4,5*). In addition to high intrinsic activity and low mammalian toxicity, some of these compounds demonstrated remarkably low toxicity to fish. Compounds of type **2**, **3**, and **5** contain a single asymmetric center. We previously reported a chemical resolution of **2** (X = Cl, Y = F) and found that the (-)-enantiomer was approximately twice as active as the racemic material (*1*).

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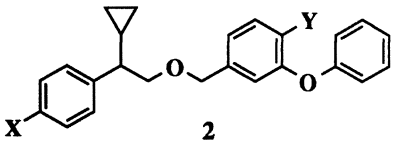

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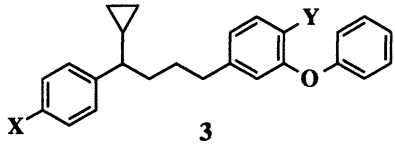

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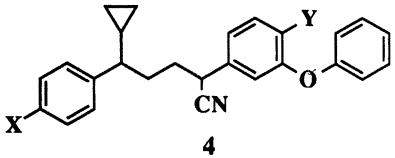

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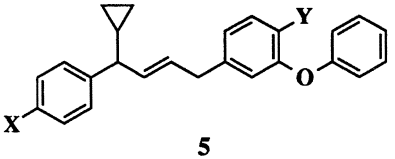

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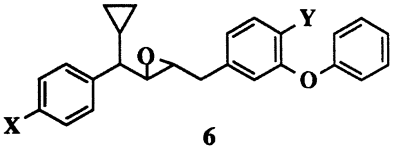

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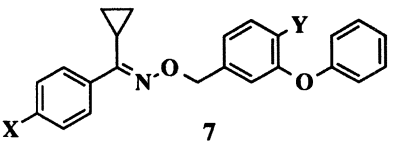


7

In most of the non-ester pyrethroid classes, substitution of CF_3O at position X increased insecticidal activity, and often gave rise to significant miticidal activity. We therefore proposed resolved **1** as an improved insecticide/acaricide. In addition, we sought to develop an improved synthesis route for preparing arylmethyl 2-cyclopropyl-2-(aryl)ethyl ethers in high enantiomeric purity.

Synthesis

Synthesis of 1 by Chemical Resolution. We initially prepared both enantiomers of **1** by chemical resolution and confirmed that the insecticidal activity resided with the (-)-enantiomer. Synthesis of the (-)-enantiomer is shown in Figure 1. It begins with 1-cyclopropyl-1-(4-trifluoromethoxyphenyl) acetic acid (**8**). Conversion of **8** to the acid chloride followed by treatment with (4S)-(-)-4-isopropyl-2-oxazolidione gave a diastereomeric mixture of amides **9** that were readily separable by silica gel chromatography. Reduction of the levorotatory diastereomer with $LiAlH_4$ in THF at 0 °C gave the resolved alcohol **10** in 24% overall yield from racemic **8**. We reacted a small sample of the alcohol with the acid chloride derived from (R)-(+)-(α-methoxy-α-trifluoromethyl)phenylacetic acid (Mosher's acid) to obtain the corresponding (α-methoxy-α-trifluoromethyl)phenylacetic ester. ^{19}F NMR analysis of the trifluoro-methyl signal showed the alcohol to have an enantiomeric excess of > 90%. Reacting the resolved alcohol **10** with 4-fluoro-3-phenoxyphenyl benzyl chloride under phase-transfer Williamson conditions gave the resolved ether in 92% yield, $[\alpha]_D$ = -13.24°. Earlier attempts at Williamson ether syntheses under homogeneous conditions (NaH, DMF) gave much lower yields of **1** (35-65%).

Synthesis of Partially-Resolved Alcohol 10 via the Ketene. Chemical resolution was suitable for preparing small amounts of **1** in high enantiomeric purity, but the low overall yield prompted us to seek a more efficient synthesis. Workers at Merck reported a method for asymmetric synthesis of 2-arylpropionic acids in high yield and enantiomeric purity (*6*). Figure 2 describes the application of this route to the synthesis of **10**. The acid **8** was dissolved in hexane, followed by slow addition of

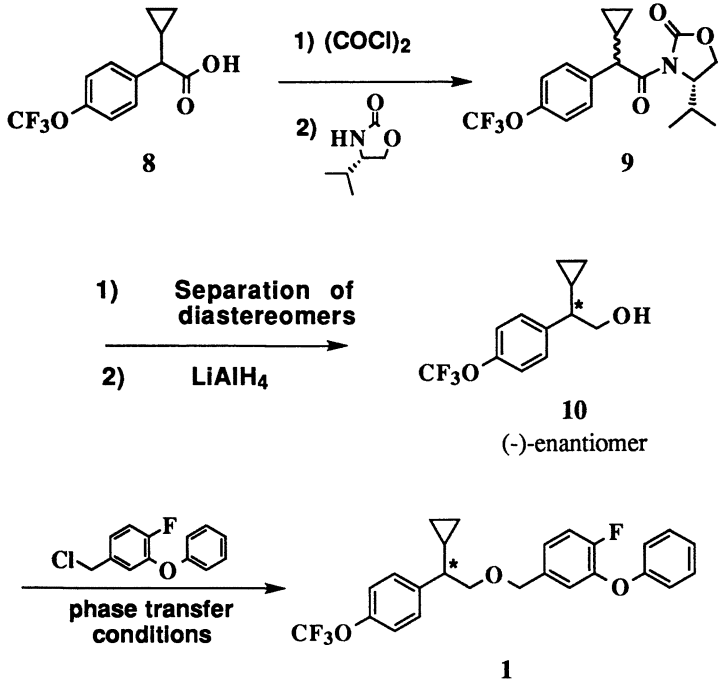


Figure 1. Synthesis of **1** by Chemical Resolution

a slight excess of thionyl chloride and a catalytic amount of DMF. We heated the reaction mixture to 55 °C for 1.25 h, cooled it to room temperature, decanted the bulk of the hexane, and then removed the residual solvent and thionyl chloride on a rotary evaporator. The acid chloride thus obtained was dissolved in dry hexane, to which we added excess *N,N*-dimethyl ethylamine slowly at room temperature. A bright yellow precipitate appeared at the end of addition of amine. The mixture stirred at room temperature for 2 h prior to cooling to -78 °C. Addition of a slight excess of isobutyl R-(+)-lactate caused the bright yellow color to disappear. The reaction mixture was allowed to warm to room temperature and stirred overnight. Workup gave the ester **11** in 95% yield. GC and NMR analysis showed a 74:26 diastereomeric ratio (48% diastereomeric excess). Treating an ether solution of **11** with $LiBH_4$ at room temperature gave the alcohol **10** in 80% yield after workup and chromatography ($[\alpha]_D$ = - 10.60 °, 36% enantiomeric excess).

We prepared the R-(-)-pantolactone derivative **12** by the procedure described above in 88% yield, as a 60:40 RR/SR ratio of diastereomers. However, attempts to reduce **12** with $LiBH_4$ gave a low yield of **10** even in refluxing THF. Reduction with $LiAlH_4$ at 0 °C in dry THF did prove to be successful, yielding the alcohol **10** in 88% yield after workup and chromatography ($[\alpha]_D$ = - 6.12 °, 22% enantiomeric excess).

Asymmetric Hydroboration. We next investigated asymmetric hydroboration as a method for converting the olefin **13** to the alcohol **10**. This reaction is depicted in Figure 3. A Schlenk tube was charged with 0.01 mmol $[Rh(COD)Cl]_2$ (COD =

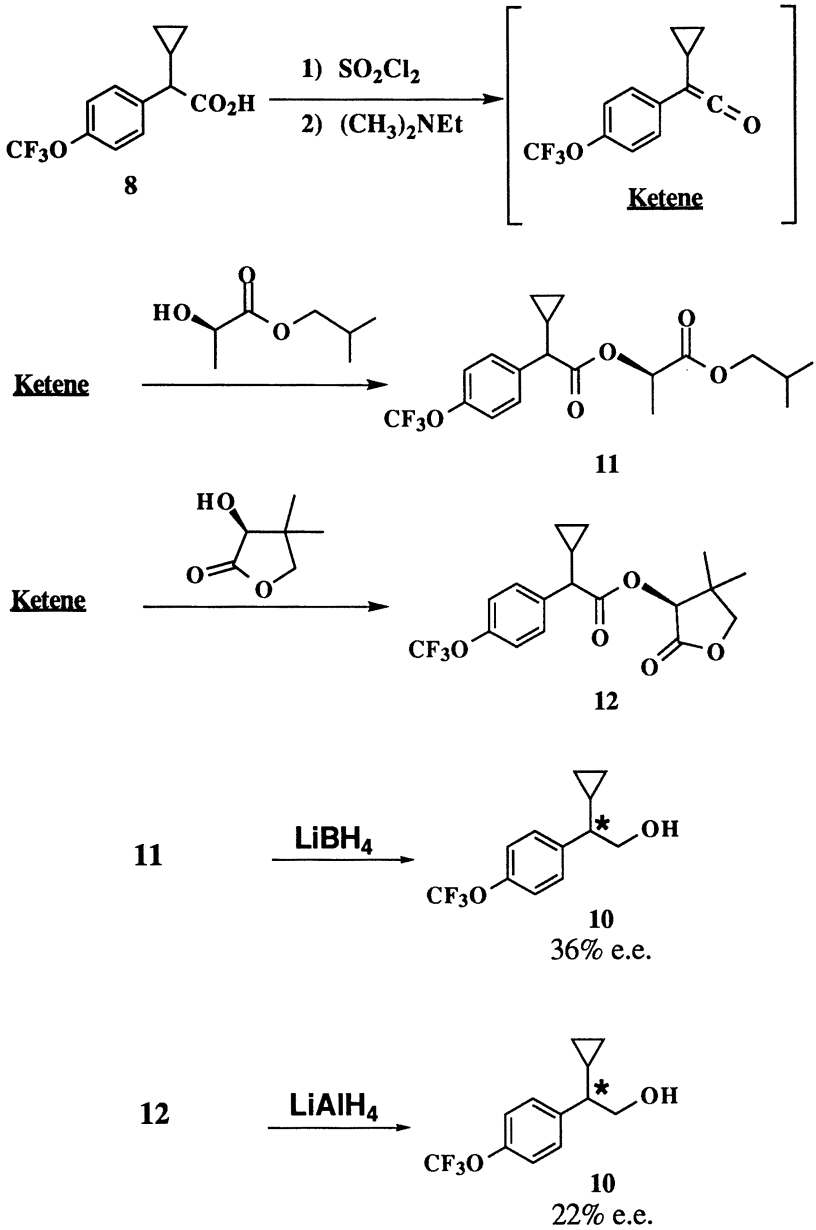
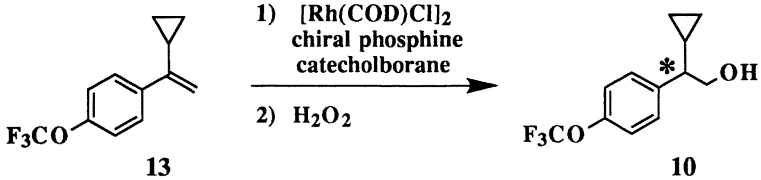


Figure 2. Synthesis of Partially-Resolved Alcohol **10** via the Ketene

cyclooctadiene) and 0.02 mmol of a chiral phosphine. We evacuated the tube and flushed it with argon prior to adding 2 mL freshly distilled anhydrous THF, followed by 1 mmol of olefin **13**. After stirring the contents for 10 min, we cooled the tube to -78 °C. Addition of 0.2 mL neat catecholborane followed, after which we warmed the reaction to 5 °C and monitored the reaction periodically by GC and TLC. When the reaction was complete we cooled the tube to 0 °C and quenched it by sequential addition of 1 mL ethanol, 1.5 mL pH 7.0 phosphate buffer (0.2 M) and 1.0 mL 30% H_2O_2. The reaction stirred for 16 h at 25 °C before extraction with diethyl ether and washing with 1 M NaOH solution and saturated NaCl solution. Drying and removal of solvent under vacuum yielded the crude product. GC analysis showed several products in all cases.



13 **10**

Figure 3. Asymmetric Hydroboration of the Olefin **13**

Enzymatic Resolution of the Alcohol (±) 10. Lipase enzymes are known to have utility in the biocatalytic resolution of alcohols (*7*). We investigated the ability of several commercially-available enzymes or crude enzyme preparations to catalyze the transformation shown in Figure 4. Reactions were generally carried out by mixing the lipase, racemic alcohol **10**, molecular sieves and vinyl acetate in hexane or heptane at room temperature. In a typical procedure, we mixed 0.5 g ground, activated molecular sieves, 0.5 g (2 mmol) (±)-**10**, 0.5 g type II crude porcine pancreatic lipase (SIGMA) and 0.68 g (8 mmol) distilled vinyl acetate in 40 ml hexane. The mixture was stirred in an open container, monitoring the course of the reaction by GC. After approximately 3 h, when 55% of the alcohol had been converted to acetate **14**, we worked up the reaction. Work-up consisted of filtration to remove molecular sieves followed by rotary evaporation to remove solvent and vinyl acetate. Chromatography on a short silica gel column readily separated the alcohol (-)-**10** from the acetate (+)-**14**. We routinely obtained isolated yields of 30-39% (-)-**10** in >95% enantiomeric excess, as determined by ^{19}F NMR analysis of the trifluoromethyl signal of the Mosher's derivative described previously.

Substituting isopropyl acetate for vinyl acetate in the reaction described in Figure 4 gave (-)-**10** in comparable yield and enantiomeric purity. However, the reaction was significantly slower, requiring 20 h at a temperature of 35-40 °C to achieve 55% conversion of alcohol to acetate.

Enzyme Recycling Experiments. We conducted a series of experiments to determine the extent to which the lipase and molecular sieve mixture used to generate (-)-**10** could be recycled for subsequent reactions. The first pass of the reaction ran as described in the preceding section. The mixture of porcine pancreatic lipase and crushed molecular sieves that had been removed by filtration was dried under vacuum at 40 °C for 40 min to remove residual solvent, vinyl acetate, and acetaldehyde. We then resuspended the lipase/sieve mixture in hexane and added a fresh charge of racemic alcohol and vinyl acetate. The reaction stirred at room temperature; periodically we withdrew aliquots for GC analysis. Workup and reactivation of the lipase/sieve mixture was done in the manner described in the preceding section for each successive cycle.

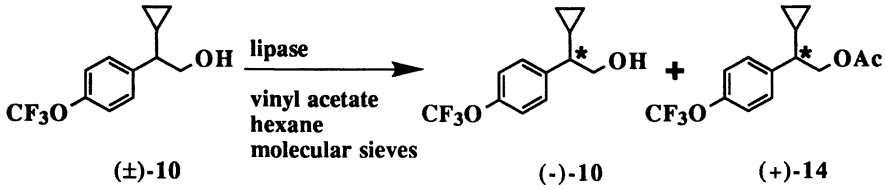


Figure 4. Enzymatic Resolution of the Alcohol (±)-**10**.

Recycling the Unwanted (+)-Acetate 14. Figure 5 describes an efficient method for recycling the unwanted (+)-enantiomer isolated from the reaction shown in Figure 4 above. Adding the acetate (+)-**14** to a suspension of 1 eq. NaH stirring in DMF at room temperature gave quantitative conversion to the alkene **13** in 2 h. The reaction could be monitored by periodically removing a small aliquot, quenching, and analyzing by GC. Dissolving the isolated olefin **13** in anhydrous THF and treating with BH_3-THF at 0 °C, followed by the standard oxidative workup with methanol, aqueous NaOH and hydrogen peroxide, gave the racemic alcohol **10** in 95% yield.

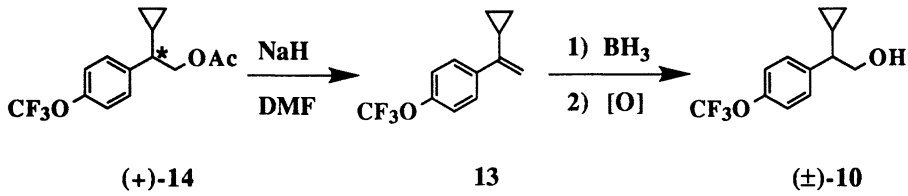


Figure 5. Recycling the Unwanted (+)-Acetate **14**.

Biological Testing

Foliar Evaluation. We screened the compounds for insecticidal and acaricidal activity against the following species: cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), Mexican bean beetle (*Epilachna varivestis*), and twospotted spider mite (*Tetranychus urticae*).

To determine the activity against cabbage looper, tobacco budworm, and Mexican bean beetle, we sprayed the upper and lower surfaces of the leaves of pinto bean plants with test solution until run-off. Second instar larvae (ten larvae for each of two replicates for each compound) infested the plants after the foliage had dried. Appropriate dilution from a stock solution of the experimental compound in 10% acetone/water gave the test solutions.

We also determined the activity against mites on pinto bean plants. We pre-infested the bean leaves with adult mites (about 75 mites for each of two replicates for each compound), then sprayed until run-off with test solution.

To prevent escape of the insects from the test site, capped cups or other appropriate containers contained the treated plant or excised leaves. Experimental protocol required holding the tests at 25°C and 50% relative humidity for an

exposure period of 48 hours. At the end of this time, we determined percent mortality and used probit analysis to determine LC_{50} values.

Foliar Evaluation for Residual Activity. We determined efficacy in residual testing by spraying the test plants to run-off with aqueous dilutions of the compounds. The treated plants were held under greenhouse conditions for the appropriate time before infestation with insects. The test was then completed as described for the initial evaluations.

Field Testing for Efficacy Against Mites. We conducted field testing against twospotted spider mites on cotton in Visalia, California. The test began when population levels reached an average of five mites per leaf. We applied **1** or a tank mix of propargite and dimethoate and recorded mite mortality through 28 days. The mite population in the untreated check increased throughout the trial. Our tests on European red mites were conducted on apples in New York. We initiated the trial when the average population levels reached six eggs and four motiles per leaf. Compound **1** or propargite was applied and the population was monitored through 48 days.

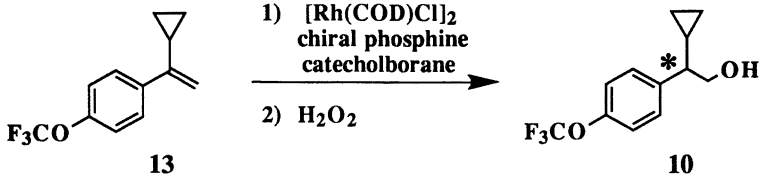
Field Testing for Efficacy Against *Heliothis*. A single trial on cotton in the Brazos Valley of Texas provided field data on both *H. virescens* and *H. zea*. Early in the season, the *Heliothis* population was > 90% *H. zea*. We compared the efficacy of foliarly-applied **1**, bifenthrin, and cypermethrin. Late in the season, the *Heliothis* population was > 90% *H. virescens* and we compared **1** to cypermethrin.

Results and Discussion

Asymmetric Hydroboration. Our efforts to prepare (-)-**10** in high yield and enantiomeric purity by means of asymmetric hydroboration were unsuccessful. Table I summarized our results with four chiral phosphines. (R,R)-DIOP (DIOP = 2,3-*O*-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane) was the most successful, giving a complete conversion of alkene **13** to products. However, only a 26% yield of alcohol **10** was obtained, and this in only 20% enantiomeric excess. (S,S)-BDPP (BDPP = 2,4-bis(diphenylphosphino)pentane) gave a slightly lower conversion of **13** and yield of **10**. (R,R)-PAMPOP (PAMPOP = 2,3-*O*-isopropylidene-2,3-dihydroxy-1,4-bis(di(3-methoxyphenyl)phosphino)-butane) and (R)-BINAP (BINAP = 2,2'-bis(diphenylphosphino)-1,1'-binapthyl) demonstrated little utility in this reaction, as both gave a conversion of 10% or less. Based on these results, we concluded that asymmetric hydroboration was unlikely to produce the alcohol **10** in sufficient yield and enantiomeric purity.

Synthesis of Alcohol 10 via the Ketene. Addition of α-hydroxy esters to ketenes derived from 2-arylpropionic acids has been shown to be an effective method of synthesizing 2-arylpropionate esters in 94-99% diastereomeric excess (*6*). The method proved to be less satisfactory when applied to the synthesis of **3**. The highest diastereomeric excess we were able to obtain was 48%, when (R)-isobutyl lactate was employed as the source of chirality (see Figure 2). The diastereomeric excess was only 20% when (R)-pantolactone was used. The reason for this reduced diastereomeric excess in our work may well be attributable to the comparative sizes of the groups attached to the ketene functionality. The method works well for aryl methyl ketenes, while our application required aryl cyclopropyl ketenes, in which the two groups are much closer to each other in size, making diastereofacial

Table I. Results of Asymmetric Hydroboration Experiments



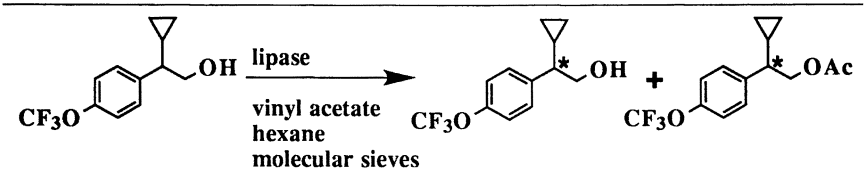
13 10

Phosphine	% Conversion	% Yield	% Enantiomeric Excess
(R,R)-DIOP	100	26	20
(S,S)-BDPP	95	23	--
(R,R)-PAMPOP	10	--	--
(R)-BINAP	2	--	--

selectivity by the approaching α-hydroxy esters much more difficult. We required a higher degree of stereoselectivity for our work, and so did not pursue this synthetic strategy further.

Enzymatic Resolution of Alcohol 10. Table II describes the results of our enzyme screening experiments using four commercially-available lipases to biocatalytically resolve alcohol (±)-**10**. All of the enzymes gave good conversion of alcohol to acetate, easily reaching or approaching the target conversion level of 55%. Resolved or partially-resolved **10** was isolated in each case. Porcine pancreatic lipase showed the greatest enantiomeric selectivity. The alcohol isolated from this reaction had an enantiomeric excess of > 95%. The unwanted (+)-isomer could not

Table II. Results of Enzyme Screening Experiments



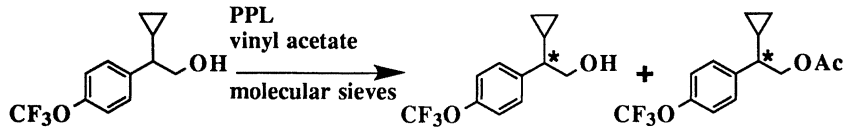
(±)-10 (-)-10 (+)-14

Lipase	Conversion %	Alcohol Yield, %	Alcohol Enantiomeric Excess, %
Porcine Pancreatic	59	21	> 95
Pseudomonas AK	62	26	75
Pseudomonas K-10	56	34	73
Candida cylindracea	48	23	40

be detected by ^{19}F NMR analysis of the Mosher's derivative. The two *Pseudomonas* lipases gave moderate resolution of the alcohol, while *Candida cylindracea* lipase was only moderately selective. Porcine pancreatic lipase was used for all subsequent experiments. While we routinely used vinyl acetate in these reactions, we demonstrated that isopropenyl acetate could also be used without loss of yield or enantioselectivity. Isopropenyl acetate offers the advantage of generating acetone as a by-product of the reaction, rather than the more-toxic acetaldehyde which is generated when vinyl acetate is used.

Enzyme Recycle Experiments. Although molecular sieves and type II crude porcine pancreatic lipase (PPL) are quite inexpensive (the enzyme preparation is commercially available for $41.20/500 g at this writing), it is desirable to recycle both as larger-scale syntheses are carried out. The sieve/PPL mixture is easily recovered by filtration at the end of each resolution reaction. We heated the mixture to 40 °C *in vacuo* for 40 min to remove solvent prior to each successive pass. PPL reportedly retains activity at temperatures up to 60 °C (*8*). The results of these experiments, reproduced in Table III, indicate that the mixture may indeed by recycled, but the catalytic activity falls off with each successive pass. A fresh mixture of sieves and PPL converted the racemic alcohol to the target conversion level of 56% in only three hours, but after three passes 24 h were required to reach the same level of conversion. The fourth-pass reaction was terminated after 24 hours, the conversion of (±)-**10** to (+)-**14** having only reached 36% at that time. However, these experiments demonstrate that the sieve/PPL mixture may be reused to some extent, and we stress that no attempts were made to optimize the conditions for regenerating the sieve/PPL mixture between successive passes of the reaction.

Table III. Results of Enzyme Recycling Experiments



(±)-**10** (-)-**10** (+)-**14**

Pass Number	Conversion to Acetate (**14**) (%)	Time Required (h)
1	55	3.0
2	52	7.5
3	56	24
4	36	24

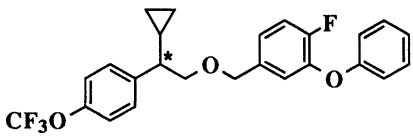
Recycling the Unwanted (+)-Acetate 14. Our work has shown enzymatic resolution to be a very simple method for preparing (-)-**10** in very high enantiomeric purity. However, in the experimental protocols already described we typically isolate (-)-**10** in 35-39% yield, the remainder of the material being the acetate (+)-**14**. Thus, we could not consider this route an economical synthesis of (-)-**10** unless an efficient method for recycling the unwanted (+)-acetate was available. We had previously noted that the alcohol (±)-**10** readily eliminated to give the

alkene **13** under conditions used for a homogeneous Williamson ether synthesis. This elimination was easily avoided by employing phase transfer conditions for the Williamson synthesis. Fortunately, the acetate (+)-**14** also eliminates rapidly under these conditions (NaH/DMF) to give **13** in quantitative yield (see Figure 5). The alkene **13** is the immediate precursor in our synthesis of the alcohol (±)-**10.** Hydroboration of **13** gives (±)-**10** in 95% yield, thereby completing the recycle of the unwanted (+)-enantiomer with very little loss of material.

We also considered pyrolysis of the acetate (+)-**14** as a potentially superior method of generating **13**. Pyrolysis of acetates to olefins are generally fast, clean, and high yielding, and so we attempted both flash vacuum pyrolysis and ambient pressure pyrolysis (N_2 atmosphere) of (+)-**14** at 250 °C, the operating limit of the equipment we had available. In both cases, pure (+)-**14** was recovered. We did no further investigation into the utility of pyrolysis for this transformation, but it should be noted that with rare exceptions, such pyrolyses require temperatures of 300-555 °C (*9*).

Biological Activity of 1. Compound **1** proved to be equivalent or superior to all pyrethroid commercial standards used in our side-by-side tests. Table IV shows that **1**, bifenthrin (Capture® insecticide/miticide), cyfluthrin (Baythroid® insecticide), and deltamethrin (Decis® insecticide) are equivalent in foliar activity against cabbage looper. Compound **1** is essentially equivalent to bifenthrin and cyfluthrin, and 5X deltamethrin, against Mexican bean beetle. Compound **1** is equivalent to cyfluthrin and deltamethrin, and 5X bifenthrin, against tobacco budworm. Compound **1** is superior, by a factor of at least 7X, to all of these standards when tested against twospotted spider mite.

Table IV. Insecticidal Activity of **1** Compared to Commercial Standards



Foliar LC_{50} Values in ppm

Compound	Cabbage Looper	Mexican Bean Beetle	Tobacco Budworm	Twospotted Spider Mite
1	0.2	0.2	0.4	0.9
bifenthrin	0.2	0.1	2.0	6.0
cyfluthrin	0.2	0.2	0.4	20
deltamethrin	0.3	1.0	0.6	> 30

Results of Field Testing 1 on Mites. Compound **1** has been shown to have good activity and spectrum in field testing against mites. Results of two field trials are shown in Table V. In both cases, **1** was substantially better (50X or more) than the commercial standard propargite (Omite® miticide, Comite® miticide), even when propargite was mixed with dimethoate (Cygon® insecticide/miticide) for testing on twospotted spider mites.

Table V. Field Test Data on Mites

- Twospotted Spider Mites on Cotton
  Compound **1** at 0.03 lbs/acre was equivalent to a mix of propargite at 1.5 lbs/acre plus dimethoate at 0.33 lbs/acre (through 28 days).
- European Red Mites on Apples
  Compound **1** at 0.02 lbs/acre was equivalent to propargite at 1.5 lbs/acre (> 90% control through 35 days).

Results of Field Testing 1 on *Heliothis* spp. Compound **1** has also been shown to have good activity in field testing against *Heliothis* spp. Results of two field trials are shown in Table VI. Compound **1** was approximately 2X-3X cypermethrin (Cymbush® insecticide or Ammo® insecticide) or bifenthrin (Capture® insecticide/miticide) against *Heliothis zea* on cotton, and was at least 3X cypermethrin against *Heliothis virescens*.

Table VI. Field Test Data on *Heliothis* spp.

- *Heliothis zea* on Cotton
  Compound **1** at 0.03 lbs/acre was equivalent to cypermethrin or bifenthrin at 0.08 lbs/acre.
- *Heliothis virescens* on Cotton
  Compound **1** at 0.02 lbs/acre was superior to cypermethrin at 0.06 lbs/acre.

Conclusions

The non-ester pyrethroid **1** has proven to be a broad spectrum insecticide and miticide with activity equal to or superior to the commercial standards cyfluthrin, deltamethrin, bifenthrin, propargite and dimethoate. The miticidal activity of **1** is particularly high for a pyrethroid, and in fact appears to be greater than that reported for any ester pyrethroid.

We have developed an efficient route for synthesizing (-)-**1** in high yield and high enantiomeric purity through enzymatic resolution of the racemic alcohol precursor (±)-**10**. The enzymatic resolution is fast, easy to carry out, and requires only relatively inexpensive reagents and enzyme. The unwanted (+)-enantiomer may be recycled in quantitative yield in a single step to the alkene **13**. This alkene is the precursor from which (±)-**10** is prepared in 95% yield through hydroboration and oxidation. This process allows the unwanted (+)-enantiomer to be recycled with very little loss of material. Our previous attempts at resolving (±)-**10** by chemical means required four synthetic steps over four days and yielded 13% (-)-**10** with an enantiomeric excess of 90-94%. The enzymatic resolution of (±)-**10** requires a single step, one-half day, and yields 39% (-)-**10** with an enantiomeric excess of >95%. Recycling the (+)-enantiomer provides an easy method to further increase the net yield of (-)-product.

Acknowledgments

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Chapter 24

Insecticidal Dibenzooxocinopyrazoline Carboxamides

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The novel dibenzooxocinopyrazoline carboxamides are conformationally restricted analogs related to the Philips-Duphar pyrazoline PH 60-42. The dibenzooxocinopyrazoline ring system was accessed via an intramolecular nitrile imine cycloaddition. The molecular modeling, synthesis, and biological activity of these compounds will be presented.

The 3-arylpyrazolines (1), such as PH 60-41, were first discovered in the early 1970's, followed by the discovery of the 3,5-diarylpyrazolines (2) and the highly active 3,4-diarylpyrazolines (3), such as PH 60-42 (Figure 1). Since then, several sub-classes of these compounds have been introduced (4-6). In an effort to further explore this area of insecticide chemistry, novel, conformationally restricted analogs of the 3,4-diarylpyrazolines were desired.

Approach

X-Ray Crystal Structure Information. The X-ray crystal structures of known pyrazolines were used as a starting point for the conception of novel pyrazolines. The crystal structure of Philips Duphar pyrazoline **I** was obtained. The crystal structure of a camphanoyl substituted pyrazoline has been recently published by Schering AG (7) (Figure 2). The key features of the crystal structures include an essentially flat molecule with the 3-aryl group, the pyrazoline heterocycle, and the aryl urea moieties in the same plane. The 4-aryl group is nearly perpendicular to the rest of the molecule. Recent QSAR and molecular shape analysis suggests that the bioactive conformation of the 3-aryl pyrazoline corresponds to the nearly all planar structure (8).

Molecular Modeling. A molecular modeling approach was used to design conformationally restricted structures corresponding to the proposed bioactive conformation. The crystal structure of **I** was used to develop a three-dimensional query. The geometric constraints were defined using the SYBYL program. A three-dimensional search of the Cambridge crystal structure database was performed.

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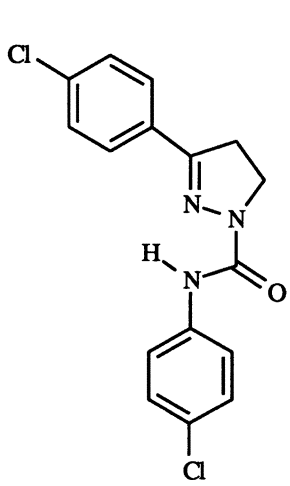
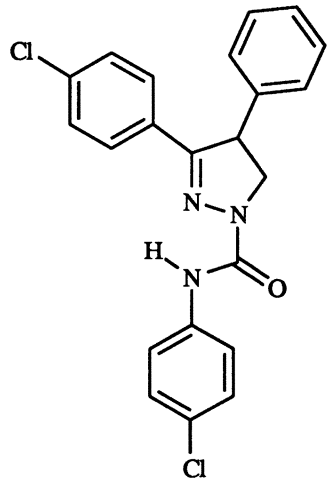
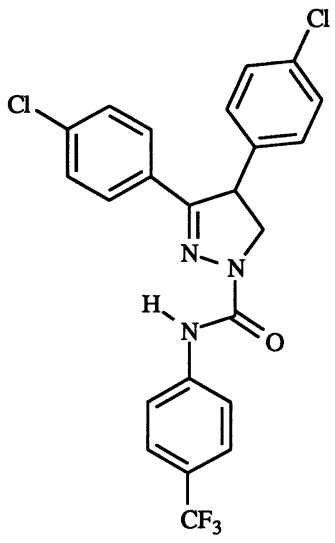


PH 60-41

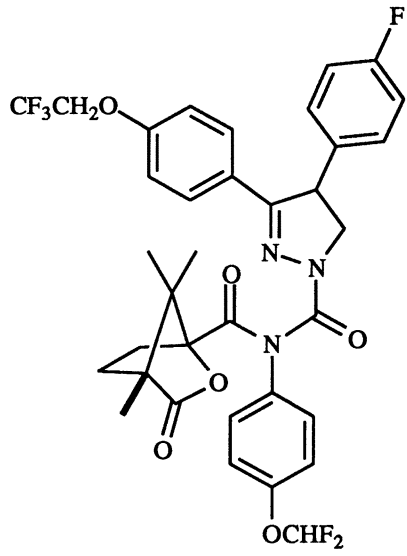


PH 60-42

Figure 1. Philips Duphar Pyrazolines.



I



Schering Pyrazoline

Figure 2. Pyrazolines With Known X-ray Crystal Structures.

Crystal structures satisfying the geometric requirements contained a twisted tub dibenzocyclooctane moiety (Figure 3). In particular, it was noted that the 3,4-diaryl groups of **I** were oriented such that the twisted tub conformation of dibenzocyclooctane (9, 10) places the aryl groups in the same region of space (Figure 4). These results guided the synthetic effort toward the conformationally restricted dibenzocyclooctapyrazoline system.

Synthesis

Dibenzocyclooctapyrazolines. The dibenzocyclooctapyrazolines were synthesized in a straight forward manner starting from dibenzocyclooctanone (11). Dibenzocyclooctanone was treated under Mannich conditions with formaldehyde, piperidine, and acetic acid in refluxing ethanol for 168 hours. The α,β-unsaturated ketone was then treated with hydrazine hydrate in refluxing ethanol to give the pyrazoline. Reaction of the pyrazoline with various aryl isocyanates in ether gave the desired products (Scheme 1). These analogs were inactive as insecticides.

Dibenzooxocinopyrazolines. Analogs with one oxygen in the ethylene bridge appeared to be synthetically attractive since these analogs could be available from 2-hydroxyphenylacetic acid and an appropriately substituted benzyl halide. In the event, 2-benzyloxyphenylacetic acid was cyclized to the ketone with polyphosphoric acid (PPA). However, attempts to form the desired α,β-unsaturated ketone under several variations of the Mannich reaction were unsuccessful (Scheme 2).

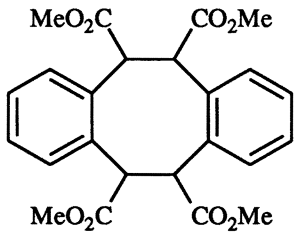
[3 + 2] Cycloaddition Approach. It was believed that the synthesis of analogs with appropriate substitution might be achieved by an intramolecular [3+2] cycloaddition of a nitrile imine and a styrene derivative. The medium sized dibenzo ring system seemed to be sufficiently rigid, due to the reduced degrees of freedom imparted by the aryl rings, to allow such a cycloaddition (Figure 5).

Treatment of 4-substituted salicylaldehydes with 2-vinylbenzyl bromide in dimethylformamide (DMF) in the presence of potassium carbonate gave the benzyloxybenzaldehydes. The 2-vinylbenzyl bromide was synthesized by Stille coupling of 2-bromobenzyl alcohol with vinyltributylstannane, followed by bromination with phosphorous tribromide. Attempts to synthesize 2-vinylbenzyl bromide by direct bromination of 2-methylstyrene were unsuccessful (Scheme 3).

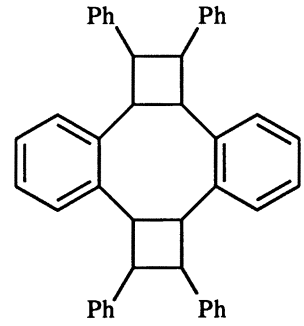
Treatment of the precursor aldehydes with diethyl phosphorohydrazidate (12) in refluxing ethanol provided the desired N-phosphoryl hydrazones. The hydrazones were treated with N-chlorosuccinimide and triethylamine in ether/dichloromethane at 0° C to induce *in situ* chlorination, nitrile imine formation, and intramolecular cycloaddition in 20-30% yield. The N-phosphoryl pyrazolines were deprotected with aqueous HCl in refluxing ethanol followed by treatment with an appropriate aryl isocyanate to give the desired products (Scheme 4). The insecticidal activity was determined against *Spodoptera frugiperda* and *Heliothis virescens* (Table I).

Encouraged by the biological activity, methods to synthesize other halogenated analogs were devised. The 3-fluorobenzaldehyde derivative was obtained by treatment of 2,3-difluorobenzaldehyde with 2-bromobenzyl alcohol in the presence of potassium carbonate in DMF at 100° C. *Bis*-halogenated analogs were obtained by bromination of 2-bromotoluene derivatives with N-bromosuccinimide (NBS) and catalytic AIBN in refluxing carbon tetrachloride, followed by benzylation of the salicylaldehydes. The aryl bromides were subjected to Stille coupling with vinyltributylstannane and a catalytic amount of $Pd(PPh_3)_4$ in refluxing toluene. It was found that performing the Stille coupling at this later stage was more practical (Scheme 5).

The desired pyrazolines were synthesized via the [3 + 2] nitrile imine cycloaddition, deprotected, and capped with the appropriate aryl isocyanate as described previously (Scheme 6). The insecticidal activity was determined against *Spodoptera frugiperda* and *Heliothis virescens* (Table II).



BINWIE
(twisted tub)



DSTYBZ
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Figure 3. Cambridge Crystal Structure Database.

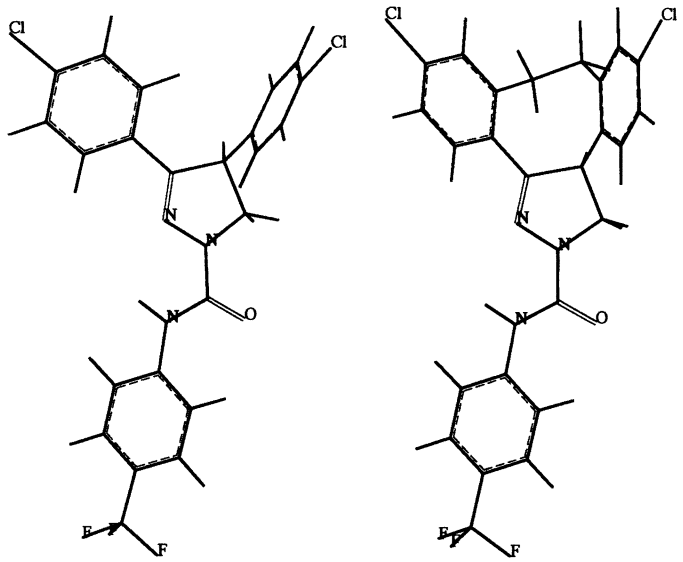
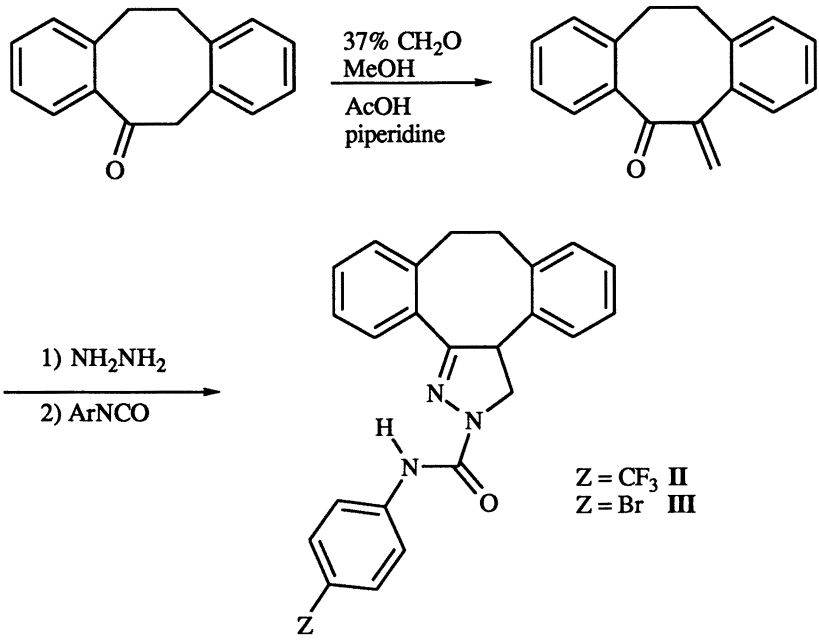
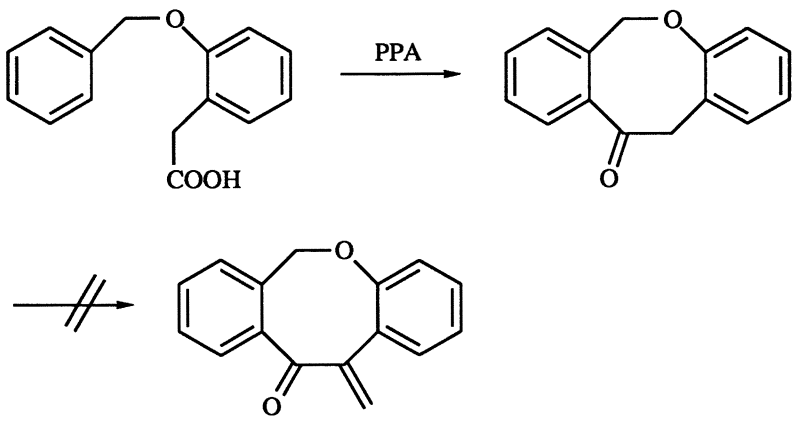


Figure 4. Modeling Comparison of I and Dibenzocyclooctapyrazoline.



Scheme 1. Synthesis of Dibenzocyclooctapyrazolines.



Scheme 2. Attempted Synthesis of Oxygen Substituted Analogs.

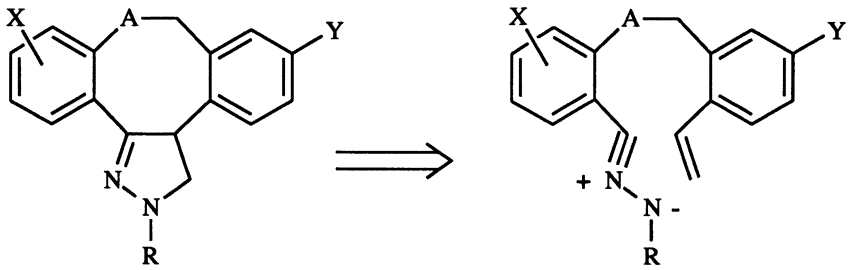
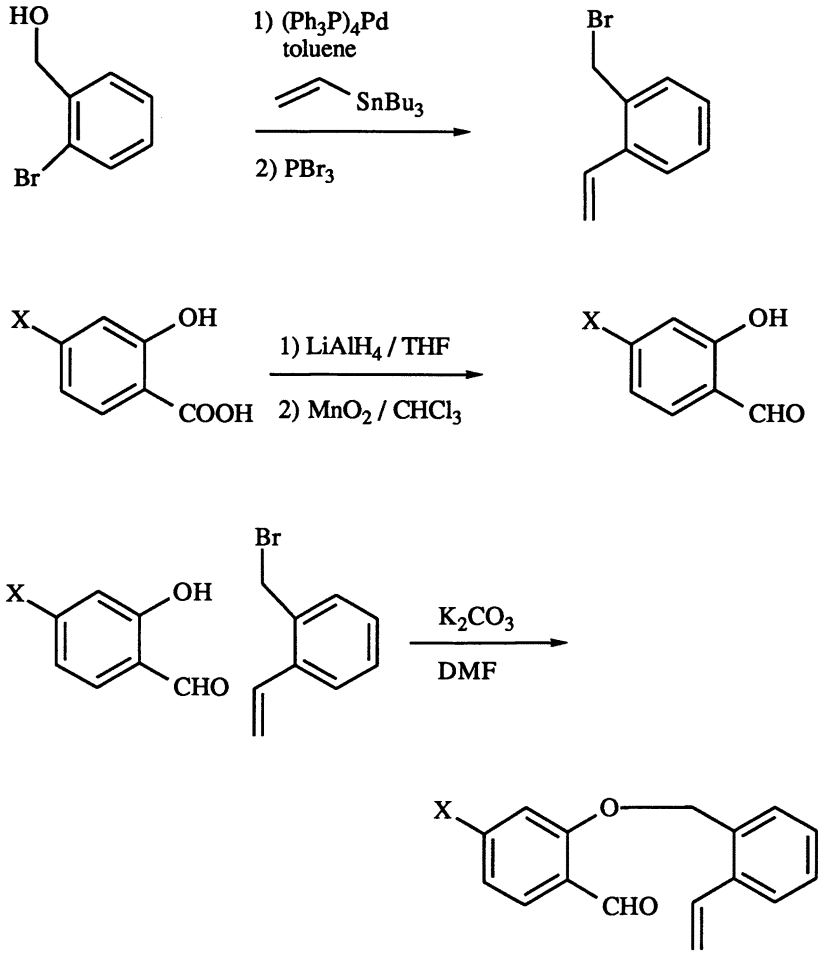
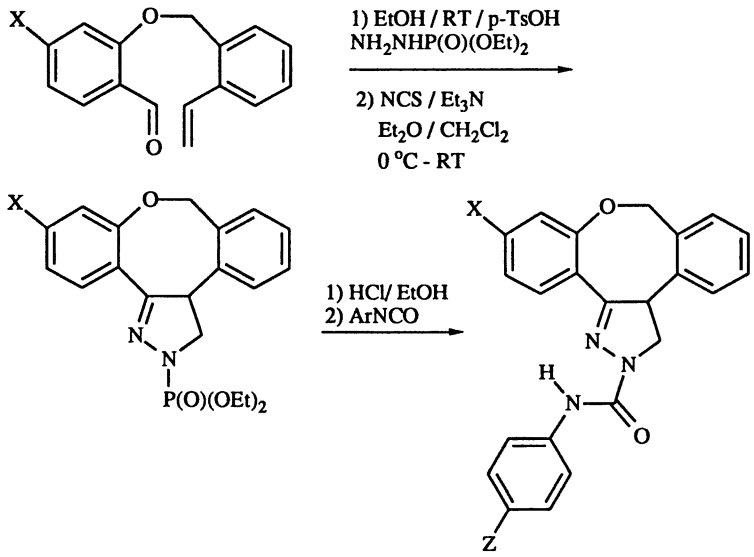


Figure 5. Proposed [3+2] Cycloaddition.

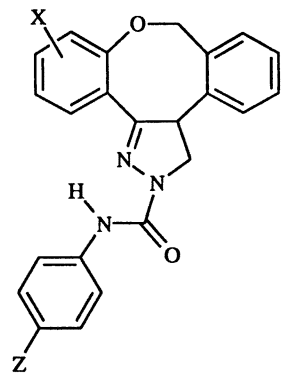


Scheme 3. Synthesis of Cycloaddition Precursors.



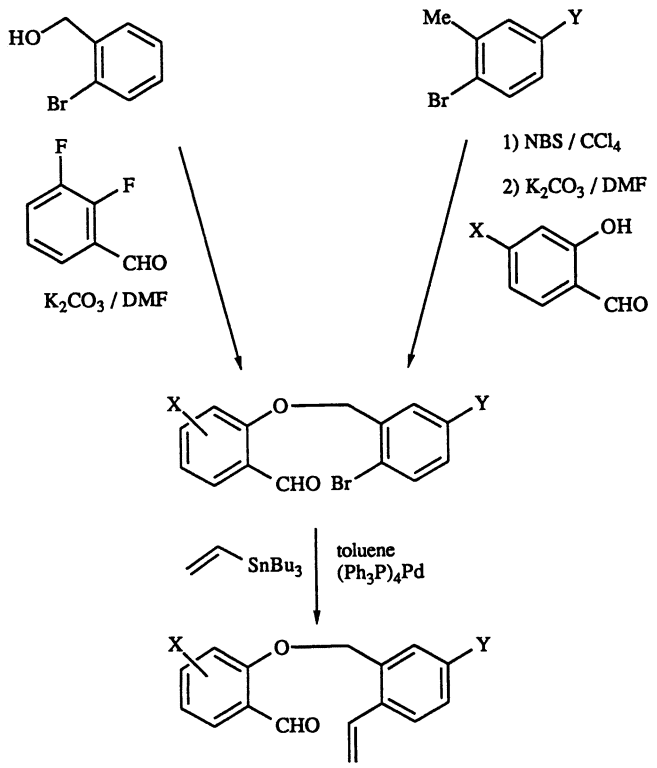
Scheme 4. Synthesis of Mono-halogenated Analogs.

Table I: Insecticidal Activity of Mono-halo Dibenzooxocinopyrazolines

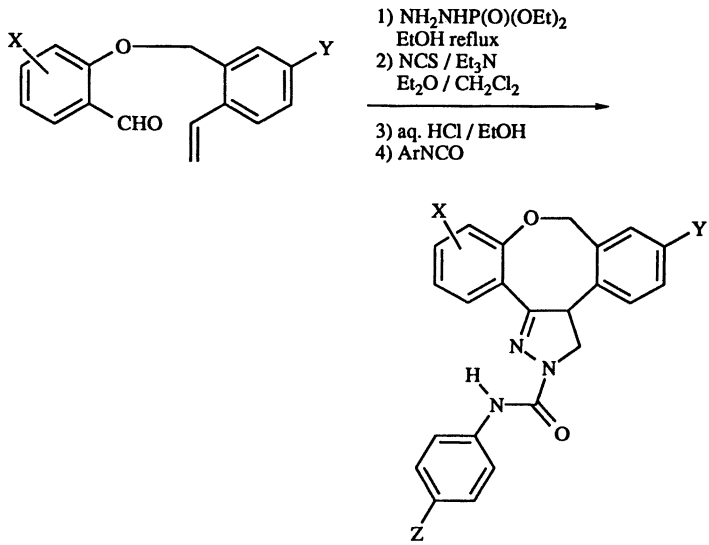


				% Mortality (48 hours)	
entry	X	Z	rate (ppm)	FAW	TBW
IV	Cl	CF_3	50	100	93
			10	100	27
V	CF_3	OCF_3	50	42	20
			10	0	20
VI	CF_3	CF_3	50	58	100
			10	0	67
VII	F	CF_3	50	8	---
			10	0	---

FAW: Fall Armyworm (*Spodoptera frugiperda*)
TBW: Tobacco Budworm (*Heliothis virescens*)

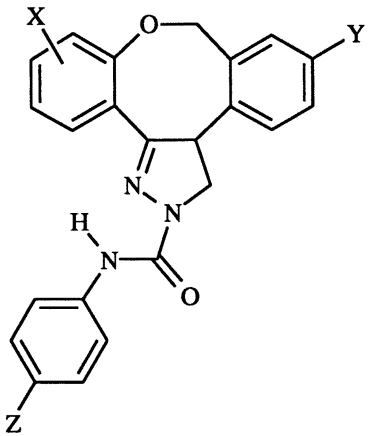


Scheme 5. Synthesis of *Bis*-halogenated Cycloaddition Precursors.



Scheme 6. Synthesis of *Bis*-halogenated Analogs.

Table II: Insecticidal Activity of Bis-halo Dibenzooxocinopyrazolines



					% Mortality (48 hours)	
entry	X	Y	Z	rate (ppm)	FAW	TBW
VIII	Cl	Cl	CF_3	50	100	87
				10	83	20
IX	Cl	F	CF_3	50	100	100
				10	100	93
X	Cl	F	Br	50	100	93
				10	42	40
XI	Cl	F	OCF_3	50	100	87
				10	83	60
XII	3-F	H	CF_3	50	100	---
				10	17	---
XIII	CF_3	F	CF_3	50	100	100
				10	75	33
XIV	F	F	CF_3	50	100	40
				10	50	47

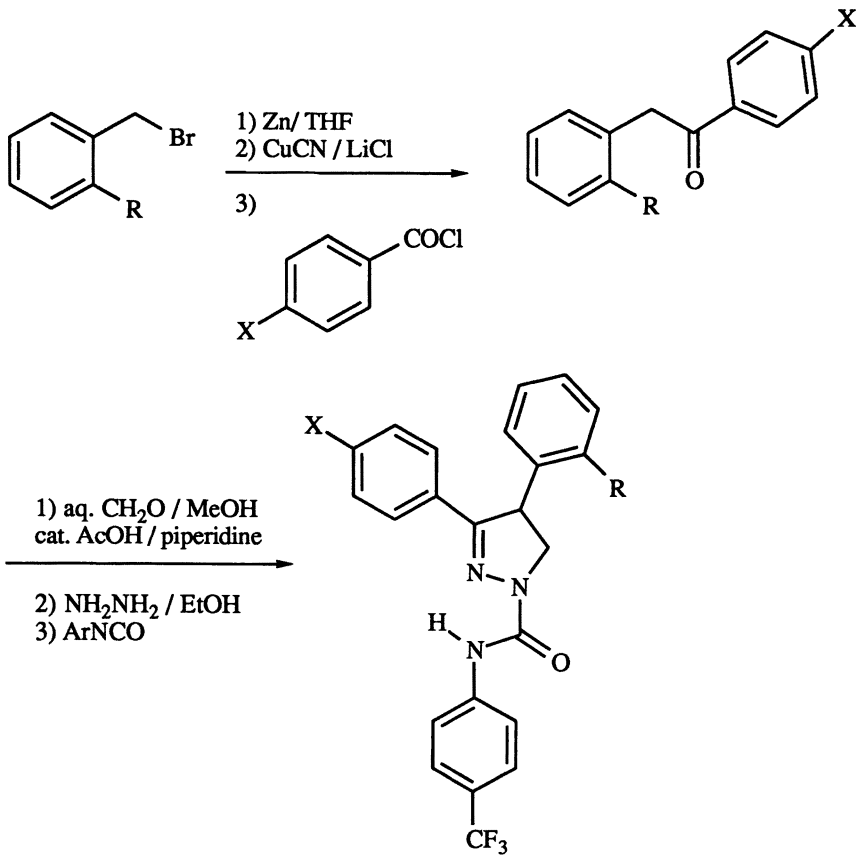
FAW: Fall Armyworm (*Spodoptera frugiperda*)
TBW: Tobacco Budworm (*Heliothis virescens*)

Effect of 4-aryl *Ortho*-substituent

The *ortho*-alkyl substituent of the 4-aryl ring may be a contributing factor to the reduced insecticidal activity of the dibenzooxocinopyrazolines. This hypothesis was tested by preparing simple 4-*o*-alkylaryl pyrazolines. In this case the required 2-aryl acetophenone derivatives were readily synthesized by reaction of the mixed zinc-cuprate with a substituted benzoyl chloride derivative (13). The 2-aryl acetophenone derivatives were then manipulated into the pyrazolines in the usual manner (Scheme 7). The insecticidal activity was determined against *Spodoptera frugiperda* and *Heliothis virescens*. As noted in Table III, the 4-*ortho*-substituted aryl pyrazolines are significantly less active than the 4-phenyl analog **XVIII**.

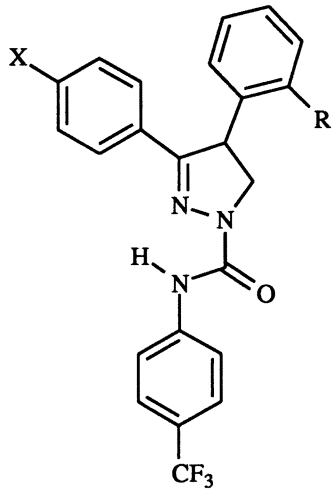
Conclusions

The insecticidal activity of the dibenzooxocinopyrazolines may be governed by factors such as conformational preferences and steric constraints. As noted in the 3, 4-diarylpyrazolines, the *ortho*-substituent of the 4-aryl ring appears to have an adverse effect on the insecticidal activity. However, in the case of the conformationally restricted dibenzooxocinopyrazolines, the effect of the *ortho*-substituted 4-aryl ring is not as detrimental to the insecticidal activity.



Scheme 7. Synthesis of 4-*o*-Substituted Aryl Pyrazolines.

Table III: Insecticidal Activity of 4-*o*-Substituted Aryl Pyrazolines



				% Mortality (48 hours)	
entry	X	R	rate (ppm)	FAW	TBW
XV	F	Me	50	100	---
			10	58	---
XVI	Cl	Me	50	100	---
			10	67	---
XVII	Cl	CF_3	50	0	---
			10	0	---
XVIII	Cl	H	50	100	100
			10	100	100

FAW: Fall Armyworm (*Spodoptera frugiperda*)
TBW: Tobacco Budworm (*Heliothis virescens*)

Acknowledgments

George Lahm for the preparation of **I** and **XVIII**. Joseph Calabrese (CR&D) for the X-ray crystal structure determination of **I**. Bonita Morrison for technical assistance.

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Chapter 25

1,5-Diphenylpyrazoline-3-carboxanilides

A New Class of Insecticides

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DuPont Agricultural Products, Stine-Haskell Research Center, Chemical Discovery, Levitt Laboratories, Newark, DE 19714

1,5-Diphenylpyrazoline-3-carboxanilides represent a new class of pyrazoline insecticides. These compounds differ from traditional pyrazoline insecticides by having the carboxanilide linkage bound to carbon rather than nitrogen. We have developed several synthetic methods for this class. For the main synthetic route to this class we have unambiguously determined the regiochemistry of cycloaddition of ethoxycarbonyl-nitrile-imines with styrenes. Heterocyclic containing pyrazolines were made by an alternative method through benzylidene pyruvanilides. Our synthetic methods as well as the insecticidal activity will be discussed.

In the early 1970's chemists at Phillips Duphar found that 3-phenylpyrazoline carboxanilides (Figure 1) were potent insecticides (1). These compounds had very high levels of activity towards coleopteran and lepidopteran insects. Insects from other orders suffered some effects from them, but recovered quickly (2). Unlike the benzoylureas (such as DIMILIN™) which Phillips Duphar had also discovered, these compounds did not inhibit the biosynthesis of chitin but appeared to be neurotoxins. Subsequently, they discovered that adding a phenyl group to the 5-position of the pyrazoline increased the activity towards lepidopteran, but not coleopteran pests (3). Moving the phenyl group to the 4-position proved optimal and high activity was seen against both lepidopteran and coleopteran pests (4). It is this class, the 3,4-diphenylpyrazoline carboxanilides, that has been the focus of research at many companies since the late 1970's. More than 40 patents have been filed to pyrazoline carboxanilides and related compounds.

The pyrazolines described above are synthesized by reaction of Mannich base derived unsaturated aryl ketones with hydrazine. The unstable pyrazolines are then capped by reaction with aryl isocyanates. A typical reaction sequence for the synthesis of the Phillips-Duphar development compound PH 60-42 is shown in Figure 2.

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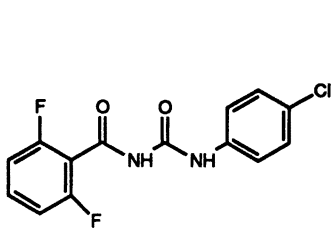
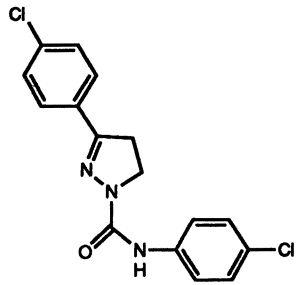
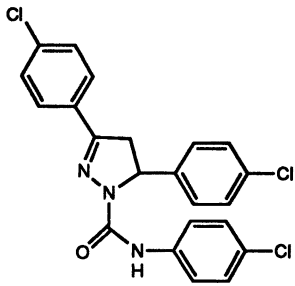


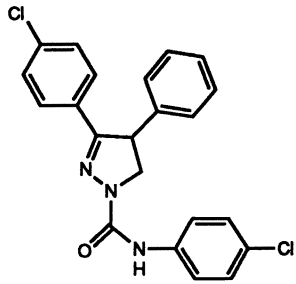
Duphar 1970
Insect Growth Regulants



DuPhar 1972
PH60-40



DuPhar 1973



DuPhar 1974
PH60-42

Figure 1. Evolution of pyrazoline insecticides.

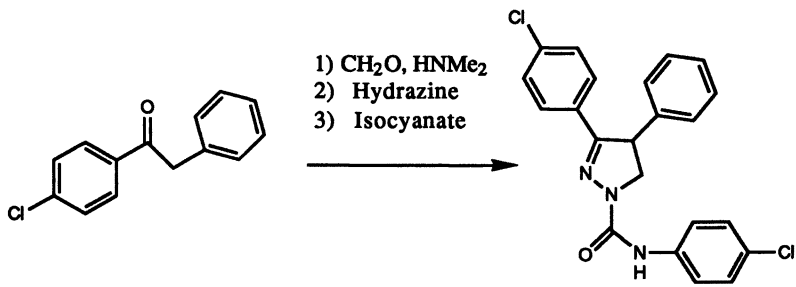


Figure 2. Synthesis of 3,4-diarylpyrazoline-1-carboxanilides.

Synthesis

We wanted to see if we could find a fundamentally different approach to the synthesis of pyrazolines incorporating dipolar cycloaddition chemistry. Our initial idea was to make a nitrile-imine that contained a N-substituted carboxanilide or a carboxanilide precursor. However, we found that such nitrile-imines do not undergo cycloaddition, but instead do a 1,5-electrocyclization (5) to oxadiazoles as shown in Figure 3.

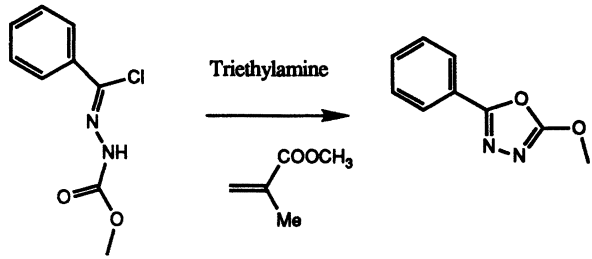


Figure 3. Electrocyclization of nitrile-imines.

Nitrile-Imine Cycloadditions. In our literature search on nitrile-imines we found that unlike N-substituted ester nitrile-imines, C-substituted ester nitrile-imines did undergo cycloaddition chemistry. Huisgen and subsequent workers showed that C-ethoxycarbonyl hydrazonyl chlorides were stable precursors to nitrile-imines and would react with a variety of alkenes, including styrene (6-8). Converting the ester to a carboxanilide function would result in compounds which resemble the Phillips Duphar pyrazolines, but have the carboxanilide function attached to carbon instead of nitrogen (Figure 4).

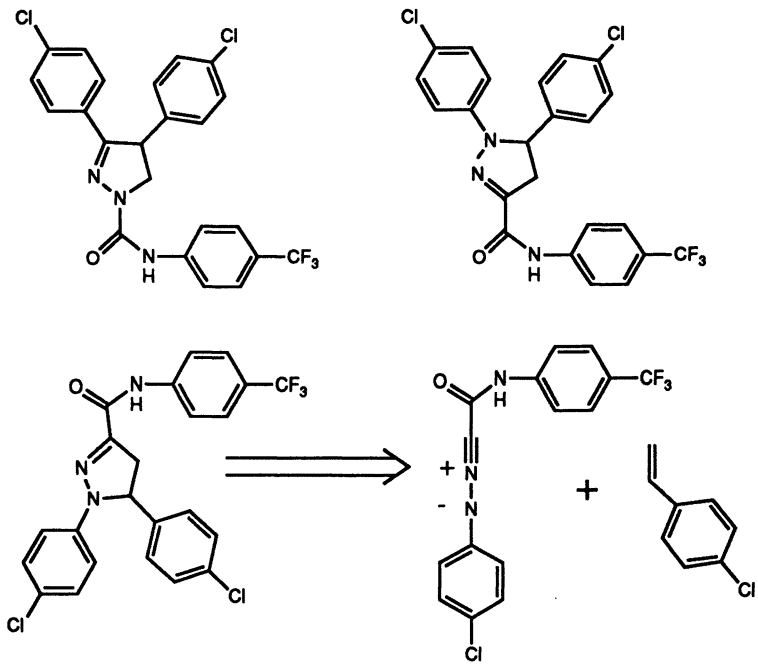


Figure 4. Retrosynthesis of 1,5-diarylpyrazoline-3-carboxanilides.

We began our synthesis in the area by using commercially available 4-chloroacetoacetanilide. Chlorination with sulfuryl chloride in methylene chloride proceeded in quantitative yield. The key step was the coupling with a diazonium salt in the presence of sodium acetate. This variant of the Japp-Klingemann reaction produces the hydrazonyl chloride in moderate yield. The cycloaddition was carried out by treating a refluxing benzene solution of the hydrazonyl

chloride and 4-chlorostyrene with triethylamine. The desired pyrazoline was isolated in 40 % yield after chromatography (Figure 5). Insecticide testing revealed activity on fall armyworm (LC_{50}, 30 ppm) and tobacco budworm (LC_{50}, 100 ppm), a typical spectrum for a pyrazoline insecticide (9).

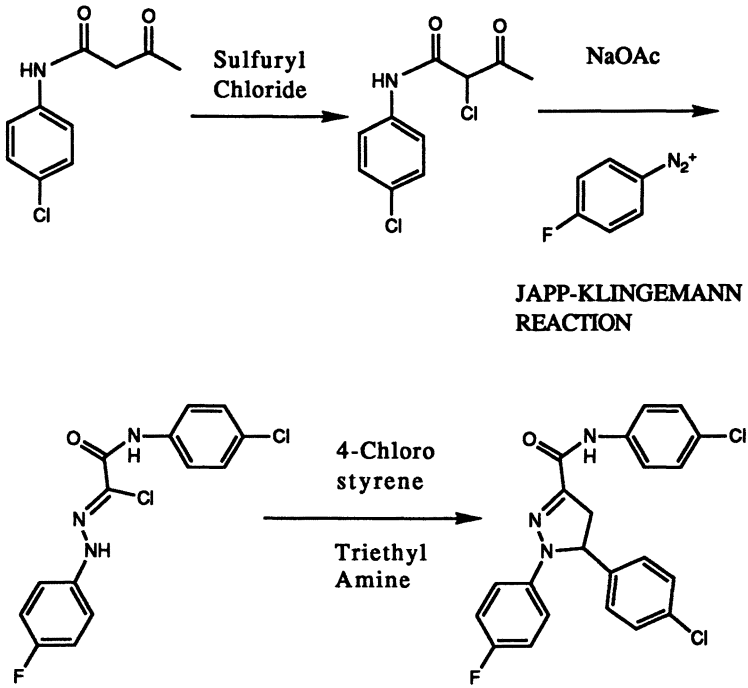


Figure 5. First synthesis of a 1,5-diarylpyrazoline-3-carboxanilide.

Analog Synthesis. In order to begin analoging in the area we decided to use ester substituted hydrazonyl chlorides to take advantage of the commercial availability of both methyl and ethyl 2-chloroacetoacetate. This not only allowed us greater flexibility in analoging of the carboxanilide function, but gave higher overall yields of products in the sequence (Figure 6). Japp-Klingemann reaction of the 2-chloroacetoacetates gave the hydrazonyl chlorides in good to excellent yield (10). The cycloaddition reaction proceeded with a very wide variety of substituted styrenes to produce the pyrazoline esters. Conversion of the esters to the carboxanilides could be done with anilines and trimethylaluminum, except for the case of 4-trifluoromethylaniline (11). Since the compounds derived from this aniline were some of the most interesting insecticides, we made the majority of carboxanilides through the acid chloride. Saponification of the ester to the acid was accomplished in quantitative yield. The acid was converted to the acid chloride using thionyl chloride in benzene. The unstable acid chloride reacted with a variety of anilines in tetrahydrofuran in the presence of triethylamine to give the desired product in good yield. In the majority of cases simply evaporating the solvent and adding methanol to the residue caused the products to crystallize, often in analytically pure form. On large scale the conversion of the esters to the carboxanilides by this method was excellent and often exceeded 90% yield. The insecticidal activity of this class was quite high. For example, an optimally substituted compound (R_1= H, Z= 4-CF_3, X= 4-Cl, and Y= 4-F) was

active on tobacco budworm (LD_{50}, 5 ppm), fall armyworm (LD_{50}, 0.5 ppm), and colorado potato beetle (LD_{50}, 4 ppm).

Structure Elucidation by Unambiguous Synthesis. Huisgen and others had reported only 5-substituted products from their cycloadditions (6-8). The basis for assigning these structures was by analogy to the regiochemistry of reactions with diphenylnitrile-imine. Since they had not done any structure proof, their findings were disputed by several other authors (12-14). Due to our interest in the insecticidal activity of this area we sought to independently verify the regiochemistry of the cycloaddition with styrene. We took advantage of the high nucleophilicity of the 3-position of pyrazolines. We independently reacted both atropaldehyde and cinnamaldehyde with phenyl hydrazine producing the isomeric 1,4 and 1,5-diphenylpyrazolines. Formylation was possible using the Vilsmaier reagent (15, 16). Oxidation to the acids was problematic due to ready oxidation of the pyrazoline ring, but freshly prepared silver oxide was highly chemoselective for the aldehyde function. Esterification with thionyl chloride and methanol provided the isomeric methyl esters. The spectral data for the 1,5-diphenyl compound matched perfectly that of the cycloaddition product (Figure 7). Close inspection of the proton NMR spectra from the crude cycloaddition product failed to show any of the resonances due to the 1,4-diphenylpyrazoline. Huisgen's structural determination for the cycloaddition products of alkoxycarbonyl nitrile-imines had been correct.

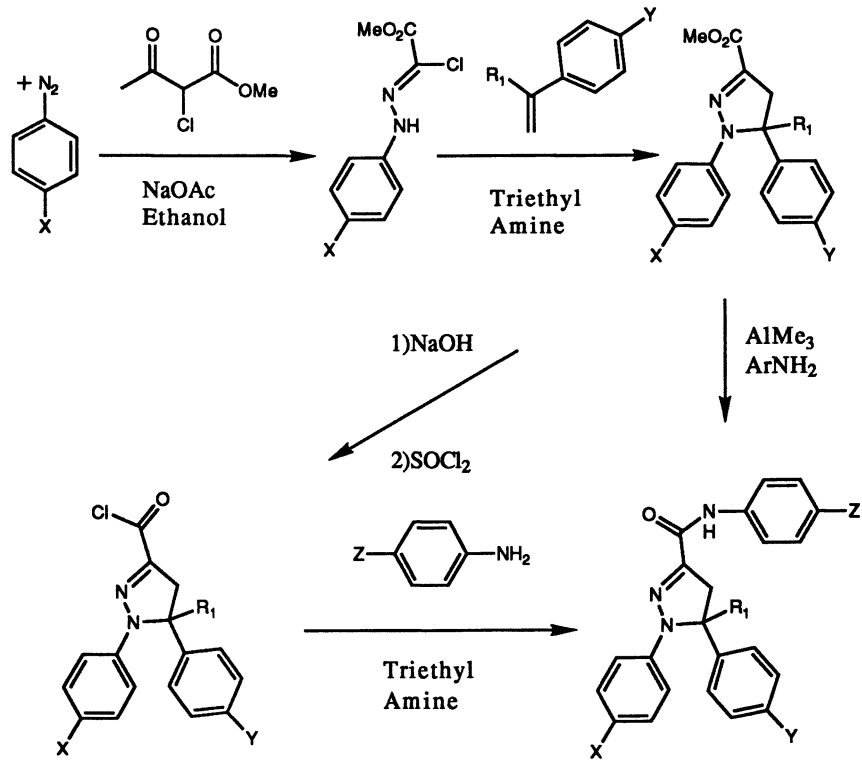


Figure 6. Synthetic scheme for the optimization program.

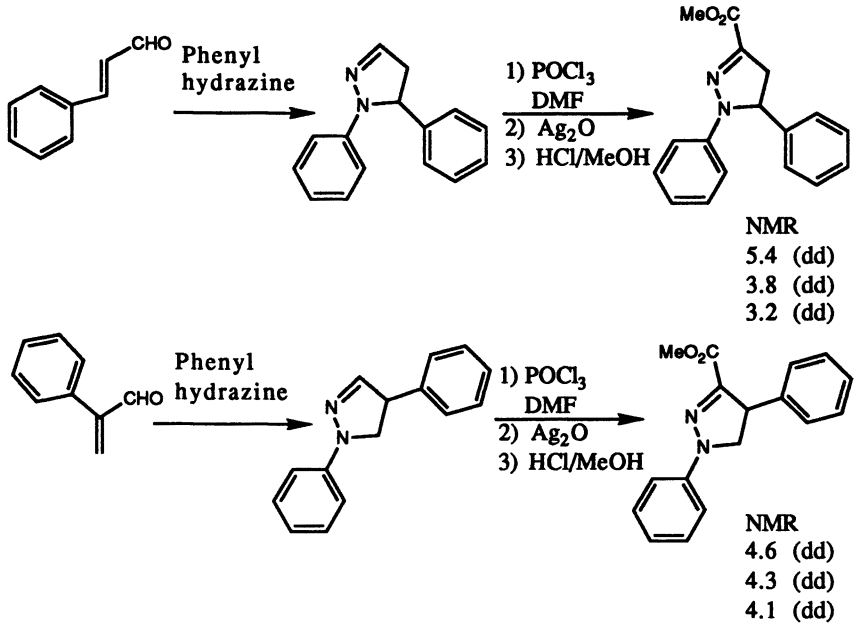


Figure 7. Unambiguous synthesis of 4- and 5-phenylpyrazolines.

Alternative Syntheses from Aryl Hydrazines. We continued to try to find convenient alternative synthetic methods for the 1,5-diphenylpyrazoline carboxanilides, since we were unable to make pyridyl substituents at the 1-position. As we had seen previously nitrile-imines are known to undergo 1,5-electrocyclization whenever possible. There are numerous examples of this in the literature such as the cyclization of the pyridyl substituted nitrile imine from the work of Wamhoff shown in Figure 8 (17).

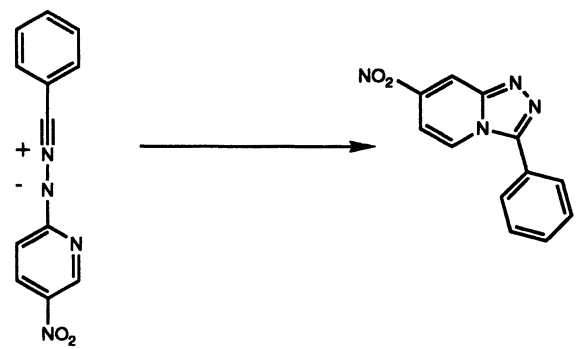


Figure 8. Electrocyclization of pyridyl nitrile-imines.

Von Auwers and coworkers had devised a synthesis of pyrazoline carboxylic acids in 1927 based on the cyclization of benzylidene pyruvic acids (18). Since benzylidene pyruvic acids are produced as their potassium salts we decided to modify von Auwers' method (Figure 9). We found that reacting an aqueous solution of the potassium salt with an aqueous solution of a phenylhydrazine

hydrochloride produced the free acid of the phenylhydrazone. Simply heating the phenylhydrazone in acetic acid for 1-3 hours produced the 1,5-diarylpyrazoline-3-carboxylic acid, but continued heating led to a significant amount of decarboxylation. This method proved convenient when the phenylhydrazine hydrochloride was commercially available.

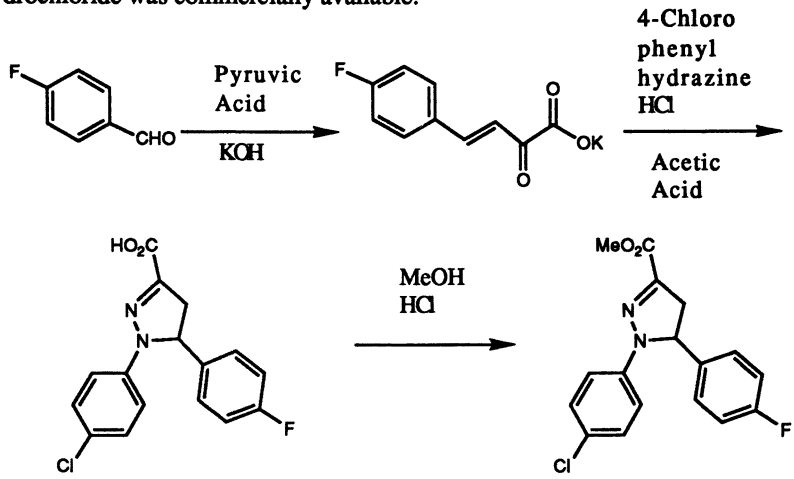


Figure 9. Alternative synthetic route to 1,5-diarylpyrazolines.

Heterocyclic Substituted Pyrazolines. To make the 1-heteroaryl pyrazolines we turned to the use of the benzylidenepyruvanilides instead of the acids. We reasoned that it might prove inconvenient to go through the acid chlorides of the heteroaryl pyrazolines for stability reasons. We incorporated the anilide function through the imidazolide of benzylidenepyruvic acid by addition of 4-trifluoromethylaniline. Heating this anilide with a heteroarylhydrazine in dimethylformamide gave the desired 1-heteroaryl-5-arylpyrazoline carboxanilides in moderate to good yields. We prepared a wide variety of compounds in this manner as shown in Figure 10.

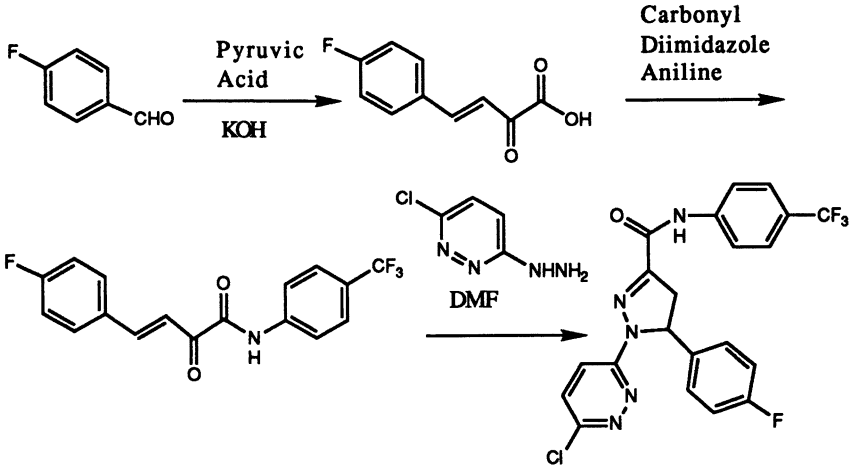


Figure 10. Synthesis of 1-heteroarylpyrazolines.

5-Heteroarylpyrazolines were made from the corresponding ethenyl-heterocycles. Palladium catalyzed reactions of heterocyclic halides proved useful for synthesis of more complicated heterostyrenes. For example 2,5-dibromopyridine selectively reacted with vinylic Grignard reagents under palladium catalysis to give 2-pyridylalkenes (Figure 11). Other heterocyclic dipolarophiles were made using Wittig olefination reactions or were commercially available. Alternatively, we were also able to convert an aldehyde directly to an oxazole by the use of tosylmethylisocyanide (19).

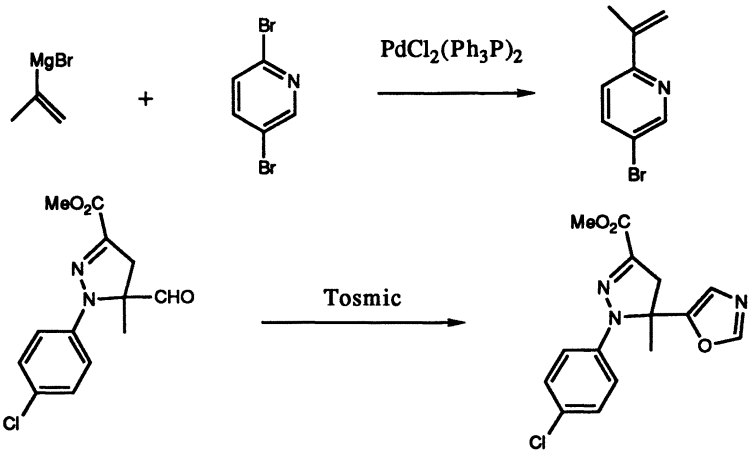


Figure 11. Synthesis of 5-heteroarylpyrazolines.

Amide Modifications. We found that the anilide function could be easily derivatized (Figure 12). Treatment of the amide with a strong base such as sodium hydride or potassium t-butoxide led followed by an alkylating or acylating agent produced the N-alkyl derivatives in high yield. Alternatively, reaction of N-alkylanilines or sodium salts of acetanilides with pyrazoline acid chlorides could also produce the derivatized materials. The amide could be readily converted to the thioamide by use of phosphorous pentasulphide in pyridine. Phenols, phenyl hydrazines, and benzyl amines also underwent reaction with the pyrazoline acid chlorides to make less active compounds in which the carboxanilide was replaced by an ester, hydrazide, or benzylamide.

Enantiomerically Pure Pyrazolines. Synthesis of enantiomerically pure pyrazolines was accomplished through separation of amides made with Evan's chiral oxazolidinones (Figure 13). The commercially available oxazolidinone was deprotonated with n-butyllithium and treated with the pyrazoline acid chloride. The diastereomers were difficult to fully separate by flash chromatography, but were completely separable by use of medium pressure liquid chromatography. The separated amides were treated with sodium methoxide to give the enantiomerically pure esters. These were converted to the pyrazolinecarboxanilides by our standard methods. Analysis on a Pirkle HPLC column showed both enantiomers to have > 99% ee. All of the insecticidal activity resided in the (-) enantiomer. Attempts to use amides derived from 1-naphthethylamine and other commercially available derivatizing agents were frustrated by our inability to cleave the separated diastereomeric products without decomposition.

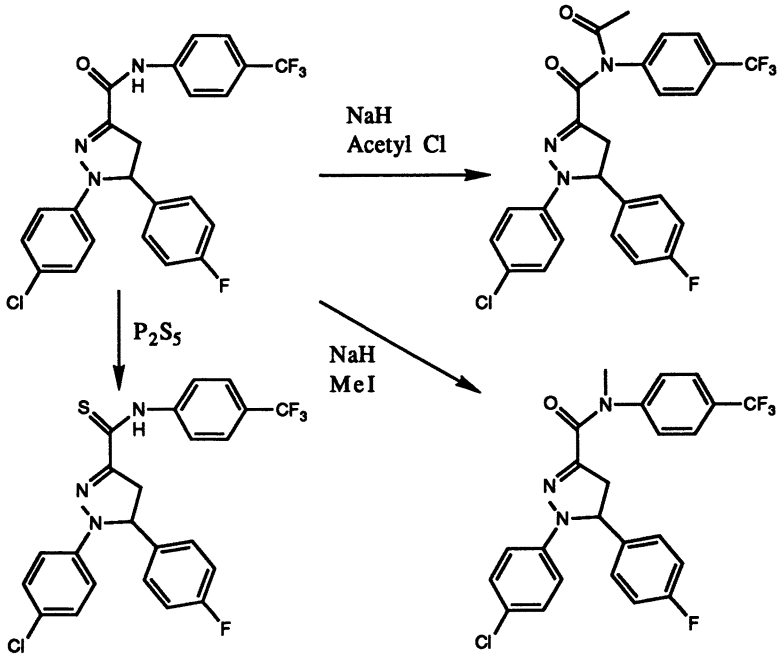


Figure 12. Modification of the carboxanilide group.

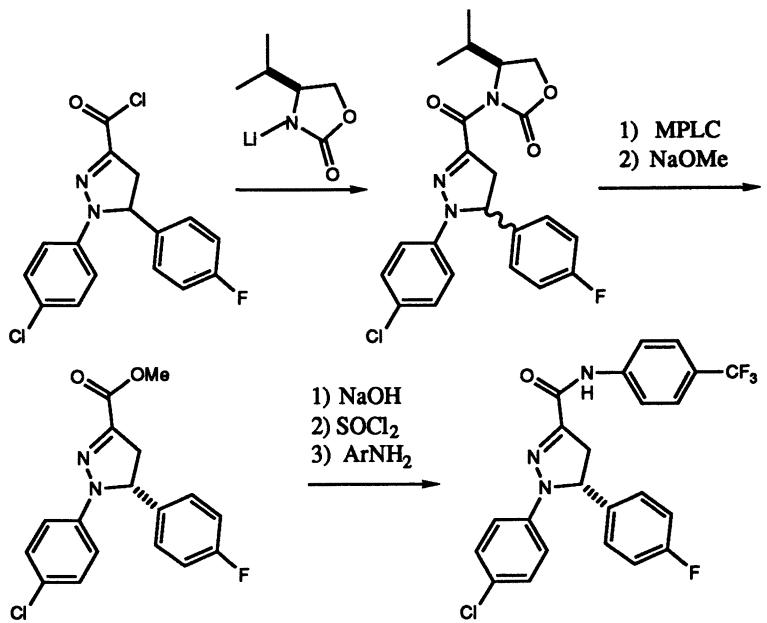


Figure 13. Synthesis of enantiomerically pure 1,5-diarylpyrazolines.

Biology

The pyrazoline carboxanilides showed very high levels of activity against coleopteran and especially lepidopteran pests (9). Many of the compounds in this area were active against fall armyworm at 2.5 ppm and below. The activity of pyrazoline insecticides is slow compared to many classes of insecticides such as pyrethroids. Mortality is best assessed at 72 to 96 hours post treatment. The level of activity and speed of onset can be improved by pre treatment of the insects with piperonyl butoxide as well as by raising the temperature of the test units (9).

As reported by Salgado pyrazolines act by blocking voltage regulated sodium channels in sensory neurons in much the same way as local anesthetics (20). The overall effect of this is a passive paralysis in which intoxicated larvae lose their grip on the leaf surface. When the larvae are disturbed they undergo tremors which Salgado attributes to overcompensation by the nervous system for the loss of sensory nervous function.

Structure-Activity Relationships

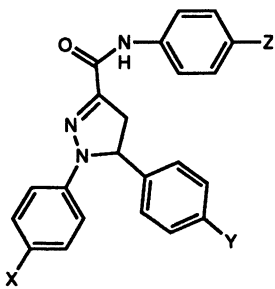


Figure 14. Generic structure of 1,5-diarylpyrazoline-3-carboxanilides.

The SAR of pyrazoline-3-carboxanilides (Figure 14) is very similar to that of traditional pyrazolines (21). The carboxanilide ring cannot be replaced by alkyl groups and insertion of spacer groups or replacement of the nitrogen with other heteroatoms decreases activity. The optimal position for mono substitution is the 4-position. A second small substituent such as a fluorine can be tolerated at the 2- or 3-positions without decreasing activity greatly. However, compounds lacking a 4-substituent have little activity.

SAR for a Z (carboxanilide) substituent in the 4-position:
CF_3 > OCF_3 > Halogen, OCF_2H > cyano, nitro > alkoxy, alkyl, H

The SAR for the 1-aryl ring is quite similar. It cannot be replaced by an aliphatic group and insertion of spacer groups also decreases activity. Again, 4-substitution is optimal, but it is not necessary in this case. In addition to the 4-substituent, substitution of a second small group at the 2- or 3-positions maintains activity. Replacement of the phenyl ring with monocyclic heterocycles such as pyridine, pyrimidine, and pyridazine leads to highly active compounds.

SAR for an X (1-phenyl) substituent in the 4-position:
Cl, CN > $OCHF_2$ > F, CF_3, OCF_3 > H > alkyl > alkoxy

The SAR for the 5-aryl ring allows for the most latitude. Although substitution at the 4-position is preferred, the 3-position can tolerate a variety of groups. Substituents in the 2 position larger than chlorine decrease activity. 2,4-

and 3,4-disubstituted compounds have very high activity. Heterocycles such as pyridines, thiophenes, and oxazoles had good activity.

SAR for a Y (5-phenyl) substituent in the 4-position:

CN, F > Cl, $OCHF_2$ > CF_3, OCF_3, nitro > alkyl, H > alkoxy

Conclusion

We have discovered a highly active class of insecticides which are especially active on lepidopteran insects. These pyrazolines share a common mode of action with traditional pyrazolines, but are chemically distinct. The structure-activity trends in this class are similar to traditional pyrazolines with some exceptions. They are active in field testing against insects which have developed resistance to other classes of insecticides.

Acknowledgments

We would like to thank all of the biologists who ran the bioassays which formed the basis for the biological data in this paper, especially Diane and Bruce Stanley. Special thanks are due John Andolaro and Dave Marsden for leading our field testing efforts. Dan Rossignol, Peter Horne and Gary Hollingshaus determined the site of action of these pyrazolines. Tariq Andrea and Dan Kleier performed structure activity calculations. Formulation work was done by Emil Shen. Finally we would like to thank Carole Beaman, Martin Currie, Ron Mattson, Mila Folgar, Bob March, Bonita Morrison, Eileen Marsilli, and Tom Boyle for technical assistance and Donna Zimmerman for technical advice and leadership.

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Chapter 26

1-Arylpyrazoline-3-carboxanilides

Novel and Selective Insecticides

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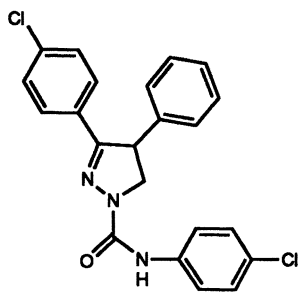
Nitrile-imine cycloaddition chemistry was utilized to synthesize a new class of pyrazoline insecticides. The structure activity relationships of the pyrazoline insecticides were explored by using a wide variety of dipolarophiles to give 5-substituted and 5,5-disubstituted products. In addition we have used dianion chemistry to produce a number of compounds which have 4-substitution opposite of the regiochemistry derived from cycloaddition.

As described in the previous chapter, 3,4-diarylpyrazoline-1-carboxanilides have been a highly investigated area in insecticide chemistry. In 1986, Jacobson at Rohm & Haas described a new series of pyrazolines, exemplified by RH3421, (Figure 1) which lacked the 4-phenyl ring (1). Jacobson made these compounds by dianion chemistry and quenching with a variety of electrophiles (Figure 2). They reported that the optimal replacement for the phenyl group was 4,4-disubstitution with a methyl group and a carbomethoxy group (2). These compounds were highly active on lepidopteran species with greatly improved activity on coleopteran pests. These compounds also had good to excellent activity on the important rice pests, plant hoppers.



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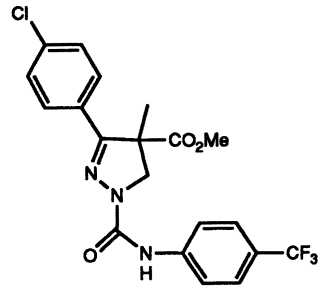

RH3421

Figure 1. 1-Aryl-5-substitutedpyrazoline-1-carboxanilide insecticides.

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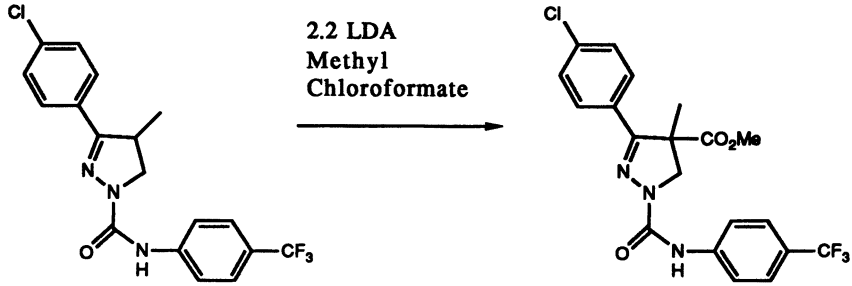


Figure 2. Jacobson's synthesis of pyrazoline-1-carboxanilides.

Synthesis

With the synthesis of 1,5-diarylpyrazoline-3-carboxanilides we had shown that the attachment of the carboxanilide group did not need to be on nitrogen in order to see insecticidal activity (3). In order to make compounds related to RH3421 we needed to use methyl methacrylate, a readily available polymer intermediate, as a dipolarophile in cycloadditions with nitrile-imines. (Figure 3).

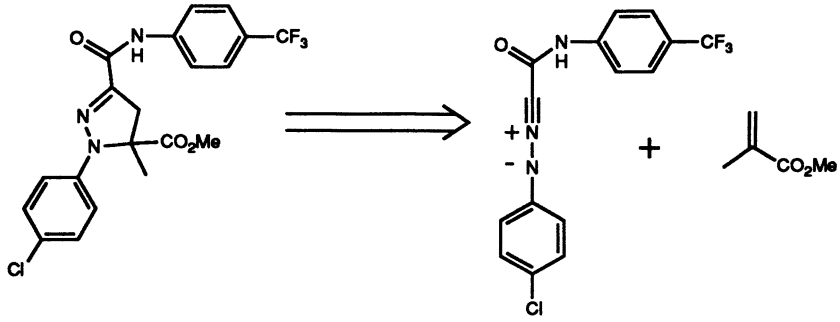


Figure 3. Retrosynthesis of 1-arylpyrazoline-3-carboxanilides via nitrile-imines.

Nitrile-Imine Cycloadditions. To synthesize the 1-aryl-3-carboxanilide analagous to the Rohm and Haas development candidate RH3421 diketene was allowed to react with 4-trifluoromethylaniline. The acetoacetanilide was chlorinated with sulfuryl chloride and then subjected to the Japp-Klingemann reaction with 4-chlorophenyl-diazonium chloride (3). The hydrazonyl chloride and methyl methacrylate were treated with triethylamine in refluxing benzene to give the desired material in good yield (Figure 4). The compound was insecticidally active on tobacco budworm (LD_{50}, 10ppm), boll weevil (LD_{50}, 12 ppm) and aster leaf hopper (LD_{50}, 40 ppm).

The hydrazonyl chlorides proved to be versatile intermediates with which to explore pyrazoline structure-activity. Dipolar cycloaddition chemistry allows the synthesis of a much wider variety of substituents than either dianion or hydrazine cyclization chemistry. Figure 5 shows some selected examples of this work. In general the nitrile-imines are reactive dipoles and most mono and disubstituted olefins will undergo cycloaddition. Most reactive are the electron deficient olefins such methyl acrylate. Electron rich olefins such as hexene are less reactive, but good yield of product can be realized by using the dipolarophile as

solvent with syringe pump addition of the triethylamine. Both dimethyl fumarate and dimethyl maleate led to the same product with the ester groups trans to each other in agreement with the work of Huisgen on diphenylnitrile-imines (4). This is due to the high acidity of the 4-position which can be easily equilibrated by the triethylamine.

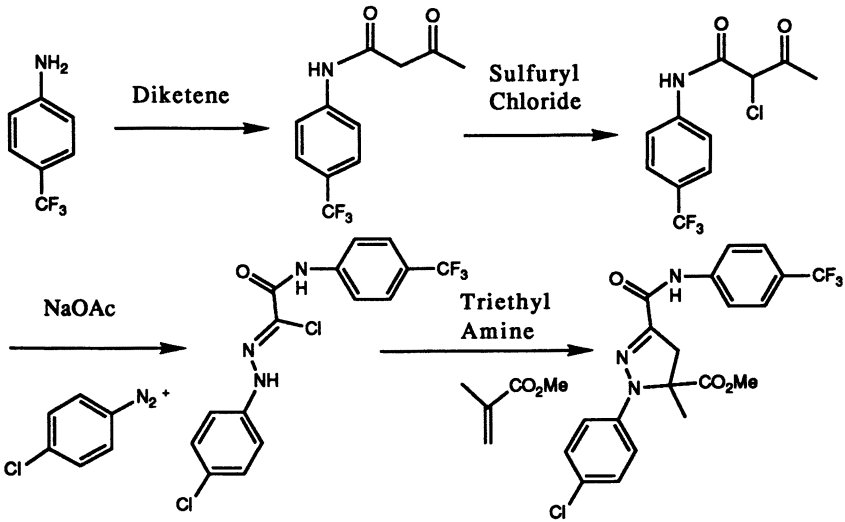


Figure 4. First synthesis of a 1-aryl-5-methyl-5-carbomethoxypyrazoline.

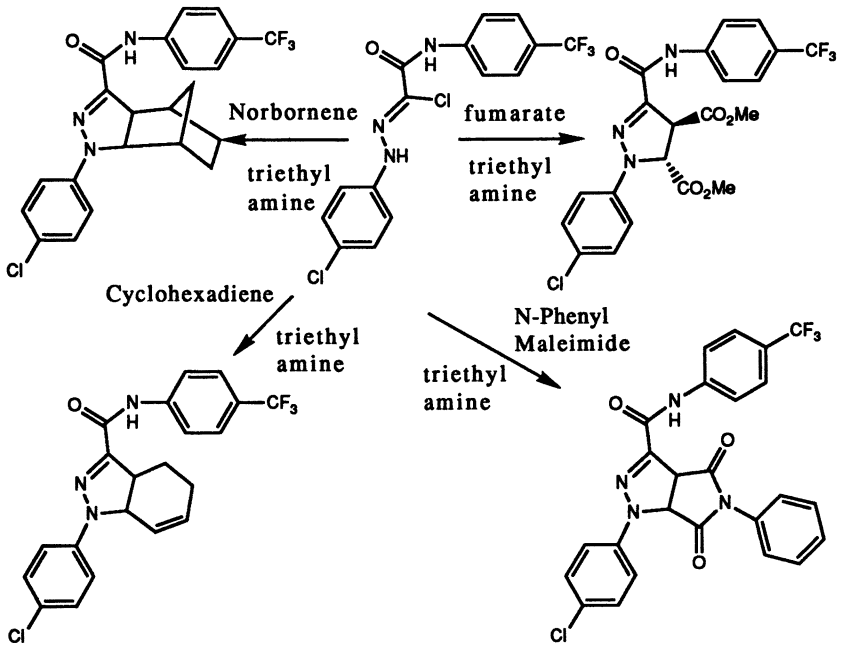
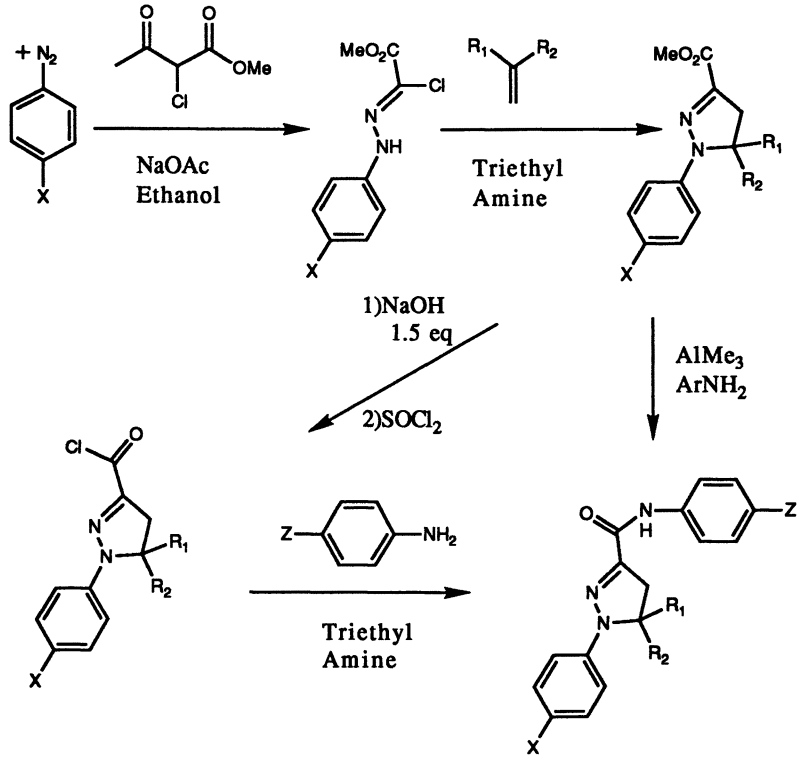


Figure 5. Dipolar cycloaddition reactions of the nitrile-imine.

Optimization Program. Since the 5-methyl-5-carbomethoxy compounds had high activity, the initial phase of our optimization program involved variation of these substitutents (Figure 6). We used commercially available methyl 2-chloro-acetoacetate as the precursor to the hydrazonyl chloride. The reaction with methyl methacrylate proceeds in excellent yield. Conversion to the anilide was more facile than expected. The 3-carbomethoxy group is much more reactive towards nucleophiles than the one at the 5-position. Reaction with aluminum amides only occurred at the 3-position. It is also possible to selectively hydrolyze the ester at the 3-position using 1.5 eq. of potassium or sodium hydroxide in methanol. This allowed the synthesis of the anilides through the acid chloride.



R1= Alkyl, Aryl, H
R2= Ester, Aldehyde, Nitrile, Alkyl, Phosphonate, Alkene, Benzyl, Benzoyl, Alkyl Ketone, Etc

Figure 6. Synthetic scheme for the optimization program.

Instability of Pyrazoline-5-Carboxylic Acids. In trying to find a common intermediate to make a variety of esters at the 5-position, we were unable to produce the free carboxylic acid at the 5-position. Attempts to prepare the free acid resulted in spontaneous decarboxylation (Figure 7). For example, saponification of the ester group produced the sodium carboxylate which evolved CO_2 on neutralization. Oxidation of the aldehyde produced the same result as did acid catalyzed deprotection of a t-butylester. Finally, we were able to solve the problem by preparing the substituted methacrylates and carrying them individually through the sequence.

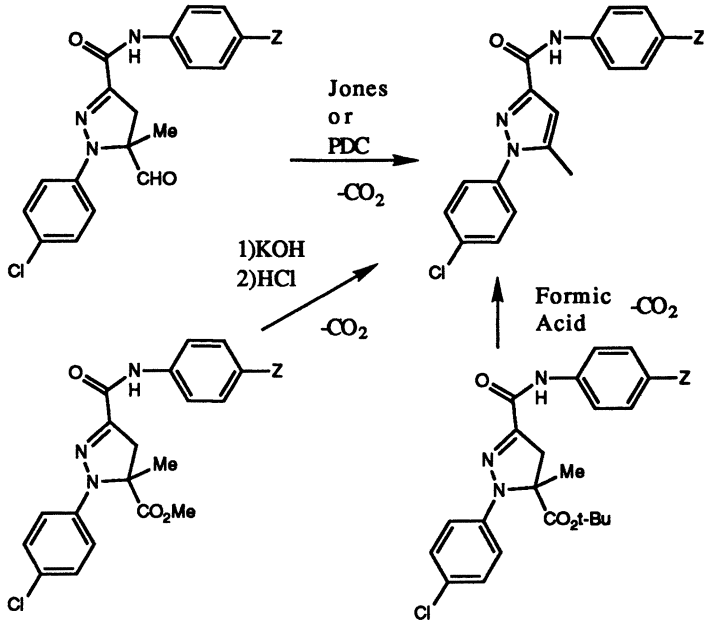


Figure 7. Chemical instability of pyrazoline-5-carboxylic acids.

Dianion Chemistry. We also wanted to see if we could complement the cycloaddition route to the pyrazolines by trying dianion chemistry on pyrazoline carboxanilides. Since the cycloaddition route led to 5-substituted products with mono substituted olefins and gave low yields with 1, 2-disubstituted olefins, we needed an alternative route to make 4-substituted pyrazolines. We began by metallating a 1,5-diphenylpyrazoline-3-carboxanilide with 2.2 equivalents of lithium diisopropylamide and quenching with methyl iodide. The methyl group was incorporated in the 4-position trans to the phenyl group. We also used a variety of other electrophiles to give trans substituted compounds (Figure 8).

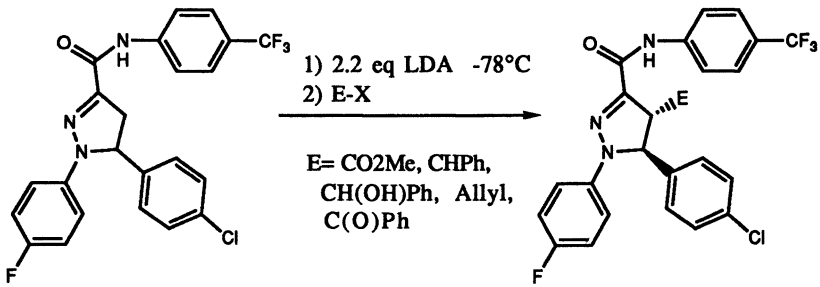


Figure 8. Use of dianion chemistry to introduce 4-substituents.

Preparation of 4-substituted compounds unsubstituted at the 5-position required the use of ethylene or an ethylene equivalent as dipolarophile followed by the dianion chemistry. Our initial attempts with ethylene either in a pressure tube or by bubbling ethylene through the solution of the hydrazonyl chloride gave very low yields (> 10%). We then turned to a reaction of hydrazonyl chlorides

with several equivalents of a sulfoxonium ylide (5). This process did produce the desired product, but in similarly low yield (Figure 9).

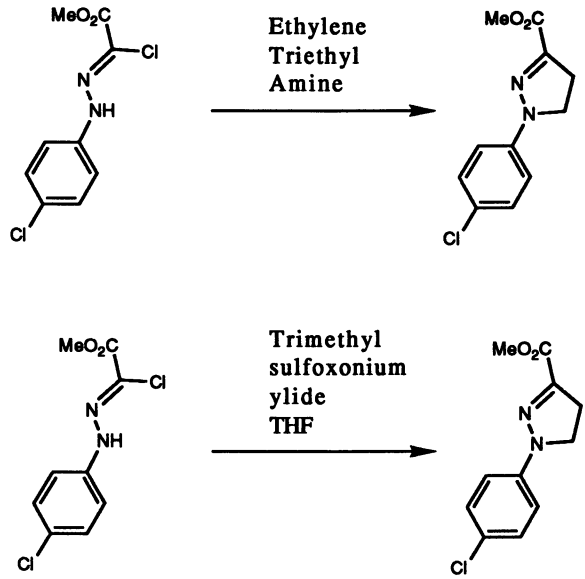


Figure 9. Attempted synthesis of 4,5-unsubstituted pyrazolines.

We were able to solve the problem by using vinyl-trimethylsilane as an ethylene equivalent. When the reaction was performed as usual and the crude product treated with fluoride the desired unsubstituted pyrazoline was produced in around 60 % yield. This intermediate was transformed to the anilide in the usual manner. The metallation sequence worked well and quenching with a variety of electrophiles was straightforward as exemplified in Figure 10. A one-pot procedure for the introduction of 2 substituents at the 4-position was also investigated. Treatment of the anilide with 3.2 equivalents of base followed by one equivalent of electrophile and then a second electrophile gave the 4,4-disubstituted products. These adducts made by dianion chemistry were chemically and physically distinct from the isomeric 5,5-disubstituted compounds made from cycloaddition chemistry. This serves as unambiguous evidence in favor of our proposed regiochemistry for nitrile-imine cycloadditions with substituted acrylates.

Ring Constrained Pyrazolines. Another class of targets we investigated were pyrazolines containing rings to restrict their conformational mobility. There are several ways to do this (Figure 11). First, we investigated indene and dihydronaphthalene as dipolarophiles which led to tricyclic pyrazolines as products. Essentially these were just 1,5-diphenylpyrazolines in which the phenyl group is restrained as part of a ring to the 4-position. Another type of constrained system was made from an unsaturated lactone. The overall effect of this is to form a bond between the methyl group and the methoxy carbon of the ester function while retaining the same number of carbons. Even though these compounds contained all the requisite functionality for activity, the anilides from both types of conformationally restricted pyrazolines had substantially reduced activity as insecticides.

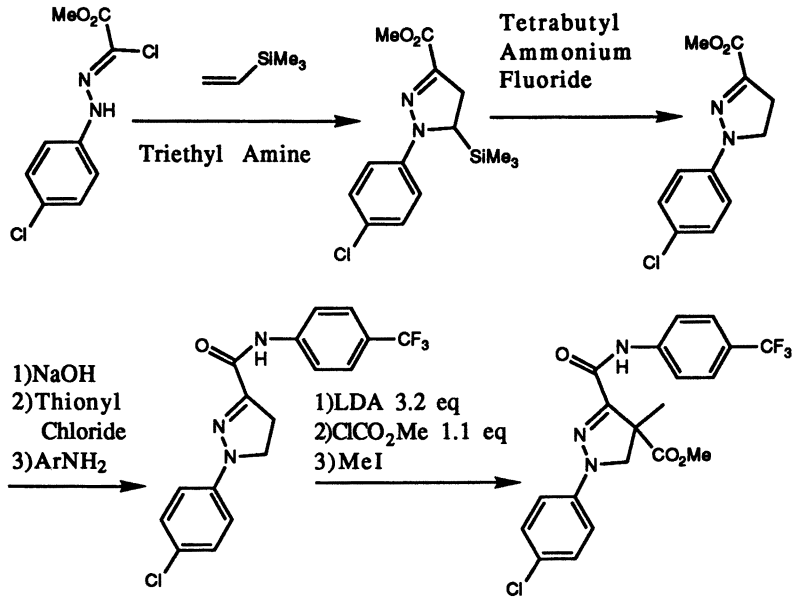


Figure 10. Synthesis of 4,4-disubstituted pyrazolines via dianion chemistry.

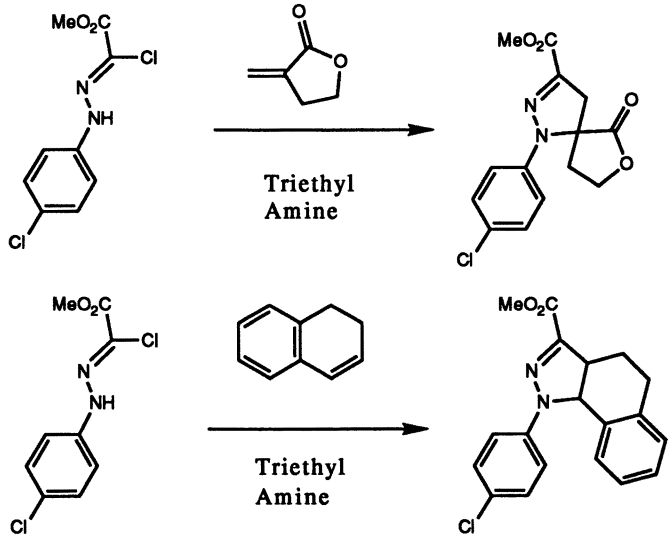


Figure 11. Conformationally restrained pyrazoline-3-carboxanilides.

Structure Activity Trends

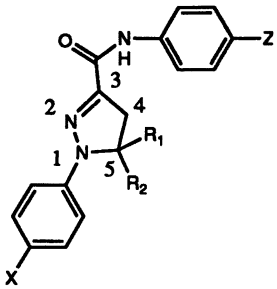


Figure 12. Generic structure of 1-aryl-5-substituted pyrazolines.

Many of the pyrazolines studied here (Figure 12) were highly active insecticides (6). Depending on the substitution pattern the optimal activity was on either coleopteran or lepidopteran pests. The structure activity trends in this area are very similar to those found in the publications of FMC (7) and Rohm and Haas (1,2) for traditional pyrazoline insecticides. For coleopteran pests the 5,5-methyl-carbomethoxy pattern was best. It is interesting to note that in spite of the wide latitude for substitution on aryl rings at the 5-position, replacement of the methyl or carbomethoxy group with an ethyl or carboethoxy group greatly reduces activity. Replacing the ester group with other small electron withdrawing groups such as an aldehyde, nitrile, phosphonate, or ketone also caused the activity to drop. The optimal substitution on the carboxanilide (Z-substituent) and 1-phenyl (X-substituent) rings was essentially the same as that reported for the 1,5-diarylpyrazoline-3-carboxanilides in the preceding chapter (3).

Of the many other substitutions we explored at the 5-position, other than the previously discussed aryl groups (3), alkyl groups showed the highest activity. The highest activity was found for 3 and 4-carbon chains. As for traditional pyrazolines (7), branching of the alkyl chain improved activity and branching adjacent to the ring was optimal. While directly substituted aryl rings had high activity inserting an alkyl or acyl spacer between the pyrazoline and the aryl ring reduced activity.

Acknowledgments

We would like to thank all of the biologists involved with the bioassays for these compounds, especially Dave Leva, Jim Gilmour and Bruce Stanley. Excellent technical support for this project was provided by Carole Beaman, Martin Currie, Kevin Poff, Barry Hart, Ron Mattson, and Kathy Russell.

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Chapter 27

Insecticidal 2-Aryl-3-trifluoromethylsulfonylpyrroles

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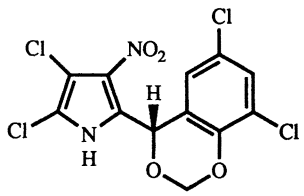
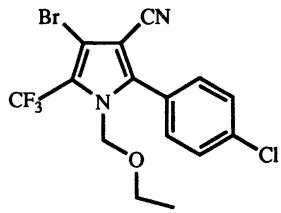
This report describes the synthesis and insecticidal activity of a series of 2-aryl-3-trifluoromethylsulfonylpyrroles containing a variety of substituents on the 4- and 5-positions of the pyrrole ring. The broad spectrum insecticidal activity of the natural product dioxapyrrolomycin was the basis for the initiation of this work.

For a number of years, a new series of insecticidal pyrroles have been under investigation at American Cyanamid (1-4). The identification of the insecticidal activity associated with a fermentation broth from a *Streptomyces* strain by our screening group, and the isolation and characterization of the active component dioxapyrrolomycin (5) by Cyanamid scientists at Lederle Laboratories, was the basis for the initiation of work in this area. At about the same time, the identical pyrrole was reported by Meiji Seika and SS Pharmaceutical in Japan (6,7), but neither of these groups mentioned the insecticidal properties associated with this material. Previously reported synthetic modifications of dioxapyrrolomycin provided AC 303,630, a member of this series currently undergoing development as a broad spectrum insecticide/ miticide (1,2,4).



Dioxapyrrolomycin



AC 303630

As has been reported, these pyrroles function via interference with respiration, in particular the uncoupling of oxidative phosphorylation (1,4). Many studies have indicated that effective uncouplers share the important properties of being highly

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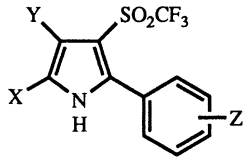
lipophilic and weakly acidic. Similarly, structure activity relationships developed in this series of highly functionalized pyrroles indicate that they too must be lipophilic and the presence of electron-withdrawing groups arrayed around the pyrrole nucleus contribute toward achieving an optimum pKa range for the parent NH pyrroles that is requisite for good insecticidal activity. With these two properties in mind we felt that the introduction of the lipophilic, strongly electron-withdrawing trifluoromethyl sulfonyl functionality onto the pyrrole nucleus should be investigated.

When considering synthetic approaches toward these targeted compounds **1**



1

efficient routes which would allow for variation of the substituents on the aryl ring as well as permitting additional functionalization of the 4- and 5-positions of the pyrrole nucleus were desired. Our synthetic approaches toward these novel compounds have involved the construction of the pyrrole nucleus from acyclic precursors containing the trifluoromethylsulfonyl moiety as well as trifluoromethylsulfenylation of appropriately substituted 2-aryl pyrroles followed by oxidation. The use of trifluoromethylsulfenyl chloride to afford the trifluoromethylthio pyrroles followed by oxidation is a well documented method for the preparation of trifluoromethylsulfonyl pyrroles. However, the utilization of acyclic precursors containing the trifluoromethylsulfonyl moiety has received little attention.

To facilitate the discussion of these compounds, they will be grouped based on the presence/absence of a trifluoromethyl group on position 5 of the pyrrole ring. The section covering 2-aryl-3-trifluoromethylsulfonylpyrroles, will describe compounds having a variety of substituents on positions 4 and 5 of the pyrrole ring, excluding a 5-trifluoromethyl group whereas the section on 2-aryl-3-trifluoromethylsulfonyl-5-trifluoromethylpyrroles will describe those having a trifluoromethyl group on position 5.

2-Aryl-3-Trifluoromethylsulfonylpyrroles

Chemistry. One of the routes developed at Cyanamid for the synthesis of 2-aryl-3-nitro or 2-aryl-3-cyanopyrroles [Figure 1 (1)], involved the condensation of the appropriate ketone , where X is a strongly electron-withdrawing group, such as nitro or cyano, with aminoacetaldehyde diethyl acetal to afford enamines as a mixture of isomers. When treated with trifluoroacetic acid, these enamines readily afforded the desired pyrroles in good yield. These enamine cyclizations fail or work poorly unless X is strongly electron-withdrawing. It was reasoned that if the analogous trifluoromethylsulfonyl enamine intermediates could be constructed, (X is trifluoromethylsulfonyl), the desired 2-aryl-3-trifluoromethylsulfonylpyrroles would be accessible via a similar cyclization.

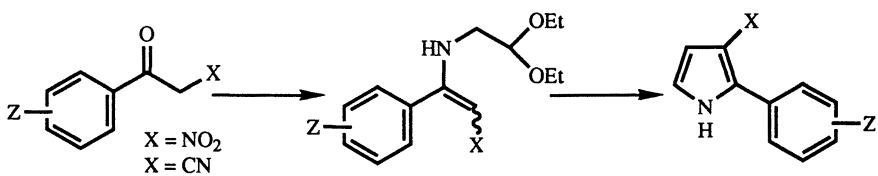


Figure 1. Synthesis of 2-Aryl-3-NO_2 and 2-Aryl-3-CN pyrroles.

As shown in Figure 2, two general routes have been developed for the preparation of the key trifluoromethylsulfonyl enamine intermediate **2**. In Method A, following the work of Hendrickson (8), potassium triflinate was reacted with 4-chlorophenacyl bromide **3** in refluxing acetonitrile for several days to afford the keto triflone **4** in 55% yield. Reaction of the triflone with aminoacetaldehyde diethyl acetal in refluxing toluene overnight afforded the crude enamine **2** as an E-Z mixture. As an alternative approach, Method B, toward the enamine, we have found that 4-chlorophenyl trifluoromethylsulfonylacetylene **5** reacts readily with amino-acetaldehyde diethyl acetal in a Michael fashion at room temperature in ether overnight to afford the enamine as a single isomer, the configuration of which has not been assigned. Several preparations of aryl trifluoromethylsulfonylacetylenes in poor to moderate yields by addition of triflic anhydride to metalo arylacetylenes have been reported (9-11). In a modification of Glass's procedure (11), we have found that inverse addition of the lithio arylacetylenes to triflic anhydride greatly improves the yields of these reactions. Due to the instability of the aryl trifluoromethylsulfonyl acetylenes, they are typically converted immediately after isolation, without purification to the enamines.

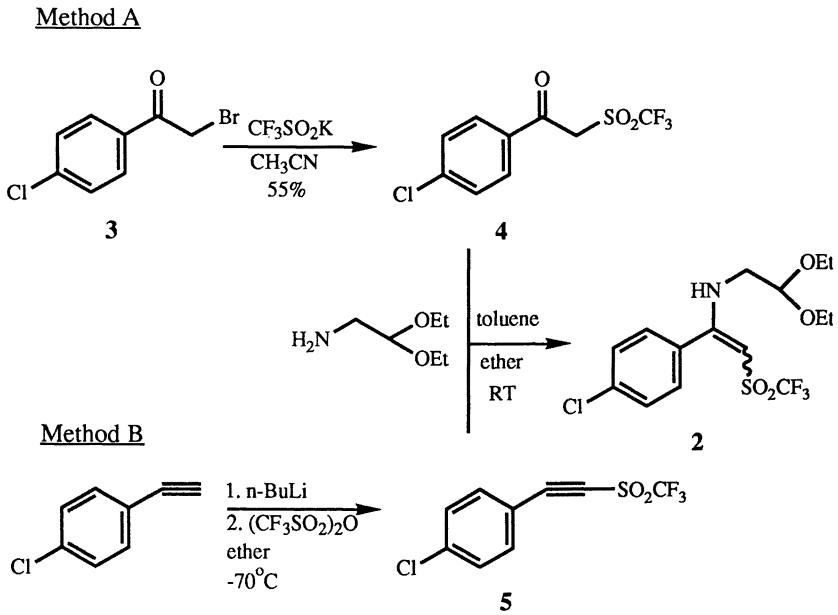
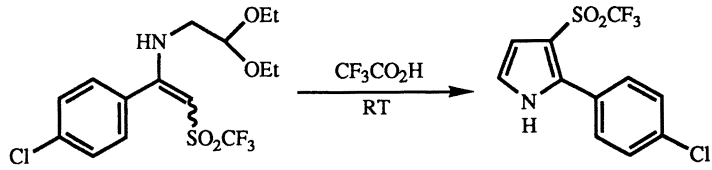


Figure 2. Synthesis of Trifluoromethylsulfonyl enamines.

After isolation, the crude enamines **2** formed by either of the two methods are cyclized (Figure 3) in good yields with trifluoroacetic acid at room temperature to afford the desired 2-(4-chlorophenyl)-3-trifluoromethylsulfonylpyrrole **6**. The overall yield of **6** from the keto triflone (Method A) is 45% and from the 4-chlorophenyl-acetylene (Method B) is 46%.

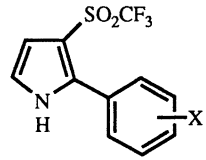
A number of 2-aryl-3-trifluoromethylsulfonylpyrroles were prepared in this manner and are listed in Table I. Preparation of the trifluoromethylsulfonyl enamine intermediates by method B was found to be the more efficient of the two methods. No attempt was made to optimize the yields of these reactions.



2 6

Figure 3. Synthesis of 2-Aryl-3-SO_2CF_3 pyrroles.

Table I



X	Method of Synthesis	% Yield *
4-Cl	A	45
4-Cl	B	46
H	B	67
Br	B	52
4-CF_3	B	23
4-$C(CH_3)_3$	B	24
3,4-di-Cl	B	15
4-F	B	10
4-CH_3	B	34
4-OCH_3	A	14
4-CN	B	3

* Overall % yield from keto triflone and arylacetylene for Method A and Method B respectively

Derivatization of the 2-aryl-3-trifluoromethylsulfonylpyrroles to afford a series of 5-substituted and 4,5-disubstituted analogs was readily accomplished as illustrated, with 2-(4-chlorophenyl)-3-trifluoromethylsulfonylpyrrole **6**, in Figures 4 and 5. As shown in Figure 4, halogenation with bromine or sulfuryl chloride could be controlled to afford either the 5-monohalo derivatives **7** and **9** or the 4,5-dihalo derivatives **8** and **10**. Nitration with nitric acid/acetic anhydride gave the 5-nitro analog **11** and cyanation to afford the 5-cyano compound **12** was accomplished with chlorosulfonyl-isocyanate. Bromination of **12** gave **13.**

The introduction of a trifluoromethylthio group at position 5 of **6** (Figure 5) was accomplished in good yield by reaction with trifluoromethylsulfenyl chloride in the presence of a catalytic amount of triflic acid for 5 days at 70°C in a pressure tube. The 5-trifluoromethylthio derivative **14** was oxidized to the sulfone **15** with excess MCPBA at room temperature and also readily brominated to afford **16**. Controlled oxidation of **16** gave the sulfoxide **17,** whereas excess MCPBA gave the sulfone **18**. Compared to the desbromo compound **14**, elevated temperatures were required to effect conversion of **16** to the sulfone.

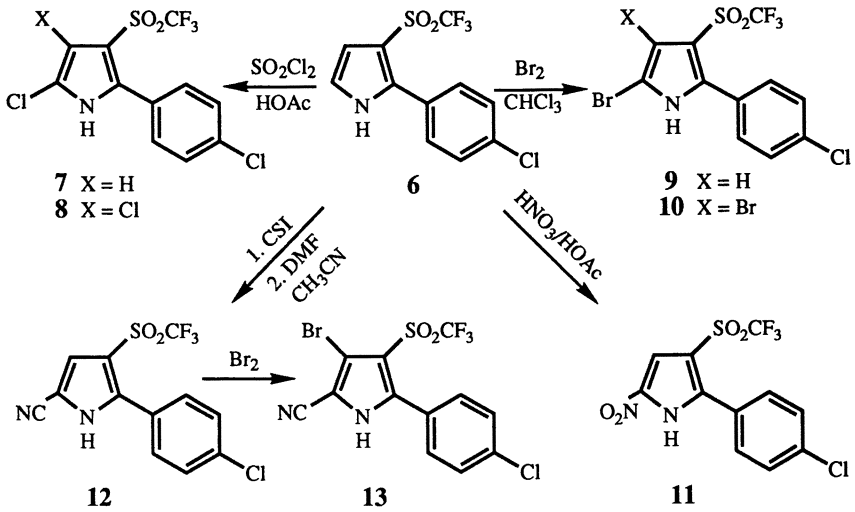


Figure 4. Derivatization of 2-Aryl-3-SO_2CF_3 pyrroles.

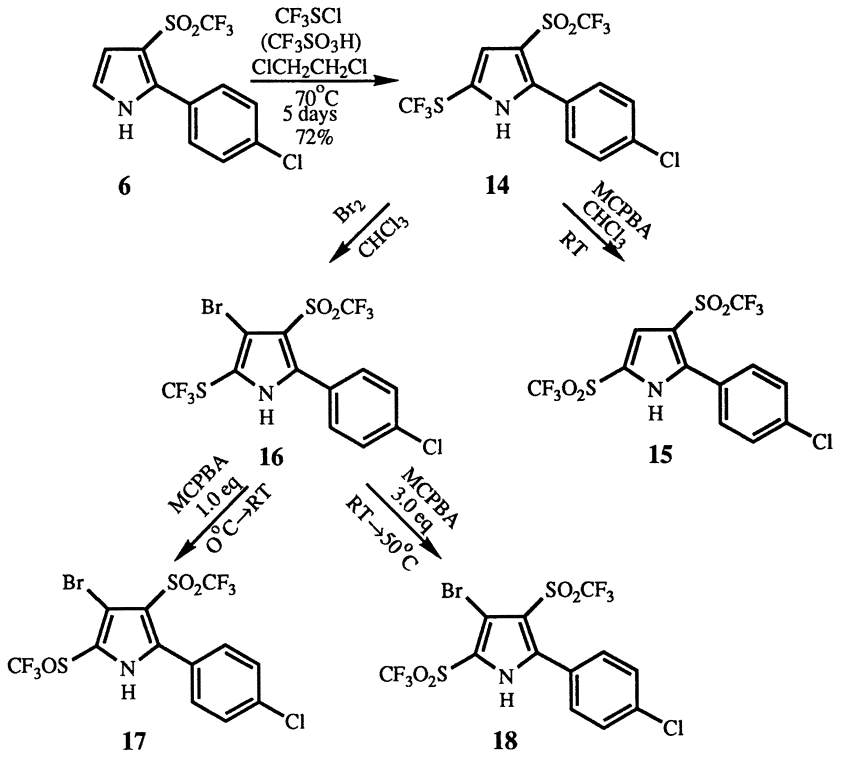
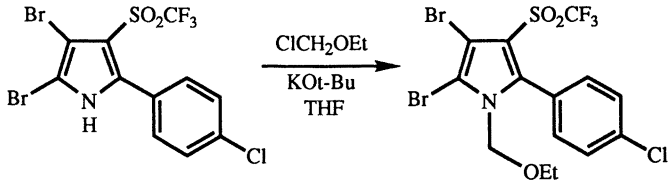


Figure 5. Derivatization of 2-Aryl-3-SO_2CF_3 pyrroles.

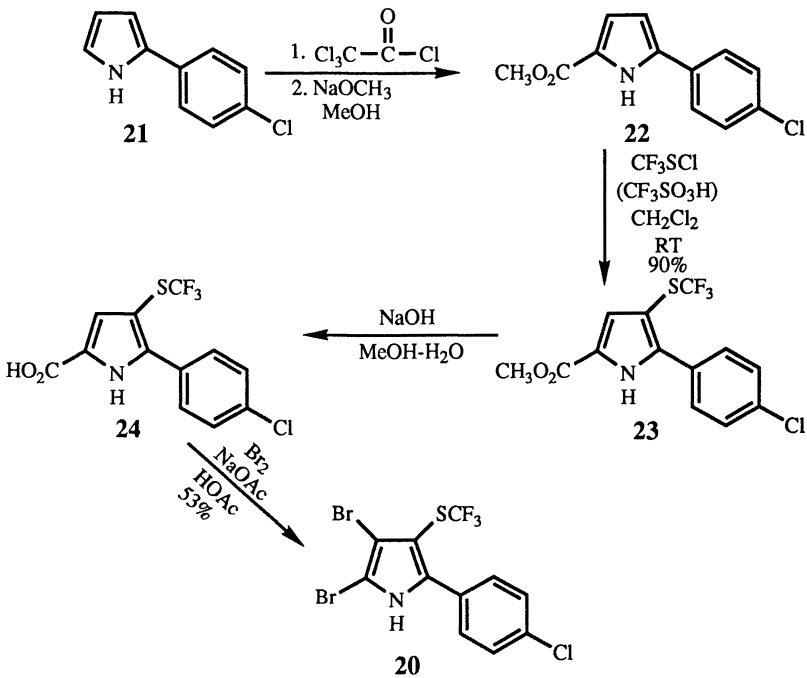
N-Derivatization. Earlier work on insecticidal pyrroles at Cyanamid (1) has shown that N-derivatization, especially with N-ethoxymethyl, can result in an increase in the level and spectrum of insecticidal activity. Consequently the N-ethoxymethyl derivatives of many of the 2-aryl-3-trifluoromethylsulfonylpyrroles were prepared as illustrated by the conversion of **10** to **19** (Figure 6).



10 **19**

Figure 6. Synthesis of N-ethoxymethyl derivatives.

2-Aryl-3-Trifluoromethylsulfenylpyrroles. Figure 7 details the preparation of **20**, the lower oxidation state analog of **10**. Conversion of 2-(4-chlorophenyl)pyrrole **21** to the 5-carboethoxy analog **22** proceeded in excellent yield via the two-step procedure. The carboethoxy group was introduced as a positional blocking/directing group for the subsequent introduction of the trifluoromethylthio functionality. The trifluoromethylsulfenylation proceeds in 90% yield to afford exclusively the 3-substituted product **23**, after overnight stirring at RT with trifluoromethylsulfenyl chloride in the presence of triflic acid. Saponification followed by brominative decarboxylation gave the desired compound **20** in 53% yield after stirring 2h at room temperature. Prolonged reaction times resulted in significantly lower yields. On standing at room temperature this material darkened, indicating some instability.



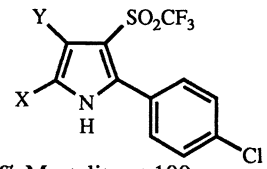
21 **22** **23** **24** **20**

Figure 7. Synthesis of 2-Aryl-3-SCF_3 pyrroles.

Insecticidal Activity. Insecticidal activity was determined using standard leaf dip assays. The compounds in this study were screened against third instar southern armyworms (SAW; *Spodoptera eridania*), third instar tobacco budworms (TBW; *Helicoverpa virescens*), western potato leafhopper (WPLH; *Empoasca abrupta*) and OP-resistant 2-spotted mites (TSM; t*etranychus urticae*). Table II shows the effect of varying the 4 and 5 substituents on 2-(p-chlorophenyl)-3-trifluoromethylsulfonyl pyrrole. The unsubstituted parent compound is devoid of insecticidal activity at 100 ppm. However mono- and especially di-halogenation afforded compounds with good broad spectrum insecticidal activities. Little difference can be noted in activity between the bromo and chloro counterparts. The 5-SCF_3-4-Br, 5-SCF_3 and 5-CN derivatives also demonstrate good activity, however the latter two lost activity at 100 ppm on TBW.

Table II

Insecticidal Activity of 2-(p-Chlorophenyl)-3-trifluoromethyl-sulfonylpyrroles. The Effect of Varying the 4- and 5-Substituents



% Mortality at 100 ppm

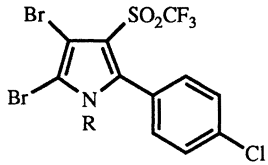
X	Y	Southern Army Worm *Spodoptera eridania* 3rd Instar	Tobacco Budworm *Helicoverpa virecscens* 3rd Instar	2-Spotted Spider Mite *Tetranychus urticae* OP-Resistant	Western Potato Leafhopper *Empoasca abrupta*
H	H	0	0	0	0
Br	Br	100	100	100	100
Cl	Cl	100	100	100	100
Br	H	100	80	100	90
Cl	H	100	50	100	90
CN	H	100	0	100	100
CN	Br	100	0	0	0
NO_2	H	0	0	0	0
SCF_3	H	100	0	100	100
SCF_3	Br	100	100	100	100
$SO2CF_3$	H	100	50	0	0
SO_2CF3	Br	100	0	0	0
$SOCF_3$	Br	0	0	0	0

The effect of varying the aryl ring substituents on insecticidal activity is shown in Table III, wherein the insecticidal activity for a series of 2-aryl-4,5-di-bromo-3-trifluoromethylsulfonylpyrroles is given. As can be seen with these 4,5-di-brominated derivatives, substitution at the para position with chlorine, bromine and trifluoromethyl imparts the highest activity. Substitution with methyl, *t*-butyl, cyano and methoxy gives significantly reduced activity.

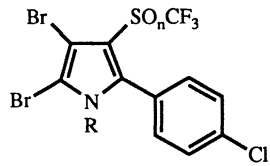
N-derivatization of the 2-aryl-3-trifluoromethylsulfonylpyrroles, generally resulted in either a decrease in the level or spectrum of insecticidal activity. For example, 100% mortality of SAW, TBW, TMS and WPLH at 100 ppm was observed

with **10**. The ethoxymethyl derivative **19,** although retaining SAW, TBW and WPLH activity at 100 ppm, lost TMS activity at 100 and 300 ppm.

The lower-oxidation-state analog of **10,** compound **20** , at 100 ppm gave 100% and 60% control of SAW and TMS respectively. No activity at 100 ppm on TBW and WPLH was observed.



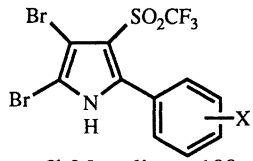
R = H **10**
R = CH_2OEt **19**



n = 2 **10**
n = 0 **20**

Table III

Insecticidal Activity of 2-Aryl-4,5-dibromo-3-trifluoromethylsulfonyl-pyrroles The Effect of Varying the Aryl Substituents



% Mortality at 100 ppm

X	Southern Army Worm *Spodoptera eridania* 3rd Instar	Tobacco Budworm *Helicoverpa virecscens* 3rd Instar	2-Spotted Spider Mite *Tetranychus urticae* OP-resistant	Western Potato Leafhopper *Empoasca abrupta*
H	100	100	90	60
4-Cl	100	100	100	100
4-Br	100	100	100	60
4-F	100	100	0	90
3,4-di-Cl	100	100	0	0
4-CF_3	90	100	100	90
4-CN	100	0	0	0
4-CH_3	0	0	0	0
4-$C(CH_3)_3$	70	0	0	70
4-OCH_3	100	0	0	0

2-Aryl-3-trifluoromethylsulfonyl-5-trifluoromethylpyrroles

From the earlier work at Cyanamid leading to the development of AC 303630, it was discovered that the replacement of the halogen at the 5-position in the 4,5-di-halo-3-cyano-2-aryl pyrroles with a trifluoromethyl group resulted in a significant increase in level and spectrum of insecticidal activity (1,2,4). With this consideration in mind, the synthesis of 2-aryl-3-trifluoromethylsulfonyl-5-trifluoromethylpyrroles was undertaken.

Chemistry. Utilizing previously described cycloaddition chemistry developed at Cyanamid (4), 2-(4-chlorophenyl)-5-trifluoromethylpyrrole **25** was prepared as shown in Figure 8. Reaction of the oxazolinone **26** prepared from 4-chlorophenylglycine **27** and trifluoroacetic anhydride with the vinyl pyridinium salt **28** in pyridine afforded **25** in excellent yield.

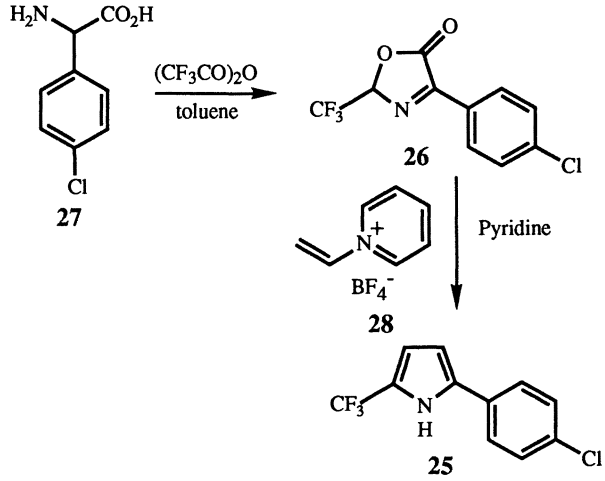


Figure 8. Synthesis of 2-Aryl-5-CF_3 pyrroles.

Reaction of **25** with trifluoromethylsulfenyl chloride (Figure 9), in methylene chloride overnight at room temperature in the presence of a catalytic amount of triflic acid gave exclusively the 3-substituted product **29** in 87% yield. In the absence of triflic acid the reaction proceeded sluggishly. Bromination of this material occurred smoothly to afford the 4-bromo analog, **30**. Attempts to oxidize **30** with MCPBA (room temperature to 70° C) or with hydrogen peroxide/acetic acid (70° to 90°C) gave no reaction. The unbrominated material **29,** however, could be oxidized with MCPBA at 50°C to afford the sulfoxide **31**. Under forcing conditions (hydrogen peroxide/acetic acid, 90°C, 3 days) **29** could be converted to the sulfone **32.**

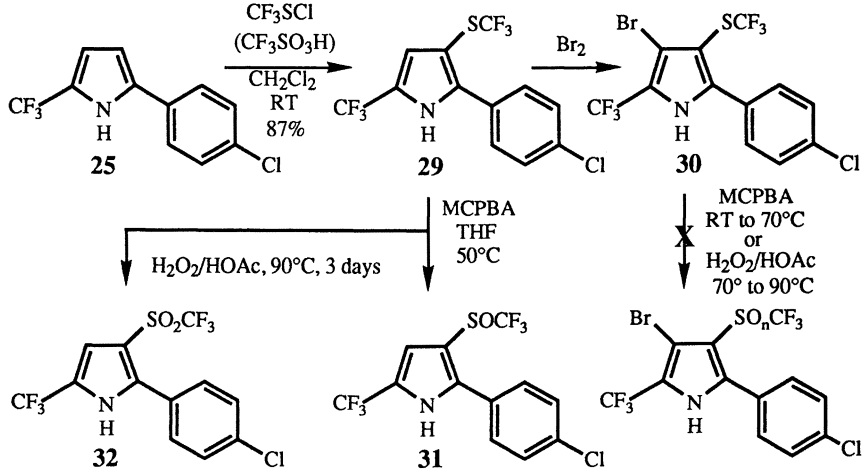


Figure 9. Synthesis of 2-Aryl-3-SO_nCF_3-5-CF_3 pyrroles.

As shown in Figure 10, the sulfone **32** could be brominated in good yield to afford **33**. Attempts to convert **31** to the 4-bromo derivative were unsuccessful. Conversion of the 5-trifluoromethylpyrroles to their N-ethoxymethyl derivatives was carried out via standard procedures.

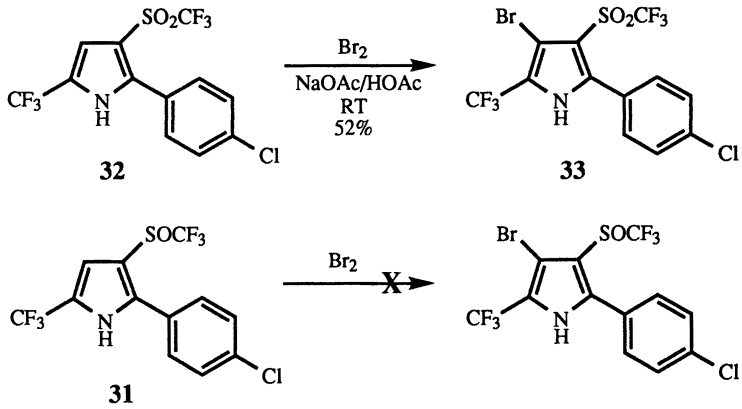
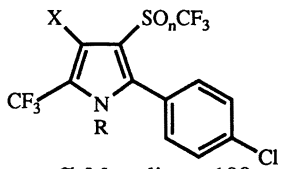


Figure 10. Bromination of 2-Aryl-3-SO_nCF_3-5-CF_3 pyrroles.

Insecticidal Activity. As shown in Table IV, all the compounds prepared having a 5-trifluoromethyl substituent gave good control of SAW and TBW at 100 ppm. N-ethoxymethylation did not greatly alter this activity; however, N-ethoxymethylation had varying effects on TSM and WPLH activity.

Table IV

Insecticidal Activity of 2-(4-Chlorophenyl)-3-trifluoromethyl-sulfonyl 5-trifluoromethylpyrroles



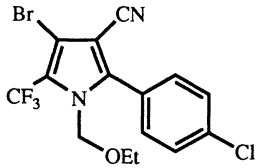
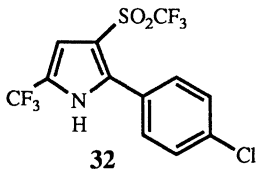
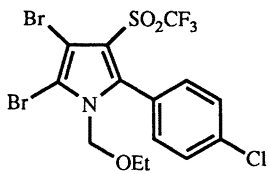
			% Mortality at 100 ppm			
n	X	R	Southern Army Worm *Spodoptera eridania* 3rd Instar	Tobacco Budworm *Helicoverpa virecscens* 3rd Instar	2-Spotted Spider Mite *Tetranychus urticae* OP-resistant	Western Potato Leafhopper *Empoasca abrupta*
0	H	H	100	90	0	0
0	H	CH_2OEt	100	100	0	100
1	H	H	100	100	0	0
1	H	CH_2OEt	100	100	90	100
2	H	H	100	100	100	100
2	H	CH_2OEt	100	100	0	100
0	Br	H	100	90	100	100
0	Br	CH_2OEt	100	100	0	100
2	Br	H	100	100	100	50
2	Br	CH_2OEt	100	90	40	0

Conclusions

The insecticidal activity described in Tables II-IV has been shown as percent mortality at 100 ppm. Many of the compounds discussed were tested at 10 ppm or lower and continued to show good activities across many of the test species at these lower rates.. However, no single compound attained the superior activity levels across as many species as AC 303630, Cyanamid's broad spectrum insecticide currently in advanced development. This is reflected in Table V which compares the activity of AC 303630 to two selected compounds. As can be seen, although **32** demonstrated better SAW and TBW activity than AC 303630, the TSM and WPLH activities were significantly lower. The 4,5-di-bromo-N-ethoxymethyl derivative **19**, showed comparable activity on SAW and WPLH as AC 303630 but had markedly decreased activity on TBW and especially TSM.

Table V

Activity Comparision of AC AC303630 to Selected 2-Aryl-3-trifluoromethylsulfonylpyrroles

	LC_{50} (ppm)			
	SAW	TBW	TSM	WPLH
 **AC 303630**	4.58	7.50	2.46	0.76
 **32**	2.06	2.14	20.0	<1>10
 **19**	5.18	>10	>300	2.02

Although none of the compounds described achieved the benchmark insecticidal activity of the development candidate AC 303630, good insecticidal activity was observed for a number of the pyrroles prepared, demonstrating the utility of the trifluoromethylsulfonyl group when a lipophilic strongly electron-withdrawing functionality, is essential for activity. Additionally, as part of this work a novel method for the construction of 2-aryl-3-trifluoromethylsulfonylpyrroles from acyclic enamine precursors containing the trifluoromethylsulfonyl group was developed.

Acknowledgments

The authors wish to thank Drs David Gange and Stephen Donovan for their work in developing an understanding of the structure-activity relationships in this area of chemistry and Dr. Roger Addor for his interest and support of this work.

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Chapter 28

Monoterpenoids and Their Synthetic Derivatives as Leads for New Insect-Control Agents

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Monoterpenoids are natural substances which are found in many higher plant species. These plant secondary metabolites are generally considered as self-defense tactics against the plants' enemies. Our study was aimed at the anticipation of improved biological activities through the synthesis of derivatives of the natural monoterpenoids. We have also developed a systematic bioassay system to evaluate the spectrum of toxicities of the monoterpenoids. The results show that the monoterpenoids, either natural or synthetic, have a relatively wide spectrum of activity against agricultural and public health insect pests. Derivatization, especially, to the acyl derivatives of the monoterpenoids, has significantly improved the acute, fumigant, larvicidal and ovicidal activities against the above insects. The ether derivatives also showed enhanced insecticidal activity. When mosquito larvae were treated with the monoterpenoids at sublethal dosages, insect growth and development activity was observed. The enhanced biological activity of the synthetic derivatives of monoterpenoids indicates that optimal chemical structures for insecticides can be possibly elucidated through the study of structure-activity relationships.

Plant secondary metabolites are a group of natural products which includes thousands of alkaloids, terpenoids, phenolics and minor secondary chemicals. The biological significance of these natural substances is not always clear. One of the theories states that these plant-origin chemical substances have no known biological functions in plant's photosynthesis, growth, or other basic aspects of plant physiology. They are considered merely by-products from the biosynthesis of essential plant hormones and metabolites (*1*). However, it is reported that terpenoids play a role in the enzyme systems of plants. They are produced during the plant's dormancy period to maintain the activity of the enzyme systems such as cytochrome P-450 monooxygenase (*2, 3*). The most dominant opinion regarding to the biological function of the plant secondary products is probably the theory that the primary purpose of these compounds is defense against the plant's enemies. They repel herbivorous predators such as insects and microorganisms and other parasitic organisms such as nematodes and other competing plant species (*4-6*). Monoterpenoids belong to the terpenoids

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which is one of the most abundant and potent groups among the naturally-occurring substances having biological activities on insects. These compounds are terminologically ten-carbon compounds based on two isoprene units, and their biosynthesis follows the same pathway (mevalonate pathway) as those of the plant hormones and steroids. Their biological activities with insects, nematodes, phytopathogenic fungi and other plant species are well documented in the recent explosion of literature in chemical ecology (*4, 6, 7*). Monoterpenoids are widely distributed in the plant essential oils, the steam-volatile, odorous constituents of many plant materials, and are industrially the most important among the various types of terpenoids. They are used as artificial flavorings, perfumes, decongestants, external analgesics and antiseptics (*8, 9*). Some of them such as *d*-limonene are used as an active ingredient of some commercially available flea shampoos (*10*) to control those insects on pets. Many of the monoterpenoids are found to possess attractant, repellent, feeding deteriorate, and ovipositional stimulant activities against various insect species (*11*). Although some of them are also acutely toxic to insects, their acute insecticidal activity is, generally, not as potent as those of the commercial insecticides. *Mentha citrata* Ehrhart oil, containing linalool and linalyl acetate has shown significant fumigant toxicity to the rice weevil, *Sitophilus oryzae* (L.) (*12*). Pulegone has prolonged larval development, delayed pupal molt, and reduced pupation success in *Spodoptera eridania* (Cramer) and the southern armyworm (*3*). *d*-Limonene has been reported to be toxic to *Dendroctonus* pine beetles (*13, 14*), and also inhibits embryonic development in the cat flea, *Ctenocephalides felis* (Bouche) (*15*). More complete reviews of the insecticidal activity of monoterpenoids have been recently published (*4, 16*). The biological activities of the monoterpenoids, as well as many other successful cases of natural products, are believed to be related to certain functional groups. It is suggested that the repellent activity of several monoterpenoid compounds are dependent upon the position of the functional groups and the molecular configuration rather than the volatility and molecular size (*17*). Chemical modification of the natural forms of monoterpenoids may lead to improved biological activity (*18*). This research was conducted to evaluate the insecticidal and other biological activities of monoterpenoids through systematic bioassays; to synthesize various analogs of the natural monoterpenoids and compare the respective bioactivities with their parent compounds; to initiate structure-activity relationships; to consider the mode of action of these compounds; and finally to explore new possible insect pest control agents through a biorational approach.

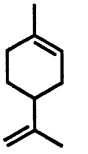
Materials and Methods

The natural monoterpenoid compounds were purchased from Aldrich Chemical Company (Milwaukee, WI). These monoterpenoids were employed in the bioassays with or without purification, depending on the purity. Some of these compounds were used as starting materials for the syntheses of the monoterpenoid derivatives. Other reagents and solvents were all commercial products. Compounds discussed in this paper are shown in Figure 1.

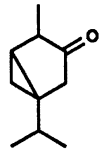
The synthetic derivatives were purified by using the preparative thin layer chromatography (TLC) plates (Whatman, No. 4861 840, 1 mm layer, fluorescent at 254 nm) and column chromatography techniques. TLC plates were visualized under ultraviolet light (254 nm) for those have UV absorption, or by spraying $KMnO_4$ solution for those have no UV absorption. Structures are confirmed using nuclear magnetic resonance (NMR) spectrum.

Syntheses of Monoterpenoid Derivatives.

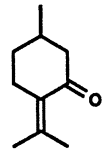
Synthesis of the Acyl Derivatives. The syntheses of acyl derivatives were achieved by using two synthetic routes depending on the starting materials used. The monoterpenoidal alcohols and phenols were used in the reactions.



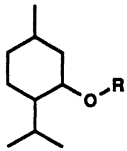
Limonene



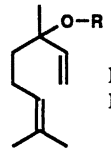
Thujone



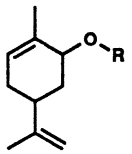
Pulegone



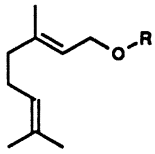
Menthol (R=H)
Menthyl pivalate (R= C(O)–t-Bu)



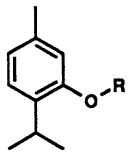
Linalool (R=H)
Linalyl acetate (R= $C(O)CH_3$)



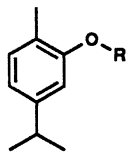
Carveol (R=H)
Carvyl acetate (R= $C(O)CH_3$)
Carvyl trichloroacetate (R= $C(O)CCl_3$)
Carvyl pivalate (R= C(O)-t-Bu)



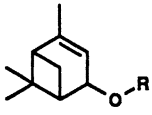
Geraniol (R=H)
Geranyl acetate (R= $C(O)CH_3$)
Geranyl chloroacetate (R= $C(O)CH_2Cl$)
Geranyl trifluoroacetate (R= $C(O)CCl_3$)
Geranyl trichloroacetate (R= $C(O)CF_3$)
Geranyl pentafluoropropionate (R= $C(O)CF_2CF_3$)



Thymol (R=H)
Ethyl thymyl ether (R= CH_2CH_3)
2-Fluoroethyl thymyl ether (R= CH_2CFH_2)
Thymyl acetate (R= $C(O)CH_3$)
Thymyl chlorodifluoroacetate (R= C(O)-$CClF_2$)
Thymyl pentafluoropropionate (R= $C(O)CF_2CF_3$)



Carvacrol (R=H)
Carvacryl 2-fluoroethyl ether (R= CH_2CH_2F)
Carvacryl isopropyl ether (R= $CH(CH_3)_2$)
Carvacryl trifluoroacetate (R= $C(O)CF_3$)
Carvacryl pentafluoropropionate (R= $C(O)CF_2CF_3$)
Allyl carvacryl ether (R= CH_2-CH=CH_2)
Carvacryl ethyl ether (R= CH_2-CH_3)



Verbenol (R=H)
Verbenyl trifluoroacetate (R= $C(O)CF_3$)

Figure 1. Chemical structures of the monoterpenoids discussed.

Haloacyl Derivatives. Acyl derivatives of the monoterpenoidal alcohols or phenols were synthesized by the reaction with acid anhydrides or acid halides in the presence of catalytic quantity of pyridine in CH_2Cl_2 at 0 °C. Usually, 0.01 mol of the starting material (monoterpenoid) was dissolved together with ca. 1 ml of pyridine in 50 ml of CH_2Cl_2 in an Erlenmeyer flask, and cooled in an ice-bath (otherwise the reaction goes too fast to give dehydration products from the monoterpenoid). Equimolar or a slight excess of the acid anhydride or the acid halide was then slowly added while the reaction solution was stirring constantly. The reaction period ranged from a few minutes to several hours, depending on the individual starting material and the reagent used in each case. The reaction solution was washed with dilute HCl to remove pyridine, and the organic layer was then concentrated and the product separated, followed by purification using TLC or column chromatography. The yield was 75-100%.

Acyl Derivatives with a Monoterpenoidal Acid Moiety. Acyl derivatives of natural monoterpenoidal acid were also synthesized from the monoterpenoidal alcohols and phenols. The reaction was achieved by using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) as a condenser and catalyst. The monoterpenoidal alcohol or phenol (0.01 mol) and the acid (0.01 mol) were dissolved or suspended in 15 ml of dry methylene chloride in a 50-ml round-bottom flask. Equimolar DCC together with or without DMAP was added under constant stirring. This reaction occurs at room temperature, and it is exothermic. DMAP is not necessarily always needed for this reaction, and the solvent CH_2Cl_2 can also be replaced by dry toluene or chloroform. The reaction period also varies from a few hours to several days depending on both the acid and alcohol used. After completion, the reaction mixture was diluted with CH_2Cl_2 and washed with dilute HCl and water. The product was also separated and purified by using TLC or column chromatography. The yield of this reaction ranges from 75 to 100%.

Synthesis of the Ether Derivatives. The syntheses of ether derivatives were carried out by using two synthetic routes depending on whether the starting material is aromatic (phenolic) or aliphatic.

Ether Derivatives of Phenolic Monoterpenoids. The ether derivatives of the phenolic monoterpenoids were synthesized from a monoterpenoidal phenol and an alkyl halide in the presence of the phase transfer catalyst, benzyltributylammonium bromide (BTAB). The starting phenol (0.01 mol) was dissolved in 50 ml of CH_2Cl_2 together with the alkyl halide (0.03 mol) and BTAB (0.0005 mol). NaOH (0.015 mol) was dissolved in 50 ml of water. The two solutions were then mixed together and stirred at room temperature. This reaction was only successful using the aromatic alcohol and the alkyl halide. The reverse, i. e. using an aliphatic alcohol and an aryl halide did not yield the desired product. The yield of this reaction varied depending on both the phenol and the alkyl halide used, and the reaction period. Some reactions need more than two weeks to reach 50% of the calculated yield. The halogenated alkyl halides slowed down the reaction and reduced the yield.

Ether Derivatives of the Aliphatic Alcohol Monoterpenoids. This reaction was carried out to make ether derivatives from two different aliphatic alcohol molecules. Two different alcohols (0.01 mol each) were dissolved in 25 ml of pyridine in a flask. Tosyl chloride (TsCl) (0.01 mol) was then added. The mixture was kept stirred at 0 °C. Another 0.01 mol of TsCl was added 2 hours later. The reaction was kept at 0 °C for at least 10 hours. This reaction occurred most readily between primary alcohols. When completed, the reaction mixture was diluted with ethyl ether and washed with saturated $NaHCO_3$. The ether layer was further washed with 1N HCl, saturated $NaHCO_3$ and NH_4Cl. Purification of the product was achieved using column chromatography. The yield of this reaction was 50-80%.

Bioassays

Bioassay is important and crucial in evaluating the insecticidal activity of a compound. Different exposure routes for one particular compound may cause different degrees or types of toxic activity against one specific insect species. Also, the same or similar activity may be observed from several different compounds in a single bioassay. In this study, the bioassays for the monoterpenoids and their synthetic derivatives include a topical application test on the house fly (*Musca domestica*) adults; fumigation tests against the house fly, the German cockroach (*Blattella germanica*), the red flour beetle (*Tribolium castaneum*), the rice weevil (*Sitophilus oryzae*), and the sawtoothed grain beetle (*Oryzeaphilus surinamensis*) adults; larvicidal tests on the western corn rootworm (*Diabrotica virgifera virgifera*) and the yellow fever mosquito larvae (*Aedes aegypti*); and an ovicidal test on the house fly eggs. These important agricultural and public health insects were mostly reared in this laboratory. The detailed procedures for the bioassays on these insect species have been published elsewhere (*18, 19*).

Results and Discussion

The monoterpenoids are generally less acutely toxic than the other natural insecticides such as pyrethrins and the conventional insecticides (*18, 19*). However, different monoterpenoidal compounds show different levels of toxicity against different insect species. Some insects are more susceptible to some of the monoterpenoids, while others may not be affected by any monoterpenoid at all. Through the relatively wide range of bioactivity survey, we found that the monoterpenoids had a relatively wide spectrum of activity, and some of them, especially the synthetic derivatives of the natural monoterpenoids had shown promising toxicities to some important agricultural and public health insect species.

Table I. Acute Toxicity of the Monoterpenoids and Synthetic Derivatives to House Fly Adults, *Musca domestica*

Compound	LD_{50} (μg/insect)	95% conf. int.
Geraniol	130	95-11
Geranyl acetate	55	50-60
Geranyl chloroacetate	39	34-43
Linalool	189	178-200
Menthol	193	171-217
Menthyl pivalate	85	73-98
Carveol	282	252-318
Carvyl pivalate	88	83-94
Carvacrol	63	60-65
Thymol	33	30-36

Acute Toxicity by Topical Application Tests. The acute toxicity of natural monoterpenoids against the house fly adults have been reported in detail elsewhere (*18*). Geraniol, carveol, linalool, menthol, and verbenol are some typical alcoholic monoterpenoids, and they had relatively low acute toxicity against the house fly adults. However, by introducing a small acid moiety to these alcoholic

monoterpenoids, a significantly enhanced topical insecticidal activity was observed. These acyl derivatives, which may or may not have one or more halogen atoms in the acid moiety, showed significantly improved topical toxicity as compared to their parent compounds (Table I).

Thujone is a naturally occurring monoterpenoid. The LC_{50} against the mosquito larvae (2nd instar) was 228±30 ppm after 24 hours of direct exposure to the aqueous solution of thujone. Its activity against the mosquito larva was dose-dependent. At higher concentrations, it showed acute toxicity while at lower concentrations, thujone showed the insect growth regulatory (IGR) activity (Table II). One example of a synthetic monoterpenoid derivative, carvyl pivalate produced an LC_{50} of 73±3 ppm in the same test. The activity of this compound was also dose-dependent and showed a tendency similar to that of thujone (Table II).

Table II. Acute Toxicity of Two Monoterpenoids to the Yellow Fever Mosquito Larvae, *Aedes aegypti* (2nd instar)

Compound	LC_{50} (ppm)	95% conf. int. (ppm)
Thujone	228	200-261
Carvyl pivalate	73	70-76

Though the QSAR has not been completed and the improvement in insecticidal activity by introducing one particular acid moiety does not always happen to other groups of the monoterpenoids, or to those even in the same group, these results still imply a great possibility that more acutely toxic monoterpenoids can be reached. This is of great importance, since it tells us that the highest acute toxicity of this group of monoterpenoids can be possibly approached by simply derivatizing the naturally occurring compounds.

The mode of action of the acute toxicity of monoterpenoids against insects is not clearly understood. However, Karr et al. (*16, 20*) in this laboratory have previously revealed that a very common monoterpene, *d*-limonene, has neurotoxic effects on the earthworm. When the worm was acutely exposed to *d*-limonene, significant decreases in conduction velocity in the medial and lateral giant nerve fiber pathways were observed. The magnitude of the decrease in conduction velocity was directly related to the concentration and duration of the exposure. This implies that the effect of monoterpenoid on the nerve system of insect might be similar.

Fumigation Tests. Some monoterpenoids and their synthetic derivatives are found to possess comparatively high fumigant properties. There was a distinct correlation between the fumigant toxicity and volatility. The more volatile monoterpenoids were the more effective fumigants (*19*). Carvacrol is an aromatic (phenolic) monoterpenoid. It showed high mortality to the house fly adult and the sawtoothed grain beetle in a fumigation test, whereas only slight or no effect was observed on the red flour beetle, rice weevil, and the German cockroach (Table III). All insects in this fumigation test were exposed to the chemical at a concentration of 50 $\mu g/cm^3$ and exposed for 14 hours. Derivatization may change the spectrum of fumigation activity. As shown in Table III, one of the ether derivatives of carvacrol, isopropyl carvacryl ether, showed greatly improved fumigation activities, especially against the German cockroach and the red flour beetle. An acyl derivative, carvacryl pentafluoropropionate, also demonstrated enhanced toxicity against the German

cockroach, while it was less toxic against the sawtoothed grain beetle as compared to the parent carvacrol (Table III). A similar phenolic monoterpenoid compound, thymol, showed less toxicity to the insect pests in the fumigation test, and the derivatization of thymol seemed not to improve its fumigation activity as greatly as it did for the carvacrol derivatives. The ether derivatives of thymol were slightly more toxic than the parent to the house fly adult, but significantly less toxic to the sawtoothed grain beetle. The position of the hydroxyl group on the benzene ring seemed not to be very important to the parent alcohols such as thymol and carvacrol, in the exertion of fumigation activity. The alkyl group of the ether derivatives might play a crucial role in the expression of the fumigant toxicity.

Table III. Insecticidal Activity of the Monoterpenoids in Fumigation Test against the House Fly (HF), the German Cockroach (GC), the Red Flour Beetle (RFB), the Rice Weevil (RW), and the Sawtoothed Grain Beetle (SGB) Adults (50 μg/cm^3, 14 hours)

	Mortality (%)				
Compound	HF	GC	RFB	RW	SGB
Carvacrol	100	0	10	10	86
Carvacryl 2-fluoroethyl ether	100	0	10	0	0
Carvacryl isopropyl ether	100	100	50	0	100
Carvacryl trifluoroacetate	90	0	0	0	14
Carvacryl pentafluoropropionate	100	50	0	0	38
Thymol	90	0	10	10	88
Ethyl thymyl ether	100	0	0	0	10
2-fluoroethyl thymyl ether	100	0	0	0	0
Control	0	0	0	0	0

Table IV. Insecticidal Activity of the Monoterpenoids in a Fumigation Test against the House Fly Adult, *Musca domestica* (14 hours)

Compound	LC_{50} (μg/cm^3)	95% conf. int.
Geraniol	>1780	-
Geranyl trifluoroacetate	11	3-38
Linalool	6.8	6.6-6.9
Menthol	3.6	2.5-5.2
Carvacrol	27	23-32
Carvacryl trifluoroacetate	27	22-34
Thymol	143	95-214
Thymyl acetate	40	19-87
Thymyl chlorodifluoroacetate	14	13-15

The fumigation activities of a variety of different types of monoterpenoids including the alcohols were surveyed (*18*). The results have shown that the fumigation activity of the alcoholic monoterpenoids can be enhanced significantly by acylating the hydroxyl group (Table IV). Both natural and unnatural esters have been tested. The enhanced fumigant toxicity may be attributed to several factors such as the increased volatility and permeability.

Larvicidal Tests. Western corn rootworm is one of the most damaging insects on corn in the corn belt area in the USA. A highly effective larvicide on the western corn rootworm would be expected to prevent a considerable loss of the corn production from the damaging of this insect. We have tested several series of monoterpenoids and their synthetic derivatives against the western corn rootworm larva in the laboratory and found that some monoterpenoid compounds were insecticidal to this agricultural insect pest. This has currently led us to a larger scale experiment in the green house (data not shown). In the laboratory tests, the phenolic monoterpenoid thymol was toxic to the second-instar western corn rootworm at >100 ppm (chemical/soil), after 24 h of exposure. An ether derivative, 2-fluoroethyl thymyl ether showed an equal or even higher larvicidal activity, while others showed reduced mortality (Table V). Another phenolic monoterpenoid, carvacrol, only showed a lower larvicidal activity as compared to the thymol, but a greater improvement in the bioactivity was obtained by modification of this monoterpenoid. For example, at 200 ppm, carvacrol killed 50% of the larvae tested, whereas its trifluoroacetate and ethyl ether killed 100% and 80% of the worms, respectively (Table VI). Neither acyl nor ether derivatives of the linear monoterpenoid geraniol showed enhanced larvicidal activity against the western corn rootworm, though only one example, geranyl pentafluoropropionate, is given in Table V.

Ovicidal Test. This test was conducted in an aqueous solution of the monoterpenoid compound in which the newly laid house fly eggs were dispersed. Generally, the alcoholic monoterpenoids were ovicidal at the test concentration (833 μg/ml) (*18*). The smaller acyl derivatives showed enhanced inhibitory activity on the egg hatching. Acetates of the alcoholic monoterpenoids were more ovicidal than the haloacetates in this test (Table VII).

Insect Growth and Development. As we discussed in the acute toxicity section, at higher doses monoterpenoids and their synthetic derivatives showed acute toxicity against the yellow fever mosquito larva when the larva was directly exposed to the aqueous solution of the monoterpenoidal chemical. However, when we treat the larva at sublethal dosages, interesting insect growth and development activity was observed. Still using thujone and (-)-carvyl pivalate as examples, on the 13th day of the treatment, in the control 55% of the larvae still remained in the larval stage, and 20% and 25% in the pupal and adult stages, respectively. However, when the mosquito larvae were treated with thujone the same day 13 showed the percentages in each stage to be disturbed. At 10 ppm, the percent of mosquitoes in the larval stage was reduced to 27%, while the pupal and adult stages were increased to 32% and 41%, respectively. This shift was further obvious at the concentration of 100 ppm. At this dosage, the percent larvae was further reduced to only 8%, and so was the percent pupae. The greatest percent (ca. 84%) of the tested mosquitoes were in the adult stage (Figure 2). This observation tells us that at the sublethal dosages, the growth (life cycle) of mosquito may be quickened by the treatment of the monoterpenoid compound, and the effect can be made stronger in a fashion with the increase of the concentration, i. e. the higher the concentration, the higher the growth effect. This seems also true to the monoterpenoid derivatives. Similar and more dramatic results were obtained with carvyl pivalate (Figure 3). In this case, when the

Table V. Insecticidal Activity of the Monoterpenoids in the Soil Application Test against the Western Corn Rootworm, *Diabrotica virgifera virgifera* (24 hours)

	Mortality (%)			
Compound	1000	100	10	1 ppm
Thymol	100	100	0	0
Thymyl pentafluoropropionate	100	60	0	0
2-fluoroethyl thymyl ether	100	100	0	20
Geraniol	100	90	0	0
Geranyl pentafluoropropionate	100	0	0	0
Control			0	

Table VI. Insecticidal Activity of Carvacrol and its Derivatives in the Soil Application Test against the Western Corn Rootworm, *Diabrotica virgifera virgifera* (24 hours)

	Mortality (%)		
Compound	1000	200	40 ppm
Carvacrol	100	50	10
Carvacryl trifluoroacetate	100	100	10
Allyl carvacryl ether	100	50	0
Carvacryl ethyl ether	100	80	10
Carvacryl isopropyl ether	100	0	0
Control		0	

Table VII. Ovicidal Activity of the Monoterpenoids on the Newly Laid Eggs (less than 12 hours old) of the House Fly (*Musca domestica*)

Compound (833 μg/ml of water)	Inhibition of egg hatch (%)
Geraniol	99
Geranyl acetate	89
Geranyl trichloroacetate	44
Geranyl trifluoroacetate	56
Linalool	87
Menthol	47
Carveol	56
Carvyl acetate	73
Carvyl trichloroacetate	24
Carvacrol	99
Carvacryl acetate	93
Carvacryl trifluoroacetate	0
Verbenol	0
Verbenyl trifluoroacetate	93
Control	0

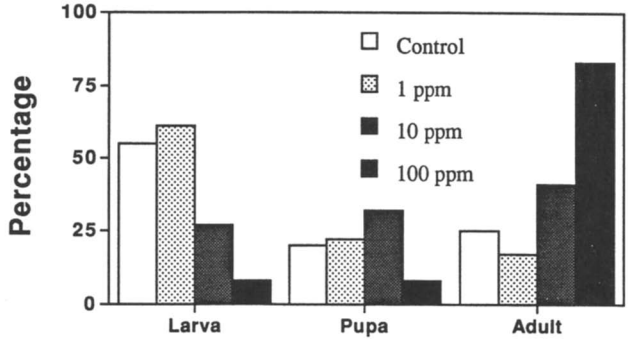


Figure 2. Insect growth and development effect of thujone on the yellow fever mosquito larva *Aedes aegypti* (2nd instar).

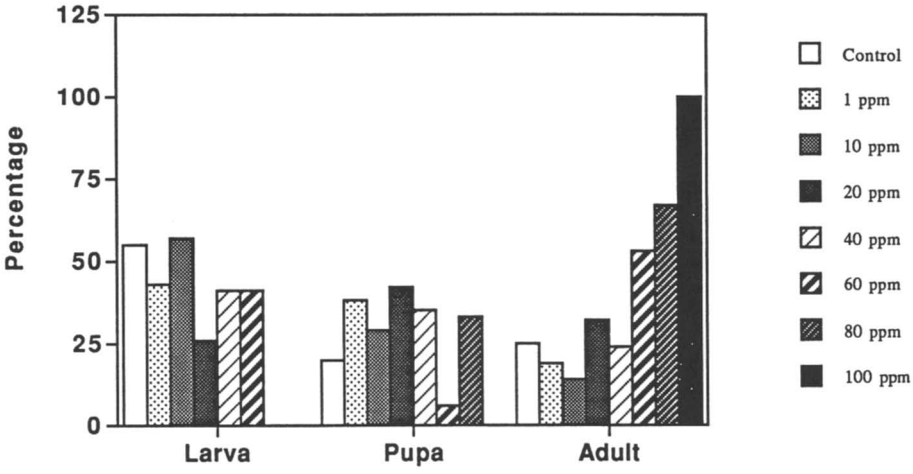


Figure 3. Insect growth and development effect of carvyl pivalate on the yellow fever mosquito larva *Aedes aegypti* (2nd instar).

concentration was higher than 80 ppm, all of the mosquitoes became pupae or adults, and when treated with 100 ppm, 100% of the treated mosquitoes emerged to adults on the 13th day. A possible insect growth regulator (IGR) effect may be involved, or the additional monoterpenoids may be supplying an essential precursor for hormone synthesis, thereby enhancing the rate of growth and development.

The above results imply a wide spectrum of insecticidal activity of the natural monoterpenoids and their synthetic derivatives, in spite of their chemical ecological properties such as attractants and repellents which have been reviewed and discussed by many researchers (*4-6, 21*). These compounds had the potential of being acute, ovicidal, fumigant, and insect growth regulatory insecticides against various insect species. The modes of action of the monoterpenoids seemed as diverse as their biological activities, and many of them are not well understood. However, we think that the neurotoxic and the IGR effect may be at least part of the mechanisms. Brattsten reported that monoterpenoids were able to induce insect cytochrome P-450, which is a very important enzyme system affecting the biosyntheses of insect hormones and pheromones. In the presence of such monoterpenoids, the disturbed hormone balance might influence the growth of the insects. Monoterpenoids also affect the reproductive success of insects (*3*).

The wide spectrum of insecticidal activity of monoterpenoids and their synthetic derivatives indicates that this is a group of natural products with latent insecticidal potential. The enhanced activities shown by some of the derivatives of the natural forms of monoterpenoids also indicate that optimal structures can be elucidated through biorational design of the derivatives. Through the establishment of the structure-activity relationships, we believe that a moderately toxic new group of insecticides is rationally achievable. There are several advantages of using monoterpenoids as lead compounds for new insecticides. The first is their availability. Monoterpenoids are an old but poorly understood group of natural products. These compounds are naturally occurring, and exist abundantly in some plants, marine algae, and insects (*4-6*). They can be relatively easily modified to various derivatives in the laboratory to improve their bioactivity. The second is their bioactivity. With more than 1000 monoterpenoids identified so far, many have been shown to possess interesting chemical ecological properties. They are chemical clues for communications between the plant and insect, within insects, and within plants.

Those monoterpenoids found in marine algae mostly contain halogen(s). These halogenated monoterpenoids generally have high biological activities (5). Our studies show that the monoterpenoid compounds, natural and synthetic, have a wide spectrum of activity against various insect species. Introducing a halogenated moiety or other group to the natural monoterpenoids may enhance their bioactivities by strengthening the affinity for a receptor or facilitating the penetration of the compound through the insect cuticle or membranes. Thirdly, monoterpenoids are relatively a safe group of natural products. Generally, they are safe to humans and other mammals, and are considered environmentally safe as well.

Conclusion

Utility of a chemical in insect control depends upon several factors which include its spectrum of activity, the potency of the chemical, and how the chemical is delivered. The spectrum of activity of the monoterpenoids, as described above, is relatively wide. The potency, however, for most natural monoterpenoids, is modest, at least when regarded as an acute toxicant to the insects. However, in our study, by adequately modifying the naturally-occurring monoterpenoids, higher insecticidal activities have been obtained. Acyl and ether derivatives of the alcoholic monoterpenoids are good examples. These esters and ethers are generally more lipophilic than their parent compounds, which might contribute to more rapid penetration of the chemical. It is possible that these derivatives, once entered into the insect body, might be hydrolyzed *in vivo* to the parent monoterpenoidal alcohol, the toxic principal, which then reacts with the receptor(s) to exert the bioactivity.

Data presented in this paper are preliminary. More biologically active monoterpenoidal analogs are being developed, with the aid of the structure-activity relationships study. The studies on the modes of action of the monoterpenoids will also provide feedback for the exploration of new possible insect control agents.

Acknowledgment

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Chapter 29

Fungicidal β-Methoxyacrylates

N-Linked Pyrroles

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Methyl β-methoxy-α-pyrrol-1-ylacrylates **7** (Figure 2), which we have termed *N*-linked pyrroles, have a broad spectrum of fungicidal activity when suitably substituted at the 2-position of the pyrrole ring. This paper describes the synthesis of a variety of acrylates of this type, and outlines structure-activity relationships for this class of compound.

We have reported previously on the origins of our interest in the β-methoxyacrylates, and the evolution of ideas which led from a family of natural products, such as strobilurin A, to ICIA5504 (Figure 1)(*1-5*). The main driving forces behind this work were the need to improve the stability and levels of activity of the natural products, and the desire to discover compounds which move systemically in plants without producing phytotoxic effects.

During the course of this project, we discovered that although most structural changes to the (*E*)-methyl β-methoxyacrylate unit found in the natural products cause a sharp fall in fungicidal activity, many modifications are permitted to the group to which this unit is attached. For example, the β-methoxyacrylate toxophore can be linked to an *ortho*-substituted benzene ring, and this is a feature of many of our most active synthetic compounds, such as ICIA5504 itself. In view of the high activity of these analogues of the natural products, it occurred to us that related compounds in which the toxophore is linked to a *heterocycle* might also be active. Of course, many possible heterocyclic systems could be envisaged, and it could not be predicted at the outset which of these would lead to active compounds. Indeed, the synthesis and testing of numerous different classes of such compound has confirmed that some have good activity, while others have little or none. Amongst those which were found to be active were pyridines **1** (*6-8*), furans **2** and **3** (*9*), thiophenes **4**, **5** and **6** (*9*), pyrroles **7** (*10-12*) and **8** (*9*), imidazoles **9** (*10*), pyrazoles **10** (*10*) and indoles **11** (*13*), where X represents a variety of substituents in each case (Figure 2).

The subject of this paper is our work in one of these areas, the synthesis and fungicidal activity of compounds in which the β-methoxyacrylate toxophore is linked

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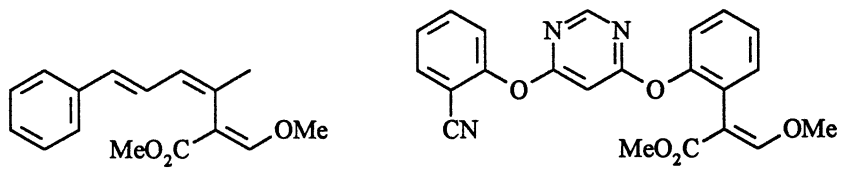


Strobilurin A ICIA5504

Figure 1. Strobilurin A and ICIA5504.

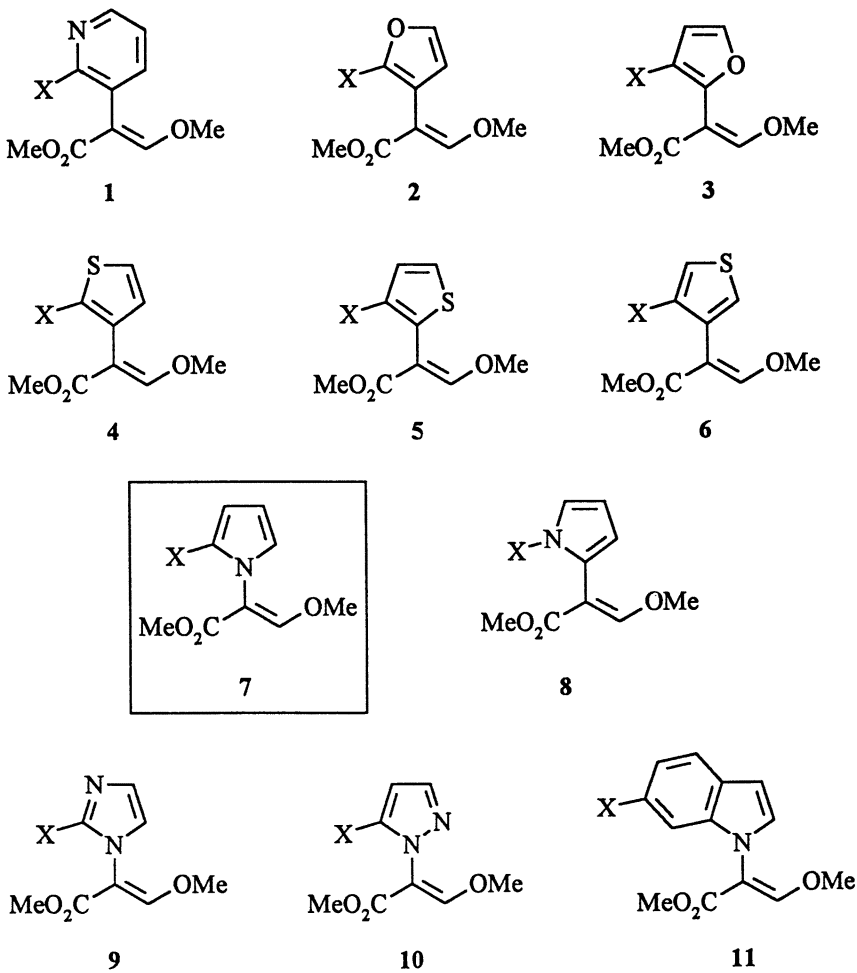


Figure 2. Heterocyclic β-methoxyacrylates.

to the nitrogen atom of a pyrrole ring to give compounds **7**, which we have termed *N*-linked pyrroles. These compounds, when the substituent X has a suitable value, have good fungicidal activity; as with strobilurin A and ICIA5504, this is a result of their ability to inhibit mitochondrial respiration in fungi. They have the added attraction of being easy to prepare.

2-Styrylpyrroles

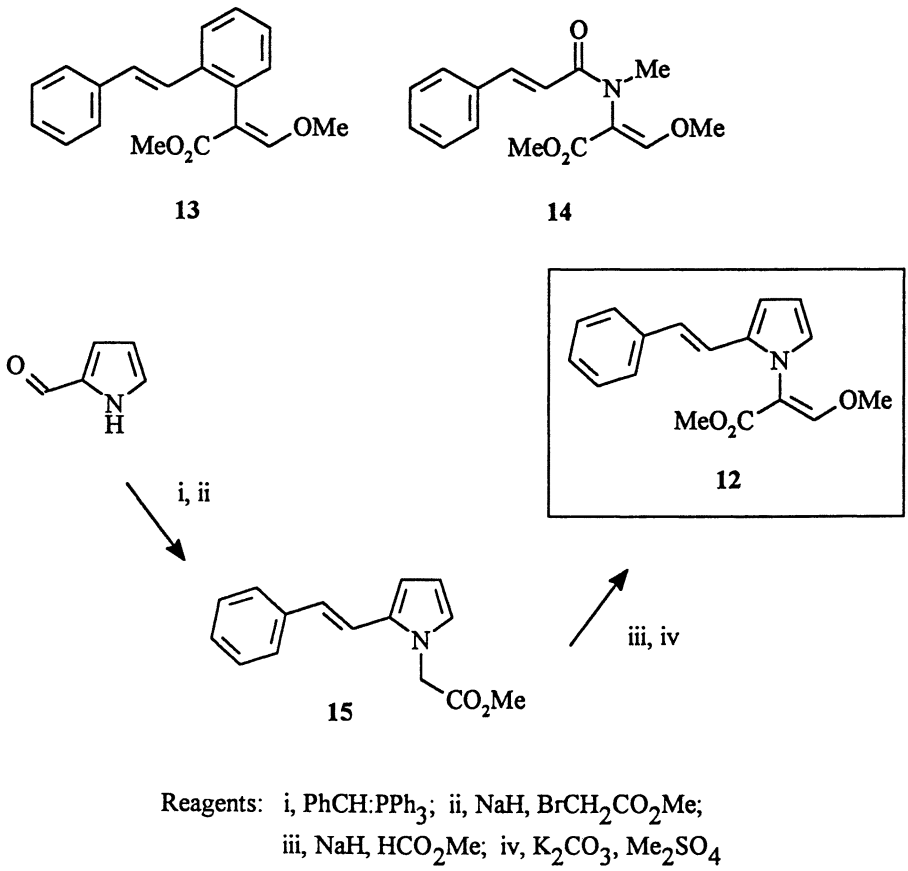
The 2-styrylpyrrole **12**, an analogue of both the stilbene **13** (*14*) and the amide **14** (*15*), both of which had earlier been shown to be good fungicides (*1*), was selected as the first target for synthesis. It was readily prepared from 2-formylpyrrole by the four steps shown in Figure 3. Thus 2-formylpyrrole reacted successively with benzylidene triphenylphosphorane and methyl bromoacetate to give, after chromatography and crystallisation, the (*E*)-styrylpyrrolylacetate **15**. Claisen condensation with methyl formate then gave a β-hydroxyacrylate which, on *O*-methylation with dimethyl sulphate, led stereospecifically to the required styrylpyrrole **12** (*10*).

Although the styrylpyrrole **12** was a potent inhibitor of mitochondrial respiration [IC_{50} = 40 nM, equivalent to the stilbene **13**, mitochondria isolated from lamb's heart tissue (*1*)], it was only weakly active in the glasshouse. This difference could be explained by the very short photochemical persistence of the styrylpyrrole: as a thin film, it was rapidly degraded on irradiation with a xenon lamp which simulated sunlight. These results were similar to those recorded earlier for the stilbene **13** (*1,2*), although the styrylpyrrole **12** was even less stable than the stilbene under the xenon lamp, with the time taken for loss of the first 50% of the two compounds being one and three minutes, respectively. The degradation products from the styrylpyrrole were not identified. Nevertheless, it is known that electron-rich pyrroles are readily photo-oxidised (*16*) and photochemically-induced electrocyclisation reactions of 2-styrylpyrroles have also been described (*17*).

2-Benzoylpyrroles

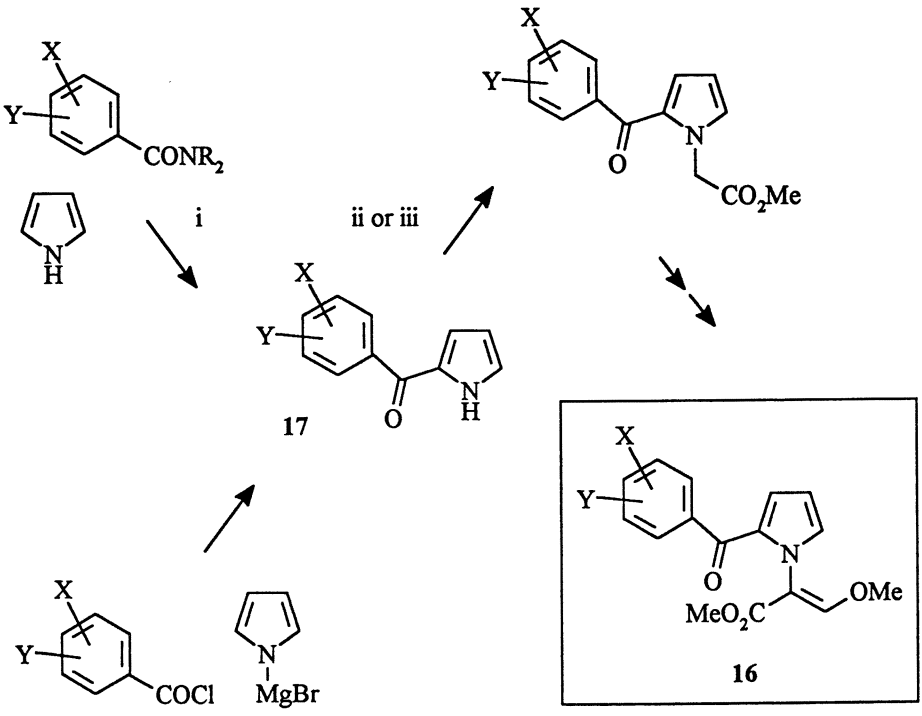
In general, pyrroles become less susceptible to photo-oxidation when substituted with electron-withdrawing groups (*16*). Consequently, 2-benzoylpyrroles **16** were chosen as our next targets for synthesis, and these were prepared by the steps shown in Figure 4. The required intermediates **17** were generally made from pyrrole itself and the Vilsmeier-Haack reagents derived from benzamides and phosphorus oxychloride (*18*). These reactions led in good yields to the required 2-benzoylpyrroles, except in cases where the substituents on the benzamide were too electron-withdrawing or sterically-demanding and the Vilsmeier-Haack reagents formed too slowly to be practicable. In such cases, an alternative route to the intermediates **17**, involving the reaction between pyrrol-1-ylmagnesium bromide and benzoyl chlorides, was used. A disadvantage of this second approach was that 3-benzoylpyrroles were usually formed in addition to the required 2-benzoylpyrroles. However, chromatographic separation of these regioisomeric products was straightforward. The intermediates **17** were then converted into the target compounds **16** using the two steps described previously for the styrylpyrrole **12**. Analogues of the benzoylpyrroles **16** in which the benzene ring was replaced with pyridine, thiophene or furan were prepared in the same way (*10*).

More than seventy benzoyl- or heteroaroylpyrroles were prepared. Many of these were tested in the mitochondrial assay and, without exception, were found to be



Reagents: i, $PhCH{:}PPh_3$; ii, NaH, $BrCH_2CO_2Me$;
iii, NaH, HCO_2Me; iv, K_2CO_3, Me_2SO_4

Figure 3. Synthesis of the styrylpyrrole **12**.



Reagents: i, $POCl_3$; ii, $BrCH_2CO_2Me$, NaH; iii, $BrCH_2CO_2Me$, Bu^tOK, 18-crown-6

Figure 4. Synthesis of benzoylpyrroles **14**.

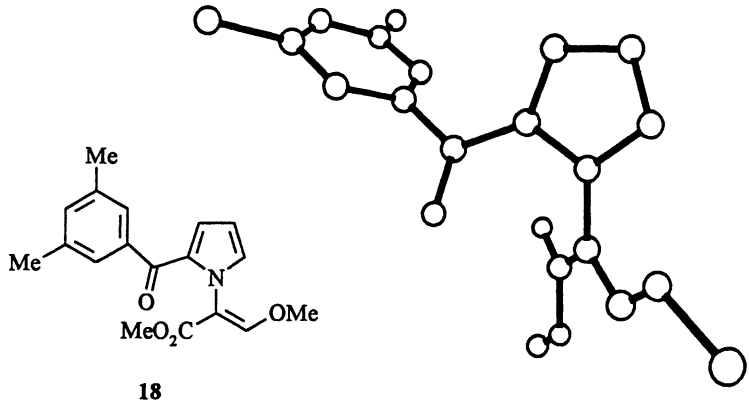
intrinsically weaker than the styrylpyrrole **12**, with the most active compounds having IC_{50}-values of about 700 nM. However, many of these benzoylpyrroles had high fungicidal activity in the glasshouse, and the best compounds were taken to field trials where they were particularly active against *Pyricularia oryzae* on rice, with useful systemic properties. Interestingly, analogues of the benzoylpyrroles with benzene in place of the pyrrole ring, *i.e.* benzophenones, are only weakly active, both in the mitochondrial assay and in the glasshouse.

One of the most active benzoylpyrroles was the 3,5-dimethyl-derivative **18**, and a single crystal X-ray structure was determined for this compound. Two crystallographically independent molecules were observed in the asymmetric unit, but these had similar conformations. It was interesting to note that the almost planar methoxyacrylate toxophore is strongly twisted in comparison to the pyrrole ring (torsion angle = 60° and 62° in the two independent molecules). This is a feature which we had previously observed with compounds in which the acrylate is linked to a benzene ring. In the stilbene **13**, for example, the corresponding torsion angle is 86° (*1*). Also noteworthy is the fact that the ketone carbonyl group is closer to coplanarity with the pyrrole ring (torsion angle = 16° and 21° in the two independent molecules) than with the dimethylbenzene ring (torsion angle = 51° and 35° respectively). Figure 5 depicts the molecule in which the torsion angles reported above are 60°, 16° and 51°. The torsion angles reported above are those between least squares mean planes calculated for the almost planar β-methoxyacrylate, carbonyl, pyrrole and dimethylbenzene units of the benzoylpyrrole **18** using Sybyl Version 6.03 (Tripos Associates, St. Louis, Missouri).

Pyrroles with other 2-Substituents: Esters, Amides and Ketones

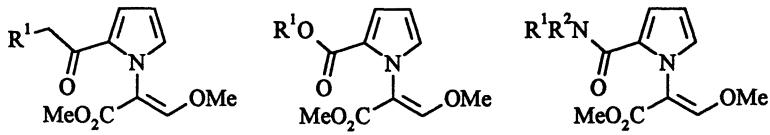
In view of the high activity of the benzoylpyrroles, other compounds, such as the ketones **19**, the esters **20** and the amides **21**, were identified as worthwhile targets (Figure 6: R^1 and R^2 represent aliphatic, aromatic or heteroaromatic groups). However, when we began to apply the methods with which we had prepared the benzoylpyrroles to the synthesis of these new compounds, problems were very soon encountered. For example, we found that although the intermediate **22**, required for the synthesis of the ketone **19** in which R^1 is *n*-propyl, could readily be made using Vilsmeier-Haack chemistry, it was converted into the indolizine **23** rather than the expected β-hydroxyacrylate **24** on treatment with sodium hydride and methyl formate (Figure 7). Furthermore, although the pyrrole **25** with a methoxycarbonyl side-chain could easily be made by the usual Claisen condensation, attempts to prepare higher homologues under the same conditions led to mixtures of products (see, for example, Figure 8).

We reasoned that one solution to these problems was to prepare the pyrrole-2-carboxylic acid **26** which, it was anticipated, could be converted into a variety of esters, ketones and amides. However, base-catalysed hydrolysis of the methyl ester **25** led, after acidification, to the β-hydroxyacrylate **27** rather than the required acid **26** (Figure 9). The structure of **27**, difficult to determine directly, was confirmed by conversion into the β-*n*-propyloxyacrylate **28**. As well as using spectroscopy to determine the structure of **28**, it was clear from its inactivity in the mitochondrial assay that the propyl substituent was part of the toxophore rather than in the side-chain as we would have liked (*1*). The regiochemistry of this hydrolysis was surprising in view of the fact that, when linked at the α-position to a benzene ring, base-catalysed



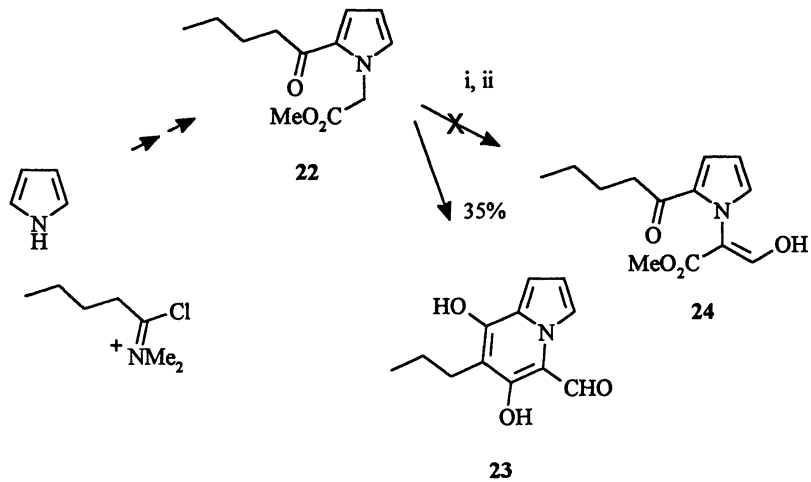
18

Figure 5. Single crystal X-ray structure of the pyrrolylacrylate **18**.



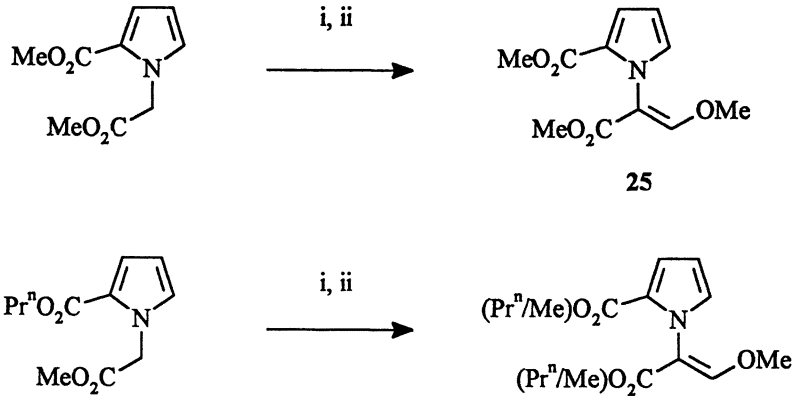
19 20 21

Figure 6. Targets for synthesis: ketones, esters and amides.



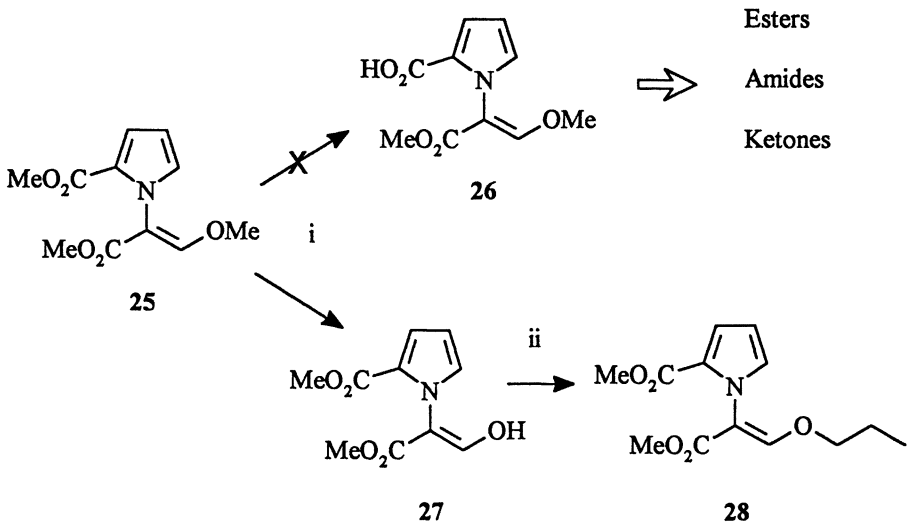
Reagents: i, NaH, HCO_2Me; ii, H_3O^+

Figure 7. Attempted synthesis of a pyrrolylacrylate with an aliphatic ketone side-chain.



Reagents: i, NaH, HCO_2Me; ii, K_2CO_3, Me_2SO_4

Figure 8. Synthesis of pyrrolylacrylates with ester side-chains.



Reagents: i, 1 equiv. KOH, aq. 1,4-dioxan; ii, Pr^nI, K_2CO_3

Figure 9. Base-catalysed hydrolysis of the pyrrolylacrylate **25**.

hydrolysis of the methyl β-methoxyacrylate toxophore takes place at the methoxycarbonyl group, leading to the corresponding α-phenyl-β-methoxyacrylic acid.

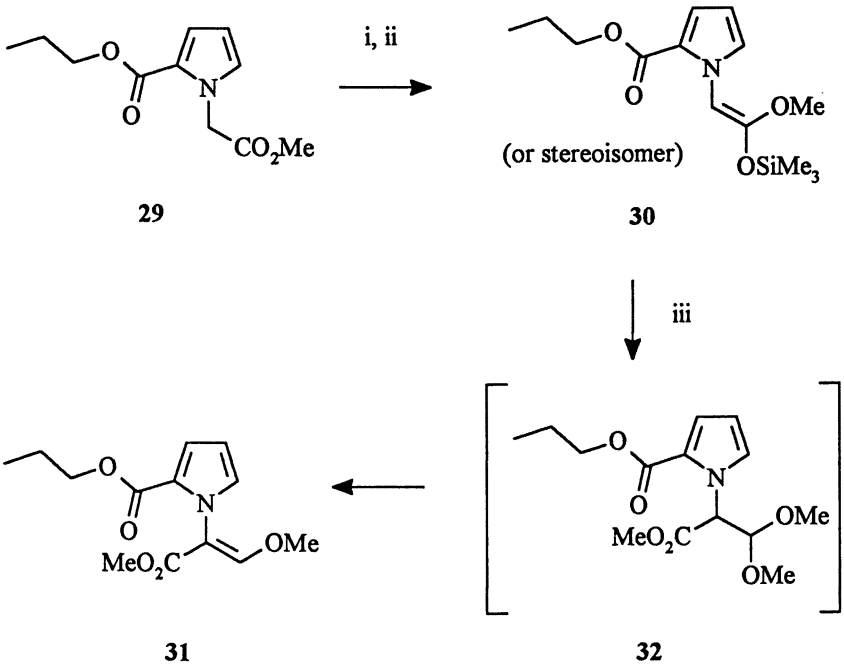
A more successful approach to the required esters **20** involved the use of a modified Claisen condensation described by Mukaiyama and his co-workers (*19*). An example is shown in Figure 10. The pyrrolylacetate **29**, on successive treatment with lithium di-isopropylamide (LDA) and trimethylsilyl chloride, reacted to form the methyl silyl ketene acetal **30** as a single unidentified stereoisomer. On exposure to a mixture of trimethyl orthoformate and titanium tetrachloride, this ketene acetal was converted into the required ester **31**, elimination of methanol from the intermediate acetal **32** occurring *in situ*, presumably triggered by the Lewis acid. One interesting feature of this sequence was the stability of the ketene acetal **30**. The related species derived from phenylacetates or 3-phenylpropanoates react with the trimethyl orthoformate-titanium tetrachloride adduct at temperatures of well below 0 °C (*19-21*), while the pyrrole derivative **30** reacted at a useful rate only in refluxing dichloromethane. In fact, in a first run the reaction was interrupted before completion and a sample of the ketene acetal **30** was isolated, unscathed after an aqueous work-up.

While this approach to the target esters was successful, it was laborious and did not lend itself to the rapid preparation of a series of compounds for testing. A better procedure was to use (*Z*)-methyl α-pyrrol-1-yl-β-methoxyacrylate **33** (Figure 11) which, we discovered, was a convenient intermediate from which to make not only the esters **20**, but also a variety of ketones such as **19**, the amides **21** and other derivatives.

The pyrrolylacrylate **33** was prepared in two steps from methyl pyrrol-1-ylacetate (Figure 11) which, in turn, was derived from 2,5-dimethoxytetrahydrofuran and the methyl ester of glycine (*10*). Claisen condensation of methyl pyrrol-1-ylacetate with methyl formate and sodium hydride in *N,N*-dimethylformamide (DMF), the solvent we had used with success in many previous cases, gave an intractable mixture of products. However, when toluene containing a few drops of methanol was used instead, the reaction mixture precipitated the sodium salt **34** which could be filtered off, dissolved in DMF and then treated with methyl iodide to give the required product **33** as a crystalline solid, melting at 88-9 °C, in an overall yield of 66%. Its (*Z*)-stereochemistry was established by the chemical shift of the olefinic proton at δ7.51 ppm (deuterochloroform). Multi-gram samples of this acrylate were readily prepared and, as described below, were derivatized to produce a multitude of pyrroles of the type **7**.

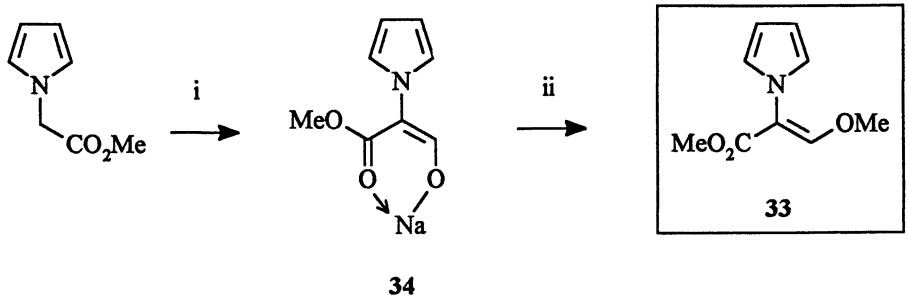
One idea was to lithiate the pyrrolylacrylate **33**, which we anticipated would occur on the pyrrole ring (*22*), and to treat the resulting organometallic species with various electrophiles. However, in a model reaction, a mixture of the pyrrolylacrylate **33** and trimethylsilyl chloride was added to LDA in tetrahydrofuran at -65 °C, and the reaction mixture was allowed to warm to -50 °C over two hours (*23*). To our surprise, clean silylation at the olefinic position occurred, giving a 90% yield of the β-silylacrylate **35**, a solid melting at 49-50 °C (Figure 12). β-Lithiated β-alkoxyacrylates are, in fact, well documented, and Schmidt, in particular, has shown that such compounds are valuable synthetic intermediates (*24*).

Much more useful were Friedel-Crafts acylations of the pyrrolylacrylate **33** which occurred exclusively on the pyrrole ring, and mainly at the required 2-position (*25*). For example, acylation with valeryl chloride in the presence of aluminium chloride gave a 95 : 5 mixture of the regioisomeric products **36** and **37** respectively



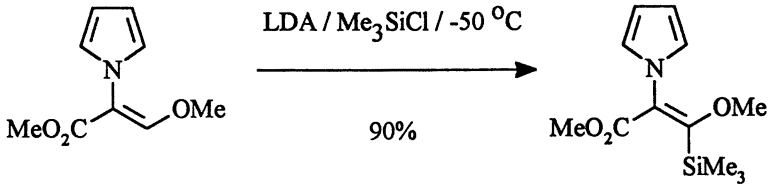
Reagents: i, LDA; ii, Me_3SiCl; iii, $(MeO)_3CH$, $TiCl_4$

Figure 10. Synthesis of a pyrrolylacrylate with an ester side-chain using the Mukaiyama modification of the Claisen condensation.



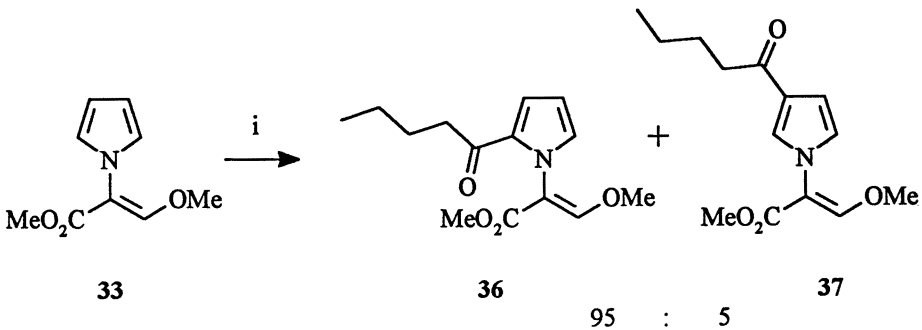
Reagents: i, NaH, HCO_2Me, PhMe, catalytic MeOH; ii, MeI, DMF

Figure 11. Synthesis of the pyrrolylacrylate **33**.



33 35

Figure 12. Silylation of the pyrrolylacrylate **33**.



33 36 37

95 : 5

Reagents: i, Bu^nCOCl, $AlCl_3$, 0 °C

Figure 13. Acylation of the pyrrolylacrylate **33**.

(Figure 13). This chemistry worked for a variety of aliphatic and aromatic acid chlorides (*12*).

Related chemistry provided access to pyrroles with ester side-chains (Figure 14). Acylation of the pyrrolylacrylate **33** with trichloroacetyl chloride, which was reactive enough to not require a Lewis acid catalyst (*25*), led to the trichloroacetylpyrrole **38** which, in turn, gave esters **20** on treatment with primary alcohols (*12*). Treatment of **38** with phenol gave a low yield of the expected ester, but secondary alcohols failed to react. Amides were not accessible *via* the trichloroacetylpyrrole **38** because amines did not displace the trichloromethyl group but, instead, the β-methoxy group from the acrylate. Thus morpholine gave the β-aminoacrylate **39**, and aniline gave the interesting 7-azaindolizine **40**. *n*-Propylamine led to an intractable mixture of products.

Acylation of the pyrrolylacrylate **33** with phosgene gave the pyrroloyl chloride **41** which was more reactive than the trichloroacetylpyrrole and led to the expected esters and amides on treatment with secondary alcohols and primary amines respectively (Figure 15). Alternatively, esters and amides could be prepared directly by acylation of the pyrrolylacrylate with chloroformates or carbamoyl chlorides (Figure 16) (*12*).

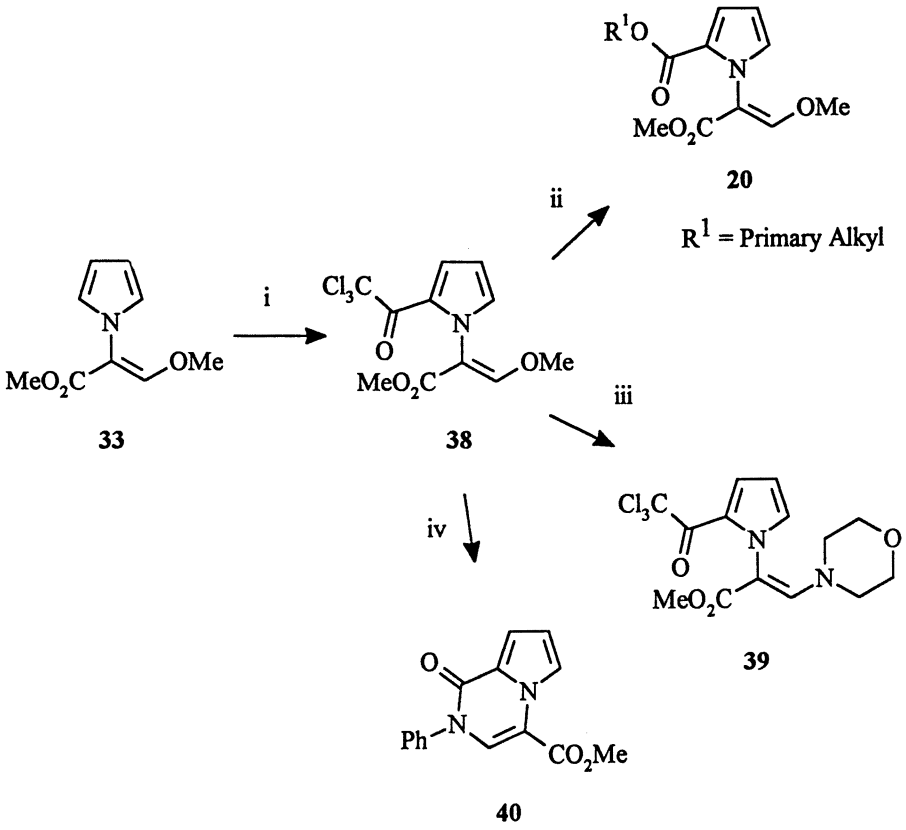
The pyrrolylacrylate **33** also reacted with a variety of other electrophiles (*7*). For instance, treatment with the simple Vilsmeier-Haack reagent derived from phosphorus oxychloride and DMF gave the 2-formyl-derivative (together with some of the readily-separable 3-formyl-isomer), a useful intermediate which, in turn, led to pyrroles with other novel side-chains. Furthermore, **33** gave Mannich products, and could be chlorinated and brominated.

Finally, it should be emphasised that the unoptimised yields of many of the reactions between electrophiles and the pyrrolylacrylate **33** described above were not high. Nevertheless, this disadvantage was outweighed by the benefits of being able to quickly prepare a variety of compounds for testing, allowing structure-activity patterns for this family of compounds to be established within a short period of time.

Structure-Activity Relationships

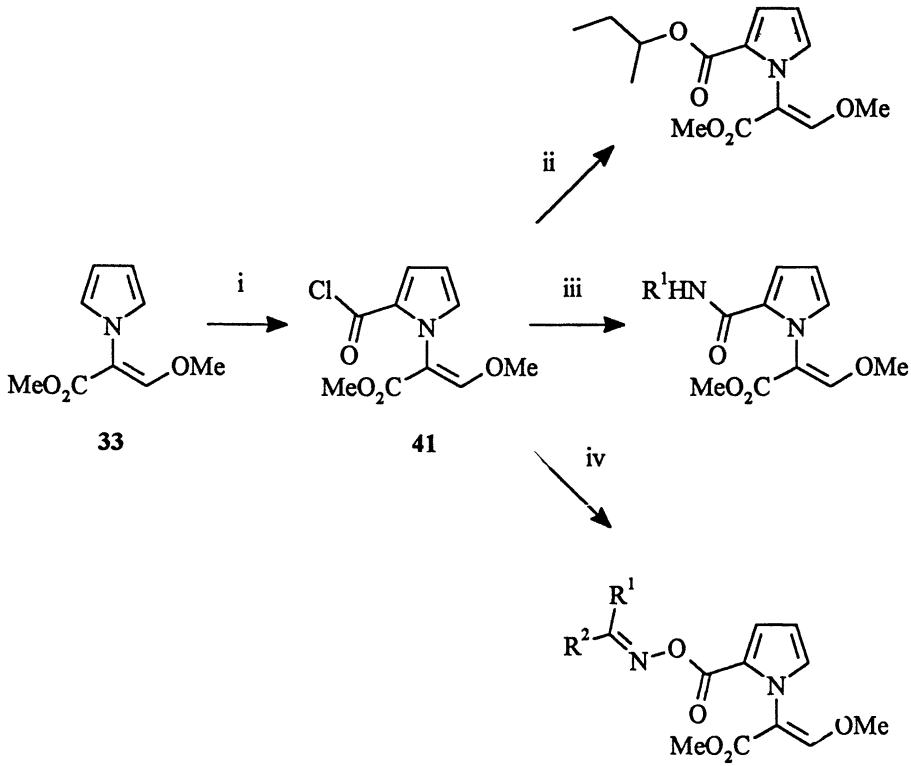
The purpose of this final section is to briefly review the structure-activity relationships which were found for the *N*-linked pyrroles, established from the synthesis and testing of more than 150 examples. The results were determined in 24-hour protectant tests in the glasshouse, *i.e.*, tests in which the plants were treated first with the acrylate, either as a foliar spray or as a root drench, and then, 24 hours later, with a spore suspension of a fungal pathogen as a foliar spray. Activity in the root drench test indicated that the acrylate was likely to have systemic movement in plants when applied as a foliar spray (*3-5*). The pathogens used were representative of those which are of global commercial importance in agriculture.

2-Benzoylpyrroles **16** were the earliest *N*-linked pyrroles to show good activity in the glasshouse (the regioisomeric 3-benzoylpyrroles **42** were uniformly weak fungicides)(Figure 17). The parent compound (**16** in which X = Y = H) had high activity, and the introduction of simple substituents at the 3-position, or the 3- and 5-positions, of the benzoyl group was often beneficial. Examples with octanol/water log P values below about 3.5 (*e.g.* **16** in which X = H and Y = H, 3-F, 3-Me or 3-MeO) were active not only when applied to the foliage, but also in the root drench test. Introduction of a 3-phenoxy group to give **43** did not result in a marked improvement



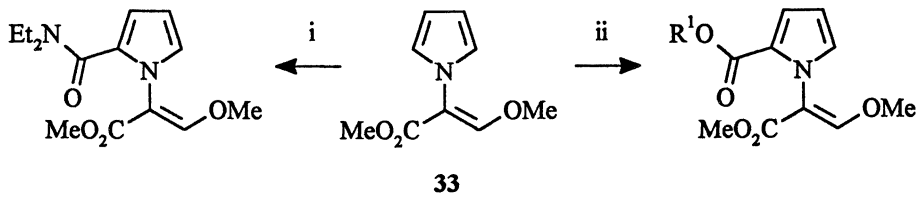
Reagents: i, $Cl_3C.COCl$, 2,6-dimethylpyridine; ii, R^1OH, K_2CO_3; iii, morpholine, K_2CO_3; iv, aniline, K_2CO_3

Figure 14. Reactions of the trichloroacetylpyrrole **38**.



Reagents: i, $COCl_2$; ii, Bu^SOH, pyridine; iii, R^1NH_2 (R^1 = alkyl, phenyl); iv, R^1R^2C:NOH, pyridine

Figure 15. Reactions of the pyrroloyl chloride **41**.



Reagents: i, Et_2NCOCl, $AlCl_3$; ii, R^1OCOCl, $AlCl_3$ (R^1 = alkyl, phenyl)

Figure 16. Reactions of the pyrrolylacrylate **33** with diethyl carbamoyl chloride and chloroformates.

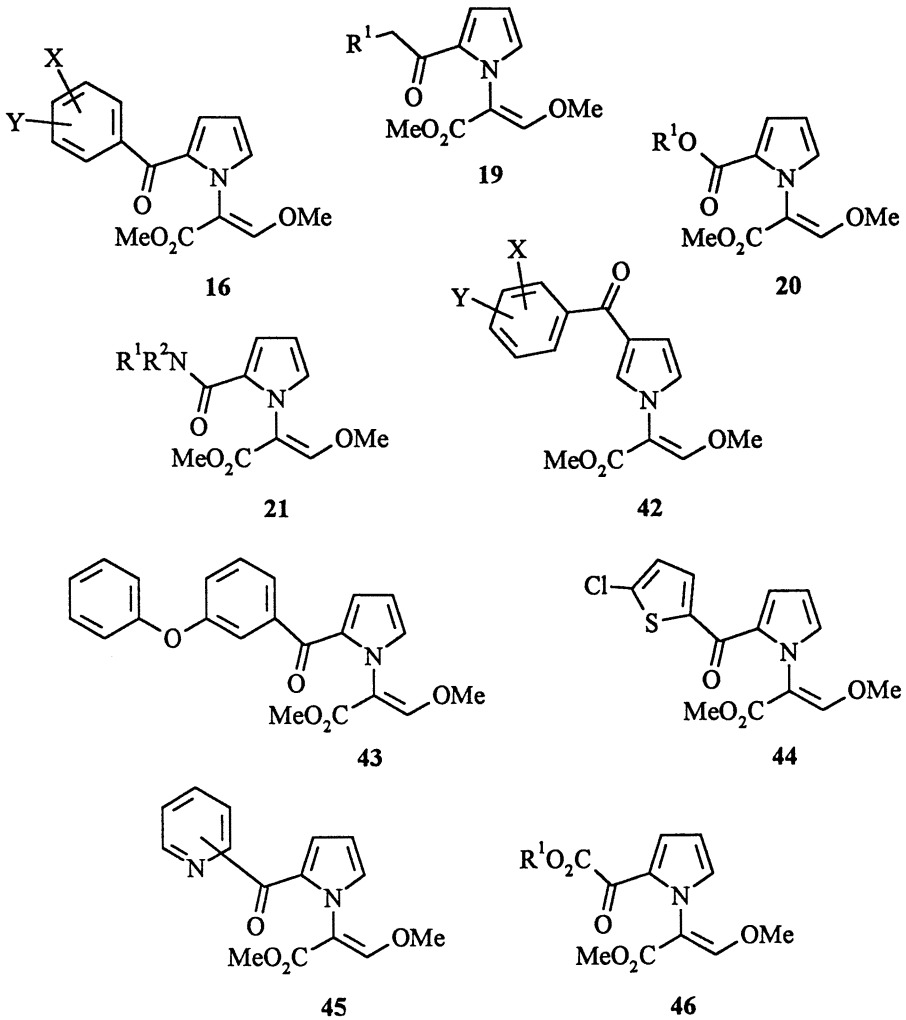


Figure 17. Key classes of *N*-linked pyrroles.

in activity in the way which had been observed in other series (*3-5*): **43** was similar in activity to the parent compound when applied as a foliar spray, and was much weaker as a root drench. The 4-position of the benzoyl group of **16** could accommodate a fluorine atom, but other substituents were detrimental to activity. The best of the 2-heteroaroylpyrroles which we prepared was the thienoylpyrrole **44**, while the simple unsubstituted pyridinoylpyrroles **45** were poorly active.

Pyrroles with simple ester side-chains **20** were highly active, rather volatile, and, as a consequence of their low partition coefficients, very systemic compounds [log P (octanol/water) = 1.8 for **20** in which R^1 = allyl, for example]. Pyrroles with simple aliphatic keto groups as side-chains **19** presented a similar picture [log P (octanol/water) = 2.1 for **19** in which R^1 = *n*-propyl]. By contrast, pyrroles with keto-ester **46** or amide side-chains **21** were poorly fungicidal.

Finally, we prepared a series of benzoylpyrroles incorporating an additional small substituent, such as a methyl group or a chlorine or bromine atom, on the pyrrole ring, but none had better activity than the corresponding compound without this substituent.

Conclusions

The *N*-linked pyrroles described in this paper are fungicides with a broad spectrum of activity and a novel mode of action. Examples with suitably low partition coefficients often exhibit systemic movement in plants. The most promising compounds in the glasshouse were taken forward for field trials, where they showed good activity, especially against *Pyricularia oryzae* on rice. Our work on the *N*-linked pyrroles was discontinued when other classes of β-methoxyacrylates, especially ICIA5504 and related tricyclic compounds, were found to have superior levels of fungicidal activity.

Acknowledgments

We wish to thank our colleagues at ZENECA Agrochemicals and ZENECA Specialties who have participated in this project, especially K. Anderton, V.M. Anthony, T.E.M. Fraser, I.R. Matthews, G.J. Sexton, B.K. Snell, R. Taylor, T.E. Wiggins and D. Youle. We thank D.J. Williams, Imperial College of Science, Technology and Medicine, London, for the X-ray crystallography.

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Chapter 30

Phenoxyphenyl Alkoxyiminoacetamides

New Broad-Spectrum Fungicides

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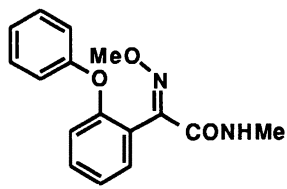
A number of substituted phenoxyphenyl alkoxyiminoacetamides were synthesized and their fungicidal activities against economically important plant diseases were examined. Our studies on the structure-activity relationships revealed that fungicidal activity was the strongest when the substituent of the oxyimino moiety was methyl and the acetamide moiety was mono-methylamide. The phenoxy group on the phenylacetamide moiety attached to the 2-position of benzene ring gave the highest activity. Introduction of various substituents on the benzene ring of the phenylacetamide moiety besides the 2-phenoxy group resulted in reduction of the fungicidal activity. However, introduction of some substituents on the phenoxy moiety increased the activity to the same extent as the unsubstituted lead compound. Among the compounds of the series, SSF-126, (E)-2-methoxyimino-N-methyl-2-(2-phenoxyphenyl)acetamide was selected as a new candidate fungicide for rice diseases because of its high activity and safety to mammals, fishes and plants.

Since Becker et al. (*1*) have reviewed the fungicidal activity of strobilurins and other natural compounds, structural modification of these lead compounds has progressed aggressively. β-Methoxyacrylates and 2-methoxyiminoacetates have been introduced as promising candidates for a new class of fungicides with a broad spectrum and new mode of action (*2-3*). Many of the current systemic fungicides are becoming less effective due to the onset of resistance by long-term repeated application. We describe herein the discovery, general synthesis and the relationship



SSF-126

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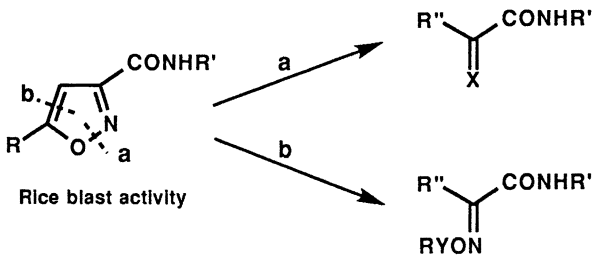
between structure and fungicidal activity of these new phenoxyphenyl alkoxyiminoacetamide derivatives (*4*).

Toxicology

The package of toxicological studies of SSF-126 is under examination, and no adverse effects have been reported so far.
Acute oral LD_{50} to rat and mouse : > 300 mg/kg
Mutagenicity (Ames test) : Negative
Chromosome aberration : Negative
Fish toxicity
LC_{50} (48 hr) to *Oryzias latipes*: 20.7 ppm
LC_{50} (48 hr) to *Cyprinus carpio*: 16.8 ppm
EC_{50} (3 hr) to *Daphnia pulex*: > 100 ppm

Discovery

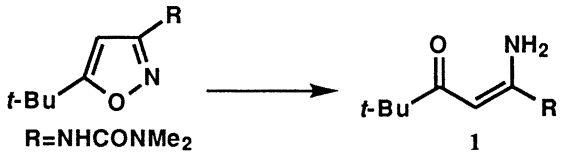
We designed ring cleaved structures (bonds **a** and **b** in Scheme 1) of the isoxazolecarboxamides in an effort to find a fungicide for rice blast on the basis of the following experimental data.



Scheme 1. Ring cleavage design of isoxazolecarboxamides

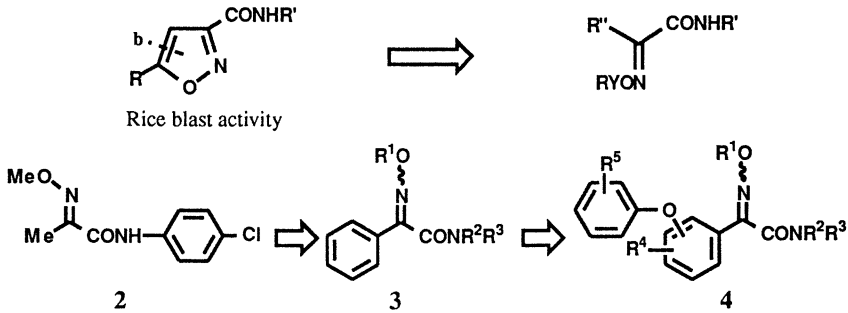
First, we had found in the past that ketoenamine **1**, which was a cleaved compound of the isoxazole ring, was the major metabolic product of the herbicide isouron, during the studies of metabolism under paddy conditions (*5*) (Scheme 2). We presumed that these carbamoyl isoxazole derivatives are activated by cleavage at bond **a** on the isoxazole ring in paddy water or soil. Secondly, some carbamoyl isoxazole derivatives were found to exhibit fungicidal activity for rice blast by submerged application (*6*). Compounds of this ring cleaved type appeared to be rather attractive and seem to be well suited for a fairly broad synthetic program.

Therefore, some α-imino (X=NH) or α-ketoamide derivatives (X=O), designed by N-O bond cleavage as shown for bond **a**, were synthesized but none of them showed activity.



Scheme 2. Cleaved metabolite of isoxazole ring

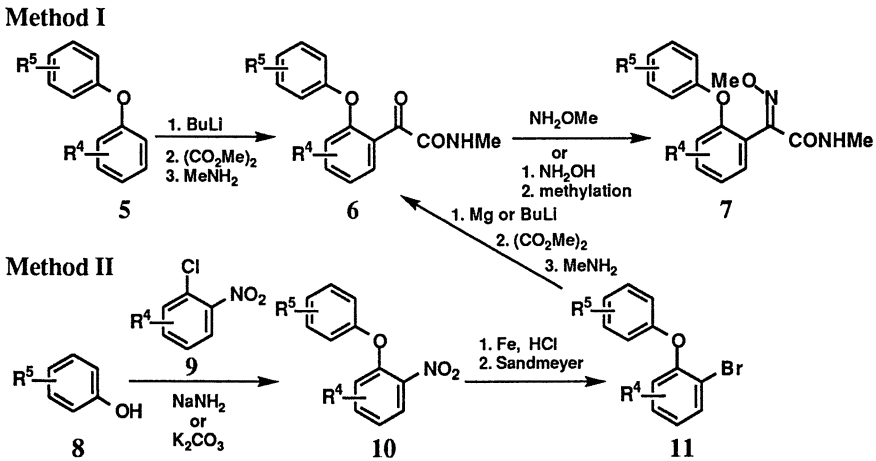
In addition, some alkoxyimino ketoamide derivatives of interest, designed by C-C double bond cleavage as shown for bond **b**, were synthesized (Scheme 3). In the course of screening tests in the greenhouse, the methyl methoxyimino benzamide **2** was found to have good systemic activity as well as preventive and curative properties on rice blast and other diseases such as gray mold and powdery mildew of cucumber. Phenyl derivatives **3** of the original methyl substituted compound also showed similar fungicidal activity. Therefore, we performed further intensive studies on the synthesis of phenyl alkoxyiminoacetamide derivatives. During the course of an extensive synthetic development, great improvement both in strength and spectrum of the fungicidal activity was obtained by the introduction of a 2-phenoxy group **4** on the phenyl ring in analogy with methoxyacrylate (*7*) (ZENECA) and methoxyiminoacetate (*8*) (BASF) strobilurine fungicides.



Scheme 3. Type b design for fungicides from isoxazolecarboxamides

General Synthesis.

All of the compounds were prepared through two synthetic routes (Scheme 4).



Scheme 4. Synthesis of phenoxyphenyl methoxyiminoacetamides

Method I. The starting diphenyl ethers **5** were allowed to react with butyl lithium, to form metal salts at the 2-position on the benzene ring. These anions were allowed to react with dimethyl oxalate to give α-keto esters according to a known procedure (*7*). The α-keto esters were then allowed to react with methylamine to give α-ketoamides **6**. Then **6** were condensed with methoxylamine hydrochloride or hydroxylamine hydrochloride followed by methylation to give the desired phenoxyphenyl methoxyiminoacetamides **7**, which were usually obtained as the favored E-isomer together with a small amount of the corresponding Z-isomer.

Method II. The starting phenols **8** were allowed to react with 2-chloronitrobenzenes **9** to give nitro substituted diphenylethers **10**, which yielded the amines on reduction. The resultant amines were converted to substituted phenoxyphenylbromides **11** by way of the Sandmeyer reaction. Then **11** were allowed to react with butyl lithium or magnesium to form metal salts. These anions were allowed to reacted in the same manner as described in Method I to give **7**.

The isomers (E/Z) of all compounds synthesized in Methods I-II were separated by silica gel column chromatography and/or recrystallization. Each of the isomers was stable at least under the process of separation and purification. Geometrical configurational assignments of the methoxime were confirmed by comparison of the proton NMR chemical shifts of both the C-6 phenyl protons of the E- and Z-isomers (Figure 1). The C-6 phenyl proton of the Z-isomer resonates in a lower magnetic field than the E-isomer. A similar relationship was also observed for other substituted 2-phenoxyphenyl derivatives as Table I shows.

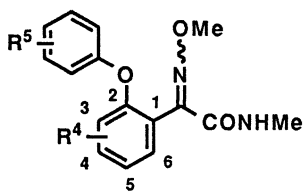


Table I. ^{1}H NMR chemical shifts of (E)- and (Z)-2-methoxyimino-N-methyl-2-(2-phenoxyphenyl)-acetamides

Compound			
R^4	R^5	Is[a)]	C_6-H NMR (ppm)
H	H	E(SSF-126)	6.70-7.47[b)]
H	H	Z	7.67
4-Me	H	E	6.83-7.33[b)]
4-Me	H	Z	7.49
5-OMe	H	E	7.23-7.29[b)]
5-OMe	H	Z	7.36
5-$SiMe_3$	H	E	7.42-7.46[b)]
5-$SiMe_3$	H	Z	7.68
H	3-Me	E	7.29-7.35[b)]
H	3-Me	Z	7.58
H	4-$SiMe_3$	E	7.44
H	4-$SiMe_3$	Z	7.60

a): Geometrical configuration (E/Z)
b): Contained together with other aromatic protons

In addition, as a general rule, the E isomers tended to be eluted forward with a mixture of hexane/ethyl acetate on silica gel column chromatography, and to show higher Rf values when developed with the same solvent system on silica gel thin layer chromatography. These generalizations were verified by X-ray diffraction analysis of both isomers as Figure 2 shows. In the case of the E-isomer, the

methoxime group is almost orthogonal to the phenyl group. On the other hand, the methoxime group in the Z-isomer is lying almost planar with the phenyl ring.

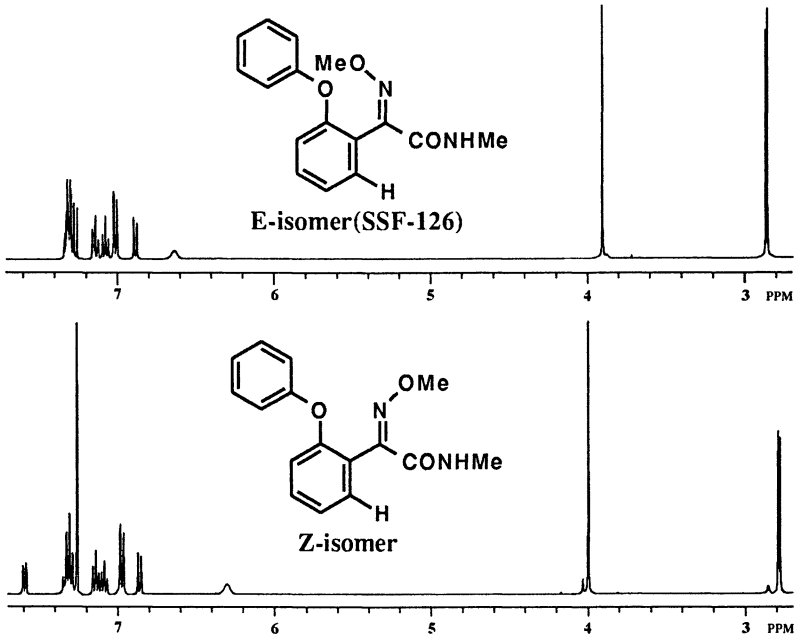
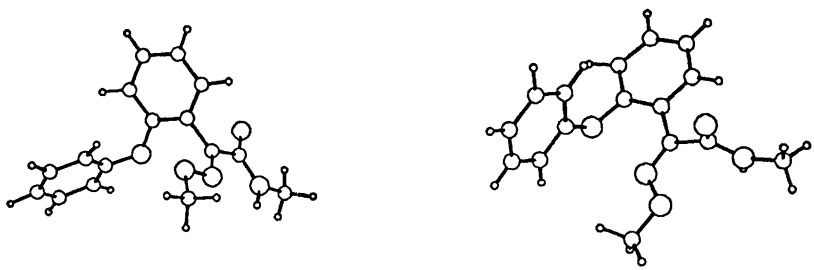


Figure 1. ^1H NMR spectra (400 MHz, $CDCl_3$) of E-isomer and Z-isomer



E-isomer(SSF-126) **Z-isomer**

Figure 2. X-Ray crystallographic structures

Assay Methods for Fungicidal Activity

***In vitro* test :**

The mycelial growth inhibition test was carried out by agar dilution methods. The mycelial disks of the test organisms, precultured on potato sucrose agar (PSA) medium, were put on the potato dextrose agar (PDA) medium with or without the test compound. The diameter of the mycelial mat of each organism was measured when

the diameter of each corresponding untreated control reached about 20-30 mm. The effective concentration for 50 % inhibition (ED_{50}, ppm) of mycelial growth was calculated by the linear regression formula obtained from the logarithm of concentration and the inhibition rate at each concentration against the untreated control.

Disease control tests in the glasshouse by foliar application:

For rice blast. Test compounds were dissolved in a small amount of N,N-dimethyl formamide (DMF) and diluted with distilled water containing sticking agent to give concentrations of 500 to 2.0 ppm . The test solutions were sprayed onto the host plant 24 hrs. before (preventive) or after (curative) inoculation. Inoculation was carried out by spraying the spore suspension of *Pyricularia oryzae* onto the rice seedlings. Five days after inoculation, the percent of the infected leaf area was assessed.

For rice sheath blight. The test compounds were applied by the same method as for rice blast as mentioned above. Inoculation was carried out by putting the mycelia of *Thanatephorus cucumeris* (teleomorph of *Rhizoctonia solani*) with the rice bran medium at the foot of the rice seedlings. Assessment was done five days after inoculation by measuring the height of the mycelia along the sheath of the rice seedlings.

For cucumber powdery mildew. The test compounds were applied by the same method as for rice blast except for the curative application, which was done 48 hrs. after inoculation. Inoculation was carried out by spraying the spore suspension of *Sphaerotheca fuliginea* onto the cucumber seedlings. Ten days after inoculation, the percent of the infected leaf area was assessed.

For cucumber gray mold. The test compounds were applied by the same method as for rice blast. Inoculation was carried out by putting the mycelial mat (4 mm dia.) of *Botrytis cinerea* previously cultured on the PSA medium, onto the cucumber leaves. Two days after inoculation, the diameter of the lesion around the inoculum was measured.

For cucumber downy mildew. The test compounds were applied by the same method as for cucumber powdery mildew. Inoculation was carried out by spotting the droplet of zoosporangial suspension of *Pseudoperonospora cubensis* onto the cucumber leaves. Ten days after inoculation, assessment was done by observing the degree of the infection around the inoculated spot.

Disease control tests by submerged application for rice blast:

Rice seeds were sown in plastic cups containing sterilized sand. Two weeks after sowing, the acetone solution of the test compound was poured into the paddy water in the plastic cup. The final concentration of acetone in the paddy water did not exceed 1 %. Seven days after application, inoculation was carried out by the same method as mentioned above. Assessment was made by the same method as for the foliar application described above. The percent control was calculated by dividing the difference of the infected leaf area, height of mycelia, diameter of the lesion or degree of the infection, between the untreated control and the treated plot by those of the untreated control.

Fungicidal Activity

***In vitro* and *in vivo* tests of SSF-126 (E) and its isomer (Z).** *In vitro* tests were carried out by growing on agar dilution methods against ten species as indicated in

Table II. The E-isomer was about 5 to 20 times more active than the Z-isomer as a whole. *In vivo* fungicidal activities were tested against 2 diseases for rice and 3 diseases for cucumber by foliar application at the concentrations of 125, 31.3, and 7.8 ppm in the greenhouse except for rice sheath blight where concentrations were 500, 125 and 31.3 ppm (Table III). The disease control activity of the E-isomer was also stronger against all 5 diseases than the Z-isomer. However the difference of activity against cucumber powdery mildew between the isomers was small compared with other diseases. This result may suggest that the Z-isomer is either isomerized into the E-isomer or equilibrated with the E-isomer on the leaf. These findings suggest that the E-configuration is favorable for fungicidal activity.

Table II. Fungicidal activities of two geometrical isomers of N-methyl-2-methoxyimino-2-(2-phenoxyphenyl)acetamide in vitro

Compound	EC_{50} (ppm)									
Is[a)]	P.a.	S.s.	G.c.	C.r.	B.c.	A.m.	F.o.	P.o.	C.l.	C.h.[b)]
E	2.8	2.0	7.2	2.5	1.1	20.8	6.2	5.6	4.7	4.5
Z	28.1	11.9	16.2	41.1	11.7	>64.0	58.1	49.1	61.8	41.8

a) Configuration (E/Z)
b) The abbreviation for the plant pathogenic fungi are as follows,
P.a.:*Pythium aphanidermatum*, S.s.:*Sclerotinia sclerotiorum*
G.c.:*Glomerella cingulata*, C.r.:*Corticium rolfsii*,
B.c.:*Botrytis cinerea*, A.m.:*Alternaria alternata* apple pathtype,
F.o.:*Fusarium oxysporum*, P.o.:*Pyricularia oryzae*,
C.l.:*Colletotrichum lagenarium*, C.h.:*Cladosporium herbarum*

Table III. Disease control activities[a)] of two geometrical isomers of N- methyl-2-methoxyimino-2-(2-phenoxyphenyl)acetamide in vivo

	Rice						Cucumber								
	Blast			Sheath blight			Powdery mildew			Gray mold			Downy mildew		
Is[b)]	125	31.3	7.8	500	125	31.3	125	31.3	7.8	125	31.3	7.8	125	31.3	7.8[c)]
E	97	90	30	70	70	50	100	100	70	95	92	73	100	100	30
Z	50	30	0	50	0	0	100	100	40	44	36	18	70	0	0

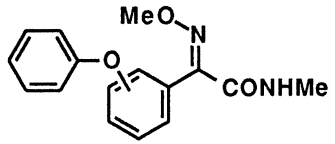
a) Percent of control by preventive application
b) Configuration (E/Z)
c) Concentration of active ingredient (ppm)

Structure Activity Relationships

Positional effects of phenoxy group. Table IV shows the positional effects of the phenoxy group against the following 5 diseases: blast and sheath blight for rice and powdery mildew, gray mold, and downy mildew for cucumber. Unless otherwise stated, the fungicidal activity is hereafter expressed as A: excellent, B: good, C: moderate, D: poor, E: no efficacy according to the percent control at less than 125 ppm by preventive and curative application in the greenhouse. When the acetamide moiety was fixed as the methoxyiminoacetamide group, the introduction of 3- or 4-

phenoxy groups gave activity consistently inferior to that of the 2-phenoxy parent compound.

Table IV. Positional effects of phenoxy group



Compound	Rice		Cucumber		
Position	Blast	Sheath blight	Powdery mildew	Gray mold	Downy mildew
2(SSF-126)	A	B	B	A	B
3	C	E	D	C	D
4	D	E	D	D	E

A: Excellent, B: Good, C: Moderate, D: Poor, E: No effect

Effects of O-substituents R^1 of alkoxyimino group. Table V shows the effects of the O-substituent R^1 against blast and sheath blight for rice. Because of the low fish toxicity of SSF-126, the main field of application of this fungicide seems to be for paddy rice. Therefore, hereafter we show the data from optimization of its structure against blast and sheath blight for foliar application in rice. When R^1 is the methyl group, the compound has an excellent activity against both diseases for rice. Ethoxyimino and *iso*-propoxyimino derivatives as well as the hydroxyimino derivative showed little or no activity.

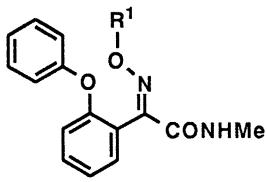
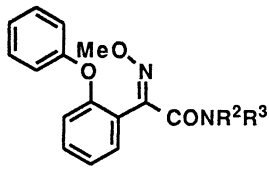


Table V. Effects of O-substituents R^1

Compound	Rice	
R^1	Blast	Sheath blight
Me(SSF-126)	A	B
Et	C	D
i-Pr	D	D
H	E	D

Effects of N-substituents R^2, R^3. Table VI shows the effects of the N-substituents R^2 and R^3 of the amide group on the disease control activity. Dimethylamino (R^2, R^3=Me) and unsubstituted amino (R^2, R^3=H) derivatives showed rather weak activity against both diseases. Moreover, the introduction of N-ethyl and N-propyl groups resulted in reduced activity against both diseases of rice. These findings suggest that inordinate bulkiness of the amide group lowers activity as does close congestion in the vicinity of the methoxyimino group.

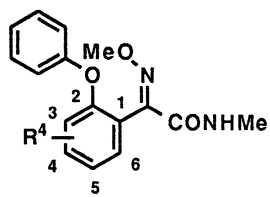
Table VI. Effects of N-substituents R^2, R^3



Compound		Rice	
R^2	R^3	Blast	Sheath blight
Me	H(SSF-126)	A	B
Et	H	B	D
Pr	H	C	D
H	H	B	D
Me	Me	C	C

Effects of substituents R^4. Table VII shows the effects of the substituents R^4 on the phenyl ring of the phenyl acetamide moiety. By introduction of these substituents on positions 3, 4, 5, and 6, the fungicidal activity decreased against both diseases compared to the lead compound, SSF-126. In particular, introduction of a nitro group in either the 3- or 5-position significantly reduced the activity of the compound.

Table VII. Effects of substituents R^4



Compound	Rice	
R^4	Blast	Sheath blight
H(SSF-126)	A	B
3-NO_2	E	E
5-NO_2	E	E
5-F	B	C
4-Me	B	C
5-Me	B	D
5-OMe	C	C
6-OMe	D	E
5-OPh	C	E

Effects of substituents R^5. Table VIII shows the effects of substituents R^5 on the phenoxy moiety. These compounds were divided into 5 groups. Group 1 consisted of alkyl substituents, group 2 of halogen substituents, group 3 of nitro substituents, group 4 of alkoxyl or phenoxy substituents, and group 5 of disubstituents.

The fungicidal activity of group 1 varied depending on the position of the substituents except on foliar application against blast. Introduction of a 3 or 4-methyl of group 1, halogen of group 2, 3 or 4-methoxy of group 4 and 3-methyl-4-chloro of group 5 gave similar levels of activity compared with the parent SSF-126 against blast for rice by submerged application. The 3,4-dichloro derivative of group 5 was slightly less effective. Only the 3-phenoxy derivative of group 4 showed similar levels of activity compared with SSF-126 against both blast and sheath blight for rice by foliar application. On the other hand, the fungicidal activity of the 4-phenoxy derivative decreased against both diseases. Again, the highly polar nitro substituents of group 4 were unfavorable in analogy with the R^4 substituents described above.

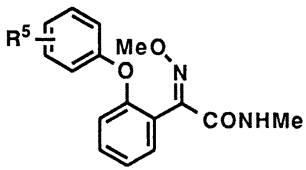


Table VIII. Effects of substituents R^5

Compound	Rice		
R^5	Blast		Sheath blight[a)]
	a)	b)	
H(SSF-126)	A	A	B
2-Me	B	C	E
3-Me	B	A	C
4-Me	B	A	C
4-*i*-Pr	B	C	C
4-Cl	B	A	C
4-Br	C	A	D
4-NO_2	D	NT[c)]	E
2-NO_2	E	NT[c)]	E
3-OMe	B	A	C
4-OMe	B	A	B
3-OPh	A	D	B
4-OPh	C	NT[c)]	D
3-Me-4-Cl	E	A	D
3,4-Cl_2	B	B	C

a) Foliar application b) Submerged application
c) NT: not tested

Field Evaluation

Disease control activity of SSF-126 on rice diseases by submerged application. The control activity of SSF-126 was tested in the field at 3 dosages by submerged application with the following reference compounds: pyroquilon for blast, flutolanil for sheath blight, and iprodion for brown spot on rice diseases (Table IX).

Table IX. Disease control activity of SSF-126 by submerged application

Compound	Dose	Percent of Control		
	g a.i./ha	Blast	Sheath blight	Brown spot
SSF-126	1000	98	84	97
	250	89	35	90
	63	15	15	50
		Pyroquilon	Flutolanil	Iprodion
Reference	1000	98	100	70
	250	74	100	30
	63	28	35	0

The results show that SSF-126 has a similar level of activity to pyroquilon for blast, and lower activity for sheath blight compared with flutolanil, and higher activity for brown spot than iprodion.

Summary

Based on the results of our present studies, SSF-126 was selected as a new candidate fungicide for rice diseases in the paddy due to its activity, ease of production and safety to mammals (*9*). A series of toxicological studies including eco-toxicology, as well as residue analysis and investigations into metabolism and mode of action are now in progress.

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Chapter 31

Retrospective Quantitative Structure–Activity Relationship (QSAR) Analysis of Tetrazolo- and Triazoloquinolines, a Series of Rice Blast Control Agents

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Although the SAR of the tetrazolo- and triazoloquinolines that culminated in the discovery of tricyclazole was carried out more than twenty years ago, a QSAR analysis of the structures had never been conducted. This paper will compare and contrast the conclusions reached in the "classical" SAR study with those suggested by modern computer-assisted design techniques.

The synthetic work upon which this paper is based was carried out more than twenty years ago and led directly to a significant body of active compounds, a number of patents (1-8) and, ultimately, to a commercial rice blast control agent, tricyclazole. Recently we decided to reexamine the SAR, utilizing some of the CAMD approaches that were unavailable earlier, to determine if the information gained by this approach would have altered our approach or changed our conclusions. In addition to the computer modeling tools now available, including Sybyl, MOPAC and modern 3D QSAR methods such as CoMFA, we now have mode-of-action information that was unavailable when the work was carried out, all of which could possibly give us better insight into how the compounds were controlling rice blast.

Retrospective analyses of old data is fraught with difficulties due to the cost and time involved in the re-synthesis or retesting of a great number of old compounds. Many of the compounds no longer were available and those available were of questionable purity. We had the additional difficulty that these compounds had a very narrow activity profile, mostly against rice blast (*Pyricularia oryzae*) *in vivo*, no biochemical *in vitro* data existed for QSAR development. Today it is known that these compounds control rice blast by inhibiting melanin formation in the germinating fungus (9-10) which is essential for the development of infection hyphae and the successful penetration of the leaf by the fungus. Therefore for this study we have relied entirely on the *in vivo* rice blast data previously generated. Since our rice blast testing has proven to be highly consistent in our screens, we had confidence in our ability to group the compounds into activity "sets" in a meaningful way.

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Classical SAR Approach to the Tetrazoloquinolines (TZQs)

The lead into the chemistry that led to tricyclazole was a third-party compound, tetrazolo(1,5a)quinoline, Figure 1, obtained from Dr. Alfred Bader of the Aldrich Chemical company.

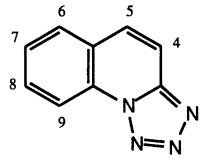

Figure 1. Tetrazolo(1,5a)quinoline

After re-synthesis and retesting confirmed that this compound controlled rice blast (*P. oryzae*) at levels of 25 ppm, a SAR action plan was developed which involved the synthesis and screening of four classes of compounds, e.g.

1.) Unfused, disubstituted tetrazoles
2.) Tetrazoles fused to heterocycles other than quinoline,
3.) Other fused quinoline azoles
4.) Substituted tetrazolo(1,5a)quinolines

1. Comparison of unfused tetrazoles. The first approach, Figure 2, was to determine whether other 1,5-disubstituted tetrazoles would control rice blast in our screens or if these tetrazoles had to be fused to another ring system.

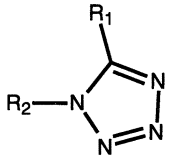

Figure 2. Unfused 1,5-Disubstituted Tetrazoles

A search of our files found hundreds of unfused disubstituted tetrazoles which had been screened for their plant disease activity. None of these compounds had any significant activity against rice blast and we concluded that the presence of a disubstituted tetrazole was ineffective in controlling rice blast.

2. Other fused tetrazoles. Our next goal was to determine whether the quinoline was unique or if other nitrogen-containing heterocycles fused to tetrazole, Figure 3, would also have rice blast activity.

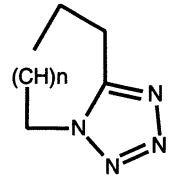

Figure 3. Other tetrazole-fused heterocycles.

We synthesized and screened a variety of monocyclic, dicyclic, and polycyclic nitrogen-containing heterocycles fused to tetrazole. We first examined simple, monocyclic heterocycles (Figure 4)[1] None of these fused tetrazoles exhibited any appreciable rice blast activity. It appears that more is required for activity than a tetrazole fused to a six-membered ring.

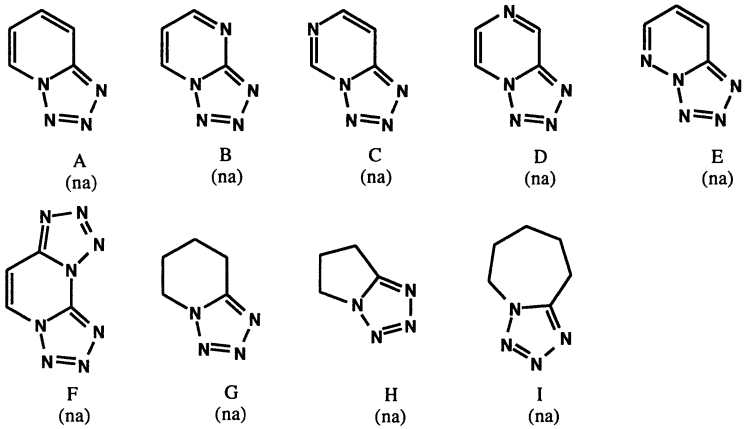

Figure 4. Rice Blast activity of monocyclic nitrogen-containing heterocycles fused to tetrazole.

We then synthesized a series of fused tetrazoles of some bicyclic heterocycles. When the tetrazole analogs of isoquinoline, quinoxalines, phthalazine, quinazolines, naphthyridin, and other 6:6 aromatic heterocycles were examined, Figure 5, only 6,7,8,9-tetrahydroquinoline, 5D, tetrazoloquinoxaline, 5B, and bis-tetrazoloquinoxaline, 5K, had significant rice blast activity.

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[1] **In all of these comparisons, rice blast activity is expressed as a RELAC value, relative to the activity of the lead tetrazolo(1,5)-quinoline(TZQ) where TZQ =1; NA= indicates that no activity against rice blast was observed at the highest rate tested.**

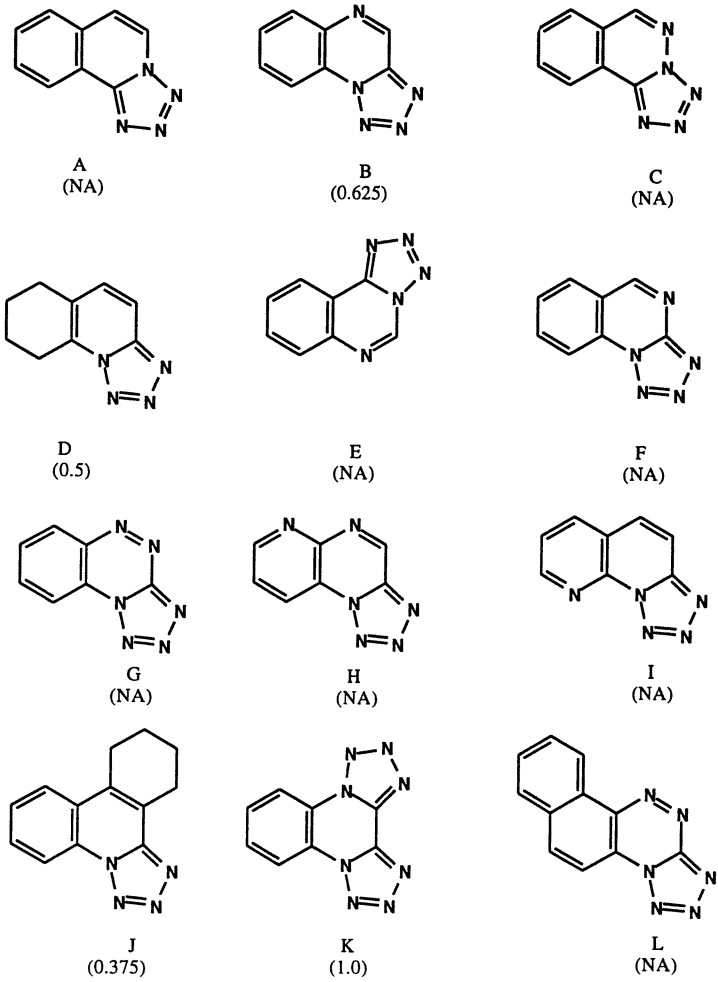


Figure 5. Rice blast activity of bicyclic and tricyclic tetrazolo heterocycles

A variety of reduced or heteroatom-substituted bicyclic heterocycles fused to tetrazoles were also synthesized and screened, (see Figure 6). The activity of the 4,5-dihydrotetrazoloquinoline, 6A surpassed that of the lead. Further expansion of this saturated ring to seven membered ring, 6B, or eight membered ring, 6C, resulted in a reduction of activity. The substitution of sulfur for carbon in the piperidine ring of 6A , e.g. 6H and 6I, also reduced activity. A number of attempts were made to synthesize fused tetrazoles from 6:5 tetrazolo-heterocycles, (i.e. indoles, benzoxazoles) and, except for tetrazolobenzothiazole, 6G, these proved very unstable, existing as an equilibrium between the tetrazole and the open-chained azido forms.

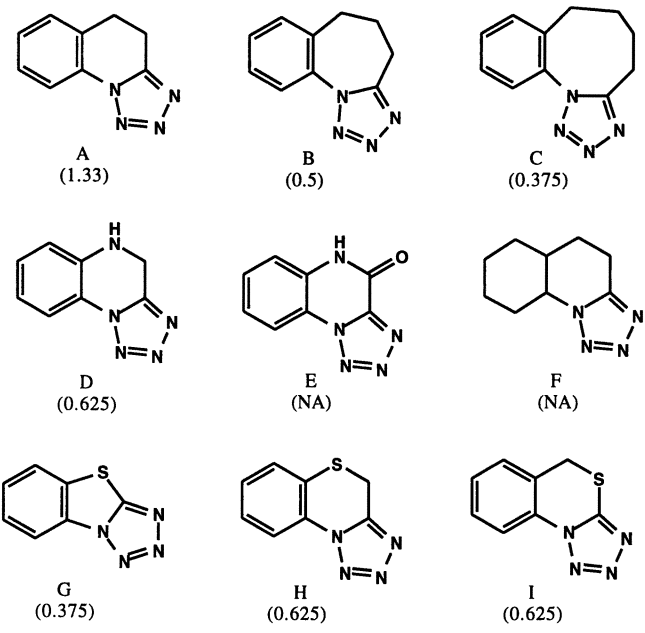


Figure 6. Rice blast activity of other heterocycles

3. **Other fused quinoline "azoles"**. We examined a number of intermediates to the tetrazoloquinolines as well as quinolines with fused "azole" rings other than tetrazole and discovered that triazolo(4,3b)quinoline, 7C, had rice blast activity comparable to but somewhat weaker than TZQ. These analogs are shown in Figure 7.

As in the case of the TZQs, we examined a series of heterocycles fused to triazoles, Figures 8 and 9, and found that for the most part, these compounds were less active than the corresponding tetrazoles. One exception was the triazolobenzothiazole, 9G, which was significantly more active than the corresponding tetrazolobenzothiazole.

At this point, we identified four major heterocycle-azole families that required synthetic attention; the tetrazolo and triazolo quinolines, quinoxalines, benzothiazines and benzothiazoles. This paper will concentrate on the tetrazolo and

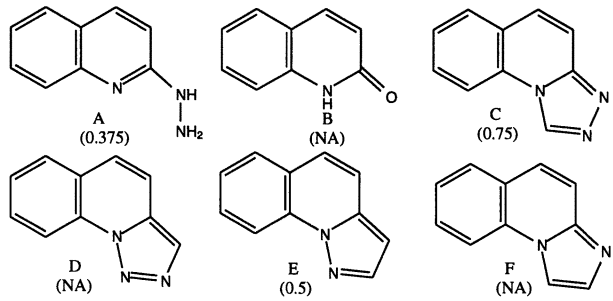


Figure 7. Other quinoline "azoles"

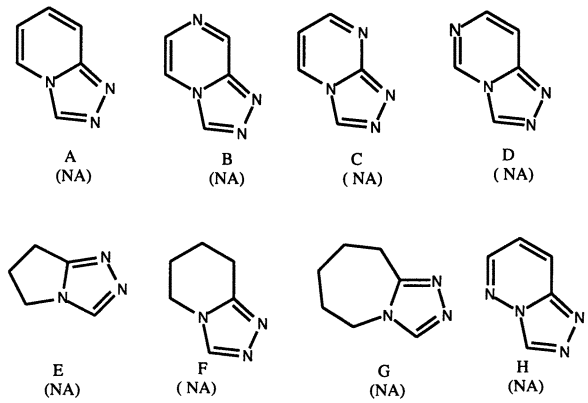


Figure 8. Rice blast activity of some triazolo heterocycles (monocyclic)

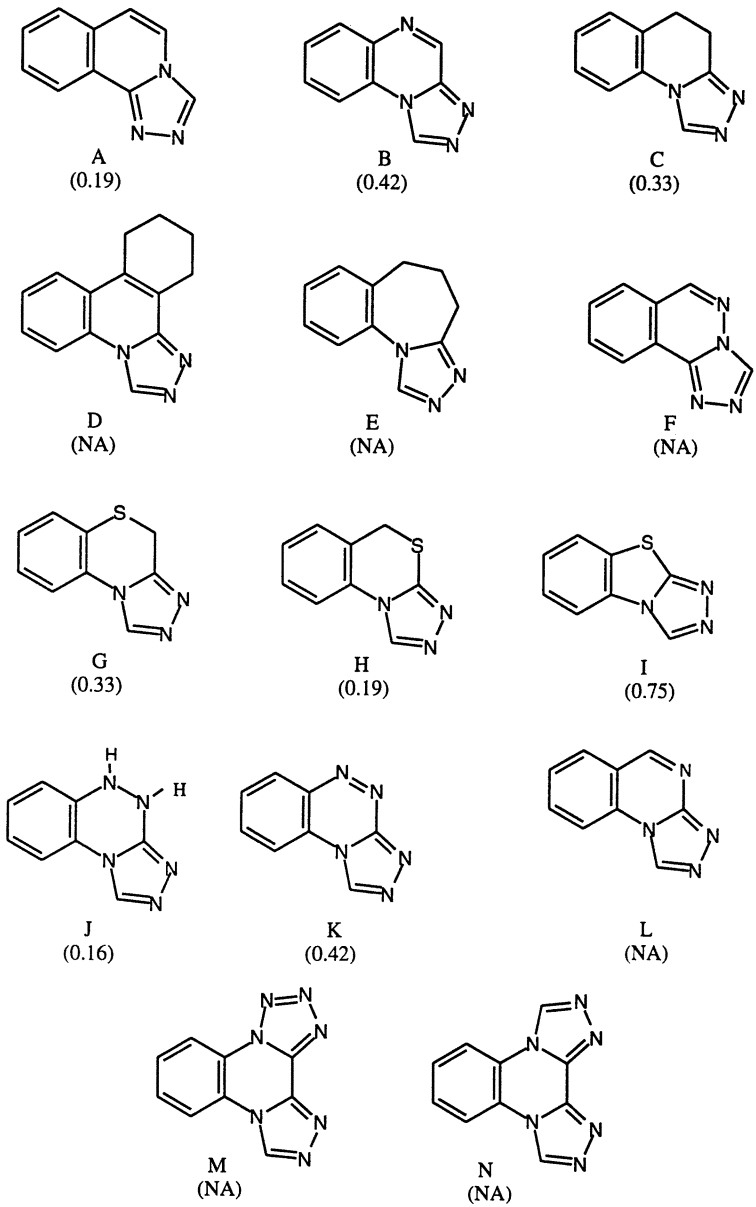


Figure 9. Rice blast activity of some bicyclic triazolo-heterocycles

triazoloquinolines, although many of the conclusions are valid for all four areas and similar SAR studies were subsequently carried out (4-8).

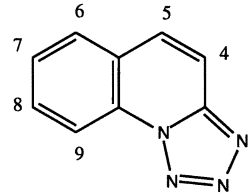
4. **Substituted tetrazolo- and triazoloquinolines.** Our approach to the SAR of the tetrazolo and triazoloquinolines was to determine which of the six possible positions for substitution on the quinoline portion had the greatest effect on rice blast activity. We then needed to determine the activity of various substituents at these positions.

Synthesis The synthetic routes to the variety of analogs we desired could be found in the literature (1, 2, 3, 11, 12) and required the conversion of the substituted 2-chloroquinoline to either the tetrazolo or triazolo quinoline. The 2-chloroquinoline intermediates were synthesized from either available quinolines or from the appropriately substituted anilines, Figure 10.

Results of screening.

Tetrazoloquinolines. A wide variety of substituted tetrazolo(1,5a)quinolines were synthesized and screened for rice blast activity. The test results indicated that the rice blast activity of the TZQs is very sensitive to both the type and position of the substitution. Substitutions at the 5 and 9 positions of the TZQ were the most favorable positions regardless of the substituent. The best substituents were small alkyls and halogens which, in the 5 and 9 positions, maintain or improve activity, whereas these substituents in other positions reduce activity, (see Table I below).

Table I. Rice Blast RELAC Values of Methyl and Chloro Substituted Tetrazolo(1,5a)quinolines



Unsubstituted	1.0		
4-Methyl	0.42	4-Chloro	0.42
5-Methyl	0.80	5-Chloro	0.91
7-Methyl	0.17	7-Chloro	0.17
8-Methyl	0.41		
9-Methyl	1.33	9-Chloro	1.0

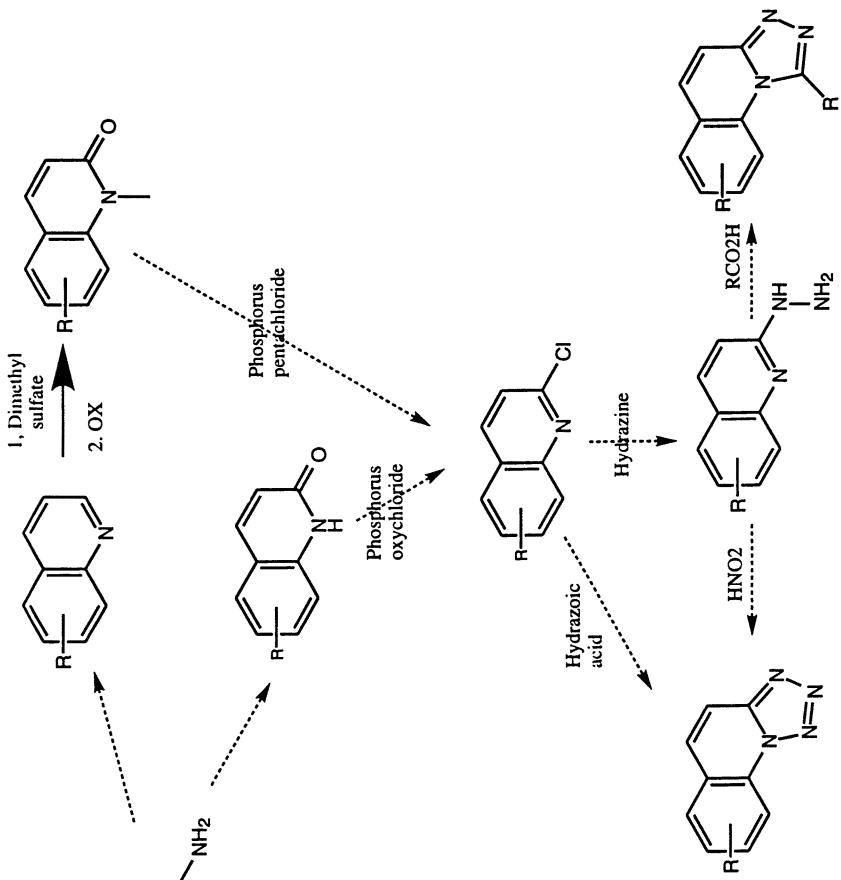


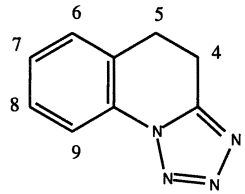
Figure 10. Generic synthetic pathway for the substituted tetrazolo-and triazoloquinolines.

Except for the small alkyls and halogens, most other substituents on tetrazoloquinoline were less active than the unsubstituted parent compound. The relative activity of other substituents in a variety of positions on the quinoline is shown below; e.g.

Cl, F, Br = CH3 > azido> substituted methyl (methanol, halomethyl, ethoxymethyl) > ethyl>>amines, alkylamines, acetamido, carboxylic acids, esters, amides, alkyl ethers, hydroxy, nitro, methylsulfide, phenyl

The combination of reduction of the 4,5 double bond plus small groups in the 9-position gave the greatest increase in activity. This is shown in Table II.

Table II. Rice Blast RELAC Values of Substituted 4,5-Dihydrotetrazolo(1,5a)quinolines Relative to the Lead



4,5-Dihydro

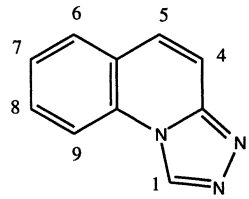
Unsubstituted	1.33	4-Methyl	NA
7-Chloro	0.42	5-Methyl	0.166
9-Chloro	1.33	6-Methyl	0.42
4,5-Dichloro	0.33	7-Methyl	0.33
4,5-Dichloro-9-ethyl	0.33	9-Methyl	1.33
7-Methoxy	0.33	6,7,8,9-Tetrahydro	NA

Triazolo(4,3b)quinolines. Similarly, in the case of the triazoloquinolines, halogens and methyls seem to be the most active substituents, and the reduction of the 4,5 quinoline bond also increased activity. Additionally, in the case of the triazoloquinolines, the 1-position was an additional point for substitution. Table III summarizes the relative activity for several of these analogs.

Conclusions on the Requirements for Activity for the Tetrazolo and Triazolo Quinolines

These studies indicated that the "window of fungicidal activity" for this series was very narrow, as indicated by the generic structure in Figure 11 where R1, R2 and R3 all must be hydrogen, methyl, or halogen, preferably chlorine and fluorine for optimum activity, and the 4,5 double bond should be reduced.

Table III. Rice Blast (RELAC) Activity of Substituted Triazolo(4,3b)quinolines



1-substituted			
Chloro	1.16	Methyl	1.0
Hydroxy	0.33	Ethyl	0.41
Carboxylic acid	0.17	i-Propyl	0.17
Carboxamide	NA	n-Propyl	0.17
Ethyl carboxylate	0.17	Cyclopropyl	0.17
Methyl sulfide	0.42	Butyl	0.33
Formate	0.33	Ethoxymethyl	0.17
Amino	0.17	Ethoxyethyl	0.17
Acetamido	NA	Methanol	NA
Mercapto	NA	Vinyl	NA
CF3	0.42	Phenyl	NA
4,5-dihydro			
Unsubstituted	0.91	1-Phenyl	NA
1-Hydroxy	0.17	1-Pyridyl	NA
1-Methyl	0.75	1(2-Thiophene)	NA
1-CF3	NA	1(2-Furanyl)	NA
9-Methyl	0.75	9-Chloro	0.91

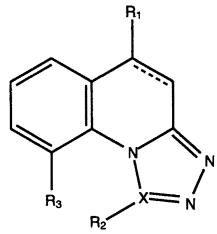


Figure 11. Optimal substitution pattern for tetrazolo-and triazoloquinolines.

QSAR and Molecular Modeling

Using the data gathered during the development of the classical SAR, we considered the ways in which we could use CAMD approaches to gain a greater understanding about the relationship between structural properties and the observed *in vivo* rice blast activity .

A QSAR strategy was developed for the TZQ molecules by examining structures for variation that could potentially be correlated with measured rice blast activity values. Although we had synthetically examined a large number of heterocyclic tetrazoles and triazoles, we decided to focus our QSAR efforts on the tetrazolo and triazolo quinolines. Figure 12 below shows the two compound classes with substitution sites identified.

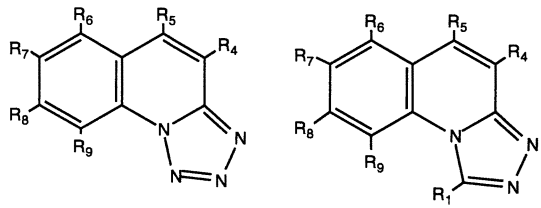


Figure 12. Substituted Tetrazolo-and Triazoloquinolines Studied

In both series, a wide variety of substituents existed at most possible substitution sites on the molecules but not enough simultaneous changes in substitution patterns had been made to perform a substituent based QSAR. Whole-molecule property QSAR methods, however, are commonly performed and properties can be calculated or estimated for most molecules. We chose this approach for our studies.

The major issue affecting the selection of QSAR strategies was the biological data available for these compounds. This data was predominately a percent control value at varying concentration levels. Incomplete dose-response rundowns were common

for many molecules and it was noted that the concentration rates were not always consistent. These inconsistencies were due to the long time period over which the data was collected and they prevented dose-response values to be calculated. However, the compounds could be placed into groups with similar activity and we could rank the groups. This ranking transformed the data from potential "interval" data to "ordinal" data which was discrete and ordered, but not normally distributed. Successful examples using the rank transform in QSAR studies have been reported by Bell et. al. (13), and Pleiss (14). Ranking the data has particular implications on what statistical techniques are most appropriate. Traditional Hansch and modern 3D QSAR methods such as CoMFA require continuous interval data. With the above limitations and data quality, we decided that CoMFA was not appropriate, but a good approach might be to perform rank transform regression and graphical pattern recognition techniques to the sets. It is interesting that once rank transformed, the biological data and descriptors can be analyzed with many of the techniques common to Hansch linear free energy relationships, (LFERs).

Methods. Whole molecule properties were calculated for the two sets of analogs in the following manner. Lipophilicity and size/bulk properties CLOGP, (Calculated LOG of the octanol-water partition coefficient) and CMR, (Calculated Molar Refractivity) were calculated with MedChem software (15). Electronic properties and optimized molecular geometries were obtained with MOPAC 6.0 (16). The MOPAC input files were prepared from CONCORD (17) geometries for each molecule using the MOPAC keywords: AM1, EF, XYZ and MULLIK. The optimized geometries, HOMO and LUMO frontier molecular orbital energies (Highest Occupied Molecular Orbital and Lowest Unoccupied Molecular Orbital) and dipole moment were compiled for QSAR analyses. When it became apparent that the dipole moment was correlating with biological activity, the dipole moment was added to the structure models from the MOPAC output files and represented as a vector superimposed on each structure in Sybyl (18). Visual comparisons and manipulations could be easily accomplished. The molecules were superimposed using a least squares fit method. The "azole" ring and fused benzene ring systems were superimposed on top of each other in the X-Y plane. The lead molecule was used as the fit template reference. New X, Y & Z dipole component coordinates for the superimposed and rotated set were written to a file and included in the QSAR analyses.

The molecules were grouped into 8 categories based on their Rice Blast activity data, (8 = very active, 1 = very weak or inactive). A rank transform was performed on all parameters and properties used in the QSAR analyses including the biological data. Ties in the ranked data were handled by using the average. Quantitative regression models were then sought using these ranked values.

Simple 2-D Pattern Recognition plots were produced for all possible pairs of QSAR descriptors with activity indicated by plot symbol. An active class plot symbol was

defined as categories = 3 - 8 and inactive as categories = 1 - 2. The SAS package (19) was used for all statistical analyses and parameter plots. Any interactions and unwanted correlations among the independent parameters were checked in this manner, as well as interesting activity patterns in the molecular properties.

Discussion. As a first attempt to identify any structure-activity trends, the two ring systems were examined together for ranked molecular properties versus biological rank. A very large set of about 120 molecules was considered. Not surprisingly, no visual trends in single parameters were evident. Too many of the compounds had the same substituent with varying levels of activity at different positions around the fused rings and whole molecule properties such as CLOGP and CMR show little or no variation for analogs like these. At this point it was decided that the two classes of ring systems should be evaluated separately to minimize confounding factors. Smaller and more specific sets of tetrazolo and triazoloquinolines were created. A set of 15 tetrazoloquinolines were selected with single substitutions at the 4, 5, 6, 7 and 9 positions that varied lipophilicity and electronics as much as possible. Steric and multiple substituted molecules were hard to consider due to the lack of structures with adequate variation and/or unavailable biological data. The same ranked biological data described above was used for the set. Fifteen structures with similar substitutions were selected for the triazoloquinolines.

No obvious trends were observed in CLOGP, CMR, HOMO, LUMO or dHL (the difference or gap between HOMO and LUMO orbital energies), for either the tetrazolo or triazoloquinoline sets. However, the plot of ranked dipole moment versus rice blast rank showed an interesting weak trend that resembled an optimal relationship. See Figure 13 below. To better understand the dipole trend, the X,Y and Z components of the dipole moment were examined by superimposing the structures as described above. This allowed for a visual comparison as well as using the three individual components quantitatively in the regression work. In Figure 14 below, the 15 tetrazoloquinolines are superimposed with their respective dipole moments represented as vectors. The dipole vectors originate near the center of the molecules (or centers of mass), and extend to the right of the structures depicting the direction as well as magnitude of the dipole moment arms. On examination of the dipole moments it is clear that there are virtually no contributions to the dipole in the Z direction. The dipole moment is almost wholly contained in the X-Y plane. The X and Y dipole components were examined as QSAR parameters and it was found that a statistically significant relationship could be developed in the X-component of the dipole moment as a quadratic relationship. Equation 1 shows the relationship with student-t significance at the 95% level in parentheses below each coefficient. Also reported are the number of compounds, N, the standard error, S, the R^2 , and model F statistics for the 95% level.

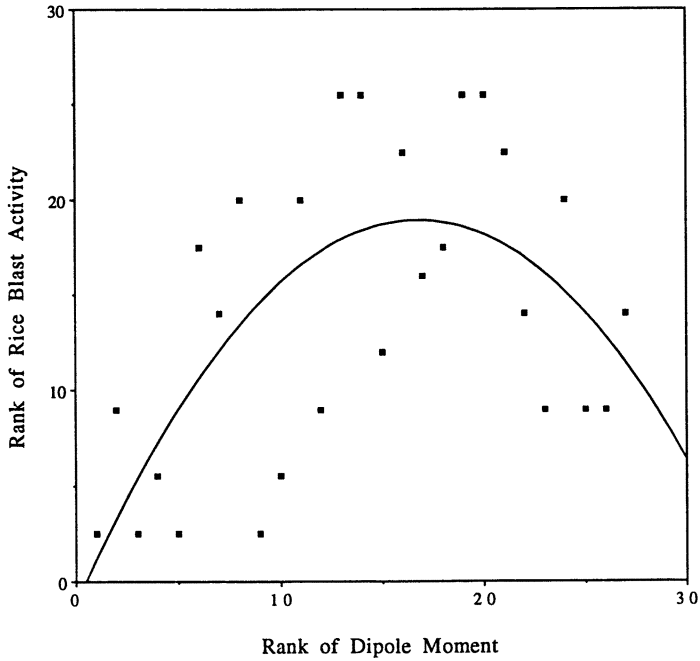


Figure 13. Plot of dipole moment vs activity showing optimal behavior.

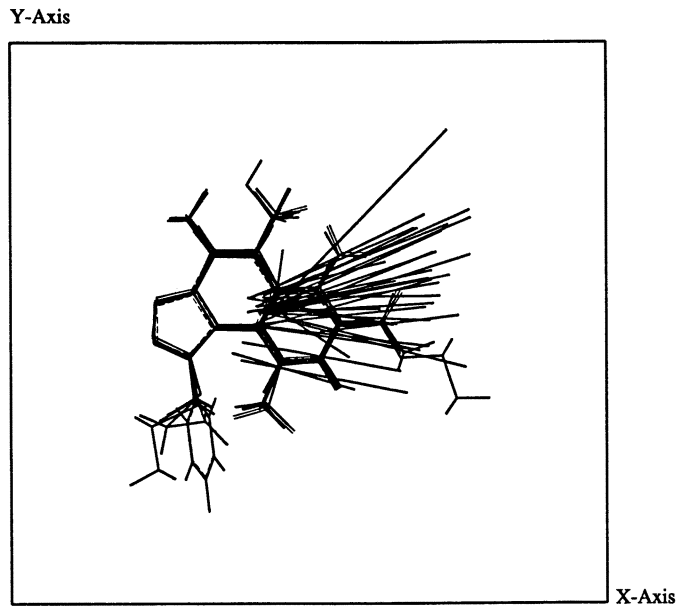


Figure 14. Triazolo- and tetrazoloquinoline structures aligned showing MOPAC calculated dipole moments as superimposed vectors

$$\mathbf{Rank\_RB} = 3.2909(\mathbf{X\text{-}Comp}) - 0.2099(\mathbf{X\text{-}Comp^2}) - 0.9736 \quad (1)$$
$$(0.0004) \qquad (0.0003) \qquad (0.687)$$

N=15 S=2.64 $R^2 = 0.68$ Prob>F 0.001

where: Rank_RB = Ranked Rice Blast Activity
X-Comp = X-Component of Dipole
$X\text{-Comp}^2$ = X-Component of Dipole Squared

Although this is an interesting finding, the QSAR is difficult to use as a predictive model because it requires a subjective alignment procedure with the dipole moment added as a structural feature. The rotated dipole components must then be ranked with respect to the model compounds, which is an estimate, further reducing the quantitative nature of the analysis. Another interesting aspect regards the traditional interpretation of the dipole moment in a QSAR equation. Dipole moment is usually found as a linear term in substituent based QSAR equations as documented by Lien et. al. (20). Typically the dipole moment has been viewed as a property that aligns the molecule prior to active site binding. This alignment interaction is difficult to interpret if it exhibits optimal (or quadratic), behavior. The QSAR described here is a whole molecule property rather than a substituent property, and whole plant *in vivo* data is used in place of *in vitro* binding data. Our findings may be due to the dipole moment being correlated with another physical property that is also strongly correlated to the *in vivo* rice blast activity. Perhaps a transport interaction is confounded with other activity requirements by the QSAR.

A complementary and alternative approach to using rank transform regression was sought in pattern recognition plots where 2 properties are plotted against each other and the biological activity rank indicated by the plot symbol. This is similar to the "parameter focusing" method described by Magee (21). Most plots of the parameters showed no significance, however the plot of the X vs Y components of the dipole moment showed that most of the active compounds for each class were grouped together, (see Figures 15-16) and inactive compounds fell outside the active groupings. The "component dipole map" shown in Figure 15 shows the pattern for triazoloquinolines, (hollow plot symbols) and Figure 16 the tetrazoloquinoline compounds (solid plot symbols). In both plots the squares represent the active compounds and the triangles the inactives. Figure 17 is the "component dipole map" for both classes of compounds together. As one can see, the active compounds group together by compound class appearing to have their own "active area", with inactive compounds surrounding them. These separate areas on the combined map suggest that other new compound classes may fall in yet a third area. This result would imply that a new series of compounds would require a separate QSAR study for optimization of activity. New target molecules are examined by calculating MOPAC electronic properties in the same manner as the training set and aligning to the same reference molecule. The X and Y dipole components are written out and plotted onto

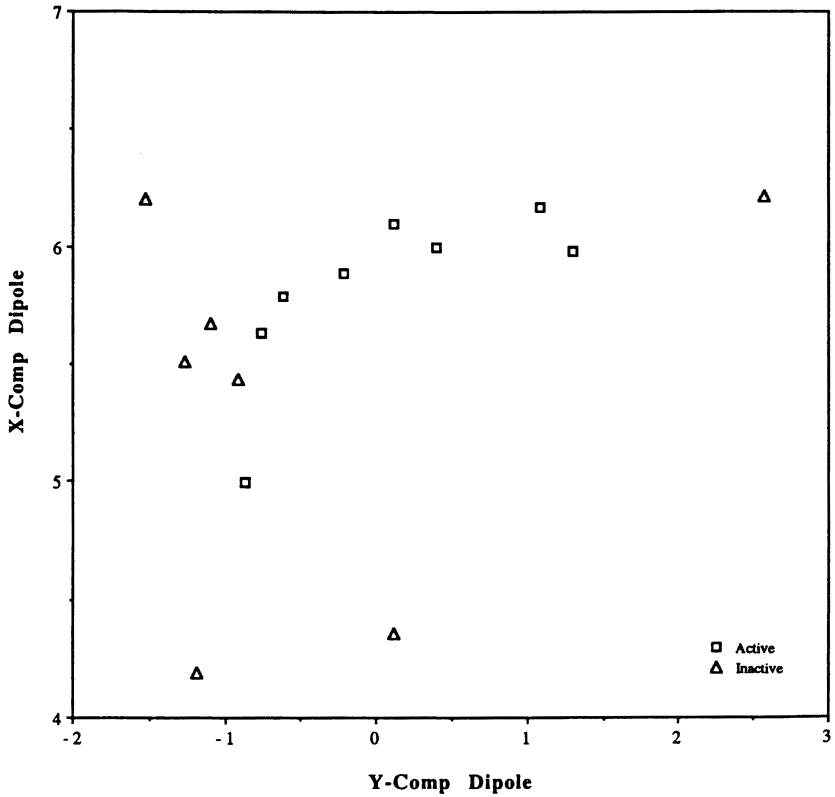


Figure 15. X & Y component dipole map for a set of triazoloquinolines

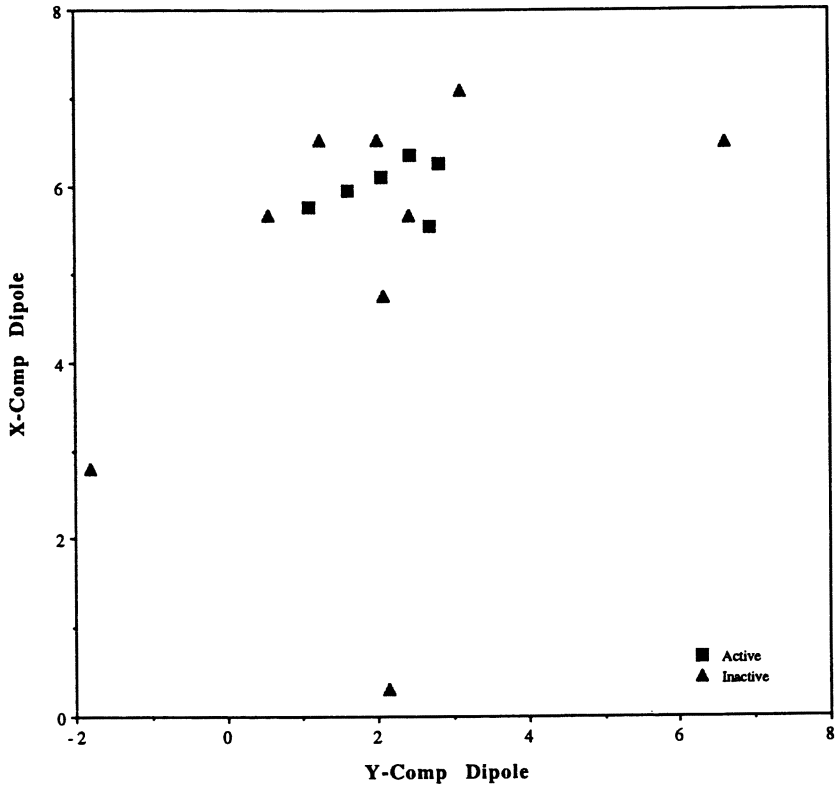


Figure 16. X & Y component dipole map for a set of tetrazoloquinolines

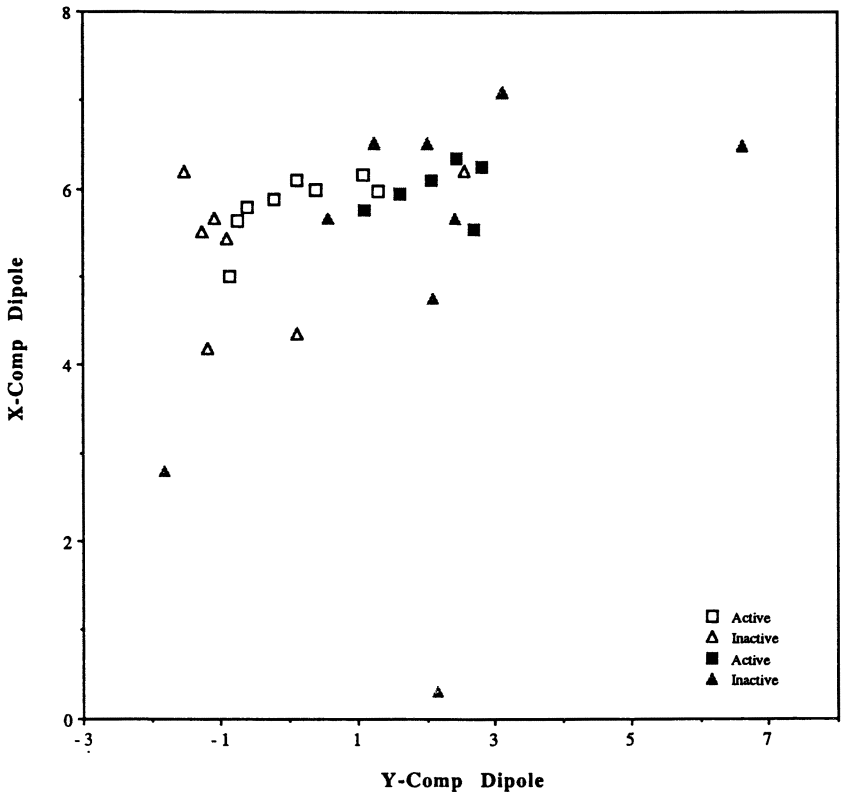


Figure 17. X & Y component dipole map for a combined set of triazolo- and tetrazoloquinolines

the training set plots. Activity can be predicted visually by locating the new point on the component dipole map. This technique was applied to a set of three tetrazolo analogs not in the original set. These molecules were chosen as a test of the general nature of the model. They differed from the test set in planarity. The central ring was modified from 6 carbons to 7 and 8 carbon saturated ring systems. The X and Y dipole components were calculated for the three and plotted onto the pattern recognition plots. The three new points all fell very close to the active center of the tetrazoloquinoline grouping, but show decidedly decreasing activity with increasing ring size. On inspection these molecules deviate from planarity as the central ring gets larger. It was found that the dipole moment was beginning to develop a significant Z component that was not present in the training set and as the degree of non-planarity increased, the activity decreased. This planarity requirement may be interpreted as affecting the dipole moment, already shown to be an important feature for activity.

Comparison of Classical SAR with QSAR. The goal of this study was to determine whether the CAMD examination of *in vivo* data would generate any information or insight not apparent during the development of the classical SAR. It was discovered that a portion of the rice blast activity appears to be governed by components of the dipole moment in the plane of the molecules. This relationship could be expressed in a quantitative equation and in pattern recognition plots. If the optimal dipole moment relationship is accurate, it suggests that further substituent changes on the triazolo and tetrazoloquinolines are not likely to result in radically better activity than what has been found to date. This is borne out by the extensive number of compounds that were synthesized over the years in the classical SAR approach. Whether the dipole moment is important in binding to the target site or is important in some transport mechanism has yet to be determined. Had this information been available earlier, we could have modeled proposed new compounds before synthesis to prioritize the order in which to make them. This would have undoubtedly been a valuable contribution to the program. In conclusion, this study emphasizes the value of using CAMD approaches in combination with classical SAR programs, even with less than perfect data.

Acknowledgments.
The authors would like to acknowledge the contributions made to this work by James D. Froyd, now with American Cyanamid, who did most of the screening, and Kenneth Kramer of DowElanco, who was responsible for the synthesis of many of the compounds described.

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Chapter 32

Synthesis and Fungicidal Activity of 2-Cyanoimidazoles

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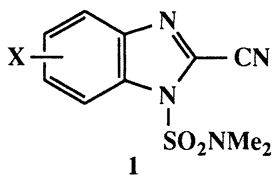
2-Cyanoimidazoles are fungicides with potential use in agriculture, particularly for the control of *Phytophthora infestans* (potato blight) and *Plasmopora viticola* (vine downy mildew). The synthesis of a range of substituted cyanoimidazoles is described with particular reference to 4-aryl and aroyl cyanoimidazoles. The biology and structure activity relationships are discussed.

As part of our research programme aimed at developing new agricultural fungicides, we became interested in 2-cyanobenzimidazoles **1** (*1*), active against *P. infestans* and *P. viticola.*



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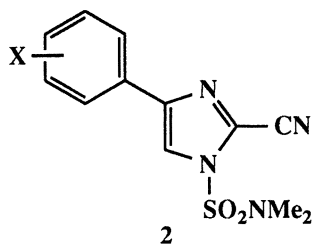

2

In considering the important features of these compounds, we wondered whether the benzimidazole ring was critical for activity. In particular, we considered whether non-fused analogues such as the arylimidazoles **2** would be fungicidally active. This paper will describe the synthesis of such compounds as well as a variety of alternatively substituted cyanoimidazoles. Their fungicide activity will also be discussed.

SYNTHESIS

4-Aryl Cyanoimidazoles. In order to test our hypothesis, a synthetic route to aryl cyanoimidazoles **2** was required. Examination of the literature revealed these to be novel structures. We envisaged that they should be accessible by the strategy outlined in Figure 1, using directed lithiation as a key step.

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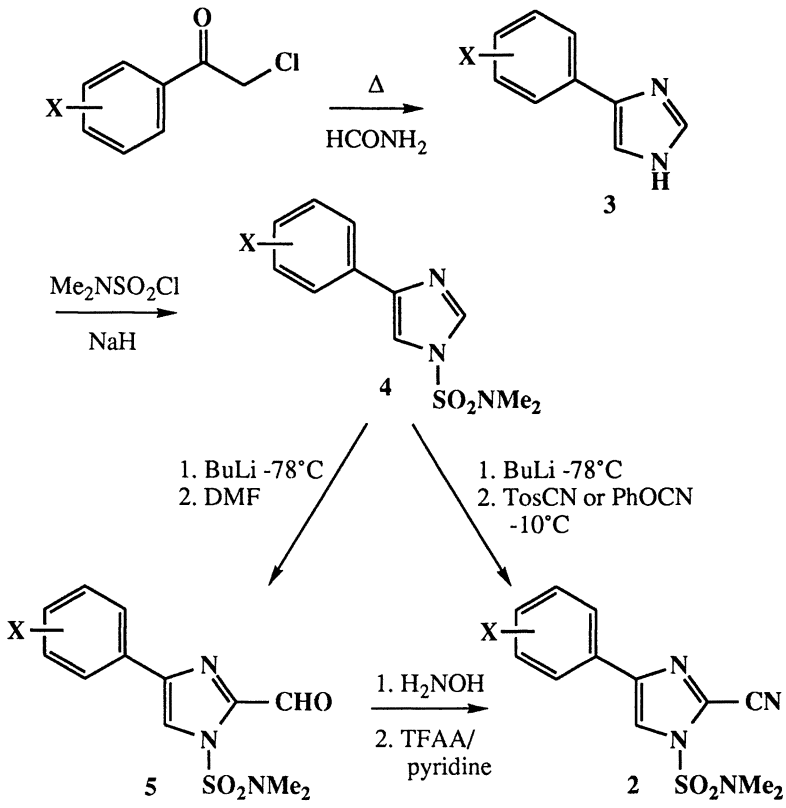


Figure 1

This approach proved to be very successful. Thus the arylimidazole **3** (X = 2,4-dichloro) (*2*) was regiospecifically sulfamoylated to give the imidazole **4**. Two approaches were then used for introducing the cyano function. In preliminary work, directed regiospecific lithiation of **4** (*3*) and reaction with DMF yielded the aldehyde **5** which was converted into the desired cyanoimidazole **2** (*4*) *via* established procedures *i.e.* formation of the oxime followed by dehydration. Although this approach proved successful, it was not sufficiently convenient or direct for wide application and we therefore developed an alternative strategy. Thus, after directed lithiation of the sulfamoylimidazole **4**, the intermediate anion was reacted with a cyanating reagent such as tosyl cyanide or phenyl cyanate (Figure 1) (*5*). This second approach proved to be successful and versatile and was used in the synthesis of a wide range of analogues including cyanopyrazoles and cyanotriazoles (*5*).

Since, gratifyingly, compound **2** (X = 2,4-dichloro) proved to be fungicidally active, we undertook a large programme of work to explore alternatively substituted aryl analogues (see later for biology). Other areas that were investigated will now be described.

5-Aryl Cyanoimidazoles. The synthesis of these is shown in Figure 2.

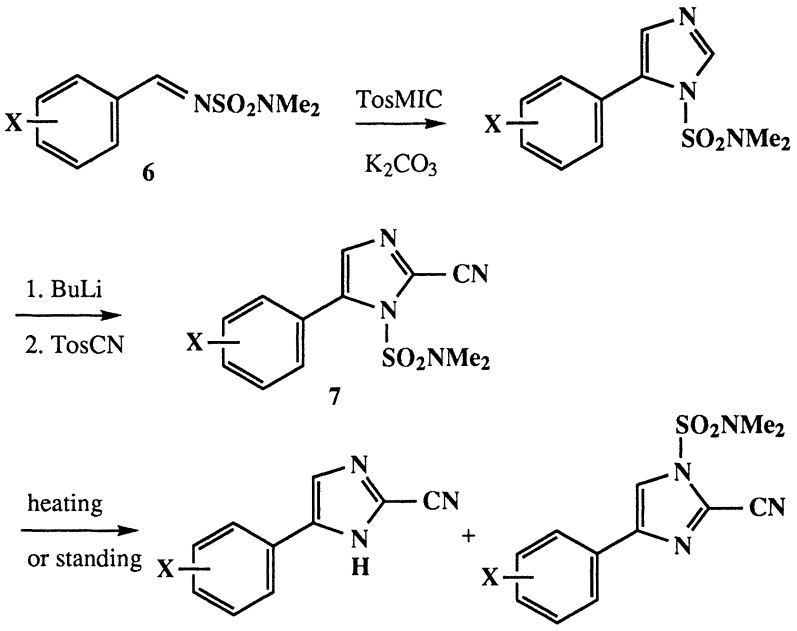


Figure 2

Reaction of the N-sulfamoyl imine **6** with TosMIC (*6*) gave a moderate yield of the 5-aryl imidazole which was converted to the desired cyanoimidazole **7** in the normal manner. However, the compounds proved to be unstable and rearranged on standing or heating to give the 4-aryl regioisomer, accompanied by some loss of the sulfamoyl group (Figure 2).

4,5-Disubstituted Cyanoimidazoles. These analogues could be prepared directly from **2** (Figure 3).

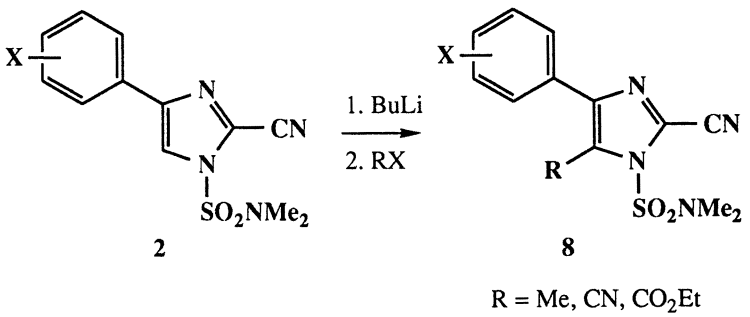


Figure 3

The important point in this approach is that blocking the 2 position of the imidazole ring with a cyano group allows directed lithiation to take place at the 5 position. Using this strategy, we could introduce a range of substituents. However, the products were also somewhat unstable, in this case *via* loss of the sulfamoyl group.

Finally, we also sought to prepare the 5-cyano regioisomer **9**. Again the synthetic strategy involved blocking the 2 position of the imidazole ring, this time by a silyl group (*3*) which would be lost under the experimental conditions (Figure 4).

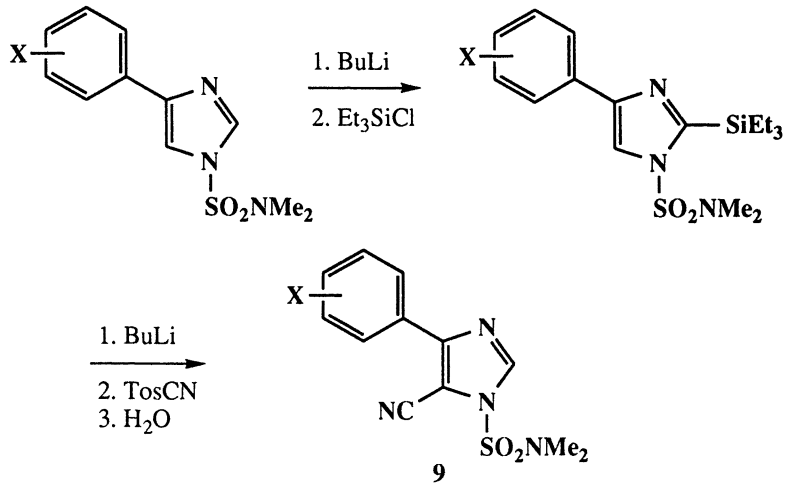
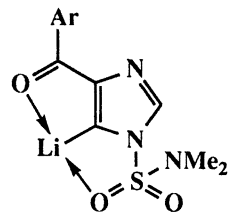


Figure 4

4-Aroyl Cyanoimidazoles. Our initial entry into this area arose by chance when we attempted to prepare an example of a 2,4,6-trisubstituted aryl cyanoimidazole using the normal procedure. When the appropriate chloroacetophenone was reacted with formamide, however, we obtained the aroyl imidazole **10** *(7)* instead of the expected aryl imidazole (Figure 5).

Seeking to exploit this finding, we converted the aroyl imidazole **10** into the aroyl cyanoimidazole **12** (Figure 5). Although the desired compound was obtained, the yield in the cyanation step was very poor. Subsequent studies showed this to be due to the fact that in the case of compound **11**, lithiation at the 5 position of the imidazole ring is favoured over that of the 2 position. This is presumably because of the extra stabilization of the 5-lithio species by the aroyl ketone as indicated below, or because of a kinetic directing effect.



Ar = substituted aryl

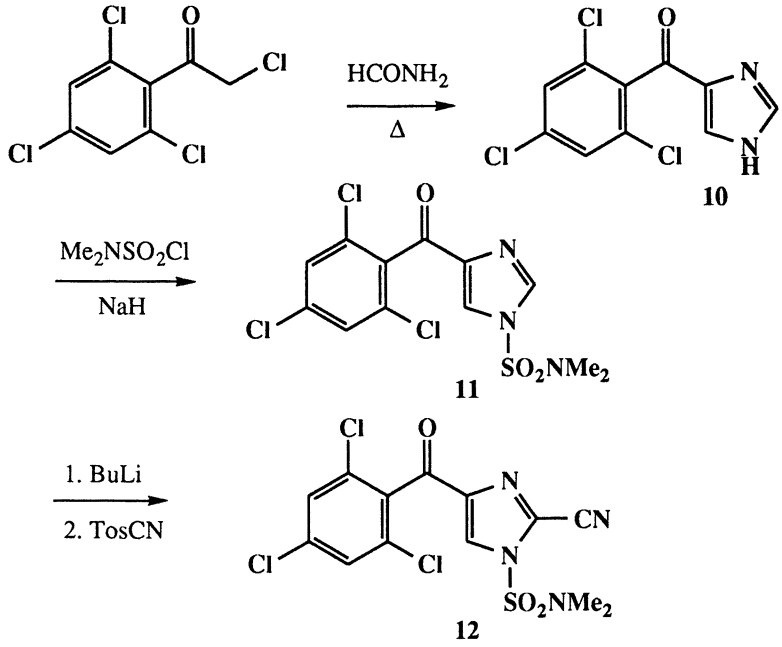


Figure 5

The finding that **12** was fungicidally active, whilst obviously pleasing, did give rise to two problems. The first was how to prepare the large amounts of compound **12**, required for field evaluation, given that the low yield in the last step made the current route impractical. The solution we developed was to reduce the aroyl ketone to the alcohol and protect as the silyl derivative **13** (Figure 6). As expected, lithiation of **13** now took place selectively at the 2 position of the imidazole. Cyanation and subsequent oxidation then provided a simple route to **12** in good overall yield.

The second problem which arose was that the route outlined in Figure 5 only applies to the preparation of 2,6-disubstituted aroyl cyanoimidazoles. Further analogues could be made by an alternative route which involved, as the key step, the addition of an aryl Grignard reagent to an imidazole carboxaldehyde (Figure 7). Using this route, we identified further promising compounds.

In summary, the preparation of a range of substituted cyanoimidazoles has been described using a diversity of synthetic approaches. We shall now consider their biological activity and the structure activity relationships within this series.

BIOLOGY AND STRUCTURE ACTIVITY RELATIONSHIPS

In general, the structure activity relationships were the same whether considering *P. viticola* or *P. infestans*.

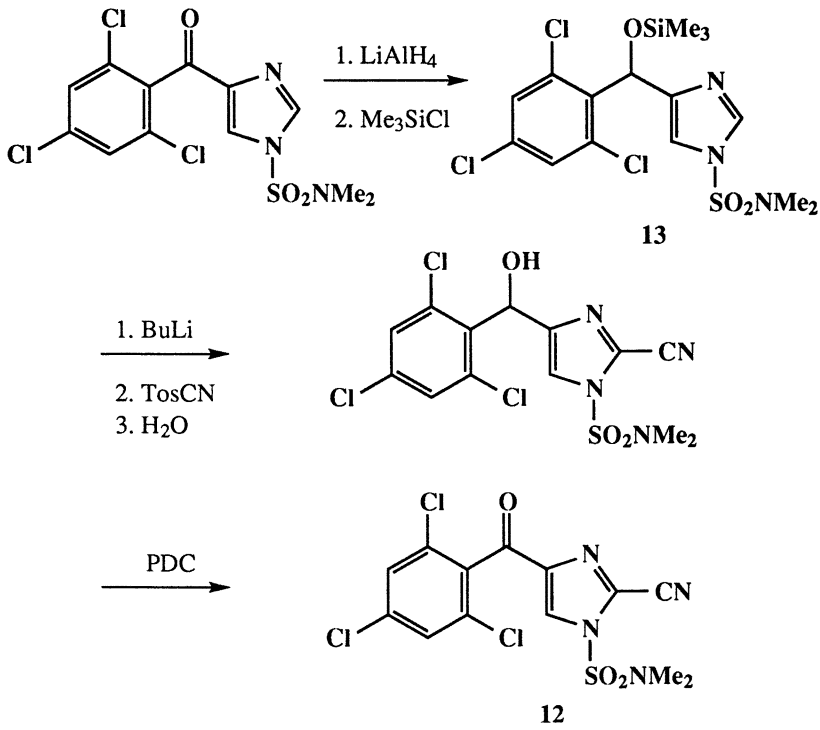


Figure 6

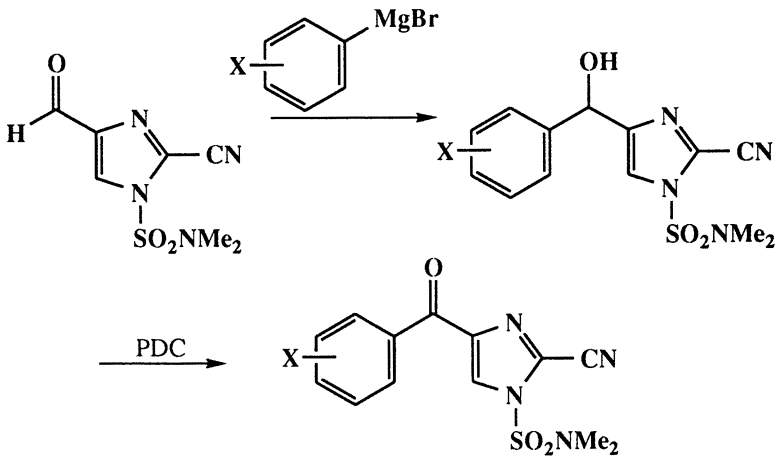
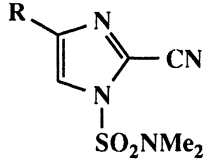
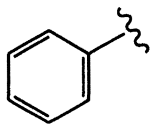
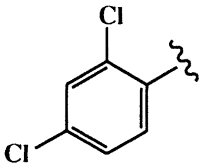
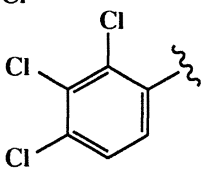
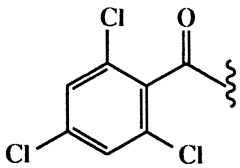


Figure 7

4-Substituted Cyanoimidazoles. Compound **2** (X = 2,4-dichloro) showed good activity against *P. infestans* and *P. viticola,* including metalaxyl resistant strains (Table I).

Table I



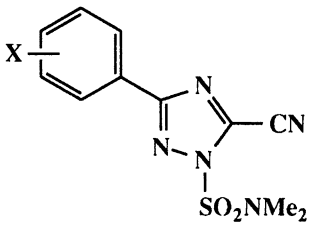
R	*Plasmopora viticola* - % control 5	2.5	1.25 ppm
	inactive		
	97	75	50
	100	100	65
	100	100	99

Subsequent studies showed that compounds in which the aryl group carried lipophilic, electron with drawing substituents such as halo or trifluoromethyl were particularly active (Table I). In addition, in the case of analogues where the aryl group is disubstituted, one of the substituents should ideally be in an ortho position.

When the 4-aryl group was replaced by an aroyl group, the compounds were more active (Table I). Again lipophilic substituents in the aroyl group are favoured although their position and electronic nature is less critical than for aryl substituents, presumably because the aroyl group itself is electron withdrawing. Finally, replacement of the 4-aryl group in **2** by alkyl, heteroaryl or trifluoromethyl gave less active compounds.

4-Aryl, 5-Substituted Cyanoimidazoles. As well as being relatively unstable, compounds **8** were less active. In addition, the 5-cyano compounds **9** were completely inactive.

Imidazole Ring Replacements. A small amount of work was undertaken to examine whether the imidazole ring could be replaced by alternative heterocycles. Fungicidal activity is retained in triazole analogues **14** although they provided no obvious advantage over the imidazole compounds.



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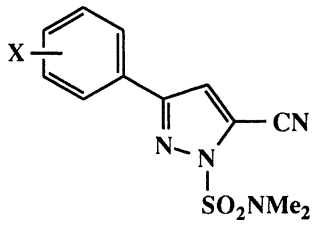

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The pyrazole analogues **15** were inactive.

Additional SAR. The cyano group appears to be essential for activity. A variety of compounds with alternative 2-substituents were investigated but all were inactive other than those with a pro-cyano group such as a thioamide. The sulfamoyl group appears to be optimal for activity although some replacements are possible, *e.g.* alkylsulfonyl.

Acknowledgment

Valuable discussions with Dr Alister Baillie during the preparation of this manuscript are gratefully acknowledged.

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Chapter 33

Novel and Selective Quinazolinyl Arylureas

Synthesis and Fungicidal Activity

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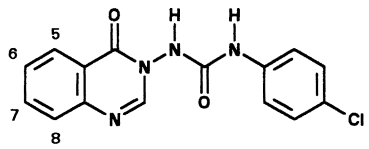
Quinazolinyl aryl ureas have been discovered to have a narrow spectrum of fungicidal activity against sugarbeet cercospora and banana black sigatoka. Compounds with 4-substituted phenyl groups were found to have the highest levels of activity. In general, substitution at the C-5 or C-8 positions on the quinazolinone did not improve activity whereas substitution at the 6 or 7 positions was detrimental. The synthesis as well as a description of the structure activity relationships for these compounds is given.

The lead compound, **1**, a urea linking a 4(3*H*)-quinazolinone to a 4-chlorophenyl group was discovered in a random screening program of the chemical library at the Agricultural Research Division. Compound **1** was tested in the primary fungicide screen and found to be active against *Cercospora beticola* (sugarbeet cercospora,



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sbc). Further testing revealed it to be active against fungal species related to sbc: *Mycosphaerella fijensis* (banana black sigatoka, bbs), *Cercosporidium personatum*, (peanut cercospora late leafspot, pcl) and *Cercospora arachidicola (*peanut cercospora early leafspot, pce). Due to the fungicidal activity and novel structure of this compound, a synthesis program was initiated to further explore this area of chemistry with hopes of increasing potency and expanding spectrum of activity.

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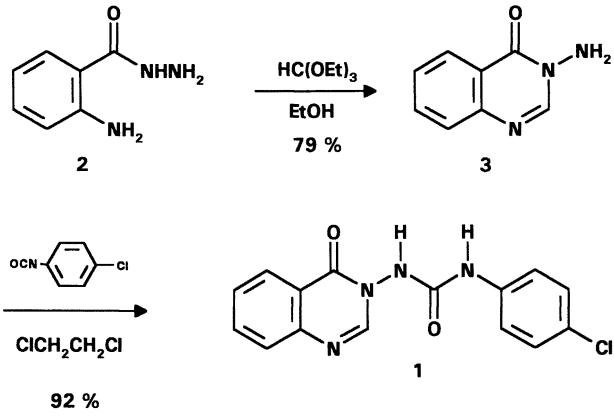
The Lead Compound

Synthesis. The synthesis began with the preparation of 3-amino-4(3*H*)quinazolinone. The procedure of Scheiner, et al.(*1*) was followed in which 2-aminobenzhydrazide (**2**) undergoes reaction with triethyl orthoformate in refluxing ethanol affording 3-amino-4(3*H*)quinazolinone (**3**) in 79 % yield. Reaction of **3** with 4-chlorophenyl isocyanate in 1,2-dichloroethane gave the urea **1** in high yield as a single crystalline product.



Biological Activity. The fungicidal activity of **1** is shown in Table I below.

Table I. Fungicidal Activity of the Lead Compound (1)

sugarbeet cercospora				banana black sigatoka				peanut cercospora early leafspot			peanut cercospora late leafspot		
rate (ppm)				rate (ppm)				rate (ppm)			rate (ppm)		
12	50	200	400	3	12	50	200	25	100	400	25	100	400
5.8	7.0	8.3	8.7	6.7	8.0	8.3	8.7	2.7	5.0	6.7	4.0	7.0	8.7

Fungicidal Activity Rating Values: The data shown are the average of 3 or more replicates.

0	0 % inhibition	5	60-74 % inhibition
1	1-14 % inhibition	6	75-89 % inhibition
2	15-29 % inhibition	7	90-95 % inhibition
3	30-44 % inhibition	8	96-99 % inhibition
4	45-59 % inhibition	9	100 % inhibition

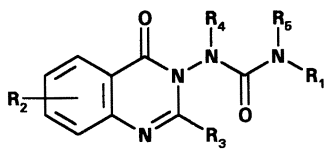
As can be seen from the data in Table I, the lead compound has moderate fungicidal activity against sbc, pce and pcl. It is more potent against bbs, with 96 % control of disease at 12 ppm. The outstanding feature of this compound, however, was subsequently discovered in advanced testing. It was found that 1 had excellent residual activity against bbs, as shown in Table II below. As one can see, fungicidal activity remained at high levels against bbs 21 days after treatment.

Table II. Residual Fungicidal Activity of the Lead Compound (1) Against Banana Black Sigatoka at Selected Rates (in ppm)

Days after Treatment	Application Rate (ppm)					
	25	50	100	200	400	800
7	5.7	6.3	7.0	7.7	8.0	8.0
14	6.0	7.3	7.3	8.0	8.3	8.7
21	5.7	6.3	8.0	8.0	8.0	8.3

Analog Synthesis

The synthesis program was divided into 5 areas of study:
1) Variation of the aryl group on the urea (R_1).
2) Substitution on the carbocyclic portion of the quinazolinone ring (R_2).
3) Substitution at the C-2 position of the quinazolinone (R_3).
4) Substitution on the urea nitrogen atoms (R_4 and R_5).
5) Replacement of the urea with a thiourea.



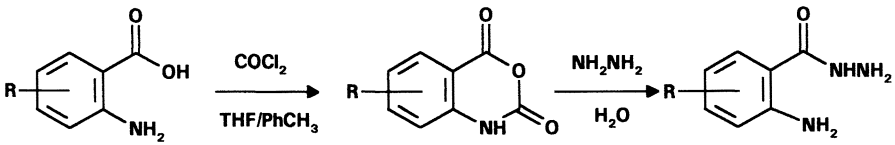
Aryl Ring Replacements, R_1

Synthesis. The synthesis of the R_1 analogs of the lead compound was quite simple. Reaction of 3-amino-4(3*H*)quinazolinone (**3**) with the appropriate isocyanate under the conditions shown for the synthesis of **1** afforded the ureas in high yield as pure crystalline products.

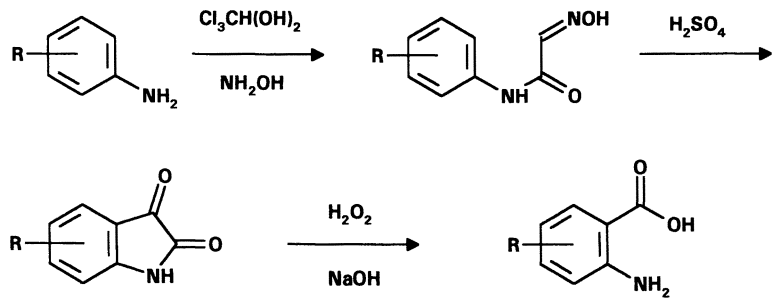
Biological Activity. The fungicidal activity of these analogs is shown in Table III. It was discovered that quinazolinyl alkyl ureas were inactive as fungicides as can be seen for the isopropyl, cyclohexyl and benzyl ureas. A phenyl or substituted phenyl group is required at this position for fungicidal activity. It was also found that compounds with halogen or trifluoromethyl substitution at C-4 on the phenyl group had the highest levels of activity. Other 4-substituted phenyl compounds such as methoxy and nitro were not active although the 4-methylphenyl group had moderate fungicidal activity. Halogen substitution on the phenyl at the C-2 or C-3 positions afforded compounds with less activity. Compounds with larger groups, such as the 2-napthyl urea, were not active as fungicides.

Quinazolinone Ring-Substituted Analogs, R_2

Synthesis. The substituted quinazolinones were synthesized starting from the anthranilic acid or isatoic anhydride as shown below. Reaction of a substituted anthranilic acid with phosgene afforded the isatoic anhydrides in good yield. Addition of hydrazine to the isatoic anhydrides then gave the substituted 2-aminobenzhydrazides, which were carried on to quinazolinones as for **1**.

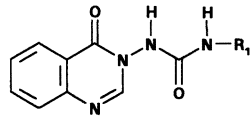


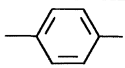
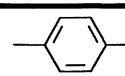
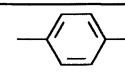
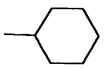
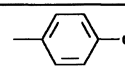
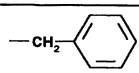
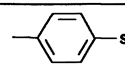
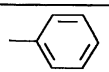
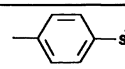
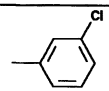
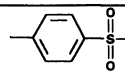
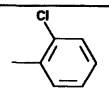
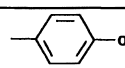
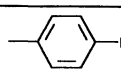
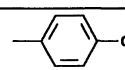
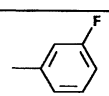
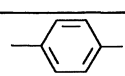
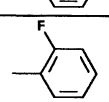
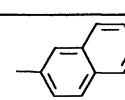
In those cases in which the substituted anthranilic acid or isatoic anhydride was unavailable, the anthranilic acid was synthesized using the route shown below. Reaction of a substituted aniline with chloral hydrate and hydroxylamine formed the α-nitrosoacetanilide which was cyclized to the isatin following the procedure of Sandmeyer (*2*). The isatin was then oxidized with hydrogen peroxide in aqueous base to afford the anthranilic acid (*3*).



Biological Activity. A number of substituted quinazolinyl 4-chlorophenyl ureas were synthesized and screened for activity. The fungicidal activity of selected substituted quinazolinone compounds is shown in Table IV.

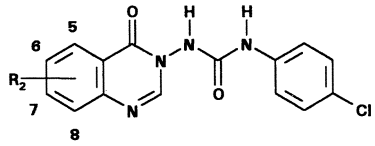
Table III. Fungicidal Activity of R_1 Analogs



	sugarbeet cercospora		banana black sigatoka			sugarbeet cercospora		banana black sigatoka	
	rate (ppm)		rate (ppm)			rate (ppm)		rate (ppm)	
R_1	100	400	50	200	R_1	100	400	50	200
	8.0	8.7	8.0	8.7		7.7	7.7	7.3	8.7
	0	0	0	0		3.3	0.7	1.7	2.7
	nt	0	0	0		6.7	7.3	7.0	8.3
	nt	0	0	0		5.0	4.0	4.0	4.7
	2.7	4.7	4.0	5.0		5.3	5.3	8.0	8.3
	4.7	5.3	1.0	1.7		5.3	6.7	7.3	7.7
	1.0	4.0	3.0	3.3		3.0	4.7	1.7	3.3
	6.0	6.7	6.7	7.7		3.3	7.3	5.3	6.3
	4.3	5.0	2.0	4.0		1.0	4.7	1.0	0.3
	6.0	6.7	4.0	5.3		2.7	3.7	0.3	1.0

nt=not tested at that rate

Table IV. Fungicidal Activity of Substituted Quinazolinones

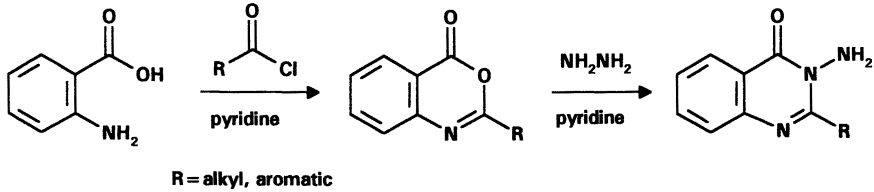


	sugarbeet cercospora		banana black sigatoka			sugarbeet cercospora		banana black sigatoka	
	rate (ppm)		rate (ppm)			rate (ppm)		rate (ppm)	
R_2	100	400	50	200	R_2	100	400	50	200
5-F	7.0	7.7	7.7	8.0	5-CH_3	3.7	4.0	5.0	6.0
7-F	2.3	1.3	0	0.3	6-CH_3	1.0	0.3	0	0
8-F	8.0	8.0	8.3	8.7	8-CH_3	5.0	6.0	5.7	6.7
5-Cl	8.0	8.0	8.0	8.3	8-OCH_3	0	0	1.3	1.0
6-Cl	0	0	4.0	1.0	5,8-di-F	7.7	8.0	7.7	8.0
7-Cl	0	0	0	3.0	5-Cl-8-CH_3	nt	0	2.7	4.0
8-Cl	1.3	2.0	8.3	8.7	6,7 	1.7	2.3	0	2.0

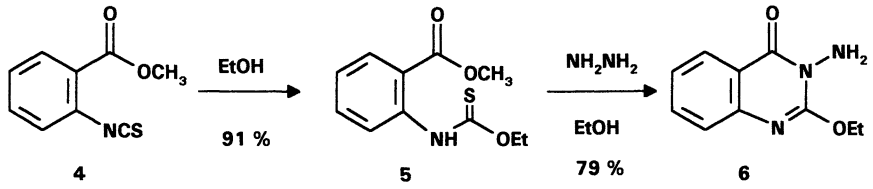
Compounds with substitution at the C-6 or C-7 positions have little or no fungicidal activity as can be seen with the 6-methyl, 7-fluoro and 6 or 7-chloro substituted quinazolinones. Compounds with substitution at C-5 or C-8 with halogen or methyl groups are active whereas the 8-methoxy substituted compound was inactive. Disubstituted compounds generally followed the same pattern, the 5,8-difluoro compound was very active although the 5-chloro-8-methyl compound was weakly active. The 6,7-napthyl compound was also found to be inactive.

C-2-Quinazolinone-Substituted Analogs

Chemistry. Quinazolinones with C-2 alkyl and aryl substituents were prepared by the reaction of anthranilic acid with the alkyl or aromatic acid chloride or anhydride to yield the benzoxazine (*4*). Reaction of the benzoxazine with hydrazine hydrate then gave the C-2 alkyl- or aryl-substituted 3-aminoquinazolinone (*5*).



The C-2 alkoxy and thioalkyl quinazolinones were synthesized following the procedure of Dean and Papadopoulos (*6*), as shown for 2-ethoxy-3-aminoquinazolinone. Reaction of methyl isothiocyanatobenzoate (**4**) with ethanol formed thiocarbamate **5**. Reaction of **5** with hydrazine hydrate gave quinazolinone **6** in 79 % yield.



The C-2 hydroxyquinazolinone was synthesized according to the procedure of Peet and Sunder (*7*), in which 2-aminobenzhydrazide (**2**) undergoes reaction with urea in refluxing decahydronapthalene to afford quinazolinone **7** in 59 % yield. The C-2 mercaptoquinazolinone was synthesized according to the procedure of Liu and Hu (*8*) in which 2-aminobenzhydrazide (**2**) is treated with base in the presence of carbon disulfide to afford quinazolinone **8** in 22 % yield. Both of these quinazolinones then underwent reaction with isocyanates under standard conditions to give the ureas.

Biological Activity. The fungicidal activity for selected C-2-substituted quinzolinyl 4-chlorophenyl ureas is shown in Table V. In general, compounds with substitution at the C-2 position of the quinazolinone were less active than the unsubstituted compound. The methyl substituted compound had reduced fungicidal activity against both pathogens, and higher alkyl groups or a phenyl group in that position gave compounds with little or no activity. Substitution with heteroatoms was also detrimental to fungicidal activity. Interestingly, the C-2 ethylthio compound was inactive against sbc, while being quite active against bbs, a feature uncommon in these compounds. The C-2 trifluoromethyl-substituted compound was moderately active against both pathogens.

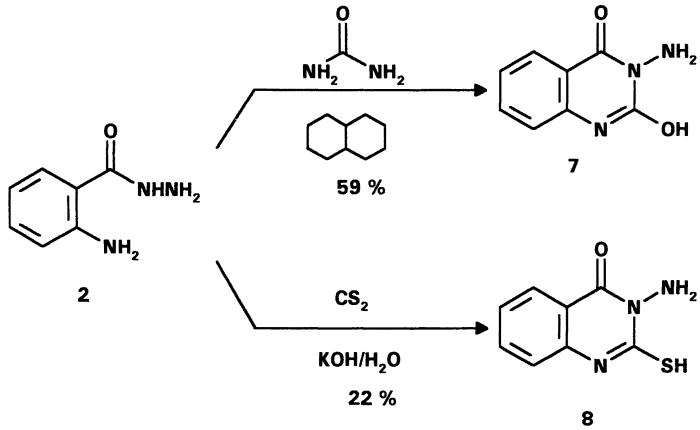
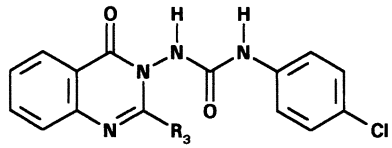


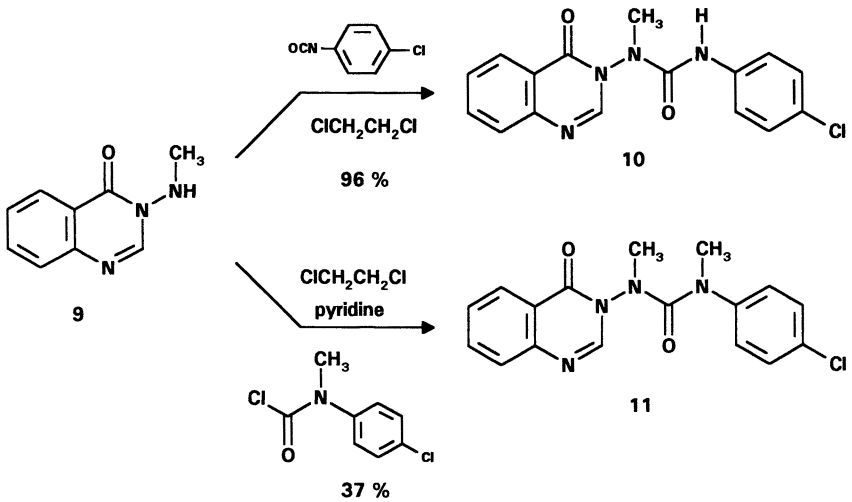
Table V. Fungicidal Activity of C-2 Substituted Quinazolinyl Ureas



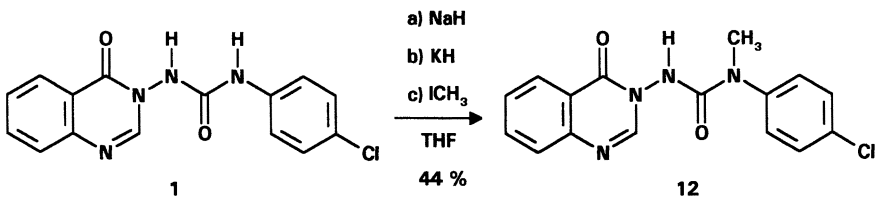
	sugarbeet cercospora rate (ppm)		banana black sigatoka rate (ppm)			sugarbeet cercospora rate (ppm)		banana black sigatoka rate (ppm)	
R_3	100	400	50	200	R_3	100	400	50	200
H	8.0	8.0	8.0	8.7	OH	nt	0	0	0
CH_3	7.3	7.3	5.3	5.0	OEt	2.3	1.0	5.3	6.3
Et	2.0	0.3	0.3	0	SH	nt	0	4.3	6.0
i-Pr	0	1.0	0	0.3	SEt	nt	0.7	6.7	9.0
Ph	4.0	5.3	0.3	0	CF_3	nt	6.0	0.3	4.0

Urea Substituted Analogs, R_4 and R_5

Synthesis. The effect of substitution on the urea nitrogen atoms was explored by synthesizing the two mono-methylated isomers and the di-methylated analog of the lead compound **1**. The N-1 methyl compound **10** was synthesized by reaction of 3-methylaminoquinazolinone (**9**) with 4-chlorophenyl isocyanate under standard conditions to afford the product in 96 % yield. The dimethyl analog **11** was synthesized by reaction of (**9**) with *N*-methyl-4-chlorophenylcarbamoyl chloride.

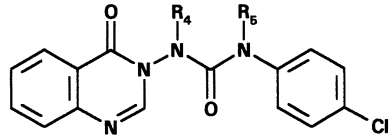


The N-3 methyl compound **12** was synthesized by reaction of the dianion of urea **1** with iodomethane to give the mono-methylated compound as the kinetic alkylation product in 44 % yield. Attempted formation of the dianion using two equivalents of sodium hydride failed. The dianion was made by the reaction of **1** with one equivalent of sodium hydride followed by one equivalent of potassium hydride.



Biological Activity. The fungicidal activity for the N-substituted compounds is shown in Table VI. It is clear from the table that methyl substitution on the urea nitrogen atoms is detrimental to fungicidal activity.

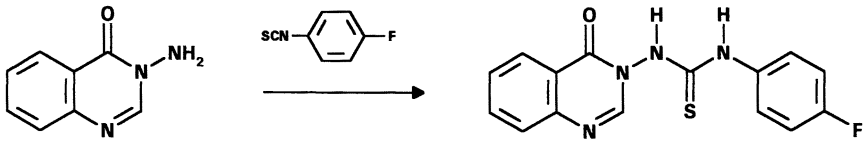
Table VI. Fungicidal Activity of N-Substituted Analogs



		sugarbeet cercospora		banana black sigatoka	
		rate (ppm)		rate (ppm)	
R_4	R_5	100	400	50	200
H	H	8.0	8.0	8.0	8.7
CH_3	H	0	0.3	0	4.0
H	CH_3	nt	0	6.3	5.3
CH_3	CH_3	0	5.3	0.7	1.3

Quinazolinyl Aryl Thioureas

Chemistry. The thioureas were synthesized by reaction of quinazolinone **3** with the appropriate isothiocyanates. Under standard conditions (refluxing 1,2-dichloroethane) the reaction gave a mixture of products which after difficult purifications, afforded the thioureas in low yields. Refluxing toluene gave a complex mixture . With pyridine, however, the thioureas were obtained in high yields, as single crystalline products after 12 hours at room temperature. For example, **13** was obtained in 90 % yield.

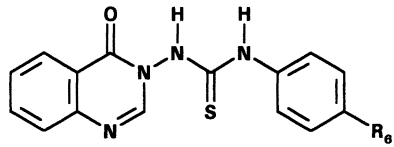


3 13

solvent	time	temp(C)	result
1,2-dichloroethane	72 h	83	50 % yield
toluene	72 h	110	<5 % yield
pyridine	12 h	25	90 % yield

Biological Activity. The fungicidal activity of the thioureas is shown in Table VII. The thioureas were found to be, in general, equal in fungicidal activity to the corresponding ureas.

Table VII. Fungicidal Activity of Quinazolinyl Aryl Thioureas



	sugarbeet cercospora		banana black sigatoka			sugarbeet cercospora		banana black sigatoka	
	rate (ppm)		rate (ppm)			rate (ppm)		rate (ppm)	
R_6	100	400	50	200	R_6	100	400	50	200
(lead compound)	8.0	8.0	8.0	8.7	Br	7.7	8.0	7.3	8.3
Cl	7.0	6.7	8.3	8.7	CF_3	6.7	6.7	7.7	8.7
F	5.3	7.3	8.3	8.3	H	nt	0	1.3	5.3

Conclusions

The synthesis program has delineated the structure-activity relationships for this class of fungicides and compounds with activity comparable to the lead have been discovered. The quinazolinyl aryl ureas are novel fungicides with a narrow spectrum of activity limited to sugarbeet cercospora and banana black sigatoka. The compounds have long residual activity against banana black sigatoka. The most active compounds have a 4-substituted phenyl group and an unsubstituted quinazolinone or a quinazolinone substituted at the C-5 and/or C-8 positions, with no substitution on the urea nitrogens. The thioureas were found to be comparable in activity to the ureas. No compounds were discovered which have substantially higher potency or wider spectrum of fungicidal activity than the lead compound **1**.

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Chapter 34

Oxime Fungicides

Highly Active Broad-Spectrum Protectants

Joseph E. Drumm, John B. Adams, Jr., Richard J. Brown, Carlton L. Campbell, David L. Erbes, William T. Hall, Stephen L. Hartzell, Mark J. Holliday, Daniel A. Kleier, Marsha J. Martin, Stephen O. Pember, and George R. Ramsey

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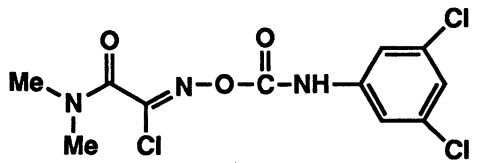
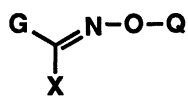
Broad spectrum contact fungicide activity was demonstrated by several oxime classes of chemistry. Biological data, chemistry, structure activity trends and the importance of hydrolysis to the effectiveness of the oxime fungicides are discussed.

The discovery of the fungicidal activity of compound **1** led to a major analog program in order to explore the range of biological activity possible for this class of chemistry (Figure 1). Control at 200 ppm of several of the economically important diseases (apple scab, peanut early leaf spot, rice blast, tomato late blight, wheat leaf rust, grape downy mildew, and cucumber botrytis) provided results similar to commercial contact standards such as mancozeb, chlorothalonil, and captan.



1

Amide Region  **Capping Group**

Leaving Group

Figure 1. Lead and Generic Structures

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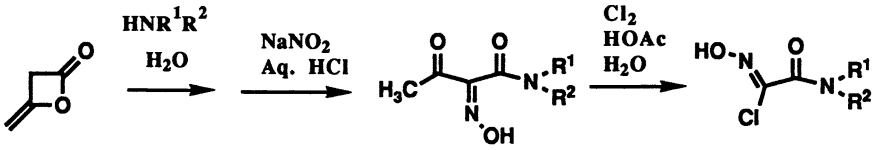
As shown in Figure 1, we have divided the molecule into three regions and will refer to them as the amide region (G-group), leaving group (X-group) and the capping group (Q-group). A discussion of some of the chemistry of the area will be followed by a presentation of some of the biological data. Structure activity relationships will be discussed, as will some of the hydrolysis (degradation) trends.

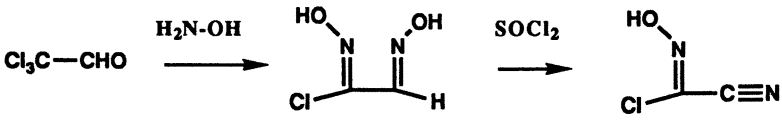
Synthesis

In many cases, it is preferable to start the synthesis from hydroximidoyl chlorides. The hydroximidoyl chlorides where G = amide or ester were readily made using Scheme 1 shown below (*1-5*). Compounds where G = phosphonate were made similarly, starting with the required β-keto-phosphonate. This method was used to generate the majority of the hydroximidoyl chlorides.



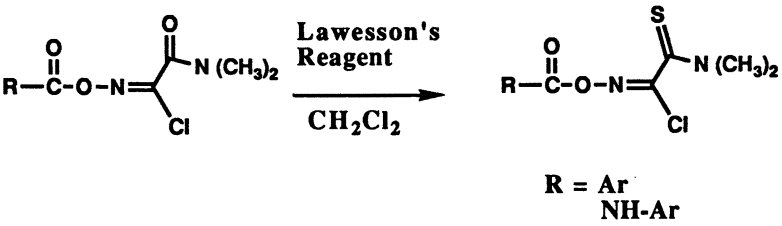
Scheme 1

Compounds where G = CN were made according to the method of Adamczyk and Kozikowski (*6*) (Scheme 2).



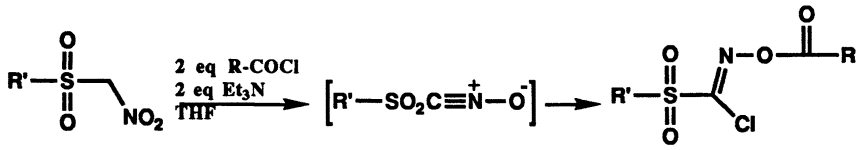
Scheme 2

Thioamide G-groups were made by reacting the appropriate carbamyl or ester-amides with Lawesson's reagent, with high selectivity for the amide carbonyl (Scheme 3).



Scheme 3

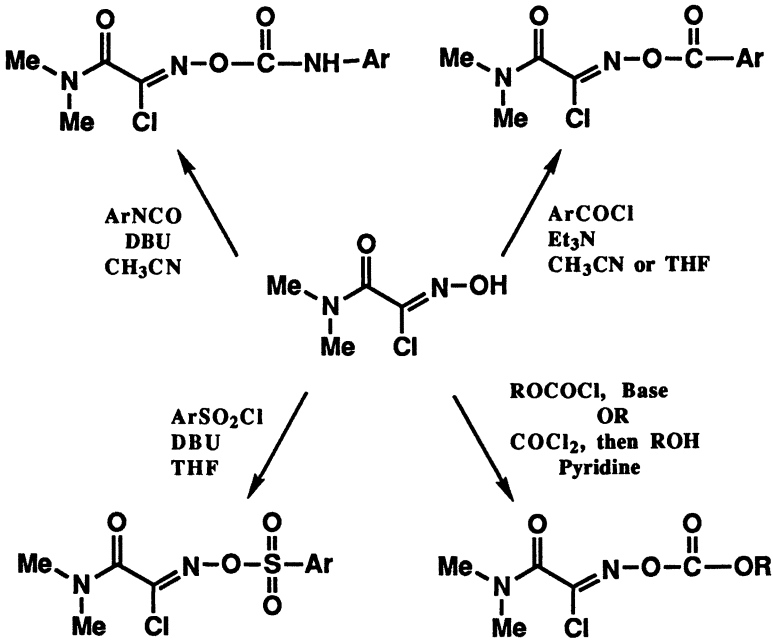
Scheme 4 shows a method adapted from Shimizu *et al* (*7*) that incorporates the X-group and Q-group in one step, for cases where G = sulfonyl. The nitronate anion, generated by deprotonation, is O-acylated. Elimination of an equivalent of the acid (corresponding to the acid chloride) provides the sulfonyl nitrile oxide, which adds across a second acid chloride equivalent.



Scheme 4

Capping methods are shown below in Scheme 5. Aliphatic isocyanates (except methyl isocyanate) failed to react under a variety of conditions. With aryl isocyanates, the use of a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was essential to the formation of the product. Other bases failed to produce the carbamates cleanly, or at all.

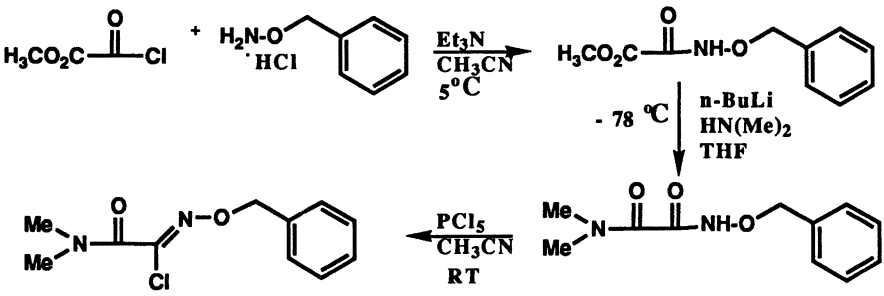
The reaction of the hydroximidoyl chlorides with other acylating groups proceeds through the reactive nitrile oxides following well-precedented pathways (*8*). Acetonitrile was the preferred solvent, although THF was frequently used; triethylamine was most frequently used as the base.



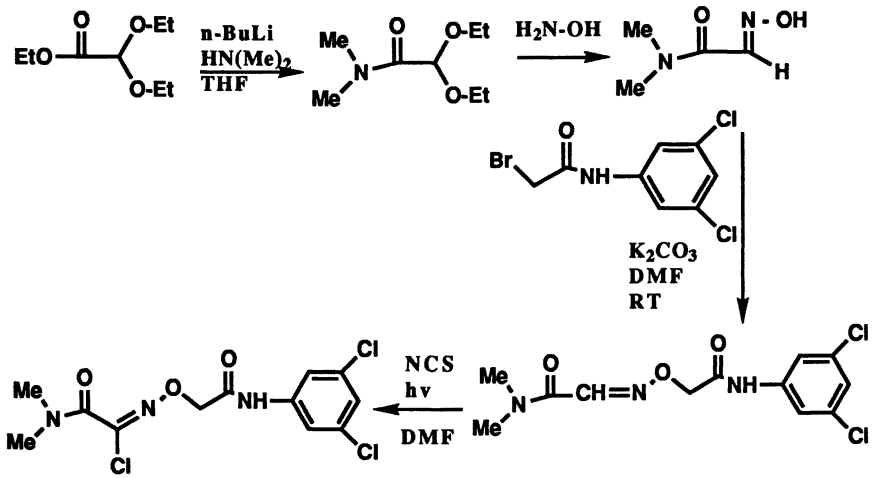
Scheme 5

O-Alkyl Derivatives

Two strategies were employed to make O-alkylated hydroximidoyl chloride derivatives (*9-11*). First, N-alkoxyoxamides were chlorinated with phosphorous pentachloride as shown in Scheme 6. The other strategy employed chlorination of the oxime with N-chlorosuccinimide to give the hydroximidoyl chloride as shown below in Scheme 7.

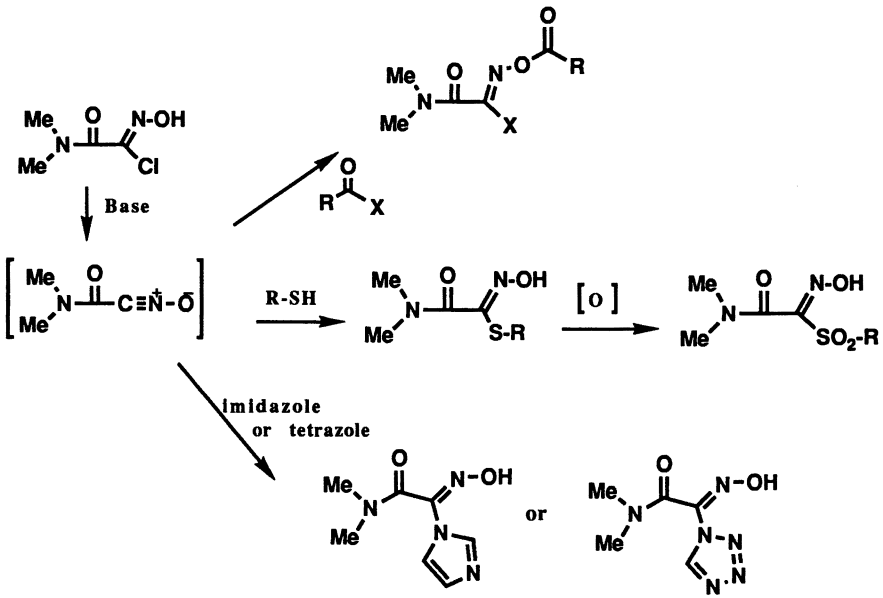


Scheme 6



Scheme 7

When different X-groups were required, they were formed prior to acylation by reaction of the nitrile oxide *(7)* with nucleophiles such as mercaptans *(12)* or heterocycles. The nitrile oxide can also be trapped with acyl halide derivatives as shown below in Scheme 8. Sulfide oxidations were carried out using either MCPBA or peroxyacetic acid.



Scheme 8

Biology - Greenhouse

The broad spectrum of activity demonstrated by these compounds is shown in Table I. This activity was limited to preventative control for most of the diseases. These compounds were equivalent to the commercial standards such as mancozeb, chlorothalonil and captan, when compared in the greenhouse. The O-alkylated oximes, by contrast, were not active. The greenhouse disease control data were used to develop the structure activity relationships.

Structure-Activity Relationships

Several elements of SAR were evident for this class of compounds (Figure 2). The criteria used for the ranking were based on the spectrum of diseases controlled, followed by the rates that disease control began to diminish (break-rate). The most consistent diseases controlled were grape downy mildew and late blight on tomatoes or potatoes.

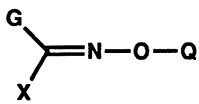
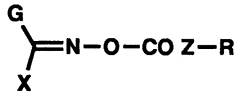
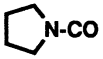
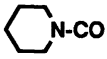
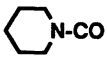


Figure 2. Generic Oxime Structure

G-group. The G-group substituents are ranked in descending order of their biological activity as shown below. The G-group was also believed to be important

Table I. Biological data



#	G	X	Z	R	Percent Disease Control at 200 ppm APS	PCS	WLR	TLB	GDM	RCB	CBT
1	Me2NCO	Cl	-	3,5-Cl-Ph	96	29	43	95	99	21	0
2	Me2NCO	Cl	NH	3,5-Cl-Ph	100	NT	90*	100	NT	NT	NT
3		Cl	-	2-Naphthyl	100	72	86	99	100	8	20
4		Cl	-	2-Naphthyl	100	82	78	99	100	0	18
5		Cl	NH	2,6-Cl-Ph	100	NT	94	99	98	88	49
6	Me2NCO	Cl	-	2-Naphthyl	100	99	85	100	100	NT	59
7	Me2NCO	Br	-	2-Naphthyl	97*	94	72	100	99*	21	0
8	Me2NCO		-	2-Naphthyl	NT	NT	NT	55	100*	0	0
9	Me2NCO	SO2-Ph	-	3-CF3-Ph	100	97	97	100	100*	53	NT
10	EtO2C	Cl	-	2-Naphthyl	99	52	63	97	99*	0	82
11	NC	Cl	-	2-Naphthyl	100	70	NT	68	99	0	0
12	Ph-SO2	Cl	-	4-Cl-Ph	99	0	0	95	70*	0	0
13	Me2N-SO2	Cl	-	Ph	NT	NT	91	100*	100*	0	91
14	Me2NCO	Cl	NH	3,4-Cl-Ph	100	88	97	100	100	93	99
15	CH3CO	Cl	-	2-Naphthyl	90*	NT	92	90	94*	NT	0
16	Me2NCO	Cl	OCH2	2-Naphthyl	100	92	85	100	100	96	99
17	Me2NCS	Cl	NH	3,5-Cl-Ph	100	80	NT	0	48*	0	0
18	(CH3O)2PO	Cl	-	3-CF3-Ph	NT	NT	23	25	80	0	0

* indicates disease control at 40 ppm; NT indicates compound not tested

APS - Apple Scab (*Venturia inequalis*)
PCS - Peanut Early Leaf spot (*Cercospora arachidicola*)
WLR - Wheat Leaf Rust (*Puccinia recondita*)
TLB - Tomato Late Blight (*Phytophthora infestans*)
GDM - Grape Downy Mildew (*Plasmopara viticola*)
RCB - Rice Blast (*Pyricularia oryzae*)
CBT - Cucumber Botrytis (*Botrytis cinerea*)

in controlling the solubilization of the compounds in water. Dissolution of the compounds in the water containing either fungal spores or actual growing fungi was found to be important for expression of disease control. A more detailed discussion will follow later. Optimal disease control was seen when the number of carbons on the amide nitrogen substituents totaled five, with the exception of the dimethylamide which provided the highest level of control.

-CO-NR1R2 > $-SO_2NMe_2$ > $-SO_2$-Ar > -CN > $-PO_3R_2$ > -S-R > $-CO_2R$

X-group. The X-group substituents demonstrated that a good leaving group is required. Table II shows the relationship of pK_a to biological activity; halogens as the X-group gave the most active compounds.

-Br, -Cl > $-SO_2R$ > $-NO_2$ >> -imidazole, -tetrazole > -CN > -S-R, -H, $-COCH_3$

Q-group. The Q-group must be hydrolyzable. The carbamates were the most active of the sub-classes, while the carbonates and benzoates (and naphthoates) were also highly active. The sulfonates were much less active, and the O-alkyl derivatives, like the uncapped hydroximidoyl chlorides themselves, were not fungicidal.

-CO-NH-R > -CO-Ar, $-CO_2R$ > SO_2R >> H, alkyl

Biology - Field

The lead compound **1** showed poor performance in early field trials due, we believe, to hydrolytic instability. Several other carbamate analogs along with an ester and carbonate were also tested, but their performance was also erratic (potato late blight, grape downy mildew and apple scab). The field results even for a given compound were not consistent, one test giving significantly more control than another, against the same disease. The results for several years of field testing for all of these compounds were much below the standards.

Hydrolysis

Many of the structural modifications reported in the synthesis section and summarized in the SAR were made in an attempt to retain the fungicidal activity while lengthening the hydrolytic stability. Investigations revealed that each of the sub-classes of capping groups fell within very specific hydrolysis ranges. The carbamates had half-lives as low as 10 minutes provided the material was completely dissolved. In most cases, an inverse relationship existed between the hydrolysis stability and the fungicidal activity in the greenhouse (Table III). The best balance between disease control and hydrolytic stability arose from the O-acyl capping group (oxime esters). We initially viewed the proclivity toward aqueous hydrolysis of the carbonyl linkage between the oxime and the capping group as an attractive feature, offering a ready mechanism for environmental degradation of these compounds. It now appears that the length of the dew periods and amounts of rainfall experienced by these hydrolytically unstable compounds greatly influences the field activity and accounts for the erratic field performance noted above.

Table II. Leaving group effects on biological activity

X	pK_a of HX	Plant Disease Control
- Br	- 8	++
- Cl	- 7	+++
- SO_2-R	2	++
- NO_2	3.5	+
Imidazole	7	-/+
-CN	9.3	-
-S-R	10	-
Acetyl	>30	-
-H, -alkyl	>40	-

Table III. Comparison of solution hydrolysis rates and biological activity

Capping group	Class	Half-life (hrs.)	Activity
R-SO2	sulfonate	12	+
R-CO	ester	2-10	++
R-O-CO	carbonate	2.5-3.5	+++
R-NH-CO	carbamate	0-2	+++

Nitrile Oxide Hypothesis

The hydrolytic instability of the O-acylated hydroximidoyl chlorides led us to speculate that they might be acting as profungicides. Greenhouse testing showed that the compounds with the fastest hydrolysis rates (carbamates) provided the highest levels of disease control when the plants were inoculated 24 hours after treatment with the chemical. Hydrolysis degradation products of **1** were isolated and identified using LC-MS. Examination of these degradation products in greenhouse testing failed to identify any active materials, including the uncapped hydroximidoyl chloride.

In vitro studies of the hydroximidoyl chlorides, however, showed that they caused lysis of *Plasmopara* zoospores within minutes. Hydrolytic stability studies within the physiological pH range on these hydroximidoyl chlorides noted a steady decomposition to other products, with an estimated half-life of several hours. Since hydroximidoyl chlorides are known to generate nitrile oxides, this led us to speculate that the nitrile oxides might be acting as the fungicidal agents (Figure 3). Zoospore lysis was observed on treatment with nitrile oxides or else, on generation of the nitrile oxides *in situ* from hydroximidoyl chlorides and base. Uncapped oximes in which the chlorine was replaced by a hydrogen or 3,5-dichloroaniline failed to cause any zoospore lysis. Thus, for *in vitro* activity, the need for a good leaving group was demonstrated. These results led us to propose the model

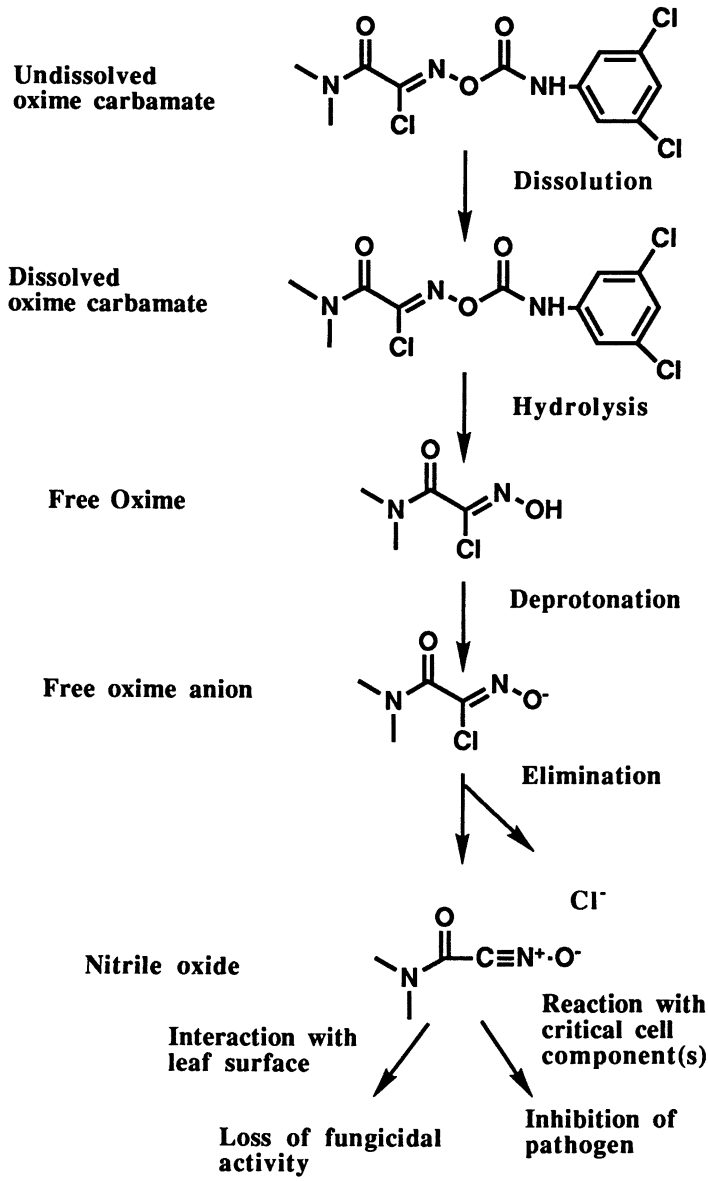


Figure 3. HYDROLYTIC CASCADE AND THE GENERATION OF NITRILE OXIDE

describing a hydrolytic cascade which generates a nitrile oxide from **1** (Figure 3). We used this as our working model to guide subsequent investigations.

We believe that the decomposition of 1 occurs on solubilization releasing first the hydroximidoyl chloride and then the nitrile oxide, the active fungicide. In the presence of a pathogen, the nitrile oxide can react with some critical cell component to inhibit its growth. In the absence of fungi, the nitrile oxide reacts indiscriminately to yield materials no longer fungicidal. Greenhouse testing with simulated dew periods showed decreased effectiveness for the capped compounds as the number and duration of the dew periods between spraying of the compound and inoculation with a pathogen were increased. These results suggested hydrolysis of the compounds was occurring on the leaf surface, followed by decomposition of the nitrile oxide prior to encountering the pathogen. This would explain the lack of residual activity that was observed.

Conclusions

The hydroximidoyl chloride derivatives discussed display broad spectrum fungicidal activity in greenhouse testing. The molecules can be divided into three portions, each of which plays a key role in the resulting biological activity. The data suggest that these compounds are profungicides, which release a reactive nitrile oxide in a hydrolytic cascade. This hydrolytic cascade, although essential to the fungicidal activity of the compounds, limits their effectiveness under field conditions where hydrolysis is influenced by uncontrollable factors such as rainfall, dew periods or other moisture. Since the hydrolysis occurs whether or not the pathogen is present, the amount of nitrile oxide available for disease control diminishes too rapidly for a standard commercial spray interval.

Acknowledgments

The authors would like to thank the many people who assisted in this work, in particular, E.A. Steel, C. Happersett, T. Tran, B. Atkins, A.E. Trivellas, F.T. Coppo and C.G. Sternberg.

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Chapter 35

2-Cyanoarylethyltriazoles as Agricultural Fungicides

Discovery of Fenbuconazole

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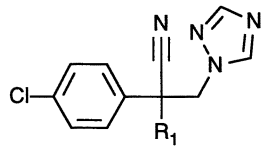
The chemical synthesis and biological studies which led to the discovery of the developmental compound RH-7592, α-(2-(4-chlorophenyl)ethyl-α-phenyl-1H-1,2,4-triazole-1-propanenitrile are described. The utilization of phenylacetonitriles as a starting point for the preparation of 2-substituted-2-cyano-phenylethyltriazoles led to the discovery and commercial development of myclobutanil, α-butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile. Further structure-activity investigations at the 2-substituent position led to the discovery of the 2-phenylethyl series of compounds which also possess high antifungal activity.

The first commercial 2-cyano-2-arylethyltriazole fungicide, myclobutanil, was introduced in 1986 for use on apples and vines (*1*). The quantitative structure-activity relationships which led to the discovery of this ergosterol biosynthesis inhibitor has been reported (*2,6*). The results of quantitative structure-activity analysis indicated



R_1 = butyl is Myclobutanil

the alkyl side chain (R_1) had an optimum length of 6-7 Å and the terminal 3 Å was optimally a hydrophobic fragment, and the *p*-chlorophenyl group was determined to be the preferred substituent for the aryl group.

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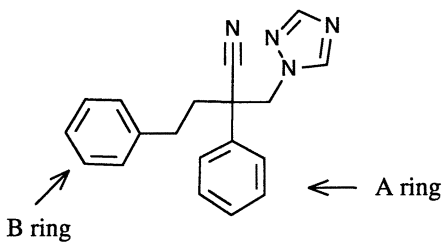
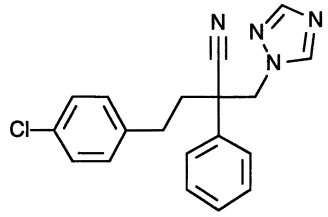
Structure activity investigations in the 2-cyano arylethyl triazoles were continued with focus on the R_1 substituent; specifically larger hydrophobic substituents which could increase biological activity were examined. Synthetic targets were chosen to determine the effect of cyclic substituents linked through a group of variable chain length to the α-cyano position. In particular, variations in ring type, ring size, chain to ring length, heteroatom position and heterocycle type were considered, eg. arylalkyl, aryloxyalkyl, heterocyclic alkyl and cycloalkylalkyl groups. At the time this work was initiated, two series of imidazole antimycotics, 1-[2-(arylalkyl) and 2-(aryloxyalkyl)]-2-phenylethylimidazoles were reported by Janssen Pharmaceutical (*3,4*). The structure-activty reported for these series indicated that chain length had little influence on *in vitro* activity. However, in these nor-cyano series, phenyl substitution focused on 2,4-dichloro which has been shown not to be an optimum substitution in the 2-cyano-2-alkyl series (*6*). Thus the implications of the chain length on antifungal activity in this 2-cyano series was unclear.

Our examination of a number of structures based on the strategy outlined above, indicated that for the 2-cyano-2-arylethyl triazoles, arylalkyl was a superior R_1 substituent to cycloalkyl, cycloalkylalkyl, arylalkenyl, aryloxyalkyl and heterocyclic alkyl. Within the 2-arylalkyl series, the β-phenylethyl substituent (*5*) was the most fungicidal, superior to benzyl, phenylpropyl and phenylbutyl and structure-activity studies of substitution on the 2-phenyl-2-(β-phenylethyl)ethyl triazole series followed.



Substitution studies on the 2-phenyl ring (A-ring) indicated that 4-fluoro or no substitution was optimum, while studies substituting on the 2-β-phenylethyl ring (B-ring), resulted in a number of substituents with excellent fungicidal properties, of which *para*-halogen and *para*-trifluoromethyl were among the best.

A number of 2-cyano-2-phenyl-2-[((substituted) phenyl) ethyl] triazoles were selected to undergo field evaluation. Results of these tests indicated a high degree of disease control on cereals, stonefruits, peanuts and other crops. The best overall level of cost-performance was shown for α-[2-(4-chlorophenyl)ethyl]-α-phenyl-1H-1,2,4-triazole-1-propanenitrile, RH-7592 (common name fenbuconazole) (*7*).



RH-7592

Chemical Synthesis

The 2-substituted-2-cyano-arylethyltriazoles were prepared by procedures previously described (*8*). The synthesis is completed in either a two or three step procedure from alkylated phenylacetonitrile intermediates as shown in Figure 1. Our synthesis

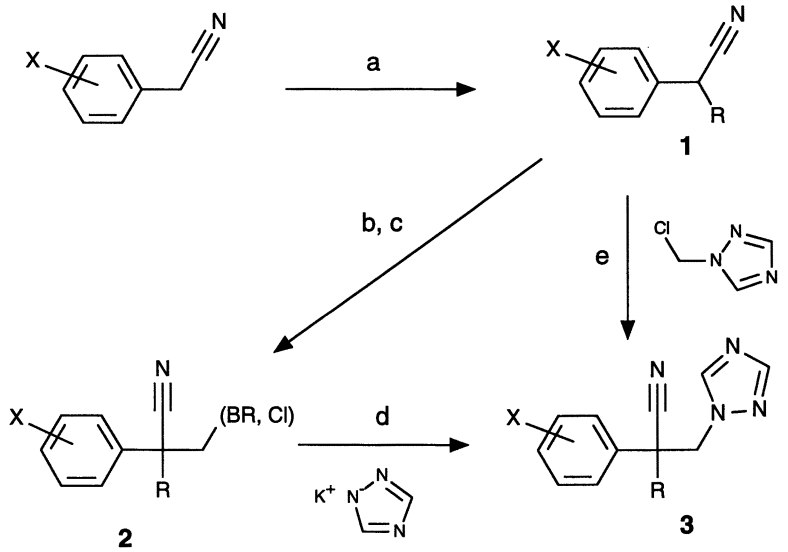


Figure 1. Synthesis of 2-Substituted-2-Cyano Phenethyltriazoles

efforts concentrated on an efficient synthesis for the key alkylated intermediate **1**. The structure-activity studies required synthesis of the alkylating reagents and the use of a diverse range of alkylating conditions to avoid dialkylation (*9*), a significant problem with reactive alkylating reagents. α-Benzyl substituents have been prepared

via a facile two step synthesis involving condensation of phenylacetonitrile with benzaldehyde followed by reduction of the double bond (*10*), while the α-(phenylethyl) phenylacetonitriles and higher homologs were prepared by alkylation of phenylacetonitriles with phenylalkyl chlorides or mesylates by phase transfer catalysis (*5*), by NaOH in DMSO, or by other strong bases (Figure 2). When NaH is utilized as the base, toluene:DMF (2:1) was employed as the solvent to minimize dialkylation (*3*).

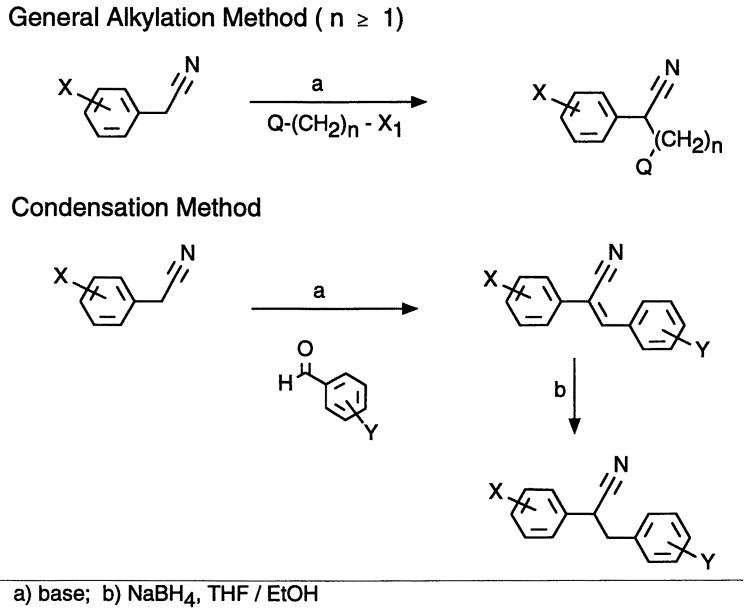


Figure 2. Synthesis of Arylalkyl Benzylcyanides

The phenylalkyl chlorides or mesylates were prepared from the corresponding alcohol using standard conditions. Some phenylalkyl alcohols were prepared by reduction of the corresponding (n-1) carboxylic acid, or as in the case of phenylethyl *via* a two carbon chain homologation with a Grignard reagent and ethylene oxide as shown in Figure 3. Phenoxyalkyl substituents (n > 2) in the R_1 position were also prepared in the manner described above using phenoxyalkyl chlorides or bromides as the alkylating reagent for reaction with phenylacetonitriles. Heterocyclic alkyl fragments with a one carbon spacer were prepared by the two step procedure utilized for α-benzyl substituents. For heterocyclic alkyl derivatives with n > 1, the heterocyclic alkyl alcohol was prepared by reduction of the acid followed by, conversion to the mesylate or chloride. These alkylating reagents were then reacted with the appropriate benzyl cyanides as described for phenylalkyl halides or mesylates.

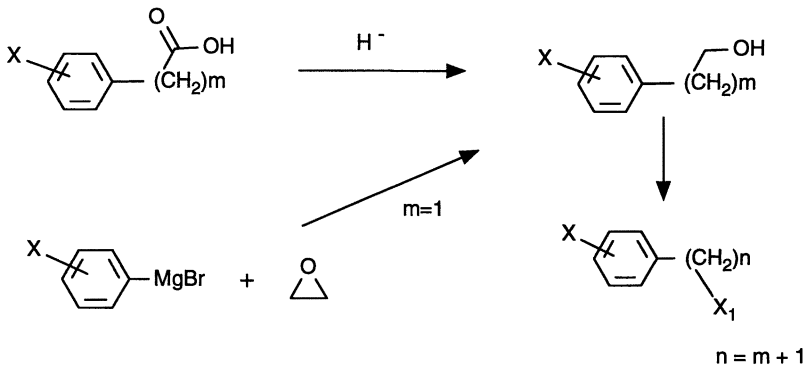
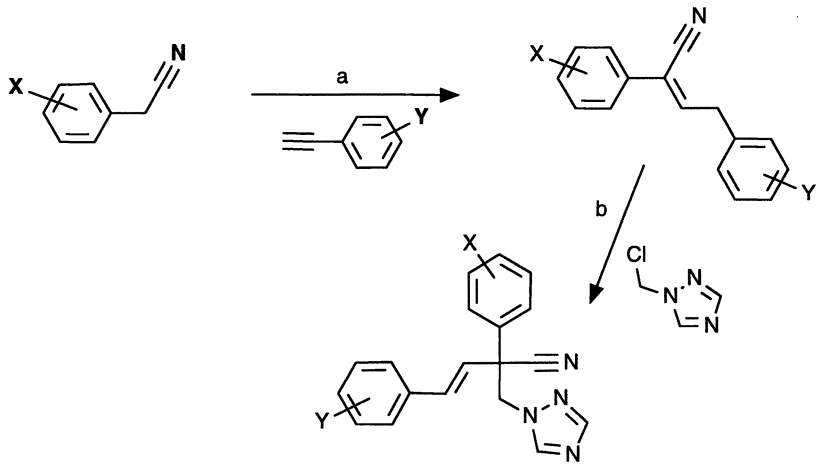


Figure 3. Synthesis of Arylalkyl Alkylating Agents

Preparation of arylethenyl substituents required the alkylation of phenylacetonitriles with phenylacetylenes. The alkylation of α-substituted phenylacetonitriles with acetylenes to prepare the tertiary nitrile has been described (*11*), however, the reaction with phenylacetonitrile was unknown. Employing the same conditions, using powdered KOH or NaOH in DMSO with a phase transfer catalyst gave an acrylonitrile derivative. Alkylation of this olefin with chloromethyltriazole, using lithium di-isopropyl amide (LDA) as the base, and gave the 2-aryl-alkenyl product as the *E* olefin (Figure 4). Biological evaluation of the compounds were carried out as described for myclobutanil (*12*).



a) NaOH / Tetrabutylammonium Chloride b) Lithium Di-isopropyl Amide/THF

Figure 4. Synthesis of 2-Arylalkenyl-2-Cyano Phenethyltriazoles

Structure-Activity Studies

Structure-activity studies of the 2-cyano-arylethyltriazoles previously described (*2,6*) led to the discovery of the plant fungicide myclobutanil. These studies of the 2-substituent position focused predominantly on acyclic hydrophobic side chains with phenyl and benzyl constituting the only cyclic substituents. In addition to the above work, a program designed to extend these studies to understand the effect of other cyclic hydrophobic side chains on fungicidal efficacy was implemented. The program was designed to examine the ring type and ring size, including heterocyclic groups, the chain length linking the cyclic system to the remainder of the molecule, and the heteroatom position on the linking chain.

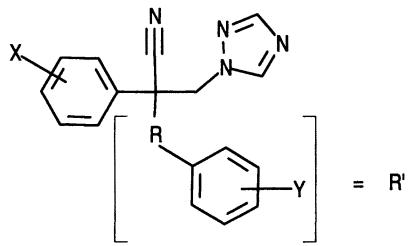
Greenhouse and *in vitro* testing of synthesized compounds were used to evaluate the influence of the structural variations on fungicidal activity. Examples of structural variations synthesized for the 2-substituent include cycloalkyl and cycloalkylalkyl, arylalkyl and arylalkenyl, aryloxyalkyl, and heterocyclic alkyl groups. A representative list of compounds and their associated greenhouse activity is given in Table I. Though the structure-activity relationships are not entirely consistent across the three diseases listed, the following conclusions are generally true. Variations of ring type and size indicated cycloalkyl and cycloalkylalkyl were less active than their aromatic counterparts. For the arylalkyl substituents in Table I, the β-phenylethyl is more active than phenyl, benzyl, or phenylpropyl; higher homologs above β-phenylethyl show a large loss in activity.

The phenylethenyl analogs, **12** and **13**, are very active and more active than the cinnamyl derivatives, **14**. Incorporation of oxygen in the chain linking the cyclic system did not improve activity. The phenoxyalkyl analogs were comparable in activity to their phenylalkyl counterparts, likewise, the cycloalkylalkyl and cycloalkoxy were similiar in efficacy. The benzyloxy compound, **15**, on the otherhand, was less active than the β-phenylethyl compound, **38**. With heterocyclic alkyl analogs, Table II, the 2-thienylethyl compound, **31**, shows greater activity than the 2-thienylmethyl compound, **30**, again indicating the requirement for a two carbon spacer. However, the heterocyclic rings were less active than phenyl. Based on these results, the β-phenylethyl fragment was selected as the 2-substituent imparting consistently good fungicidal properties to the 2-cyano-2-arylethyl triazoles.

Phenyl and β-Phenethyl Structure Activity Studies. Studies to determine the optimum phenyl substitution in both phenyl and β-phenylethyl rings were investigated next. A three phase program was used. In the first phase, the β-phenylethyl side chain was left unsubstituted, and the 2-phenyl substitution was varied. This was followed by a study of substituent effects for the β-phenylethyl ring using 2-phenyl as the base. Finally, simultaneous substitution of both rings was investigated and the fungicidal efficacy compared to mono ring substitution.

Phenyl Substitution. A representative compound list of 2-cyano-2-(substituted) phenyl-2-(β-phenylethyl)ethyltriazoles is given in Table III. In foliar green house

Table I. Control of Diseases in Foliar Green house Pot Tests

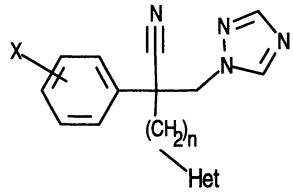


= R'

No.	R	X	Y	Rate giving 90% disease control (ug/ml) WPM[a]	WLR[b]	WSR[c]
4	-	H	H	10	200	38
5	CH_2	H	H	12	40	19
6	CH_2	H	4-Cl	5	32	2
7	$(CH_2)_2$	H	H	1	50	15
8	$(CH_2)_2$	H	4-Cl	0.6	3	25
9	$(CH_2)_3$	H	H	>300	-	125
10	$(CH_2)_3$	H	4-Cl	50	>200	-
11	$(CH_2)_4$	H	H	35	-	400
12	C=C (*E*)	H	H	1	400	-
13	C=C (*E*)	H	4-Cl	3	1.5	-
14	C=CCH_2 (*E*)	4-Cl	H	150	150	-
15	CH_2O	4-Cl	H	75	>300	-
16	$(CH_2)_2O$	4-Cl	H	100	250	200
17	$(CH_2)_2O$	H	4-Cl	450	-	33
18	$(CH_2)_3O$	H	H	30	-	>300
19	$(CH_2)_4O$	H	H	>300	-	>300
	R'	X				
20	O-cyclohexyl	4-Cl		15	>200	-
21	O-cyclopentyl	4-Cl		8	100	-
22	CH_2-cyclopropyl	2-OCH_3		15	>300	40
23	$(CH_2)_2$-cyclopentyl	4-Cl		115	100	25
24	cyclohexyl	H		25	40	60
25	cyclohexyl	4-Cl		5	-	5
26	CH-cyclohexyl	4-Cl		50	-	33
27	$(CH_2)_2$-cyclohexyl	4-Cl		400	100	150

[a] wheat powdery mildew (*Eryisphe graminis* f.sp. *tritici*)
[b] wheat leaf rust (*Puccinia reconditia* f.sp. *tritici*)
[c] wheat stem rust (*Puccinia graminis* f.sp. *tritici*)

Table II. Control of Diseases in Foliar Greenhouse Pot Tests



No.	Het	n	X	Rate giving 90% disease control (ug/ml) WPM[a]	WLR[b]	WSR[c]
28	3-pyridyl	1	4-Cl	125	>100	-
29	2-furyl	1	4-Cl	-	>100	>100
30	2-thienyl	1	4-Cl	5	>100	25
31	2-thienyl	2	4-Cl	5	50	15
32	2-pyridyl	2	H	250	>200	-
33	N-morpholine	2	4-Cl	>200	-	>200
34	N-2,6 dimethyl-morpholine	2	4-Cl	>200	-	200
35	N-piperidine	3	2,4-Cl_2	250	>200	-

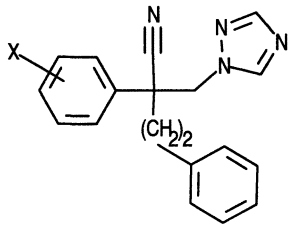
[a] wheat powdery mildew (*Eryisphe graminis* f.sp. *tritici*)
[b] wheat leaf rust (*Puccinia reconditia* f.sp. *tritici*)
[c] wheat stem rust (*Puccinia graminis* f.sp. *tritici*)

tests, the compounds showed good activity on wheat powdery mildew and stem rust, with moderate activity on leaf rust. The activity on rice blast was generally weak. For wheat powdery mildew and wheat stem rust, the 4-chloro, 4-fluoro and the unsubstituted ring were better than any substituent at the 2 or 3 position, or any disubstitutient combination. This is not the case for wheat leaf rust which appears to have a slightly different structure-activity pattern from the other two diseases. Also, the optimum substituent is disease dependent. The 4-fluoro substituent is stronger on rust diseases, while for rice blast the 3-halogen (3-F, 3-Br) and 2-alkoxy substituents were better than other substituents.

β-Phenylethyl Substitution. The next study involved variation in substitution on the β-phenylethyl side chain. The biological activity of selected 2-cyano-2-phenyl-2-[2-(substituted) phenyl)] ethyltriazoles which were prepared in this study is shown in Table IV. Again in many cases, excellent greenhouse activity was achieved on foliar wheat diseases. The 4-halogen and 4-trifluoromethyl substituents had the highest levels of activity, 3,4-dichloro was not far behind and better than 2-halogen, 2-alkoxy, 4-methyl, all 2,4-disubstitution and hydrogen. In most cases, substitution of the β-phenylethyl ring provided consistently higher levels of disease control than the analogous substitution of the 2-phenyl ring.

Simultaneous Substitution. Having determined that 4-halogen substitution of the β-phenylethyl side chain provided excellent fungicidal activity, we again focused our attention on the 2-phenyl group (A-ring). Keeping the phenylethyl (B-ring) substituted primarily with 4-halogen, mixed A-ring phenyl substituted compounds were prepared and their biological activity evaluated. Biological data for a representative set of these compounds on wheat powdery mildew is shown in Table V. For 4-bromo (B-ring) there was no increase in powdery mildew control and with 4-chloro (B-ring)

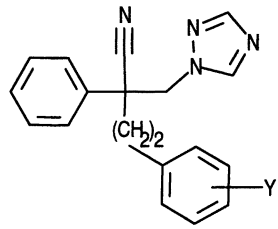
Table III. Control of Diseases in Foliar Greenhouse Pot Tests
A-Ring Phenyl Substitution



		Rate giving 90% disease control (ug/ml)			
No.	X	WPM[a]	WLR[b]	WSR[c]	RB[d]
7	H	1	50	15	250
36	2-Cl	250	200	-	-
37	3-Cl	90	150	150	300
38	4-Cl	35	80	3	>600
39	2-F	200	225	-	-
40	3-F	200	300	-	25
41	4-F	1	15	5	200
42	2-Br	300	300	-	300
43	3-Br	2	600	25	25
44	4-Br	75	30	19	300
45	2-OCH_3	25	130	5	80
46	4-OCH_3	50	200	-	>500
47	3-CF_3	2	300	75	>500
48	2,4-Cl_2	17	125	17	>500
49	2-Cl, 6-F	200	600	-	>500
50	2-OC_2H_5	3	200	75	50
51	4-C_6H_5	300	80	25	-

[a] wheat powdery mildew (*Eryisphe graminis* f.sp. *tritici*)
[b] wheat leaf rust (*Puccinia reconditia* f.sp. *tritici*)
[c] wheat stem rust (*Puccinia graminis* f.sp. *tritici*)
[d] rice blast (*Pyricularia Oryzae*)

Table IV. Control of Diseases in Foliar Greenhouse Pot Tests B-Ring Phenyl Substitution

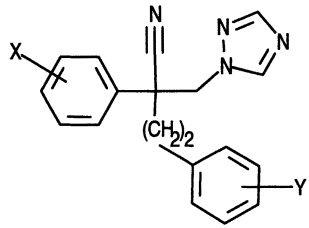


No.	Y	Rate giving 90% disease control (ug/ml) WPM[a]	WLR[b]	WSR[c]
7	H	1	50	15
52	2-Cl	15	150	-
53	3-Cl	2	85	25
8	4-Cl	0.6	3	25
54	3-F	1	150	100
55	4-F	15	15	125
56	3-Br	2	25	200
57	4-Br	3	4	100
58	2-CF_3	40	100	-
59	3-CF_3	4	75	100
60	4-CF_3	5	4	50
61	4-CH_3	6	>200	50
62	2-OCH_3	400	>200	-
63	3-OCH_3	50	200	100
64	4-OCH_3	30	100	125
65	3,4-$(OCH_3)_2$	125	>100	-
66	4-OH	>200	>200	>200
67	2,4-Cl_2	19	300	150
68	3,4-Cl_2	6	4	25
69	3,5-Cl_2	60	50	150
70	4-C_6H_5	>200	>200	>200

[a] wheat powdery mildew (*Eryisphe graminis* f.sp. *tritici*)
[b] wheat leaf rust (*Puccinia reconditia* f.sp. *tritici*)
[c] wheat stem rust (*Puccinia graminis* f.sp. *tritici*)

there was little positive effect on wheat leaf rust or wheat glume blotch (*septoria nodorum*) by the addition of 2-phenyl substituents. With the 4-fluoro (B-ring) substituent, adding a 4-halogen (A-ring) improved the powdery mildew control while adding a 4-fluoro increased the leaf rust and wheat glume blotch efficacy. The biological data for wheat leaf rust, on this series of X and Y cross substituted compounds is shown in Table VI, and in Table VII for wheat glume blotch. The substituent effects on the two diseases were generally parallel, but 3-substituents on the B-ring tended to have greater activity on Wheat Glume Blotch than on Wheat Leaf Rust. For this chemistry, additivity of substituent effects between the A and B rings is exhibited

Table V. Control of Wheat Powdery Mildew[a] in Foliar Greenhouse Pot Tests - A and B-Ring Phenyl Substitution



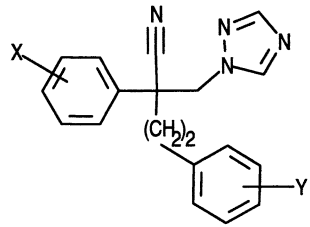
	Rate giving 90% disease control (ug/ml)			
	Y			
X	4-Cl	4-F	4-Br	3-CF_3
H	0.6	15	3	4
2-Cl	8	20	-	-
3-Cl	33	6	125	-
4-Cl	5	4	19	75
2-F	10	100	-	-
3-F	15	6	25	-
4-F	1.5	1	4	14
2-Br	300	-	-	100
4-Br	10	4	-	-
3-CF_3	300	6	4	125
2-OCH_3	5	6	-	-
4-OCH_3	115	-	-	-
4-OH	250	-	-	-
2,6-Cl_2	10	-	-	-
3,4-Cl_2	200	-	-	-
2-Cl,6-F	115	-	-	-
2-OC_2H_5	30	125	4	4

[a] *Eryisphe graminis* f.sp. *tritici*

to the extent that combining the best substituents from the individual ring studies yields the best overall compounds.

The highest level of activity seems to cluster around pseudohalogen groups as the substituent in the 4-position of the phenylethyl (B-ring), and hydrogen or flourine as the substituent on the 2-phenyl (A-ring). However, for wheat powdery mildew, when the A-ring contains a 3-CF_3, there is an unusual inexplicable enhancement of activity from B-ring substitution with 4-F and 4-Br which is not exhibited by 4-Cl.

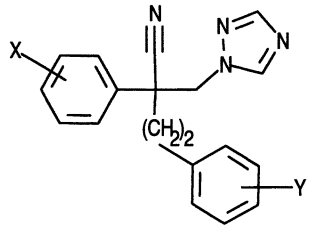
Table VI. Activity Matrix for Control of Wheat Leaf Rust in Foliar Greenhouse Pot Tests - A and B-ring Phenyl Substitution



	X									
Y	H	4-F	4-Cl	4-OCH_3	3-F	3-Cl	3-CF3	2-F	2-Br	2-OCH_3
H	++	+++	++	+	+	+	+	+	+	++
2-F	.	++	+	.	.	.	.	.	.	.
2-Cl	+	.	.	.	.	.	.	.	.	.
3-F	+	.	+	.	+	0	.	.	.	.
3-Cl	++	++	++	.	+	+	.	.	.	++
3-Br	+++	+	.	.	+	+	.	.	.	.
3-CF_3	+	+++	+	.	++	+	+	.	+	.
3-OCH_3	+		.	.	.	.	.	.	.	.
4-F	+++	+++	+	.	+	+	+	+	.	+
4-Cl	++++	++++	++	0	+	.	+	+++	+	+++
4-Br	++++	+++	++	.	+++	+++	+	.	.	+++
4-CF_3	++++	++++	++	.	.	.	.	.	.	+++
4-CH_3	0	.	++	.	.	.	.	.	.	.
4-OCH_3	+	.	++	.	.	.	.	.	.	.

KEY: EC_{90} range ++++ = <10 ppm
+++ = 10-49 ppm
++ = 50-99 ppm
+ = 100-300 ppm
0 = >300 ppm

Table VII. Activity Matrix for Control of Wheat Glume Blotch in Foliar Greenhouse Pot Tests - A and B-ring Phenyl Substitution



Y	X									
	H	4-F	4-Cl	4-Br	3-F	3-Cl	3-CF3	2-F	2-Cl	2-OCH_3
H	++	.	++	++	.	.	0	.	.	.
2-F	.	++++	+	.	.	.	.	.	.	.
3-F	+	.	.	.	.	0	.	.	.	.
3-Cl	++++	++++	++	++	+	0	.	.	.	++++
3-Br	+	++++	++	++	.	0	.	.	.	.
3-CF_3	++	+++	++	.	+	.	.	.	.	.
4-F	++	++++	++	++	+	0	0		+	+
4-Cl	++++	+++	+++	++	++++	+++	.	++	++	+++
4-Br	++	++++	0	.	.	.	0	.	.	.
4-CF_3	++++	++	0	.	.	.	.	.	.	++
4-OCH_3	++	.	++	.	.	.	.	.	.	.

KEY: EC_{90} range ++++ = <10 ppm
+++ = 10-49 ppm
++ = 50-99 ppm
+ = 100-300 ppm
0 = >300 ppm

The most active β-phenylethyl compounds were subjected to additional studies, including systemic, curative, and residual tests. RH-7592, α-[2-(4-chlorophenyl)-ethyl]-α-phenyl-1H-1,2,4-triazole-1-propanenitrile, provided the best overall cost performance and was selected as a developmental compound.

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Chapter 36

Synthesis and Biological Properties of Fenbuconazole Metabolites

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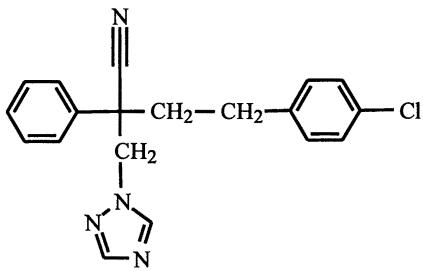
The commercial development of fenbuconazole required an extensive series of residue, metabolism and environmental studies, and in support of these studies, standards for metabolite identification and analytical methods development were prepared. The metabolites synthesized included single and double site oxidation products followed by intramolecular cyclization to form butyrolactones as well as benzylic and phenolic oxidations, each followed by conjugation.

A major step in the development of a commercial agrochemical is the isolation and identification of key animal, plant and soil metabolites. Traditional spectroscopic methods such as mass spectrometry and NMR analysis are used to identify these metabolites. To aid in the identification of complex metabolites comparison to synthesized standards is deemed highly desirable. Fenbuconazole **1**, α-[2-(4-chlorophenyl)-ethyl)]-α-phenyl-1H-1,2,4-triazole-1-propanenitrile, an ergosterol biosynthesis inhibiting fungicide was introduced in the European cereal fungicide market in 1991 as Indar (*1*). During the development of fenbuconazole **1**, the identification of an extensive series of plant, animal and soil metabolites was required.



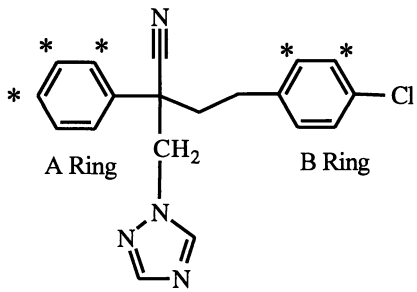
Fenbuconazole, 1

0097–6156/95/0584–0420$12.00/0

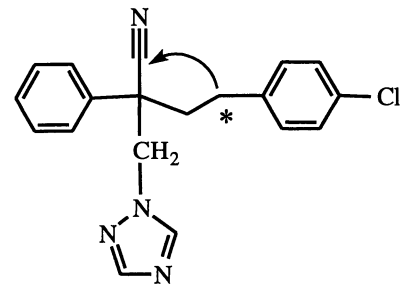
A synthesis program was initiated to facilitate the identification of key fenbuconazole metabolites by comparison to authentic standards. Synthesis focused on the most probable sites of fenbuconazole metabolism. Of the many classes of triazole containing fungicides the 2-cyano-2-arylethyltriazole structure is unique with a cyano group at the quaternary center, β to the triazole (*2*). In addition, fenbuconazole possesses two aromatic rings, a benzylic position and a methylene α to the triazole, all of which could be metabolically transformed via a series of oxidations and intramolecular cyclizations. A further objective of this program was to evaluate the biological activity of the key metabolites. Biological comparsion to fenbuconazole would provide insight into the roles these metabolites may play in the overall performance of the fungicide.

Synthesis Strategy

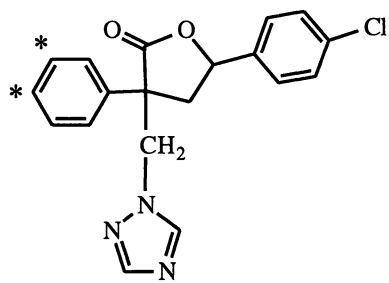
Single site and double site oxidation (hydroxylation) products alone or in combination with intramolecular cyclization to the nitrile were all envisioned as possible candidates for synthesis (Figure 1). In addition, conjugation to a sugar or sulfate at the benzylic hydroxyl or an aryl hydroxyl were considered possible metabolites.



Single Site Oxidation: A or B Ring



Benzylic Oxidation / Cyclization

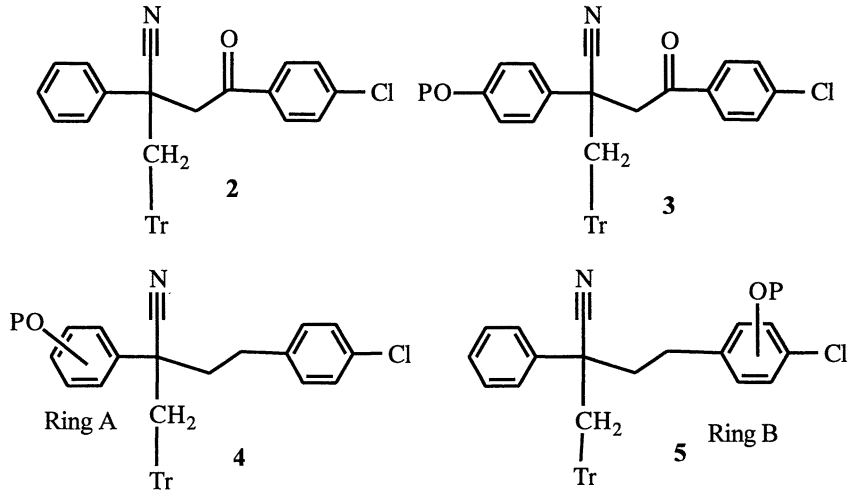


Double Site Oxidation: Aryl and Benzylic

Figure 1: Oxidative Sites of Fenbuconazole (* Sites)

A multifunctional synthesis strategy was undertaken wherein key common intermediates would provide the basis for further elaboration to many of the possible metabolites. The key intermediates would provide access to compounds with single site oxidation on either aromatic ring or at the β-phenethyl benzylic position. Double site oxidation compounds derive from intermediates having oxidation on one ring and

oxidation at the benzylic center. Four key intermediates were considered priority targets for synthesis: the benzylic ketone **2**, benzylic ketone in combination with ring A phenol **3**, hydroxylation of the phenylacetonitrile ring A **4**, and hydroxylation of the β-phenethyl ring B **5**. Targeted phenolic intermediates **4** of ring A are monosubstituted at C2, C3 or C4 and C3,4 disubstituted. Targeted phenolic intermediates **5** of ring B can be monosubstituted at C2 and C3. For **3**, the double site oxidation intermediate, aryl oxidation at C4 of ring A was targeted (See Figure 2.)



P = protecting group
Tr=1H-1,2,4-triazole

Figure 2: Key Synthesis Intermediates

Chemical Synthesis

Two major synthetic strategies were employed to provide both single site and double site oxidation products. The first strategy utilized two separate synthetic sequences to provide single site oxidation products. The first sequence provided products resulting from benzylic oxidation of the side chain. The second sequence provided phenolic A or B ring derivatives.

The second strategy generated double site oxidation products having aryl hydroxylation and benzylic oxidation. Higher levels of oxidation, such as oxidation of both rings and benzylic oxidation, were not initial synthetic targets of great interest but are well accommodated by our proposed synthetic routes.

Single Site Oxidation, Benzylic Oxidized Metabolites. Structure elucidation of plant, soil and animal metabolites, by our metabolism group utilizing classical magnetic resonance and mass spectrometric methods indicated benzylic oxidation of the β–phenethyl side chain and elaboration to further products. Benzylic ketone **2** was the first key intermediate targeted for synthesis as a precursor to a variety of potentially important metabolites (Figure 3). The benzylic ketone would provide access to the reduced benzylic alcohol **6** and possible conjugates as well as the nitrile cyclized products, butyrolactones **7**, and hydrolyzed metabolites such as the keto acid **8** and unsaturated lactam **9.**

Benzylic Ketone. Direct benzylic oxidation of fenbuconazole was considered; however, results provided non-selective oxidation and a total synthesis of the unequivocal benzylic ketone **2** was undertaken as described in Figure 4. A facile synthesis was developed starting with the condensation of 4-chloroacetophenone and benzaldehyde in the presence of NaOH. Addition of HCN to the unsaturated ketone provided the β-cyanoketone **10** which was protected as the ketal in an overall 65% yield. The ketal could be elaborated to the 2-cyanoarylethyltriazole derivative via two previously reported procedures (*3*). Direct coupling with chloromethyltriazole and NaH gave the ketal **11** in 70% yield; alternatively, a two step procedure utilizing CH_2Br_2 followed by reaction with potassium triazole proceeded smoothly. The ketal was carefully deprotected with 25% H_2SO_4 in ethyl acetate at 50°C to give the benzylic ketone **2** in 85% yield. This ketone, in addition to being a key fenbuconazole plant, soil and fish metabolite, was further elaborated to a wide array of potential metabolites.

γ-Iminolactones/γ-Butyrolactones. Major metabolites isolated from soil, plants and animals were spectroscopically identified as the diastereomeric γ-butyrolactones **7**. These metabolites are formed by benzylic oxidation and intramolecular cyclization to the nitrile which, after hydrolysis, provides the lactones. This family of 2,4-disubstituted-γ-butyrolactone triazoles has been previously described as plant fungicides (*4*).

The synthesis of the lactones (Figure 5) proceeded by reduction of the benzylic ketone **2** with $NaBH_4$ in EtOH at room temperature affording a diastereomeric mixture of iminolactones **12** in 95% yield. The iminolactones were hydrolyzed with conc. HCl in DMF at 50°C, providing after chromatographic separation, the γ butyrolactone isomers **7A** and **7B** in a 1:2.5 ratio. The relative configuration of the 4-chlorophenyl ring at C4 to the methylenetriazole at C2 was established by both X-ray crystal determination of lactone isomer **7A** and by long range proton coupling NMR experiments of both isomers **7A** and **7B**. Both studies indicated that for **7A** the 4-chlorophenyl and methylenetriazole are in a *cis* relationship about the five membered γ-butyrolactone. In addition, the crystal structure demonstrated the 4-chlorophenyl substituent at C4 and the methylenetriazole at C2 occupy equatorial positions. The *trans* lactone **7B** the major product of reduction after mild hydrolysis of the iminolactone mixture, can be thermodynamically equilibrated by treatment with conc. HCl at 90°C to provide a 3:1 mixture of the *cis:trans* isomers **7A:7B**. These synthesized γ-butyrolactones confirmed the structure of the isolated lactone metabolites which were found to be major fenbuconazole metabolites.

Benzylic Alcohol and Conjugation. The benzylic alcohol **6** was envisioned as simply arising from the reduction of the ketone **2**. However, as noted previously sodium borohydride reduction gave exclusively the cyclic iminolactones. Intramolecular cyclization of the benzylic hydroxy is very facile; therefore, the preparation and isolation would have to account for its very labile nature. Previous studies with 2,4-disubstituted-γ-butyrolactones (*5*) and 2,4-diphenyl-γ-butyrolactones (*6*) have demonstrated the difficulties in isolating the γ-hydroxynitrile. Experimentation with a wide variety of reducing agents gave either ketone **2** or iminolactones **12** except when tetrabutylammonium borohydride (*7*) was utilized.

With tetrabutyl ammonium borohydride the benzylic alcohol **6** could be isolated from a mixture of ketone **2** and iminolactones **12**. However, the reduction was plagued from complex mixtures of ketone **2**, iminolactones **12,** lactones **7** (after hydrolysis) and alcohol **6**. Careful study of this reaction by varying solvent, temperature, equivalents of reducing reagent and reaction time eventually provided only ketone **2** and alcohol **6** when using a large excess (8 eq) of reducing reagent in CH_2Cl_2 at room temperature (Figure 6).

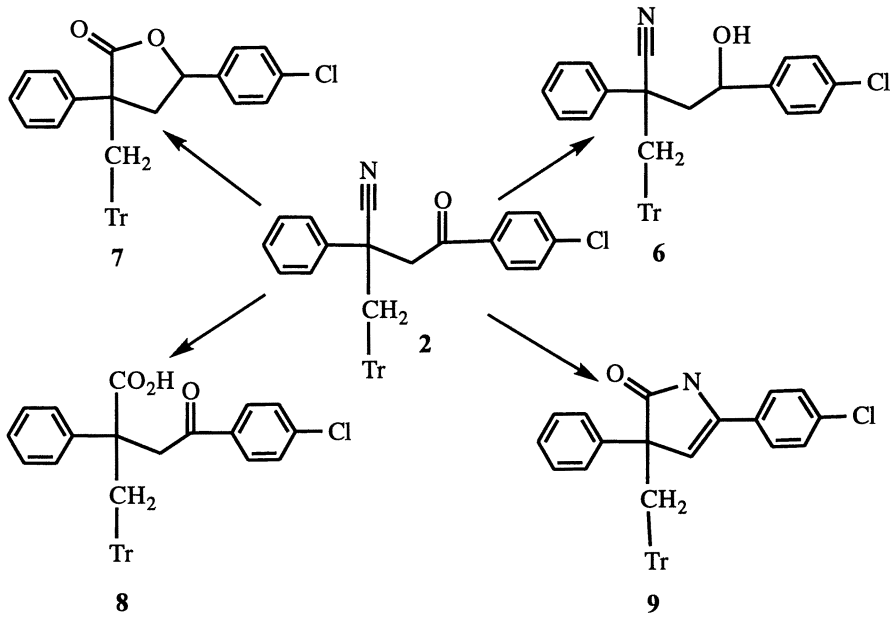


Figure 3: Benzylic Ketone Derived Products

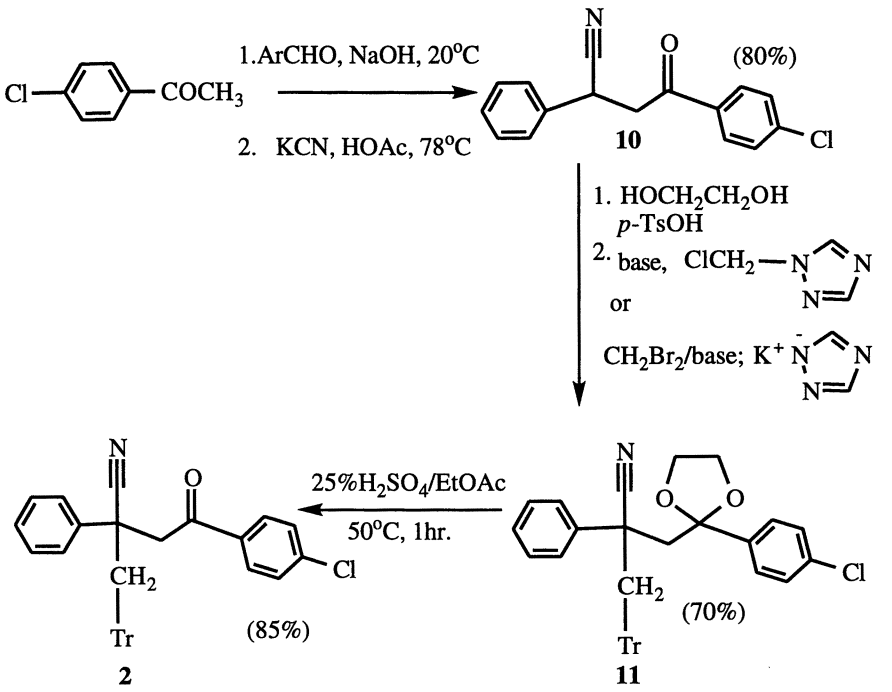


Figure 4: Synthesis of Benzylic Ketone 2

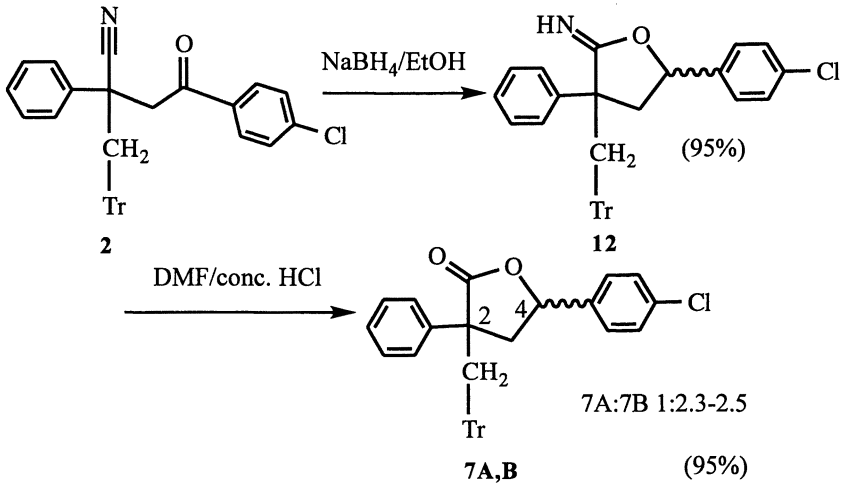


Figure 5: Synthesis of Isomeric γ-Butyrolactones

The final adjustment for optimization affording alcohol **6** in 90% yield was accomplished by reducing and quenching in one operation via direct addition of ketone and reducing agent in methylene chloride to a two phase system of 10%HCl and methylene chloride. Animal metabolism studies indicated the presence of a sulfate conjugate of the benzylic alcohol. The benzylic alcohol **6** was treated with dicyclohexylcarbodiimide (DCC) in H_2SO_4 at 5°C (*8*) to provide, after basic extraction with KOH in *n*-butanol, the sulfate potassium salt **13** in 60% yield.

Keto Acid, Butenolide and Unsaturated Lactam. The benzylic ketone oxidation state was the starting point for a series of reactions that lead to metabolites formed at this same oxidation state. Metabolic hydrolysis of the nitrile of **2** or metabolic oxidation of the butyrolactone at C4 of **7** would lead after proton transfer to keto acid **8**. Cyclization of **8** would then provide the unsaturated lactone **14**. In addition, hydrolysis of the nitrile of **2** to the corresponding amide would provide, after cyclization, unsaturated lactam **9**. The synthesis of each of these compounds starting from the benzylic ketone **2** is described in Figure 7.

Hydrolysis of the tertiary cyano group in the 2-cyano-2-arylethyl triazole series in acidic or basic media requires very vigorous conditions (*9*). Fenbuconazole can be hydrolyzed to the corresponding acid with refluxing 48% HBr or in a two step process in which the amide, formed with 50% NaOH, is hydrolyzed at reflux in 25% H_2SO_4. With the benzylic ketone **2,** this hydrolysis is extremely facile and was conducted with 25% H_2SO_4 below 25°C to give the keto acid **8** in 80% yield. The milder hydrolysis conditions are presumably due to participation of the ketone. The keto acid **8** was converted by activation of the acid with acetic anhydride at 135°C to the unsaturated lactone **14** in 46% yield. This butenolide formation was reversed by treating with 50% HCl at 95°C to give **8**. The unsaturated lactam **9** could be prepared by *in situ* trapping of the keto amide formed from benzylic ketone **2**. Treatment of the ketal **11** (Figure 4) with concentrated H_2SO_4 in ethyl acetate at 50°C for 15 min caused removal of the ketal,

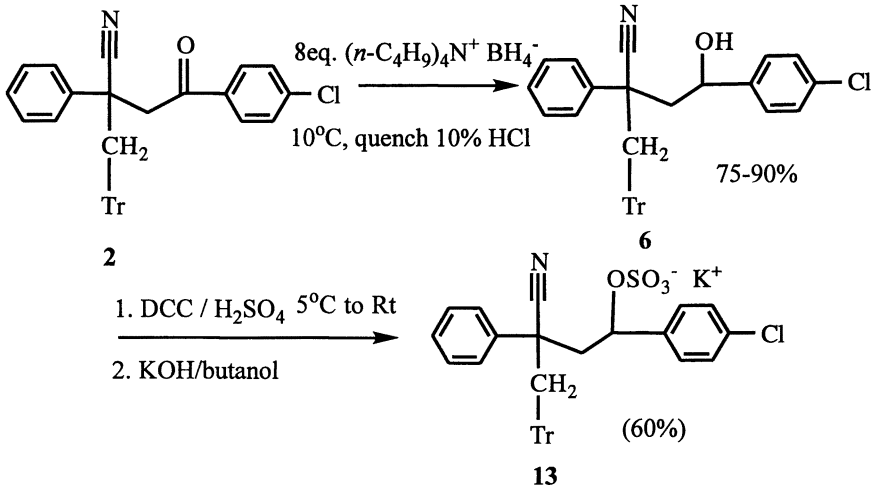


Figure 6: Synthesis of Benzylic Alcohol and Sulfate

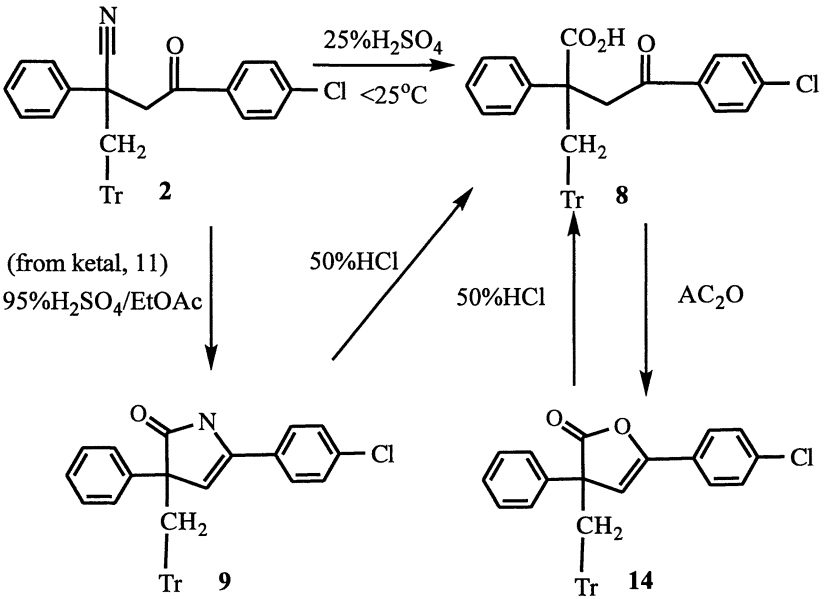


Figure 7: Synthesis of Keto Acid, Butenolide, Unsaturated Lactam

hydrolysis of the nitrile to the amide, cyclization of the amide to the benzylic ketone and dehydration to give the unsaturated lactam **9** in 45% yield. This lactam could also be hydrolyzed to the keto acid **8** under acidic conditions.

Single Site Aryl Hydroxylation. Aryl ring hydroxylated products were synthesized during traditional structure-activity studies of fenbuconazole (*10*). Ring A and B hydroxylated analogs of fenbuconazole were prepared from their methoxy precursors via the standard synthesis procedures for 2-cyano-2-arylethyl triazoles (*2*). Ring A 4-hydroxy and 3,4-dihydroxy derivatives were prepared along with ring B 3,4-dihydroxy and 4-hydroxy (replacing 4-Cl) compounds.

Ring A Hydroxylation. Ring A hydroxylation at C4 utilized 4-methoxyphenylacetonitrile as the starting material; the methyl ether product **15** was deprotected in the final step with 57% HI at 90-95°C to give phenol **16** (Figure 8). Phenol **16** was derivatized via DCC/H_2SO_4 to the corresponding sulfate **17** in 92% yield. Both the 4-phenol and sulfate were confirmed as rat metabolites. The *o*-hydroxy product was targeted by hydrolysis of the 2-methoxy precursor **18**. However, under acidic conditions the benzofuranone derivative **19** formed. This benzofuranone was not observed as a metabolite and the *o*-phenol derivative, if formed in biological systems, would likely cyclize. From the 3,4-di-OMe precursor **20** the final ring A oxidized product, 3,4-di-OH **21**, was synthesized.

Ring B Hydroxylation and Sugar Conjugation. Ring B hydroxylated products were not initially targeted for synthesis beyond initial structure-activity studies in the fenbuconazole series (*10*). However, a plant metabolite was identified which, by mass spectrometry indicated a β–4-chlorophenethylphenolic glycosidic conjugate. The 2-OH,4-Cl phenyl and the isomeric 3-OH,4-Cl phenyl analogs of fenbuconazole were the possible aglycone targets for synthesis. Proton NMR analysis of the acid hydrolyzed plant metabolite also indicated a phenolic product; however, the chemical shifts were inconclusive to establish the isomer identity. The 2-OH,4-Cl-phenethyl side chain synthesis started with 4-chloro-*o*-anisic acid which was converted to the mesylate in two steps. Completion of the synthesis in three steps (*3*) and deprotection with HI gave phenol **22.** Unfortunately, neither the proton NMR spectrum nor HPLC analysis matched the plant derived metabolite. The 3-OH,4-Cl-phenethyl mesylate alkylating reagent was prepared from 3-methoxy-4-chlorotoluene in five steps, treated with benzylcyanide and processed to the phenolic product **23** as described in Figure 9. This product matched the hydrolyzed plant metabolite by 500MHz NMR and HPLC analysis.

Following structure confirmation, glycosidic conjugation of the 3-OH,4-Cl phenethyl derivative was investigated. 4-(4-Chlorophenyl)-2-(4-hydroxyphenyl)-butanenitrile and 3-hydroxy-4-chlorotoluene were model substrates, and α-bromotetra-O-acetylglucose and α and β-glucosylpenta-O-acetate used as sugar reagents. Classical Koenigs-Knorr conditions (*11*) employing various catalysts such as tin(IV) chloride, silver triflate, and $BF_3.Et_2O$ provided mostly starting material. Coupling of both model substrates with α-bromotetra-O-acetylglucose in the presence of silver oxide in acetonitrile/sieves gave the desired tetraacetate conjugate in good yield. Coupling of the target substrate 3-OH,4-Cl-phenyl **23** was optimized by adjusting the reaction temperature to 70-75°C affording the acetylated glycoside **24** in 62% yield. Deprotection with 25% $NaOCH_3$ in methanol at room temperature and after lyophilization gave the desired glycoside **25**, as a mixture of α and β isomers, in 52% yield (Figure 9).

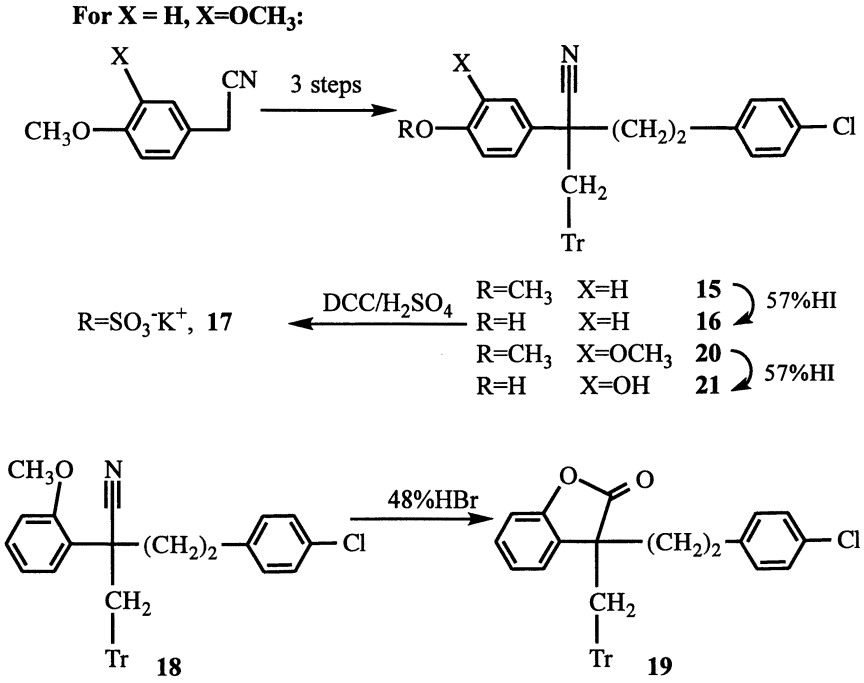


Figure 8: Synthesis of Ring A Hydroxylation Products

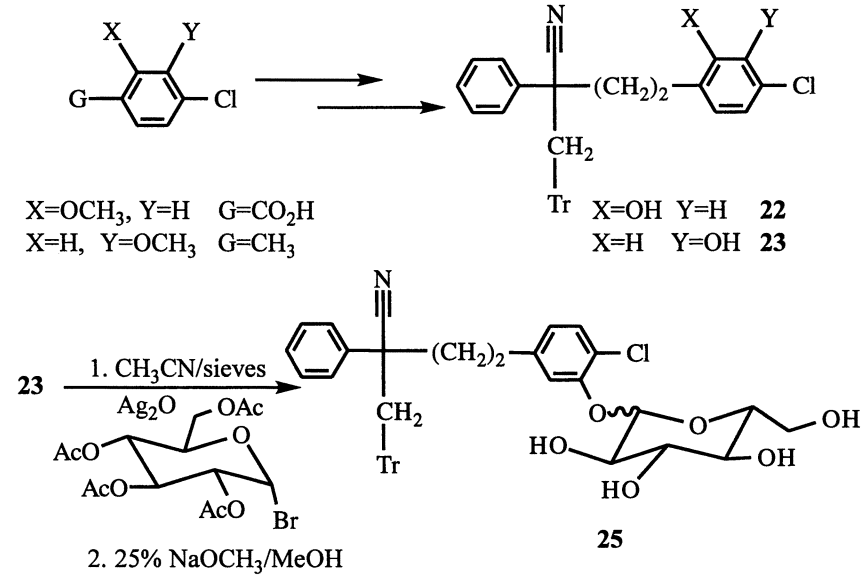


Figure 9: Ring B Hydroxylated and Conjugated Metabolites

Double Site Oxidation. Aryl ring hydroxylation and benzylic oxidation of fenbuconazole provides double site oxidized metabolites. These products can be formed metabolically in either of two sequences of oxidation events. The first sequence is initiated by benzylic oxidation to form the benzylic ketone **2** or the butyrolactones **7** followed by ring A hydroxylation. The second is initiated by aryl ring hydroxylation followed by benzylic oxidation to the ketone or to the benzylic alcohol which cyclizes to the butyrolactone.

Ring A 4-Phenol Butyrolactone. Animal metabolism studies indicated the presence of double site oxidation products. The major animal single site aryl hydroxylated metabolite is the ring A phenol **16** and therefore the most probable double site oxidation product was the ring A phenol with benzylic oxidation. Since the benzylic alcohol **6** and butyrolactones **7** are animal metabolites and the benzylic ketone **2** is not, the most likely targets for synthesis are the 4-phenol butyrolactones **29** (Figure 10).

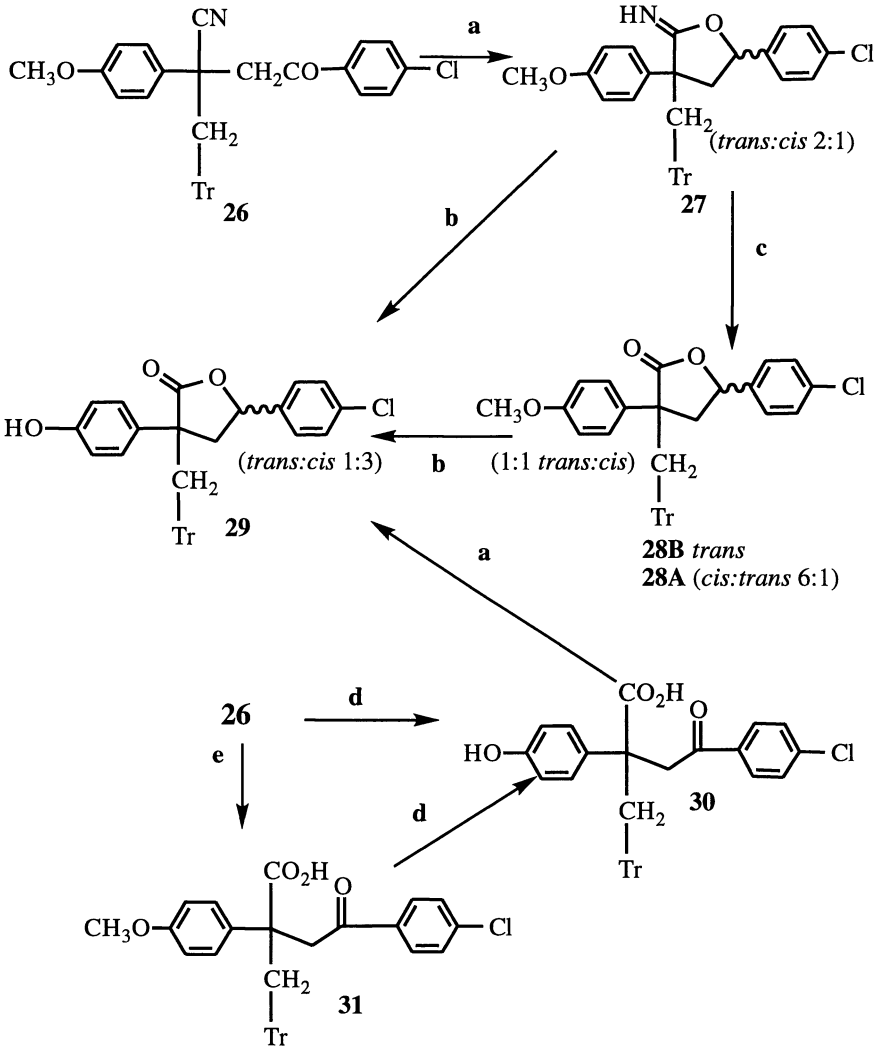
The key intermediate, 4-methoxyphenyl benzylic ketone **26**, was prepared in six steps starting from 4-methoxybenzaldehyde and 4-chloroacetophenone. The ketone **26** was reduced with sodium borohydride to give the 4-methoxyphenyl iminolactone isomers **27** (*trans:cis* 2:1). The iminolactone **27** was hydrolyzed with 10% HCl to give lactones **28** from which the *trans* isomer **28B** and an enriched *cis* isomer (6:1 *cis:trans*, **28A**) was isolated. A 50:50 mixture of the lactones **28** were treated with 57% HI/HOAc at 105°C to give the 4-phenol lactone **29** as a 3:1 *cis:trans* mixture in 64% yield. Alternatively, in a one step procedure, **27** (*trans:cis* 2:1) was treated with 57% HI/HOAc at 95-105°C to give the lactones **29** as a *cis:trans* 3:1 mixture in 88% yield.

Ring A 4-Phenol Keto Acid. The final double site oxidation product targeted for synthesis was the keto acid **30**. This potential metabolite can be formed *in situ* by benzylic oxidation of the butyrolactone **29** in a fashion similar to the keto acid **8**. Treating ketone **26** in 47% HI/HOAc at 95-105°C provided the keto acid **30** in a 66% yield. At this temperature both deprotection of the methyl ether and hydrolysis of the nitrile occurred. At 50°C only the nitrile hydrolyzed to provide the keto acid **31** in a 65% yield. Heating **31** at higher temperature gave the keto acid **30**. Finally, reduction of **30** with $NaBH_4$ and workup with HCl gave the lactone **29** as a 3:1 *cis:trans* mixture.

Biological Results

Table I compares the *in vitro* growth inhibition activity of fenbuconazole with eight key metabolites while Table II describes *in vivo* protectant greenhouse data for eleven key metabolites. Both *in vitro* and *in vivo*, lactone **7A** (*cis* isomer) is more active than **7B** and is the most fungicidally active fenbuconazole metabolite synthesized. In greenhouse protectant screening, lactone **7A** is equal in activity to fenbuconazole on wheat glume blotch but less active on wheat leaf rust.

Butenolide **14** is less active *in vitro* than **7A** but is generally more active than the lactone **7B**. The iminolactone mixture of isomers **12** is less active than the lactones **7** both *in vivo* and *in vitro*. Likewise the unsaturated lactam **9** is less active than the butenolide **14**. Ketone **2** is devoid of fungicidal activity both *in vitro* and *in vivo* while the benzylic alcohol **6** has similar greenhouse efficacy to iminolactone **12**. This result is not unexpected since the benzylic alcohol is labile and easily cyclizes to **12**. Phenols **16**, **22** and **23** show poor *in vitro* and *in vivo* activity.



a) $NaBH_4$/EtOH, 25°C b) 57% HI / HOAc, 105°C c) 10%HCl, 40°C
d) 47% HI / HOAc, 95-105°C e) 47% HI / HOAc, 50°C

Figure 10: Ring A 4-Phenol Lactone and 4-Phenol Keto Acid

Table I: *In vitro* Data of Fenbuconazole & Key Metabolites

		Rate giving 75% control (ug/ml)					
		BOT[a]	CER[b]	MON[c]	PSH[d]	SEP[e]	PYR[f]
1	fenbuconazole	0.1	<0.1	<0.01	0.03	0.08	0.05
7A	'*cis* ' lactone	0.6	0.8	<0.2	<0.2	0.2	6
7B	'*trans*' lactone	>25	25	0.8	12	3	12
7	*cis:trans* lactone(1:2)	12	3	<0.2	0.8	3	12
12	iminolactone	25	6	0.4	0.8	3	12
14	unsatd. lactone	>25	3	0.2	1.0	5	>25
9	unsatd. lactam	>25	12	12	20	6	>25
2	benzylic ketone	>25	>25	>25	>25	>25	25
16	4-OH phenyl	>12	>12	12	>12	>12	>12

[a] *Botrytis cinerea* (causes vegetable grey mold)
[b] *Cercospora beticola* (causes sugar beet cercospora)
[c] *Monilinia fructicola* (causes brown rot)
[d] *Pseudocercosporella herpotrichoides* (causes eyespot of wheat)
[e] *Septoria nodorum* (causes wheat glume blotch)
[f] *Pyricularia oryzae (causes* rice blast)

Table II: *In vivo* Greenhouse Data of Fenbuconazole & Key Metabolites

		Rate giving 90% control(ug/ml)		
		WPM[a]	WLR[b]	SNW[c]
1	fenbuconazole	6	6	25
7A	'*cis* ' lactone	1.5	10	25
7B	'*trans*' lactone	12	40	>100
7	*cis*:trans lactone (1:2)	12	25	150
12	iminolactone	50	150	150
14	unsatd. lactone	10	12	250
9	unsatd. lactam	300	>200	>200
6	benzylic alcohol	50	25	200
2	benzylic ketone	>200	>200	>200
16	4-OH phenyl	300	>200	-
22	2-OH,4-Cl phenethyl	25	>200	>200
23	3-OH,4-Cl phenethyl	75	>200	>200

[a] Wheat powdery mildew (*Erysiphe graminis* f.sp.*tritici)*
[b] Wheat leaf rust (*Puccinia recondita* f.sp. *tritici*)
[c] Wheat glume blotch (*Septoria nodorum)*

Metabolic Pathways

The fenbuconazole metabolic pathways with the key triazole containing metabolites are shown in Figure 11. Four major metabolic pathways are depicted. Three pathways involve single site oxidation and the fourth pathway involves a double site oxidation. Benzylic oxidation followed by cyclization to the nitrile is a major pathway in animals, plants and soil with the *cis* lactone predominating. In addition, the benzylic alcohol is conjugated to a sulfate in the rat metabolic pathway. Further oxidation of the benzylic alcohol to the ketone occurs in plants and soil. The ring A 4-OH sulfate is formed in the rat metabolic pathway while the ring B 3-OH,4-Cl glycoside is a plant metabolite and the ring B 3-OH,4-Cl is an animal metabolite. Benzylic oxidation followed by ring A hydroxylation, a double site oxidation pathway, occurs during metabolism in the rat.

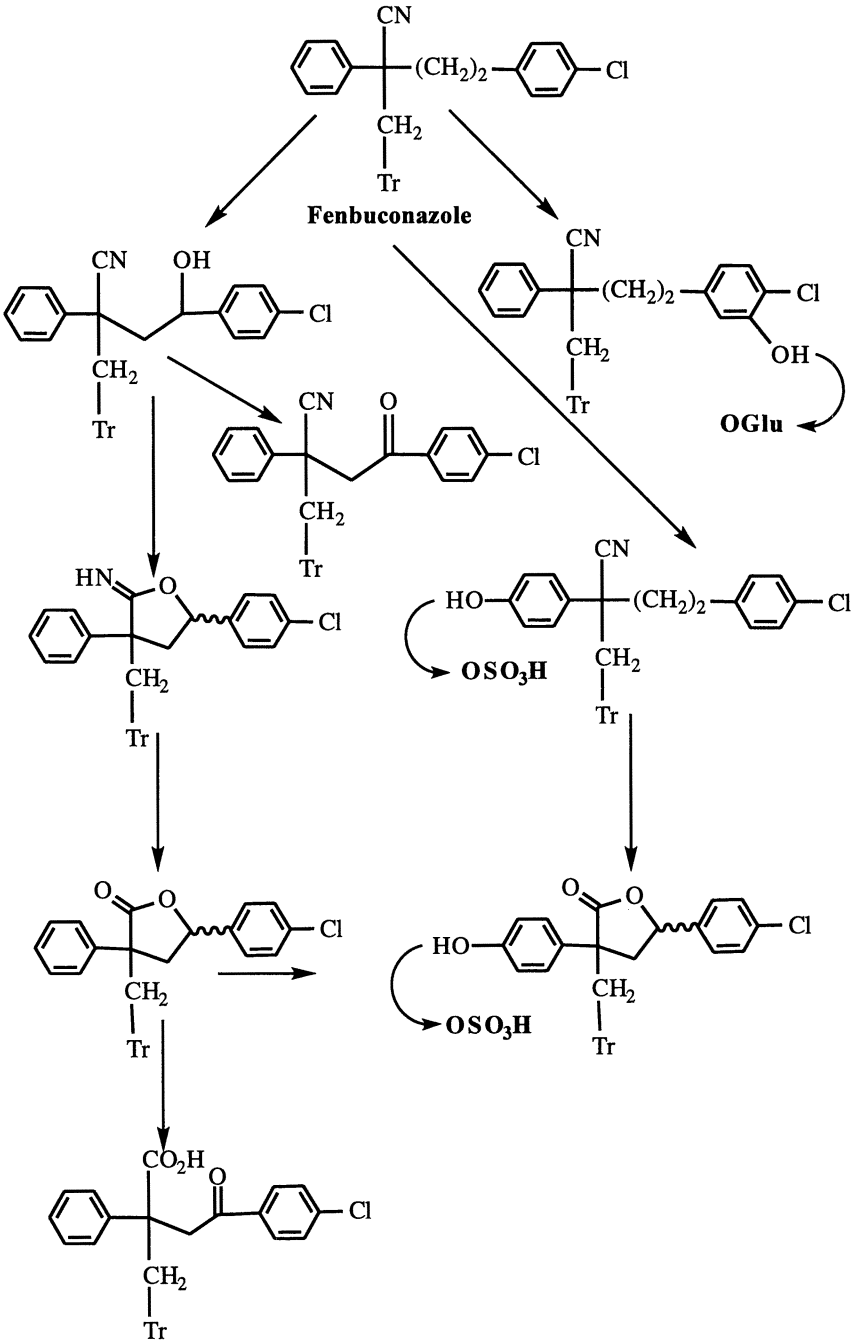


Figure 11: Fenbuconazole Metabolic Pathways

Summary

All of the major fenbuconazole metabolites were synthesized to confirm identification and when necessary purified as analytical standards. The synthetic approach allowed for incorporation of single and double site oxidation metabolites. Reduction conditions were developed to allow for isolation of the sensitive benzylic alcohol. In addition, specific glycosylation conditions for phenolic fenbuconazole metabolites were developed. *In vitro* and *in vivo* fungicidal testing of the key metabolites demonstrated that the γ-butyrolactones are highly active fungicides with the *cis* isomer possessing activity comparable to fenbuconazole.

Acknowledgments

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Chapter 37

Novel Class of Pyridyl Fungicides

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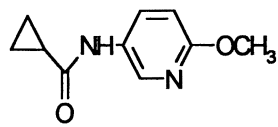
These novel cyclopropane carboxamides came about as the result of a new *in vivo* fungicide test for *Botrytis cinerea*. The test compounds were applied to rose petals which were later innoculated with the fungal spors and then incubated at room temperature. Among the first compounds tested were these compounds as exemplified by Compound 1. The various structural types related to these compounds were prepared and tested for their antifungal activity. There seems to be a fairly narrow structure activity relationship for this new class of fungicides.



Compound 1

During the last 40 years there have been major changes in the science associated with agrochemicals (1). There has been a revolution in the analytical tools such as nmr, gas chromatography, and mass spectral analysis. There are many new specialized reactions and reagents. There are a variety of chromatography tools. The first generation of organic agrochemicals came about as a result of the random screening approach. Here all manner of organic compounds were tested for action on plants, insects or fungi. By the 1950s it became apparent that certain groups such as the phosphates, carbamates, or amides conferred certain toxic responses when a part of certain compounds. It then became a race to attach such groups to just about any kind of backbone molecule imaginable and to then test the result. Active natural products such as the pyrethrins became models for synthesis. Here we see that over a period of many years many groups have added to our knowledge of what constitutes the synthetic pyrethroids.

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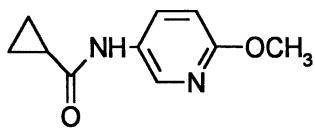
The mimicking of auxin in the plant by 2,4-D and its herbicidal effect led to many modifications. This mimicking process whether applied to natural products or to other active materials has often been referred to as "me too chemistry" and has yielded a variety of commercial materials.

Another interesting approach is the design of new compounds which interact with a critical enzyme of interest. This strategy has been labeled biochemical design. It has shown commercial success in the pharmaceutical field, however, as yet there have been no commercial agrochemicals which have come through this route. Molecular modeling is another similar approach which is currently widely used in the design of new agrochemicals.

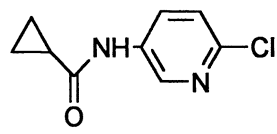
Lead Discovery

How do you find that new active compound with that unexpected superior biological activity. Most often this comes about by what some have called "serendipity." However, this is usually much more than luck. It usually comes about with individuals prepared to recognize the unexpected. The lead compounds in this series came about as a result of an intermediate in a herbicide synthesis program being tested in a new series of fungicide tests (2-5). Here two white rose petals are placed in a petri dish lined with wet filter paper. The compound to be tested is diluted with a 50/50 acetone /water solution to produce decreasing concentrations. One half ml of test solution is atomized onto the petals, and allowed to dry. Inoculum of *Botrytis cineria* of 20 microliters is placed on each petal . The petri dishes with inoculated petals are stored in sealed plastic boxes to maintain saturated humidity. Results are read four days following inoculation as a percent reduction in necrotic area compared to the acetone/water controls. Compound concentrations which provide 90% disease control are determined from dosage/dilution curves. The results are presented in parts per million.

Among the first compounds tested in this new test were Compounds 1 and 2:



Compound 1

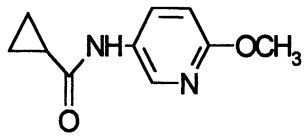
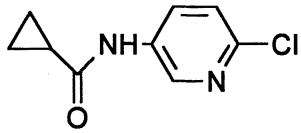


Compound 2

Synthesis and Testing

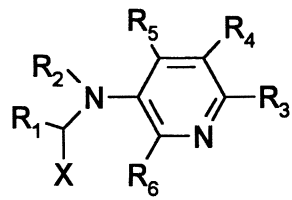
The data below shows the IC_{90} from this new test for these two compounds. These compounds were also active on a variety of other organisms, such as leaf rust, powdery mildew, apple scab, and rice blast. However, here we will only report the results coming from this new *in vivo* type test.

The keys to developing new agrochemical materials which meet the criteria of the 21st century are tests which provide the synthesis chemist with data as to how new test compounds meet the requirements of the marketplace. In addition to efficacy, safety and environmental factors are of prime concern. Also properly designed screening tests are very important. In a standard *in vitro* screening test the lead compounds would not have been found. In this particular example an *in vivo* test with *Botrytis cineria* provided an indication of the high activity of the compounds.

	Botrytis Bud Blight Test IC_{90} (ppm)
 Compound 1	20
 Compound 2	25

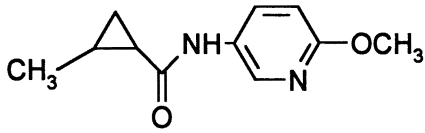
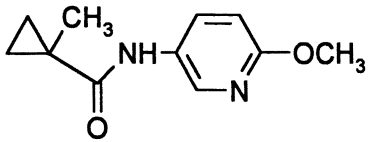
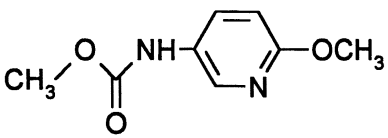
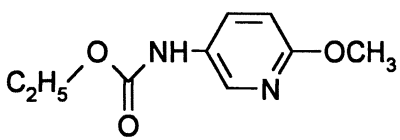
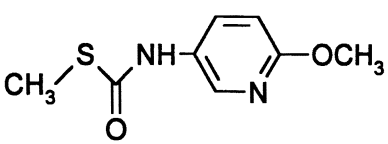
Potential Sites for Structure Modification

Once that new active compound is identified modifications can be made in the basic structure of the molecule to determine the range and scope of the biological action. Structure Activity Relationships can be determined. Molecular modeling is employed to gain insight into possible modifications which may be effective in design of new compounds. The following structure indicates potential modifications which can be made in order to determine the scope of the biological activity.

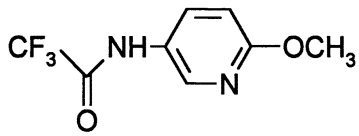
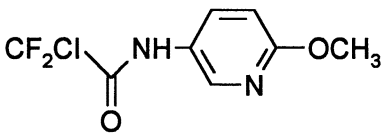
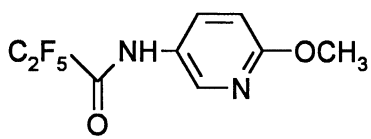


R_1 Types of Modifications

There are a wide variety of substituents which can be tried for R_1. As can be seen below, substituents in this position greatly modify the fungicidal effect.

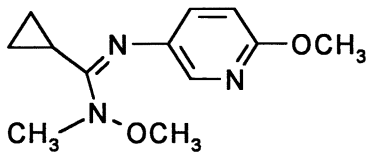
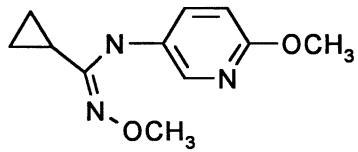
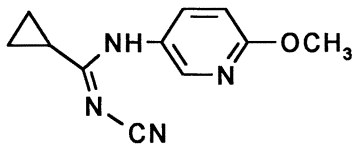
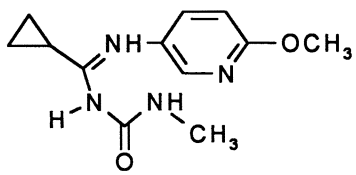
	Botrytis Bud Blight Test IC_{90} (ppm)
 Compound 3	750
 Compound 4	200
 Compound 5	25
 Compound 6	80
 Compound 7	500

Since this class of compounds seemed reminiscent of many old PSII inhibitors, a variety of previously prepared materials were tried in this new test. The various R-groups were modified by a variety of substituents. Even minor changes had a profound effect in the biological activity. The optimum substitution on the pyridine ring was 2-halogen and 2-methoxy. For the sake of the discussion data is reported for the 2-methoxy compounds. These modifications and their effect on biological activity will be discussed.

	Botrytis Bud Blight Test IC_{90} (ppm)
 Compound 8	150
 Compound 9	50
 Compound 10	2250

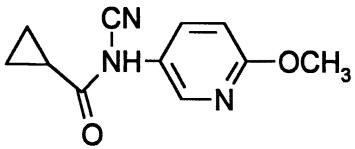
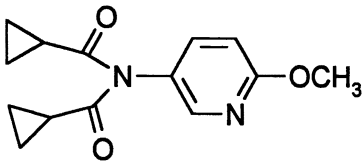
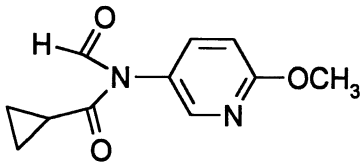
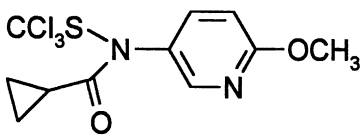
Carbonyl Modifications

The carbonyl group can be modified in a variety of ways. The listing below indicated some of the possibilities.

	Botrytis Bud Blight Test IC_{90} (ppm)
 Compound 11	70
 Compound 12	700
 Compound 13	600
 Compound 14	750

R_2 Types of Modifications

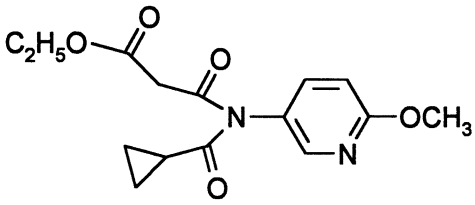
Groups attached to the amide nitrogen are represented as modifications of R_2. The following examples represent this type of modification.

	Botrytis Bud Blight Test IC_{90} (ppm)
 Compound 15	25
 Compound 16	30
 Compound 17	70
 Compound 18	70

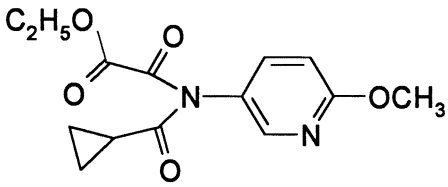
Pro Drug Type Compounds

The compounds prepared with substituents on the amide nitrogen suggest that a Pro-Drug type approach might be applied to nitrogen substituted compounds. The following are examples of this type compound.

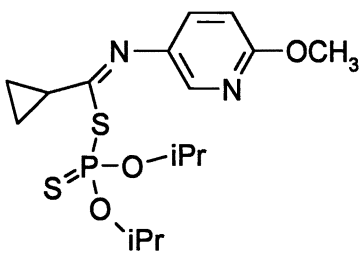
Botrytis Bud Blight Test
IC_{90} (ppm)



Compound 19 — 30



Compound 20 — 30



Compound 21 — 25

Conclusions

The best antifungal activity in this class of compounds is obtained when halogen or methoxy are in the 2-position of the pyridyl ring. For substituents on the nitrogen, cyclopropanecarbonyl and methoxycarbonyl are the most active. These basic structures may be modified by a variety of groups which might be expected to yield the parent compound on hydrolysis or metabolism.

In this way a variety of physical and chemical properties can be obtained for the compounds. Using this approach the structures may be modified in such a manner to give the optimum properties necessary for good fungal control.

Acknowledgments

We would particularly like to thank our numerous colleagues within the research laboratories of the Zeneca organization who provided helpful suggestions and support during the course of this project.

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1. Baker, D. R.; Fenyes, J. G.; Moberg, W. K.; Cross, B. *Synthesis and Chemistry of Agrochemicals*; Baker, D. R.; Fenyes, J. G.; Moberg, W. K.; Cross, B. ; Eds.; American Chemical Society: Washington, D. C., **1987;** pp 1-8.
2. Baker, D. R.; Brownell, K. H. U. S. Patent 4,766,134, **1988.**
3. Baker, D. R.; Brownell, K. H. U. S. Patent 4,766,135, **1988.**
4. Baker, D. R.; Kezerian, K.; Brownell, K. H. U. S. Patent 4,767,772, **1988.**
5. Baker, D. R.; Brownell, K. H. U. S. Patent 4,808,600, **1989.**

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Chapter 38

Synthesis and Fungicidal Activity of *N*-Phenylpyridinamines

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[2]Central Research Institute, Ishihara Sangyo Kaisha Ltd., 2–3–1 Nishi-shibukawa, Kusatsu, Shiga 525, Japan

A series of N-phenylpyridinamines were prepared and evaluated as agrochemicals. Among the N-phenylpyridinamines which showed fungicidal and acaricidal activity, fluazinam **1** (IKF-1216, 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine) was selected as the final candidate to be developed as a fungicide. Fungicidal activity against cucumber gray mold (*Botrytis cinerea*) was used for structure-activity relationships. The role of substituents both on the pyridine ring and on the phenyl ring was well understood. Optimal substituents patterns of fluazinam were well recognized. Background of invention, and synthesis and chemistry of fluazinam are also described.

In a continuing effort to find new compounds for use in agriculture, research at Ishihara Sangyo Kaisha, Ltd. has uncovered a new class of highly active fungicides represented by N-phenylpyridinamines of the general formula **2**, which were prepared originally with an attempt to improve the activity profile of the diphenylamine acaricide fentrifanil **3**. Although some of the scouting compounds thus prepared showed weak acaricidal activity, they showed good activity against *Botrytis cinerea* (gray mold) on cucumber. Initial studies suggested that an in-depth investigation of various substituted N-phenylpyridinamines would be a promising area to investigate. The reasons were: 1) Although pesticidal activities of diphenylamines were well known in the patent literature, few studies on N-phenylpyridinamines were conducted; 2) There was a constant need for new plant protection chemicals because of the development of resistance (especially fungicides against *B. cinerea*); and 3) A number of pyridinamines **4**, precursors for N-phenylpyridinamines, were easily prepared from our rich stock of pyridine chemi-

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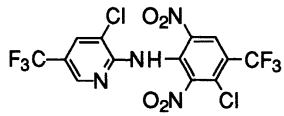
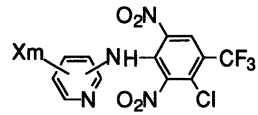
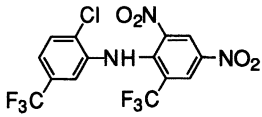


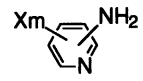
Fluazinam **1**



N-phenylpyridinamines **2**

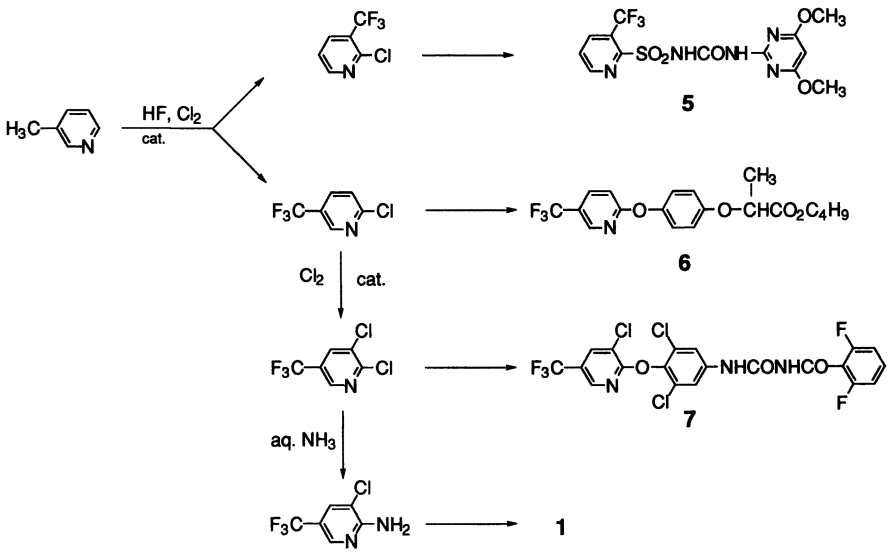


Fentrifanil (ICI) **3**



Pyridinamines **4**

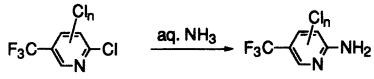
cals (especially halo-trifluoromethylpyridines). The utility of the trifluoromethylpyridinyl group as a building block for valuable agrochemicals was reviewed *(1,2)* and flazasulfuron **5**, fluazifop butyl **6** (both herbicides), and chlorfluazuron **7** (insect growth regulator) were well documented (Scheme 1). In this chapter, synthesis and fungicidal activity of N-phenylpyridinamines is discussed, especially from the view point of the utility of trifluoromethylpyridines as valuable agrochemical intermediates.



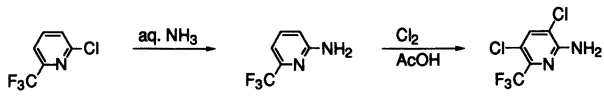
Scheme 1 Agrochemicals derived from trifluoromethylpyridines

Syntheses of N-phenylpyridinamines

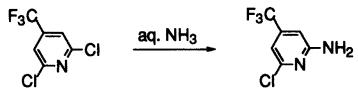
Most of 2-pyridinamines were prepared by amination of corresponding 2-halopyridines *(3,4)* (Schemes 2-4). 3-Pyridinamines were prepared by reduction of the corresponding 3-nitropyridines (Scheme 5). Some chloropyridinamines were prepared by chlorination. Most of **2** were synthesized by nucleophilic reaction of **4** with 2,4-dichloro-3,5-dinitrobenzotrifluoride (Scheme 6). N-phenylpyridinamines whose 5-position of the pyridine ring were substituted by carboxylic groups such as carboxylic ester or acid, were prepared by photoreaction of fluazinam in an appropriate solvent (Scheme 7).



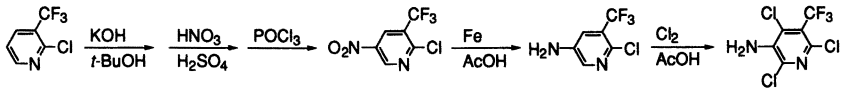
Scheme 2



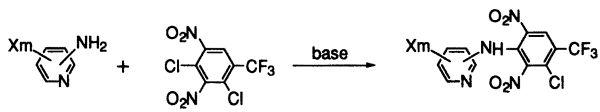
Scheme 3



Scheme 4

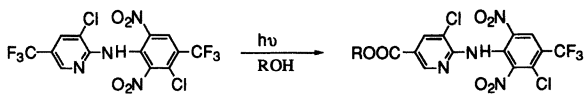


Scheme 5



X : CF_3, NO_2, Halogen, alkyl, cyano, alkylamino, carboxylic groups
m = 0-4

Scheme 6



Scheme 7

Fungicidal Activity

Preventive activity of N-phenylpyridinamines was investigated against *B. cinerea* on cucumber. In this experiment, cucumber plants (cultivar : suyo; 1 leaf stage) were treated with a solution of N-phenylpyridinamines at different rates and kept in a greenhouse at 25 ℃ for one day. Then, the leaves of cucumber plants were inoculated with a mycelial agar disc of *B. cinerea*. Three days after inoculation in a moist chamber at 25 ℃, a length of lesion was measured and evaluated. Results are reported as EC_{90}, the ranges of <16 ppm, 16-500 ppm, and >500 ppm.

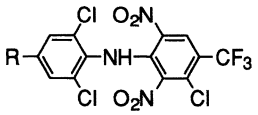
Structure-activity Relationships on N-phenylpyridinamines and Diphenylamines

Effects of substituents in the pyridine ring of fluazinam on the fungicidal activity against *B. cinerea* are summarized in Table I. The structural requirement for high activity seems rather simple. The best fungicidal activities were observed in those compounds substituted with one trifluoromethyl group and one or two halogens (preferably chlorine), or two trifluoromethyl groups. Compounds substituted with cyano or nitro showed a tendency towards diminished activity, and substituted hydrophilic carboxylic acid groups or methylamino groups showed no activity at 500 ppm. These results can be explained as a hydrophobicity and/or by an electron balancing effect. Both substituents, the trifluoromethyl group and chlorine, are hydrophobic with rather small volumes.

QSAR analysis (Akagi T., in preparation) was carried out to investigate substituent effects quantitatively. Several combinations of electronic, steric, and hydrophobic parameters were examined. In fluazinam, electronic effect with hydrophobic character was important. The role of trifluoromethylpyridine in fluazinam presented a good contrast to the role of trifluoromethylpyridines in fluazifop butyl **6** and chlorfluazuron **7**, where QSAR study revealed that (chloro) trifluoromethylpyridine moiety improved biological activity mainly through its hydrophobic character *(1)*.

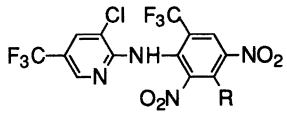
Through extensive syntheses, we also investigated many diphenylamine analogues, some of which showed activity. For example, diphenylamine **8** (R=CF_3) showed good activity. However, it was accompanied by enhanced phytotoxicity. Diphenylamine **8** (R=Cl) showed no activity at 500 ppm with phytotoxicity. Thus, 3-chloro-5-trifluoromethyl-2-pyridyl moiety was selected as the best fungicidal compartment of N-phenylpyridinamine.

Through other extensive syntheses, we also investigated many diarylamine analogues. Again, activity was often compromised by phytotoxicity. For example, N-phenylpyridinamine **9** (R=H, Cl) showed good activity although this was accompanied by enhanced phytotoxicity. Thus, 3-chloro-2,6-dinitro-4-trifluoromethylphenyl moiety was selected as the optimal fungicidal compartment of N-phenylpyridinamine.



diphenylamine (R=Cl, CF_3)

8



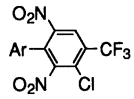
(R=H, Cl)

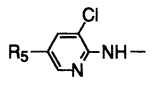
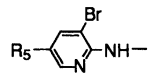
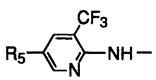
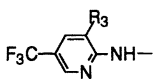
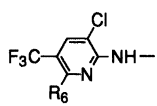
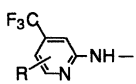
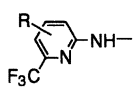
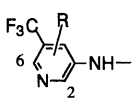
9

Synthesis and Chemistry of Fluazinam

After extensive and intensive studies, **1** was chosen as a final candidate to be developed as a fungicide. **1** was prepared through several synthetic routes. One of the typical laboratory procedures is:

Table I Effect of substituents in the pyridine ring of fluazinam on the fungicidal activity against *Botrytis cinerea*



Ar	Fungicidal activity EC_{90} (ppm)		
	<16	16-500	>500
	R_5 : CF_3 (fluazinam)	CN; NO_2	Cl; Br; COOH; $COOCH_3$; $COOC_2H_5$; $CONH_2$
	R_5 :		Cl; Br
	R_5 : Cl; CF_3	Br	H
	R_3 : F; Br		H; NO_2
	R_6 : F; Cl		$NHCH_3$; $N(CH_3)_2$
	R : 6-CF_3; 3,5-Cl_2	6-Cl; 3,5,6-Cl_3	
	R : 3,5-Cl_2		H
	R : 2-Cl	6-Cl; 2,4,6-Cl_3	

A 3.22 g quantity of 2-amino-3-chloro-5-trifluoromethylpyridine *(3)* in 60 ml of tetrahydrofuran was treated gradually with 2.0 g of powdered potassium hydroxide at 0 ℃. While still at 0 ℃, a solution of 5.0 g of 2,4-dichloro-3,5-dinitrobenzotrifluoride in 40 ml of tetrahydrofuran was added dropwise over 10 minutes. Upon completion of the addition, the reaction mixture was warmed to room temperature and stirred for 3 hours. The reaction mixture was then poured into 500 ml of water and 150 ml of ethyl acetate was added. Upon acidification of the aqueous layer of this bi-phasic mixture to pH 5 and agitating, the organic phase was removed, washed twice with 500 ml water each, dried over anhydrous sodium sulfate and concentrated. The resulting crude material was then purified by silica gel chromatography (10:1 *n*-hexane:ethyl acetate, eluent) to afford 6.5 g of fluazinam, 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.

Fluazinam is yellowish and odorless solid with molecular weight of 465.1 ($C_{13}H_4Cl_2F_6N_4O_4$), and melting point of 116-117 ℃. Fluazinam is a weak acid with p*K*a value of 7.22 in 50% ethanol. Water solubilities of fluazinam are 0.071 ppm and 350 ppm at pH 7.0 and, pH 11.0, respectively. Partition coefficient (log P) of fluazinam is 3.56. Fluazinam shows safe mammalian acute toxicity, LD_{50} (rat oral) >5000 mg/kg. This is in good contrast with fentrifanil, with a mammalian acute toxicity reported to be LD_{50} (rat oral) 136 mg/kg *(5)*.

Biological Properties of Fluazinam

Fluazinam has a broad antifungal spectrum and showed good preventive effect against plant diseases. Fluazinam had little curative and systemic activity; however, it showed good residual effect and rain fastness. Fluazinam showed good activity against benzimidazole and/or dicarboximide resistant strains of *B. cinerea*. Field tests demonstrated excellent activity of fluazinam against potato *Phytophthora infestans (6)*. It is also noteworthy that fluazinam significantly reduced the population of mites by repeated treatments in the field, although fluazinam showed low acaricidal activity in a greenhouse test.

Acknowledgments

The authors are grateful to Dr. Ryuzo Nishiyama for his supervision and continued guidance throughout this work. The authors wish to thank Mr. K. Fujikawa, Mr. I. Yokomichi, Dr. F. Kimura, Mr. T. Toki and Mr. R. Nasu for invaluable discussion.

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RECEIVED October 5, 1994

Chapter 39

Antimicrobial Properties of Natural Volatile Compounds

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Macerated foliage from several plant species including tomato emits volatile compounds which inhibit germination of pollen and growth of hyphae of the economically important plant pathogenic fungi *Alternaria alternata* and *Botrytis cinerea*. The compounds emitted from the wounded tomato tissue consist of lipoxygenase-lyase derived aldehydes and alcohols and also terpene hydrocarbons as shown by Tenax headspace vapor trapping and GC-MS analysis. Unsaturated aldehydes such as (*E*)-2-hexenal, at sub-μmol / L air concentrations, inhibited fungal growth in bioassays whereas the terpenes tested did not. The aldehydes appear to account for the antifungal activity exhibited by vapors from the wounded leaf tissue. Lipoxygenase-lyase derived volatile compounds also inhibited growth of plant pathogenic bacteria (*Pseudomonas* species) and *E. coli*. (*E*)-2-Hexenal was active against *Pseudomonas syringae* pv *angulata* at a sub-μmol / L air concentration. (*E*)-2-Hexenal was relatively toxic to *Salmonella typhimurium* tester strains in the Ames test but did not appear to be mutagenic when tested in the vapor phase. Possible applications of volatile compounds, which are human dietary constituents, to reduce microbial populations on foods and plants are discussed.

It is well-known that volatile organic compounds are ubiquitous in plant species and are emitted by leaves, flowers, fruits, stems, and roots. Hundreds of volatile compounds have been identified as plant components including substances such as alcohols, aldehydes, ketones, esters, lactones, ethers, amines, and carboxylic acids. These compounds are important constituents of food flavor (*1,2*) and are thought to function in biological processes such as pollination (*3*) and host-pest

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interactions including the growth of pathogenic microorganisms on plants (*4,5*). The role of volatiles in flavor chemistry and physiology has been investigated more thoroughly than their roles in pollination or plant-pest interactions.

In this chapter an overview is presented of several studies which we have conducted and published (*6-8*) on the inhibitory effects of natural volatile compounds, emitted from "wounded" leaves, on the germination of pollen and the growth of certain fungi and bacteria. The studies summarized herein were initiated as a follow-up to a report by French and coworkers (*9*) that certain synthetic volatile compounds stimulated germination of pollen from several species of pine. We investigated the effects of volatile compounds emitted from both intact and crushed flowers and leaves on germination of apple pollen. None of the plant material tested had an effect on pollen, except for crushed or wounded leaves, which partially or completely inhibited germination. Since economically important fungi and bacteria which cause serious diseases may come in contact with vapors from damaged or wounded plant material, studies were conducted to determine the effects of commonly occurring natural volatile compounds on representative pathogenic microorganisms which attack plants and their products.

Experimental

Bioassays - Pollen, Fungi, Bacteria. The bioassay system has been described previously (*7, 8*). Briefly, it consisted of a 5 cm Petri dish containing a block of water agar placed within a 9 cm Petri dish. Apple pollen grains or fungal (*Alternaria alternata, Botrytis cinerea*) spores were placed on the surface of the agar block. Leaves or flower petals, crushed or intact, were placed in the 9 cm dish around the edges of the 5 cm dish. Solutions of synthetic compounds were tested by placing them in a sample dish contained within the 9 cm dish. The lid of the 9 cm dish was then placed over the assembly (120 ml volume). Pollen germination or fungal hyphal length after an exposure period (1.5 hrs for pollen; 5-10 hrs for fungi) was measured using a microscope with a net micrometer ocular.

Bacterial bioassays using *Pseudomonas syringae* pv *angulata, P.s.* pv *tabaci* and *E. coli* TB 1 were carried out as above except that overnight broth cultures were placed on nutrient agar. Bacteria exposed to compounds for 22.5 hrs were serially diluted, plated, and the number of colony forming units was counted.

Isolation of Volatile Compounds. The method for isolation of headspace compounds has been described earlier (*6*). Briefly, crushed tomato leaves were placed in a flask and high purity air was used to entrain the emitted volatile compounds which were subsequently trapped on Tenax. The Tenax was washed with hexane and the tomato components were then separated on a polar capillary column using GC. Compounds were identified by GC-MS analysis and co-chromatography.

Direct headspace quantitation of components in the atmosphere within the Petri dish bioassay system (fitted with a septum in the lid) was achieved by

withdrawing an air sample using a syringe. The sample was analyzed directly using a GC with a capillary column. A similar sampling method was used to measure the concentration of (*E*)-2-hexenal in the Ames bioassay desiccator system described below.

Ames Bioassay. Five strains of histidine deficient *Salmonella typhimurium*, TA 97a, TA 98, TA 100, TA 102, and TA 104, were used in the bioassays which were carried out following the procedures of Maron and Ames (*10*) as modified for volatile compounds using a 9 L desiccator according to Simmon (*11*). 2-Aminofluorine, activated with S-9 was used as a positive control for the tester strains.

Results and Discussion

Plant Vapors and Pollen Germination. The flowers and the intact leaves bioassayed did not affect apple pollen germination. However, vapors emitted by crushed tomato, apple, and strawberry leaves did inhibit germination whereas chrysanthemum leaf components did not (*6,12*). Five grams of 'Mountain Pride' tomato leaves completely inhibited pollen germination during the 90 minute bioassay whereas the control pollen germinated (data not shown).

Identification of Volatile Compounds from Crushed Tomato Leaves. To determine the types of volatile compounds which were emitted from damaged leaves that inhibited pollen germination, a dynamic headspace apparatus was set up to isolate the leaf vapors. Compounds were entrained in air and trapped on Tenax to obtain large enough quantities for analysis by gas chromatography-mass spectrometry. A chromatogram of the compounds emitted from tomato leaves is shown in Figure 1. Sixteen compounds were identified (Table I) which fall into two main categories. These comprise six-carbon aldehydes and alcohols derived from the lipoxygenase-hydroperoxide lyase pathway and terpene hydrocarbons (mono- and sesquiterpenes) formed from the mevalonic acid pathway. The concentrations of several of these were subsequently determined by direct sampling of the atmosphere within the Petri dish bioassay system using a gas-tight syringe followed by GC analysis (Table I).

Pollen Bioassays using Individual Components of Crushed Tomato Leaf Vapor. Plant components identified, except α-phellandrene and α-terpinene, were tested individually against pollen at three vapor phase concentrations. The test concentrations were selected to include a vapor phase level corresponding to that measured in vapor emitted from crushed tomato leaves in the bioassay system. Plots of the vapor phase concentration versus the percent germination of pollen were made from the bioassays and an ED_{50} value was estimated for each tomato component evaluated (Table II). These data indicated that the most inhibitory compounds from crushed tomato leaves were the lipoxygenase-lyase products (*E*)-2-hexenal and (*Z*)-3-hexenal. The estimated ED_{50} for (*E*)-2-hexenal, for example, was 0.25 μmol / L air which was close to the vapor phase concentration of this compound, 0.19 μmol / L air, measured from crushed tomato leaves in the

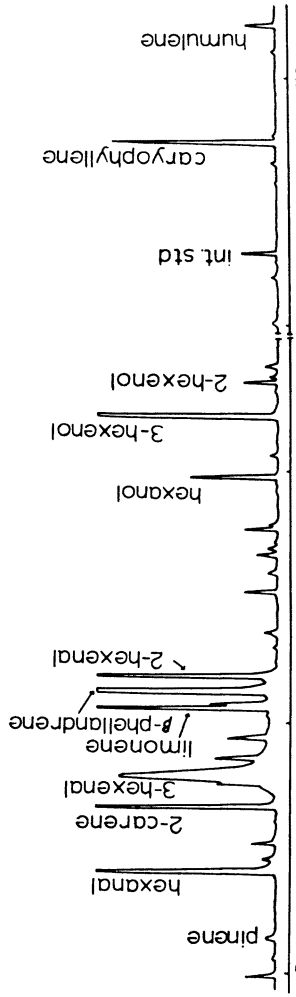

Figure 1. Chromatogram of crushed tomato leaf headspace compounds obtained using a 60 m X 0.32 mm Supelcowax GC column.

Table I. Identification and Quantitation of Headspace Compounds from Crushed Tomato Foliage

compound	evidence[a]	recovery from Tenax, % of total[b]	direct sampling of concn above leaves in bioassay, µmol / L air[c]
hexanal	MS,RT	5.3	0.34 ± 0.19
(*E*)-2-hexenal	MS,RT	9.0	0.19 ± 0.08
(*Z*)-3-hexenal	MS,RT	14.9	0.29 ± 0.15
1-hexanol	MS,RT	1.4	
(*E*)-2-hexen-1-ol	MS,RT	0.7	
(*Z*)-3-hexen-1-ol	MS,RT	8.9	0.07 ± 0.01
(*Z*)-3-hexenyl acetate	MS,RT	0.2	
2-carene	MS,RT	6.1	0.21 ± 0.02
limonene	MS,RT	6.3	0.10 ± 0.01
α-phellandrene	MS,RT	1.5	
β-phellandrene	MS[d]	27.2	0.42 ± 0.08
α-pinene	MS,RT	0.5	0.05 ± 0.01
α-terpinene	MS,RT	1.4	
caryophyllene	MS,RT	3.0	0.03 ± 0.01
α-humulene	MS,RT	0.6	
benzyl alcohol	MS,RT	0.1	

[a]Identification based on comparison of mass spectral and GC retention time data of plant components with those of authentic compounds.
[b]Tenax trapping period was 2 hrs; total yield of volatile compounds trapped was 24 µg/g of leaves.
[c]Results from direct headspace sampling, using syringe, of atmosphere in bioassay system containing 5 g of crushed tomato leaves 1 hr after set up of assembly.
[d]Spectrum consistent with that published by Buttery *et al.* (*24*).
(Reproduced in part from Ref. *6*, Copyright 1991, American Chemical Society.)

Table II. Estimated ED_{50}s of Compounds Assayed for Activity against Apple Pollen (*Malus* x *domestica* cv Red Delicious)[a]

compound	ED_{50}, µmol / L air	compound	ED_{50}, µmol / L air
(*Z*)-3-hexenal	0.19	(*E*)-2-hexen-1-ol	5.0
(*E*)-2-hexenal	0.25	hexanal	7.8
(*Z*)-3-hexenyl acetate	3.6	1-hexanol	8.3
(*Z*)-3-hexen-1-ol	4.8		

[a]ED_{50}s estimated from plots of vapor-phase concentrations of compounds measured by GC versus percent pollen germination. Tests with the terpene hydrocarbons, 2-carene, limonene, and α-pinene, at vapor-phase concentrations similar to those of the lipoxygenase-lyase products, did not inhibit pollen germination.

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bioassay system (Table I). Thus the lipoxygenase-lyase derived aldehydes appear to contribute to the inhibition of pollen germination observed. With regard to natural occurrence, (*E*)-2-hexenal appears to be a ubiquitous compound found in all vegetative tissues examined, in varying amounts, and is referred to as leaf aldehyde. (Z)-3-Hexenal is a more labile natural product and deteriorates relatively rapidly.

In contrast to the results with the aldehydes, the terpene hydrocarbons had little or no effect on pollen germination even at the highest concentrations evaluated. The highest concentrations of the monoterpenes, (D)-limonene, 2-carene, and (D)-α-pinene tested, which were 4.8, 7.6, and 9.9 μmol / L air, respectively, did not significantly inhibit pollen germination. Likewise, the sesquiterpenes caryophyllene and α-humulene at 0.6 and 0.7 μmol / L air, respectively, had no effect on germination. These concentrations exceeded those detected from crushed tomato leaves. Based on these results it was concluded that of the two major groups of compounds emitted from wounded tomato leaves, the lipoxygenase-lyase unsaturated aldehydes effectively inhibited pollen germination whereas the terpene hydrocarbons had no significant effect.

Effects of Volatile Compounds on Fungal Pathogens. Following the investigations of the marked inhibitory effects caused by leaf wound vapors on pollen germination, studies were undertaken to determine if these natural compounds affect another important type of micropropagule, that is, spores of pathogenic fungi which parasitize plants. Two fungal pathogens were chosen for this study, *Botrytis cinerea* which causes gray mold disease on fruit and flowers and *Alternaria alternata* which causes a foliar disease. Spores of these fungi were placed on an agar block as in the pollen experiments and exposed to volatile compounds from leaves. Vapors emitted from wounded tomato leaves inhibited hyphal growth of *Alternaria alternata* as shown in Table III. Tests with two representative terpenes, 2-carene and (D)-limonene, revealed that neither of these compounds inhibited growth of *A. alternata.* The concentrations of the terpenes tested exceeded the concentrations measured above crushed tomato leaves (Table I). In contrast, the aldehydes tested inhibited hyphal growth which corresponded to the results obtained with pollen. (*E*)-2-Hexenal inhibited hyphal growth at a concentration (0.23 μmol / L air) which was in the same range as that detected in crushed tomato leaves (0.19 μmol / L air). Hexanal also inhibited the growth of *A. alternata.* The nine-carbon analogs, (*E*)-2-nonenal and nonanal which are also naturally occurring plant constituents, caused inhibition similar to that observed with the six-carbon aldehydes (Table III). Bioassays using the same aldehydes and terpenes described above and *Botrytis cinerea* as the test fungus gave similar results (Table III) as were obtained with *A. alternata.*

The α, β-unsaturated aldehydes were more inhibitory than were the saturated aldehydes. At the lowest concentrations tested, the saturated aldehydes, hexanal and nonanal, appeared to stimulate hyphal growth in *B. cinerea.* This is consistent with the results obtained with saturated aldehydes and other volatile synthetic flavor compounds using certain other fungi, for example, *Puccinia* species which cause rust disease on grains such as wheat [see review by French (*13*)].

Table III. Effects of Natural Volatile Compounds on Hyphal Lengths of the Fungal Pathogens *Alternaria alternata* and *Botrytis cinerea*

source	concn, µmol / L air	relative hyphal length *A. alternata*	*B. cinerea*
no leaf		100 A[a]	100 A
crushed tomato leaf (5g)		18 B	20 B
(D)-limonene	0	100 A	100 A
	4.8	100 A	110 A
2-carene	0	100 A	100 A
	7.6	92 A	108 A
(*E*)-2-hexenal	0	100	100
	0.02	94	23
	0.23	25	0
	4.6	6 L[b]	0 L
hexanal	0	100	100
	0.04	100	132
	0.32	67	15
	12.8	<5 L	0 L
(*E*)-2-nonenal	0	100	100
	0.01	95	100
	0.37	0	43
	2.1	0 L	0 L
nonanal	0	100	100
	0.02	100	118
	0.27	69	76
	2.0	6 L	0

[a]Means followed by the same letter are not different by Student t test at $P<0.05$

[b]For a given compound, L denotes a significant linear correlation ($R^2 >0.90$) between hyphal length and concentration.

Overall, the results showed that wound vapors from macerated tomato leaves inhibited growth of two pathogenic fungi. As with pollen, aldehydes, especially α, β-unsaturated derivatives, inhibited growth whereas the terpene hydrocarbons tested did not.

Effects of Volatile Compounds on Bacterial Pathogens. Preliminary bioassays indicated that crushed tomato leaves were not active against the bacterium *E. coli.* However, inhibitory lipoxygenase-lyase products from the tests above, (*E*)-2-hexenal and hexanal, were evaluated for their effects on *E. coli* and two bacterial plant pathogens. The tobacco bacterial pathogens *Pseudomonas syringae* pv *angulata*, which causes angular leaf spot disease and *Pseudomonas syringae* pv *tabaci*, which produces wildfire disease were used as test organisms. The results obtained with the two *Pseudomonas* pathogens using (*E*)-2-hexenal and hexanal (Table IV) show that the former compound was more inhibitory than the latter to both bacteria. (*E*)-2-Hexenal significantly inhibited the proliferation of *P. syringae* pv. *angulata* at 0.4 μmol/L air which is in the concentration range for this compound measured in crushed tomato leaf vapors. (*E*)-2-Hexenal was also more active than hexanal against *E. coli.* The concentration of (*E*)-2-hexenal tested which reduced growth of *E. coli* was 9.5 μmol/L air which was considerably greater than the level emitted from plant material. Tests with the alcohols, (*E*)-2-hexen-1-ol and 1-hexanol, also showed that the unsaturated alcohol was more active against the bacteria than was its saturated counterpart (data not shown).

Ames Test of (*E*)-2-Hexenal. Bioassays were conducted to determine the effects of (*E*)-2-hexenal in the Ames test which shows the capacity of volatile compounds to cause mutagenesis in strains of the bacterium *Salmonella typhimurium.* Compounds active in this bioassay are frequently carcinogenic and positive responses may preclude their development and use in applications involving human exposure.

The test system for evaluating volatile compounds in the Ames bioassay employed a desiccator containing Petri dishes with the histidine deficient *S. typhimurium* strains. The results obtained with the tester strain TA 100 and (*E*)-2-hexenal are given in Table V. The highest vapor phase concentration of (*E*)-2-hexenal tested, 26 μmol/L air, was toxic to the *S. typhimurium* strain as shown by the absence of colony formation. TA 100 also was inhibited by (*E*)-2-hexenal at 6 μmol / L air. This contrasts to results with methylene chloride, a known mutagen (*14*), which exhibited no toxicity at 1040 μmol / L air but did produce more than four times the number of revertant colonies as a control. At a concentration where no *S. typhimurium* inhibition was observed, 0.6 μmol / L air, no evidence was obtained for mutagenesis by (*E*)-2-hexenal using standard assay conditions, a three-fold increase in bacteria as suggested by Eder (*15*), or with the S-9 mutagen activator preparation. Similar results were obtained for (*E*)-2-hexenal, with or without S-9, using four additional tester strains of *S. typhimurium* (TA 97a, TA 98, TA 102, and TA 104) when vapor phase Ames tests were conducted (*16*).

Marnett *et al.* (*17*) tested (*E*)-2-hexenal as a liquid using a modification

Table IV. Effects of (*E*)-2-Hexenal and Hexanal on the Growth of Two *Pseudomonas syringae* Bacterial Pathogens

compound	conc. µmol / L air[a]	no. of bacteria ($x10^9$)[b] *P.s. angulata*	*P.s tabaci*
(*E*)-2-hexenal	0	2.1 A	5.4 A
	0.05	2.3 A	5.2 A
	0.4	0.8 B	4.4 A
	5.7	0 C	0 B
hexanal	0	2.1 A	5.3 A
	0.12	1.8 A	4.2 A
	1.5	2.5 A	4.3 A
	21	2.0 A	1.5B

[a]Vapor phase concentration of compound in bioassay assembly.
[b]Means followed by the same letter for a given compound and pathogen are not different by LSD at P<0.05.

Table V. Ames Desiccator Test - Response of *Salmonella typhimurium* to (*E*)-2-Hexenal[a]

concn, µmol / L air	revertant colonies per plate ± S.D. treatment	control
	CH_2Cl_2	
1040	823 ± 188	172 ± 41
	(*E*)-2-hexenal	
26	0	146 ± 4
6	18 ± 8	124 ± 3
0.6	203 ± 10	188 ± 3
0.6[b]	172 ± 21	195 ± 6
0.6[c]	155 ± 12	174 ± 18

[a]*S. typhimurium* tester stain TA 100 used in bioassays.
[b]Test done with three-fold more bacteria than standard Ames test (*15*).
[c]S-9 preparation added to bioassay system.

of the Ames bioassay and TA104. Their experiments involved pre-incubation of the compound with the bacteria followed by treatment with glutathione to ameliorate the toxicity of (*E*)-2-hexenal. Under these conditions, (*E*)-2-hexenal gave more than twice the revertants per dish as did the control. This experiment was repeated in our laboratory using liquid (*E*)-2-hexenal and results similar to those of Marnett et al. (*17*) were obtained.

In conclusion, (*E*)-2-hexenal, a major leaf wound volatile compound found to be inhibitory to certain fungi and bacteria, was relatively toxic to *S. typhimurium* stains but was not found to be mutagenic when tested in the vapor phase. This compound was mutagenic to *S. typhimurium* when tested as a liquid using bioassay modifications (*17*).

Biochemistry of Lipoxygenase - Lyase Products. The six-carbon aldehydes, (*E*)-2-hexenal and hexanal, are formed in plants from the ubiquitous C_{18} fatty acids, linoleic and linolenic acid, by lipoxygenase and hydroperoxide lyase (*18*) as shown in Figure 2. The other principal product of this pathway, formed from the carboxylic acid end of the fatty acid, is thought to be 12-oxo-(*E*)-10-dodecenoic acid. This compound is commonly known as traumatin and contains an α, β-unsaturated aldehydic moiety like that of (*E*)-2-hexenal and thus might be expected to be inhibitory to microorganisms.

The α, β-unsaturated aldehydes are believed to react in biological systems by formation of 1,4-addition products with sulfhydryl groups such as the -SH of cysteine or formation of Schiff bases with amino groups such as the ε-amino group of lysine (*19,20*). These and other adducts may disrupt the structure of peptides and proteins and other essential molecules in cells.

Possible Applications of Volatile Compounds to Reduce Microbial Populations. There are several ways in which volatile natural products such as those described here (which are constituents of the human diet) might be used to reduce populations of harmful microorganisms on foods and plants. These include fumigation of foods with these compounds to reduce microbial populations prior to marketing. An example is strawberry fruit that is infected shortly after pollination with the fungus *Botrytis cinerea* which leads to the development of gray mold disease on ripe fruit. Fumigation of the fruit, after harvest, with natural compounds such as (*E*)-2-hexenal or (*E*)-2-nonenal might reduce the levels of this economically important disease organism and, due to the volatility of the natural compounds, leave insignificant residues of these materials on the treated fruit. Vaughn *et al.* (*21*) have recently reported success from in vitro tests in reducing gray mold on small fruits using volatile compounds.

Alternatively, since the biochemical pathway for synthesis of the lipoxygenase-lyase products is well established it should be possible to increase the endogenous levels of these compounds in species where their concentrations are low. Recently, Deng *et al.* (*22*) transferred lipoxygenase isozyme 2 from soybean seeds to tobacco leaves and increased production of (*E*)-2-hexenal in the leaves by 2-to 3-fold. The transgenic plants have not been evaluated to determine if they are more resistant to pathogens.

A third method for using these natural products might be to synthesize

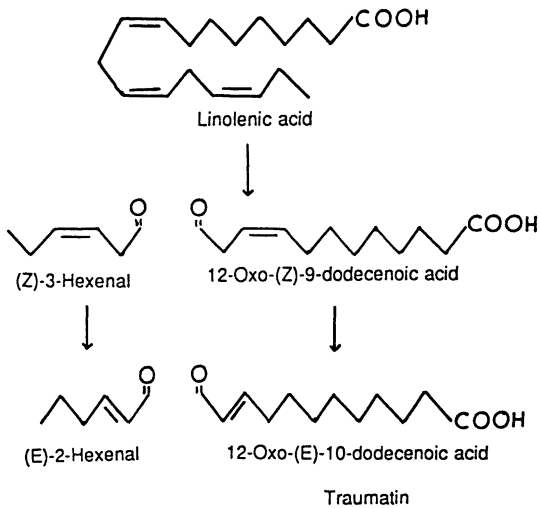

Figure 2. Biosynthetic origin of (*E*)-2-hexenal and traumatin in wounded plant material.

their glycosides, many of which also occur in nature and are probably human dietary constituents [for example, the glucoside of (*E*)-2-hexenal was detected in tomato fruit (*23*)]. These compounds which are nonvolatile and water soluble might be applied topically to plants and subsequently taken up by deleterious fungi and bacteria. Hydrolysis of the glucosides by ubiquitous β-glycosidases would release inhibitory aglycones such as (*E*)-2-hexenal as shown by Buttery *et al.* (*23*) or traumatin. In this manner, the populations of pathogenic microorganisms on plants might be reduced to help protect against economically important diseases without substantial negative impact on the environment.

Conclusions

Volatile compounds emitted from wounded or crushed leaf material inhibited pollen germination and growth of pathogenic fungi and bacteria in *in vitro* bioassays. Among the most inhibitory natural products identified were aldehydes, especially α, β-unsaturated compounds, from the lipoxygenase-hyproperoxide lyase pathway. The ubiquitous plant volatile compound, (*E*)-2-hexenal, inhibited growth of the fungi, *Botrytis cinerea* and *Alternaria alternata*, and the bacteria, *Pseudomonas syringae* pv *angulata*, *P. s.* pv *tabaci*, and *E. coli*. Ames tests with (*E*)-2-hexenal in the vapor phase indicated that the plant component was relatively toxic to strains of *Salmonella typhimurium* but did not exhibit mutagenic activity. Possible ways in which the plant produced volatile compounds might be used to reduce microbial populations include fumigation, genetic transformation to enhance endogenous concentrations in foods and plants, and topical applications of glycosides of these compounds.

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Chapter 40

Nitrosation of Tobacco Alkaloids During Storage Reduced by Antimicrobial (*E*)-2-Hexenal Vapor

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Release of volatiles by the lipoxygenase enzyme system (LOX) in plants may be a primary defense mechanism against microbial infection. We showed that among LOX C-6 aldehydes, an α,β double bond adjacent to the carbonyl moiety enhanced antimicrobial activity. Smokeless tobaccos during storage may have undergone nitrosation by microbial action causing accumulations of alkaloid-derived nitrosamines. Some nitrosated alkaloids related to nicotine are carcinogenic and it is desirable to prevent their formation. Storage of snuff tobaccos with *E*-2-hexenal (which generally has the highest relative abundance among LOX-derived C-6 aldehydes) in the gaseous phase resulted in reduced accumulations of nitrite and nitrosamines compared to controls without *E*-2-hexenal.

Pathways have been elucidated for the formation of volatile C-6 aldehydes and alcohols in plant tissue via the lipoxygenase enzyme (LOX) pathway (*1*). The formation of C-6 aldehydes and alcohols are illustrated in Figure 1 for the oxidative cleavage of linolenic acid which may serve as the starting material. Naturally-occurring volatile C-6 and C-9 ketones are also emitted from plants and may play a role in plant defense against microbial infection, but it is not believed that these are LOX products (*2*). Wounding of plant tissues has been shown to stimulate the oxidative cleavage of unsaturated fatty acids yielding volatile components in the headspace at the wound site. The biological activity of LOX product components in the vapor phase has been demonstrated with several assay systems. Recently, it was shown that *Z*-3-hexenal and *E*-2-hexenal inhibited apple pollen germination (*3*). Estimates of the effective dose, ED_{50}, of several compounds that inhibited pollen germination by 50% were made. Oxygenated C-6 and C-9 aldehydes and ketones generally inhibited apple pollen germination. The unsaturated aldehydes were considerably more inhibitory than the saturated aldehyde hexanal. Comparison of the two saturated aldehydes hexanal and

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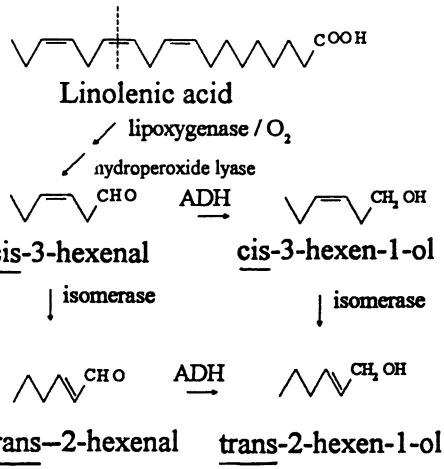

Figure 1. Biosynthetic pathway of C-6 aldehydes and alcohols by the LOX enzyme system pathway. Abbreviations used: LOX = lipoxygenase; ADH = alcohol dehydrogenase.

nonanal showed that the C-9 compound was more inhibitory than the C-6 compound when the amount of each compound in the vapor phase was taken into account. In addition to LOX product aldehydes and alcohols and structurally-related ketones, several aromatic compounds were generally inhibitory to pollen germination. The third group of compounds investigated, namely, terpenoids exhibited little or no inhibition of the apple pollen germination. In addition to the inhibition of pollen germination, C-6 and C-9 aldehydes were also found to inhibit fungal growth (*4*).

Biosynthetic pathways have been determined for carcinogenic nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in tobacco during postharvest processing by nitrosation reactions as shown in Figure 2 (*5*). Either the secondary amine-alkaloid nornicotine or the tertiary amine-alkaloid nicotine act as precursors and are formed and accumulate in tobacco during growth. Nitrosation occurs during postharvest treatments via formation of nitrite from the reduction of ubiquitous nitrate stored in tobacco as a result of fertilization. Nitrate reductase present in bacteria and other microorganisms is thought to effect the nitrite production during storage and its subsequent reactivity with tobacco alkaloids.

The purpose of our study reported herein is to determine whether a naturally-occurring C-6 aldehyde (LOX product) exogenously added as a vapor phase component would inhibit the biosynthesis of undesirable nitrosated alkaloids in tobacco. The selected aldehyde should have anti-microbial activity in at least one bioassay test system and it should either be nonmutagenic or have low mutagenic potential, factors which may favor development of the compound as an antimicrobial agent.

Materials and Methods

Synthetic samples of volatile compounds investigated in the bioassays were either obtained from Aldrich (Milwaukee, WI) or from Bedoukian Research, Inc. (Danbury, CT). 4-(*N*-Methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), nornicotine and anatabine were obtained from Chemsyn Science Laboratories, Lenexa, KS. *N'*-Nitrosonornicotine (NNN) and *N'*-nitrosoanatabine (NAT) were synthesized by nitrosation of nornicotine and anatabine, respectivley, in a manner described for the nitrosation of morpholine (*6*). Isolates of *Alternaria alternata* (Fr.) Keissl. were obtained from lesions of tobacco leaves as previously described (*4*). A reference smokeless tobacco research product, namely, moist snuff (1S3) was obtained from the Tobacco and Health Research Institute, University of Kentucky, Lexington, KY. The principal components of the product (wt. %) were moisture (53%), dark fire-cured leaf (26%), dark air-cured leaf (7.8%), burley stems (3.7%).

Quantitative estimation of LOX-derived compounds in the vapor phase (headspace) were carried out in a bioassay system previously described (*4*); 9-cm petri dishes contained a microcup with water and a solution of test compound (but without the 5-cm dish and agar block). The bioassay dish illustrated in the reference was wrapped with Parafilm and incubated at a specified temperature and

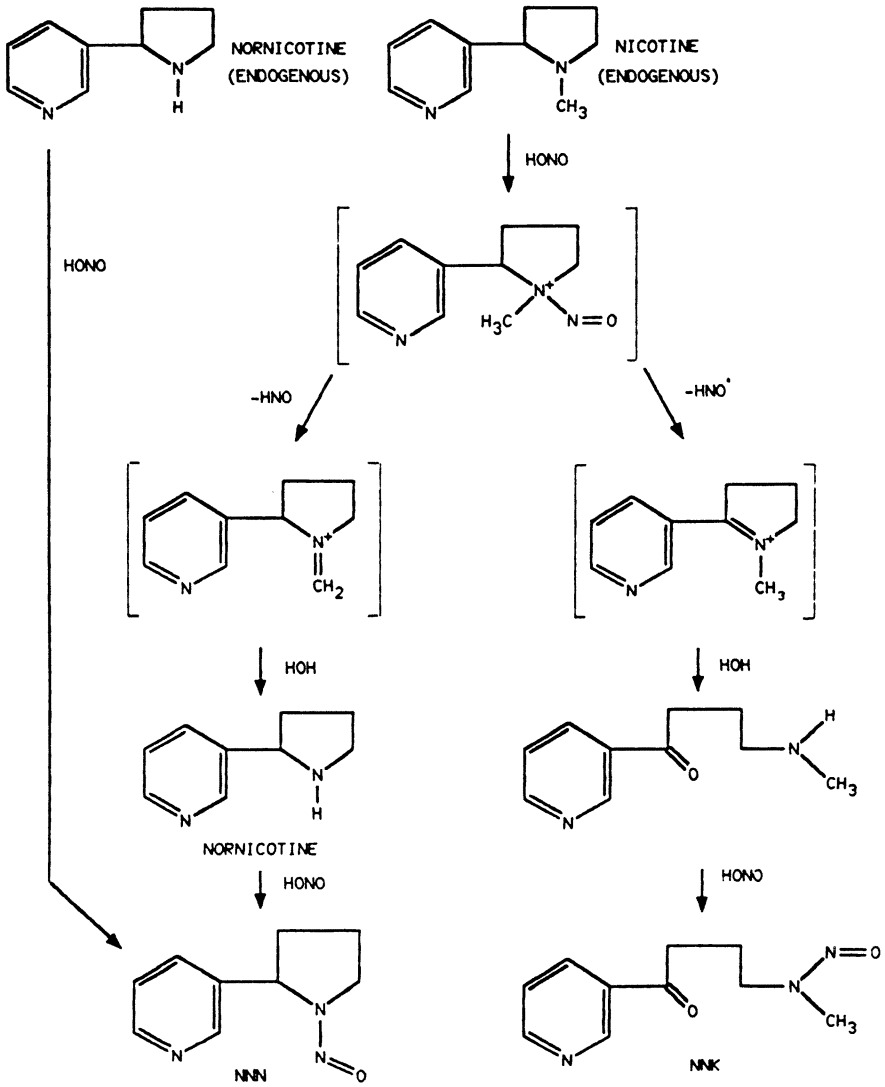

Figure 2. Formation schemes of NNN and NNK during tobacco postharvest processing. (SOURCE: Adapted from ref. 5.)

time period. Subsequently, a sample of vapor was withdrawn from the system through the septum with a syringe and injected into a GC injection port. A Varian 3700 GC equipped with a 30 m x 0.53 mm x 1-μm film DB-WAX (polyethylene glycol) column and a 1 m x 0.53 mm fused silica precolumn was used for direct injection. Operating conditions were as previously described (*4*). A flame ionization detector was used. Response factors for authentic reference compounds were determined using a 2-L static dilution bottle following an EPA method (*7*) employing argon for dilution.

The system used to determine effects of volatiles on the growth of conidiospores from *Alternaria alternata* was previously described (*4*). A 9-cm petri dish contained a 1-cm^3 2% water agar block, and a 1-cm diameter glass sample dish contained 1 mL of water. For the bioassay, spores were spread on the surface of the agar block and 10 μL of a solution of the volatile test compound was placed in the sample dish containing water. The cover of the 9-cm petri dish was then placed over the system (internal volume=120 mL) which was immediately wrapped with Parafilm. After 4-6 h, germ tube lengths were measured using a microscope with a net micrometer ocular at 100 x magnification.

A colorimetric method for determination of nitrite in tobacco was developed that stabilizes and decolorizes tobacco extracts for automated continuous flow analysis at ppb or greater (*8*). It avoids the problem that nitrite measurement in unsterilized tissue is error-prone because of bacteria-mediated reduction of nitrate to nitrite. Spectrophotometric measurement of nitrite is made after derivatization with 0.1% *N*-(1-naphthyl)-ethylene diamine.

Nitrosated pyridine alkaloids in tobacco were extracted and separated from the bulk of the "parent" alkaloids by liquid-liquid partitioning with ethyl acetate-pH 5 buffer, and were determined by a GC procedure employing a fused silica non-polar DB5 column and a thermionic nitrogen-phosphorus detector (NPD) as previously described (*9*).

Results and Discussion

Anti-Fungal Activities of Aldehydes vs *Alternaria alternata*. The effects of vapor concentrations of seven aldehydes ranging from 0 to 12 μmole per liter of air on fungal germ tube lengths were determined for at least three vapor concentrations of each aldehyde. The acronym GTL_{50} was defined as the value in terms of headspace concentration of a specific compound that caused a 50% reduction in the germ tube elongation of *A. alternata* as compared to the respective bioassay control treatment. Values of the seven aldehydes are given in Table I, and chemical structures of these compounds are illustrated in Figure 3. These compounds were also ranked in the order of decreasing anti-fungal activity as determined by their relative GTL_{50} values. The most biologically active aldehydes contained an α,β-unsaturated bond, and it appeared that a carbonyl group adjacent to the double bond was required for the larger increases of anti-fungal activity observed compared to the saturated aldehydes, namely, hexanal and nonanal. It is probable that the presence of an α,β-unsaturated bond increases the electrophilic properties of the carbonyl compounds that contain this bond structure

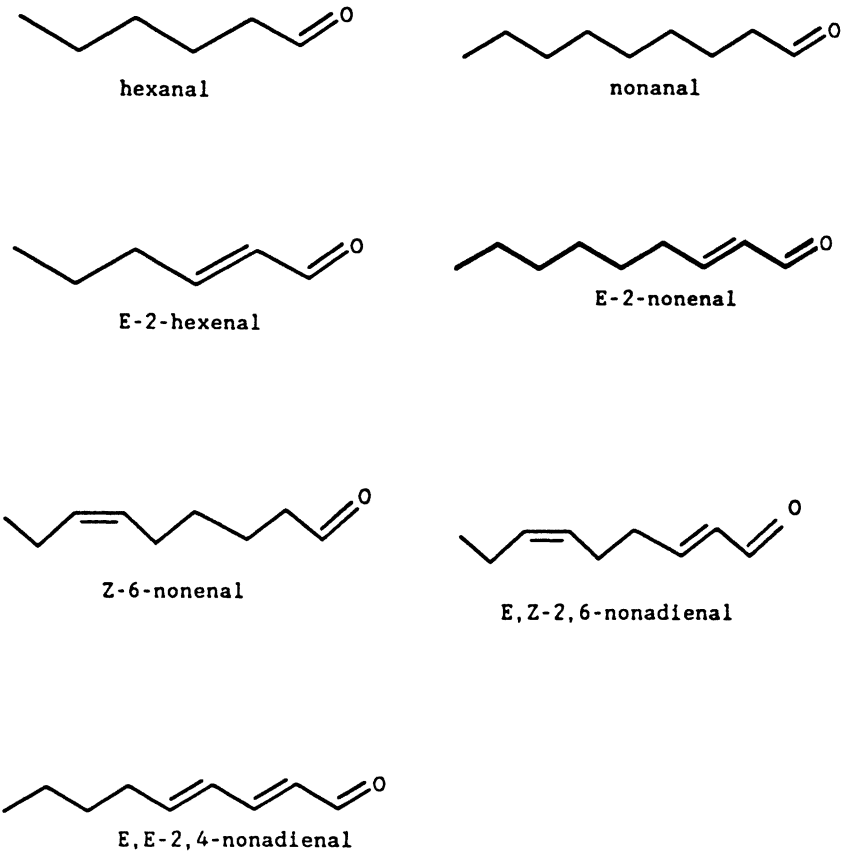

Figure 3. Chemical structures of volatile C-6 and C-9 aldehydes.

compared to their saturated counterparts (*10*). Consequently, the inhibitory properties of these compounds and their effectiveness against *A. alternata* may be increased due to their increased propensity to react with thiols and amino groups of the target fungi. *Z*-6-Nonenal possesses a double bond that is not adjacent to the carbonyl functional group. Therefore, it does not meet the requirement for increased electrophilic properties of the carbonyl group that are the case for the more strongly

Table I. Relative Anti-Fungal Activities of Volatile C6 and C9 Aldehydes vs *Alternaria alternata*

Compd	*Headspace Concn Causing 50% Reduction of Control Germ Tube Length (GTL_{50}), nmol x 10^{-2}/L air*[a]
ALDEHYDES (rank in order of decreasing anti-fungal activity)	
hexanal (7)	54
E-2-hexenal (6)	10
nonanal (5)	6
E-2-nonenal (1)	1
Z-6-nonenal (4)	4
E,E-2,4-nonadienal (3)	3
E,Z-2,6-nonadienal (2)	2

[a]An x-y plot of germ tube lengths vs headspace concentrations for a given compound was used to generate a best curve fit. A GTL_{50} value was obtained from the curve by interpolation. (SOURCE: Adapted from ref. *11*).

inhibitory (anti-fungal) structural analogs such as *E*-2-nonenal. Our results indicate that at the quantities investigated, C-9 aldehyes are generally more potent than their C-6 counterparts as agents versus *A. alternata* spore germination and subsequent germ tube elongation. It is noteworthy, however, that C-6 aldehydes were more effective than their C-9 counterparts versus *Botrytis cinerea* (*4*).

Although *E*-2-nonenal was a stronger anti-fungal compound than *E*-2-hexenal (Table I), the latter aldehyde was selected for further study in terms of its possible anti-microbial effect on tobacco during storage. The reason for this choice was based on the knowledge that *E*-2-hexenal is generally present in much higher concentrations than *E*-2-nonenal in emissions from green plants, and it was already tested in the volatile state and found to be nonmutagenic in the Ames test (*11*).

Nitrite and Nitrosated Alkaloid Concentrations in Smokeless Tobacco After Storage. The effects of moisture and temperature on the accumulation of nitrite nitrogen and two nitrosated alkaloids in moist snuff are given in Table II.

Table II. Effect of Moisture and Temperature on Concentrations of Nitrite and Nitrosated Alkaloids in Moist Snuff after 24 Weeks Storage[a]

Moisture, %	*Temp., °C*	*NO_2-N, μg/g*	*Nitrosonornicotine, μg/g*	*NNK, μg/g*
22	24	5 C	13 C	2 C
56	24	1204 B	100 B	27 B
22	32	4 C	11 C	2 C
56	32	1604 A	198 A	44 A

[a]Mean values in a vertical column followed by no corresponding letter are significantly different at P = 0.05. (SOURCE: Adapted from ref. *14*).

Nitrosated alkaloid derivatives and nitrite nitrogen generally accumulated at higher rates during storage up to 24 weeks at high moisture (56%) and high temperature (32°C) compared to low moisture (22%) and low temperature (24°C). In this tobacco, high moisture resulted in larger increases of nitrosated alkaloids and nitrite during storage than those increases attributed to higher temperature. The moisture-temperature effects appear to lend credence to the hypothesis that microbial growth determines the degree of nitrosation of carcinogenic tobacco alkaloids. Ghabrial (*12*) determined that air-cured burley tobacco incubated at 35°C and 30-40% moisture underwent a sharp increase in bacterial counts. To test the hypothesis that increases of the growth of microbial populations may lead to increased contents of nitrosated alkaloids and nitrite in a smokeless tobacco (moist snuff) we heated moist snuff in a manner analagous to pasteurization prior to storage to determine whether chemical and pH changes associated with accumulations of nitrosamines would lessen after heating. As shown in Table III,

Table III. Storage Effects of 30-Min Heat Treatment at 0-Time Storage on Moist Snuff (55.5% Moisture-24°C)

Heat Treat? yes/no	°C	*Weeks Stored*	*pH*	*Nitrite-N*	*Total Nitrosated Alkaloids*
				----------μg/g----------	
no	ambient	0	6.9 A*	12.8 A*	38.0 A*
yes	24	24	6.8 A	5.8 A	9.9 A
no	24	24	7.2 B	1203 B	532 B

*Means followed by different letter in vertical column differ at P = 0.05. (SOURCE: Adapted from ref. *15*).

significant increases in pH, total nitrosamines and nitrite were found for nonheat-treated moist snuff stored at 55.5% moisture and 24°C compared to 0-time controls. Results obtained after heat pretreatment of moist snuff and then stored in the same manner did not differ from 0-time controls.

Effect of *E*-2-Hexenal Vapor on Accumulations of Nitrite and Nitrosated Alkaloids in Smokeless Tobacco during Storage. Moist snuff maintained at

55% moisture was stored in sealed glass canning jars for 19 weeks at 24°C. Each treatment jar was presterilized and contained 60 g of tobacco. There were four treatments as follows: 1) control at 0-time storage, no vapor phase treatment; 2) control at 19 weeks storage, no vapor phase treatment; 3) *E*-2-hexenal treatment as one dose (100 μL) placed in a test tube in the canning jar at 0-time storage, sampled at 19 weeks; and 4) *E*-2-hexenal treatment as 15 doses (100 μL each) placed in a test tube at 0-time storage and successively after 2,3,4,6,7,8,9,11, 12,13,14,16,17, and 18-weeks storage, sampled at 19 weeks. Results of these treatments in terms of nitrite, nitrosated alkaloids and pH are given in Table IV. Large increases of nitrite-*N*, NAT, NNK, and total nitrosated alkaloids (sums of NNN, NAT and NNK) were observed for the 19-week stored treatment with no *E*-2-hexenal added to the vapor phase (Treatment 2) compared to the 0-time control treatment counterpart (Treatment 1). Much smaller increases in these chemical components occurred in Treatment 3 for 19-week stored tobacco that had received only one dose of *E*-2-hexenal at 0-time storage. The increase of tobacco pH, however, was unchanged from that of Treatment 2 versus Treatment 1.

Table IV. Effect of *E*-2-Hexenal Vapor Treatment on Accumulation of Nitrite-*N* and Nitrosated Alkaloids in Moist Snuff (56% Moisture) Incubated 18 Weeks[a]

Vapor Phase Treatment	*Weeks of Incubation at 24°C*	*Nitrite-N*	*Nitrosated Alkaloids*				*pH*
			NNN	*NAT*	*NNK*	*Total*	
		μg/g					
none	0	49 B	7 A	24 A	0.1 A	31 A	7.3
none	19	1031 D	129 C	773 C	7.0 B	909 C	7.8
E-2-hexenal, one dose	19	462 C	51 B	317 B	0.5 A	292 B	7.8
E-2-hexenal, 15 doses	19	13 A	7 A	22 A	0.0 A	29 A	6.9

[a]Means followed by different letter in vertical column differ at P = 0.05.

No increases were observed in nitrite, NNN, NAT, total nitrosated alkaloids and pH in Treatment 4 for 19-week stored tobacco that had received 15 doses of *E*-2-hexenal compared to the control at 0-time storage (Treatment 1). In fact a substantial decrease in nitrite N was observed for Treatment 4 compared to the 0-time storage control.

The significance of the results summarized in Table IV may derive from the effectiveness of *E*-2-hexenal in preventing the formation of undesirable, carcinogenic nitrosamines in tobacco during prolonged storage. The use of this aldehyde may afford a safe means of preventing the nitrosation reaction in tobacco and possibly in some food products. We found that this aldehyde was nonmutagenic in the vapor phase when bioassayed using a modification of the

Ames test. However, there is a possibility that oxidative decomposition products may accumulate from specific aldehydes that are undesirable from a toxicological or anti-quality point of view. *E*-2-Hexenal under oxygen atmosphere yielded predominant formations of acids such as *E*-2-hexanoic acid as well as 3-hydroxyhexanal and small amounts of 4-hydroxy-*E*-2-hexenal (13).

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