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Herbicidal Derivatives of Aminomethylenebisphosphonic Acid. Part IV. Hydroxyalkylidenebisphosphonates, Iminomethylenebisphosphonates and Ureidomethylenebisphosphonates

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Abstract. Derivatives of aminomethylenebisphosphonic acids constitute a class of promising herbicides. Replacement of the amino group by hydroxyl, ureido, thioureido, or imino moieties leads to compounds of significant herbicidal properties. This indicates that protonated amino function is not a requirement for phytotoxic action of bisphosphonates.

Key Words. Glyphosate—Bisphosphonates—Amino acid biosynthesis

N-substituted aminomethylenebisphosphonic acids, developed in Japan (for example, compounds 1 and 2, Suzuki et al. 1979) and in the United States (Cromartie and Fisher 1995) constitute a new class of promising herbicides (Fig. 1). These acids possess two strongly acidic residues and one positively charged amino group, and thus closely resemble the popular herbicide glyphosate. Most members of this new class of herbicides exert remarkable phytotoxic effects at both the plant and cell culture level. (Lejczak et al., 1996). Although studies have attempted to define their mechanism of action (Cromartie and Fisher 1995; Forlani et al. 1996; Lejczak et al. 1996; Oberhauser et al., 1998; Cromartie et al., 1999), it remains unclear. Physiologic activity of the structurally related series of compounds is routinely examined to define the structural requirements of target receptors. Thus, in trying to better understand these requirements we have synthesized a short series of analogs in which a substituted amino group was replaced by a hydroxyalkyl moiety (compounds 3 and 4), ureido (or thioureido) group (compounds 5), or in which an aminomethylene fragment was replaced by an iminomethylene group (compounds 6). The resulting compounds were screened for their phytotoxic properties against cress and cucumber.

Materials and Methods

Chemical Syntheses

Compounds 3, 4, and 5 were obtained according to the previously described procedures (Chuiko et al. 1991, Chuiko et al. 1995; Chuiko et al. 1996). Melting points and yields of these compounds are given in Table 1.

N-(3-Morpholino-2-hydroxypropyl)aminomethylenebisphosphonic acid (6) was obtained by reacting 10 g (0.052 mole) of aminomethylenebisphosphonic acid with 7.6 mL (0.056 mole) of morpholinoepoxypropane for 3 days in 100 mL of 2 N sodium hydroxide solution at room temperature. The resulting mixture was passed through KU-28 (analog of Dowex-50X8, H^+ form) resin and the eluate left in the refrigerator overnight. The precipitated product was recrystallized from water (Table 1).

Evaluation of Herbicidal Activity of Aminomethylenebisphosphonic Acids

The physiologic activity of the studied compounds was tested using common cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L. cv. Wisconsin) seedlings. Each experiment was replicated four times.

Effects of Studied Compounds on the Growth of Test Plants

Seeds were germinated at 33°C for 1.5–4 days in darkness. Groups of 10–40 uniform seedlings (depending on the species used) were transferred to Petri dishes (9 cm) lined with two disks of Whatman No. 2

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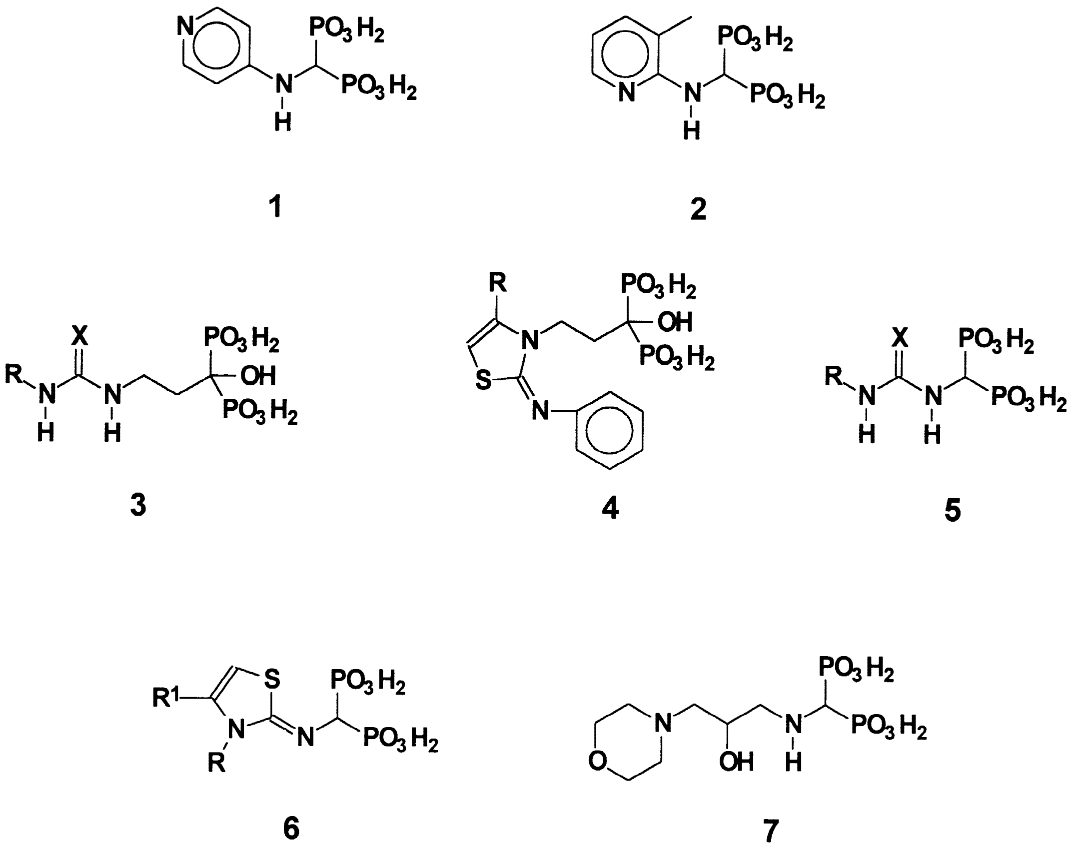


Fig. 1. *N*-(Pyridylamino)aminomethylenebisphosphonic acids (compounds 1 and 2) discovered by Suzuki et al. (1979) and their analogs—hydroxyalkylidenebisphosphonic acids (compounds 3 and 4); ureido- or thioureidemethylenebisphosphonic acids (compounds 5); derivatives of iminomethylenebisphosphonic acid (compounds 6) and compound 7.

filter paper wetted with 10 mL of distilled water (control) or solutions of the test compounds to give final concentrations of 0.05, 0.15 or 1.5 mM. Plants were grown at 25°C with a 12-h day length for 5 to 9 days (depending on species) under fluorescent tubes (about 300 μE $m^{-2}s^{-1}$ at plant level). Separated roots and shoots were then weighed on a torsion balance. The herbicidal effect was expressed as percentage change in plant root and shoot fresh weight in relation to untreated control.

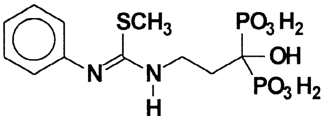
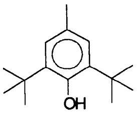
Statistical Treatment

Dixon’s *Q*-test was used to reject the unreasonable results. The means for samples and controls were compared by testing the null hypothesis at the 5% significance level. (Miller and Miller 1984). Results not statistically significant are marked in tables as “N.”

Results and Discussion

Previous studies on herbicidal activity of derivatives of aminomethylenebisphosphonic acids revealed that for compound to be active, two strongly acidic residues and one positively charged substituted amino group is required (Kafarski et al. 1997). We attempted to determine how structural modifications of the amino moiety influence herbicidal action of these compounds. Thus, compounds 3 and 4 represent those in which an amino group was replaced by a hydroxyl, in compounds 5 an amino group was replaced by ureido- or thioureido-function, whereas compounds 6 contain an imino group (an oxidized form of the amino group). Physiologic effects on growth of cress are given in Table 2. The influence of the tested compounds on plant root development was usually more pronounced than their action on shoots. The main indicators of phytotoxicity observed with the most active compounds were browning and swelling of shoots, and strong hypocotyl chlorosis with no effect on their width and branching. These indicators are similar to those exerted by the popular herbicide glyphosate used as a positive control. As Table 2 shows, most of the studied compounds, with the exception of compounds 4a, 4b, and 5a, which were inactive, exhibited quite significant phytotoxicity expressed as a decreased change in shoot and hypocotyl length in relation to control plants. Some compounds exhibited exceptionally strong herbicidal action.

Table 1. Compounds 3–7.

Compound	X	Structure R	R^1	Yield (%)	m.p. (°C)
3a	S	phenyl		98	126–128
3b	S	*p*-bromophenyl		62	172
3c				88	<320[a]
4a		Methyl		95	212
4b		Phenyl		83	211
5a	O	Methyl[b]		64	<360[a]
5b	S	Methyl[b]		79	<360[a]
5c	O	Phenyl[b]		80	<360[a]
5d	S	Phenyl[b]		75	350[a]
6a		Methyl	Methyl	51	350–355[a]
6b		Methyl	Phenyl	57	<270[a]
6c		Allyl	Phenyl	67	230–240[a]
6d		Allyl		45	250–258[a]
7				63	184

[a] Decomposition at this temperature was observed.
[b] As disodium salt.

Table 2. Effect of analogs of aminomethylenebisphosphonic acid on the growth of cress, measured as percentage change in root and shoot weight compared to that of control.

Compound	Root or shoot	Concentration (mM) 0.05	0.15	0.5	1.5
Glyphosate	R	−(86 ± 8)	−(88 ± 4)	−(90 ± 7)	−(94 ± 9)
	S	−(13 ± 1)	−(15 ± 2)	−(20 ± 2)	−(44 ± 2)
1	R	N	−(42 ± 7)	−(50 ± 3)	−(84 ± 8)
	S	N	−(15 ± 2)	−(36 ± 7)	−(49 ± 4)
2	R	−(76 ± 7)	−(82 ± 13)	−(89 ± 9)	−(95 ± 15)
	S	−(27 ± 6)	−(42 ± 9)	−(51 ± 7)	−(58 ± 9)
3a	R	−(64 ± 8)	−(51 ± 12)	−(78 ± 2)	−100
	S	N	+(17 ± 4)	−(84 ± 1)	−100
3b	R	N	−(52 ± 4)	−(86 ± 1)	−100
	S	N	−(43 ± 3)	−(86 ± 1)	−100
3c	R	N	N	−(47 ± 7)	−(59 ± 2)
	S	N	N	−(19 ± 5)	−(33 ± 2)
4a	R	N	N	N	N
	S	N	N	N	N
4b	R	N	N	N	N
	S	N	N	N	N
5a	R	N	N	N	N
	S	N	N	N	N
5b	R	N	N	−(26 ± 15)	−(76 ± 8)
	S	N	N	N	−(40 ± 4)
5c	R	N	N	−(45 ± 8)	−(51 ± 4)
	S	N	N	−(17 ± 2)	−(47 ± 3)
5d	R	N	+(31 ± 5)	−(18 ± 2)	−(36 ± 6)
	S	N	N	N	−(40 ± 3)
6a	R	−(72 ± 4)	−(89 ± 1)	−100	−100
	S	−(34 ± 2)	−(62 ± 2)	−100	−100
6b	R	N	N	−(72 ± 6)	−(83 ± 1)
	S	N	N	−(32 ± 1)	−(44 ± 2)
6c	R	−(15 ± 6)	−(17 ± 3)	−(14 ± 4)	−(12 ± 4)
	S	N	N	N	N
6d	R	N	N	N	−(66 ± 5)
	S	N	N	N	−(34 ± 3)
7	R	N	N	−100	−(94 ± 2)
	S	N	N	−100	−100

Thus, compounds 3a and 6a were comparable to the most herbicidally active controls, namely glyphosate and compound 2, whereas two other (compounds 3b and 6b) were comparable in activity to the third control compound 1. The relatively strong activity of compound 7 when applied in higher concentrations was not surprising because it represents a classical derivative of aminomethylenebisphosphonic acid.

The synthesized compounds represent at least three structurally diverse groups of analogs. The first group (compounds 3 and 4) contains a hydroxyl moiety in place of the amino group of aminomethylenebisphosphonate herbicides. Two compounds from this group (3a and 3b) appeared to be strongly herbicidal. This finding alongside the observed moderate herbicidal activity of compounds 5b, 5c, and 5d was somewhat surprising because it seems to indicate that a positively charged amino group is not a required structural fragment of bisphosphonate herbicides. Replacement of the amino moiety with an imino group (which also might be protonated) resulted in compounds 6, the closest structural analogs to parent compounds 1 and 2. As might be expected, they showed potent or moderate herbicidal activity. In summation, our results indicate that a substituted, protonated amino group is not a required structural feature of phytotoxic bisphosphonates. Similarly, as in previous studies, we were unable to find any reasonable structure-activity relationship for the synthesized compounds (Kafarski et al. 1997).

The compounds most active against cress were also tested on cucumber. As in our previous studies, cucumber was less sensitive than cress (Lejczak et al. 1996), and again the influence on plant root growth was usually more pronounced than the action on hypocotyls. The main indicators of phytotoxicity (similar to those observed on cress) were visible only when the highest concentrations of active compounds were applied. Results presented in Table 3 show that compound 3a, which exhibited exceptionally strong herbicidal activity on cress, exhibited typical plant growth regulatory activity on cucumber being stimulatory at lower concentrations and herbicidal at higher. Compound 6a was still most strongly phytotoxic being as active as glyphosate. The herbicidal activity observed for the studied representatives of bisphosphonic acids indicates their potential usefulness as a new class of herbicides.

Table 3. Effect of analogs of aminomethylenebisphosphonic acid on the growth of cucumber, measured as percentage change in root and shoot weight compared to that of control.

Compound	Root or shoot	Concentration (mM)			
		0.05	0.15	0.5	1.5
Glyphosate	R	−(60 ± 4)	−(77 ± 4)	−(78 ± 7)	−(84 ± 2)
	S	N	N	N	−(22 ± 2)
1	R	−(62 ± 5)	−(74 ± 5)	−(81 ± 9)	−(81 ± 3)
	S	−(34 ± 2)	−(34 ± 6)	−(47 ± 3)	−(49 ± 5)
2	R	−(76 ± 11)	−(88 ± 12)	−100	−100
	S	−(48 ± 10)	−(66 ± 9)	−(68 ± 7)	−100
3a	R	+(70 ± 0.3)	+(60 ± 0.2)	+(20 ± 0.2)	−(80 ± 0.2)
	S	+(10 ± 0.1)	N	+(17 ± 09.2)	−(36 ± 0.2)
3b	R	+(8 ± 0.6)	+(16 ± 0.4)	−(25 ± 0.2)	−100
	S	+(25 ± 0.2)	+(34 ± 0.2)	N	−(50 ± 0.2)
6a	R	N	(−83 ± 0.2)	−100	−100
	S	−(7 ± 0.1)	N	−100	−100
6b	R	N	−(22 ± 0.3)	−(45 ± 0.1)	−(61 ± 0.5)
	S	N	−(18 ± 0.2)	−(18 ± 0.1)	−(15 ± 0.1)
7	R	N	N	−(14 ± 0.3)	−(18 ± 0.5)
	S	−(14 ± 0.2)	−(14 ± 0.4)	−(18 ± 0.2)	−(21 ± 0.2)

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References

Chuiko AL, Filonenko LP, Borisevich AN, Lozinsky MO (1991) Reaction of aminoalkyliden-1,1-bisphosphonic acids with iso(thio)cyanates and tetramethyl thioureidosulphite (in Russian). Zh Obsch Khim 61:2552–2557

Chuiko AL, Filonenko LP, Borisevich AN, Lozinsky MO (1991) Synthesis and properties of thiouronium-substituted alkylidene-1,1-bisphosphonic acids. Zh Obsch Khim 66:1479–1483

Chuiko AL, Filonenko LP, Borisevich AN, Lozinsky MO (1995) Synthesis and properties of heterosubstituted alkylidene-1,1-bisphosphonic acids. I. Preparation and structure of thioazole-substituted alkylidene-1,1-bisphosphosphonic acids. Zh Obsch Khim 65:1332–1337

Cromartie TH, Fisher KJ (1995) Method of controlling plants by inhibition of farnesyl pyrophosphate synthase. PCT (Patent Cooperation Treaty, World Intellectual Property Organization International Bureau) Int Appl WO 95/34207

Cromartie TH, Fisher KJ, Grossman JN (1999) The discovery of a novel site of action for herbicidal bisphosphonates. Pestic Physiol Biochem 63:114–126

Forlani G, Lejczak B, Kafarski P (1996) *N*-Pyridyl-aminomethylene-bisphosphonic acids inhibit the first enzyme in the shikimate pathway, 3-deoxy-*D*-*arabino*-heptulosonate-7-phosphate synthase. Pestic Physiol Biochem 55:180–188

Kafarski P, Lejczak B, Forlani G, Gancarz R, Torreilles C, Grembecka J, Ryczek A, Wieczorek P (1997) Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acids. Part III. Structure-activity relationship, J Plant Growth Regul 16:153–158

Lejczak B, Boduszek B, Kafarski P, Forlani G, Wojtasek H, Wieczorek P (1996) Mode of action of herbicidal derivatives of aminomethylenebisphosphonic acids. I. Physiological activity and inhibition of anthocyanin biosynthesis, J Plant Regul 15:109–113

Miller JC, Miller JN (1984) Statistics for analytical chemistry. Ellis Harwood Ltd., Chichester

Oberhauser V, Gaudin J, Fonne-Pfister R, Schar H-P (1996) New target enzyme(s) for bisphosphonates: inhibition of geranylgeranyl diphosphate synthase. Pestic Physiol Biochem 60:111–117

Suzuki F, Yoshihiro F, Yamamoto S, Mizutani H, Funabashi C, Ohya T, Ikai T, Oguchi T (1979) *N*-Pyridylaminomethylenediphosphonsaureverbindungen. Ger Offen 2.831.578