



AUXIN-LIKE ACTIVITY OF 1,2-BENZISOXAZOLE-3-ALKANOIC ACIDS

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Key Word Index—1,2-Benzisoxazole-3-alkanoic acids; auxin-like activities; pea stem elongation; flax root growth; *in vitro* shoot regeneration.

Abstract—A series of benzisoxazole-alkanoic acids, differing in the length of the side-chain, have been synthesized and their activity tested on pea stem elongation, flax root growth and shoot regeneration from tomato cotyledon explants. All compounds had little or no effect on cell elongation or root growth, but a stimulating effect on shoot induction *in vitro*, thus showing that their activity, like that of 1,2-benzisoxazole acetic acid, is partly independent of the side-chain and is linked to the structure of the benzisoxazolic ring.

INTRODUCTION

In previous papers [1, 2], we demonstrated that 1,2-benzisoxazole acetic acid (BOA), a new synthetic growth regulator, differs in activity from other auxin-like compounds, because it has little or no effect on cell elongation or root growth, but a powerful stimulating effect on shoot regeneration *in vitro*. Besides, if deprived of the acidic side-chain it partly retains the activity, which is not enhanced by the presence of a Cl atom on the benzene ring [3, 4]. These observations suggest that the peculiar behaviour of BOA depends almost exclusively on the structure of the heterocyclic nucleus. To test this hypothesis, we have synthesized a homologous series of compounds, having the same nucleus but different side-chain (Table 1), since it is known that auxin-like activity of

many other compounds is a function of the number (even or odd) of carbon atoms in the side-chain [5]. We have tested the activity of the new compounds on pea stem elongation, flax root growth (Tables 2 and 3) and shoot regeneration from tomato cotyledon explants (Fig. 1).

RESULTS AND DISCUSSION

Effects on pea stem elongation

All compounds were ineffective at a concentration lower than 1 mM. At this concentration the only compound that showed some activity was BOIP, which has the carboxylic group at the same distance from the nucleus as BOA, but where a methyl group is substituted

Table 1. Physiochemical data of 1,2-benzisoxazole-3-alkanoic acids

	R	Mp (°)	S.f.c.	IR (cm ⁻¹)	¹ H NMR (δ)
1	CO ₂ H	145–148*	Ligroin	1700 (CO)	7.50–8.50 (4H, <i>m</i> , arom. protons)
2	CH ₂ CO ₂ H	125–127†	H ₂ O	1715 (CO)	12.40–13.10 (OH, <i>br</i>); 7.30–8.05 (4H, <i>m</i> , arom. protons); 4.12 (2H, <i>s</i> , CH ₂ CO)
3	(CH ₂) ₂ CO ₂ H	126–128‡	H ₂ O	1690 (CO)	12.20–12.60 (OH, <i>br</i>); 7.20–8.15 (4H, <i>m</i> , arom. protons); 2.60 3.40 (4H, <i>m</i> , CH ₂ CH ₂ CO)
4	CH(Me)CO ₂ H	83–85	Ligroin	1730 (CO)	12.25–12.75 (OH, <i>br</i>); 7.20–8.05 (4H, <i>m</i> , arom. protons); 4.32 (1H, <i>q</i> , CHCO); 1.60 (3H, <i>d</i> , Me)
5	(CH ₂) ₃ CO ₂ H	122–124	H ₂ O	1690 (CO)	12.20 (OH); 7.30–8.20 (4H, <i>m</i> , arom. protons); 3.20 (2H, <i>t</i> , δCH ₂); 2.60 (4H, <i>m</i> , βCH ₂ ; CH ₂ CO)

*Ref. [6].

†Ref. [7].

‡Ref. [9].

S.f.c. = Solvent for recrystallization.

1 = 1,2-Benzisoxazole-3-carboxylic acid (BOC); 2 = 1,2-benzisoxazole-3-acetic acid (BOA); 3 = 1,2-benzisoxazole-3-propanoic acid (BOP); 4 = 1,2-benzisoxazole-3-methylacetic acid (BOIP); 5 = 1,2-benzisoxazole-3-butanoic acid (BOB).

Table 2. Effect of 1,2-benzisoxazole-3-alkanoic acids on pea stem elongation

	BOC	BOA	BOP	BOIP	BOB	IAA
1.0 mM	5.1	16.1*	4.4	11.6*	6.1	17.3*
0.1 mM	6.0	6.9	4.3	5.6	8.6	22.2*
10 μ M	7.3	5.5	6.7	5.7	11.2	24.1*
1 μ M	7.5	6.8	5.0	5.3	11.2	20.6*
Control	13.6	13.2	13.5	10.5	11	10.1

Increase of fresh weight of the third internode of etiolated pea stem, as referred to 100 mg of initial fresh weight, after 3 hr of treatment. The SEM was lower than 10% in each experiment.

Control = distilled water.

*Significant at $P < 0.01$ (Student's *t*-test).

Table 3. Effect of 1,2-benzisoxazole-3-alkanoic acids on flax root growth

	BOC	BOA	BOP	BOIP	BOB	IAA
1.0 mM	12.1	4.9*	7.9	2.9*	4.2*	2.0*
0.1 mM	12.8	10.7	11.5	8.8	7.9	2.1*
10 μ M	10.9	112.3	11.2	8.3	8.6	2.6*
1 μ M	10.0	13.5	10.9	9.3	9.3	3.2*
Control	10.0	10.3	10.1	10.5	8.6	9.7

% increase of root length after 24 hr of treatment.

Control = distilled water.

*Significant at $P < 0.01$ (Student's *t*-test).

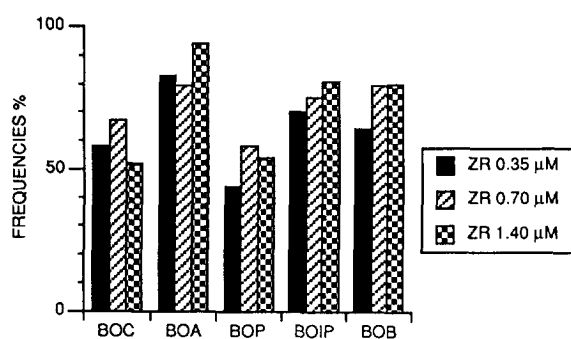


Fig. 1. Effect of 20 μ M 1,2-benzisoxazole-3-alkanoic acids on shoot regeneration from cotyledon explants, in the presence of different concentrations of zeatin riboside (ZR).

for a hydrogen on the δ -carbon of the side-chain. This shows that activity can be modified not only by changing the length of the side-chain, but also by substituting one of its hydrogens. BOP and BOB showed a weak inhibiting activity, which could be ascribed to a toxic effect owing to the high dose employed.

Effects on flax root growth

The compounds with an odd number of carbons in their side-chain were inactive, those with an even number

(BOIP, BOB) inhibited root growth at 1 mM, like BOA. It seems, therefore, that in this case Thimann's rule can be applied also to benzisoxazolic compounds; only those having an acetic side-chain, or a chain that can be metabolically transformed to an acetic chain have this kind of auxin-like activity.

Effects on shoot regeneration

All compounds induced a good number of shoots, with maximum activity at 20 μ M, independently of zeatin concentration in the culture medium. At lower or higher doses, their activity diminished (data not shown). This kind of activity was slightly reduced when the side-chain was composed of an odd number of carbons (BOC and BOP), but, unlike that of other auxins, was not totally suppressed. As with other auxins, maximum activity depended on the distance between the carboxylic group and the lipophilic nucleus.

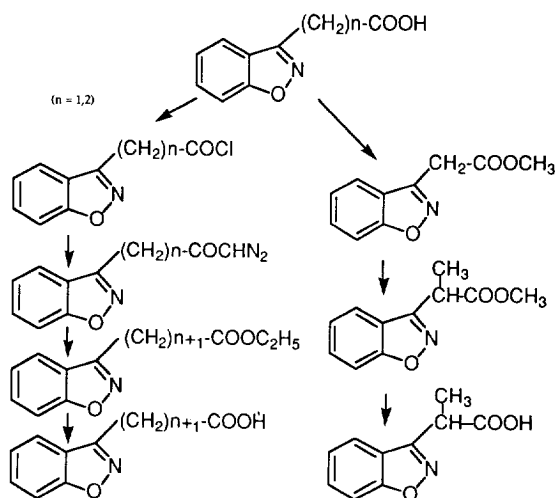
These findings prove once more that the shoot-inducing activity of BOA and related compounds is linked to the structure of the benzene ring, being partly independent of the presence or length of the side-chain [3]. Changes in the length of the latter influence, to some extent, only the activity of these compounds on cell elongation and root growth. In this case, however, though the general rule is followed that only compounds with an even number of carbons are active, their activity remains low, as they work only at high concentrations. The reverse is true for shoot-regenerating activity, as all compounds work better at very low concentrations, irrespective of the length of their side-chain. These results show that BOA homologues also behave like BOA, i.e. they have little effect on cell elongation or root growth inhibition, but stimulate shoot regeneration, thus confirming that the activity of benzisoxazolic compounds is more specific than that of other auxins, and that this activity is linked to the structure of the benzisoxazolic ring.

EXPERIMENTAL

Tomato cotyledons were obtained and cultivated *in vitro* as previously described [1]. Stem elongation, root growth inhibition and shoot regeneration tests were performed as described in ref. [4]. Each expt was done in triplicate and repeated $\times 3$.

Chemicals. 3-Carboxy-1,2-benzisoxazole was synthesized from *o*-nitrophenylacetic acid, via ethyl 2-oximino-2-(*o*-nitrophenyl) acetate, according to ref. [6]. The other compounds tested were obtained from 1,2-benzisoxazol-3-ylacetic acid [7]: 2-(1,2-benzisoxazol-3-yl) propanoic acid, via alkaline hydrolysis of the methyl ester [8]; 3-(1,2-benzisoxazol-3-yl) propanoic [9] and 4-(1,2-benzisoxazol-3-yl) butanoic acids by the Arndt-Eistert reaction (Scheme 1). Analytical data of these compounds are reported in Table 1.

The various reactions were checked by TLC on silica gel, with EtOH-C₆H₆ (10:1) or EtOAc-HOAc (49:1) as eluents for acids and CHCl₃-EtOH (99:1) for diazo-



ketones. Mps: uncorr. Only the most significant peaks of IR (KBr) are reported. ^1H NMR spectra were recorded with TMS as int. standard, $\text{DMSO}-d_6$ as solvent for acids (for 3-carboxy-1,2-benzisoxazole, unstable in DMSO, CD_3OD was used) and CDCl_3 for diazoketones. The results of elemental analysis (C, H, N) were within ± 0.3 of the theoretical values.

1-Diazo-2-oxo-4-(1,2-benzisoxazol-3-yl)butane. Prepd from the respective acyl chloride [10], by usual methods [4]. Mp $72-74^\circ$ (C_6H_6 -petrol), yield 90%. IR $\nu \text{ cm}^{-1}$: 1620 (CO) 2100 ($=\text{N}^+=\text{N}-$); ^1H NMR: δ 12.4–13.1 (OH, br), 7.1–7.9 (4H, m, arom. protons), 5.35 (1H, s,

CHN_2), 2.8–3.5 (4H, m, $\text{CH}_2\text{CH}_2\text{CO}$). From this diazoketone, 4-(1,2-benzisoxazol-3-yl)butanoic acid was obtained, via the respective Et ester [9].

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