tography is not satisfactory for isolation of urinary BAIB. The method of SAINI[13], although utilizing three runs, appears to be the method of choice, since it avoids losses and inconvenience of the desalting procedure.

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Separation of growth regulators from *Picea abies* Karst. on Sephadex LH-20

The occurrence of endogenous growth regulators in plants is usually investigated by means of various biological assays. The separation of the active substances from substances interfering with their determination is an important problem. Partition between organic solvents and water[1] and paper chromatography[2] are classical methods which have been used for this purpose. Some plant species, especially conifers, contain large quantities of growth inhibitors which can be difficult to separate from the auxins if only these methods are used[3,4].

The results reported here were obtained during an investigation of endogenous growth regulators in sprouting buds and seedlings of Norway spruce (*Picea abies* Karst.) The plant material was frozen and extracted with cold methanol. The *acid ethyl ether fraction* obtained by conventional methods[1,5] and a *butanol fraction* obtained by extraction of the aqueous acid solution, remaining after the ether extraction, with *n*-butanol were used in the experiments. The Avena straight-growth coleoptile test was used to detect biological activity.

Examples of the growth response of coleoptile sections to R_F zones from paper chromatograms of the above fractions developed in isopropanol–ammonia–water (100:14:6) are shown in Fig. 1. The ether fraction had considerable inhibitory activity but a certain stimulation was usually obtained at the R_F of indole-3-acetic acid (IAA). The butanol fraction often gave growth stimulation over a broad R_F range (Fig. 1B) but in certain cases only inhibition was obtained (Fig. 1C).

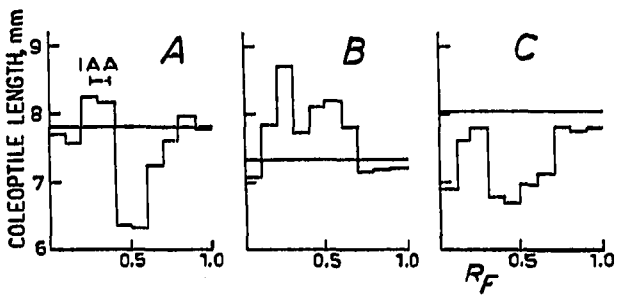

Fig. 1. Activity in the Avena coleoptile test of paper chromatograms of the acid ether fraction (A) and the butanol fraction (B, C). Extracts equivalent to 3–4 g fresh weight of buds (A, B) or 4 weeks old seedlings (C) of Norway spruce. Horizontal lines = control growth. R_F of IAA indicated.

The fractions were subjected to gel filtration on a Sephadex LH-20 column: diameter 2.5 cm, length 30 cm, flow rate 30 ml/h, temperature 21–23°. The solvent was 96 or 70% ethanol to which was added HCl to a concentration of 0.001 M. Addition of acid or a buffer solution was found to be necessary in order to obtain reproducible elution volumes for weak acids of the kind present in the extracts. Transmission of UV at 280 mμ was recorded with an LKB Uvicord II. The solution was collected in 10 ml fractions. Each fraction was evaporated to dryness and the residue was assayed in the coleoptile test with or without preceding paper chromatography.

The results obtained in an experiment with the acid ether fraction are shown in Fig. 2. Elution volumes for abscisic acid (ABA) and IAA on the same column are denoted. It is suggestive that the main inhibitory activity is obtained at about the same elution volume as ABA. The substance causing growth stimulation at the elution

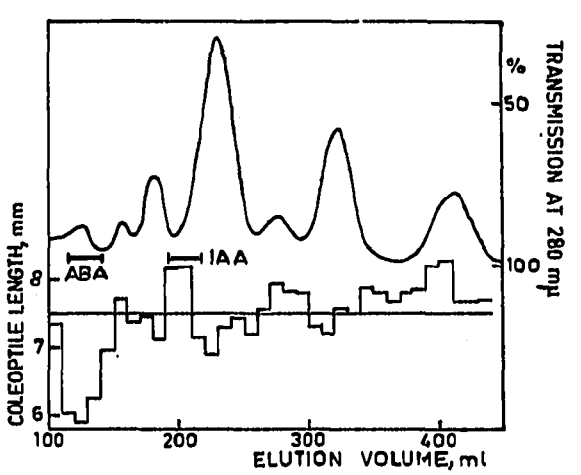

Fig. 2. UV absorption and biological activity of 10 ml fractions from gel filtration of the acid ether fraction. Extract equivalent to 10 g fresh weight of buds.

volume of IAA has been tentatively identified as IAA by its R_F values in five different solvent systems, by electrophoresis and by its color reaction with Ehrlich reagent. The growth stimulators with higher elution volumes were not identified. The extract contains several UV-absorbing components which, however, need not be identical with the biologically active substances.

The growth stimulation obtained in the paper chromatograms of the butanol fraction (Fig. 1B) at R_F 0.1–0.7 was shown by Sephadex filtration to be due to several components with elution volumes from 80 to 270 ml. Gel filtration followed by paper chromatography of 20 ml fractions as well as the extract of Fig. 1C could be shown to contain growth stimulators admixed with inhibitors (Fig. 3). The nature of the growth stimulators of the butanol fraction is not known. Possibly complexes of IAA are involved as some IAA is released by heating the fraction with 1 *M* NaOH.

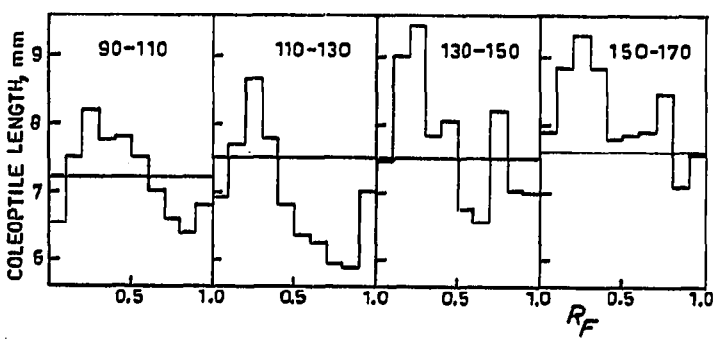

Fig. 3. Biological activity of paper chromatograms of 20 ml fractions from gel filtration of the butanol fraction. The same extract as in Fig. 1C. The figures denoted elution volumes. Extract equivalent to 10 g fresh weight of tissue.

The experiments show that Sephadex LH-20 is a useful tool for the separation of growth-regulating compounds in plant extracts. The possibility of using an organic solvent in this separation is of importance because of the greater solubility of the compounds and the greater stability of some active compounds in an alcoholic solution than in water solution. A full account of the investigation will be published elsewhere.

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