STUDIES ON HONEY AND POLLEN VI. ON THE SUGAR COMPOSITION OF SEVERAL KINDS OF POLLEN

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By

Yoshie MOTOMURA, Toshiyuki WATANABE
and Kiyoshi ASO

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University Sendai Japan.

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Since a few years, we (1-5) have studied on the sugar composition of honey, nectar and nectar from the stomach of honeybees by paper partition chromatography (PPC), carbon column chromatography (Carbon CC) and Magnesol-Celite column chromatography (Mag CC), and also on pollen. As reported in Part III (3), on the pollen of cattails(*Typha latifolia L.*) analyses, PPC of sugars, fractionation and determination of sugars by Carbon CC were carried out. From these results, sucrose, glucose, fructose, rhamnose, xylose, arabinose, kojibiose, nigerose, maltose, isomaltose, turanose, leucrose, maltotriose, raffinose and four oligosaccharides were detected in the pollen of cattails.

The constituents of pollen were studied widely, Lundén (6) and Chauvin (7) published a review of the chemical composition. Ichikawa (8), Vivino (9), Sekine (10), Kubo (11) and Sakata *et al.* (12) have reported on the analyses of many kinds of pollen.

On the sugar composition of pollen, Miyake (13), Weygant (14), Ueno (15), Nilsson (16), Mizuno (17) and Togasawa *et al.* (18) have examined by PPC mainly, and detected glucose, fructose, sucrose, xylose, arabinose, rhamnose, galactose, dextrin, hemicellulose, raffinose, stachyose and galacturonic acid. Particularly, Nilsson (16) examined the pollen of pine by Carbon CC followed by PPC, and detected glucose, fructose and sucrose. Sucrose was confirmed as free sugar and crystalline acetate.

In the present report, on the pollen of pine, gold-banded lily, tiger lily, pumpkin and evening primrose, analyses (only three kinds of pine, gold-banded lily and tiger lily), PPC of sugars, determination of sugars by PPC, and frac-

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tionation of sugars in the pollen of pine by Carbon CC and determination were carried out.

Experimental

1. Analyses of pollens.

Table 1 shows the properties of five kinds of pollen.

Table 1. Source, Crop year, Productive place, Appearance of Pollen.

Source of Pollen	Botanical name	Crop year	Productive place	Color	Appearance
Pine	*Pinus Thunbergii Parl.*	1961. 5	Idohama, Miyagi Pref.	yellow	fine powder
Gold-banded lily	*Lilium auratum Lindl.*	1960. 7	Sendai Miyagi Pref.	red-orange	fine powder adhesive
Tiger lily	*Lilium lancifolium Thumb.*	1960. 8	Sendai Miyagi Pref.	red-brown	fine powder adhesive
Pumpkin	*Cucurbita moschata Duch var. Toonas Makino.*	1961. 8	Nopporo Hokkaido	yellow	fine powder adhesive
Evening primrose	*Oenothera Lamarckiana Ser.*	1960. 8	Sendai Miyagi Pref.	yellow	fine powder adhesive

The collective method was as follows. In the case of gold-banded lily, tiger lily and evening primrose, anthers were cut off from the stamen of the opening flower, dried under reduced pressure, then pollen was sweeped and collected. In the case of pumpkin, from the field of pumpkin at Nopporo in Hokkaido the opening flowers were picked up, pollen was sweeped off from the stamen and dried under reduced pressure. In the case of pine, 31.29 kg of pine cones were collected and dried in the shade indoors. The pollen was passed through a sieve of 100 mesh and 2.7 kg of pollen was obtained.

On the pollen of pine, gold-banded lily and tiger lily, analyses was carried out by the same method as previously reported (Part III) (3), as shown in Table 2.

On the pollen of pumpkin and evening primrose, analyses could not be made because of the few samples. In Table 2, as comparison analyses of pollen of cattails are shown collectively. The pollen of pine and cattails belong to anemophilous pollen, and that of gold-banded lily and tiger lily to entomophilous pollen.

To describe on the componential differency, crude fat, total invert sugar and reducing sugar were larger in the entomophilous pollen than in the

Table 2. Analyses of Pollen.

	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude fat (%)	Crude starch (%)	Total invert sugar (%)	Reducing sugar (%)
Cattails	16.00	3.70 4.40	18.90 22.50	1.16 1.38	11.31 13.46	6.00 7.14	5.61 6.68
Pine	9.12	4.46 4.91	17.01 18.72	2.50 2.75	2.46 2.71	4.72 5.19	1.91 2.10
Gold-banded lily	4.20	3.87 4.13	25.10 26.76	17.05 18.18	1.36 1.45	10.51 11.20	10.09 10.76
Tiger lily	2.68	4.11 4.22	21.00 21.58	12.26 12.60	3.57 3.65	11.46 11.77	11.31 11.62

anemophilous pollen. As shown in Table 1, in the case of entomophilous pollen, the adhesive power was remarkably large, this adhesion is due to the fatty substances which cover the surface of the pollen, because, when the surface fatty substances were removed with ether, the entomophilous pollen had no adhesion being just the same as the entomophilous pollen.

2. PPC of sugars in pollen.

To the above five kinds of pollen, ten times of water was added, and

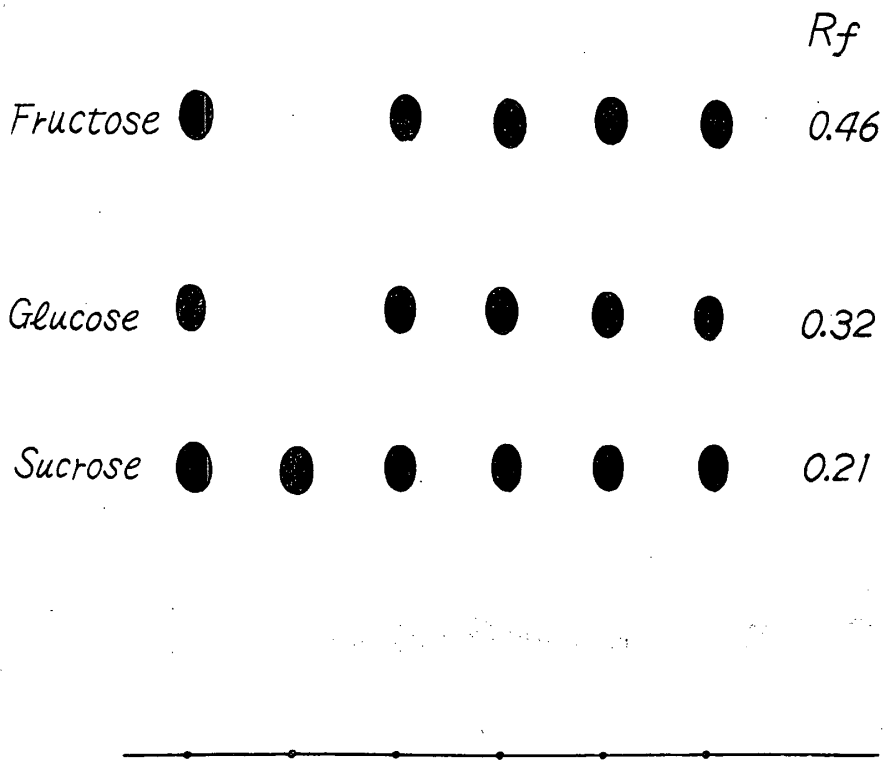

Fig. 1. PPC of sugars in pollen.

heated on the boiling water bath for 30 minutes, and filtered. The filtrate was concentrated under reduced pressure, and spotted on the Tôyo filter paper No. 2, and developed three times with the supernatant of phenol : butanol : acetic acid : water (20 : 20 : 8 : 40) and located by spraying with aniline hydrogen phthalate (AHP) as shown in Fig. 1.

From the results of PPC, in the pollen of pine only sucrose was detected and in the others glucose, fructose and sucrose as in the cattails.

The hot water extracts from the above five kinds of pollen were concentrated to syrup respectively under reduced pressure and ethanol was added to 85 per cent concentration(v/v). Precipitation was filtered off. The filtrate was concentrated under reduced pressure again, PPC of sugars was carried out and the same results were obtained as with the hot water extract.

3. Separative determination of sugars in pollen.

As the main sugars in pollen fructose, glucose and sucrose were detected, separative determination of these three sugars were carried out. To the above five kinds of pollen ten times of water was added and heated on the boiling water bath and filtered. The filtrate was concentrated to syrup under reduced pressure and ethanol was added to 85 per cent concentration. Produced precipitation was filtered off and the filtrate was concentrated under reduced pressure again and spotted on the Tôyo filter paper No. 51 and developed three times by the ascending method with the supernatant of phenol : butanol : acetic acid : water (20 : 20 : 8 : 40) as the developing solvent.

Guid strips were located with AHP and the sections corresponding to each sugar spots shown by the guid strips were cut off. These sections were eluted successively with distilled water and each samples of eluates were determined. Glucose and fructose fraction were determined by the Somogyi method, and sucrose fraction was determined by the same method after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes. Percentages of each sugars to total sugars were calculated as shown in Table 3.

Table 3. Separative determination of sugars in pollen.

	Fructose (%)	Glucose (%)	Sucrose (%)
Cattails	43.84	34.62	21.54
Pine	3.19	3.27	93.54
Gold-banded lily	25.96	23.83	50.21
Tiger lily	21.52	24.81	53.68
Pumpkin	42.21	21.11	36.68
Evening primrose	27.12	20.29	54.04

From the results of Table 3, in the pollen of pine sucrose contents was larger remarkably, in that of pumpkin and cattails it was less comparatively.

It was observed that in the pollens except tiger lily, fructose content was larger than that of glucose but in the pollen of lily both sugar contents were almost equal. In the pollen of pumpkin, fructose contents was larger than glucose remarkably (being nearly two times). The proportion of sugar content in these pollens will be different according to the time of crop or the methods of storage.

4. Fractionation of sugars in pollen of pine by Carbon CC.

From the results of PPC of sugars in the pollen of pine, the main sugar was sucrose, and small quantities of glucose and fructose were detected. Fractionation of sugars in pollen of pine by Carbon CC was carried out to examine the sugar composition carefully. 1.65 kg of pollen of pine was shaked with ether, followed by filtration and the air dried residue was extracted with 85 per cent ethanol overnight at room temperature and filtered. The filtrate was concentrated to syrup under reduced pressure. The precipitation occured by the addition of water was filtered off. The filtrate was concentrated under reduced pressure to 500 *ml* and determined. The sugar content in 500 *ml* was as follows: total invert sugar 99.60 g, reducing sugar 82.80 g.

The extract of pollen of pine was poured on a column composed of the same amount of active carbon (Takeda, 500 g) and Celite (No. 545, 500 g) and eluted with water and ethanol successively. Eluate was fractionated every 3 *l* and concentrated to 10 *ml* then examined by PPC as shown in Table 4(a).

In this case, for the admixture except sugar was contained largely in the extract. As it was difficult to detect clearly the sugar spot on PPC, the fractions over disaccharides were collected and fractionated again by Carbon CC.

From fraction No. 12 which was the end of 3 per cent ethanol fraction, to No. 47 were collected and concentrated under reduced pressure and the sugar content was determined as follows: total sugar 4.56 g (as glucose), reducing sugar 1.26 g (as glucose).

The concentrate was filled up to 250 *ml* and to except the protein, neutral lead acetate was added. After removal of the precipitation by centrifugation, the supernatant was neutralized and H_2S gas was passed through it. Precipitation was filtered off and supernatant was neutralized, concentrated to 100 *ml* and determined. The results were as follows: total sugar 2.69 g, reducing sugar 0.72 g.

This was poured on a column composed of the same amount of active carbon (Takeda, 65 g) and Celite (No. 545, 65 g) and eluted with water and ethanol successively. The eluate was fractionated every at 500 *ml* and concentrated to 10 *ml* and examined by PPC as shown in Table 4(b).

PPC was carried out as follows: after irrigating the chromatogram with pyridine : butanol : water (4 : 6 : 3), for three times, sugars were located by spraying with AHP and urea phosphate reagent.

The numbers in parentheses represent the Rf values. Analyses of sugars

Table 4. (a) Fractionation of sugars in pollen of pine by Carbon CC.

Fraction No.	Volume of effluent (*l*)	Solvent used for elution	Sugar components by PPC	Yield (g)
1-2	6	Water	Fructose, Glucose, Sucrose	81.28
3-6	12	〃	Unknown(0.99), Fructose, Glucose, Sucrose	8.63
7-10	12	Water 2-3% EtOH	Unknown (0.99), Sucrose	2.04
11	3	3% EtOH	Unknown (0.99), Fructose, Glucose, Sucrose	1.08
12-47	108	3-30% EtOH	Oligosaccharides	4.57

(b) Refractionation of oligosaccharides fraction by Carbon CC.

Fraction No.	Volume of effluent (*l*)	Solvent used for elution	Sugar components by PPC
1-13	6.5	Water 2.5-5% EtOH	Sucrose
14-15	1	5% EtOH	Turanose, Sucrose
16-18	1.5	〃	Turanose, Sucrose, Maltose, Isomaltose
19	0.5	〃	Turanose, Oligo. (0.57), Sucrose, Maltose, Oligo. (0.38), Isomaltose
20	0.5	10% EtOH	Turanose, Oligo. (0.57), Sucrose, Maltose, Oligo. (0.38), Isomaltose, Raffinose, Oligo. (0.23)
21	0.5	〃	Turanose, Oligo. (0.57), Sucrose, Maltose, Oligo. (0.38), Raffinose, Oligo. (0.23)
22-23	1	〃	Turanose, Oligo.(0.57), Sucrose, Oligo.(0.38) Raffinose, Oligo. (0.23)
24-25	1	〃	Turanose, Oligo.(0.57), Sucrose, Oligo.(0.38)
26-27	1	15% EtOH	Turanose, Oligo. (0.57), Oligo. (0.38)
28-29	1	〃	
30-33	2	15-20% EtOH	Oligo. (0.25)
34-50	8.5	20-30% EtOH	

in each fraction were carried out. In the first fractionation by Carbon CC, sucrose was detected in addition to glucose and fructose in the water fraction, each fraction was heated with 0.1 per cent HCl on the boiling water bath and neutralized with NaOH and determined by the Bertrand-Henmi method and calculated as glucose. The results are shown in Table 4(a).

In the second fractionation by Carbon CC, analyses of sugars in each fraction were not carried out. From the results of Table 4, sucrose, which was main sugar in the pollen, was eluted in 3 per cent ethanol fraction. In the first fractionation, glucose and fructose were eluted in water mainly and a little were in 3 per cent ethanol fraction.

From the result of refractionation of oligosaccharides, sucrose, turanose, isomaltose, maltose and two oligosaccharides were eluted in 5 per cent and 10

per cent ethanol fraction, raffinose and a oligosaccharide were eluted in 10 per cent ethanol fraction.

In 15 per cent ethanol fraction turanose and two oligosaccharides, in 15-20 per cent ethanol a oligosaccharide was eluted. In higher ethanol fraction no sugars was detected.

From the results of Table 4 (a) and (b), unknown substance (*Rf* 0.99), fructose, glucose, sucrose, turanose, maltose, isomaltose, raffinose and four oligosaccharides (two of them were sugars containing ketose) were detected in the pollen of pine.

5. Comparison of sugar composition of pollen of pine with cattails.

As mentioned above, in the pollen of pine fructose, glucose, sucrose, turanose, maltose, isomaltose, raffinose, unknown substance and four oligosaccharides were detected, the sugar composition of pollen of pine and cattails were compared.

In the pollen of cattails 18 kinds of sugars were detected and five of them were sugars containing ketose, on the other hand in the pollen of pine 12 kinds of sugars were detected and six of them were sugars containing ketose.

Summary

Three kinds of pollen of pine, gold-banded lily and tiger lily were analysed. From these results, crude fat, total invert sugar and reducing sugar in entomophilous pollen were larger than in anemophilous pollen.

PPC of sugars in five kinds of pollen of the above three and pumpkin and evening primrose were carried out. In the pollen of pine only sucrose was detected, and in the others fructose, glucose and sucrose were detected.

Separative determination of the above three sugars in five kinds of pollen was carried out by PPC followed by the Somogyi method. The contents of the three sugars were different by the kind of pollen.

The sugars in the pollen of pine was fractionated by Carbon CC and examined by PPC, fructose, glucose, sucrose, turanose, maltose, isomaltose, raffinose and other five sugars were detected.

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