STUDIES ON HONEY AND POLLEN III. ON THE SUGAR COMPOSITION IN THE POLLEN OF TYPHA LA TIFOLIA LINNE

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III. ON THE SUGAR COMPOSITION IN THE POLLEN OF *TYPHA LATIFOLIA LINNÉ.**

By

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Previously, we (1, 2) have reported on the sugar composition of honey. From the results, it was found that honey contains about 30 kinds of sugars and 20 of them contained ketose. It is not certain whether these sugars are originally contained in nectar from the flower or nectar from the stomach of honeybees, or produced in the beehive by the effect of temperature, or by the action of enzyme excreted by honeybees.

For the present report, we studied on the pollen of *Typha latifolia Linné*; it has yellow color and is easily collected in our country for which reason it is often sold mainly as the nutritious food of honeybees. Its chief production district is Aichi Prefecture and it is collected directly by person.

The analyses on the pollen of *Carpinus laxiflora Blume* was reported by Ichikawa (3), and the pollen of pine by Sekine (4). Vivino *et al.* (5) have reported on the general analyses and vitamin content in the pollen collected by honeybees. Kubo (6) has reported, recently, on the analyses of the pollen of an azalea plant. Miyake (7) has reported on the sugar composition of pollens. From the above results, the pollen contains glucose, fructose, sucrose, dextrin, starch and hemicellulose composed of xylose and glucose. Weygant *et al.* (8) has carried out the separative determination of glucose and fructose in pollens. Ueno (9) has detected fructose, glucose, sucrose, raffinose and stachyose in the pollen of pine by paper chromatography (PPC). Nilsson (10) has reported that when water extract of pine pollen was passed through Amberlite IR-120 and Amberlite IR-4B and fractionated by carbon-Celite column chromatography (Carbon CC), glucose, fructose and sucrose were detected by PPC. Sucrose was confirmed as free sugar and crystalline acetate. As to the tri- and

* The original Japanese report was published in the Nippon Nogei-Kagaku Kaishi **34**, 704 (1960).

tetrasaccharides, no reference was made though these sugars were detected by PPC.

When the bee takes pollen of *Typha latifolia Linné*, as food, it causes more production of eggs. It is also used as medicine, and therefore, some reports on it are based on the pharmaceutical view. In our country, for instance, Kimura (11) has isolated α-Typhasterin from it. Kuwata (12) has reported that this substance is identical with Sitosterin in the plant of high class. Hattori *et al.* (13) have also confirmed its identity with Sitosterin ($C_{29}H_{50}O$) from the mixed melting point and ultimate analysis. However, no study has hitherto been carried out on the sugar composition of pollen.

We now report on the analyses of the pollen of *Typha latifolia Linné*, PPC of sugars in pollen and on the fractionation and determination of the sugars by Carbon CC.

Experimental

I. Analyses of Pollen of *Typha latifolia Linné*.

The sample of pollen was sorted out with a 200 mesh screen. Analytical methods were as follows. Total sugar, after heating with 2.27 per cent HCl on the boiling water bath for 2.5 hours followed by neutralization with NaOH, determined by Bertrand-Henmi method (estimated as glucose); total invert sugar, after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes followed by neutralization with NaOH, determined by the Bertrand-Henmi method (estimated as invert sugar); reducing sugar, Bertrand-Henmi method (estimated as glucose). The results of analyses are shown in Table 1.

Table 1. The analyses of pollen of *Typha latifolia Linné*. (%)

Moisture	Crude ash	Crude protein	Crude fat	Crude starch	Total sugar	Total invert sugar	Reducing sugar
16.00	3.70	18.90	1.16	11.31	6.47	6.00	5.61
—	4.40	22.50	1.38	13.46	7.70	7.14	6.68

The pollen of *Typha latifolia Linné* was extracted with water and concentrated under reduced pressure. This concentrated sample was spotted on Toyo filter paper and developed with pyridine: butanol: water (4 : 6 : 3). The sugars were located by spraying aniline hydrogen phthalate and resorcinol reagent. The spots of glucose and fructose were detected.

II. Fractionation of sugars in pollen of *Typha latifolia Linné* by Carbon CC.

Two hundred grams of pollen was shaked with three portions of 1 liter of ether. The air dried residue was then extracted with 2 liter of distilled water at room temperature for six hours and filtered by suction. Lead acetate was added into the filtrate and allowed to stand overnight. After removal

of the precipitate by filtration, the filtrate was treated with H_2S to remove the residual Pb ion. The precipitated PbS was then filtered off and the filtrate was neutralized with NaOH to adjust to pH 6.0. The neutralized solution was then concentrated under reduced pressure. Ethanol was added to the concentrated solution so that the ethanol concentration became about 70 per cent, and after the whole solution was kept overnight, the supernatant was passed through a column of Amberlite IR-120 and IRA-410. The deionized solution was again concentrated to 100 *ml*. The sugar content in 100 *ml* was as follows: total invert sugar 4.37 g, reducing sugar 4.23 g.

The extract of pollen of *Typha latifolia Linné* containing 4.10 g of total invert sugar was poured on a column (400×75 mm) composed of the same amount of active carbon (Takeda 80 g) and Celite (No. 545, 80 g) and eluted with water (6 1), 2.5 per cent (2 1), 5 per cent (5 1), 10 per cent (5 1), 15 per cent (4 1), 20 per cent (3.5 1), 25 per cent (3.5 1) and 30 per cent (5 1) ethanol successively. Each eluate was concentrated to 10 *ml* and examined by PPC and paper ionophoresis (PI) as shown in Table 2.

Table 2. Fractionation and determination of the sugars in pollen of *Typha latifolia Linné*.

Fraction No.	Volume of effluent (l)	Solvent used for elution	Sugar component by PPC and PI	Yield (g)
1-2	1	Water	No sugar	—
3	0.5	〃	Fructose, Glucose	3.64
4	0.5	〃	Xylose, Arabinose, Fructose, Glucose	
5-10	3	〃	Rhamnose, Xylose, Arabinose, Fructose, Glucose	
11-12	1	〃	Rhamnose, Fructose, Glucose	
13-14	1	2.5%EtOH	Rhamnose, Fructose, Glucose	0.04
15-16	1	〃	Rhamnose, Pentose (0.44), Fructose, Glucose	
17	0.5	5%EtOH	Pentose (0.44), Glucose, Oligo. (0.18), Isomaltose	0.06
18	0.5	〃	Turanose, Sucrose, Oligo. (0.18), Isomaltose	
19	0.5	〃	Turanose, Sucrose, Leucrose, Isomaltose	
20	0.5	〃	Turanose, Sucrose, Kojibiose, Leucrose, Isomaltose	
21-24	2	〃	Turanose, Oligo. (0.21), Kojibiose, Leucrose, Isomaltose	
25	0.5	〃	Turanose, Oligo. (0.21), Kojibiose, Isomaltose	
26	0.5	〃	Oligo. (0.21), Kojibiose, Isomaltose	
27-31	2.5	10%EtOH	Nigerose, Maltose, Maltotriose, Raffinose, Oligo. (0.02)	0.02
32-33	1	〃	Nigerose, Maltose, Oligo. (0.17), Maltotriose, Raffinose, Oligo. (0.02)	
34-36	1.5	〃	Turanose, Nigerose, Maltose, Oligo. (0.17), Maltotriose, Raffinose, Oligo. (0.02)	
37-42	3	15%EtOH	Maltose, Maltotriose	0.01
43-44	1	〃		—
45-51	3.5	20%EtOH	Oligo. (0.07), Oligo. (0.02)	0.002
52-58	3.5	25%EtOH	Oligo. (0.07), Oligo. (0.02)	0.008
59-68	5	30%EtOH	Oligo. (0.07), Oligo. (0.02)	0.01

(The numbers in parentheses represent the Rf values.)

PPC and PI were carried out by the usual methods. For the clear separation of arabinose and fructose on paper chromatogram, phenol : butanol : acetic acid : water (20 : 20 : 8 : 40) was used as the developing solvent (the *Rf* value of arabinose is 0.55 and that of fructose is 0.51).

From the result of Table 2, rhamnose, xylose, arabinose, fructose, glucose, kojibiose, nigerose, maltose, isomaltose, sucrose, turanose, leucrose, maltotriose, raffinose and four oligosaccharides were detected in pollen of *Typha latifolia Linné.*

Analyses of sugars in each fraction were carried out. The effluents of water and 2.5 per cent ethanol were determined by Bertrand-Henmi method (as glucose). The effluents of 5-30 per cent ethanol were determined by the Somogyi method (as glucose) after heating with 2.27 per cent HCl on the boiling water bath for 2.5 hours followed by neutralization with NaOH.

From the result of the above experiment, the behavior of Carbon CC is shown in Table 3.

Table 3.

Solvent used for elution	Sugar component by PPC	Yield (g)	(%)
Water	Rhamnose, Xylose, Arabinose, Fructose, Glucose	3.64	96.04
2.5%EtOH	Rhamnose, Fructose, Glucose	0.04	1.06
5%EtOH	Kojibiose, Isomaltose, Sucrose, Turanose, Leucrose	0.06	1.58
10%EtOH	Nigerose, Maltose, Maltotriose, Raffinose	0.02	0.53
15%EtOH	Maltose, Maltotriose	0.01	0.26
20%EtOH	Oligosaccharides	0.002	0.05
25%EtOH	Oligosaccharides	0.008	0.21
30%EtOH	Oligosaccharides	0.01	0.26

From Table 3, the five sugars, fructose, glucose, rhamnose, xylose and arabinose, which were eluted with water and 2.5 per cent ethanol, occupied about 97 per cent of the total sugar. Kojibiose, isomaltose, sucrose, turanose, leucrose from 5 per cent ethanol is about 1.5 per cent of the total sugar; nigerose, maltose, maltotriose, raffinose from 10-15 per cent ethanol is about 1 per cent and all the oligosaccharides from 20-30 per cent ethanol is so small in amount as 0.5 per cent.

The recovery of sugars in pollen of *Typha latifolia Linné* by carbon CC is shown in Table 4.

Table 4.

Before passing through the Carbon CC	4.10g
After passing through the Carbon CC	3.79g
Recovery of sugars in pollen	92.54%

Carbon column chromatographic behavior of sugars in pollen of *Typha latifolia Linné* are shown in Fig 1.

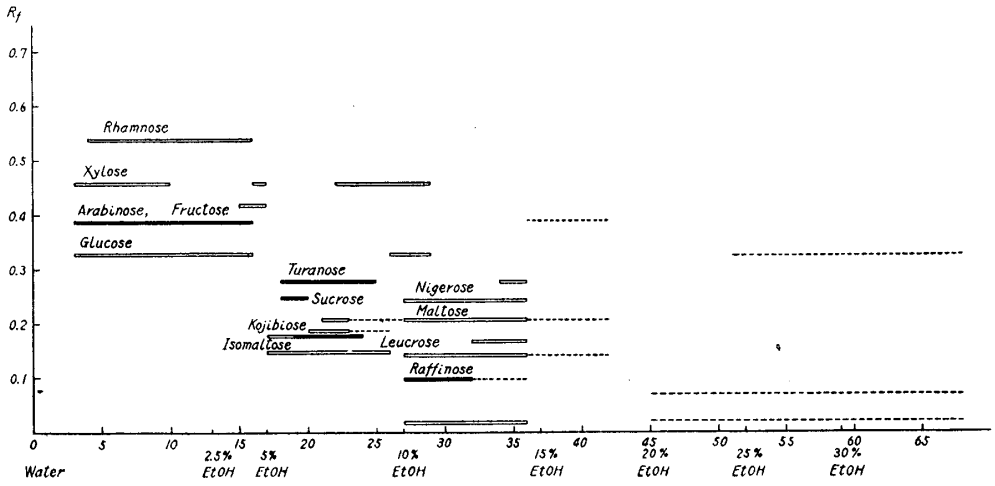

Fig 1. Carbon column chromatographic behavior of sugars in pollen of *Typha latifolia Lenné*.
═══ AHP, positive. ——— AHP, positive; Resorcin, positive.

From the results of Fig. 1, glucose, fructose, rhamnose, xylose and arabinose (monosaccharides) were eluted in water and 2.5 per cent ethanol fraction; kojibiose, isomaltose, sucrose, turanose and leucrose (disaccharides) in 5 per cent ethanol fraction; nigerose, maltose, maltotriose and raffinose (di- or trisaccharides) in 10 per cent ethanol fraction, a small amount of maltose and maltotriose in 15 per cent ethanol, and all the rest of oligosaccharides were eluted in 20-30 per cent ethanol fraction.

From the above results, these gluco-bioses were eluted in turn to 1, 6-, 1, 2-, 1, 4-, 1, 3- linkage just as the same behavior as the previous reports (2, 14).

As compared with the sugar composition of honey, the main sugar components were glucose and fructose, and four kinds of gluco-bioses (kojibiose, nigerose, maltose and isomaltose), disaccharides containing ketose (sucrose, turanose and leucrose) and raffinose are common in the pollen and honey. Rhamnose, xylose, arabinose and maltotriose were detected in pollen only. The tetra- and higher oligosaccharides fractions in honey had more kinds of sugars and its amount was larger than the sugars in the pollen of *Typha latifolia Linné*. Honey contains 30 kinds of sugars and 20 of them are of the ketose type; but the pollen contains 18 kinds of sugars and five of them contains ketose. Namely, the pollen contains less ketoses than honey.

There may be some question whether all these sugars exist originally in pollen or are produced in part by the enzymic conversion during the procedures, because the extraction in the above experiment has been carried

out only with water. Therefore, the authors consider that the extraction with alcohol must be necessary for the future studies.

Summary

1) The pollen of *Typha latifolia Linné* was analysed as follows: moisture, 16.00 per cent; crude protein, 18.90 per cent; carbohydrate, 17.78 per cent; crude fat, 1.16 per cent and crude ash, 3.70 per cent.

2) Glucose, fructose, rhamnose, xylose, arabinose, kojibiose, nigerose, maltose, isomaltose, sucrose, turanose, leucrose, maltotriose, raffinose and four oligosaccharides were detected in the pollen by PPC and PI.

3) The results of the estimation of sugars in each fraction by Carbon CC were as follows. The five sugars, glucose, fructose, rhamnose, xylose and arabinose, which occupied about 97 per cent of the total sugars were eluted with water and 2.5 per cent ethanol. Kojibiose, isomaltose, sucrose, turanose and leucrose (1.5 per cent of total sugar) were eluted with 5 per cent ethanol; nigerose, maltose, maltotriose and raffinose (1 per cent of total sugar) were eluted with 10-15 per cent ethanol ; and the oligosaccharides containing very small amount as 0.5 per cent were eluted with 20 to 30 per cent ethanol.

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