TECHNICAL PAPERS

A Qualitative Analysis of Amino Acids in Pollen Collected by Bees[1]

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Pollen is collected by honeybees from a great variety of plants and is stored in cells of the brood comb, where it undergoes a lactic acid fermentation. This material provides an essential part of the diet for the larvae and adults, as it contains their only natural source of proteins and vitamins.

In an analysis of 34 species of pollen, the majority of which were bee collected, Todd and Bretherick (*5*) found the crude protein content to vary from 7.02 to 29.87%. Vivino and Palmer (*6*), using four samples of mixed pollen taken from the hive, reported the crude protein content on a fresh basis to average 20.15%, while Elser and Ganzsmüller (*3*) found the crude protein of *Pinus* spp. to be 12.5%; *Alnus* spp., 23.44%; and *Corylus* spp., 16.19%. Heyl and Hopkins (*4*) identified the amino acids arginine, histidine, lysine, and tyrosine in the pollen of ragweed (*Ambrosia artemisifolia* L.), and Anderson and Kulp (*1*) reported proline in corn pollen.

The present work comprises a qualitative analysis of the amino acids in a few pollen samples, and the information obtained should be useful in the study of the nutrition of the honeybee.

Pure samples of dandelion and willow pollen were removed from a brood comb of a colony in the spring. A sample of mixed pollen, largely composed of dandelion, willow, maple, plum, alder, and a few other plants which were unidentified was also obtained. From 0.1 to 0.2 gm of each sample was moistened with water and ground in a small mortar with powdered glass until many of the pollen grains had been crushed. The material was diluted approximately 1: 10 with 95% ethyl alcohol to precipitate the proteins, centrifuged, and the supernatant (extract) poured off. The extraction was repeated several times until the supernatant gave a negative ninhydrin test. The extracts were concentrated by evaporation almost to dryness and diluted to approximately 500 microliters with water. The free amino acids in these extracts were determined qualitatively by partition chromatography on filter paper according to the method described by Consden, Gordon, and Martin (*2*). Separation was accomplished on two-dimensional chromatograms, using 50 and 250 microliters of each extract.

The residue from the alcoholic extraction was analyzed qualitatively for the amino acids in the proteins. Half of the residue from each sample was hydrolyzed in 5 N NaOH and the remaining half in 5 N H_2SO_4. Hydrolysis was carried on for 10 hrs in small ampules in an autoclave at 120° C. The hydrolysates were then neutralized, extracted with 95% ethyl alcohol, and processed in the same manner as above. Aliquots of 50 microliters of each hydrolysate were used for the separation of the amino acids on two-dimensional chromatograms. Table 1 contains a list of the amino acids identified in the samples.

TABLE 1

Amino acids and amides	Dandelion		Willow		Pollen mixture	
	Free amino acids	Protein hydrolysate	Free amino acids	Protein hydrolysate	Free amino acids	Protein hydrolysate
Alanine	++	+	++	+	++	+
β-Alanine			+		+	
α-Amino-*n*-butyric acid			+	+	+	+
Arginine	+	+	+	+	+	+
Asparagine	+		++		+++	
Aspartic acid	+	+	+	+	+	+
Cystine		+		+	+	+
Glutamic acid		+	+	+	+	+
Glutamine	+		+		++	
Glycine	+	+	+	+	+	+
Histidine	++		+		+	
Hydroxyproline	+	+	+	+	+	+
Isoleucine and/or leucine	+	+	+	+	+	+
Lysine		+		+	+	+
Methionine	+	+	+	+	++	+
Proline	+++	+	++	+	+++	+
Serine	+	+	+	+	++	+
Threonine		+		+	+	+
Tryptophane			+		+	+
Tyrosine		+	+	+	+	+
Valine	+	+	+	+	+	+

(++) and (+++) indicate relative quantities of the compounds appearing on the same chromatogram.

The results indicate that pollen contains a large number of amino acids which varies somewhat with different pollen species. There are also variations in the same pollen species between the free amino acids and those which are constituents of the protein molecules. If the amides asparagine and glutamine are present in the proteins, they are converted to their respective amino acids during the hydrolysis and are therefore lacking in the hydrolysates. The presence of α-amino-*n*-butyric acid in two of the protein hydrolysates is not significant, since it is known that methionine may partially break down to that compound during hydrolysis. The separation of leucine from isoleucine is rather difficult when using collidine-lutidine as the second solvent; however, the evidence points strongly to the fact that both are present in the proteins of the three samples analyzed and as free amino acids in the pollen mixture. The two-dimensional

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chromatograms also contained a few unidentified spots, one of which may represent histamine, observed on the free amino acid chromatograms of the pollen mixture.

An analysis of pollen collected directly from dandelion plants gave the same results as those obtained from the dandelion collected in the beehive.

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The Formation of Monoiodotyrosine From Radioiodine in the Thyroid of Rat and Man[1]

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A further investigation of the multiplicity of radioactive substances found in filter paper chromatograms of thyroid hydrolysates (*1*) has indicated that monoiodotyrosine acts in the metabolism of iodine by the thyroid.

Ten rats were given an intravenous or intraperitoneal injection of 0.05–1 mc of carrier-free I^{131} and sacrificed from 1 min to 10 days later. The thyroids received from about 0.5 to 40,000 rep (roentgen equivalents, physical) of beta radiation, delivered at rates varying from about 30 to 10,000 rep/hr. Portions of the thyroids were hydrolyzed in sealed tubes at 100° C with 8% $Ba(OH)_2 \cdot 8H_2O$ for 6 hrs, 2N NaOH for 10 hrs, or 6N HCl for 24 hrs. The hydrochloric acid hydrolysate was evaporated as a small spot directly on a sheet of filter paper. The sodium hydroxide hydrolysate was adjusted to approximately pH 4 with 6N H_2SO_4, and a butyl alcohol extract of this solution was applied to the paper. The $Ba(OH)_2$ hydrolysate was adjusted to approximately pH 8 with 6N H_2SO_4, centrifuged, and the supernatant used for preparing the chromatogram.

Two-dimensional chromatograms were developed at 26° C with phenol as the first solvent and the upper phase from a secondary butyl alcohol, tertiary butyl alcohol, and water mixture (4, 1, and 4½ vols, respectively) as the second solvent. After the phenol run, the paper was washed with methyl alcohol except for a narrow strip below the original spot.

Radioautographs were prepared to show the exact positions of radioactive substances, the corresponding portions of the filter paper cut out for Geiger counter measurements, and the chromatograms then sprayed with ninhydrin. In each of the 40 chromatograms prepared as outlined a highly radioactive spot appeared with R_F values between those of tyrosine and diiodotyrosine. When monoiodotyrosine[2] was added either before hydrolysis or directly to the filter paper, it gave a ninhydrin spot which corresponded exactly both in *position* and *shape* with that of the above-mentioned radioactive spot.[3] The total radioactivity in the monoiodotyrosine spot ranged from about one-third to two-thirds of that shown by the adjacent diiodotyrosine spot.

A chromatogram was prepared from a $Ba(OH)_2$ hydrolysate of a thyroid biopsy specimen taken from a patient with an adenoma of the thyroid 8 days after the oral administration of 12 mc of I^{131} (*5*). It likewise showed a radioactive spot corresponding to monoiodotyrosine and containing about half the amount of radioactivity present in the diiodotyrosine spot.

As a check on the possibility that radioactive monoiodotyrosine might have been formed by exchange with, or decomposition of, other iodine-containing substances of the thyroid during the processing of the tissue, normal rat thyroids were hydrolyzed with 2N NaOH in the presence of carrier-free I^{131} or in the presence of various radioactive substances isolated from chromatograms. Radioiodide under these conditions gave a chromatogram similar to those obtained when iodide is chromatographed alone, mono- and diiodotyrosine yielded small amounts of iodide, and about 10% of the thyroxine broke down to diiodotyrosine and iodide. A mixture of two or more radioactive compounds with R_F values between those of diiodotyrosine and thyroxine yielded small amounts of iodide, monoiodotyrosine, and diiodotyrosine. The relatively slight amount of radioactivity associated with monoiodotyrosine in these control studies, in contrast to the *in vivo* findings described above, strongly indicates that the compound was present before the processing of the tissue. The possibility remains, however, that iodine-containing amino acids bound in their normal peptide linkage may be more subject to decomposition than were the free amino acids used as markers in these control experiments. This question is under investigation.

Considering the large amount of radioactivity associated with the monoiodotyrosine spot after administration of radioiodine and the reportedly low concentration of this compound in thyroglobulin (*4*), it seems probable that monoiodotyrosine attains a high specific activity. Preliminary tests indicate that by use of radioactive reagents it may be possible to determine the concentration of a number of the iodine-containing compounds on an

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[2] The authors are indebted to S. Woislawski for preparing a supply of 3-iodotyrosine according to the method of Harington (*2*) and to C. E. Dent for sharing a sample of the compound given to him by C. R. Harington.

[3] From the data available there seems little doubt that the substance in question actually is monoiodotyrosine, but it is hoped that additional evidence may be obtained by means of the electron diffraction pattern (*3*) when the installation of an electron microscope at this laboratory is completed.