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AUXIN-LIKE ACTIVITY OF 1,2-BENZ*ISO*THIAZOLE DERIVATIVES

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Key Word Index—*Pisum sativum*, Leguminosae; pea; *Helianthus tuberosus*; Compositae; Jerusalem artichoke, synthesis, auxin-like activity, 1,2-benz*iso*thiazol-3-yl-derivatives, acids, ethyl esters, amides, nitriles.

Abstract—The 1,2-benz*iso*thiazol-3-yl-acetic and -3-yl-butyric acids and their ethyl esters, amides and nitriles are generally active in the split pea stem test, induce an increase in both length and fresh weight of pea internodes, inhibit the development of pea roots, and, with some exceptions (1,2-benz*iso*thiazol-3-yl-butyric amide and nitrile), induce the production of ethylene by pea segments. Moreover they stimulate cell multiplication and raise the degree of hydration of *Helianthus tuberosus* explants grown *in vitro*. These activities are often similar or sometimes higher than those of IAA. By contrast, the 1,2-benz*iso*thiazole derivatives having a side chain with an odd number of carbon atoms (-3-yl-carboxylic and propionic acids, amides, ethyl esters and nitriles) are inactive or show a far lower activity.

INTRODUCTION

Recently it was reported that 1,2-benz*iso*thiazol-3-yl-acetic acid (BIA) and some of its derivatives form a new class of synthetic growth substances with activity similar to that of indol-3-yl-acetic acid (IAA)[1–10]. The strong analogies found between IAA and BIA suggested that it would be of interest to analyze the possible hormonal activities of some benz*iso*thiazole acids with a variety of side chain substituents. For this purpose we have developed methods of synthesis of the 1,2-benz*iso*thiazol-3-yl-carboxylic (BIC), -acetic (BIA), -propionic (BIP), -butyric (BIB) acids and the respective ethyl esters, amides and nitriles (Table 1). The present paper gives the activities of these compounds on the curvature, growth (as cell enlargement and division), ethylene production and root growth inhibition.

RESULTS AND DISCUSSION

Pea tissues

In the curvature of split internodes of etiolated peas, the derivatives having a side-chain with an even number of carbon atoms (BIA, BIB and their ethyl esters, amides and nitriles) were in general active while those with an odd number of carbon atoms (BIC, BIP and their ethyl esters, amides and nitriles) were completely inactive (Fig. 1). In the group of active compounds a greater activity was shown by the ethyl esters (BIAE and BIBE); on the contrary, the amides (BIAA and BIBA) generally induced a lesser curvature than the acids (BIA, BIB); the

Table 1 The 1,2-benzisothiazole derivatives tested

Compound	R =	Compound	R =
BIC	$-COOH$	BIP	$-(CH_2)_2-COOH$
BICE	$-COOC_2H_5$	BIPE	$-(CH_2)_2-COOC_2H_5$
BICA	$-CONH_2$	BIPA	$-(CH_2)_2-CONH_2$
BICN	$-CN$	BIPN	$-(CH_2)_2-CN$
BIA	$-CH_2-COOH$	BIB	$-(CH_2)_3-COOH$
BIAE	$-CH_2-COOC_2H_5$	BIBE	$-(CH_2)_3-COOC_2H_5$
BIAA	$-CH_2-CONH_2$	BIBA	$-(CH_2)_3-CONH_2$
BIAN	$-CH_2-CN$	BIBN	$-(CH_2)_3-CN$

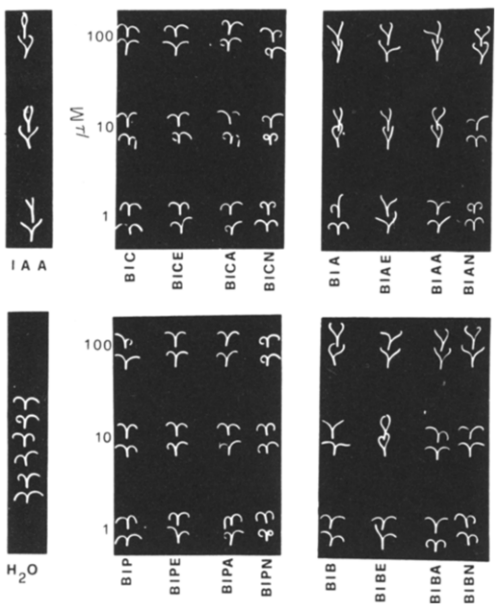


Fig 1 Effects of 1,2-benz*iso* thiazole derivatives at different concentrations on the split pea internode curvature test.

respective nitriles (BIAN, BIBN) acted only at the maximum concentration assayed (100 μM).

We subsequently investigated the benz*iso*thiazole derivatives on cell enlargement in intact pea segments. The compounds having a side-chain with an even number of carbon atoms stimulated the increase in both fr. wt (Fig. 2) as well as length (Fig. 3); the most active are the BIA and BIB whereas the BIAN and BIBN are the least effective; of the ethyl esters, BIBE seems to be more active in respect to BIAE.

It is reported[11] that the natural auxins at high concentrations induce the production of ethylene by epicotyl sections of etiolated pea. In our experiments the optimum concentration for obtaining the maximum production for all the substances was 100 μM. Lesser or higher doses stimulated ethylene production to a lesser degree. Figure 4 reveals the observed alternation of activity among the compounds (100 μM) with an even or an odd number of carbon atoms. For the acetic derivatives the activities decrease in the sequence: acid, ethyl ester, amide and nitrile, which agrees with the assays previously reported for this series, whereas the ethylene inducing capacity of the butyric derivatives is quite different.

Figure 5 shows the capacity of the acetic and butyric derivatives to inhibit root growth in pea seedlings in contrast to the carboxylic and propionic derivatives, which were inactive in this respect.

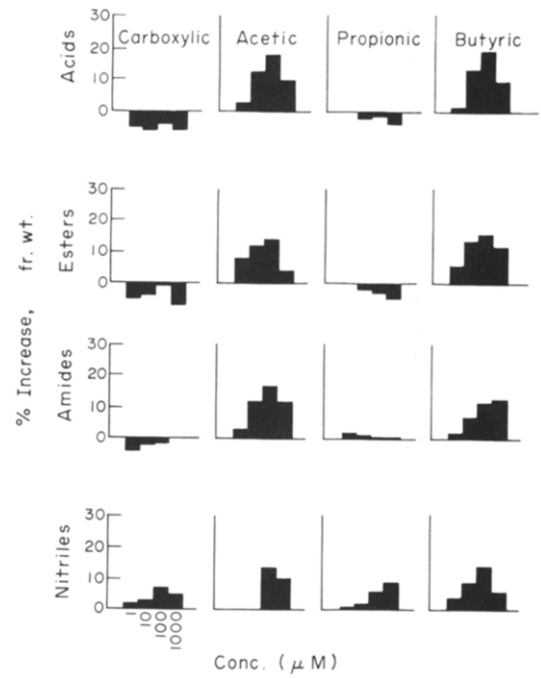


Fig 2 Effects on fresh weight of pea internodes induced by different concentrations of 1,2-benzisothiazole derivatives Control (H_2O) = 0

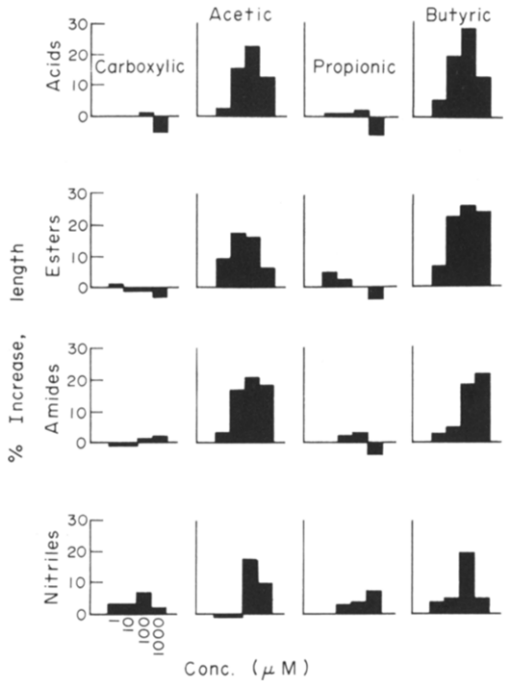


Fig 3 Effects on the length of pea internodes induced by different concentrations of 1,2-benzisothiazole derivatives. Control (H_2O) = 0

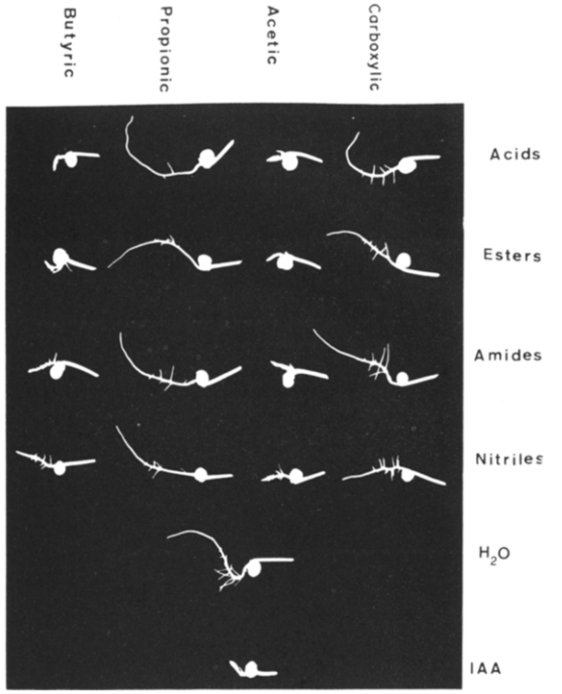


Fig. 5 Inhibition of pea root development caused by 1,2-benzisothiazole derivatives at 100 μM concentration compared with H_2O and 100 μM IAA

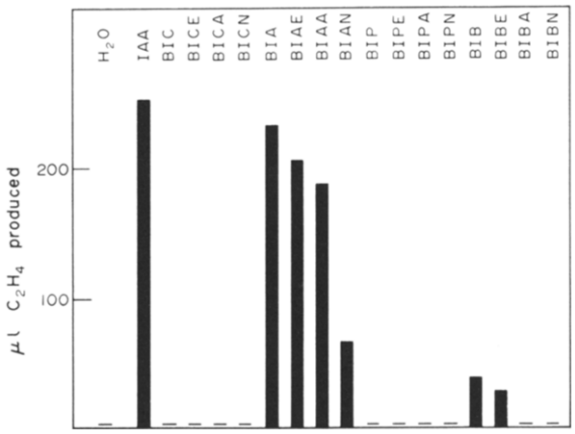


Fig 4 Effects of 1,2-benzisothiazole derivatives at 100 μM concentration on ethylene production by pea internodes compared with H_2O and 100 μM IAA.

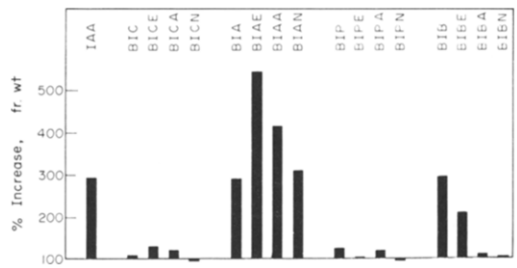


Fig 6 Effects of 1,2-benzisothiazole derivatives at optimal concentrations (1 or 10 μM) on the growth *in vitro* of *Helianthus tuberosus* explants compared with 2 μM IAA Control on basal medium alone = 100 Optimal concentrations = 10 μM BIAN, BIP, BIPE, BIBE, BIBA, BIBN

Jerusalem artichoke tissues

The growth *in vitro* of the explants was stimulated to a much greater degree by the benzisothiazole derivatives having an even number of carbon atoms in the side-chain compared to those with an odd number (Fig. 6). The optimum concentrations (between 1 and 10 μM) of BIA, BIAE, BIAA, BIAN are at least as stimulatory as IAA (2 μM); BIB and BIBE have an activity similar to that of IAA; on the contrary, BIBA and BIBN are virtually inactive. These results are analogous to those found with the test for ethylene induction. In the series of the acetic and butyric derivatives, the compounds active on cell proliferation are also able to raise the hydration of the tissues to the same degree as that induced by IAA (Table 2).

Table 2 Modification of the hydration (%) of *Helianthus tuberosus* explants induced by 1,2-benz*iso*thiazole derivatives at optimal concentrations (1 or 10 μM)

μM	Compound	Hydration (%)
	O	77 0
2	IAA	87 6
1	BIC	76 2
1	BICE	75 7
1	BICA	74 0
1	BICN	75 7
1	BIA	87 6
1	BIAE	89 7
1	BIAA	88 7
10	BIAN	87 7
10	BIP	78 0
10	BIPE	79 0
1	BIPA	71 8
1	BIPN	78 8
1	BIB	88 2
10	BIBE	86 1
10	BIBA	79 4
10	BIBN	73 9

Some results (split pea test, increase in fr. wt and length, root growth) might be explicable on the basis of β-oxidation· BIB and its functional derivatives, as well as BIP and related compounds, could give rise respectively to BIA (active) and BIC (inactive) by enzymatic breakdown However, in the case of ethylene induction and cell proliferation, such hypotheses do not seem easily applicable. In fact BIB and its functional derivatives show an unexpected behaviour: only BIB and BIBE are active, while BIBA and BIBN are inactive

Our data are similar to those obtained previously [12, 13], in regard to the series of indole compounds, in regard to the series of aryloxyalkanecarboxylic derivatives and in particular in regard to the α-naphthyl acids of which the 1,2-benz*iso*thiazole derivatives can be considered isosteric.

EXPERIMENTAL

TLC was effected on Si gel GF_{254} with PhOH–EtOH (7 1) C_2H_4 production was determined by GLC using FID on a 150 × 0 2 cm column of 30–60 mesh Si gel, activated for 24 hr at 150° The column was operated at 60° with N_2 as carrier gas at 54 ml/min The peaks were integrated by triangulation and standardized with a mixture of 9 6 ppm C_2H_4 in air The elemental analyses were within ±0 3% of the theoretical values

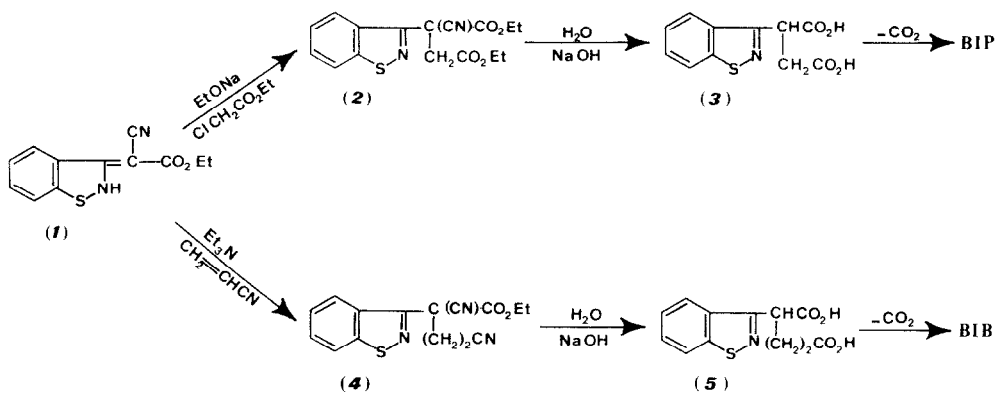
Synthesis The BIC and BIA, and their relative functional derivatives (Table 1) were prepared according to published methods [3–6, 14–18]

The BIP (**3**) *and BIB* (**4**) were synthesized according to the Scheme from the ethyl-2-cyano-2-(1,2-benz*iso*thiazol-3-ylidene) acetate (**1**), the functional derivatives were obtained from the corresponding acids according to common procedures

Ethyl-2-cyano-2-(1,2-benzisothiazol-3-yl) succinate (**2**) 5 4 g of (**1**) were added to a soln of EtONa (0 7 g of Na in 50 ml of EtOH) The reaction mixture was evaporated to dryness and the residue dissolved in 150 ml of ethylchloroacetate and refluxed for 1 hr The compound, obtained by filtration was evaporated to dryness and the residue extracted with Et_2O The crude product obtained by concentration of the Et_2O extracts was crystallized from ligroin White needles, mp 54–55° yield 85% Anal (Found C 58 10, H, 4 86, N, 8 65, $C_{16}H_{16}N_2O_4S$ requires C, 57 82, H, 4 85, N, 8 43%) IR (KBr) ν 2750 cm^{-1} (w CN) 1740 cm^{-1} (s CO)

2-(1,2-benzisothiazol-3-yl) succinic acid (**3**) 6 4 g of (**2**) were boiled (10 hr) in 50 ml of NaOH (10%) in 50% EtOH The residue, obtained by concentration, was dissolved in H_2O and treated with charcoal The soln was acidified with dil HCl and the ppt was crystallized from C_6H_6 White needles, mp 154° decomp , yield 70% Anal (Found C, 52 58, H, 3·54, N 5 77 $C_{12}H_{10}NO_4S$ requires C 52 58, H 3 61, N 5 58%), IR (KBr) ν 3030–2600 cm^{-1} (*m* OH bonded) 1710 cm^{-1} (s CO)

BIP 3 6 g of (**3**) were decarboxylated at 154°, the cooled product was crystallized from C_6H_6 White needles, mp 134–135°, yield 80% Anal (Found C, 58 10, H, 4 37, N, 6 82, $C_{10}H_9NO_2S$ requires C, 57 95, H 4 83, N, 6 76%), IR (KBr) ν 3060–2560 cm^{-1} (w, OH bonded), 1730 cm^{-1} (s CO)



BIPE This was prepared by esterification of BIP according to Fischer Pale yellow oil, bp 0 05 103°/mm, yield 80%; Anal. (Found C, 61 52, H, 5 62, N, 5 99, $C_{12}H_{13}NO_2S$ requires C, 61 25, H, 5 56, N, 5 95%); IR: ν 1725 cm^{-1} (*s* CO)

BIPA This was prepared by ammonolysis of the corresponding ester (*BIPE*) with dry NH_3 in MeOH. White needles from H_2O; mp 133–135°, yield 80% Anal (Found C, 58 41, H, 4 90; N, 13 36, $C_{10}H_{10}N_2OS$ requires C, 58 23, H, 4 88; N, 13 58%); IR (KBr) ν 3410–3210 cm^{-1} (*s*.NH), 1660 cm^{-1} (*s* CO)

BIPN 1·07 g of BIPA dissolved in 20 ml of $CHCl_2$–$CHCl_2$ were added to 1 33 g of P_2O_5. The mixture was boiled under reflux for 1 hr, after cooling the supernatant was separated by filtration and the residue re-extracted with $(CHCl_2)_2$ The collected extracts were evaporated to dryness and the solid was crystallized from petrol White needles, mp 85–87°, yield 77% Anal (Found C, 63 89, H, 4·33; N, 15 04, $C_{10}H_8N_2S$ requires C, 63 80, H, 4 28, N, 14 88%); IR (KBr) ν (2250 cm^{-1} w.CN)

2-*Carboxyethyl*-2-(1,2-*benzisothiazol*-3-*yl*)*glutaric dinitrile* (**4**). 1 1 g of acrilonitrile and 0·5 ml of Et_3N were added to 2 46 g of (**1**) suspended in 20 ml of EtOH at room temp The mixture was stirred for 4 days to complete dissolution Solvent was removed by evaporation and remaining oil extracted with Et_2O, the Et_2O soln was washed successively with NaOH (10%), dil HCl, and H_2O and then dried A yellow oil, slowly solidifying, was obtained by evaporation of the solvent. R_f 0 89 (R_f of (**1**) 0 75) White needles from EtOH, mp 68–69°, yield 70%, Anal (Found: C, 60 24, H, 4 41, N, 13·81, $C_{15}H_{13}N_3O_2S$ requires C, 60 18, H, 4 37, N, 14 03%); IR (KBr) ν 2250 cm^{-1} (w CN), 1740 cm^{-1} (*s* CO)

2-(1,2-*benzisothiazol*-3-*yl*)*glutaric acid* (**5**) was prepared, as (**3**), by hydrolysis in soln of NaOH in EtOH 50% White needles from Et_2O–petrol , mp 125–126° decomp , yield 80% Anal (Found C, 54 35, H, 4 30; N, 5·24; $C_{12}H_{11}NO_4S$ requires. C, 54 33, H, 4 18, N, 5·28%), IR (KBr) ν 2950–2500 cm^{-1} (*m* OH bonded), 1730, 1670 cm^{-1} (*s*.CO)

BIB This was prepared by decarboxylation of (**5**) at 125° The melted mass after cooling was crystallized from C_6H_6 White needles, mp 97 5–98°, yield 80%, Anal (Found C, 59·92, H, 5·07, N, 6 42, $C_{11}H_{11}NO_2S$ requires C, 59·70, H, 5 01; N, 6 33%), IR (KBr) ν 3075–2525 cm^{-1} (w, OH bonded), 1720 cm^{-1} (*s* CO)

BIBE This prepared in a similar manner to BIPE, by esterification of BIB Yellow oil, 0 04; bp 140°/mm, yield 80%, Anal (Found C, 62 92, H, 6·10; N, 5 52 $C_{13}H_{15}NO_2S$ requires C, 62·62, H, 6 06, N, 5 61%), IR ν 1740 cm^{-1} (*s* CO).

BIBA This was obtained in a similar manner to BIPA, by ammonolysis of BIBE White needles from H_2O; mp 103–104°, yield 75%, Anal (Found: C, 60 27, H, 5 70, N, 12 85; $C_{11}H_{12}N_2OS$ requires C, 59 97, H, 5 48, N, 12 71%), IR (KBr) ν 3390 and 3210 cm^{-1} (*s* NH), 1660 cm^{-1} (*s* CO)

BIBN This was obtained in a similar manner to (BIPN), by dehydration of BIBA. Yellow oil, 0·005, bp 170°/mm; yield 80%, Anal (Found· C, 65 21, H, 5 04, N, 13 60, $C_{11}H_{10}N_2S$ requires: C, 65 32; H, 4 98, N, 13·85%), IR ν 2250 cm^{-1} (w. CN)

Biological tests Pea seeds (*Pisum sativum* cv Alaska) were washed in running H_2O for 5 hr then germinated in moist sand at 25° in the dark After 7 days germination, the apical internodes (3rd internode) were dissected from the seedlings After washing for 30 min in H_2O, the randomised internodes were divided into bathces to be used in the different tests *Split stem test* a 10-mm longitudinal cut was made in 30-mm long internodes After 1 hr in H_2O, batches of 10 split internodes were placed in Petri dishes containing 10 ml of the solns to be tested and incubated for 20 hr at 25° in the dark The results were determined from photographic records *Pea test* batches of 12 10-mm long internode sections were placed in Petri dishes containing 10 ml of the solns to be tested After 3 hr at 25° in the dark, the segments were collected, dried on blotting paper and their length and fr wt measured The increase in fr wt was expressed as percentage of the initial wt *C_2H_2 production.* Batches of 20 10-mm long pea sections were placed in 125-ml conical flasks containing 20 ml of the solns to be tested. The flasks were fitted with rubber stoppers having two stopcocks The incubation was carried out at 25° for 16 hr in the dark with agitation of 110 rpm The benzisothiazole derivatives were used at concentrations between 10 and 1000 μM *Root inhibition test* Pea seeds were washed for 5 hr in running H_2O and placed on wet filter paper to germinate at 25° in the dark When the roots were about 1 cm long, statistically significant batches of the randomised seeds were treated with 10 μM solns of the substances to be tested The seeds were then incubated for 5 days and the results recorded by photography *Growth* in vitro Dormant tubers of *Helianthus tuberosus* (Jerusalem artichoke) var OB1 were sterilized with Na OCl soln for 40 min and washed 3 × with sterile H_2O. Cylindrical explants (3 mm diam, 4 mm height) of the homogeneous medullary parenchyma were placed in sterile culture *in vitro* on a medium described in ref [19] with glucose 5% and purified agar 1% The different 1,2-benzisothiazole derivatives were used between 100 and 0 1 μM with controls in basal medium alone and basal medium plus 2 μM 15 replications were used for every concn. The cultures were randomized in a culture room at 24° in a 12 hr day (3200 lx) and grown for 20 days

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