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Note

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Chromatography of five cytokinins on an insoluble polyvinylpyrrolidone column

Polyclar AT, an insoluble form of polyvinylpyrollidone (PVP) forms insoluble complexes with phenols, which may thus be removed from plant extracts. Columns of PVP have been used[1,2] to purify and to separate plant hormones including the cytokinin zeatin (6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine), but other cytokinins have not been investigated in this way. However, a number of purines have been separated on similar columns[3] and this report describes the separation of five cytokinins on a PVP column.

Materials and methods

The column was prepared by pouring a slurry of Polyclar AT in distilled water into a 2 × 30 cm glass column by packing it to a height of 20 cm by gravity flow. It was allowed to settle and then washed thoroughly with distilled water followed by M/75 phosphate buffer at pH 6.4. A mixture of five cytokinins in 6 ml of buffer was applied to the column and then eluted with further phosphate buffer at pH 6.4. The cytokinins were 6-(γ,γ-dimethyl-allylamino) purine (DMAA), 6-furfurylaminopurine (kinetin) and 6-benzylaminopurine (BA) at $1.25 \times 10^{-4}M$, zeatin at $1.15 \times 10^{-4}M$ and zeatin riboside at $1.8 \times 10^{-5}M$. The column flow-rate was 35 ml/h. The cytokinin elution profile was obtained by monitoring the transmittance of ultraviolet light at 254 nm through the eluate using an LKB Uvicord II absorptiometer. For quantitative determinations each cytokinin was chromatographed

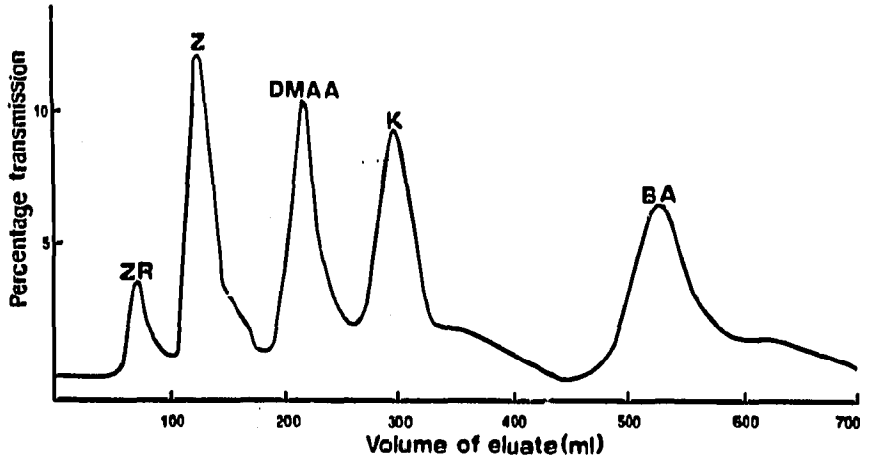

Fig. 1. Separation of a mixture of cytokinins on a PVP column eluted with M/75 phosphate buffer at pH 6.4 as measured by percentage transmission at 254 nm. Key: ZR = zeatin riboside, Z = zeatin, DMAA = 6-(γ,γ-dimethylallylamino) purine, K = kinetin, BA = 6-benzylaminopurine.

separately and the optical density of the total eluate was recorded at $\lambda_{max.}$ for the cytokinin.

Results and discussion

Fig. 1 shows that separation of the cytokinins into five discrete peaks was obtained. The percentage recoveries of the cytokinins when applied singly to the column were 80, 78, 95 and 85 % for DMAA, kinetin, zeatin and BA, respectively. The quantity of zeatin riboside available for investigation was too small for any reliable determination to be made.

PVP may thus be used to separate synthetic applied cytokinins *viz.* BA and kinetin, from those cytokinins which may occur naturally in the plant *viz.* zeatin, zeatin riboside and DMAA, and from phenolic contaminants existing in plant extracts.

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