

Abundant references in the literature state, and a casual inspection of the data presented may lead to the conclusion, that the concentration of an insecticide in milk will rise rapidly for a matter of a few hours or days following initiation of the experiment and then level off at a plateau characteristic for each concentration of chemical in the diet. A closer inspection of the data suggests the possibility that the concentration of insecticide may continue to show slight increase over a period of several months. In considering DDT as a typical example, Figure 1 illustrates the trends developed during the periods of storage and dissipation.

Figure 2 is presented to show graphically the relative propensities for storage

and/or excretion of the insecticides tested at the end of the 16-week feeding period.

Weight gains and milk production of treated cows were comparable to those of untreated animals. Throughout the feeding period, and at slaughter, in spite of some of the high levels of insecticide fed, an examination of organs and tissues failed to show any evidence of pathology.

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## HERBICIDE STRUCTURE AND ACTIVITY

# The Action of Alkyl *N*-Phenylcarbamates on the Photolytic Activity of Isolated Chloroplasts

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Inhibition of the photolytic activity of isolated turnip green (*Brassica* spp.) chloroplasts by various alkyl *N*-phenylcarbamates was investigated. All highly active carbamates possessed a free imino hydrogen atom. Substitution of this hydrogen by an alkyl or aryl group resulted in a loss in, or decrease of, inhibitory power. Possible roles played by the various substituents of the carbamate molecule in inhibiting the photolytic reaction are discussed. Responses obtained were correlated with the herbicidal activity of the same compounds reported by other workers from greenhouse studies. In both the laboratory and greenhouse studies maximum activity was obtained with the propyl and butyl esters and when the ring was halogenated in the meta position.

THE ACTION OF ESTERS of carbamic acid and *N*-substituted carbamic acids on animals and plants has held the interest of investigators for many years. Initially the compounds most intensively studied were ethyl carbamate (urethan, EC) and ethyl *N*-phenylcarbamate (phenylurethan, EPC). Research on this group of compounds was given new impetus when the herbicidal properties of isopropyl *N*-phenylcarbamate (IPC) and isopropyl *N*-(3-chlorophenyl)carbamate (3-CIPC) were discovered. The last two compounds are currently being used to control weeds in certain crops.

Carbamates such as EPC and IPC have a profound effect on cell division and mitosis, and are most active as herbicides when applied to the soil and subsequently absorbed by roots. Monocotyledonous plants are more susceptible to their action than dicotyledonous plants. These effects, together with their history and development, have been concisely and adequately reviewed in the literature.

The exact mechanisms through which the carbamates (urethans) exert their

phytotoxic effects are not known. EC and EPC are reported to inhibit photosynthesis of microorganisms (25-28). More specifically, they inhibit the photolytic cleavage of water in photosynthesis. In this photolytic (Hill) reaction (12) oxygen is evolved by isolated chloroplasts or chloroplast fragments under the influence of light in the presence of a suitable hydrogen acceptor. This reaction can be considered as "photosynthesis with a substitute oxidant" or photosynthesis without carbon dioxide fixation (2). As chloroplasts (8, 13, 16, 29) and chloroplast fragments (3, 4) have been obtained from different sources and different techniques have been used to follow the course of the photolytic reaction, the amount of inhibition obtained by different investigators with a particular concentration of an inhibitor has shown some variation.

The herbicidal properties, together with the selective action of many of the *N*-phenylcarbamates, have been studied and reported by Shaw and Swanson (20) and George *et al.* (10, 11). The compounds were applied by Shaw and

Swanson as pre-emergent sprays. George *et al.* reported results based on studies with both pre- and postemergent applications.

The specific objectives of this study were to investigate relationships between structural configurations of alkyl *N*-phenylcarbamates and their ability to inhibit the photolytic activity of isolated chloroplasts, and to correlate these responses with the herbicidal properties of the same compounds which have been evaluated by other workers under field and greenhouse conditions.

#### Materials and Methods

Chloroplasts were isolated from turnip greens (*Brassica* spp.), obtained through commercial sources, by the procedure of Spikes *et al.* (27). After the initial isolation, the chloroplasts were washed three times with 0.5*M* sucrose.

The photochemical activity of the isolated chloroplasts was measured in 50-ml. beakers. A 10-ml. reaction mixture which had the following composition was used: 0.0002*M* potassium ferricyanide, 0.01*M* potassium chloride,

0.17M sucrose, 0.05M potassium phosphate buffer (pH 6.85), 2% ethyl alcohol by volume, and an aliquot of chloroplasts that contained about 0.3 mg. of chlorophyll. Prior to use in the reaction, the suspension of chloroplasts was maintained in an ice bath in the dark. Chlorophyll concentration was determined by the method of Arnon (1). Tank nitrogen, after being passed through alkaline pyrogallol, was continuously bubbled through the reaction mixture to keep the chloroplasts stirred and in suspension. To trap the heat emitted by the lamp, a water filter was interposed between the lamp and the reaction vessel. Running tap water was passed through this filter. With this arrangement, the temperature of the reaction mixture varied less than 1° C. during the 4-minute illumination period. The lower surface of the chloroplast suspension was illuminated at an intensity of 2800 foot-candles. Under the conditions used in this study, maximum reduction occurred at an intensity of about 750 foot-candles. Consequently, light should not have been limiting the rate of the reaction.

The activity of the chloroplasts was followed potentiometrically at room temperature, about 26° C., with platinum and saturated calomel electrodes connected to a Beckman Model H-2 pH meter. In making measurements, the system without the chloroplasts was permitted to equilibrate in the dark for 2 minutes. The aliquot of chloroplasts was added, the light turned on, and millivolt readings were subsequently recorded over a 4-minute period. These values were converted to micromoles of ferricyanide reduced by means of the relationship given by Spikes *et al.* (21).

Some chloroplast preparations effected a slight reduction of ferricyanide in the dark. This was assumed to be non-enzymatic in character, because it was also obtained with boiled chloroplast preparations. Appropriate corrections were made for this slight dark reduction, whenever it occurred.

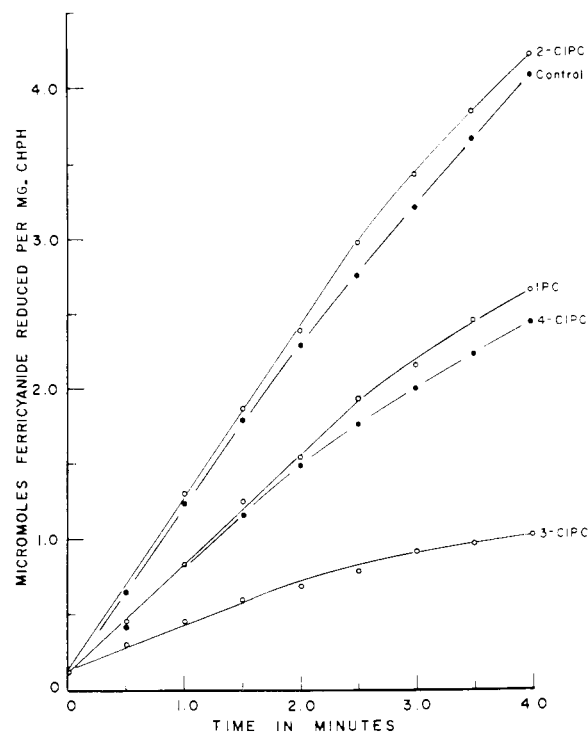
Because most of the alkyl *N*-phenylcarbamates are relatively insoluble in water, solutions of the desired strength were prepared in absolute ethyl alcohol. The final concentration of ethyl alcohol in all the reaction mixtures was held constant at 2% by volume. This concentration of ethyl alcohol had no detectable effect on the reaction.

All determinations were made in either duplicate or triplicate with chloroplasts obtained from at least two different lots of turnip greens. Data are presented as the arithmetic averages of the individual determinations.

## Results

An  $I_{50}$  value of  $1.7 \times 10^{-3}M$  for EPC was obtained under the conditions established for this study. This value falls

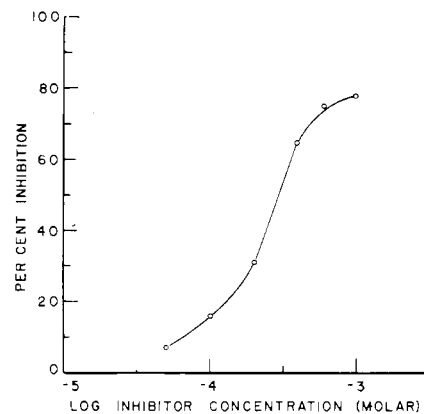
**Figure 1. Effect of isopropyl *N*-phenylcarbamate and its monochlorinated derivatives, at  $6 \times 10^{-4}M$ , on the Hill reaction of isolated chloroplasts**



within the range of those previously reported for this compound.

**IPC and Monochlorinated Derivatives.** Because IPC and 3-CIPC are the *N*-phenylcarbamate derivatives being used as herbicides, their influence on this photochemical reaction was examined initially. The ortho (2-CIPC) and para (4-CIPC) chlorinated isomers were also included in this test, so that the effect of chlorine position could be determined. Responses expressed by these four compounds, together with a control curve obtained in the absence of an inhibitor, are shown in Figure 1. The micromoles of ferricyanide reduced per milligram of chlorophyll were plotted as a function of time during a 4-minute period. All compounds were present at a concentration of  $6 \times 10^{-4}M$ . 3-CIPC produced the greatest amount of inhibition at this concentration. 4-CIPC was slightly more inhibitory than the unchlorinated derivative (IPC). The slight stimulation shown for 2-CIPC was obtained repeatedly. At low concentrations up to 15% stimulation has been noted. However, at higher concentrations some inhibitory action was expressed.

For these carbamates, maximum inhibitory action was obtained at concentrations which saturated the reaction mixture. 3-CIPC and 4-CIPC appeared to possess similar solubility characteristics. The reaction mixture was saturated with both compounds at the  $6 \times 10^{-4}M$  concentration. However, 2-CIPC and IPC were both slightly more soluble. At  $2.5 \times 10^{-3}M$ , a concentration which was on the threshold of saturation for both compounds, inhibition of 39 and 76%, respectively, was obtained.



**Figure 2. Effect of different concentrations of isopropyl *N*-(3-chlorophenyl)carbamate on the Hill reaction of isolated chloroplasts**

Shaw and Swanson (20) compared the herbicidal activity of these monosubstituted derivatives of the isopropyl series. 3-CIPC was the most active member; 2-CIPC was slightly more active than 4-CIPC, but both compounds were much less active than 3-CIPC and possessed very low activity as herbicides. IPC was more active than 4-CIPC. In comparing these results with those obtained with the Hill reaction, some deviation is obtained in the behavior of 2-CIPC.

Wessels and van der Veen (29) also found that the addition of chlorine to the benzene ring of EPC in the meta or the para position enhanced the inhibitory properties as expressed against the Hill reaction. However, they reported identical  $I_{50}$  values for ethyl *N*-(3-chlorophenyl)carbamate (3-CEPC) and ethyl *N*-(4-chlorophenyl)carbamate (4-CEPC).

**Dilution Curve for 3-CIPC.** The inhibition obtained with different concentrations of 3-CIPC is shown in Figure 2. These data were obtained by comparing the micromoles of ferricyanide reduced over a 4-minute period in the presence of various concentrations of 3-CIPC with the amount reduced under identical conditions in the absence of the compound. The decrease in activity was expressed on a percentage basis and is plotted as a function of the log of the molar concentration of the inhibitor. The concentration of 3-CIPC required to reduce the photochemical activity of the chloroplasts by 50% ( $I_{50}$ ) was  $2.9 \times 10^{-4}M$ .

A sigmoidal curve of this general type was obtained with all the inhibitory compounds. Maximum inhibition was obtained with the phenylcarbamates, when the reaction mixture became saturated with the test compound.

#### Dichlorinated Derivatives of IPC.

Of the six benzene ring-substituted dichlorinated isomers, five were available for testing. The 2,6 isomer could not be obtained. The effects of the five dichlorinated isomers, at concentrations of  $1 \times 10^{-4}M$  and  $6 \times 10^{-4}M$ , expressed against the Hill reaction of isolated chloroplasts are presented in Table I.

Compounds substituted at the 2 position were not strong inhibitors. The 2,4 and 2,5 isomers stimulated the reaction even at concentrations which saturated the reaction mixture. Stimulation was obtained with the 2,3 isomer at the lower concentration, but some inhibitory influence was expressed at the higher concentration. Considerable in-

hibition was produced at the higher concentration by the 3,5 isomer. The 3,4 isomer was a more potent inhibitor of the reaction than 3-CIPC.

Wessels and van der Veen (29) gave  $I_{50}$  values for only two dichlorinated derivatives of EPC: ethyl *N*-(3,4-dichlorophenyl)carbamate (3,4-DCEPC) and ethyl *N*-(2,5-dichlorophenyl)carbamate (2,5-DCEPC). 2,5-DCEPC was slightly more active than the unsubstituted EPC. However, 3,4-DCEPC was reported as being the most inhibitory derivative of EPC tested. Based on  $I_{50}$  values, Wessels and van der Veen assigned relative activity ratings to EPC, 3-CEPC, and 3,4-DCEPC of 1, 5, and 25, respectively. By applying the same type of analysis in the present study, relative activity ratings of 1, 4, and 36 could be assigned to IPC, 3-CIPC, and isopropyl *N*-(3,4-dichlorophenyl)carbamate (3,4-DCIPC), respectively (Table II). In the two studies, and with different esters, dichlorination in the 3,4-positions of the ring produced compounds that were much more inhibitory to the photochemical activity of isolated chloroplasts than were the respective meta-chlorinated or nonchlorinated derivatives.

Shaw and Swanson (20) also compared the phytotoxicity of four of the dichlorinated isomers. Their results placed these isomers in the following order of decreasing phytotoxicity: 3,6 (identified as 2,5 in this paper); 3,4, 2,4, and 3,5. Deviation is again encountered between the greenhouse and laboratory studies with the ortho-substituted derivatives. The 2,5 (3,6) isomer and the 2,4 isomer showed no inhibitory activity against the Hill reaction at the concentrations reported in Table I. The 3,4 and 3,5 isomers were active in both studies.

#### Meta-Substituted Derivatives of IPC.

Determinations were made only at a concentration of  $6 \times 10^{-4}M$  with the meta-substituted derivatives of IPC. The objective was to obtain a relative indication of the influence of the radical in inhibiting the reaction. Radicals substituted in the meta position can be ranked in their order of decreasing effectiveness as follows (Table III): bromo = trifluoromethyl, chloro, methyl, ethoxy, hydrogen (IPC), nitro, hydroxy, aceto, and cyano. The most inhibitory derivatives contained one or more halogen atoms. The cyano derivative did not

inhibit the reaction at this concentration.

The ranking given here compares closely with that presented by Shaw and Swanson (20), who reported on the phytotoxic properties of many of these derivatives. They presented the following order based on decreasing activity within the isopropyl series: chloro, methyl, methoxy, trifluoromethyl, nitro, aceto, and hydroxy. The unsubstituted derivative, IPC, would probably be placed after the methoxy derivative in their listing. The only compound that showed a strikingly different response in the two tests was the trifluoromethyl derivative. This proved to be a very strong inhibitor of the Hill reaction. Shaw and Swanson (20), however, reported that this compound was very effective in inhibiting chlorophyll formation in intact plants, but rated it as being only "slightly active" as a preemergent herbicide.

**Alkyl Esters of *N*-(3-chlorophenyl)carbamic Acid.** The inhibitory effects of several alkyl esters of *N*-(3-chlorophenyl)carbamic acid on the photochemical activity of isolated chloroplasts were also compared (Figure 3). The test compounds were present at a concentration of  $6 \times 10^{-4}M$ . The micromoles of ferricyanide reduced per milligram of chlorophyll at the end of 4 minutes for the control and each of the esters are shown on the bar graph. At this concentration the shorter alkyl esters expressed considerable activity. Of the esters tested, the four-carbon *sec*-butyl ester was the most inhibitory derivative. Inhibitory effectiveness decreased as the number of carbons in the side chain increased above four. The eight-carbon capryl and the 12-carbon lauryl esters had essentially no effect on the reaction, at least within the 4-minute period over which the activity was measured. At this concentration, the reaction mixture was saturated with the longer alkyl esters; consequently, maximum inhibitory activity should have been expressed.

The action of these esters relative to

**Table I. Effects of Dichlorinated Isopropyl *N*-Phenylcarbamates on the Hill Reaction of Isolated Chloroplasts**

(Expressed as per cent of control activity)

Ring Substitutions	Concentration	
	$1 \times 10^{-4}M$	$6 \times 10^{-4}M^a$
2,3	105	78
2,4	117	112
2,5	119	106
3,4	27	18
3,5	102	60

<sup>a</sup> Reaction mixtures were saturated with the test compounds at this concentration. Consequently, the actual molarities were not known, but were less than  $6 \times 10^{-4}M$ .

**Table II. Inhibition of the Hill Reaction of Isolated Chloroplasts by Some Isopropyl *N*-Phenylcarbamates**

Compound	Concentration at which 50% Reduction Occurred, Molarity	Relative Activity
Isopropyl <i>N</i> -phenylcarbamate, IPC	$1.2 \times 10^{-3}$	1
Isopropyl <i>N</i> -(3-chlorophenyl)carbamate, 3-CIPC	$2.9 \times 10^{-4}$	4
Isopropyl <i>N</i> -(3,4-dichlorophenyl)carbamate, 3,4-DCIPC	$3.3 \times 10^{-5}$	36

**Table III. Comparison of Inhibitory Action Expressed, on the Hill Reaction of Isolated Chloroplasts, at  $6 \times 10^{-4}M$  by meta-Substituted Derivatives of Isopropyl *N*-Phenylcarbamate**

Derivative	Control Activity, %
Trifluoromethyl	23
Bromo	23
Chloro, CIPC	25
Methyl	49
Ethoxy	58
Hydrogen, no substitution, IPC	61
Nitro	64
Hydroxy	88
Aceto	97
Cyano	101

herbicidal and plant-growth-regulatory potentials has been examined by at least two groups of workers. Both groups also found that maximum activity was expressed by some of the lower alkyl esters. The *sec*-butyl and isopropyl esters of *N*-(3-chlorophenyl)carbamic acid were the most active compounds tested by Shaw and Swanson (20). George *et al.* (10) reported that the lower alkyl esters were generally active. They obtained maximum activity with the ethyl and isopropyl esters. The *sec*-butyl ester was slightly less active in their tests than the isopropyl ester. George *et al.* also obtained a decrease in activity with alkyl esters longer than four carbons. The lauryl ester displayed little or no activity. The isopropyl derivative is the most active of the non-chlorinated alkyl *N*-phenylcarbamates (9, 22, 23).

**Additional *N*-Phenylcarbamates.** To obtain information concerning the roles exercised by various portions of the molecule, the action of certain other *N*-phenylcarbamates was studied. The results obtained at selected molar concentrations of these compounds are presented in Table IV.  $I_{50}$  values for EPC, IPC, and 3-CIPC have also been included in the table for comparative purposes.

Replacement of the imino hydrogen of EPC with an ethyl, phenyl, or benzyl radical resulted in loss of inhibitory activity (Table IV). The herbicidal properties of these compounds have not been reported. However, Norman and Weintraub (17) reported that some repression of the root growth of seedlings was obtained with *N*-phenylcarbamates in which a methyl group was substituted for the imino hydrogen atom. Templeman and Sexton (22, 23) found that the compound formed by the replacement of the imino hydrogen of EPC with a carboethoxy group to give *N,N*-di(carboethoxyaniline) [ $C_6H_5N(COOC_2H_5)_2$ ] was as active in their tests as EPC.

Substitution of a sulfur atom for the carbonyl oxygen of IPC to form isopropyl *N*-phenylthionocarbamate reduced the inhibitory activity shown against the chloroplast reaction (Table IV). It was much less active than the closest related derivative, IPC. This compound was classified as being "very active" by Shaw and Swanson (20).

3-Chloroisobutyranilide proved to be a potent inhibitor of the Hill reaction (Table IV). It was more active than the closely related 3-CIPC and could be assigned a relative activity rating of 18, if its activity was compared with that of IPC. However, it was not as active as the most inhibitory *N*-phenylcarbamate tested (3,4-DCIPC). 3-Chloroisobutyranilide was found to be relatively inactive against intact plants. Shaw and Swanson (20) assigned it a rating of "slightly active."

## Discussion

Many of the alkyl *N*-phenylcarbamate derivatives found to inhibit strongly the photochemical activity of isolated chloroplasts in this study are the ones that have been reported to be highly active as herbicides. Maximum inhibitory activity in both this laboratory investigation and the greenhouse studies of other investigators was expressed when the ring was chlorinated in the meta position in combination with either a *sec*-butyl or isopropyl side chain. Certain derivatives, however, have manifested greater activity in one type of study than they did in the other.

Correlations between responses observed in greenhouse studies with intact plants and laboratory studies with components extracted from plant tissue can only be made with certain reservations. These reservations are introduced by factors associated with field and greenhouse studies. In these latter studies precise quantitative data are rather difficult to obtain and rates at which chemicals are applied cannot be controlled accurately. Because investigators use different species of plants, comparisons between different studies are difficult to make. Other factors that

influence the action of compounds tested under field and greenhouse conditions are the volatility characteristics of a compound, soil moisture and temperature requirements, and ability to withstand microbial decomposition. Some investigators have correlated the volatility of the carbamates with herbicidal activity. Many of these factors are either easier to control in, or do not enter into, laboratory studies. Nevertheless, cognizance must be given to them in the development of correlations between laboratory, greenhouse, and field studies.

The action of the carbamates is probably not simple. Consequently, interference with more than a single system is probably involved in the control of growth by the *N*-phenylcarbamates. If more than one system is sensitive to the action of any inhibitor, the most sensitive system should be affected first. However, if the sensitivity differential between systems is rather small, several may be affected simultaneously. Under a given set of conditions, photosynthesis may be one of the systems sensitive to the *N*-phenylcarbamates. Conceivably, interference with photosynthesis may be involved in the growth-controlling action of these compounds.

The successful entry into, and sub-

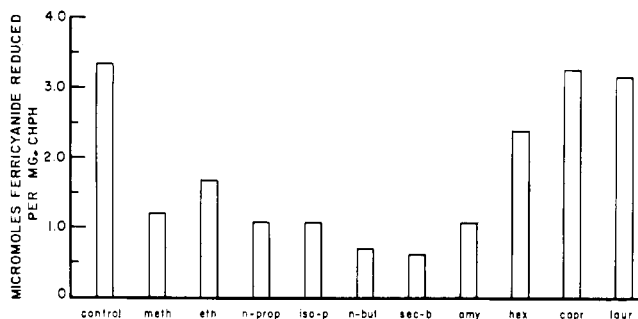


Figure 3. Interference with the Hill reaction of isolated chloroplasts by alkyl esters of *N*-(3-chlorophenyl)carbamic acid at  $6 \times 10^{-4}$  M in 4 minutes

Table IV. Effects of Various Alkyl *N*-Phenylcarbamates on the Hill Reaction of Isolated Chloroplasts

Compound	Concentration, M	Control Activity, %
Ethyl <i>N</i> -phenylcarbamate, EPC	$1.7 \times 10^{-3}$	50
Ethyl <i>N</i> -ethyl <i>N</i> -phenylcarbamate	$2.0 \times 10^{-2a}$	64
Ethyl <i>N,N</i> -diphenylcarbamate	$1.8 \times 10^{-3}$	127
	$1.8 \times 10^{-3a}$	137
	$6.0 \times 10^{-4a}$	132
	$1.0 \times 10^{-4}$	111
Ethyl <i>N</i> -phenyl <i>N</i> -benzylcarbamate	$1.8 \times 10^{-3a}$	110
	$6.0 \times 10^{-4a}$	119
	$1.0 \times 10^{-4}$	116
Isopropyl <i>N</i> -phenylcarbamate, IPC	$1.2 \times 10^{-3}$	50
Isopropyl <i>N</i> -phenylthionocarbamate	$1.2 \times 10^{-3a}$	79
	$6.0 \times 10^{-4a}$	89
	$1.0 \times 10^{-4}$	100
Isopropyl <i>N</i> -(3-chlorophenyl)carbamate, 3-CIPC	$2.9 \times 10^{-4}$	50
3-Chloroisobutyranilide	$6.6 \times 10^{-5}$	50

<sup>a</sup> The reaction mixture was saturated with the test compound at this concentration. Consequently, the actual molarity was not known, but was less than that indicated.

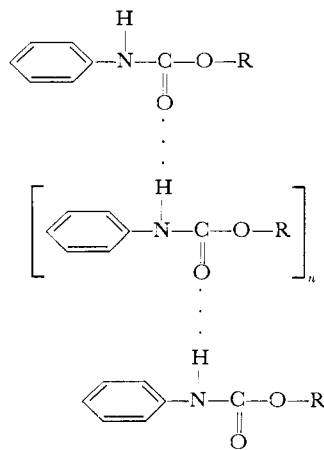
sequent movement within, a plant is considered to be dependent upon the molecular configuration and chemical and physical properties possessed by a particular compound. However, once within a plant the same configuration and chemical and physical properties may not necessarily be required to produce a maximum effect at the reactive site(s). Correlations such as those attempted in this paper may help differentiate these requirements.

EC and EPC are classified as narcotics in the literature. Classically, narcotics have been considered to act as inhibitors by being adsorbed on catalytically active surfaces. The carbamates may act by indiscriminately covering the surface of the chlorophyll-protein complex in the Hill reaction or through specific adsorption at an active site. Because adsorption is possibly involved in this action, consideration can be given to the forces involved and the roles played by the different constituents of the alkyl *N*-phenylcarbamate molecule. The chemical behavior of this group of compounds and the response shown in the chloroplast system are such as to eliminate the involvement of ionic or covalent bonds. The carbamates can be removed from the chloroplasts by washing and photolytic activity is subsequently restored. The binding forces involved are more than likely relatively weak. Hence, hydrogen bonding and van der Waals electrostatic attraction forces are possibly involved. The atoms of the alkyl *N*-phenylcarbamate molecule that can take part in hydrogen bond formation are the imino hydrogen, carbonyl oxygen, and the ring-substituted chlorine atom(s).

The imino hydrogen appears to play an important role in this reaction. It may take part in hydrogen bond formation with some electronegative constituent located either at or near the reactive center in the chloroplasts. Evidence in support of this suggestion is derived from two sources: compounds in which the imino hydrogen is replaced are not potent inhibitors of the photochemical reaction and derivatives with chlorine substituted at an ortho position of the benzene ring lack inhibitory properties. The chlorine in the ortho position may be able to form a hydrogen bond intramolecularly—chelate-ring formation—with the imino hydrogen, or through steric influence it could prevent an electronegative group from approaching close enough to the imino hydrogen to form a hydrogen bond. Chlorines substituted at the meta and para positions of the ring are too far removed from the imino hydrogen to exert these influences.

Barker *et al.* (5) made an extensive study of hydrogen bonding involving the imino hydrogen of carbamic acid esters. They found that compounds

such as EPC could undergo association by means of hydrogen bonds to form polymers of the following type:



The carbonyl oxygen of one molecule is able to bond with the imino hydrogen of a second molecule. This can be repeated to form a polymer composed of several carbamate molecules. Association was essentially prevented by chelate-ring formation with the imino hydrogen by suitable ortho-substituents, steric influences introduced by other ortho-substituents, and replacement of the imino hydrogen by an alkyl or phenyl group. These observations may explain the low potency expressed by ortho-chlorinated and imino-substituted phenyl carbamates in the present study.

The tendency to form associations is a function of the concentration of the compound—i.e., the greater the concentration, the longer the polymers formed. Possibly, the polymers formed are more efficient inhibitors of the photochemical reaction than the monomers. That associated products may be important in this reaction can be postulated from the observation that marked inhibitory activity was obtained as the reaction mixture approached saturation with the carbamate tested.

Replacement of the carbonyl oxygen with a sulfur atom to form a thionocarbamate—isopropyl *N*-phenylthionocarbamate—produced a compound that was only a weak inhibitor of the photochemical reaction. Sulfur may not form hydrogen bonds. However, if it did participate in hydrogen bond formation, the bonds formed could be expected to be very weak (15). Because of this behavior, orientation and action of the carbamate molecule may also involve the formation of hydrogen bonds between the carbonyl oxygen of the carbamate group and the imino hydrogen on the peptide nitrogen at the reactive site. Bonding which involves these groups occurs in proteins and is considered to be responsible for maintaining the secondary and tertiary structure of proteins—i.e., holding together the postulated coils and folds of protein

molecules. The carbonyl oxygen of the carbamate molecule can participate in the formation of hydrogen bonds. This was shown in the association studies reported by Barker *et al.* (5).

The chlorophyll molecules and associated factors which mediate the photolytic reaction are probably located within the highly organized chloroplasts. Within the chloroplast, the chlorophyll molecules are concentrated in areas called grana. These grana, in turn, are embedded in a matrix known as the stroma. Both grana and stroma are composed primarily of regularly oriented lipoidal and proteinaceous materials. Because the sites involved in the inhibitory action are probably located on or near the chlorophyll molecules, the inhibitor molecules must possess properties that will enable them to pass through, or partition into, the lipoprotein matrices. Consequently, a balance in the molecule between lipophilic and hydrophilic groupings must be obtained. Such a balance has been recognized as a requirement for the expression of auxin activity (6, 7, 24), and for the penetration of spores by fungicides (14, 18, 19). In the present study, this requirement seems to have been satisfied by the addition of chlorine to the meta position of the benzene ring and by the shorter alkyl groups. Maximum inhibitory activity was manifested when the alkyl group contained either three or four carbon atoms (propyl and butyl derivatives). The decreasing inhibitory activity expressed by derivatives with alkyl groups containing more than four carbon atoms may be due to solubility characteristics which prevented them from reaching the reactive sites.

It is not known whether the ring-substituted chlorine plays a direct or an indirect role in inhibiting the photochemical reaction. Conceivably, the chlorine atom could influence the reaction by affecting the liposolubility of the molecule, participating in hydrogen bond formation, influencing the strength of the hydrogen bond formed with the imino hydrogen, controlling the spatial configuration assumed by the molecule, and orienting more precisely the carbamate molecule at or near the reactive center.

The exact role of, or requirement for, the ester oxygen remains to be determined. 3-Chloroisobutyranilide was the only compound tested which was structurally related to the carbamates and did not contain an ester linkage. This compound was more inhibitory to the photochemical reaction than 3-CIPC, the closely related ester. Actually, it was second in potency to 3,4-DCIPC, which was the most inhibitory alkyl *N*-phenylcarbamate tested. This compound expressed only limited herbicidal activity against intact plants (20).

Undoubtedly, a particular steric configuration is required for an inhibitor to approach the reactive site successfully. The configuration assumed by a molecule is the result of the various actions exerted by its different substituents. The substitution of a bulky chlorine atom at the ortho-position of the benzene ring would prevent the ring from assuming certain spatial relationships relative to the linear portion of the molecule. Consequently, the molecule may not have a steric configuration that would enable it to approach the reactive site. This may also explain the decreased inhibitory activity shown by the ortho-chlorinated derivatives.

Until more information becomes available relative to the mechanisms involved in the Hill reaction, it will not be possible to determine where in the reaction, or how, the alkyl *N*-phenylcarbamates interfere.

Whether interference with photosynthesis is one of the primary ways through which the alkyl *N*-phenylcarbamates exercise their control over plant growth remains to be ascertained. While interference with this system may not be the main way through which control over growth is exercised, it might be a contributory one. If the carbamates do reach the chloroplasts within the plant, interference with photosynthesis should result. The finding by other workers concerning inhibition of photosynthesis by EC and EPC in microorganisms suggests that similar responses could be expected in higher plants. Some of the carbamates used as herbicides should be even more potent inhibitors of this reaction in microorganisms than the ones available to the earlier investigators.

Correlations such as the ones attempted in this paper between laboratory and greenhouse studies should help

elucidate the contribution made by the various parts of a molecule in terms of the over-all effect. Progress in the comprehension of the mechanism of action of herbicidal materials should be accelerated as information of this type becomes available to the investigator.

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## PLANT GROWTH REGULATORS

### Preparation and Plant Growth-Regulating Activity of Crude Protein Hydrolyzate Derivatives of DL-2-(2,4-Dichlorophenoxy)-propionic Acid

THIS STUDY is a continuation of research on amino acid amides of plant growth-regulating acids which has been concerned with basic studies on specific purified amino acid derivatives (3-6, 8). The objective of the present work

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was to modify advantageously the growth-regulating properties of conventional, regulating compounds utilizing readily available, inexpensive mixtures of amino acids and peptides. These mixtures come from the simple acid, alkaline, or enzymatic hydrolysis of abundant proteins.

Previous preliminary findings with amino acid amides have shown that

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amino acid coupling with phenoxy-type regulators produces from little or no effect to marked effect upon various growth-regulating properties of this type of compound. On selected test plants this effect depends upon the kind and optical configuration of the amino acid and the kind of phenoxy acid coupled. It also depends upon the specific plant used.