

BIOLOGICALLY ACTIVE NATURAL PRODUCTS: Agrochemicals

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CRC PRESS

Boca Raton London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

Cutler, Horace G., 1932-

Biologically active natural products: agrochemicals / Horace G. Cutler, Stephen J. Cutler.

p. cm.

Includes bibliographical references and index.

ISBN 0-8493-1885-8 (alk. paper)

1. Natural products in agriculture. 2. Agricultural chemicals. 3. Bioactive compounds. I. Cutler, Stephen J.
II. Title.

S587.45.C881999

631.8—dc21

99-20202

CIP

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International Standard Book Number 0-8493-1885-8

Library of Congress Card Number 99-20202

Printed in the United States of America 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

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Agrochemicals and Pharmaceuticals: The Connection

Horace G. Cutler and Stephen J. Cutler

CONTENTS

1.1	Introduction.....	1
1.2	Benzodiazepines	2
1.2.1	Bioassay	5
1.2.2	Synthetic Benzodiazepines.....	5
1.2.3	Phenoxy Compounds	6
1.2.4	Organophosphates	9
1.3	Epilogue.....	12
	References.....	13

ABSTRACT Three categories of agrochemicals (or potential agrochemicals) and pharmaceuticals are discussed both in the context of historical discovery and utilitarian development. The benzodiazepines include the natural products cyclopenin and cyclophenol, their potential as plant growth regulators, fungicides, and tranquilizers vs. the synthetics lorazepam, oxazepam, clorazepate dipotassium, temazepam, prazepam, flurazepam dihydrochloride, triazolam, and alprazolam. All exhibited activity in the etiolated wheat coleoptile bioassay. The phenoxy compounds include 2,4-dichlorophenoxyacetic acid, and its 2,4,5-trichloro congener, 2-(3-chlorophenoxy)-propanoic acid, and *p*-chlorophenoxyacetic acid, all of which are plant growth regulators. Juxtaposed to these is the development of clofibric acid, clofibrate, and their derivatives which reduce blood serum cholesterol. Finally, the organophosphates metrifonate, initially used to control schistosomiasis, and dichlorvos are examined. Dichlorvos, a catabolite of metrifonate, was an important insecticide but now finds use in controlling Alzheimer's disease.

1.1 Introduction

There is a certain pleasure that comes from migrating across scientific disciplines. At first, there is suspicion that the translation will dilute the fund of knowledge rather than construct a highly viable hybrid and, furthermore, it is difficult to keep abreast of the discoveries in one discipline let alone taking on a new vocabulary and findings of another. Surprisingly, in this transition it happens that chemical structures and their congeners,

which one has known for many years, turn up like old friends. To put it tritely, different play, different characters, same actors. Perhaps another metaphor for the relationship between agrochemicals and pharmaceuticals also exists. The common thread between the two disciplines is chemistry, both synthetic and natural product chemistry. And, while the thread may be singularly strong, it is only when it is woven into cloth that it becomes exceptionally durable and has major utilitarian value. Paradoxically, the threads in a piece of cloth consist of a warp and a weft, and they are diametrically opposed. It is the precise opposition that maximizes the strength.

Both agrochemicals and pharmaceuticals are two of the pillars upon which modern civilization stands. And *mens sana in corpore sano*, a sound mind in a sound body, is certainly the ideal product of that interdisciplinary marriage. The first set gives rise to an abundant supply of food, the second set keeps the body and mind in a healthy, functioning state. From the economic point of view, pharmaceuticals are lucrative value-added products. Agrochemicals, on the other hand, while they may yield a solid return on investment, do not generate as much income on a “weight to sale” proportion. That is, a few milligrams of a medicinal may sell for several dollars, while several pounds of an agrochemical may sell for the same price. However, between the two disciplines there is an apparently vast no-man’s land. As we shall see, there are a number of chemical templates that have found use in both disciplines. But surely, in the multitude of pharmaceuticals that have been discovered as natural products, their synthetic derivatives, and the logical sequence by which one arrives at an active product that no longer resembles its progenitor, there must be compounds that have alternate uses. Of special interest in agriculture are those natural products that do not adversely affect the environment because they are target specific, have high specific activity, and are biodegradable — all attributes of natural products and, to a certain extent, their synthetically modified products.

It may be argued by the reader that some of the examples presented herein are not all natural products and that the symposium, after all, involved natural product agrochemicals and pharmaceuticals. Like all true penitents we have to say *mea culpa* to the charge, and while we have not kept to the strict letter of the law we have certainly kept to the spirit. And most certainly the phenoxy compounds, for example, had their origin in a natural product. Nature absolutely preceded the synthetic chemist in the biosynthesis of the benzodiazepines. But above all, the intention is to weave the disciplines of agrochemicals and pharmaceuticals to the benefit of all.

1.2 Benzodiazepines

It is erroneously believed that the discovery of the benzodiazepine structure was first revealed in the second half of 1955.¹ In fact, the events leading up to the use of this family of compounds as anxiolytic agents was completely separate to the elucidation and first disclosure of the structure. In retrospect, it is difficult to understand how much literature was ignored by those involved in agrochemistry and pharmaceuticals, and it can only be assumed that the two were operating in isolation. From the pharmaceutical perspective the history is charged with the elements of chance, genius, and geography. In the 1930s, Leo Sternbach had been working in Cracow, Poland as a post-doctoral assistant. At that time, he was looking at the properties of the benzheptoxdiazines for use as dyestuffs, in a very unsettled world. Hitler was already rattling the sabres of war, Poland was becoming a bone of contention in the gathering political storm, and the fortuitous opportunity arose for Sternbach to make his way to America to work at the Roche laboratories.

During the 1950s, the first tranquilizers were made available to the public. They were, in chronological order of the patents that were granted in the U.S., the following: chlorpromazine, 2-chloro-10-[3-(dimethyl amino) propyl] phenothiazine, commonly known as thiorazine (U.S. Patent 2,645,640 to Rhône-Poulenc, 1953) and marketed as the hydrochloride. In 1951, a minor tranquilizer, meprobamate, 2-methyl-2-propyl-1,3-propanediol dicarbamate, had been synthesized by Ludwig and Piech² and this was later patented by Carter Products (U.S. Patent 2,724,720) in 1955. There followed the root of the Indian plant *Rauwolfia serpentina* L. (= *Ophioxylon serpentium* L.) which had been used in the East for generations as an antihypertensive and as a “mood altering” agent with tranquilizing properties. The chemical composition of the root extract was shown to be reserpine, reserpinine, yohimbine (a purported aphrodisiac), ajmaline, serpentine, and serpentinine.³⁻⁵ Of these, reserpine, 11,17 α -dimethoxy-18 β -[(3,4,5-trimethoxybenzoyl) oxy]-3 β , 20 α -yohimban-16- β -carboxylic acid methyl ester, was the constituent which possessed antihypertensive and tranquilizing properties.⁵ Most importantly, it contained the indole structure, a key molecule in both psychomimetic and plant growth regulating substances. The material was patented and marketed (U.S. Patent 2,833,771 to Ciba, in 1958).

Ironically, the soma described by Aldous Huxley in 1932 as the opiate of the select masses in his book, *Brave New World*, came in a multitude of guises in the early 1950s with the exception that these were highly useful medicinals. The pharmaceutical industry was quick to realize the potential of these materials and rightly so. One should bear in mind that in 1950 World War II had only been over for 5 years. Horrific events still haunt some of us who lived in European cities a half century later, and the reasons for developing those types of drugs were critical to post-war stabilization. The game was afoot.

So it was that Leo Sternbach found himself placed on a project at Roche to find new tranquilizers. His analysis of the research task went through some dichotomous thinking. None of the available tranquilizers, or sedatives, or hypnotics were structurally similar and, subsequently, there was no common chemical denominator. That left him with the option of either using those compounds that existed and synthetically modifying them or trusting to luck and empirically producing biochemicals on the off chance that they might have the desired activity. On the positive side of the equation, all the necessary bioassays were in place. Fortuitously, he decided that it would be far more profitable if he pursued some chemistry that he knew, namely the benzoheptoxdiazines, the compounds that he had worked on in Cracow which, since that time, had remained in relative obscurity. First synthesized by Auwers and von Meyenburg, and reported in 1891,⁶ the ground had been well cultivated for the prepared mind of Sternbach and, furthermore, for the element of serendipity to come into play.

Oddly, the progression of the research took an odd turn. The chemistry of certain benzoheptoxdiazines was such that hydrogenation produced quinazolines and, more specifically, quinazoline-3-oxides. Once again, fate intervened. First, the pharmacological properties of these were, to overstate the case, uninteresting. Second, in the latter part of 1955 the project was halted because other unrelated, more important research avenues were opening up for the chemical company. Then, as Sternbach so eloquently relates, the laboratory had reached a critical stage by April 1957. The clutter was such that there was no space on which to place anything. And for those who have used coffee cups to hold test tubes, when all the racks have been expended, it is a familiar story. But during the laboratory cleanup two crystalline products, a base and a hydrochloride salt, were found in separate containers and the water soluble material was tendered for bioassay. That product was patented in July, 1959 (U.S. Patent 2,893,992 to Hoffman-LaRoche) and given the trademark Librium®.⁶ The entire process from discovery to application had taken only 2 years which is something of a miracle by present day standards. From that point on, many thousand benzodiazepines were synthesized.

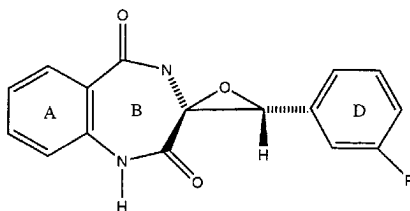


FIGURE 1.1

Structures for cyclophenin and cyclophenol.

R = H Cyclophenin

R = OH Cyclophenol

However, in 1954 a paper was published which, at the time, was of little interest to those working with tranquilizers. The title of the paper, "Studies in the biochemistry of microorganisms. 93. Cyclophenin, a nitrogen-containing metabolic product of *Penicillium cyclopium* Westling," was largely ignored with the exception that the metabolite had demonstrated slight antibiotic activity against *Micrococcus pyogenes* var. *aureus*, and *Escherichia coli*. At best, it could be hoped that synthetic modification would produce a practical antibiotic. But, what was important was the disclosure of the structure,⁷ a benzodiazepine (Figure 1.1). In addition, a second benzodiazepine, cyclophenol, was discovered in 1963^{8,9} and it had its genesis in *Penicillium viridicatum*. Again, the significance of the benzodiazepine structure appears to have fallen on barren ground. It is a point of irony that the original sample of cyclophenin, extracted from *P. cyclopium*, was later shown to be composed of cyclophenin and cyclophenol. It was not until another 20 years had elapsed following the initial discovery that the extent of the biological properties of both cyclophenin and cyclophenol were published.¹⁰ First, it was shown that crude extracts from an aberrant strain of *Penicillium cyclopium* (NRRL 6233) contained cyclophenin and cyclophenol in a ratio of 1:4 and that the crude extract inhibited the growth of etiolated wheat coleoptiles. Upon purification and identification, cyclophenin was shown to inhibit coleoptiles at 10^{-3} and 10^{-4} M, 100 and 33%, while cyclophenol inhibited only 20% at 10^{-3} M, relative to controls. In other intact plant bioassays, cyclophenin induced malformations in the first set of trifoliate leaves of 9-day-old bean (*Phaseolus vulgaris* L. cv. Black Valentine) at treatments of 10^{-2} M and stunting and necrosis in 1-week-old corn (*Zea mays* L. cv. Norfolk Market White). But, there were no visible effects on 6-week-old tobacco plants (*Nicotiana tabacum* L. cv. Hick's). On the other hand, cyclophenol had no apparent effect on bean, corn, or tobacco plants.¹⁰ Conversely, cyclophenol was highly active at rates of 0.025%, while cyclophenin was moderately active at the same concentration, against *Phytophthora infestans*, late blight of potato (*Solanum tuberosum* L.).¹¹ Significantly, this fungus was responsible for the Great Famine in Ireland during the years 1846 and 1847 and led to a massive emigration of those surviving starvation and typhus.¹² Fully one quarter of the population left home aboard the coffin ships, so-called because many souls were lost at sea, the most notable being, quite by chance, the Titanic upon which many emigrants were sailing to the New World in 1912. Oddly, the organism had only been recorded in the U.S. and Europe in 1840,¹³ but it is now a perennial problem in the potato-growing areas of the world and the applications of fungicides required to control the organism ranges from 8 to 18 per season in the U.S.

Pharmaceutically, cyclophenin induced drowsiness in day-old chicks within 2 h when dosed in corn oil *via* crop intubation, at doses of 250 mg/kg. At rates of 500 mg/kg chicks exhibited intoxication, ataxia, prostration, but were not paralyzed, within 1 h. Doses of 25 and 125 mg/kg failed to produce any external symptoms. In all cases, the chicks had fully recovered within 18 h, although those treated at the highest rate remained slightly drowsy. However, cyclophenol induced no response.¹⁰ This poses the question as to the utility

of the hydroxyl on the D ring of the latter. And does a substitution, either electron withdrawing or donating, appreciably affect the performance of the molecule as a plant growth regulator, fungicide, or anxiolytic agent? A further proposition arises in that the possibility of other benzodiazepines being present in other microorganisms also exists. Also intriguing is the observation that benzodiazepines without a C3-OH generally have long half-lives and are converted to this species by hepatic oxidation, while compounds with C3-OH possess short half-lives because they conjugate with glucuronide and are then excreted in the urine.¹⁴ So, does the epoxide form the C3-OH, and if it does in both cyclophenin and cyclophenol, why then is the latter inactive as a tranquilizer? It is possible that the polar nature of the hydroxyl group on the D ring prevents cyclophenol from entering the central nervous system in therapeutic levels. Obviously, the nature of the substituent at C15 is critical and, perhaps, other sites on the D ring would play an important role.

1.2.1 Bioassay

It must be emphasized that the discovery of the plant growth regulating properties of cyclophenin on intact plants and the tranquilizing properties were predicated on the growth inhibiting effects induced in the coleoptile bioassay, a plant bioassay. Briefly, the assay consists in sowing wheat (*Triticum aestivum* L. cv. Wakeland) on moist vermiculite in plastic dishpans, sealing them with aluminum foil and placing them in the dark at $22 \pm 1^\circ\text{C}$.¹⁵ When the seedlings are 4 days old, they are harvested, the caryopses and roots cut from the shoots, discarded, and the shoots are fed tip first into a Van der Weij guillotine. The apical 2 mm are cut away and the next 4 mm are retained for bioassay. Only one 4 mm section is excised from each shoot. Ten sections are then placed in a test tube, with the crude extract or compound to be tested, in 2 mL of phosphate-citrate buffer, pH 5.6, supplemented with 2% sucrose as a carbon source.¹⁶ Test tubes are placed in a roller-tube apparatus and rotated at 0.25 rpm. Following incubation at 22°C the sections are removed, blotted, placed on a glass sheet that is introduced into a photographic enlarger, and the images ($\times 3$) are recorded and the data statistically analyzed. All manipulations are carried out at 540 nm.¹⁷

1.2.2 Synthetic Benzodiazepines

Because cyclophenin was active in the assay, the synthetic benzodiazepines became an obvious area to explore. Diazepam was the first tested and it inhibited coleoptiles at 10^{-3}M , 100% relative to controls.¹¹ When week-old corn plants (*Z. mays* L. cv. Norfolk Market White) were treated with 10^{-2}M solutions of diazepam, the treated portions of the plants became bleached after 4 days, but a week later the plants had recovered and were green.¹¹ Beans (*P. vulgaris* L. Black Valentine) were not affected by diazepam at concentrations that ranged from 10^{-2} to 10^{-4}M . Other benzodiazepines tested in the coleoptile bioassay included lorazepam, oxazepam, clorazepate dipotassium, temazepam, prazepam, flurazepam dihydrochloride, triazolam (Halcion®), alprazolam (Xanax®), and chlordiazepoxide (Figure 1.2). Of these, diazepam, lorazepam, clorazepate dipotassium, temazepam, prazepam, and flurazepam dihydrochloride significantly inhibited ($P < 0.01$) coleoptiles 100% at 10^{-3}M . Triazolam and alprazolam, which are structurally very similar and differ only by the substitution of a fluorine for a chlorine in the latter, significantly inhibited ($P < 0.01$) 42% at 10^{-3}M , while chlordiazepoxide inhibited 40% at 10^{-3}M , and oxazepam inhibited 34% at 10^{-3}M , relative to controls. Of all the compounds tested, lorazepam also was significantly active at 10^{-4}M . However, when applied to week-old corn plants (*Z. mays* L. cv. Norfolk Market White) only flurazepam dihydrochloride and chlordiazepoxide

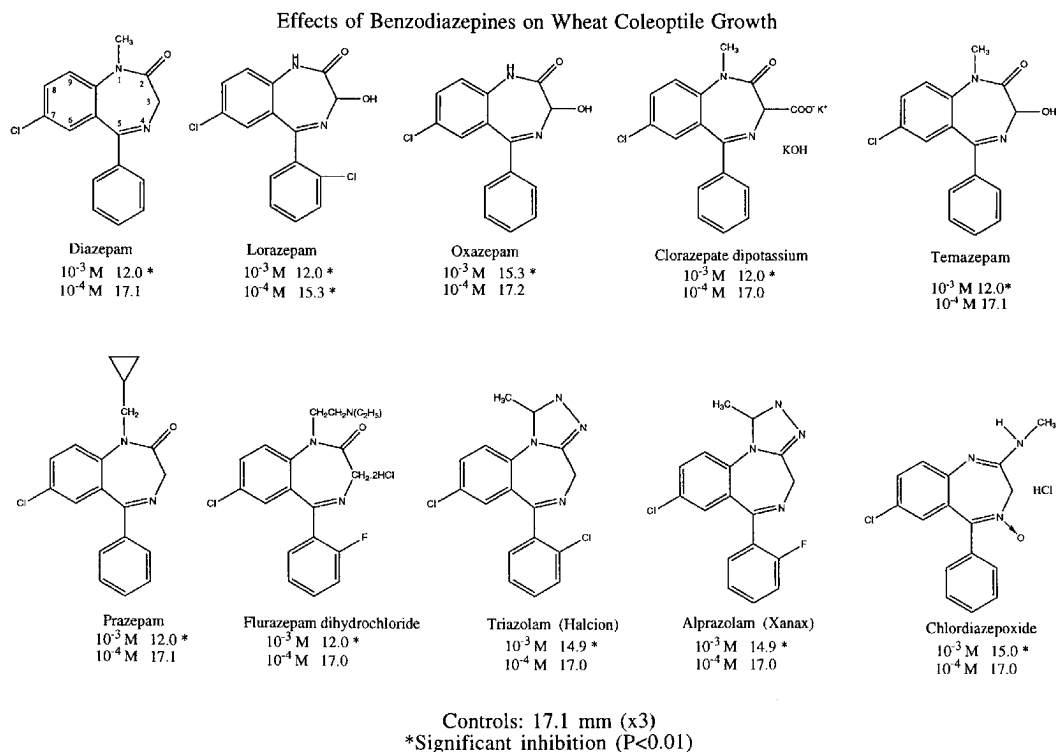


FIGURE 1.2

Structures for assorted benzodiazepines.

induced necrosis within 48 h when applied at 10⁻²M, but the plants resumed normal growth a week later. None of the compounds affected week-old bean plants (*Phaseolus vulgaris* L. cv. Black Valentine).¹⁸

It is of interest that the etiolated wheat coleoptile bioassay detected biological activity with the benzodiazepines which, for the most part, are medicinals. It is also capable of detecting antimicrobials, immunosuppressants, anti-amoebic, and “other” biologically active materials.¹⁷ But, it does not detect neurotoxins. Furthermore, an amplification effect has been observed in that if biological activity is noted in the bioassay then when the “correct” niche for the active metabolite is found, the specific activity of that substance increases by a factor of several hundred times.¹⁸ Hence, while the assay was originally designed to detect plant growth promoting and inhibiting compounds, it also is a very powerful tool for detecting pharmaceutical and other compounds. The mechanism of action by which the bioassay detects structurally diverse, biologically active materials is not known, but it is highly probable that a quick detection *in vitro* system can be developed, thus reducing the assay time from 18 h to less than 1 h.

1.2.3 Phenoxy Compounds

The phenoxy compounds have a long and illustrious history in the field of agrochemicals and much has been written concerning their application. The most famous are 2,4-dichlorophenoxyacetic acid (2,4-D) and its congener 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,4-D had its genesis in troubled times which accelerated its development. In World War II (1939 to 1942), the Allies found themselves fighting a jungle war in Asia. While the jungle

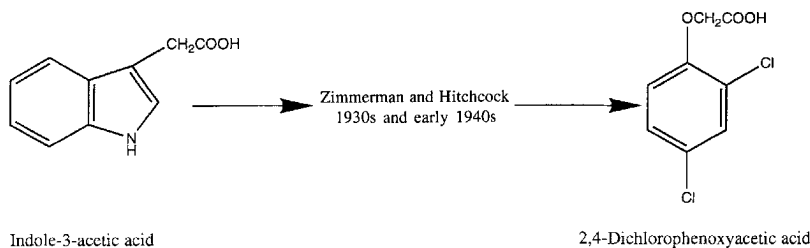


FIGURE 1.3

Schema for transition of indole-3-acetic acid to 2,4-D.

is an excellent environment to fight a guerilla war and hide equipment, it becomes difficult terrain for the hunter to find his quarry and to take a straight shot at an enemy through undergrowth and foliage. 2,4-D was extremely active at low concentrations in killing dicotyledonous plants and its derivative, 2,4,5-T, was most effective against shrubs and defoliating trees. These were still the chemicals of choice during the Vietnam conflict and were used under the name Agent Orange.¹⁹ Unfortunately, the demand exceeded the available supply during that conflict and in order to meet production demands the reaction temperature was increased to raise the Q_{10} of the synthesis rate with disastrous results because a side product, the potent teratogen dioxin (2,3,7,8-tetrachlorodibenzo [*b,e*] [1,4] dioxin), was produced.²⁰ One of the major industrial accidents with this material occurred in Seveso, Italy on July 10, 1976, with disastrous result to the inhabitants.

The history of the development of 2,4-D and 2,4,5-T is somewhat murky. Discussions with Zimmerman and Hitchcock indicate that they realized that the structure of indole-3-acetic acid (IAA) might be a good template for synthesizing agriculturally useful compounds. IAA was a phenyl pyrrole and making substitutions on the phenyl or pyrrole ring might give active compounds (Figure 1.3). Also, naphthalene acetic acid had been shown to have high specific activity as a plant growth regulator. As an interjection, but cogent point, Lawrence J. King who worked at the Boyce Thompson Institute (the same location as Zimmerman and Hitchcock) proposed that a carbamate derivative of naphthalene acetic acid would possibly be a herbicide, or plant growth regulator, and asked his colleague Joseph Lambrecht to make..." six novelty carbamates".²¹ One of these turned out to be not a herbicide but, rather, an insecticide marketed as Sevin®. So the indole ring, the acetic acid group, and probably some other features combined in the minds of Zimmerman and Hitchcock to produce 2,4-D. Their work was carried out in the late 1930s and early 1940s. At about the same time, work was progressing in England on 2,4-D but it was cloaked in the Official Secrets Act, because of the war. The first disclosure of the synthesis of 2,4-D and 2,4,5-T was in 1941²² by Pokorny in an exceptionally brief, 27-line, one quarter page report from the C. B. Dolge Company and the patents issued were: British Patent 573,476 in 1945, and U.S. Patent 2,471,575 to U.S. Rubber in 1949. Obviously the pursuit of patents was rather loose in the early years of agrochemical discovery!

Apart from being used as a broadleaved postemergence herbicide, 2,4-D also has been used to control grasses in sugarcane at very high application rates²³ even though it is not considered to be a grass herbicide. It is also used as a yield enhancer at rates of 4 to 200 mg/L in grapefruit, lemons, and oranges when the fruit is approaching one in. in diameter, which causes the fruit to increase in size. Additionally, it is used to control fruit drop in citrus trees that are over 6 years old. The storage life of lemons may be increased and ripening inhibited by treating fruit with solutions containing 500 mg/L.²⁴

Because of their high specific activities, 2,4-D and 2,4,5-T became model templates for the production of a number of plant growth regulators. In 1945, 2-(3-chlorophenoxy)-propanoic

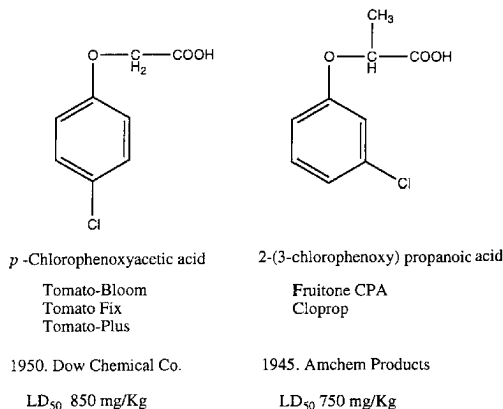


FIGURE 1.4

Structures of chlorophenoxy plant growth regulators.

acid (Figure 1.4) appeared on the market under the trade name Fruitone[®] and it was used in pineapple to reduce crown growth, increase fruit size and weight, and to lower shipping costs because of injury reduction in the shipping process.²⁴ A congener of Fruitone[®], called Tomato Bloom[®], was *p*-chlorophenoxy acetic acid and it found use as a compound to increase fruit set in tomatoes.²⁴ The material is applied at 500 mg/L to tomato flowers as they open although it is not necessary to spray all the flowers to induce fruit set. By way of an oddity, it also is used as a soak for mung beans (*Phaseolus aureus*) to inhibit root growth, but the seed has to be washed with water following the 5 to 8 h treatment.²⁴

One of the first pharmaceutical phenoxy compounds was clofibrate, ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate (Figure 1.5). It is administered to control type III hyperlipoproteinemias, where the triglycerides and total cholesterol are elevated, although it is moderately active against type IIb and type IV cases. In the former, triglycerides are slightly elevated and cholesterol levels are high while, in the latter, triglycerides are moderately-to-highly elevated and cholesterol is normal-to-elevated. Upon ingestion, esterases act upon clofibrate to yield the free acid which then binds to serum albumin in the blood. Clofibric acid (Figure 1.5) also can be given, but it absorbs more slowly into the bloodstream relative to the ethyl ester. It has been reported that the most efficient form of the medicinal is the aluminum salt.²⁵ The mode of action of clofibrate is inhibition of *sn*-glyceryl-3-phosphate acyltransferase, the enzyme necessary for the conversion of acetate to mevalonate. It also acts in the liver where it inhibits 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and subsequently controls cholesterol biosynthesis.¹⁴

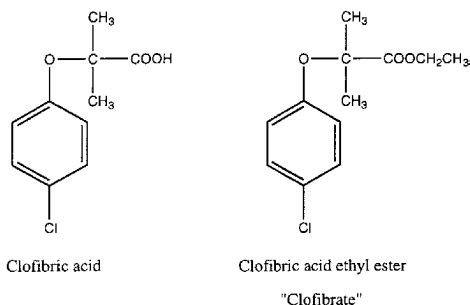


FIGURE 1.5

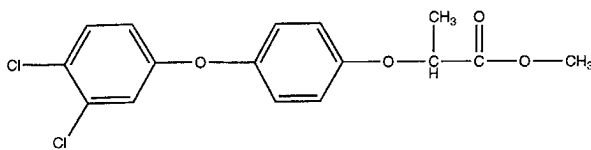
Structures of clofibric acid and clofibrate.

For those involved with agricultural chemicals, the structure of clofibrate and clofibric acid is an intellectual magnet because of the similarities to those compounds already discussed and, therefore, it was surmised that both had plant growth regulatory properties. On examining them in the etiolated wheat coleoptile bioassay it was determined that clofibrate significantly inhibited ($P < 0.01$) 100% at both 10^{-3} and 10^{-4} M. On the other hand, clofibric acid inhibited 100 and 60% at 10^{-3} and 10^{-4} M, relative to controls. This was interesting in that wheat is a monocotyledonous plant, and 2,4-D mainly inhibits the growth of dicotyledonous plants. 2,4-D inhibits the growth of wheat coleoptiles at 10^{-3} and 10^{-4} M, 100 and 50%, respectively, and significantly promotes growth at 10^{-5} and 10^{-6} M, 37 and 34% respectively. At 10^{-2} M, clofibrate induced the production of leathery, malformed leaves in week-old bean plants (*P. vulgaris* L. cv. Black Valentine) 10 days following treatment and they were inhibited approximately 50 and 10%, at 10^{-2} and 10^{-3} M, respectively, compared with controls. With clofibric acid, bean plant first trifoliates were even more leathery and malformed than those treated with clofibrate at 10^{-2} M; intact plants were inhibited 50% relative to controls at the same concentration, 1 week following treatment.¹⁸ Clofibrate, at 10^{-2} M, induced chlorotic streaks on the leaves of week-old corn plants (*Z. mays* L. cv. Norfolk Market White) within 48 h and, after 10 days, plants were inhibited approximately 50% at 10^{-2} M, but the new leaves exhibited no necrosis. With clofibric acid, at 10^{-2} M, week-old corn plants were inhibited and necrotic at 72 h following treatment. Jones et al. were awarded the patent that included both clofibrate and clofibric acid (British Patent 860,303 which corresponds to U.S. Patent 3,262,850 to ICI in 1961 and 1966). But whether the chemistry of these products for use as pharmaceuticals was based on the earlier herbicide discovery impetus with phenoxy compounds remains quite unclear. One point is certain and that is that the aryloxyphenoxypropionate herbicides inhibit the formation of fatty acids thereby interrupting lipid biosynthesis which is essential for the growth of the plant. The susceptible enzyme is acetyl-CoA carboxylase. However, only perennial and annual grasses are affected, while broadleaved plants are not.

The most recent phenoxy herbicides to reach the market are: 2-(4-dichlorophenoxy) phenoxy-methyl propanoate, Hoelon®, 1974; Butyl 2-[4-(5-trifluoro-2-pyridyloxy)phenoxy] propionate, Fusilade®, 1980; Methyl 2-(4-((3-chloro-5-trifluoromethyl)-2-pyridinyloxy)-phenoxy) propanoate, Verdict/Gallant®, 1981; and Ethyl (*R*)-2-[4-[(6-chloro-2-benzoxazolyl)-oxy]-phenoxy] propanoate, Whip®, 1982 (Figure 1.6). All are grass herbicides, in contrast to their progenitor 2,4-D, and it remains to be determined as to whether their structures will influence pharmaceutical applications.

1.2.4 Organophosphates

An odd chemical marriage has taken place between agrochemistry, pharmaceuticals, and the modern disease Alzheimer's. And, like some unions, it has come as something of a surprise to the parties concerned, although the sequence of events is quite logical. Alzheimer's is one of the major diseases affecting the elderly and it is manifest by the loss of short-term memory, cognitive skills, and a general dementia to a greater or lesser degree. The emotional stress on families is incalculable and the institutional costs are exorbitant. While the emotional and social consequences are extremely complex, the chemistry by which the disease progresses is, in comparison, relatively simple. In the aging process, neurons in the brain degenerate, especially in the hippocampus where they play a special role in the accession of new memory. Chemically, the neurons are acetylcholine receptors (cholinergic), but the enzyme acetylcholinesterase also is present in the chemical equation and it plays a role in keeping the acetylcholine in balance under normal conditions. As a protein, it has certain properties and its activity is governed by specific attributes. Among these are the anionic

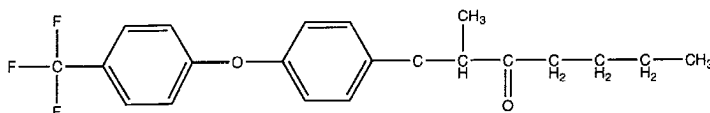


2-(4-(2,4-dichlorophenoxy)phenoxy)-methyl propanoate

Hoelon

1974 Hoechst AG

Preemergence and postemergence grass herbicide

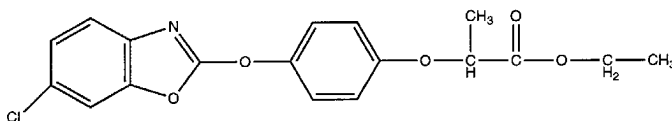
LD₅₀ 2140 mg/kg

Butyl 2-[4-(5-trifluoro-2-pyridyloxy)phenoxy] propionate

Fusilade

1980 Ishihara Sangyo Kaisha Ltd of Japan

Control grass weeds

LD₅₀ 3328 mg/kg

Ethyl (R)-2-[-4[(6-chloro-2-benzoxazolyl)oxy]phenoxy] propanoate

Whip

1982 Hoechst AG

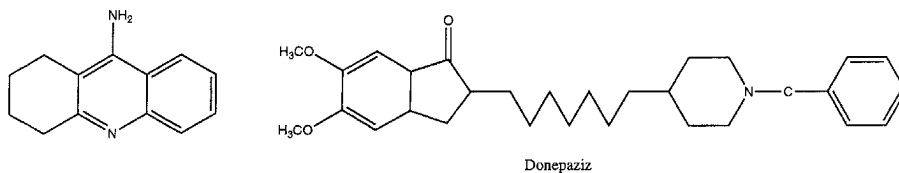
Grass herbicide

LD₅₀ 2357 mg/kg

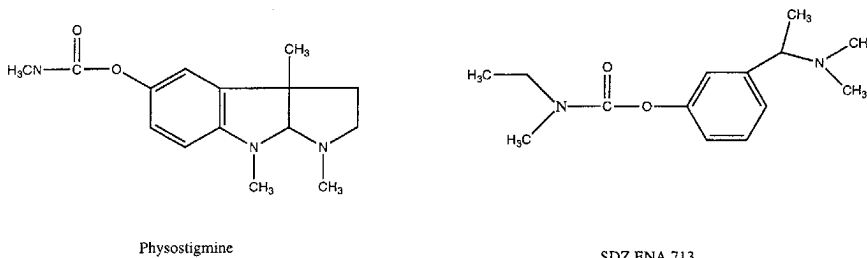
FIGURE 1.6

Structures for Hoelon, Fusilade, and Whip.

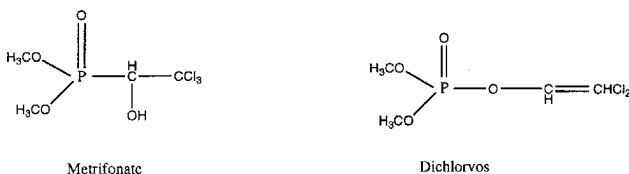
and esteratic sites. Glutamic acid, with its γ -carboxylic group, has a free COO^- function which constitutes the anionic segment, while the esteratic segment is constituted from a tyrosine residue, two imidazole groups from histidine residues, and a serine residue.¹⁴ Because of the dynamics involved which include pH and, therefore, pKas, the molecular formation and conformation of interacting compounds fall into three categories. First are the reversible inhibitors of acetylcholinesterase and include tachrine and donepezil (Figure 1.7), while the second group includes the pseudo- or semireversible compounds such as physostigmine from the dried seeds of *Physostigma venenosum* and SDZ ENA 713 (Figure 1.7). The third group are irreversible and include sarin, the nerve agent released by terrorists in a Tokyo subway in 1995, and dichlorvos. The reason that the latter are considered to be irreversible is because certain organophosphates esterify the serine residue on acetylcholinesterase, but hydrolysis of the phosphorylated serine species to phosphoric acid and the free acid is extremely slow. Enzymes that have been exposed to organophosphates undergo aging, that is, the phosphorylated moiety becomes permanently affixed to the enzyme generating a fully inactivated species so that the original enzyme cannot be



Reversible Acetylcholinesterase Inhibitors



Pseudo-/Semireversible Acetylcholinesterase Inhibitors



Irreversible Acetylcholinesterase Inhibitors

FIGURE 1.7

Classes of cholinesterase inhibitors.

recovered. Conversely, physostigmine is a competitive inhibitor with acetylcholine for acetylcholinesterase and is, thus, a reversible inhibitor.

If there is reduced production of acetylcholine in Alzheimer-compromised patients then selective inhibition of acetylcholinesterase would seem to be an appropriate control measure. In fact, one of the first drugs used to treat patients was metrifonate, *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate (Figure 1.7) an organophosphate that was originally prescribed for controlling schistosomiasis. Its mode of action consisted in paralyzing the organism rendering it susceptible to phagocytosis.¹⁴ In addition, the compound was an acetylcholinesterase inhibitor and it produced parasympathetic nerve stimulation, all of which were considered side effects relative to its main purpose. Following oral administration, the major metabolic product was dichlorvos (Figure 1.7). The disclosure that dichlorvos, phosphoric acid 2,2 dichloroethenyldimethyl ester, has pharmaceutical properties comes as a surprise to agricultural chemists because of its extensive use as an insecticidal fumigant whose LD₅₀ in rats is 70 mg/kg. It was sold under various trade names including dichlorvos, DDVP, dichlorophos, and vapona. The U.S. Patent 2,956,073 was granted to Shell in 1960.

Recent experiments have been conducted in rats and rabbits. With respect to the former, it has been shown, *in vitro*, that metrifonate induces acetylcholinesterase inhibition through the slow release of dichlorvos in rat brain and blood serum.²⁶ Proof of this biotransformation

lies in the fact that the metrifonate-induced acetylcholinesterase inhibition had the same pH dependence as its dehydrochlorination to dichlorvos, while the cholinesterase inhibition induced by dichlorvos was not pH dependent. Dichlorvos cholinesterase inhibition also was governed by a competitive interaction with the catalytic site of the enzyme and this resulted in irreversible inhibition within minutes; that is, addition of more substrate did not improve drug dissociation or enzyme activity. While the same effects were noted with physostigmine and tetrahydroaminoacridine, their effects were reversible although the means by which this was achieved was different in each case. *In vivo* experiments also have been carried out on 3- and 19-month-old rats, with metrifonate and dichlorvos, measuring cholinesterase in forebrains, erythrocytes, and blood plasma. A single dose of either compound induced cholinesterase inhibition in both brain and blood in conscious rats, and the effects were dose dependent and completely reversible. At the same time, there was good correlation between brain and blood cholinesterase activity. Oral doses of 10 mg/kg of dichlorvos in 3-month-old rats induced maximum inhibition within 15 to 45 min in the brain, and 10 to 30 min in erythrocytes and plasma, with recovery of cholinesterase activity in the plasma within 12 h. However, brain and erythrocyte cholinesterase did not reach control levels after 24 h. Metrifonate had similar, but delayed activity, so that peaks were reached in 45 to 60 min in the brain, and 20 to 45 min in the blood, after dosing, followed by complete recovery in 24 h. Notably, 19-month-old rats were more sensitive to both compounds.²⁷

Rabbit assays also have been useful in evaluating metrifonate as a cholinesterase inhibitor.^{28,29} The compound improved eyeblinking in aging rabbits without inducing the undesirable side effects observed with other acetylcholinesterase inhibitors and, additionally, the dose-dependent results were predictable. Associative learning also was improved from 30 to 80%.²⁸

While rat and rabbit bioassays have given promising results, experiments also have been conducted with Alzheimer's patients using both oral and transdermal delivery systems. Both the pharmacokinetics and pharmacodynamics have been evaluated in oral treatments with metrifonate and dichlorvos, the net results being that metrifonate and, subsequently, dichlorvos because it is the catabolic product, is potentially useful as a treatment.³⁰ Transdermal patches also show promise for metrifonate, dichlorvos, and other cholinesterase inhibitors such as physostigmine, eptastigmine, and tacrine.^{31,32}

1.3 Epilogue

While the connection between certain agrochemicals and pharmaceuticals has been presented, there is another area of chemistry that has not been covered. Briefly, the indole nucleus appears in both plant and animal products, although it may be argued that the latter are the result of the ingestion of plants. Research with indole-3-acetic acid (IAA), sometimes referred to as auxin, has generated hundreds of research articles over the past 50 years and the compound is considered to play a major role in plant growth and development. The indole nucleus also appears in tryptophane, a precursor for IAA by way of deamination, which of itself is an essential amino acid for animals. Coincidentally, one of the original isolations of IAA was from human urine when Fritz Kögl (1897–1959) was working with schizophrenic patients who appeared to metabolize indole in an atypical fashion. Serotonin, 5-hydroxytryptamine, also is synthesized from tryptophane in the brain where it plays a part in regulating central and peripheral nervous systems.³³

Another natural product that has fascinated plant scientists since its discovery in the 1950s is abscisic acid. First found in the buds of *Acer pseudoplatanus*^{34,35} and cotton bolls, *Gossypium hirsutum* L.,³⁶ it plays a significant role in keeping buds dormant, controlling abscission of cotton bolls, opening and closing of stomata in leaf surfaces to control gas exchange, and it protects plants from drought.³⁶ It also mediates other plant functions. It is ubiquitous and it has also been found in various fungi including *Cercospora rosicola*,³⁷ *Cercospora cruenta*,³⁸ and *Botrytis cinerea*,³⁹ although its function is not known. Oddly, it has been found in animal brains including pigs, rats,⁴⁰ rodents, and ruminants,⁴¹ where it may be an integral part of the chemistry, but no role has been ascribed to it.

In the final analysis the relationship between agrochemistry and pharmaceuticals will prove to be an interesting association, not only from an academic perspective, but also a financial one.

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