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BIOLOGICAL ACTIVITY OF NEW AMINOPHOSPHONATES

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Potential biological activity of binary mixtures of some new aminophosphonates with phosphonomethylglycine (PMG) was studied. The hemolysis of erythrocytes induced by individual compounds and their mixtures with PMG was a measure of this activity since it is commonly accepted that toxic action of many compounds, including erythrocyte membrane hemolysis, is the result of their interaction with the lipid phase of biological membranes. The experiments were expected to show if the new compounds exhibited good biological activity and if this activity can be improved when they are used in mixtures with the well-known herbicide PMG.

The analysis of the effects of binary mixtures was carried out by constructing graphs for both components of binary mixtures that give the same 50% hemolysis – the so-called "isobole method".

The results obtained show that the individual hemolytic, *i.e.* potential biological, activity of the aminophosphonates is quite good. When used in binary mixtures with PMG, they exhibit enhanced (synergistic) or weaker (antagonistic) hemolytic ability compared with individual compounds. The interaction type seems to be dependent on the structural features of the aminophosphonates since synergistic type of the interaction was found for cyclic aminophosphonates with hexane ring.

A final conclusion is that all the newly synthesized aminophosphonates could be applied as herbicides and some of them also in binary mixtures with phosphonomethylglycine.

INTRODUCTION

Aminophosphonates constitute an important group of compounds, some of which are widely used in agrochemistry as pesticides. They are known to be influencing various biochemical processes in plants, modifying or inhibiting them [Forlani *et al*., 1996, 1997; Lejczak *et al*., 1997]. This is why new aminophosphonates are synthesized and assayed for potential bioactivity. There are different approaches to determine that activity. One of the quickest is to study the interaction between aminophosphonates and biological or model lipid membranes.

These latter are especially useful because the aminophosphonates studied exhibit high lipophilicity and may freely interact with the membranes. On the other hand, hemolysis of erythrocytes is a good measure of toxicity of various compounds, including aminophosphonates, since the hemolytic process is commonly regarded as taking place in the lipid phase of the erythrocyte membrane. Our earlier studies on other compounds of the aminophosphonate group confirmed the approach to be right [Kleszczyńska *et al*., 2000a, b, 2001a, b]. The widespread use of herbicides constitutes danger also to humans, which is an important motivation for the use of erythrocytes for testing purposes.

The mixtures of two or more bioactive compounds are nowadays very often used. The most important reasons are to fight various diseases simultaneously and to exploit possible interaction between mixture compounds which may result in reducing concentrations of compounds without the loss of activity [Gisi, 1996]. In this case, it was to verify whether the interaction between mixture components leads either to an increase in mixture activity in comparison with that found for individual compounds (synergism), or to its decrease (antagonism) or whether no interaction is taking place (additivity). As it was mentioned, studies of pesticide mixtures are nowadays quite popular and many compounds were found to be more potent when used in mixtures [Oruç & Uner, 2000; Pape-Lindstrom & Lydy, 1997; Tripathi & Agarwal, 1997].

MATERIALS AND METHODS

All the aminophosphonates, the general structures of which are presented in Table 1, were synthesized at the Institute of Organic Chemistry, Biochemistry and Biotechnology, University of Technology, Wrocław, Poland. They were purified by column chromatography.

Pig erythrocytes were used in the hemolytic experiments. Erythrocytes (RBC) were washed four times in phosphate buffer (pH 7.4) and incubated in the same buffer solution with the addition of chosen concentrations of aminophosphonates or their binary mixtures with phosphonomethylglycine (PMG) at 37°C. The samples were then centrifuged and hemoglobin content in supernatant was measured with a Specol 11 spectrophotometer. The hemolytic curves obtained enabled determining concentrations of the compounds causing 50% hemolysis (C_{50}).

To evaluate the binary mixture effects, the isobole method was used [Kortenkamp & Altenburger, 1998]. The values of C_{50} obtained for particular components were marked on X and Y axis and connected with straight lines. The points

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TABLE 1. The structure and substituent groups of the aminophosphonates. N = 0 stands for acyclic aminophosphonate, N = 1 and 2 for cyclic ones with pentane or hexane rings, respectively.

N	Compound	R^1	R^2	R^3	R^4
0	1	CH_3	CH_3	n-C_8H_{17}	n-C_4H_9
1	2			n-C_4H_9	n-C_4H_9
2	3*	t-C_4H_9		n-C_4H_9	n-C_4H_9
2	4			n-C_8H_{17}	n-C_4H_9
2	5			n-$C_{14}H_{29}$	n-C_4H_9

* compound 3 has tert butyl group incorporated in hexane ring

on the line predicted the combinations of both components that yield the same effect (50% hemolysis). If binary mixtures gave a point that belonged to this line, the interaction between the components was defined as additive. In such cases, the components can be viewed as behaving like dilutions of each other. Experimental points above or below the isobole may be defined as antagonistic and synergistic interaction, respectively.

RESULTS AND DISCUSSION

The results of the hemolytic experiments are collected in Table 2 which contains the values of concentrations of particular compounds and their binary equimolar mixtures with N-phosphonomethylglycine (PMG) that cause 50% hemolysis (C_{50}). The example of hemolytic curve that enabled determination of C_{50} values is shown in Figure 1. It can be seen that the ability of the aminophosphonates to hemolyse erythrocyte (RBC) membranes differs significantly.

TABLE 2. The concentrations of aminophosphonates and their equimolar binary mixtures with phosphonomethylglycine (PMG) inducing 50% hemolysis of erythrocytes (C_{50}).

Compounds	C_{50} (mmol)	Binary mixtures	The interaction type
1	0.080	1 + PMG	antagonism
2	0.86	2 + PMG	antagonism
3	0.77	3 + PMG	synergism
4	0.42	4 + PMG	synergism
5	0.83	5 + PMG	synergism
PMG	2.50		

Standard deviation did not exceed 5%.

The weakest hemolytic activity was found for PMG, which may suggest that herbicidal action of this compound may not be directly connected with the lipid phase of RBC membrane. It was already mentioned that hemolysis is commonly regarded as the result of interactions of lipophilic xenobiotics with membrane lipids. The results obtained for the other aminophosphonates showed that their hemolytic efficiencies are good enough to regard them as potential pesticides, especially compound 1.

Studies on binary mixtures of aminophosphonates with PMG revealed that the interactions between mixture components were of two types. Compounds 1 and 2 reacted antagonistically with PMG and hemolysing efficiencies of these both mixtures weakened substantially (Table 2) in comparison with those found for individual components. It must be noted here that there are different approaches to the problem of determining what kind of interaction between mixture components is taking place [Gisi, 1996; Pape-Lindstrom & Lydy, 1997; Kortenkamp & Altenburger, 1998]. We have selected isobole method and the results are presented in Figure 2 which contains isobole presenting the synergistic interaction for compound 5.

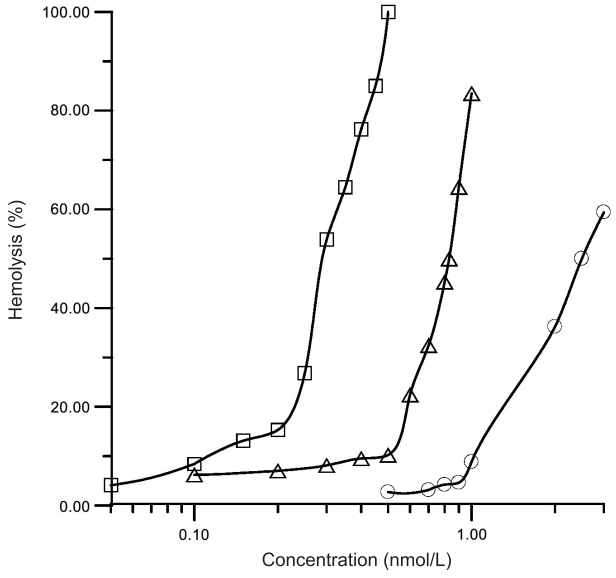

FIGURE 1. The hemolysis of erythrocytes induced by compound 5 (Δ), phosphonometylglicyne (PMG) (○), and their binary mixture (□).

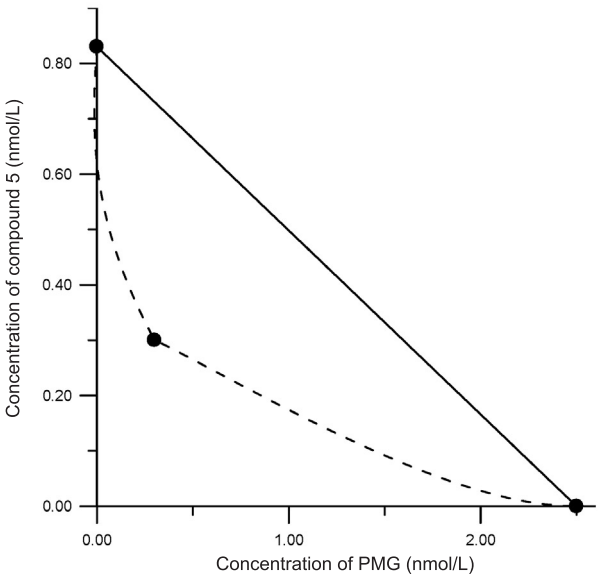

FIGURE 2. Expected (——) and obtained (- - -) isoboles for equimolar binary mixtures of PMG with compound 5.

CONCLUSION

It is obvious, on the basis of the results obtained, that the binary mixtures of compounds 3, 4 and 5 with phosphonomethylglycine fulfill expectations because synergistic interactions between them were observed.

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