Pesticide Synthesis Through Rational Approaches

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

This book is dedicated to the memory of
John Brian Siddall
1939–1981

IN MEMORIAM

JOHN BRIAN SIDDALL, a brilliant young scientist and member of the Executive Committee of the American Chemical Society's Division of Pesticide Chemistry, died from a sudden attack of acute leukemia on April 26, 1981, at the age of 41, during the course of organizing the symposium on which this book is based. This book and that symposium are dedicated to his memory.

John Siddall was born in Chesterfield, England, in 1939, and obtained his Ph.D. degree at Manchester University under Arthur Birch in 1963. In the same year, he began work in pharmaceutical chemistry, in Mexico, for the Syntex Company, becoming senior chemist in 1964 and group leader in 1967 at the new research facilities of Syntex in Palo Alto. While at Syntex, he headed a group working on the chemistry of insect molting hormones (the ecdysones) and insect juvenile hormones, work that led to the establishment of Zoecon Corporation. John served as the first director of research at Zoecon, then as vice president of research, and later as its first senior scientist. The work on juvenile hormones was a prelude to the discovery of the first commercially produced and registered insect growth regulator, Methoprene, as well as to other useful homologs.

John Siddall was a prolific scientist who published a large number of articles and patents. He always maintained a complementary interest in insect physiology, as well as in chemical synthesis, particularly of chiral compounds. In fact, just before he entered the hospital, he and Steven Fung completed a total synthesis of the plant growth regulator Brassinolide, a complex steroid with many asymmetric centers.

In addition to being a member of the American Chemical Society and an officer of its Division of Pesticide Chemistry, John was also a member of the Chemical Society of London, the American Association for the Advancement of Science, and the Federation of American Scientists, and was on the editorial staff of the *Journal of Chemical Ecology.* He was a gentleman in manner and in action. He had a keen analytical mind and an excellent sense of humor. He enjoyed outdoor sports, particularly sailing and skiing, at which he excelled, and he maintained a long and active interest in the Boy Scouts. John was also a loving husband and father.

John Siddall had intense scientific interest in two areas covered in this book. The chemical structures of the new bioactive substances discussed in the first section reveal configurations in which biological activity and specificity are highly dependent on isomeric differentiation, an area of synthesis that he understood and avidly pursued. He also advocated more extensive use of

approaches to quantitative structure–activity relationships such as those discussed in the first and third sections of the book.

It is entirely fitting that we dedicate this book on the discovery of new pesticides to the memory of this brilliant young scientist: a fine gentleman, a man of sterling qualities, an inspiring colleague, and a friend to many of us.

GUSTAVE K. KOHN
Palo Alto, California

February 8, 1984

PREFACE

MOST PESTICIDE AND DRUG COMPANIES depend on the premise that
commercial success is directly related to the number of new compounds they
synthesize and test. The size of nearly every synthesis group in industry has
been determined largely by management decisions based on this premise; the
compounds to be synthesized and tested are determined by the perceived
odds for success. The number of compounds tested worldwide for each new
commercial pesticide has been increasing over the past two decades, how-
ever. Experience with the newer pesticide compositions has been that
synthesis (or its fermentation-derived equivalent) followed by careful and
sometimes imaginative screening does yield highly useful agricultural prod-
ucts; the synthesis and screening process is currently less productive, how-
ever, and has a lower probability of success per structure submitted and
assayed than in the past. More rational approaches are needed to improve
the odds for success, and the interactions of several hitherto discrete fields of
inquiry hold much potential for developing them.

Enormous progress is being made in understanding the basic biology,
biochemistry, and physiology of organisms both as a whole and on the
molecular level. Much of the current accumulation of knowledge, and its
potential for use in rational design, comes from interdisciplinary interactions
of physical and organic chemistry and biochemistry, biology, and mathemat-
ics and computer science, as well as from the development of regression
functions that are the basis of quantitative structure–activity relationship
(QSAR) methods.

QSAR itself is a new and still developing discipline, perhaps 20 years
old, that has been employed mostly for optimization after initial discoveries
of activity. Its more visionary practitioners, however, foresee combinations
of quantum chemistry, computerization, and QSAR merging to form
advanced instruments for the design both of artificial intelligence and of
chemical structures that fit parameters predefined by some biochemical
processes. Combined, such knowledge should soon be put to use in the
rational design of novel and selective plant and animal protection chemicals.

Inherent in this book is the promise of a methodology that will make
rational design of safe and target-specific pesticide compounds a reality. We
look forward to seeing whether and how rapidly that promise is fulfilled.

PHILIP S. MAGEE   GUSTAVE K. KOHN   JULIUS J. MENN
Vallejo, California   Palo Alto, California   Palo Alto, California

April 1984

The Discovery of Ivermectin and Other Avermectins

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The avermectins were initially detected in a
program in which thousands of microbial fermenta-
tion products were tested in mice for activity
against the nematode, Nematospiroides dubius.
Among the few preparations showing activity in this
assay, was the product of a microorganism isolated
from soil by workers at The Kitasato Institute.
The microorganism was classified as a new species
of actinomycete, Streptomyces avermitilis. Its
anthelmintic activity was shown to reside in 8
closely related macrocyclic lactones, named aver-
mectins, which were also found to possess activity
against free-living and parasitic arthropods. One
of the natural components, avermectin B_1, is now
being evaluated as a pesticide for the control of
mites of citrus and cotton crops and control of the
Red Imported Fire Ant. A chemical derivative,
22,23-dihydroavermectin B_1, or ivermectin, has been
developed as an antiparasitic agent. It is being
marketed for use in cattle, horses and sheep and is
expected to become available for swine and dogs.

The title of this session, Guided and Serendipitous Discovery
(within the symposium, Approaches to Rational Synthesis of
Pesticides) focuses attention on the nature of the discovery,
rather than the outcome. The discovery of the avermectin
family of compounds was by no means serendipitous; those who
were seeking found what they sought. It is the purpose of
this paper to record the manner of the seeking and the manner

of assessing and enhancing what was found. The discovery of
ivermectin is part of the larger story of the discovery of the
avermectin family of compounds: the avermectins are produced
by a microorganism, and ivermectin is a chemical modification
of one of those substances.

The initial objective of the search was an anthelmintic
with properties radically different from those of known
anthelmintics. What was found was an anthelmintic whose
properties were indeed markedly different -- not only in terms
of chemical structure and efficacy against helminths, but also
in the extension of the potential utility of the class to the
control of arthropod parasites of animals. Further, the
compounds turned out to have striking activity against a
variety of free-living and plant-parasitic nematodes and
arthropods -- and so it has come about that a new livestock
anthelmintic has become an agenda item in this symposium on
agricultural pesticides.

Primary Screening and Microbiology

Several in vitro assays for detecting fermentation products
with anthelmintic activity had been run without success,
primarily because of the large number of toxic compounds which
had to be eliminated. Finally, the decision was made to use
an in vivo assay in mice with the hope that the mice would
screen out the non-specific toxic compounds.

The nematode, Nematospiroides dubius, was selected for
the assay. Infected mice were fed for six days with milled
Purina Lab Chow which had been mixed with the fermentation
product to be tested. The mice were then fed a normal diet
and, at 14 days postinfection, fecal pellets were examined for
the presence of eggs. If eggs were absent on three successive
days, the mice were sacrificed and their small intestines
examined for the presence of worms.

This assay was relatively successful in achieving the
goal of eliminating nonspecific toxic activities. Among the
many thousands of cultures tested in this assay, about 1% were
active in the first test. All active cultures were regrown,
and about 20% of the cultures initially selected were active.
Among the cultures confirmed active, most were still extremely
toxic and were not pursued further. However, during the
testing of a large collection of cultures which had been
received from The Kitasato Institute of Tokyo, Japan, the
choice of the in vivo assay was finally justified. Among one

group of 50 Kitasato cultures submitted for assay was a culture bearing the number OS-3153. The results of the early assays of this culture are summarized in Table I. It was scored as fully active (no eggs, no worms) in the first test. Marked reduction in mouse body weight indicated that the fermentation product was either unpalatable or toxic.

Culture OS-3153 was regrown on the original medium (designated KH) and on a second, unrelated medium (JH) (Table I, Experiment No. 2). The culture grown on the second medium was very toxic, resulting in host death on the day that the first fecal pellet was examined for eggs. That grown on the original medium was also toxic, causing severe suppression of weight gains; but, again, there were no eggs or worms.

In the third test, the activity was titrated using serial 2-fold dilutions. All four levels were fully active, and this time there was little sign of toxicity. A few cultures had been confirmed active previously, but none had exhibited a separation of toxicity from activity, let alone activity over at least an 8-fold range. After an inauspicious start, the activity of culture OS-3153 had been firmly established. Success had been achieved in the quest for a fermentation product with anthelmintic activity. In fact, as events would soon prove, the newly found product had an even broader activity than had been anticipated.

The culture, now bearing the product number C-076 and the Merck culture collection number MA-4680, was submitted for taxonomic studies. Its characteristics, including a brownish-gray spore mass color, smooth spore surface, spiral sporophores born as side branches on the aerial mycelia and the production of melanoid pigments, were unlike those of any previously described species of <u>Streptomyces</u>. The culture was named <u>Streptomyces</u> <u>avermitilis</u>, the <u>Streptomyces</u> "capable of separating from worms".

Based on a correlation of anthelmintic activity and HPLC analysis of the total avermectin complex, it was estimated that the third fermentation contained a minimum of 9 µg/ml. Improvement of the medium increased the yield by the original culture (MA-4680) to 120 µg/ml. A high-producing isolate (MA-4848) obtained from this culture produced nearly 500 µg/ml of total avermectins. Thus, this culture yielded readily to medium improvement and isolate selection to produce relatively large amounts of the avermectins. Accounts of the early fermentation studies and taxonomy have been published (<u>1-2</u>).

Table I. Summary of the First Assays of
          Culture OS-3153.

Expt. No.	Medium	Dose[1]	Feed Eaten (g)	Mouse Weight (g)	Activity[2]
1	KH	50	13	14	A
2	KH	50	25	15	A
	JH	50	13	–	Dead
3	KH	50	25	22	A
		25	25	25	A
		12	25	29	A
		6	25	28	A
	JH	50	25	22	SA
		25	25	29	SA
		12	25	–	NA

(1) Ml of broth per 25 g of feed.

(2) A, active – no eggs or worms; SA, slightly active – no
    eggs, worms present; NA, not active.

Chemistry

Isolation. The avermectin complex, consisting of four major
components designated A_{1a}, A_{2a}, B_{1a}, B_{2a} and four lower
homologs designated A_{1b}, A_{2b}, B_{1b}, B_{2b}, was extracted with
acetone from the mycelia of Streptomyces avermitilis (3).
Solvent partition and adsorption on granular carbon produced
an oily residue containing 5% A_1, 16% A_2, 20% B_1 and 15% B_2.
Separation of the A components from the B components was
achieved by partition chromatography with hexane-methylene
chloride-methanol (10:10:1) over Sephadex LH-20. A_1 was
separated from A_2 using Sephadex LH-20 and a solvent system
containing hexane-toluene-methanol (6:1:1). B_1 was separated
from B_2 either by crystallization from ethylene glycol or by
Sephadex LH-20 chromatography using hexane-toluenemethanol
(3:1:1). Similar chromatography was also used to separate the
lower homologs and purity was established by reverse-phase
HPLC analysis.

Structure Determination. The structures of a new group of
pesticidal sixteen-membered lactones named milbemycin B_1, B_2,
and B_3 were described in 1975 (4) on the basis of X-ray
analysis. A later publication (5) gave details of isolation
and structures of thirteen milbemycins, with spectral data.
The avermectins were discovered in 1975 and part-structures,
deduced from proton and ^{13}C NMR spectra and their mass
spectral fragmentation patterns, suggested a close
relationship with the milbemycins (6). Methanolysis of aver-
mectin A_2 gave an aglycone and a 6:1 mixture of -and
-methyl-Loleandroside. The recovery of this glycoside in more
than 100 mol. % yield demonstrated the presence of two
identical sugars in the molecule. Further spectral exami-
nation indicated the attachment of an α-L-oleandrosyl-α-L-
oleandrosyloxy disaccharide to the 13-α-position of the
macrolide ring. A chemical proof for the point of attachment
and identity of the disaccharide was provided by ozonolysis
and isolation of the disaccharide attached to the fragment C_{11}
through C_{14}. The structures of the eight components are shown
in Fig. 1 and all contain the same disaccharide substituent at
the 13-α-position. They vary at C-5 with hydroxy or methoxy
groups, at C-23 with an axial β-hydroxy group on a 22,23-olefin
and at C-25 with isopropyl or sec-butyl groups in contrast to
the methyl and ethyl substituents at the 25-position of the
milbemycins. X-ray analysis (7) of B_{2a} aglycone and B_{1a} both

A_2 R_5 = CH_3
B_2 R_5 = H

A_1 R_5 = CH_3
B_1 R_5 = H

a series R_{25} = $CH(CH_3)C_2H_5$
b series R_{25} = $CH(CH_3)_2$

H_2 (1 atm) $(Ø_3P)_3$ RhCl
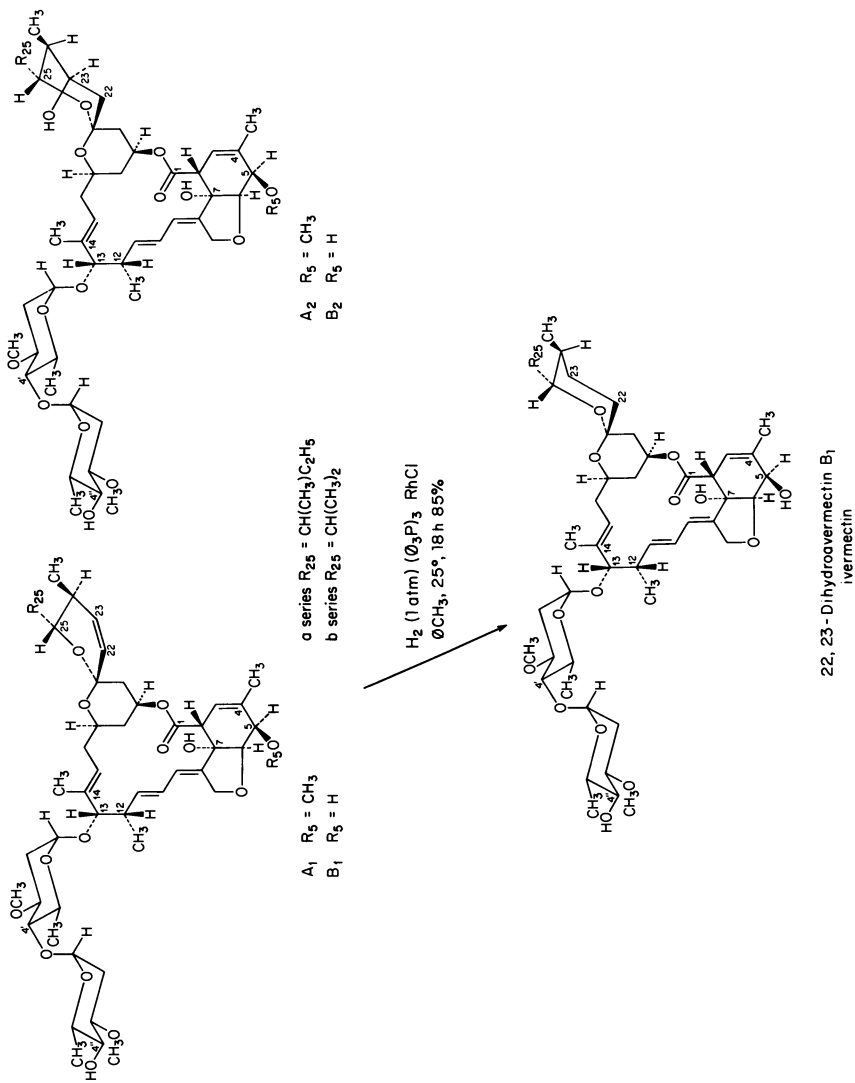$ØCH_3$, 25°, 18 h 85%

22, 23 - Dihydroavermectin B_1
ivermectin

Figure 1.   Structures of the Avermectins.

confirmed the structure and, through the L-oleandrose, established the absolute stereochemistry.

Parasitological Evaluation

Following the demonstration of efficacy in the Nematospiroides-mouse assay, and the associated microbiological and chemical research described above, much work was needed to determine the relative anthelmintic efficacy of both the natural avermectin components and the derivatives. For this purpose two types of bioassay were employed. In one, compounds were tested in small laboratory animals infected with nematodes other than N. dubius. For example, test materials were given to jirds (Meriones unguiculatus) infected with Trichostrongylus colubriformis, and the animals were subsequently killed for determination of worm burden (8). The use of that host-parasite combination for anthelmintic testing had been reported by Panitz and Shum (9) and has proved useful in the evaluation of a variety of anthelmintics, including the avermectins. In the other, compounds were tested against a variety of nematodes in sheep. These were small scale tests, done in conjunction with the small-animal testing and providing important information on the efficacy of the test substances in a ruminant host. Compounds of special interest were similarly tested against helminths in sheep, and occasionally in other hosts, using larger numbers of test animals (10-12).

As the remarkable potency and unique structure of the avermectins became apparent, testing was extended to organisms other than helminths. The first test against an insect was done using the Confused Flour Beetle, Tribolium confusum, and the incorporation of the test substance into the flour in which the beetles lived. In this fashion the insecticidal activity of the avermectins was demonstrated (13) and was followed independently and almost immediately by the demonstration of efficacy against a parasitic insect (14). Efficacy against parasitic insects was further established by tests using the rodent bot, Cuterebra sp., in mice (D. A. Ostlind, unpublished) and was shown to extend to some parasitic acarines (15-16). Tests against the trematodes Schistosoma mansoni and Fasciola hepatica and the cestode Hymenolepis diminuta in laboratory animals failed to show efficacy (D. A. Ostlind, unpublished data). This is in accord with reports that the avermectins disrupt GABA-mediated nerve

transmission in nematodes and arthropods and that flukes and
tapeworms do not employ GABA as a neurotransmitter (17).

Agricultural Chemical Evaluation

The results (above) against the Confused Flour Beetle
(Tribolium confusum), rodent bot (Cuterebra spp.), and the
ectoparasitic larva of the sheep blowfly (Lucilia cuprina)
were sufficiently encouraging to suggest that the avermectins
may possess general biological activity against arthropod
pests and, in particular, those of importance in crop
protection (18). To investigate this potential and to expand
our in-house capabilities, a research program was established
with the Boyce Thompson Institute for Plant Research to test
the avermectin derivatives in their miticide and insecticide
screens. In all, nearly seventy-five related structures,
natural products and semisynthetic derivatives, were evaluated
in the greenhouse for toxicity to a spectrum of arthropod
pests. Of these, avermectin B_1, the major component of the
fermentation process, was determined to be the most promising
candidate as an agricultural pesticide.

Results from these laboratory studies demonstrated that
avermectin B_1 had high toxicity for the twospotted spider mite
(Tetranychus urticae) on bean plants. When applied in
solution directly onto adult and nymphal spider mite
populations on foliage, avermectin B_1 was shown to be 50-200
times as potent as commercially available acaricides, with an
LC_{90} of 0.02-0.03 ppm. Additional tests on foliage with
insects in the order Lepidoptera, Coleoptera, Homoptera,
Orthoptera, Diptera, Isoptera and Hymenoptera confirmed the
broad spectrum activity and potency of the avermectin family
of compounds and avermectin B_1 in particular. Table II pro-
vides LC_{90} values for avermectin B_1 for the control of larval
forms of several of these insects in foliar residue assays
(18).

Table II. Efficacy of Foliar Residues of Avermectin
B_{1a} Against Adult mites and Larval
Insects.

Insect	LC_{90} (ppm)
Twospotted spider mite	0.02-0.03
Tomato hornworm	0.02
Colorado potato beetle	0.03
Mexican bean beetle	0.2
Cabbage looper	0.75-1.2
Southern armyworm	6.0

On the basis of the efficacy demonstrated in the green-
house and laboratory studies avermectin B_1 was selected for
development and assigned the Merck development code number
MK-936. Avermectin B_1 has been evaluated worldwide for
efficacy against mites and insects affecting a number
of agricultural crops including citrus, cotton, apples, pears,
vegetables, potatoes, tree nuts, and grapes. Under field use
conditions it has been observed that excellent control of a
number of economically important pests including the citrus
rust and red mite, twospotted spider mite, broad mite,
Colorado potato beetle, diamond back moth, pear psylla, and
Liriomyza leafminers can be achieved at extremely low
application rates of MK-936 in the range of 0.005-0.03 lb per
acre (5.5 - 33 g per hectare). For foliage applications a
0.15 EC (1.8% w/v) emulsifiable concentrate formulation has
been developed. Field studies have shown that the formulation
is non-phytotoxic to all target crops on which it has been
evaluated including many varieties of sensitive ornamental
plants.
     During the course of the development program, samples of
avermectin B_1 were provided to a number of outside agencies
for evaluation in specialized assays. As a consequence, it
was discovered in testing conducted by the USDA laboratory for
Insects Affecting Man and Animals, Gainesville, Florida, that
the red imported fire ant (Solenopsis invicta) is among the
most susceptible species of insects to the toxic action of
avermectin B_1. When applied in a corn grit bait, avermectin

B_1 at rates as low as in 25 to 50 mg per acre has been
effective in controlling fire ant infestations in large scale
trials in the southern United States. A submission for
registration of MK-936 for this application has been made.

The avermectin natural products are pesticides possessing
novel chemistry and mode of action. Cross-resistance has not
been observed in laboratory or field studies with mites
andinsects tolerant to commercially available organophosphate,
carbamate, chlorinated hydrocarbon and pyrethroid pesticides.

Synthetic Program

Structure-Activity Relationships. Compounds of the B series
were generally more potent than those of the A series. Thus
an unsubstituted hydroxy group at the 5-position is activity
enhancing (19). Differences in potency between the 1- and 2-
series varied among parasites, but in most instances the 1-
series was more potent. Reduction of the 22,23-olefin had
little effect on activity but further reduction caused a
substantial decrease in activity. The monosaccharides were
two- to fourfold less active than the parent compounds while
the aglycones were more than thirtyfold less active, Table III.
Acetylation at the 4"-position caused no change in activity
whereas acetylation at the 5- or 23- position caused a
considerable decrease in activity. Diacetates and triacetates
showed similarly reduced activity, Table IV (20).

Ivermectin. Early biological studies demonstrated that while
avermectin B_1 was more active than avermectin B_2 by oral
administration, the converse was true when the compounds were
given parenterally. Furthermore, avermectin B_1 was much less
effective against Cooperia species when given parenterally
than by oral treatment. Avermectin B_2 had generally lower
activity against Haemonchus species. Examination of the B_1
and B_2 structures revealed that the differences centered on
the 22,23-position. Avermectin B_1 is a 22,23-olefin whereas
in avermectin B_2 this bond is hydrated with the hydroxyl group
at the 23-position. The conformation of the ring bearing
these functionalities is different and it was reasoned that
bioactivity might be linked to conformation. It therefore
became an important objective to synthesize 22,23-
dihydroavermectin B_1 which required for its synthesis the
selective reduction of one of five olefins. However, only the

Table III. Activity of Avermectin Derivatives against Adult Gastrointestinal Helminths of Experimentally Infected Sheep on Oral Administration.

Structure[b]	Dose, mg/kg	Efficacy[a]					
		H.c.[c]	O.c.	T.a.	T.c.[c]	C. spp.	O.c.
A_1	0.1	2	2	0	0	2	0
A_2	0.1	3	3	3	3	0	3
B_1	0.05	3	3	3	3	3	3
B_2	0.1	0	3	3	3	3	3
H_2A_1	0.3	3	2	0	1	0	3
H_2B_1	0.1	3	3	3	3	3	3
B_1MS	0.15	2	2	3	3	3	0
B_2MS	0.2	1	1	3	3	3	3
H_2B_1MS	0.3	3	3	3	3	2	3
H_2B_1AG	3.0	1	2	3	3	1	3
H_4B_1	0.2	0	0	1	0	0	3

[a] 0 = < 50%, 1 = 50-74%, 2 = 75-90%, 3 = > 90% efficacy. Abbreviations used: H.c. Haemonchus contortus; O.c., Ostertagia circumcincta; T.a., Trichostrongylus axei; T.c., Trichostrongylus colubriformis; C. spp., Cooperia spp.; O.c., Oesophagostomum columbianum.

[b] MS = monosaccharide, AG = aglycon, H_2 = 22,23-dihydro derivative, H_4 = 3,4,22,23-tetrahydro derivative.

[c] Benzimidazole resistant.

Table IV. Derivatives of Avermectin A_{2a} and B_{2a} and Anthelmintic
Activity against <u>Trichostrongylus colubriformis</u> in Gerbils
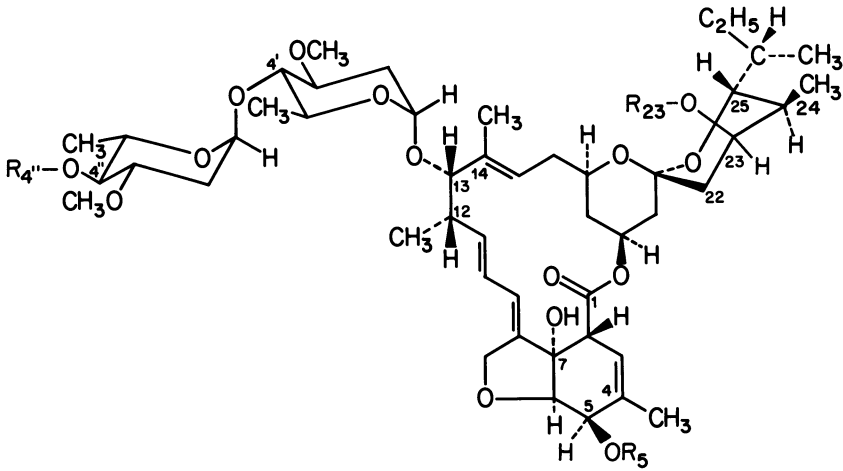
R₄"	R₅	R₂₃	anthelmintic act.[a]
H	CH_3	H	0.05
H	H	H	0.0125
CH_3CO	CH_3	H	0.0625
H	CH_3	CH_3CO	0.25
CH_3CO	CH_3	CH_3CO	0.5
CH_3CO	CH_3CO	CH_3CO	0.5

[a]Minimal dose (mg/kg) needed to remove > 83% of the worm burden.

22, 23-olefin is <u>cis</u>-substituted, suggesting the use of Wilkinson's homogenous catalyst $(Ph_3P)_3RhCl$ known to be very sensitive to the steric environment of an olefin. Hydrogenation of avermectin B_1 for 20 hours using Wilkinson's catalyst in benzene or toluene at 25°C under one atmosphere of hydrogen gave 22,23-dihydroavermectin B_1 in 85% yield (Figure 1). This compound was selected for development as a broad-spectrum antiparasitic agent for animals on the basis of its overall efficacy by oral and parenteral routes and for its improved safety profile (<u>19-21</u>). 22,23-Dihydroavermectin B_1, containing at least 80% of 22,23-dihydroavermectin B_{1a} and not more than 20% of 22,23-dihydroavermectin B_{1b} has been assigned the non-proprietary name ivermectin. The compound was subjected to a large international program of development, which lies beyond the scope of this paper, and which included efficacy trials and safety assessment in sheep, cattle, horses, swine and dogs. This development program resulted in the introduction of ivermectin as a commercial antiparasitic agent in 1981. For cattle, sheep and horses, the dosage recommended for general antiparasitic use is 0.2 mg/kg; for swine the dosage is 0.3 mg/kg. The compound is used both orally and parenterally -- the formulation and route of administration depending on the host species being treated.

Discussion

The discovery of the avermectins, by virtue of the wide spectrum of the compounds, and their extreme potency and novel mode of action, met the initial objective of finding an anthelmintic with radically different characteristics. The avermectins are not active against all groups of helminths -- they have not been reported active against flukes or tapeworms -- but they are active against all nematode groups that have been tested, and indeed there is no clear evidence that any species of any genus of nematode is refractory to the action of ivermectin. In at least one instance (adult <u>Dirofilaria immitis</u>) a particular life cycle stage is refractory while other stages of the same species are susceptible. The occurrence of antinematodal and antiarthropod activity in a single chemical class, is not entirely unprecedented. The organophosphates are active against parasites of both groups, but their spectrum of activity against nematodes is relatively narrow. The salicylanilide compounds are active against certain nematodes and arthropods but are used primarily against flukes.

The discovery of avermectins resulted from the deliberate choice of fermentation products as the prime source of substances to be submitted for anthelmintic screening. Many factors were critical to the success of the venture, including the submission of novel actinomycte isolates by workers at The Kitasato Institute in Japan, the selection of the in vivo screen, the rapid isolation and identification of the active principle, the assessment of antiparasitic properties, and the enhancement of biological properties by synthetic chemical modification of the structure. The names of those responsible for these contributions may be found in the early papers published on the subject, and are listed elsewhere according to scientific discipline (2). The discovery of the avermectins thus rested on empirical testing -- as did the discovery of all other successful anthelmintics and ectoparasiticides. Such discoveries are nevertheless attributable to deliberate and far-from-arbitrary choices made during the initial conception and subsequent operation of the screening program.

The identification of a biochemical mode of action that appears to differ profoundly from that of previous antiparasitic agents has provided a tool for new approaches to understanding and exploiting the basic biochemical pathways of animal and plant parasites. The biological properties of the avermectins have opened new possibilities for the study of low-dose drug delivery systems, and many aspects of nematodology, entomology and acarology, as well as contributing directly to the control of many livestock parasites and agricultural pests.

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Sulfonylureas: New High Potency Herbicides

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The sulfonylureas are new herbicides which exhibit
both preemergence and postemergence activity at extra-
ordinarily low rates of application. Two of these
compounds have been commercialized by Du Pont. Glean
weed killer controls most broadleaf weeds and some
grasses in wheat at 5-35 g a.i./ha. The active
ingredient in this product is chlorsulfuron. Oust
weed killer is especially useful for control of seed-
ling and rhizome johnsongrass with safety to Bermuda-
grass. The active component of this product is
sulfometuron methyl.
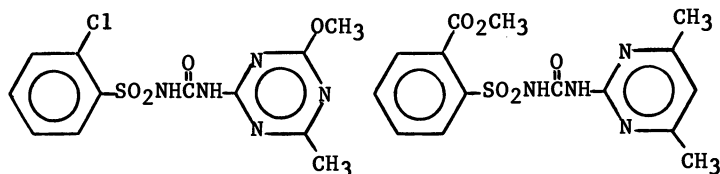
Chlorsulfuron   Sulfometuron methyl

The discovery, mode of action, and empirical structure-
activity relationships of these new herbicides will be
reviewed.

The sulfonylureas described here are new herbicides with unpre-
cedented activity. Activity at extraordinarily low rates of
application has been demonstrated by both preemergence and post-
emergence applications (Ref. 1). These rates are best described
in grams per hectare compared to kilograms per hectare for most
herbicides currently marketed. Furthermore, the combination
of low application rates, half lives of generally less than
two months in the soil (Ref. 2) and excellent safety to mammals
[LD$_{50}$'s of generally >5000 mg/kg for technical material in male
rats] (Ref. 1C) makes these compounds attractive products from
an environmental viewpoint.

The DuPont Company has commercialized two of these compounds. The first of these is chlorsulfuron (Fig. 1), formerly DPX-W4189, the active ingredient in Glean weed killer. It is being marketed by DuPont for use in small grains such as wheat and barley. This compound is especially effective for the control of most broadleaf weeds and some grasses found in these crops. Recommended use rates vary from 5 to 35 g a.i./ha depending on the nature of weeds present, the type of soil involved, and intended rotational crops. The second candidate is sulfometuron methyl (Fig. 1), formerly DPX-T5648, the active ingredient in Oust® weed killer. It is a potent herbicide especially useful for long term control of johnsongrass (Sorghum halepense) with safety to Bermudagrass (Cynodon dactylon). Oust is not selective on most agronomic crops.

DPX-W4189   DPX-T5648
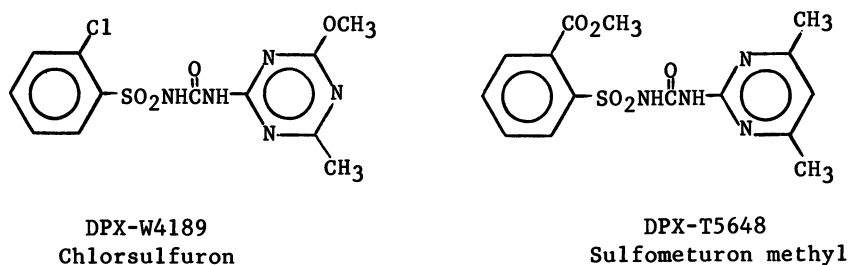Chlorsulfuron   Sulfometuron methyl

Figure 1.   Commercialized sulfonylureas

Synthesis

Both chlorsulfuron and sulfometuron methyl can be prepared by the reaction of the appropriate arylsulfonyl isocyanate and the substituted heterocyclic amine as shown in Fig. 2.
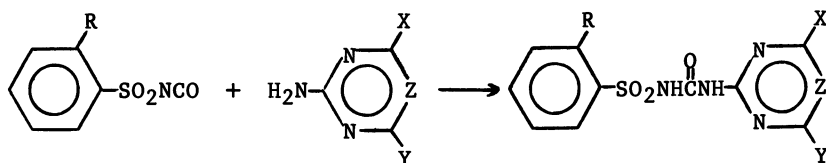

Figure 2.   Synthesis of sulfonylureas

This reaction is typical for the synthesis of sulfonylureas; it is mildly exothermic and proceeds smoothly in a variety of inert aprotic solvents. The product is usually obtained in very high yield, as a fine crystalline precipitate. The sulfonyl isocyanates are readily prepared from the substituted benzene sulfonamides by reaction with phosgene, Fig. 3, in the presence of an alkyl isocyanate, for example, butyl isocyanate in an inert solvent at 120 to 140°C according to the general procedure of H. Ulrich and A. A. R. Sayigh (Ref. 3).
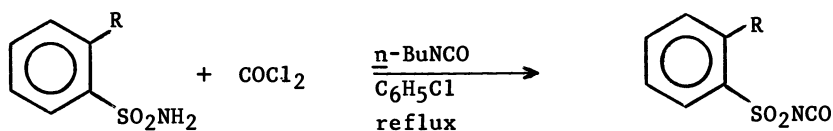
Figure 3. Sulfonyl isocyanate formation

o-Chlorobenzenesulfonamide can be prepared from o-chloroaniline by diazotization and treatment of the resulting diazonium salt with sulfur dioxide and hydrochloric acid in the presence of cuprous or cupric chloride to yield the sulfonyl chloride (Ref. 4) which is converted to the sulfonamide with ammonia.
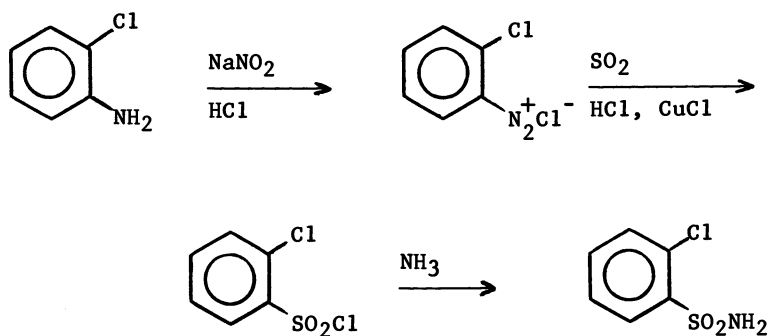

Figure 4. Chlorsulfuron intermediate sulfonamide synthesis

This procedure is satisfactory for the synthesis of a variety of aryl sulfonamides. The heterocyclic intermediate used in the synthesis of chlorsulfuron is prepared according to K. R. Hoffmann and F. C. Schaeffer (Ref. 5) as shown in Figure 5.
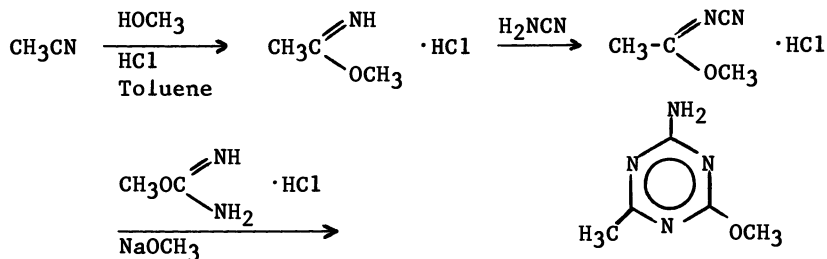

Figure 5. Chlorsulfuron heterocycle synthesis

Condensation of guanidine carbonate and acetylacetone yields
the required 2-amino-4,6-dimethylpyrimidine (Ref. 6) for sulfo-
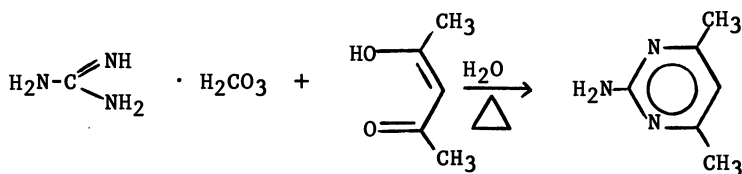meturon methyl (Figure 6).

Figure 6. Sulfometuron methyl heterocycle synthesis

Mode of Action

Extensive mode of action studies have been carried out (Ref.
7) with chlorsulfuron. Chlorsulfuron enters plants either through
the foliage or roots and moves rapidly through the entire plant.
Chlorsulfuron is a potent inhibitor of plant cell division and
quickly causes the plant to stop growing. Even nanomolar concen-
trations have been found to inhibit plant cell division within
six hours. Seeds germinate, however, seedling growth is inhib-
ited. Other life processes in the plant such as photosynthesis,
respiration, cell elongation and protein and RNA synthesis do
not appear to be initially affected. Susceptible plants die
slowly showing chlorosis, vein discoloration, terminal bud death
and finally necrosis. This type of action appears typical for
these sulfonylurea herbicides. Resistant plants such as wheat,
barley or oats have been shown to rapidly metabolize chlorsul-
furon. P. B. Sweetser and J. M. Hutchison (Ref. 8), using phenyl
C^{14} labeled chlorsulfuron characterized the major metabolite
as a nonphytotoxic compound in which the phenyl ring had under-
gone hydroxylation in the 5-position followed by conjugation
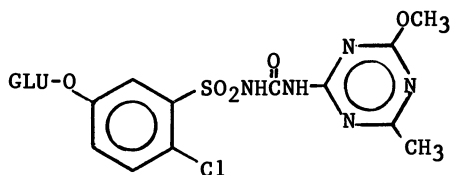with glucose.

Figure 7. Glucose conjugate of the major metabolite
of chlorsulfuron

They found a good inverse correlation between sensitivity of
a plant to chlorsulfuron and the rate of metabolism to this
metabolite. For purposes of their study, plants such as wheat,
barley and oats which showed only slight to moderate temporary
growth inhibition by a foliar spray of 50-400 ppm solution of

the compound were considered tolerant. Sensitive plants such as sugarbeet, mustard, rape and galium showed severe growth retardation from a 0.1 to 0.5 ppm spray. Twenty-four hours after treatment of sugarbeet leaves with ^{14}C-labeled chlorsulfuron, nearly 97% of the radioactivity could be recovered as chlorsulfuron, whereas with wheat 95% of the radioactivity had been converted to the nonphytotoxic metabolite.

Discovery and Empirical Structure-Activity Relationships

The original lead was N-(p-cyanophenylaminocarbonyl) benzenesulfonamide. This compound showed weak plant growth regulant activity. The synthesis of compounds with variations in substitutions on the phenyl rings and the bridge failed to substantially increase this activity. A review of the sulfonylureas previously investigated by us revealed only a limited amount of work with sulfonylureas in which heterocycles replaced the p-cyanophenyl of the lead compound. This appeared to be a potentially fruitful area for synthesis since it was reasoned that each different heterocyclic type might produce a very different biological response.
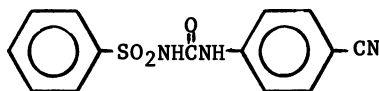
Weak Growth Retardant at 2 kg/ha

Figure 8. Lead Compound

Benzenesulfonylureas were prepared from 2-aminopyrimidines substituted in the 4- and 6-positions. Growth retardant activity was found in the 4-methyl-6-chloro compound (Figure 9). This activity was lost when both substituents were chlorine. However, a substantial improvement in growth retardant activity was observed with the 4,6-dimethyl analog.
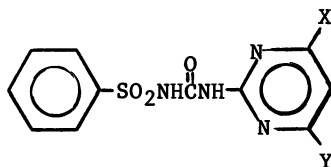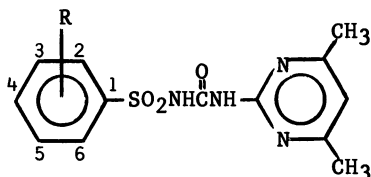

X	Y	Activity
CH$_3$	Cl	Growth Retardant
Cl	Cl	Inactive
CH$_3$	CH$_3$	Improved Growth Retardant

Figure 9. Analogs of the lead compound

When the dramatic improvement in activity was found with the
4,6-dimethylpyrimidine heterocycle, sulfonylureas of this system
were prepared from the three isomeric tolyl sulfonyl isocyanates.
The para compound, which was a literature compound (Ref. 9),
was devoid of activity; whereas, the meta compound was found
to have substantial activity. When the ortho tolyl compound
was prepared, another major boost in activity was observed
(Figure 10).

R	Activity
4-CH$_3$	Inactive
3-CH$_3$	Active Herbicide
2-CH$_3$	Highly Active Herbicide

Figure 10. The effect of aryl substitution

Further modifications of the heterocycle showed that 4,6-disub-
stituted sym-triazines were also active, and that replacement
of a methyl group with a methoxy group gave another slight boost
in activity (Figure 11). More importantly, the triazine compounds
were found to have safety on wheat. The addition of an ortho
chloro substituent to the aromatic ring gave a compound-chlor-
sulfuron-possessing both high herbicidal activity and good safety
on wheat and barley.
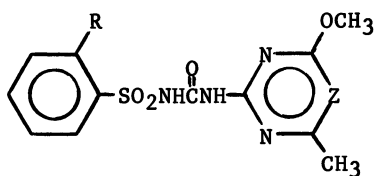
R	Z	
H	N	Active Broadleaf Herbicide-Wheat Safety
Cl	CH	Highly Active Herbicide-Phytotoxic to Wheat
Cl	N	Chlorsulfuron - Highly Active and Safe on Wheat, Barley

Figure 11. Discovery of chlorsulfuron

Turning to disubstituted benzenesulfonylureas we found they
retained activity. In fact, the 2,6-dichloro derivative was
almost equivalent to the 2-chloro analog. The activity lowering
effect of 4 substitution is seen again by comparing the 2,4
with the 2,3 or 2,5 substituted compounds. This is further
confirmed by comparing the 3,4 with the more active 3,5 compound
(Figure 12). The comparison of 2,4 with 3,5 is not clear cut.
The 2,4 has the potentiating effect of a 2-substituent countered
by the activity reducing effect of the 4-substituent whereas
the 3,5 has the minor improvement due to the meta substituents.
The 3,4 derivative, not being helped by ortho substitution and
hindered by para, is the weakest of these compounds.

Isomer: 2,6   2,3   2,5   2,4   3,5   3,4

ACTIVITY: ◄─────────────────────────────

Increasing Activity
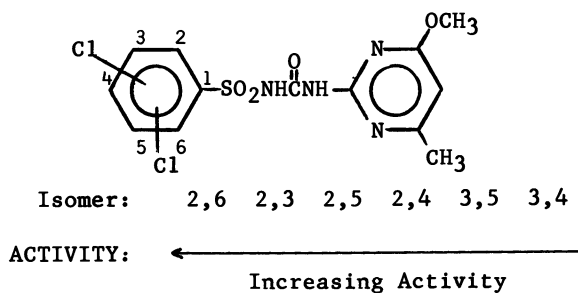
Figure 12.   Relative overall plant response of isomeric
             dichlorophenylsulfonylureas

Many functional groups have been found which potentiate activity
when present in the ortho position (Figure 13). A variety of
electron-withdrawing and electron-donating groups have a poten-
tiating effect. The free carboxylic acid group is one group
that does not have a potentiating effect.
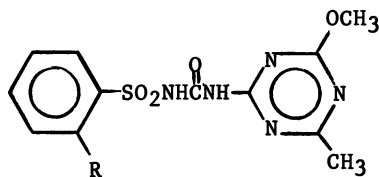
R =
Activating Groups: CO_2CH_3, NO_2, F, Br, Cl,
                     SO_2CH_3, SCH_3, $SO_2N(CH_3)_2$, CF_3
                     CH_2Cl, OCH_3, OCF_3, CH_3
Nonactivating: CO_2H

Figure 13.   Examples of potentiating ortho substituents
             on benzene (relative to hydrogen)

In addition to benzene compounds, pyridine, thiophene, furan
and naphthalene sulfonylureas are also active. Compounds with
the unmodified sulfonylurea bridge are generally more active
than compounds with substituted bridging groups. Compounds
with the bridges shown in Figure 14 have shown some activity.
The relative activity of compounds for each bridge type is deter-
mined by the substituents on the aryl and the heterocyclic ring.

ARYL —— | BRIDGE | —— HETEROCYCLE

$$-SO_2NH\overset{\overset{O}{\|}}{C}NH-$$

| S |
$-SO_2NH\overset{\overset{S}{\|}}{C}NH-$

| S-Alk |
$-SO_2N=\overset{\overset{S-Alk}{|}}{C}-NH-$

| O-Alk |
$-SO_2N=\overset{\overset{O-Alk}{|}}{C}NH-$

$-SO_2\overset{\underset{|}{CH_3}}{N}\overset{\overset{O}{\|}}{C}NH-$

$-SO_2NH\overset{\overset{O}{\|}}{C}\overset{\underset{|}{CH_3}}{N}-$

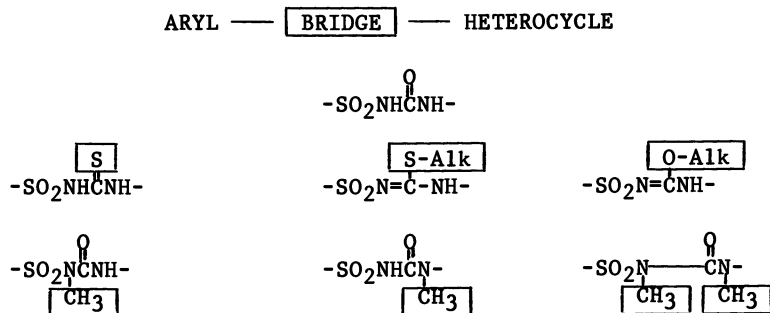$-SO_2\overset{\underset{|}{CH_3}}{N}\overset{\overset{O}{\|}}{C}\overset{\underset{|}{CH_3}}{N}-$

Figure 14.   Bridge systems showing activity

Summary

In summary, the sulfonylureas are new herbicides which exhibit
activity at extremely low rates of application and show very
low mammalian toxicity. Exceptionally high activity is shown
by compounds containing a benzene ring substituted in the ortho
position, an unsubstituted sulfonylurea bridge, and a pyrimidine
or triazine heterocycle substituted with methyl or methoxy groups.

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o-(5-Oxo-2-imidazolin-2-yl)arylcarboxylates: A New Class of Herbicides

MARINUS LOS

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This paper traces in some detail the path which led
to the discovery of a new class of herbicides, the
2-(5-oxo-2-imidazolin-2-yl)arylcarboxylates. The
journey started when it was found that a phthalimide,
α-isopropyl-α-methyl-1,3-dioxo-2-isoindoline-
acetamide, had sufficient herbicidal activity to
warrant further synthesis effort. This work led to
a series of analogs essentially devoid of herbicidal
activity yet possessing interesting plant growth
regulating effects. Further chemical modifications
resulted in the synthesis of two new groups of
compounds, imidazoisoindolediones and dihydro-
imidazoisoindolediones, and the return of her-
bicidal activity. The imidazoisoindolediones
were in turn transformed into o-(5-oxo-imidazo-
lin-2-yl)benzoates, the first members of a very
interesting new class of herbicides.
     This initial discovery has since led, by ap-
propriate molecular modifications, to the synthesis
of three compounds currently in advanced testing.
The synthesis of these compounds, coded AC 222,293,
AC 252,214 and AC 252,925, will be described
together with their biological activities.

Three approaches may be taken to discover new herbicides. They
are: (a) the random screening of chemicals, (b) the use of known
herbicides as lead compounds for a synthesis program, and (c)
the design of compounds interfering with known metabolic pro-
cesses - the "rational" approach. There are no publications
in the field of herbicide discovery that would suggest that
the latter approach has advanced very much beyond the theoretical
stage. The second approach, sometimes referred to as the "me-
too" approach, has both positive and negative features. The

introduction of an "n^{th}" analog with much the same activity as
the first can have its problems. Yet, it is always hoped that
the next analog will have some unusual properties not shown by
the parent compound. Although not discovered in this way, who
would have predicted the grass herbicidal activity of the
phenoxyphenoxy herbicides given the broad leaf herbicidal act-
ivity of the phenoxy herbicides? Finally, by the random screen-
ing approach, thousands of chemicals can be examined for their
herbicidal activity on the species one wishes to affect. This
is the method most likely to afford the truly exciting result,
the discovery of an entirely new class of herbicides.

    The discovery of the imidazolinone herbicides had its be-
ginnings in a random screening test some 12 years ago. The
phthalimide 1 prepared originally as an anticonvulsant at
Cyanamid's Lederle Laboratories had sufficient herbicidal act-
ivity to warrant the synthesis of additional members of this
series. One of these, the chloro analog 2, was essentially
devoid of herbicidal activity but had instead a pronounced
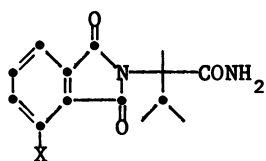
1 (X = H)
2 (X = Cl)
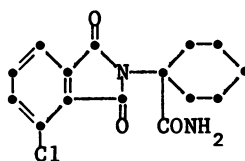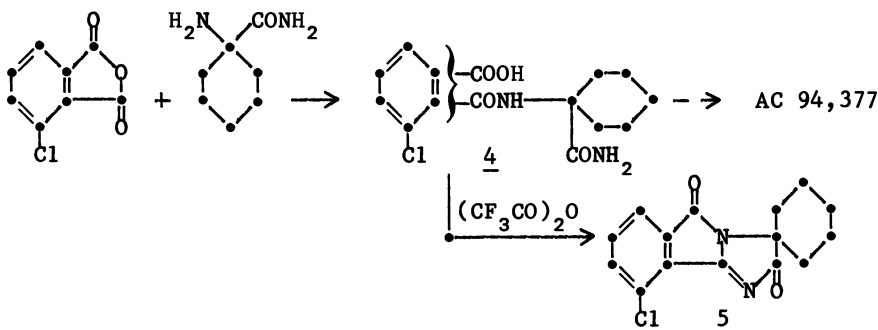
3 (AC 94,377)

plant growth stimulating effect not unlike that of gibberellic
acid. Particularly noteworthy was internode elongation. This
serendipitous discovery led to yet another synthesis program
and resulted in the preparation of 3, coded AC 94,377, one of
the most active growth stimulants in this series that has been
estimated to have approximately one tenth the activity of $GA_7(1)$.

Requests for larger samples for field testing necessitated
the examination of alternate routes to AC 94,377. One of these
started with 1-amino-1-cyclohexanecarboxamide and 3-chloro-
phthalic anhydride. Attempted cyclization of the intermediate
phthalamic acid 4 with cold trifluoroacetic anhydride gave,
albeit in low yield, the readily isolated imidazoisoindole 5.
Biological evaluation of 5 indicated that it had very similar
PGR properties to that of AC 94,377 yet was perhaps slower
acting. This was attributed to the slow hydrolysis of 5 back
to AC 94,377.

These findings did encourage us to examine further the
biological properties of the imidazoisoindoles, particularly
since their physical properties were also quite different from
their phthalimide precursors. The first requirement was an
improved procedure for the synthesis of the imidazoisoindoles.
A number of reagents, both acidic and basic, were found that
would effect cyclization of these phthalimides. One of the
most consistent methods utilized sodium hydride in hot toluene
or xylene. Some large-scale preparations, e.g. of 6 were suc-
cessfully run using sodium hydroxide pels in xylene.
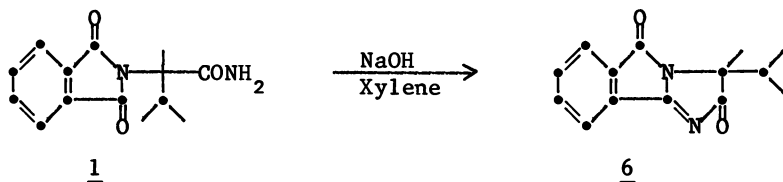
It should be noted that when a substituted phthalimide is
converted to the corresponding imidazoisoindole, a mixture
results since cyclization can occur on both imide carbonyl
groups.

Of the many phthalimides converted to imidazoisoindoles,
one of the more interesting, from a biological standpoint, was
6, derived from 1, the compound that started this program.
The product 6 is a broad-spectrum, non-selective herbicide par-
ticularly effective against perennial weeds such as nutsedge
and bindweed. Since the effect of changes in structure on bio-
logical activity is similar in several series of compounds,
this topic will be discussed later.

These imidazoisoindoles were novel heterocyclic compounds
and this, together with the associated biological activity,
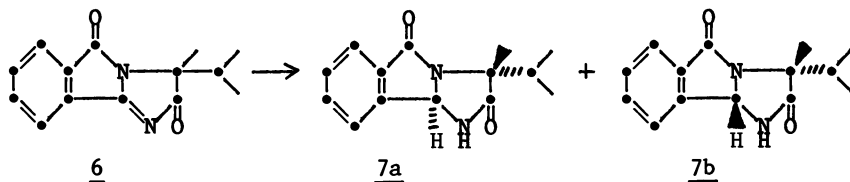
was reason enough to look more closely at the chemistry of the
system. Hydride (NaBH$_4$) reduction of, for example, 6 gave a
mixture of diastereomers, 7a and 7b. These could be separated
by fractional crystalization and either individually or as a
mixture showed high herbicidal activity. It was further found
that 6 would add a variety of alcohols, amines and thiols across
the imine double bond.
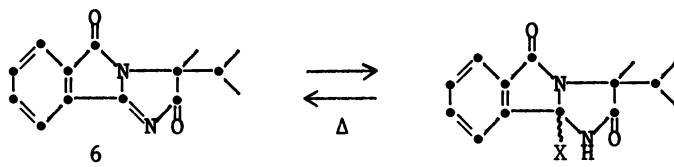
When X is a small group such as OCH$_3$, NH$_2$, etc., the
reaction can be reversed by heating. These compounds also have
good herbicidal activity but whether this is due to their re-
version to 6 or to their own intrinsic activity has not been
determined.

The reaction of compound 6 with methoxide in methanol was
found to give yet another type of product. In this case, the
reaction occurred not at the imine bond but at the isoindole
carbonyl group. The product was thus a (5-oxo-imidazolin-2-yl)
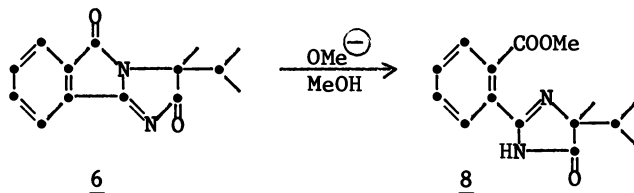
benzoate 8, the first member of this new class of herbicides
to be prepared. Other alcohols plus their alkoxides as well
as amines could be used in the above reaction to give a variety
of benzoates and benzamides. The corresponding acids could
be prepared most conveniently either by base hydrolysis of the
methyl esters or hydrogenolysis of their benzyl esters. Once
again these compounds had good herbicidal activity and it was
possible to study in some detail the effects of change in
structure on biological activity. Biological activity here
means general phytotoxicity.
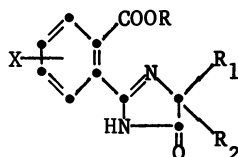
    In general, highest biological activity was found in the
parent acid of any given series. Esters such as propargyl,
allyl, furfuryl and benzyl which could be considered acid-labile
in a chemical sense were also highly active, followed by the
methyl ester. Activity fell off quite rapidly with increasing
chain length.

    Changes in R_1, R_2, and X had very similar effects whether
these changes were in the imidazoisoindoles, the dihydroimidazo-
isoindoles or the benzoates. Optimum activity was associated
with R_1 = CH_3 and R_2 = $CH(CH_3)_2$. There was nevertheless still
considerable activity in compounds in which R_1,R_2 were methyl,
methyl or methyl, ethyl or methyl, cyclopropyl. Other com-
binations of R_1 and R_2 usually resulted in compounds with lower
levels of activity. Substitution in the aromatic ring also re-
duced biological activity.

    Table I shows the herbicidal activity of a number of
structures already discussed. These are greenhouse data and,
although not obtained in a side-by-side test, they do give some
indication of their relative activity. The term "control rate"
as used in Tables I-III is defined as the amount of chemical
required to produce a 91-100% reduction of growth of the weed
when compared to the control plant.

    It is of interest to note that these were not the first
benzoates substituted in the ortho position with a heterocyclic
ring to show effects in plants. Invariably, however, in these
earlier cases the heterocyclic ring was an aromatic one such as
a pyrazole or oxazole substituted with a phenyl group (2-4).
None of these had pronounced herbicidal activity but rather PGR
activity. Compound 9, for example, has been shown to be an
auxin transport inhibitor, a property probably shared by the
other members of this class also. A structure activity analysis
for this group of compounds has been reported by Katekar (5).
The imidazolinone benzoates probably do not fall within this
class.
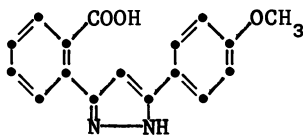
9

    Although the compounds described above were non-selective
herbicides and aromatic substitution resulted in compounds of
lower activity, it was gratifying to find that the mixture,
coded AC 222,293, and prepared as shown in Scheme I, had good
activity against wild oats, black grass and mustards yet was
well tolerated by most winter and spring wheats and barleys.

Table I

Herbicidal Activity of Various Benzoate Derivatives

Control Rates in Kg/Ha

Weed	Pre				Post			
	A	B	C	D	A	B	C	D
Field Bindweed	1/8	1/32	1/2	ND	1	1	>1	ND
Purple Nutsedge	1	1	1	4	1	1	1/4	>4
Morning Glory	2	1/2	1/4	10	1/2	1/2	1/2	4
Velvet Leaf	1	1/8	1/4	>1	1/2	1/4	4	>1
Ragweed	2	1	4	>10	>2	>2	>10	>4
Barnyard Grass	2	1/2	ND	>10	1	2	4	>4
Crabgrass	>2	2	4	>10	1A	1A	10	>4
Green Foxtail	>2	1	1/2	>10	1	1/4	4	>4

A. =

B. =

C. =

D. =
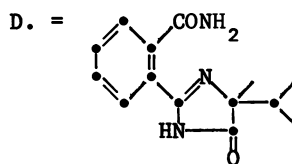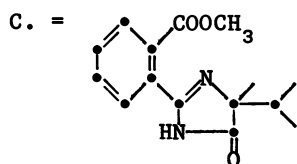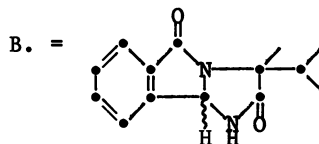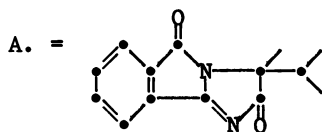
Table II
Comparison of AC 222,293 with its Optical and Positional Isomers
Postemergence Test - Rates in Kg/Ha

| Compound | Controls At | | | | Safe At |
	Black Grass	Green Foxtail	Wild Oats	Wild Mustard	Spring & Winter Wheat & Barley
Rac-AC 222,293	0.6	2.0	0.6	0.4	4.0
R-AC 222,293	0.3	2.0	0.3	0.3	2.0
Rac-p-isomer	2.0	2.0	2.0	0.3	4.0
R-p-isomer	0.5	0.7	0.7	0.1	3.0
Rac-m-isomer	0.6	> 4.0	0.4	> 4.0	4.0
R-m-isomer	0.3	> 4.0	0.2	> 4.0	4.0
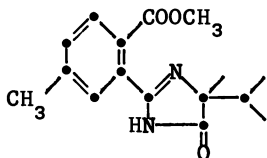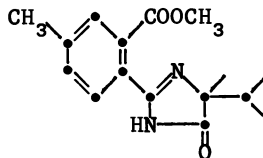
p-isomer   m-isomer

Table III
Herbicidal Activity of AC 243,997 and its Enantiomers
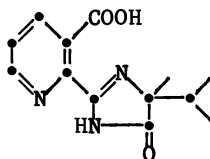
Control Rates in g/Ha
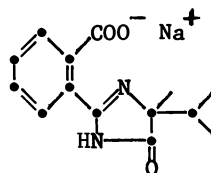
Weed	AC 243,997		S-isomer		R-isomer			
	Post	Pre	Post	Pre	Post	Pre	Post	Pre
Purple Nutsedge	63	4	125	32	32	4	125	< 63
Field Bindweed	16	4	32	< 2	2	2	ND	ND
Quackgrass	16	2	63	32	8	4	ND	ND
Matricaria	32	2	63	8	16	< 2	ND	ND
Velvet Leaf	16	2	63	16	8	< 2	500	500
Morning Glory	8	4	63	16	4	4	250	125
Barnyard Grass	63	32	250	63	16	16	250	1000
Green Foxtail	16	2	16	16	4	8	500	125
Ragweed	63	16	125	32	32	8	500	ND

Scheme I
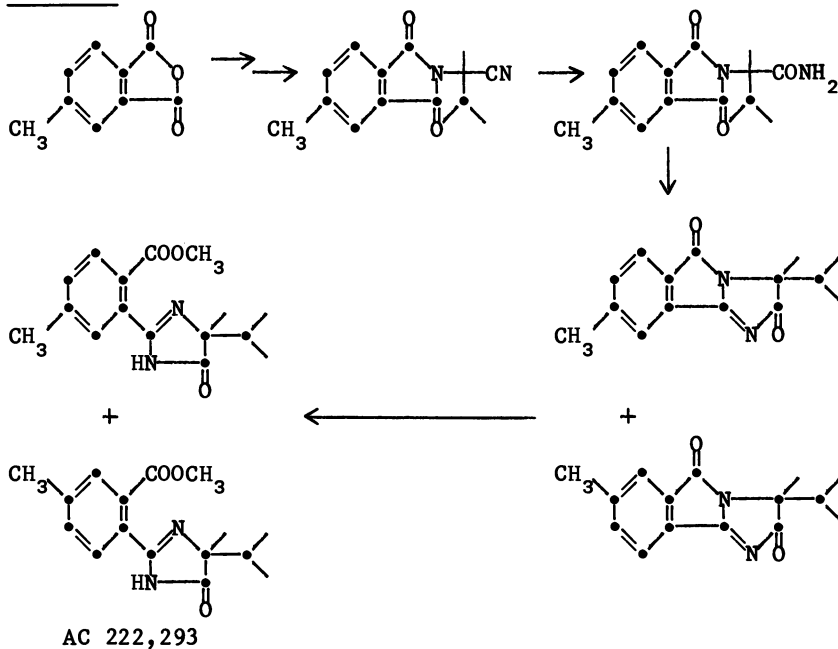
AC 222,293

It now became of interest to determine the biological activity of the individual isomers in AC 222,293 and this required an alternate regio-selective synthesis. The synthesis for the m-toluate is shown in Scheme II.

Scheme II

1. 2.2 sec-BuLi
2. CO_2
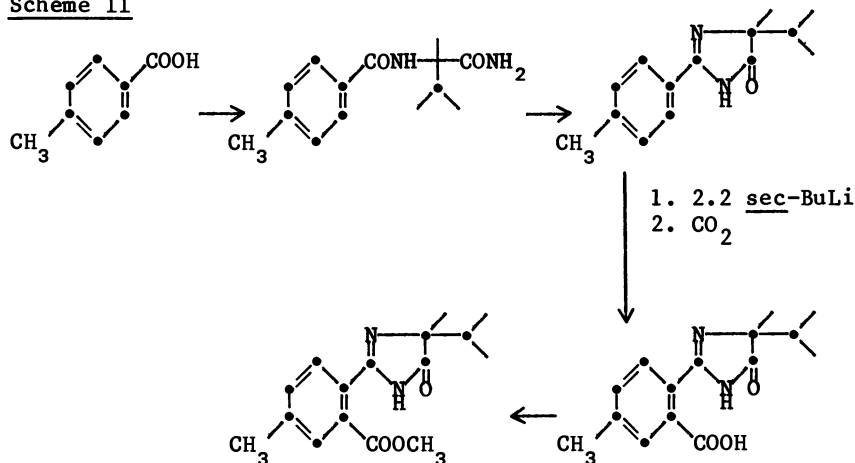
A similar sequence starting with m-toluic acid then gave the
p-toluate.

    One further question needed to be resolved. The carbon
atom in AC 222,293 bearing the methyl and isopropyl group is
chiral. Does all the biological activity reside in one en-
antiomer? The preparation of a suitable chiral intermediate
was achieved by means of an enzymatic resolution:

$$CH_3CONH\text{---}COOH \quad \xrightarrow[\text{acylase}]{\text{hog kidney}} \quad H_2N\text{---}COOH \ [S\text{-}(-)]$$

$$+$$

$$H_2N\text{---}COOH \ [R\text{-}(+)] \ \longleftarrow \ CH_3CONH\text{---}COOH \ (-)$$

The resolution of α-methylvaline had not been reported at the
time this work was done. Subsequently, Turk, et al. published
the resolution of trifluoroacetyl-α-methylvaline by carboxy-
peptidase A (6). Some comments on the absolute configuration
of the amino acids will be made later.

    It was now possible to compare the biological activity
of AC 222,293 with that of its regio- and optical isomers.
Table II summarizes these results. Most noticable is the good
activity of the m-toluate against wild oats and black grass
but poor activity against mustard. On the other hand, mustard
is very sensitive to the p-toluate, whereas wild oats and black
grass are quite tolerant. Careful studies with the more active
enantiomer of AC 222,293 showed it to be approximately twice
as active as AC 222,293. It should be noted that the select-
ivity shown by AC 222,293 is dependent on the presence of the
methyl ester function. The acids are not selective but studies
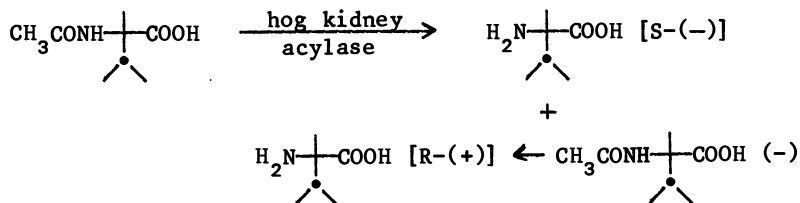(7) indicate that it is, in fact, the acid which is the toxicant
and this is liberated at different rates from the ester in the
sensitive weeds and the crops.

    After this considerable effort in the benzene series, it
was decided to examine the effect of replacing the benzene ring
with a pyridine ring. It was at this point that good fortune
played a role. There was the possibility of preparing four
isomeric analogs. Had any one of three of these possibilities
been prepared first, it is unlikely that the project would have
continued much longer. Be that as it may, the synthesis of
the first pyridine analog was carried out. The route shown
gave a somewhat ambiguous result and is shown in Scheme III.

Scheme III
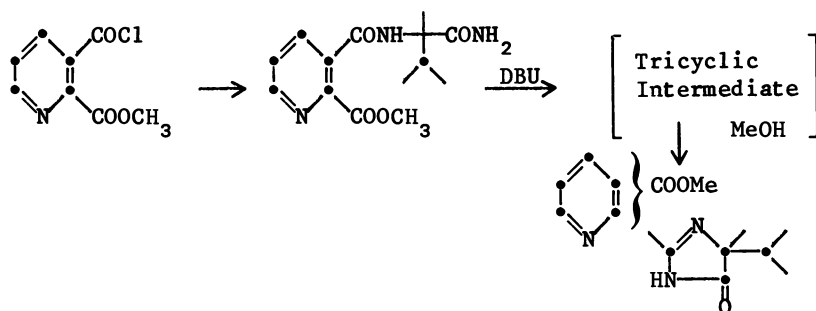
Herbicide evaluation of the product from this sequence of re-
actions showed that it was a very active but non-selective her-
bicide. We were unable to determine with certainty at this
point the regiochemistry of the product.

The synthesis of this material was repeated by a sequence
frequently used in the benzoate series. This started with quino-
linic anhydride and is shown in Scheme IV.

Scheme IV
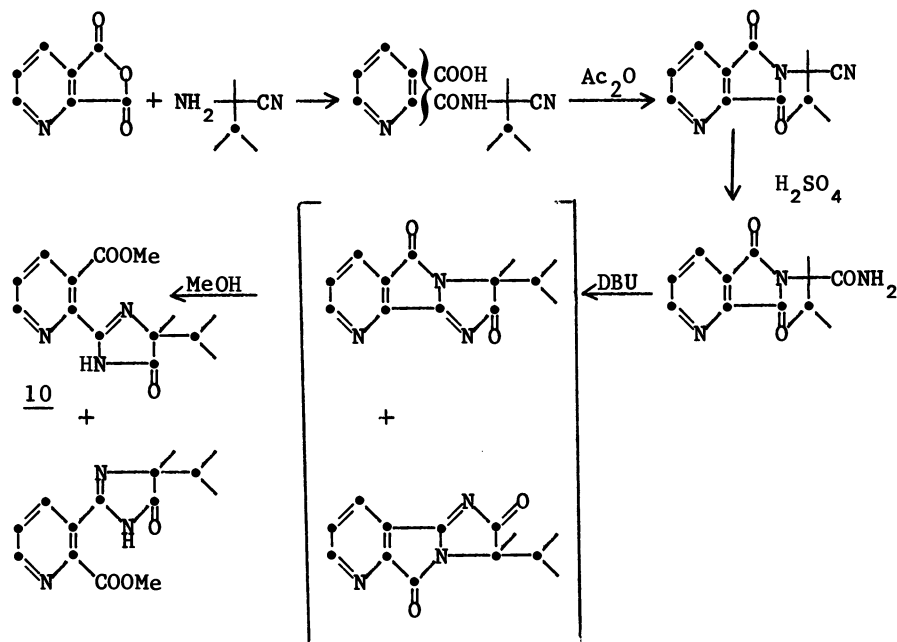
As would be anticipated by the method of synthesis, two
isomeric products were formed in a ratio of about 3:1 and these
were easily separated by column chromatography. The compound
formed in greater amount proved to be identical to the material
isolated from the first preparation. A study of the ^{13}C nmr
spectra indicated that the more active isomer was the nicotinate
10 and this was based primarily on the coupling of the carboxyl
carbon atom with the C-4 proton.

The corresponding acid was expected to be more active than
the methyl ester and it was prepared in two ways -- by hydrolysis
of the methyl ester or hydrogenolysis of the benzyl ester:

AC 243,997
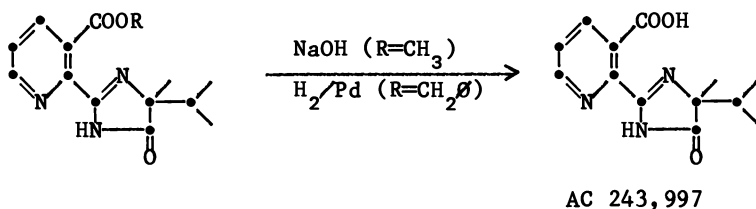
It was now important to again examine the biological activity
of the enantiomers in this series. These were prepared using
the chiral amino acids and the activity of these compounds par-
ticularly when compared to the benzoate analogs is striking.
These data are shown in Table III.

Examination of molecular models of the imidazolinyl
nicotinates indicated that both substituents could not be
coplanar with the pyridine ring. Further, the absolute con-
figuration of the more active enantiomer was not known. In
order to obtain this information, a crystallographic analysis
was performed on the p-bromobenzyl ester 11 of the more active
enantiomer. This analysis showed that (a) the more active com-
pounds are definitely nicotinates, not picolinates; (b) in the
solid state, the carbonyl group of the ester function is 67° out
of the plane of the pyridine ring; (c) the imidazolinone ring is
only 17° out of that plane; (d) the imidazolinone ring is
oriented so that the NH of the NH-CO group is near the pyridine
nitrogen; and (e) the absolute configuration of the more active
enantiomer is R.

11
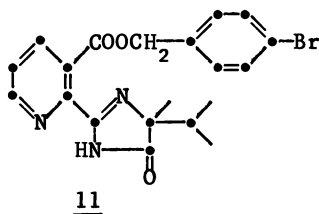
Further work was now directed toward the synthesis of analogs of this highly active herbicide. Substituted quinolinic acids are not readily available and these are usually obtained in poor yield by the oxidation of the appropriate quinoline:
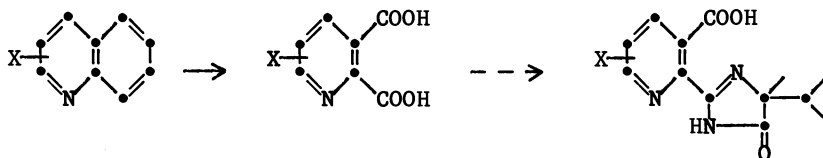
A route valuable for the synthesis of 6-substituted analogs utilized the N-oxide 12.

12

R = NR$_2$, OR, SR
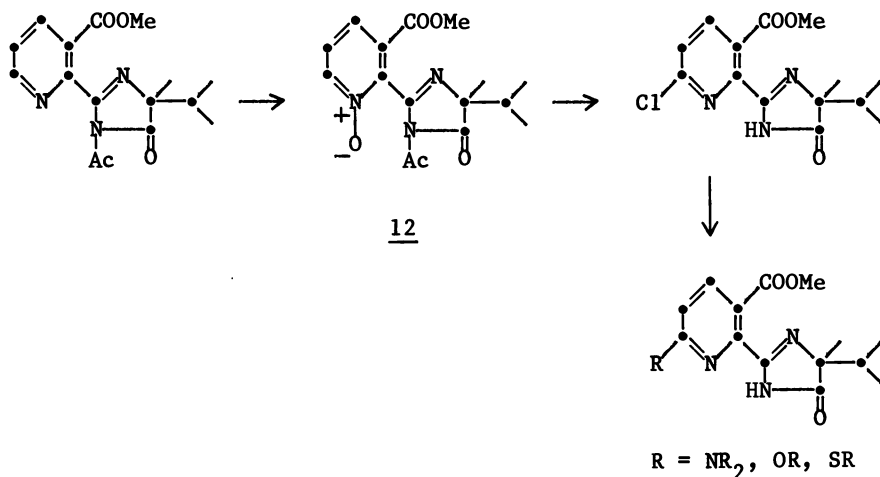
A number of groups have been found to direct and stabilize o-metallation in aromatic systems since Meyers (8) introduced the oxazolidine group for this purpose. It was reasoned that the imidazolinone group should also serve this purpose. It was gratifying to find that treatment of the pyridyl imidazolinone 13 with 2.2 equivalents of butyl lithium followed by carbon dioxide gave a good yield of the nicotinic acid 14 (R=H).

1. BuLi
2. CO$_2$

13   **14**
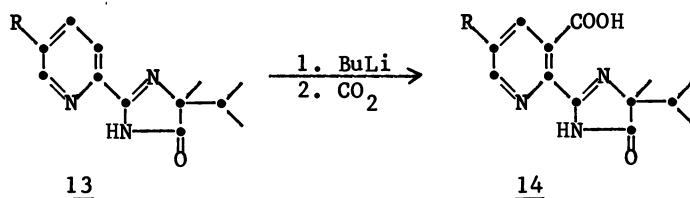
This synthesis was applied to the preparation of a variety of
analogs in which R = alkyl, alkoxy and dialkylamino.

Based on these and other compounds prepared in this series,
several conclusions on the effect of structure on herbicidal
activity can be drawn:

    a.   The combination of methyl and isopropyl group in the
imidazolinone ring is preferred. Compounds with the
R configuration are more active than those with the S.

    b.   For a given substituent, herbicidal activity varies
with the position of the substituent in the order
$$C\text{-}5 > \quad C\text{-}6 \gg \quad C\text{-}4.$$

    c.   When the substituent X is at any one position and X
is one of a series such as R, OR or $N(R)_2$, the her-
bicidal activity decreases as the size of R increases.

Probably the most active compound in this series is the
parent which, as its isopropylamine salt, is coded AC 252,925.
It is a broad-spectrum herbicide active both pre- and post-
emergent. Studies with radiolabelled compounds (9) show it
to be rapidly translocated particularly to the meristematic
regions. Translocation to the underground storage organs of
perennial weeds prevents regrowth of these weeds. Most her-
baceous and woody plants are controlled in the field at 0.4–1.0
kg a.e./ha whereas most woody plants require 0.7–3.0 kg a.e./ha
(10).

The natural extension of this series to the quinoline analog
of AC 252,925 was then undertaken. The synthesis route used is
based on the work of E. C. Taylor which employed anthranil as
a precursor to a quinoline-2,3-dicarboxylic acid derivative (11).
The sequence is shown in Scheme V.

Scheme V
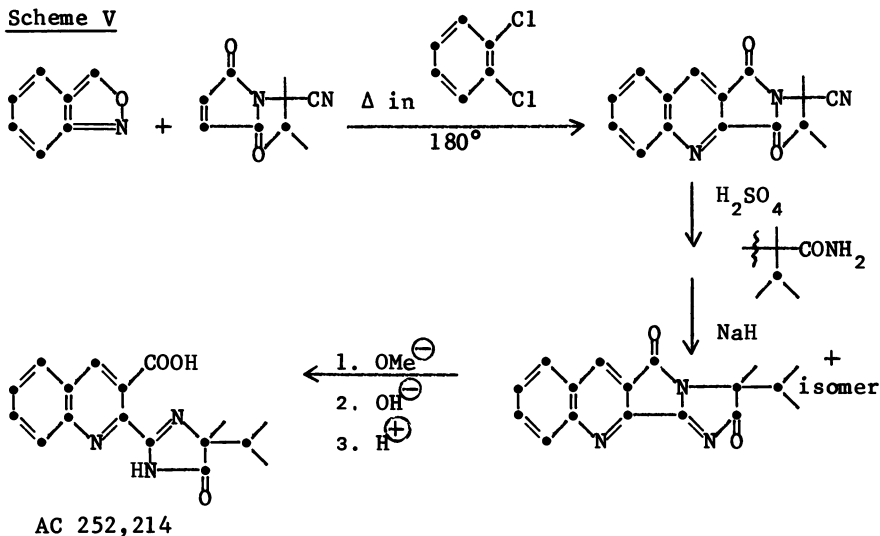
AC 252,214

From the earliest greenhouse studies, it was obvious that AC 252,214 was an interesting compound. Remarkably, it was well tolerated by legumes, especially soybeans, yet showed a broad-spectrum of herbicidal activity, killing broad-leaved, grassy and perennial weeds. Further it was shown that AC 252,214 could be used both as a preemergent and postemergent herbicide. Rates for field application are 125-250 g/ha (12).

From the synthesis of a large number of analogs, a number of conclusions can be drawn on the effect of changes in structure on biological activity.
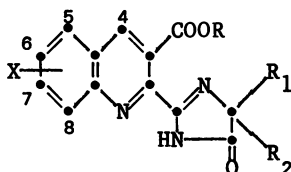
The most active compound is AC 252,214 with R = H, R_1 = CH_3, R_2 = $CH(CH_3)_2$, and X = H. Any other change decreases activity although the soybean selectivity is usually retained. Substitution at C-4 generally led to poor herbicides, while substitution at other positions reduced biological activity to varying degrees. For example, biological evaluation of all the monochloro derivatives of AC 252,214 gave results as follows:

$$C-5 > C-6 > C-8 > C-7 \gg C-4.$$
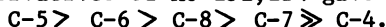
What is evident from the above discussion is that the imidazolinylarylcarboxylates constitute an exciting new class of herbicides and that within this class, three members, AC 222,293, AC 252,925, and AC 252,214 have been shown to have strikingly different and useful biological properties.
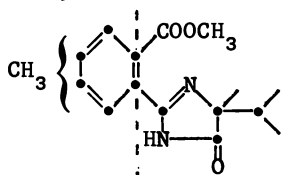
AC 222,293
(Cereals)
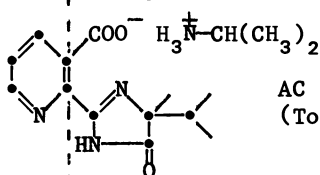
AC 252,925
(Total Vegetation Control)
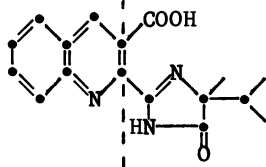
AC 252,214
(Legumes)

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    Ciarlante, D. R. <u>Proceedings 1983 Weed Science Society
    of America</u>.

The Discovery and Development of Bromethalin, an Acute Rodenticide with a Unique Mode of Action

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Because rodent populations world-wide were becoming
resistant to the widely used Warfarin-type anticoagulant
poisons, a search was initiated to find a rodenticide with
a different mode of action; one that would be effective
against these resistant rodents. This search led to the
discovery of the toxic nature of a family of diphenyl
amines which act as uncouplers of oxidative phosphory-
lation. A structure-activity relationship (SAR) study was
undertaken to choose a derivative that would be both
poisonous to rodents but still readily consumed by them.
This approach led to the discovery of bromethalin,
N-methyl-2,4-dinitro-N (2,4,6-tribromophenyl)-6-(trifluoro-
methyl)benzeneamine, which had all the desired rodenticidal
properties.

History. For centuries man has recognized that rodent pests
destroy his habitat, consume his food, and cause the spread of
virulent diseases. Throughout the same centuries man has sought
to eliminate these pests with a variety of poisons such as
strychnine, arsenious oxide, and red squill - a steroidal
glycoside extracted from the bulb of a lily-like plant, Urginea
maritima.
     These materials, as well as more recent poisons like zinc
phosphide and fluoroacetic acid, work as acute rodenticides and
can be effective when a rodent consumes a lethal quantity of
poison in a single dose in its food or drink. Unfortunately,
animals often consume less than a lethal dose, which produces
side effects which the animals associate with the bait. The
result is that they become "bait shy" and are likely to be wary
of the same poison bait a second time.
     The discovery of the anticoagulant properties of dicoumarin
(1) led to the development of the more potent anticoagulant
warfarin (2), (Structure 1). The subsequent discovery that the
anticoagulants can be successfully used as multiple dose

rodenticides(3) signalled a shift from the development of acute
rodenticides to the chronic, anticoagulant materials. These
compounds all work by suppressing the activities of vitamin-K
dependent clotting factors in the target animal's blood which
consequently causes the animal to bleed to death. The anti-
coagulants avoid the development of bait shyness because they
don't produce poisoning symptoms that cause feeding to stop.
Because they are effective at low dosages(0.005-0.25%) they are
relatively non-toxic to domestic animals and man.
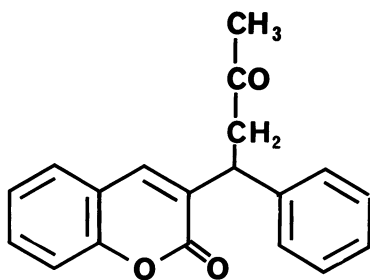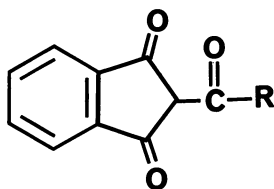
| 1 | 2 |
| Warfarin | Indandiones |

    Very rapidly, a number of other anticoagulants, including the
indanediones (4), (Structure 2), were developed as rodenticides.
Warfarin first came into wide usage as a rodenticide in 1950 and
virtually supplanted all other materials then in use. In the
case of all these early materials, multiple bait applications
were needed to control rodent populations which, while making the
materials safer to use than the available acute poisons,
curtailed their use in underdeveloped and less affluent countries
because of the large quantities of bait that must be placed to
destroy the populations of rodents.
    Within the first decade after it and the other anti-
coagulants were first successfully employed, reports(5) were
received that Norway rats in a farming area in Scotland were
"remarkably tolerant" to warfarin and other anticoagulants.
Additional cases of resistance were reported in Norway, Denmark,
England, Wales, Germany, and the Netherlands.(6) The first
evidence of anticoagulant resistance in the United States came
from a study of Norway rats living on a farm near Raleigh, North
Carolina.(7) They have since been found in a great number of
other sites in the U.S.(8) Soon resistant mice populations also
began to be discovered.(9) The anticoagulant resistant
populations were found to be cross resistant to the various,
chronic rodenticides.

Discovery of bromethalin

Initial search for a rodenticide. Interest at Lilly in the discovery of a rodenticide from among our extensive file of biologically active compounds first began with N. J. A. Gutteridge, a Lilly chemist working in England. When he became aware of the resistance problem he started a search for some rodenticidally active materials that would control resistant rodent populations. He appreciated the fact that although toxicity is an essential prerequisite for an effective rodenticide, it was not the sole criterion upon which an ideal rodenticide is based. He outlined, in one of the most extensive reviews of rodenticides to date(10), other features of an ideal rodenticide as follows:

   (i)   The toxic action should be slow to allow animal to consume a lethal dose.
   (ii)   The poison should not be unpalatable, and should preferably be odorless.
   (iii) Symptoms of acute poisoning should be absent; no bait shyness.
   (iv)   The manner of death should not arouse suspicions in surviving animals.
   (v)   The poison should be specific to the species to be controlled.
   (vi)   No difference in susceptibility due to age, sex, or strain should be present.
   (vii) There should be no danger of secondary poisoning through animals eating poisoned rodents.
   (viii) No immunity or build-up of tolerance to the poison should develop.
   (ix)   The chemical compound in the bait should be stable under varied environmental conditions.

Although Gutteridge was unsuccessful in finding a compound that would satisfy these criteria, his work sparked our interest in this problem. This interest in rodenticides was further increased by the discovery of the anticoagulant activity of a series of imidazo(4,5b)pyridines (Structure 3), which were structurally so dissimilar to the known anticoagulants that they were first thought to have a different mode of action. Subsequent studies(11) indicated that in spite of the totally different chemical structure of the imidazo(4,5b)pyridines, these compounds suppressed the same four vitamin K dependent procoagulants that warfarin supresses. Further, rodents resistant to the warfarin-type of anticoagulants were also resistant to these materials. This continues to be true for all the so-called "second generation" anticoagulants now available.
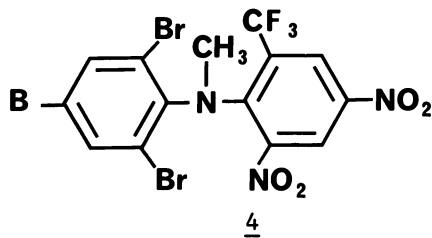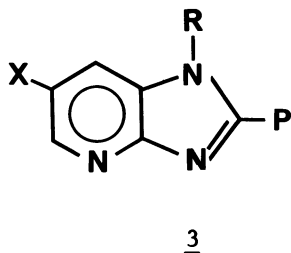
X = Halogen, Trifluoromethyl
R = Hydrogen, Hydroxyl, O-alkyl   Bromethalin
P = Perfluoroalkyl

<u>The discovery of the activity of the diphenyl amines</u>. At this
point there began a sequence of events that finally led to the
the discovery of the diphenyl amine rodenticide bromethalin
(Structure 4). Our interest in the chemistry of the diphenylamines
initially came about because of the discovery that they had
fungicidal activity against a number of plant diseases (12). A
large number of these compounds were synthesized in order to
develop a fungicidal structure-activity relationship. One of the
most promising of these fungicides in greenhouse tests, was
trichloro-trinitro diphenyl amine (Structure 5).
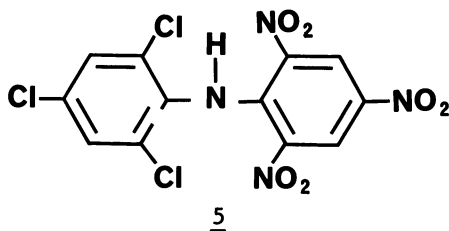
        This compound, targeted as a foliar applied fungicide, was
active against diseases of grape, especially downy mildew, and so
a large sample was prepared for possible evaluation under field
conditions. Before a potential fungicide is tested on a large
scale in a field test program, an evaluation is carried out to
determine if there is any potential danger to the applicator.
While undergoing this routine toxicological hazard evaluation, it
was noted that this compound had an oral LD_{50} of 3.2 mg/kg in
Harlan rats. On the basis of this result, we decided that
compound 5 was too hazardous to be evaluated as a foliar

fungicide. Instead we submitted the compound to a rodenticide
feeding study. It proved to be toxic to rats in their diet at
25 ppm and it appeared that the onset of toxicity was delayed.
     With a testing system already in place from our work with the
anticoagulants, we began a search for other rodenticidal diphenyl
amines from among the diphenyl amines synthesized for their
fungicidal activity. Since the relative toxicity of the other
analogs made for the fungicide program was unknown, we set out to
determine what aspects of the structure of diphenyl amines made
the compounds toxic. To accomplish this we utilized a screen
originally established to determine the toxicity of drug
candidates. The test used was one to determine the toxicity of
compounds to mice, in which groups of three mice were injected
intraperitoneally (I.P.) with an acacia suspension of the test
compound until a level was reached where no toxicity was
observed. This screen avoids many of the pitfalls of feeding
studies since the test compounds cannot be rejected. By means of
this test, we were able to semi-quantify the toxicity of a large
number of diphenyl amines very rapidly. Some of the results
obtained can be seen in Appendix I.
     Once we determined the toxicity by this I.P. route, we then
tested the most active compounds in a non-choice feeding test in
which the test compounds were incorporated into a rat diet and
fed to weanling and adult Sprague-Dawley rats for ten days. This
test required five rats per dose level and the levels were from
200 ppm to 10 ppm. Daily feed consumption data as well as
necropsy data on the dead and moribund rats were collected.
There appeared to be a good correlation between the mouse
toxicity data from an I.P. route and the rat feeding study
(Appendix II). With the results from these tests we were able to
define the "A" ring substitution requirements required to make
the trinitro diphenyl amines toxic to rats.
     Since toxicity is a necessary but not sufficient quality of a
rodenticide, the next phase of the testing was conducted to
determine the acceptability of the treated diet to rats and
mice. The test consisted of twenty rats per treatment level with
at least twenty control animals. The tests were run from three
to ten days, with the feed consumption data and necropsy data
recorded for each animal. The desirable candidate compounds
should comprise at least 30% of the total diet of the rats and
should have a mortality of 90% or more.
     The most active rodenticidal compound turned out to be our
initial lead, 2,4,6-trichloro-2',4',6'-trinitrodiphenyl amine
(Structure 5). This was tested under a variety of conditions with
white laboratory rats and mice. We were able to determine from
these tests that, even given a choice, laboratory rats and mice
would consume enough of the toxicant to cause 80% lethality.
     To determine how activity against laboratory rodents
translated into activity against wild rodents, we asked
Dr. William B. Jackson at the Center for Environmental Research

and Services, Bowling Green University, to conduct a study using compound 5 on some of his wild rodent populations. The trials he ran on wild rats and mice parallelled our own studies on laboratory rats and mice, but his results were quite different from ours. In his studies with wild Norway rats (Rattus norvegicus), the rats totally rejected the treated diet, actually choosing not to eat rather than eat the toxicant. The results with house mouse (Mus musculus) proved equally unsatisfactory. What we had was a very toxic material that was not readily acceptable to wild rodents, even in a non-choice test.

We realized that in order to develop one of these diphenylamines as a rodenticide, we had to find a way to get rodents to eat a toxic amount of the toxicant. We were convinced that the novel rodenticidal mode of action of these materials (i.e. non-anticoagulant) would be valuable if we could find a way to get rodents to consume them, so we approached the problem as one of either finding a analog of 5 that would be more toxic or more acceptable. If this approach failed, as a last resort we could look at ways to make formulations more acceptable. The search for a more active diphenyl amine concentrated on the substituents on the "B" ring, since we were confident that we had defined the substitution patterns on the "A" ring. A structure-activity relationship study (SAR) was carried out with the results seen in Appendix III. Only one substitution seemed to be superior to the trinitro; the substitution of one of the ortho nitro groups with a trifluoromethyl. A new SAR using the Mouse Toxicity Test as a screen was conducted, holding "B" ring constant with 2-trifluoromethyl-4,6-dinitrophenyl and substituting the "A" ring. The result indicated that the same substitution pattern that led to the most active trinitro compounds held for the 2,4-dinitro-6-trifluoromethyl compounds, except that the latter compounds were more active (Appendix IV). However, even these more toxic compounds were discriminated against in our choice-efficacy studies.

Before we got involved in the extensive delays and expense incurred in screening a large number of these compounds in free choice efficacy tests, tests which might not translate into efficacy against wild rodents, a means was sought to generate a more acceptable compound by choosing the most "rodent-acceptable atoms" in the "A" ring from among the most active substituents and by substituting on the amine nitrogen.

We initiated a literature search to determine the kinds of functional groups that attract rodents. The object of the search was to try to define the aromatic substituents on ring "A" that have been shown to have attractant properties. This effort met with no success. Much has been published on the types of feed grains and mixtures thereof that are preferred by rodents but little on the types of chemicals.

While searching the literature for such positive information,

it became evident that while the work on chemical attractants had been very qualitative, the search for repellents had been put on a rather formal scientific basis.   Working for the Fish and Wildlife Service, U.S. Dept. of the Interior, Bellack and DeWitt(13) developed an equation for a repellency Index, K, whose final form was:

$$K=100-1/100W(8T_1+4T_2+2T_3+T_4)(U_1+U_2+2U_3+4U_4+8X)$$

where $T_1....T_4$ represents the daily consumption of treated food; $U_1...U_4$ represents the daily consumption of untreated food, X the residual untreated food and W the kilogram body weight of the animals.

Many compounds were tested using this equation at both the Fish and Wildlife Service and at the Army's Natick Laboratory, and the its usefulness in determining repellency was verified. On examining their results, we were struck by the fact that, periodically, negative K values occurred.   Examination of the equation shows that this can only happen when large amounts of the treated diet were eaten early and/or untreated material was untouched.   We interpreted the fact that compounds had large negative K values as an indication that they might actually act as attractants.

Although a variety of structures were represented by these negative K values, among the substituted aromatic compounds a pattern was observed.   Whereas polychlorination often was associated with the repellency of compounds, polybromination appeared to confer "antirepellency" effects.   The K-values shown in Table I illustrate this point.   Although "antirepellency" isn't necessarily a direct measure of attractiveness of compounds, it gave us a rationale for our choice of substituents on the "A" ring.

Table I

Structures with a negative repellency index (K = negative value)

COMPOUND	K Value
Acetic acid, 1,3,6-tribromo-2-naphthalenyl ester.....	−63
2,4-Dibromo-1-(2,4-dibromophenoxy)benzene............	−213
1,3,5-Tribromo-2-(2,4,6-tribromophenoxy)benzene......	−137
2-Amino-1,3-dibromo-4-methyl-9,10-anthracenedione....	−88
9-Bromoanthracene...................................	−77
4-Amino-2,6-dibromophenol...........................	−134

We also recognized that compound 5 possessed a very acidic proton on the amine nitrogen and that many known compounds that exhibit odor and taste contain acidic SH, OH, or NH protons. We thought it desirable to replace the proton on the amine with an

alkyl group to mask the acidity. We hoped to retain the toxicity
of the compound while avoiding the taste and/or odor character-
istics that we suspected rodents associated with these compounds.
Because the most active diphenyl amines are substituted in the
2,6- and 2'6' positions, causing a steric barrier to replacement
of the amine proton, the methylation could not be carried out on
the already substituted diphenyl amine. The synthesis of these
materials required new, stepwise chemical approaches (Scheme I).
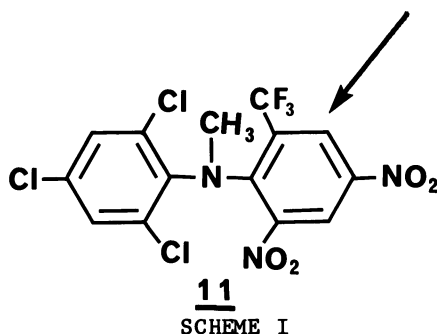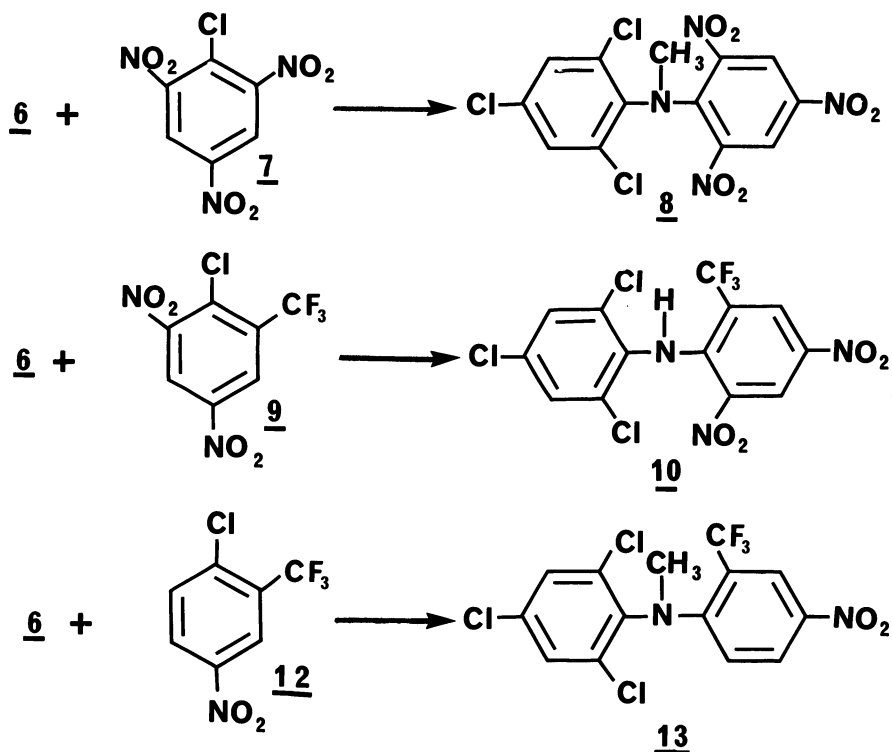
SCHEME I

    In the case of the picryl derivatives, the coupling of 2,4,6-trichloro-N-methyl aniline, 6, with picryl chloride,(Figure 7), gave the desired N-methyldiphenyl amine in the trinitro series (Figure 8). However, the same methylated aniline, when reacted with 2-chloro-3,5-dinitrobenzotrifluoride, (Figure 9), consistently demethylated to the N-H material, (Figure 10), instead of giving the desired product, (Figure 11). This was true regardless of the bases, solvents, and conditions used. Attempts to use other N-alkylated anilines, including N-methyl aniline itself also led to the dealkylated material. Finally an approach was developed whereby 11 could be generated by first reacting 6 with the des-nitro compound 12 to form the des-nitro diphenyl amine 13, and then nitrating under mild conditions with ammonium nitrate in trifluoroacetic acid.

    Side-by-side comparisons of the acceptability of the N-methyl material 11, versus its N-H analog 10, indicated that by blocking the free amine we could make the diphenyl amine far more acceptable to rats, (i.e. Table II).

Table II

10

	(a)	
	Treated Diet	Untreated Diet
5 Males-	14g	17g
5 Females-	11g	24g
Total-	25g	41g
% of Diet	37.9%	62.1%

11

	Treated Diet	Untreated Diet
5 Males	15g	5g
5 Females	29g	21g
Total	44g	26g
% of Diet	62.9%	37.1%

10   vs   11

	(b)	
	10ppm 10 in Diet	10ppm 11 in Diet
5 Males	30g	63g
5 Females	38g	62g
Total	68g	125g
% of Diet	35.6%	65.4%

(a)Toxicant level 100 ppm in treated diet
(b)The animals were given a choice of toxicants

On the basis of the information we had developed, it appeared
that the compound of choice should be polybrominated in the "A"
ring, be substituted with a 2-trifluoromethyl-4,6-dinitrophenyl
group in ring "B", and have a methyl group on the amine
nitrogen. Synthetically this was a problem since the
appropriately substituted N-H diphenyl amine cannot be directly
converted to the N-methyl analog, and the routes used in the case
of the trichloro-N-methyl material led to poor results. A
synthetic route was finally developed which resolved most of the
problems (Scheme II). A diphenyl amine unsubstituted in the "A"
ring was synthesized from 2-chloro-3,5-dinitrobenzotrifluoride,
9 and aniline, and this was methylated using very mild
methylating conditions; i.e. dimethyl sulfate and sodium
carbonate in acetone to form the des-brominated compound,
(Structure 15). The bromination of 15 took place in two steps. The
first two bromines added readily while the third bromine required
the use of N-bromosuccinimide (NBS) in sulfuric acid, to afford
the desired material 4 in high yield.
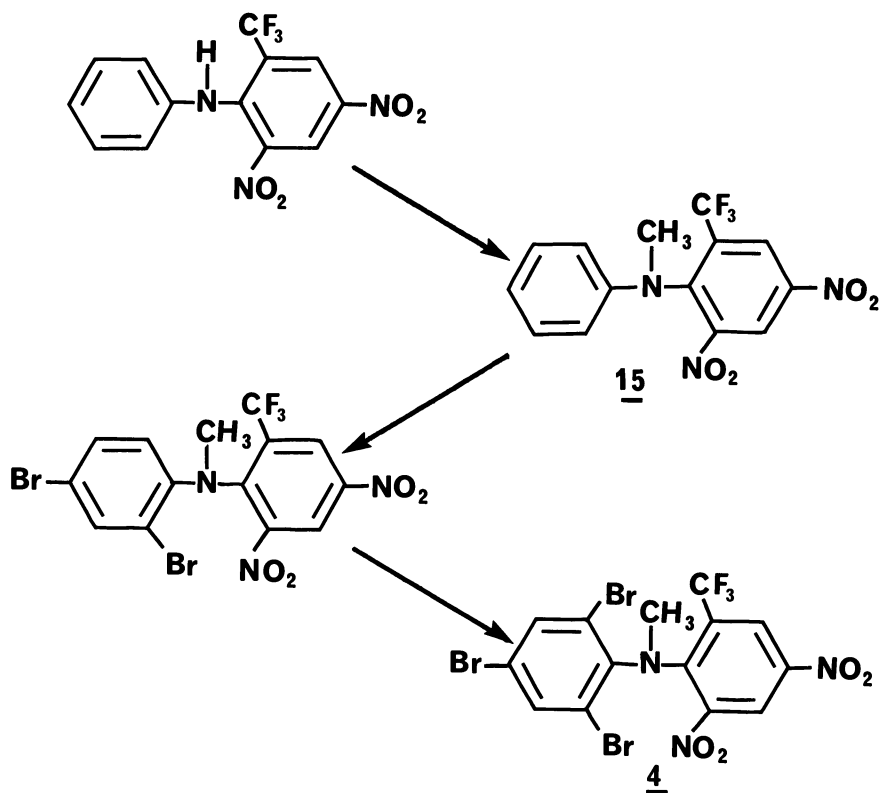
SCHEME II

Finally, choice tests were initiated both at Lilly and at Bowling Green on the acceptability and efficacy of this material as a rodenticide(14). All laboratory tests indicated that compound 4 was readily accepted by rats and mice (Appendix V). In some cases rodents actually seemed to prefer the treated diet over the basal diet. Compound 4 was assigned the Lilly code EL-614, and, somewhat later, the generic name bromethalin.

Development of bromethalin as a rodenticide

Once bromethalin had been identified as a compound that was both toxic to rodents at low dose levels and acceptable to them in bait, it was necessary to develop information on its performance under field conditions and to learn more about the mode of action.

Field evaluation of bromethalin. Both indoor and outdoor field trials against Norway rat and house mouse populations were conducted under an EPA experimental use permit in a number of geographical locations in the U.S.(15,16) In all cases 0.005% bromethalin was used in a bait containing 65% corn meal, 25% rolled oats, 5% sugar and 5% corn oil(E.P.A. diet).

Three census techniques were used before and after bromethalin treatment in order to estimate control of the rodent population, followed by snap trapping at the end of the test as an additional measure of control.

Field results indicate that bromethalin bait was exceptionally effective against both Norway rats and house mouse populations. Bait acceptance was excellent with no signs of bait-shyness observed.

It was also essential to determine how effective bromethalin was against warfarin-resistant rats and mice. Such animals, whose resistance to anticoagulants had been determined by World Health Organization tests,(17) were subjected to a standard EPA choice feeding efficacy test with bromethalin at 0.005% in the treated diet. The results indicate (Appendix 6) that 90% of the animals were killed and that consumption patterns were similar to those observed in other choice tests.

A field trial was conducted using 0.005% bromethalin bait against a resistant population of house mice in a poultry house. Resistance had been verified by using the standard WHO laboratory procedure. About two-thirds of the mice tested were resistant to warfarin. Control was achieved under extremely difficult conditions (i.e., extremely large population with an abundant food supply in a complex physical environment).

Toxicology of bromethalin

Acute toxicity of bromethalin. Acute LD_{50}s were determined in various target and non-target species by gavage administration of bromethalin solublized in polyethylene-glycol 200. These data

indicate that similar doses were required to produce lethality in
all species tested. Nevertheless, species selectivity is
obtained with bromethalin baits since rodents will consume larger
quantities of food per unit body weight than larger animals.

Secondary toxicity. In a study run to determine if bromethalin
treated rats pose any threat to animals that happen to eat the
treated carcass, rats that had been fed bromethalin bait for
16 hours at a level of 0.005% in their diet sufficient to kill
95%, were killed, ground into "ratburger", and fed to dogs
conditioned to eating rat meat. The dogs were fed this diet for
two weeks. At the end of this time, none of the dogs showed any
signs of toxicity. It was concluded that dogs consuming rats
that had consumed a lethal dose of bromethalin did not receive
enough toxicant to produce any signs of toxicity.

Mode of action of bromethalin. Signs of toxicity observed in
laboratory or field efficacy studies using large, single doses
include tremors, one or two episodes of clonic convulsions, and
prostration, with death usually occurring within 36 hours. In
contrast, sublethal doses from ingestion of repeated small
quantities of bait or from doses less than the LD_{50} can produce
lethargy, hind-leg weakness, loss of muscle tone and
paralysis.(18) These effects have been observed in laboratory
studies and have been shown to be reversible.
    Experiments on the physiological and biochemical mechanisms
of action suggest that bromethalin uncouples oxidative
phosphorylation in central nervous system mitocondria(19). This
could lead to a decreased production of ATP, a diminished
activity of Na^+/K^+ ATPase, and a subsequent fluid build up
manifested by fluid-filled vacuoles between the myelin sheaths.
This vacuole formation in turn leads to an increased
cerebrospinal fluid pressure and increased pressure on the nerve
axons, yielding a decrease in nerve impulse, paralysis, and death.
    At present it is not clear whether the same sequence of
action is responsible for bromethalin's convulsive and paralytic
actions observed at different doses; however, it is likely that
some areas of the brain would be more sensitive to ATP depletion
and fluid imbalance than others.
    The cerebral edema produced by sublethal doses of bromethalin
can be ameliorated by treatment with an osmotic diuretic and
corticosteroids. Pathology from sublethal doses, even without
treatment have been shown to be reversible.

Conclusion

    Bromethalin is a unique, highly potent rodenticide which
satisfies most of the criteria of an ideal rodenticide. It offers
a distinct advantage over currently used acute and anticoagulant
rodenticides because of its unique mode of action. Bromethalin

provides a lethal dose to rodents in a single feeding with death generally delayed two or three days. Rodents do not discriminate against bromethalin bait, therefore excellent bait acceptance is achieved. It has been shown to have a mode of action different from the anticoagulants and is very effective against anticoagulant-resistant rats and mice. Properly used it poses no danger to non-target species.

The discovery and development of bromethalin was the result of both serendipity and the timely application of the scientific method. The contributions of scientists from many disciplines were necessary for its success.

Acknowledgments

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Appendix I
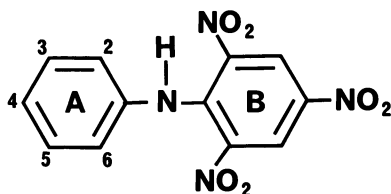
Substitution on Ring "A"	(a) Dose Levels in Mouse Toxicity Test (PPM)					
	1000	300	100	30	10	3
Mono substituted Cl, Br, F, CN, methyl, ethyl, nitro, methoxy. hydroxyl, Butyl.................	0/3					
2,3-Dichloro.....................	3/3	2/3	0/3			
2,4-Dichloro.....................	3/3	3/3	0/3			
2,5-Dichloro.....................	3/3	1/3	0/3			
2,6-Dichloro.....................	3/3	2/3	0/3			
2,4-Difluoro.....................	3/3	2/3	0/3			
2,3-, 2,4-, 2,5-, 2,6-Dimethyl...............	0/3					
3,5-Di Trifluoromethyl...........	3/3	3/3	3/3	3/3	0/3	
3-Methyl-4-bromo.................	0/3					
2,4-Dimethoxy....................	0/3					
2-Chloro-4-methyl...............	0/3					
2-Methyl-5-nitro.................	0/3					
2,4,6-Trichloro..................	3/3	3/3	3/3	3/3	3/3	0/3
3,4,5-Trichloro..................	0/3					
2,4,5-Trichloro..................	3/3	3/3	3/3	0/3		
2,3,4-Trichloro..................	3/3	3/3	0/3			
2,4,6-Tribromo...................	3/3	3/3	3/3	0/3		
2,4,6-Trifluoro..................	3/3	3/3	0/3			
2,4,6-Trimethyl..................	0/3					
2,4,6-Trinitro...................	3/3	3/3	0/3			
2,4-Dichloro-4-nitro.............	3/3	3/3	3/3	3/3	0/3	
2,3,4,5,6-Pentachloro............	3/3	3/3	3/3	2/3	0/3	
2,3,4,5,6-Pentafluoro............	3/3	3/3	3/3	3/3	3/3	

(a).The Mouse toxicity data is expressed as the ratio of the number of dead mice over the total tested at each level

Appendix II
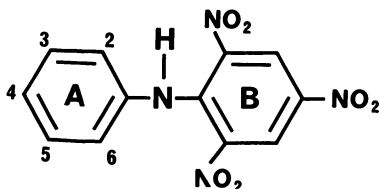
Substituents on Ring "A"	(a) MTT(mg/kg)	(b) Feeding Study(PPM)
3,4-Dichloro.....................	2/3 (300)	0/5 (200)
2,4-Difluoro.....................	2/3 (300)	0/5 (200)
2,3-Dimethyl.....................	0/3 (1000)	0/5 (200)
3,5-Di trifluoromethyl..........	3/3 (3)	5/5 (15)
2,4,6-Trichloro.................	3/3 (3)	5/5 (15)
3,4,5-Trichloro.................	0/3 (1000)	0/5 (200)
2,4,5-Trichloro.................	3/3 (30)	5/5 (100)
2,3,4-Trichloro.................	3/3 (300)	0/5 (200)
2,4,6-Tribromo..................	3/3 (10)	5/5 (30)
2,4,6-Trifluoro.................	3/3 (300)	0/5 (200)
2,4,6-Trimethyl.................	0/3 (1000)	0/5 (200)
2,4,6-Trinitro..................	1/3 (100)	0/5 (200)
2,3,4,5,6-Pentachloro..........	2/3 (30)	5/5 (100)

(a)Mouse Toxicity Test expressed as a ratio of the number of
dead mice over the total tested at the lowest level toxicity
was observed.   (b)Norway rat feeding study expressed as a
 ratio of the number of dead rats over the number tested at
lowest level toxicity was observed.

Appendix III

$$\text{Cl} \quad \text{Cl} \quad \overset{H}{\underset{|}{N}} \quad \text{X} \quad \text{B} \quad \text{Y}$$

Structure: ring A with Cl at three positions (2,4,6), linked via N–H to ring B bearing substituents X, Y, Z.
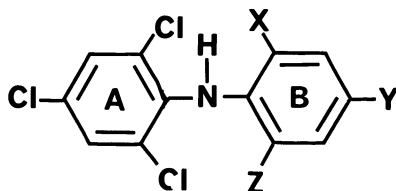
X	Y	Z	MTT[a] (Mg/Kg)	FEEDING[b] (ppm)
NO_2	NO_2	NO_2	3/3 (3)	5/5 (15)
NO_2	CF_3	NO_2	2/3 (1000)	0/5 (200)
NO_2	NO_2	H	0/3 (1000)	
H	NO_2	H	0/3 (1000)	
NO_2	CH_3	NO_2	0/3 (1000)	
NO_2	i-Propyl	NO_2	0/3 (1000)	
NO_2	t-Butyl	NO_2	0/3 (1000)	
NO_2	CN	NO_2	0/3 (1000)	
NO_2	SO_2NH_2	NO_2	0/3 (1000)	
NO_2	SO_3K	NO_2	0/3 (1000)	
CH_3	NO_2	NO_2	0/3 (1000)	
SO_2NH_2	NO_2	NO_2	0/3 (1000)	
CO_2H	NO_2	NO_2	0/3 (1000)	
CO_2Et	NO_2	NO_2	0/3 (1000)	
NO_2	Cl	NO_2	0/3 (1000)	
Cl	NO_2	NO_2	0/3 (1000)	
CF_3	NO_2	NO_2	3/3 (3)	5/5 (10 ppm)

(a)Mouse Toxicity Test expressed as the ratio of the number of dead mice over the total number of animals at the lowest level toxicity was observed. (b)Norway rat feeding study expressed as dead/total at the lowest level in feed that toxicity was observed.

Appendix IV
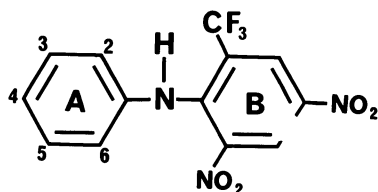
SUBSTITUENTS ON "A"	MTT[a] (mg/kg)
3,5-Dichloro.................................	1/3 (3)
3,4-Dichloro.................................	3/3 (10)
2,4-Dibromo..................................	2/3 (3)
2,6-Dibromo..................................	1/3 (30)
2,4-Difluoro.................................	2/3 (30)
2,4-Dinitro..................................	2/3 (30)
2,5-Difluoro.................................	2/3 (30)
3,5-Ditrifluoromethyl........................	3/3 (3)
3,4-Dimethyl.................................	0/3 (1000)
2,5-Dimethoxy................................	0/3 (1000)
2,4,6-Trichloro..............................	3/3 (10)
2,4,6-Tribromo...............................	1/3 (3)
2,4,6-Trifluoro..............................	3/3 (10)
2,6-Dichloro-4-bromo.........................	2/3 (10)
2-Chloro-4,6-dibromo.........................	2/3 (1)
2,4,6-Trimethyl..............................	1/3 (1000)
3,4,5-Trimethoxy.............................	0/3 (1000)
2,6-Dibromo-4-trifluoromethyl................	2/3 (10)

Appendix V

Choice Efficacy Studies with 0.005% Bromethalin Bait

Species	Strain	Test	Dead Total	a/	b/	c/
Rattus norvegicus	Wistar	3-day	136/140	3.4	51.2	3.04
Rattus norvegicus	Wild	3-day	19/20	2.8	48.9	1.79
Rattus norvegicus	Wild	1-day	20/20	3.6	56.2	2.65
Mus musculus	ICR	3-day	19/20	3.1	54.4	7.47
Mus musculus	Wild	3-day	20/20	3.3	45.5	5.10
Mus musculus	Wild	1-day	19/20	3.4	47.5	3.47
Rattus rattus	Wild	3-day	20/20	2.5	46.0	2.27

a/ Average days until death; b/ Percent acceptable;
c/ Bromethalin consumed mg/kg.

Appendix VI

Efficacy of 0.005% Bromethalin Against Warfin-Resistant Rodents Laboratory 3-Day Choice Test With Warfarin-Resistant Wild Norway Roats and House Mice

Species	No. Sex	a/ EPA Placebo	a/ Bromethalin	Mortality	Bromethalin Consumed (mg/kg)
Rattus norvegicus	10 M	8.2	7.0	10/10	1.0
	10 F	21.3	7.7	9/10	1.5
Mus Musculus	10 M	3.4	3.3	8/10	5.9
	10 F	1.6	1.3	10/10	2.6

a/ Average 3-Day Consumption (g)

Synthesis-Directing Structure–Activity Relationships of Some Fungicides Inhibiting Ergosterol Biosynthesis

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Substances of considerably diverse chemical structure all inhibiting ergosterol biosynthesis in numerous fungi have achieved considerable success as fungicides in many important agricultural crops. These chemicals can be characterized generally as broad spectrum locally systemic fungicides. Information regarding synthesis directing structure activity relationships of 1,2,4-triazolyl-1-aroyloxbutanes [2-aryl-2-(1,2,4-triazolylmethyl)1,3-dioxolane], α,α-diaryl-5-pyrimidinemethanols will be presented.

The discovery of the antifungal activity of α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine methanol (EL-273, triarimol) (Fig. 1) as reported by Brown, Taylor and Hall (1) and the initial determination by Ragsdale and Sisler (2) of its inhibition of biosynthesis of the fungal sterol ergosterol was the forerunner to the introduction of a succession of several sterol inhibiting (SI) fungicides of quite diverse chemical structure. Several of these sterol inhibiting fungicides have been marketed commercially within the last few years for the control of a wide range of fungus incited plant diseases.

Although many of the fungitoxicants described as SI compounds inhibit the same metabolic reaction in ergosterol biosynthesis, the biological activity of these compounds differ in many respects. This difference suggests that apart from the aspects influencing the basic inhibitory action against the fungus, the molecular structure influences other parameters associated with or responsible for the activity spectrum of the compound. For instance, certain members of the SI fungicide group demonstrate strong apoplastic systemicity within plant tissue whereas others do not. Some compounds are more active against certain pathogens and less active against others. The structure of the molecule as it influences the numerous

parameters associated with penetration and distribution within the host and pathogen is critical.

Although termed "SI" or "EBI" compounds, the latter referring to ergosterol biosythesis inhibitors, these compounds do not all inhibit ergosterol biosynthesis at the same metabolic site (Fig. 2). For instance, the fungicide tridemorph, unlike most EBI compounds, does not inhibit demethylation at C-14 but rather it apparently prevents the $\Delta^8 \rightarrow \Delta^7$ isomerization resulting in the accumulation of Δ^8 containing sterols in treated cells (3).

In this report we will limit our discussion of chemical structure and biological activity to substituted pyridine and pyrimidine methanols which have been shown or are believed to inhibit demethylation at carbon 14, an action which leads to inhibition of demethylation, also at carbon 4.

Fungal cells treated with an EBI fungicide are generally characterized by short germ tubes demonstrating some abnormal morphological form such as bulbous swellings often resulting in cytoplasmic leakage (4). In Figure 3 is shown a conidium of the fungus Helminthosporium sativum treated with 10 ppm of fenarimol. Typical of EBI compounds, the spore germination and initial development of the germ tubes is not visibly influenced. However, growth and development of the germ tube is ultimately arrested by the apparent disintegration and functionality of cytoplasmic membrane. Ergosterol is an integral structural component of the membranes of fungal cells. Diminution of ergosterol availability is believed to be the primary mechanism by which EBI fungicides inhibit fungal growth.

The schematic presentation of ergosterol biosynthesis and EBI sites of inhibition is available in references (Siegel [5]; Kato [6]) and only that segment directly related to the interests of this report will be presented here (Figure 4).

An early precursor in the synthesis of ergosterol is mevalonic acid which is condensed by several steps to squalene. The cyclization of squalene leads to the formation of lanosterol and 24-methylenedihydrolanosterol. This latter compound accumulates in EBI treated cells because of the inability of these cells to remove the C-14 methyl group. Demethylation would yield 4,4-dimethylfecosterol, the next sterol in the biosynthetic pathway leading to ergosterol. Ragsdale (7) showed the ergosterol biosynthesis in Ustilago maydis was greatly inhibited within 30 minutes after treatment with triarimol. The ergosterol content of total declined from a normal 70-80% of total to less than 4% within 6-9 hours following treatment.

The specific action of the EBI fungicides in preventing demethylation at C-14 appears to be directed at a cytochrome P-450 enzyme involved in the demethylation process (8). Gadher et al. (9) provide information that indicates the interaction of

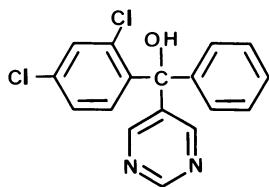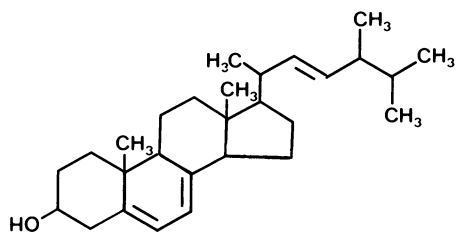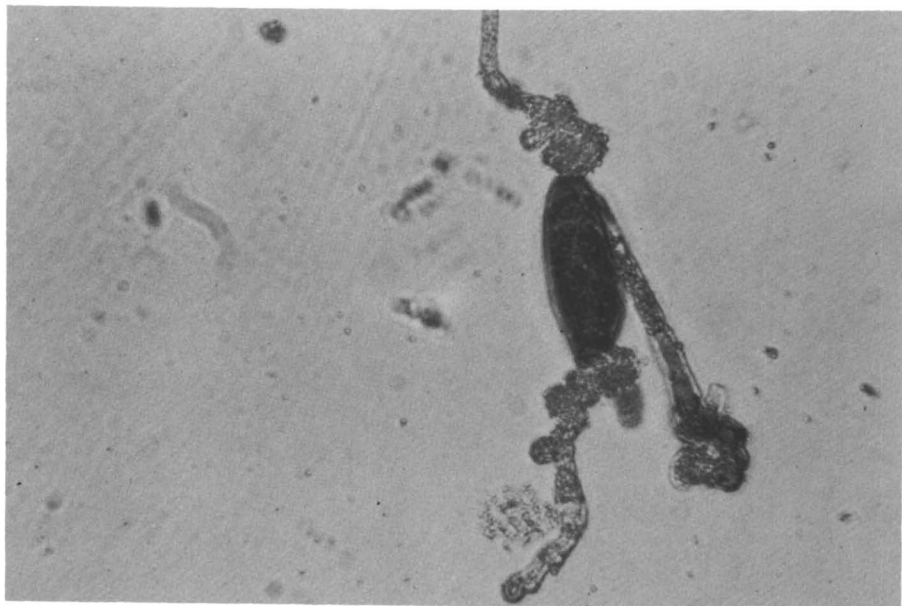Figure 1. α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol (EL-273, triarimol).

Figure 2. Ergosterol

Figure 3.   Conidium of <u>Helminthosporium sativum</u> Treated
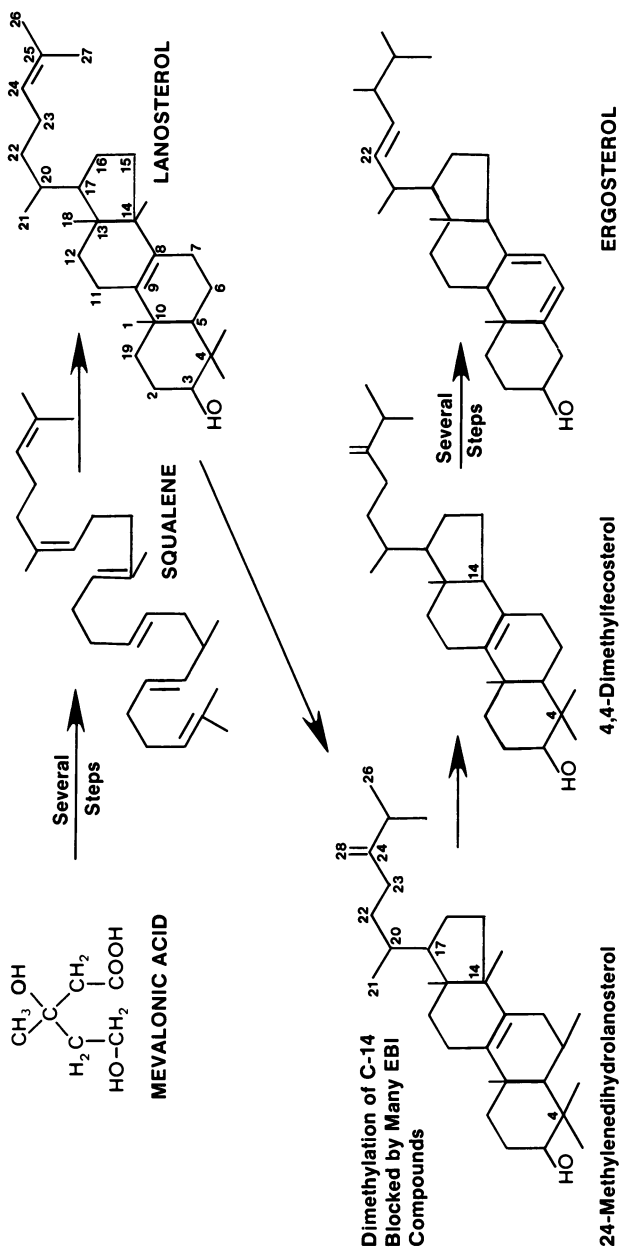With 10 ppm of Fenarimol.

Figure 4. Brief Schematic of Biosynthesis of Ergosterol.

an EBI compound and cytochrome P-450 involves an association of
a heterocyclic nitrogen atom with the protoheme iron of
cytochrome P-450. The demethylation at C-14 of the sterol
nucleus involves three reaction steps. These are shown in
Figure 5. The data of Gadher et al. indicate that the C-14
demethylation inhibiting compounds attack the first step of the
reaction. This reaction step utilizes cytochrome P-450 (10).
The inhibition of C-14 demethylation is thought to be due to the
binding of a heterocyclic nitrogen atom (probably N-4) in
triazole containing EBI fungicides to the protoheme iron atom
which in turn results in the exclusion of oxygen that would
normally take part in the reaction. The authors further suggest
that the non-heterocyclic portion of the fungitoxicant binds to
the lipophilic sites of cytochrome P-450. The structure of
several EBI fungicides are presented in Fig. 6.

The recent reviews by Jager (11) and Kramer et al (12)
describe the synthetic routes and biological activity of
selected tritylimidazoles, trityltriazoles and other variously
substituted N-azole fungicides. The information that follows
will describe certain structural activity relationships of
selected EBI fungicides containing pyridine and pyrimidine
heterocyclic moieties.

The greenhouse methods used to evaluate our compounds are
similar to those used by most companies. Generally candidate
fungicides are (1) applied to the foliage of test plants, and
(2) allowed to dry for varying periods of time. Test plants are
(1) inoculated with the appropriate pathogen, (2) placed in
environmental chambers for expression fo disease symptoms or
signs; and (3) evaluated.

In the early 1960's we came across the antifungal activity
of certain α,α-diphenyl-3-pyridine methanols and α,β-diphenyl-
3-pyridine ethanols. Generic structures are shown in Fig. 7.
These pyridines are primarily active against fungal genera
causing powdery mildew diseases. Depending on the aryl ring
substitution, some activity is seen against rice blast and
cucumber anthracnose. The pyridines are not systemic but do
demonstrate a measure of translaminar movement as indicated by
the control of powdery mildew on the upper leaf surface when the
fungitoxicant is applied to the lower leaf surface only (Fig.
8).

The significance of heterocyclic binding to cytochrome
P-450 as indicated by Gadher et al. was not recognized in the
early 1960's when the structure/activity relationships of the
lead compounds was being developed. However, numerous
α,α-diphenylmethane heterocyclic compounds were evaluated. The
activity of these compounds against bean powdery mildew is
presented in Table I. Critical for activity is the positioning
of the methane substitution beta to a nitrogen atom in the
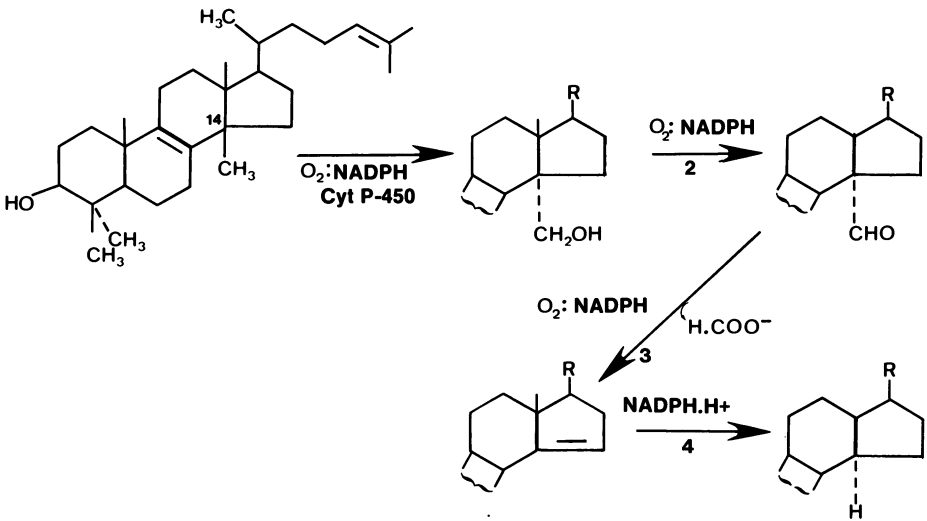
Figure 5. Outline of the Sterol 14-Demethylation Reaction Sequence (9).

I. Pyrimidine

II. Pyridine

R	R_1	Common Name	Manufacturer
2,4-Cl	H	triarimol	Elanco
2-Cl	4-Cl	fenarimol	Elanco
2-Cl	4-F	nuarimol	Elanco
4-Cl	4-Cl	parinol	Elanco
		buthiobate	Sumitomo

Figure 6. Ergosterol Biosynthetic Inhibitors.

Manufacturer	Common Name	R	R$_1$	R$_2$
Janssen	imazilil	H	-OCH$_2$-CH=CH$_2$	
Rohm & Haas	fenapanil	-CN	n-C$_4$H$_9$	
Fisons-Boots	prochloraz			
Bayer	triadimefon	H	H	
Ciba-Geigy	propiconazol		H	
Bayer	biloxazol		H	
ICI	diclobutazol		H	

IV. Triazole

Figure 6. Continued.

R,R$_1$ = 4Cl
Parinol

R, R$_1$ = halogens
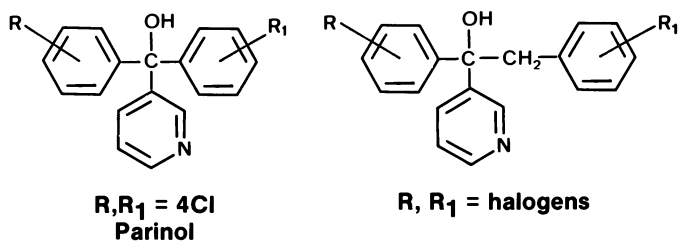
Figure 7.   3-Pyridine Methanol/Ethanol.

Figure 8.   Systematic Movement of Parinol.

Table I. Control of Bean Powdery Mildew (BPM) by α,α-Diphenylmethaneheterocyclic Compounds Applied at 400 ppm
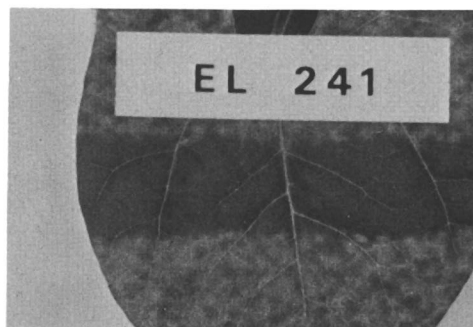
DISEASE CONTROL

Z =						
BPM	6	6	0	0	4	0	2

DISEASE CONTROL

Z =					
BPM	0	8	0	10	0	10

Rating Scale: 0 = no control; 10 = complete control.

heterocyclic ring. This requirement is clearly shown by the
inactivity of the -2-pyridine and 4-pyridine compounds compared
to the high level of activity of the 3-pyridine compound.
Further support for this statement is seen in the inactivity of
the 2-pyrimidine in which both nitrogen atoms are alpha to the
methane substitution as compared to the highly active
5-pyrimidine in which both nitrogen atoms are beta. The
heterocycles 1,3-imidazol-1-yl and 1,2,4-triazol-1-yl both
contain a nitrogen beta to the methane substitution and
demonstrated good activity against the bean powdery mildew
fungus. These heterocycles are used in many of the EBI
fungicides marketed today. Whereas the 4-pyridazine compound
demonstrated some activity the 3-pyridazine was inactive. Both
derivatives have a heterocyclic nitrogen beta to the methane
substitution. However, in the inactive 3-pyridazine compound
another nitrogen atom is positioned between the beta nitrogen
and the methane substitution. The presence of this nitrogen
might interfere with the binding of the beta nitrogen to
cytochrome P-450. The presence of a nitrogen atom alpha to the
methane substitution does not destroy activity as long as it is
not adjacent to the meta positioned nitrogen atom. Examples of
this are 1,2,4-triazole and 2-pyrazine.

    Another parameter investigated was the usefulness of
different substituents other than hydrogen or hydroxyl on the
α-carbon. Table II provides the relative activity of several
diphenylmethyl substituted 5-pyrimidines. Clearly, the hydroxyl
substituted compound is the most active against bean powdery
mildew.

    The activity of α-carbon alkyl and cycloalkyl substitutions
is presented in Table III. These data indicate that the
α,α-dicyclohexyl moiety is very active against bean powdery
mildew. Further, the data indicate that the α-cycloalkyl-α-
phenyl moieties are not only superior to α-alkyl-α-phenyl
substitutions for powdery mildew control, but that they also
confer some activity against cucumber anthracnose. Based on
these and other data, the α,α-diphenyl substitution was
considered to be the best choice.

    The next logical step was to determine the influence of
phenyl ring substitution on the scope and degree of antifungal
activity. The activity of some of these compounds is presented
in Table IV. When one phenyl ring is substituted and the other
ring unsubstituted, foliar protectant activity is optimized with
halogens at the 2 or 4 position. Specifically, the
4-fluoro-substitution (cmpd. 6) appears more active than other
mono substitutions of the phenyl ring and equal to the
2,4-dichlorophenyl moiety which is the original pyrimidine
fungicide, triarimol. Ortho-chloro or ortho-fluoro-substitution
imparts excellent systemic activity against the bean rust

Table II. Activity of Diphenylmethyl–Substituted 5–Pyrimidines Against Bean Powdery Mildew
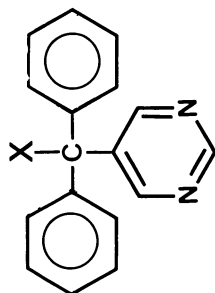
DISEASE CONTROL

X =	Parts Per Million			X =	Parts Per Million		
	400	80	16		400	80	16
-CL	10	10	5	-NHCOCH$_3$	10	6	2
-NH$_2$	10	6	5	-NHC$_6$H$_5$	10	6	4
-CN	10	9	4	-H	10	4	2
-OC$_2$H$_5$	10	9	4	-OH	10	10	10

Disease Rating: 0 = no control; 10 = complete control.

Table III. Activity of α-Phenyl-α-alkyl or Cycloalkyl-5-pyrimidine Methane or Methanols

Compound	Disease Control ppm	Bean PM	Cuke A
dicyclohexyl methane (H–C with cyclohexyl, H)	400	4	4
	80		
	16		
dicyclohexyl methanol (OH–C with cyclohexyl, H)	400	10	0
	80	10	
	16	5	
phenyl cyclohexyl methanol (OH–C with cyclohexyl, phenyl)	400	10	5
	80	9	2
	16	5	0
phenyl cyclobutyl methanol (OH–C with cyclobutyl, phenyl)	400	10	6
	80	5	2
	16	0	0

Compound	Disease Control ppm	Bean PM	Cuke A
phenyl methyl carbinol (OH–C–CH$_3$, phenyl)	400	4	0
	80	0	
	16	0	
phenyl hexyl carbinol (OH–C–C$_6$H$_{13}$, phenyl)	400	6	0
	80	0	
	16	0	
diphenyl methanol (OH–C with two phenyl)	400	10	0
	80	10	
	16	9	

PM = Powdery Mildew   Disease Rating: 0 = no control;   10 = complete control.
A = Anthracnose

Table IV. Comparative Activity of Substituted Diphenyl-5-pyrimidine Methanols
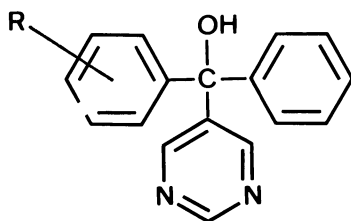
			Beam PM		Cuke A		Rice Blast	Apple Scab		Bean Rust (Systemic)	
Cmpd.	R	ppm:	16	3.2	400	80	400	100	25	20	10
#1	H		9	2	0	0	0	1	0	10	9
#2	2-Cl		8	5	4	0	0	-	-	10	10
#3	2-F		6	4	6	2	0	9	0	10	10
#4	3-Cl		1	0	0	0	0	1	0	0	0
#5	4-Cl		10	5	9	3	0	9	2	10	6
#6	4-F		10	5	10	3	7	10	10	10	10
#7	3-CF3		7	0	3	0	6	-	-	2	0
#8	4-CF3		5	0	4	0	0	-	-	5	0
#9	4-OCH-3		5	0	7	2	0	-	-	10	7
#10	4-NO2		9	4	0	0	0	-	-	0	0
#11	2,4-DiCl		10	9	9	5	5	10	10	10	8
#12	2,5-DiCl		7	7	8	5	0	10	10	0	0
#13	3,4-DiCl		10	7	9	2	0	-	-	0	0

PM = Powdery Mildew   Disease Rating: = no control;

  A = Anthracnose   10 = complete control

fungus. In this test, compound is added as a one-time soil drench to pots containing rust inoculated bean plants. The meta-chloro phenyl compound (cmpd. 4) was not as fungitoxic as the ortho- or para-chloro phenyl derivatives and probably not systemic although in the absence of sufficient antifungal activity, systemic movement is difficult, if not impossible, to assay. However, the 2,5 and 3,4 dichlorophenyl compounds, which contain a halogen meta to the α-carbon substitution, demonstrated no systemicity although some antifungal activity is indicated.

The scope and degree of antifungal activity is increased when substitutions are made on each phenyl ring. Results of our greenhouse evaluations are presented in Table V. The importance of an ortho-halo substitution for degree of antifungal activity is readily observed. For instance, compound 15 (2-chlorophenyl-α-3-chlorophenyl) shows considerably more activity than compound 17 which is bis(3-chlorophenyl). A similar observation is made between compounds 19 and 20. Of interest is the lower level of activity in the bis (4-chlorophenyl) derivative (18). The poor activity of α-bis-(4-chlorophenyl)-5-pyrimidine methane (cmpd. 18) is somewhat surprising since the similarly substituted 3-pyridine methanol (parinol, Parnon) was the compound selected and positioned in the U.S. ornamental crop market. Generally, fluoro substitutions on the phenyl rings provide slightly better activity than the corresponding chloro substitutions.

From our research two compounds were selected from this group for marketing. They are the α-(2-chlorophenyl)-α-(4-chlorophenyl) (cmpd. 16) and α-(2-chlorophenyl)-α-(4-fluorophenyl) (cmpd. 25) substituted 5-pyrimidine methanols, known as fenarimol and nuarimol, respectively.

DISCUSSION AND CONCLUSIONS

The work of Gadher et al and the awareness of the diversity of the biological activity of EBI fungicides suggests that the structure of the fungitoxicant must satisfy more than one requirement. The ability to bind to the protoheme iron of cytochrome P-450 appears to be a required function for activity. The fitness to bind could be dependent upon several facets such as:

1.   The heterocycle itself and the position of the nitrogen atoms within the ring. For example, in Table 1 we showed that α,α-diphenylmethyl-3-pyridazine was inactive despite the presence of a beta positioned nitrogen. We supposed that the presence of the alpha nitrogen atom adjacent to the beta nitrogen was responsible for the lack of activity.

Table V. Comparative Activity of Disubstituted Diphenyl-5-pyrimidine Methanols
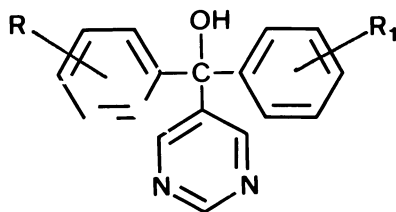
DISEASE CONTROL

Cmpd.	R	R1	ppm:	Bean PM		Cuke A		Rice Blast	Apple Scab		Bean Rust (Systemic)	
				16	3.2	400	80	400	50	10	20	10
#14	2-Cl	2-Cl		9	4	4	0	0	10	10	10	7
#15	2-Cl	3-Cl		10	9	10	10	5	10	10	9	10
#16	2-Cl	4-Cl		9	8	10	5	6	10	10	10	8
#17	3-Cl	3-Cl		6	7	8	0	4	4	2	0	0
#18	4-Cl	4-Cl		1	0	0	-	0	0	0	-	-
#19	2-Cl	4-OCH3		10	9	8	0	0	10	2	10	10
#20	4-Cl	4-OCH3		4	0	5	-	4	0	-	0	-
#21	4-CH3	4-CH3		-	-	0	-	0	-	-	0	-
#22	2-F	3-F		9	5	10	6	5	10	10	0	-
#23	3-F	3-F		3	1	8	2	4	-	-	6	3
#24	4-F	4-F		9	6	10	7	0	-	-	10	10
#25	2-Cl	4-F		10	10	10	9	5	10	10	10	10
#26	3,4-DiCl	3,4-DiCl		4	0	0	-	0	-	-	0	-

PM = Powdery Mildew
A = Anthracnose
Disease Rating: 0 = no control; 10 = complete control.

This supposition may be valid for pyridazine but in the Bayer U.S. Patent 4,098,894, they show good antifungal activity for 1-(3-trifluoromethyltriphenyl) 1,2,3-triazole, a compound which also contains a nitrogen atom alpha to the substitution and adjacent to the beta nitrogen.

2. The "spaghetti" attached to the heterocyclic ring could greatly influence the degree of association with cytochrome P-450 as well as many of the other facets of EBI fungicide activity.

The ability of these chemicals to penetrate the cuticle, the cell membrane, move into the protoplast and distribute in the plant is to a large measure dependent upon the hydrophilic/lipophilic balance, the steric configuration and its stability in or on the plant. Similarly, these same chemical and physical factors are critical for toxicity of the chemical to the fungus.

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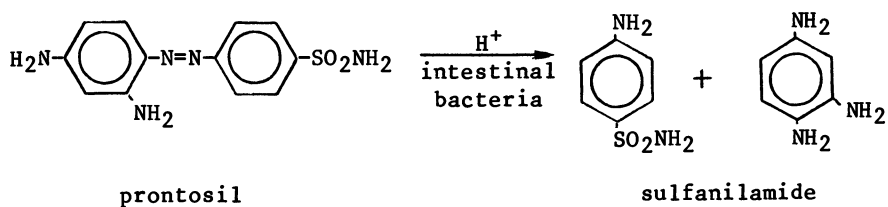
Propesticides

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During the past decade a number of new derivatives
of toxic methylcarbamate insecticides with improved
toxicological properties have been discovered and
several are now close to reaching commercial promi-
nence. These proinsecticides have in common a
sulfur atom which bridges the nitrogen atom of the
carbamyl moiety to the derivatizing group. The
toxicological properties of the original methylcar-
bamate insecticide may be markedly affected by deri-
vatization, resulting generally in substantial
improvement in mammalian toxicity and retention or
improvement in insecticidal activity. Derivatiza-
tion may greatly alter the physical properties of a
pesticide, e.g. from polar to non-polar, and this
change often affects the spectrum of activity of the
compound. The development of chlorosulfenyl- and
chlorosulfinylmethylcarbamates as intermediates in
derivatization opens up many possibilities for the
design and synthesis of new proinsecticides with
unusual properties. The intermediates may be used
in derivatizing a wide variety of other pesticidal
compounds.

A propesticide is a pesticidally active material or compound
which in its original form is inactive and is transformed into an
active state by a plant, animal, or microorganism. In most
cases, the target organism which is being affected unwittingly
carries out a self-inflicting lethal synthesis by chemically or
biochemically converting an inactive compound into an active pro-
duct. The concept behind the development of propesticides has
been known for half a century and has been exploited for many
years in the design of drugs by the pharmaceutical industry. An
early example of a synthetic prodrug is prontosil, a drug used

for the treatment of streptococcal and pneumococcal infections.
Recognition for the discovery of the chemotherapeutic value of
prontosil was given to Domagk (1) who was awarded the Nobel Prize
in Medicine for 1938. Following the discovery of prontosil,
scientists at the Pasteur Institute showed that prontosil was
metabolically converted into sulfanilamide (p-aminobenzenesul-
fonamide) and that it was sulfanilamide which was responsible
for the therapeutic value of prontosil (2,3). Prontosil itself
was first synthesized as a possible azo dye. Since the discovery
of p-aminobenzenesulfonamide as an antibacterial agent, vast num-
bers of sulfanilamide derivatives have been synthesized and

prontosil   sulfanilamide

evaluated and several have attained clinical importance (4).
     The discovery of prontosil was fortuitous and was not based
on rationale design. There are a large number of pesticides
which fall in the same category as prontosil, i.e., they are
active by virtue of their susceptibility to metabolic or chemical
modification to active intermediates. The classical example of
an insecticide of this type is parathion, a phosphorothionate
ester which in animals or plants is oxidatively desulfurated to
the potent anticholinesterase paraoxon (5). The insecticidal
activity of parathion was known for several years before the
purified material was shown to be a poor anticholinesterase and
that metabolic activation to paraoxon was necessary for intoxica-
tion.
     In contrast to "pro" compounds of the type described above,
a large number of propesticides have also been developed in
recent years which were designed on the basis of their antici-
pated activation in biological systems. In these cases, active
compounds were modified by derivatization into products which
reverted back to the original compound within the target orga-
nism. This kind of approach to the design of new pesticides has
many virtues and the most important of these are described
briefly as follows.
     The activity spectrum of a pesticidal compound is often
determined by the physical properties of the compound. For
example, systemic insecticides usually require both water and
lipid solubility since it is necessary for them to pass through

aqueous and lipid phases while moving through a plant or animal
(6). Therefore, by attaching an appropriate functional group to
an insecticide, it is possible to convert a non-systemic compound
into one which is systemic. Moreover, by proper selection of the
derivatizing moiety, the physical properties of an insecticidal
compound may be manipulated to obtain products with other
selected types of activity. However, it should be added that
derivatization may also lead to a partial or complete loss of
insecticidal activity.

Another important virtue to derivatization of insecticides
to proinsecticides is the significant improvement in the toxico-
logical properties often observed in the derivatized product.
This has been particularly evident with the methylcarbamate and
phosphoramidothioate insecticides where in almost all cases deri-
vatization has resulted in improved mammalian toxicity. Further,
in many cases, equal or greater insecticidal activity has also
been observed although in some cases insecticidal activity may be
substantially reduced. Improvement in mammalian toxicity has
been attributed to the delayed factor provided by the deriva-
tizing group, giving the animal the opportunity to metabolize the
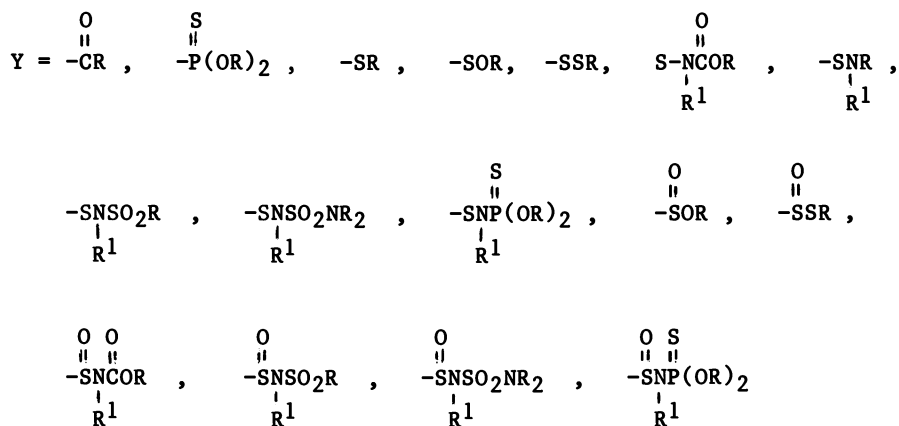compound to non-toxic products.

Derivatization, therefore, has been widely applied to
improve the toxicological properties of known insecticides and
this paper will attempt to review accomplishments and discuss
possibilities for future strategy for the design of new compounds
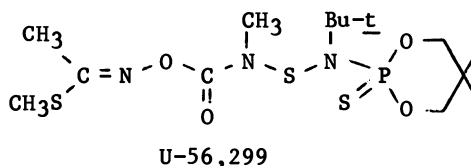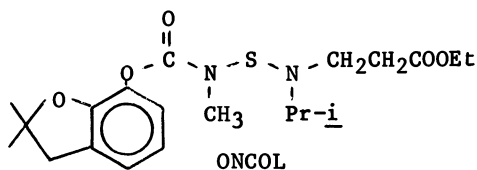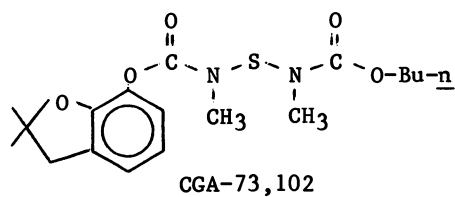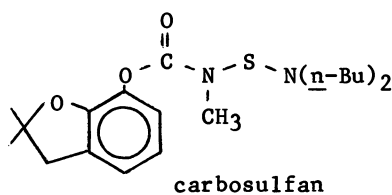with this group of pesticides.

Methylcarbamates

The methylcarbamate insecticides are particularly suitable to
derivatization owing to the availability of the replaceable pro-
ton on the methylcarbamyl nitrogen atom. The proton may be
replaced by a wide variety of groups to give reasonably stable
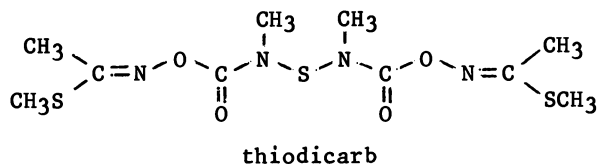products, as indicated by the general scheme below (7,8).
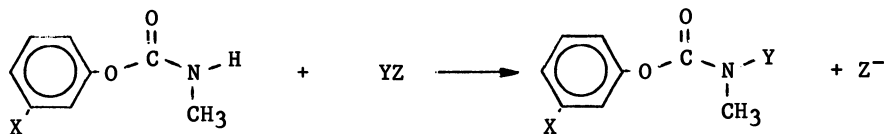
The number of groups represented by Y which give active products
is very large and include the following (R, R^1, etc. = alkyl or
aryl).

$$Y = -\overset{\overset{\text{O}}{\|}}{C}R \;,\quad -\overset{\overset{\text{S}}{\|}}{P}(OR)_2 \;,\quad -SR \;,\quad -SOR, \quad -SSR, \quad S-\underset{\underset{R^1}{|}}{N}\overset{\overset{\text{O}}{\|}}{C}OR \;,\quad -\underset{\underset{R^1}{|}}{S}NR \;,$$

$$-\underset{\underset{R^1}{|}}{S}NSO_2R \;,\quad -\underset{\underset{R^1}{|}}{S}NSO_2NR_2 \;,\quad -\underset{\underset{R^1}{|}}{S}N\overset{\overset{\text{S}}{\|}}{P}(OR)_2 \;,\quad -\overset{\overset{\text{O}}{\|}}{S}OR \;,\quad -\overset{\overset{\text{O}}{\|}}{S}SR \;,$$

$$-\underset{\underset{R^1}{|}}{S}N\overset{\overset{\text{O O}}{\| \|}}{C}OR \;,\quad -\underset{\underset{R^1}{|}}{S}N\overset{\overset{\text{O}}{\|}}{S}O_2R \;,\quad -\underset{\underset{R^1}{|}}{S}N\overset{\overset{\text{O}}{\|}}{S}O_2NR_2 \;,\quad -\underset{\underset{R^1}{|}}{S}N\overset{\overset{\text{O S}}{\| \|}}{P}(OR)_2$$

Group Y must be of such a structure to give a product with a
moderately labile N–Y bond so that activation to the toxic
methylcarbamate may occur readily within the target organism.
A number of proinsecticides of methylcarbamate esters have
attained commercial importance or are currently being developed
for commercial use. The structures of some of these are given
below.

carbosulfan

CGA-73,102

ONCOL

U-56,299

$$CH_3-C(=N-O-C(=O)-N(CH_3)-S-N(CH_3)-C(=O)-O-N=C(CH_3)(SCH_3))$$
$$CH_3S$$

thiodicarb

Carbosulfan, CGA-73,102, and ONCOL are derivatives of carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) which have broad spectrum activity similar to that of carbofuran but are substantially less toxic to mammals. CGA-73,102 is particularly effective as a soil insecticide by both contact and systemic action (9). Thiodicarb (10) and U-56,299 (11) are highly effective lepidopterous larvicides but are less toxic to plants and mammals than methomyl. U-56,299, with a rat acute oral LD_{50} of 8000 mg/kg, is very safe to mammals.

An example of the profound effect which change in physical property may have on the toxicological properties of a methylcarbamate insecticide is made apparent from toxicological data for a series of thiocarbamate derivatives of carbofuran (Table 1) (12).

Table I. Toxicity of Thiocarbamate Derivatives of Carbofuran to House Flies, Mosquito Larvae and Mice

No.	R	R^1	log P	Housefly LD_{50}, μmol/g	Mosq. larv. LC_{50}, μM	Mice LD_{50}, μmol/kg
	(carbofuran)		–	0.030	0.235	9
1	Me	Me	2.28	0.027	0.068	132
2	Et	Me	2.78	0.025	0.045	203
3	Me	Et	2.78	0.025	0.045	175
4	i-Pr	Me	3.08	0.024	0.024	141
5	Pr	Me	3.28	0.025	0.045	238
6	Et	Et	3.28	0.025	0.027	231
7	Et	i-Pr	3.58	0.031	0.015	327
8	Bu	Me	3.78	0.026	0.011	340
9	C_5H_{11}	Me	4.28	0.027	0.0056	353
10	C_7H_{15}	Me	5.28	0.026	0.0024	412
11	C_8H_{17}	Me	5.78	0.027	0.0019	433
12	$C_{10}H_{21}$	Me	6.78	0.030	0.0026	5.36

The compounds are arranged in order of increasing log P values (P
= octanol/water partition coefficient). Increase in log P values
had little effect on house fly toxicity with all of the compounds
showing equal toxicity although there was a broad range in
octanol/water partitioning properties. In contrast, toxicity to
mosquito larvae and the white mouse changed markedly with change
in log P with mosquito larvae toxicity increasing and mouse toxi-
city decreasing with increase in log P. In the case of mosquito
larvae, toxicity of the carbofuran derivatives increased to a
maximum level when log P was in the range of 5-6 and then
decreased. The relationship between log P and mosquito larvae
toxicity for derivatives of carbofuran, propoxur and m-isopropyl-
phenyl methylcarbamate is shown in Figure 1.
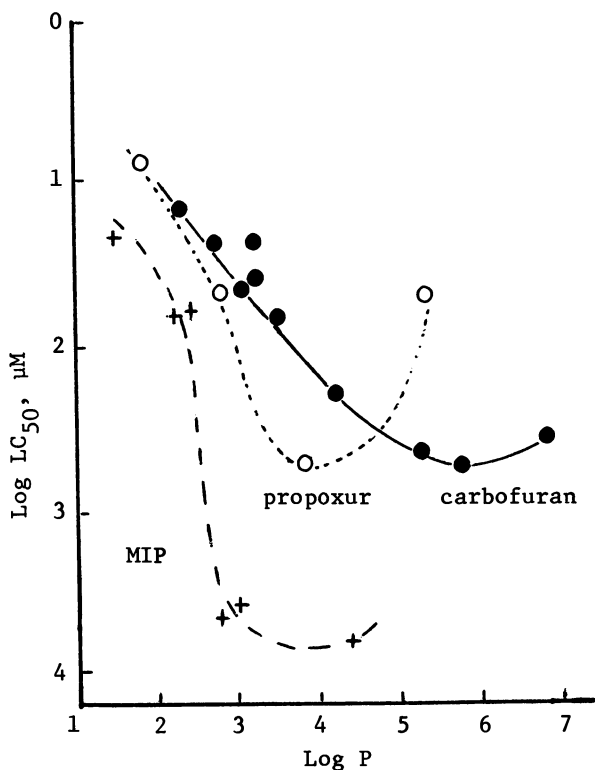
Figure 1. Relationship between the mosquito larvicidal toxicity
(log LC$_{50}$) of the thiocarbamate derivatives of carbofuran, pro-
poxur and m-isopropylphenyl methylcarbamate (MIP) and logarithm
of the octanol/water partition coefficient (log P).

The $\underline{N}$-chlorosulfenyl and $\underline{N}$-chlorosulfinyl derivatives of methylcarbamate esters are useful intermediates for the synthesis of a large variety of new derivatives having favorable toxicological properties (12,13,14). Examples of the types of compounds which may be synthesized are indicated in Figure 2. The $\underline{N}$-chlorosulfenyl and $\underline{N}$-chlorosulfinyl intermediates react with an array of nucleophiles in the presence of a proton acceptor, e.g. alcohols, thiols, carbamates, sulfonamides and phosphoramidates, to give the respective derivatives. The $\underline{N}$-chlorosulfenyl intermediates also react with amines to give stable products analogous to carbosulfan and ONCOL. These intermediates provide the means to modify carbamate insecticides in many different ways and the potential for the design of tailored-made compounds by application of the reactions depicted in Figure 2 is enormous.
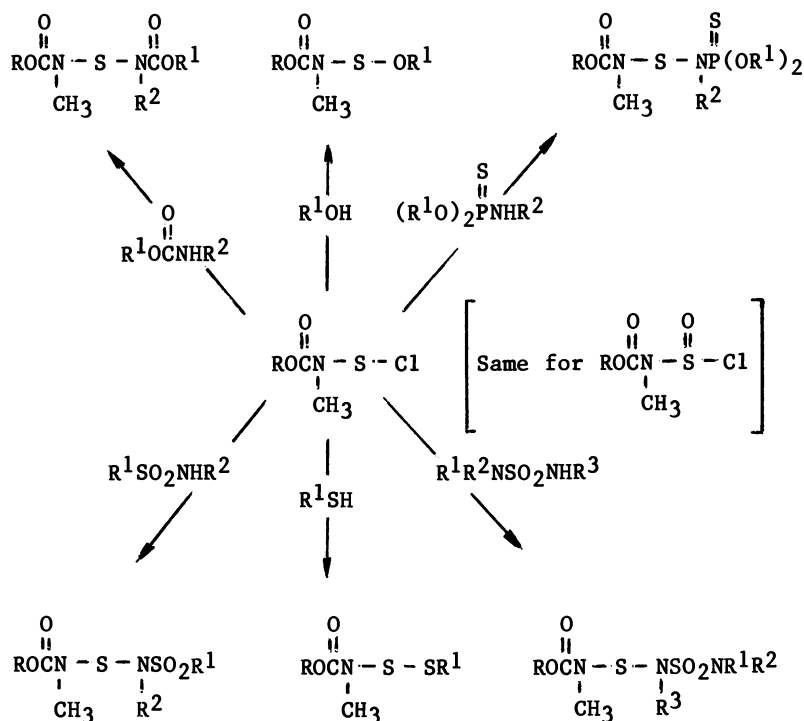

Figure 2. Equations showing the reaction between an $\underline{N}$-chlorosulfenyl-$\underline{N}$-methylcarbamate intermediate (or corresponding $\underline{N}$-chlorosulfinyl-N-methylcarbamate) and different nucleophilic agents.

Hypothetical examples of the possible exploitation of this kind of chemistry are given as follows.
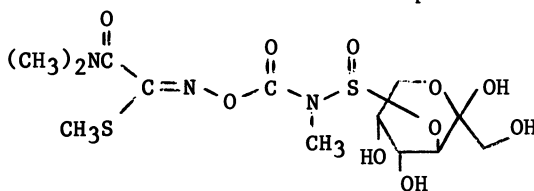
The usefulness of a downward-moving systemic insecticide or nematicide is obvious. It would be highly advantageous to control root-damaging insects and nematodes by systemic action, i.e., by foliar application of a pesticide which will move downward into the roots. While there have been reports of the downward movement of methylcarbamate pesticides in plants following foliar application (15,16), the extent of their downward movement has not been large enough for them to effectively control root parasites by systemic action. It is possible that these methylcarbamates, e.g. carbofuran, methomyl and oxamyl, all of which are potent insecticides and nematicides, require assistance in getting into the root zone in effective concentrations.

There are a number of substances which are phloem mobile, i.e., are downward moving. These include carbohydrates, amino acids, organic acids and, in general, compounds of high polarity (17). These substances may act as a carrier group in assisting an active compound into the root zone. We have recently demonstrated the feasibility of using reactions described in Figure 2 to attach a carrier group to a methylcarbamate ester for this purpose (18). A protected sugar, e.g., a diisopropylidene-fructose or glucose, was reacted with the chlorosulfinyl intermediate obtained from several methylcarbamate insecticides to give the respective sulfinyl-sugar derivatives. A typical example using the chlorosulfinyl intermediate of oxamyl and 1,2,4,5-di-$\underline{O}$-isopropylidene-α-$\underline{D}$-fructose is shown below.
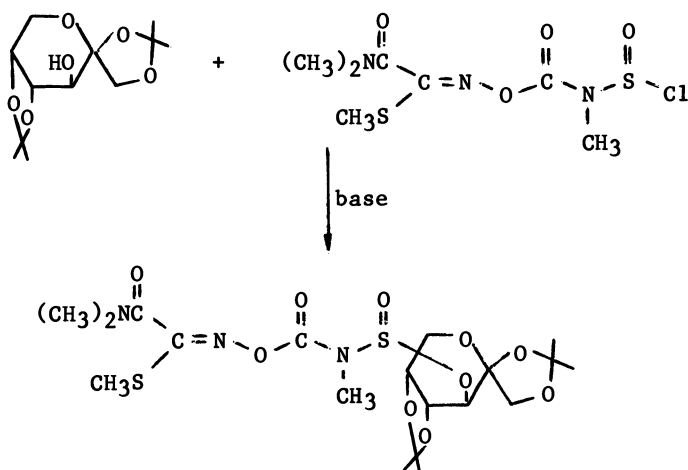
The rationale for the synthesis of this compound and others like it is made apparent from the equation below.

$$\text{oxamyl} - \overset{\overset{\displaystyle O}{\|}}{S} - \text{diisopropylidenefructose}$$

$\xrightarrow{\text{penetration into plant}}$ $\xrightarrow{\text{removal of isopropylidene}}$
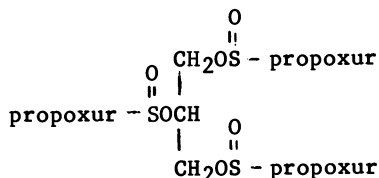

(phloem mobile)

Protection of fructose by the labile isopropylidene groups was considered necessary for two reasons, (1) it facilitates the sulfinylation reaction because of the much higher solubility of the protected sugar in the organic reaction medium and (2) derivatization with a less polar protected sugar would give a derivative which could penetrate the waxy cuticle of the plant leaf. The rationale is that the protected sugar derivatives must first penetrate into the plant, and following penetration hydrolytic removal of the isopropylidene groups would generate the methylcarbamate containing the highly polar deprotected sugar which would assist in phloem mobility. Because of the lability of the N-S bond the toxic methylcarbamate should eventually be regenerated in the roots or within organisms feeding on the roots. Owing to the unavailability of an adequate bioassay, unfortunately these derivatives were not tested for downward moving systemic activity. However, some of them proved to be effective against different insects by contact action. Further work along these lines is in progress.

The downward systemic movement of ONCOL (structure given earlier), a new insecticide derived from carbofuran, has been observed (19). A significant amount of radioactivity was observed in the roots of cotton and bean plants treated topically at the base of bifoliate or trifoliated leaves with [carbamate carbonyl[14]C]ONCOL. Downward movement of the radiolabeled material may be explained by hydrolytic degradation of the ethoxycarbonyl moiety in ONCOL to the carboxylic acid derivative, the acid function serving as a downward moving carrier.

The N-chlorosulfinyl intermediates of insecticidal methylcarbamate esters also reacted with polyalcohols to form the polysulfinylmethylcarbamate derivatives (18). For example, three moles of the N-chlorosulfinyl intermediate of propoxur (2-isopropoxyphenyl methylcarbamate) and one mole glycerol resulted in a good yield of the tris(oxysulfinyl)propoxur derivative.

$$\text{propoxur} - \overset{\overset{\displaystyle O}{\|}}{S}\overset{\overset{\displaystyle \overset{\displaystyle O}{\|}}{CH_2OS - propoxur}}{C}H_{\underset{\underset{\displaystyle CH_2OS - propoxur}{\|}}{O}}$$

This compound was essentially non-toxic to house flies by topical application but showed moderate activity against the green rice leafhopper and brown planthopper as a residual spray. These results raise the possibility of applying derivatization reactions for the synthesis of polymeric insecticidal materials which may act as slow release agents.

Formamidines

There is evidence to indicate that chlordimeform [N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine] requires metabolic activation to demethylchlordimeform (DCDM) and that it is DCDM which is responsible for the behavioral and toxicological properties of chlordimeform (20).
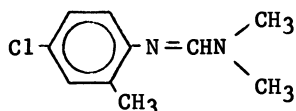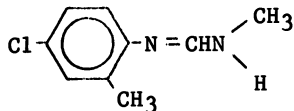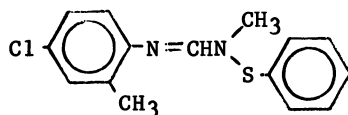

chlordimeform   demethylchlordimeform
                                          (DCDM)

Support for this is found in the substantially greater octopa-minomimetic activity of DCDM compared to chlordimeform (20) and the observation that mixed function oxidase inhibitors, e.g. piperonyl butoxide and sesamex, strongly antagonized the toxicity of chlordimeform to the southern cattle tick larvae and synergized the toxicity of DCDM (21). Therefore, chlordimeform may be considered to be a propesticide of DCDM.

Insecticidal and acaricidal formamidines such as DCDM possess a replaceable hydrogen and therefore are suitable for derivatization. DCDM and a number of related formamidines have been converted to the corresponding N-arylthio derivatives and these have been examined for activity against eggs, larvae and adults of insects and spider mites (22). In general, the N-arylthio derivatives retained the activity exhibited by the parent formamidine and in some cases were superior in activity. For example, the two spotted spider mite LC$_{50}$ (ppm) of the phenylthio derivative of DCDM is 6 compared to 12 for DCDM and 19

$$Cl-\langle\bigcirc\rangle-N=CHN\begin{smallmatrix}CH_3\\\\S-\langle\bigcirc\rangle\end{smallmatrix}$$
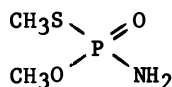
CH$_3$

phenylthio derivative

for chlordimeform (23). The phenylthio derivative was also superior to chlordimeform against certain insects, e.g. cabbage looper eggs and green peach aphid (22).
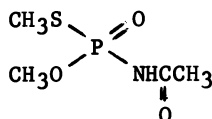
Needless to say, it would be interesting to apply the same type of chemistry described in Figure 2 for the methylcarbamate esters to the synthesis of derivatives of the formamidine insecticides. However, additional work with the formamidines, particularly those related to chlordimeform, has been discouraged because of the mutagenic and carcinogenic potential of the arylamine metabolic products.

Phosphoramidothioates

As in the case of the methylcarbamate esters, the organophosphorus insecticide methamidophos (O,S-dimethyl phosphoramidothioate) may be derivatized by substitution of a hydrogen atom on the phosphoramido nitrogen atom. An outstanding example of the benefit from this type of substitution is found in acephate, the
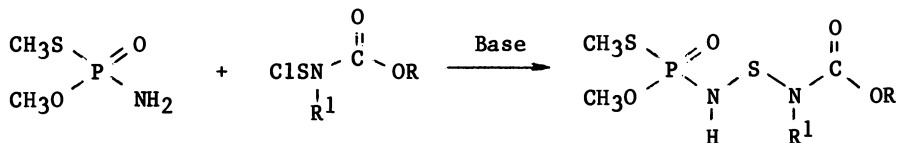
$$\begin{matrix}CH_3S\\CH_3O\end{matrix}P\begin{smallmatrix}O\\\\NH_2\end{smallmatrix}$$

methamidophos

$$\begin{matrix}CH_3S\\CH_3O\end{matrix}P\begin{smallmatrix}O\\\\NHCCH_3\\\ \ O\end{smallmatrix}$$

acephate

acetylated product of methamidophos, which is 45-fold less toxic to the rat than methamidophos while still retaining approximately the same insecticidal activity (24). In insects, acephate is converted into methamidophos which is believed to be responsible for poisoning (25).

The reaction between a phosphoramidothioate and N-chlorosulfenylcarbamate described in Figure 2 has been applied to methamidophos. In Figure 2, the reaction was used to derivatize a toxic methylcarbamate ester by a non-toxic phosphoramidothioate; however, in the case of methamidophos the reaction was used to derivatize a toxic phosphoramidothioate with a non-toxic carbamate moiety. The N-alkoxycarbonyl-N'-alkylaminosulfenyl derivatives of methamidophos thus prepared, where R
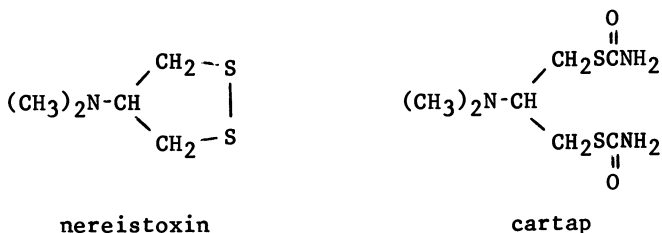
$$CH_3S\diagdown \diagup O \atop CH_3O \diagup P \diagdown NH_2 \quad + \quad ClSN \diagup \overset{O}{\overset{\|}{C}} \diagdown OR \atop \underset{R^1}{|} \quad \xrightarrow{\text{Base}} \quad CH_3S\diagdown \diagup O \atop CH_3O \diagup P \diagdown \underset{\underset{H}{|}}{N} \diagdown S \diagdown \underset{\underset{R^1}{|}}{N} \diagdown \overset{O}{\overset{\|}{C}} \diagdown OR$$

ranged from methyl to n-hexyl and R^1 ranged from ethyl to t-
butyl, all show good activity against house flies along with a 2-
to 3-fold reduction in mouse toxicity (26).

Attempts to react methamidophos with the corresponding
chlorosulfinylcarbamate intermediate, however, resulted in pro-
ducts which were unstable and of no value as proinsecticides. In
general, phosphoramidothioates such as methamidophos are more
difficult to derivatize than the methylcarbamate esters and
therefore are less useful as precursors to proinsecticides.

Nereistoxin

Nereistoxin or 4-(N,N-dimethylamino)-1,2-dithiolane is a
naturally occurring substance found in the marine annelid
Lumbriconereis heteropoda Marenz (27). Nereistoxin causes para-
lysis of insects by a ganglionic blocking action on the central
nervous system. Neurophysiological studies have shown that
nereistoxin suppresses the sensitivity of the nicotinic post-
synaptic membrane to acetylcholine. It is categorized as a non-
depolarizing neuromuscular blocking agent (28).

Cartap or S,S'-[2-(dimethylamino)-1,3-propanediyl]dicarbamo-
thioate is a proinsecticide of nereistoxin which was discovered
by analog synthesis following elucidation of the structure of
nereistoxin (27). In insects, cartap is rapidly converted into
nereistoxin by breakdown, probably hydrolytic, of the thiocar-
bamate moiety to eventually give nereistoxin (29). Nereistoxin
evidently binds to the acetylcholine receptor site (nicotinic
receptor) and blocks the depolarizing action of acetylcholine,
probably in a manner similar to that proposed for nicotine.

$$(CH_3)_2N\text{-}CH \diagup \overset{CH_2 -\, S}{\underset{CH_2 - S}{\diagdown} |} \qquad\qquad (CH_3)_2N\text{-}CH \diagup \overset{\overset{\overset{O}{\|}}{CH_2SCNH_2}}{\underset{\underset{\overset{\|}{O}}{CH_2SCNH_2}}{\diagdown}}$$

          nereistoxin   cartap

A variety of derivatives of 2-dimethylamino-1,2-propanedi-
thiol have been synthesized and evaluated as proinsecticides of
nereistoxin (29). The most prominent of these at present is
S,S'-[2-(dimethylamino)-1,3-propanediyl] bis(benzenethiosulfo-

nate) (TI-78 or bensultap) (30).   Bensultap has shown excellent
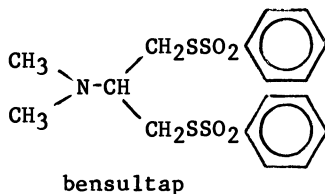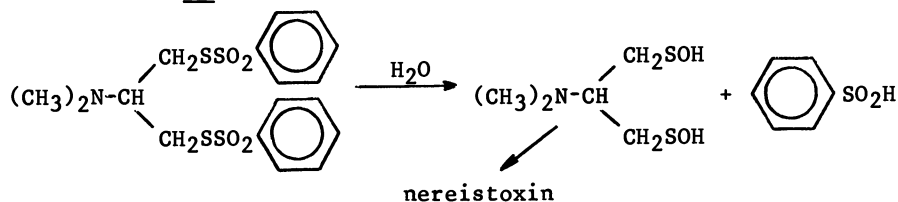

bensultap

activity against many insects as a contact and stomach poison and
is especially effective against the Colorado potato beetle, rice
stem borer and different lepidopterous larvae. Bensultap, with
an acute rat oral LD_{50} of 1120 mg/kg, is relatively non-toxic to
mammals. The acute rat oral LD_{50} of cartap is 325-345 mg/kg and
the acute mouse oral LD_{50} of nereistoxin is 118 mg/kg.
     In searching for other proinsecticides of nereistoxin it
must be kept in mind that the compound must possess labile groups
bonded to the thiolsulfur atoms so that nereistoxin may be formed
readily. Cartap and bensultap represent two types of compounds
which may be transformed into nereistoxin by two different pro-
cesses. A plausible mechanism for cartap is split of the S-C(O)
bond by attack of a nucleophile, e.g. water, on the carbonyl car-
bon atoms, generating the 2-dimethylaminopropanedithiol which is
then oxidized to nereistoxin.
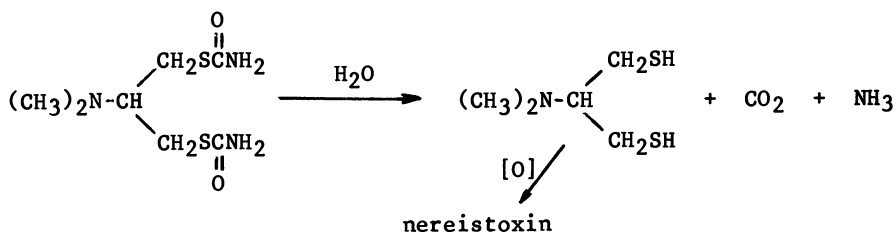
nereistoxin

In contrast, attack of water on bensultap should take place
through a bis-sulfenic acid intermediate to eventually give
nereistoxin (30).

nereistoxin

Thus, the thiol sulfur may act as either a leaving group in car-
tap or as an electrophile in bensultap on route to the formation
of nereistoxin. Therefore, the number of nereistoxin analogs
which may be prepared for insecticidal evaluation is very large.
This includes the possibility of derivatives formed from the
reaction between 2-dimethylamino-propane-1,3-dithiol and the
chlorosulfenyl and chlorosulfinyl intermediates described in
Figure 2.

<u>Summary</u>

In the preceding discussion, examples are given with four dif-
ferent classes of insecticides, i.e., the methylcarbamate esters,
formamidines, phosphoramidothioates and nereistoxin analogs
where conversion to proinsecticides has resulted in products with
improved toxicological properties. Derivatization of active in-
secticides provides an easy means of generating a variety of new
compounds with different kinds of insecticidal activities and
some of these possibilities are described. The type of chemistry
used for derivatization of methylcarbamate insecticides should
have wide applicability to other types of pesticides and biologi-
cally active compounds, including drugs.

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Aspects of the Biochemistry and Toxicology of Octopamine in Arthropods

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The occurrence, physiological significance, and biochemistry of octopamine (1-[4-hydroxyphenyl]-2-aminoethanol; OA) in arthropods are reviewed with emphasis on the applications of this information to the discovery of new pesticides. The functions of OA in arthropods parallel those of norepinephrine in vertebrates, with probable involvement in stress responses, arousal, and feeding. The best current prospect to disrupt these functions is to design compounds that affect octopamine receptors. Recent results on the structure-activity relations (SAR) of three groups of OA agonists, phenylethylamines, amidines, and imidazolines are presented. These cause symptoms in arthropods congruent with stimulatory actions on OA receptors. A variety of OA antagonists are known, but the data are currently insufficient for SAR analysis or to indicate whether such compounds may be useful for the control of arthropods in the field.

Prominent among the approaches to pesticide discovery which can legitimately be termed rational is biochemically-aided design. This approach is based on the thoughtful selection of a biochemical target site in a group of important pests, the study of its properties, and the synthesis of compounds which might be capable of interfering with its normal functions. Such an approach is increasingly interesting, not because of its inherent high probability of success or ease of pursuit, but because of the decreasing efficiency of traditional synthesis and screening approaches, particularly in the area of arthropod control agents (1-3), and the increasing sophistication of our knowledge of pest physiology and biochemistry.

Desirable Characteristics of a Biochemical Target

Some of the most important considerations in selecting a target for a biochemically-aided design program are presented in Table I.

Table I.   Important Criteria in Choosing a Biochemical Target

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1. Target is likely to be essential for the survival of one or more economically important species.
2. Target has been 'validated'.
3. Rapid response to disruption of target is predicted.
4. High potential for selectivity/low vertebrate toxicity.
5. Low potential for resistance.
6. Compounds are known which can affect the target and act as leads for new synthesis.
7. Pharmacokinetics of lead compounds are favorable.
8. Convenient assays for in vitro and in vivo activity exist.
9. Background biochemistry and physiology of target system is reasonably well understood.
10. Concept is novel.

All these requirements are desirable in a proposed target, but not all are critical. Some are certainly more important than others, and no target system is likely to satisfy all of them. Most are self-evident, but others deserve a word of explication. In regard to item 2, a 'validated' target is one where there is direct or good indirect evidence that if compounds are found that can disrupt the target, they will have a potent physiological effect which can be translated into effective arthropod control under field conditions. These effects may kill the insect or other arthropod directly (insecticides) or may prevent damage by inducing non-lethal effects such as altered behavior (insectistats). For example, compounds which inhibit acetylcholinesterase certainly act on a validated target, although they may be subject to excessive resistance problems, lack intrinsic selectivity, and this is not a novel target. On the other hand it is possible to design inhibitors for the enzyme which synthesizes acetylcholine, choline acetyl-transferase, but it is not clear that such inhibitors would yield successful control agents. In fact there is evidence that they may not (4); choline acetyltransferase is a target which has not yet been validated.

The need for lead compounds (item 6) is a particularly critical one since our ability to design compounds to affect enzymes, receptors, or other biochemical targets ab initio is very limited. However, there is an important constraint in respect to the physical properties of lead compounds. The need for favorable pharmacokinetics (item 7) is a factor which may be overlooked.

Simply put, potential toxicants must be able to get to the target
site within the insect. The insect integument is generally
impermeable to polar compounds. If compounds must act within the
central nervous system (CNS), there are additional penetration
barriers that exclude charged and highly polar compounds from the
synaptic areas in the neuropile. It is therefore important in
choosing a target to consider whether lead compounds are known that
will penetrate to the site of action. There are several examples
where the lack of such favorable leads has impeded progress in
discovering effective control agents. One of these is glutamate as
the excitatory transmitter at the insect neuromuscular junction.
Active compounds so far have contained at least three polar groups
and few have shown high potency (5). Further consideration of the
desirability, strategies and problems associated with the biochem-
ical design of pesticides will be found in references 2,3,6 and 7.
    It is the purpose of this chapter to discuss the neuroeffector,
octopamine, and its related biochemistry in the arthropod nervous
system as a target area for the rational discovery of control
agents in the light of the criteria listed in Table I.

The Occurrence of Octopamine in Arthropods and Vertebrates

Octopamine (OA) is 1-(4-hydroxyphenyl)-2-aminoethanol (Figure 1).
As such it is one of several biogenic amines which are regarded as
established or potential neuroeffectors in invertebrates (8,9). The
close resemblence of OA to its catecholamine analog, norepinephrine
(NE: Figure 1), is notable. Norepinephrine and its N-methyl analog,
epinephrine (E), are known to have a number of important functions
in vertebrates. In the peripheral nervous system, NE is the major
transmitter at the sympathetic effector junctions, while in the
central nervous system (CNS) it is believed to influence such
states as alertness, hunger, learning and memory, and emotional
responsiveness (10). Epinephrine acts as a circulating hormone that
increases blood pressure through cardiac stimulation and vasocon-
striction in response to excitation and stress. It also increase
metabolic rates in several tissues, leading to hyperglycemia,
hyperlipemia and increased oxygen consumption.
    Whereas NE is abundant in the vertebrate nervous system, OA is
relatively scarce. The reverse is true in insects where NE is
thought to be absent or present only at low concentration (8). It
has been suggested (9,11,12) that OA carries out functions in these
invertebrates which parallel those of NE and E in vertebrates. Al-
though it is not possible to say that OA has no important functions
in vertebrates, such functions have yet to be established (13). If,
as it now seems, OA is a much more critical neurotransmitter in
arthropods than mammals, there is reason to hope that compounds
which affect octopaminergic systems will show some degree of
selective toxicity between insect and mammals, although the rather
slight structural difference between OA and NE indicates that this
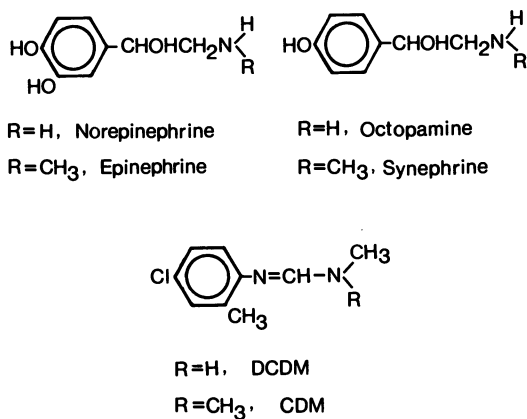window for selectivity may not be a wide one.

R=H, Norepinephrine   R=H, Octopamine

R=CH₃, Epinephrine   R=CH₃, Synephrine

R=H, DCDM

R=CH₃, CDM

Figure 1. Structures of octopamine, norepinephrine, the pesticide/pestistat chlordimeform (CDM), and related compounds.

The Functional Significance of Octopamine in Arthropods

The known and postulated actions of OA in arthropods have been the subject of several recent reviews (8,9,12,13) and will be mentioned here only in as far as it helps to indicate the kinds of responses which may occur when octopaminergic drugs are presented to an arthropod, and how some of these responses may be utilized to develop assays for the discovery and evaluation of such agents.

The neuroeffector roles of OA may be broadly classified as those in which it acts as a neurotransmitter, as a neuromodulator, and as a neurohormone (12). The distinction between these actions is not absolute, but a neurotransmitter, released into a synapse, tends to have a rapid, highly localized action on a neighboring cell, while a neurohormone tends to have a slower, more prolonged action on a large number of cellular elements, often at a considerable distance from the point of its release. A neuromodulator is a neurohormone, released locally or at a distance from its site of action, that regulates the excitability of another nerve, muscle or gland cell.

Firefly lantern. One probable action for OA as a neurotransmitter is in the lanterns of larval and adult fireflies (9,12,13). Here it is likely that OA acts as the excitatory transmitter which causes the lantern photocytes to glow. This has proved to be a useful system for testing the octopaminergic actions of compounds both in vivo and in vitro. The lighting response is easily seen and can be evaluated either by a visual rating scale (14) or recorded more quantitatively and permanently with a simple photocell attached to a strip chart recorder (14,15). Perfused tails and isolated light organs also respond by lighting (15), but so far have proved difficult to develop as a quantitative and reproducible tool to evaluate octopaminergic potency. The receptors for OA are linked to an adenylate cyclase system which may be used to assay for octopaminergic activity in broken cell preparations from adult (15,16) or larval (17) lanterns. Freeze-dried adult lanterns can be stored at $-20°C$ for many months with very little loss of this OA-sensitive adenylate cyclase activity. Unfortunately, adult fireflies are seasonal in occurrence and cannot be conveniently reared in the laboratory, so that in vivo testing is limited to the summer months. However, the larvae of some species can be collected in the fall and held in the laboratory for long periods (18). These offer a more regularly available source for bioassays. Tests with whole insects may be necessary to detect prodrugs which act on octopaminergic systems with greater potency after metabolic activation e.g. the formamidine insecticide/insectistat chlordimeform (CDM, Figure 1), which is N-demethylated in vivo to the more potent octopaminergic agonist, DCDM. In vitro methods such as adenylate cyclase assays will not detect such pro-compounds, or will seriously underestimate their true in vivo potency.

Locust corpus cardiacum. A second system in which evidence for a
neurotransmitter role for OA is persuasive resides in the corpus
cardiacum of the locust. Orchard and his coworkers (12,19) have
shown that hyperlipemic hormones that elevate blood lipid levels
are released from neurosecretory cells in the glandular lobe of the
cardiacum by an OA-dependent mechanism. Hormone is released by
exogenously-applied OA at concentrations (0.1 μM) which can arise
in the hemolymph of excited animals, thus suggesting that
circulating OA may initiate hormonal release. However, in addition
to this possible neurohormonal action of OA, there is also
excellent evidence that specific neurones that arise in the brain
and innervate the gland cells are octopaminergic and that OA is the
normal neurotransmitter by which they excite the gland cells to
release hormone. Recently it has been shown that OA mimics the
effect of stimulation of the nerves innervating the glandular lobe
by inducing an increase in its cyclic AMP (cAMP) level, and that
exogenously applied cAMP analogs also cause hormone release (20).

Locust DUMETi neurone. Octopamine also acts as an effector at some
neuromuscular sites in the locust. It is synthesized and stored in
neurones whose cell bodies are located in the dorsal midline of the
metathoracic ganglion. These neurones branch bilaterally to
innervate the large extensor tibiae muscles of the metathoracic
(jumping) legs (9,11,12). Because of their location and morphology
these are termed DUMETi cells i.e. dorsal unpaired median (DUM)
cells innervating the extensor tibiae (ETi) muscle. In this muscle
it seems very likely that glutamate acts as the excitatory
transmitter for both the slow and fast contractile response
systems, while GABA acts as an inhibitory transmitter. Octopamine
is released from blind-ending terminals of the DUMETi neurone in
proximity to the ETi muscle and has two quite distinct effects on
it. A small group of fibers in the proximal part of the muscle
undergoes an intrinsic rhythmical contraction, the function of
which is unknown, but which might aid in the pumping of hemolymph
down the narrow distal parts of the leg (9). Stimulation of DUMETi
or the application of OA to this proximal bundle causes the
rhythmical contractions to cease. No such obvious effects are seen
if OA is applied to the rest of the muscle. However if OA is
present when the slow extensor motorneurone (SETi) is stimulated,
several changes in the response of the muscle are observed in
comparison to the situation when OA is absent. These OA-induced
alterations include an increase in the twitch tension and in the
relaxation rate of the muscle, and a change in its 'catch tension'.
As a result of the action of OA, the muscle response becomes less
adapted for the maintenance of body posture while the insect is
immobile and more suitable for locomotor movements (9,21). The
inhibitory effect of OA on the contractions of the proximal bundle
can also be interpreted as an adaptation of the muscle for
controlled movement. In this regard, OA is acting as a
neuromodulator rather than as a neurotransmitter. These dual
responses of the ETi muscle are relatively easy to observe and make

a convenient assay system for putative octopaminergic agents once
the experimental animal is available. Similar systems probably
exist in insects other than the locust, but their biochemistry and
functions have not been investigated extensively (9).

Octopamine as a neurohormone. Octopamine has a number of actions
which would fit it for a neurohormonal role in insects e.g. it
increases the heart rate and stimulates metabolic rates in nerve
and muscle tissues (9,12). However, it remains to be established
that such events occur in the living insect as a result of the
release of OA into its circulatory system. One example of a
neurohormonal role for OA is better established. When locusts are
excited by handling or injection a rapid rise in blood OA occurs
followed shortly by a prolonged elevation of lipid in the blood
(12). Although the site(s) of release of the OA are not certain, it
appears that the lipid is released from the fat body. When applied
to the isolated locust fat body, OA causes an elevation of cAMP in
the tissue and a release of lipid into the medium. The level of OA
in the blood of excited locusts exceeds the threshold needed to
cause lipid release from the fat body in vitro ($0.2 \mu M$) (22). Thus
it is reasonable to suppose that the circulating OA in the stressed
animals activates receptors on the fat body cells, and the
resulting rise in cAMP causes the cells to release lipid. As
pointed out previously, it is also likely that the elevated OA
level during stress enhances the release of hyperlipemic
hormones from the corpus cardiacum which would then cause an
additional increase in blood lipid levels. A parallel situation
exists in the early stages of flight in the locust when blood OA is
elevated and lipid is released to the hemolymph as a fuel source
before any rise is seen in the level of hyperlipemic hormones (12).
Another parallel is found in the American cockroach where excita-
tion and stress cause an elevation in blood sugar levels, probably
through an OA-induced release of trehalose from the fat body (23).
It is noteworthy that in the lobster, OA is released from the
second roots of the thoracic ganglia into the hemolymph where it is
believed to initiate a number of neurohormonal responses (24,25).

Octopamine in the CNS. It is reasonable to suppose that this
handful of varied systems where OA has effector functions is only
the tip of the iceberg and that more will be discovered. All of
these known systems are located peripherally since the demonstra-
tion of a specific transmitter role for any compound within the CNS
is very challenging. However, there is every reason to believe that
OA has important transmitter or modulator functions in the CNS of
arthropods. It is synthesized and stored there in plausible amounts
(8,9,13) and OA-sensitive adenylate cyclase activity has been found
within the CNS of several arthropods. In addition to the examples
cited in the recent review by Bodnaryk (26), this activity has been
demonstrated in the tobacco hornworm (27,28), Drosophila
melanogaster (29), the tick, Amblyomma hebraeum (27), and the
crayfish (30,31). Neurones specifically sensitive to OA have been

observed in the CNS of the American cockroach (32), and ligand binding studies have shown that receptor-like binding sites for OA are present in the head of Drosophila (33). The further development of such binding assays would provide a very desirable tool for the rapid screening of compounds which bind to OA receptors.

Although the functional significance of OA in the invertebrate CNS is unknown, a number of behavioral responses are seen when OA or its mimics are injected, which again suggest that it has specific functions e.g. honeybees show enhanced responsiveness to olfactory stimuli after injection of OA into the brain (34). Octopamine causes anorexia (a central response) in the cockroach after injection into the hemocoel (35). On the other hand, black blowflies develop a decreased threshold for the intake of sucrose solutions and consume a much greater amount after the injection of OA and related compounds (36). Formamidines and imidazolines, which mimic OA, cause a range of behavioral effects in insects and acarines, most frequently involving increased excitation and locomotor activity, that are likely to arise through actions in the CNS (37). Although we do not yet understand the exact genesis and specificity of these behavioral responses, some, such as flight excitation or disruption of oviposition patterns in lepidopterans, or the blowfly feeding responses, may prove to be useful in assaying compounds believed to have octopaminergic actions.

Crayfish Behavioral Assay. A problem which hampers the study of the central effects of some octopaminergic agents, including OA itself, is that the insect CNS is protected from charged compounds by an ion barrier system (38). Insects also may have well developed mechanisms for removing circulating biogenic amines from the blood (9,39). This explains why in many cases very high levels of OA must be injected into the insect's hemolymph in order to see any behavioral response. The same is not necessarily true for other arthropods e.g. several crustaceans have been shown to have poorly developed blood-brain barriers of this type (40). Also, in contrast to insects, crustaceans such as the crayfish have an extensive arterial system that penetrates and ramifies within the CNS, carrying blood directly to the synaptic regions in the neuropile (41). Compounds injected into the pericardial sac surrounding the heart are therefore rapidly carried deep into the CNS, and such crustaceans offer considerable advantages compared to insects in assessing the central effects of aminergic agents. Kravitz and coworkers (42,43) injected OA into crayfish and lobsters in this way, and observed a rapid response characterized by a raising of the tail, a change in posture of the legs, activation of the swimmerets, and, in some cases, defecation and regurgitation. These investigators also observed that 5-hydroxytryptamine caused an opposing response where the tail is curled tightly under the body. These responses probably arise from the effects of the amines on the CNS, perhaps at the level of 'command fibers' which are able to program complex co-ordinated behavioral responses (43).

We have attempted to characterize this response further and to define its pharmacology and its utility as a rapid in vivo assay for octopaminergic agents, particularly for actions on the CNS (31). The response to OA is specific in that none of the other putative neuroeffectors tested induced a comparable effect with the exceptions of NE and dopamine, both of which are known to have octopaminergic actions of their own. The response to OA was blocked by typical octopaminergic antagonists such as phentolamine, metoclopramide and mianserin (Table II).

Table II. Potency of Octopamine and DCDM in the Crayfish Behavioral Assay and the Effect of Selected Octopaminergic Antagonists on their Action.

Antagonists	Dose[a] (mg/kg)	Octopamine		DCDM	
		ED50[b] (mg/kg)	% Change in ED50	ED50[b] (mg/kg)	% Change in ED50
None	--	0.81 $\pm$ 0.29	--	1.44 $\pm$ 0.40	--
Mianserin	1.25	7.80 $\pm$ 1.86	+875	2.36 $\pm$ 0.35	+64
Phentolamine	10	3.21 $\pm$ 0.33	+601	2.19 $\pm$ 0.46	+56

[a] Antagonists injected 3 minutes before agonist.
[b] Concentration for 50% response to agonist. Mean $\pm$ SD for four independent determinations. ED50 after antagonist treatment is significantly higher than control ED50 at $P = 0.05$ in every case.

As discussed later, two other groups of compounds which induced this response were the amidines and imidazolines. These compounds are known to be octopaminergic agonists in other invertebrate systems. The behavioral action of the formamidine, DCDM, closely resembled that of OA and was also antagonized by the compounds which blocked the effect of OA, although they were less effective against DCDM (Table II). In conjunction with the up-and-down assay method of Dixon (44), the crayfish assay can give an estimate of the ED50 for this octopaminergic behavioral response with as few as six animals in about one hour.

General Functions of Octopamine in Arthropods. The actions of OA in invertebrates therefore are multiple and probably involve both central and peripheral sites. Moreover, as pointed out previously, many of the known actions of OA are comparable to those of NE and E in the vertebrate central and sympathetic nervous systems. These multiple effects are concerned with arousal and stress responses, increasing the responsiveness to outside stimuli, and alerting the resting animal and priming it for action and movement. It is interesting, though inconclusive, in this context to note that ants

with low endogenous activity rates have low OA levels in the brain
while those which are naturally hyperactive have higher than normal
levels (45). A related observation has been made with a mutant
strain of Drosophila melanogaster (46) that has a specific
deficiency in octopamine biosynthesis in the brain. These mutants
show abnormal diurnal activity rhythms. Furthermore, many of the
behavioral effects described in insects, mites and ticks exposed to
formamidine pesticides may be related to an underlying increase in
excitability and locomotor activity (37,47). A second area in which
OA and octopaminergic agents appear to play a role is in the
control of hunger and feeding responses in insects. Reports so far
indicate both an anorectic effect (cockroach; 35) and increased
feeding and water consumption (blowfly; 36). Interestingly, NE in
the mammalian CNS is also believed to have a role in governing
hunger, and has been shown capable of either increasing or
decreasing food and water consumption depending on the type of NE
receptor affected (48).

Biochemistry of Octopamine

If one accepts that octopaminergic events in arthropods provide
interesting possibilities for the biochemically-aided design of
pesticides and pestistats, an immediate question is how this
dependence on OA may best be exploited. The diagram in Figure 2
shows the general biochemical events believed to important for
octopaminergic transmission. The major elements in Figure 2 are:

1. Biosynthesis of OA from aromatic amino acids.
2. Storage of OA in the presynaptic terminal.
3. Release of OA into the synaptic cleft (or hemolymph in the case
   of a neurohormone).
4. Interaction of OA with a specific receptor located on the
   surface of the responding cell or on the presynaptic membrane
   of the releasing cell.
5. A sequence of biochemical responses to this interaction. In the
   cases so far studied, the initial biochemical response to stim-
   ulation of OA receptors is an increase in adenylate cyclase
   activity which in turn leads to a temporarily enhanced level of
   intracellular cAMP. The elevation of the cAMP level in its turn
   leads to a cascade of responses which eventually alter the
   physiological status of the receptive tissue. The cAMP is de-
   stroyed by phosphodiesterase activity which, in part, is re-
   sponsible for limiting and terminating the response (5A in
   Figure 2). This model of the mechanism by which stimulation of
   the OA receptor leads to a response is not the only one pos-
   sible, although others, such as a reduction in cAMP synthesis,
   altered synthesis of cyclic GMP, or the direct opening of an
   ionophore channel through the postsynaptic membrane, have not
   yet been clearly demonstrated with OA in arthropods. By analogy
   with other aminergic synapses, presynaptic autoreceptors may be
   involved in the feedback regulation of further transmitter
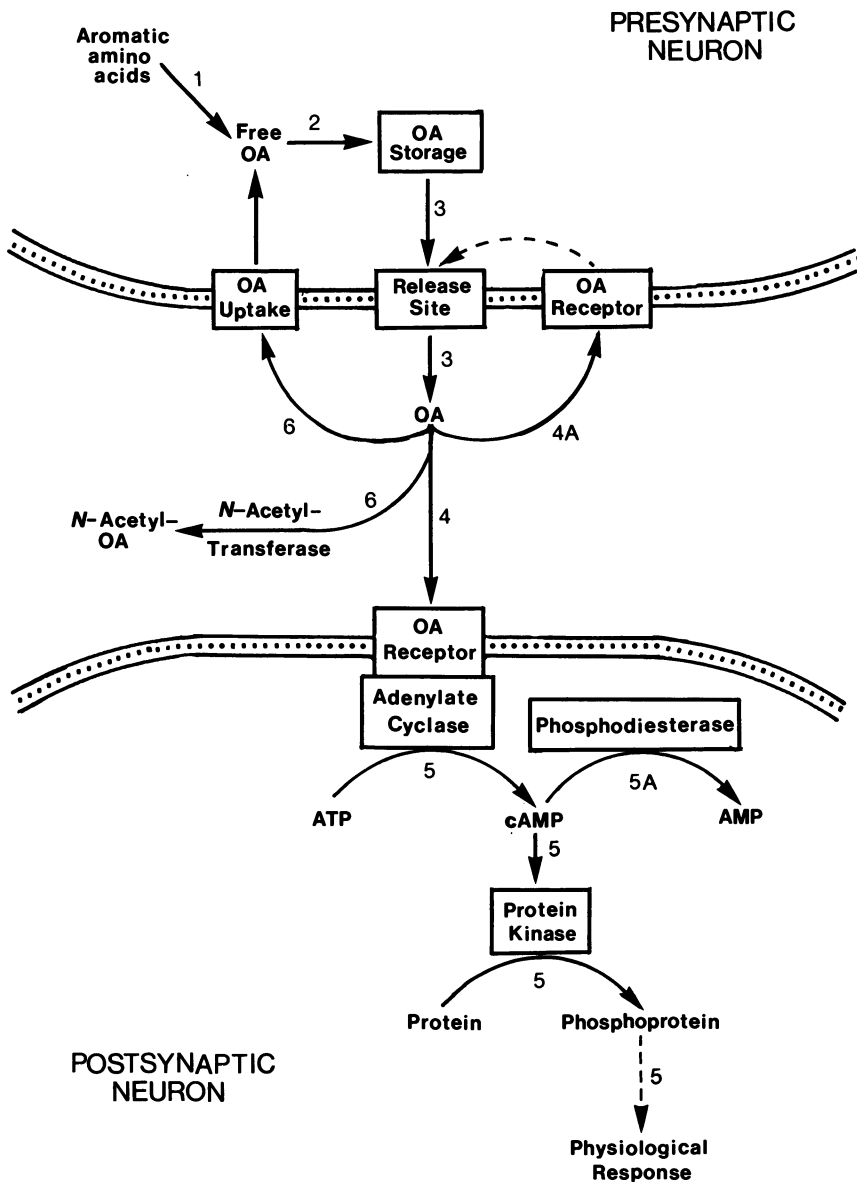
Figure 2. Diagrammatic representation of the biochemical components of an octopaminergic synapse mediated by cyclic AMP. Reproduced with permission from Ref. 37. Copyright 1982, Academic Press.

release (4A in Figure 2), but this also has yet to be demon-
strated with octopamine in arthropods.

6. The removal of OA from the region of the receptor by diffusion,
reuptake into neighboring cells, and subsequent metabolism e.g.
by N-acetylation or perhaps by the action of monoamine oxidase
in the Malpighian tubules.

Each of these events in theory might be disrupted so that octo-
paminergic transmission is either prevented or augmented. However,
some possibilites are more attractive than others e.g. the role of
the N-acetyltransferase in the removal of OA is not well studied,
but it seems likely that it is a significant one (9,49), and that
in this regard insects are different from vertebrates where the
N-acetylation of biogenic amines is not a major means for their
destruction. Inhibition of this process therefore offers some hope
for selective toxicity. Unfortunately, relatively little is known
regarding this enzyme activity and no specific inhibitors are
available. This problem plagues most of the other possible
approaches to disrupting specific steps in octopaminergic trans-
mission - there is a paucity of data on their properties and
significance to the organism, and little or no information on what
types of chemicals may provide leads for further study and develop-
ment. For example, currently there is only one published study on
the uptake of OA by insect nervous tissues (50). In this case, up-
take by the cockroach CNS appeared to have pharmacological
properties comparable to those of related systems for the uptake of
biogenic amines in vertebrates, but this may partly reflect the
fact that in such studies on arthropods the pharmacological agents
assayed are inevitably drawn from compounds found to be active in
prior mammalian studies. Similarly it has been shown that methyl-
xanthine inhibitors of phosphodiesterase synergize the activity of
octopaminergic agonists in their ability to excite locomotor ac-
tivity in lepidopterous adults (28) and lantern illumination in the
firefly (51), perhaps through enhanced elevation of cAMP levels.
However, in these cases also, the inhibitors employed were those
familiar from previous mammalian studies and cannot be expected to
be selective. The same criticism can be made of the limited studies
with compounds reputed to deplete biogenic amines including OA from
their presynaptic storage sites, such as reserpine (14,20) or
amphetamine (14). These are effective in insects but not selective.

Another strategy of some interest is to deplete biogenic amines
such as OA by inhibiting their biosynthesis. Inhibitors of such
enzymes in the biosynthetic pathway as aromatic amino acid
decarboxylase which converts tyrosine to tyramine, or dopamine
β -hydroxylase which converts tyramine to OA are known and have
interesting effects in insects (e.g. see 52,53), but a discussion
of this area lies outside the scope of this paper. Nevertheless, it
is a particularly interesting one since these or related enzymes
are also needed to produce catecholamines for cuticular sclerotiza-
tion, thus offering dual routes to the discovery of compounds with
selectively deleterious actions on insects.

Octopamine Receptors

The only aspect of octopaminergic transmission for which a
relatively large amount of structure-activity data is available
relates to the properties of OA-receptors themselves. Agonists
that stimulate these systems, and antagonists that block them,
are known and such compounds exist in several structural groups.
The three major groups of agonists currently identified are
phenylethylamines, amidines, and imidazolines.
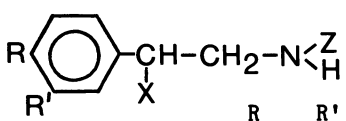
Phenylethylamines. The properties of phenylethylamines have been
investigated in some detail as stimulators of cAMP synthesis in
homogenates of the CNS of the American cockroach (54,55), in the
crayfish behavioral assay (31) and as inhibitors of the myogenic
contraction of the locust ETi muscle (56), as shown in Table III.
    It is very important to note that none of these assays measures
the interaction with the receptor directly and each may be subject
to interferences from such factors as competition from amine uptake
and metabolic systems, the presence of penetration barriers, and
possible indirect effects on the receptor by release of natural
amines from presynaptic storage sites. This is particularly true
for the behavioral assay with the crayfish. The results therefore
cannot be taken as a completely reliable guide to the relative
activity of these compounds at the OA receptor. However, the three
studies show a number of similarities as well as some differences.
    On comparing the activities of the five compounds for which
numerical estimates are available in all three assays (synephrine,
octopamine, phenylethanolamine, norepinephrine and tyramine) the
rank orders of potency in the three systems are : Crayfish,
1,2,3,4,5; Cockroach, 2,1,3,4,5; Locust 1,2,3,5,4. This indicates a
basic similarity in the responses of these preparations. In each
case it was found that ring hydroxylation of the phenylethylamine
nucleus was not essential for activity, although p-hydroxylation
does yield the best activity. This is particularly evident in the
crayfish study where α-MAMBA (α-methylaminomethyl benzyl alcohol),
the analog of synephrine which lacks ring substitution, was one of
the most active compounds tested, and β-phenylethanolamine, the
corresponding analog of OA, is almost as active as OA. The base
compound for this series, phenylethylamine, also shows appreciable
activity, but only in the crayfish assay.
    In every case and with each assay, β-hydroxylation improved
activity (X = OH in Table III) e.g. compare tyramine with
p-octopamine or phenylethylamine with phenylethanolamine. With the
crayfish and locust, N-methylation also improved potency with both
octopamine (yielding synephrine) and norepinephrine (yielding
epinephrine). This situation is often found in octopaminergic
systems (9,57), but was not observed with the cockroach CNS in this
study. Extending the N-alkyl group to i-propyl (isoproterenol, a β
-adrenergic agonist) completely eliminated activity in the crayfish
and cockroach studies.

Table III. Comparative Activity of Substituted Phenylethylamines
           in Three Octopamine-sensitive Systems from Arthropods.

R—C₆H₄(R')—CH(X)—CH₂—N(Z)(H)					Crayfish behavior ED50 (mg/kg) [a]	Cockroach CNS cAMP EC50 (μM) [b]	Locust ETi Inhibn. (%) [c]
	R	R'	X	Z			
Synephrine (dl)	OH	H	OH	Me	0.22	11	100
						d	d
α-MAMBA (dl)	H	H	OH	Me	0.26	ND	ND
						e	
Epinephrine (l)	OH	OH	OH	Me	0.65	(<50%)(dl)	52.1
p-Octopamine (dl)	OH	H	OH	H	0.81	2.7	100
Phenylethanolamine	H	H	OH	H	0.84	32	100
Phenylethylamine	H	H	H	H	1.50	(<50%)	0
Norepinephrine (l)	OH	OH	OH	H	3.35	240 (dl)	20.9
Dopamine	OH	OH	H	H	3.60	ND	11.4
Tyramine	OH	H	H	H	8.90	290	36.9
m-Octopamine (dl)	H	OH	OH	H	11.10	(<50%)	ND
Normetanephrine (dl)	OH	OMe	OH	H	ND	66	27.5
Isoproterenol (dl)	OH	OH	OH	i-Pr	>20	(<50%)	ND

[a] Data from Ref. 31
[b] Data from Ref. 55
[c] % Inhibition of ETi myogenic rhythm at 1 μM; data from Ref. 56
[d] ND = Activity not determined
[e] Maximum response was less than 50%
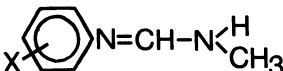
     A problem in trying to use these results in developing
potential pest control agents is that none of these compounds is
likely to penetrate either the cuticle or the CNS of insects
effectively, since all are fully ionized at physiological pH.
Derivatization of the polar groups would be one possible solution
to this problem. The formamidines and imidazolines generally do not
suffer from this problem since they have pKas which are lower than
those of the related phenylethylamines and thus are at least partly
in the free base form at pH 7.

Amidines. After the initial observation that formamidines are
agonists for OA receptors in the firefly (14,15,58), these
compounds have been shown to mimic OA in virtually every insect
system known to contain OA receptors, including the DUMETi system
(59), the glandular lobe of the corpus cardiacum (60) and the fat
body (61) of the locust, and various CNS adenylate cyclase
preparations (26-28). The structure-activity relations of amidines
as octopaminergic agonists have been examined in several systems
including adenylate cyclase stimulation in the firefly lantern and

the CNS of Manduca sexta (27), the excitation of locomotor activity
in adult Heliothis virescens (27,28) and the crayfish behavioral
assay (31). The results from these systems are in general
agreement, although some differences exist, particularly in
comparing the two in vitro systems with the crayfish responses.
   The general requirements for activity either in the stimulation
of firefly tail adenylate cyclase activity or in the crayfish
behavioral test are that the compound should be a formamidine.
Substitution on the amidine carbon greatly reduces activity. The
amino nitrogen atom should be either unsubstituted, or, preferably,
monomethylated. Longer alkyl groups or disubstitution are generally
unfavorable. From Table IV it can be seen that, in the
N-methylformamidine series, the ring substituents are optimal when
they are 2,4-located alkyl groups or halogens. These are also the
general requirements for compounds which are active in practical
pest control in the formamidine series (62), a fact that strongly
supports the hypothesis that a significant part of the action of
formamidines in the field arises through the stimulation of OA
receptors. In the crayfish behavioral assay, these requirements
hold except that in one case (2-Cl analog) monosubstitution of the
ring gave an active compound, and 2,3-disubstitution also yields an
active material when the ring substituents are methyl groups.

Table IV.   Stimulation of Adenylate Cyclase and Arthropod Behavior
            by Substituted-Phenyl N-Methylformamidines.

$X-\langle C_6H_4\rangle-N=CH-N\langle^H_{CH_3}$ X	Adenylate Cyclase (% increase over basal rate)		Behavioral Excitation (ED50, mg/kg)	
	Firefly[a]	Manduca[b]	Heliothis[c]	Crayfish[d]
2-Me,4-Br	659±213	53±18	1.26	--
2-Me,4-Cl	559±172	61±10	0.86	1.44
2,4-diMe	353±128	33±9	4.32	0.52
2,4-diCl	269±75	37±11	4.79	1.90
4-Cl	69±44	--	20	>40
2-Me,5-Cl	59±47	9±6	34	5.52
2-Me	56±47	--	21	4.96
2-Cl	50±16	5±4	--	0.48
4-Me	41±25	6±6	--	--
3,4-diCl	41±34	8±5	>100	--
2,3-diMe	39±23	6±5	69	1.57

[a]Formamidines at 1.0 μM.
[b]Formamidines at 0.02 μM.
[c]Induction of locomotor activity.
[d]Behavioral syndrome.
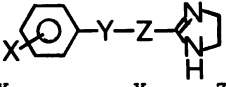
Imidazolines. As shown in Tables V and VI, the data so far available for the imidazoline series are too incomplete to allow many structure–activity generalizations, but some interesting relationships do appear. Apparently more structural variations are possible for these agonists than is the case with the formamidines. Ring substituents do not have to be located in the 2,4–positions; 2,3–disubstitution produces highly active compounds also (Table V). Activity is also found whether there are one or two atoms bridging the phenyl and imidazoline ring systems. The nature of these bridging atoms does not appear to be critical, although it is likely that more extensive comparisons would reveal that some combinations are more favorable than others. In addition to the stimulation of adenylate cyclase, these imidazolines also elicit formamidine–like behavioral responses in Heliothis and crayfish. Their potency in the crayfish assay often considerably exceeds that of OA or DCDM, but they are notably less active than DCDM in stimulating locomotor activity in Heliothis. This is in agreement with their lower potency than DCDM in stimulating adenylate cyclase activity in the CNS of another lepidopteran species, Manduca, but it is still to be proved that this type of locomotor stimulation is a direct result of actions on OA receptors.

Table V. Stimulation of Adenylate Cyclase and Arthropod Behavior by Selected Substituted–Phenyl Imidazolines.

X=	Y=	Z=	Adenylate Cyclase (% over basal rate)[a]		ED50 (mg/kg) Behavioral Excitn.	
			Firefly tail	Manduca CNS	Heliothis	Crayfish
2,6–diCl	NH	—	19	18.3	80	2.74
3,4–diCl	NH	—	122	—	—	—
2–CH$_3$,4–Cl	NH	—	345	—	—	—
2,3–diCH$_3$	NH	—	277	28.7	>300	7.34
2,4–diCH$_3$	NH	CH$_2$	135	16.1	330	0.67
2,3–diCH$_3$	NH	CH$_2$	642	73.8	13	0.015
2,6–diCl	O	CH(CH$_3$)	92	28.1	110	0.82
2,3–diCH$_3$	O	CH(C$_2$H$_5$)	674	—	—	—
DCDM			368	121.5	0.86	1.44
Octopamine			157	38.4	—	0.81
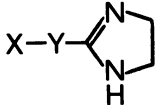
[a]Compounds assayed at concentration of 1 µ M

More limited data are available for the interactions of imidazolines with the locust ETi muscle (Table VI), but some compounds show extremely high potencies in the inhibition of the myogenic contractions of the proximal bundle. In some cases the

imidazolines are considerably more potent than OA in these assays, although relative potencies vary widely between them. The imidazolines appear to be a group with considerable room for the further study of novel structures. Compounds of this type have shown good activity in controlling acarines (e.g. see 63 and 64).

Antagonists. Structure–activity studies of the compounds which act as antagonists of OA are very limited, and once again, initial choices of compounds have been made from those which are already known to be antagonists of monoaminergic systems in vertebrates. However, a broad range of structures is capable of yielding antagonists, as shown in Figure 3. In terms of their major activity in mammalian systems, these antagonists are rather diverse, e.g. cyproheptadine and mianserin are generally categorized as serotonergic and/or histaminergic blockers, while chlorpromazine is classed as a dopamine antagonist, and phentolamine and yohimbine and its analogs are categorized as α–adrenergic blockers. However, there often is overlap in the activity of aminergic blockers in such systems, and these categorizations are not absolute, e.g. chlorpromazine also has distinct α–adrenergic blocking actions.

Table VI.   Comparative Activities of Several Phenyl Imidazolines on Locust Extensor Tibiae (ETi) Muscle and in the Crayfish Behavioral Assay.

X–Y– (imidazoline)		Locust Muscle			ED50 Crayfish Behavior d (mg/kg)
		I50 Myogenic Rhythm a (nM)	SETi (EC50)		
X	Y		Amplit. b (nM)	Relaxn. c (nM)	
1-Naphthyl	CH_2	12	13	220	0.32
Phenyl	CH_2	1.5	3,200	600	1.19
2,6-DiCl-phenyl	NH_2	0.68	6,400	20,000	2.74
5,6,7,8-Tetra- hydro-1-naphthyl	NH	1.7	6,000	900	7.34
dl-Octopamine		15	3,300	2,000	1.44

[a] Concentration reducing frequency of contraction by 50%. Type 1 receptors, Ref. 65.
[b] Concentration causing 50% of maximal increase in SETi twitch tension amplitude. Type 2A receptors, Ref. 65.
[c] Concentration causing 50% of maximal increase in twitch tension relaxation rate. Type 2B receptors, Ref. 65.
[d] Dose causing 50% behavioral response; Ref. 31.
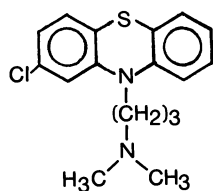
Chlorpromazine
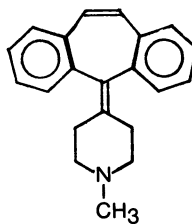
Cyproheptadine
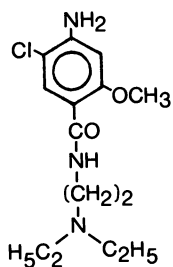
Metoclopramide
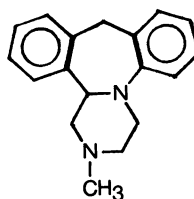
Mianserin
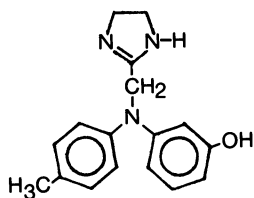
Phentolamine
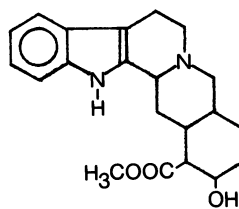
Yohimbine

Figure 3. Structures of some octopaminergic antagonists.

From the differential responses of the OA-sensitive functions
of the locust ETi system to these antagonists and selected agonists
(e.g. see Table VI), Evans (65) concluded that two broad categories
of OA receptors were present. Those responsible for depressing the
myogenic rhythm in the proximal bundle were antagonized in the
order: phentolamine > chlorpromazine > yohimbine > mianserin >>
metoclopramide, and were termed Type 1 receptors. The responses to
OA of the SETi twitch tension in the rest of the muscle were
designated as due to Type 2 receptors. These were antagonized in
the order mianserin = metoclopramide = phentolamine >>
chlorpromazine > yohimbine. Evans also distinguished between two
subclasses of Type 2 receptors on the basis of their differential
sensitivities to imidazoline agonists (Table VI) and selected
antagonists. The receptors mediating the increase in twitch tension
amplitude (Type 2A) are believed to be located presynaptically and
to act by modulating transmitter release, while those associated
with the increased relaxation rate of tension (Type 2B) are
probably located postsynaptically on the muscle.

Most other systems so far studied tend to follow the Type 2
pattern, although differences exist. These include the firefly
lantern and Manduca CNS adenylate cyclase activities (26) and the
crayfish postural response (Table II; 31). In these systems the
most active antagonists in each instance is mianserin. In some
cases the potency of these antagonists is considerable e.g. several
compounds antagonize the activity of OA on the locust ETi myogenic
rhythm with EC50 values in the range of 1-10 nM (65).

Whether such antagonists represent useful models for the
discovery of new pest control agents is unclear. Their immediate
effects on insects in a laboratory setting are not impressive.
However, since OA appears to be involved in the arousal and stress
responses of insects, it might be expected that OA antagonists
would not elicit strong generalized symptoms of excitation
comparable to those caused by many current insecticides. On the
other hand, significant behavioral and physiological effects are
observed if more subtle and specific tests are conducted. For
example, the antagonist cyproheptadine, even applied topically,
blocked the ability of the firefly lantern to light (14). Although
the firefly is not a pest species, this action, if prolonged, would
clearly interfere with its reproductive behavior and success. This
illustrates two principles - that octopaminergic antagonists may
have potentially deleterious effects on insects, and that specific
behavioral tests will probably be necessary to detect them. A
drawback to virtually all the compounds now used as antagonists is
again their ionized nature at physiological pH which presumably
minimizes their effects on the CNS of insects. Clearly there is
much more to learn regarding the actions and structure-activity
relations of octopaminergic antagonists and their potential for
applications in invertebrate control. Analogs which would readily
enter the insect CNS are particularly desirable.

Heterogeneity of Octopamine Receptors. Evidence is outlined above
from the work of Evans (65) that there are several types of OA
receptors which may differ considerably, not only in function, but
also in the pharmacology of the compounds which stimulate or block
them. This is a complicating factor in attempts to design and
screen agents with commercially useful effects on such receptors,
but at the same time raises the prospect that agonists and
antagonists may be found which will be relatively selective against
pest species.
    The fact that OA receptors vary in their specificity from
tissue to tissue is also illustrated by the observation that
the N,N-dimethylformamidine, chlordimeform, is quite active com-
pared to its N-monomethyl analog (DCDM) in releasing hyperlipemic
hormones from the locust corpus cardiacum (60). In most other
systems, including lipid release from the locust fat body (61) and
the examples in Table IV, this compound is much less potent than
DCDM. Further, while OA itself has comparable potencies in
stimulating the adenylate cyclase activities in firefly tails and
the CNS of Manduca, DCDM is about 15 times more active in the
Manduca preparation than that of the firefly (27).
    Another difference between OA receptors from different tissues
lies in their apparent affinities for OA and other agonists. Those
which have been shown to be linked to adenylate cyclase appear to
have rather low affinities for ligands (0.1 to 10 μM; 26,27,29,56).
A similar micromolar potency range was observed for OA and typical
agonists and antagonists in their effects on the twitch tension of
the locust ETi muscle (65; Table VI). On the other hand, the
activity of these compounds in the myogenic contraction assay of
the ETi is much higher (1-10 nM) and comparable to that seen for
the binding of OA to insect head homogenates (33). There are
several possible explanations for these apparently large variations
in the potency of octopaminergic ligands in different assays, e.g.
in some cases single receptors are known to demonstrate both high
and low affinity states. However, this situation is reminiscent of
that with some other biogenic amines in mammals where both high and
low affinity receptor types are known e.g. low affinity dopamine
receptors in the brain (D-1 receptors; micromolar affinity) mediate
an increase in adenylate cyclase, while high affinity receptors (D-
2 receptors; nanomolar affinity) cause a decrease in adenylate
cyclase activity (66). In such a situation, high affinity receptors
offer the better theoretical prospect for the development of
physiologically active ligands with high potency.

Conclusion

Returning to the criteria listed in Table I, it appears that octo-·
paminergic neurotransmission, particularly OA receptors themselves,
make an attractive theme for attempts at the biochemically-aided
discovery of insecticide/acaricides. Sensitive sites of action are
present in important pest species, and the results of interacting
with these sites are often likely to be both rapid and effective in

controlling the pest. There are reasonable prospects for selectivity at the vertebrate-invertebrate level, although this may not be a major strength of OA as a target choice. More favorable is the existence of many lead compounds, some of which at least are capable of penetrating to their proposed sites of action. The pharmacology of OA receptors in many ways resembles that of α2-adrenergic receptors in vertebrates (9,57,65). New lead compounds applicable to arthropod control may arise from the extensive research on α2-agonists conducted by the pharmaceutical industry. Both in vivo and in vitro assays are available, and growing in number regularly as our understanding of the biochemistry and physiology of OA as a neuroeffector advances rapidly. Novelty is a relative concept, but, octopaminergic biochemistry is an area which, although not new, has not been explored extensively in the search for new pesticidal structures. Overall it is safe to commend this topic for serious consideration by anyone weighing the prospects for a biochemical approach to the rational discovery of pesticides.

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Harnessing Insect-Specific Enzymes to Activate Novel Proinsecticides

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The 29-fluorophytosterols, members of a new class
of selective pro-insecticides, have been
synthesized and examined in vivo in tobacco
hornworms. Dealkylation at C-24 of the steroid
side chain by insects releases the latent poison
fluoroacetate, resulting in dose-dependent
reductions in growth rate, maximum weight, and
survival when fed at 1 to 100 ppm to Manduca sexta.
Larval steroid composition is unaffected. The
effects of six related 29-monofluorosterols show
that both the 22,23-and 24,28-double bonds increase
toxicity dramatically.
Inhibitors of sterol dealkylation suppress the
toxicity of 29-fluorostigmasterol, whereas a
cholesterol supplement does not alleviate the toxic
effects. Metabolism of [29-^{3}H]-29-fluorostig-
masterol releases tritiofluoroacetate, as
demonstrated by the isolation and identification of
[2-^{3}H]-erythro-2-fluorocitrate from in vivo
experiments with 5th instar larvae. Rates of
steroid dealkylation measured with [29-^{3}H]-phyto-
sterols are higher in younger larvae, with
sitosterol being more efficiently dealkylated than
stigmasterol in all stages.

The biorational design of pesticides in the agrochemical industry
lags over a decade behind the analogous biorational design of
drugs in the pharmaceutical industry. This lag reflects two
primary problems: (1) poor understanding of insect biochemistry
vis a vis mammalian biochemistry, and (2) difficulty in
implementing the desirable switch away from cheap, broad-spectrum
biocides. Rationally-designed compounds which are substrate
analogs for insect-unique enzymes satisfy the prime criteria for
the development of future insect control agents(1,2).

In analogy to the use of prodrugs in chemotherapy(3),
enzyme-activated pro-insecticides are possible, in which the
target enzyme would convert a less toxic precursor into an
inactivator of a specific protein (see, for example, the chapter
by T. Fukuto in this volume). There are two forms such
proinsecticides might take. Suicide substrates(4) can be
designed such that a latent reactive functionality is both
unmasked by and then irreversibly binds to the enzyme active site.
Alternatively, the enzyme may cause the release of a latent or
less reactive toxicant from a nontoxic precursor, such that the
enzyme system involved in the liberation of the toxicant is
different from that actually suffering the biochemically adverse
effects of the unmasked poison. We have recently reported the
successful harnessing of the insect phytosterol dealkylation
pathway to release fluoroacetate from 29-fluorophytosterols in
Manduca sexta larvae in vivo(5,6). In this paper we present
further evidence for (a) the dealkyation rates of fluorinated and
nonfluorinated phytosterols by hornworms, (b) the suppression of
29-fluorostigmasterol toxicity by chemical inhibition of
dealkylation, and (c) the isolation of tritiated fluorocitrate as
the ultimate biochemical lesion resulting from 29-fluoro-
stigmasterol dealkylation.
     Insect steroid metabolism has two biochemically distinctive
components: dealkylation of phytosterols to cholesterol and
polyhydroxylation of cholesterol to ecdysone. We will focus on
the first of these. Lacking the ability to synthesize sterols de
novo, insects instead have evolved a dealkylation pathway to
convert plant sterols to cholesterol(7-10). The dealkylation
pathways are apparently absent in most other higher and lower
organisms, which can convert mevalonate to squalene and thence
into sterols(11). Specific insecticides are possible based on
these biochemical differences.
     Specifically, many phytophagous insects degrade sitosterol
(1,X=H) via fucosterol (2), fucosterol epoxide (3), and
desmosterol (4) to cholesterol (5). Stigmasterol (6) is
degraded analogously via the stigmastatrienol 7 (Fig. 1). The
evidence for the steps indicated (dehydrogenation, epoxidation,
fragmentation, and Δ^{24}-reduction) has been obtained using
azasterols as inhibitors of Δ^{24}- reductase, or using allenic and
imino fucosterol analogs as inhibitors of epoxidation and
dealkylation(7-10).
     Although dealkylation is restricted to arthropods, not all
insects possess this ability, nor do all insects employ the same
steroid nucleus. In general, phytophagous insects are capable of
dealkylation while zoophagous insects lack this ability.
Although about twelve different insects(7,8,12) have been
examined, the distribution of dealkylative enzymes both within
and outside the class Insecta is poorly known. This is largely
due to the cumbersome task of following the conversion of a
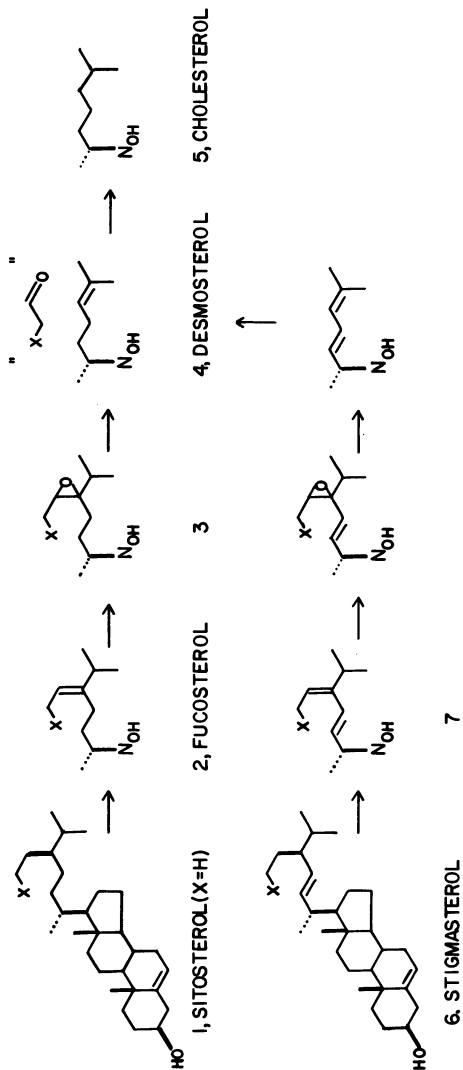
Figure 1. Steroid dealkylation pathway in insects.

[14]C-ring labelled phytosterol to the dealkylated form. We have attempted to remedy this situation by developing rapid, more sensitive assay using [29-^{3}H] phytosterols and doubly-labelled [29-^{3}H], [26-^{14}C]-sterols.

Partition assay for dealkylation

A rapid and sensitive method for determining the extent and rate of phytosterol dealkylation in vivo or in vitro has been developed recently in our laboratories(13). The method is based on the use of [29-^{3}H]-phytosterols (Fig. 2) as substrates which then release [^{3}H]-acetate (or its metabolic equivalent) upon dealkylation. Following in vivo or in vitro incubation with the [29-^{3}H] sterol substrate, the insect or tissue is homogenized and partitioned between water and ethyl acetate. Liquid scintillation counting of the two layers gives a direct measure of dealkylation, since the net change due to dealkylation is transformation of lipid-soluble [^{3}H]-sterol to aqueous [^{3}H]-acetate (and its subsequent entry into other pathways). With doubly-labelled sterols containing ^{14}C in the steroid nucleus, the change in ^{3}H/^{14}C ratio can define the degree of dealkylation even more precisely.

To test this method, we first employed Manduca sexta third- and fifth-instar larvae and pupae, using both [29-^{3}H]-sitosterol (specific activity 0.55 mCi/mmole or 2.91×10^6 dpm/mg) and [29-^{3}H]-stigmasterol (specific activity 0.55 mCi/mmole or 2.90×10^6 dpm/mg) synthesized in our laboratories(14). These relatively low specific activities were chosen to optimize total recovery of radioactivity, which was poor at 100-fold higher specific activities. Pupae were injected abdominally and larvae were injected perorally with 5 μl of a dimethylformamide (DMF) solution of the [29-^{3}H] sterol (100,000 dpm per individual for pupae and 5th instars, 20,000 dpm X 5 larvae for third instars) and then frozen at the times indicated. Dealkylation was calculated on a per gram fresh weight basis from the aqueous dpm at each time point corrected for the aqueous dpm at zero time. "Percent dealkylation" was also calculated by dividing the corrected aqueous dpm by the total recovered dpm. Recovery of applied radioactivity was initially >95% and declined in a roughly linear manner to 80% after 8 hr, reflecting excretion and redistribution. Less than 1% of injected [^{3}H]-acetate was converted into lipids during an 8 hr incubation. High aqueous dpm at zero time (up to 4% of applied dpm for pupae) were attributed to the presence of DMF and proteins in the aqueous layer which could solubilize the labelled sterol.

Table 1 shows the preliminary results at two time points for the nanomoles of two [29-^{3}H]-sterols dealkylated per gram fresh weight (mean ±1 S.D.) for four replicates. For third instars, approximately 23% dealkylation of sitosterol and 11% dealkylation of stigmasterol was observed at 4 hours, which indicates rapid
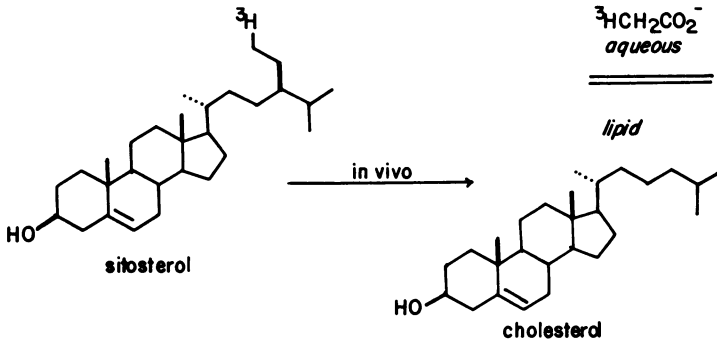
Figure 2. [29-³H]-Phytosterol partition assay for rapid measurement of dealkylation in vivo.

Table 1. Dealkylation of [29-^{3}H]-phytosterols *in vivo* by *Manduca sexta*.

Stage	Dealkylation, nmole sterol/gram fresh weight, mean (S.D.)			
	[29-^{3}H]-sitosterol		[29-^{3}H]-stigmasterol	
	1 hr	4 hr	1 hr	4 hr
3rd Instar	7.54 (0.37)	19.7 (1.1)	1.74 (0.27)	6.37 (0.47)
5th Instar	0.57 (0.03)	3.85 (0.86)	0.19 (0.04)	0.58 (0.09)
Pupa	0.18 (0.03)	1.59 (0.27)	0.11 (0.01)	0.28 (0.02)

and efficient turnover of the injected [29-^{3}H]-phytosterols. Pupal and fifth instar values are 3-4% for sitosterol and 2-3% for stigmasterol (these numbers indicate aqueous dpm 2-3 fold higher than the zero time value). For both [29-^{3}H]-sitosterol and [29-^{3}H]-stigmasterol, the turnover per gram insect is approximately ten fold higher in third instars than in fifth instars or pupae, suggesting a greater level of dealkylation activity in the more rapidly growing life stages. Also, an increased rate of dealkylation for sitosterol relative to stigmasterol is apparent, consistent with the greater abundance of sitosterol in the host plants of <u>Manduca</u> larvae. We are now refining this technique to coadminister the [^{14}C]-ring labelled sterols with their [29-^{3}H] counterparts in order to more clearly define the fates of both fragments of the dealkylation pathway, and to separate true dealkylation from conjugation and passive transport processes. A full report on these methods as applied to all life stages of <u>Manduca</u> and <u>Tenebrio</u> is forthcoming(13). We feel that this technique can be adapted to give quantifiable results on the utilization of sterols with different A/B ring and side chain functionalities by a wide range of insects.

Toxicity of 29-Fluorophytosterols

Substitution of fluorine for hydrogen at C-25 and C-26 of phytosterols and at C-20, C-22, C-24 or C-25 of cholesterol provided compounds which did not affect <u>Manduca sexta</u> growth or development significantly at 50 ppm in the diet(15). In contrast, we predicted that the C-29 fluorophytosterols (Fig. 1, X=F) would release the metabolic equivalent of fluoroacetate as a result of dealkylation(5,6).

The 29-fluorophytosterols (Fig. 3) all showed significant impairment of growth and development of larval tobacco hornworms when fed at 1 to 100 ppm to <u>Manduca sexta</u>[5]. It is clear that the Δ^{22} sterols 8 and 11 were more toxic and caused more severe stunting than the 22,23-dihydro analogs 9 and 12 (Fig. 4). Moreover, C-24 stereochemistry was not as important in determining relative toxicity as the absence or presence of the Δ^{22} olefinic bond. The 29-fluorofucosterol 13 was found to be intermediate in its effects between the 29-fluorositosterol 9 and 29-fluorostigmasterol 8. Each of the three 29-fluorophytosterols examined in detail (8, 9, 13) showed a clear dose dependency for the reduction of the larval growth rate, maximum larval weight reached, larval mortality, and percent pupation(5). As expected, the 29,29-difluorostigmasterol 10 was significantly less toxic than 29-fluorostigmasterol 8. Larvae fed 29-fluorosterols matured slowly but progressed through the normal instar molts, as determined by daily observations to detect apolysis. Severely poisoned larvae had trouble shedding skins and developed abnormally large 5th-instar heads on only 2nd or 3rd instar-sized bodies. The ability of 29-fluorosterols to
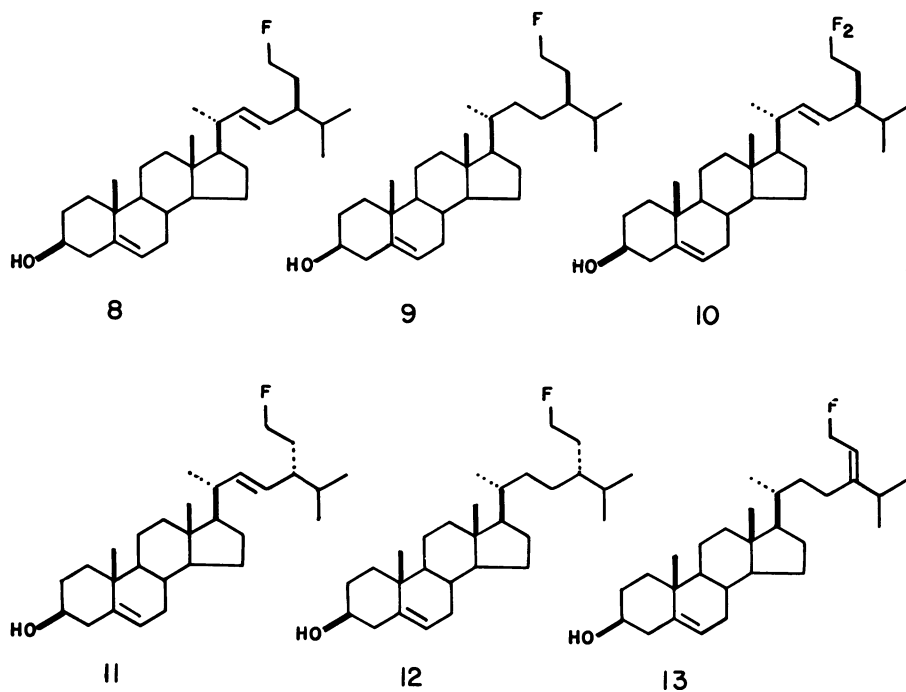
Figure 3.   29-Fluorophytosterols assayed _in vivo_.
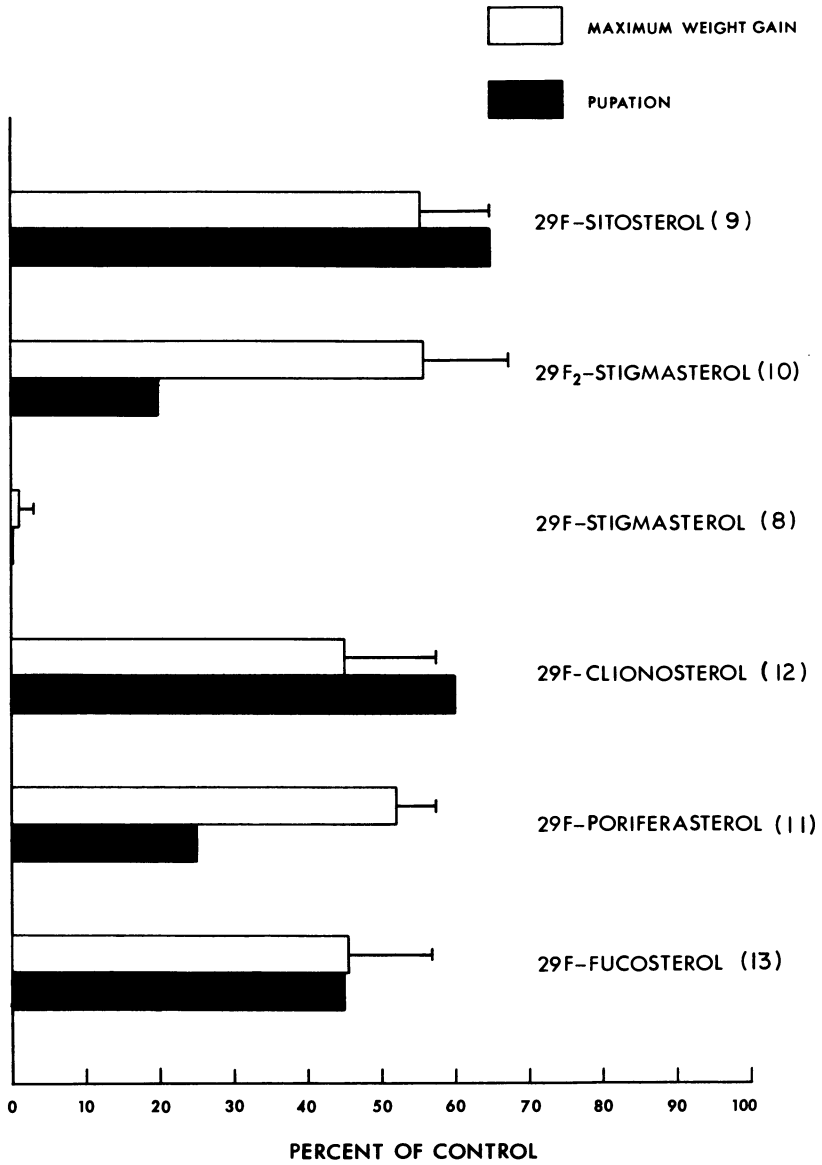
Figure 4. Effects of 29-fluorophytosterols on growth and pupation of <u>Manduca</u> <u>sexta</u>, fed from second instar on 50 ppm 29F-sterol in diet.

overcome the usual gating phenomenon in Manduca is noteworthy.

We reasoned(5,6) that the slow steps in sterol dealkylation were (a) 24,28-desaturation to give 29-fluorofucosterol 13 or the corresponding 29-fluorostigmastatrienol, and (b) epoxidation of the 24,28 double bond. The substitution of the weakly electron withdrawing fluoromethyl for methyl causes desaturation and epoxidation reactions to proceed sluggishly on the 29-fluoro analogs, while proceeding unimpaired with nonfluorinated sterols. With the Δ^{22} bond present, electron release into an incipient conjugated diene can compensate for the electron withdrawing effect of the fluoromethyl group. Preparation and bioassay of the unsaturated fluorides and their epoxy derivatives is in progress to verify their intermediacy in the the dealkylation pathway.

Importantly, larval steroid composition is unaffected by the monofluorophytosterols and monofluorocholesterols(5,15). That is, none of these materials interfere with the dealkylation process. In fact, of the fifteen fluorinated sterols tested, the 29-fluoro derivatives were unique in exhibiting toxicity.

For these explanations of 29-fluorosterol toxicity to be valid, we needed to demonstrate that an inhibitor of dealkylation would protect larvae from the latent toxicity of an ingested 29-fluorosterol. As the inhibitor, we chose the 24,28-allene 14, previously prepared(16) and tested in vivo in Bombyx mori(17) by Ikekawa and his associates. This material was shown to interfere with the sitosterol to fucosterol and the fucosterol to fucosterol epoxide conversions, but not to affect silkworm larvae fed on fucosterol epoxide, desmosterol, or cholesterol. Our experimental results are summarized in Fig. 5. Hornworm larvae fed diet containing 300 ppm cholesterol developed normally, in contrast to those fed 29-fluorostigmasterol (30 ppm) and 300 ppm cholesterol. A protective "dilution" effect was found when 300 ppm stigmasterol was fed with the 29-fluorostigmasterol, but mortality was still high. Most dramatically, addition of 100 ppm of allene 14 to the 29-fluorostigmasterol plus cholesterol treatment completely reversed the effects of the 29-fluorostigmasterol. With dealkylation blocked, no activation of the latent poison occurred.

Toxicity and isolation of fluorocitrate

Fluoroacetate undergoes a "lethal synthesis"(18) to 2-fluoro-citrate which may reversibly inhibit aconitase and which irreversibly binds to a membrane-associated citrate transport protein(19,20). Insecticidal and other biocidal uses of fluoroacetate (or its metabolic precursors) received considerable attention twenty-five years ago(21) but most uses have been abandoned due to high nonspecific vertebrate toxicity of these compounds. We have reported the use of ω-fluoro fatty acids and their derivatives as delayed-action toxicants for targeted
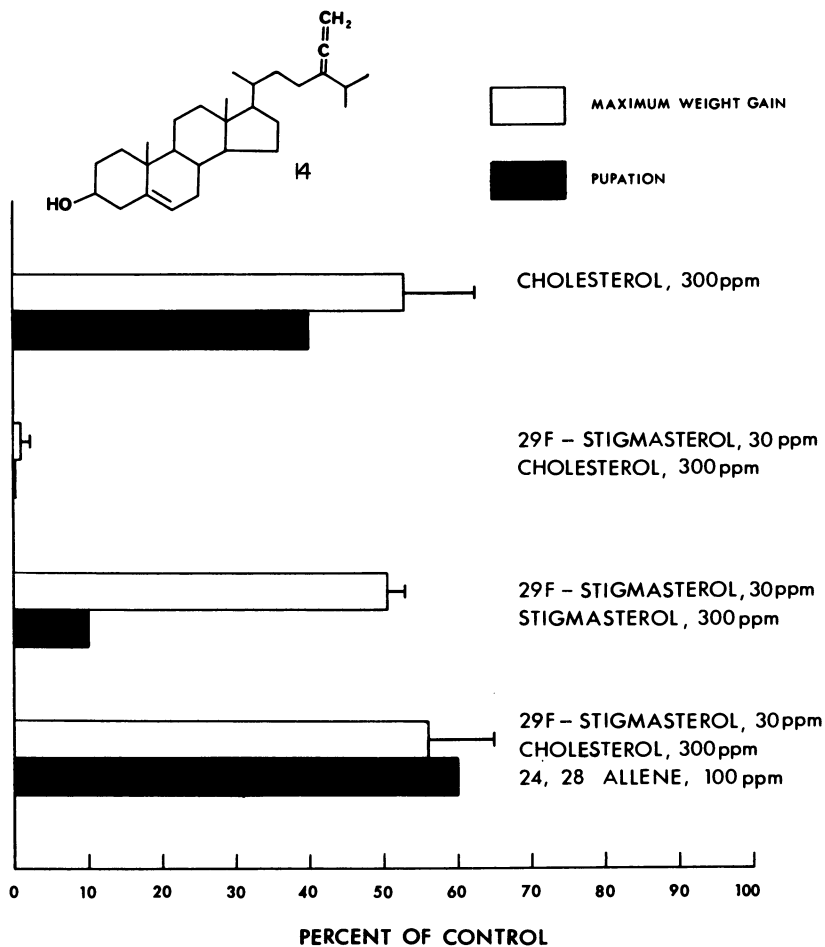
Figure 5. Protection from toxicity of 29-fluoro-
stigmasterol (8) by 24,28-allene 14. Data
show growth and pupation for Manduca sexta.

termite control using the bait-block method(22). Selected
fluorolipids give acceptably long delay times, attractancy, and
high kill at low oral doses. Selectivity for termites is
achieved in this scheme by a targeted delivery of the poisons in
a food source. In the case of the 29-fluorophytosterols, the
release of fluoroacetate from a masked poison which could not be
activated by vertebrates would seem to be a superior strategy for
achieving selective toxicity.

In addition to the circumstantial comment — Fluorocitrate and several metabolic precursors (e.g.,
fluoroacetate, (E)-16-fluorohexadec-9-enoic acid) produced
effects in hornworms analogous to those seen for 29-fluoro-
stigmasterol, albeit at lower doses(5). The active (2R, 3R)
steroisomer(23), (-)-erythro-2-fluorocitrate was 100-1000 fold
more toxic than 29-fluorostigmasterol and 10-100 fold potent than
fluoroacetate or (E)-16-fluorohexadec-9-enoic acid. The
conversion of fluoroacetate to the coenzyme A thioester and then
condensation with oxaloacetate (Fig. 6) gives only a single
fluorocitrate isomer in mammalian systems(19). Both enzymic
reactions have significantly lower velocities relative to the
normal acetate to acetyl CoA to citrate conversion. For example,
pig heart citrate synthase has the same K_m for acetyl CoA and
fluoroacetyl CoA, but the V_{max} for fluoroacetyl CoA is only
1/300th of the V_{max} for acetyl CoA(19). Also, we expected the
ω-fluorofatty acid to be more efficiently converted to
fluorocitrate than either fluoroacetate or 29-fluorostigmasterol,
since the β-oxidation pathway provides the CoA derivative
directly, whereas the dealkylation pathway formally gives
fluoroacetaldehyde as the released fragment.

In addition to the circumstantial in vivo evidence for
fluorocitrate as the ultimate biochemical lesion we desired to
demonstrate unambiguously that it was produced as a metabolite of
29-fluorostigmasterol. The toxicity of fluorocitrate and the
resulting lethal accumulation of citrate in mouse, fly and
cockroach tissues have been shown in early experiments with
fluoroacetamide and fluoroacetate(24). However, to our
knowledge, complete characterization of (2R,3R)-2-fluorocitrate
as the lethal metabolite in vivo has not previously been reported.
We thus prepared(25) [29-^{3}H]-29-fluorostigmasterol,
[29-^{3}H]-29-fluorositosterol and [16-^{3}H]-16-fluorohexadec-9-enoic
acid to enable isolation of [2-^{3}H]-2-fluorocitrate from in vivo
incubations using Manduca sexta.

Four early fifth instar larvae (1-2 g each) were starved for
24 hr and then each was fed on a 20 mg slab of diet treated with
5 μl of a DMF solution containing ca. 3 x 10^{6} dpm of [29-^{3}H]-29-
fluorostigmasterol (specific activity 150 mCi/mmol), [29-^{3}H]-29-
fluorositosterol (specific activity 240 mCi/mmol), or
[16-^{3}H]-16-fluorohexadec-9-enoic acid (specific activity 33
mCi/mmol). After 8 hrs, the larvae were frozen and later
homogenized individually in ethyl acetate-water (1:1).
Unlabelled (-)-erythro-2-fluorocitrate as the tris(cyclohexyl-
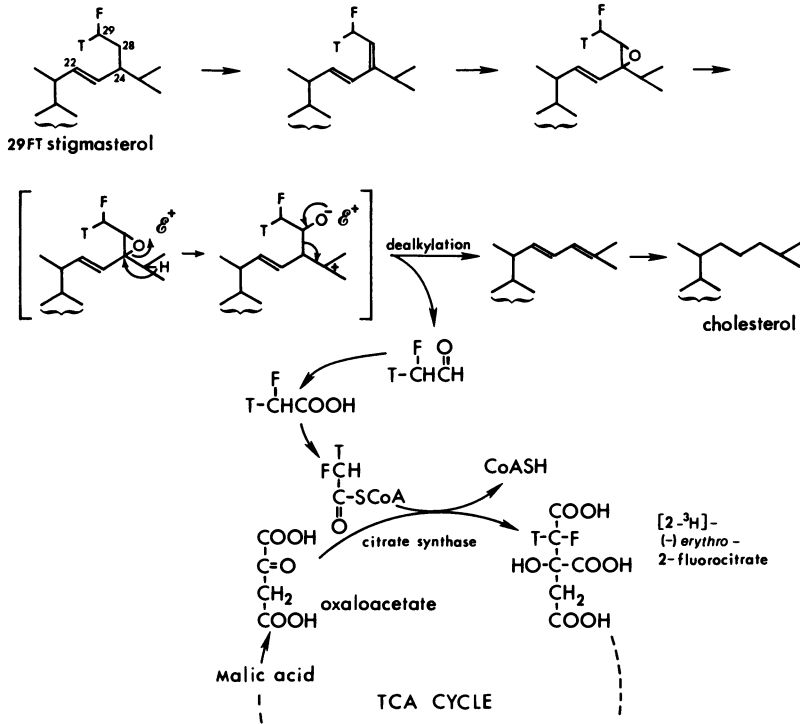
Figure 6. Metabolic conversion of [29-^{3}H]-29-fluoro-stigmasterol to [2-^{3}H]-2-fluorocitrate *in vivo*.

ammonium) salt (1 mg) was added to the aqueous layer, since we
estimated that only several nanograms of labelled fluorocitrate
per insect would be produced. The lengthy purification
scheme(25) consists of the following key features: (1) elution
of isocitrate, citrate, and fluorocitrate from ion exchange
column with 2 N ammonium formate; (2) acidification,
lyophilization and diazomethane methylation followed by silica
gel chromatography to give the trimethyl esters of citrate,
isocitrate, and fluorocitrate (fractions checked by capillary GC
analysis); (3) benzoylation and silica gel chromatography of the
benzoates; (4) separation of the diastereomeric trimethyl ester
benzoates by reverse phase HPLC (C_8-PXS 10/25, 34% CH_3CN-H_2O).
At each stage, the location of the labelled material was followed
by LSC of chromatographic fractions, with the dpm in steps (2)
and (3) moving only with the fluorocitrate-containing fractions.
In addition, capillary GC (30 m DB-5) was used to quantify
citrate, isocitrate and fluorocitrate trimethyl esters and
benzoates in each fraction. Finally, dpm were observed only in
the _erythro_ isomer of trimethyl 2-fluorocitrate benzoate. The
progress of the purification is given in Table 2. Full details
will be published separately(25).

We observed that the ω-fluorofatty acid was much more
efficiently converted to fluorocitrate (0.57% of applied dpm)
than was the 29-fluorostigmasterol (0.005% of applied dpm).
Conversion of [29-^{3}H]-29-fluorositosterol to fluorocitrate was
examined, but insufficient label remained after step (2) for
further purifications(5,25). This suggests much lower
dealkylation efficiency for this analog (<0.001% of applied dpm)
and is consistent with its reduced toxicity _in vivo_.

Summary

Phytosterol dealkylation can be harnessed in insects to release a
fluoroacetate equivalent from a 29-fluorinated sterol. Moreover,
the fluorocitrate which then results from the "lethal synthesis"
can be isolated and chemically characterized. We hope that the
range of insects susceptible to the 29-fluorophytosterols and
more commercially viable analogs will be further explored.
Furthermore, we urge wider scrutiny of insect biochemical
pathways in search of possible targets for suicide substrates or
latent toxin release.

Table 2. Purification of [2-^{3}H]-erythro-2-fluorocitrate from $\underline{in\ vivo}$ experiments.

Stage of Purification	Radioactivity (dpm) remaining for precursor fed		
	[29-^{3}H]-29-fluoro-stigmasterol	[29-^{3}H]-29-fluoro-sitosterol	[16-^{3}H]-(E)-16-fluoro-9-hexadecenoic acid
Applied	8.74×10^6	9.88×10^6 dpm	5.40×10^6 dpm
H$_2$O layer	1.08×10^6	1.22×10^5	1.71×10^6
Ion exchange (2N Ammonium formate)	9.80×10^4	4.60×10^3	4.60×10^5
SiO$_2$ column of trimethyl esters (40% EtOAc-hexane)	5.00×10^3	$<1 \times 10^2$	9.82×10^4
SiO$_2$ column of benzoates (20% EtOAc-hexane)	2.20×10^3	—	5.23×10^4
HPLC, C$_8$ of benzoates (34% CH$_3$CN-H$_2$O)	4.10×10^2	—	3.10×10^4
Percent conversion to [2-^{3}H]-erythro-2-fluorocitrate	0.005%	—	0.57%

Acknowledgment

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Substituted Pyridazinone Herbicides: Structural Requirements for Action on Membrane Lipids

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Substituted pyridazinones induce decreases in linolenic acid (18:3) and increases in linoleic acid (18:2) in plant membranes. These changes are associated with biological response of plants to low temperature, high temperature, and water status. Multivariate n-dimensional cluster analysis of large volumes of data indicated that the pyridazinones could be grouped into five clusters with maximum homogeneity in structure and physiological activity within clusters, but with maximum inhomogeneity between clusters. The cluster of 4-chloro-5-dimethylamino-pyridazin-3-ones had the strongest effect on the ratio of 18:2/18:3 fatty acids in membrane lipids of wheat (Triticum aestivum L.) shoots. Compounds in this cluster formed the basis for the first successful application of a QSAR (quantitative structure activity relationship) approach to studies of the interactions of chemicals with plant membrane lipids. On the basis of QSAR analysis, a Hansch equation was developed with the Hammett electronic parameter σ and the hydrophobic parameter π as variables.

Contemporary studies of the response of plants to temperature changes using modern techniques including electron-spin resonance, nuclear magnetic resonance, fluorescence spectrometry, and differential scanning calorimetry emphasize the importance of membrane lipids. Substituted pyridazinones induce decreases in linolenic acid (18:3) and increases in linoleic acid (18:2) in plant membranes (1). These changes in membrane lipids are associated with response of plants to low temperature (2, 3), high temperature, and water status (4). Additional biological

actions, dependent on pyridazinone structures, include blocking
the Hill reaction and photosynthetic CO_2 fixation, blocking the
formation of chloroplast pigments, prevention of the formation of
chloroplast ribosomes, and dramatic alterations in chloroplastic
ultrastructure (5). The potential benefits of being able to
chemically tailor a plants' response to its environment are
enormous, hence our interest in developing structure-activity
correlations for interactions of agrichemicals with the lipids of
plant membranes.

Structure-activity correlations are usually quantitated on
the basis of "biological" data obtained in vitro. For example,
the values for 50 percent inhibition (pI_{50}) of the Hill
reaction in isolated chloroplasts have been used to derive
equations describing the action of herbicides which inhibit
photosynthetic electron flow (6-8). In our studies, the ideal
index of activity would be the pI_{50} value for the enzyme
linoleic acid desaturase. Unfortunately, none of the enzymes
responsible for sequential introduction of double bonds into 18
carbon acyl chains have been isolated and even their localization
in vivo is uncertain and remains an intensely researched subject
in plant lipid biochemistry. Any quantitative study requiring
the use of in vivo data faces formidable problems. The ratio of
18:2/18:3 in membrane lipids in an in vivo system is determined
by complex processes. Faced with these complexities, we
subjected the data from our experiments aimed at changing the
lipid composition of chloroplast membranes in vivo to cluster
analysis prior to a quantitative structure activity approach.

Cluster Analysis

Over 50 different pyridazin-3-ones were evaluated for biological
activity in a wheat (Triticum aestivum L.) test system described
previously (1). Briefly, seeds were germinated in 9-cm petri
dishes on three layers of filter paper. Pyridazinones were
dissolved in acetone and the filter papers were impregnated with
1 ml of acetone solution. After the soluent evaporated, 10 ml of
distilled water were added to form an inhibitor concentration of
100 µM. Seeds were planted directly on the moist papers and
germinated for 4 days in a controlled environment chamber on a
16-hr photoperiod with $27\pm1C$ day temperature and $21\pm1C$ night
temperature. Light intensity from both fluorescent and
incandescent bulbs was 28 klux at dish level. Lipids were
extracted and recovered from 1 g of lyophilized shoot tissue,
separated into membrane and non-membrane lipids, and analyzed by
gas chromatography as described (1).

The data ultimately used in the cluster analyses were: the
linoleic/linolenic acid ratios, the fresh and dry weights, and
the length of the shoots. The cluster analyses have been
described in detail recently (9) and will only be summarized
herein.

Initially, univariate two-dimensional plots of the data were obtained with a plot program in BASIC on a Wang 220C5 computer. The x-axis in all two-dimensional plots was the ratio of 18:2 to 18:3. This ratio was plotted against a single variable (y-axis) such as fresh weight or dry weight. The two-dimensional graphs did not allow a clear differentiation of pyridazinones with homogeneous properties; however, these plots did allow us to select 23 substituted pyridazinones from an initial group of 49. The data obtained with these 23 pyridazinones was then subjected to cluster analysis.

Of the four different methods of cluster analysis applied, the method of Ward described in the Clustan User Manual (10), worked best when compared to the single-, complete-, or average-linkage methods. Using Ward's method, two clusters, Gn and Gm, are fused when by pooling the variance within two existing clusters the variance of the so formed clusters increases minimally. The variance or the sum of squares within the classes will be chosen as the index h of a partition.

The use of the partition index (h = 1.97) in the dendogram of the Ward method resulted in the identification of five different clusters of pyridazinones (Table I). A comparison of the means and standard deviations of the biological measurements before and after cluster analyses demonstrates the advantages of n-dimensional cluster analysis in order to group the 23 substituted pyridazinones in one or several clusters according to different activity. Cluster 1 (Figure 1) consists of the 5-dimethylamino-substituted 2 phenyl-pyridazin-3-ones, with a shift in the ratio of 18:2/18:3 in favor of 18:2 as the single most characteristic biological action. Pyridazinones in clusters 2, 3, and 4 had no single characteristic action discernable from these data. Cluster 5 contains pyridazin-3-ones with the maximum ability to reduce fresh weight and longitudinal growth.

Examination of the structures grouped into cluster 4 (Figure 1) by the computer reveals one of the most exciting results of these studies. Cluster 4 consists exclusively of pyridazinones substituted in 2-position by phenyl moieties containing either trifluoromethyl- or trifluoromethyoxy-groups in 3-position of the phenyl ring. Pyridazinones carrying these specific structures have been shown to be potent inhibitors of the biosynthesis of chloroplast pigments in algae (11, 12) and higher plants (13, 14). Although we had analyzed the effect of these pyridazinones on chloroplast pigments in the present study. We did not use these data for the cluster analyses. Nonetheless, these analyses were still able to tightly cluster these pyridazinones, indicating a correlation between structure and activity (not defined to the computer and not identifiable in Table I). This serves to emphasize the strong correlations between structures and activity within clusters and demonstrates the capacity of these cluster analyses to arrive at meaningful clusters through unbiased procedures. These analyses confirmed multiple modes of

Table I. Means and Standard Deviations of the Data for 23 Differentially Substituted Pyridazinones Before and After Analysis by Ward's Method.

	Fresh wt (g)	Dry wt (g)	Length (mm)	18:2/18:3 ratio
Before Ward's Method				
Pyridazinones: n = 23	7.01 ± 1.78	0.80 ± 0.19	36.52 ± 7.98	2.71 ± 1.71
After Ward's Method				
Pyridazinones:				
Cluster 1: n = 4	9.06 ± 0.55	0.97 ± 0.09	46.35 ± 2.84	5.78 ± 0.61
Cluster 2: n = 2	9.60 ± 0.08	1.11 ± 0.03	48.28 ± 2.94	1.71 ± 1.14
Cluster 3: n = 7	6.76 ± 0.98	0.82 ± 0.09	35.85 ± 4.82	2.03 ± 1.29
Cluster 4: n = 8	6.45 ± 0.55	0.67 ± 0.06	32.87 ± 2.31	2.37 ± 0.65
Cluster 5: n = 2	3.35 ± 1.09	0.47 ± 0.12	22.00 ± 2.94	1.21 ± 0.19

Pyridazinone concentration = 100 μM.
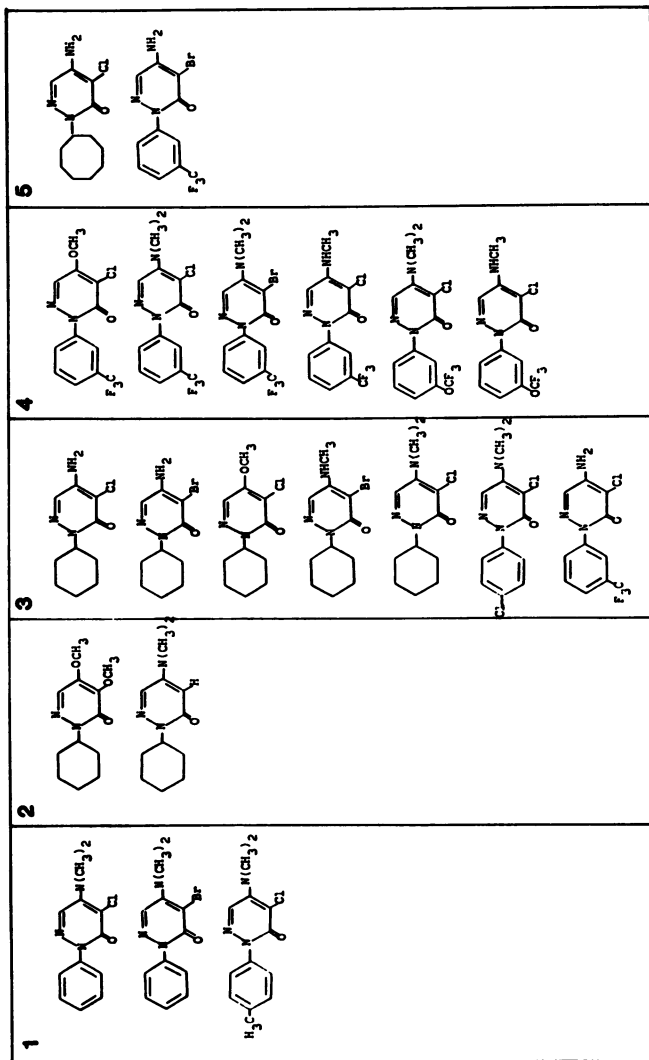
Figure 1. Pyridazinone structures belonging to clusters identified by the method of Ward (partition index of 1.97).

action for the pyridazinones, with action being dependent on pyridazinone structure. The cluster analysis results predict that 4-chloro-5-dimethylamino-pyridazin-3-ones will have the strongest effect on the 18:2/18:3 ratio. The compounds of this cluster formed the basis for our quantitative approach.

Quantitative Structure Activity-Relationship (QSAR) of 4-Chloro-5-dimethylamino-pyridazin-3-ones

The general formula for the pyridazinones evaluated by QSAR methods is:
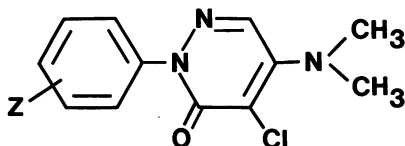
Five new pyridazinones were synthesized with substitutions in the two-position of the phenyl ring as given in Table II. Using the Hansch approach, correlations were made between the experimentally determined 18:2/18:3 ratios and the π values and σ, σ^2 values taken out of the data collection of Hansch and Leo (15) (Table II and Equations 1-3). The hydrophobic parameter π is derived from the 1-octanol/water partition coefficient and σ is the Hammett electronic parameter.

Table II. Experimentally Determined 18:2/18:3 Ratios of Differentially Substituted 4-chloro-5-dimethylamino-pyridazin-3-ones and the Corresponding π and σ Values.

Z	18:2/18:3	π	σ	Code Number
H	6.38	0	0	LAB 13 338
4 CH_3	5.53	0.51	−0.17	LAB 79 044
4 Cl	4.62	0.71	0.23	LAB 77 013
3 CF_3	3.21	0.88	0.43	LAB 77 817
3 OCF_3	3.18	1.04	0.38	LAB 107 421

$$18:2/18:3 = 6.68 \ (\pm1.45) - 3.33 \ (\pm2.01) \ \pi \quad n=5 \quad r=0.95 \quad s=0.51 \quad F=27.9 \quad (1)$$
$$18:2/18:3 = 5.44 \ (\pm1.35) - 4.92 \ (\pm4.71) \ \sigma \quad n=5 \quad r=0.89 \quad s=0.75 \quad F=11.1 \quad (2)$$
$$18:2/18:3 = 6.00 \ (\pm1.03) - 17.68 \ (\pm9.81) \ \sigma^2 \quad n=5 \quad r=0.96 \quad s=0.47 \quad F=32.9 \quad (3)$$

In these and subsequent equations, the figures in parenthesis express the 95% confidence intervals. The correlation

coefficients for Equations 1 and 3 of r = 0.95 and r = 0.96, respectively, are quite high, but may lead to completely different interpretations (compare Figure 1 and Figure 2, respectively). Equation 1 suggests that the ability of compounds to shift the 18:2/18:3 ratio is a linear function based on the hydrophobicity of the molecule, whereas Equation 3 suggests the function is parabolic and based on electronic characteristics of the molecule. Therefore, there is no clear distinction in the relation of π or σ values to the 18:2/18:3 ratio.

The statistical equivalence of Equation 1 and Equation 3 is caused by the collinearity between π and σ (r = 0.74) or π and σ^2 (r = 0.84), respectively. To break this collinearity and to be able to select the best equation out of Equation 1-3, the 4-OH and 4-NO$_2$ substituted 2-phenylpyridazinones were synthesized and evaluated in the wheat test, resulting in the following 18:2/18:3-ratios:

Table III.   18:2/18:3-Ratios of Differently Substituted 4-chloro-5-dimethylamino-pyridazin-3-ones and the Corresponding π- and σ-Values.

Z	18:2/18:3	π	σ	Code Number
4NO$_2$	0.54	-0.28	0.78	LAB 138 050
4OH	0.84	-0.67	-0.37	LAB 138 048

Repeating regression analysis with these newly established data, the equation with σ^2 was optimal, although the statistical significance was worse, r = 0.77 for Equation 6 versus 0.96 for Equation 3.

$$18:2/18:3 = 2.97 \, (\pm 2.38) + 1.59 \, (\pm 3.55) \, \pi \qquad n\ 7 \quad r\ 0.46 \quad s\ 2.17 \quad F\ 1.33 \quad (4)$$
$$18:2/18:3 = 3.79 \, (\pm 2.52) - 1.76 \, (\pm 6.20) \, \sigma \qquad 7 \quad 0.31 \quad 2.32 \quad 0.53 \quad (5)$$
$$18:2/18:3 = 4.82 \, (\pm 2.00) - 8.23 \, (\pm 7.9) \, \sigma^2 \quad 7 \quad 0.77 \quad 1.56 \quad 7.2 \quad (6)$$

An excellent correlation coefficient, r = 0.98, results when a multiple regression equation is formed with the three parameters π, π^2, and σ^2 (Equation 7).

$$18:2/18:3 = \hspace{10cm} (7)$$
$$5.82 \, (\pm 1.28) + 2.65 \, (\pm 1.57) \, \pi - 4.55 \, (\pm 2.51) \, \pi^2 - 6.90 \, (\pm 3.66) \, \sigma^2$$
$$n = 7 \qquad r = 0.98 \qquad s = 0.55 \qquad F = 32.0 \qquad \pi \text{ optimal} = +0.29$$

The statistical relevance is questionable, however, when such an equation is formed with just seven test compounds. Nevertheless, the indicated high correlation coefficient for this
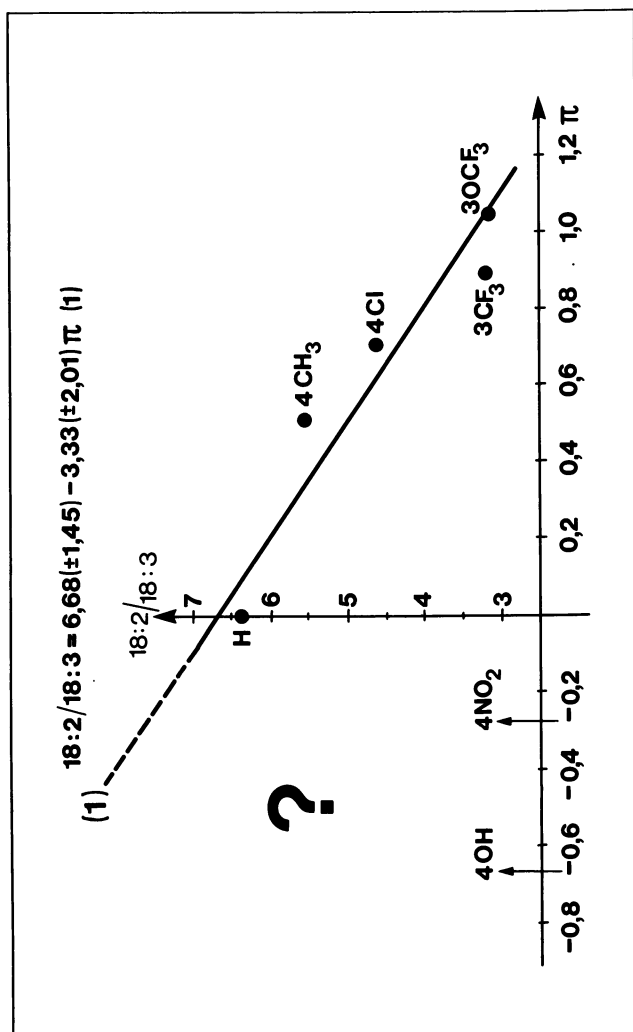
Figure 2. Equation 1 correlating the relation between the 18:2/18:3 ratio and π values.
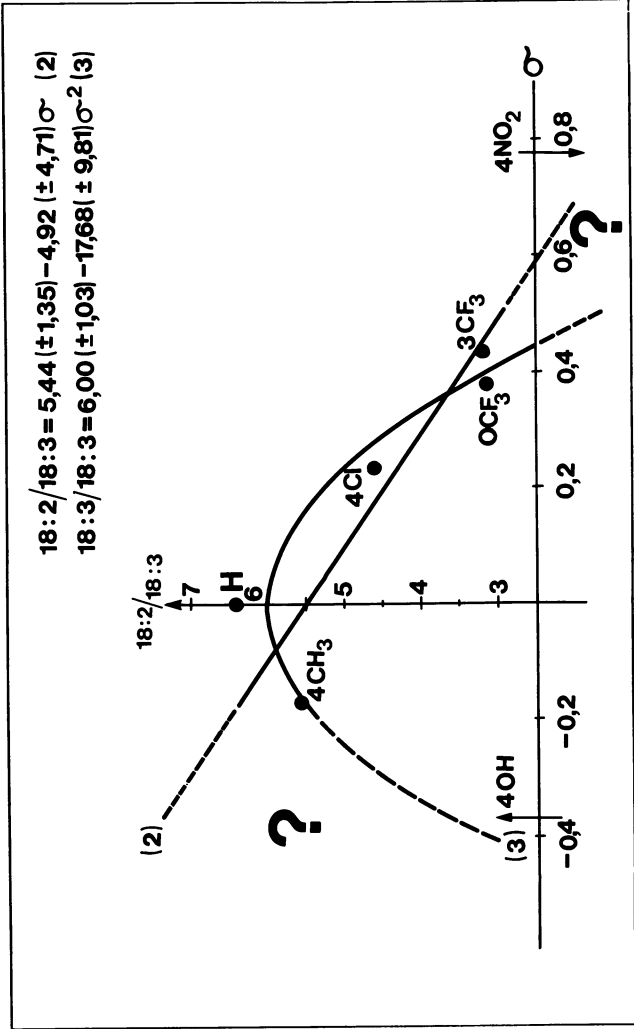
Figure 3. Equations 2 and 3 correlating the relation between the 18:2/18:3 ratio and σ values.

equation and the recent suggestions for inclusion of σ^2 variables in Hansch equations applied in herbicide research (Singer, M. S., unpublished results; Magee, P., private communication) led us to collect additional evidence for this equation. Nine more 2-phenyl-pyridazinones were synthesized and the 18:2/18:3 ratios were determined experimentally. The π values ranged from -1.63 to 2.80 and σ values ranged from -0.83 to 0.72 (Table IV).

Table IV. Experimentally Determined 18:2/18:3 Ratios of Differently Substituted 4-chloro-5-dimethylamino-pyridazin-3-ones and the Corresponding π and σ Values.

Z	18:2/18:3	π	σ	Code Number
4 OCH_3	7.44	-0.02	-0.27	LAB 139 287
4 $OCH(CH_3)_2$	2.62	0.85	-0.29	LAB 152 136
4 $OCH_2CH=CH_2$	1.97	1.00	-0.25	LAB 152 134
4 SO_2CH_3	1.03	-1.63	0.72	LAB 138 047
4 $OC_4H_9(n)$	0.98	1.55	-0.32	LAB 152 137
4 OCH_2O	0.87	1.66	-0.42	LAB 152 135
4 00	0.63	2.08	-0.03	LAB 164 248
4 N $(CH_3)_2$	0.58	0.18	-0.83	LAB 152 139
4 NH_2	0.53	-1.23	-0.66	LAB 152 138

Sixteen compounds (Tables II, III, and IV) may be combined to give an equation similar to 7:

$$\text{18:2/18:3} = \tag{8}$$
$$5.34\ (\pm1.56) - 0.43\ (\pm1.06)\ \pi - 0.96\ (\pm0.72)\ \sigma^2 - 7.24\ (\pm4.35)\ \sigma^2$$
$$n = 16 \quad r = 0.81 \quad s = 1.49 \quad F = 7.8 \quad \pi\ \text{optimal} = -0.22$$

Applying Equation 8, the calculated 18:2/18:3 ratio was very similar to the experimentally determined ratio for all compounds except 2-(4-hydroxyphenyl)-4-chloro-5-dimethylamino-pyridazin-3-one (4.18 calculated vs. 0.84 experimental, Table III). This large difference could be explained by the well known ability of chemicals with phenolic HO- groups to form conjugates which result in bound residues. Therefore, repeating the process of calculation as shown in Equation 8, excluding the 4-chloro-5-dimethylamino-pyridazin-3-one with the 4-hydroxyphenyl substituent in two-position of the heterocycle, results in Equation 9:

$$\text{18:2/18:3} = \tag{9}$$
$$6.15\ (\pm1.24) - 0.91\ (\pm0.83)\ \pi - 0.97\ (\pm0.52)\ \pi^2 - 8.85\ (\pm3.43)\ \sigma^2$$
$$n = 15 \quad r = 0.91 \quad s = 1.07 \quad F = 18.4 \quad \pi\ \text{optimal} = -0.47$$

This equation shows an improved correlation coefficient of
r = 0.91 compared to r = 0.81 for Equation 8.   The data in
Table V show close agreement between the experimental ratios of
18:2/18:3 and those calculated using Equation 9.

Table V.   18:2/18:3-Ratios of Differently Substituted
2-phenyl-4-chloro-5-dimethylamino-pyridazin-3-ones
Experimentally Found and Calculated after Equation 9.

Z	18:2/18:3 Exp. Found	18:2/18:3 calculated	Δ	Code Number
4 OCH_3	7.44	5.55	1.89	LAB 139 287
4 H	6.38	6.15	0.23	LAB 13 338
4 CH_3	5.53	5.17	0.36	LAB 79 044
4 Cl	4.62	4.57	0.05	LAB 77 013
3 CF_3	3.21	3.01	0.20	LAB 77 817
3 OCF_3	3.18	2.91	0.27	LAB 107 421
4 $OCH(CH_3)_2$	2.62	3.97	1.35	LAB 152 136
4 $OCH_2CH=CH_2$	1.97	3.73	1.76	LAB 152 134
4 SO_2CH_3	1.03	0.45	0.58	LAB 138 047
4 $OC_4H_9(n)$	0.98	1.51	0.53	LAB 152 137
4 $OCH_2\emptyset$	0.87	0.35	0.52	LAB 152 135
4 $O\emptyset$	0.63	0.03	0.60	LAB 164 248
4 $N(CH_3)_2$	0.58	-0.15	0.58	LAB 152 139
4 NO_2	0.54	0.93	0.39	LAB 138 050
4 NH_2	0.53	1.91	1.38	LAB 152 138

The close agreement between the experimental and calculated
(Equation 9) ratios of 18:2/18:3 support exclusion of the
4-hydroxylphenyl analogue from the calculations.   Examination of
Equation 9 shows an interdependence between the biological
activity and the hydrophobic properties of the chemical used,
commonly found with many QSAR equations.   This interdependent
relationship is determined by the π and π^2 terms, respectively.
These terms control phenomena of hydrophobic interactions with
receptors and phenomena of transport and distribution within the
total biological systems.   The occurrence of squared terms of the
hydrophobic parameter in structure-activity correlations has been
explained on the assumption that the compound has to penetrate
several lipophilic-hydrophilic barriers or compartments on its
way to the site of action (16, 17).   This is consistent with the
uptake of pyridazinones by roots and sbsequent translocation to
the shoots (chloroplast) as the site of action (13).
    The σ term describes the overall electronic characteristics
of the phenylpyridazinones.   X-ray analysis of the parent
compound LAB 13 338 was conducted to find out whether the

insertion of substituents withdrawing or releasing electrons
into the phenyl ring would affect electronic characteristics of
the pyridazinone moiety.

X-ray Structural Analysis and Electron Density Calculations.

X-ray structural analysis was applied to crystals of LAB 13 338
(Figure 4, Table VI).

Table VI.   X-ray Structural Analysis of Crystals of 2-chloro-5-
            N,N-dimethylamino-2-phenylpyridazin-3-one (LAB 13 338).

Dihedral angle between	Solvent		
	Diethelether	Diisopro-pylether molecule A	Diisopro-pylether molecule B
pyridazinone ring/ phenyl ring	58.4°	40.5°	40.8°
pyridazinone ring/ dimethylamino group	27.2°	8.8°	30.7°
phenyl ring/ dimethylamino group	83.6°	45.2°	13.8°

Depending on the solvents used, three crystalline varieties
were obtained with different interatomic distances, bond angles,
and dihedral angles between the heterocyclic moiety and the
benzene ring or the dimethylamino group respectively. This means
that even in the optimal case, with only a dihedral angle of
40.5°, a strong interaction between the electrons of the
benzene ring and of the rest of the molecule and its substituents
may be weakened.
     Nevertheless, a planar molecule with a dihedral angle of
0° between the phenyl ring and the pyridazinone ring was used
for calculating the electron densities (Figure 5). The results
describing the effect of substituents on the electron density in
various parts of the basic molecule are listed in Table VII. The
calculations reflect the maximum potential for electronic
interaction between the phenyl ring and the pyridazinone ring.
     Depending on the insertion of substituents with donor or
acceptor characteristics, the electron density within the phenyl
ring strongly changed (Δ max up to 0.162).
     The changes in the electron density show excellent
correlation with the electronic parameter σ used in Equation 9:
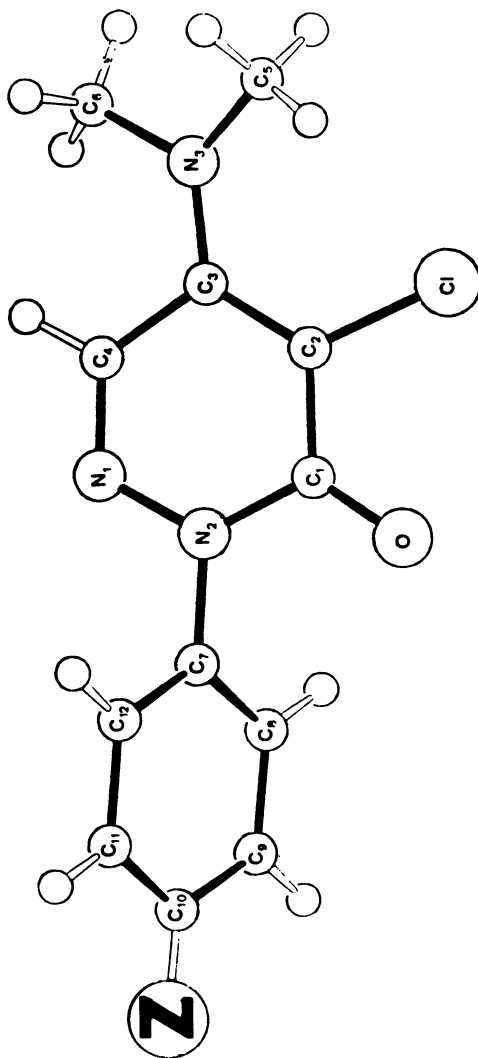
Figure 4. Interatomic distances and bond angles after x-ray structural analysis of LAB 13 338.
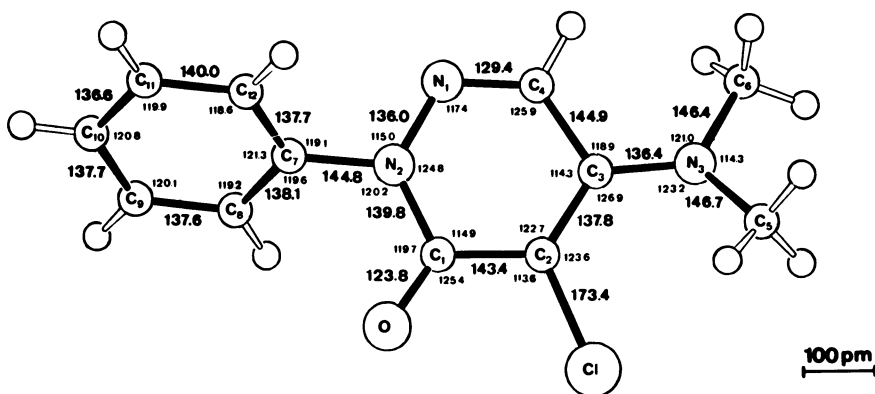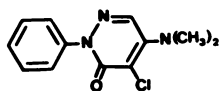
Figure 5.   Planar molecule used to describe electron
densities depending on the insertion of a substituent Z.

Table VII. Calculated Electron Densities of Various
Differently p-Substituted 2-phenyl-pyridazinones

	$Z^{1/}$				
	SO_2ME	$-H$	$-OCH_3$	$-NMe_2$	Δ max
N_1	1.097	1.098	1.100	1.103	0.006
N_2	1.500	1.507	1.507	1.506	0.007
C_4	1.008	1.018	1.021	1.025	0.017
C_3	0.880	0.884	0.886	0.889	0.009
C_2	1.176	1.177	1.177	1.179	0.003
C_1	0.784	0.789	0.791	0.794	0.010
0	1.657	1.663	1.665	1.666	0.009
Cl	1.812	1.815	1.817	1.818	0.006
N_3	1.987	1.988	1.988	1.988	0.001
C_7	0.899	0.964	0.990	1.020	0.101
$C_{8/12}$	1.038	1.036	1.032	1.029	0.009
$C_{9/11}$	0.915	0.988	1.037	1.077	0.162
C_{10}	1.088	1.027	0.978	0.952	0.136

$\underline{1/}$LAB Codes: 138 047: Z = $-SO_2Me$; 13 338: Z = $-H$; 139 287: Z = $-OCH_3$; 152 139: Z = $-NMe_2$.

$$C_{9/11}\text{-electron density} = -0.107 \; (\pm 0.05) \; \sigma + 0.994 \; (\pm 0.03) \qquad (10)$$
$$n = 4 \qquad r = 0.991 \qquad s = 0.012 \qquad F = 105.1$$

$$C_{10}\text{-electron density} = 0.091 \; (\pm 0.06) \; \sigma + 1.020 \; (\pm 0.03) \qquad (11)$$
$$n = 4 \qquad r = 0.980 \qquad s = 0.014 \qquad F = 49.8$$

$$C_{7}\text{-electron density} = -0.079 \; (\pm 0.03) \; \sigma + 0.961 \; (\pm 0.02) \qquad (12)$$
$$n = 4 \qquad r = 0.991 \qquad s = 0.008 \qquad F = 114.4$$

By contrast, the insertion of substituents with donor or acceptor characteristics changed the electron density within the heterocyclic moiety only to a minor extent (Δ max up to 0.017), and did not change the electron density at the 5-dimethylamino group. Thus, the σ^2 term out of Equation 9 in essence describes the electronic characteristic of the phenyl ring.

An overall view of the results may be gained by plotting π versus σ values together with their corresponding experimentally determined 18:2/18:3 ratios and using Equation 9 to calculate the contour for an 18:2/18:3-ratio of 4.5.

It can be observed from Figure 6 that Equation 9 is a significant and accurate equation for correlating chemical parameters with biological function of compounds altering the 18:2/18:3 ratio. The contours using Equation 9 to calculate 18:2/18:3 ratios and the placement of the experimentally determined 18:2/18:3 ratios define the π/σ area to be used as a guide to the development of novel compounds having maximum ability to shift the 18:2/18:3 ratio. A rational approach to synthesis of compounds affecting the relative proportions of 18:2 to 18:3 in plant membranes is now possible.
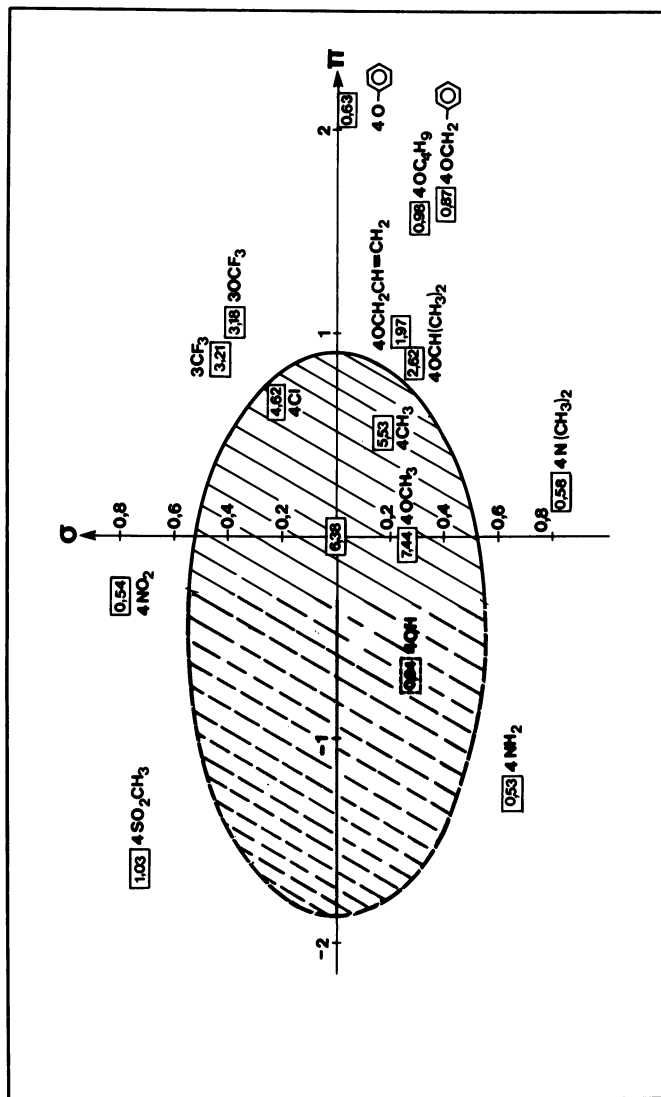
Figure 6. Diagram for 18:2/18:3-ratio depending on π and σ values. Most of the experimentally determined ratios lie in the area of π>-0.3. The area with π<-0.3 contains only two points and one misleading measurement. Therefore, the dotted lines indicate the paucity of experimentally determined values to support the predicted activity in this area of the calculated contours.

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Insecticidal and Molluscicidal Activities of Isobutylamides Isolated from *Fagara macrophylla* and Their Synthetic Analogs

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Five isobutylamides were isolated as insect growth inhibitors and toxicants from Fagara macrophylla and identified from their spectroscopic and chemical data. Synthesis and bioassay of the five natural products plus four analogs showed pellitorine to be the most active against a variety of insects, but not against a species of snail.

Biologically active isobutylamides have been isolated from plants of the Compositae and the Rutaceae.[1,2] Some of the isobutylamides were found to have paralytic and toxic activities against insects, especially when applied topically to several species of Coleopterans and Dipterans.[1,3,4,5] The present work describes the isolation, spectral identification, synthesis, and insect and snail bioassays of five isobutylamides from the Rutaceae plant, Fagara macrophylla. In addition, the synthesis and bioassay of four analogs of the isobutylamide natural products are described.

F. macrophylla is an East African medicinal tree known to be relatively free from insect attack. In order to test for chemical factors involved in this observed resistance, pieces of root bark were extracted with methanol, followed by evaporation of the methanol and suspension of the extract in water. A chloroform extraction of the suspension was chromatographed on silica gel 60 (30-70 mesh ASTM) in methylenechloride-methanol. Cuts from the silica gel column were incorporated into artificial diets optimized for several economically-important agricultural pest insects, the pink bollworm Pectinophora gossypiella, the tobacco budworm Heliothis virescens, the corn earworm H. zea and the fall armyworm Spodoptera frugiperda.[6,7] Monitoring with this artificial diet bioassay, further column chromatography and preparative TLC on silica gel in diethylether-petrol yielded five

insect growth inhibitors and/or toxicants. The five compounds
were identified as the isobutylamide compounds fagaramide
[N-isobutyl-3-(3,4-methylenedioxyphenyl)-2E-propenamide] (1),
piperlongumine [N-isobutyl-5-(3,4-methylenedioxyphenyl)-2E,4E-
pentadienamide] (2), 4,5-dihydro-piperlongumine [N-isobutyl-5-
(3,4-methylenedioxyphenyl)-2E-pentenamide] (3), pellitorine
(N-isobutyl-2E,4E-decadienamide) (4), and N-isobutyl-2E,4E-
octadienamide (5) based on spectroscopic and chemical data. In
addition, during the course of the isolation of the isobutylamide
compounds, the lignan "sesamin" [tetrahydro-1,4-bis(3,4-
methylenedioxyphenyl)-1H,3H-furo[3,4-C]-furan] (6) crystallized
from solution and was spectrally identified.   Sesamin
is a well known synergist for pyrethrins.[8]
     The most abundant of the isolated amides was fagaramide (1).[9]
The structure of fagaramide has long been known,[10] but the
geometry of its side chain double bond has not been clearly
established. This has now been confirmed as <u>trans</u> based on the
large coupling constant (16 Hz) in the 400 MHz ^{1}H-NMR spectrum.
     The artificial diet feeding assay mentioned above was
employed to monitor the chromatographic separation of the 5
bioactive principles. Once separated, purified, and spectrally
identified, the active principles were synthesized and tested in
the same artificial diet feeding assay in order to obtain
ED_{50}-values, the effective doses for 50% growth inhibition.
The growth-inhibitory activity of the 5 amides on 4 species of
Lepidopteran larvae of agricultural importance is shown in
Table I. Pellitorine, the second most abundant amide isolated
from <u>F</u>. <u>macrophylla</u>, was the most active of the isolated amides,
especially against <u>P</u>. <u>gossypiella</u> (ED_{50}=15 ppm). Pellitorine
also caused death (LD_{90}=25 ppm) to <u>P</u>. <u>gossypiella</u> larvae, but
not to those of <u>H</u>. <u>zea</u>, <u>H</u>. <u>virescens</u>, and <u>S</u>. <u>frugiperda</u>. The
isolated compound that was closely related to pellitorine,
N-isobutyl-2E,4E-octadienamide (5), also caused mortality to <u>P</u>.
<u>gossypiella</u> only (LD_{90}=100 ppm). Attempts to synergize the
insect growth inhibitory activity of the isolated isobutylamides
with the co-occurring sesamin were unsuccessful.
     Four isobutylamide analogs of the natural products were
synthesized in order to compare their insecticidal efficacy with
that of the natural products. The synthetic scheme for the
preparation of <u>cis</u>-fagaramide (7) is shown in Figure 1. The
syntheses of the <u>cis</u> (9) and <u>trans</u> (8) isomers of N-isobutyl-
cinnamamide were accomplished by procedures similar to those
utilized in the preparation of the <u>cis</u> (7) and <u>trans</u> (1) isomers
of fagaramide, with the exception that benzaldehyde was used as
starting material in the former case, piperonal in the latter.
Isobutylbenzamide (10) was synthesized from benzoyl chloride and
isobutylamine.
     Table I shows the growth inhibitory activity of the four
synthetic isobutylamides on the four lepidopterous larvae. The
activity of the synthetic <u>cis</u> isomer (7) of fagaramide (1) was
2- to 4-fold greater, depending on the insect species tested,
than was fagaramide itself. Bioassay of synthetic amides identi-

Table I. Insect growth inhibitory activity (ED_{50}[a] in ppm) of natural isobutylamides and synthetic analogs.

Compounds	Insect Species (First Instar)			
	Pectinophora gossypiella	Heliothis virescens	H. zea	Spodoptera frugiperda
Fagaramide (1)	440	350	510	530
Piperlongumine (2)	430	370	—	500
4,5-Dihydropiperlongumine (3)	800	—	—	1700
Pellitorine (4)	15[b]	270	210	230
N-Isobutyl-2E,4E-octadienamide (5)	70[c]	600	600	280
cis-Fagaramide (7)	135	175	135	150
N-Isobutyl-3-phenyl-2E-propenamide (8)	600	800	720	270
N-Isobutyl-3-phenyl-2Z-propenamide (9)	3100	800	1000	360
N-Isobutylbenzamide (10)	N.E.	N.E.	N.E.	N.E.

[a] ED_{50}-values are the effective doses for 50% growth inhibition.

[b] LD_{90}-value, the lethal dose for 90% death, for pellitorine against P. gossypiella is 25 ppm.

[c] LD_{90}-value, the lethal dose for 90% death, for N-isobutyl-2E, 4E-octadienamide against P. gossypiella is 100 ppm. No lethal effect was observed by any of the compounds to 200 ppm against the other tested insect species.
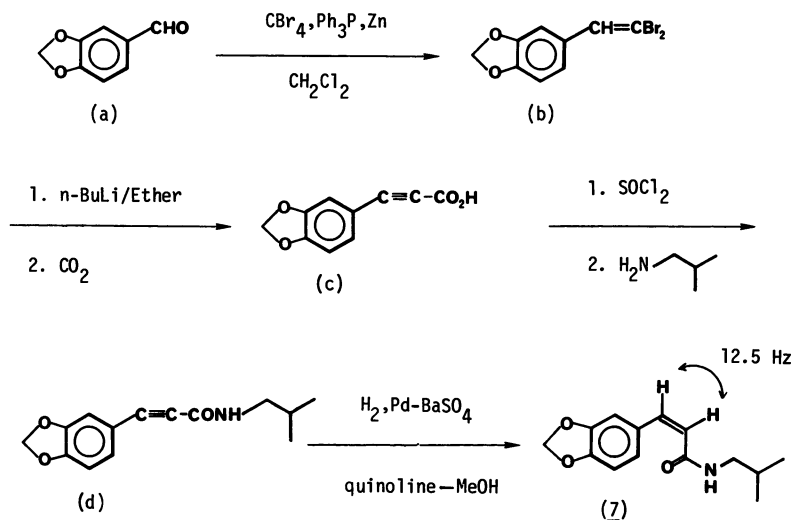
Figure 1.   The preparation of cis-fagaramide (7).

The cis-fagaramide (7) was synthesized as outlined below. The required acetylenic acid (c) was prepared from piperonal (a) by the Corey's procedure.[17] Treatment of piperonal with carbon tetrabromide, triphenylphosphine and zinc gave the bromo olefin (b) as an oil in 71% yield. The bromo olefin (b) was treated with 2 equivalents of n-butyl lithium followed by quenching with dry ice to give acetylenic acid (c) in 54% yield. Treatment of (c) with excess thionyl chloride without solvent at 50 °C followed by addition of isobutyl amine in benzene gave the acetylenic amide (d) as a viscous oil in 96% yield. Partial reduction of (d) gave cis-fagarmide (7) in 89% yield.

cal to the cis and trans isomers of fagaramide, but without the
methylenedioxy moiety, showed that the trans isomer (8) was from
1- to 5-fold more active than was the cis isomer (9) (Table I).
Except against S. frugiperda, the removal of the methylenedioxy
moiety decreased the insect growth inhibitory activity (Table I).
Isobutylbenzamide (10) was found to be inactive as an insect
growth inhibitor.

Thus, the most potent of the natural isobutylamides was
pellitorine (4), while the most potent of the synthetic
isobutylamides was the cis isomer (7) of fagaramide (1).
Through a comparison of the activities of the nine natural and
synthetic compounds in Table I, certain functionalities appeared
to be important in the efficacy of the isobutylamides to inhibit
the growth of the lepidopterous larvae. These functionalities
included the chain length, presence of the methylenedioxy moiety,
the number of the side chain double bonds, and the stereochemis-
try of the side chain. Combinations of these functionalities
would likely enhance the activity. For instance, Miyakado et
al.[11] found that addition of the methylenedioxy moiety to
pellitorine (4) increased its toxicity when topically applied to
adults of the beetle, Callosobruchus chinensis.

Pellitorine (4) has long been known for its toxicity when
topically applied to adults of the beetle, Tenebrio molitor.[12]
We found that 10 µg doses of topically applied pellitorine caused
a paralytic action on adults of the confused flour beetle,
Tribolium confusum (unpublished data). However, all of the
affected beetles recovered within 24 hrs posttreatment. Similar
topical applications of up to 20 µg/beetle of fagaramide (1),
piperlongumine (2), and N-isobutyl-2E,4E-octadienamide (5) proved
ineffective.

Previous work with extracts of various plant species contain-
ing isobutylamide compounds indicated the larvicidal effects of
the extracts against several species of mosquito.[1,13] Other
work has shown the molluscicidal activity of some unsaturated
aliphatic isobutylamides against Physa occidentalis.[14]
Therefore, we conducted additional bioassays of four of the F.
macrophylla isobutylamides with the house mosquito, Culex pipiens
and the freshwater snail, Biomphalaria glabratus (Tables II, III).
Both species are of medical importance.

The lethal activity of the isobutylamides on C. pipiens is
shown in Table II. The amides were dissolved in 0.1% acetone in
distilled water to give concentrations of 1-20 ppm. Third-instar
C. pipiens were transferred (5 larvae/10 ml test solution) into
1 oz. plastic cups using a 1 x 1-inch circle of ordinary window
screen. Each treatment was replicated 4 times and the minimum
concentration of each compound which caused 100% mortality
(LD_{100}) within 48 h at 25°C and 16L/8D photoperiod was deter-
mined. In a result similar to that found with the artificial
diet bioassay with lepidopterous larvae, pellitorine proved to
be the most toxic of the assayed amides (LD_{100} = 5 ppm).

Natural
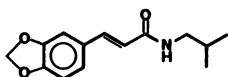
Fagaramide
(1)
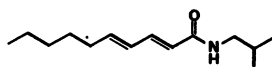
Pellitorine
(4)
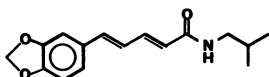
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(2)
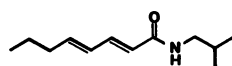
N-Isobutyl-2E,4E-octadienamide
(5)
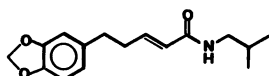
4,5-Dihydropiperlongumine
(3)
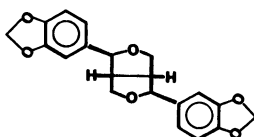
d-Sesamine
(6)

Synthetic
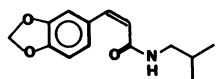
cis-Fagaramide
(7)
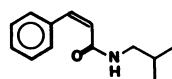
N-Isobutyl-3-phenyl-2Z-propenamide
(9)
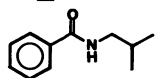
N-Isobutylbenzamide
(10)
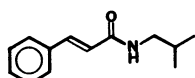
N-Isobutyl-3-phenyl-2E-propenamide
(8)

Table II. Lethal activity (LD_{100}[a] in ppm) of natural isobutylamides[b] against third-instar larvae of Culex pipiens.

Compounds	LD_{100} (ppm)
fagaramide (1)	15
piperlongumine (2)	10
pellitorine (4)	5
N-isobutyl-2E,4E-octadienamide (5)	15

[a] LD_{100}-values are the lethal doses for 100% death in a 48-h bioassay.

[b] 4,5-dihydropiperlongumine was not tested due to insufficient quantities available.

The lethal activity of the isobutylamides on B. glabratus is shown in Table III. Molluscicidal activity was monitored as described previously.[15] Briefly, snails of uniform sizes (average diameter of the shell 9 mm) were placed 2 snails/ concentration into deionized water solutions containing known concentrations of the isobutylamides. Unlike the relative activities of the isobutylamides on the tested insect species, fagaramide (1) and N-isobutyl-2E,4E-octadienamide (5) were more potent molluscicides than were pellitorine (4) or piperlongumine (2).

The mode of action of the isobutylamides is unknown, although Miyakado et al.[11] found that several isobutylamides (i.e. pi, percide and related compounds) caused repetitive discharge when the nerve cord of the cockroach, Periplaneta americana was stimulated. We found that fagaramide was inactive as an acetyl- choline esterase inhibitor in an in vitro assay[16] (unpublished data).

In summary, we have isolated and identified through spectral data and synthesis five natural isobutylamides from root bark of Fagara macrophylla as having moderate to weak activity against several species of lepidopterous larvae, a species of larval mosquito, and a species of snail. In addition, we have attempted to enhance the biological activity of the natural isobutylamides through synergism and synthesis. Although these latter attempts have not resulted in a compound as potent as the natural product pellitorine, they have served to illustrate the importance of certain functionalities in the structure-activity relationships of the isobutylamides.

While the F. macrophylla isobutylamides, especially pellito- rine, are active against several species of pest organisms of medical and agricultural importance, their activity must be enhanced before they can be used on a commercial basis. Hope- fully, the results of the present work will direct future studies leading to synthetic isobutylamides of sufficient activity to warrant their practical use in insect and snail control.

Acknowledgments

Table III. Lethal activity (LD$_{50}$[a] in ppm) of natural isobutylamides against the snail Biomphalaria glabratus.

Compounds	LD$_{50}$ (ppm)
fagaramide (1)	200
piperlongumine (2)	>200
pellitorine (4)	>200
N-isobutyl-2E,4E-octadienamide (5)	200

[a]LD$_{50}$-values are the lethal doses for 50% death.

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The Design of Triazole Fungicides

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The use of high performance computer graphics with theoretical calculations is demonstrated to be an imaginative approach in the study of triazole fungicides. As an example, theoretical work is presented which when taken with the results from x-ray, infra-red, and n.m.r. experiments can, with the help of computer graphics, be used to construct a model of the cytochrome P-450 active site. In this way the triazole fungicides can be compared directly with the natural substrate, 24-methylene-24,25 dihydrolanosterol, to provide insight into the design of novel inhibitors.

Let us begin with a fundamental question. What are the research departments of the major drug and crop protection companies trying to do? They are trying to invent small, biologically active molecules whose effects have commercial worth or advantage. In that case, what makes a molecule biologically active? Usually a molecule possesses activity primarily because it binds to an active site on a biological macromolecule, most commonly a three-dimensional protein structure. In the past, the discovery and subsequent development of biological activity has been done in three conceptual ways: (a) large scale empirical screening; (b) close analogue chemistry; (c) the more rational design of biologically active molecules at the molecular level. Although of greater financial potential, the great difficulty with the latter approach has always been our stark inability to answer several crucial questions concerning the binding of a small molecule to its protein receptor. For instance, what do (a) the substrate and (b) the active-site of the receptor actually look like as the one approaches the other. The free energy changes associated with the removal of a molecule from its solvent sheath are to some extent amenable to experimental evaluation, but the exact nature and geometry of the recognition process, collision and chemical binding of a small substrate to a protein is still largely a mystery.

In molecular terms, how one molecule appears to another, whether it be the substrate or the binding site, is really two questions.

Where are the nuclei?. This is not just a question of equilibrium shape as measured by n.m.r., x-ray or neutron spectroscopy, but also concerns what possible shapes the molecule can assume as it interacts with its partner; in general, what flexibility it possesses. Flexibility is clearly a property of both small molecules and the protein binding sites.

Where are the electrons?. This question too can only be studied experimentally for molecules in equilibrium and in a roughly homogeneous environment such as a crystal or in solution. What we really want to know is how the distribution of these electrons around the nuclei determine the likelihood of effective collision and how they then behave during the interaction. Since molecules interact most strongly at their accessible surfaces, it is important to know what these surfaces look like.

Advances in theoretical methods and computer technology mean that both these questions can now be answered using a computer and any number of easily obtained programs. Having obtained the answer to our problem theoretically however, there is a further difficulty. How can these often complex molecular properties be displayed. This is really the crux of the matter for the world's drug and crop protection companies. It is a fact of life that the scientists trained to make molecules will not be influenced by those trained to design them unless the proposed rationale can be seen to be obvious. Computer Molecular Graphics provides this link between a chemist's intuition and the vast array of chemical, physical and biological information. As an example of the use of computer graphics and theoretical methods this paper describes a study in the design of the triazole fungicides, for instance the ICI compounds diclobutrazol ('Vigil') and the new PP450 ('Impact'), and the Bayer compound triadimefon ('Bayleton'). The Eli Lilly material triarimol ('Elancocide') though not a triazole fungicide is equivalent in its mode of action. This general class of fungicide is now attracting wide commercial interest in both the crop protection and pharmaceutical industries as inhibitors of fungal ergosterol biosynthesis (Fig 1).

This design work can be divided into three stages :-

1. Assembly of biochemical, physical and biological information mostly from the literature, but also experiment, to construct a crude two dimensional picture of the site of action of these compounds.

2.   A computer graphics facility, was then used with available
     crystal data, molecular orbital and molecular mechanics
     calculations, infra-red and n.m.r. studies to construct a
     three dimensional model of the target enzyme active site (a
     cytochrome P-450) designed specifically to accommodate both
     the natural substrate (24 methylene 24,25 dihydrolanosterol)
     and these known antagonists in their minimum or low energy
     forms.

3.   This model was then used to suggest new structure activity
     relationships and contribute towards novel fungicide
     design.

The Qualitative Enzyme Model

It has been shown (1) that the triazole fungicides inhibit the
14α-demethylation of 24-methylene 24,25-dihydrolanosterol,
the ergosterol precursor. This is a crucial step in ergosterol
biosynthesis which has to be completed before a number of other
steps can begin more or less in parallel. This inhibition is
brought about by the compounds binding to the heme prosthetic
group of the cytochrome P-450 oxidase enzyme system which
catalyses this transformation. When added to a rat liver
cytochrome P-450 preparation, for instance, an unmistakable Type
II Soret difference spectrum is produced indicating that the
triazole 4-nitrogen coordinates to the heme ferric ion which
maintains its ferric (Fe^{3+}) low spin resting state. In so
doing the antagonist has to displace the natural sixth ligand of
the heme which is probably a water molecular (2) or possibly an
imidazole group derived from a protein histidine. The other
axial ligand, below the heme plane is believed to be a cysteine
sulphur as first suggested by Murakami and Mason (3).

The 14α-demethylation of dihydrolanosterol proceeds in three
main stages with the two intermediates - the alcohol, 5α-
lanost-8-ene-3β,32-diol, and the aldehyde, 3β-hydroxy-5α-
lanost-8-en-32-al, being tightly protein bound. The cytochrome
P-450 is the component of the enzyme system required to initiate
oxidation of the 14α-methyl group, but not of that responsible
for the subsequent oxidation steps required for its
elimination as formic acid (4). This initial oxidation also
seems to be directly inhibited by the alcohol and aldehyde
metabolities.

Consequently in computer modelling the antagonism of these heme binding fungicides it seemed necessary to consider only the first oxidation of the parent lanosterol to the 14-methyl alcohol. It is only in the last few years that a plausible mechanism for this oxidation has been suggested (5). On binding the substrate the ferric porphyrin is converted from low to high spin due to the displacement of the high field sixth ligand. Studies with spin labelled substrates have shown that this substrate binding site places the bound molecule very close to the iron (6). This complex is then reduced and as Fe^{2+} can then bind molecular oxygen. Further one electron reduction yields a species which is less well defined but corresponds to the hypothetical state $[Fe^{3+} O_2^{2-}]$ which has all the electron equivalents required for methyl hydroxylation, water production and regeneration of the ferric resting state. This final step, however, requires an effector molecule – a free acylating group, provided in bacterial hydroxylase by the carboxy terminal tryptophan, or the penultimate glutamine of putidaredoxin. This acyl group is responsible through a peracyl group of generating the final iron-oxene intermediate.

Figure 2 shows the crude two dimensional model of the P-450 active site. In designing an inhibitor for this process there are three central features to consider :-

1. The heme prosthetic group available for complexation.

2. The hydrophobic substrate binding site specific for lanosterol. Indeed a recent paper by Dus (7) implicates two binding sites for various cytochrome P-450's – one for substrate and the other for nascent product, and both with activated thiol groups.

3. The occurence of hydrophilic groups in an otherwise grossly hydrophobic environment. The porphyrin propionate side chains as suggested by Peterson et al. (8) and the acyl effector group could both intervene between the bound substrate and the plane of the heme. There is also the possibility of hydrogen bonding with the displaced histidine (if present) and also a general polar interaction with the polar interface which exists by virtue of the enzyme sitting in a membrane.

The task now was to locate the natural substrate and the flexible inhibitors in a three dimensional computer model of the enzyme site to examine if interactions with these features could go some way to providing plausible structure/activity relationships.
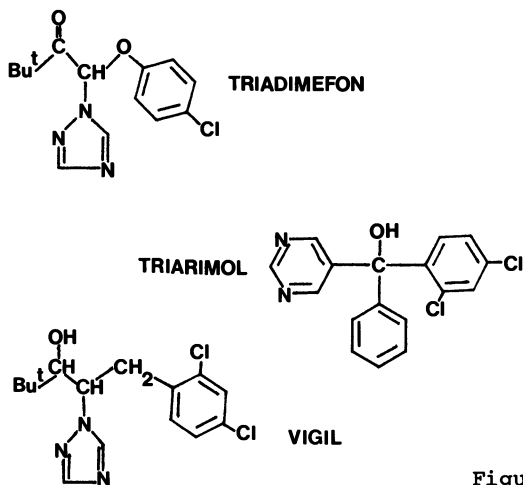
Figure 1. Triazole fungicides.
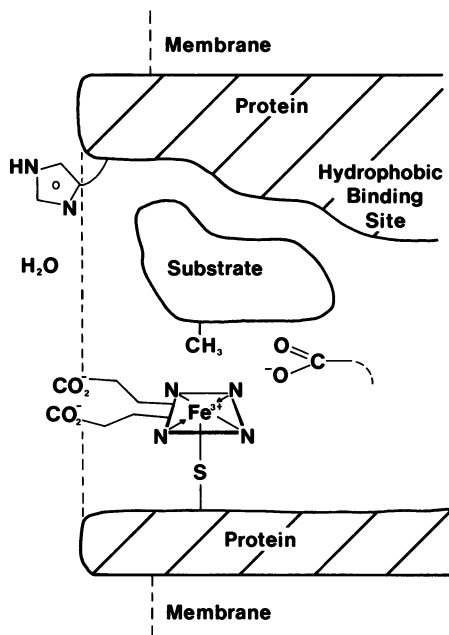
Figure 2. Model enzyme site.

Computer Modelling Of Enzyme Site

The crystal structures of the protoporphyrin IX and the
lanosterol nucleus were obtained directly by computer link to
the Crystal Structure Search and Retrieval (CSSR) library
provided by the SERC on the Edinburgh Dec 10 Computer. The
approach and interaction of the lanosterol with the iron-oxene
system was then modelled on the graphics screen. Ideally, one
might prefer to model some transition state for the reaction of
the oxene with the 14-methyl group. However, since the
intermediary alcohol could well be an inhibitor for this enzyme,
the alcohol ground state geometry was chosen with an iron-oxygen
distance of 1.9A° and a carbon-oxygen-iron angle of 130°. These
values are those obtained theoretically by Loew for an iron-
carbene system (9). There are now three single bonds :- iron
oxygen, oxygen-carbon and carbon-carbon about which the bound
lanosterol can exercise internal rotations. The computer
graphics facility could now be used to investigate the possible
orientations of the lanosterol relative to the porphyrin ring
and calculate sumultaneously, by molecular mechanics, the total
internal energy of interaction.

Figure 3, for example, places the lanosterol so as the 3β
hydroxyl polar group lies over the propionate side chains. To
reduce the complexity of this picture one can now replace the
lanosterol structure by a surface canopy to represent the extent
of the hydrophobic substrate binding site. There is also the
facility to code this surface to signify the electronic
properties of the substrates such as their electron density,
electrostatic potential, or HOMO/LUMO values. Theoretical work
of this type is currently suggesting quite remarkable
complementarity of electron properties between bound substrates
and protein binding sites. (10).

The Shapes of Bound Antagonists

At the beginning of this study the crystal structures of
specimen antagonists were unknown. Theoretically the task of
calculating all the low energy shapes for just one molecule of
interest is considerable. A complete global minimisation for a
typical triazole fungicide eg. diclobutrazol with five axes of
rotation, sampled at 30° intervals, involves a quarter of a
million individual calculations. Even with a large computer
this severely degades the quality of calculation which can be
done at each point. A strategy was used, therefore, which
attempted to reduce this number to a manageable level. Firstly,

a crude molecular mechanics method based on Van der Waals contacts was used to eliminate from a full conformational search all those shapes which are sterically too high in energy to be considered for further analysis. All the remaining steric minima were then analysed using semi-empirical molecular orbital methods and subject to a single full <u>ab-initio</u> calculation to obtain the absolute minimum energy conformation. The calculated structure for RR-diclobutrazol, for example, agrees very well with the crystal structure as determined by Branch and Nowell (11). Good agreement between calculated and x-ray was also observed for the less fungicidally active isomer (RR-) of triadimenol (12). The calculated structure for both diclobutrazol and triadimenol (Bayer) also seemed consistent with measured n.m.r. coupling constants.

<u>Hydrogen bonding</u>. In setting up these calculations the hydroxyl proton was placed so as to be unavailable for possible hydrogen bonding with the 2-N of the triazole. Theoretically it is well known that extraordinary lengths have to be undertaken to account for this phenomenon properly, even for simple molecules. It seemed more sensible to calculate the other energy contribution theoretically but to look for the formation of internal hydrogen bonding in a dilution experiment in the infra-red. Intra-molecular hydrogen bonds are not observed in the available crystal structures. Infra-red dilution studies show internal hydrogen bonds in both diclobutrazol diastereoisomers but in neither the active RS-, SR- or the less active RR-, SS-triadimenol. In short, the presence of an intra-molecular hydrogen bond between the hydroxyl group and the triazole 2-nitrogen does not in itself relate directly to activity.

A Comparison of the Antagonists with the Natural Substrate

As an example of the techniques, Figure 4 shows a comparison of the fungicidally active RR- diclobutrazol with the natural substrate lanosterol. The sterol C-32 alcohol is chelated to the iron porphyrin. The three central features of the model cytochrome P-450 can be elucidated. The hydrophobic binding site, the polar region between this hydrophobic region and the heme plane, and a common complexation to the porphyrin iron.
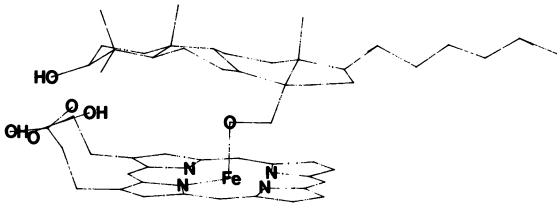
Figure 3.   Lanosterol on P-450 porphyrin ring.
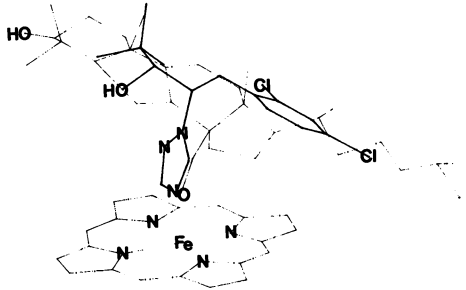
Figure 4.   Comparison of diclobutrazol and lanosterol
in P-450 active site.

Three features might be noted :

1.   The hydrophobic substrate binding site consists of three
     volumes :-

     a)   a region corresponding to the lanosterol A ring which
          terminates in a polar group, the 3β-hydroxyl. The
          inhibitor makes no use of this space in the enzyme
          cleft.

     b)   a bulky volume occupied by the inhibitor tertiary butyl
          group and in part by the sterol 6α-methyl. It might
          be expected therefore that extension of the t-butyl
          group other than onto the A ring would reduce
          activity. In fact, in vitro activity has been shown to
          be highly sensitive to the size of the this lipophilic
          moiety.

     c)   a deep cavity into which the lanosterol molecule
          protudes its side chain and the triazole fungicide
          projects the benzyl group. This suggests that the
          benzyl group could be greatly extended, which again
          agrees with in vitro data. The paraphenyl benzyl
          compound for example, shows good activity.

2.   The antagonist hydroxyl function lies at a distance relative
     to the heme group which would make it a candidate for
     hydrogen bonding to either a heme propionate side chain or
     an effector acyl group. More generally the polar hydroxyl
     and the triazole 2-N could mark the interface with polar
     protein, membrane phospholipid head groups or solution.
     This agrees very much with the model proposed by Peterson et
     al. (8) for the 5-exo hydroxylation of d and l camphor in
     mammalian cytochrome P-450, and is also consistent with the
     relationship they noted from steroid metabolism by
     cytochrome P-450, between the position hydroxylated and its
     relation to a polar funtional group.

3.   The triazole group binds perpendicularly to the heme group
     and gauche to the iron-nitrogen bonds in the porphyrin
     plane.

Structure and Activity

Figure 5 now summarises what the model requires for _in vitro_
anti 14-demethylase activity. A gauche conformation is required
between the polar function and the iron chelating group leading
to restrictions on the substitution pattern at A, B, C and D.
Logically this leads to the possibility of other substitution
patterns which will achieve this gauche conformational
requirement with groups of the right kind. Substitution at A
and B, for example, leaving C and D as hydrogen yields a series
of compounds which have all the correct requirements for
activity and yet are different in overall appearance.

The new ICI fungicide PP450 has orthofluorophenyl and
parafluorophenyl in these two positions. Theoretical
calculations on PP450 give excellent agreement with a recent
crystal structure determination by Kendrick and Owsten at the
Polytechnic of North London, which again shows no intra-
molecular hydrogen bonding but a gauche relationship between the
hydroxyl function and triazole.

lipophilic backbone

A   B   C   D

X
polar function

Fe
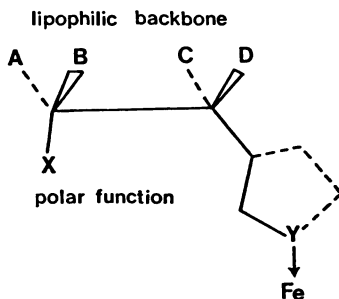
A,B :- rigid, limited length
C,D :- articulated, extended length

Figure 5.   Model P-450 14-demethylase inhibitor.

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Optimization of Physicochemical and Biophysical Properties of Pesticides

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Pesticide performance depends on the intrinsic toxicity of the chemical concerned and on the amounts which reach the sites of toxic action. There is substantial scope for improving selection of candidate compounds for development and increasing efficiency of utilisation by optimising physico-chemical and biophysical properties which underlie these determinants of performance. This is illustrated by considering the dynamics of toxicant behaviour within receiving organisms and delivery of the toxicant from the point of application to the recipient. Analysis of the free energy relationships describing processes within the organism demonstrates the dangers of misinterpreting data on relative toxicity of different compounds and the importance of designing appropriate tests for comparing compounds. In considering delivery to the receiving organism it is now possible to define the optimum physico-chemical properties for many crop protection purposes. Much can also now be done to predict molecular structures which give these properties on the basis of partition relationships and knowledge of additive molecular characteristics such as paraquat. These principles are illustrated with specific examples.

There are compelling reasons for seeking to optimise pesticide properties in relation to several criteria, including selective activity against the target organism, cost effectiveness, reliability against the target organism and avoidance of harmful effects in the environment. A common objective is to achieve the greatest degree of toxic effect to the intended recipient with the smallest amount of chemical:optimisation of

physico-chemical properties will be discussed here largely on this basis. For such optimisation it is necessary to understand the physico-chemical processes which contribute to toxicity or to establish empirical relationships between physico-chemical properties and observed effect. The former approach clearly provides a more secure framework for prediction and selection of candidate components.

The toxic effect produced by a chemical agent on a susceptible organism depends on the nature and magnitude of its interactions with the vital processes which it disrupts (which may be termed its intrinsic toxicity) and on the amounts which reach the sites of interaction. Both these determinants of toxic effect are expressions of biophysical and physico-chemical factors. The effectiveness of the lethal interaction which is equivalent to the intrinsic toxicity depends on the affinity of the toxicant for the molecular configuration of the target site and possibly on its stability to enzymatically regulated reactions. This is reflected for example, in the relationships between steric parameters and activity of a wide range of pesticides (1) or between chemical reactivity and anticholinesterase activity of organo-phosphorus insecticides (2).

Quantitative treatment of the second determinant of toxic effect, dosage transfer, is often based on the principle that relative injury is some function of (in many cases proportional to) the product of concentration (C) and time (t) in situations where a constant concentration can be maintained. More generally the Ct product should be replaced by the integral $\int_0^\infty C dt$ which Hartley (3) termed the availance. In practice, two components of the availance can be distinguished : first the processes of penetration, transport and chemical modification occurring within the organism which govern the way in which the dose received at the surface of the organism is transported to the site of action and secondly the transfer processes which must take place between the point of application and the receiving organism under any practical conditions of use and which determine the pattern of concentration in space and time to which the organism is exposed. In many laboratory studies and screening tests factors of the second type are completely excluded as the compounds are applied direct to the organism, while factors of the first type are unavoidably integrated with the intrinsic toxicity in the overall measure of response. However, it should be noted that even under such controlled conditions the relative activity of different compounds may vary according to the dynamics of the treatment (for example optimum LC_{50} for indefinite exposure, optimum $L(CT)_{50}$, LC_{50} for a standard exposure time or LD_{50} for a discrete dose). This is not merely of academic interest:

it is desirable to understand the dosage transfer processes within the organism, which underlie such differences, in order to predict optimum properties and structures and interpret correlations found empirically.

There is an even stronger case for giving more attention to dosage transfer from the point of application to the receiving organism. With most practical pesticide treatments, only a very small proportion (usually less than 1%) of the applied dose achieves its intended effect : the remainder is dissipated ineffectually in the environment. This situation is likely to be aggravated with the trend to progressively more potent compounds because the powerful weathering, inactivating and redistribution processes which attenuate the applied dose are likely to have proportionately greater effects as rates of application fall (4). Given the practicalities of pesticide application and the dispersed nature of most target organisms, highly efficient utilisation cannot be expected, but it should be possible to achieve substantial improvements by optimising the physico-chemical properties which determine response to the attenuating processes. The optimal properties are unlikely to correspond precisely with those for most effective internal transfer to the target site and there is good evidence that they are not related to molecular structure in the same way as intrinsic toxicity. For example Briggs (5) found that the electronic and partition properties of herbicidal N,N-dimethyl N^1 phenyl ureas influenced their relative inhibitory activity in the Hill reaction in the opposite direction to their effects on soil adsorption which determines availability for uptake by plant roots and mobility. It follows that compounds found to be most active when applied directly to the organism in screening tests may differ considerably from those which would perform best in practice because physico-chemical properties have not been sufficiently considered and are therefore suboptimal. The implication is that the most effective practical compounds could be missed.

To seek compounds with optimal characteristics for external and internal transfer on a rational basis requires an understanding of pesticide availance and how this is influenced by physico-chemical and biophysical properties; to predict the most effective compounds then requires a knowledge of the relationship between these properties and molecular structure. This paper briefly reviews the considerable progress which has been made in these directions and the prospects for future advance.

Optimal properties for internal transfer to the target site.
The intense interest in the discovery of potent biologically active compounds has prompted various attempts to relate toxicity to molecular structure and to codify the effects

of substituents on the toxicity of a parent structure. Effects
related to the chemical reactivity of the compound have
been successfully described in terms of the familiar free
energy parameters σ and σ^* for electronic or polar influences
and E_s for steric influences introduced by Hammett (6) and
Taft (7). A more sophisticated modern treatment of steric
influences is the 'STERIMOL' approach of Verloop (1). However
we are here more concerned with penetration into the organism
and translocation to the site of action. Probably the most
productive approach to systematising the properties influencing
these processes is that formulated by Hansch (8) which has
subsequently generated much research in this field and was
the subject of a multi-author review (9). The Hansch approach
is based on a physical model which envisages the toxicant
reaching the target site by a random walk through the various
internal tissues and phases interposed between this site
and the points of penetration. During this passage the toxicant
molecule is presumed to be subject to numerous partitions
between phases of different polarity. It would be expected
therefore that effectiveness of transfer would be influenced
by the partitioning properties of the molecule concerned
and there is a widely held view that there is an optimum
efficiency of transfer at a finite value of the partition
coefficient.

        The influence of partition and the effects of substituents
are treated quantitatively by means of the substituent constant
π defined as $\log P_X - \log P_H$ where P_X is the partition coeffi-
cient in favour of octanol from water of derivative X and
P_H is the partition coefficient of the parent molecule.
This constant like σ and E_s is, to a first approximation, an
additive property of the molecular groups comprising the
molecule as discussed more fully below. A major achievement
of structure-activity studies in recent years has been the
demonstration that the results of many simple toxicity tests
can be correlated to structure by equations involving various
combinations of these constants (10, 11). It has been considered
that correlations over a wide range of partition coefficients
require the inclusion of a π^2 term, the generalised correlation
equation having the form:-

$$\log (1/C) = K_1\pi - K_2\pi^2 + K_3\sigma + K_4E_s + K_5$$

where C is the concentration giving a standard biological
response and K indicates a constant. The π^2 term implies
a parabolic relationship between efficiency and the logarithm
of the partition coefficient and reflects the concept that
there is an optimal value of the partition coefficient.

        There are some striking examples of the parabolic
relationship, for example the relationship between relative
toxicity of pyrethroid insecticides and mobility on reverse-
phaseTLC plates shown by Briggs et al (12). However, there

are many other cases where the parabolic relationship does not obtain. In seeking a better insight into the influence of partition properties on toxicity and an improved basis for prediction of structure-activity relationships it is instructive to examine further the concepts and models which underlie the expectations of a parabolic relationship. The detailed mathematical working for the arguments set out below is given by Hartley and Graham-Bryce (13).

Transfer across alternate aqueous and lipid layers. The general characteristics of the "random walk" concept may be illustrated using the simplified model of a multilaminate septum consisting of alternate aqueous and lipid layers having equal thickness. At first sight the requirement for optimum polarity may seem valid on the argument that if partition of the toxicant in favour of the lipid phase is low, permeation will be slow because the chemical can only slowly cross the lipid layers whereas if partition is high the chemical will be held up in the lipid layers: hence transfer should be maximal at an intermediate polarity. The second part of this argument can however only apply at a sufficiently early stage of the permeation process: once the capacity of the lipid phases has been saturated there is no further hold-up. The flux per unit area (F/A) across the septum (thickness X) when this steady state is reached, assuming a constant concentration C_o on the supply side of the septum, is given by:-

$$F/A = C_o(D/X)(2P/1+P)$$

where P again denotes partition coefficient and D is the diffusion coefficient. The flux is thus a monotonic function of partition coefficient, the partition coefficient term being zero for P = 0 and having a limit of 2 for this model as P $\rightarrow \infty$. This general conclusion would not be altered in principle for more realistic assumptions of different thicknesses and diffusion coefficients and clearly is not consistent with a parabolic relationship between partition co-efficient and toxicity.

        In the crescent state, before the steady state is reached, however, the flux is given by relationships of the form:

$$F/A = (4P/(1+P)^2 \ D\bar{C}) \ X \ F/A = \bar{C} \ (D/X) \ (4P/[1+P^2])$$

where $\bar{C}$ is the mean concentration. This flux does go through a maximum, tending to zero for both P $\rightarrow$ 0 and P $\rightarrow \infty$, as a result of the permeability and capacity properties of the septum being influenced differently by partition coefficient. The position is summarised in Figure 1 which shows the flux passing through the model septum as a function of partition coefficient at different times after application of a constant concentration to the input side.
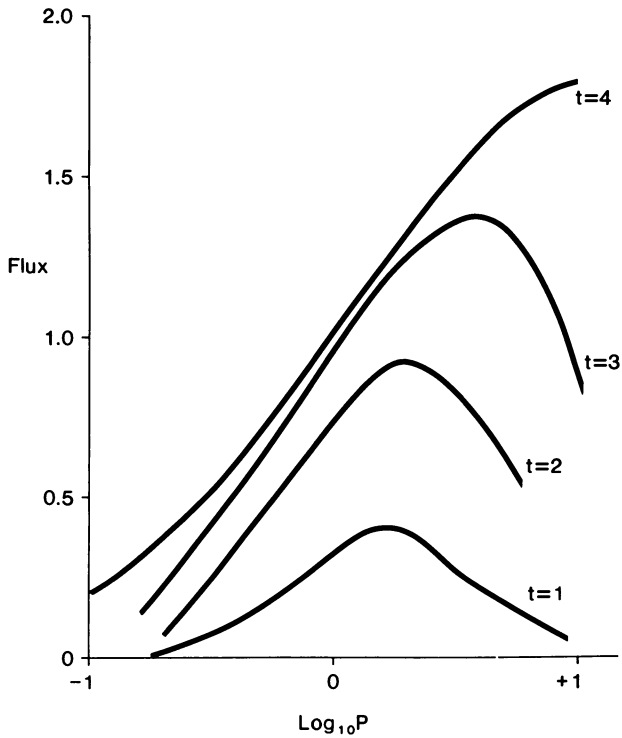
Figure 1.   Flux passing through model septum as a function
of partition coefficient at different times (t) after appli-
cation.   Redrawn from Hartley and Graham-Bryce (13).

It should be emphasised that the dependence on partition coefficient occurs only in the earliest stages of the transfer which is unlikely to apply to the cases examined in structure/activity studies. It should be noted also that if the constant supply concentration in the model is replaced by a discrete dose application the availance within the organism would also depend on partition coefficient, but would pass through a minimum with changing P rather than a maximum.

The conclusions indicated above were also reached from computer analysis of the rather more arbitrary model of Penniston et al (14) by Dearden and Townend (15). In view of the large variations with P of time to maximal concentration, they expressed concern at the widespread practice of measuring biological effects of a series of congeners at a fixed time interval after dosage.

This excursion into models suggests that the random walk process in itself is unlikely to lead to parabolic relationships between partition coefficient and toxicity and that other processes must also be involved. Some of these are considered below.

Differential effects on uptake and excretion. If the tissue in which the lethal process occurs is separated from the source of the toxicant and from an excretory or detoxifying process by septa with permeabilities influenced differentially by partition coefficient, then the concentration in that tissue will go through a maximum with change of partition coefficient under steady state conditions. For example if the septum on the input side is of the type described above and that on the sink side consists of a group of equal parallel aqueous and lipid paths, the concentration in the central tissue is given by

$$C = 4C_o P/(1 + 6P + P^2)$$

which is maximal at $P = 1$ as shown in Figure 2 where values of C predicted by this relationship are plotted against log P for $C_o = 20$ arbitrary units.

Interaction of permeation with decomposition. The effects of detoxifying reactions can be considered using the multilaminate model and assuming that first order reaction takes place only in the aqueous phase with a rate constant K which becomes $K/(1+P)$ when applied to the greater concentration in the multilaminate septum. The variation with partition coefficient of the availance at the mid point of the model septum following application of a discrete dose and assuming that the inner face is maintained at zero concentration is shown in Figure 3 for $K = 1$ and $K = 0.04$. The general parabaloid shape will be apparent.
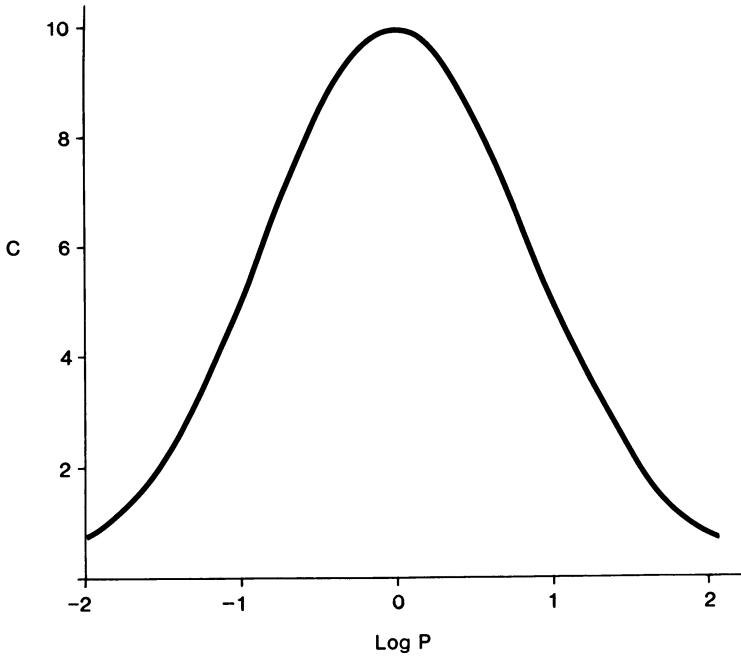
Figure 2. Effect of partition coefficient on concentration
within tissue bounded by barriers with different permeability
characteristics.
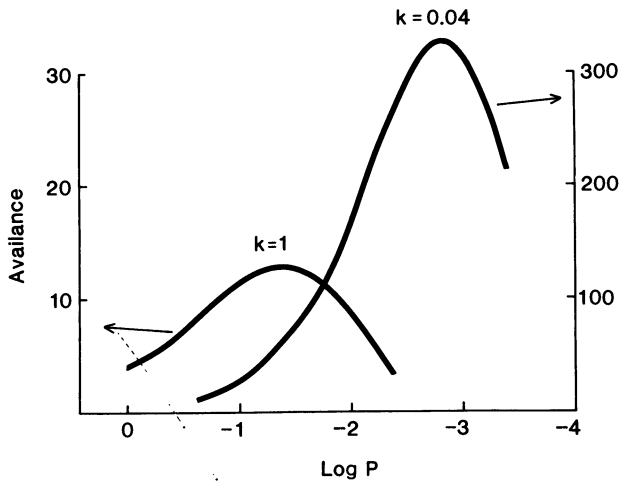
Figure 3. Effect of partition coefficient (P) on availance
within a multilaminate septum in which detoxifying reactions
occur with rate constants (k) = 1 and (k) = 0.04. Redrawn
from Hartley and Graham-Bryce (13).

Effects of partition coefficient on form of C-t curve.

The analysis presented so far demonstrates that the effective-
ness of steady-state transfer is probably always a monotonic
function of partition coefficient, but that combination
with other processes can result in the total availance passing
through a maximum. Permeability can however have a much
greater influence on the shape of the C-t curve, as compared
with the influence on its area which represents the availance.
Because the capacity for the toxicant of the tissues intervening
between point of application and target site can increase
indefinitely with partition coefficient whereas the increase
of permeance will be limited, the pulse curve of the toxicant
arriving at an internal site will always become flatter
and more diffuse with increase of P. The shape of the pulse
can be of considerable importance in determining biological
response as the following argument shows.

   Although it is generally considered that response
to a toxicant is determined by the availance $\int_{0}^{\infty} Cdt$, it must
clearly also be expected that there is some threshold of
concentration below which the toxicant can be tolerated
indefinitely: the toxicant arrives so slowly that replacement
and repair reactions can compensate for any damage. At the
other extreme if the pulse is too rapid the temporary disruption
of the vital process may be too short to produce irreversible
effects. There is thus likely to be only a defined range
of pulse forms producing the specified biological response
for any applied dose and since pulse form depends so strongly
on P. an optimum value for the partition coefficient within
a series of related compounds.

   It may be concluded from these various arguments
that the random walk concept will not in itself give rise
to an optimum partition coefficient for biological effect,
except in the early stages of the build up to the steady
state. A maximal internal availance at an optimal value
of P only arises when uptake competes with chemical decay
or an excretion process with a different permeability relation-
ship. The explanation of an optimum P value is more likely
to involve effects on the form of the pulse of toxicant
reaching the site of action, which result in the effect
of the chemical transferred, rather than the availance,
being greatest at a particular polarity.

   If these conclusions are valid they have important
implications for the design of tests to evaluate relative
toxicity and also for structure/activity theories; for example
if the relationship between polarity and toxicity is strongly
influenced by decay processes then it is important that
these are given appropriate consideration in designing candidate
compounds. The analysis presented above also gives a strong
support for further pharmacodynamic studies of pesticide action

such as those undertaken by Soderlund (16) with pyrethroid
insecticides, which would provide the essential information
to clarify the relative importance of the different processes.

Toxicant delivery to the receiving organism

Since in practical use by far the most significant factors
reducing amounts of toxicant reaching the target site operate
outside the organism, it is perhaps surprising that more
attention has not been given by those seeking more effective
pesticides to characterising the processes occurring between
the points of application and uptake, and to optimising
physicochemical properties to minimise losses. However,
the principles of dosage transfer outside the organism are
now reasonably well defined and the required properties
can be specified within reasonable limits. Furthermore,
analysis of the transfer processes can suggest more sophisti-
cated approaches to improving pesticide performance such
as the use of precursors or progenitors having more favourable
properties for efficient delivery than the toxicant itself.
The underlying objective is to achieve a pattern of concen-
tration in space and time which will maximise uptake by
the intended target species and minimise exposure of unintended
recipients. Much can be achieved in this direction by manipu-
lation of application methods and formulation, but these
lie outside the scope of this paper. The following sections
illustrate some of the possibilities for specifying and
obtaining appropriate properties in the toxicant molecule
for efficient delivery. Space only allows a few examples
from a large subject which is more comprehensively covered
by Hartley and Graham-Bryce (13).

Behaviour in soil. The mobility and biological availability
of pesticides in soil is determined essentially by the way
in which they partition between the solid, liquid and air
phases, which in turn depends on their volatility, solubility
and polarity. Variation in these properties, together with
adjustment of initial placement by method of application
and rate of release through formulation can give a wide
range of dosage delivery patterns in relation to differently
distributed receiving organisms.
    In broad summary, a reasonable measure of residual
effect for uptake by plant roots or control of soil-borne
organisms requires moderate adsorption (which expresses
the partition between solid and liquid phases) and a small
fraction in the air-phase to allow some movement in dry
soil conditions and some degree of vapour action. This last
property is particularly important in ensuring a robustness
of performance under varying climatic conditions which may

outweigh the advantages of an intrinsically more active
but more variable compound.

Optimal values for adsorption properties may be deduced
by considering effects on the two transport processes, diffusion
and mass flow. To estimate detailed figures, it is necessary
to solve transport equations for the particular boundary
conditions of the system under study, but in general terms
the effects on mass flow can be illustrated by considering
the downwash through soil of a thin band of pesticide. From
simple chromatographic theory it would be expected that
such a band would be leached downwards as a bell-shaped
curve which becomes flatter and more diffuse with distance
travelled. The rate of descent of the peak concentration
is f times the rate of descent of the soil water, where
f is the fraction of pesticide not adsorbed, equal to
$\theta/(\theta + K_d)$ where θ is the moisture content and K_d is the
slope of the adsorption isotherm, assumed linear. The effect
on diffusion is to reduce the diffusion coefficient to
$1/(K_d + \theta)$ times that in the free liquid of the pore system.
Detailed consideration of these principles suggests that
K_d values of up to about 40 are satisfactory for most soil
applications. For example, calculations by Graham-Bryce
(17)indicated that mass flow and diffusion were capable
of supplying adequate quantities of the systemic insecticides
disulfoton (K_d = 20) and dimethoate (K_d = 0.03) to wheat
roots following soil treatment to give satisfactory control
of aphids. As a further example, in practice most residual
soil-acting herbicides have K_d values in the range 1 - 20.
Inactivation in soil by adsorption, as occurs with the bipyridi-
num herbicides would require values exceeding 10^3 and preferably
10^4.

In the case of partition between solution and vapour
phases, because vapour movement is so much faster than movement
in solution, a partition coefficient between vapour and
aqueous phases (P_{water}^{air}) of 10^{-4} is normally sufficient to
ensure adequate vapour effects. Many soil-acting pesticides
have significantly lower partition coefficients but they
tend to be less effective in dry conditions. For fumigant
action, a higher value (> 0.01) is of course desirable, and
adsorption should also be slight. The vapour/solution partition
may be calculated from the ratio of the solubility to the
concentration in the saturated vapour which may in turn
be calculated from the vapour pressure. Table I summarises
properties required for different patterns of behaviour
in soil and may be compared with values for representative
pesticides in Table II. The requirements are of course not
absolute also favourable air/water partition may partly
compensate for unfavourable adsorption and vice versa.

Table I. Properties required for specified types of action in soil

	$P \frac{air}{water}$	K_d
Fumigant action	> 0.01	< 5
Residual action: moderate mobility and ready availability to roots and soil-borne organisms	$10^{-3} - 10^{-6}$	$0.5 - 20$
Localised action: seed treatments and placed granules	$10^{-3} - 10^{-6}$	$20 - 50$
Inactivation by adsorption: no residual action	negligible	$> 10^3$

Table II. Properties of some representative soil-applied pesticides

Compound	Type	$P \frac{air}{water}$	K_d
Ethylene dibromide	fumigant	0.02	0.5
Dichloropropene	fumigant	0.05	2.8
EPTC	residual herbicide	5.3×10^{-4}	6.0
atrazine	residual herbicide	3.1×10^{-7}	2.9
dimethoate	soil insecticide	4.0×10^{-9}	0.3
disulfoton	soil insecticide	1.8×10^{-4}	20
lindane	soil insecticide	5×10^{-5}	25
ethirimol	fungicide: seed treatment	1.1×10^{-7}	33
paraquat	non-residual herbicide	neg.	$> 10^5$

<u>Uptake by plants, insects and fungi</u>. Penetration into plants for systemic action and through the outer membranes of insects and fungi is a complex process which may involve compound-specific metabolic or active processes. Rigorous relationships with physico-chemical properties cannot therefore be expected. Nevertheless some broad general guidelines can be deduced.

Uptake from soil by the underground parts of plants involves a series of steps. Firstly the compound must be readily available from soil which implies moderate adsorption as discussed above. The chemical must then be capable of entering the free space, passing through the lipophilic barriers in the endodermis and have sufficient water solubility to move in significant quantities through the apoplast without being sequestered into lipoidal materials which it encounters in its path. Hence some compromise between hydrophilic and lipophilic character is essential for optimum uptake and mobility with a tendency for hydrophilic properties to be more important. Thus compounds such as dimethoate ($P_3 = 6.3$) are readily translocated whereas lindane ($P_4 \simeq 10^3$) shows slight systemic properties; a value of $P = 10^4$ can be regarded as the limit for apoplastic systemic action. Uptake and trans-location in the symplast almost certainly involve compound-specific processes so that no general relationship with polarity can be deduced although it is clear that the plasmalemma separating the apoplastic and symplastic systems is much more permeable to lipophilic than to hydrophilic molecules.

There is, however, one means of achieving the conflicting properties required for apoplastic transport without resort to compromise. This is by using precursors which have character-istics favouring uptake and are then converted within the plant to active toxicants which are more readily translocated. The principle is best illustrated by the long-established organophosphorus insecticides of the systox type such as demeton, disulfoton and phorate which are relatively lipophilic (log $P \simeq 5$) favouring uptake by the plant. Once absorbed however they are oxidised at thioether and $P = S$ groups to give more polar products (log $P \simeq 2.5$) which, as indicated above, have polarity more appropriate for translocation; they are also more effective toxicants and incidentally, more readily degraded. Many other chemical conversions to give either more polar or more lipophilic products can be suggested, and it should also be recognised that the principle can be elaborated advantage-ously by exploiting characteristics of dosage transfer. For example experimental measurements and computation of uptake of organophosphorus insecticides from soil (17) suggest that for some compounds mass flow in response to transpiration may bring larger quantities of toxicant to the root surface than can be taken up, resulting in an accummulation at the root surface. It is also know that there is a gradient of pH across the rhizosphere and that the pH at the root surface

may often differ from the pH a few mm away by 1-2 units (18). It should therefore be possible to devise pH-sensitive precursors which would be converted effectively only in the required region for uptake to compounds of appropriate polarity: this would be especially beneficial where the products were labile and required protection against degradation.

Penetration into insects is greatly influenced by the manner in which the compound is presented. Classic studies by Treherne (19) indicated that when the toxicant is supplied to detached insect cuticle in aqueous solution penetration decreased with increasing polarity, explicable on the assumption that partition into and passage through the lipoidal epicuticle is the rate determining step. In contrast several studies (20 - 22) have shown that when the toxicant is dissolved in a suitable organic solvent penetration decreases with increasing oil/water partition coefficient. This is consistent with the hypothesis that the organic solvents introduce the toxicant directly into the hydrophobic epicuticular wax so that penetration is determined by the rate at which the chemical moves from the wax through the underlying more polar layers of the cuticle: this would obviously be favoured by polar character. Finally it should not be forgotten that insecticides may be received by insects in the absence of carrier solvents, either as vapour or in certain ULV applications. The vapour route in particular may be of considerable importance for uptake both from soil and from leaf surfaces as discussed further below. Under these conditions uptake appears to be proportional to partition coefficient (23,24) which would be expected as the compounds must first pass unaided through the lipophilic epicuticular layer.

Penetration into and accumulation by fungi are considered to be key factors in the selective toxicity of many fungicides and again polarity appears to be a determining property. Indeed selectivity between fungitoxicity and phytotoxicity may well be achieved by attaining the appropriate polarity in the fungicide molecule so that it can readily penetrate the fungus but not the plant cuticle. Rough calculations from some of the published results for alkyl imidazolines and ethylenethioureas (25,26) suggest that the optimum for uptake by fungal cells may be in the region of log P=5.5 as compared with log P=4.5 for uptake of the same compounds by plant surfaces. More recent studies by Brown and Woodcock with formamide fungicides (27) have also established the importance of polarity in a Hansch analysis. Caution should be exercised in seeking generalised conclusions because of differences between fungal species in cell wall characteristics.

Residual action on plant surfaces. Many crop protection agents exert their effects by residual action following application to plant surfaces. In addition to photochemical stability,

the properties required for such applications include reasonable affinity for the plant surface to give rainfastness, although not to the extent that the chemical is rendered unavailable to damaging organisms if the toxicant acts by contact effects. For control of organisms on the plant surface sufficient volatility to ensure vapour transfer is also desirable, particularly as most spray or granule applications leave a deposit in the form of discrete spots and a large proportion of the surface is effectively untreated. Approximate values may be assigned to the key properties in the light of experience with representative pesticides. Rainfastness requires that the compound does not partition readily into water from the material of the cuticular waxes: the example provided by some of the best known and most effective contact insecticides such as DDT and the synthetic pyrethroids suggests Log P values of 6 to 7 are optimal: this may be compared with the value of <4 for translocated compounds discussed above.

The optimal vapour pressure will be such as to allow some vapour transfer, but not give rise to rapid loss to the atmosphere by evaporation. This implies surprisingly low vapour pressures. The evaporative potential of the atmosphere is substantial and could easily dissipate 2 kg/ha/month of a pesticide with a vapour pressure of 10^{-5} mm Hg (28). The positive effects of vapour transfer can be exemplified by calculating the quantity of toxicant (M) taken up in time t by an idealised model insect, assumed to be a sphere of radius a. For practical purposes this is given by:-

$$M = 4 \pi a \, DtC$$

where D is the vapour diffusion coefficient and C is the uniform vapour concentration to which the insect is exposed, which would approximate to the saturated vapour concentration (SVC) close to a pesticide source. For illustration we may consider phorate which has a SVC of about 1.2×10^{-10} g ml^{-1} at temperatures typically occurring in the field and for which D is approximately 0.1 cm^2 s^{-1}. For a model aphid, a can be taken as 0.5mm, giving a potential uptake over one hour of roughly 3×10^{-8} g which is of the same order as the observed LD_{50}, consistent with experimental evidence for vapour effects with this compound. In the case of fungicides, Bent (29) found clear evidence for vapour action with drazoxolon (v.p. 4×10^{-6} mm Hg) and oxythioquinox (v.p. 2×10^{-7} mm Hg). Taken with other evidence, such results suggest that vapour pressures in the range $10^{-5} - 10^{-7}$ are appropriate: it should be recalled that the volatility of the active ingredient may be modified by formulation or by sorption into receiving surfaces.

The various properties favouring particular types of action are summarised in Table III. It must be stressed that these can be characterised only in very broad terms and that the type of activity may be shown to some degree by compounds falling outside the specified range, in some cases as a result

of individual factors such as metabolic or chemical conversion. Nevertheless pesticides having the properties listed can be expected to give the corresponding patterns of behaviour; Table IV presents values for some representative translocated and contact pesticides for comparison, to complement those shown in Table II.

Prediction of structures having optimal properties

The various broad guidelines indicated in the preceding sections are illustrative only and would have to be refined by detailed

Table III. Physico-chemical properties favouring different types of pesticide action

Activity	Properties required
Effective apoplastic translocation in plants	$\log P < 3$
Persistent residual action on foliage	$\log P > 5$; v.p. $< 10^{-5}$ mm Hg
Vapour action by residual deposit	v.p. $10^{-5} - 10^{-7}$ mm Hg
Contact action : direct absorption through insect cuticles	$\log P > 5$
Selective uptake by fungal cells	? $\log P \sim 5.5$.

Table IV. Properties of representative translocated and contact
pesticides

Compound	$\log^{p}$	Vapour pressure (mm Hg at 20°C)	Type of action
dimethoate	0.8	4.1×10^{-6}	systemic insecticide
ethirimol	1.3	9.4×10^{-7}	systemic fungicide
aldicarb	1.57	5.3×10^{-5}	systemic nematicide and insecticide
ioxynil	1.65	6.2×10^{-7}	contact herbicide with systemic activity
carbaryl	2.32	2.1×10^{-5}	contact insecticide; slight systemic properties
captan	2.54	5.0×10^{-6}	general fungicide
linuron	2.76	8.6×10^{-6}	translocated soil-applied herbicide
lindane	3.2	9.4×10^{-6}	soil-applied and contact insecticide with some fumigant action; minimal systemic action
parathion	3.93	3.8×10^{-5}	non-systemic contact insecticide with detectable vapour action
DDT	6.0	1.9×10^{-7}	non-systemic, persistent contact insecticide
permethrin	6.6	1.5×10^{-7}	non-systemic, residual contact insecticide.

(data from Hartley and Graham-Bryce (13) and Briggs (33))

consideration of the dosage transfer objectives for the parti-
cular situation envisaged in order to arrive at a detailed
specification for appropriate molecular properties. They
demonstrate, however, that such specifications can now be
produced and that this can and should be as much a part of
directed rational synthesis as the consideration of toxico-
logical properties.

Having specified the required properties, consideration
must then be given to devising molecular structures which
will provide them. In this connection a most valuable physico-
chemical principle is that the free energy increase, ΔG,
in the transfer of a molecule between two phases is an approxi-
mately additive property of the component groups. The partition
coefficient is given by the exponent of $\Delta G/RT$ according to
Boltzmann's law so that the log of the partition coefficient
should also be an additive property. This principle found
earliest expression in Traube's rule for surface tension.
Where a molecule contains polar groups some modification
of the simple additive relationship may be necessary to allow
for their interactions.

Provided, therefore, the additive contributions for
different structural components can be quantified, the partition
coefficient can be readily computed. A long established and
convenient means for such quantification is already available
in the form of the parachor, which is equivalent to the molar
volume of a substance when its surface tension is unity.
Parachor is primarily an additive property and there are
extensive tabulations of parachor equivalents for various
structural elements, such as that by Quayle (30). Parachor
(H) can be related to partition coefficient (P) using the
relationship of McGowan (31):-

$$log P = 0.012H + E_a$$

where E_a is a term to allow for interactions between hydrogen-
bonding groups where these are present. The parachor concept
was first related to pesticide behaviour in soil by Lambert
(32) and subsequently further developed by Briggs (33). Briggs
further showed that the ability to calculate partition coeffi-
cients as an additive property was particularly powerful
when coupled with another physico-chemical principle ennunciated
by Collander (34) which shows that any pair of partition
coefficients, P_1 and P_2, can be related by the expression:-

$$log P_2 = A log P_1 + B.$$

The reason why this combination of principles is so powerful
is that Briggs was also able to demonstrate that the key
properties discussed in earlier sections which determine
pesticide behaviour can be regarded as partitions. Thus solubility
may be envisaged as a partition between the compound itself
and water, soil adsorption can be treated as a partition

between soil organic matter and water and so on. Hence if the partition coefficient for one system can be calculated using additive values, it should then be possible to compute the other properties from the Collander relationship. Octanol/water partition (P) is an obvious reference system in view of its widespread use in structure/activity studies and knowledge of the parachor relationship indicated above. The appropriate equations relating this reference system to key properties found by Briggs were:

Water solubility (WS) For organic liquids:-

$$\log WS = 1 - \log P$$

For solids it is necessary to introduce a further term to allow for the energy needed to break up the crystal structure. The necessary correction long established by thermodynamic theory is $L \cdot T/R(T_1 \cdot T_m)$ where ΔT is the temperature difference between the melting point T_m and the temperature of measurement T_1 and L is the latent heat of fusion. For most compounds L increases with melting point and Briggs found the following expression a satisfactory approximation:

$$\log WS = -0.38 - \log P - (0.01 \, T_m - 0.25).$$

Soil adsorption

$$\log P_W^{OM} = 0.52 \log P + 0.41$$

where P_W^{OM} is the partition coefficient between soil organic matter (which is the soil component having the dominant influence on adsorption of most non-ionic pesticides) and water.

Vapour pressure. Vapour pressure (vp) of non-associated liquids can be related to temperature (T) using the impirical equation of McGowan (35) which is based on a combination of Trouton's rule and the Clapeyron-Clausius equation as follows:-

$$\log vp = 5.6 - 2.7 \, (T_b/T)^{1.7}$$

where T_b is the boiling point. Vapour pressure at any temperature can thus be derived from a measurement of boiling point. Where the boiling point is not readily available, or for consideration of hypothetical structures, approximate boiling points can be calculated by adding increments for substituents to parent structures of known boiling point as shown by Briggs (36).

For solids, the crystal factor must again be included giving the expression:-

$$\log vp = 5.6 - 2.7 \, (T_b/T)^{1.7} - (0.01 \, T_m - 0.25).$$

Comparison of measured and predicted values

The concepts of defining the physico-chemical properties required for various pesticide treatments and then predicting structures which will have the intended characteristics may be illustrated by reference to dosage transfer in soil which

is often particularly demanding. This will be exemplified by reference to diffusion of three compounds of contrasting properties listed in Table II: the fumigant ethylene dibromide and the systemic organophosphorus insecticides disulfoton and dimethoate (28).

It has already been pointed out that fumigant action requires favourable air/water partition and weak adsorption: ethylene dibromide owes its success to these properties which allow it to move rapidly in soil air space. Limited adsorption is also essential for availability to plant roots. The very weak adsorption of dimethoate thus makes it readily taken up from soil by plants, although it is also vulnerable to downwash. The air/water partition properties of dimethoate rule out any possibility of vapour action and also make the compound very sensitive to soil moisture content, becoming virtually immobile in dry soils. The advantages of a vapour component are demonstrated by disulfoton where the air/water partition ensures a balancing of air and water pathways so that movement and availability are more or less independent of moisture content. The stronger adsorption also protects against leaching and retains the chemical in the intended zone without rendering the chemical unavailable. The physico-chemical properties of disulfoton may be regarded therefore as approaching the optimal specification for a soil applied systemic compound.

To ascertain how far it might have been possible to anticipate the properties of these compounds in advance, and predict those for hypothetical structures, measured values are compared in Table III with values calculated from the relationships given above.

Vapour pressures for the organophosphorus compounds were estimated by applying boiling point increments for substituent groups to the known value for $(EtO)_2$ PSSEt.

The agreement may be regarded as satisfactory, except for the calculated vapour pressure of dimethoate. This may be due to inappropriate allowance for the melting point effect or it may be that the measured value is approximate bearing in mind the difficulties of measuring low vapour pressures. Even allowing for this discrepancy however the calculations are adequate to classify the different compounds and predict broad patterns of behaviour.

Clearly much more elaborate treatments are possible, particularly if simulation modelling is employed, but such results give encouragement for further development of this approach and for giving more attention to these physico-chemical factors which have such a profound influence on efficacy and performance.

I believe that John Siddall, whom this symposium commemorates would have approved of this view as representing a further component of the rational approach to pesticide design which his work so clearly represented.

Table V. Comparison of measured and calculated properties for representative soil-applied pesticides

	Ethylene dibromide		Dimethoate		Disulfoton	
	measured	calculated	measured	calculated	measured	calculated
Solubility mg l^{-1}	4300	4700	2.5×10^4	1.3×10^4	25	32
Vapour pressure mm Hg	11.0	11.3	8.5×10^{-6}	8.2×10^{-5}	1.8×10^{-4}	3.4×10^{-4}
K_d	0.5	2.3	0.28	0.34	25.1	37.3
$P \frac{water}{air}$	40	41.1	2.5×10^8	1.27×10^7	5500	6500

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Partitioning in Pesticide Mode of Action and Environmental Problems

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The hydrophobic parameter, as measured by logP (octanol/
water), has been used to optimize transport or binding
of bioactive molecules to bacteria, organelles and in-
tact animals. It now appears that the optimal log P is
the same for animals as for plants (2.0-2.5). The direct
relationship between logP (o:w) and logP (oc) (the or-
ganic component of soil) and between logP (o:w) and bio-
accumulation in aquatic organisms makes this a useful
parameter to model environmental transport and fate,
especially since good progress is being made in calculat-
ing it from structure. The capabilities and current
problems of both manual and computer calculation pro-
cedures is discussed.
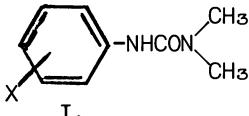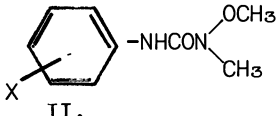
As the title of this symposium suggests, it is becoming rare in-
deed when "ein glücklisher zufall" (1) turns up a marketable pest-
icide. It is almost a certainty that, in this field, the future
belongs to those who develop some sort of design rationale.
Rational design, however, implies some knowledge of mode of action,
and this information usually falls far short of the ultimate; i.e.,
knowledge of the three-dimensional structure of a target enzyme,
of the active or allosteric sites which can prevent normal sub-
strate processing, and of the difficulties which an inhibitor
might encounter in being transported, unmetabolized, to the target
site. In this paper the focus is on some of the simpler aspects
of mode of action. These can be helpful in rational pesticide
design even before much of the mechanistic details have been
elucidated. More specifically, the discussion will cover some
current efforts to apply the knowledge of hydrophobic forces at
the molecular design stage: to insure optimized passive transport
to the active site, to increase the binding of inhibitor to enzyme,
and to predict some increasingly important environmental effects,
such as bioaccumulation and soil transport. Thus this paper will
not address the problem of *discovering* new toxiphores, but, rather
the optimizing of some new lead with respect to these factors. At
first these factors might seem peripheral, but nonetheless they

have often spelled the difference between commercial success and
failure.

QSAR in the Hill Reaction

In the search for more effective post-emergent herbicides, many
laboratories have measured the inhibition of photosystem II in
chloroplasts; i.e., the Hill reaction. In a continuing investi-
gation of this system, (2) Corwin Hansch's group at Pomona College,
in cooperation with BASF in Germany, analyzed two sets of phenyl
substituted ureas: 17 1,1-dimethyl-3-phenyl, and 38
1-methoxy-1-methyl-3-phenylurea analogs acting on spinach
chloroplasts (Table I). In all cases, including comparisons

Table I   Ureas as Hill Reaction Inhibitors

I. Substituents

1. H	4. 3-OCH$_3$	7. 3-OH	10. 4-COPh	13. 4-t-Bu
2. 3-NO$_2$	5. 3-nBu	8. 3-NH$_2$	11. 4-F	14. 4-i-Pr
3. 3-CF$_3$	6. 3-O(CH$_2$)$_2$Ph	9. 3,4-Cl$_2$	12. 4-CyHex	15. 4-O-n-Dec

16. 3-OCH$_2$PH-2',4'-Cl$_2$ 17. 3-Cl,4-CO$_2$CH(CH$_2$-i-Pr)$_2$

II. Substituents

1. H	14. (Flourenyl)	27. 4-i-Pr
2. 3-t-Bu	15. 4-NO$_2$	28. 4-NHSO$_2$CH$_3$
3. 3-F	16. 4-NH$_2$	29. 4-Cy-Hex
4. 3-CN	17. 4-OCH$_2$Ph	30. 4-O(CH$_2$)$_2$-CyHex
5. 3-O-CH$_2$Ph	18. 4-O(CH$_2$)$_2$Ph4'-Me	31. 4-OCH(CH$_3$)CH$_2$-CyHex
6. 3-CH$_2$OH	19. 4-CH=C(CN)$_2$	32. 4-O(CH$_2$)$_4$OPh
7. 3-OH	20. 4-NHEt	33. 4-OCH$_2$COAdm
8. 3-NO$_2$	21. 4-N(Et)$_2$	34. 4-O(CH$_2$)$_2$-α-Naph
9. 3-CF$_3$	22. 4-COCH$_3$	35. 4-(CH$_2$)$_2$Ph
10. 3-O(CH$_2$)$_4$OPh	23. 4-Br	36. 4-(CH$_2$)$_4$Ph
11. 3-OCH$_2$O-Adm	24. 4-F	37. 4-(CH$_2$)$_3$Ph-4'-Cl
12. 3-Cl, 4-OCH$_2$Ph	25. 4-CO$_2$Et	38. 4-(CH$_2$)$_3$Ph-4'-Ph
13. 3-Cl,4-OCH$_2$COAdm	26. 4-COPh	

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with other toxiphoric groups, the biological activity is expressed
in terms of I$_{50}$, which is the molar concentration of inhibitor
causing a 50% inhibition of the Hill reaction (Table II). Basic-
ally, this work confirmed an earlier study which found the hydro-
phobic effect of the phenyl substituents to be the dominant

feature in the QSAR, and no electronic term proved to be significant. A bilinear term for log P and a branching factor for substituents in the 4-position did prove marginally significant and could be applied to the methoxyureas also (Table III). It

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Table II Inhibition of Hill Reaction in Spinach Chloroplasts

$pI_{50} =$ n = 17

	r	s	log P_O	$F_1,..$
1. 0.54(±.17)logP + 4.32(±.53)	.875	.584		(15)48.7

2. $0.66(\pm.20)\log P - 1.18(\pm 1.3)\log(\beta_{10}{}^{\log P}+1) + 4.11(\pm.55)$

                                                     .906 .549 5.83 (13) 1.98

3. $0.81(\pm.17)\log P - 1.08(\pm.59)\log(\beta_{10}{}^{\log P}+1) - 0.11Br(\pm.07) + 4.01$
                                                                              (± .41)

                                                     .957 .390 5.21 (12)13.8

'Br' is branching factor for 4-position; equals MR for smaller of

R_1 or R_2 in-Y$\begin{smallmatrix}R_1\\R_2\end{smallmatrix}$

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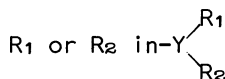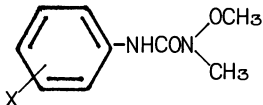
will be noted that in Equation 2, Table II for example, the upward slope of the initial linear portion of the relationship at low log P values is given by the coefficient, +0.66. The downward slope at high log P values is given by the sum of the coefficients of the first two terms: +0.66 - 1.18 = -0.52. Thus a simple parabolic equation given by: a log P -b(log P)^2 would serve very well for Equation 2, but it would poorly fit Equation 3 in either the dimethyl (Table II) or methoxymethyl (Table III) series.

It will be noted that in isolated spinach chloroplasts, one hardly needs to worry about making an inhibitor too hydrophobic; i.e. optimal log P = 5.2 for the N,N-dimethyl- and 5.4 for the methoxymethyl-ureas. In contrast to the isolated chloroplast studies, one sees from a list of commercially successful herbicides for which log P values have been measured or calculated, (Table IV) that getting the herbicide to the chloroplast in the living plant places much greater restrictions on its hydrophobic-hydrophilic balance. Indeed, the average log P of this set is only 2.54.
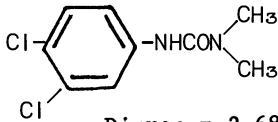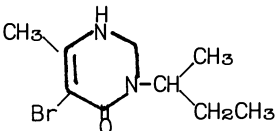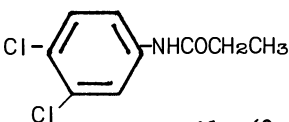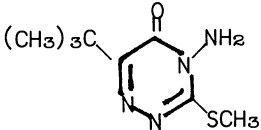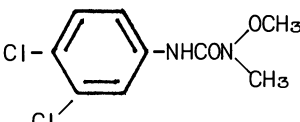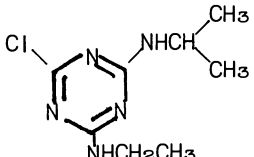
Table III   Inhibition of Hill Reaction in Spinach Chloroplasts by
N-methoxyureas

$p\mathrm{I}_{50} =$ n = 38

	r	s	$\log P_0$	$F_{1,..}$
1. $0.65(\pm.15)\log P + 3.73(\pm.47)$	.832	.749		(36) 80.9
1a. $0.84(\pm.27)\log P* + 3.39(\pm.61)$	.907	.555		
2. $0.67(\pm.11)\log P - 0.14(\pm.06)Br + 3.85(\pm.37)$	.907	.577		(35) 25.6
3. $1.02(\pm.18)\log P - 0.95(\pm.43)\log(\beta_{10}^{\log P}+1)-0.17(\pm.05)Br$				
$+ 3.37(\pm.37)$	.944	.465	5.40	(33) 10.3

log P* = measured partition coefficients;   n = 12
'Br' = branching factor as defined in Fig. 2.

Table IV   Log P of Commercial Herbicides

Diuron = 2.68

Bromacil = 2.02

Propanil =(3.50)

Sencor = 1.70

Linuron = 2.76
3.20

Atrazine = 2.60
2.75

Average log P = 2.54
( ) = calculated log P

Figure 1.   Optimizing both Transport and Hill Reaction Inhibition
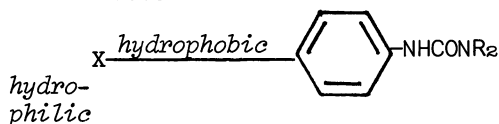
Hydrophobic chain should extend to limit of enzyme's hydro-
phobic area; polar group (X) does not then decrease binding
but enhances transport.

This information suggests a design rationale which may
satisfy both requirements. It is pictured in Figure 1. One starts
with a toxiphore with reasonable intrinsic activity (i.e., with
isolated chloroplasts). Hydrophobic binding to the enzyme can
then be increased with a chain or ring-chain combination attached
to the para position. The hydrophobic region of the enzyme cannot
extend indefinitely, and there is a good possibility that it opens
into solvent space. Therefore a polar fragment, X, can be placed
far enough out so it cannot affect this binding (and therefore
Hill inhibition) but it still could bring the solute log P into
the optimal 2.0 to 3.0 range.
    A matter of philosophical rather than practical significance
is the close similarity in the optimal hydrophobicity for the
random-walk process in plants and animals. In a series of papers
dating bact to 1968, Hansch (3) has shown that drugs acting rather
non-specifically in the animal central nervous system, such as
anesthetics and barbiturates, also have an optimal log P in the
2.0 to 2.5 range (Table V).

Table V   Log P of some CNS Depressants

Pentobarbital = 2.10

Methoxyflurane = 2.21

Ethrane = 2.10

Halothane = 2.30

The eleven equations shown in Table VI express the activity
of a group of Hill Reaction inhibitors (4-11). A common structural
feature, in all the sets except Number 5, seems to be a nitrogen
atom having considerable double bond character. Activity is

strongly dependent on hydrophobicity, while the role of electronic effect is minimal or absent. Just the intercept itself is a fair measure of intrinsic activity, keeping in mind that differing experimental conditions could account for as much as 0.5 difference in the intercept value. The intercept of 0.56 for benzimidazoles is in the range of non-specific toxicity caused by alcohols and other simple neutral molecules; i.e., 0 to 1.0 (12). So it would seem unwarranted to speak of a 'toxiphore' in the 2-CF3-benzimidazole moiety. On the other end of the scale are the 1,2,4-triazinones investigated by Draber with an intrinsic activity 10,000 fold greater (11).

Table VI   Intrinsic Activity in Hill Reaction Inhibition

$pI_{50} = (a) \log P + (b)$

	Coefficient (a)	Intercept (b)	n	r
1. 2-CF₃-benzimidazoles (4)	1.35	0.56	25	.93
2.*N-Ph-i-propylcarbamates (5)	0.71	0.87	9	.95
3.*N-Ph-ethylcarbamates (5)	0.77	1.34	7	.957
4. i-butyric acid anilides (5)	1.23	1.74	10	.935
5. diphenyl ethers (6)	0.46	2.30	18	.927
6. N-aryl-pyrrolones (7)	0.75	3.15	32	.852
7.**phenoxyphenyldimethyl ureas (6)	1.07	3.20	14	.935
8.**3-alkoxyuracils (8)	1.12	3.78	23	.991
9. 1-Ph-3-methyl ureas (9)	1.03	4.27	15	.957
10. azidotriazines (10)	0.85	4.27	17	.857
11. 1,2,4-triazinones (11)	0.86	4.84	11	.864

 * Has sigma term; probably corrects a calculated log P.
** Has small bi-linear component; (7.) includes DCMU also.

Environmental Effects

Obviously it is important to know as much as possible about the mode of action of pesticides on NON-target organisms if the differential between them (pest and non-pest) is to be maximized. But again some intelligent choices can be made based on knowledge of some simpler 'modes of action', such as: how does an organism collect a higher concentration of a pesticide than is in the environment in which it is living? And how does a chemical travel through soil, water and air and arrive at locations far from any site of application?

In a classic study, Neely, Branson and Blau (13) showed that the rate of uptake and elimination of chemicals in trout was related to the chemical's octanol/water partition coefficient. Just as a drop of octanol would equilibrate with one million times the concentration of some PCBs as the water which surrounded it, so

would a trout in Lake Michigan. One of the most thorough studies
of the bioaccumulation of solutes in various aquatic organisms
has been undertaken by the Environmental Research Laboratory at
Duluth, Minn. (14). In the effort of modeling transport through
soils, attention has been focused on the partitioning of solutes
between water and the organic component of soil. Kenaga (15) and
Briggs (16) have accumulated much valuable data to support the
role of a hydrophobic parameter in soil transport and have shown
that there is a relationship between Log P (octanol/water) and
Log P (org. comp. soil/water), but the relationships differ some-
what for different classes of chemicals.

Predicting Hydrophobicity from Structure

In spite of considerable effort to simplify it by HPLC and other
techniques (17, 18), measurement of octanol/water partition
coefficients remains a demanding and costly activity. Even though
the *number* of chemicals for which hydrophobic parameters are
needed for environmental hazard assessment is huge, fortunately
the *precision* necessary for bioaccumulation or transport modeling
is not so demanding. For a quantitative structure activity re-
lationship by regression analysis, we expect a parameter
precision of ± 0.1. For most environmental assessment, a precision
of ± 0.3 is adequate. It appears possible to reach this lower
level of precision with a rapid, cheap computer calculation based
on a well-tested manual procedure (19). The program, CLOGP, will
operate on structures individually supplied by the operator, or
else it will accept connection tables normally a part of a large
structurally-diverse chemical file. It is designed to operate on
the new generation of desk-top computers using either Fortran-77
or Unix language.
     Before illustrating the operation of CLOGP-3, the point
should be made clear that calculation and measurement should be
considered as *complimentary* tools rather than one being a replace-
ment for the other. Of course calculation is the only alternative
for the pesticide design chemist when he must make a decision
about what analogs to synthesize. Measurement 'after the fact'
might show that a compound, synthesized at some great expense,
was out of the desirable log P range. When both measurements
and calculations are available, discrepancies between the two
values can raise some interesting possibilities: Not infrequently
the measured value is wrong; sometimes the measured value is lower
because of an appreciable amount of ionization was not allowed
for; or else a tautomeric form was not considered. Sometimes a
positive deviation (Calculated-Measured) can indicate that con-
formation in the aqueous phase results in overlapping of hydro-
phobic portions of the solute, while a negative deviation might
be interpreted as hydrophilic overlap. An example of the latter
is seen with adenosine, which has a negative deviation even though
adenine and ribose are reasonably well calculated. It should be

obvious, therefore, that to get the maximum benefit from use of
the CLOGP program one must at least be familiar with the theory
upon which the calculations are based. It is quite an investment
in time to learn *all* the rules necessary for manual calculation
but in the process one may learn a great deal about the relative
solvation forces which compete in the aqueous and the lipid phases.
     The most advanced computer version, CLOGP-3, is really a
log P modelling system; that is, all the numerical data to operate
it resides in tables which can conveniently be changed or updated.
Figure 2 illustrates two kinds of structure entry which can
provide the suitable connection table input for benzoic acid:

Figure 2.   Computer Storage of 2-dimensional Structure as Linear
            Array
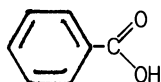
Benzoic Acid

1) WLN: QVR

2) SMILES: clccccclC(=O)O   or,
              Cl=CC=CC=ClC(=O)O   or,
              OC(=O)clccccccl   (starting point arbitrary)

---

1) Wiswesser Line Notation (WLN), (20) or 2) SMILES. The latter
method, developed by David Weininger, consists of a linear array
of atomic symbols and numbers which indicate ring bonds 'broken'
to maintain linearity. This character string, hydrogen-suppressed
to save space, is converted to a unique format by means of graph
theory and thence to a connection table (21). In contrast to the
effort required to learn to write WLN, where the encoder is re-
sponsible for uniqueness, SMILES can be mastered by a chemist in
five minutes. Used to enter new structural 'fragments' into the
program, SMILES promotes both speed and accuracy in this most
critical step.
     Table VII illustrates the entry format for the carboxamido
fragment. Measurements from a number of solutes have established
the values for many of its bond 'environments' and its suscepti-
bility to proximity effects. Table VIII shows the fragment data
for N-oxy-urea. It is immaterial which direction the operator
chooses to enter the fragment structure, because the program de-
velops a unique sequence for fragments just as it does when deal-
ing with complete structures. Of course the operator must use
care to associate the correct bonding designations with the
appropriate data. Two calculations using this fragment will serve
to illustrate some features of the program.

Table VII Data Entry for Carboxamido Fragment

Fragment Structure:

$$\overset{O}{\underset{\longleftarrow}{\overset{\parallel}{C}}\text{-NH}\longrightarrow}$$
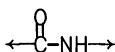
Entered SMILES: *C(=O)N*   (one of several alternates which program converts to GSMILE)

GSMILE: C(=O)(N*)* Attchm: 1, 3

English: NH-Amide   Bond types:

SIGMA	1	0.32	aa	M -1.06	a = to aromatic Isol. Carbon
RHO	1	0.72	Aa	M -1.81	A = to aliphatic I.C.
RHO	3	1.08	AA	M -2.71	Y = to styryl I.C.
PROXTYPE		2.0	aA	M -1.51	V = to vinyl I.C.
OCLASS	1	12	AY	M -1.52	Z = to benzyl I.C.
OCLASS	3	18	AV	E -2.26	
			VA	E -2.11	
			ZA	C -2.5	

Table VIII Data Entry for N-oxy-Urea Fragment

Fragment Structure:

$$\overset{O}{\underset{\longleftarrow\text{NH}\overset{\parallel}{C}\text{-N}\longrightarrow}{}}\overset{O\longrightarrow}{}$$
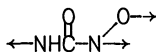
Entered SMILES: *NC(=O)N(*)O*

GSMILE: C(=O)(N(O*)*)N*

English: 1-oxy-1,3-Urea

PROXTYPE		2.0	AAa	M -2.13	
OCLASS	7	17.0			Note: Bond types 'AAa' are given in order
RHO	7	1.08			of asterisks in GSMILE; i.e., aliphatic
					to -O- and -N and aromatic to -NH-

Other factors being equal, the larger the solute molecule the higher its log P. But the fragment values listed in the computer computation of log P for the p-nitrophenyl-N-methoxyurea and its 2,4-dichloro analog (Table IX), clearly show that the polarity of the nitro group overcomes its bulkiness; i.e., the nitro is negative while the smaller Cl is strongly positive. The nitro group does enhance hydrophobicity by an electronic effect (22, 23) because of the presence of the very susceptible urea substituent with its -NH- attachment. It is evident that two chlorine substituents also produce a sizeable electronic enhancement of hydrophobicity. However this is more than cancelled when

Table IX Examples of CLOGP-3 OUTPUT

A.
NAME: 3-(p-nitrophenyl)-1-methoxy-1-methylurea
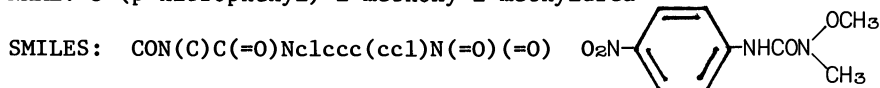
SMILES: CON(C)C(=O)Nc1ccc(cc1)N(=O)(=O) O_2N—〈 〉—NHCON(—OCH$_3$, —CH$_3$)

Class	Type	Value	Comment	Contribution Description
EXFRAGMENT	CARBON	0.40		2 Aliphatic isolating carbon(s)
EXFRAGMENT	CARBON	0.78		6 Aromatic isolating carbons
EXFRAGMENT	HYDROG	2.30		10 Hydrogens on I.C.
EXFRAGMENT	BONDS	-0.24	(combined)	2 chain and 0 alicyclic (net)
FRAGMENT #1		-2.13	MEASURED	1-Oxy-1,3-urea
FRAGMENT #2		-0.03	MEASURED	Nitro
ELECTRONIC	SIGRHO	0.648	InRing	1 Potential interact.; 1 used
		1.728	ANSWER	CLOGP Version 3.04
		1.74	(Measured)	

B.
NAME: 3-(2,4-dichlorophenyl)-1-methoxy-1-methylurea
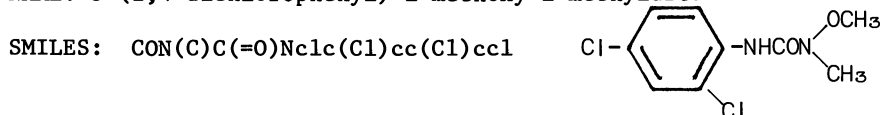
SMILES: CON(C)C(=O)Nc1c(Cl)cc(Cl)cc1   Cl—〈 〉—NHCON(—OCH$_3$, —CH$_3$), Cl

Class	Type	Value	Comment	Contribution Description
EXFRAGMENT	CARBON	0.40		2 Aliphatic isolating carbon(s)
EXFRAGMENT	CARBON	0.78		6 Aromatic isolating carbons
EXFRAGMENT	HYDROG	2.07		9 Hydrogens on I.C.
EXFRAGMENT	BONDS	-0.24	(combined)	2 chain and 0 alicyclic (net)
FRAGMENT #1		-2.13	MEASURED	1-Oxy-1,3-Urea
FRAGMENT #2		0.94	MEASURED	Chloride
FRAGMENT #3		0.94	MEASURED	Chloride
ELECTRONIC	SIGRHO	0.454	InRing	2 Potential interact.; 1.5 used.
ORTHO	RING 1	-0.56		1 normal ortho interact.
		2.654	ANSWER	

Log P not measured,   but 3,4-dichloro isomer = 3.20; and
applying ortho corr.=2.64 (second estimate)

---

one chlorine is in the 2-position (24). This negative ortho
effect appears related to both the steric and sigma inductive
values of the two substituents involved, but as yet it cannot be
calculated de novo, and so it must be looked up in a table when
log P is manually calculated. The ability of the computer to
both save time and prevent errors is readily apparent to anyone
who has attempted calculation of a diverse set of structures.

    A final example in Table X shows the log P computation which
CLOGP-3 performs on atrazine. If the three 'Isolating Carbon'
atoms in the ring were truly isolating, the negative fragments
would predominate and a value of -1.15 would be obtained. The

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Table X   Example of CLOGP-3 OUTPUT

NAME: Atrazine

SMILES: CC(C)Nclnc(NCC)nc(Cl)nl
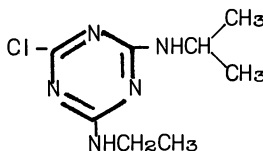
Class

```
EXFRAGMENT CARBON 1.00          5 Aliphatic isolating carbon(s)
EXFRAGMENT CARBON 0.39          3 Aromatic isolating carbons
EXFRAGMENT HYDROG 2.76         12 Hydrogens on I.C.
EXFRAGMENT BRANCH-0.22 (Group) 1 non-halogen, polar group branch
EXFRAGMENT BONDS -0.60          5 chain and 0 alicyclic (net)
FRAGMENT #1       -1.03 MEASURED  Secondary amine
FRAGMENT #2       -1.12 MEASURED  Aromatic nitrogen (Type 2)
FRAGMENT #3       -1.03 MEASURED  Secondary amine
FRAGMENT #4       -1.12 MEASURED  Aromatic nitrogen (Type 2)
FRAGMENT #5        0.94 MEASURED  Chloride
FRAGMENT #6       -1.12 MEASURED  Aromatic nitrogen (Type 2)
FRAGMENT REDUCE    0.84          3 Delocalized TYPE 2 fragments*
ELECTRONIC SIGRHO 3.232 InRing 8 Potential interact.; 3.69 used*
                   2.922 ANSWER    *see text
                   2.75 MEASURED
```

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strong inductive effect of the ring nitrogens on the amino sub-
stituents increase hydrophobicity by a programmed factor of 4.07
log units,* bringing the calculated value in good agreement with
the measured.

Electronic parameters are frequently important in QSARs for
whole organisms, but it may be relevant to distinguish electronic
influences in the transport process, when the pesticide is merely
a solute, from those acting on the target enzyme when the chemical
is a ligand.

Summary

Hydrophobicity is often an important, or even a dominant, param-
eter in herbicidal activity. At the present state of the art, it
might be fair to state that de novo calculation of log P may *not*
be sufficiently accurate for regression analysis, but coupled with
measurements of one or two key analogs, it may eliminate the need
to measure each and every chemical in the set. Certainly de novo
calculation can be used as a predictive tool prior to synthesis,
and it can serve quite adequately for the less demanding require-

ments of bioaccumulation and environmental transport models. At the present time, the use of the hydrophobic parameter in rational pesticide design seems to be limited more by the lack of reliable partitioning data than by the lack of experience or the unfamiliarity of workers in this field. It will be some time before published measurements can overcome this lack, but in combination with reliable calculation, the future of this approach looks quite promising.

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The Treatment of Ionizable Compounds in Quantitative Structure–Activity Studies with Special Consideration to Ion Partitioning

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The use of distribution coefficients for the QSAR treatment of ionizable compounds has been extended to consideration of ion-pair partitioning into biolipid phases. Two experimental methods for determining ion-pair partition coefficients are described. One is a single-phase titration in water-saturated octanol, in which case (for acids) $\log P_i = \log P + pKa - pKa'$. The other is a two-phase titration (octanol/water) from which the ratio $(P + 1)/(P_i + 1)$ can be calculated. An example outcome is that the uncoupling activity of phenols can be represented by an equation in $\log D_i$ instead of $\log D$ and pKa.

A high percentage of biologically active compounds are ionized at physiological pH. In most cases (exceptions will be noted below), the partitioning of the ionized species has been assumed to be negligible and has been neglected. We neglected this consideration ourselves in our earlier work on the use of distribution coefficients in QSAR. A problem one faces in this area is the paucity of data on ion-pair partition coefficients. I would like to describe two relatively simple means for determining this property by titration and present some examples where I think the ionic species may be the active form.

This symposium is an appropriate place to discuss ion partitioning because many herbicides have the potential to act in the ionized form in biolipid phases. That is to say, at physiological pH, the biolipid phases of membranes, organelles, etc., will contain a high percentage of ion, and in fact, the ion may be the predominant species. Examples of such herbicides are collected in Figure 1. There are three attributes of these agents that favor ion partitioning: (1) low pKa, (2) high lipophilicity, and (3) the potential for chelation in the ion pair. First, note that these acids are not just acidic, but

ioxynil

2, 3, 6 - TBA

aciflurfen

2, 4, 5 - T

tricamba

dichloropicolinate

perfluidone

US 4,370,480 (1983)
to DuPont

US 4,367,339 (1983)
to American Cyanimide
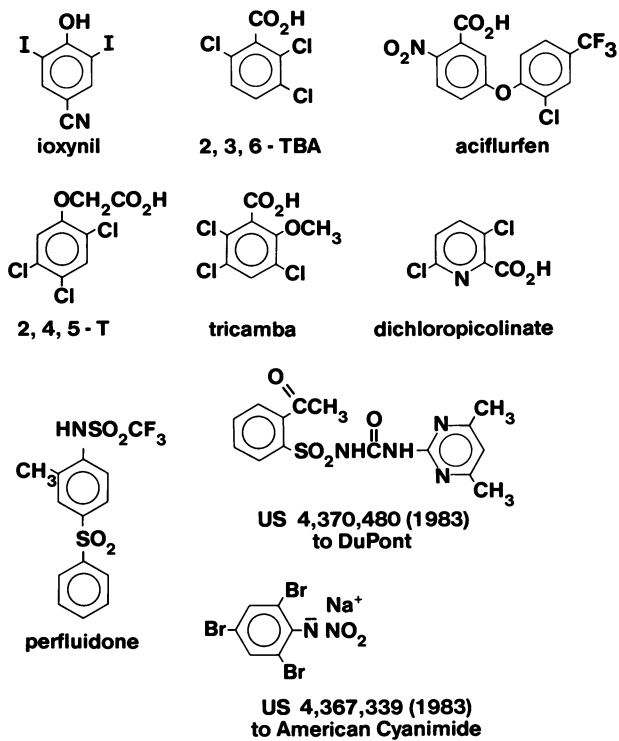
Figure 1.   Herbicides with lipophilic anions.

they are <u>highly</u> acidic, with <u>o</u>-halo and <u>o</u>-nitrocarboxylic acid
groups, multiple halogen substitution, and the trifluoromethane-
sulfonanilide function. Second, many are also highly
lipohophilic, such as aciflurfen with a trifluoromethylchloro-
phenoxy substitution, the tribromoanilide, and again, all the,
polychloro-substituted compounds. A third feature that some
possess is a heteroatom at a favorable distance from the anionic
site for chelation in the salt form, such as in the oxyacetic
acid and the sulfonylurea. R. Sauers pointed out earlier in
this symposium that in chlorsulfuron and related compounds the
<u>ortho</u> aromatic nitrogens are critical for activity.
Electron-donating groups on the heterocyclic ring [which would
enhance chelation] favor activity. The oximated β-trione
derivatives described by I. Iwataki in this volume can also form
chelated salts. These are properties which favor high lipid
concentrations of ion pairs. They will be elaborated on as we
go along.

Distribution Coefficients

In developing some of the relationships, it is helpful to use a
four-quadrant diagram in which each quadrant represents a
species in a lipid or water phase. The diagram below shows a
typical distribution of an acid, AH, between two phases where
ion partitioning is assumed to be negligible. The partition
coefficent, P, is the ratio of the concentration of AH in the
octanol to the concentration of AH in the aqueous phase. The
distribution coefficient, D, is the ratio of the concentration
in the octanol to that of all forms in the water. This is also
called the apparent partition coefficient.

$$AH_o$$

OCTANOL

WATER

$$AH_w \quad \rightleftharpoons \quad A^- + H^+$$

$$P = \frac{[AH_o]}{[AH_w]} \qquad\qquad D = \frac{[AH_o]}{[AH_w] + [A^-]}$$
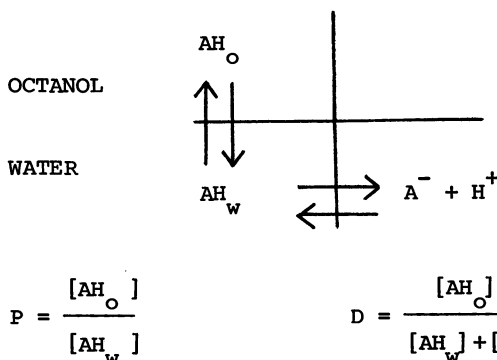
Log D is given by Equation 1 where α is the degree of
ionization, but the approximations, Equations 2 and 3, can be
used when the pH is more than a unit away from the pKa toward
ionization. A convenient table is available for determining α
for various pH-pKa differences (<u>1</u>).

$$\log D = \log P + \log (1 - \alpha) \qquad (1)$$

(for acids) $\qquad \log D = \log P + pKa - pH \qquad (2)$

(for bases) $\qquad \log D = \log P - pKa + pH \qquad (3)$

Regression Analyses Using Log D

Log D accounts for where the "dose" is, so to speak. The distribution depends on the pKa as well as the pH of the medium. The advantage of using log D is that it incorporates these factors, so, for simple processes such as absorption, distribution coefficients "explain" the whole process. An example from our earlier work (2) is the colonic absorption of acids ranging from phenols to strong carboxylic acids. The absorption rate is given by an equation involving only log D terms.

$$\log \% \text{ ABS} = -0.079(\log D)^2 + 0.236 \log D + 1.503 \qquad (4)$$

$$(n = 10; \ r = 0.982; \ s = 0.096; \ F = 96)$$

Kubinyi (3) used distribution coefficients of the same series of compounds with his bilinear model for absorption to obtain an even closer correlation, Equation 5. β is a constant related to the model.

$$\log \% \text{ ABS} = 1.033 \log D - 0.921 \log (\beta D + 1) + 2.953 \qquad (5)$$
$$(n = 10; \ r = 0.994; \ s = 0.063; \ F = 154.71)$$

We've looked at many other analyses of "simple" processes, but my favorite is a correlation for the absorption of an acid and a base in the same equation, -- not only that, but with each one at six different pH's, (Figure 2). The data are from Schurmann & Turner (4); the base is propranolol and the acid, 4-n-hexylphenylacetic. Only a single parameter is required, log D, Equation 6. Kubinyi's bilinear equation gives an even better correlation (5), Equation 7. (This is a special version of the bilinear model which sets the coefficients of each term equal.)

$$\log K_{abs} = 0.348 \log D - 1.651 \qquad (6)$$
$$(n = 12; \ r = 0.966; \ s = 0.163; \ F = 140)$$

$$\log K_{abs} = 0.448 \log D - 0.448 \log (\beta D + 1) - 1.689 \qquad (7)$$
$$\log \beta = -2.792$$
$$(n = 12, \ r = 0.988; \ S = 0.102)$$

In more complex processes correlation equations may require electronic, steric, or other factors which contain information

about the mechanism of action. For example, Equation 8 shows
the correlation of uncoupling of oxidative phosphorylation by
the phenols in Table II. This is a property possessed by many
herbicides (6) but is usually an undesired property when it
occurs in drugs. One can say from the coefficients (1) that the
more acidic the phenol of a given log P, the more active it will
be as an uncoupler, even though there will be less compound in
the lipid phase. We will see later that these two terms can be
replaced by the single parameter, log D of the sodium salt.

$$\log \frac{1}{C} = 0.471 \log D - 0.618 \text{ pKa} + 7.58 \qquad (8)$$
$$(n = 23; \ r = 0.946; \ s = 0.351; \ F = 86)$$

Ion Partitioning

Martin (7) has written a perceptive analysis of the possible
ways in which an ionized species may behave in various models
and contribute to or be responsible for a given activity. QSAR
studies that have dealt with ion-pair partitioning include a
study of fibrinolytics (8) and the effect of benzoic acids on
the K^+ ion flux in mollusk neurons (9). Schaper (10) recently
reanalyzed a large number of absorption studies to include terms
for the absorption of ionized species. Because specific values
were not available for log P_i, he let the relation between log
P_i and log P be a parameter in a nonlinear regression analysis.
In most cases he used the approximation that the difference
between the two values is a constant in a given series. This
same assumption was made in the earlier studies (8,9). Our work
suggests that the pKa of an acid can influence this differential
(see below). The influence of structure on the log P_i of
protonated bases or quaternary ammonium compounds is much more
complex (11,12) and points out the desirability of being able to
easily measure these values.

Sodium Salicylate Partitioning. Our earlier work was based on
the assumption that the ionized species does not significantly
partition into the biolipid phase. It turns out that even at
low levels of partitioning, lipid ion concentrations can be
significant. Salicylic acid provides a good example. Figure 3
shows the log D, or apparent log P, for salicylic acid over a
broad pH range. There is a flat portion at low pH where log D =
log P of the unionized acid. There is an inflection at the pKa
and a decreasing proportion of acid in the lipid phase with
increasing pH, following the equation indicated. Figure 4 shows
the distribution of sodium salicylate. Its log P_i measured in
pH 8.5 phosphate buffer is −1.44 (11). Again there is an
inflection at the pKa with a declining log D with decreasing pH
following the equation for bases.
    It is interesting to superimpose these two plots (Figure
5). Even though the difference in log P between the acid and
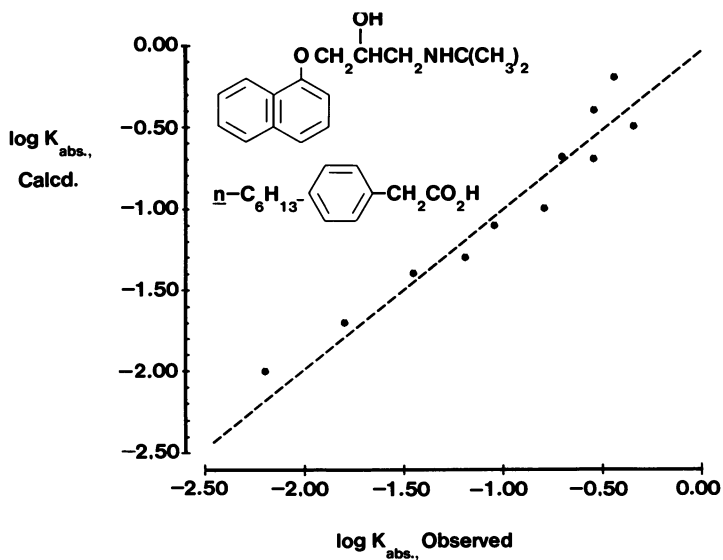
Figure 2. Buccal absorption at six pH's of an amine and
an acid, Equation 6.
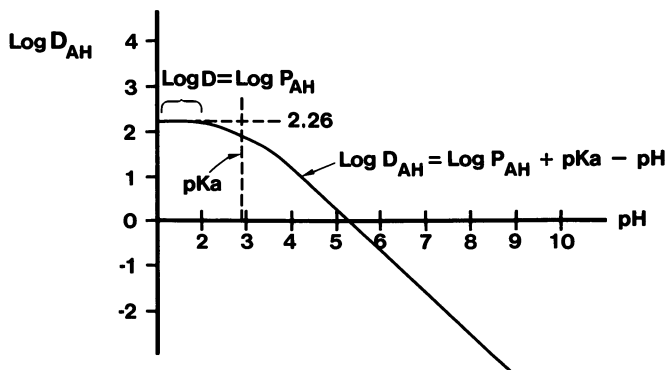
Figure 3. Octanol/water distribution of salicylic acid
over a range of pH values.
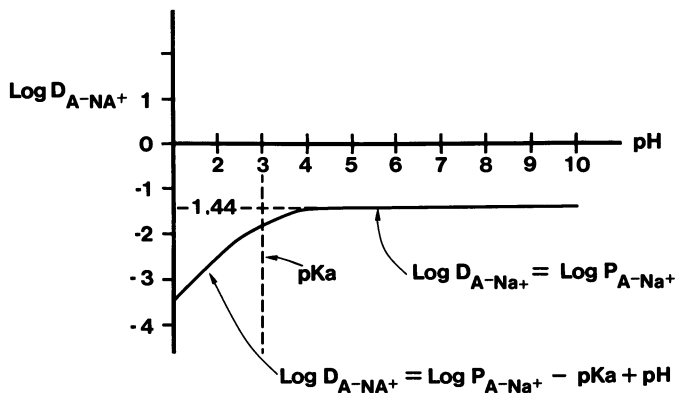
Figure 4.   Octanol/water distribution of sodium salicylate over a range of pH values.
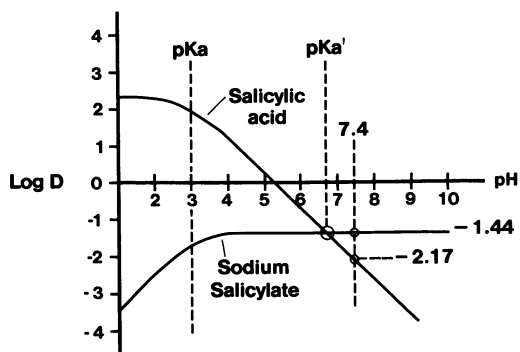
$$D = \frac{[\text{species in octanol}]}{[ArCO_2H]_w + [ArCO_2^-]_w}$$

Figure 5.   Overlaying Figures 3 and 4 shows that the anion is the dominant octanol-soluble species at pH 7.4. the pKa' is 6.7.

the ion is 3.70, the concentration of sodium salicylate in the
lipid at pH 7.4 is 5X greater than the neutral form. Could the
anion be the active species for some of the properties of
salicylic acid? It turns out, interestingly, that a similar
analysis of the antiinflammatory agents indomethacin (13) and
phenylbutazone (14) shows that in octanol at pH 7.4 the sodium
salts will be the dominant species by factors of 5.3 and 10,
respectively. Note from Figure 5 that the more acidic a
compound, the further to the left the declining phase begins and
the less favored the acid form at physiological pH. This bears
on the highly acidic herbicides in Figure 1.

Again referring to Figure 5, where the two lines cross at
pH 6.7, the concentrations of acid and anion in the octanol
phase are equal. What is this other than the pKa in octanol? I
define the pKa in octanol to be equal to the pH of the aqueous
phase which is in equilibrium with the system when the octanol
concentrations of the two species are equal. It is a challenge,
though, to see if this pKa can be determined directly.

When the ion-pair partitioning is indicated in the quadrant
diagram (below) it becomes obvious that a circle of equilibria
is present. Knowing the octanol pKa, the log P and the aqueous
pKa should allow one to calculate the partition coefficient of
the ion pair. From these equilibria one can write that the
difference in log P between the acid and its salt is the same as
the difference between the pKa's (Equation 9). The closer the
pKa's, the more lipid soluble the ion pair will be, relative to
the acid. Internal hydrogen bonding or chelation that
stabilizes an ion pair will affect the octanol stability more
than the aqueous stability, where it is less needed, and so will
decrease the delta pKa. Chelation should therefore favor
biolipid solubility of ion pairs. Ultimate examples are
available in some ionophores. This is one of the properties of
some of the herbicides I pointed out earlier.
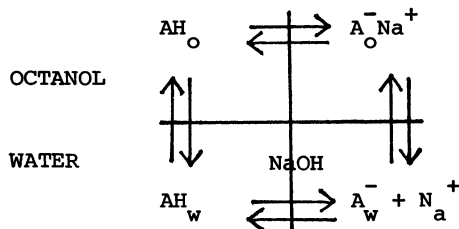
$$\log P_{AH} - \log P_i = pKa' - pKa. \qquad (9)$$

Incidentally, the relationship in Equation 9 was discussed by
Kolthoff et al. (15) back in 1938. In their case the partition

coefficients were for ethanol/water and were determined by solubilities, since they could not be directly measured.

Log P_i by Titration in Water-Saturated Octanol

We decided to try a direct titration in water-saturated octanol. What we hoped to achieve, if not duplicating literature values, was to obtain partition coefficients proportional to "true" values so that regression analyses could be run and would be meaningful. Secondly, this should be a rapid method to assess structural features in a series for their effect on ion-pair partitioning.

In collaboration with Jon Belisle, octanol pKa values were measured for a series of benzoic acids and phenols. A coupled electrode calibrated in aqueous buffers was used. The half-neutralization potential was measured since the Henderson-Hasselbalch equations would not apply. The titrant was 0.1 $\underline{N}$ sodium hydroxide in isopropanol:methanol 4:1. The titrant was only 6% of the total volume at half-neutralization, so the medium was essentially octanol-like. The results are listed in Table I and some benzoic acid values are plotted in Figure 6.

The calculated log P_i values in Table I are slightly higher than the literature reported values. The pKa of benzoic acid in octanol is 3.55 units higher than in water. From Equation 9, the Δ log P is 3.55 compared with the measured difference of 4.14 in 0.1 $\underline{N}$ sodium hydroxide. The Δ log P for salicylic acid is calculated to be 3.35 compared with the directly measured value 3.70. The most interesting finding is that the slope of the benzoic acid line is 1.49 and not 1.0 (Equation 10). These results suggest that, contrary to the current assumption (11), the Δ pKa, and therefore the Δ log P, for benzoic acids is not a constant. The stronger the acid, the less the Δ pKa and the more lipophilic the salt will be in relation to its acid. Taft (16) has observed that the pKa's of the strongest acids in a series are often less affected by changes in solvent, likely because their ions are more stable and need less solvation. This is another reason for saying the highly acidic herbicides in Figure 1 will tend to have lipophilic salts.

Exceptions to the general pattern in Figure 6 are nitrosalicylic acids (Table 1) and the $\underline{o}$-chlorobenzoic acids. Nitrosalicylic acid salts are more lipophilic than expected from their aqueous pKa. The $\underline{o}$-chlorobenzoic acids are less lipophilic than a $\underline{m}$ or $\underline{p}$-benzoic acid would be of the same pKa. The 2,6-dichlorobenzoic acid point should perhaps be on a separate line parallel to the $\underline{m}$ and $\underline{p}$-benzoics. From the values in Table I, the following correlations can be made for predicting the pKa', and therefore log P_i, for similar compounds. The 95% confidence limits are indicated.

Table I. Half-Neutralization pH for Benzoic Acids and Phenols
in Water-Saturated Octanol.

Benzoic acid substitution	pKa^a	pKa'^b	pKa'-pKa (Corresponds to log P-log P_i, Eq 9)
H	4.19	7.74	3.55
3-Br	3.82	7.23	3.41
3-OH	4.08	7.65	3.57
4-Cl	3.98	7.31	3.33
3,4-Cl$_2$	3.66	6.90	3.24
4-CH$_3$	4.37	7.93	3.56
3-NO$_2$	3.47	6.53	3.06
4-NO$_2$	3.41	6.64	3.23
2-Cl	2.94	6.71	3.77
2,5-Cl$_2$	2.47	6.26	3.79
2,6-Cl$_2$	1.59	5.61	4.02
2-OH	2.97	6.32	3.35
2,4-(OH)$_2$	3.33	7.05	3.72
2,3-(OH)$_2$	2.91	6.14	3.23
2,5-(OH)$_2$	2.97	6.21	3.24
2,6-(OH)$_2$	1.08	3.80	2.72
5-Br, 2-OH	2.62	5.76	3.14
5-Cl, 2-OH	2.63	5.79	3.16
3,5-Cl$_2$, 2-OH	2.30	5.15	2.85
5-OCH$_3$, 2-OH	2.96	6.20	3.24
5-NO$_2$, 2-OH	2.12	3.91	1.79
3,5-(NO$_2$)$_2$, 2-OH(ppt)	0.70	2.19	1.49
Phenol substitution			
3-CH$_3$	10.10	12.66	2.56
2-Cl	8.56	11.23	2.67
4-Cl	9.38	11.94	2.56
3,5-Cl$_2$	8.18	10.78	2.60
2,4-Cl$_2$	7.85	10.50	2.65
2,6-Cl$_2$	6.79	9.55	2.76
4-CN	7.95	10.40	2.45
4-NO$_2$	7.16	9.70	2.54
2,6-Cl$_2$,4-NO$_2$	3.54	5.92	2.38
2-NO$_2$	7.23	9.66	2.43
2,4-(NO$_2$)$_2$	4.10	5.82	1.72
2,5-(NO$_2$)$_2$	5.22	7.03	1.81
2,6-(NO$_2$)$_2$	3.58	5.20	1.62

[a]Literature values. [b]Titrant: 0.1 N NaOH in isopropanol:methanol
4:1; 1 equivalent of titrant was ca. 12% of final volume.
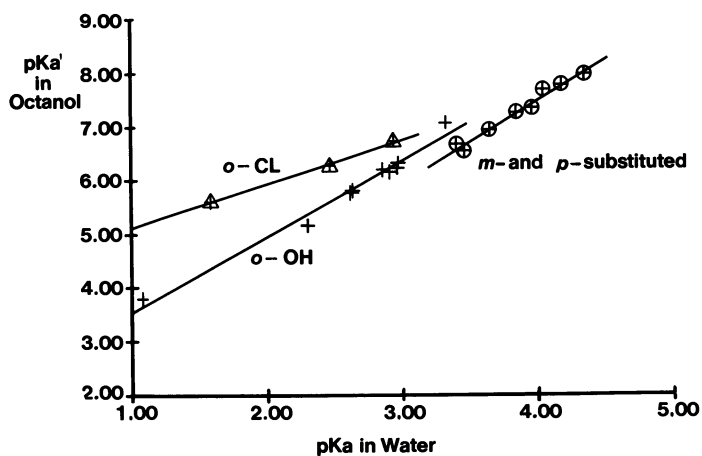
Figure 6. Half-neutralization potentials of benzoic acids in water-saturated octanol vs. their corresponding aqueous pKa values.

m- and p- substituted benzoic acids

$$pKa' = 1.49 \ (\pm 0.24) \ pKa + 1.48 \ (\pm 0.93) \qquad (10)$$
$$(n = 8; \ s = 0.888; \ r^2 = 0.975)$$

salicylic acids, except nitro-substituted

$$pKa' = 1.38 \ (\pm 0.19) \ pKa + 2.17 \ (\pm 0.50) \qquad (11)$$
$$(n = 9; \ s = 0.146; \ r^2 = 0.978)$$

o-nitrophenols

$$pKa' = 1.22 \ (\pm 0.17) \ pKa + 0.79 \ (\pm 0.82) \qquad (12)$$
$$(n = 4; \ s = 0.104; \ r^2 = 0.998)$$

phenols (not o-nitro-substituted)

$$pKa' = 1.02 \ (\pm .05) \ pKa + 2.39 \ (\pm 0.40) \qquad (13)$$
$$(n = 9; \ s = 0.112; \ r^2 = 0.977)$$

Test of Octanol Titration Procedure. In order to see how close
we were to reality with these titrations, the antiinflammatory
sulfonanilide, R-805, nimesulide, was examined because the anion
has a distinctive UV absorption. R-805 has pKa 5.9, log P 2.6,
and a pKa' in octanol of 8.82. With this data the log P_i for
the sodium salt is calculated from Equation 9 to be -0.3. R-805
was equilibrated between two phases at pH's such that the
octanol contained ionized species (e.g. 39% of the 805 was in
the octanol and 69% of this was the ion). The log P_i's observed
at two pH's were -0.37 and -0.35.
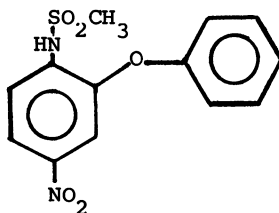
R-805, nimesulide

QSAR Using Log D_i and Log P_i. The uncoupling activity of the
phenols from which Equation 8 was derived, was reanalyzed using
log D_i (Table II). The activity highly correlates with the
lipid concentration of the ion pair, Equation 14. This is
satisfying in view of a proposed mechanism of uncoupling (17)

Table II. Uncoupling of Oxidative Phosphorylation by Phenols at pH 7.5 and Physicochemical Constants Used[a]

Substitution	Log P	pKa	pKa' octanol[b]	Log P_i[c]	Log D	Log D_i[d]	Uncoupling, log 1/C Obsd.	Uncoupling, log 1/C Eq.14
H	1.48	9.99	12.62	-1.15	1.48	-3.64	2.10	2.00
2-Cl	2.17	8.56	11.23	-0.50	2.17	-1.59	2.79	3.26
4-Cl	2.41	9.43	11.94	-0.10	2.41	-2.03	3.05	2.98
3-Cl	2.52	9.12	11.69e	-0.05	2.52	-1.67	3.38	3.21
2,4-Cl$_2$	3.08	7.98	10.50	0.56	2.96	-0.04	4.17	4.21
2,5-Cl$_2$	3.22	7.51	10.05e	0.68	2.92	0.38	4.17	4.47
2,4-Br$_2$	3.24	7.94	10.49e	0.69	3.11	0.11	4.17	4.30
2,4,6-Cl$_3$	3.64	6.22	8.73e	1.13	2.36	1.13	4.34	4.93
2,4,6-Br$_3$	3.98	6.44	8.96e	1.46	2.89	1.46	5.14	5.13
2,3,4,6-Cl$_4$	4.12	5.74	8.24e	1.62	2.36	1.62	5.48	5.23
Cl$_5$	4.12	4.95	7.44e	1.63	1.57	1.63	5.70	5.23
2-NO$_2$	1.81	7.23	9.66	-0.62	1.35	-0.80	2.84	3.74
4-NO$_2$	1.98	7.16	9.70	-0.56	1.47	-0.72	3.96	3.79
3-NO$_2$	2.02	8.36	10.91e	-0.53	2.02	-1.45	3.49	3.34
2,4-(NO$_2$)$_2$	1.52	4.10	5.82	-0.20	-1.88	-0.20	4.72	4.12
2,5-(NO$_2$)$_2$	2.02	5.22	7.03	0.21	-0.26	0.21	4.32	4.36
2,6-(NO$_2$)$_2$	1.57	3.71	5.20	0.08	-2.22	0.08	3.94	4.28
3-CH$_3$	1.98	10.10	12.66	-0.58	1.98	-3.18	2.30	2.28
4-CH$_3$	1.97	10.28	12.88e	-0.63	1.97	-3.41	2.28	2.14
2-naphthol	2.72	9.59	12.17e	0.14	2.72	-1.95	3.17	3.03
F$_5$	3.25	5.33	7.83e	0.75	1.08	0.75	4.47	4.69
2,6-Cl$_2$-4-NO$_2$	2.96	3.54	5.92	0.58	-1.00	0.58	4.80	4.59
2,6-Br$_2$-4-NO$_2$	3.07	3.39	5.85e	0.61	-1.04	0.61	5.11	4.61

[a]Original data of Stockdale, M., and Selwyn, M., Eur. J. Biochem. 1971, 21, 565. Physical constants, Ref. 1.   [b]From Table I.   [c]Calculated from Equation 9.   [d]From Equation 1; log (1-) values for bases from Ref. 1.   [e]Calculated from Equation 13.

which involves short circuiting the energy storage capacity of a
cell by transferring cations in one direction and protons in the
other. From Equation 14 one could say that the rate-limiting
step is transfer of the cation.

$$\log \frac{1}{C} = 0.471 \log D - 0.618 \, pKa + 7.58 \tag{8}$$
$$(n = 23; \; r = 0.946; \; s = 0.351; \; F = 86)$$

$$\log \frac{1}{C} = 0.614 \log D_i + 4.23 \tag{14}$$
$$(n = 23; \; r = 0.940; \; s = 0.361; \; F = 160)$$

The data of Levitan and Barker (9) on the ability of carboxylic
acids to promote potassium ion conductance in mollusk neurons
(Table III) was reexamined. One can write Equations 15 and 16
for simple benzoic acids, but salicylic acids do not give a good
correlation in log P_i alone (r = 0.785).

$$\log RA = 1.04 \log D - 1.09 \, pKa + 5.67 \tag{15}$$
$$(n = 13; \; r = 0.985; \; s = 0.122; \; F = 159)$$

$$\log RA = 0.984 \log P_i + 1.52 \tag{16}$$
$$(n = 13; \; r = 0.968; \; s = 0.162; \; F = 165)$$

The Levitan and Barker series has also been examined by Hansch
(18), who reported that the benzoic acids, salicylic acids and
four miscellaneous acids could all be correlated using log P_i,
Equation 17. The difference is in the calculation of log P_i
values which were obtained by subtracting constants from the log
P values, 3.69 for salicylic acids and 4.36 for benzoic acids.

$$\log \frac{1}{C} = 0.839 \log P_i + 331 \tag{17}$$
$$(n = 30; \; r = 0.979; \; s = 0.177)$$

Log P by a Two-Phase Titration

At this point I want to emphasize that the procedure I am about
to describe bears no relation to the previous titration method,
though confusingly similar. The latter is a single-phase
titration in octanol, while this is an aqueous-phase titration
in the presence of octanol.
     The two-phase titration was first described in 1963 (19) as
a means to simultaneously determine a partition coefficient and
pKa. Several variations have appeared since (20-24), but none
has seemed to gain favor. I like the simplified approach of
Clarke (25,26) who uses the method to determine partition
coefficients after separately determining (or knowing) the pKa.
     Figure 7 shows a typical titration. The aqueous titration
curve is on the left. In the presence of an equal volume of
octanol, the pKa shifts 1.9 pK units, which is about the log P
of benzoic acid. The exact relationships, correcting for volume

Table III. Membrane Conductance and Physicochemical Constants of Benzoic Acids Used

Substitution	Log P[a]	pKa	pKa'	Log $D_{7.8}$[a]	Log P_i[b]	Activity Obs	Activity Calcd[c]	Activity Calcd[d]
H	1.87	4.19	7.74	-1.74	-1.68	-0.70	-0.75	-0.71
3-OH	1.50	4.08	7.65	-2.22	-2.07	-1.00	-1.11	-1.09
3-Br	2.84	3.82	7.23	-1.14	-0.57	0.48	0.27	0.32
4-Br	2.86	3.98	7.41[e]	-0.96	-0.57	0.40	0.27	0.33
2-Cl	1.98	2.94	6.71	-2.88	-1.79	-0.70	-0.85	-0.53
3-Cl	2.68	3.82	7.17[e]	-1.30	-0.67	0.18	0.18	0.15
4-Cl	2.65	3.98	7.31	-1.17	-0.68	0.00	0.17	0.11
3,4-Cl_2	3.46	3.66	6.90	-0.68	0.22	0.93	1.00	0.97
3-I	3.13	4.0	7.44[e]	-0.67	-0.31	0.60	0.51	0.61
4-I	3.02	3.91	7.31[e]	-0.87	-0.38	0.54	0.45	0.50
4-CH_3	2.27	4.37	7.93	-1.16[f]	-1.29	-0.52	-0.39	-0.30
3-NO_2	1.83	3.47	6.53	-2.50	-1.23	-0.70	-0.33	-0.71
4-NO_2	1.89	3.41	6.64	-2.50	-1.34	-0.52	-0.44	-0.65

[a] From Ref. 2. [b] Calculated from Equation 9. [c] Calculated from Equation 16. [d] Calculated from Equation 15. [e] Calculated from Equation 10. [f] Value in Ref. 2 is in error.

of the phases, are given in Equations 18 and 19, where pK' is
the apparent pKa in the presence of the organic phase, and V_w
and V_o are the volumes of the aqueous and organic phases.

$$\text{(for acids)} \qquad P = \frac{V_w}{V_o} (10^{pK'-pKa} - 1) \qquad (18)$$

$$\text{(for bases)} \qquad P = \frac{V_w}{V_o} (10^{pKa-pK'} - 1) \qquad (19)$$

[The reader who doesn't continue to the end of this chapter
should be cautioned that Equations 18 and 19 hold only when
ion-pair partitioning is not significant.]

Clarke (26) has written a microcomputer curve-fitting
program to analyze titration curves for pKa. It analyzes the
curve for each of twenty points, fitting it to the
Henderson-Hasselbalch equation. It is especially valuable to
ascertain that equilibrium conditions prevailed during the
titration and there were no anomalous factors. I have also used
a nonlinear simultaneous weighted lease squares curve-fitting
program KINFIT (27). My titrations are usually run on 0.2 meq.
of compound in 50 ml of degassed distilled water and 50 ml of
octanol (each previously saturated with the other phase), using
0.1 N acid or base, a Metrohm 536 automatic titrator and an
Orion Research, Ross combination electrode. A microtitrator
would allow one to work at one tenth of this scale (25,26).

Ion-Pair Partition Coefficients by a Two-Phase Titration

Sometimes the log P from a two-phase titration using Equations
18 or 19 is low, compared with shake-flask values. We attribute
this to ion-pair partitioning. The quadrant diagram, Figure 8,
is helpful for developing the pertinent equations. The amount
of each species in each phase is shown in the appropriate
sector.

If [X] is the concentration of AH in the aqueous phase
(Figure 8), the concentration of AH in the octanol is P times
this. The aqueous-phase concentration of the ion is [X] times
the degree of dissociation. Multiplying this product by the
ion-pair partition coefficient P_i (or P_{A-}) gives the
concentration of the ion pair in the octanol. The actual amount
of species in a phase is given by its concentration times the
volume of the phase. At the pK', the amount of neutral species
equals the amount of ionized species. Setting the sum of the
two terms in the left quadrants equal to the sum of the two
terms on the right, one can derive Equation 20. The equivalent
expression for bases is Equation 21.
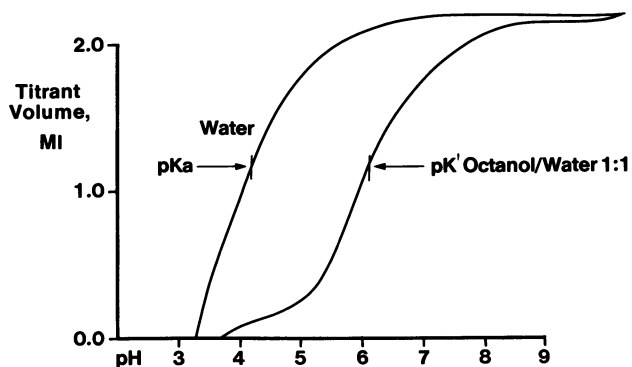
Figure 7. Benzoic acid titration curves in water and in the presence of an equal volume of octanol.
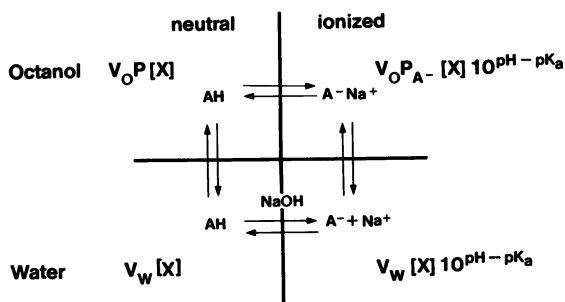
Figure 8. A quadrant diagram to aid derivation of equation 20. V is the volume of octanol or water phase; [X] is the concentration of AH in the aqueous phase; P is the partition coefficient of AH; P_{A-} is the partition coefficient of the ion pair A^-Na^+; pKa is the aqueous single-phase pKa; pK' is the two-phase pKa.

$$\text{(for acids)} \qquad \frac{\dfrac{V_o P}{V_w} + 1}{\dfrac{V_o P_i}{V_w} + 1} = (10^{pK'-pKa}) \qquad (20)$$

$$\text{(for bases)} \qquad \frac{\dfrac{V_o P}{V_w} + 1}{\dfrac{V_o P_i}{V_w} + 1} = (10^{pKa-pK'}) \qquad (21)$$

That this titration method provides a _ratio_ of partition
coefficients was not recognized here-to-fore. One must know one
value to calculate the other. When P_i is zero, the equations
reduce to the original derivations, Equations 18 and 19. The
value of this ratio is very sensitive to P_i. When it is 1 (log
P_i = 0), the value is reduced by half. This is easily in the
range of sensitivity to detect (corresponding to a pK'
difference of 0.3 units).

Results. Examples of partition coefficients measured by the
two-phase titration method are shown in Table IV. A striking
feature is the marked influence of counter ion concentration and
type. This supports the proposition that we are measuring
ion-pair partitioning. We chose 0.15 M sodium chloride to
approximate physiological ion concentrations, but for a closer
match one might use a mixture of ions, depending on the
objective. Human plasma ion concentrations (28), in mmoles/L
include sodium 152, potassium 5, chloride 113 and bicarbonate
27. Intracellular levels of ions (28) include sodium 14,
potassium 157, magnesium 13, bicarbonate 10 and phosphate 37
mmoles/L. It seems that the effect of counter-ion concentration
may not be the same for different classes of compounds,
comparing propranolol and 4-t-butylpyridine (Table IV).
    The recent work of Wang and Lien (29) illustrates that ion-
pair partitioning occurs to a greater extent than previously
realized. Partition coefficients calculated from measurements
made on partially ionized compounds depend not only on the pH,
but on the buffer used. They may vary by more than one log
unit. The authors derived equations to correct log P to
octanol/water values, but these can still be off by several
tenths of a log unit. A preferable solution would be to know
the log P_i and account for ion-pair partitioning.

Table IV. Partition Coefficients of Ion Pairs Determined by Two-Phase Titrations[a]

	pKa	Log P[b]	Log Pi 0.004M NaCl	Log Pi 0.15M NaCl	Log Pi .04M CF3SO3Li	Log Pi n-C6H13SO3Na .004M	Reported[b] Log Pi
Propanolol	9.45	3.56	0.12	0.91	1.56	1.18	-0.45
4-t-Butyl-pyridine	6.36[c]	2.98	0.21	0.33	0.44		(0.25)[d]
Diphenhydramine	9.1	3.5 (1it 3.27)	-[e]	0.2	1.0		-0.12
Verapamil	9.2	5.0[f] (1it 2.51)[g]	0.6	1.3			
4-CF3-Trifluoro-methane-sulfonanilide	3.35	4.47		0.55			

[a]See text for conditions. [b]Ref.11. [c]Calculated from Hammet equation. [d]Calculated from log Pi 4-n-butylpyridine = 0.4; Yeh, J. C.; Higuchi, W. I. J. Pharm. Sci. 1976, 65, 80-86. [e]Log P by titration in .004M NaCl. This is greater than the literature value so Pi cannot be calclated for .004M NaCl. Subsequent calculations are therefore minimum values. [f]Calculated by the computer program of A. Leo, preceding chapter. [g]The low vale 2.51 (32) may result from hydrolysis in 0.1 N NaOH.

Propranolol. At physiological pH, the octanol concentration of the protonated β-blocker propranolol is 25% of the concentration of the neutral species (based on its aqueous pKa, the reported log P, and log P_i from 0.15 $\underline{M}$ sodium chloride, (Table IV). Henry et al. (30) recently developed models to explain the pH dependence of propranolol binding to plasma proteins which required the presumption of ion-pair partitioning into organic phases, or that both ionized and nonionized species bind to protein. The ammonium ion could be pharmacologically important, but to gain more information on this point one would need to test a series of compounds covering a range of pKa's, or conduct evaluations over a range of pH values.

Verapamil. Verapamil (Table IV), is a Ca^{++} channel blocker. A recent report on an analog (31) describes its increased biological activity with pH changes favoring protonation. It is reasonable that a cation might be the active form to block Ca^{++}. The reported literature log P (32) is too low (hydrolysis in the 0.1 $\underline{N}$ NaOH?), based on our titration. The calculated log P from fragment constants using the computer program described by A. Leo in the preceding paper in this symposium is 5.0. Against 0.15 $\underline{M}$ sodium chloride the log P_i is 1.3. The octanol concentration of protonated verapamil is 20 times the aqueous concentration and is about 1% of the drug form at pH 7.4.

The question of the differences (Table IV) between log P_i's determined using standard conditions, 0.1 $\underline{N}$ HCl and octanol, and titration determined values must be addressed. At this point they cannot be explained. It remains to be seen which method more nearly represents the biolipid concentration of ionized species under physiological conditions.

Conclusions

These new methods for determining the partition coefficients of ionized species are still experimental, but they are presented in a spirit that they may stimulate thinking and further refinement. Single-phase titrations with HCl in octanol have only recently been run. A possible concentration dependency of pKa' in the single-phase titrations has been suggested by a referee and will be looked for. Further refinement of the two-phase titrations may incorporate ion-pair association constants.

I hope it will prove useful to think of ion-partitioning of compounds in relation to their pKa's in lipids. If there is one final message, it has to be that for many drugs and pesticides, the ionized species can be present in biolipid phases in significant concentrations. To test their pharmacological significance requires either testing a series of compounds with a range of pKa's, or testing a given compound over a range of pH values.

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Bulk and Steric Parameters in Binding and Reactivity of Bioactive Compounds

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The use of steric parameters such as
and of methods such as the branching equ-
ations to represent steric effects on bio-
activity is justified. Transport para-
meters are composite; they are a function
of differences in intermolecular forces.
The function of bulk and area parameters
is to provide the proper mix of intermol-
ecular forces required by a particular
mode of bioactivity. In the absence of
parabolic or bilinear behavior bioactiv-
ity can be modeled by an equation based
on intermolecular forces and steric ef-
fects.

Correlation analysis is the most effective, simple,
generally applicable method for the quantification of
structural effects on chemical, physical, or biologi-
cal properties. It was first successfully applied to
biological activities by Hansch and his coworkers([1])
in an equation of the form

$$BA_X = T \Pi_X + \rho \sigma_X + h \tag{1}$$

where BA_X represents the bioacticity of the substrate
bearing the substituent X; ρ, T, and h are coeffici-
ents; σ is the Hammett electrical effect parameter. Π
is a transport parameter defined by the equation

$$\Pi_X \equiv \log P_X - \log P_H \tag{2}$$

in which P_X and P_H are n-octanol-water partition co-
efficients for X-substituted and unsubstituted sub-

strates respectively. It was soon apparent that suc-
cessful quantification of bioactivity frequently re-
quired more than electrical and transport parameters.
Steric effect parameters that had been developed for
the quantification of chemical reactivities and physi-
cal properties were then incorporated in the correla-
tion equation. The Hansch equation then takes the form

$$BA_X = \rho\sigma_X + T_1\tau_X + T_2\tau_X^2 + s\upsilon_X + h \qquad (3)$$

where τ is a transport parameter such as log P or π,
σ is a composite electrical effect parameter, υ a
steric parameter. The τ^2 term is introduced to account
for the parabolic dependence on the transport para-
meter which is frequently observed for bioactivities.
Separation of the electrical effect into localized
(field and/or inductive) and delocalized(resonance)
effects gives

$$BA_X = L\sigma_{IX} + D\sigma_{DX} + s\upsilon_X + T_1\tau_X + T_2\tau_X^2 + h \qquad (4)$$

In Equations 3 and 4; ρ, L, D, S, T_1, T_2 and h are co-
efficients.
     It is convenient in correlation analysis to des-
cribe a data set as having the general structure X-G-Y
in which X is a variable substituent, Y the active site
at which some measurable phenomenon occurs, and G is a
skeletal group to which X and Y are bonded.
     As the search for improved quantitative structure-
activity relationships(QSAR)went on, new parameters
were continually introduced, generally on a trial and
error basis. Among these were a group of parameters
which represent a measure of substituent volume and
are called bulk parameters. Although thousands of ex-
amples of QSAR involving steric and/or bulk parameters
are now extant in the literature, their interpretation
has remained open to question. Thus, it has been
pointed out that steric parameters such as and E_S are
at least in part derived from intramolecular inter-
actions whereas those in biological systems are gener-
ally intermolecular. Much disagreement as to the mean-
ing of correlations with bulk parameters has appeared
in the literature(2). Some authors have argued that
they are a measure of steric effects. A number of
cases have now been reported in which a receptor site
on a biopolymer undergoes conformational changes to
better accomodate a substrate. Thus, in place of the
fixed shape lock of the "lock-and-key" theory, in
which the lock is the receptor site and the key the
substrate that binds to it, we now have a flexible
lock and flexible key.

It is argued that volume is a better measure of steric effects for a flexible lock receptor than are $\mathcal{V}$ or E_S. Alternatively, it has been suggested that bulk parameters are actually a measure of polarizability and represent London(dispersion)forces in substrate-receptor site binding.

QSAR are useful in the design of pesticides and medicinal drugs, and in environmental problems such as the prediction of toxicity and biodegradability. An empirical relationship can be properly used only for interpolation whereas one based solidly on well-established theory can be used at least to some extent for extrapolation as well. It seems of real importance, then, to determine the nature and significance of steric and bulk parameters in QSAR.

Steric Parameters

The Nature of Steric Effects.
Steric effects result from electrostatic repulsions between electrons in orbitals on non-bonded atoms. Such repulsions always result in an increase in the energy of a system. We define steric effects which result in an increase in some measurable physical, chemical, or biological property as steric augmentation, those which result in a decrease in the property as steric diminution(3). There are several categories of steric effects. They include:
1. Steric effects due to change in coordination number and hybridization of a reacting atom.
2. Steric inhibition of resonance.
3. Steric inhibition of solvation.
4. Steric determination of conformation.
5. Steric shielding of the active site.

The Minimal Steric Interaction(MSI) Principle.
As all steric interactions result in an increase in the energy of a system, the observed steric effect in the system will be the smallest possible. Thus, when the steric effect of a group X depends on its conformation, the group will prefer that conformation which results in the smallest possible steric interaction. This is the MSI principle(3,4).

Conformational dependence of the steric effect.
Some groups show little or no conformational dependence of their steric effects(3). Non-conformationally dependent groups are monatomic (halogen or hydrogen). MZ_3 symmetric top (M is hybridized sp3), $:MH_2$ or $:MH$, MZ_4,

and MZ_5 substituents are minimally conformationally
dependent. Problems in characterizing steric effects
are generally due to substituents which show a consid-
erable dependence of their steric effect on conforma-
tion. Among groups of this type are $MZ_2{}^1Z^3(CH_2Br)$,
$MZ_1Z_2Z^3$ (CHMeCl) and planar π bonded($Xp_{\pi}-$) substituents
such as aryl, vinyl, nitro, and carbonyl. Most alkyl
and substituted alkyl groups fall in this category.
Xp groups when attached to planar π bonded skeletal
groups, Gp_{π}, will exhibit a variable delocalized ele-
ctrical effect related to their variable steric effect
(5).

Van der Waals Radii And Related Steric Parameters. Van
der Waals radii have long been considered a valid meas-
ure of atomic size. Taft proposed the first valid set
of steric parameters for correlation analysis defined
from acid hydrolysis of esters. Charton derived equ-
ations for the calculation of Van der Waals radii, r_V,
of symmetric top MZ_3 groups(6). These values of the
Van der Waals radii were used, together with that for
H, to show that E_S is a linear function of r_V.

$$E_{S,X} = a_1 r_{V,X} + a_0 \tag{5}$$

Thus, E_S is an r_V based steric parameter. The r_V val-
ues themselves have been used as steric parameters.
Finally, Charton has defined the υ steric parameter
as (7)

$$\upsilon_X \equiv r_{VX} - r_{VH} - 1.20 \tag{6}$$

Groups which exert conformationally dependent(CD) ster-
ic effects cannot have steric parameters defined for
them in this way. Steric parameters for these groups
are generally obtained indirectly from chemical react-
ivities. The steric demands of a reaction vary from
one reaction to another and no signle set of steric
parameters will suffice for all reaction types.
    For almost all group types, three different Van
der Waals radii are of importance, leading to three
different υ values(3,6,7). These r_V values are:
1. $r_{V,min}$; the minimum Van der Waals radius perpendi-
    cular to the group axis.
2. $r_{V,mx}$; the maximum Van der Waals radius perpendi-
    cular to the group axis.
3. $r_{V,ax}$; the Van der Waals radius parallel to the
    group axis.
    The group axis is collinear with the bond joining
substituent X and skeletal group G. For monatomic

groups the minimum, maximum and parallel Van der Waals radii are equal to each other. For cylindrically symmetric groups such as CN or C=CH the maximum and minimum radii are equal. From Equation 6 we obtain $\mathcal{V}_{mx}$, $\mathcal{V}_{mn}$ and $\mathcal{V}_{ax}$ parameters. The $\mathcal{V}_{ef}$ constants, determined from chemical reactivities for groups with conformationally dependent steric effects, are probably close to $\mathcal{V}_{mn}$ values.

<u>Topological Parameterization of Steric Effects</u>. We have noted that different rv based steric parameters will be required for conformationally dependent groups in order to account for phenomena with different steric requirements. This must result in a multiplicity of steric parameters for different types of phenomena. The difficulty can be avoided by the use of topological methods which represent steric effects as the result of contributions of all atoms other than H in the group. Various topological methods have been proposed. The discussion here will be restricted to the branching equations (<u>4,8,9</u>). The simple branching (SB) equation is defined by

$$Q_{Ak} \equiv \sum_{i=1}^{m} a_1 n_i + a_0 \tag{7}$$

where Q_{Ak} is the quantity to be correlated for the substrate bearing the alkyl substituent Ak. The variable n_1 is equal to the number of branches (C-C bonds) at the ith C atoms of the Ak group. The numbering is begun at the substituent C atom bonded to the skeletal group (Figure 1). a_1 and a_0 are coefficients. The SB equation can be extended to substituents other than Ak groups. It has a great advantage over $\mathcal{V}$, E_S and related parameters. When it is restricted to acyclic groups $MZ_f^1 Z_r^2 Z^3$ the n_i are exact and free of error. The SB equation suffers from a serious disadvantage in its assumption that the effect of branching is independent of the order of attachment of the branches. This is equivalent to averaging the effect of the branches at some ith atom.

We may account for the effect of the order of branching by means of the expanded branching (XB) equation,

$$Q_{Ak} \equiv \sum_{i=1}^{m} \sum_{j=1}^{3} a_{ij} n_{ij} + a_{00} \tag{8}$$

in which n_{ij} represents the number of jth branches on atoms designated i. a_{ij} and a_{00} are again coefficients.

As in the SB equation groups are numbered starting wi~
th the atom bonded to the skeletal group (Figure 2).
The XB equation is a very good model of steric effects
and is indeed generally more effective than the SB
equation. This is to be expected from the operation of
the MSI principle. Thus, for example, an n-amyl group
can choose a conformation which leads to a small ster-
ic effect. A 3-amyl group can do so to a much smaller
extent. A t-amyl group cannot choose a conformation
which will minimize the steric effect of the branches
at C^1.

The XB equation has two disadvantages relative
to the SB equation. The first is that the much larger
number of variables requires a much larger data set
for good results. The second is that for all alkyl
groups other than methyl n_{11} must equal 1. Direct de-
termination of a_{11} is therefore impossible as n_{11} is
essentially constant throughout the data set and can-
not be used as a variable. Then from Equation 8,

$$Q_{Ak} = a_{11} + a_{12}n_{12} + a_{13}n_{13} + \sum_{i=2}^{m} \sum_{j=1}^{3} a_{ij}n_{ij} + a_{00} \tag{9}$$

or

$$Q_{Ak} = a_{12}n_{12} + a_{13}n_{13} + \sum_{i=2}^{m} \sum_{j=1}^{3} a_{ij}n_{ij} + a'_{00} \tag{10}$$

The Nature of Steric Effects on Bioactivity

The MacFarland Model of Bioactivity. In order to dis-
cuss the nature of the steric effects that are likely
to be encountered in bioactivity studies it is necess-
ary to have a model of substrate bioactivity. The mod-
el presented here is based on that proposed by MacFar-
land (10). Consider a bioactive substrate (bas) which
has been introduced into some organism or component
thereof. Its bioactivity results from some combination
of the following steps:
1.   Transport - The bas moves from the point of entry
      to some receptor site. In the course of its trav-
      els it may cross one or more biomembranes (Figure
      3). The bas may diffuse through the first aqueous
      phase (ϕ_1) to the anterior membrane surface (ams)
      or it may bind to plasma protein (plp) which
      transports it to the ams. It may then diffuse
      through the membrane or alternatively form a com-
      plex with a lipid soluble membrane carrier mole-
      cule (mcm) which carries it to the posterior mem-

$$
\begin{array}{c}
\text{C}^3 \\
/ \\
\text{C}^2 \!-\! \text{C}^3 \qquad\qquad \text{C}^5 \\
/ \qquad\qquad\qquad\qquad\quad / \\
\text{G} \!-\! \text{C}^1 \!-\! \text{C}^2 \!-\! \text{C}^3 \!-\! \text{C}^4 \!-\! \text{C}^5 \\
\backslash \\
\text{C}^2 \!-\! \text{C}^3 \!-\! \text{C}^4 \\
\backslash \\
\text{C}^4
\end{array}
$$
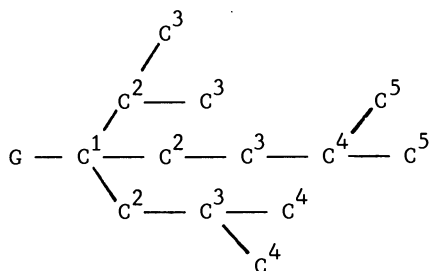
Figure 1. Values of n_i for use with the SB equation and relationships derived from it. $n_1 = n_3 = 3$, $n_2 = 4$, $n_4 = 2$. $n_b = 4$.

$$
\begin{array}{c}
\text{C}^{31} \\
/ \\
\text{C}^{21} \!-\! \text{C}^{32} \qquad\qquad \text{C}^{51} \\
/ \qquad\qquad\qquad\qquad\qquad / \\
\text{G} \!-\! \text{C}^1 \!-\! \text{C}^{22} \!-\! \text{C}^{31} \!-\! \text{C}^{41} \!-\! \text{C}^{52} \\
\backslash \\
\text{C}^{23} \!-\! \text{C}^{31} \!-\! \text{C}^{41} \\
\backslash \\
\text{C}^{42}
\end{array}
$$
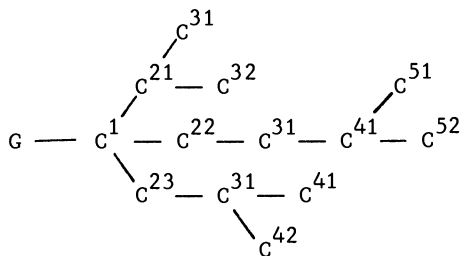
Figure 2. Values of n_{ij} for use with the XB equation and relationships derived from it. $n_{21} = n_{22} = n_{32} = n_{42} = n_{51} = n_{52} = 1$, $n_{31} = 3$, $n_{41} = 2$. $n_b = 4$.
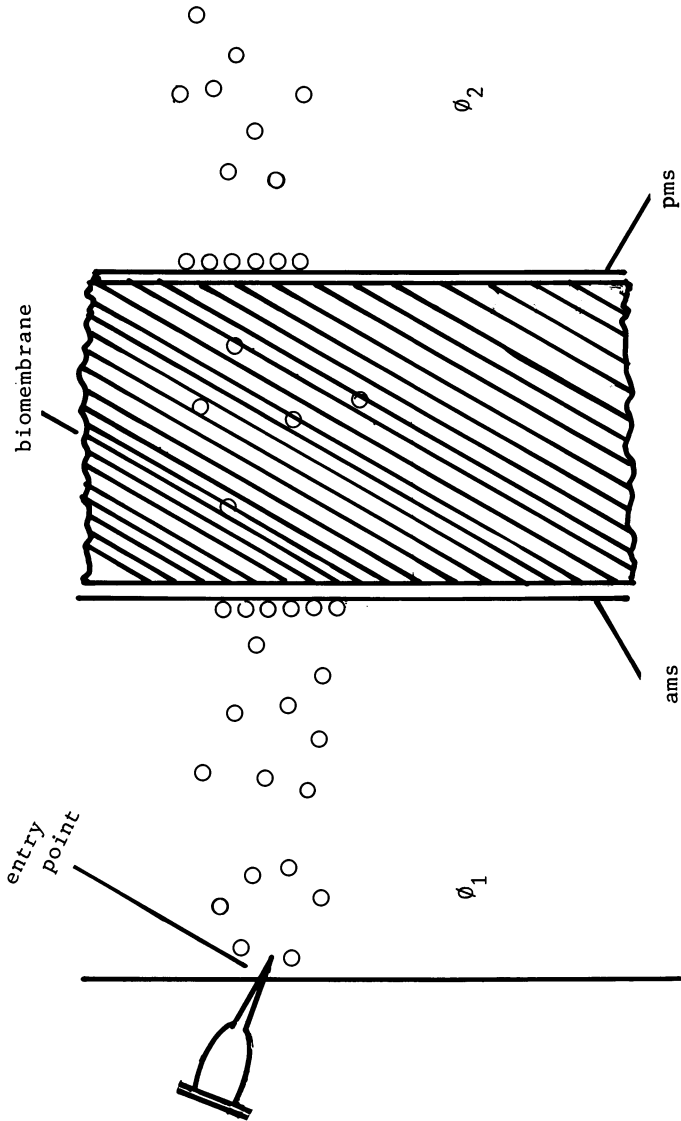
Figure 3. Step 1 in the modified MacFarland bioactivity model: Diffusion or carriage by plasma protein from the entry point to the anterior membrane surface (ams); transfer from the first aqueous phase ($\emptyset_1$) to the ams; passage through the membrane by diffusion or by lipid soluble membrane carrier molecule; bound to the posterior membrane surface (pms) transfer from the pms to the second aqueous phase ($\emptyset_2$).

brane surface (pms). The bas is then transferred
to the second aqueous phase (ϕ_{2}).

2. Receptor-bas binding - a. <u>Recognition</u>. The bas
and the receptor (rcp) form a weak complex.(Figure 4)(bas · · rcp). b. <u>Binding</u>. Conformational
changes may occur in either or both the bas and
the rcp. The bas will align itself to form a
strongly bound complex (Figure 5)(bas · rcp).

3. Chemical reaction - The receptor reacts with
and/or catalyzes reactions of the bas forming
product (prd).

The model is summed up in Scheme 1.

Scheme 1. The MacFarland Model of Bioactivity

1.
$$\text{bas}(\phi_1) \xrightleftharpoons{\text{1a}} \text{bas} \cdot \text{ams} \xrightleftharpoons{\text{1d}} \text{bas} \cdot \text{pms} \xrightleftharpoons{\text{1g}} \text{bas}(\phi_2)$$

with 1b, 1c, 1e, 1f branches:
bas · plp   bas · mcm

2,3.
$$\text{bas}(\phi_1) \xrightleftharpoons{\text{2a}} \text{bas} \cdot \cdot \text{rcp} \xrightleftharpoons{\text{2b}} \text{bas} \cdot \text{rcp}$$

$$\text{rcp} + \text{prd} \xrightleftharpoons[\text{3b}]{\text{3a}} \text{bas} \cdot \text{rcp}^{\ddagger}$$
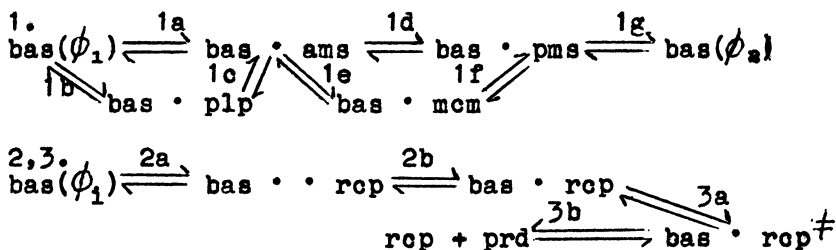
bas, bioactive substrate; rcp, receptor; ams, anterior membrane surface; pms, posterior membrane surface;
plp, plasma protein; mcm, lipid soluble membrane carrier molecule; prd, product; ϕ_i, ith aqueous phase;
bas · · rcp, weak complex; bas · rcp, strong complex;
bas · rcp‡, transition state.

In the transport step steric effects can result
from steric inhibition of binding or steric inhibition
of solvation while in the binding step steric hindrance can decrease the strength of binding to the receptor. The steric effects encountered in the chemical
reaction step are of the same types as those found in
any abiotic chemical reaction. We have noted above
that it has been suggested that steric effects in the
binding step may not be well represented by parameters
such as v and E_S as they are intra-, not intermolecular. We can test the validity of this argument by examining the correlation of data for abiotic systems
involving intermolecular interactions with v or E_S. As
the argument is presented as justification for a steric interpretation of the results of correlations with
bulk parameters it also rules out any topological par-

receptor in
initial
conformation

substrate
in initial
conformation
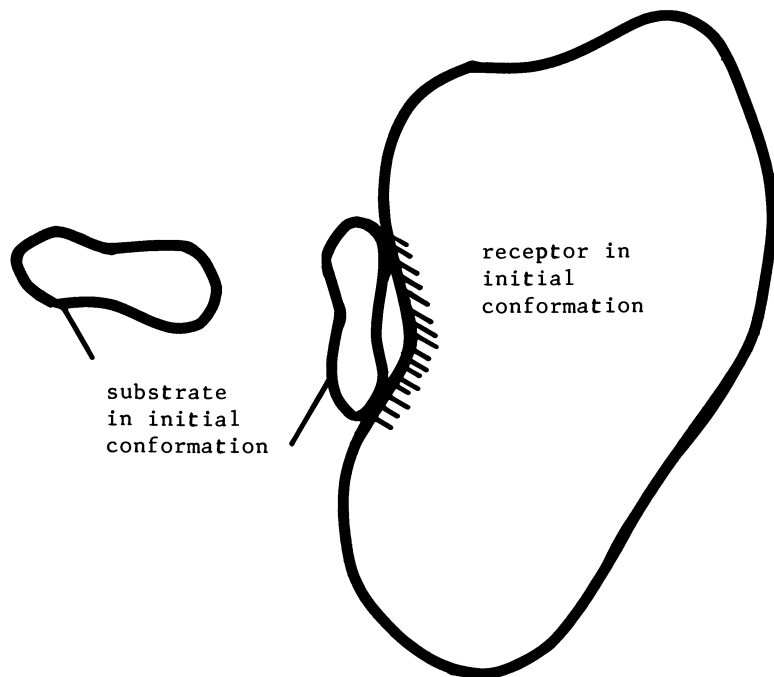
Figure 4.   Step 2a in the modified MacFarland bioactivity
model.   Recognition involving transfer from $\emptyset_i$ to the re-
ceptor surface with formation of a weak complex.

substrate
in final
conformation

receptor
in final
conformation
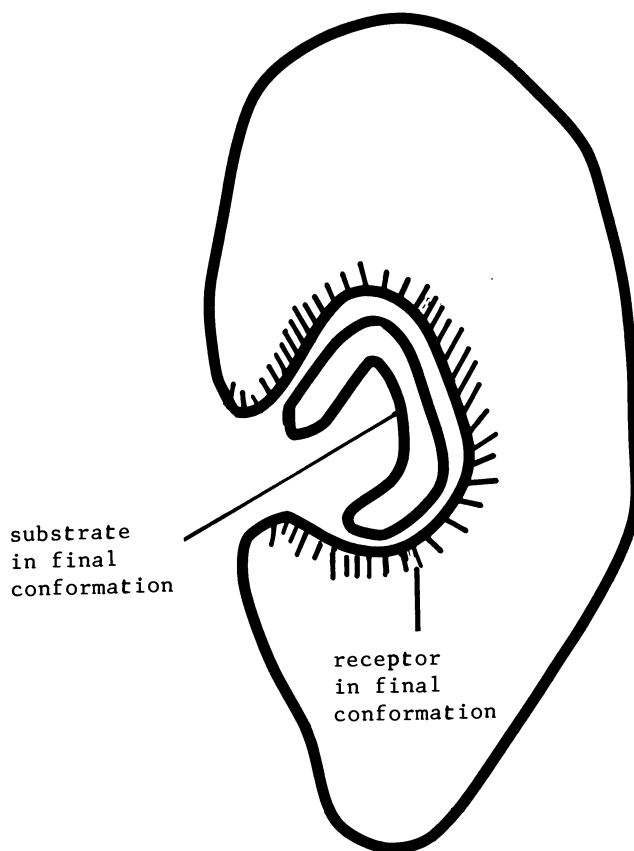
Figure 5.   Step 2b in the modified MacFarland bioactivity
model.   Binding, involving the change from a weak to a
strong complex.   Conformational changes in both substrate
and receptor site may take place.

ameters which do not represent both steric effects and polarizability.

<u>Abiotic Model Systems.</u> Possible abiotic model systems are listed in Table I. We have recently studied steric on charge transfer (ct) complex formation ($\underline{11}$). Both $\mathcal{V}$ and $\Delta\mathcal{V}$ were considered as steric parameters. $\Delta\mathcal{V}$ is defined by the expression

$$\Delta\mathcal{V} \cong r_{V,X} - r_{V,mn,Gp\pi} = \mathcal{V}_X - \mathcal{V}_{Gp\pi} \qquad (11)$$

when $\mathcal{V}_X$ is greater than or equal to $\mathcal{V}_{mn,Gp}$. When $\mathcal{V}_X$ is less than $\mathcal{V}_{mn,Gp}$

$$\Delta\mathcal{V} \equiv 0 \qquad (12)$$

$\Delta\mathcal{V}$ is intended for use when X is bonded to a planar bonded skeletal group (Gp) as if $\mathcal{V}_X$ is less than or equal to the half thickness of Gpπ ($\mathcal{V}_{mn,Gp\pi}$) it should exert no steric effect on ct complex formation. Generally, best results were indeed obtained with $\Delta\mathcal{V}$ as the steric parameter.

Table I. Abiotic Model Systems

System	Data	Models steric effects on
ct complex formation	log Ke	B
molecular association	log Ke	B, S
intermolecular hydrogen bonding	log Ke	B, S
reactivity in heterogeneous catalysis	log kr	B
chromatographic data	log V_G, I, log Rf, log t_R	B, S
binding to abiopolymers	log Ke	B
artificial enzyme models	log kr	R

Ke, equilibrium constant; kr, rate constant; Rf, rate of flow,; t_R, retention time; V_G, specific retention volume; I, retention index,; B, binding; S, solvation; R, reactivity; ct, charge transfer.

We have studied data for one molecular associa-
tion set, the self association of dialkyl ketones, us-
ing the XB equation with the addition of a term in the
number of C atoms (n_C) as a measure of polarizability.
Thus,

$$Q_{Ak^1Ak^2} = a_c n_c + \sum_{i=1}^{m} \sum_{j=1}^{3} a_{ij} n_{ij} + a_{oo} \qquad (13)$$

Although good correlation was obtained the results are
suggestive rather than conclusive due to the collin-
earity of the n_{ij} with n_C (12). We have successfully
correlated log K_c values for hydrogen bonded (hb) com-
plex formation between methanol and 2-alkylpyridines
or 2,6-dialkylpyridines with Equation 14. Significant
results were obtained for correlations of ΔV_{OH} values
for hb complexes of alcohols with heteroaromatic bases
using the SB equation modified as was Equation 13 to
give Equation 15.

$$Q_{Ak} = s V_{Ak} + a_o \qquad (14)$$

$$Q_{Ak} = a_c n_c + \sum_{i=1}^{m} a_1 n_1 + a_o \qquad (15)$$

Logarithms of retention indices of alkyl benzenes;
specific retention volumes of esters, aldehydes and
alcohols; and retention times of alkanes and alkenes
have been correlated with Equations 13 and 15 or rela-
tionships derived from them (13). Logarithms of reten-
tion times of allyl alkyl ethers on various column
packings have also been successfully correlated with
Equation 15 (14).
        Logarithms of relative rate constants for the
hydrogenolysis of alkyl alkanoates were successfully
correlated with a relationship derived from Equation
13 (14). The results suggested a dependence on branch-
ing but due to collinearity were not conclusive.
        We have examined the correlation of binding con-
stants for 4-substituted phenols with five abiotic
polymers using an equation based on intermolecular
forces and steric effects which will be described lat-
er in the section on transport parameters. In the case
of poly(ethylene glycol) a borderline dependence on V_Z
was observed (12). The substituent was considered to
have the form ZW where Z is the atom bonded to the
ring.
        Rate constants for the hydrolysis of alkyl or
cycloalkyl 2,4-dinitrobenzoates catalyzed by 1 are
well correlated by the equation

$$Q_{Ak} = a_c n_c + a_c \cdot n_c^2 + \sum_{i=1}^{m} a_i n_i + a_0 \qquad (16)$$

A highly significant dependence on branching at C^1 and C^2 was observed.
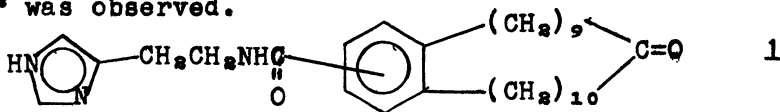
The results obtained for these abiotic systems support the conclusion that $\mathcal{V}$ and the branching equations are effective in modelling steric effects on intermolecular complex formation and the interaction of molecules with surfaces. We conclude that the use of these methods to represent steric effects occurring in steps 1 and 2 of the bioactivity model is justifiable.

The Choice of $\mathcal{V}$ Type for Bioactivity Correlations.
The nature of the steric effect exerted in binding to a receptor site depends on the shape of the receptor site and the manner in which the bas binds to it (15). If the receptor surface and substituent group axis are parallel then $\mathcal{V}_{mn,X}$ should be required. If they are perpendicular then $\mathcal{V}_{ax,X}$ ought to give best results.

Bulk Parameters

Over the past twenty years many volume or "bulk" parameters have been proposed, including Van der Waals (V_W), Traube (V_T), molar (V_M), and molal (V_{ag}°) volumes parachor (Pch), group molar refractivity (MR) and molecular weight (W_M) (2). The use of surface areas such as the Van der Waals area (A_W) as parameters has also been proposed. As we remarked previously there has been much disagreement as to the interpretation of correlations with these parameters.

Relationships among Volume, Area and Bulk Parameters.
It has been shown that MR, V_M, V_T and Pch values are all highly linear in V_W values (2,16). They are therefore all linear functions of each other. Thus,

$$Q_X = a_1 V_{W,X} + a_0 \qquad (17)$$

where Q is V_M, V_T, MR or Pch. W_M values are highly linear in the total number of electrons in the group, n_e. As MR values are also roughly linear in n_e W_M is crudely related to MR. From Equation 17 it follows that W_M must also be related to the other volume parameters.

$$Q_X = a_{11}n_{e,X} + a_{10} \qquad (18)$$

where Q is W_M or MR.

Numbers are generally linear in their squares and in their cubes.

$$m^2 = a_2 m + a_{20} \qquad (19a) \qquad m^3 = a_3 m + a_{30} \qquad (19b)$$

It follows then that

$$m^2 = a_4 m^3 + a_{40} \qquad (20)$$

Thus some degree of linearity of A_W in V_W is to be expected.

Molar Refractivity and Polarizability. Molar refractivity is an additive property with the dimensions of volume. For any member of a data set with the structure XGY we may write

$$MR_{XGY} = MR_X + MR_{GY} \qquad (21)$$

Then if only X is varied and GY is held constant throughout the data set MR_X may be used as a physicochemical parameter of the group X. The relationship between MR and polarizability is given by the equation

$$MR = (4/3)\pi N_A \alpha' = b\alpha' \qquad (22)$$

where N_A is Avogadro's number and α' is the polarizability. As all the other volume parameters are linear in MR they are also proportional to polarizability. W_M and AW which are significantly linear in MR must also be linear in polarizability.

Volume and Steric Parameters. We have noted above that steric effects are directed quantities. This is the underlying basis of the MSI principle. In contrast to the vectorial behavior of steric effects volume and bulk parameters are scalars. In order to clarify the difference between steric and volume parameters we shall make use of substituent sets which are isochoric (constant volume) or isosteric (constant $\mathcal{V}$). We define the quantities

$$f_U \equiv \mathcal{V}_X / \mathcal{V}_{X^o} \qquad (23)$$

where $\mathcal{U}_X{}^\circ$ is the steric parameter for the smallest group in the set, $\mathcal{U}_X$ is that for any other member of the set; and

$$f_B \equiv \bar{n}_{1,X} / \bar{n}_{1,X}{}^\circ \qquad (24)$$

where

$$\bar{n}_1 \equiv \sum_{i=1}^{m} n_i / m \qquad (25)$$

and m is equal to the number of atoms in the longest chain in X minus 1. Again, $n_{1,X}{}^\circ$ is the smallest value of $n_{1,X}$ for any member of the set. Finally, we define

$$f_V \equiv V_{W,X} / V_{W,X}{}^\circ \qquad (26)$$

where $V_{W,X}{}^\circ$ is the smallest V_W value for any member of the set. Values of f_U, f_B, and f_V for isochoric and isosteric sets are given in Table II. They clearly show the difference between steric and volume parameters.

Transport Parameters

Most of the QSAR that have been obtained in the last twenty years have included one or more transport parameters ($\mathcal{T}$). In fact it is this dependence on such parameters which most often distinguishes correlations of bioactivities from thos of chemical reactivities or physical properties. Among the primary types of transport parameters are the logarithms of the partition coefficient P, the molar solubility S_M, and chromatographic R_f values. Secondary $\mathcal{T}$ values are π, defined in Equation 2, fragment constants obtained in a similar manner, and R_M values. R_M is defined as

$$R_M \equiv \log [(1/R_f) - 1] \qquad (27)$$

The Composition of $\mathcal{T}$.

All of the $\mathcal{T}$ parameters represent a difference in intermolecular forces (imf). This difference results from a transfer of some substrate from one phase ϕ to another. For partition the change is from ϕ(aq) to ϕ(nonaq). For solubility it is from ϕ(s) to ϕ(soln), while for chromatographic quantities it is from ϕ(mobile) to ϕ(fixed). Thus,

$$\mathcal{T} = f(imf_s - imf_1) = f(\Delta imf) \qquad (28)$$

Table II.   Values of f_U, f_B and f_V

1. C_4 alkyl groups, V_W = 44.35$\pm$0.01. Ak, f_U, f_B; Bu, 1.00, 1.00; iBu, 1.44, 1.50; sBu, 1.50, 1.50; tBu, 1.82, 3.00

2. C_5 alkyl groups, V_W = 54.58$\pm$0.01. Ak, f_U, f_B; Am, 1.00, 1.00; iPrCHMe, 1.90, 2.00; Et_2CH, 2.22, 2.00; $EtMe_2$C, 2.40, 2.00; iAm, 1.00, 1.00; PrMeCH, 1.54, 1.33; $tBuCH_2$, 1.97, 2.00; $sBuCH_2$, 1.47, 1.33

3. C_6 alkyl groups, V_W = 64.805$\pm$0.015. Ak, f_U, f_B; Hx, 1.07, 1.00; $tBuCH_2CH_2$, 1.03, 1.67; iBuMeCH, 1.60, 1.67; tBuMeCH, 3.10, 2.50; $iAmCH_2$, 1.00, 1.00; BuMeCH, 1.57, 1.25; iPrEtCH, 3.10, 2.50

4. Groups with V_W = 15.49$\pm$0.79. X, f_U; CH_2OH, 1.51; Br, 1.86; CN, 1.14; PH_2, 1.71; NO_2, 1.00, 3.97; CHO, 1.43, 2.71

11. Groups with V= 0.68. X, f_V; Pr, 1.00; Bu, 1.30; Am, 1.60; iAm, 1.60; $iAmCH_2$, 1.90; Oc, 2.50; No, 2.80; Ud, 3.40, Trd, 4.00; Pnd, 4.60; Hpd, 5.20

12. Groups with V= 0.55$\pm$0.03. X, f_V; CH_2NH_2, 1.73; Me, 1.14; CH_2OH, 1.30; Cl, 1.00; C=CH, 1.63; OPr, 3.15; OBu, 4.01; Et, 1.99; OAm, 4.86; OCH_2CH_2tBu, 5.71

Sets 1-4 are isochoric, sets 11 and 12 are isosteric. In set 4 values of f_U are given for both V_{mn} and V_{mx} for Xpπ groups. Abbreviations are Hx, hexyl; Oc, octyl; No, nonyl; Ud, undecyl; Trd, tridecyl; Pnd, pentadecyl; Hpd, heptadecyl.

Possible imf are shown in Table III together with the physicochemical parameters which represent them. The correlation equation for T then takes the form

$$T = A\sigma_X + L\sigma_{IX} + D\sigma_{DX} + H_1 n_{HX} + H_2 n_{nX} + Ii_X + B_0 \tag{29}$$

It is also necessary to account for the possibility of steric inhibition by the X group of the solvation of the remainder of the substrate. For this purpose we introduce a steric term into Equation 29. This steric

effect can be parameterized by $\mathcal{V}$ or by branching. The use of $\mathcal{V}$ gives

$$\mathcal{T} = A\alpha_X + L\sigma_{IX} + D\sigma_{DX} + S\mathcal{V}_X + H_1 n_{HX} + H_2 n_{nX} + $$

$$I i_X + B_0 \tag{30}$$

From the SB equation we have

$$\mathcal{T} = A\alpha_X + L\sigma_{IX} + D\sigma_{DX} + \sum_{i=1}^{m} a_i n_i + H_1 n_{HX} + $$

$$H_2 n_{nX} + I i_X + B_0 \tag{31}$$

while from the XB equation we obtain

$$\mathcal{T} = A\alpha_X + L\sigma_{IX} + D\sigma_{DX} + \sum_{i=1}^{m} \sum_{j=1}^{3} a_{ij} n_{ij} + $$

$$H_1 n_{HX} + H_2 n_{nX} + I i_X + B_{00} \tag{32}$$

We have examined the correlation of 18 sets of $\mathcal{T}$ values for amino acids with Equation 30 with very good results (17,18).

Table III.   Intermolecular Forces

imf	abbr.	parameter
hydrogen bond	hb	n_H, n_n
dipole - dipole	dd	σ_I, σ_D
dipole - induced dipole	di	σ_I, σ_D, α
induced dipole - induced dipole	ii	α
charge transfer	ct	σ_I, σ_D
ion - dipole	Id	i
ion - induced dipole	Ii	i

$n_H \equiv$ number of OH or NH bonds in X.
$n_n \equiv$ number of lone pairs on N or O atoms in X.
σ_I and σ_D are localized(field and/or inductive) and delocalized(resonance) electrical effects respectively.
$\alpha \equiv$ (MR$_X$ - MR$_H$) / 100. It is a measure of polarizability.
i = 1 for charged X; i = 0 for uncharged X.

A further test of the validity of Equations 30,31, and 32 is obtained by considering sets of $\mathcal{T}$ values in which X is limited to alkyl(Ak) groups. As electrical

effects of Ak groups are constant, τ_{Ak} is a linear function of the number of C atoms in the Ak group, and for Ak groups n_H, n_n and 1 are all equal to zero, Equations 30, 31 and 32 simplify to

$$\tau_{Ak} = a_c n_c + s\mathcal{U}_{Ak} + B_{10} \tag{33}$$

$$\tau_{Ak} = a_c n_c + \sum_{i=1}^{m} a_1 n_1 + B_{20} \tag{34}$$

$$\tau_{Ak} = a_c n_c + \sum_{i=1}^{m} \sum_{j=1}^{3} a_{1j} n_{1j} + B_{30} \tag{35}$$

Correlation of 20 sets of partition data, 4 sets of solubility data and 17 sets of chromatographic data with Equations 34 and 35 or relationships derived from them gave excellent results providing further support for the validity of the model (14).

Finally, 15 sets of τ values for both aromatic and aliphatic systems have been correlated with Equations 30 - 32 with generally good results. The data sets studied are set forth in Table IV, the statistics obtained are reported in Table V. These results supply further support for the imf model of transport parameters. It seems highly likely then that Equations 30 - 32 are generally applicable and that τ values are indeed composite parameters.

Table IV.   Values of τ

LP 1,2,3 log P, Ph(CH$_2$)$_n$X, n = 1,2,3. X, log P$_1$, log P$_2$
     log P$_3$[a]; H, 2.69, 3.15, 3.68; F, -, -, 2.95; Cl,
     -, 2.95, 3.55; Br, -, 3.09, 3.72; OH, 1.10, 1.36
     1.88; NH$_2$, 1.09, 1.41, 1.83; CO$_2$Me, 1.83, 2.32,
     2.77; CO$_2$H, 1.41, 1.84, 2.42; CN, 1.56, 1.72,
     2.21; Ac, 1.44, -, 2.42; CONH$_2$, 0.45, 0.91, 1.41
     OAc, 1.96, 2.30, 2.77; OMe, -, -, 2.70; NMe$_2$,
     -, -, 2.73; Me, 3.15, 3.68, -.

LP 4 log P, PhX. X, log P[a]; NH$_2$,0.90; OMe, 2.11; H,
     2.13; Cl, 2.84; CO$_2$Et, 2.62; NO$_2$, 1.85; OH, 1.46
     C≡CH, 2.53; CH$_2$CONH$_2$, 0.45; CH$_2$OH, 1.10.

LP 11,12,13 log P, 2-, 3-, 4-C$_5$H$_4$N. X, log P$_2$, log P$_3$,
     log P$_4$[a]; H, 0.65, 0.65, 0.65; Me, 1.11, 1.19,
     1.22; Et, 1.69, -, -; tBu, 2.40, -, -; Cl, 1.27,
     1.33, 1.28; Br, 1.38, 1.58, 1.51; I, -, 1.80, -;
     NO$_2$, 0.48, 0.60, 0.33; CN, 0.50, 0.36, 0.46; Ac,
     0.83, 0.43, 0.54; Bz, 1.96, 1.83, 1.98; CO$_2$Me,

Table IV. Continued.

0.27, 0.81, 0.87; CO_2Et, 0.87, 1.36, 1.43;
$CONH_2$, 0.29, -0.34, -0.28; NH_2, 0.58, 0.20,
0.26; NMe_2, 1.43, -, 1.34; NHAc, 0.61, 0.41,
0.50; OMe, 1.34, -, 1.00; OPh, 2.45, -, -; SMe,
1.71, -, -; CH_2NH_2, -, -0.10, -; Pr, -, -, 2.10

PI 1 π aliphatic. X,π^a; OH, -1.16; OMe, -0.47; SMe,
0.45; F, -0.17; Cl, 0.39; Br, 0.60; I, 1.00;
NH_2, -1.19; NHMe, -0.67; NMe_2, -0.32; Me, 0.50;
C=CH, 0.48; $CONH_2$, -1.71; Ac, -0.71; CN, -0.84;
CO_2Me, -0.27; OAc, -0.27; NO_2, -0.85; H, 0

PI 11, 12, 13 π, 2-, 3-, $4-XC_6H_4O_2CNHMe$. X, π_2, π_3, π_4^c;
H, 0, 0, 0; Me, 0.31, 0.54, 0.50; Et, 0.77,
1.04, 1.08; iPr, 1.15, 1.47, 1.64; Pr, 1.25,
1.48, 1.56; tBu, 1.44, 1.77, 1.90; sBu, -, -,
2.04; F, 0.10, 0.33, 0.12; Cl, 0.49, 0.87, 0.85
Br, 0.62, 1.09, 1.01; I, 0.80, 1.36, 1.30; OMe,
-0.35, 0.14, 0.04; OEt, 0.08, 0.60, 0.48; OiPr,
0.36, 0.80, -; OBu, -, 1.80, 1.66; SMe, -, -,
0.77; CHO, -, -0.24, -0.17; Ac, -, -0.26, -0.15
COEt, -, 0.33, 0.39; CN, -0.04, -0.18, -0.21;
CF_3, -, 1.21, -; NO_2, -0.14, 0.23, 0.30; SO_2Me,
-, -, -0.13^x; NMe_2, -, 0.27^x, -

PI 51 π aromatic. X,π^d; Br, 0.86; Cl, 0.71; I, 1.12; F,
0.14; H, 0; CF_3, 0.88; CN, -0.57; NH_2, -1.23^x;
OMe, -0.02^x; SMe, 0.61; NHMe, -0.47^x; C=CH,
0.40; OEt, 0.38^x; Me, 0.56; Et, 1.02; iPr, 1.53
tBu, 1.98; $SiMe_3$, 2.59; SO_2Me, -1.63; NMe_2,
0.18; SF_5, 1.23; CH_2Cl, 0.17; CH_2OH, -1.03;
CH_2OMe, -0.78; CH_2Br, 0.79; CH_2I, 1.50; $NHNH_2$,
0.11; SEt, 1.07

PI 101,102 π, 3-, $4-XC_6H_4NO_2$. X, π_3, π_4^e; H, 0, 0; Cl,
0.61, 0.54; Br, 0.79, -; Me, 0.57, 0.52; CH_2OH,
-0.65, -0.60; CO_2H, -0.02, 0.03; Ac, -0.43,
-0.36; CN, -0.68, -0.66; OH, 0.15, 0.11; OMe,
0.31, 0.18; NH_2, -0.48, -0.46; NO_2, -0.36,
-0.39; $CONH_2$, -1.08, -1.03

FR 1 Fr, aliphatic. X, Fr^d; Br, 0.20; Cl, 0.06; F,
-0.38; I, 0.59; H, 0.23; OH, -1.64; NH_2, -1.54;
CCl_3, 1.61; CF_3, 0.29; CN, -1.27; Me, 0.77;
C=CH, 0.01; tBu, 2.22; OMe, -1.59; SMe, -0.02;
NHMe, -1.38; NMe_2, -0.64; CH_2Br, 0.74; CH_2I,
1.13; CH_2Cl, 0.60; Et, 1.43; iPr, 1.84; Pr,
1.97; OEt, -0.51; SEt, 0.52; CH_2OMe, -0.23^x;
CH_2CN, -0.73^x; $SiMe_3$, 2.96; NO_2, -1.16; Ac,
-1.13; CO_2Me, -0.72; $CONH_2$, -2.18; CO_2Et, -0.18
$CH=CH_2$, 0.88; Ph, 1.90

Table IV. Continued.

    a. Tute, M. S. Adv. Drug Res. 1971, 6, 1
    b. Lewis, S. J.; Mirrlees, M. S.; Taylor, P. J. Quant. Struct.-Act. Relat. in press
    c. Fujita, T.; Kamoshita, K.; Nishoka, T.; Nakajima, M. Agr. Biol. Chem. 1974, 38, 1521
    d. Hansch, C.; Leo, A. J. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979.
    e. Fujita, T. Prog. Phys. Org. Chem. 1983, 14, 75
    x. Excluded from the correlation.

Table V. Results of Correlations

Set	A	L	D	S	H_1
LP1	-	-2.62	-	-	-0.727
LP2	-	-2.79	-	0.193	-0.813
LP3	-11.1	-3.27	-	3.92	-0.933
LP4	8.92	1.20	-	-1.08	0.620
LP11R	5.71	-	-4.79	0.472	-0.271
LP12Z	4.88	-	-1.94	-	-0.674
LP13R	4.57	-0.739	-0.301	0.890	-0.540
PI1	-3.64	-1.10	-	2.46	-0.695
PI11R	-	-0.508	0.403	1.37	-
PI12Z	6.48	-	-0.686	0.841	-
PI13R	6.40	-	-0.484	0.873	-
PI51	2.30	-0.916	-0.561	1.46	0.253
PI101Z	-8.72	-	-1.14	-1.79	-0.454
PI102R	-11.3	-0.757	-0.646	1.98	-0.537
FR1	2.73	-2.48	-	1.46	-0.906

Set	H_s	B_0	$100R^2$ [a]	F [b]	r_{1s} [c]
LP1	-	2.77	86.83	23.08	-
LP2	-0.168	2.91	91.94	17.11	-
LP3	-0.111	3.38	81.15	6.889[J]	0.011
LP4	0.281	2.222	94.25[1]	13.12[1]	0.080
LP11R	-0.197	0.54	87.25	17.80	-

Table V.   Continued.

LP12Z	-	0.55	87.39	25.40	-
LP13R	-0.112	0.59	86.44	9.560	0.013
PI1	-0.145	-0.37	85.07	14.81	0.038
PI11R	-0.114	0.00	86.83	16.48	-
PI12Z	-0.161	-0.34	81.28	16.28	-
PI13R	-0.175	-0.32	80.28	15.26	-
PI51	-0.761	-0.26	91.31	29.76	0.346
PI101Z	-	-0.25	76.37	6.464^l	-
PI102R	0.103	-0.11	80.70	3.385^n	0.357
FR1	-0.213	-0.08	85.45	31.72	0.370^m

Set	r_{13}^c	r_{14}^c	r_{15}^c	r_{16}^c	r_{23}^c
LP1	-	-	-	-	-
LP2	-	-	-	-	-
LP3	-	0.692^n	0.158	0.535	-
LP4	-	0.720	0.062	0.598	-
LP11R	0.007	0.465^n	0.176	0.276	-
LP12Z	0.315	-	0.138	-	-
LP13R	0.147	0.414	0.130	0.362	0.263
PI1	-	0.715^l	0.154	0.315	-
PI11R	-	-	-	-	0.018
PI12Z	0.128	0.774^h	-	0.042	-
PI13R	0.146	0.774^h	-	0.042	-
PI51	0.013	0.667^l	0.144	0.028	0.071
PI101Z	0.362	0.721^J	0.035	-	-
PI102R	0.383	0.700^n	0.093	0.461	0.244
FR1	-	0.695^h	0.256	0.105	-

Set	r_{24}^c	r_{25}^c	r_{26}^c	r_{34}^c	r_{35}^c
LP1	-	0.069	-	-	-
LP2	0.554^n	0.197	0.081	-	-
LP3	0.520	0.322	0.170	-	-
LP4	0.102	0.502	0.301	-	-
LP11R	-	-	-	0.034	0.220

Table V.   Continued.

LP12Z	-	-	-	-	0.329
LP13R	0.152	0.121	0.328	0.001	0.270
PI1	0.261	0.284	0.182	-	-
PI11R	0.289	-	0.406	0.085	-
PI12Z	-	-	-	0.126	-
PI13R	-	-	-	0.070	-
PI51	0.078	0.164	0.105	0.400^n	0.403^n
PI101Z	-	-	-	0.042	0.141
PI102R	0.216	0.238	0.327	0.167	0.311
FR1	0.226	0.050	0.351^m	-	-

Set	r_{86}^c	r_{45}^c	r_{46}^c	r_{56}^c	R_1^d
LP1	-	-	-	-	-
LP2	-	0.110	0.294	0.282	-
LP3	-	0.021	0.397	0.264	-
LP4	-	0.316	0.249	0.102	-
LP11R	0.302	0.076	0.137	0.200	-
LP12Z	-	-	-	-	0.317
LP13R	0.316	0.004	0.031	0.172	-
PI1	-	0.164	0.014	0.186	-
PI11R	0.186	-	0.201	-	-
PI12Z	0.255	-	0.306	-	0.834^h
PI13R	0.144	-	0.306	-	0.838^h
PI51	0.052	0.221	0.085	0.412^n	-
PI101Z	-	0.048	-	-	0.795^j
PI102R	0.241	0.153	0.213	0.332	-
FR1	-	0.243	0.344^m	0.257	-

Set	R_2^d	R_3^d	R_4^d	R_5^d	R_6^d
LP1	-	-	-	-	-
LP2	0.573	-	0.614^n	0.371	0.435
LP3	-	-	-	-	-
LP4	-	-	-	-	-
LP11R	-	-	-	-	-

Table V. Continued.

LP12Z	–	0.427	–	0.338	–
LP13R	–	–	–	–	–
PI1	–	–	–	–	–
PI11R	0.459	0.226	0.323	–	0.456
PI12Z	–	0.312	0.847^h	–	0.587^k
PI13R	–	0.274	0.850^h	–	0.568^k
PI51	–	–	–	–	–
PI101Z	–	0.501	0.758^j	0.170	–
PI102R	–	–	–	–	–
FR1	–	–	–	–	–

Set	s_{est}^e	s_A^e	s_L^e	s_D^e	s_S^e
LP1	0.325	–	0.632^j	–	–
LP2	0.324	–	0.674^j	–	0.717^m
LP3	0.384	0.465^m	1.21^m	–	1.50^m
LP4	0.287	4.62^p	0.766^p	–	0.989^r
LP11R	0.282	1.15	–	0.234^n	0.347^p
LP12Z	0.254	1.11^j	–	0.433	–
LP13R	0.369	1.49^k	0.467^p	0.268^q	0.551^p
PI1	0.327	3.11^q	0.522^n	–	0.771^j
PI11R	0.235	–	0.283^p	0.287^p	0.234
PI12Z	0.358	2.53^m	–	0.410^p	0.587^p
PI13R	0.367	2.63^m	–	0.352^p	0.608^p
PI51	0.327	2.12^q	0.403^m	0.375^p	0.363
PI101Z	0.336	5.91^p	–	0.484^m	0.923^n
PI102R	0.316	5.83^p	0.542^q	0.344^p	0.907^n
FR1	0.531	2.42^q	0.605	–	0.483^j

Set	$s_{H_1}^e$	$s_{H_2}^e$	$s_{B_0}^e$	s^{*f}	n^g
LP1	0.129	–	0.210	0.434	10
LP2	0.134	0.0630^m	0.291	0.384	11
LP3	0.185^j	0.0874^q	0.332	–	14
LP4	0.188^m	0.108^n	0.258^j	–	10
LP11R	0.115^m	0.0540^j	0.194^k	–	19

Table V. Continued.

LP12Z	0.0922	-	0.141^j	0.415	15
LP13R	0.129^j	0.0656^p	0.269^n	-	16
PI1	0.130	0.0655^m	0.254^p	-	19
PI11R	-	0.571^n	0.181	0.444	15
PI12Z	-	0.0848^n	0.244	0.500	20
PI13R	-	0.0856^n	0.251^q	0.513	20
PI51	0.140^n	0.0769	0.210^q	-	24
PI101Z	0.127^j	-	0.288^s	0.620	13
PI102R	0.154^k	0.0844^q	0.305^v	-	12
FR1	0.197	0.0828^k	0.310^w	-	33

a. Per cent of the variance of the data accounted for by the correlation equation. R is the multiple correlation coefficient. The superscript indicates the confidence level of R (CL) if it is <99.9 %.

b. F test for the significance of the correlation. The superscript indicates the CL if it is <99.9%

c. Partial correlation coefficients. The matrix below gives the parameters corresponding to the subscripts.

	σ_I	σ_D	υ	n_H	n_n
α	12	13	14	15	16
σ_I		23	24	25	26
σ_D			34	35	36
υ				45	46
n_H					56

The superscript indicates the CL if it is >80.0%

d. Multiple correlation coefficients of the ith independent variable with all of the other independent variables. Variables corresponding to the subscripts are 1, α; 2, σ_I; 3, σ_D; 4, υ; 5, n_H; 6, n_n. The superscript indicates the CL if it is >80.0%.

e. Standard errors of the estimate and the regression coefficients. The superscripts indicate the CL if it is <99.9%.

f. Corrected standard error. $s^* \equiv s_{est}/(\text{root mean square of the data})$

g. Number of data points in the set
h. 99.9% CL i. 99.5% CL j. 99.0% CL
k. 98.0% CL l. 97.5% CL m. 95.0% CL
n. 90.0% CL p. 80.0% CL q. 70.0% CL
r. 60.0% CL s. 50.0% CL v. 20.0% CL
w. 10.0% CL

Table V.   Continued.

The letters Z and R after set designations indi-
cate correlations with σ_R^o and σ_R respectively. All
σ_I and σ_D constants are from Charton, M. **Prog.**
Phys. Org. Chem. 1981, **13**, 119. υ values are from
ref. 3. α values are obtained from the equation
$\alpha = (MR_X - MR_H)/100$ using MR values given in foot-
note c, Table 4 or were estimated assuming addi-
tivity.

Of considerable interest is a quantitative com-
parison of the composition of τ values. Consider a hy-
pothetical reference group X^o for which we define

$$\alpha = \sigma_I = \sigma_D = \upsilon = n_H = n_n = 1 = 1 \qquad (36)$$

Then we may write the per cent of the total substituent
effect due to the kth independent variable v_k as

$$P_k = V_k \cdot 100/(\sum_{k=1}^{q} Vk) \qquad (37)$$

where V_k is the coefficient of the independent variable
v_k. Values of P_k for the sets studied are reported in
Table VI.

Table VI.   Values of P_k

Set	P_1	P_2	P_3	P_4	P_5	P_6
LP1	-	78.3	-	-	21.7	-
LP2	-	70.4	-	4.9	20.5	4.2
LP3	57.4	16.9	-	20.3	4.8	0.6
LP4	73.7	9.9	-	8.9	5.1	2.3
LP11R	49.9	-	41.9	4.1	2.4	1.7
LP12Z	65.1	-	25.9	-	9.0	-
LP13R	63.9	10.3	4.2	12.4	7.6	1.6
PI1	45.3	13.7	-	30.6	8.6	1.8
PI11R	-	21.2	16.8	57.2	-	4.8
PI12Z	79.3	-	8.4	10.3	-	2.0
PI13R	80.7	-	6.1	11.0	-	2.2
PI51	36.8	14.7	9.0	23.4	4.0	12.2
PI101Z	72.0	-	9.4	14.8	3.8	-
PI102R	73.7	4.9	4.2	12.9	3.5	0.7
FR1	35.0	31.8	-	18.7	11.6	2.7

Variables corresponding to the subscripts are:
1, α ; 2, σ_I; 3, σ_D; 4, υ ; 5, n_H; 6, n_n.

The Significance of the Composition of $\mathcal{T}$. The general
equation for the correlation of bioactivities in the
absence of parabolic or bilinear behavior is

$$BA_X = L^\circ\sigma_{IX} + D^\circ\sigma_{DX} + S^\circ\upsilon_X + T\mathcal{T}_X + B \qquad (38)$$

Our results support the conclusion that $\mathcal{T}$ is a compo-
site parameter described by Equation 30 or relation-
ships derived from it. Then combining Equations 30 and
38 gives

$$BA_X = A\sigma_X + L\sigma_{IX} + D\sigma_{DX} + S\upsilon_X + H_1 n_{HX} + H_s n_{nX} +$$
$$I i_X + B^1 \qquad (39)$$

where $L = L^\circ + TL$, $D = D^\circ + TD$, $S = S^\circ + TS$ and $B^1 =$
$B + B_0$. There are several vital implications of
Equation 39.
1. A given transport parameter has a fixed composi-
    tion of imf. The existence of electrical effect
    terms in Equation 39 does not necessarily mean
    that they are due to Step 3 of the bioactivity
    model. The electrical effects observed may also
    be due to imf involved in Steps 1 and 2.
2. The same argument applies to a steric effect term
    in Equation 39.
3. We have noted that all bulk parameters are a lin-
    ear function of polarizability. Their introduct-
    ion into the correlation equation is most easily
    rationalized as a means of correcting $\mathcal{T}$ to ob-
    tain the appropriate composition of imf.
4. In the absence of parabolic or bilinear behavior,
    Equation 39, or relationships derived from it can
    be used for the correlation of bioactivities.

Binding in Biopolymers

We have noted above that Equation 39 or relationships
derived from it can be applied to the correlation of
bioactivities. An example of such a correlation has
already been reported(18). We have now studied the
correlation of four data sets involving the binding of
substrates to proteins with relationships derived from
Equation 39. The data used are presented in Table VII,
the results of the correlations in Table VIII. Sets
BP1 and BP2 do not include a wide range of substituent
types and show a high degree of parameter collinearity.
Sets BP101 and BP102 have a wider range of substituent
type and much less collinearity but still do not in-
clude groups that are electron acceptors by the deloc-

alized electrical effect. Thus, although very good results were obtained, we may only conclude that they are in accord with the validity of Equation 39 but do not prove it beyond doubt. Much further work is required for that purpose.

Table VII.   Biopolymer Binding Data

BP 1,2 Equilibrium constants, 10^6Ke, m^{-1}, rate constants 10^5 ka($m^{-1}s^{-1}$) 4-$XC_6H_4SO_2NH_2$ + human carbonic anhydrase isozyme C.   X, Ke, ka[a]; H, 4.93, 8.80; Me, 12.2, 11.2; Et, 34.0, 33.0; Pr, 59.0, 50.2; Bu, 200, 100; Am, 724, 246; CO_2Me, 96.3, 45.3; CO_2Et, 313, 106; CO_2Pr, 590, 117; CO_2Bu, 1300, 325; CO_2Am, 2450, 466; CO_2Hx, 2440, 586; CO_2NHMe, 12.0, 5.90; CO_2NHEt, 33.8, 8.60; CO_2NHPr, 12.0, 11.3; CO_2NHBu, 308, 17.6; CO_2NHAm, 566, 25.5; CO_2NHHx, 762, 38.1; CO_2NHHp, 846, 43.1

BP 101 Equilibrium constants, 5-X-2-(4 -$H_2NC_6H_4SO_2NH$)-3-OH-C_5H_2N + albumin, X, log Ke[b]; H, 0.114; Me, 0.851; Et, 1.215; iPr, 1.699; Am, 2.645; Ph, 2.684; OMe, 0.94; OEt, 1.519; OPr, 2.017; OiPr, 1.40; OBu, 2.318; SMe, 1.516; Cl, 1.468; Br, 1.771; I, 2.312

BP102 Percent albumin binding(PEB) 4-XC_6H_4-$OCH_2CH_2NEt_2$. X, log PEB[b]. H, 1.028; Me, 1.137; OMe, 0.822; OEt, 0.906; Cl, 1.458; Br, 1.452; I, 1.458; Pr, 1.456; SEt, 1.437; NH_2, 0.695; NHMe, 0.549; NHEt, 0.728; OH, 0.779

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Table VIII. Results of Correlations of Biopolymer Binding Data

Set	A	L	D	S	H_1
BP1	7.36	0.898	-	-	-0.875
BP2	4.59	0.538	-	-	-1.25
BP101	9.89	1.74	1.14	-	-
BP102	-	0.453	0.948	0.613	-

Table VIII. Continued.

Set	B^1	$100R^{2a}$	F^b	$r_{12}{}^c$	$r_{13}{}^c$
BP1	0.862	95.40	103.7	0.578^j	-
BP2	1.075	95.87	116.1	0.578^j	-
BP101	0.398	93.01	48.79	0.073	0.201
BP102	1.051	95.23	59.89	-	-

Set	$r_{15}{}^c$	$r_{23}{}^c$	$r_{24}{}^c$	$r_{25}{}^c$	$r_{34}{}^c$
BP1	0.441^n	-	-	0.458^m	-
BP2	0.441^n	-	-	0.458^m	-
BP101	-	0.497^h	-	-	-
BP102		0.037	0.366	-	0.161

Set	$R_1{}^d$	$R_2{}^d$	$R_3{}^d$	$R_4{}^d$	$R_5{}^d$
BP1	0.611^j	0.621^j	-	-	0.507^m
BP2	0.611^j	0.621^j	-	-	0.507^m
BP101	0.283	0.528^n	0.552^m	-	-
BP102	-	0.378	0.191	0.405	-

Set	$S_{est}{}^e$	$S_A{}^e$	$S_L{}^e$	$S_D{}^e$	$S_S{}^e$
BP1	0.190	0.512	0.398^m	-	-
BP2	0.138	0.373	0.290^n	-	-
BP101	0.210	0.836	0.371	0.327	-
BP102	0.0873	-	0.161^m	0.0930	0.116

Set	$S_{H_1}{}^e$	$S_{B_1}{}^e$	S^f	n^g
BP1	0.105	0.0975	0.241	19
BP2	0.761	0.0710	0.299	19
BP101	-	0.138^k	0.309	15
BP102	-	0.0762	0.263	13

For footnotes, see Table V.

Conclusions

On the basis of the results presented here we may
arrive at the following conclusions:
    All of the evidence we have collected so far on
abiotic model systems either strongly supports or is at

least in accord with the validity of both the γ para-
meter and the branching equations as a means of repre-
senting steric effects on intermolecular phenomena. We
have so far found no exceptions. As bioactivities are
the result of physicochemical properties associated
with molecular structure the sue of the υ parameter
and the branching equations to model steric effects on
bioactivity is justified.

Based on our correlations of γ values for amino
acids(12, 17, 18) peptides(12), alkyl substituted com-
pounds(14) and the results reported here it seems
clear that transport parameters are composite and are
a function of differences in intermolecular forces.
It is quite true that collinearity is frequently a pro-
blem in these correlations. Furthermore, our present
model of intermolecular forces seems less effective
when substituents are bonded to aromatic skeletal
groups than when they are bonded to aliphatic groups.
The model is probably in need of improvement. Never-
theless, the composite nature of transport parameters
seems certain. Less certain but very likely is the
conclusion that the composition of transport para-
meters varies with the type of quantity (partition co-
efficient, solubility, chromatographic retention index,
...) and the structure of the parent compound of a set.

The function of volume, "bulk" and area parameters
is to correct the transport parameter composition so
that the composition required by the bioactivity is
obtained. To argue that when collinearity between
steric and volume parameters is absent the latter meas-
ure steric effect is to accept that the steric effects
of an n-heptyl and a triethylcarbinyl (which have the
same bulk).are the same. This is equivalent to re-
quiring identical effects of one group shaped roughly
like a pencil and another shaped roughly like a plum.
As this is clearly false we can reject a steric inter-
pretation of correlations with volume, area, and bulk
(VAB) parameters.

Some workers contend that in a flexible key -
flexible lock type of bas - rcp interaction the VAB
parameters are a measure of the acceptable "size" of a
group. This cannot be the case. If the receptor site
is infinitely flexible it can accomodate any group no
matter what its dimensions may be. In that highly im-
probable event no steric effect is possible. In order
to wrap itself around a substrate the receptor must
undergo conformational changes which can be achieved
only at the expense of some quantity of energy. If a
bas with diminsions larger than some limiting size is
then bound to the receptor site true vectorial steric

effects will be observed, dependent on the shape of
both bas and rcp. We therefore conclude that a steric
interpretation of correlation with VAB parameters is
unacceptable.

On the basis of our results for amino acid and
peptide bioactivities and those for binding to bio-
polymers we tentatively conclude that in the absence
of parabolic or bilinear behavior Equation 39 and re-
lationships derived from it are useful for the correl-
ation of bioactivities.

Acknowledgement

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Use of STERIMOL, MTD, and MTD* Steric Parameters in Quantitative Structure–Activity Relationships

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STERIMOL and MTD (Minimum Topological Difference) steric parameters to account for steric influences in QSAR of pesticides have been described and applied in the literature. We have recently developed an improved version of the Simon MTD method, i.e. MTD* parameters. The MTD and MTD* methods will be described. The scope and limitations of the STERIMOL, MTD and MTD* approaches will be discussed and compared using QSAR of insecticidal benzoylphenyl-ureas, DDT-type analogs and benzylchrysanthemates, herbicidal benzonitriles and nitrophenols, and plant-growth regulating phenoxypropionic acids.

In 1976 we have published the STERIMOL approach (1); including a table containing 5 substituent constants of about 250 different groups which could be used in QSAR studies where steric effects were expected. Recently this method has been developed further, with a "second generation" set of STERIMOL parameters as a result (2). These STERIMOL parameters have been compared with other steric constants, such as Es (1), molar refractivity (3) and the MSD (minimum steric difference) method of Simon (4). From these studies it could be concluded that the predictive power of the MSD method was less with regard to the STERIMOL approach. In general it could be stated that STERIMOL parameters were preferable in describing steric effects if a great variation in the substituents exists; variation in the sense of deviations from spheric shape (3,5).

MTD and MTD* approaches

In 1976 Simon developed a new method to describe steric effects in QSAR (6). He defined the minimum steric difference (MSD)

between a molecule and the natural substrate of a biologcal
receptor as the non-overlapping volume of the conformations which
allow of a maximum spatial overlap of the 2 molecules. In practice
the planar structural formulae of the molecules are super-imposed
and the unsuperposable atoms are counted. An example is given in
Figure 1.

The MSD parameters might be characterized as providing a rough
measure of the deviation from "ideal" bulk. Of course some
problems immediately arise, i.e.:

1. The superposition of the molecules is often rather subjective
    and it takes hardly into account the flexibility of the
    molecules studied.
2. The natural substrate is unknown in many studies of QSAR.
    Simon et al. adopt in those cases the most active molecule of
    a series as the standard, assuming that its shape is closest
    to the "natural" substrate.
3. The greatest problem of the MSD approach is that it does not
    discriminate between differences in shape of the parts of the
    molecules directed to the walls of the receptor which would
    be relevant, and differences in shape towards the outer
    region which would be irrelevant.

Balaban et al. (7) recently published a method which partly
overcomes the above-mentioned problems. This MTD (minimum
topological difference) approach is trying to develop an optimal
standard molecule by systematically analyzing the shapes of the
members in a series in relation to their biological activities.
The method can be briefly described as follows.

1. A so-called hypermolecule is developed, which can be
    considered as the assembly of all atomic positions of all
    molecules in the series. Figure 2 and 3 show the
    procedure for 32 substituted benzonitriles.
2. An initial standard molecule is chosen, for example the most
    active member, and the MTD values of all the members are
    calculated in the same way as in the Simon method. Now all
    the positions in the hypermolecule are changed one by one in
    such a way that not only favourable and unfavourable positions
    are taken into account, but also indifferent positions, which
    do not contribute to the MTD value at all. After every change
    the obtained MTD values are correlated with the biological
    activity and the correlation coefficient is optimized by the
    so-called steepest ascent method. If no single change in the
    final standard can deliver MTD values which correlate better
    with the biological activity, an optimal standard is derived.
3. To avoid the finding of only a local optimum of the standard,
    the procedure is repeated several times, starting from
    different other initial standards, e.g. the total hyper-
    molecuie, or initial standards which are generated at random,
    until most optimizations have led to the same standard.

In the Balaban MTD approach the additional influence of electronic
and hydrophobic effects is accounting for by adding e.g. π and σ
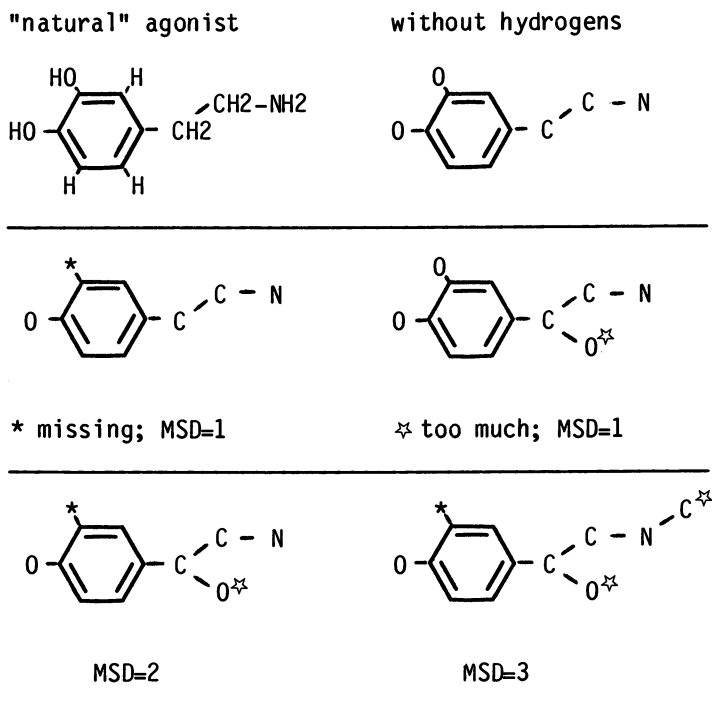
Figure 1. Calculation of the original MSD parameter, according to the method of Simon.

structure　　　　　hypermolecule
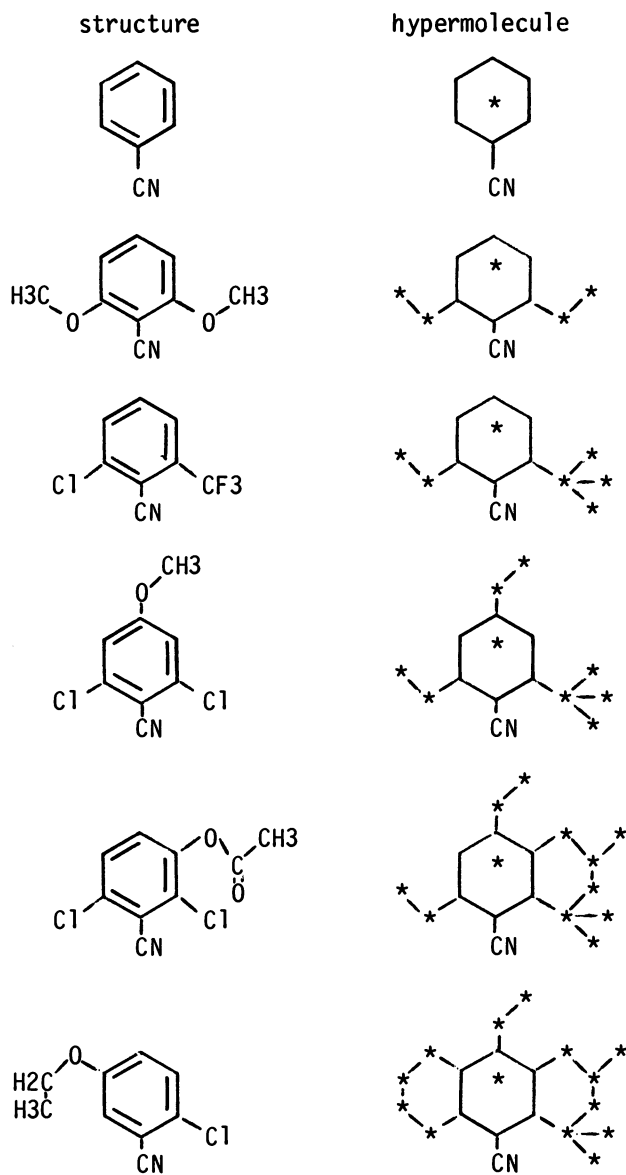
Figure 2.　Construction of the hypermolecule of 31 substituted benzonitriles.
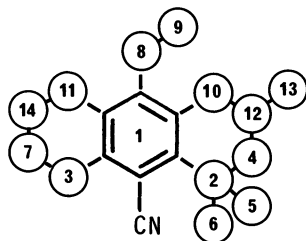
all the compounds can be covered by the hypermolecule, none of the positions is superfluous.

structure	positions
H	1
2-Cl	1 2
2,6-F_2; 2,6-Cl_2; 2,6-Br_2; 2,6-I_2	1 2 3
2-F,6-Cl; 2-Cl,6-OH; 2,6-$(CH_3)_2$	1 2 3
2-Cl,6-Br; 2-Cl,6-CH_3;	1 2 3
4-Cl	1 8
3-Cl	1 10
2-OCH_3	1 2 4
2-CF_3,6-Cl	1 2 3 4 5 6
2-OC_2H_5,6-Cl	1 2 3 4 12
2,6-$(OCH_3)_2$	1 2 3 4 7
2,4,6-Cl_3; 2,6-Cl_2,4-OH	1 2 3 8
2,4-Cl_2; 2-Cl,4-CH_3	1 2 8
2,6-Cl_2,4-OCH_3	1 2 3 8 9
2,6-Cl_2,3-OCH_3	1 2 3 10 12
2,6-Cl_2,3-$OCOCH_3$	1 2 3 4 10 12 13
2,3,6-Cl_3	1 2 3 10
2,3,5,6-Cl_4	1 2 3 10 11
2,5-$(OC_2H_5)_2$	1 2 4 7 11 12 14
3,4,5-Cl_2	1 8 10 11
2,3,4-Cl_3	1 2 8 10
2,3,4,5,6-Cl_5	1 2 3 8 10 11
3,4-Cl_2	1 8 10
2,4,5-Cl_3	1 2 8 11

Figure 3. Atomic positions in the hypermolecule of 31 substituted benzonitriles.

terms to the regression after the development of the optimum standard. We considered that a better approach would be to include hydrophobic and electronic parameters already in the procedure to obtain the optimum standard (8). The resulting steric parameters are indicated as MTD* values. Options in the procedure make it possible to connect the favourable atomic positions to each other and to the basic structure.

Comparison of STERIMOL, MTD and MTD*

The results of the studies will be summarized. Details of the QSAR analyses are or will be published elsewhere, including inter-correlation matrices of the steric parameters mentioned. But relevant conclusions from e.g. intercorrelations will be dicussed. At this moment the STERIMOL method has been applied successfully in about 50 publications; often with better results than other steric approaches, including MTD and MTD*, especially in series with few substituent positions. A recent example is our study of DDT analogs. Brown et al. (9) analysed a series of 21 derivatives using the van de Waals (Vw) volumes as steric parameters. In Table I the equations are given in which the steric parameters are compared.
The MSD approach gave no significant results. Addition of σ* to Equation 1-3 did not improve the correlation significantly. STERIMOL gave the best results and MTD and MTD* were comparable with Vw. The obtained optimal standard from the procedure that gave Equation 4 is shown in Figure 4, and it indicates that di-substituted carbon atoms gave the best fit.
    The STERIMOL approach however, has also some weaknesses and limitations (2). One aspect is that 5 STERIMOL parameters at each substitution position might absorb too many degrees of freedom so that the problem of "chance correlations" might arise, especially when many substituent positions are involved. This was one of the reasons for decreasing the number of STERIMOL parameters to 3 in the "second generation" STERIMOL approach. Still the method is difficult to use when the number of observations is small in relation to the number of substitution positions, sothat other steric methods would be needed, e.g. the MSD and MTD approaches. An example is the QSAR study of the herbicidal activity of substituted benzonitriles. Our first analysis using the STERIMOL parameters showed steric effects to be very important, but because of the large number of 5 substituent positions many parameters, sometimes even in quadratic form were necessary, which resulted in an equation of too low statistical significance. The biological activity is expressed as the inhibition of root growth of Panicum miliaceum grown on agar. The biological and physico-chemical data are published elsewhere (5) and the resulting equations when applying MTD and MTD* parameters are shown in Table II.

Table I.   Comparison of Vw, MSD, MTD, MTD* and STERIMOL parameters in a series of 21 DDT analogs with activity against Culex fatigans.

$$H_5C_2O-\bigcirc-\underset{X}{\overset{|}{C}}-\bigcirc-OC_2H_5$$
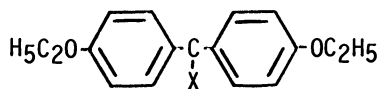
		t	r	s	F	
$-\log LC_{50} =$	$+0.500$ VwX	5.40	0.789	0.513	14.82	(1)[β]
	$-0.006(VwX)^2$	5.26				
	-0.859					
$-\log LC_{50} =$	-0.29 MSD[α]	2.04	0.424	0.735	4.17	(2)
	$+1.44$					
$-\log LC_{50} =$	-1.33 MTD	4.76	0.731	0.554	21.85	(3)
	$+0.95$					
$-\log LC_{50} =$	-1.13 MTD*	4.96	0.782	0.520	14.19	(4)
	$+0.31$ σ*	2.52				
	$+0.93$					
$-\log LC_{50} =$	$+20.21$ B1	8.86	0.921	0.345	22.37	(5)
	-4.68 (B1)^2	8.86				
	-0.96 B5	3.67				
	$+0.44$ σ*	5.19				
	-17.35					

[α] MSD values calculated from the most active member; $X=CH(C_2H_5)NO_2$
[β] Equation 1 according to Brown et al. (9)

Table II.   Inhibition of root growth of <u>Panicum miliaceum</u> (pC_{50}) under the influence of 31 benzonitriles.
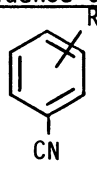
		t	r	s	F	
pC_{50} =	$+1.37\ \pi$	2.32	0.454	0.886	2.33	(6)
	$-0.36\ \pi^2$	2.35				
	$+0.16\ \sigma$	0.26				
	-1.50					
pC_{50} =	-0.59 MTD	5.36	0.705	0.680	28.73	(7)
	$+0.27$					
pC_{50} =	$+1.21\ \pi$	3.45	0.856	0.524	17.82	(8)
	$-0.42\ \pi^2$	4.34				
	$+0.67\ \sigma$	1.84				
	-0.62 MTD	5.02				
	-0.51					
pC_{50} =	$+1.02\ \pi$	3.66	0.913	0.414	32.35	(9)
	$-0.46\ \pi^2$	5.98				
	$+1.95\ \sigma$	5.83				
	-0.70 MTD*	9.87				
	$+0.31$					

Without steric parameters Equation 6 was obtained, which was of no significance. The MTD values used in Equation 7 and obtained after optimisation were added to the variables from Equation 6, resulting in Equation 8. If the optimisation took place in the presence of the physical parameters (MTD* approach) the best result was achieved, shown in Equation 9. Electron withdrawing groups increase the activity at an optimal π-value of about 2. The steric requirements are given in Figure 5, showing that di-ortho substitution gave the best fit.

Another example of the use of the MTD and MTD* approaches can be found in a series of optically active α-phenoxypropionic acids with auxin-like activity, partly published in (5). The R-stereo isomers are much more active than the S-analoges. Both series were analyzed by Lien et al. (10) and a correlation with π, π^2, σ and the Van der Waals volume was found. The Pfeiffer rule is explained in terms of different structural requirements for the substituents as measured by π and van der Waals volume. Analysing the series using STERIMOL delivered equations containing too many parameters. In Table III the equations are given as a result of
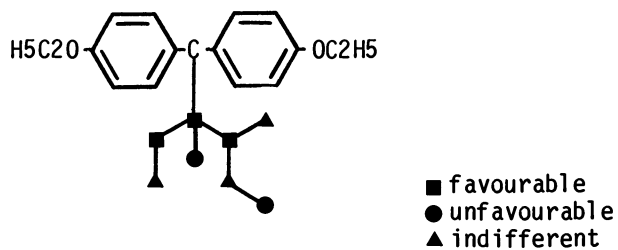
Figure 4. Standard used in Equation 4 to obtain MTD* values in a series of DDT analogs.
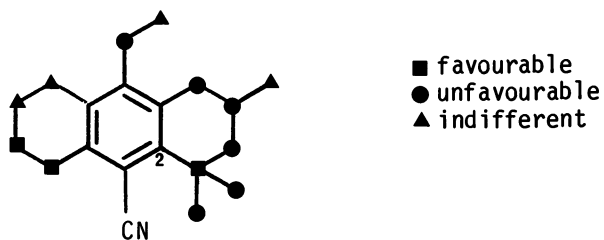
Figure 5. Standard used in Equation 9 to obtain MTD* values in a series of bezonitriles.

Table III.   Auxin-activity of phenoxypropionic acids (pC_{50})

$$\underset{CH_3}{\overset{H}{HOOC-C-X^\alpha-}}\!\!\left\langle\!\!\bigcirc\!\!\right\rangle^{\!R}$$

			t	r	s	F	n	
$pC_{50} =$	+2.18	π	1.84	0.475	0.941	1.94	24	(10)
	−0.45	π^2	2.15					
	+0.98	σ	1.28					
	+3.69							
$pC_{50} =$	−0.88	MTD	5.80	0.778	0.641	33.66	24	(11)
	+8.10							
$pC_{50} =$	+2.04	π	2.86	0.855	0.569	12.90	24	(12)
	−0.38	π^2	2.96					
	+0.31	σ	0.65					
	−0.84	MTD	5.97					
	+5.38							
$pC_{50} =$	+1.62	π	2.81	0.910	0.455	22.89	24	(13)
	−0.43	π^2	4.22					
	+1.14	σ	3.06					
	−0.78	MTD*	8.16					
	+7.32							
$pC_{50} =$	+1.84	π	5.24	0.865	0.269	16.79	21	(14)
	−0.33	π^2	5.09					
	+1.02	σ	3.93					
	+1.92							
$pC_{50} =$	−0.62	MTD	5.48	0.783	0.315	30.07	21	(15)
	+5.40							
$pC_{50} =$	+1.41	π	4.21	0.910	0.229	19.19	21	(16)
	−0.26	π^2	4.20					
	+0.66	σ	2.56					
	−0.32	MTD	2.72					
	+3.01							
$pC_{50} =$	+1.77	π	7.76	0.949	0.175	36.09	21	(17)
	−0.25	π^2	5.57					
	+0.35	σ	1.62					
	−0.28	MTD*	4.96					
	+1.95							

R- (rows 10–13); S- (rows 14–17)

α X=O or S

the MTD and MTD* methods. The Equations 10-13 are related to the series of the R-analogs and 14-17 to the S-derivatives. Equation 10 shows that the physical parameters π and σ cannot describe the activity significantly. MTD alone gives already better results as shown by Equation 11 and combining the MTD-values with π and σ results in Equation 12, in which the σ parameter doesn't give a significant contribution
(see t-value). The best results were obtained with the MTD* approach shown in Equation 13. Electron withdrawing substituents with a π-optimum of 1.9 are favourable. The ideal standard is given in Figure 6. With the compounds having a S-configuration a good correlation could already be obtained without steric parameters as shown in Equation 14. Indeed, the addition of the MTD parameter (Equation 15-16) or MTD* (Equation 17) did improve the correlation but the difference was not as pronounced as with the R-stereo isomers. The explanation for this difference in steric influence might be the fact that the R-analogs fit excellently at the receptor and small changes in the molecule disturb this fit rather easily, in contrast with the S-compounds which do not fit well sothat the same changes have only a small effect on the already poor fit. Also in this example MTD* gave better results than MTD, especially in the R series.

When less substituent positions are present STERIMOL can be used but in those cases there is often hardly any difference in results if compared with the MTD* method. This is illustrated by our version of the QSAR of the insecticidal activity against American cockroaches of 36 substituted benzyl chrysanthemates. The biological data were published by Nakagawa et al. (11) and we used for this example the minimum molar dose to cause death (MLD) without addition of synergists. The authors split up the series in ortho, meta and para substituted subseries and they found that the biological activities could be correlated with the Van der Waals volume in a way which was dependent on the substitution position. We have put all the compounds together and compared the different steric approaches. The results are summarized in Table IV.
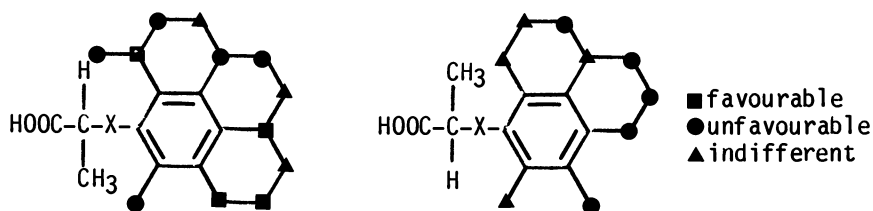
Figure 6.   Standards used in Equation 13 (left) and in Equation 17 (right) to obtain MTD* values in a series of stereo isomers of phenoxypropionic acids.

Table IV.   Insecticidal activity against American cockroaches of 36 substituted benzyl chrysanthemates.
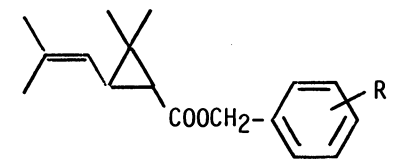
		t	r	s	F
$\log(1/MLD)$ =	+0.44 dVw ortho	6.28	0.915	0.377	40.21 (18)
	+0.62 dVw meta	12.56			
	+0.86 dVw para	4.88			
	-0.14 dVw^2 para	3.21			
	+4.98				
$\log(1/MLD)$ =	+0.41 B5 ortho	5.50	0.896	0.409	43.61 (19)
	+0.63 B5 meta	11.34			
	+0.46 L para	4.83			
	+3.09				
$\log(1/MLD)$ =	-0.40 MTD	12.19	0.902	0.386	148.5 (20)
	+9.62				

Equations 18-20 give about the same results and the standard obtained in the procedure that led to Equation 20 is shown in Figure 7. Since these equations contain only steric parameters, the picture obtained from the MTD method can directly be used to compare the biological activity of the compounds. MTD* and MTD are in principle the same in this example because no electronic and hydrophobic parameters are involved. However, extrapolation outside the hypermolecule is not permissible.

Another example is the herbicidal activity of substituted nitrophenols, expressed as the inhibition of the Hill reaction. In a series of 28 compounds with 3 substitution positions, MTD MTD* and STERIMOL were compared (5). The biological values were taken from the work of Trebst and Draber (12) and the results are summarized in Table V. There is already a significant correlation with π alone, as can be seen from Equation 21, but it can be improved by adding B1 terms for both ortho substituents. Equation 22 shows the result. The more steric hindrance of the hydroxyl group, the more the inhibition increases. The MTD* approach led to Equation 23 and it is statistically as good as Equation 22. The standard obtained with this procedure is shown in Figure 8. Optimization in the absence of π yielded Equation 24. The MTD values used in Equation 24 turned to be highly correlated with the π values (r=0.924), so that combination of the parameters gave no significant improvement in Eq. 25. The intercorrelation
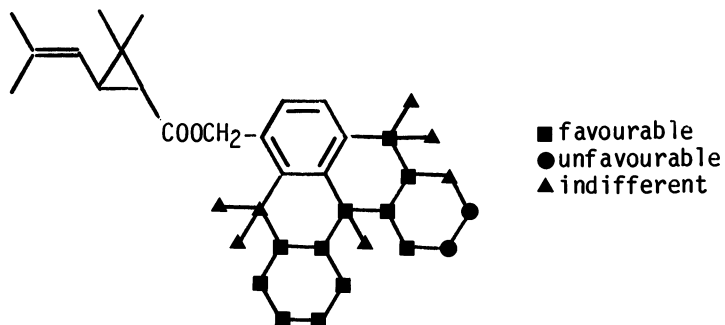
Figure 7. Standard used in Equation 20 to obtain MTD values in a series of benzyl chrysanthemates.
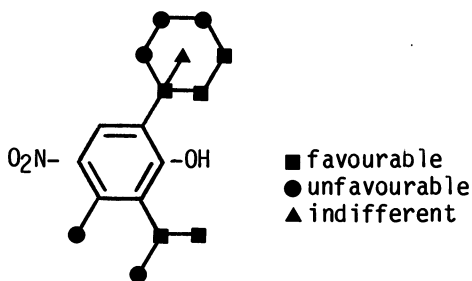
Figure 8. Standard used in Equation 23 to obtain MTD* values in a series of nitrophenols.

of π and MTD* in Equation 23 however is very low (r=0.001). So, we conclude that if a variable (in this example π) is already correlated with the biological activity, a MTD optimization without this variable often produces a MTD parameter that is intercorrelated with that variable. On the other hand an optimization in the presence of this variable (MTD*) will reduce the residual sum of squares and will prevent an intercorrelation.

Table V.   Inhibition Hill reaction by 28 nitrophenols

$$O_2N-\text{(benzene ring)}-OH \quad \text{with } R1, R2, R3$$

		t	r	s	F	
$pI_{50} =$	+0.98 π	8.61	0.860	0.589	74.15	(21)
	+3.38					
$pI_{50} =$	+0.63 π	7.37	0.958	0.344	89.75	(22)
	+0.88 B1 ortho R1	4.22				
	+1.34 B1 ortho R2	6.82				
	+0.23					
$pI_{50} =$	+0.98 π	12.80	0.942	0.396	98.28	(23)
	-0.75 MTD*	5.70				
	+6.37					
$pI_{50} =$	-1.22 MTD	9.40	0.879	0.551	88.42	(24)
	+7.83					
$pI_{50} =$	+0.37 π	1.36	0.888	0.542	46.60	(25)
	-0.80 MTD	2.38				
	+6.21					

When the number of observations increases and more variables are allowed, STERIMOL appears to be better because of its more precise description of the shape of substituents. A QSAR analysis of the larvicidal activity of 61 substituted benzoylureas is an example of this. The activity against Pieris brassicae is expressed as LD_{50} (in ppm) and these values are published by Wellinga et al. (13). The QSAR results are given in Table VI. Equation 26 shows that only the indicator parameter D1, which discriminates between the 2,6-Cl_2 and the 2,6-F_2 derivatives, gives a significant contribution to the regression if steric parameters are omitted. MTD on it's own gives only a poor correlation which can be seen from Equation 27. Combination with

Table VI. Larvicidal effect against <u>Pieris brassicae</u> of 61
           substituted benzoyl phenylureas.

		t	r	s	F
$-\log(LD_{50}) = $	$+0.17\ \Sigma\pi$	1.34	0.710	0.805	14.27 (26)
	$+0.60\ \sigma R$	1.67			
	$+1.91\ D1^{\delta}$	6.75			
	$-0.30\ D2^{\epsilon}$	0.93			
	-0.88				
$-\log(LD_{50}) = $	$-0.61\ MTD$	4.61	0.515	0.956	21.28 (27)
	$+1.09$				
$-\log(LD_{50}) = $	$-0.68\ MTD$	7.02	0.862	0.585	31.84 (28)
	$+0.52\ \Sigma\pi$	5.01			
	$+0.48\ \sigma R$	1.82			
	$+1.56\ D1$	7.34			
	$-0.44\ D2$	1.91			
	$+0.25$				
$-\log(LD_{50}) = $	$-0.64\ MTD*$	7.10	0.861	0.589	31.56 (29)
	$+0.55\ \Sigma\pi$	5.15			
	$+0.37\ \sigma R$	1.41			
	$+1.56\ D1$	7.33			
	$-0.50\ D2$	2.13			
	$+0.18$				
$-\log(LD_{50}) = $	$-0.03\ L^2$ para	4.86	0.899	0.514	32.08 (30)
	$-0.20\ B5$ para	2.87			
	$-1.31\ L$ meta	8.63			
	$+0.82\ \Sigma\pi$	7.16			
	$+1.76\ \sigma R$	6.50			
	$+1.48\ D1$	7.60			
	$-0.53\ D2$	2.58			
	$+2.57$				

δ D1=0 for 2,6-Cl_2 compounds and D1=1 for 2,6-F_2 derivatives
ϵ D2=0 for N(H) and D2=1 for N(CH_3) compounds

the variables used in Equation 26 leads to an improved result:
Equation 28. The MTD* procedure gives no better correlation, for
the Equations 28 and 29 are about the same. The main reason for
the lack of difference between MTD and MTD* is found in the low
intercorrelations between these parameters and the other
physical parameters. Equation 30 shows the result of the STERIMOL
approach. Both indicator parameters and the σ term give a
significant contribution to the regression and it can be
concluded that electron withdrawing groups with a lipophilic
character and substituted at the para position are preferable.
When the substituents become too large, the square of L becomes
more important, which results in a decrease in activity. The
2,6-F_2 compounds are far more active than the chloro analogs.
N-methylation causes a slight decrease in activity.

Discussion and Conclusions

We tried to summarize the QSAR studies dicussed in a rough way in
Table VII

Table VII. Comparison of usefulness of MTD, MTD* and STERIMOL
parameters.

Series	Number of		Relative quality of equation with		
	members	substitution positions	MTD	MTD*	STERIMOL
DDT analogs	21	1	−	±	+
Benzonitriles	31	5	±	+	−
Phenoxypropionic acids	21/24	5	±	+	−
Benzyl chrysanthemates	36	3	+	(+)	+
Nitro-phenols	28	3	±	+	+
Benzoylphenylureas	61	2	±	±	+

These results give some insight in the scope and limitations of
the MTD, MTD* and STERIMOL parameters. Let us first compare the
MTD and MTD* methods. In the example of the benzyl
chrysanthemates the regression equations have only steric terms,
sothat there is no difference between the two methods in
principle. In the case of the benzoylphenylureas the
intercorrelation between the MTD values and the other parameters
is very low, so it is understandable that there is hardly any
difference. But in the four other studies there was much more
intercorrelation between the MTD values on the one hand and the
electronic and/or hydrophobic parameters on the other hand, and
in these cases the MTD * method gives slightly better results.
Our preliminary conclusion from the examples discussed, is that
the MTD* is the preferable one, both from fundamental and

practical point of view. We expect that this conclusion will be confirmed in other QSAR studies.

The choice between the STERIMOL and MTD* approaches is dependent on some properties of the series studied, e.g. the number of members in relation to the number of substitution positions In those cases where the resulting degrees of freedom are sufficient, the STERIMOL approach gives the most satisfactory results (e.g. in the studies on the DDT analogs and to a lesser extent in benzoylphenylureas; Table VII). But in opposite cases, i.e. the QSAR's of the benzonitriles and the phenoxypropionic acids, the STERIMOL parameters cannot be used. The studies on the chrysanthemates and the nitrophenols are of an intermediate character. But in such cases the STERIMOL approach is often still preferable because of its higher predictive power; we have illustrated that in an earlier study on the plant growth regulating activity of substituted phenoxyacetic acids (4). This phenomenon is probably caused by the fact that the STERIMOL parameters are independent geometric measures, in contrast to the MTD* parameters which are initiated by the biological data and thus not independent. Besides the MTD* values are obtained after an optimization with many regression analyses which might more easily give rise to chance correlations or overrating of the statistical criteria. Therefore randomization or splitting up the data in a training and an evaluation set are needed to verify a MTD* approach (8). But if the obtained results are significant, the standard molecule can help to visualize the steric aspects of QSAR.

The choice between the MTD* and STERIMOL approaches is influenced by still other factors such as the reproducibility and the computer time needed (5). Our tentative conlusion is that the STERIMOL approach has the greatest advantages provided that the chemical series studied allows its application. If that is not the case then the MTD* method can be useful, notwithstanding its restricted applicability of predictive purposes.

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Biphenylmethyl Pyrethroids

A Quantitative Structure–Activity Relationship Approach to Pesticide Design

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A major portion of successful pesticide design comes from continued work in areas where there is already a considerable body of knowledge. The probability of success for these programs can be improved significantly by adopting the systematic approach embodied in current QSAR (Quantitative Structure Activity Relationship) strategies. These strategies not only provide techniques for rapid optimization of leads but when applied to their fullest capabilities can provide models from which new lead areas can be generated.

Synthetic pyrethroid insecticides represent a particularly timely example of a mature pesticide area that has received continued interest well after the pioneering works of Michael Elliott and others had established seemingly complete structure-activity relationships(1,2). In 1976 our laboratory began a program aimed at finding and developing novel synthetic pyrethroid insecticides. Following basic QSAR strategies we were able to not only identify a novel lead area but to quickly optimize activity in that area, and ultimately to identify additional novel structures that possess the broad spectrum insecticidal and acaricidal activity that differentiates this class of pyrethroid insecticides from other synthetic pyrethrin analogs. Our application of QSAR strategies to this problem is the subject of this report.

First two important elements must be present for a QSAR strategy to succeed; accurate biological data and a readily accessible, easy to use data analysis system.

FMC is primarily interested in the development of insecticides for agricultural applications. Since the largest single class of insect pests of agriculture are the lepidopterans we chose a representative of this class, the southern armyworm, for development of our structure activity relationships. Although foliar application and evaluation of insecticides more accurately represents field conditions, we found that more reliable data could be obtained using topical application (Figure 1). Not only is the exact amount of compound applied to the insect known in topical tests but the amount reaching the active site depends only on intrinsic characteristics of the organism not on the interaction of the compound with the host plant and its environment nor on how much the insect consumes before it dies. All tests were run in the presence of a standard, permethrin, and the results are expressed as relative potency. This value was converted to its negative logarithm for regression analysis.

Although southern armyworms were used for the development of QSARs the other insects listed in Figure 2 were used in topical or foliar assays to monitor spectrum and performance under field conditions.

As this study was in progress we were also in the process of developing the necessary data analysis system. The computer based system that appears in Figure 3 was developed largely as a result of the pyrethroid program and thus can be viewed as a secondary product.

This system was constructed on the concept that structure activity studies are best performed by the scientist responsible for the design and synthesis of target pesticides. We found that one of the major impediments to the laboratory chemist's application of a QSAR strategy is the tedium of assembling physicochemical parameters and biological data in a form that can be submitted to the applicable statistical program. To overcome this barrier we have written a simple computer process that automatically writes the necessary tables for submission to our statistical package, the University of California's BMDP(3) Statistical Software. Molecular Design Limited's MACCS system(4) is used to assemble a file of compounds of interest. Using FMC reference numbers as keys this file is used to consult our biological data base for the required data. The same MACCS file is used to search a separate chemical file where substituents on FMC numbered compounds are identified with a simple Wisswesser Line Notation code. This latter informa-

Topical

• 1 μl Acetone Solution

• 24 Hour Mortality

Foliar

• Plant Sprayed With Water/Acetone Solution

• 48 Hour Mortality

$$\text{Relative Potency} = \frac{\text{LD}_{50} \ (\text{LC}_{50}) \ \text{FMC 33297 (Permethrin)}}{\text{LD}_{50} \ (\text{LC}_{50}) \ \text{Test Compound}}$$

Figure 1. Evaluation of biological response.

Species

Southern Armyworm (Spodoptera Eridania)	(SAW)
Cabbage Looper (Trichoplusia Ni)	(CL)
Tobacco Budworm (Heliothis Virescens)	(TBW)
Mexican Bean Beetle (Epilachna Varivestis)	(MBB)
Large Milkweed Bug (Oncopeltus Fasciatus)	(MWB)
Twospotted Spider Mite (Tetranychus Urticae)	(TSM)
Pea Aphid (Acyrthosiphon Pisum)	(PA)

Figure 2. Evaluation of biological response.
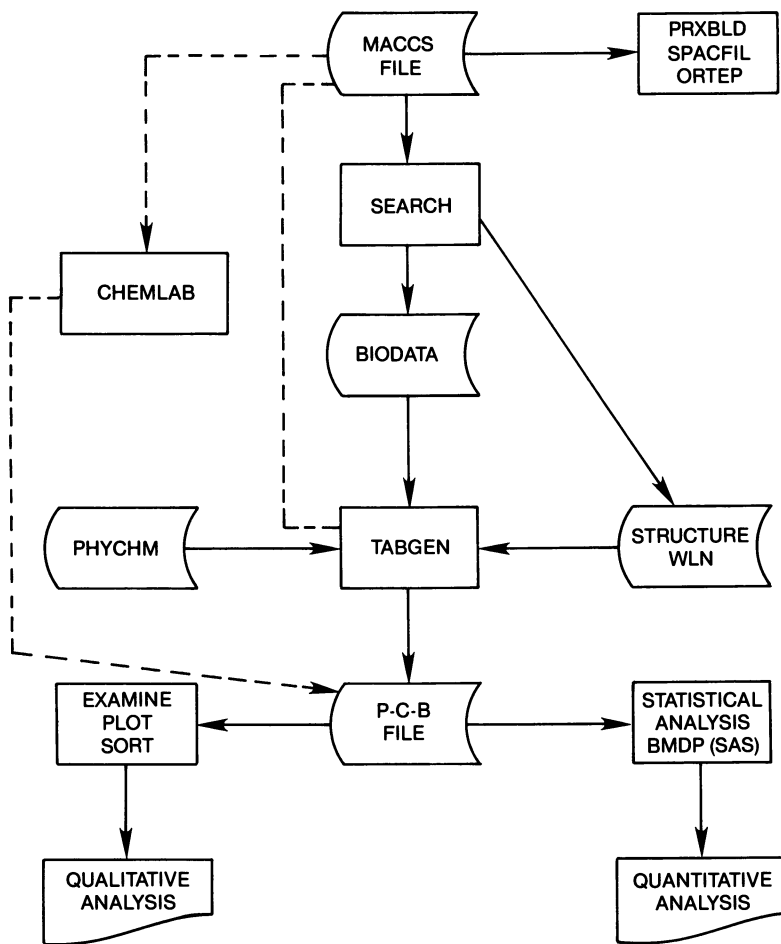
Figure 3.   QSAR system.

tion is used to consult a table of physiocochemical
parameters. In this table we store data for pi,
sigma, F, R, and MR from Hansch's tabulations(5) and
the STERIMOL parameters L, Bl and B4(6). This data
is used to generate a table that includes parameter
data for each position on an aromatic ring, the sum
of parameters for one or more rings when appropriate
and the relevant biological response data.

Quantitative and qualitative analysis facilities
are available through the BMDP programs and the cap-
abilities of our data managment system, the A.D.M.
Data Retrieval System (DRS)(7). We find it parti-
cularly important to have the plotting programs readi-
ly available since this is, as I will illustrate by
several examples, an important first step in analysis
of structure-activity data.

· We have also added the capabilities of Dr. Hop-
finger's CHEMLAB package(8) which can be used to
generate new parameters for analysis or can be used in
a more qualitative fashion for conformational analysis
based on molecular mechanics.

Our search for a new lead in the pyrethroid area
started with a simple proposition: It has been sug-
gested that for a pyrethroid ester to be active it
must have an alcohol with the specific feature of two
centers of unsaturation separated by a bridging group
(1,2) (Figure 4). In the case of allethrin and res-
methrin the bridging atom is a methylene group. In
permethrin it is an oxygen atom. If this is a re-
quirement, one should be able to express it with some
physicochemical parameter or set of parameters.

In the initial phase of our work we prepared a set
of benzyl esters of the 3-(2,2-dichloroethenyl)-2,2-
dimethylcyclopropanecarboxylic acid (DVA) which were
monosubstituted in the meta position. The substi-
tuents were chosen to cover a broad spectrum of physi-
cochemical parameter space by selecting them from
Hansch's cluster sets(9). In addition, substituents
with no bridging group, one atom and two atom bridges
were included to probe the need for a bridging atom.

As we have previously reported(10) regression anal-
ysis of the data for all compounds gave poor correla-
tions on all our tabulated parameters. But on examina-
tion of the plot (Figure 5) of lipophilicity versus
southern armyworm activity we found that, with the ex-
ception of four two-atom bridged compounds, this para-
meter could explain up to 86% of the variation in the
biological data. At this point we considered the two
atom bridged substituents outliers, primarily because
of their common feature. By doing so we had a useful
model from which to continue our design work.
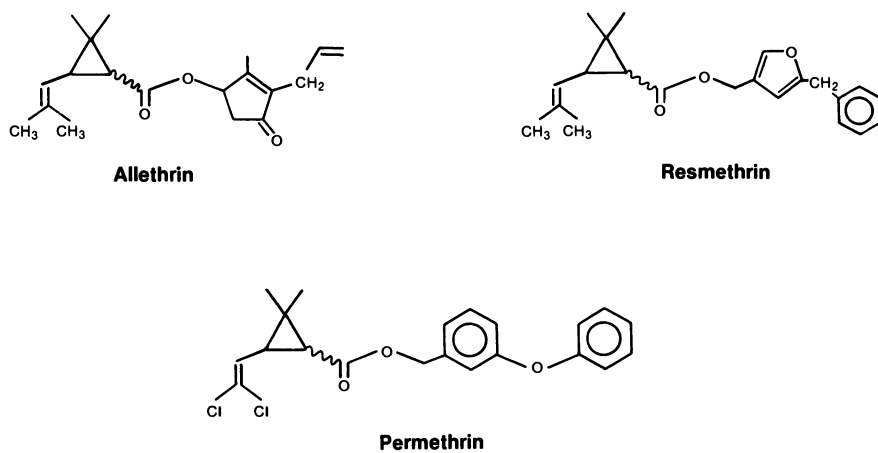
Figure 4. Bridging atoms of pyrethroid alcohols.
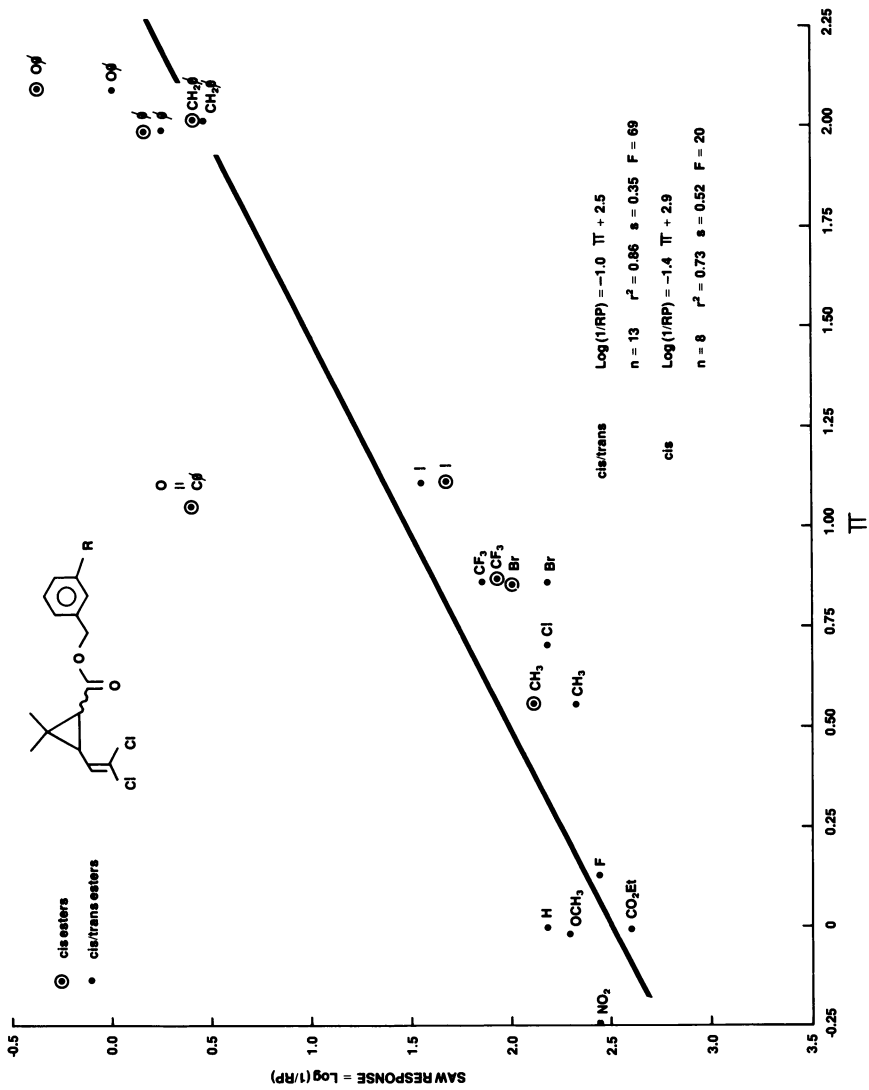
Figure 5.

We concluded that phenoxy, benzyl, benzoyl and
phenyl substituents were all members of a congeneric
series of substituents to which simpler members, say
fluorine or a methyl group also belonged. In other
words, for these simple benzyl esters, no bridging
atom was required for insecticidal activity.
     A relatively large gap existed in this data be-
tween iodine and the phenyl group. We were thus con-
cerned that the correlation that we observed was only
one cluster of compounds pointing at another. If no
bridging atoms were required for activity one would
anticipate that heteroaromatic rings should fall on
this line and would generally have activity reflect-
ing their lower lipophilicity. As Figure 6 indicates
this was the case. An equation of essentially the
same form as the smaller group of substituents was
found. The equation indicates that 80% of the varia-
tion for this set of _meta_ monosubstituted benzyl
esters could be explained by variation in the lipo-
philicity of the substituent(11).
     Since additional activity was not to be found in
the less lipophilic heteroaromatic rings our study
turned to the effect of substitution on the biphenyl
aromatic rings(12).
     There are seven nonequivalent aromatic positions
in the biphenyl alcohol. The strategy we adopted to
optimize the substitution pattern included mono sub-
stituting each of these positions with fluorine,
chlorine and a methyl group. Although mono methoxy
and nitro substituents, as well as several poly sub-
stituted compounds were prepared, the substitution
trends that resulted from this simple strategy pre-
vailed (Figure 7). With the possible exception of the
6-fluoro compound, substitution in the 4,5,6,3' and
4' position lead to a significant loss of activity.
Only substitution in the 2 and 2' position seemed to
significantly improve activity.
     It is interesting to reinvestigate our decision
to ignore the two-atom bridged esters in our earlier
study of _meta_ monosubstituted benzyl esters in the
light of the effect of substitution in the biphenyl
series.
     If, as shown in Figure 8, one plots the length of
the _meta_ substituents, now including the 4'-substi-
tutedbiphenyl(13) compounds, versus southern armyworm
topical activity one sees first, an apparent lack of
correlation below 5.0 angstroms. However, above that
length a negative correlation of length with activity
exists. Since they fit this trend, the two-atom
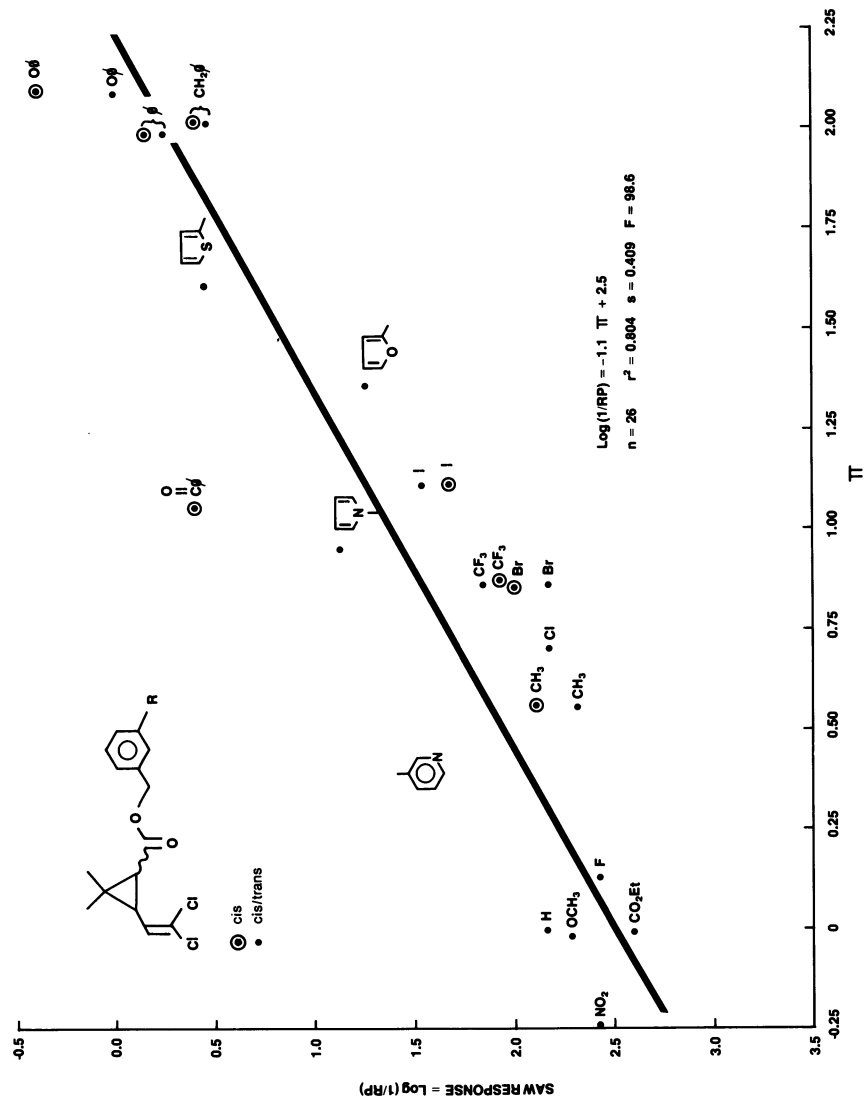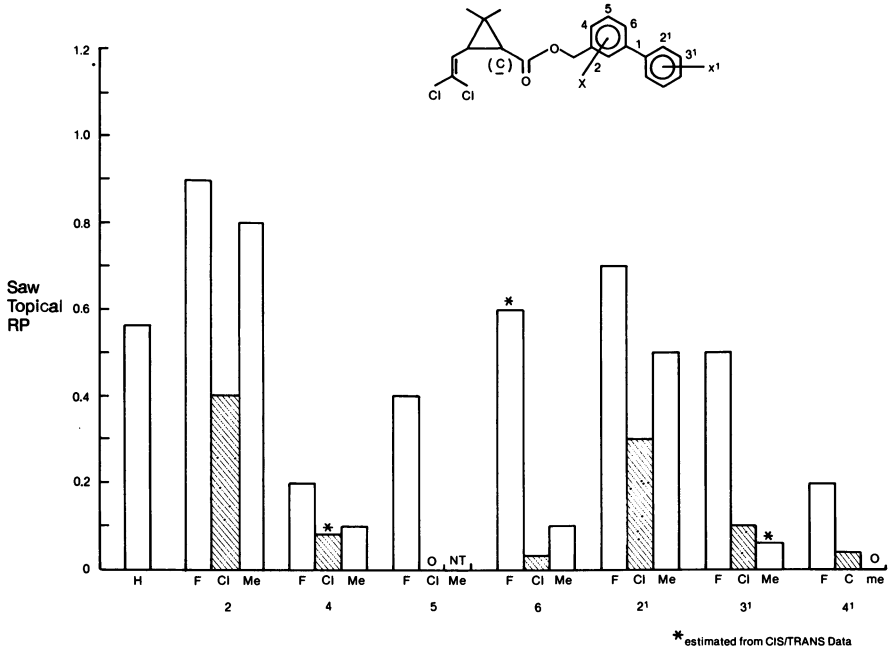
Figure 6.

Figure 7.

Figure 8.

bridged compounds are no longer to be considered out-
liers.

As indicated earlier, substitution in the 6- posi-
tion of the biphenyls generally leads to a loss of
activity. This is consistent with the effects of <u>para</u>
substitution in benzyl esters. Although regression
analysis does not show a linear relationship of length
to activity for 12 <u>para</u> substituted benzyl esters,
when one plots (Figure 9) the length to activity re-
lationship, for all but two substituents increasing
length beyond a methyl group leads to a loss of acti-
vity. The phenylethyl and phenyl groups, both of
which are very long, are essentially inactive.

The data we have accumulated to date suggests the
partial model for a composite (combining transport
and metabolism as well as active site interation)
pyrethroid active site shown in Figure 10. The low
activity of the 5 and 6 substituted biphenyls suggests
a steric barrier at those positions. In addition, the
limit on the length of a <u>meta</u> substituent suggests
that a hydrophobic pocket in this position has a
specific length of approximately 5 to 6 angstroms.

At this point it was clear that the highest po-
tential for increased activity was by substitution in
the 2-position of the biphenyl alcohol. We prepared
the sequence of compounds shown in Table 1. Substi-
tuents were again chosen to maximize the parameter
space covered within the relatively stringent synthe-
tic limitations of the biphenyl substitution pattern.
The application of regression analysis to the data
for these compounds provided no clear relationship
between structure and activity when the parameters in
our standard data base were used. The best linear
fit was found for B4, the STERIMOL maximum radius.
However, the correlation coefficient was only 0.625.
It was clear, from even a qualitative inspection of
the data, that the presence of a methyl or fluoro
group in the meta position had the effect of not only
increasing activity versus the design insect, the
southern armyworm, but also lead to dramatic increases
in level of topical activity against other species.

We thus turned to a study of the effect of substi-
tuting a methyl group on the activity of a set of <u>meta</u>
substituted benzyl esters that closely resembled the
original data set. Most of our original work was done
on monosubstituted benzyl esters of DVA while during
later work we concentrated our efforts on the gener-
ally more active <u>cis</u>-3-(2-chloro-3,3,3-trifluoro-1-
propenyl)-2,2-dimethylcycopropanecarboxylic acid (TFP)
esters. In order to include more compounds in com-
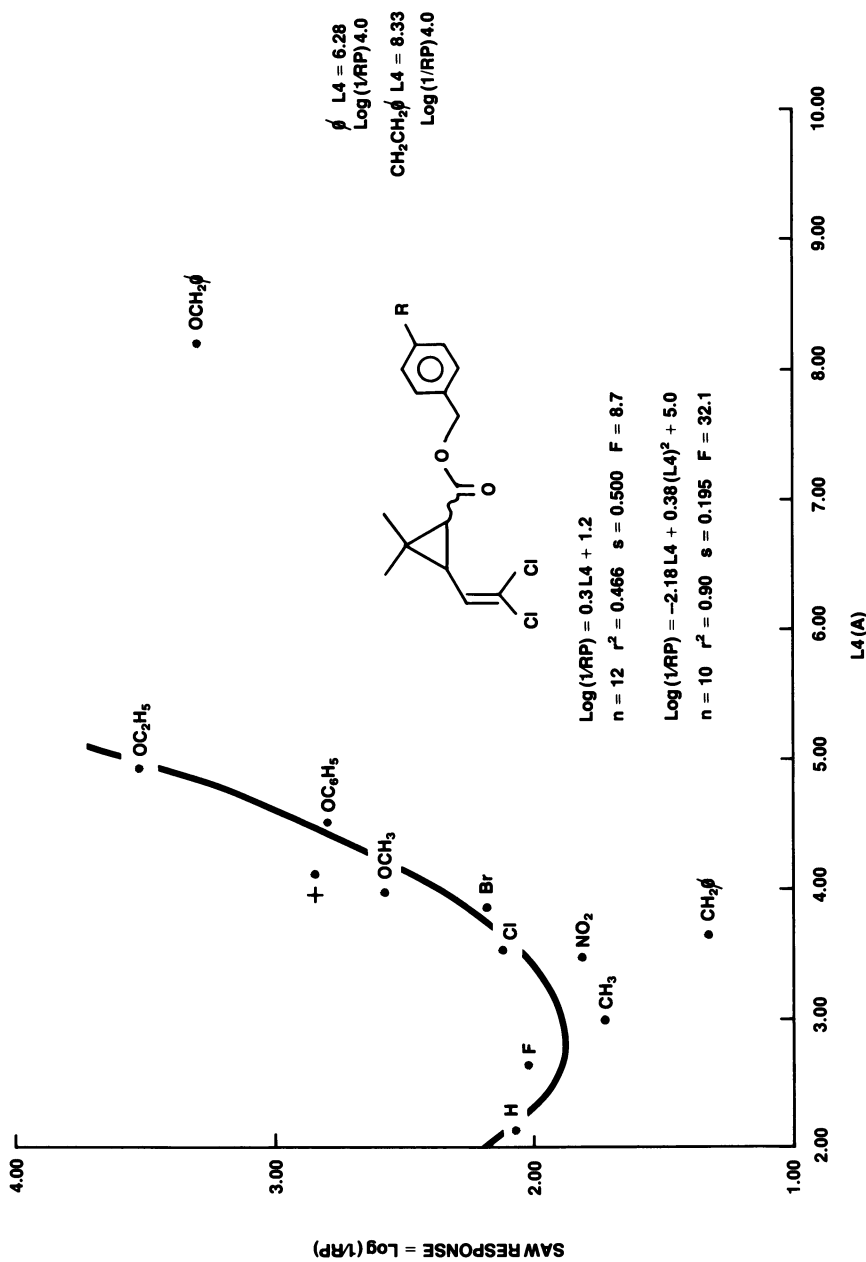
Figure 9.
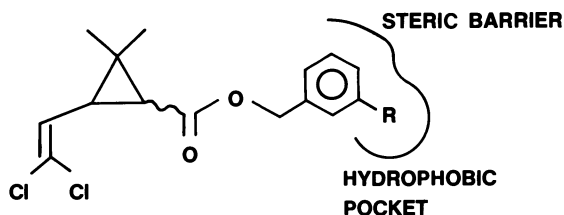
Figure 10. Model of composite "active-site."

Table I. (2-Substituted [1,1'-Biphenyl]-3-yl)methyl cis-3-(2,2-Dichloroethylenyl)-2,2-Dimethylcyclopropane Carboxylates
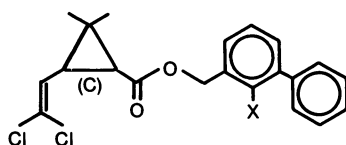
Topical Relative Potency (X = H = 1)

X	SAW	CL	TBW	MBB	MWB
F	1.40	1.70	1.70	0.60	4.20
CH$_3$	1.10	8.10	3.10	1.70	3.40
Cl	0.60	2.40	0.80	1.00	2.10
Br	0.30	1.40	0.40	1.00	1.40
CH$_2$CH$_3$	0.30	5.20	0.30	1.50	1.00
OCH$_3$	0.30	—	—	0.30	Inactive
CH = CH$_2$	0.10	1.00	Inactive	0.10	0.50
NO$_2$	0.80	0.80	Inactive	0.10	0.10
CN	0.07	0.20	0.07	0.70	0.50
CO$_2$CH$_2$CH$_3$	Inactive	—	—	Inactive	Inactive

parisons of the two studies the data sets were combined and an indicator variable used (1 = DVA esters and 2 = TFP esters) to account for the type of acid. In Figure 11 a plot of the hydrophobic substituent constant versus southern armyworm activity for this series is presented. A considerable change has occurred in the relationship of activity to the lipophilicity of the substituents. The best equation that could be generated from this data is shown along with the regression line. For this series the hydrophobic substituent constant explains far less of the biological variation than it did for the series without a 2-methyl substituent. This is all the more a problem since the line was generated ignoring three substituents, the one atom bridged compounds with phenoxy, benzyl and benzoyl groups, which with the 2-methyl substituent are all much less active than their unsubstituted parents. The benzoyl substituted compounds were in fact, essentially inactive.

The fact that these compounds are outliers helps to support a model that can be used to understand the enhanced activity of the 2-methyl substituted compounds, particularly those containing substituents with aromatic rings.

The conformation of biphenyls has been studied extensively. As indicated in Table 2 biphenyl itself is essentially planar even in the solution phase (14, 15). The substitution of a group in the 2 position leads to an increase in the dihedral angle between the two rings in proportion to the size of the substituent. Qualitatively there is, with the possible exception of the ethyl group, a change in activity with increasing twist angle which reaches a maximum at or between the fluoro and methyl groups. One possible explanation of this phenomena is that the preferred conformation of the 2-fluoro- or 2-methyl-biphenyl ester has a better fit at the active site in the target species or that the change in electronic distribution in the two phenyl rings that results from the reduction in conjugation (16) when the twist angle increases causes improved target site interaction.

Elliott (2) has suggested that the bridging atom in pyrethroid alcohols is responsible for holding the two centers of unsaturation in a non-planar configuration. Such a configuration could be the optimum configuration for fit of one atom bridged alcohols at the active site. The presence of a methyl group could stabilize a conformation that had a poor fit at the active site or it could prevent the second aromatic ring from reaching a binding site. Using the facilities of the
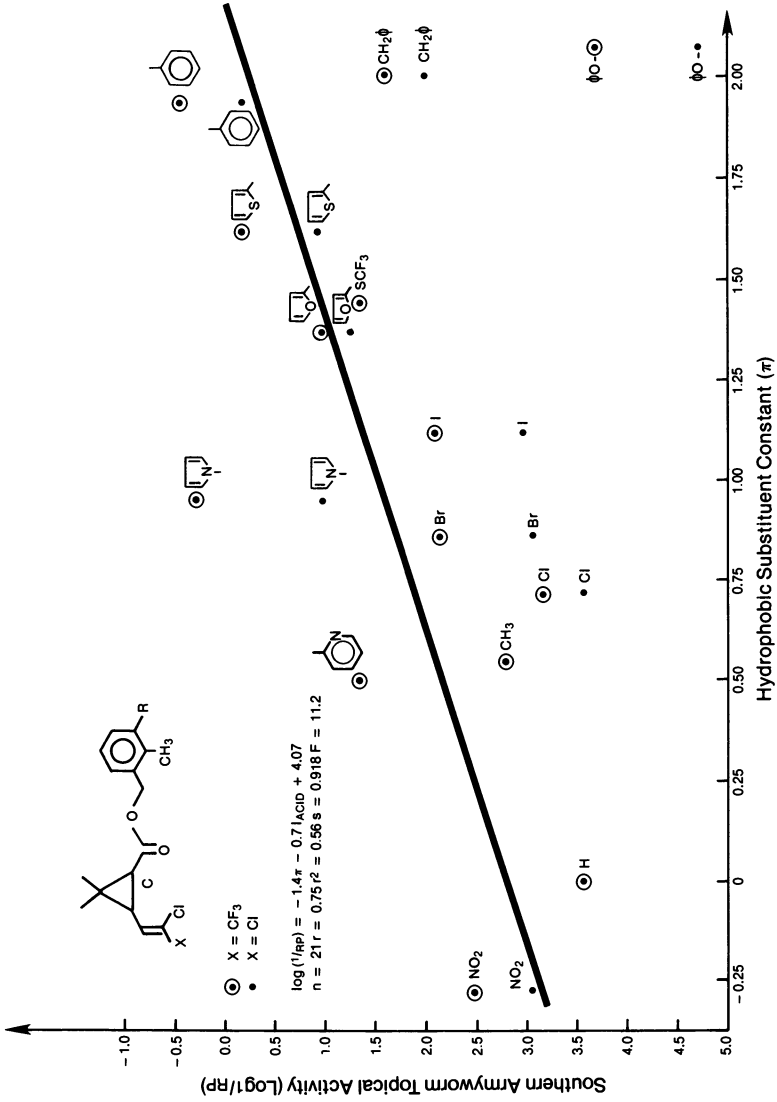
Figure 11.

Table II. Biological Activity vs. Biphenyl Twist Angle
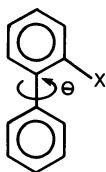
X	Θ	Southern Armyworm Topical Activity			
		DVA esters		TFP esters	
		SAW	MWB	SAW	MWB
H	20°	0.7	1.3	1.0	3.6
F	48-49°	0.9	5.2	2.6	6.5
CH$_3$	53-58°	0.8	4.2	2.0	11.6
CH$_2$CH$_3$	53-61°	0.2	1.2	0.5	4.4
Cl	63°	0.4	2.7	1.1	3.6
Br	—	0.2	1.8	0.4	2.1

CHEMLAB programs developed by Dr. Hopfinger(8) we
determined the lowest energy conformation of 3-benzyl-
benzyl alcohol.

In this configuration the two twist angles are
alpha = 34° and beta = 35°. When a methyl group is
added at the 2-position (Figure 12) and all rotable
bonds are minimized, the lowest energy conformation
has alpha = 48° and beta = 146°. Clearly with the
2-methyl group present the lowest energy conformation
is different. In addition, the previous low energy
configuration is destablized by about 10 kcal.

In both the biphenyl case and the benzyl case the
aromatic ring is free to rotate and if sufficient
energy is available at the active site the molecule
could simply twist to an active configuration. How-
ever, this would not be true if the 2- and 2'-posi-
tions of the biphenyl alcohol were bridged with an
alkyl chain where the conformation is fixed within
narrow limits that depend on the length of the chain
(17).

With this model in hand we prepared the series of
bridged biphenyl alcohols shown in Figure 13. The
esters prepared from these alcohols using the TFP acid
had the activities shown against southern armyworm and
the milkweed bug. The most active esters are prepared
from the 3-carbon bridged alcohol. Literature data
indicates a twist angle of from 46°-51° for this com-
pound(17,18,19). It is particularly interesting that
the twist angle anticipated for the 2-methylbiphenyl
(53°-58°)(14,15,16) lies between the 3- and 4 carbon
bridged alcohols. Since the maximum activity and
optimum spectrum is found in the three carbon bridged
compounds, one must conclude that the optimum twist
angle for the biphenyl alcohols lies at the low side
of the 2-methylbiphenyl range i.e. around 50°-53°.
It is thus interesting that the 2-fluorobiphenyl
esters are equal to or in some cases more active than
the equivalent 2-methyl compound since the twist angle
for the former has been reported to lie between 48°-
49^O(16,17).

In Table 3 a summary is presented of the biologi-
cal activity of a series of bridged biphenyl and
bridged heteroaromatic pyrethroid esters. The bio-
logical activity of these compounds is a function of
chain length, increasing as the length increases from
1 to 3 carbon atoms and then decreasing when it
reaches 4 atoms. The three-carbon bridged esters have
the greatest activity and broadest spectrum of the
compounds tested. Like many synthetic pyrethroid
esters these compounds have good lepidopteran acti-
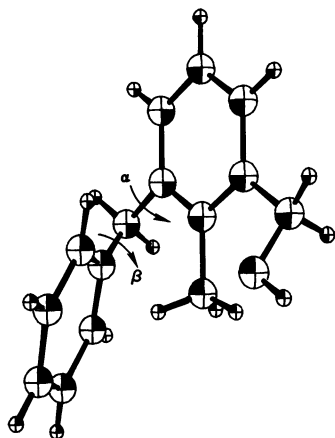
Figure 12. CHEMLAB minimized 3-benzyl-2-methylbenzylalcohol.
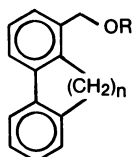
n	⊖	Southern Armyworm Topical Activity of TFP Esters (RP)	
		SAWTOP	MWBTOP
1	0-13°	0.01	<0.10
2	20-39°	0.50	4.30
3	46-51°	0.54	6.50
4	58-62°	0.06	1.50

Figure 13. Biological activity vs. bridged biphenyl twist angle.

Table III.   Biological Activity of Bridged Biphenyl and Heteroaromatic Bridged Biphenyl Esters of <u>cis</u>-3-(2-Chloro3.3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylic acid
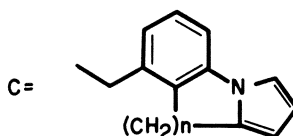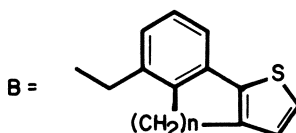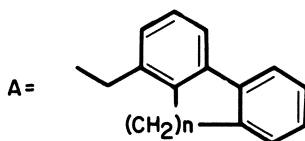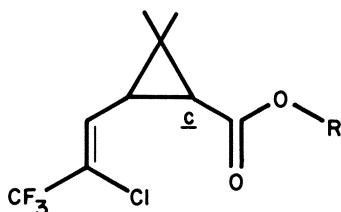
Compound Number	R	n	Tropical Relative Potency			Foliar Relative Potency	
			S.[a] eridania	E.[b] varivestis	O.[c] fasciatus	A.[d] pisum	T.[e] urticae
I	A	1	0.01	0.03	<0.10	0.20	<0.002
II	A	2	0.50	2.30	4.30	4.90	0.050
III	B	2	0.70	—	1.70	—	<0.050
IV	A	3	0.50	3.00	6.50	77.00	0.700
V	B	3	1.03	0.80	13.10	33.00	0.100
VI	C	3	0.80	0.20	2.06	1.25	<0.002
VII	A	4	0.06	0.60	1.50	3.55	0.090
VIII	C	4	0.20	0.20	1.20	8.49	<0.002

a Standard Permethrin LD_{50} = 23.1 (13.9 — 32.2) ng insect^{-1}
b Standard Permethrin LD_{50} = 12.8 (7.8 — 17.8) ng insect^{-1}
c Standard Permethrin LD_{50} = 660.0 (311 — 1008) ng insect^{-1}
d Standard Permethrin LC_{50} = 21.1 (3.6 — 38.6) mg litre^{-1}
e Standard Monocrotophos LC_{50} = 3.0 (0.27 — 5.37) mg litre^{-1}
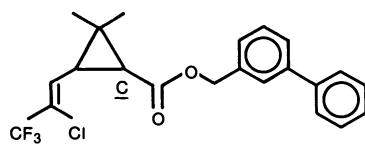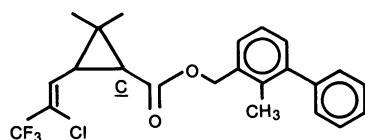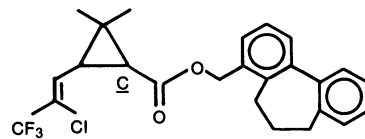
vity, although somewhat reduced compared to the bi-
phenyl pyrethroids, but like the 2-substituted bi-
phenyl esters the bridged esters have excellent co-
leopteran and aphid activity as well as being acari-
cides.

   We conclude from this data that the improved acti-
vity of the 2-methyl and 2-fluorobiphenyl pyrethroids
is indeed a direct function of the conformational pre-
ference of these molecules for a twist angle of about
50°.   Since the loss in activity of the phenoxy-,
benzyl- and benzoylbenzyl alcohols is also linked to
the conformation of the second aromatic ring, it is
suggested that this second ring is involved with
binding at the active site. It further appears that
the three dimensional position of this ring is criti-
cal. Although the general dependence of activity for
meta substituted benzyl esters on lipophilicity does
not exclude a non-specific lipophilic binding, the
angular dependence does suggest a rather specific fit
with a binding site. Similarly, our original QSAR
study of meta substituted benzyl esters did not ex-
clude the possibility that the dependence of activity
on lipophilicity was merely a reflection of improved
mobility of the molecule in the target organism. How-
ever, both the fact that substitution of more lipo-
philic groups elsewhere on the molecule does not gen-
erally improve activity and the apparent dependence
of activity on the conformational preference of the
meta substituent suggests that transport is not what
is being affected.

   Regardless, the application of a QSAR strategy to
the problem of novel discovery in the area of pyre-
throid insecticides has provided not only a new lead
area that was efficiently optimized but also provided
a new class of bridged esters. In Table 4 I have
summarized the activity gains that have been achieved
in this study.

Table IV. Comparative Activity of Biphenyl
Pyrethroids

Foliar Relative Potency

	SAW	MBB	PA	TSM
20	1.0	1.0	1.0	1.0
20	1.2	20.0	3.0	10.0
	0.4	14.0	13.4	8.0

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Regression Approaches to Structure–Activity Relationships in Miticidal 2-Aryl-1,3-cycloalkanediones and Enol Esters

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While retrospective quantitative structure/activity
relationships (QSAR) serve to document that critical
factors in the relationship between structural fea-
tures/physiochemical factors and biological potency
are well understood, quantitative structure/activity
trends developed during the course of synthesis lend
direction and efficiency to the property optimiza-
tion effort. Synthesis planning for a novel class
of miticides, 2-aryl-1,3-cycloalkanediones and enol
esters was supported by development of regression
equation models during the course of the project
which provided the basis for optimizing the shape
and size of substituents at the 5,5-position of
the dione nucleus, tailoring of substituents on the
2-phenyl group, and adjustment of the shape and
size of the acyl moiety.

Regression equations descriptive of multi-dimensional structure/
activity relationships in quantitative terms too frequently are
intellectual curiosities developed retrospectively after work in
optimization of the biological properties of a series by analog
or homolog synthesis has been completed. Retrospective analysis
serves well to document that critical factors in the relationship
between structural features/physiochemical factors and biological
potency are well understood and that optimum compounds have been
achieved. Structure/activity understanding developed during the
course of a synthesis project, however, lends direction and
efficiency to the property optimization effort.
    Optimization of biological properties in a series of mitici-
dal and mite ovicidal 2-aryl-1,3-cycloalkanediones, Ia,b, and
enol esters, II, was achieved through analog synthesis and test-
ing supported by the development of quantitative structure/
activity trends during the course of the project. QSAR equations
developed during an initial phase provided the basis for both
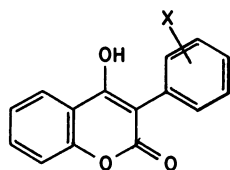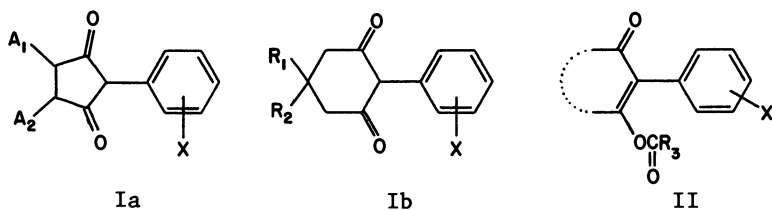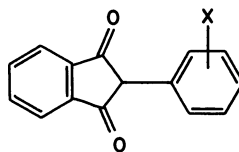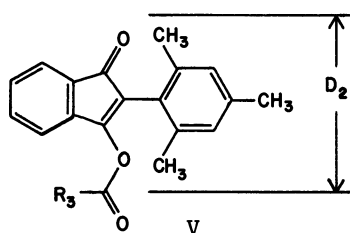
	R_3	LC$_{50}$ (ppm)	
		MITE	EGG
Va	CH_3	9	8
Vb	$C(CH_3)_3$	5	2

directing new work to advantageous structural areas and for se-
lecting critical compounds to be made for the purpose of challen-
ging and refining the specifics of early models. The final data-
set embodied not only highly active miticides but also the basis
for a more correct quantitative expression of key structure/
activity elements in the series.

Background

In the early 1970's potent acaricidal activity in 3-aryl-4-hy-
droxy-coumarins, III, and 2-aryl-1,3-indanediones, IV, and their
enol esters was discovered at Union Carbide (1,2). Extensive
synthesis and screening of analogs in the dione and enol ester
series led to the field test candidates Va and Vb, active against
both motile forms (mite) and eggs. Va and Vb embodied certain
structural and physicochemical features shown by published QSAR
studies (2,3) to be important in determining the level of activity
in the 1,3-indanedione series: (1) the dimension D_2 across the
aryl ring must be near 7A and symmetry is preferred; (2) the

4-position of the aryl ring may be substituted and the substituent
should be small in size; (3) enol esterification is critical for
effectiveness under field conditions, presumably by protecting
the compounds against photodegradation.

Cycloalkanedione Program

Although effective compounds were found in the indanedione series,
the class was not without significant shortcomings: the compounds
were highly colored, thus capable of staining, and had very limit-
ed solubility in conventional formulation media; furthermore,
highly active compounds tended to be phytotoxic. Work continued,
therefore, in the search for materials in which these undesirable
features were minimized or eliminated entirely. Molecules gener-
alized by Ia,b and II seemed attractive candidates since removing
the benzo- moiety from the indanedione, expanding the dione ring
to six members, adding substituents, etc. would yield compounds
having greater flexibility, diminished color, higher pKa's,
different susceptibility to metabolism, and an opportunity to
study anew the effects of substitution on the aromatic ring.
     Following the synthesis and testing of some 80 compounds in
the new series, data inspection and preliminary regression analy-
sis provided the following structure/activity guidelines: (1)
cyclohexanediones substituted at the 5-position with small alkyl
groups have better activity than unsubstituted cyclopentane or
cyclohexane compounds; implication: bulk and/or lipophilicity
at the backside of the dione ring may be important; (2) a single
ortho group may be superior to two ortho groups or no ortho groups
in the 2-phenyl moiety, a surprising departure from SAR observed
in the indanedione series where two ortho groups and symmetry were
documented to be important; (3) chlorines are probably equivalent
to methyl groups as aryl substituents in the new series; implica-
tion: their steric and lipophilic properties matter more than
electronic effects; (4) strongly electron withdrawing function-
ality on the aryl ring is disadvantageous; (5) chain length in the
acyl group can modify activity; (6) alpha branching in the acyl
group, advantageous because it reduces foliage phytotoxicity in
certain plants, apparently also detracts from acaricidal activity.
Preliminary structure/activity trends were observed to be similar
for both motile forms (mite) and eggs.

Model. These guidelines provided concepts or operating hypotheses
on which the synthesis of approximately 120 additional compounds
was based. Towards the end of this phase of the project, here-
after referred to as Phase I, our concepts had crystallized to the
point where they could be expressed using the discrete quantita-
tive parameters described in Table I. Parameters illustrated in
the table bearing the "I" tag are indicator variables set equal to
unity when the indicated feature is present, zero when it is not;
I[56] = 1 indicates compounds in which the entire dione moiety is

TABLE I

Concepts and Parameters for Phase I Model
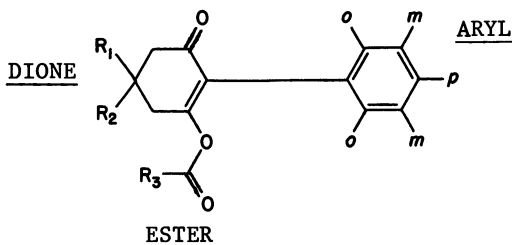
DIONE:

  DICARB: Carbons in R_1, R_2
  I[5-phenyl]: R_1 or R_2 is phenyl
  I[unsubst]: R_1 and R_2 are H
  I[56]: Perhydroindanedione

ARYL:

  I[ortho-1]: Single ortho
  I[ortho-2]: Two ortho
  I[meta]: Both meta not H
  I[para]: Para not H

ESTER:

  ESCARB: Carbons in R_3
  I[alpha]: α-branch in R_3
  I[parent]: Free enol

MOLECULAR:

  Log P, ΣMR, etc.

perhydroindane-1,3-dione. DICARB and ESCARB, log P, and ΣMR are
continuous variables, the latter two estimated from literature
tabulations (4) in the conventional manner.
    Parameters other than simple carbon counts for substituents
at the 5-position of the dione ring and the size of the ester
chain were examined in preliminary studies. For example,
Charton's steric parameter, ν, (5) was examined as an alternative
to DICARB for bulk at R_1 and R_2; Σπaryl, for the substituents on
the aryl ring. Preliminary regression equation results achieved
using such alternatives were similar but not superior to those
outlined below; the carbon counts for DICARB and ESCARB have the
further advantage of conceptual simplicity and computational
accuracy.

Objectives. Given an extensive databank of more than 200 com-
pounds, many of which had excellent miticidal and ovicidal potency
and the opportunity to continue the synthesis of permutations in
pursuit of even better compounds (but with the probability of
encountering more of the same), what did we hope to achieve by
multidimensional analysis at this stage of the project? Answers

were sought for the following questions: (1) Can consistent
structure/activity relationships be demonstrated using the para-
meters cited above as expressions of our thinking; how much of
the variation in biological response for the entire dataset can be
explained by our model? (2) Which compounds show activity not
explained well by the best statistical expressions of the model;
do discrepancies between observed vs. predicted activity levels
for such compounds (residuals) form a pattern suggesting refine-
ment in our thinking or our model? (3) Are the best compounds in
hand? In what areas of structure/activity "space" can equivalent
activity or even superior activity be expected? (4) Do the
results of the analysis provide the basis for a decision to stop
research in this area?

Multiple regression analysis provided the basis for objective
answers to these questions.

Methods. Synthetic methods used to create the compounds described
in this study and protocols used in the determination of toxicity
to motile forms (mites) and eggs (activity expressed as LC50, ppm
of foliar spray) have been published (6-10, 1). The test organism
was Tetranychus urticae (two-spotted mite).

Biological activity for mite (motile forms) and egg kill used
in regression studies was expressed in log molar "potency" units
derived from foliar spray LC50's (ppm) as follows: POTENCY = 3-
log (LC50/MW) where MW is the molecular weight of each compound;
the "log 1/C" potencies are thus activity expressed on a log scale
with higher numbers representing greater activity.

Before use in regression analysis, all indicator variables
were centered by transforming to $(X_i - \overline{X})$; this causes each
indicator variable's effect on the regression to sum to zero over
the dataset such that term coefficients do not change as terms
are entered into or removed from equations during prospective
trials. DICARB and ESCARB were offered to regression in both
linear (X_i) and quadratic forms $(X_i - \overline{X})^2$ to look for the effect
of curvature; the mean was subtracted prior to squaring to break
X/X^2 correlation. All equations discussed in this report are
algebraically expanded to their transformation-free equivalents so
that the sense of terms is easily visualized and simple substi-
tution allows for predictive calculations; the dependent variable
remains 3-log (LC50/MW) throughout.

In each new regression experiment, the complete list of inde-
pendent variables was first offered to SAS (11) STEPWISE regres-
sion routines without bias to allow the computer to add variables
having the highest possible significance; the entry of the first
variable was accepted only if an F test showed P >0.95. Forcing
regression was then used in followup runs to test possible com-
petitive combinations of variables intuitively expected to be
important. No variable was accepted if the "F-level" for the
equation having (n + 1) variables was not improved over that for
equations having (n) variables.

    The residuals (log 1/C observed - log 1/C predicted) for the
entire dataset were examined in detail for each key regression
equation. Analysis of major residuals allowed not only perception
of true "outliers" but also provided insights for improving the
model; i.e., sharpening hypotheses and concepts.

Phase I Results

Overview of the Databank. A profile of the Phase I databank of
compounds is presented in Tables II-IV; 202 of the 2652 permuta-
tions possible from 12 dione moieties, 13 aryl substitution
patterns, and 17 different enol ester acyl groups constituted the
Phase I dataset. The structures and data given below exemplify
how activity varied with structure throughout the dataset.

Regression Equations. Elements of four regression equations,
Equations 1-4, embodying the structure/activity relationships
inherent in the Phase I dataset are illustrated in Table V.
Equations 1 and 2 were derived from all the observations in the
202 compound Phase I dataset except five compounds inactive at
500 ppm in the mite test and six compounds inactive at 500 ppm
in the egg test. The r^2 terms, in each case the fraction of bio-
logical data variation in the regression datasets accounted for
by the model, are satisfactory considering the precision of the
test protocols and the size and diversity of the datasets;
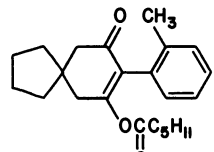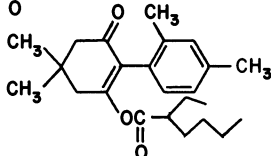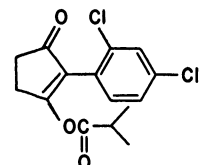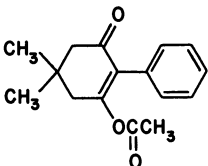
	Mite		Egg	
	LC_{50}	Potency	LC_{50}	Potency
	4	4.9	3	5.1
	4	5.0	4	5.0
	$\sim$500	2.8	$\sim$500	2.8
	$\sim$500	2.7	$\sim$500	2.7

TABLE II
Phase I DIONE Substitution Patterns
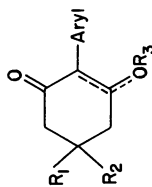
R_1, R_2	DICARB	COMPOUND COUNT
Unsubstituted	0 (-)[a]	18
Methyl, H	1 (+)	16
Phenyl, H	6 (+)	4
Methyl, Methyl	2 (++)	82
Methyl, Ethyl	3 (++)	9
Ethyl, Ethyl	4 (+)	5
Methyl, Propyl	4 (+)	6
Methyl, Amyl	6 (-)	5
Methyl, Phenyl	7[b] (-)	6
Spirocyclopentyl	3 (++)	13
Unsubstituted		
Cyclopentanedione	0[c] (-)	19
Perhydroindanedione	3[d] (++)	19

a) General Contribution to Activity (+: positive; -: negative)

b) DICARB = 3: "Compact" Diethyl

c) DICARB = 0: Set equivalent to unsubstituted cyclohexanedione

d) DICARB = 3: Fused - $(CH_2)_4$ - Moiety

TABLE III
Phase I ARYL Substitution Patterns
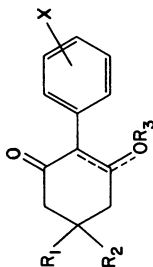
ARYL SUBSTITUENTS	COMPOUND COUNT
Unsubstituted	7 (?)[a]
2-Methyl	38 (+)
2-Chloro	21 (+)
2,4-Dimethyl	68 (+)
2,4-Dichloro	20 (+)
2,4,6-Trimethyl	6 (?)
2,5-Dimethyl	20 (?)
2,6-Dichloro	3 (?)
2,6-Dimethyl	1 (?)
2-Chloro-6-methyl	1 (?)
4-Methyl	10 (-)
3,4-Dimethyl	6 (-)
2,4,6-Trichloro	1 (?)

a) General Contribution to
Activity (+: positive;
-: negative)

TABLE IV
Phase I Acyl Groups
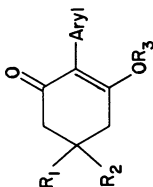
R_3	ESCARB	COMPOUND COUNT
Parent Enol	1*	29
Acetate	1	23
n-Propionate	2	2
n-Pentanoate	4	2
n-Hexanoate	5	18
n-Heptanoate	6	1
n-Octanoate	7	8
n-Nonanoate	9	10
n-Hexadecanoate	15	11
n-Octadecanoate	17	9
I [alpha] = 1		
Isobutyrate	3	15
Cyclopropanecarbox.	3	2
Cyclohexanecarbox.	6	3
2-Ethylbutyrate	5	3
Pivalate	4	11
2-Ethylhexanoate	7	34
Benzoate	6	11

* I[Parent] distinguishes from acetate

TABLE V

Coefficients and Parameters From Phase I Equations

EQUATION:	1	2	3	4
TARGET:	MITE	EGG	MITE	EGG
NOTE:	Actives Only	Actives Only	Optimized	Optimized
n	197	196	177	166
r^2	0.69(0.69)[a]	0.64(0.67)[a]	0.83(0.61)[a]	0.81(0.66)[a]
s	0.36	0.44	0.25	0.31
F	35.0	36.0	66.5	72.0
Intercept	2.312	1.751	2.257	1.657

ARYL VARIABLES:

I[ortho-1]	0.98 (11)	0.97 (9)	1.00 (16)	1.02 (13)
I[ortho-2]	0.52 (4)	0.39 (2)	0.52 (5)	0.42 (4)
I[meta]	-0.27 (3)	---------	-0.35 (6)	---------
I[para]	0.41 (7)	0.38 (6)	0.40 (10)	0.39 (8)

DIONE VARIABLES:

DICARB	0.33 (7)	0.74 (8)	0.37 (10)	0.75 (12)
$DICARB^2$	-0.075(7)	-0.121(10)	-0.087(10)	-0.120(13)
[DICARB(opt.)]	[2.2]	[3.0]	[2.1]	[3.1]
I[unsubst.]	-0.37 (5)	---------	-0.26 (4)	---------
I[5-phenyl]	0.45(94%)	0.84 (3)	0.65 (4)	0.68 (3)
I[56]	0.31 (3)	---------	0.40 (6)	---------

ESTER VARIABLES:

ESCARB	0.182(7)	0.194(8)	0.190(10)	0.210(12)
$ESCARB^2$	-0.0095(7)	-0.0091(5)	-0.0099(9)	-0.0094(7)
[ESCARB(opt.)]	[9.6]	[10.7]	[9.6]	[11.2]
I[parent]	0.16(92%)	---------	0.14 (2)	---------
I[alpha]	-0.23 (3)	-0.25 (3)	-0.25 (5)	-0.28 (5)

a) r^2 for full 202 compound dataset; all other numbers in paren-
   theses are coefficient t-levels (coefficient ÷ s.e.)

(r^2 noted in parentheses in the table are % accountabilities for
the underline entire 202 compound Phase I datasets where the LC50 for com-
pounds inactive at 500 ppm was set to 550 ppm). All terms in
Equations 1 and 2 had t-ratios (in parentheses adjacent to each
coefficient) greater than the critical t for 95% significance ex-
cept I[5-phenyl] and I[parent] (significance levels are indicated
for these two) for Equation 1. As one might expect, the size,
magnitude, and significance of coefficients for the "correcting"
I[unsubst], I[5-phenyl], and I[56] indicator variables are sensi-
tive to the specifics of the functions for DICARB.

   The similarity between Equations 1 (mite) and 2 (egg) is re-
markable considering each was derived from independent biological
datasets; this result is consistent with the concept, verified by
independent experiments (12) on rate of kill vs. egg age, that
"egg kill" with this series of compounds involves kill of pharate
larvae rather than prevention of emergence.

   The roles of ortho and para substituents are similar for mite
and egg; a meta substituent is disadvantageous for mite; toxicity
to the egg is apparently unaffected by substitution at this posi-
tion.

   The coefficients for linear and squared DICARB and ESCARB
terms of Equations 1 and 2 describe parabolas having calculated
optima as indicated in Table V. Details on these functions will
be discussed later in the context of the "refined" Equations 3 and
4.

   The intercepts for Equations 1 and 2 can be considered as the
potencies for a theoretical compound having zero carbons on the
dione ring or in the ester chain, no aryl substituents and all
indicator variables set equal to zero. The sense of the equations
in adjusting activity against an intercept is illustrated in
Table VI. For the compound in the illustration, the ortho and
para methyl groups increase potency relative to the reference by
0.98 and 0.41 potency units, respectively; compounds having
DICARB = 2 (near the optimum) have potency up 0.36 units relative
to an unsubstituted analog; five carbons in ESCARB promotes
activity over a theoretical compound having ESCARB = 0 by 0.67
potency units. The illustrated compound has potency 2.42 log
units or 263-fold higher activity on the LC50 scale than the
"theoretical reference" compound. All four terms are critical to
understanding SAR in the series.

Major Residuals and Refined Equations. Observed vs. predicted
potencies for mite (Equation 1) and egg (Equation 2) for the Phase
I dataset are plotted in Figure 1.

   A detailed overview of the major residuals is beyond the
scope of this report; highlights of such an overview, however,
include the following points: (1) the compounds inactive at 500
ppm fit predictions well if their "observed" LC50 is assigned as
550 ppm; (2) extreme residuals occur more frequently in the egg
test than in the mite test and activity less than predicted
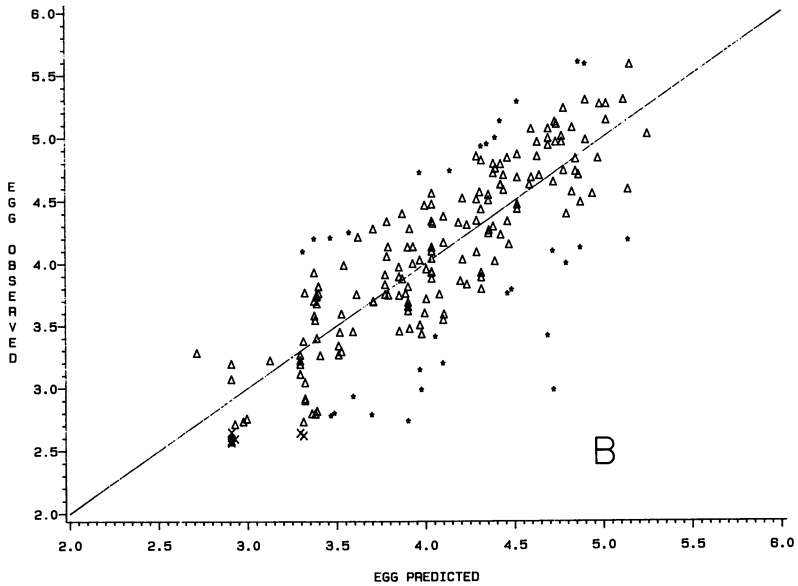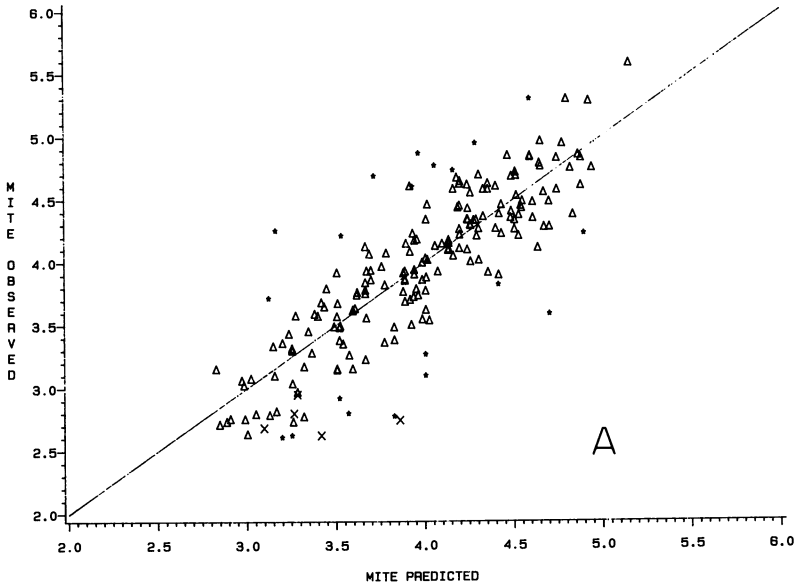
Figure 1. Observed vs. predicted potencies: (A) Mite by
Equation 1; (B) Egg by Equation 2. Key: compounds inac-
tive at 500 ppm (X); residuals removed prior to generation
of Equations 3 and 4 (★); other Phase I compounds (Δ).

TABLE VI
Illustration of the Sense of Terms of Equation 1

Mite Potency = $[3 - \log(LC_{50}/MW)]$ =

+ 2.312	
+ 0.98	[ortho-1]
+ 0.41	[para]
+ $[-0.075(2)^2 + 0.33(2)$	[DICARB]
+ $[-0.0095(5)^2 + 0.18(5)$	[ESCARB]
= 4.732	
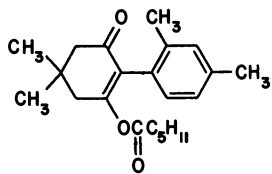
Mite LC_{50} = 6.6 ppm; Observed: 5 ppm

predominates among the major egg residuals (this suggests compound delivery anomalies in the egg test possibly related to egg age and whether the eggs are breathing direct or through their shells); (3) while the major residuals were for the most part random with respect to structure, examination of all residuals suggested the following considerations for model refinement: (a) pivalates tend to be less active than predicted (I[alpha] does not properly handle doubly-branched acyls?); (b) cyclohexanediones bearing a spirocyclopentyl substitution at the 5,5-position (DICARB was set equal to "3") tend to be slightly more active than predicted (setting DICARB equal to "2" might even better account for the compactness or rigidity of these structures); (c) activity of free enol compounds may be influenced in a special way by the presence and count of ortho substituents in the aryl ring. These points will be expanded later.

Because equations from this Phase of the study were destined for use in predictive work, refined versions of Equations 1 and 2, Equations 3 and 4 (Table V), were developed by subjecting abbreviated Phase I datasets to regression analysis: the dataset used to create Equation 3 was built by removing the five compounds inactive against mite at 500 ppm and the twenty compounds having residuals larger than 0.55 (Figure 1A) from the original dataset; removal of the six compounds inactive against egg and thirty compounds having egg residuals larger than 0.60 (Figure 1B) yielded Equation 4.

As expected, Equations 3 and 4 convey the same message as did Equations 1 and 2 and have improved t-ratios on term coefficients; they are less confounded by the noise characteristic of the full datasets and are thereby more suitable for predictive work.

As illustrated in Figure 2A, and as was true for Equations 1 and 2, the DICARB and ESCARB functions for Equations 3 and 4 describe, respectively, precipitous and flat parabolas having the optima cited in Table V. The DICARB curves clearly document the superiority of carbon counts near 2.5 over compounds having fewer
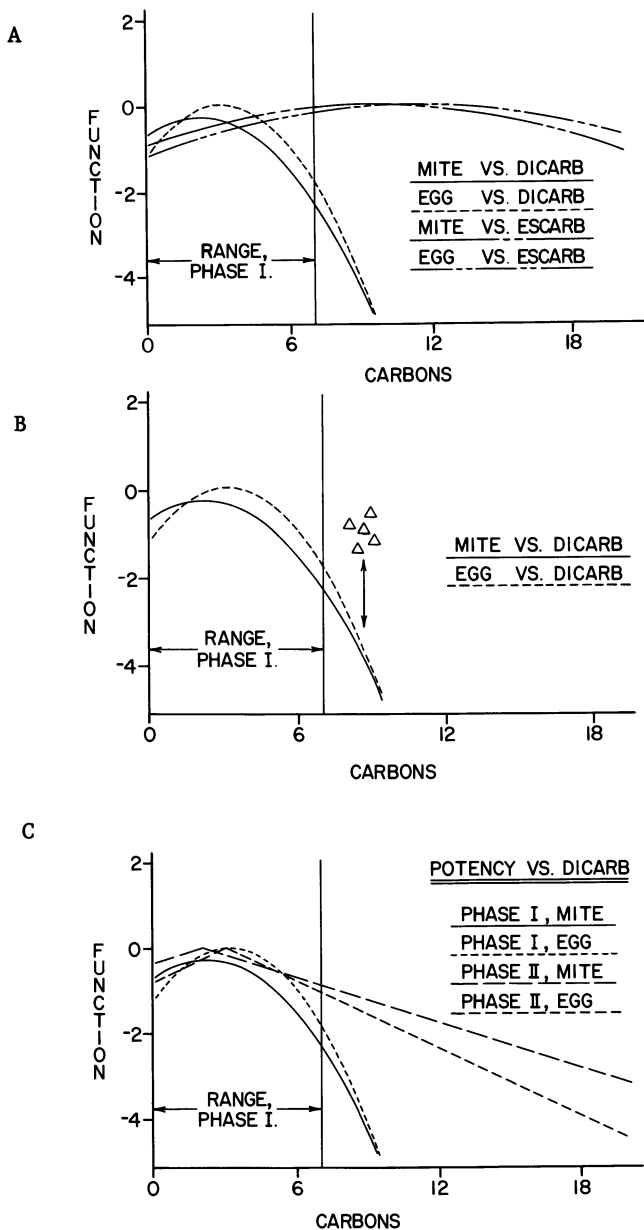
Figure 2. Potency vs. carbon count functions. (A) DICARB and ESCARB functions for Phase I mite (Eq. 3) and egg (Eq. 4); (B) Schematic illustration of underprediction for Phase I DICARB quadratic functions outside Phase I range in DICARB; (C) Phase II DICARB functions vs. Phase I DICARB functions.

or greater numbers of carbons at the 5,5-position: within the
range of DICARB studied in Phase I, two orders of magnitude in
activity are controlled by this parameter. The ESCARB functions
are considerably less dramatic. They are real, however, (note
solid t-levels in Table V) and document clearly the advantage of
acyl residues having ESCARB near 10.0.

Both the I[unsubst] and I[5-phenyl] terms of the Phase I
equations "correct for" inadequacies in the curvature of the two-
term DICARB parabolas. This point is developed further below.

Phase II Dataset: Pragmatic Predictions and Problems

During and subsequent to the Phase I QSAR work, 103 new compounds
were synthesized and tested; 72 of these were expected to have
good to excellent activity based on the concepts embodied in the
Phase I models; 32 served to challenge the Phase I models. As
illustrated in Table VII, 29 compounds having DICARB <1 or >4 were
synthesized in this second phase of the work, hereafter referred
to as Phase II. These materials provided an opportunity to re-
fine our understanding of the roles of extremes of DICARB. The
remainder of the compounds were aimed at areas of DICARB predicted
by Phase I models to contain highly active compounds.

Found vs. Predicted Activity Classes in Table VII confirm
that the Phase I models served well in forecasting general activi-
ty levels for the Phase II compounds. Data in Table VIII under-
score this conclusion in more quantitative terms: for mite
(motile forms) only one compound of the 66 expected on the basis
of Equation 3 to have LC50 <40 ppm had LC50 >120 ppm; none of the
28 expected to have LC50 $\geq$150 ppm had LC50 <55 ppm; similar re-
sults were found for egg. The test dataset validated concepts
expressed in the Phase I models and documented the utility of the
models as useful tools in guiding continuing synthesis: highlight
compounds are illustrated below.
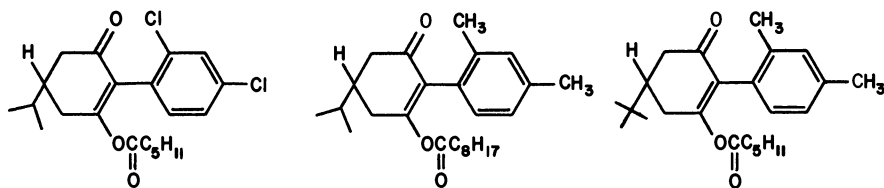
Predicted/Observed LC$_{50}$'s

Mite:	8/7	5/5	12/7
Egg:	3/7	2/1	4/1

TABLE VII
Phase II DIONE Substitution Patterns
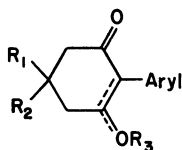
R_1, R_2	DICARB	COMPOUND COUNT	PREDICTED ACTIVITY CLASS*	FOUND ACTIVITY CLASS
Unsubstituted	0	3	–	–
Methyl, H	1	6	+	+
Ethyl, H [NEW]	2	7	++	++
Propyl, H [NEW]	3	4	++	++
Isopropyl, H [NEW]	3	37	++	+++
Isobutyl, H [NEW]	4	2	+	+
t-Butyl, H [NEW]	4	6	+	++
3-Amyl, H [NEW]	5	3	±	–
Cyclohexyl, H [NEW]	6	2	–	–
Trifluoromethyl, H [NEW]	"2"	2	?	+–
Methyl, Methyl	2	6	++	++
Methyl Isopropyl [NEW]	4	4	+	+
Methyl, Amyl	6	5	–	––
Methyl, Nonyl [NEW]	10	14	––	––
Phenyl, H	6	2	?	––

*Phase I Equations

TABLE VIII
Statistics on Phase II Activity Classes

		$LC_{50} < 40$ ppm	$LC_{50} \geq 150$ ppm
Mite	Predicted	66 Compounds[a]	28 Compounds[b]
	Found	54 Compounds (82%)	22 Compounds (79%)
Egg	Predicted	66 Compounds[c]	28 Compounds[d]
	Found	53 Compounds (80%)	24 Compounds (86%)

a) One of 66 had $LC_{50} > 120$ ppm
b) None had $LC_{50} < 55$ ppm
c) One of 66 had $LC_{50} > 120$ ppm
d) Two had $LC_{50} < 30$ ppm

Contrary to the practical results reviewed above, statistics from correlation work revealed a serious deficiency in the accuracy with which Phase I Equations 3 and 4 predicted for the Phase II dataset: r^2 for Equation 3 predictions for the 103 compound Phase II data was only 0.45; r^2 for Equation 4 predictions for the Phase II dataset was only 0.44. An analysis of the residuals for the Phase II dataset [Potency(observed)-Potency(predicted by Phase I models)] immediately identified the source of the problem: of the 26 Phase II compounds having DICARB >4, 17 had potency for adult observed more than one log unit better than predicted; 15 had egg potency observed more than one log unit better than predicted. As schematically shown in Figure 2B, the parabolic functions for DICARB for the Phase I models underpredict at values of DICARB extrapolated beyond those represented in the Phase I dataset.

Model Refinement

There is no doubt that the DICARB function must describe an optimum near 2 (mite) or 3 (egg) carbons. It is also clear that the exponential aspect of a parabolic function exaggerates the detrimental aspects of extreme values of DICARB away from an optimum. The solution to the problem is the creation of transforms of DICARB according to the following expressions:

$$\text{Mite: DICARB} = \mid 2.0 - \text{DICARB} \mid$$
$$\text{Egg : DICARB} = \mid 3.0 - \text{DICARB} \mid$$

These transforms offer the regression analysis the opportunity to ask of the data the following question: "How sensitive is activity to linear movement of DICARB away from the stipulated optima?" The lines plotted in Figure 2C illustrate the concept. Regression runs against the combined Phase I and II datasets confirmed the utility of this approach and the suitability of 2.0 (mite) and 3.0 (egg) as constants in the transform.

Two other refinements to the model were explored in our final experiments. Detailed examination of residuals for both Phase I and Phase II compounds had revealed two classes of compounds generally mispredicted by the Phase I models (other than compounds having DICARB >4): pivalates (generally less active than Phase I model predictions) and parent enols bearing two aryl ortho substituents (generally more active than predicted). Two new indicator variables, I[pivalate] (=1 if a compound is a pivalate) and I[twopar] (=1 if a compound is a parent enol having two aryl ortho groups) were created to test the general applicability of these concepts.

The complete 305 compound Phase I and Phase II datasets were combined and subjected to progressive regression analysis to develop final expressions. Compounds inactive at 500 ppm were left in the datasets for these regression runs with LC50's set at 550 ppm.

TABLE IX
Coefficients and Parameters From Phase II Equations

EQUATION:	5	6	7	8
TARGET:	MITE	EGG	MITE	EGG
NOTE:	Full Dataset	Full Dataset	Cluster Group	Cluster Group
n	299	296	100	100
r^2	0.72(0.68)[a]	0.74(0.69)[a]	0.74(0.65)[a]	0.78(0.65)[a]
s	0.34	0.39	0.34	0.38
F	61.8	74.0	22.4	28.5
Intercept	2.865	3.047	2.781	2.740
ARYL VARIABLES:				
I[ortho-1]	0.98 (13)	0.97 (12)	0.90 (8)	0.81 (7)
I[ortho-2]	0.49 (5)	0.38 (4)	0.63 (5)	0.47 (4)
I[meta]	-0.33 (4)	---------	-0.15(87%)	---------
I[para]	0.40 (9)	0.41 (8)	0.43 (6)	0.56 (7)
DIONE VARIABLES:				
[X-DICARB]	-0.18 (15)	-0.26 (17)	-0.18 (9)	-0.28 (10)
[X=]	[2.0]	[3.0]	[2.0]	[3.0]
[DICARB(opt.)]	[defined]	[defined]	[defined]	[defined]
I[unsubst.]	-0.40 (7)	-0.43 (6)	-0.46 (5)	-0.29 (3)
I[5-phenyl]	-0.24 (2)	---------	---------	0.94 (3)
I[56]	0.54 (6)	---------	0.68 (5)	0.41 (3)
ESTER VARIABLES:				
ESCARB	0.144(8)	0.160(4)	0.171(5)	0.280(8)
$ESCARB^2$	-0.008(7)	-0.007(11)	-0.009(4)	-0.014(5)
[ESCARB(opt.)]	[9.0]	[11.6]	[9.8]	[9.8]
I[parent]	---------	-0.19 (25)	---------	---------
I[alpha]	-0.21 (4)	-0.20 (3)	-0.26 (3)	-0.38 (3)
NEW VARIABLES:				
I[pivalate]	-0.32 (3)	-0.23 (2)	-0.38 (3)	-0.24(92%)
I[twopar]	0.20(91%)	0.44 (3)	---------	---------

a) r^2 for full 305 compound dataset; all other numbers in paren-
   theses are coefficient t-levels (coefficient ÷ s.e.)

Elements of the final equations achieved after removing only six major residuals (stars in Figure 3A) from the 305 to create a final mite dataset and nine major residuals (stars in Figure 3B) from the 305 to create a final egg dataset are given in Table IX (Equations 5 and 6).

Note the homogeneity of terms in the two equations. The new DICARB functions (plotted in Figure 2C) eliminated the inconsistency in the I[unsubst] terms characteristic of the Phase I models and minimized leverage in the "correcting" I[5-phenyl] term of the Phase I models. Both the I[pivalate] and I[twopar] terms were validated.

Final observed vs. predicted plots for all 305 Phase I and Phase II compounds are given in Figure 3.

Conclusions. The objectives cited earlier were met. Solid trends do exist in this series, can be documented, and can be used to chart new work. Significant trends in residuals encountered after the first phase of the study provided the basis for model refinement. Good support exists for the premise that we have thoroughly traversed the most attractive areas of structure/activity space in this series.

Could this information and the highlight compounds have been achieved more efficiently? Retrospection indicates unequivocally that the answer to this question is yes. This history and the following experiment argue strongly for good series design at the beginning of synthesis (which we did not have) as well as good series design midway in a program.

If one performs cluster analysis on the final 305 compound dataset using the variables I[oneorth], I[twoorth], I[meta], I[para], I[unsubst], I[alpha], I[pivalate], and I[dibig] (an indicator variable set "on" if DICARB is greater than 4), the dataset can be compressed to 38 groups with zero information loss. If 100 compounds are then selected randomly from the 38 groups with the provisos that all groups are represented fairly evenly and that "parent enols", "short", "midlength", and "long" chain esters are fairly represented and if this dataset of 100 compounds is subjected to progressive regression analysis, Equations 7 and 8 (Table IX), essentially identical to the final equations from the 305 compound study, are achieved.
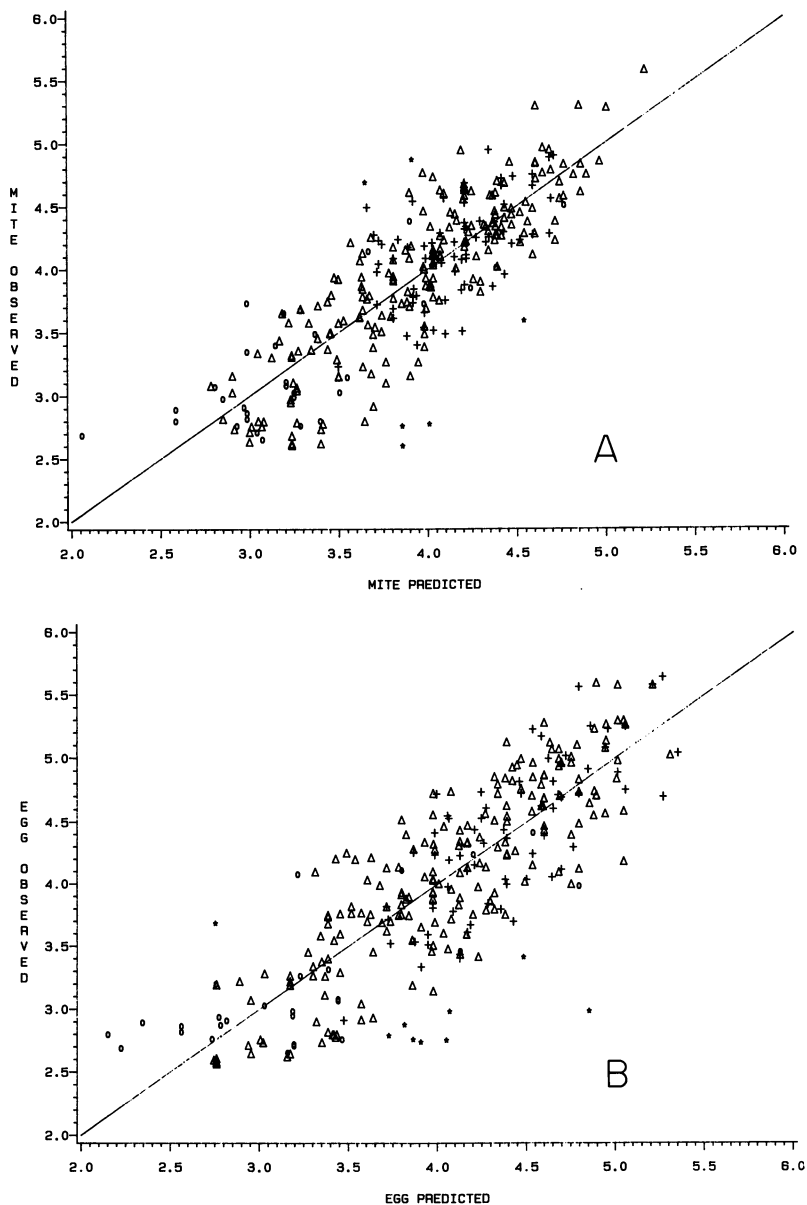
Figure 3. Observed vs. predicted potencies: (A) Mite by
Equation 5; (B) Egg by Equation 6. Key: Phase I compounds
(Δ); Phase II compounds expected on the basis of Phase I
equations to have good activity (+); other Phase II com-
pounds (O); residuals removed prior to generating Equations
5 and 6 (★).

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