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Constituents of Pollen

I. Low-molecular Carbohydrates in Pollen from *Pinus montana* Mill.

MARTIN NILSSON

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

Sucrose, glucose, fructose, myo-inositol, sequoitol and pinitol have been isolated from pollen of *Pinus montana* Mill. Of these substances pinitol and sequoitol have not been previously found in pollen.

The literature on pollen chemistry has been recently reviewed by Lundén [1]. Not very much systematic work has been carried out on the low-molecular weight carbohydrates in pollen and only a few substances have been isolated in the crystalline state: sucrose [2], lactose [3] and myo-inositol [4]. However, paper chromatographic investigations indicate the presence of glucose [5], fructose [5], raffinose [6] and stachyose [6] also.

For *Pinus silvestris* various authors (for detailed references and discussion see Ref.[7]) have determined the contents of "reducing" and "non-reducing sugars" as 0—2 % and 8—12 % in uncontaminated pollen. The non-reducing sugars were shown to consist mainly of sucrose. Recently Nielsen, Grömmer and Lundén [8] investigated various pollen using microbiological methods and found after acid hydrolysis 0.9 % myo-inositol in pollen from *Pinus montana*.

The pollen samples used in this investigation were collected in 1954 by *A. B. Cemille*, Vegeholm, Sweden, and contained at least 99 % of pollen from *Pinus montana* Mill.[8] (subgenus Diploxylon). The water-soluble part of the methanol-extract from the pollen was, after a preliminary treatment, fractionated by chromatography on a carbon-celite column [9]. Sucrose (9 %), myo-inositol (0.1 %) and sequoitol (0.05 %) were thus obtained directly in the crystalline state. Pinitol, fructose and glucose could not be separated on the carbon column. From the mixture of these substances pinitol (1.2 %) was separated by crystallisation. The remaining sugars were isolated as *iso*propylidene derivatives as described by Bell [10]. Thus crystalline derivatives of fructose (0.7 %) and glucose (0.3 %) were obtained.

Parts of these sugars might originate from sucrose and other saccharides present by hydrolysis during the storage of the pollen and perhaps also during the extraction and purification process. However, it was found that the first

extracts showed strong spots corresponding to glucose and fructose on paper chromatograms.

Pinitol and sequoitol have not been previously reported to occur in pollen. Pinitol appears to occur in the needles of all conifers [11] and has also been found in the heartwood of pines belonging to the subgenus *Haploxylon* [12]. Sequoitol has only been encountered once in pines; in the heartwood of *Pinus Lambertiana* [13] (Haploxylon).

EXPERIMENTAL

All melting points are uncorrected. Evaporations were carried out *in vacuo* at 40–50°. Paper chromatograms were run in *n*-butanol, ethanol, water (5:1:4) and/or ethyl acetate, acetic acid, water (3:1:1) and developed with the silver nitrate-sodium ethoxide reagent.

Air-dried pollen from *Pinus montana* Mill. (250 g) was extracted with ether in a Soxhlet apparatus for two weeks and then with methanol for three weeks. As the solvent flow through the pollen layer was very slow the extraction temperature was about 25°. The methanol extract on evaporation yielded a semi-solid mass (72 g), which was extracted with water (1.5 l, in portions). The solution was filtered (celite), the filtrate extracted with ether (2 × 300 ml) and purified by the lead acetate method [14]. It was shown by paper chromatography that practically no cyclitols were precipitated or adsorbed under the conditions chosen (pH 5). The aqueous solution was deionized (IR 120 and IR 4 B) and evaporated to dryness. The resulting syrup (42 g) was dissolved in water (400 ml) and fractionated on a carbon-celite column (50 × 6 cm. Ethanol 0–20 %, 15 l, afterwards washed with ethanol, 50 %, 10 l). The eluate was divided into fractions which were investigated by paper chromatography and checked for optical rotation. The first run gave 12.9 g of easily eluted components, 28.0 g of sucrose fraction and 1.3 g of higher sugars. The sucrose fraction on treatment with methanol yielded crystalline sucrose (19.6 g). The easily eluted components were refractionated on the same column (Ethanol 0–10 %, 16 l) giving crystalline myo-inositol (0.23 g), sequoitol (0.12 g) and sucrose (2.2 g). From fractions containing a mixture of pinitol, fructose and glucose (8.1 g), pinitol (3.0 g) was obtained by crystallisations from ethanol. The material in the mother liquors was treated with acetone and sulphuric acid converting the sugars to di*iso*propylidene derivatives, the remaining pinitol being unaffected. Mild hydrolysis of the chloroform-soluble substances and partition of the products obtained gave crystalline 2,3–4,5-di(O-*iso*propylidene)-fructopyranoside (1.2 g) and 1,2-O-*iso*propylidene-glucofuranoside (0.48 g), corresponding to fructose (0.8 g) and glucose (0.4 g).

A reference experiment showed 80 % and 50 % recovery of the fructose and glucose derivatives, respectively, from a mixture of equal parts of pinitol, glucose and fructose. Paper chromatographic investigation of the water-soluble material after the acetone treatment showed the presence of pinitol and glucose only.

The fraction of higher sugars and the sucrose mother liquors were investigated by paper chromatography and were found to contain in addition to sucrose several components with lower R_F-values. Some of the spots could be developed with the anisidine-phosphoric acid reagent and some also with resorcinol-hydrochloric acid. These facts point to the presence of tri- and tetrasaccharides. The isolation of these substances was not, however, attempted.

The *sucrose* m. p. 178–184° was homogeneous on the paper chromatograms. $[\alpha]_D$ +68° (*c* 1.8, water). Octa-acetate (acetic anhydride, pyridine), m. p. and mixed m. p. 89–90°, $[\alpha]_D$ +59° (*c* 6,4, chloroform).

The crude *pinitol* m. p. 180–90° was sublimed and recrystallised from water-ethanol, m. p. and mixed m. p. 188–189°, $[\alpha]_D$ +63° (*c* 6,2, water); pentaacetate (acetic anhydride, perchloric acid), m. p. and mixed m. p. 99–100° $[\alpha]_D$ −6,9 (*c* 6,4, chloroform).

The *myo-inositol* m. p. 219–224° was recrystallised from water-ethanol, m. p. and mixed m. p. 225–228°; hexaacetate (acetic anhydride, perchloric acid) m. p. and mixed m. p. 216–218°.

The *sequoitol* m. p. 233—238° was recrystallised from water-ethanol, m. p. and mixed m. p. 238—239°; pentaacetate (acetic anhydride, perchloric acid), m. p. and mixed m. p. 196—197°.

2,3—4,5-di(O-isopropylidene)-fructopyranoside, m. p. 83—98°, was recrystallised from ether-petrol, m. p. and mixed m. p. 95—96°, $[\alpha]_D$ −21° (*c* 1.4, chloroform).

1,2-O-isopropylideneglucofuranoside, m. p. 100—120°, was recrystallised from ethyl acetate, m. p. and mixed m. p. 159—160°, $[\alpha]_D$ −4.2 (*c* 7.3, ethanol).

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