Isolation of Zeatin from Larvae of *Dryocosmus kuriphilus* Yasumatsu[1]

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Abstract. **Cytokinin active compounds were isolated from larvae of *D. kuriphilus* within galls of Japanese chestnut, *Castanea crenata*, Sied. et Zucc. One of the compounds coincided with zeatin by T L C, G L C and Sephadex LH_{20} column chromatography.**

D. kuriphilus lays its eggs during summer in buds of Japanese chestnut which hatch from late summer through autumn. Small galls are formed which increase in size as the larvae within the buds develop along with vegetative growth during the following spring. The formation of galls on resistant cultivars in recent years caused large grower losses in Japan.

This report describes the isolation and identification of a larval cytokinin which may play a role in gall initiation and development in Japanese chestnut.

Larvae of *D. kuriphilus* picked from the galls of Japanese chestnut were homogenized in mortar containing a small quantity of 70% ethanol and sea sand. The homogenate was extracted with 100ml of 70% ethanol 3 times and filtered (Fig. 1). The volume of the combined ethanolic filtrate was reduced to 40ml *in vacuo* at 50°C with a rotary evaporator and then the residue adjusted to pH 8.4 with 1N NaOH and extracted with 50ml of n-butanol 3 times. The combined n-butanol fraction was evaporated to dryness *in vacuo* at 50° with a rotary evaporator and taken up in 15ml of water. The aqueous extract was added to a Dowex 50 (H^+form) column (3.5cm × 40cm) and eluted with 500ml of water and 5N NH_4OH solutions successively. The water and 5N NH_4OH solutions were evaporated separately to 50ml *in vacuo* and each bioassayed for cytokinin activity with tobacco ('Wisconsin No. 38') pith callus (8). High cytokinin activity was detected in the 5N NH_4OH fraction (H-fraction) of n-butanol extract, but not in the water eluate (Fig. 2)

II-fraction was further separated on a Sephadex LH_{20} column (2.6cm × 60cm) with 33% ethanol and each sub-fraction analyzed by UV absorption at 260nm. Five peaks were obtained with this method one of which coincided with authentic zeatin. A 6th peak, considered to be zeatin riboside, was detected on the hydrolyzed II-fraction with hydrochloric acid (Fig. 3).

II-fraction was analyzed by gas liquid chromatography (G L C) after silylation with bis(trimethyl silyl) acetamide and acetonitrile using a Nihon Denshi Model 1100 G L C equipped with flame ionization detector. A stainless steel column (3ϕ × 2m) was packed with 3% SE_{52} on 80 – 100 mesh Diatoport S. Column temperatures were 210° and 250°C. T M S derivatives of II-fraction, authentic zeatin, its riboside and kinetin analyzed by G L C revealed that one of 2 peaks obtained from this fraction coincided with zeatin (Table 1).

II-fraction was chromatographed on microcrystalline cellulose using water as solvent. This technique separated the constituents of this fraction into 5 spots of which one had the same value as authentic zeatin (Fig. 4). This cytokinin has been demonstrated capable of inducing galls in plant tissue by several investigators. Fasciation of pea stem

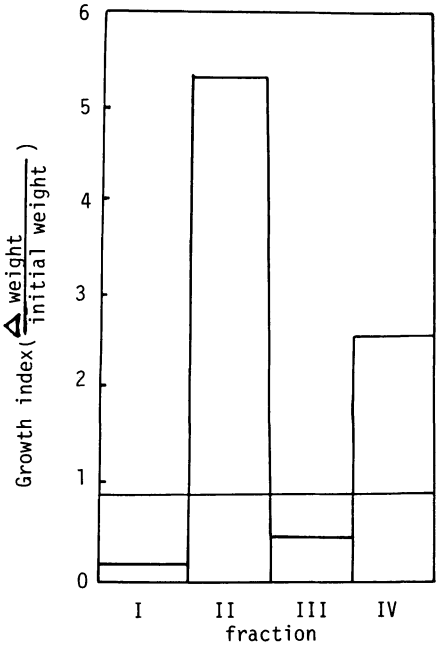

Fig. 2. Cytokinin activity of separated fractions by tobacco pith callus assay. Control is indicated as horizontal line.

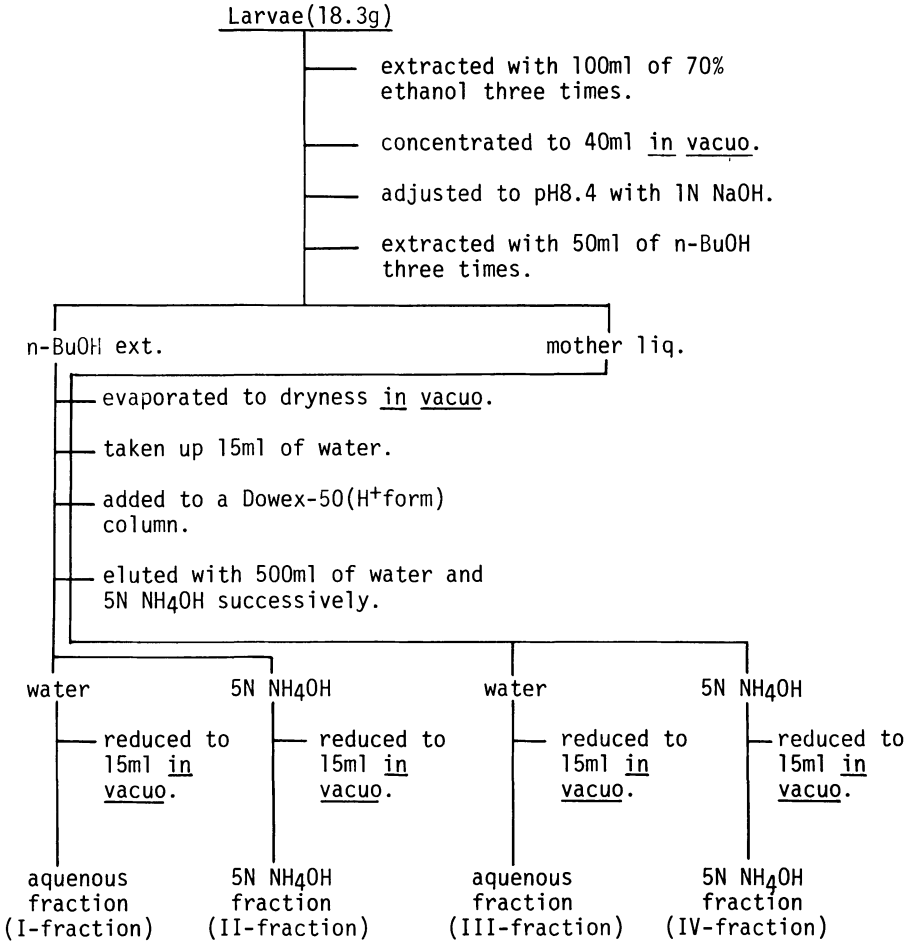

Fig. 1. Separation scheme of cytokinin active fractions from larvae of *Dryocosmus kuriphilus*.

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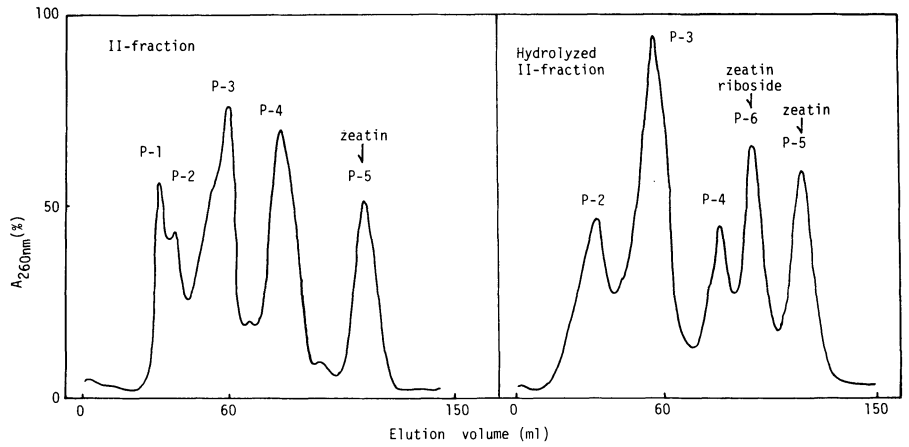

Fig. 3. Column chromatogram of II-fraction on Sephadex LH_{20} with 33% ethanol.

tissue has been induced by *Corynebacterium fascians* (4, 7, 11).

Table 1. GLC retention times of peaks from II-fraction and authentic compound on 3% SE_{52}.

Peak	Isothermal at 210°		Isothermal at 250°	
	Time (min)	Re (Kinetin)	Time (min)	Re Kinetin)
Peak-I	4.30	1.30	–	–
Peak-II	5.80	1.81	–	–
Kinetin	3.20	1.00	0.90	1.00
Zeatin	5.80	1.81	–	–
Zeatin riboside	–	–	9.40	10.44

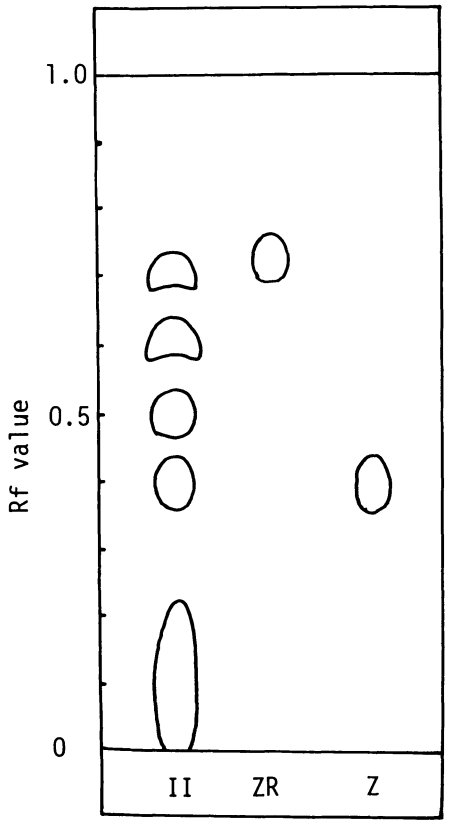

Fig. 4. Thin layer chromatogram of II-fraction. After developed with water, each spot was detected under UV at 366nm. Z:zeatin, ZR: zeatin riboside.

The factors isolated from liquid cultures of this organism were identified as 6-(γ,γ-dimethylallyl amino)purine, nicotinamide and 6-methylaminopurine. Likewise gall formation of cabbage and rape tissues is inducible with *Plasmodiophora brassicae* (5, 6, 9, 10, 12). The infected gall tissue contained significantly greater amounts of cytokinins than non-infected ones. The active factor involved in gall formation has also been identified earlier as cytokinin.

"Green islands" are well known symptoms of birch larvae of *Stigmella argentipedella.* These isolated larvae were reported to contain zeatin-like compound and its ribonucleotide which may have their origin in the labial glands of the larvae (1, 2, 3). The actual compounds were not identified.

Preliminary experiments in this laboratory indicate that zeatin injected into chestnut buds initiated gall formation. From these examinations, it is postulated that the larvae releases zeatin from its salivary glands similar to *S. argentipedella.* The continuous secretion of hormones by the larvae induced a profusion of undifferentiated cells leading to gall formation which inhibits terminal shoot growth and subsequent flower formation.

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Manganese Chelate Sprays Increase Growth and Yields of Peas[1]

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Abstract. **Manganese chelate sprays at bud formation, first bloom and a split application at bud formation and full bloom, increased vine growth of peas (*Pisum sativum* L.) Shelled pea yields increased with the following Mn chelate rates and timing: 134 g Mn/ha (1 lb. Mn chelate/acre) at first bloom, 269g Mn/ha (2 lb. Mn chelate/acre) at bud formation and first bloom; and with the split application of 134g Mn/ha at bud formation and 134g Mn/ha at full bloom. Tissue analyses showed that redistribution of foliar applied Mn to the non-sprayed new growth was negligible.**

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