Aminophosphonate-Induced Changes of Betacyanine and Ionic Efflux

Edward Grzyś[a], Krzysztof Bielecki[a] and Janusz Sarapuk[b,*]

[a] Department of Botany and Plant Physiology, Agricultural University, Cybulskiego 32, 50–205, Wrocław

[b] Department of Physics and Biophysics, Agricultural University, Norwida 25, 50–375 Wrocław, Poland. Fax: (+48)-071-32-05-172. E-mail: JS@ozi.ar.wroc.pl

* Author for correspondence and reprint requests

Z. Naturforsch. **56 c**, 349–352 (2001); received November 27, 2000/February 4, 2001

Aminophosphonates, Physiological Activity

Betacyanine and ionic leakage from red beet (*Beta vulgaris ssp. L. rapacea*) roots and lilac (*Syringa vulgaris L.*) leaves under the influence of new aminophosphonates were studied by spectroscopic and conductometric methods. It was found that the leakage of dye or electrolytes depended both on the concentration of the compounds used and their structural features. The results compared to those obtained for the well known herbicide Buminafos® (dibutyl 1-butylamino-1-cyclohexanephosphonate) enabled to conclude that some of the compounds studied exhibited comparable or better activity than this herbicide. That makes them potentially good herbicides. It is possible that the effects observed are the result of action on cell membranes of the tissues used. The possible role of the structural features of aminophosphonates in this action is discussed.

Introduction

Aminophosphonates are new promising group of compounds of interesting biological properties. Some representatives are widely applied as plant growth regulators as well as potent herbicides. The best known of the latter are glyphosate, the active substance of which is N-phosphonomethylglycine and Buminafos® (dibutyl 1-butylamino-1-cyclohexanephosphonate). The mechanism of their action is not sufficiently known, however, some studies revealed that buminafos modified cell membranes (Linsel *et al.*, 1988).

To estimate the potential phytotoxicity of the new compounds synthesized for herbicidal application some standard tests have been performed. These are plant growth tests and studies on the permeation of plant cell membranes. Usually, various aquatic plants, like *Egeria densa* or *Spirodela polyrrhiza* (Crowley, 1980; Kafarski *et al.*, 1997; Linsel *et al.*, 1988; Marré *et al.*, 1998; O'Brien, 1979) or plants and weeds (Forlani *et al.*, 1997; Lejczak *et al.*, 1996; Lydon and Duke, 1988; Ruiter *et al.*, 1987; Shimabukuro, 1994; Sterling, 1994) are used in those tests. Disks cut from red beet (*Beta vulgaris ssp. L. rapacea*) roots and lilac (*Syringa vulgaris L.*) leaves were used in this work. Betacyanine or electrolyte efflux from both tissues under the influence of aminophosphonates was performed in order to estimate their abilities to modify transport properties of membranes. The measured parameters were the betacyanine concentration in the exudate and the conductivity of the leaked electrolyte. Changes of conductivity in the internal layer of cells that protect plants or isolated tissue are often caused by damages to cell membrane structure as shown elsewhere (Crowley, 1980; Di Tomaso, 1993; Linsel *et al.*, 1988; Lydon and Duke, 1988; Marré *et al.*, 1998; O'Brien, 1979). The consequence is permeation into the cell interior and subsequent damages to various cell organelle membranes. The result is disturbance in many metabolic processes, especially in transport of amino acids, plant hormones, inorganic ions and/or of synthesis and functioning of membrane-localized lipids and proteins, including enzymes and proton pumps, also ATPases. Lack of ATPase leads to inhibition of ATP production and active transport inhibition. In addition, the result of damages to cellular structures are changes like inhibition of photosynthesis, cell development and growth disturbance, blocking of cell division and biosynthesis of amino acids, dyes and fatty acids (Crowley, 1980; Duke, 1989; Kenyon *et al.*, 1988; Lydon and Duke, 1988; Shimabukuro, 1994; Sterling, 1994).

0939–5075/2001/0500–0349 $ 06.00 © 2001 Verlag der Zeitschrift für Naturforschung, Tübingen · www.znaturforsch.com · D

Materials and Methods

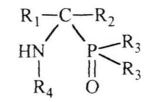
Red beet root test

The efflux of betacyanine from disks cut from roots of *Beta vulgaris L. ssp. rapacea* (1 mm thick and 15 mm diameter) was measured. The disks were washed repeatedly in distilled water, dried on filter paper, put into vessels (10 disks in a vessel of volume 50 cm^3) containing 10 ml of aqueous solutions of the compounds studied. Concentrations of aminophosphonates were 0.25 mM, 0.5 mM and 1.0 mM. Additionally, one set of disks was put into distilled water and boiled for 5 min. Vessels were thermostated at 25 °C. The amount of effluxed betacyanine was determined spectrophotometrically by measuring absorbance at 560 nm after 24 h. It was then compared to betacyanine efflux from boiled disks that was taken as 100%. Experiments were repeated 4 times.

Lilac leaf test

Disks of diameter 15 mm were cut off from leaves of *Syringa vulgaris L.* and repeatedly washed with distilled water. Sets of 15 disks were then put in vessels containing 50 ml solutions of the aminophosphonates studied. Their concentrations were the same as in red beet root tests. Vessels were kept at 26 °C and illuminated continuously with artificial light. Conductivity of exudate was measured by conductometric method (Linsel *et al.*, 1988; Marré *et al.*, 1998; Ruiter *et al.*, 1987) with the conductometer OK.-102/1 (Radelkis, Hungary) and compared to control conductance of boiled disks (= 100%). Experiments were repeated 4 times.

Table I. The substituents of the compounds studied.



b - Buminafos®

Compound no.	R_1		R_2	R_3	R_4
1	CH_3		n-C_4H_7	n-C_4H_9	n-$C_{10}H_{21}$
2	CH_3		CH_3	n-C_4H_9	n-$C_{10}H_{21}$
3		-C_4H_8-		i-C_3H_7	n-C_8H_{17}
4		-C_5H_{10}-		C_2H_5	n-C_8H_{17}
5		-C_5H_{10}-		n-C_4H_9	n-$C_{14}H_{29}$
6		-C_5H_{10}-		n-C_4H_9	n-C_8H_{17}
7		-C_5H_{10}-		n-C_4H_9	i-C_4H_9
8	CH_3		CH_3	n-C_4H_9	n-C_8H_{17}
9	CH_3		C_2H_5	n-C_4H_9	n-C_8H_{17}
10		-C_4H_8-		n-C_4H_9	n-C_8H_{17}
11	CH_3		CH_3	i-C_3H_7	n-C_8H_{17}
12	CH_3		n-C_4H_9	i-C_3H_7	n-C_8H_{17}
13		-C_6H_{12}-		i-C_3H_7	n-$C_{10}H_{21}$
14		-C_5H_{10}-		i-C_3H_7	n-$C_{10}H_{21}$
15		-C_5H_{10}-		n-C_4H_9	n-$C_{10}H_{21}$
16	CH_3		CH_3	n-C_4H_9	n-$C_{14}H_{29}$
17	CH_3		n-C_4H_9	i-C_3H_7	n-$C_{10}H_{21}$
18	CH_3		CH_3	i-C_3H_7	n-$C_{10}H_{21}$
19		-C_4H_8-		n-C_4H_9	n-$C_{10}H_{21}$
20		-C_5H_{10}-		n-C_4H_9	C_2H_4OH
b		-C_5H_{10}-		n-C_4H_9	n-C_4H_9

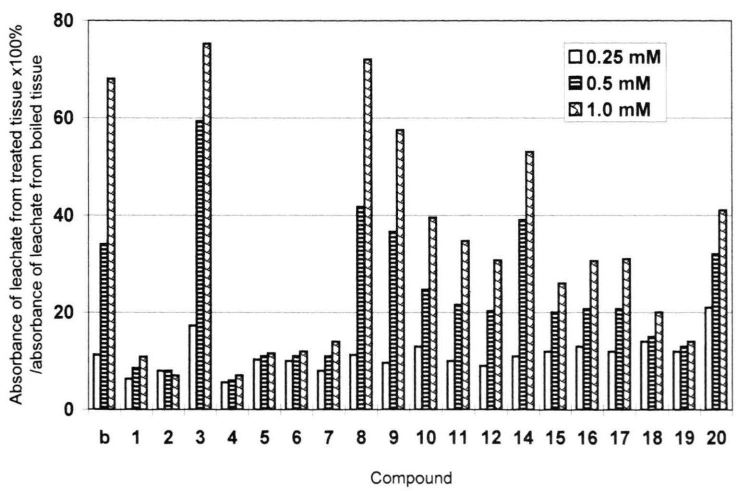


Fig. 1. Efflux of betacyanine from red beet (*Beta vulgaris ssp. L. rapacea*) roots. Standard deviation did not exceed 12%.

The aminophosphonates studied represent both cyclic and acyclic forms and were synthesized at the Department of Organic Chemistry, Technical University of Wrocław. Their general structure and particular substituents are documented in Table I. Details of synthesis as well as spectral data are reported elsewhere (Wieczorek *et al.*, 2000a; Wieczorek *et al.*, 2000b).

Results and Discussion

The betacyanine efflux from red beet root was most actively influenced by aminophosphonates nos. 3, 8, 9 and 14. When used at the concentrations specified earlier (see Materials and Methods) they were inducing a bigger or comparable efflux of dyes to that caused by Buminafos® (see Fig. 1). Some of the compounds studied (nos. 10 and 20) were only slightly less active than this herbicide. All the other aminophosphonates exhibited poor modifying properties of the tissue used.

Similar results were obtained from lilac leaf tests (Fig. 2). Compounds 3, 8 and 14 were found to exhibit a slightly better efficiency in inducing ionic leakage than that observed for Buminafos®, while compounds 4 and 9 caused a somewhat smaller efflux. In contrast to the red beet root test, some compounds proved to be moderate inducers of ionic efflux (nos. 6 and 20).

The similarity of the results obtained for the most active compounds indicates that their action relates to the same membrane phase of the tissues studied. It is reasonable to assume that aminophosphonates cause mechanical damage to the lipid phase of the membrane thus inducing dye or ionic leakage from the tissues. However, some quantitative differences were observed for both effluxes, especially with the less active compounds. The ionic efflux induced by some aminophosphonates was significantly greater than that of betacyanine. This may be due to the consequence of the larger dimension of the dye molecule in comparison to the hydrated ion radii, and/or to the difference of constituents between the tissue membranes.

The most active compounds (nos. 3, 8, 9 and 14) represent both cyclic and acyclic forms. So, it seems that the substituent at the carbon atom is not decisive for the efficacy of compound. The same conclusion seems to be valid for substituents at the phosphorus atom. Acyclic compounds have a butyl while cyclic ones have a branched propyl substituent. However, in the case of all the rest of compounds, those with branched propyl substituents had a significantly weaker influence on betacyanine efflux from red beet roots. For all the compounds studied, apparently too long or too short alkyl substituents at the nitrogen atom do not improve the modifying properties. Exception is compound 20 with a short alkyl chain, where the terminal methylene group was replaced by hydroxyl (see R_4 in Table I).

Apparently a combination of all the structural features of aminophosphonates and/or their stereochemistry makes some of them suitable for use

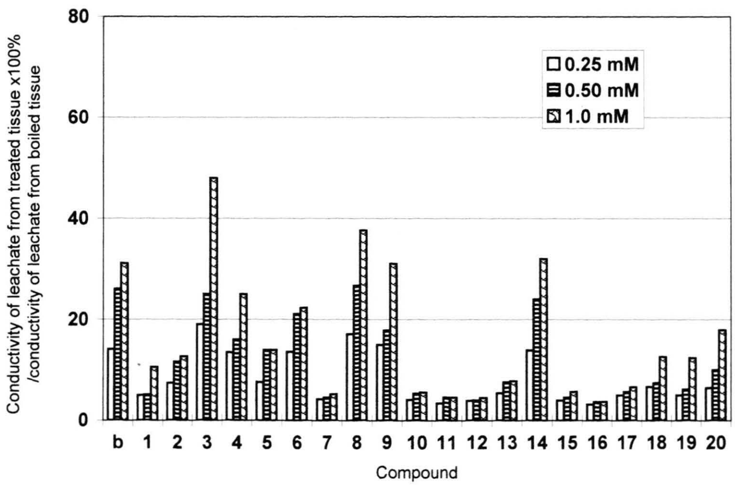


Fig. 2. Efflux of electrolyte from lilac (*Syringa vulgaris L.*) leaves. Standard deviation did not exceed 12%.

as herbicides. So far, of all the compounds studied in this work only four exhibit biological properties similar to those of Buminafos®.

Acknowledgements

This work was supported by grant no. 6PO4 G 050 17 from the State Committee for Scientific Research (KBN).

Crowley J. (1980), Effects of herbicides of different modes of action on leaf-cell membrane permeability in *Phaseolus vulgaris*. Canad. J. Plant Sci. **60**, 613–620.

Di Tomaso J. M. (1993), Evidence against a direct membrane effect in mechanism of action of graminicides. Weed Sci. **42**, 302–309.

Duke S. O. (1989), Oxadiazon activity is similar to that of p-nitro-diphenyl ether herbicides. Weed Sci. **37**, 152–160.

Forlani G., Kafarski P., Lejczak B. and Wieczorek P. (1997), Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acid. Part II. Reversal of herbicidal action by aromatic amino acids. J. Plant Growth Regul. **16**, 147–152.

Kafarski P., Lejczak B., Forlani G., Gancarz C., Torreilles C., Grembecka J., Ryczek A. and Wieczorek P. (1997), Herbicidal derivatives of aminomethylenebisphosphonic acid. Part III. Structure-activity relationship. J. Plant Growth Regul. **16**, 153–158.

Kenyon W. H. Duke S. O. and Paul R. N. (1988), Effects of temperature on the activity of the p-nitrosubstituted diphenyl ether herbicide acifluorfen in cucumber (*Cucumis sativus L.*). Pestic. Biochem. Physiol. **30**, 57–66.

Lejczak B., Boduszek B., Kafarski P., Wojtaszek H and Wieczorek P. (1996), Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acid. Part I. Physiological activity and inhibition of anthocyanin biosynthesis. J. Plant Growth Regul. **15**, 109–113.

Linsel G., Dahse I. and Muller E. (1988), Electrophysiological evidence for the herbicidal mode of action of phosphonic acid esters. Physiol. Plant. **73**, 77–84.

Lydon J. and Duke S. O. (1988), Porphyrin synthesis is required for photobleaching activity of p-nitrosubstituted diphenyl ether herbicides. Pestic. Biochem. Physiol. **31**, 74–83.

Marré M. T., Amicucci E., Zingarelli L., Albergoni F. and Marré E. (1998), The respiratory burst and electrolyte leakage induced by sulfhydryl blockers in *Egeria densa* leaves are associated with H_2O_2 production and are dependent on Ca^{2+} influx. Plant Physiol. **118**, 1379–1387.

O'Brien M. C. (1979), Effect of herbicides on cell membrane permeability in *Lemna minor*. Weed Res. **19**, 331–334.

Ruiter H., Verbeek M. A. M. and Uffing A. J. M. (1988), Mode of action of a nonionic and cationic surfactant in relation to glyphosate. In: Pesticide Formulations – Innovations and Developments (B. Cross and H. B. Scherr) Amer. Chem. Soc. Symp. Series, Washington DC. **371**, 44–55.

Shimabukuro R. H. (1994), Effects on transmembrane proton gradient and lipid biosynthesis in mode of action of diclofop-methyl. Pestic. Biochem. Physiol. **48**, 85–97.

Sterling T. M. (1994), Mechanisms of herbicide absorption across plant membranes and accumulation in plant cells. Weed Sci. **42**, 263–276.

Wieczorek J. S., Gancarz R., Bielecki K., Grzyś E. and Sarapuk J. (2000a), Synthesis and physiological activities of new acyclic aminophosphonates. Phosphorus, Sulfur and Silicon, in press.

Wieczorek J. S., Gancarz R., Bielecki K., Grzyś E. and Sarapuk J. (2000b), Synthesis of some new cyclic aminophosphonates and their physiological activities. Phosphorus, Sulfur and Silicon, in press.