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1,2 Benzisothiazol-3-ylacetic acid, a new synthetic auxin

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Fitofarmacologia. — *1,2 Benzisothiazol-3-ylacetic acid, a new synthetic auxin.* Nota di CAMILLO BRANCA e ENRICO GAETANI, presentata (*) dal Corrisp. E. MARRÈ.

RIASSUNTO. — Il composto di sintesi acido 1,2 benzisotiazol-3-ilacetico (BIA) risulta indurre, sul test segmenti di internodio di piantine eziolate di pisello, uno stimolo alla crescita per allungamento e un effetto curvatura (« split pea test ») molto simile a quelli indotti dall'auxina naturale, acido indolil-3-acetico (IAA). L'effetto del BIA sulla crescita per distensione cellulare è – come quello dell'IAA – inibito da inibitori della sintesi proteica e accompagnato, a concentrazioni relativamente elevate, da stimolo alla produzione di etilene.

Il BIA induce inoltre sul test allungamento di radici di pisello un effetto di inibizione quantitativamente molto simile a quello indotto dallo IAA.

Questi risultati, insieme a considerazioni di ordine strutturale, permettono di includere il BIA tra le auxine sintetiche.

A new phytoactive molecule: BIA, has been prepared through processes of controlled drug synthesis [1]. This compound showed remarkable herbicidal properties [2] and the power and selectivity of the acid as well as of other related compounds have been investigated *in vivo*. It has also been observed that the herbicidal action of this group of compounds was often accompanied by morphological alterations of the hormone induced kind [3].

The above mentioned facts and the structural similarity between BIA and IAA have prompted a more detailed investigation into the biological effects of BIA and their relationship with the effects induced by growth factors.

Taking into account what has recently been shown by other workers [4], we summarise in this paper the results of experiments on the action of BIA in curvature tests, cell expansion, formation of secondary roots and production of ethylene.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum.* var. Alaska) were washed in running water for 5 hrs then placed to germinate in moist sand at 25° C in the dark. After seven days germination, the apical internodes (3rd internode) were dissected from the seedlings. After washing for 30 mins. in distilled water, the randomised internodes were divided into batches to be used in the different tests.

(*) Nella seduta del 10 febbraio 1973.

Split test. – A 20–mm longitudinal cut was made in 30–mm long internodes. Batches of 10 split internodes were placed in Petri dishes containing 10 ml of the solutions to be tested and incubated for 20 hrs at 25° C in the dark. The results were determined from photographical exposures.

Pea test. – Batches of 12 10–mm long internode sections were placed in Petri dishes containig 10 ml of the solutions to be tested. After 3 hrs at 25° C in the dark the segments were collected, dried on blotting paper and their length and fresh weight measured. The increase in fresh weight was expressed as percentage of the initial weight.

Determination of ethylene production.

Batches of 30 10–mm long sections were placed in 125–ml conical flasks containing 10 ml of the solutions to be tested. The flasks were stoppered with rubber stoppers in which two glass tubes with stopcocks were fitted.

The incubation was carried out in a shaking water bath at 25° C for 8 hrs in the dark with an agitation of 110 rpm. From each flask a sample of gas (6 ml.) was then collected by introducing distilled water through one of the two glass tubes and connecting the other to the gas chromatograph.

The gas chromatography equipment used was a C. Erba Fractovap model C fitted with a flame ionisation detector. A 150×0.20 cm column of 30–60 mesh silica gel, activated for 24 hrs at 150° C was used. The column was operated at 60° C with nitrogen as carrier gas; gas flow was 54 ml/min.

The peaks on the chromatogram were integrated by triangulation and the figures obtained were normalised to a standard mixture of 9.6 p.p.m. ethylene in air.

Root inhibition test.

Pea seeds were washed for 5 hrs in running water and placed on wet filter paper to germinate at 25° C in the dark. When the roots were about 1 cm long, statistically significant batches of the randomised seeds were treated with 10^{-5} M solutions of the substances to be tested. The seeds were then incubated for 20 days and the results determined from photographic exposures.

In the experiments referred to above, we have used BIA obtained by synthesis as described [2]; melting point: 153–54° C. "Analar" grade chemicals have been used throughout. Indolyl–3–acetic acid (IAA) was obtained from Merck and cycloheximide from Schuchardt.

Results and discussion

The observation of the morphological changes that accompany the herbicidal action of BIA drew our attention initially towards the activity of BIA in inducing the curvature of split internodes of etiolated peas and the inhibitory effect of the acid towards the formation of secondary roots in intact seedlings.

The results of this set of experiments support, as a whole, previous findings of other researchers [4] and of ourselves [3].

Figs. 1 and 2 show how the effects of BIA compare with those induced by IAA. On the basis of these results we have successively investigated the action of BIA on growth by cell enlargement. This is the choice test to detect auxin-like activity in the material used. We have measured the growth of pea internodes as increase in both length and weight.

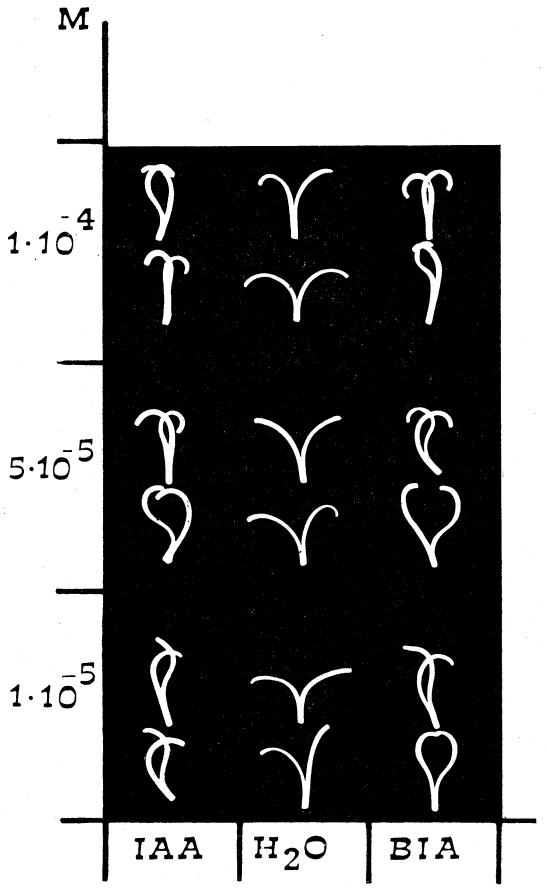

Fig. 1. – Effects of IAA and of BIA on the split pea internode curvature test.

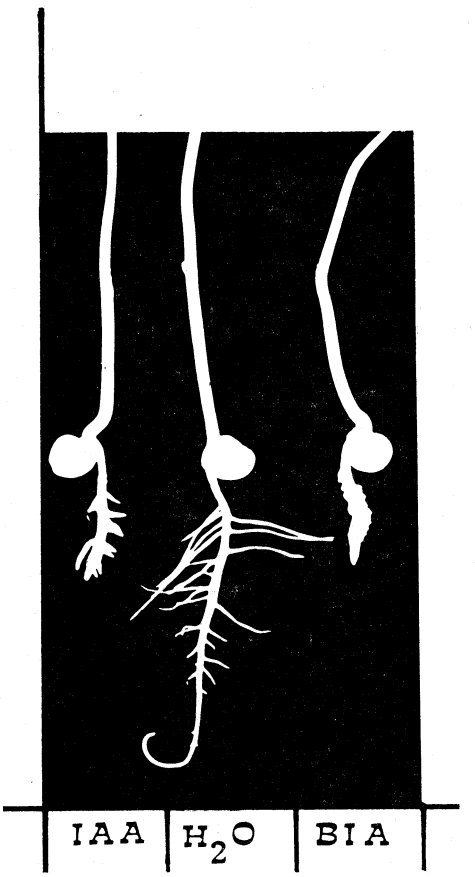

Fig. 2. – Effects of IAA and of BIA on pea root development.

Figs. 3 and 4 show the effects on growth by cell enlargement obtained with BIA and with IAA as a control. From these results the following considerations can be made:

1) BIA has a very strong effect on growth both in length and in weight. The optimum concentration of 10^{-4}M causes an increase in length of 34.3 % and an increase in fresh weight of 34.7 %, figures these that fall in the same range as those obtained in the presence of IAA at the same concentration.

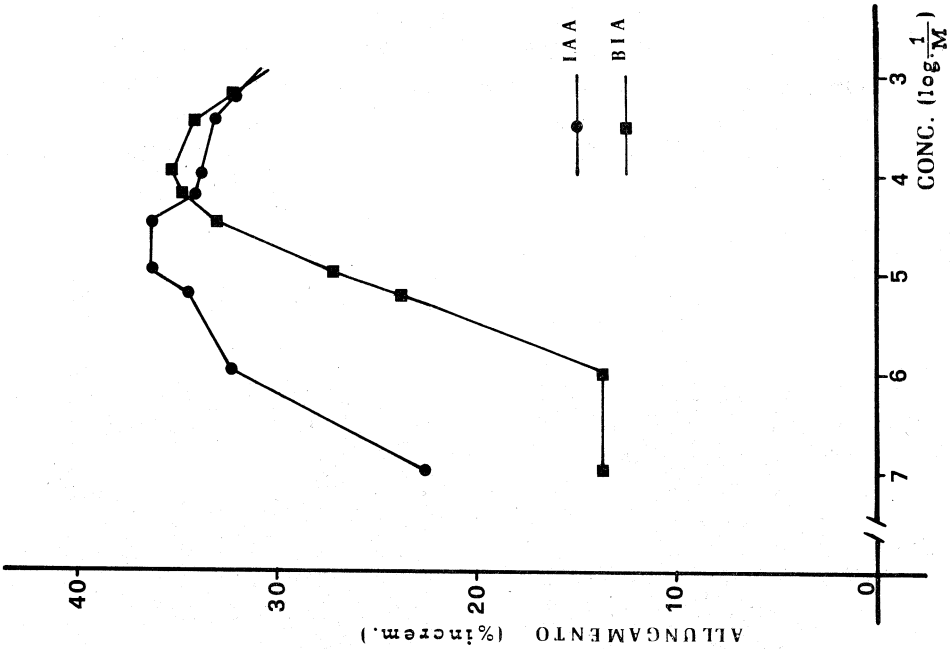

Fig. 4. – Effects of IAA and BIA on the elongation of pea internode segments.

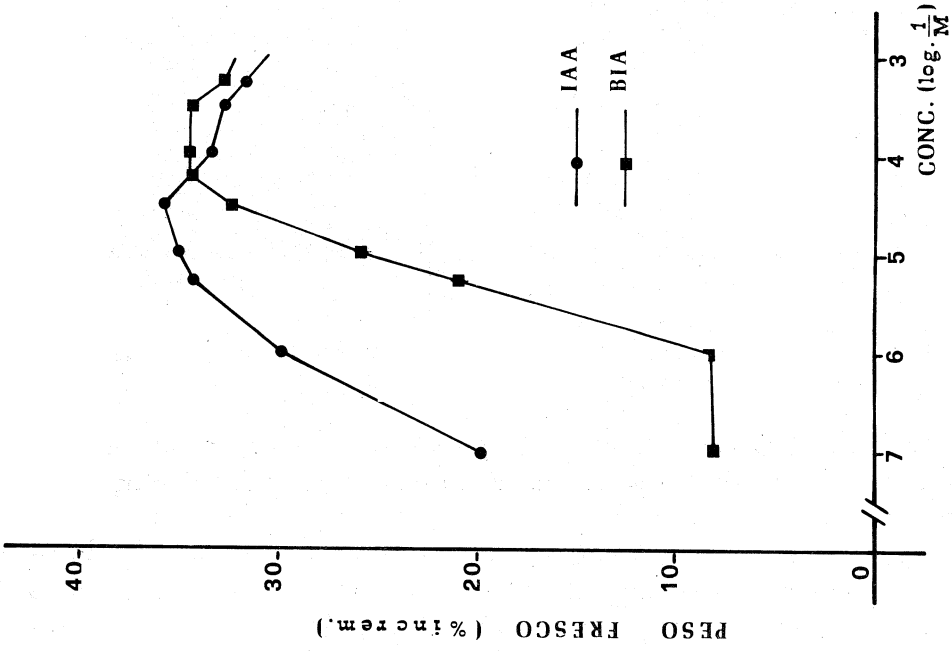

Fig. 3. – Effects of IAA and BIA on the increase in fresh weight of pea internode segments.

2) The minimum active concentration of BIA is 10^{-6} M, markedly higher than the concentration of IAA.

3) At concentrations higher than the optimum BIA shows a progressive inhibitory effect on growth but to a noticeably lesser extent than the one shown by IAA.

The data in Table I show that the capacity of BIA to induce water uptake and cell enlargement is dependent upon protein synthesis in the same way as for IAA.

TABLE I

The effects of cycloheximide on the IAA-*and* BIA-*stimulated increase in fresh weight and elongation of pea internode segments.*

	Control		Cycloheximide 100 γ/ml	
	% increase in fresh weight	% increase in length	% increase in fresh weight	% increase in length
IAA 3×10^{-5} M . . .	35.8	36.4	2.6	0
BIA 10^{-4} M	34.3	34.7	1.7	0

In the presence of an inhibitor of protein synthesis such as cycloheximide (100 γ/ml) growth is completely blocked.

It has recently been shown [5] that the natural hormones of the auxin group induce the production of ethylene in etiolated pea internodes.

The results in Table II show that BIA as IAA at concentrations above 10^{-5} M induces a substantial production of ethylene. Under our experimental conditions the maximum effect is obtained, with both BIA and IAA, at the concentration of 10^{-4} M.

TABLE II

The effects of IAA *and of* BIA *on ethylene production by pea internode segments.*

Concentration (Molar)	μl C_2H_4 produced in 8 hours	
	BIA	IAA
10^{-3}	0.37	0.38
10^{-4}	0.46	0.60
10^{-5}	0.23	0.34
10^{-6}	0.02	0.04
10^{-7}	0.00	0.00

Conclusions

On the basis of the results obtained some general conclusions can be drawn:

1) BIA shows an obvious auxin-like activity.

2) The maximum effect on growth by cell enlargement is obtained with a concentration of BIA three times higher than the one required with IAA. This might suggest a lower affinity of the benzothiazol compound for the active sites involved in the general pattern of growth by cell enlargement.

3) The production of ethylene induced by BIA supports the suggested analogy between the latter and IAA as far as their mechanism of action is concerned.

The above mentioned results provide a good background for future investigations on the phytotoxic action of benzothiazol compounds.

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