Short Communications / Kort Mededelings

Transport and metabolism of hypoxoside in intact plants of *Hypoxis hemerocallidea*

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Received 2 March 1994; revised 3 May 1994

Following application of [^{14}C] hypoxoside to the leaves or corms of vegetative *Hypoxis hemerocallidea* plants, most of the radioactivity recovered in ethanolic extracts was detected in the corms. When applied to the corms, the radioactivity recovered from this organ co-chromatographed predominantly with authentic hypoxoside. Where it was applied to the leaves, however, most of the radioactivity detected in the corm co-chromatographed with cinnamic acid and only a small amount with hypoxoside. The results suggest that hypoxoside is not hydrolysed in the corm and that only a limited amount is exported from this organ. Hypoxoside is rapidly hydrolysed in the leaf tissue and the end products are exported to the corm and roots.

Na aanwending van [^{14}C] hipoksosied op die blare of knolle van vegetatiewe *Hypoxis hemerocallidea* plante is die meeste van die herwinde radioaktiwiteit in etanoliese ekstrakte in die knolle van die plante gevind. Waar aanwending deur die knol geskied het, het die radioaktiwiteit hoofsaaklike met outentieke hipoksosied gekochromatografeer. In the geval van blaaraanwending het die radioaktiwiteit wat in die knol vasgestel is, egter in hoofsaak met sinnamiensuur gekochromatografeer en slegs 'n klein hoeveelheid met hipoksosied. Die resultate dui daarop dat hipoksosied nie in die knol gehidroliseer word nie en dat slegs 'n beperkte hoeveelheid vanaf die orgaan geëksporteer word. Hipoksosied word vinnig in blaarweefsel gehidroliseer en die eindprodukte word na die knol en wortels afgevoer.

Keywords: *Hypoxis hemerocallidea*, hypoxoside, transport.

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The phenolic diglucoside, hypoxoside, is composed of two phenolic nuclei linked by a five-carbon bridge which contains two positions of unsaturation (Figure 1). This compound is found in high levels in *Hypoxis hemerocallidea* corms (Drewes *et al.* 1984). Similar types of compounds have been isolated from other *Hypoxis* species (Marini-Bettolo *et al.* 1985; Galeffi *et al.* 1987; Drewes *et al.* 1989). These compounds are of potential medicinal importance, particularly for the treatment of urogenital diseases (Drewes *et al.* 1984). Phenylalanine and t-cinnamic acid have been found to be effective precursors for hypoxoside in intact *H. hemerocallidea* plants (Bayley & Van Staden 1990). The corms are the most effective with respect to biosynthesis (Bayley 1989). A question that needs to be addressed is what role this secondary product could fulfil in the intact plant. Examination of the levels of hypoxoside in the different plant components and the transport and metabolism of this compound within the plant, may give a clue as to its role in the plant. There is some indication that the levels of hypoxoside in the plant components change during plant development and during different seasons (Bayley 1989). The relatively high levels present in the corms in particular, however, may mask small but significant changes. Experiments were conducted to address this issue.

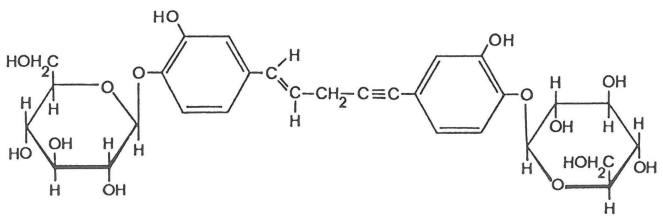

Figure 1 Structure of hypoxoside.

In order to conduct meaningful experiments it was necessary to obtain radiolabelled hypoxoside. Previous work has indicated that t-[^{14}C] cinnamic acid is incorporated into hypoxoside (Bayley & Van Staden 1990, 1991). [^{14}C] Cinnamic acid (specific activity 1.77 GBq mmol^{-1}) was applied via a microsyringe to the apical region of 25 18-month-old *H. hemerocallidea* plants at weekly intervals over a period of 8 weeks. Approximately 18.5 kBq of radiolabelled cinnamic acid was applied each week. One week after the last treatment the corms were harvested, freeze-dried and subsequently extracted and the hypoxoside was purified as previously outlined (Page & Van Staden 1987; Bayley & Van Staden 1990, 1991). The radiolabelled hypoxoside was finally further purified by analytical HPLC and the collected product was used for experimentation.

Once the purified [^{14}C] hypoxoside was available it was injected by microsyringe into the leaves or corms of 18-month-old juvenile plants that had been produced by tissue culture. The plants were left in the greenhouse for 96 h whereafter they were harvested and divided into leaf, corm and root components. The three plant fractions were subsequently freeze-dried, ground to fine powders and extracted with 80% ethanol. The radioactivity associated with each ethanolic fraction was assessed in order to obtain an indication of how the radiolabel was distributed. The remainder of the extracts of each plant organ was streaked onto Merck60 F_{254} silica gel thin-layer plates and developed using the upper phase of 2-butanol: benzene:H_2O:methanol (4:3:2:1) for 45 min, air-dried and re-developed a second time. All plates were divided into 10 R_f fractions and the radioactivity associated with each fraction was determined.

Following the application of [^{14}C] hypoxoside to either the leaves or corms of intact *H. hemerocallidea* plants, most of the radioactivity recovered in the ethanolic extracts was present in the corms 96 h after application (Table 1). When applied to the leaves, 29% of the radioactivity also ended up in the roots.

Table 1 Radioactivity detected in the different components of *Hypoxis* plants 96 h after application of [^{14}C] hypoxoside to either leaves or corms of intact plants. The radioactivity is expressed as a percentage of that recovered for the whole plant

Plant part treated	Component analysed		
	Leaves	Corm	Roots
Leaves	22	49	29
Corms	16	82	2

This was not the case when hypoxoside was applied to the corm, the major storage organ of the plant. Of interest is that 16% of the radioactivity of the corm-treated plants ended up in the leaves. This suggests that some of the radioactivity is exported from the corm to the leaves.

Thin-layer chromatographic analysis of the various ethanolic extracts indicated that very little of the radioactivity detected in leaf and root extracts co-chromatographed with authentic hypoxoside (Figure 2). When leaf-applied, the radioactivity recovered from the leaves co-chromatographed with cinnamic acid, a precursor in the biosynthetic process (Bayley & Van Staden 1990). Although not very pronounced, most of the radioactivity detected in the leaves of corm-treated plants also chromatographed with this compound (Figure 2B). This suggests that the leaves contain enzymes that readily hydrolyse hypoxoside. The corms do not seem to contain such enzymes. When applied to the corm directly, most of the recovered radioactivity from the corm extracts co-chromatographed with hypoxoside (Figure 2B). This had been expected as this is the area of hypoxoside storage and one would assume a paucity/ inactivity of the enzyme(s) involved in hypoxoside hydrolysis. Leaf application of hypoxoside resulted in the detection of two radioactive peaks in the corm (Figure 2A). The major peak co-chromatographed with cinnamic acid, the other with hypoxoside. This result could be due to the fact that hypoxoside hydrolysis occurred in the leaves and that both it and cinnamic acid were then transported to the corm. Alternatively, cinnamic acid could have been transported to the corm and subsequently utilized for the synthesis of hypoxoside in the corms. As no radioactivity was associated with hypoxoside in the roots, it means that either the roots cannot convert cinnamic acid to hypoxoside or that none of the applied compound was exported to the roots. From the present results it would appear that hypoxoside is unstable if applied to the leaves and that it and one of the breakdown products, cinnamic acid, can be transported to the corm. There is no clear evidence that hypoxoside is exported to the leaves from the corm and roots. However, as radioactivity was detected in the leaves which co-chromatographed with cinnamic acid, it is possible that hypoxoside is transient in the leaves. If hydrolysed rapidly then it may well serve as a source of intermediates for the shikimic-acid pathway.

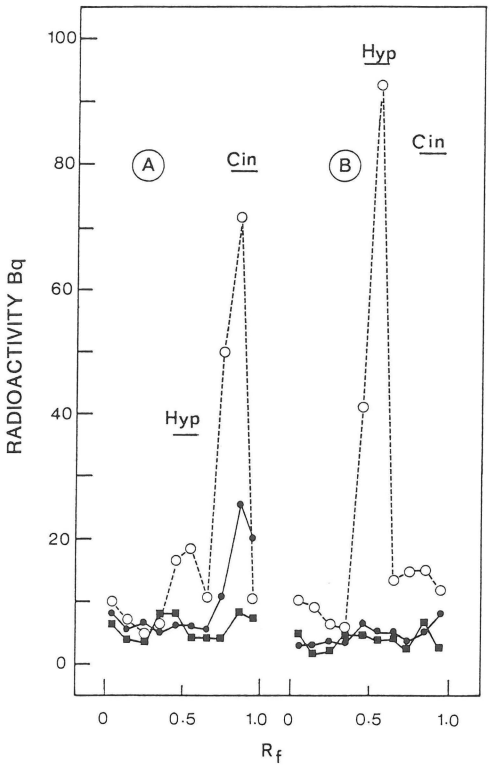

Figure 2 Radioactivity detected in ethanolic extracts from the leaves (●), corms (○) and roots (■) of *Hypoxis hemerocallidea* plants 96 h after the application of [^{14}C] hypoxoside to the leaves (A) or corms (B) of 18-month-old plants. Extracts were separated by TLC and the radioactivity associated with each R_f fraction was determined. Hyp = hypoxoside; Cin = cinnamic acid.

Acknowledgements

Dr A.D. Bayley is thanked for a supply of [^{14}C] hypoxoside, and the University of Natal for financial support.

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