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Constituents of Pollen

II.* Long-chain Hydrocarbons and Alcohols

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Some waxy, crystalline fractions isolated from pollen have been investigated by mass spectrometry. This method has proved to be most convenient for the investigation of these mixtures of long-chain natural products.

Pollen from *Pinus montana* contains *n*-tetracosanol-1, *n*-hexacosanol-1 and *n*-octacosanol-1, while pollens from *Zea mays* and *Alnus glutinosa* have been found to contain paraffins; maize *n*-pentacosane and *n*-heptacosane, alder mainly *n*-heptacosane and *n*-nonacosane.

For the investigation of pollen constituents carried out at the Royal Institute of Technology, Stockholm, some representative plants have been chosen; the gymnosperm *Pinus montana*, Mill. and the angiosperms *Zea mays*, L. and *Alnus glutinosa*, Gaertn., representing mono- and dicotyledons respectively [1].

The present paper deals with the ether-extractable material from these pollens. The amount of ether-extract was approximately the same in pine and alder pollen (around 9 %), but rather small (2 %) in maize pollen. This difference is, however, not so significant as the amount of ether-extract in the maize pollen apparently varies widely from year to year [1]. The extracts were divided into acid and neutral fractions; the neutral fractions were semisolid and crystalline material deposited when the hot solutions in acetone (ethanol) were left to cool. These crystalline products had a waxy consistency and appeared to consist of long-chain aliphatic compounds.

X-Ray powder photographs of the different long-chain mixtures were taken in a Guinier camera using Cu-$K\alpha$ radiation. They were all very similar and one of them is shown in Fig. 1. The appearance and distribution of the strong diffraction lines indicate that the mixtures consist of long straight-chain hydrocarbons with no substituents or only smaller ones (*e. g.* paraffins and alcohols). The chains are packed in the common *ortho*-rhombic structure [2-4] with the chain-plane of each molecule approximately perpendicular to that of the next.

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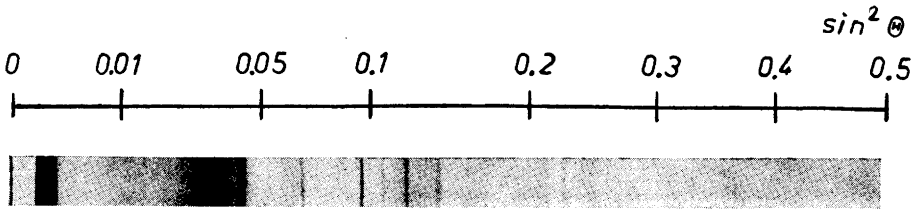

Fig. 1. Guinier X-ray powder photograph of the crude long-chain mixture from *Alnus glutinosa* pollen. Cu-*Kα*-radiation. Exposure 4 h.

To show the nature of the long-chain mixtures they were investigated using the mass spectrometer described by Ryhage [5], which has been used in structural research on long-chain compounds and mixtures [6,7].

Mountain pine gave a large amount (1 %) of a material, m. p. 70—74°, which when treated with urea in methanol formed a complex [8]. Purification by recrystallisation and by complex formation did not raise the melting point appreciably (72—74°). The elementary analysis was consistent with the composition $C_{24}H_{50}O$ and hence the product was presumed to be a mixture of straight chain alcohols in the C_{24}—C_{26}-region.

Similar fractions have frequently been isolated from plants. On careful fractionation and X-ray investigation they have generally proved to be mixtures of closely related alcohols, sometimes contaminated with hydrocarbons [9,10]. The mass spectrometric investigation, discussed in detail in a later section, showed that the product from *P. montana* contained *n*-tetracosanol-1, *n*-hexacosanol-1 and *n*-octacosanol-1. The mass spectrum of the crude product was easily interpreted and the investigation of the purified material merely served to confirmed the result.

The crude materials from maize and alder pollen were therefore investigated directly although their melting point intervals were very broad. The mass spectra showed them to consist largely of paraffins and also revealed the presence of other compounds so they were purified by filtration through alumina and the preliminary results confirmed by an additional mass spectrometric analysis. The maize pollen contained *n*-pentacosane and *n*-heptacosane and the alder pollen *n*-heptacosane, *n*-nonacosane and smaller amounts of *n*-tricosane and *n*-pentacosane.

DISCUSSION

The investigation has revealed a marked difference between the gymnosperm pollen, which contains large quantities of longchain alcohols and the angiosperm pollens, which contain smaller amounts of paraffins.

Paraffins have been found in angiosperm pollen earlier; "tricosane" in hazel pollen [11], "heptacosane" in pollen from sugar beet [12] and as a mixture in maize pollen [10,13]. A fraction closely related to "ceryl alcohol" has been found in pollen from a pine [12] and in an investigation of *Pinus silvestris* pollen a waxy substance, m. p. 80°, has been obtained [14], which can now be supposed to be a wax alcohol mixture.

If the ether-extracted pollens were extracted with alcohol a further quantity of lipid material was obtained, which was obviously retained by the pollen membrane during the ether extraction (*c. f.* Ref.[15]). It may be presumed that the waxes dealt with in this paper are located on or near the surface of the pollen grains in the same way as plant waxes in general are located in the outer layer of the tissues [16]. The function of the pollen waxes is apparently the same as that of plant waxes in general — a barrier against loss of water — as shown by the striking parallel between the waxy conifer-needles [17] and the heavily wax-impregnated pine pollen.

The gymnosperms are wind-pollinated whereas the angiosperms are generally pollinated by insects and this might have some connection with the differences between the long-chain compounds in the gymnosperm and the angiosperm pollen.

EXPERIMENTAL

Extraction, isolation and purification

The melting points were determined on a Kofler block. The pollen samples were collected in 1954 by *A. B. Cemille*, Vegeholm, Sweden, and contained at least 99 % of the right pollen [1].

Air-dried pollen (500 g) was exhaustively extracted with ether in a Soxhlet apparatus (*ca.* 2 weeks); the extraction temperature was *ca.* 25°. The ether was evaporated, the residue dissolved in chloroform and extracted with 2 M potassium hydroxide. After appropriate back extractions an acid fraction and a neutral fraction were obtained. The neutral fractions were dissolved in a minimum amount of hot acetone (for maize ethanol) and the crystalline precipitate obtained after cooling collected.

Pinus montana gave 8.0 % ether-extract giving 3.6 % neutral material, from which 1.0 % crystalline material, m. p. 70—74°, was obtained. The corresponding figures for *Zea mays* were 2.0, 1.1, 0.2 %, m. p. 48—58° and for *Alnus glutinosa* 10.0, 8.5, 0.6 % and m. p. 57—64°, all percentages being calculated on an air-dried pollen basis.

The crude product from *P. montana* was recrystallised from acetone and dissolved in a hot solution of urea in methanol (160 g/l). On cooling long needles separated. The urea complex was collected and decomposed with chloroform and dilute hydrochloric acid, the chloroform layer was separated, evaporated and the product recrystallised from acetone, m. p. 72—74°. (Found: C 81.4; H 13.8. Calc. for $C_{24}H_{50}O$: C 81.4; H 14.1.)

For purification of the maize and alder products, samples (*ca.* 1 g) were dissolved in methyl *cyclo*hexane (50 ml), passed through a column of alumina (10 × 1.5 cm, 20 g) and the column was washed with the same solvent (150 ml). On evaporation colourless waxes were obtained; from maize 65 % of input with a melting point range of 45—60°, from alder 55 %, m. p. 55—70°. Elution with benzene (200 ml) gave 10 and 15 %, m. p. 45—55° and 60—75°, respectively, of pale yellow waxy material. Further elution with ethyl acetate and methanol gave discoloured, heterogeneous material. The methyl *cyclo*hexane fractions are referred to in the next section as purified products, the benzene fractions were investigated with the mass spectrometer but the spectra were too complicated for interpretation presently. The other fractions were not further investigated.

Mass spectrometric study

The mass spectrometer used is that described by Ryhage [5]. For typographic reasons only the high mass regions of the mass spectra are shown in the figures. The mass spectrum of the crude product from *Pinus montana* is shown in Fig. 2. This is a spectrum typical of long-chain compounds. In the C_{28}-, C_{26}- and C_{24}-alkyl groups of peaks there are two comparatively strong peaks corresponding to $C_nH_{2n}^+$ and $C_nH_{2n-2}^+$, which is typical of a long-chain alcohol with n carbon atoms [6]. Such an alcohol gives, however, also

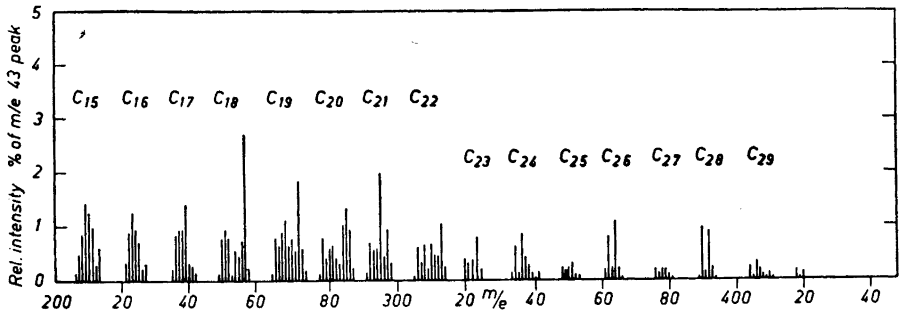

Fig. 2. Mass spectrum of the crude long-chain mixture from *Pinus montana.*

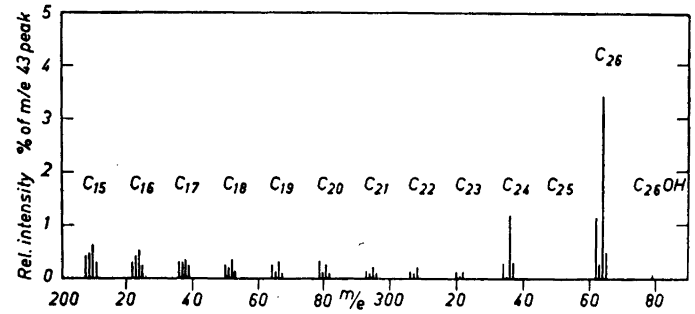

Fig. 3. Mass spectrum of pure *n*-hexacosanol-1.

peaks at *m/e* corresponding to $C_{n-2}H_{2n-4}^+$ and $C_{n-2}H_{2n-6}^+$ but these are much lower. For comparison the spectrum of pure *n*-hexacosanol is shown in Fig. 3. In the case of *P. montana* there are peaks at *m/e* = 392 ($C_{28}H_{56}^+$) and *m/e* = 390 ($C_{28}H_{54}^+$) indicating the presence of *n*-octacosanol-1. There are also peaks at *m/e* = 364 ($C_{26}H_{52}^+$) and 362 ($C_{26}H_{50}^+$) which to a small part are due to fragmentation of *n*-octacosanol-1 and to a larger extent to fragments from *n*-hexacosanol-1. The same thing is valid for the peaks in the C_{24}-group, which indicate smaller amounts of *n*-tetracosanol-1, as the peaks in the C_{22}-group are lower. There are no obvious signs of any other long-chain compounds. The purified mixture from *P. montana* was also analysed and found to contain more *n*-octacosanol-1 and less *n*-tetracosanol-1 than the crude product, which is of course consistent with the larger solubility of the shorter alcohols.

The mass spectra of the products from *Zea mays* and *Alnus glutinosa* are also typical of long chain compounds, but in this case of *n*-hydrocarbons. The mass spectrum of the purified product from *Zea mays* is shown in Fig. 4 and that of the crude product from *Alnus glutinosa* in Fig. 5. Mass spectra of heavy normal and branched chain hydrocarbons have been extensively studied by O'Neal and Wier [18]. A normal chain hydrocarbon spectrum always contains a strong parent peak corresponding to the unfragmented molecule and a series of peaks corresponding to $C_nH_{2n+1}^+$ with *n* up to the number of carbon atoms in the molecule.

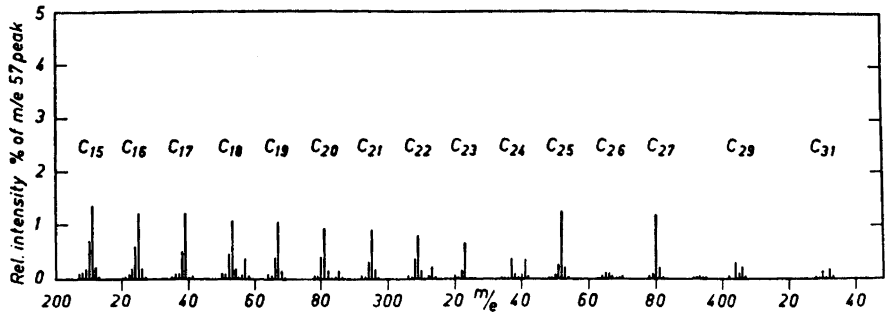

Fig. 4. Mass spectrum of the purified product from *Zea mays*.

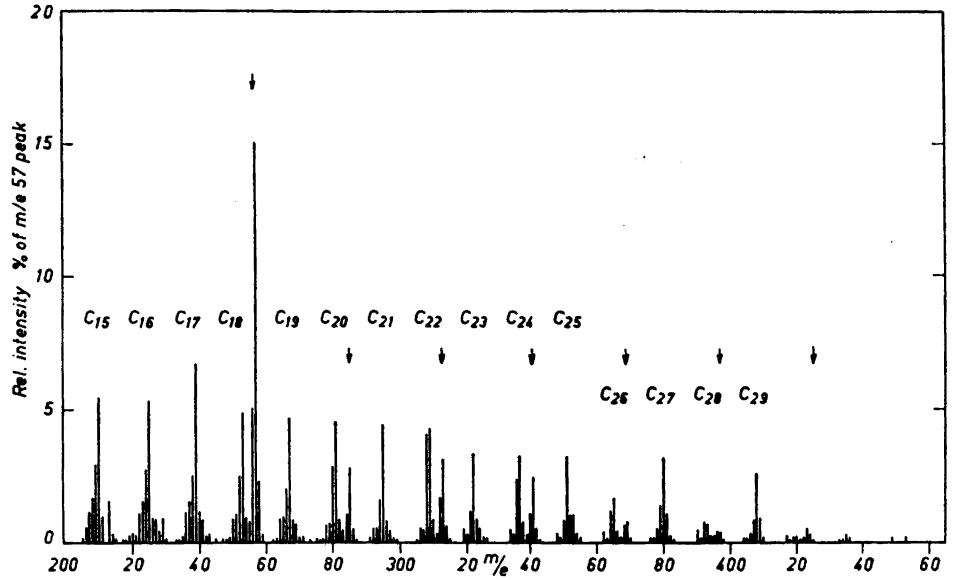

Fig. 5. Mass spectrum of the crude product from *Alnus glutinosa*.

It can be seen that the spectra of the mixtures from *Zea mays* and *Alnus glutinosa* (Fig. 4 and 5) are characteristic for hydrocarbons. The first one contains only *n*-pentacosane and *n*-heptacosane while the last one contains *n*-heptacosane, *n*-nonacosane and traces of *n*-tricosane and *n*-pentacosane. There are peaks in both spectra, which cannot be referred to fragments of these hydrocarbons, but which indicate the presence of other compounds. Thus the spectrum of the crude product from *A. glutinosa*, which had a faint yellow colour, had strong peaks at $m/e = 257 + n \times 28 = 285, 313, 341$ .... (marked with arrows in Fig. 5). This might indicate some sort of conjugated system attached to a fragment with a molecular weight approximately equal to 257. The same series of peaks was also recognised in the spectrum of *P. montana* although the intensities were lower.

The weak peaks in the C_{29}-and C_{31}-regions of the maize spectrum cannot be due to saturated hydrocarbons or alcohols but possibly to unsaturated hydrocarbons. In lack of reference substances no conclusions should be drawn however. In the crude products from maize and alder there are also smaller amounts of other compounds, probably containing oxygen. The investigation of the purified mixture from alder gave no further information.

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