

# *n*-Alkanes – Common Surface Constituents of Pollen from Gymno- and Angiosperms

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Pollen of Gymno- and Angiosperms, Surface Waxes, *n*-Alkanes

The surface waxes of several gymnosperm and angiosperm pollen were separated into different lipid classes. *n*-Alkanes were found to be common constituents of these waxes. They appeared as a homologous series ranging from C<sub>20</sub> to C<sub>33</sub>. The distribution patterns of *n*-alkanes from gymnosperm pollen displayed little variation whereas those of angiosperm pollen exhibited pronounced differences from one another. It is noteworthy that especially for the gymnosperm species, homologues with even and odd numbers of carbons occurred in similar amounts.

## Introduction

The exine, the outer pollen wall, is one of the most complex wall systems occurring in the life cycle of higher plants. It consists of sporopollenin, an extremely resistant biopolymer of largely unknown structure. The exine protects the pollen, the carrier of the male gametophyte, from various environmental factors. The protective effect of this wall system may be enhanced by several soluble substances such as phenolic compounds [1] and waxes [2–4] which are accumulated at and/or within the structure of the exine. In leaves for example it has been shown recently that cuticular waxes play a crucial role as the diffusion barrier limiting the transport of water and solutes [5–7]. Multiple roles of lipids in the general economy of the pollen are discussed [4].

Surface waxes of pollen grains are a mixture of long chained and lipophilic components. They are composed of different lipid classes such as hydrocarbons, wax esters, aldehydes, primary alcohols, free fatty acids [2, 3] and triterpenoids [4].

Current knowledge about the distribution patterns of *n*-alkanes in surface waxes of pollen grains from gymno- and angiosperms is very limited. In this paper we therefore examine the distribution of *n*-alkanes from the pollen of a number of gymno- and angiosperm species.

## Materials and Methods

### Plant material

Pollen from *Picea abies*, *Pinus mugo*, *Taxus baccata*, *Torreya nucifera*, *Cucurbita maxima*, *Corylus avellana* and *Narcissus pseudonarcissus* were collected in the Botanical Garden and in the region around Münster.

### Isolation of surface waxes

200 mg to 400 mg of pollen were extracted with chloroform (1 ml solvent per mg pollen) through a 2 cm diameter filter (frit size: 4). The extract, representing the total wax fraction, was dried under vacuum and resolved in 5 ml pentane. The total wax fraction was separated into individual lipid classes on a 15 cm × 2 cm column of silica gel 60 (0.06–0.2 mm, Merck, Darmstadt, F.R.G.) suspended in pentane. The column was successively eluted with 70 ml of pentane, 2-chloro-propane, methanol and chloroform [8]. Each solvent was freshly distilled prior to use. Each eluate was reduced to dryness and resuspended in 50 to 200 µl hexane.

### Characterization of hydrocarbons

Pentane fractions, including the lipid class of hydrocarbons, were subjected to thin-layer and gas chromatographic analyses.

### Thin-layer chromatography

Thin-layer silica gel plates (20 cm × 20 cm; 0.2 mm silica gel thickness; silica gel 60, Merck

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Darmstadt, F.R.G.) were developed in benzene. Bromothymol blue (0.04 g/100 ml 0.01 N NaOH) was used for staining.

#### Gas chromatography

Between 1 and 3 µl of each pentane fraction was analyzed using a Shimadzu GC-9A gas chromatograph with FID, integrator and a 25 m fused silica capillary column OV-101 (Macherey-Nagel, Düren, F.R.G.). The carrier gas was N<sub>2</sub>. The temperature program began with 160 °C for 2 min, followed by an increase up to 280 °C at a rate of 4 °C/min. The final temperature was held for 60 min.

Lipid classes, individual compounds and their chain lengths were identified by comparison with standard mixtures.

#### Gas chromatography-mass spectrometry

Results were confirmed by GC-MS-analyses (Finnigan MAT 4515), carried out by Dr. L. Witte (Institute of Organic Chemistry, University of Braunschweig, F.R.G.).

### Results and Discussion

The *n*-alkane distribution data from the surface waxes of several gymno- and angiosperms are given in Table I. The results show that the *n*-alkanes of gymnosperm pollen formed a homologous se-

ries in the range from C<sub>20</sub> to C<sub>33</sub>. The distribution patterns of *n*-alkanes from *Pinus mugo* and *Taxus baccata* pollen were characterized by a number of main components (C<sub>25</sub>–C<sub>29</sub> and C<sub>24</sub>–C<sub>27</sub>, respectively). In *Picea abies*, pentacosane and heptacosane were the main components while those of *Torreya nucifera* were penta-, hepta- und nonacosane. It is remarkable that homologues with even and odd numbers of carbons appeared in similar amounts. This distribution pattern was consistent among the *n*-alkanes of all the gymnosperm pollen investigated here.

The *n*-alkanes of angiosperm pollen comprised a homologous series ranging in chain length from C<sub>20</sub> to C<sub>33</sub> as well (Table I). The distribution patterns of the angiosperm species investigated, however, were rather different from one another. The distribution pattern of *n*-alkanes from *Narcissus pseudonarcissus* exhibited a well-defined maximum at 23 carbon atoms whereas those of *Cucurbita maxima* and *Zea mays* were each characterized by two main components; heptacosane and nonacosane, and pentacosane and heptacosane, respectively. The *n*-alkanes from the pollen of *Corylus avellana*, on the other hand, displayed quite a different distribution pattern. Homologues with even and odd numbers of carbons appeared in similar amounts, only tricosane and pentacosane predominated as main components.

Table I: Composition (% peak area) of *n*-alkanes from different pollen species.

No. of carbon atoms	Gymnosperms				Angiosperms			
	<i>Pinus mugo</i>	<i>Taxus baccata</i>	<i>Picea abies</i>	<i>Torreya nucifera</i>	<i>Cucurbita maxima</i>	<i>Corylus avellana</i>	<i>Narcissus pseudonarc.</i>	<i>Zea* mays</i> B37
20			2.2				0.3	
21			3.4				13.9	
22	3.9	8.8	5.7	9.0		2.0	9.7	
23	7.9	9.5	9.9	9.0		18.5	69.4	2
24	9.7	14.7	8.1	8.3		12.9	1.4	1
25	11.3	16.4	12.3	12.1	14.4	17.9	2.8	30
26	10.5	14.4	9.1	7.9	2.3	9.1	1.8	2
27	16.2	12.8	16.8	15.6	37.9	9.4	0.4	46
28	10.3	9.7	7.7	6.5	4.2	7.6	0.1	2
29	12.6	9.3	8.3	10.9	34.8	8.0	0.2	10
30	7.4	4.4	6.1	6.9	0.7	5.5		–
31	10.2		5.5	9.3	5.7	4.6		7
32			2.9	4.5		2.6		
33			2.0			1.9		

\* Extended according to [4].

Recently, it has been shown that pollen accumulate a complex mixture of waxes on the surface of the grains and/or within the structure of the exine [2, 4]. In addition to the other lipid classes described in the introduction, this mixture of surface waxes includes hydrocarbons. In order to elucidate the composition of *n*-alkanes in surface waxes from pollen of different taxonomic sources, the distribution patterns of the *n*-alkanes from several gymno- and angiosperm pollen were analyzed. Our results indicate that the distribution patterns of *n*-alkanes from gymnosperm pollen display little variation whereas those of angiosperm pollen exhibit pronounced differences from one another. However, considering the small number of pollen species investigated as yet, this fact implies no quality for a chemotaxonomic analysis as often shown for the patterns of epicuticular waxes of leaves and needles [9–11]. The high level of *n*-alkanes with an even number of carbons in the surface waxes of the gymnosperm pollen is note-

worthy, where hydrocarbons with even and odd numbers of carbons occurred in similar amounts. A similar distribution pattern has been described for *n*-alkanes from epicuticular waxes of gymnosperm needles [9]. In contrast to these results, *n*-alkanes with a odd number of carbons dominate in the epicuticular waxes of other sources (*i.e.* leaves of angiosperms) [11, 12].

Once again it could be shown by our studies that the outer pollen wall, the exine, is an important accumulation site of secondary plant products of different structures.

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