Binary Mixtures of (*N*-phosphonomethyl)-glycine with New Aminophosphonates

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The potential biological activity of binary mixtures of some new organophosphorous compounds, aminoalkane- and aminofluorenephosphonates, with (*N*-phosphonomethyl)-glycine (glyphosate, PMG) was studied. The inhibition of growth of wheat (*Triticum aestivum*) induced by individual compounds and their equimolar mixtures with PMG was a measure of that activity. The experiments were expected to show if the new compounds exhibited good biological activity to be used for agrochemical applications and if this activity can be improved when they are used in mixtures with glyphosate which is the active component of the well-known herbicide Roundup.

The results obtained show that aminofluorenephosphonates inhibited wheat growth when used in micromolar concentrations. Thus, their efficiency can be compared to that of PMG. The efficiency of aminoalkanephosphonates was one order of magnitude weaker. The measure of the efficiency was the effective concentration inhibiting wheat growth by 50% (EC_{50}). The most demanded interaction, *i.e.*, a synergistic was observed for only one of binary mixtures of the compounds studied with PMG. Mostly they showed antagonistic or strong antagonistic interactions. Some of them were of the additive type. Such results exclude the possibility of potential use of all the compounds studied in binary mixtures with phosphonomethylglycine, especially as the mentioned synergistic interaction found was rather weak. The influence of structural features of aminophosphonates on the results obtained is discussed.

Key words: Aminophosphonates, Biological Activity, Binary Mixtures

Introduction

Organophosphorous compounds are an important group of compounds. Some of them are herbicidally active and are widely used in agrochemistry. They are known to interfere with various biochemical processes in plants, which may lead to the death of the biological object against which they are applied. Recently, their use has markedly increased (Suwalsky *et al.*, 1999) and the main purpose is to synthesize new, efficient and cheap compounds that easily undergo degradation in the environment and can thus be used as pesticides.

In this work we have studied some recently synthesized acyclic and cyclic aminoalkanephosphonates and aminofluorenephosphonates. Some of them, as well as others belonging to these groups, were intensively studied in the last years and found to influence membrane properties and growth of different biological objects (Gancarz and Dudek, 1996; Wieczorek *et al.*, 2000, 2001; Bielecki *et al.*, 2001, 2003; Sarapuk *et al.*, 2002). Also, some of the compounds exhibited antioxidative activity (Kleszczyńska and Sarapuk, 2001). Generally, they were found to be biologically active and their activity was in some cases similar to that of (*N*-phosphonomethyl)-glycine (glyphosate, PMG), which is the active component of the very popular herbicide Roundup. PMG was chosen as partner of the compounds studied in equimolar binary mixtures. The reasons for such a choice was the low toxicity of PMG (Pieniążek *et al.*, 2004) and the fact that it can be used as a good reference compound comparing its activity with that of organophosphorous compounds.

Mixtures of two or more bioactive compounds are very often used nowadays. The reasons are multiple. The most important are to fight various diseases simultaneously and to exploit the possible interactions between mixture compounds. These interactions may result in reduced concentrations of the compounds without loss of activity or in a significantly increased mixture activity in comparison with that of individual compounds (syner-

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gism). There are also other possible interactions where the activity of a mixture may be drastically reduced (antagonism) or the additive effect results (additivity or zero interaction) (Gisi, 1996; Pape-Lindstrom and Lydy, 1997; Tripathi and Agarwal, 1997; Kortenkamp and Altenburger, 1998; Oruç and Üner, 2000; Mora and Earle, 2001). In the present case the aim was to check if the interaction between mixture components leads to the most desired synergistic effect.

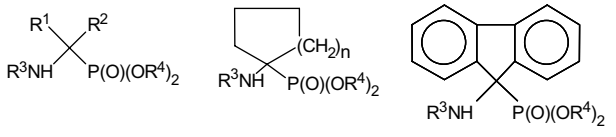
Materials and Methods

All the aminophosphonates, of the general chemical structures presented in Table I, were synthesized in the Institute of Organic Chemistry, Biochemistry and Biotechnology, University of Technology, Wrocław, Poland. They were purified by column chromatography. The synthesis methods were described previously (Wieczorek *et al.*, 2000, 2001; Gancarz and Wieczorek, 1980)

We used the modified toxic unit approach to model joint toxicity. In the toxicity unit (TU) model, the value of 1 TU is assigned to a 50% effective concentration (EC_{50}) value of contaminant. The sum of the TU contributed by each component describes the toxicity of a mixture as follows: $TU_{mixture} = Cw_1/EC_{50} + Cw_2/EC_{50}$, where Cw_i are the concentrations of chemicals in the mixture and EC_{50} is the EC_{50} value for the respective component chemicals of the mixture. The empirically measured toxicity was compared with the expected toxicity (as predicted by the $TU_{mixture}$, which was generated using EC_{50} values determined by tests of individual toxicants).

Tests with binary mixtures were conducted in a similar manner as the individual compound tests. Concentrations of each compound were added at proportions of their respective EC_{50} values so that the summation of concentrations of the combination of pesticides were equivalent to the five con-

Table I. The structures and substituent groups of the compounds studied. n = 1 or 2 for pentane and hexane rings, respectively.



Ring	Compound	R^1	R^2	R^3	R^4
		Acyclic aminoalkanephosphonates			
	1	CH_3	CH_3	*n*-C_8H_17	*iso*-C_3H_7
	2	CH_3	CH_3	*n*-C_8H_{17}	*n*-C_4H_9
	3	CH_3	*n*-C_4H_9	*n*-C_3H_7	*n*-C_4H_9
	4	CH_3	*t*-C_4H_9	*n*-$C_{10}H_{21}$	*n*-C_4H_9
	5	*n*-C_4H_9	CH_3	*n*-C_6H_{13}	*n*-C_4H_9
	6	*n*-C_5H_{11}	CH_3	*n*-C_4H_9	C_2H_5
	7	CH_3	*n*-C_4H_9	-$CH(CH_2)_5$	C_2H_5
	8	*n*-C_5H_{11}	CH_3	*n*-C_4H_9	*n*-C_4H_9
		Cyclic aminoalkanephosphonates			
Pentane n = 1	**9**			*n*-C_4H_9	*n*-C_4H_9
	10			*n*-C_8H_{17}	*iso*-C_3H_7
Hexane n = 2	**11**			*n*-C_4H_9	*iso*-C_3H_7
	12*			*n*-C_4H_9	*n*-C_4H_9
	13			*n*-C_8H_{17}	*n*-C_4H_9
	14			*n*-$C_{14}H_{29}$	*n*-C_4H_9
		Aminofluorenephosphonates			
	15			*n*-C_4H_9	*n*-C_8H_{17}
	16			*n*-C_4H_9	*n*-$C_{11}H_{23}$

* Compound **12** has a *tert*-butyl group incorporated into the hexane ring.

centrations: Σ0.5 TU, Σ0.75 TU, Σ1.0 TU, Σ1.5 TU, Σ2.0 TU. Therefore, a binary mixture of compound **13** + PMG contained 350 mM (= 0.5 TU) **13** and 240 mM (= 0.5 TU) PMG to give a nominal value of Σ1.0 TU.

The 96-h growth tests with individual compounds were conducted with wheat (*Triticum aestivum* L. cv. Henika; supplier: Małopolska Hodowla Roślin, Cracow, Poland) in a SANYO® growth chamber with continuous light at 25 °C. Five concentrations of each substance were used in three replicates. Values of EC_{50} are given as nominal concentrations of the compounds. Seeds were germinated at 25 °C for 2 d in darkness. 15 uniform seedlings were transferred to Petri dishes (9 cm) with two discs of Whatman No. 2 filter paper wetted with distilled water (control) or water solutions of tested compounds. The shoot length of the plants was measured after 4 d.

Results and Discussion

The results of the growth experiments are collected in Table II, which contains the values of concentrations of (*N*-phosphonomethyl)-glycine (PMG) and particular compounds needed to inhibit wheat growth by 50% (EC_{50}). As expected, the best herbicidal property was observed for PMG. Somewhat weaker activity exhibited both aminofluorenephosphonates (**15** and **16**). All these compounds were used in micromolar concentrations. EC_{50} values determined for acyclic and cyclic aminoalkanephosphonates show that their activities were about 3–6 times weaker in comparison to the activity of PMG. There were no big differences between activities of acyclic compounds, regardless of their lipophilicity. It seems that the inhibiting efficiency is rather connected with geometric dimensions of a molecule than its general lipophilicity. A confirmation of that may be the EC_{50} value determined for compound **4**. Its bulky *tert*-butyl group at the carbon atom is probably the reason why this compound, the most lipophilic under the acyclic aminophosphonates, exhibited the weakest inhibiting efficiency. Contrary, the same group attached to an hexane ring seems to improve the efficiency of compound **12**. Also, the *iso*-propyl group substituted at the phosphorus atom improves that efficiency if accompanied by high enough lipophilicity of compound **10**. The tendency of efficiency to decrease with the dimension of the molecule seems to confirm the EC_{50} values obtained for cyclic aminoalkanephosphonates. All the compounds with a hexane group (**11**–**14**) were found to be clearly weaker inhibitors than compound **9** with a pentane group substituted at the carbon atom. That rule does not work in the case of aminofluorenephosphonates which, as mentioned above, very actively inhibited wheat growth. The good membrane activity of the compounds of this group was found earlier (Gancarz and Dudek, 1996).

The results of experiments with equimolar binary mixtures of aminophosphonates with (*N*-phosphonomethyl)-glycine are collected in Table III.

Table II. The concentrations of compounds studied inducing 50% inhibition of growth of wheat (EC_{50}).

Compound	EC_{50} [mM] (± 95% confidence limits)
1	2900 (2670–3130)
2	1560 (1460–1660)
3	1750 (1610–1890)
4	2700 (2550–2850)
5	2100 (1942–2257)
6	1550 (1451–1649)
7	3400 (3180–3620)
8	2000 (1860–2140)
9	1650 (1520–1780)
10	1200 (1130–1270)
11	2100 (1970–2230)
12	1950 (1810–2050)
13	3050 (2840–3260)
14	2300 (2160–2440)
15	700 (658–742)
16	930 (874–985)
PMG	480 (446–514)

Table III. Toxicity of equimolar binary mixtures of the compounds studied with (*N*-phosphonomethyl)-glycine (PMG) and the type of combined action.

Binary mixtures	Toxic units (TU)	Combined action
PMG + **1**	1.5 (1.38–1.62)	Antagonistic
PMG + **2**	1.15 (1.09–1.21)	Additive
PMG + **3**	1.5 (1.38–1.62)	Antagonistic
PMG + **4**	1.1 (1.0–1.2)	Additive
PMG + **5**	1.4 (1.29–1.5)	Antagonistic
PMG + **6**	1.7 (1.59–1.81)	Strong antagonistic
PMG + **7**	1.88 (1.74–2.02)	Strong antagonistic
PMG + **8**	1.2 (1.12–1.28)	Antagonistic
PMG + **9**	1.3 (1.2–1.4)	Antagonistic
PMG + **10**	1.4 (1.0–1.2)	Antagonistic
PMG + **11**	2.3 (2.16–2.44)	Strong antagonistic
PMG + **12**	0.87 (0.82–0.92)	Weak synergistic
PMG + **13**	2.2 (2.05–2.45)	Strong antagonistic
PMG + **14**	1.6 (1.49–1.71)	Antagonistic
PMG + **15**	0.95 (0.89–1.01)	Additive
PMG + **16**	1.1 (1.01–1.19)	Additive

As it is shown, the interaction between binary mixture components gave, as the final result, inhibitory effects that may be defined as additive or antagonistic (also strong antagonistic). Only in one case that effect may be treated as weakly synergistic. It must be noted here that there are different approaches to the problem of determining what kind of interaction between mixture components occurs and how it can be expressed in mathematical terms (Gisi, 1996). We have used the approach where synergism is stated when the ratio of expected and observed EC_{50} values is greater than 1; for additivity and antagonism these values are 1 or < 1, respectively. To model joint toxicity, the toxic unit approach was used (Pape-Lindstrom and Lydy, 1997; Cooper, 2001).

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Bielecki K., Dziamska A., and Sarapuk J. (2001), Physiological activity of some aminophosphonates. Z. Naturforsch. **56c**, 995–998.

Bielecki K., Dziamska A., and Sarapuk J. (2003), Assessment of phytotoxicity of α-aminoalkanephosphonic acids derivatives. Biol. Plantarum **46**, 467–470.

Cooper C. (2001), Use of microtox for assessing heavy metal complex formation with the organic solvents acetonitrile and dimethyl sulphoxide: a preliminary study. Bull. Environ. Contam. Toxicol. **67**, 526–534.

Gancarz R. and Dudek M. (1996), Structure-activity studies of aminophosphonic derivatives of fluorene. Phosphorus, Sulphur and Silicon **114**, 135–142.

Gancarz R. and Wieczorek J. (1980), Synthesis of phosphonic analogues of morphactines. J. Prakt. Chem. **322**, 213–222.

Gisi U. (1996), Synergistic action of fungicides in mixtures. Phytopathology **86**, 1273–1279.

Kleszczyńska H. and Sarapuk J. (2001), New aminophosphonates with antioxidative activity. Cell. Mol. Biol. Lett. **6**, 83–91.

Kortenkamp A. and Altenburger R. (1998), Synergisms with mixtures of xenoestrogens: A reevaluation using the method of isoboles. Sci. Total Environ. **221**, 59–73.

Mora A. and Earle E. D. (2001), Combination of *Trichoderma harzianum* endochitinase and membrane-affecting fungicide on control of *Alternaria* leaf spot in transgenic brocolli plants. Appl. Microbiol. Biotechnol. **55**, 306–310.

Oruç E. O. and Üner N. (2000), Combined effects of 2,4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Oreochomis niloticus*. Comp. Biochem. Physiol. C **127**, 291–296.

Pape-Lindstrom P. A. and Lydy M. J. (1997), Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. Environ. Toxicol. Chem. **16**, 2415–2420.

Pieniążek D., Bukowska B., and Duda W. (2004), Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. Pestic. Biochem. Physiol. (in press).

Sarapuk J., Bielecki K., and Trela Z. (2002), Influence of some new aminophosphonates on electrical properties and stability of plasma membranes of plant cells. Acta Soc. Bot. Poloniae **71**, 223–226.

Suwalsky M., Quevedo L., Norris B., and Benites M. (1999), Toxic action of the herbicide 2,4-D on the neuroepithelial synapse and on the nonstimulated skin of the frog *Caudiverbera caudiverbera*. Bull. Environ. Contam. Toxicol. **62**, 570–577.

Tripathi A. M. and Agarwal R. A. (1997), Synergism in tertiary mixtures of pesticides. Chemosphere **35**, 2365–2374.

Wieczorek J. S., Gancarz R., Bielecki K., Grzyś E., and Sarapuk J. (2000), Synthesis and physiological activities of new acyclic aminophosphonates. Phosphorus, Sulphur and Silicon **166**, 111–123.

Wieczorek J. S., Gancarz R., Bielecki K., Grzyś E., and Sarapuk J. (2001), Synthesis of some new cyclic aminophosphonates and their physiological activities. Phosphorus, Sulphur and Silicon **174**, 119–128.