β-(6-Bromopyridin-3-yl)alanine. A new plant growth regulator

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Summary. The heterocyclic amino acid, β-(6-bromopyridin-3-yl)alanine [DL-α-amino-β-(6-bromopyridin-3-yl)propanoic acid], interferes with the morphological development of the crucifer *Arabidopsis thaliana* when incorporated in the growth medium. It appears to overcome the normal apical dominance control mechanism within the seedling, producing multi- rather than single-stemmed plants.

The growth of the crucifer *Arabidopsis thaliana* on nutrient agar has proved a useful indicator of plant growth regulatory (PGR) activity[1], since it completes its life cycle from seed germination to seed set in 21 days and the effects of compounds incorporated in the medium on general plant morphology, and in particular, on seed, root, shoot, flower and foliage development, can be readily assessed. A number of natural and synthetic growth regulators including indol-3-ylacetic acid, gibberellic acid, kinetin, abscisic acid, 2,3,5-triiodobenzoic acid and chlormequat chloride have been shown[1] to produce characteristic morphological responses with which the effects of test compounds can be compared. The subject of this communication, β-(6-bromopyridin-3-yl)alanine (**I**) [DL-α-amino-β-(6-bromopyridin-3-yl)propanoic acid], attracted interest because of its unusual effects on the morphological development of the *Arabidopsis* plant.

Materials and methods. Arabidopsis thaliana [L.] was seeded on a solidified agar nutrient medium (pH 6.0) and grown at 25 °C under continuous fluorescent lamp illumination of 15,000–20,000 lx as described by Brown[1]. A fully developed 9-leaf rosette was formed after 15 days, followed by the rapid growth (8–10 cm over a 3-day period) of a single shoot from the stem apex, before flowering occurred (day 18) and seed was set (day 21).

β-(6-Bromopyridin-3-yl)alanine (**I**), synthesized from 2-amino-5-methylpyridine following the procedure of Sullivan and Norton[2], was incorporated at various concentrations into the agar nutrient medium in which the *A. thaliana* was grown and the effects on plant development noted. Controls and treatments were replicated on 25 plants.

Results and discussion. When the bromopyridylalanine was incorporated in the agar at concentrations ranging from 8 to 128 μmoles dm^{-3}, seed germination and rosette formation proceeded normally but bolting of the stem from the apex on day 15 was accompanied by the development of several shoots from the region of the rosette leaf axils. These secondary shoots grew more slowly than the single apical shoot in untreated plants and flowering, pod formation and seed set were delayed by up to 5 days. Root development appeared normal over this concentration range but at higher concentrations of compound **I** (> 250 μmoles dm^{-3}) retardation of root growth was observed.

Figure 1 shows a photograph of a single-stemmed untreated plant 26 days after sowing, compared with a corresponding multi-stemmed plant which had been grown on

Fig. 1. Typical *Arabidopsis thaliana* plants 26 days after sowing. *a* Grown in nutrient agar containing 128 μmoles dm^{-3} of compound (**I**). *b* Grown in nutrient agar.

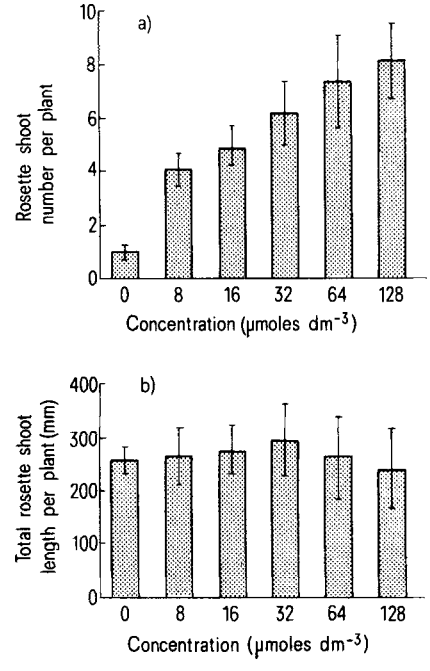
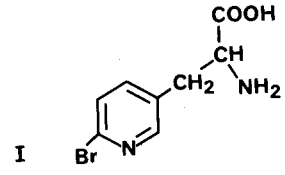


Fig. 2. The concentration of the pyridylalanine (**I**) in the nutrient medium in relation to *a* the average number and *b* the average total length of shoots from the rosette region of an *Arabidopsis* plant measured 26 days after sowing. Each figure is based on a mean of 25 replicates with SE limits as shown.

128 μmoles dm^{-3} of compound **I**. As figure 2, a, indicates, the average number of shoots from the leaf rosette increased with increasing concentration of compound, from 1, in the case of untreated seedlings, to around 8 in the case of seedlings grown on nutrient containing 128 μmoles dm^{-3} of the pyridylalanine. In contrast there was no significant difference in overall growth, as measured by total rosette shoot length, between single-stemmed untreated plants and multi-stemmed treated plants (figure 2, b).



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When apical dominance in a normal single-stemmed *Arabidopsis* plant is suppressed by decapitating the stem, the 1st shoot to emerge from the rosette leaf axils quickly reestablishes dominance, so that only 1, or at the most 2, shoots develop; in the latter case one usually becomes dominant. The observation that several shoots arise more or less simultaneously from plants grown in the presence of the pyridylalanine (**I**) suggests that this compound overcomes the normal apical dominance control mechanism and allows the various axillary buds to initiate shoots independently of each other. The lack of a significant difference in total shoot length between single-stemmed untreated and multi-stemmed treated seedlings suggests that nutrient uptake could well be a growth limiting factor.

Although a large number of compounds have been assayed for potential PGR activity using *A. thaliana* as the test species, the bromopyridylalanine (**1**) was the first to produce multi-stemmed seedlings. Subsequently 2 closely related derivatives, β-(6-chloropyridin-3-yl)alanine and β-(5,6-dibromopyridin-3-yl)alanine were found to behave similarly. Since both growth-enhancing auxins and cell-division promoting kinins have been implicated in apical dominance control[3] it could be suggested that these pyridylalanines act by interference with either the biosynthesis, transport or activity of one or other of these hormones within the plant.

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Temperature acclimation of $Mg^{2+}Ca^{2+}$-myofibrillar ATPase from a cold-selective teleost, *Salvelinus fontinalis*: a compromise solution

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Summary. Brook trout (*Salvelinus fontinalis*, Mitchill) were acclimated over 15 weeks to either +4 °C or +24 °C. The effects of temperature on myofibrillar $Mg^{2+}Ca^{2+}$-ATPase activities were investigated. In contrast to goldfish, temperature acclimation does not alter the kinetic properties of the brook trout myofibrillar ATPase. Activation energy ($\Delta G^{\ddagger}$) is lower and substrate turnover number is higher than values previously reported for cold-adapted stenotherms. Properties of brook trout ATPase appear to be a compromise enabling function across a broad temperature range. The different strategies of adapting to seasonal temperature variations are briefly discussed.

Many species of fish show a partial or complete compensation in their locomotory capacities following acclimation from summer to winter temperatures[2]. Studies on the eurythermal goldfish *(Carassius auratus)* have shown differences in the properties of skeletal muscle $Mg^{2+}Ca^{2+}$ myofibrillar ATPase following temperature acclimation[3-6]. Cold acclimation is associated with a significant increase in ATPase activity and modifications in thermodynamic activation parameters[3,4].

In contrast to goldfish, a number of eurythermal fish will select (wherever possible) a relatively narrow temperature range in their natural habitat and yet retain a wide temperature tolerance. In the present study the effect of temperature acclimation on the properties of $Mg^{2+}Ca^{2+}$ myofibrillar ATPase has been determined on one such species (brook trout) and compared with previous findings on goldfish.

Materials and methods. Brook trout (*Salvelinus fontinalis*, Mitchill) approximately 220–270 mm standard length were obtained during April from the West of Scotland Trout Farm (Renfrewshire, Scotland). Groups of about 20 fish were acclimated to +4 °C or +24 °C over a period of a least 15 weeks. Experiments were carried out approximately 6 weeks after the final temperatures were attained.

Fish were stunned by a blow to the head and killed by spinal cord transection. Myofibrils were prepared from the fast (white) trunk muscle as previously described[7]. Contamination with nonfibrillar ATPases was reduced to a low level (< 0.1%) by treatment with triton-X 100[7]. $Mg^{2+}Ca^{2+}$-activated ATPase activities were assayed over a temperature range of 0–31 °C in a volume of 1 ml of 40 mM Tris-HCl pH 7.4 (at 10 °C); 5 mM $MgCl_2$; 100 μM $CaCl_2$; 6 mM disodium-ATP, and at a myofibril concentration of 0.4–0.6 mg · ml^{-1} and ionic strength of 0.12 (adjusted with KCl). Preparations were 90–95% Ca^{2+} sensitive (assayed in presence of 5 mM EGTA). Reactions were started by the addition of ATP to preincubated myofibrils, and terminated at intervals by the addition of 1 ml of 15% (w/v) trichloroacetic acid. Precipitated protein was removed by centrifugation at 3000 × g for 5 min and inorganic phosphate (P_i) determined in an aliquot of the supernatant[8] Protein concentrations of the myofibril suspensions were determined by a biuret method[9].

ATPase activities at different assay temperatures were represented as Arrhenius plots and thermodynamic parameters calculated as described previously[10].

Results. Temperature dependence of the brook trout ATPase does not alter significantly between groups of fish acclimated to +4 °C or +24 °C ($p > 0.10$) (table; fig. 1). Substrate turnover number is higher, and activation energy ($\Delta G^{\ddagger}$) is significantly lower than values previously reported for stenothermal species adapted to either of these