Short Communication

Yoriko OHWAKI* : Thin-Layer Chromatography of Diffusible Auxin of Corn Coleoptiles**

大脇頼子*: トウモロコシ子葉鞘拡散性オーキシンの薄層クロマトグラフィー**

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Thin-layer chromatography (TLC) seems to be far more superior to paper chromatography in separating small quantities of indole compounds, as demonstrated by Stahl and Kaldewey^{1,2)}. But silica gel, which was used by them, is considered to be unsuitable for quantitative study of auxins, since on this layer indoleacetic acid (IAA) is partly destroyed and the powder interferes with the *Avena* coleoptile section test. The present paper describes the TLC on cellulose and its application to the investigation of the diffusible auxin of corn coleoptiles. In this chromatography IAA of 0.01-0.3 μ g can be recovered completely.

One g of cellulose powder (Tōyō Rōshi Co.), which had been washed with concentrated HCl-water (1:1, v/v) and then with distilled water, was mixed with 3 ml water. On a 5×20 cm glass plate the slurry was spread evenly without using any special applicator. The air-dried chromatoplate was heated in an oven at 105° for one hour, and the sample was applied to it. Before development, the plate was equilibrated for 30 minutes in the bottle saturated with solvent vapours. Of many solvents tried, the mixture of methyl acetate: acetonitrile: 28% ammonia: water in the ratio of 40:10:5:4 (v/v) was found to be the best for the separation of acidic indole compounds. The solvent front was allowed to ascend 16 cm for about 15 minutes. IAA, indolepropionic acid and indolebutyric acid showed the average R_f values of 0.44, 0.53 and 0.63, respectively. Neutral indole compounds ran to the solvent front.

The activities of chromatograms were examined by the *Avena* coleoptile section test or the modified *Avena* curvature test. The cellulose powder was scraped from each zone to be tested, and placed in each small Petri dish, to which was added 1 ml of M/100 potassium phosphate buffer (pH 4.5) containing 1% sucrose for the section test, or 0.2 ml of distilled water and agar blocks for the curvature test.

The diffusible auxin was obtained from coleoptiles of *Zea mays* L. var. Golden Bantam. Four mm tips cut from the coleoptiles grown to about 3 cm in the dark were allowed to stand on an agar plate in a small Petri dish in a dark moist thermostat at 25°. After 3 hours, the agar was cut into pieces and extracted with freshly distilled ether. The ether extracts were evaporated to dryness, dissolved in methanol and chromatographed. Fig. 1 shows that the chromatograms of diffusates exhibited a growth promoting activity at the R_f value corresponding with that of IAA both in the section test and in the curvature test. In the former test, the zone at R_f 0.9-1.0 also caused a growth promotion, although this was not always the case with the materials from a different source. The active substance in the zone corresponding to the IAA zone was identified as IAA from its R_f values in other solvent systems

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and by the following colour reactions. In the chromatograms of diffusates from about 400 coleoptile tips, this zone gave crimson colour with Salper reagent (1 ml of 0.5 M FeCl_3 in 50 ml of 35% HClO_4), purple with *p*-dimethylaminocinnamaldehyde (DMCA) reagent (2 g of DMCA in 100 ml of 6 N HCl and 10 ml of ethanol) and yellow fluorescence with Procházka reagent (mixture of 10 ml of 35% formaldehyde, 10 ml of 25% HCl and 20 ml of ethanol).

Fig. 1. Histograms showing the activities in the *Avena* coleoptile section test (a) and in the *Avena* curvature test (b) of the chromatograms of diffusates from corn coleoptile tips. Section test: 4 mm sections were incubated for 5 hours. Curvature test: agar blocks immersed in the solution for 2 hours were tested. UV fluorescence on the chromatogram is shown below. b: blue, v: violet.

Fig. 2. Histograms showing the activities in the *Avena* coleoptile section test of the chromatograms of diffusates from the upper and the lower halves of corn coleoptiles kept horizontal for 3 hours. — lower half, ---- upper half.

Next, the diffusible auxin was separately obtained from the upper and lower halves of the coleoptile tips held horizontal. As shown in Fig. 2, IAA diffused in greater amounts from the lower than from the upper half, but there was no difference in activity of the other promoting zone of higher R_f between the two halves.

These results suggest that, at least in the corn coleoptile, the endogenous auxin responsible for the geotropic curvature is IAA. Details will be published elsewhere.

References

- 1) Stahl, E., and Kaldewey, H., Zeitschr. physiol. Chem. **323**: 182 (1961).
- 2) Kaldewey, H., and Stahl, E., Planta **62**: 22 (1964).