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Investigations on the Chemical Composition of Pollen from some Plants

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Pollen from *Zea mays* (two samples), *Alnus glutinosa*, *Alnus incana* and *Pinus montana* has been investigated. The pollen samples were collected directly from the plants and were of a very high degree of purity. The contamination with foreign pollen was not greater than 1 %.

Pollen from *Pinus montana* contained only 13 % protein, while the other pollen species contained about 25 %. The content of water-soluble components was also low in *Pinus montana* pollen. The contents of fats, ash, carbohydrates, sulphur and phosphorus were determined. The different samples showed considerable variations in these components.

The amino acid composition was investigated qualitatively by paper chromatography and 7 amino acids were also quantitatively determined by microbiological methods. No great difference between the pollen species could be shown either qualitatively or quantitatively (the amino acid content calculated as percent of the protein content).

The vitamin contents with respect to riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin and inositol were determined. The two samples of *Zea mays* proved to be rich in pantothenic acid and inositol, whilst pollen of the *Alnus* species had a high riboflavin content and a low inositol content. Pollen from *Pinus montana* was relatively low in pyridoxine.

The chemical composition of pollen has already been the object of several investigations. A short summary of the literature in this field was recently compiled by Lundén [1]. Most of the investigations have been made in connection with studies on the importance of pollen as bee food. In most cases, pollen carried by bees has been collected in pollen traps and the material investigated has thus consisted of mixtures of pollen from various plants. Only in a few cases has pollen from a single species been subjected to investigation, the pollen having been collected directly from the plants. Since it has been pointed out (*cf. e. g.* Maurizio [2]) that there are certain differences, with respect to bee food value, between pollen collected directly from the plants and pollen collected by bees from the same plants, there must occur either certain changes in the pollen collected by the bees or a mixing of the pollen with other

substances by the bees. In an investigation on the chemical composition of pollen, it is therefore preferable to use pollen collected directly from the plants.

The methods for collecting pollen have recently been improved so that considerable amounts of pure pollen can be taken from individual plants *. In the investigations described in the following, we have used pollen from four different plants, *viz. Zea mays*, *Alnus glutinosa*, *Alnus incana* and *Pinus montana*. All the samples were collected in 1954. In addition, pollen from *Zea mays* collected in 1953 was investigated in order to determine to what extent pollen from the same plant but from different years would show variations in the chemical composition. The two *Alnus* species were selected to determine whether two closely related species show any differences in their chemical composition. The investigation thus includes examples of monocotyledons, dicotyledons and conifers. (It is generally believed that pollen from conifers has a lower bee food value [2].) The pollen samples used had been dried, immediately after collection and purification, to a moisture content of 5—7 % and were kept cool and dry. The investigations were carried out during the autumn of 1954. The samples from 1954 had thus been stored only a few months and the samples from 1953 somewhat more than a year. In the case of the 1954 samples, any changes in the composition resulting from storage must be considered improbable, at least with respect to the substances investigated here.

The presence of other pollen species in the samples was determined by Professor Gunnar Erdtman, head of the Palynological Laboratory, Bromma, Sweden, and was found to be greatest in the samples of *Zea mays*, about 1 %. In the other samples, considerably less was found, only about 0.1 %. The presence of foreign pollen is so insignificant that it cannot affect the results of the analyses.

In our investigations we have performed certain general chemical analyses, investigated the amount of amino acids present and also determined the content of certain vitamins belonging to the B group.

GENERAL CHEMICAL COMPOSITION

For each of the pollen samples, determinations were made of the content of nitrogen (protein = N × 6.25), ash (sulphate ash), reducing sugars (as glucose), carbohydrates after acid hydrolysis (calculated as glucose), water-soluble components (extraction in Soxhlet apparatus) and lipoids (ether-extractable substances, Soxhlet). In addition, sulphur and phosphorus analyses were made. The values obtained are shown in Table 1.

Pollen from *Pinus montana* shows a low nitrogen content corresponding to approximately 14 % protein while the nitrogen contents in the other pollen species correspond to about 26 % protein. The lipoid content varies considerably, *e. g. Zea mays* pollen contains 2—5 % ether-soluble components while the *Alnus* species have a higher content (almost 10 %), *Pinus* pollen occupies an intermediate position in this respect. The sum of water-soluble and ether-soluble components is low for pollen from *Pinus montana*, which may possibly

* These methods are based on an invention by Mr. G. Carlsson at AB Cernelle, Vegeholm, Sweden.

Table 1.

	Zea mays 1953	*Zea mays* 1954	*Alnus glutinosa*	*Alnus incana*	*Pinus montana*
N %	4.1	4.2	4.1	4.2	2.2
Protein % (N × 6.25)	25.6	26.3	25.6	26.2	13.8
Sulphate ash %	4.9	4.9	2.4	2.8	3.0
P %	0.58	0.75	0.42	0.28	0.30
S %	0.43	0.30	0.24	0.32	0.18
Reducing sugars (as glucose) %	10.3	7.3	8.4	5.7	2.7
Total carbohydrates (as glucose) %	35.1	34.6	27.4	22.5	29.5
Water-soluble substances %	35.9	49.7	41.2	33.3	31.9
Ether-soluble substances %	5.0	1.8	9.4	13.2	7.1

be due to the higher content of resistant membrane substances (pollenine) in pollen from conifers [3].

Both the sulphur and the phosphorus contents show considerable variations. Especially, the large difference between the sulphur contents of the two samples from *Zea mays* should be observed. *Pinus montana* pollen has a low sulphur as well as phosphorus content. Sosa-Bourdouil [4] found in general a lower phosphorus content in gymnosperm than in angiosperm pollen.

AMINO ACIDS

In three of the samples, *viz. Zea mays* 1954, *Alnus glutinosa* and *Pinus montana*, the amino acid composition was investigated using paper chromatography. The pollen samples were hydrolyzed by boiling with 5 *N* hydrochloric acid for 16 h. The results of these analyses are shown in Table 2. In the chromatographical examination of pollen hydrolyzed with NaOH, tryptophane could not be detected. However, as the presence of this amino acid was demonstrated in the quantitative microbiological determinations (see below), it has been included in the table.

Table 2.

Amino acids	*Zea mays* 1954	*Alnus glutinosa*	*Pinus montana*
Alanine	+	+	+
α-Amino-butyric acid	+	−	−
Arginine	+	+	+
Aspartic acid	+	+	+
Cystine	+	+	+
Glutamic acid	+	+	+
Glycine	+	+	+
Histidine	+	+	+
Hydroxyproline	+	−	−
*Iso*leucine	+	+	+
Leucine	+	+	+
Lysine	+	+	+
Methionine	+	+	+
Phenylalanine	+	+	+
Proline	+	+	+
Serine	+	+	+
Threonine	+	+	+
Tryptohane	+	+	+
Tyrosine	+	+	+
Valine	+	+	+

The same eighteen amino acids have been found in each of the three pollen samples. In addition, the presence of hydroxyprolin and aminobutyric acid was demonstrated in pollen from *Zea mays*. Consequently, there seems to be no great difference in the amino acid composition.

In the case of certain amino acids, quantitative determinations were made by microbiological methods (Table 3). The pollen samples were hydrolyzed by autoclaving at 120° for 7 hours with 5 *N* H_2SO_4. For the determination of tryptophane, the pollen was hydrolyzed in the same way with 5 *N* NaOH.

Table 3. Amino acid content of the pollen samples (g of amino acid per 100 g protein).

Amino Acid	*Zea mays* 1953	*Zea mays* 1954	*Alnus glutinosa*	*Alnus incana*	*Pinus montana*
Arginine	6.3	5.7	9.8	6.2	6.4
Leucine	7.6	5.6	6.0	7.1	6.5
Lysine	5.9	5.0	4.7	5.0	5.1
Methionine	1.6	1.6	1.4	1.6	1.5
Phenylalanine	2.9	2.3	2.3	3.0	2.1
Tryptophane	0.6	0.6	0.8	0.4	0.8
Tyrosine	1.9	1.9	1.7	1.9	2.1

No determinations of tyrosine have previously been made on pollen. As far as the other amino acids investigated here are concerned, two previous investigations have been carried out — one with pollen from *Zea mays* [5] and another with some bee-collected pollen species and a mixed pollen [6]. Our determinations agree well with these earlier investigations and the results indicate that no great qualitative or quantitative differences exist with regard to the amino acid contents of the pollen proteins. The amino acid content per gram of pollen is of course less in the case of *Pinus montana*, owing to the low protein content of this pollen.

VITAMINS

The following vitamins were determined: riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin and inositol. With regard to riboflavin, nicotinic and pantothenic acid, values from a number of previous investigations are available, mostly concerning pollen mixtures[7-10]. As to pyridoxine, biotin and inositol in pollen only a few investigations have been made [10,11]. In the case of inositol, no quantitative determinations have previously been carried out.

In our investigations, all determinations have been carried out by microbiological methods.

Riboflavin was determined with *Lactobacillus casei* according to Snell and Strong [12], although the test was performed with the help of the paper-disc method. Samples (5 g) were hydrolyzed by autoclaving at 120° for 60 min in 50 ml of 0.1 N HCl.

Nicotinic acid was determined with *L. arabinosus* according to Snell and Wright [13] using the paper-disc method. Samples (5 g) were hydrolyzed with 50 ml of 1 *N* HCl at 120° for 15 min. In this test the whole nicotinic acid complex is determined.

Pantothenic acid was determined with *L. arabinosus* according to Skeggs and Wright [14] using the paper-disc method. Pantothenic acid determinations present certain difficulties owing to the fact that the liberation of pantothenic acid often requires special extrac-

tion methods. From a series of experiments involving different sample treatments, it was found that boiling with water gave as high values as enzymatic extraction. Pearson [9] even states, with regard to his investigations on the pantothenic acid content of pollen, that this extraction method gives maximum values. Samples (5 g) of pollen were consequently extracted three times with 100 ml of water and the combined extracts obtained tested as described above.

Pyridoxine was determined with *Neurospora sitophila* according to Stokes *et al.*[15]. Samples (2.5 g) were hydrolyzed with 20 ml 1 *N* NaOH at 120° for 60 min. By this method the whole pyridoxine complex is determined.

Biotin was determined with *L. arabinosus* according to Lynes and Norris [16]. Samples (1 g) were hydrolyzed with 50 ml 3 *N* H_2SO_4 at 120° for 30 min according to Barton-Wright [17].

Inositol was determined partly with *Saccharomyces carlsbergensis* according to Atkin *et al.* [18] and partly with *Neurospora crassa according to Beadle* [19]. The samples were hydrolyzed by boiling with 50 ml 18 % HCl per gram of pollen at 120° for 6 h. The values obtained with both methods were well in agreement.

The results obtained have been summarized in Table 4. The values shown are the averages of 6—10 analyses.

Earlier determinations of the riboflavin content are derived from Vivino and Palmer [7], Ridi and Aboul Wafa [8] and Kitzes *et al.*[10] Ridi and Aboul Wafa investigated pollen from one single species, collected (probably directly) from the monocotyledon *Phoenix dactylifera*. They found the riboflavin content to be 6.5 μg/g, *i. e.* approximately the same as we have found for pollen from *Zea mays* and *Pinus montana*. The other two investigations [7,10] which were made on mixed pollen collected by bees, gave considerably higher values, *viz.* 16—19 μg/g pollen. In one of the reports [7], the main components of the pollen mixture are indicated. All of the plants were dicotyledons. In our investigation the two pollen samples from dicotyledons gave considerably higher values for the riboflavin content than the pollen from *Zea mays*. It is thus possible that pollen from monocotyledons contain smaller amounts of riboflavin than pollen from dicotyledons. The material studied, however, is not sufficient to serve as a basis for any general conclusions. As regards the riboflavin content in conifer pollen, no previous values are available.

The nicotinic acid content is fairly similar in the different pollen samples with exception of *Zea mays* pollen from 1953 where the nicotinic acid content is only about half that of the other samples. This low content may be due to the relatively long storage period and it is therefore intended that the analyses on the 1954 samples are to be repeated after one year in order to determine the influence of storing.

In the earlier investigations, Ridi and Aboul Wafa [8] found approximately the same values for pollen from *Phoenix dactylifera* as we have found in our pollen samples. Investigations made on pollen collected by bees [7,10] showed considerably higher values for the nicotinic acid content.

The pantothenic acid content in the two samples from *Zea mays* is about double that in the others. Values reported from other investigations show variations from 16 to 51 μg/g.

In the case of pyridoxine, only one previous investigation has been reported, apparently made on pollen collected by bees. This mixed pollen had a pyridoxine content of 9 μg/g.

The biotin content is very similar for the different pollen samples, about 0.6 μg/g. Only one determination [10] has previously been carried out and it showed that mixed pollen contained 0.25 μg/g.

Quantitative determinations of the inositol content have not previously been made. That inositol is present in pollen is indicated in an investigation by Anderson and Kulp [11] who isolated inositol from *Zea mays* pollen. This investigation showed that the content of free inositol is probably about 10 mg/g. We have determined the sum of free and bound inositol and our values for the inositol content in *Zea mays* pollen, 30 mg/g, are therefore not necessarily at variance with Anderson and Kulp's result. Pollen from *Zea mays* has a much higher inositol content than pollen from the other plants examined. Pollen from *Pinus montana* also has a fairly high content of inositol.

As the above investigations indicate, the two pollen samples from *Zea mays* are very similar except as regards their nicotinic acid contents. Also the two pollen samples from the *Alnus* species show a great deal of similarity, while the pollen from *Pinus montana* diverges from all of the others. *Zea mays* pollen is characterized by very high contents of pantothenic acid and inositol. Pollen from *Alnus* has a high riboflavin content. Finally, *Pinus montana* pollen has a considerably lower pyridoxine content than the other pollen samples. In view of its content of pantothenic acid and inositol, *Pinus montana* pollen occupies a position between pollen from *Zea mays* and pollen from *Alnus*. With regard to riboflavin, previous investigations [7,8,10] as well as our own have shown low values for monocotyledons and high values for dicotyledons. There consequently seems to exist a certain relationship between the systematic position of the plants and the vitamin content of their pollen. The number of pollen species examined, however, is too limited to permit any definit conclusions.

It is remarkable that pollen from *Pinus montana* does not in general show a lower vitamin content than the other pollen species. The only vitamin which occurs in smaller amount is pyridoxine. As far as the other vitamins are concerned, the contents are not significantly lower than the average values for the other pollen species. As Table 1 indicates, pollen from *Pinus montana* has a very low content of protein and water-soluble components. The protein content is only half that of the other pollen species. If the vitamin content is expressed as percent of the protein content, then the pyridoxine content will be about the same as for the other pollen species while the other vitamin contents will be about double. The vitamin content is also high when expressed as percent of the water-soluble components.

Table 4. Vitamins in the pollen samples (μg/g pollen dry weight).

	Zea mays 1953	*Zea mays* 1954	*Alnus glutinosa*	*Alnus incana*	*Pinus montana*
Riboflavin	5.7	6.2	11.2	12.1	5.6
Nicotinic acid	40.7	71.8	82.7	82.3	79.8
Pantothenic acid	14.2	12.7	4.2	5.0	7.8
Pyridoxine	5.9	5.5	5.7	6.8	3.1
Biotin	0.52	0.55	0.65	0.69	0.62
Inositol (mg/g)	30	30	3.0	3.5	9.0

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