LIPIDS IN UNGERMINATED POLLEN OF PETUNIA

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Investigations on the lipid content of pollen are extremely scarce (reviews: LUNDÉN 1954, 1956; LINSKENS 1967; STANLEY & LINSKENS 1969) and cover mostly over-all analysis. More recently CHING & CHING (1967) found a total fatty acid content between 0.79 and 1.33% of the dry weight in pollen of some coniferous species, but no acids with uneven carbon numbers. The occurrence of fatty acid methylesters in corn pollen, which can be considered as growth substances (FUKUI c.s. 1958), has been confirmed (FATHIPOUR c.s. 1967).

As far as the reserve material is concerned, pollen is divided in two groups: fat pollen and starch pollen. *Petunia* pollen is considered to belong to the type which contains mostly lipids as reserve material. This is confirmed by electron microscopical observations (SASSEN 1964), which demonstrated a high amount of lipid droplets in ripe pollen grains.

Pollen was extracted following the method of FOLCH c.s. (1954); after grinding with quartz-sand it was hydrolyzed in methanolic KOH and fatty acid determination was done by the method of HEINEN & DE VRIES (1966). The averaged results are given in *table 1*. It is seen that more than 4% of the dry weight is present in the form of fatty acids; the majority of these are in the bound form. Phospholipids amount to 4.2% of the total weight of dry pollen.

Table 1. Lipid analysis in *Petunia* pollen. Fatty acid content in mg per gram pollen.

	total fatty acids	free fatty acids	bound fatty acids	fatty acids in triglycerides	phospholipids
number of analyses	8	6	calculated	1	7
amount in mg/g pollen	42.3	10.26	32.04	4.8	42.16

Thin layer chromatography of polar lipids (JAMES & MORRIS 1964) and neutral lipids (PARKER c.s. 1968) resulted in as many as 11 spots, most of which could be identified (*tables 2* and *3*) using color reactions and comparing Rf-values of standard substances. Kephaline, lecithine, and inositides have always been found, as well as free cholesterols and cholesterol esters.

Table 2. Chromatographic analysis of the phospholipids in ungerminated *Petunia* pollen. Solvent: chloroform-methanol-acetic acid-water, 85:15:10:4 v/v).

spot	Rf-value	molybdenum blue	Dragendorff	Ninhydrin	compound:
a	0.82				
b	0.74				
c	0.67	+		+	kephaline
d	0.41	+	+		lecithine
e	0.17	+			inositides

Table 3. Chromatographic analysis of neutral lipids in ungerminated *Petunia* pollen. Solvent: hexane-diethylether-acetic acid, 90:30:2 v/v).

spot	Rf-value	reacting with $SbCl_3$	compound:
a	0.85	+	cholesterol esters
b	0.72	+	
c	0.52		triglycerides (see *fig. 1*)
d	0.4		fatty acids (see *fig. 2*)
e	0.2	+	free cholesterol
f	0.1		unidentified

The spots of triglycerides and fatty acids were eluted from the silicagel in 10 ml chloroform, and after condensation the residue was saponified and methylated (method: VAN WIJNGAARDEN 1967). The resulting residue was redissolved in heptane and introduced into the gas chromatograph (Carlo Erba, column length 77 cm, filled with 10% apiezon on Chromosorb W, 100/120 mesh, input temperature 295°C, column temperature 240°C). The resulting vapor phase chromatogram (BURG 1962) of the free fatty acids is given in *fig. 1*, of the bound fatty acids in *fig. 2*.

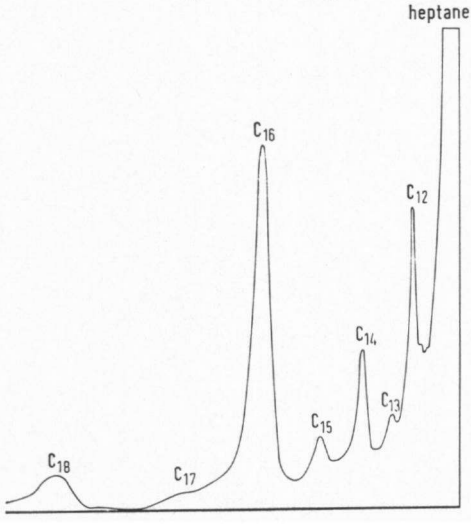

Fig. 1. Vapour phase chromatography elution diagram of the free fatty acids from ungerminated *Petunia* pollen.

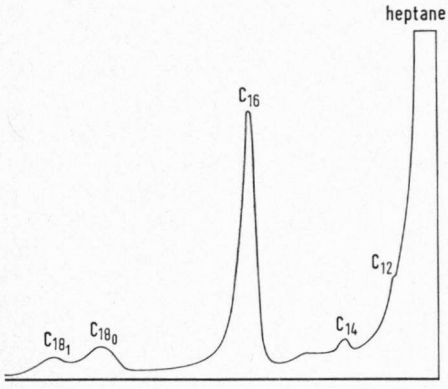

Fig. 2. Vapour phase chromatography elution diagram of the bound fatty acids in ungerminated *Petunia* pollen.

The amount of the separated acids was determined by calculating surfaces beneath the peaks and the values of various determinations are averaged. As seen from *table 4* the main free fatty acid is palmitic acid, being 42% of the

Table 4. Quantitative evaluation of the fatty acid peak areas after vapour phase chromatography (*fig. 1* and *2*). Surfaces beneath the peaks are measured by planimetry and percentages calculated.

peak	fatty acid	total content in free fatty acids	%	number of experiments	total content of bound fatty acids	%	number of experiments
C-11	undecylic acid	1.074	2.1	4	0.0	0.0	5
C-12	lauric acid	6.452	12.4	4	2.098	3.0	5
C-13	tridecanic acid	1.813	3.5	4	0.888	1.3	5
C-14	myristic acid	5.505	10.6	4	30.432	43.3	5
C-15	pentadecanic acid	2.922	5.8	4	2.118	3.0	5
C-16	palmitic acid	22.001	42.2	4	25.289	35.9	5
C-17	heptadecanic acid	4.120	7.9	4	0.210	0.3	5
$C\text{-}18_0$	stearic acid	8.053	15.5	4	3.292	4.7	5
$C\text{-}18_1$	oleic acid				6.008	8.5	5

total. Furthermore, it is striking that the acids with even numbers of C-atoms occur in higher amount than the uneven ones. That is true also for the bound fatty acids, among which myristic and palmitic acids are the most important ones.

The C-18 peak of the chromatogram of bound fatty acids was double, which means the occurrence of an acid with a double bond. The fact that other acids have no double peak is not significant for the absence of unsaturated acids, because differences in the retention time are very small.

Comparing the observation in *Petunia* pollen with earlier reported occurrences of fatty acids in other pollen species, it is noticeable that the major compounds in coniferous pollen were oleic, palmitic, and linoleic acids. Furthermore, in *Petunia* pollen we observed the occurrence of odd-numbered fatty acids, which were not found in coniferous pollen. The total amount of fatty acids in *Petunia* is 4 times that of conifers. It seems, therefore, justified to call *Petunia* pollen fat pollen.

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