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Herbicidal Activity of Phosphonic, Phosphinic, and Phosphonous Acid Analogues of Phenylglycine and Phenylalanine

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Abstract. A series of phosphonic, phosphinic, and phosphonous acid analogues of phenylglycine and phenylalanine was synthesized and tested as herbicides against *Lepidium sativum* and *Cucumis sativus*. Aminobenzylphosphonic acids exhibited notable herbicidal activity and thus represent a group of the most active herbicides found among aminophosphonic acids.

The biosynthesis of aromatic amino acids in plants is a multistep pathway that gives rise not only to amino acids but also supplies precursors for the production of numerous secondary plant products that contain aromatic groups. This process involves a common route to chorismate known as the shikimate pathway, followed by three separate pathways from chorismate to phenylalanine, tyrosine, and tryptophan. These amino acids are the key substrates for the production of lignins, coumarins, tannins, alkaloids, and flavonoids as well as for many plant defense substances. It is well established that a number of herbicidally active substances act by inhibition of aromatic amino acid metabolism. Indeed, several compounds have been shown to have profound and specific effects on certain enzymes involved in the synthesis and catabolism of these acids. The most clear cut results were obtained with glyphosate (Fig. 1), which has been shown to elicit its herbicidal activity by the inhibition of 5-enolpyruvoylshikimate-3-phosphate synthase, thus blocking the shikimate pathway (Grossbard and Atkinson 1985). The identification of glyphosate as an extremely effective and environmentally friendly herbicide stimulated intensive studies on the synthesis of phosphonic acid analogues of aromatic amino acids and evaluation of their herbicidal activity. These efforts resulted in the discovery of numerous highly active compounds. These include, at least, herbicidal (*N*-pyridylamino)methylenebisphosphonic acids (**2**) being developed in Japan (Suzuki et al. 1979); 2-aminoindan-2-yl-phosphonic acid (**3**), which is a potent inhibitor of phenylalanine ammonia-lyase (Zoń and Amrhein 1992); plant growth-regulating phosphonic acid analogues of morphactins as, for example, compound **4** (Wojtasek et al. 1991); and the phosphonic acid analogue (**5**) of dopa (Kafarski et al. 1989).

However, the generation of an equivalent structure-activity relationship within this class of compounds is still in its infancy. This stimulates an intensive search for new structural variants of aromatic amino acid analogues. In this paper we have investigated the herbicidal activity of phosphonic acid analogues of phenylglycine and phenylalanine against the test plants *Lepidium sativum* (cress) and *Cucumis sativus* (cucumber).

Materials and Methods

Chemical Syntheses

Phosphonous acid analogues (**6**) of substituted phenylglycines were available from previous studies or were prepared using standard procedures (Tyka et al. 1991). Derivatives of aminobenzylphosphonic (**7**) and aminobenzyl(*p*-methyl)phosphinic (**8**) acids, phosphonic and *p*-methylphosphinic acid analogues (**9**) of phenylalanine as well as 1-aminoalkylphosphonic and phosphinic acids (**10**) were prepared by amidoalkylation of phosphorus trichloride or methyldichlorophosphine with aldehydes or ketones and acetamide (Soroka 1990). Compounds **11** were available from previous study (Tyka and Hagele 1989). Melting points and yields of these compounds are given in Tables 1–5.

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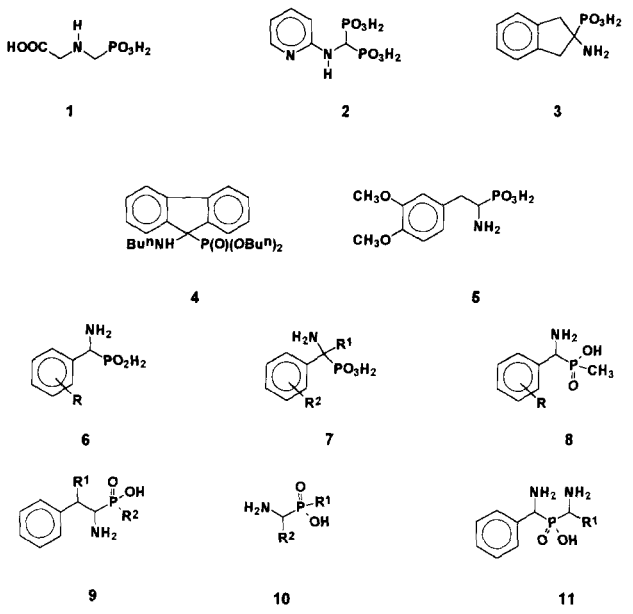


Fig. 1. Glyphosate (**1**); (*N*-pyridylamino)methylenebisphosphonic acid (**2**); 2-aminoindan-2-yl-phosphonic acid (**3**); phosphonic acid analogue of morphactin (**4**), phosphonic acid analogue of dopa (**5**); phosphonous acid (**6**), phosphonic acid (**7**) and phosphinic acid (**8**) analogues of phenylglycine; analogues (**9**) of phenylalanine; 1-aminoakylphosphonic and -phosphinic acids (**10**); and phosphinic acids (**11**).

The structures of all of the compounds were supported by their infrared and proton magnetic resonance (^{1}H NMR) spectra as well as by elemental analyses.

Biologic Assays

The herbicidal activity of the synthesized compounds was evaluated in two sets of experiments on *L. sativum* and *C. sativus* var. *Wisconsin*. Each experiment was replicated three times.

Effects of Studied Compounds on the Growth of L. sativum. Groups of 40 seeds of *L. sativum* were placed in Petri dishes (9 cm) filled with cotton wool and were kept damp by occasional spraying with distilled water until germination occurred (2 days, 4–6 mm total length of the plants). Distilled water (control) or aqueous solution of test compound (to give a final concentration of 0.05, 0.15, or 1.5 mM; 10 ml each) was then applied to the roots. Plants were grown for 7 days at 25°C with a 9-h day length under fluorescent tubes (2,500–3,000 lux at plant level), and the lengths of the roots and shoots were then measured.

Test on C. sativus. Seeds were germinated at 33°C for 1.5 days in darkness. Groups of 10 uniform seedlings (7–8 mm total length) were transferred to Petri dishes (9 cm) lined with two discs of Whatman no. 2 filter paper wetted with distilled water (control) or solutions of test compounds to give a final concentration of 0.05, 0.15, or 1.5 mM; 10 ml each. The plants were grown at 25°C with a 12-h day length for 9 days under fluorescent tubes (2,500–3,000 lux at plant level). Separated roots and hypocotyls were weighed at a torsion balance.

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).

Non–statistically significant results are marked in Tables as *N*.

Results and Discussion

Simple phosphonic acid analogues of phenylalanine were found to be effective inhibitors of phenylalanine ammonia-lyase (Janas et al. 1985, Janas and Olechnowicz 1994, Knypl et al. 1986, Laber et al. 1986, Zoń and Amrhein 1992) and plant growth regulators (Kafarski et al. 1989, Knypl et al. 1986, Zoń and Amrhein 1992). In this work we have synthesized a series of phosphonic, phosphinic, and phosphonous acid analogues of phenylalanine and phenylglycine and screened for their herbicidal activity against *L. sativum* and *C. sativus*. Our intention was to determine how the structural changes introduced to the molecule of the phosphonic acid analogue of phenylalanine (compound **9**, $R^1 = R^2 =$ H) influence their herbicidal activity.

Data shown in the Table 6 concern those compounds that exhibited either significant herbicidal or growth stimulatory action toward *L. sativum*. Compounds not included in the table were either weakly active (less than 20% of inhibition or promotion of growth) or completely inactive. Herbicidally active 1-aminopropylphosphonous acid (compound **6**) (Baylis et al. 1984) was used as a positive control in this study.

With the exception of compound **7e**, all of the aminobenzylphosphonic acids (compounds **7**) displayed significant activity against *L. sativum*, with compounds **7d**, **7g**, **7i**, **7j**, and **7k** being equal in activity to a reference compound **6a**. Reflecting the mode of application, their influence on the growth of plant roots was usually more pronounced than their action on shoots. However, unlike compound **6a**, which did not affect the shoot growth, there were also significant effects on the plant shoots with **7g** exhibiting the strongest effect. It is also evident that the compounds bearing the substituent in *para*-position of the aromatic ring were generally the most active. In contrast, most of the aminobenzylphosphonous (compounds **6**) and all of the (*p*-methyl)aminobenzylphosphinic acids (compounds **8**) were practically inactive.

Table 1. Aminobenzylphosphonous acids (**6**).

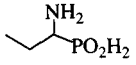
Compound	Structure (R)	Yield (%)	m.p. (decomp) (°C)	Ref.
6a		75	223–224	Baylis et al. 1984
6b	H	70	242–243	Tyka and Hagele 1989
6c	*o*-CH_3	30	228–230	Tyka and Hagele 1989
6d	*o*-Cl	31	229–231	Tyka and Hagele 1989
6e	*m*-Cl	48	235–236	Tyka and Hagele 1989
6f	*p*-Cl	41	240–250	Tyka and Hagele 1989
6g	*p*-Br	56	246–247	Tyka and Hagele 1989
6h	*m*-O_2N	51	247–248	Tyka and Hagele 1989

Table 2. Aminobenzylphosphonic acids (**7**).

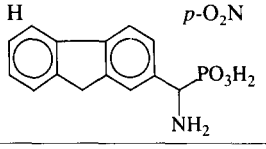
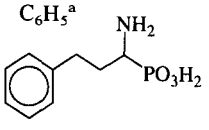
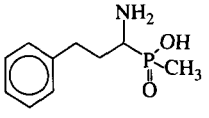
Compound	Structure R^1	Structure R^2	Yield (%)	m.p. (dec.) (°C)	Ref.
7a	H	*o*-F	52	256–258	
7b	H	*m*-F	72	279–280	
7c	H	*p*-F	67	284–285	
7d	CH_3	*p*-F	46	211–213	
7e	H	*o*-Cl	47	239–242	
7f	H	*m*-Cl	38	245–247	
7g	H	*p*-Cl	67	268–270	Oleksyszyn et al. 1978
7h	H	*o,p*-Cl_2	74	249–252	
7i	H	*o*-Br	39	222–223	
7j	H	*p*-Br	68	272–273	Lukszo and Tyka 1977
7k	H	*p*-HOOC	68	204–207	
7l	H	*p*-O_2N	100	200–201	Lukszo and Tyka 1977
7m			20	218–220	

Table 3. (*p*-Methyl)aminobenzylphosphinic acids (**8**).

Compound	Structure R	Yield (%)	m.p. (decomp) (°C)	Ref.
8a	H	40	246–248	Oleksyszyn and Gruszecka 1981
8b	*o*-Cl	24	229–231	
8c	*p*-Cl	51	256–257	
8d	*o,p*-Cl_2	83	210–211	

Table 4. Analogues of phenylalanine (**9**) and compounds **11**.

Compound	Structure R^1	Structure R^2	Yield (%)	m.p. (decomp) (°C)
9a	OH	OH	55	191–193
9b	CH_3	OH	67	251–253
9c	CH_3	CH_3	37	205–207
11a	CH_3[a]		35	234–236
11b	C_6H_5[a]		46	244–246
12a			71	286–287
12b			43	199–200

[a] Isolated as monohydrochloride.

Among analogues of phenylalanine containing longer alkyl chain (compounds **9**, **11**, and **12**) only compound **12b** displayed activity similar to the reference compound **6a**. Compounds **9c** and **12b** represent rare examples of herbicidally active phosphinic acids. Taking into account that compound **12a** acts as inhibitor of phenylalanine ammonia-lyase (Janas and Olechnowicz 1994) and is significantly less herbicidally active than its phosphinic counterpart (compound **12b**) the molecular mode of action of **12a** is worthy of further study.

Table 5. 1-Aminoalkylphosphonic and -phosphinic acids (**10**).

Compound	Structure R[1]	Structure R[2]	Yield (%)	m.p. (decomp) (°C)
10a	OH	*tert*-Butyl	30	235–236
10b	OH	Adamantyl	43	224–226
10c	CH_3	Adamantyl	41	150–151

Finally phosphinic acids (compounds **11**), which were recently found to be promising inhibitors of HIV proteases (Peyman et al. 1994), also exhibited moderate herbicidal activity on the roots of *L. sativum*.

To determine the role of planarity of aromatic ring we synthesized compounds **10** containing sterically large groups and evaluated their influence on the growth characteristics of *L. sativum*. As seen from Table 6 all three 1-aminoalkylphosphonic acids exerted quite high herbicidal activity in higher concentrations. They were, however, significantly weaker than aminobenzylphosphonic acids.

The compounds most active against *L. sativum* were also tested on *C. sativus*. Results presented in Table 7 show *C. sativus* to be more tolerant of the action of the compounds studied than of the reference compound **6a.** The effect of compound **6a** was different in this test plant as it influenced significantly both stem and hypocotyl growth at a whole range of applied concentration. Also in the case of *C. sativus* the best herbicidal activities were achieved for the aminobenzylphosphonic acids **7c, 7f,** and **7j.** Again, the influence on the growth of plant roots was usually more pronounced than the action on hypocotyls. However, there is no simple correlation between the effects observed in *L. sativum* and *C. sativus*. Compound **7j** appears to be promising candidate for further studies. Among other compounds studied aminobenzylphosphonous acid **6g** (stimulatory in *L. sativum*) and compound **12a** (moderately herbicidal in *L. sativum*) attained quite high herbicidal potency. Nearly all of the herbicidally compounds studied in this work caused the death of *C. sativus* if applied at a concentration of 1.5 mM. This effect was due to both the herbicidal action of the applied compound and to the high susceptibility of the plant to fungal infections. The fact that plants treated by analogues of phenylalanine were infected by fungi may be explained by the hypothesis that the analogues interact with aromatic acid biosynthesis or catabolism pathways, thereby decreasing the plant's ability to produce aromatic defense substances (Brammall and Higgins 1988, Johal and Rahe 1984).

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Table 6. Effect of phosphonic, phosphinic, and phosphonous acid analogues of phenylglycine and phenylalanine on the growth of *L. sativum*, measured as percentage change in root and shoot length compared with that of the control.

Compound	Root or shoot	Concentration (mM) 0.05	0.15	0.5	1.5
6a	R	−60	−76	−83	−85
	S	N[a]	N	N	N
6d	R	N	−18	−21	−41
	S	N	−13	−10	−10
6e	R	−33	−38	−42	−52
	S	N	N	N	−13
6g	R	N	+51	+39	N
	S	N	+20	+20	+15
7a	R	N	−26	−60	−88
	S	N	−13	−16	−43
7b	R	N	−36	−85	−92
	S	N	N	−47	−65
7c	R	N	−47	−58	−82
	S	N	−12	−34	−71
7d	R	−59	−68	−72	−90
	S	N	N	N	−20
7f	R	N	N	−50	−57
	S	N	N	N	−28
7g	R	−50	−86	−81	−100
	S	N	−58	−55	−100
7h	R	N	+51	−45	−70
	S	N	N	−43	−60
7i	R	−54	−61	−81	−100
	S	N	N	−18	−100
7j	R	−57	−79	−92	−100
	S	N	−23	−59	−100
7k	R	−47	−71	−83	−94
	S	+25	N	−10	−38
7l	R	−24	−70	−80	−78
	S	N	N	−15	−36
7m	R	N	−23	−38	−37
	S	N	N	N	N
9b	R	−32	−31	−46	−96
	S	N	N	N	−26
9c	R	N	−22	−49	−96
	S	N	N	N	−65
10a	R	N	N	−68	−84
	S	N	N	N	−14
10b	R	N	N	−46	−99
	S	N	N	−16	80
10c	R	N	N	N	−84
	S	N	N	N	N
11a	R	N	−22	−23	−57
	S	N	N	N	N
11b	R	N	−33	−37	−34
	S	N	N	N	N
12a	R	N	N	−32	−90
	S	N	N	N	−74
12b	R	−48	−47	−55	−100
	S	N	N	N	N

[a] N, no effect.

Table 7. Effect of aromatic aminophosphonates on the growth of *Cucumis sativus*, measured as percentage change of root and hypocotyl weight compared with that of the control.

Compound	Root or hypocotyl	Concentration (mM) 0.05	0.15	0.5	1.5
6a	R	−77	−75	−85	−100
	H	−30	−38	−52	−100
6d	R	N[a]	N	N	+37
	H	N	N	N	N
6g	R	N	N	−98	−100
	H	N	N	−99	−100
7a	R	N	+39	N	−21
	H	N	N	N	N
7b	R	N	N	−65	−76
	H	N	N	N	−42
7c	R	N	−40	−77	−90
	H	N	N	N	−15
7d	R	N	N	−22	−95
	H	N	N	N	N
7f	R	−33	−45	−51	−100
	H	N	N	−26	−100
7g	R	N	N	−48	−54
	H	N	N	N	N
7h	R	N	N	−39	−100
	H	N	N	+55	−100
7i	R	N	N	−36	−100
	H	N	N	+31	−100
7j	R	N	−25	−66	−100
	H	N	N	N	−100
7k	R	N	N	−65	−98
	H	N	N	N	−100
7l	R	N	N	−48	−100
	H	N	N	N	−100
9b	R	+84	+61	−30	−100
	H	N	−13	−17	−100
9c	R	N	N	N	−98
	H	N	N	N	−94
10a	R	N	N	N	N
	H	N	N	N	N
10b	R	N	N	N	−100
	H	N	N	N	−100
11a	R	N	N	−39	−63
	H	N	N	N	−13
12a	R	−23	−31	−100	−100
	H	N	N	−100	−100
12b	R	N	N	N	−68
	H	N	N	N	N

[a] N, no effect.

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