Growth-regulating substances from *Pinus silvestris* separated on Sephadex LH-20

In conjunction with studies concerning *Melampsora pinitorqua*, a parasitical fungus of importance to forestry in the whole of Europe[1,5], elucidatory tests have been carried out into the growth-regulating substances inherent in *Pinus silvestris*. This subject has not hitherto been studied to any extent. The purpose of this paper is to show that gel filtration in an organic solvent, in this instance ethanol, is a very practical method of separating the extract from pine shoots.

The extract was prepared by using the methods described by FRANSSON[3,4] and BOYSEN JENSEN[2]. Young terminal leaders (needles removed) were cut into small pieces and extracted with 96 % ethanol. This extract was filtered and evaporated, the residue then being extracted with chloroform. This solution was decanted and evaporated and the residue was extracted with distilled water. The water phase was made slightly alkaline (pH 8–9) and fractionated with ether. The water phase was then acidulated (pH 3.4) and again fractionated with ether. This acid ether phase was used in the experiments.

Sephadex LH-20, intended for organic solvents, was used for the gel filtration. A laboratory column SR 25/45, solvent resistant (Pharmacia Fine Chemicals AB, Uppsala), 70 % alcohol and a fraction collector and siphon were used. Bed diameter: 2.5 cm. Bed volume: approx. 135 ml. Flow rate: approx. 0.5 ml/min. Void volume: approx. 50 ml. Spectrophotometer: Beckman DB.

The ether extract from pine was intended in the first place for use in bioassays, the so-called straight growth test. The pure extract showed itself to have a pronounced growth-inhibiting effect on the elongation growth of the Avena coleoptile section test. The germinative capacity of the basidiospores of *Melampsora pinitorqua* was also clearly inhibited.

To identify any other components which may have been present in the extract, elucidatory chromatographical tests using standard methods were made. Without going into detail, it can be mentioned that the extract showed itself to contain a considerable number of components.

It proved possible with the help of gel filtration to separate the extract into five fractions, which were easily identified in a spectrophotometer. The fractions contain a far more suitable number of substances for chromatography than the pure extract, which can initially contain 20-odd substances. Of these at least five can be coloured with Ehrlich's reagent and are therefore probably indole compounds. Subsequent studies will be concerned with identifying as many substances as possible, and to determining which of them have a biological effect on, and eventual connection with, the occurrence of *Melampsora pinitorqua*. The fractions leaving the column can be described as follows:

First fraction: 1.3–2 void volume; extinction max. at 242 mμ and 200 mμ.

Second fraction: indistinctly separated from the former, 2.2–2.6 void volume; extinction max. at 275 mμ, 242 mμ and 202 mμ and also indications of maxima at 285 mμ, 250 mμ, 235 mμ and 220 mμ.

Third fraction: 2.8–3.5 void volume; extinction max. at 265 mμ, 231 mμ and possibly also 200 mμ.

Fourth fraction: 4.3–4.6 void volume; extinction max. at 255 mμ.

Fifth fraction: 4.9–6.1 void volume; extinction max. at 309 mμ, 272 mμ and 218 mμ. The maximum at 309 mμ is sometimes double that at 309 mμ and 300 mμ.

All that can be said at this early stage is that the first fraction, without further purification, has a very marked inhibitory effect on the elongation growth of the Avena coleoptile section tests. Quantities equal to 0.2 g of pine shoots give a clear reading. The germination of spores of *Melampsora pinitorqua* (basidiospores on water agar) is also inhibited.

The total quantity of extract substances and the internal quantitative relationship between the fractions varies in different pine material. Pine varieties which are resistant to *Melampsora pinitorqua* contain greater quantities of extract substances than the non-resistant types.

The first and second fractions appear to be the most complex and contain at least three components that can be coloured with Ehrlich's reagent.

Several fluorescent substances are present in the fractions. This is most apparent in the fifth fraction, where the solution taken straight from the column fluoresces in ultra-violet light.

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