The action of some derivatives of phenylurethan and of 3-phenyl-1,1-dimethylurea on the Hill reaction

One of the most interesting herbicides of the last years is CMU (3-(4-chlorophenyl)-1,1-dimethylurea)[1]. When plants are sprayed with this substance most of them show no symptoms during the first week. In the second week after spraying they usually die quickly after a severe wilting.

When studying the physiology of the action of CMU we were struck by the fact that photosynthesis in sprayed plants, as measured with the diaferometer[2], is immediately inhibited. By application of CMU to only a part of a leaf it appeared that the substance is rather firmly held by this part. Although after two weeks and more no outward symptoms of any damage are visible on the treated leaf, the part that has absorbed some CMU is still incapable of any photosynthesis, while the rest of the leaf is quite normal. So there is probably no transport of CMU within the green leaf. On the other hand, when CMU is given to the roots it is readily transported throughout the whole plant. This is in accordance with the experiments of HAUN AND PETERSON, who found the same interesting way of transportation by using ^{14}C ring-labeled CMU[3]. It therefore seems probable that CMU is adsorbed on an active site which is essential for photosynthesis.

This activity of CMU and the structural relationship between this compound and phenylurethan prompted us to investigate the action of this substance and of some closely related derivatives on the Hill reaction. The experiments were performed with 2,6-dichlorophenolindophenol as hydrogen acceptor. The photochemical reduction of this dye by isolated chloroplasts was studied by measuring the oxidation-reduction potential during illumination[4,5].

The results obtained indicate that replacement of the ethoxy group in phenylurethan by the dimethylamino group and introduction of a chlorine atom or a nitro or trifluoromethyl group into the benzene nucleus result in an enhanced inhibitory action upon the Hill reaction (see Table I). Introduction of a hydroxyl, dimethylamino or acetylamino group into the benzene nucleus, on the other hand, decreases the activity. Substitution of a chlorine atom or a hydroxyl group in the *meta* position is more effective than substitution in the *para* position. With the nitro derivative, on the other hand, the reverse is observed.

TABLE I

INHIBITION OF THE HILL REACTION BY SOME DERIVATIVES OF PHENYLURETHAN AND OF 3-PHENYL-1,1-DIMETHYLUREA

Substance	*Concentration at which 50% inhibition occurs*	*Relative activity*
Ethyl-N-phenylcarbamate (phenylurethan)	$5 \cdot 10^{-4}$ *M*	1
Ethyl-N-(3-chlorophenyl)-carbamate	10^{-4} *M*	5
Ethyl-N-(4-chlorophenyl)-carbamate	10^{-4} *M*	5
Ethyl-N-(4-nitrophenyl)-carbamate	$2 \cdot 10^{-4}$ *M*	2.5
Allyl-N-phenylcarbamate	$5 \cdot 10^{-4}$ *M*	1
Allyl-N-(4-chlorophenyl)-carbamate	$8 \cdot 10^{-5}$ *M*	6
Ethyl-N-(3,4-dichlorophenyl)-carbamate	$2 \cdot 10^{-5}$ *M*	25
Ethyl-N-(2,5-dichlorophenyl)-carbamate	$3 \cdot 10^{-4}$ *M*	1.7
Benzyl-N-phenylcarbamate	$2 \cdot 10^{-4}$ *M*	2.5
Ethyl-N-(4-hydroxyphenyl)-carbamate	$3 \cdot 10^{-3}$ *M*	0.2
Ethyl-N-(3-hydroxyphenyl)-carbamate	10^{-3} *M*	0.5
3-Phenyl-1,1-dimethylurea	$4 \cdot 10^{-5}$ *M*	12.5
3-(4-Chlorophenyl)-1,1-dimethylurea (CMU)	$4 \cdot 10^{-6}$ *M*	125
3-(3-Chlorophenyl)-1,1-dimethylurea	$2 \cdot 10^{-6}$ *M*	250
3-(3,4-Dichlorophenyl)-1,1-dimethylurea	$2 \cdot 10^{-7}$ *M*	2500
3-(3,4,5-Trichlorophenyl)-1,1-dimethylurea	$2 \cdot 10^{-7}$ *M*	2500
3-(4-Nitrophenyl)-1,1-dimethylurea	$8 \cdot 10^{-6}$ *M*	63
3-(3-Nitrophenyl)-1,1-dimethylurea	$1.5 \cdot 10^{-5}$ *M*	33
3-(4-Trifluoromethylphenyl)-1,1-dimethylurea	$4 \cdot 10^{-6}$ *M*	125
3-(3-Trifluoromethylphenyl)-1,1-dimethylurea	$6 \cdot 10^{-6}$ *M*	83
4-(3,3-Dimethylureido)-S-trichloromethylphenylthiosulfonate	$4 \cdot 10^{-7}$ *M*	1250
3-(4-Methylphenyl)-1,1-dimethylurea	$3 \cdot 10^{-5}$ *M*	17
3-(4-Methoxyphenyl)-1,1-dimethylurea	$5 \cdot 10^{-5}$ *M*	10
3-(4-Dimethylaminophenyl)-1,1-dimethylurea	$2 \cdot 10^{-4}$ *M*	2.5
3-(4-Acetylaminophenyl)-1,1-dimethylurea	$2 \cdot 10^{-3}$ *M*	0.3

The narcotics, of which phenylurethan is a classical example, are generally supposed to act as inhibitors by being adsorbed on catalytically active surfaces. However, CMU and some other 3-phenyl-1,1-dimethylurea derivatives inhibit the Hill reaction at lower concentrations than *o*-phenanthroline and dicoumarol, which were up to the present the most potent inhibitors. This added to the fact that CMU does not affect the respiration of leaf discs at concentrations at which photosynthesis is completely blocked, suggests that this substance should be considered as a specific inhibitor and not as a narcotic. As the inhibitory action can be removed by simply washing away the CMU, the latter probably exerts its influence by specific adsorption on an active site. It is quite possible that phenylurethan acts in the same manner as CMU rather than that the inhibition of the Hill reaction is due to an indiscriminate surface-blocking action.

In a previous paper[6] we postulated that vitamin K plays a part as energy acceptor and hydrogen donor in photosynthesis. It was suggested that phenylurethan, like vitamin K, might associate with the active cyclopentanone ring of chlorophyll by way of hydrogen bonds, thus preventing the transfer of excitation energy from chlorophyll to vitamin K (*cf.*[7]). Such an association is expected to be stronger since the hydrogen atom of the amino group and the oxygen atom of the carbonyl group are more electrophilic and nucleophilic, respectively. Replacement of the ethoxy group in phenylurethan by the more powerful electron-releasing dimethylamino group and introduction of an electron-attracting group into the benzene nucleus would then result in an enhanced inhibitory action upon the Hill reaction. Introduction of an electron-releasing group into the benzene nucleus, on the other hand, would decrease the activity. The experimental results, summarized in Table I, show that in general these effects are actually observed. The fact that ethyl-N-(2,5-dichlorophenyl)-carbamate is less effective than ethyl-N-(3,4-dichlorophenyl)-carbamate may be attributed to intramolecular hydrogen bonding with the *ortho* chlorine atom in the former compound.

It is possible that in addition to the electron-releasing or electron-attracting properties of the substituent, the liposolubility of the substance also affects its inhibitory activity. This factor may for instance account for the fact that chloro derivatives are slightly more active than nitro derivatives, and for the enhanced inhibitory effect brought about by substitution of methyl and phenyl groups.

The fact that 3-(3,4-dichlorophenyl)-1,1-dimethylurea and 3-(3,4,5-trichlorophenyl)-1,1-dimethylurea inhibit the Hill reaction at a concentration of $2 \cdot 10^{-7}$ molar, whereas the concentration of chlorophyll in our experiments was about hundred times higher, may be explained by assuming that the poisons described in this paper are preferably adsorbed on definite "active" chlorophyll molecules. The latter would then be present in an amount of one per hundred or more chlorophyll molecules. These "active" chlorophyll molecules may be different from the others by being associated with vitamin K, which occurs in green leaves at a concentration which is some hundred times smaller than that of chlorophyll. The cyclopentanone ring of the chlorophyll molecules not associated with vitamin K might be enolized and chelated, thus being unable to combine with the inhibitor (*cf.*[8]).

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