The Application of a Lipophilic Dextran Derivative to Analysis of Plant Terpenoids

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Gels of hydroxyalkoxypropyl-dextran ('modified LH-20'), made by reaction of terminal epoxyalkanes with Sephadex LH-20 (Ellingboe, Nyström & Sjövall, 1968), swell in non-polar solvents such as benzene. Our observations indicate that the separating properties of the benzene–gel preparation involve gel filtration. The selective retention of aromatic compounds, observed for Sephadex G-25 and LH-20 (Janson, 1967), is absent: there is, however, a powerful hydrogen-bonding effect, the gel acting exclusively as the basic component. Hydroxyl groups markedly increase retention to a degree dependent on their number and steric environment. Carboxylic acids are severely retained. The incorporation of an alcohol in the benzene permits selective displacement of such samples, apparently by competitive hydrogen bonding. Propan-2-ol–benzene (1:3, v/v) gives negligible retention of monohydroxylic compounds. Columns (100cm. × 0·9cm. diam.) of 'modified LH-20' had theoretical plate heights 0·12–0·14mm. The composition of eluates was determined by gas–liquid chromatography, which demonstrated that the compounds were eluted as symmetrical peaks. Examination of radioactive samples indicated the virtual absence of 'tailing'. We define standard elution volume (S.E.V.) as the elution volume for a column of bed volume 100 units; this allows the correlation of results from columns of various dimensions. Standard elution volumes are found to be reproducible, to S.E.M. ± 0·5% (internal standard: cholesteryl acetate, S.E.V. 56·3). Examples include isopetasyl acetate (a sesquiterpenoid ester), S.E.V. 59·1; lanosterol (a hindered alcohol), 82·3; cholesterol, 110·2; and isopetasol, 119·6.

Crude plant extracts have previously shown poor separations on unmodified Sephadex LH-20: for example, selective retention of highly conjugated compounds causes pigments to be eluted with material of lower molecular weight (Ruzicka, Thomson, Wheals & Wood, 1968). By using columns of 'modified LH-20' in benzene, these pigments are eluted largely in early fractions. Most terpenoids are eluted later by the same solvent. Highly polar compounds (glycosides, free acids etc.) remain until eluted by alcohol–benzene mixtures.

As shown by Sjövall, Nyström & Haahti (1968), the application of gel chromatography offers a number of advantages. Analytical scale (10μg.–10mg.) results may be reproduced preparatively (10mg.–1g.) with little loss in efficiency. The mild conditions are particularly suited to samples susceptible to decomposition, and it is unnecessary to prepare derivatives of highly polar or non-volatile compounds.

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The Use of Cellulose Phosphate in the Extraction of Free Nucleotides from Plant Tissue

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The quantitative extraction of certain nucleotides from plant tissues requires the use of a metal-complex-forming agent (Cole & Ross, 1966; Isherwood & Barrett, 1967; Keys, 1968). For this purpose 1,1,1-trifluoro-3-(2-thenoyl)acetone was used (Keys, 1968) because it and its metal complexes are soluble in methanol and may be washed away, leaving nucleotides in the tissue residue. Difficulties were encountered in this procedure and an insoluble polymer was sought to which metal-binding groups were attached. Enquiries lead to the successful use of the cellulose phosphate derivative, Whatman Column Chromedia P11.

Freeze-dried samples of powdered tobacco leaf were treated with boiling ethanol and phenazine methosulphate (Keys, 1968). Cellulose phosphate (0·5g.), previously washed and freed from fine particles, was added and the suspension transferred to a chromatography column that already contained 0·5g. of cellulose phosphate mixed in acetone with 0·5g. of Whatman CF1 cellulose powder. Extraction followed the method of Keys (1968) except that 1,1,1-trifluoro-3-(2-thenoyl)acetone and ammonium acetate were omitted from extracting solvents. Inclusion of ammonium acetate, pH 7·4, especially where this was sufficient to saturate the cellulose phosphate with ammonium ions, caused up to 90% of the UDP-glucose in samples to be lost. No UDP or UMP was formed to account for the loss of UDP-glucose. Added ^{14}C-labelled UDP-