Acta Bot. Croat. *33* (1974) 111—116

GROWTH INHIBITORY ACTIVITY OF ALDONOLACTONES IN *AVENA* COLEOPTILE SECTION TEST

MIROSLAV POKORNY

(National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.)

Received October 31, 1973.

Introduction

Aldonolactones are known as powerful, competitive and highly specific inhibitors of enzymes which catalyze the hydrolysis of glycosides derived from the same aldoses. The high affinity of the aldonolactones for these enzymes might be due to their flexible half chair conformational similarity to the hypothetical transition state in an enzyme-catalyzed pyranoside hydrolysis (Levvy and Snaith 1972). Aldonolactones may exist as five-membered or six-membered rings and in water solution they spontaneously equilibrate into mixtures containing both lactones and the corresponding free acid. It is presently believed that the 1,5-lactones are more active than the corresponding 1,4-lactones.

There is little known about the activity of aldonolactones in higher plants although they are not completely foreign in the plants: D-glucono-1,5-lactone is an intermediary in carbohydrate metabolism and D-galacturono-1,4-lactone, L-galactono-1,4-lactone and 2-keto-L-galactono-1,4-lactone are involved in the biosynthesis of ascorbic acid which is also a lactone (Staněk et al. 1963). The inhibitory properties of aldonolactones have been applied in some cases for characterization and identification of plant glycosidases but no one has considered their activity in a bioassay test, although it is known that some non-sugar lactones obtained from plant material, may have antifungal, antibacterial, antitumorous, as well as inhibitory and promotive growth activities in plants (Iino et al. 1972., Cambie and Russel 1973). In the present paper the inhibitory activity of several aldonolactones on elongation of *Avena* coleoptile sections is reported.

Materials and Methods

Lactones were synthesized in this laboratory by standard procedure or were commercially available. Solutions rich in 1,5-lactone (the so called "the most inhibitory solutions") were prepared from the corresponding acids as described by Levvy et al. 1962).

Oat seeds (*Avena sativa* var. Golden rain) were germinated in the dark at 25—26° (Keglević and Pokorny 1969). For assay, coleoptiles (2.8—3.0 cm) were cut 3 mm below the tip in the lengths of 5 mm and 20 sections were incubated with various concentrations of aldonolactones in 10 ml 2% saccharose (Nitsch and Nitsch 1956) at 26° in the dark. After 24 h the sections were measured under a dissection microscope. Inhibition growth of treated coleoptiles was calculated as percentage of control growth.

For extraction of glycosidases, a butch of 100 coleoptiles (2.8—3.0 cm) were collected and each was cut into four sections from the tip down successively in lengths of 3, 5, 10 and 10 mm. Each batch of sections was homogenized in 5—8 ml of 0.05 M sodium citrate buffer (pH 4.3) which contained 0.1 M NaCl (Levvy and Conchie 1966). After 2 h at 0°, the homogenates were centrifuged and the precipitates were washed with 2—3 ml of buffer and centrifuged again. Each combined supernatant were made up to 10 ml with the same buffer and stored at 0° for several days without loss of activity.

Enzyme assay. The activity of the various glycosidases was assayed in aliquots which represented 20 mg fresh weight coleoptile sections, by using the corresponding p-nitrophenyl glycosides. The aliquots were incubated either in 2 ml 0.2 M McIlvaine buffer or in 2 ml 0.2 M acetate buffer at optimal pH values for 20 min at 38°. At the end of incubation 5 ml of 0.4 M glycine-NaOH buffer pH 10.5 was added and the liberated p-nitrophenol was measured spectrophotometrically at 430 nm. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of p-nitrophenol per minute at 38° under the assay conditions. The specific activity is expressed as enzyme unit per mg of fresh plant material.

Results and Discussion

A number of aldonolactones were screened at various concentrations for growth-inhibitory activity in the standard *Avena* coleoptile section test (Table 1). It can be seen that all the lactones tested, with the exception of D-ribono-1,4-lactone and L-galactono-1,4-lactone, showed inhibitory activity. However the ratio of inhibition varied as to ring size of of the lactone as well as to the type and configuration of the sugar from which the lactone had been synthesized. The results showed that in the same series of sugar the 1,5-lactones were better inhibitory agents than the corresponding 1,4-lactones. One might consider that part of the activity of 1,4-lactones can be assigned to their corresponding 1,5-lactones that spontaneously arise in the proces of equilibria. Only in the case of D-glucaro-1,4-lactone similar inhibitions were observed as in its 1,5-lactone (solution rich on 1,5-lactone). However this can be attributed to a rapid equilibrium (Levvy and Snaith 1972).

Table 1. Growth inhibition of aldonolactones in *Avena* coleoptile section

Aldonolactones	Concn. (mM/l)		
	0.5	1.0	2.0
D-Allono-1,4-lactone	0	3	8
D-Allono-1,5-lactone	5	8	22
D-Galactono-1,4-lactone	6	14	16
D-Galactonolactone*	86	100	100
L-Galactono-1,4,-lactone	0	0	0
L-Galactonolactone*	11	16	18
D-Glucono-1,4-lactone	20	24	61
D-Glucono-1,5-lactone	36	63	100
L-Glucono-1,5-lactone	18	38	70
D-Gulono-1,4-lactone	0	18	18
D-Gulonolactone*	8	28	36
D-Mannono-1,4-lactone	0	14	28
D-Mannonolactone*	44	50	60
L-Mannono-1,4-lactone	0	5	8
L-Mannonolactone*	10	13	18
D-Galactarolactone*	65	86	100
D-Glucaro-1,4-lactone	25	96	100
D-Glucarolactone*	26	96	100
D-Glucuronolactone*	27	31	48
D-Galacturonolactone*	24	32	54
2-Acetamido-2-deoxy-D-galactono-1,4-lactone	6	13	24
2-Acetamido-2-deoxy-D-galactonolactone	16	22	75
2-Acetamido-2-deoxy-D-glucono-1,4-lactone	6	6	27
2-Acetamido-2-deoxy-D-glucono-1,5-lactone	31	44	76
2-Acetamido-2-deoxy-D-mannono-1,4-lactone	7	40	43
2-Acetamido-2-deoxy-D-mannonolactone*	18	52	62
2-Acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone	0	3	9
2-Acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone	0	2	3
D-Glucoheptonolactone*	13	21	21
D-Mannoheptonolactone*	4	6	8
α-Metasaccharinolactone*	6	18	40
β-Metasaccharinolactone*	4	6	24
D-Arabinolactone*	53	71	98
L-Arabinolactone*	23	43	63
D-Ribono-1,4-lactone	0	0	0
D-Ribonolactone*	8	14	21
3-Deoxy-D-glucono-1,4-lactone	5	8	8
3-Deoxy-D-gluconolactone*	8	12	16

+ Growth inhibition was calculated as percentage of control experiments in which coleoptile sections grew approximately 5 mm.

* »The most inhibitory solutions« have been used.

Certain herbicides having a lactone structure are known to inhibit IAA activity in the coleoptiles (Brown 1972, Sequeira et al. 1968). However no significant difference was found in the effect of aldonolactones on the growth inhibition of coleoptiles when followed either in 2% saccharose or various concentrations of IAA.

Tests were also made to determine the nature of the inhibition growth of coleoptiles by aldonolactones. For this purpose sections were placed in a 1 mM solution containing either D-glucono-1,5-lactone or D-glucaro-1,4-lactone for 3 h. After being thoroughly washed with water and incubated as usual in 2% saccharose solution for 24 h, the treated sections did not show any significant inhibition of extension. Therefore it seems that aldonolactones may be considered as reversible growth inhibitors, a behavior observed in other compounds possessing a lactone group.

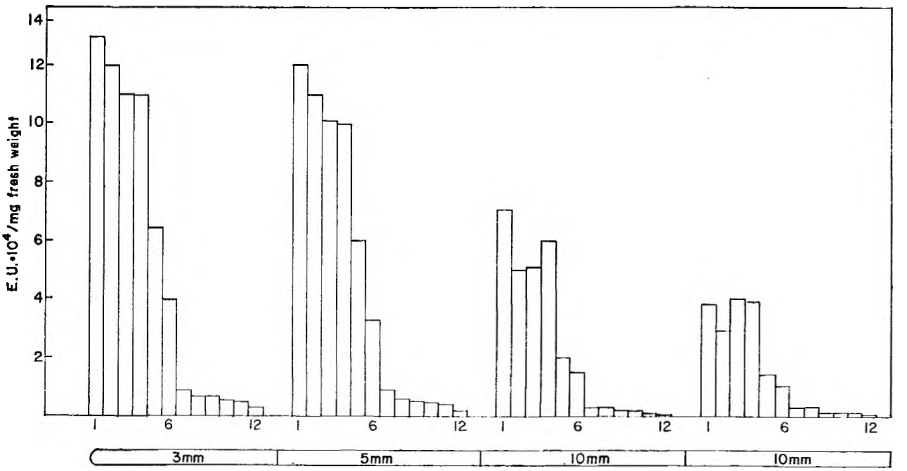

Fig. 1. Distribution of several glycosidases over the length of coleoptiles. The height of the columns represent enzyme units x 10^4 per mg fresh weight of corresponding sections. 1. α-mannosidase, 2. 2-acetamido-2-deoxy-β-D-glucosidase, 3. β-galactosidase, 4. β-glucosidase, 5. α-galactosidase, 6. 2-acetamido-2-deoxy-β-D-galactosidase, 7. β-glucuronidase, 8. β-xylosidase, 9. L-β-fucosidase, 10. α-glucosidase, 11. L-α-fucosidase and 12. 2-acetamido-2-deoxy-α-D-glucosidase.

Finally, it has been found that the activity of most of the glycosidases is predominant in the tip and the elongation zone of the coleoptiles (Fig. 1) and this suggests that they might play a considerable role in the growing tissues. Furthermore it was shown that these enzymes are strongly inhibited by the corresponding aldonolactones (Table 2). The results indicate to some extent that retarded coleoptile growth in the presence of aldonolactones is the consequence of a blocked glycosidases system in the tissue.

Table 2. The inhibition of glycosidases by corresponding aldonolactones

Enzymes	50% inhibition (μM)
β-Glucosidase	9.9*
2-Acetamido-2-deoxy-β-D-galactosidase	10.1★
2-Acetamido-2-deoxy-β-D-glucosidase	18.7*
β-Galactosidase	101.5★
α-Mannosidase	109.8★

* »The corresponding crystalline 1,5-lactone has been used.
* »The most inhibitory solution« has been used.

Summary

The growth inhibitory activity of a number of aldonolactones have been assayed by a standard *Avena* coleoptile section test. This activity, which is believed to be due to the consequence of a blocked glycosidases system, varies with the nature of the sugar molecule as well as the size of the lactone ring.

References

Brown, B. T., O. Johansen and *W. H. F. Sasse,* 1972: New inhibitors of auxin transport. Experimentia *28,* 1290—1291.

Cambie, R. C. and *G. B. Russel,* 1973: A norditerpene lactone from *Podocarpus hallii.* Phytochem. *12,* 2057—2057.

Iino, Y., A. Tanaka and *K. Yamashita,* 1972: Plant growth inhibitory activities of synthetic α-methylene-γ-butyrolactones. Agr. Biol. Chem. *36,* 2505—2509.

Keglević, D. and *M. Pokorny,* 1969: The chemical synthesis of 1-0-(Indol-3'-ylacetyl-)-β-D-glucopyranoside. Biochem. J. *114,* 827—832.

Levvy, G. A., A. McAllan and *A. J. Hay,* 1962: Inhibition of glycosidases by aldonolactones of corresponding configuration. Biochem. J. *82,* 225—232.

Levvy, G. A. and *J. Conchie,* 1966: Mammalian glycosidases and their inhibition by aldonolactones. In *Collowick, S. P.* and *N. O. Kaplan,* (Eds.) Methods in Enzymol. *8,* 571—584.

Levvy, G. A. and *S. M. Snaith,* 1972: The inhibition of glycosidases by aldonolactones. Advan. Enzymol. *36,* 151—181.

Nitsch, J. P. and *C. Nitsch,* 1956: Studies on the growth of coleoptile and first internode sections, a new sensitive, straight-growth test for auxins. Plant Physiol. *31,* 94—111.

Sequeira, L., R. J. Hemingway and *S. M. Kupchan,* 1968: Vernolepin : a new reversible plant growth inhibitor. Science *16,* 789—790.

Staněk, J., M. Černý, J. Kocourek and *J. Pacák,* 1963: The Monosaccharides, Academic Press, New York — London.

SADRŽAJ

Niz aldonolaktona je testirano na inhibiciju produžnog rasta koleoptila zobi. Nađeno je da stupanj inhibicije ovisi o prirodi šećerne komponente kao i o veličini laktonskog prstena. Istraživanja ukazuju na to da je smanjeni rast posljedica blokiranih glikozidaza u tkivu kleoptila.

Dr Miroslav Pokorny
National Institute of Arthritis, Metabolism and Digestive Diseases,
National Institutes of Health, Public Health Service,
U.S. Department of Health, Education and Welfare,
Bethesda, Maryland 20014, U. S. A.