THE TOXICITY OF SOME AMINES FOR DUCKWEED, *LEMNA MINOR*

FRITZ FROMM[1]

Department of Chemistry, Mount Mercy College, Pittsburgh, Pennsylvania

The relation of the chemical structure of selective herbicides to their phytotoxicity has been extensively investigated, but relatively few studies have been published on the chemical groups responsible for nonselective phytotoxicity. This paper is concerned with the nonselective weed-killers, specifically, those connected with ammonium sulfamate. In a previous paper (Fromm and O'Donnell, 1951) the sulfonamide radical has been shown to be toxic in combination with a number of organic and inorganic groups which modify the magnitude of its phytotoxicity. Arenesulfonamides, e.g., benzenesulfonamide, were much more toxic than alkanesulfonamides, e.g., ethanesulfonamide. The toxicity of both, benzenesulfonamide and ethanesulfonamide, for duckweed became about ten times greater after introduction of an amino group in the organic radical (Fromm and O'Donnell, 1955). The question of whether this increased toxicity was caused by an additive or synergistic action of the two toxic groups (NH_2 and SO_2NH_2) or whether the amino group had no action of its own and served only as an auxotoxic agent, i.e., enhanced the action of the sulfamide group, was not answered.

For a better understanding of the effects of the two active groups in the same molecule, information about the phytotoxicity of aniline and ethylamine for duckweed seemed desirable. Cyclohexylamine and ammonium ion were also included in this investigation to give some idea about the action of amines in general. The test plant was a strain of *Lemna minor*, which had been used in previous research; the procedure was that described earlier (Fromm, 1955), but the experiments with ammonium chloride were preformed with a new strain of duckweed because the Mount Mercy strain did not acclimatize in Puerto Rico and had to be abandoned. These experiments with ammonium chloride were performed in the greenhouse of the Department of Botany and Plant Pathology, The Ohio State University, at an average temperature of 28°C and much better lighting than that available at Mount Mercy College. The average growth rate of the controls at Columbus was nearly twice that of the experiments at Pittsburgh, though it did not reach the optimum rate of 0.087 to 0.125 which Clark (1925) recorded under much better controlled conditions for the growth constant of *Lemna major*. The chemicals used were commercial products of c.p. quality; the aniline was applied as acetate, the other bases as chlorides since it was assumed that the toxic action of the salts depended only on the cation.

The results were expressed in terms of the equation $\log (N/N_0) = kt$, in which N_0 is the average number of fronds at the beginning of the experiment, N is their average number at the time t (in days), and k is a constant which represents the rate of growth. If k_0 is the constant of growth in the control solution and k that of the experimental solution, the value 100 (k/k_0) gives the growth in the experimental solution in percent of the control. Table 1, which includes also the probable error (σ') and the standard deviation (σ) of the average k and the T value as measure of the significance of the difference $k-k_0$ or k_0-k, shows the results of representative series of experiments.

Aniline acetate in concentrations of 0.01M or more killed duckweed in 7 days or less. A solution containing 10^{-4}M aniline acetate had no significant effect

[1]Present address: Chemical Abstracts, The Ohio State University, Columbus 10, Ohio.

while intermediate concentrations were growth-inhibiting. Hydrogenation of the benzene ring decreased the phytotoxicity slightly: the lethal action of 0.01M cyclohexylamine hydrochloride was slower than that of aniline of the same concentration, and the growth inhibition by a 10^{-3}M solution was less marked. A 10^{-4}M solution of the compound produced 127 percent of the growth of the control; i.e., it probably showed the effect of increased nitrogen supply. The pH of the solution affected the results. At pH 5.5, cyclohexylamine hydrochloride was definitely more toxic than at pH 6.8. Also, ethylamine hydrochloride killed duckweed in concentrations of 0.01M or more, but a 10^{-3}M solution did not

TABLE 1

Growth rate k and relative growth in per cent for Lemna minor *fronds in Clark solution with amines*

Compound	Molar Con.	k ± σ′	σ	T	100 (k/k₀)	pH
†*$PhNH_2$ • AcOH	0	0.0283±0.0027	0.0089	——	100	6.7
$PhNH_2$ • AcOH	$7x10^{-2}$	all dead on the 7th day		——	0	6.8
$PhNH_2$ • AcOH	10^{-2}	begin to die on the 4th day; 2% of fronds surviving on 14th day			——	6.7
$PhNH_2$ • AcOH	10^{-3}	0.0058±0.0016	0.0052	8	20	6.7
$PhNH_2$ • AcOH	10^{-4}	0.0221±0.0031	0.0097	1.2	78	6.7
$C_6H_{11}NH_2$ • HCl	0	0.0268±0.0011	0.0034	——	100	6.8
$C_6H_{11}NH_2$ • HCl	10^{-2}	begin to die on 7th day; 60% of fronds surviving on 15th day			——	6.7
$C_6H_{11}NH_2$ • HCl	10^{-3}	0.0262±0.0007	0.0020	0.46	98	6.9
‡$EtNH_2$ • HCl	0	0.0238±0.0016	0.0045	——	100	6.7
$EtNH_2$ • HCl	10^{-1}	all dead on 9th day ——		——	0	6.8
$EtNH_2$ • HCl	10^{-2}	all dead on 9th day ——		——	0	6.8
$EtNH_2$ • HCl	10^{-3}	0.0220±0.0015	0.0043	0.82	93	6.8
$EtNH_2$ • HCl	10^{-4}	0.0286±0.0009	0.0023	2.75	120	6.7
NH_4Cl	0	0.0508±0.0017	0.0037	——	100	6.5
NH_4Cl	10^{0}	all dead on 7th day ——		——	0	6.4
NH_4Cl	10^{-1}	56% of fronds surviving on 9th day			——	6.4
NH_4Cl	10^{-2}	0.0212±0.0023	0.0052	10.33	42	6.4
NH_4Cl	10^{-3}	0.0335±0.0020	0.0045	5.83	70	6.4

†Ph—C_6H_5—
*Ac—CH_3COO—
‡Et—C_2H_5—

show any growth-inhibition and a 10^{-4}M solution produced a significant increase of the number of fronds over the control, presumably again on account of the better supply of nitrogen. Ammonium chloride also killed *Lemna minor* in concentrations of 0.1M or more and inhibited frond growth down to 10^{-3}M solutions. A 10^{-4}M ammonium chloride solution supplied additional nitrogen for increased growth.

The toxic effect of all four compounds for duckweed has thus been established. Qualitatively, the result is not entirely unexpected. Aniline has long been known to pharmacologists as a poison (Goodman and Gilman, 1955). It has also been reported to be poisonous to bean plants (Ciamician and Ravenna, 1920) and roots of *Lupinus albus* (Mary Chrysostom, 1936), where it leads to exosmosis and death. Cyclohexylamine is less toxic to man than aniline (Watrous and Schulz, 1950) and has been included in mixtures for thinning apples (Kenworthy, 1947) and for control of aquatic plants (Schmidl, 1950). Ciamician and Ravenna (1921) observed also phytotoxic action of ethylamine which was much more effective than methylamine or isoamylamine. Quantitatively, the toxicity of these amines was surprisingly high.

It may be concluded that the amino group as such is phytotoxic. Its activity is modified by the organic molecule to which it is attached and increases in the order alkyl-, cycloalkyl-, arylamine. Hence, in the case of the amino-substituted sulfonamides, tauramide and sulfanilamide, two phytotoxic groups are present in the molecule. Previous investigations (Fromm and O'Donnell, 1953) of the simultaneous action of *p*-aminobenzoic acid and sulfanilamides on duckweed have already shown that there is little antagonism between the two compounds in their effects on plants. This is interpreted as indicating that the major source of phytotoxicity is the sulfonamide group [which possibly interferes with the plant carbonic anhydrase (Fromm and O'Donnell, 1955)] while the amino group is an additional agent which might compete with *p*-aminobenzoic acid. It is tempting to speculate whether the approximately tenfold increase of phytotoxicity produced by the amino group in organic sulfonamides represents a quantitative relation in the activity of the two groups or not, but the available data are not ample enough for a conclusion.

SUMMARY AND CONCLUSIONS

Lemna minor was killed by M ammonium chloride, 0.01M ethylamine, 0.01M aniline, and about the same concentration of cyclohexylamine. The amino group as such is phytotoxic; its effect increases with the organic group to which it is attached in the order ethyl, cyclohexyl, and phenyl radical. In compounds substituted by both amino and sulfonamide groups, both radicals contribute to the toxic action but the effect of the sulfonamide prevails.

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