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Purification of plant auxin by polyamide thin-layer chromatography

Various methods have been employed for the purification and separation of indole-like growth regulators from plant tissues. These include gel filtration on columns of Sephadex LH-20 (refs. 1,2), paper partition chromatography[3-6] and thin-layer chromatography (TLC) on cellulose[7] and silica gel[8,9]. Separation by chromatography on thin layers of silica gel has been avoided in some studies because of the rapid oxidizability of indole auxins under these conditions. Furthermore silica gel powder is unsuitable for estimations of levels of auxin by bioassay techniques[7].

Estimates of the levels of auxins are usually achieved by bioassay with coleoptile segments. A substantial amount of data has been accumulated to show that indole-3-acetic acid (IAA) can interact either synergistically or antagonistically in coleoptile assays with a variety of phenolic substances occurring widely in plant tissues[10-15]. Furthermore, the observation that amounts of such phenolic substances vary in level with photoperiod[16-18] introduces a complicating factor into daylength studies on levels of plant auxin.

The usual chromatographic procedures for separating endogenous auxin, i.e., paper chromatography (PC) using isopropanol–ammonium hydroxide–water (10:1:1) do not adequately separate such substances from the bulk of plant phenols[19] and the possibility arises that interactions between the phenols and auxin may result in an unreliable estimate of auxin activity. It is important therefore that phenolic materials should be removed from extracts where auxin is to be bioassayed.

Polyamide is a hydrogen bonding resin which has been advantageously employed for the separation of indoles[20] and phenols[21] and for the removal of phenols from plant extracts[22].

The possibility of employing polyamide chromatography for purifying auxin was investigated during studies on the daylength control of hormone levels in relation to tuberization in a potato variety, *Solanum andigena*. Preliminary attempts were made to separate phenolics from auxin using short polyamide columns. However, this procedure was found to be inadequate since a substantial amount of extracted phenolic material co-chromatographed with the auxin and a series of synthetic phenols had the same elution profile also. Therefore, a TLC technique was developed which separated a considerable amount of phenolic material from auxin.

Experimental

Thin-layer foils of MN Polygram polyamide (polyaminodecanoic acid) were purchased from Macherey-Nagel and Company, Düren, G.F.R. (available in Great Britain from Camlab (Glass) Ltd., Cambridge) and were pre-washed before use with the chromatographic solvent system with which it was intended to develop the foils. It was found that this removed a zone of pronounced ultraviolet absorbance which migrated with the solvent front.

MN Polygram polyamide is available in sheets 20 cm × 20 cm. On their own these sheets were not rigid enough to stand upright in a chromatography tank and so they were fixed to glass chromatography plates. A strip of substrate (2.0 cm) was removed from parallel sides of the foils in order to prevent a capillary pull between

the layer and the strips of polythene adhesive tape used to fix the foils to the glass support plate. The foils were heated in an oven at 80° for 15 min (ref. 23) prior to fixing on the glass support plates.

The diethyl ether soluble plant acids containing auxin were divided into two and were either strip loaded in a small volume of methanol on the surface of the polyamide foil or directly onto Whatman No. 1 chromatography paper sheets. A chromatographic technique was employed in which extracts on foils were first chromatographed in one direction in a solvent system consisting of ethyl methyl ketone–methanol (99:1) which moved IAA almost to the solvent front. The polyamide sheet was then removed from the solvent once the solvent front had migrated 15 cm beyond the origin and was dried for half an hour in a cold stream of air. The sheet having been turned through 180° was then placed in a solvent system consisting of benzene–hexane (50:50) and developed twice, the new solvent front being located at the original start line. Once chromatography was completed, the excess benzene and hexane were evaporated from the foil and a zone opposite an IAA marker spot was scraped from the plastic backing into a 50-ml conical flask and eluted three times with a mixture of ethanol–acetic acid (10:2) on a shaking platform in the dark at 2°. After a brief centrifugation to pellet the chromatographic substrate, the supernatant was removed with a Pasteur pipette and reduced to dryness *in vacuo* at 34° in the dark. The residue was taken up in a small volume of methanol for chromatography on Whatman No. 1 chromatography paper using the solvent system isopropanol–ammonium hydroxide–water (10:1:1). Levels of auxin activity were estimated with the Avena coleoptile assay[24].

Results and discussion

Using synthetic IAA as a standard auxin and loading known amounts of only this compound on polyamide TLC sheets and subjecting them to the procedure described above followed by bioassay, indicated that between 68% and 70% of the IAA could be recovered (Table I). As a result of the first development in ethyl methyl ketone–methanol (99:1) most of the fluorescing phenolic material spread between R_F 0 to R_F 0.6 and IAA migrated with the solvent front. Traces of pigmented material and some phenolic substances co-chromatographing with the auxin were removed

TABLE I

REVERSE CHROMATOGRAPHY OF IAA ON POLYGRAM POLYAMIDE TLC SHEETS.

IAA loaded (μg)	*Support medium*	*Solvent system*	R_F	*Recovery* (%)
10	Polyamide	Ethyl methyl ketone–methanol (99:1)	0.92	—
10	Polyamide	Benzene–hexane (50:50)	0.75	—
10	Whatman No. 1 paper	Isopropanol–ammonium hydroxide–water (10:1:1)	0.35	70.2

by rechromatographing the foils in the reverse direction with the benzene–hexane solvent system.

Using sprays to detect phenolic compounds on paper chromatograms, or relying on zones of fluorescence when observed under ultraviolet light, a marked reduction of phenolic substances was observed when extracts had been chromatographed on polyamide TLC sheets prior to PC.
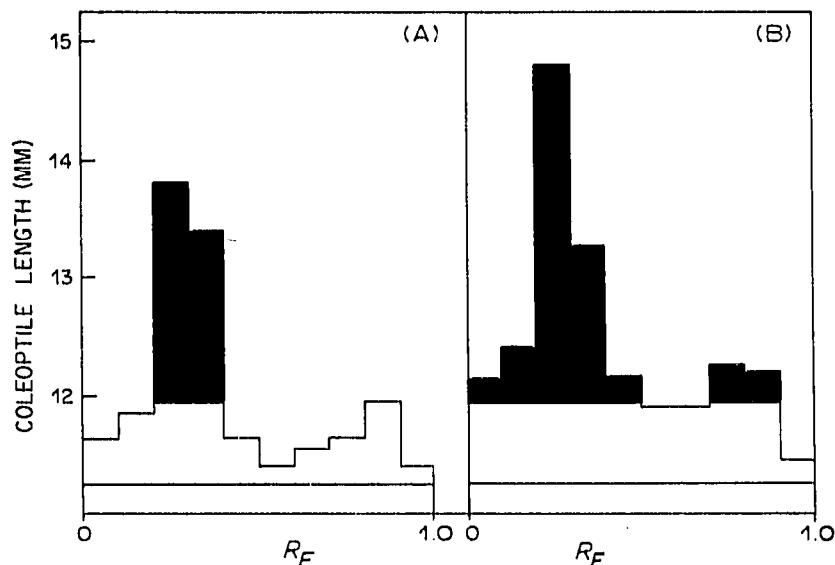

Fig. 1. Activity in the Avena coleoptile test of paper chromatograms of the acidic ether fraction purified with (A) and without (B) polyamide TLC sheets. Extracts equivalent to 20 g freshweight of leaves. Horizontal lines = control growth; darkened area = promotion significant at 1% level of probability. R_F of IAA = 0.32.

After correcting for auxin losses during the purification procedures, using the data obtained above with synthetic IAA, the results presented in Fig. 1 indicated that in unpurified, auxin-containing extracts, high amounts of phenolic substances brought about an interaction in the coleoptile bioassay which exaggerated the actual amounts of auxin present. These results therefore strongly suggested that removal of phenolic materials by hydrogen bonding to polyamide brought about a reduction of a synergistic interaction between phenols and auxin in the Avena coleoptile assay.

The technique adopted here was used solely for the purification of auxin-containing extracts from the potato species, *Solanum andigena* and it is highly likely that some modification of the technique will be required for use on other tissues.

Nevertheless, it was evident from the results presented here that assessment of auxin activity in plant extracts should be treated with a certain amount of caution where there is an inadequate separation of such growth regulators from phenolics.

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