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Movement to bark and metabolism of xylem cytokinins in stems of *Lupinus angustifolius*☆

Ren Zhang[a,b], David S. Letham[a,*], David A. Willcocks[a]

[a]*Research School of Biological Sciences, The Australian National, University, PO Box 475, Canberra, ACT 2601, Australia*
[b]*Department of Biological Sciences, University of Wollongong, NSW 2522, Australia*

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Abstract

Following uptake of [^{3}H]zeatin riboside and [^{3}H]dihydrozeatin riboside by girdled lupin (*Lupinus angustifolius* L.) stems via the transpiration stream, rapid lateral movement of the radioactivity from xylem to bark was observed. Short-term studies with intact stems, and other studies with excised stem tissues, revealed that the ribosides and/or the corresponding nucleotides were the cytokinin forms which actually moved into the bark tissues. Relative to cytokinin metabolism in xylem plus pith, metabolism in bark was both more rapid and more complex. Riboside cleavage and formation of the *O*-acetylzeatin and *O*-acetyldihydrozeatin ribosides and nucleotides were almost completely confined to bark tissues. Exogenous ^{3}H-labelled *O*-acetylzeatin riboside was converted to zeatin riboside in bark tissue, but the presence of the acetyl group suppressed degradation to adenine metabolites. The sequestration and modification of xylem cytokinins by stem tissues probably contributes significantly to the cytokinin status of the shoot. New cytokinins identified by mass spectrometry in lupin were: *O*-acetyldihydrozeatin 9-riboside, a metabolite of exogenous dihydrozeatin riboside in stem bark; *O*-methylzeatin nucleotide and *O*-methyldihydrozeatin 9-riboside, metabolites of endogenous cytokinins in stem bark; *O*-methylzeatin nucleotide and *O*-methylzeatin 9-riboside, metabolites of exogenous zeatin riboside in excised pod walls.

Keywords: *Lupinus angustifolius* L.; Leguminosae; Cytokinin translocation and metabolism; Stem tissues

1. Introduction

A considerable proportion of exogenous radioactive cytokinins which move to the shoot in the transpiration stream becomes localised in the stems and this appears to apply to isoprenoid cytokinins, such as zeatin 9-riboside (ZR; **1**, R = H; (*E*)-6-(4-hydroxy-3-methylbut-2-enyl)aminopurine 9-β-D-ribofuranoside), and also to 6-benzylaminopurine and kinetin (see references in Letham, 1994). Stems may provide a cytokinin reserve for supply to the leaves (Carmi and Van Staden, 1983) and appear to have the ability to sequester and modify cytokinins moving in the xylem (Letham, 1994). In studies with lupin (*Lupinus angustifolius* L.) plants (Jameson et al., 1987), direct lateral movement of ZR, or of a metabolite with an intact zeatin (Z) or dihydrozeatin (DZ) moiety, from xylem to bark was established by stem girdling (removal of a bark ring). In bark tissue, a new nucleotide metabolite of ZR was detected (Jameson et al., 1987) and dihydrozeatin 9-riboside (DZR; **2**, R = H; 6-(4-hydroxy-3-methylbutyl)-aminopurine 9-β-D-ribofuranoside) yielded an analogous metabolite in bark which was identified as *O*-acetyldihydrozeatin riboside 5′-monophosphate (AcDZNT) (Letham and Zhang, 1989).

The above studies involved supply of ZR and DZR to the xylem of intact stems for periods of over 5 h. Because of the apparent importance of the stem tissues in the cytokinin status of shoots, short-term studies of the translocation and metabolism of xylem-supplied ZR and DZR within lupin stems are now reported together with some complementary studies with excised tissues. These ribosides have previously been quantified by mass spectrometry in the lupin xylem sap where they are the principal endogenous cytokinins (Jameson et al., 1987).

2. Results

2.1. Metabolism in excised stem tissues

In initial studies, [^{3}H]ZR and [^{3}H]DZR were supplied to excised bark tissue at 2 μM for 2.5 h. Although the

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* Corresponding author. Fax: +61-2-6125-4331.

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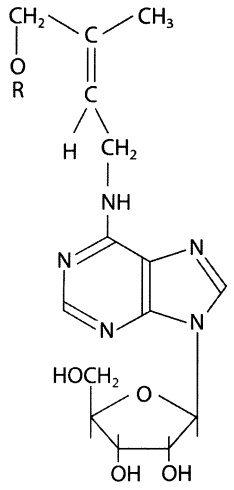
^{3}H-labelled nucleotide metabolite AcDZNT and the corresponding Z nucleotide (AcZNT) were detected, ^{3}H-labelled metabolites with the chromatographic properties of the corresponding ribosides (*O*-acetylzeatin 9-riboside, AcZR, **1** R = acetyl; *O*-acetyldihydrozeatin 9-riboside, AcDZR, **2** R = acetyl) were more prominent. Formation of AcDZR in excised bark as a metabolite of exogenous DZR was confirmed by mass spectrometry. Extracts of excised bark supplied with DZR (25 μM) for 3 h were fractionated by EtOAc extraction yielding extracts termed fraction A in Experimental. After cation exchange on cellulose phosphate, TLC, HPLC and HPTLC, fraction A yielded a UV-absorbing compound (0.77 μg/100 g fresh weight) which exhibited an EIMS ([M]$^+$ 395) in accord with that recorded (Letham and Zhang, 1989) for AcDZR. In the above experiment in which DZR was supplied to excised bark, a purified nucleotide fraction was prepared from the EtOAc-extracted aqueous phase (see Experimental). Hydrolysis with alkaline phosphatase then yielded a riboside fraction in which AcDZR was detected by mass spectrometry (1.10 μg/100 g fresh weight) after TLC, HPLC and further TLC. This confirmed the earlier report (Letham and Zhang, 1989) that AcDZNT was a metabolite of DZR in lupin bark.

When purifying AcDZR as a metabolite of DZR in bark, it was noted that some HPLC fractions gave an EIMS with fragment ions which suggested the presence of an *O*-methyldihydrozeatin moiety (ions observed: m/z 264, 236, 235, 220, 204, 190, 162, 148, 135 and 119). During purification of AcDZR in the phosphatase hydrolysate of the nucleotide fraction, HPLC fractions were obtained with EIMS indicative of an *O*-methylzeatin (MeZ) moiety (ions observed: *m*/*z* 233, 160, 148, 135, 119 and 98). *O*-Methylzeatin 9-riboside (MeZR, **1** R = methyl) and *O*-methyldihydrozeatin 9-riboside (MeDZR, **2** R = methyl) were synthesized and EIMS determined for comparison with the spectra of the purified fractions. This comparison and chromatographic data established that both MeZ nucleotide and MeDZR occurred in bark supplied with DZR, the yields being about 2.0 and 0.9 μg/100 g fresh weight respectively. There was negligible incorporation of DZR radioactivity into these compounds indicating that they were produced largely endogenously and were not derived from exogenous DZR in short term experiments.

ZR (100 μM) was also supplied to excised pod walls for 24 h and the resulting extracts were purified by the methods used for bark extracts. This yielded two fractions with EIMS in accord with that of MeZR. One fraction (0.80 μg/100 g fr. wt) was derived from the fraction (A) prepared by EtOAc extraction; the other (0.70 μg/100 g fr. wt) was purified from the phosphatase hydrolysate of the nucleotide fraction eluted from DEAE-cellulose. Hence both MeZR and MeZ nucleotide occurred in pod walls as metabolites of exogenous ZR.

^{3}H-labelled metabolites present in excised bark and xylem plus pith were compared after uptake of [^{3}H]ZR and [^{3}H]DZR for 2.5 h. This time was selected because the levels of ^{3}H-labelled acetyl ribosides were approximately maximal at this time. These studies showed that metabolism occurred more rapidly in bark than in xylem plus pith and the pattern of metabolites differed appreciably between the two tissues (Table 1). In bark, prominent metabolites of ZR and DZR were the *O*-acetyl ribosides, and to a lesser degree, *O*-acetyl nucleotides, but both types of metabolites were negligible in xylem plus pith where zeatin nucleotide (ZNT) and/or DZ nucleotide (DZNT) were the principal metabolites. Similarly, metabolites formed by deribosylation (DZ and Z) were prominent in bark but not in xylem plus pith. Glucoside metabolites were not detected or made a negligible contribution to radioactivity in all extracts.



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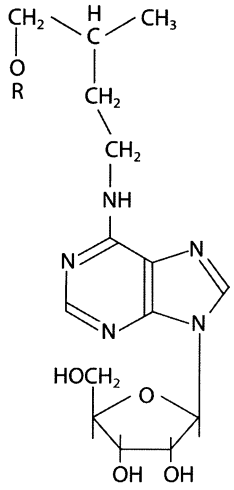

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[^{3}H]AcZR (10 μM) was also supplied to excised bark for 20 h. In addition to [^{3}H]AcZNT, the following metabolites were detected (% of extracted ^{3}H in parentheses): ZR (15.8%), DZR (6.5%), Z (2.3%), DZ (6.5%), adenine (Ade) + adenosine (Ados) (12%). Hence, while AcZR is formed in bark tissues, it and probably AcZNT and the corresponding dihydro compounds are also cleaved to yield the free ribosides in these tissues. In a comparison of the metabolism of [^{3}H]AcZR and [^{3}H]ZR (2 μM, uptake for 22 h) in excised bark, the two cytokinins exhibited the same uptake and the ^{3}H due to Ade + Ados + AMP was determined and expressed as a percentage of the total ^{3}H extracted. The percentage for ZR (14.3%) was significantly ($P < 0.05$) greater than that for AcZR (9.0%). Hence acetylation of the Z sidechain appeared to reduce degradation to Ade type compounds.

2.2. Metabolism in intact stems

In the initial experiments, [^{3}H]ZR and [^{3}H]DZR were supplied through the transpiration stream to derooted

Table 1
Radioactivity due to cytokinin metabolites in extracts of excised stem internode tissues supplied with [^{3}H]ZR or [^{3}H]DZR (2 μM) for 2.5 h

Compound supplied and region of stem	Dpm mg^{-1} fr. wt[a]	Radioactivity (%)									
		DZ	Z	Ade	DZR	ZR	Ados	AcZR + AcDZR	DZNT	ZNT	AcZNT + AcDZNT
[^{3}H]ZR											
Bark	547.2	6.7	7.7	2.8	9.2	15.2	3.2	15.9	7.6	7.9	4.4
Xylem + pith	206.5	<0.7	1.5	nd	7.0	39.9	1.5	0.9	9.9	14.3	2.1
[^{3}H]DZR											
Bark	657.9	17.6	nd	nd	23.7	nd	nd	16.6	11.4	nd	7.2
Xylem + pith	233.8	2.9	nd	nd	52.9	nd	nd	0.7	24.3	nd	1.3

nd denotes not detectable.
[a] Extracted with MeOH–H_2O–HCO_2H (15:4:1 by vol).

Table 2
Radioactivity due to cytokinin metabolites in extracts of internodes of intact stems supplied with [^{3}H]ZR via the transpiration stream[a]

Region of stem	Radioactivity (%)										
	DZ	Z	Ade	DZR	ZR	Ados	AMP	AcZR + AcDZR	DZNT	ZNT	AcZNT + AcDZNT
Experiment A											
Bark	12.9	1.5	1.2	7.5	1.0	6.8	4.3	nd	6.5	0.8	9.6
Xylem + pith	6.0	0.5	1.3	6.3	4.3	8.6	8.5	nd	11.8	3.5	2.1
Experiment B											
Bark	4.5	4.8	1.7	11.9	12.0	1.2	<2.0	8.6	9.6	9.6	nd
Xylem + pith	<0.7	1.4	0.4	8.5	35.6	0.5	<2.0	<0.4	4.8	24.6	nd

nd: denotes not detectable.
[a] In Experiment A, the ZR was supplied for 1.5 h, followed by water for 3.5 h; in Experiment B, the corresponding times were 30 and 10 min respectively.

plants with girdled bark for 1.5 h followed by water for 3.5 h (Experiment A). Of the total ^{3}H recovered from the main stem internodes by solvent extraction, 11.7 and 15.1% were derived from the bark tissues after [^{3}H]ZR and [^{3}H]DZR application respectively. A higher proportion of radioactivity moved into the bark of the girdled first internode of the lateral stems and the corresponding percentages were 24.1 and 18.9% respectively. There was no difference between nodes and internodes in terms of ^{3}H distribution between bark and xylem plus pith. A complex of metabolites was present in both xylem plus pith and bark of the main stem internodes when [^{3}H]ZR was supplied (Table 2, Experiment A) and DZ, DZR, and DZNT were the dominant xylem-plus-pith metabolites which retained the N^6-sidechain. These metabolites and the acetyl nucleotides AcZNT and AcDZNT were prominent in the bark and accounted for 37% of radioactivity recovered from this region of the internodes. In Experiment A, the pattern of metabolites of [^{3}H]ZR in the xylem plus pith and bark of the first internode of the lateral stems were very similar to those recorded for the internodes of the main stem (Table 2).

Because a number of metabolites of [^{3}H]ZR were found in xylem plus pith in Experiment A, the identity of the cytokinin(s) that actually moved into bark was uncertain. In an attempt to clarify this, [^{3}H]ZR and [^{3}H]DZR were supplied to the derooted plants with girdled bark for only 30 min followed by uptake of water for 10 min (Experiment B). These times were selected because at them the radioactivity in the bark first reached a level which permitted chromatographic identification of metabolites. In xylem plus pith, ZNT was the dominant identified metabolite of ZR with only relatively small amounts of DZR and DZNT (Table 2). Metabolism of DZR in xylem plus pith (data not shown) resembled that in excised xylem plus pith (Table 1), DZNT being the dominant metabolite.

Although only 1–2% of the radioactivity extracted from main stem internodes in Experiment B was derived from the bark, a range of metabolites was present in these tissues after the uptake of [^{3}H]ZR or [^{3}H]DZR for only 30 min. The pattern of [^{3}H]ZR metabolites in bark (Table 2) and that found for excised bark (Table 1) differed only in the absence of AcZNT in the former. However, both bark tissues contained AcZR and

AcDZR as prominent metabolites. These acetylated ribosides, DZR, ZNT, DZNT and unmetabolised ZR were the principal labelled compounds detected in bark of Experiment B. Unmetabolised DZR, AcDZR and DZ were the main forms of ^{3}H in bark of stems supplied with [^{3}H]DZR (data not shown).

3. Discussion

In previous studies, the nucleotide AcDZNT was identified as a metabolite of DZR in lupin bark (Letham and Zhang, 1989). The present studies with excised bark, in which [^{3}H]ZR and [^{3}H]DZR were taken up for a relatively shorter time (2–3 h), established that DZR was also converted to the riboside AcDZR while ZR yielded compounds which cochromatographed with AcZR and AcDZR as well as the acetyl nucleotides, AcZNT and AcDZNT. During normal phase TLC on silica gel (solvent A), the above acetyl ribosides exhibited an R_f value (0.59) greater than that of ZR and DZR (R_f 0.53) but less than that of N^6-(2-isopentenyl)adenosine (iPA; R_f 0.63) and were purified from this fraction. However, other apolar cytokinin ribosides also occurred in this chromatographic zone derived from free riboside and nucleotide fractions of bark and pod wall extracts. Mass spectrometry indicated these ribosides were *O*-methyl derivatives of Z and DZ ribosides, compounds not detected previously in plants.

O-Methylzeatin is known to be less active than Z in bioassays (Letham, 1972) and the *O*-methyl ribosides characterised in this paper are presumably less effective as cytokinins relative to the unmethylated compounds. MeZR and iPA exhibit similar chromatographic properties and MeZR would be expected to cross-react with antibodies raised against iPA which show lower specificity than those raised against ZR and DZR (Badenoch-Jones et al., 1987). This again emphasises the importance of adequate purification of iPA fractions for quantification by immunoassay (cf. Nandi et al., 1989).

While acetyl cytokinin ribosides and nucleotides have been identified as cytokinin metabolites in lupin bark, ZR has been shown to be converted to AcZR in tobacco tumour tissue (Mornet and Laloue, 1989) indicating that the acetyl-type of metabolite occurs in other plant species. However the present report describes the first studies of the metabolism of acetyl ribosides. In a preliminary study [^{3}H]AcDZR (prepared from AcDZNT formed by biosynthesis in lupin bark from exogenous [^{3}H]DZR) was supplied to lupin shoots at 2 μM via the transpiration stream (data not presented). In lupin leaves, three main metabolites occurred—DZR, DZ and OGDZ. The metabolic fate of chemically synthesized [^{3}H]AcZR in excised lupin bark was then studied in detail. It was phosphorylated to produce AcZNT and deacetylated to yield ZR as the major metabolite. This is consistent with the view that the acetylated cytokinins are transient metabolites (Letham and Zhang, 1989). The comparison of the metabolism of [^{3}H]AcZR and [^{3}H]ZR in excised lupin bark showed that conversion to Ade, Ados and AMP was partially suppressed by acetylation of the zeatin sidechain. Hence this substitution, like *O*-glucosylation (McGraw and Horgan, 1983), appears to confer resistance to the enzyme, cytokinin oxidase which cleaves the N^6-isopentenyl group of cytokinins. This may explain the greater activity of *O*-acetylzeatin relative to Z in bioassays (Letham, 1978).

The studies presented established that ZR and DZR, which occur naturally as major cytokinins in lupin xylem sap (Jameson et al., 1987), move rapidly from xylem into bark tissues where they are actively metabolized. Relative to metabolism in xylem + pith, metabolism in the bark was both more rapid and more complex. Studies of metabolites formed in intact and excised stem tissues showed that release of cytokinin bases (Z, DZ) from ribosides and formation of acetyl metabolites (AcZR, AcDZR, AcZNT and AcDZNT) was largely confined to the bark tissues. Reduction of the Z sidechain also appeared to occur preferentially in the bark.

From the study of metabolites formed during the short-term uptake of [^{3}H]ZR and [^{3}H]DZR by intact stems, and metabolites formed by excised stem tissues, some conclusions can be made concerning the [^{3}H]-labelled cytokinins, which actually move from xylem to bark. Cytokinin glucosides were not formed in these experiments while the bases Z and DZ and the acetyl riboside metabolites were almost completely confined to bark tissues (Table 1; Table 2, Experiment B); hence these metabolites do not appear to be involved in cytokinin movement from xylem to bark. In xylem plus pith, DZNT is essentially the only metabolite of DZR and hence either or both of these compounds could move into bark. DZR, ZNT and DZNT are the only metabolites of ZR formed in appreciable amounts in xylem plus pith. Since DZ-cytokinins are not converted into Z-cytokinins in bark, ZR and/or ZNT must move into bark to account for the metabolites with an intact Z moiety in these tissues. Hence ZR, DZR, ZNT and DZNT are the only compounds which could be directly responsible for the lateral cytokinin movement from xylem to bark. In bark, the acetyl ribosides derived from these compounds appear to be the precusors of the acetyl nucleotides, AcZNT and AcDZNT (cf Experiments A and B, Table 2). The significance of acetyl metabolites remains unclear. However it is relevant that relative to ZR, AcZR was found to be less readily degraded (inactivated) by cytokinin oxidase. Hence the acetyl compounds AcZR and AcZNT could be partially protected cytokinins for movement through phloem and other living tissues and a role in cytokinin translocation was suggested previously (Jameson et al., 1987).

In lupin stems, DZ is formed from ZR exclusively in the bark. However when ZR is supplied to intact stems via the xylem, DZ is present in xylem plus pith. Hence DZ in bark, derived from xylem ZR, is translocated into xylem, providing evidence for cytokinin interchange between xylem and bark. Bark tissues are probably involved in the control of the cytokinin status of the shoot by sequestering and modifying xylem cytokinins and releasing cytokinins back into the xylem.

4. Experimental

4.1. Synthesis of cytokinins

[2-^{3}H]Zeatin riboside ([^{3}H]ZR) and [8-^{3}H] *R,S*-dihydrozeatin riboside ([^{3}H]DZR), 141 and 189 TBq mol^{-1} respectively, were prepared as described previously (Young et al., 1990; Zhang and Letham, 1990). [8-^{3}H]*O*-acetylzeatin 9-riboside ([^{3}H]AcZR; 5 TBq mol^{-1}) was synthesized by enzymic ribosylation of [8-^{3}H]*O*-acetylzeatin with nucleoside phosphorylase (Letham and Zhang, 1989).

O-Methylzeatin and *O*-methyldihydrozeatin were synthesized by condensing 6-chloropurine with the corresponding amine (Letham et al., 1969) and then converted to 9-ribosides with nucleoside phosphorylase. The products were purified by TLC. *O*-Methylzeatin 9-riboside, EIMS (probe) 70 eV, *m/z* (rel. int.): 365 $[M]^+$ (7), 333 $[M-CH_3OH]^+$ (18), 276 (5), 262 (5), 233 (4), 232 (11), 218 (12), 201 (30), 160 (14), 148 (100), 135 (30), 98 (33; N^6-sidechain fragment, H transfer to adenine moiety). *O*-Methyldihydrozeatin 9-riboside, EIMS (probe) 70 eV, *m/z* (rel. int): 367 $[M]^+$ (4), 352 (5), 336 (17), 278 (32), 264 (61), 236 (17), 235 (12), 220 (36), 204 (21), 190 (12), 162 (62), 148 (100), 135 (47), 119 (22), 108 (10).

4.2. Supply of radioactive cytokinins to tissues

Lupin (*L. angustifolius* L., cv Unicrop) plants were grown as described previously (Zhang and Letham, 1990) and were used about 20 days after the primary inflorescence was in full bloom. At this stage, the pods of this inflorescence had nearly completed seed fill and the plants had developed two laterals each of which terminated in an inflorescence bearing pods about to undergo seed fill. The mean weight per organ and mean organ number in plants at this stage have been presented (Jameson et al., 1987). The leaves of the main stem were then excised leaving 1-cm long petiole segments. Narrow rings of bark were peeled off just below and above all nodes so that the bark tissues were disconnected along the stem. Roots were then excised and the stem bases without bark were placed in aqueous solutions (2 μM) of [^{3}H]ZR or [^{3}H]DZR. Periods of cytokinin uptake and subsequent water uptake are given in the Results section. Bark and xylem plus pith, as 3-cm segments, were also excised from the internodes of stems and incubated on filter paper with [^{3}H]ZR and [^{3}H]DZR solutions in petri dishes at 23 °C for 2–3 h; the inner surface of bark segments was in contact with the solution. In longer term (20–22 h) experiments with [^{3}H]ZR and [^{3}H]AcZR, stems were surface sterilized with hypochlorite and washed with sterile water prior to excision of bark segments. ZR was also supplied to segments of pod walls in petri dishes as described above for 24 h. The seeds (mean weight 90 mg) were excised from the surface-sterilized pods and the segments were dipped in the ZR solution; the exterior surface was then placed in contact with this solution on the filter paper.

4.3. Tissue extraction and metabolite identification

Tissue supplied directly with cytokinin was first washed with water for 30 s. All tissue samples were extracted with MeOH–H_2O–HCO_2H (15:4:1 by vol) after enzyme inactivation at −20 °C (Singh et al., 1988). Radioactivity due to cytokinin metabolites was determined by TLC of the crude extracts with authentic metabolites using a combination of normal and reversed phase methods (Jameson et al., 1987; Letham et al., 1992).

Extracts of stem bark and pod walls were purified by methods which minimized exposure to alkaline pH values and thus permitted recovery of *O*-acetyl cytokinins for mass spectrometry. The evaporated extracts were suspended in water, clarified by centrifugation and extracted four times with an equal volume of EtOAc giving an extracted fraction (A) containing cytokinin ribosides such as AcZR. The aqueous phase was further extracted with *n*-BuOH (three times with an equal volume; extracts discarded) giving an aqueous phase (fraction B) containing cytokinin nucleotides.

To purify the cytokinins in fraction A, an aqueous solution (pH 2.9) was passed through a column of cellulose phosphate (pyridinium ion form, equilibrated to pH 2.9) which was washed with 0.05 M acetic acid and then eluted with 5% (v/v) aqueous pyridine (pH to 7.5 with acetic acid). The eluted fraction was subjected to TLC on silica gel (Merck PF_{254}; solvent *n*-BuOH–H_2O–HOAc 12:5:3 by vol., solvent A) and the zone between cochromatographed DZR and the top of iPA was eluted (50% EtOH) and the resulting fraction was subjected to HPLC (Nova-Pak C_{18} column; Letham and Zhang, 1989). Each UV-absorbing fraction suspected of being a cytokinin from UV absorption characteristics was purified by HPTLC (Merck silica gel 60 F_{254}, solvent H_2O-satd *n*-BuOH containing 0.3 M NH_4OH) and, when necessary, by additional HPLC (Zorbax C_8 column; Letham and Zhang, 1989). The EIMS (probe) of purified compounds were determined at 70 eV.

Nucleotides in fraction B were purified by retention on, and elution from, DEAE-cellulose (HCO_3^- form; Badenoch-Jones et al., 1984); the eluted fraction was hydrolysed to ribosides by alkaline phosphatase (*Escherichia coli*, Sigma Chemical Co; 0.2 mg protein ml^{-1}, pH 9.6 for minimum time) giving a riboside fraction derived from nucleotides. These ribosides were purified by the methods used for fraction A and EIMS determined.

References

Badenoch-Jones, J., Letham, D.S., Parker, C.W., Rolfe, B.G., 1984. Quantitation of cytokinins in biological samples using antibodies against zeatin riboside. Plant Physiology 75, 1117–1125.

Badenoch-Jones, J., Parker, C.W., Letham, D.S., 1987. Use of isopentenyladenosine and dihydrozeatin riboside antibodies for quantification of cytokinins. Journal of Plant Growth Regulation 6, 159–182.

Carmi, A., Van Staden, J., 1983. Role of roots in regulating the growth rate and cytokinin content in leaves. Plant Physiology 73, 76–78.

Jameson, P.E., Letham, D.S., Zhang, R., Parker, C.W., Badenoch-Jones, J., 1987. Cytokinin translocation and metabolism in lupin species I. Zeatin riboside introduced into the xylem at the base of *Lupinus angustifolius* stems. Australian Journal of Plant Physiology 14, 695–718.

Letham, D.S., 1972. Regulators of cell division in plant tissues XIII. Cytokinin activities of compounds related to zeatin. Phytochemistry 11, 1023–1025.

Letham, D.S., 1978. Cytokinins. In: Letham, D.S., Goodwin, P.B., Higgins, T.J.V. (Eds.), Phytohormores and Related Compounds—A Comprehensive Treatise, Vol 1. Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 205–263.

Letham, D.S., 1994. Cytokinins as phytohormones- sites of biosynthesis, translocation, and function of translocated cytokinin. In: Mok, D.W.S., Mok, M.C. (Eds.), Cytokinins—Chemistry, Activity and Function. CRC Press, Boca Raton, FL, pp. 57–80.

Letham, D.S., Zhang, R., 1989. Cytokinin translocation and metabolism in lupin species II. New nucleotide metabolites of cytokinins. Plant Science 64, 161–165.

Letham, D.S., Singh, S., Willcocks, D.A., 1992. Reversed phase thin-layer chromatographic methods for separation of cytokinins. Phytochemical Analysis 3, 218–222.

Letham, D.S., Mitchell, R.E., Cebalo, T., Stanton, D.W., 1969. Regulators of cell division in plant tissues VII. The synthesis of zeatin and related 6-substituted purines. Australian Journal of Chemistry 22, 205–219.

McGraw, B.A., Horgan, R., 1983. Cytokinin oxidase from *Zea mays* kernels and *Vinca rosea* crown gall tissue. Planta 159, 30–37.

Mornet, R., Laloue, M., 1989. Synthesis of 6-[(*E*)-4-acetoxy-3-methylbut-2-enylamino]-9-β-D-ribofuranosylpurine, a new metabolite of zeatin riboside. Journal of Chemical Research S382–383.

Nandi, S.K., Letham, D.S., Palni, L.M.S., Wong, O.C., Summons, R.E., 1989. 6-Benzylaminopurine and its glycosides as naturally occurring cytokinins. Plant Science 61, 189–196.

Singh, S., Letham, D.S., Jameson, P.E., Zhang, R., Parker, C.W., Badenoch-Jones, J., Noodén, L.D., 1988. Cytokinin biochemistry in relation to leaf senescence IV. Cytokinin metabolism in soybean explants. Plant Physiology 88, 788–794.

Young, H., Letham, D.S., Hocart, C.H., Eichholzer, J.V., 1990. Synthesis of radioactive zeatin riboside and related compounds by alkylation of purine moieties. Phytochemistry 29, 385–386.

Zhang, R., Letham, D.S., 1990. Cytokinin translocation and metabolism in lupin species III. Translocation of xylem cytokinin into the seeds of lateral shoots of *Lupinus angustifolius*. Plant Science 70, 65–71.