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BRIEF COMMUNICATION

Substances with Cytokinin Activity Extracted from Growing Apices of Apple-Tree Shoots

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Abstract. The presence of native cytokinins was studied in growing apices of apple-tree annual shoots. During June the presence of two compounds showing cytokinin activity in biotest was observed and their R_F value was determined in three chromatographic systems.

After the efforts of many investigators, LETHAM (1963) succeeded in discovering first the native cytokinin in *Zea mays*. Later the same author together with his co-workers determined its chemical structure (LETHAM *et al.* 1967). ZWAR (1963, 1970) extracted a substance with cytokinin activity from young apple-tree fruitlets. LUCKWILL and WHITE (1968) extracted a compound showing cytokinin activity from the xylem sap of the apple-tree. This substance is probably transported from the roots.

At present the number of newly discovered substances with cytokinin activity from different material is increasing and already the first valuable data of its dynamics during plant development are being obtained (BLUMENFELD and GAZIT 1970).

In this paper the presence of substances with cytokinin activity in the growing apices of annual shoots of apple-tree is studied.

The growing apices of annual shoots of apple-trees with two or three of the youngest leaves were used as material. They were collected during June, mostly from the apple-trees cv. Berlepsch's rennets. The apices were immediately frozen and kept for 2—3 days at —30 °C. Altogether 8.5 kg of fresh material was collected.

In the cold room with a temperature under 0 °C the shoots were crushed and 3 to 5 times extracted with cooled acetone excess. Each extraction lasted about 4 h.

The acetone extract so obtained was distilled to dryness in a vacuum evaporator. The distillation residue was reextracted with hot water, treated with HCl to obtain pH 2 in the water extract, which was immediately purified by centrifugation. By this operation chlorophyll and other ballast were removed.

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One part of this crude extract was put on chromatographic paper Whatman MM3 and washed with water. A part of the coloured components moving with the front were removed. The chromatogram was then cut into 10 strips, which were tested on bioassay.

The other part of the crude extract was further cleaned. The pH of the solution was adjusted by NaOH to a neutral reaction and shaken out with an equal quantity of a butanol and ammonia mixture (9 : 1). This extraction was repeated, if needed 3—5 times, whereupon the butanol extract was collected and again shaken out with water as long as coloured components passed from butanol into the water.

Then the butanol extract was distilled to dryness in a vacuum evaporator (3 to 5 times) and the residue reextracted with a small quantity of hot water. The remainders of the coloured components were precipitated by adding HCl to pH 2. The precipitate was removed by centrifugation. Then the aqueous acid extract was repeatedly distilled in a vacuum until its pH became neutral. If the traces of coloured substances were not completely removed, the aqueous extract was neutralized by NaOH and shaken out into basic butanol, leaving the coloured substances in the aqueous phase. The clarified extract was distilled to dryness, dissolved in a minimum of 50% basic ethanol and put on chromatographic paper Whatman MM3. The chromatograms were developed with distilled water.

On the chromatogram the spots were identified in UV light and eluated with 50% basic ethanol. The aliquot of the eluated compounds was assayed in the biotest.

The remaining aliquot of the identified compounds was gradually eluted and chromatographed in the following systems:

a) n-butanol : water : ammonia — 86 : 14 : 5,
b) isopropanol : HCl : water — 680 : 170 : 144,
c) isopropanol : water : ammonia — 7 : 2 : 1.

The biotest was made on a MURASHIGE and SKOOG (1962) nutrient substrate, where, instead of kinetin, the chromatographic paper eluate was added. As a control a substrate without cytokinin and with N6-benzyladenine was used. For the biotest of the first crude extract the pith tissue of tobacco "Wisconsin 38" was inoculated. In the second biotest, where the clarified extract was assayed, the callus of the Antonovka apple-tree was inoculated. The callus is known not to grow without cytokinin and is taxonomically near to the extracted material.

Both biotests were evaluated by comparing the weight increase of the dry matter of the calluses with the control without cytokinin.

The first biotest of the crude extract (see material and methods) did not show any biological activity.

Fig. 1. shows the increase of dry matter in the biotest performed on the Antonovka callus after the described procedure of clarification.

From the above diagram it may be seen that spots No. 1 and 4 display an apparent biological cytokinin activity.

Table 1. indicates the R_F values of the mentioned compounds in water and the described solvent systems.

As can be seen from the Table, compound No. 4 splits into two components after rechromatography in a basic solvent. The same applies to an acid solvent.

The problem of naturally occurring cytokinin activity is also a problem of the metabolism and mechanism of its action. From the different results ob-

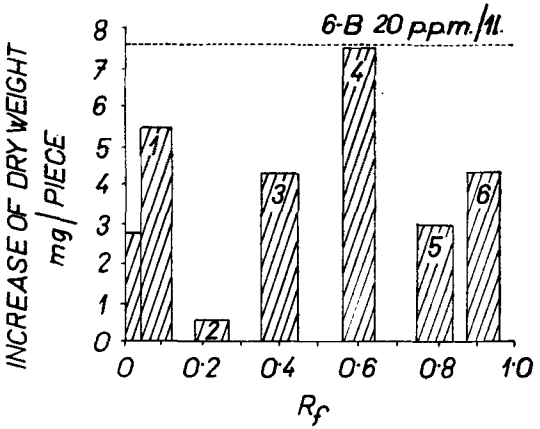

Fig. 1. Increase of dry matter of the Antonovka callus in biotest of clarified extract from growing apices of apple-tree shoots (6-B = N^6-benzyladenin).

tained by LUCKWILL from the xylem sap of the apple-tree and from our results obtained from the youngest tissue of apple-tree shoots, it may be deduced that there is not only one cytokinin, which is specific for a certain plant species. A plant probably contains several components with cytokinin activity, which may change metabolically during growth and this changed form may have a stimulating effect on that cell division which is specific for cytokinins. The question of a possible modification of the substance No. 1 during the clarification will be studied with more precision in future.

TABLE 1

R_F VALUES of compounds No. 1 and 4

Solvent: / Spot No.:	water	n-butanol: H_2O : NH_3OH (86 : 14 : 5)	2-propanol: HCl : H_2O (680 : 170 : 144)	2-propanol: H_2O : NH_3OH (7 : 2 : 1)
1	0.11	0.50	—	0.84
4	0.63	0.95	0.80 0.53	0.84 0.90

An important factor in the method of native cytokinin isolation is the height level of native inhibitors, blocking cytokinin activity in biotests. This is described for instance by GAZIT and BLUMENFELD (1970) who found such an inhibitor in avocado fruit. This phenomenon may explain why our first biotest with crude extract was not successful.

Another problem is the connection of native cytokinin with other components, blocking their cytokinin activity. *E.g.* in the work of the mentioned authors the cytokinin effect of the plant extract appeared only after the acid hydrolysation of the clarified extract.

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