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Note

Poly-N-vinylpyrrolidone column chromatography of plant hormones with methanol as eluent

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Phenolic compounds seriously interfere with analysis of plant hormones, particularly in bioassay detection, and the affinity of poly-N-vinylpyrrolidone (PVP) for phenolic compounds has been used to remove them during the purification of plant hormones from tissue extracts[1]. However, a major disadvantage of PVP column chromatography is that with the aqueous buffer solutions commonly used as eluents, partitioning of column fractions into an organic solvent is necessary prior to further purification steps. In addition, with the cytokinins, considerable fractionation occurs[2], which limits the usefulness of PVP chromatography as a group purification step. Moreover, degradation or rearrangement of gibberellins may occur during chromatography in alkaline buffer[3].

This report describes an investigation into the feasibility of eluting plant hormones from PVP columns with a volatile organic solvent (methanol) in an attempt to overcome the difficulties mentioned above.

EXPERIMENTAL

PVP (Polyclar AT), obtained from BDH, Poole, Great Britain, was used without separation of the finer particle sizes. Small columns (10 × 1.5 cm I.D.), packed under gravity flow with 2.9 g PVP slurried with methanol gave unrestricted flow-rates of 40–60 ml h^{-1}.

Authentic plant hormones were purchased from Sigma London, Poole, Great Britain; the sample of gibberellin $A_4 + A_7$ mixture was a gift of ICI, Plant Protection Division, Yalding, Great Britain.

Samples (10–100 μg) were applied to the columns in 1 ml methanol. The columns were eluted with methanol and 1.25-ml fractions collected. Elution of solutes was monitored by absorbance measurements at 270 nm (cytokinins), 254 nm (abscisic acid) and 280 nm (indolyl-3-acetic acid, IAA). For gibberellins A_3, A_4 and A_7 fractions were heated with 3 ml sulphuric acid–ethanol (5:95, v/v) at 50° for 15 min and the absorbance then measured at 255 nm. Fractions containing [^{14}C]-IAA were mixed with 15 ml scintillation fluid (0.4% w/v, 2,5-diphenyloxazole in toluene–2-ethoxyethanol, 3:2, v/v) and counted for radioactivity in a Packard TriCarb liquid scintillation spectrometer, applying corrections for counting efficiency (by use of an external standard) and for background radioactivity.

RESULTS AND DISCUSSION

The elution of five cytokinins, gibberellins A_3, A_4 and A_7, (±)*cis,trans*-abscisic acid (ABA) and IAA is summarized in Table I.

TABLE I

CHROMATOGRAPHY OF PLANT HORMONES ON PVP COLUMNS ELUTED WITH METHANOL

Hormone	*Elution range (ml)*
Isopentenyladenine	15.0–19.0
trans-Zeatin	15.0–20.0
Isopentenyladenosine	17.5–22.5
Ribosyl-*trans*-zeatin	20.0–25.0
6-Furfuryladenine	19.0–24.0
Gibberellin A_3	17.5–22.5
Gibberellins A_{4+7}	20.0–25.0
ABA	17.5–22.5
IAA	35.0–45.0

The rapid elution of the cytokinins tested is similar to that with pH 3.5 buffer as eluent[2]. Of the known naturally occurring cytokinins only 6-(2-hydroxybenzyl)-adenine and its riboside would be expected to bind strongly to the PVP matrix, and little separation of individual cytokinins occurred; thus the column is suitable for group purification.

The elution order of the monocarboxylic acid types of plant hormone is identical with that observed with pH 8 buffer (1): gibberellin A_3 preceding gibberellins $A_4 + A_7$, ABA and the gibberellins preceding IAA. Di- and tricarboxylic gibberellins would elute later than gibberellins $A_4 + A_7$ but before IAA and glycosyl esters in advance of free gibberellins[1]. The slower elution of IAA may reflect hydrogen bonding between the nitrogen in the indole ring and the PVP matrix, and the separation of ABA and IAA could be of value in the analysis of both hormones from the same initial tissue extract.

Column efficiencies were higher than with the coarser-grained PVP used with buffer solutions: approximately 250 theoretical plates for a 10 × 1.5 cm column as compared with approximately 50 for a 30 × 1.9 cm column eluted with phosphate buffer[1].

The chromatographic behaviour of IAA in the presence of a plant tissue extract was tested by chromatographing 50,000 cpm of [2-^{14}C] IAA (54 mCi mmole^{-1}; Radiochemical Centre, Amersham, Great Britain) alone and mixed with a crude acidic diethyl ether-soluble fraction prepared by the method of Mousdale *et al.*[4] from 1.5 g fresh weight of apple (*Malus domestica* Borkh. cv. "Miller's Seedling") leaf tissue. Both samples were applied in 1 ml methanol. Recovery of the applied IAA was quantitative (Table II).

Binding of phenolic pigments occurred in the top 1 cm of the column, and a dry weight reduction of approximately eight-fold was obtained by passage through the column.

A PVP column eluted with methanol therefore affords an effective and rapid purification step as part of the purification of plant hormone-containing extracts.

TABLE II

PVP COLUMN CHROMATOGRAPHY OF [^{14}C]IAA IN THE PRESENCE OF AN APPLE LEAF EXTRACT

V_e/V_t = Peak elution volume/column bed volume.

Sample	V_e/V_t	*Recovery of applied [^{14}C]IAA in 35–45-ml fraction (%)*
IAA alone	2.24	97.3
IAA + extract	2.18	96.8

Solvent can be quickly removed from column fractions under mild conditions (for example, rotary evaporation under reduced pressure at room temperature), and there is the added advantage that methanol is a superior solubilizing agent for organic solvent fractions to aqueous buffer solutions.

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