THE CYTOKININS IN A LIQUID SEAWEED EXTRACT: COULD THEY BE THE ACTIVE INGREDIENTS?

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Abstract

A complex of cytokinins is present in the commercial liquid seaweed extract "Maxicrop". Zeatin, dihydrozeatin, isopentenyladenine and isopentenyladenosine have been unequivocally identified by gas chromatography-mass spectrometry (GC-MS). Cytokinin glucosides have also been detected by the tobacco callus bioassay. Indole-3-acetic acid has been unequivocally identified by GC-MS in freshly made-up Maxicrop.

1. Introduction

The commercial liquid seaweed extract, Maxicrop, is applied as a foliar spray to both agricultural and horticultural crop plants in over 23 countries. The manufacturers claim that the extract, which is prepared from Norwegian *Ascophyllum nodosum*, enhances plant growth and/or product quality in a broad range of crops. How the extracts enhance growth is not understood although the manufacturers suggest that the results are a response to foliar absorption of plant growth substances and chelated trace elements.

As the reported levels of trace elements in the product appear insufficient to be of importance, we are investigating the manufacturers' claim that cytokinins are present in the extract.

2. Materials and Methods

Samples of dried Maxicrop powder were dissolved in water (20% w/v) at 25°C.

2.1 Cytokinin analysis

The cytokinins were extracted twice into ethanol with a final concentration of 80% (v/v). The solution was reduced to the aqueous phase by rotary film evaporation (RFE) at 35°C. The aqueous solution was then adjusted to pH 3 with 2N HCl and centrifuged prior to application to a cellulose phosphate cation exchange column (NH_4^+ form) (e.g. Palni et al., 1983).

The acidic wash was discarded and the alkaline fraction was retained, reduced to near dryness by RFE, redissolved in 20% (v/v) ethanol, centrifuged and the equivalent of 2 g Maxicrop applied to a column of Sephadex LH-20 (850 mm x 26 mm ID). The column was eluted with 20% ethanol and 30 ml fractions collected, evaporated to dryness and assayed by the tobacco callus bioassay (Murashige and Skoog, 1962). In additional analyses the early-eluting fractions (3 to 19) were combined and reduced to dryness by RFE. The extract was dissolved in 0.1 M sodium acetate buffer (pH 5.4) and combined with β-glucosidase (from sweet almonds - Boehringer) to give a final concentration of

enzyme of 0.3 mg ml^{-1}. Incubation was for 24h in darkness at 27°C. The released cytokinin bases and ribosides were partitioned against butan-1-ol at pH 8 and subsequently re-chromatographed and bioassayed.

For GC-MS analysis 10 g samples of Maxicrop powder were extracted, purified and chromatographed as described above. Selected fractions, corresponding to the elution volumes of authentic cytokinins, were combined, reduced in volume and dried over P_2O_5 prior to derivatisation. Details of derivatisation procedures will be published elsewhere but essentially followed the procedures outlined in the Pierce Handbook and General Catalogue (1984).

2.2 Auxin analysis

The auxins were extracted twice into methanol with a final concentration of 80% (v/v) (Morgan and Durham, 1983). The solution was reduced to the aqueous phase by RFE at 35°C. Indole-3-acetic acid (IAA) was subsequently extracted following a partitioning sequence adapted from Bandurski and Schulze (1974) and Badenock-Jones et al., (1982, 1983). The final dichloromethane phase was evaporated to dryness, the extract dissolved in 100% methanol, transferred to a Pierce Reacti-vial, dried over P_2O_5 and redissolved in 25 µl pyridine. Trimethylsilyl (TMSi) derivatives were prepared using 75 µl N, *O*- *bis*-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (Pierce Chemical Co.) with heating at 90°C for 30 min.

2.3 Gas chromatography-mass spectrometry

The derivatised compounds were analysed on a Hewlett Packard 5985 GC-MS system. The compounds were separated on a 10 m x 0.35 mm ID SE-54 column with the helium carrier gas flow at 2 ml min^{-1}. Following on-column injection of sample, the column temperature was maintained at 150°C for 5 min, subsequently increased from 150 to 300°C at 10° min^{-1}, and finally held for 10 min at 300°C. The MS was operated in the selected ion monitoring mode.

3. Results

3.1 Cytokinins

Cytokinin-like activity exhibiting chromatographic properties similar to zeatin riboside, zeatin and isopentenyladenine was detected in the tobacco callus bioassay (figure 1). Any cytokinin-like activity eluting earlier than zeatin riboside (Fraction A) may have been masked by impurities. Following treatment of Fraction A with β-glucosidase, cytokinin-like activity was detected at the elution volumes of zeatin and zeatin riboside confirming the presence of at least two *O*-glucosides (e.g. Palni et al., 1983). From the bioassay data we estimate (without correcting for losses during purification) that the cytokinin-like activity is equivalent to 5.4 µg kinetin per gram of Maxicrop powder. This is equivalent to approximately 1.3 mg cytokinin per litre of Maxicrop (Triple Concentrate).

The presence of zeatin, dihydrozeatin, isopentenyladenine and isopentenyladenosine was confirmed by GC-MS (details will be published elsewhere). The activity contributing to the "zeatin riboside" peak remains unidentified.

3.2 Auxins

IAA was unequivocally identified by GC-MS of the TMSi-derivatives. McDougall and Hillman (1980) considered that the high mass ions at m/e 319 and 202 are characteristic of $TMSi_2$-IAA. We detected these ions, as well as those at m/e 276, 203 and 147 which are also characteristic of $TMSi_2$-IAA.

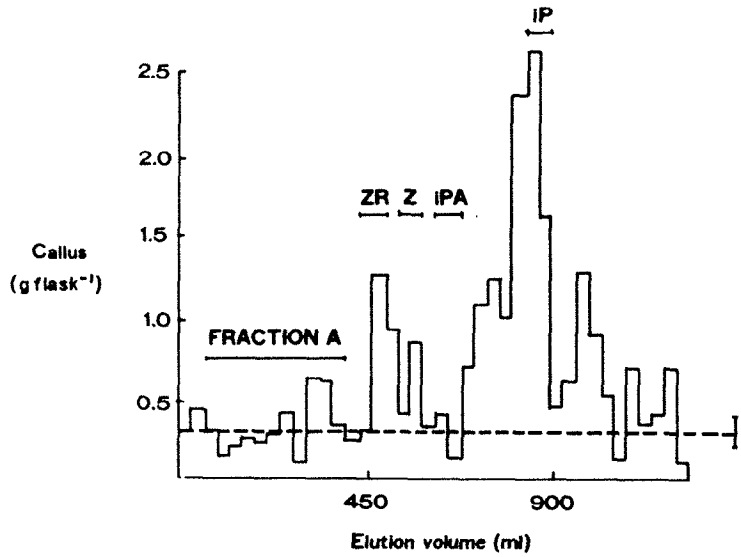

Figure 1 - Tobacco callus bioassay of 2 g Maxicrop powder. The extract was resolved by partition chromatography on Sephadex LH-20, eluted with 20% (v/v) ethanol. Callus growth by the control is indicated (---). Elution volumes of zeatin riboside (ZR), zeatin (Z), isopentenyladenosine (iPA) and isopentenyladenine (iP) are indicated (⊢⊣).

4. Discussion

A number of the claimed responses to Maxicrop are similar to those obtained following application of known plant growth substances, for example, increased fruit set (auxins and gibberellins), reduced fruit drop (auxins), improved fruit quality (auxins, cytokinins and gibberellins) (e.g. Looney, 1979).

Data on the cytokinin content of Maxicrop published by Williams et al., (1981) indicated extraordinarily high levels of cytokinin (up to 200 mg kinetin equivalents per litre of Maxicrop). Our bioassay data indicate approximately 1.3 mg kinetin equivalents per litre of Maxicrop which, taking into account the rate at which Maxicrop is applied in horticulture and agriculture, is still of sufficient concentration to be physiologically active.

The presence of *O*-glucosides and dihydroderivatives indicates that Maxicrop contains cytokinins of potential physiological activity which, if taken up by the plant, would not immediately be degraded to inactive compounds (Parker et al., 1978, McGaw and Horgan, 1983).

Kingman and Moore (1982) detected IAA in "dehydrated *A. nodosum*

powder concentrate" by gas-chromatography. We have unequivocally identified IAA in dried Maxicrop powder by GC-MS.

The presence in Maxicrop of IAA, along with a complex of cytokinins, supports some of the claims made by the manufacturers, although whether these compounds are physiologically active in the field situation is not yet proven.

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