STUDIES ON HONEY AND POLLEN
IV ON THE SUGAR COMPOSITION OF NECTAR AND NECTAR FROM THE STOMACH OF HONEYBEES*

By

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In 1953, Wykes (1) has analysed the sugar composition of 12 kinds of nectars from different floral sources by paper chromatography (PPC) and by the determination methods of Somogyi and Nelson and reported that the ratio of glucose and fructose are different according to the places of floral source. Shaw (2) has classified 40 kinds of plants into three groups according to their content of nectar determined by refractometer. Namely, 12 of them contained more than 40 per cent, 16 samples 30-40 per cent and 12 samples less than 30 per cent. Bailey *et al.* (3) have determined fructose, glucose and sucrose in the nectars from satsuma orange (*Citrus nobilis var. unshiu*), white clover (*Trifolium repens L.*), red clover, alfalfa, honeysuckle, pear and cotton. They (4) have also determined the content of fructose, glucose and sucrose in the nectar from the stomach of honeybees working satsuma orange and white clover.

Since a few years, we (5, 6) have studied on the sugar composition of honey and found that honey contains about 30 kinds of sugars and 20 of them contains ketose.

To ascertain whether the nectar collected by honeybees contain so many kinds of sugars or these sugars are produced by the enzymic conversion or merely by the effect of temperature in beehive, the sugar composition of the nectar from the flower of tobacco was analysed. The nectar from tobacco is easily collected and sugar analysis of which has not been hitherto reported. The total invert and reducing sugars in the nectar from tobacco were at first determined, and then detection of sugars by PPC, separative determination by PPC, fractionation and determination by carbon-Celite column chromatography

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(Carbon CC) were carried out. And the sugar composition of the nectars from the flowers of evening primrose (*Oenothera Lamarckiana Ser.*), buddleja (*Buddleja insignis Carr.*), wild pink (*Dianthus superbus L.*), wild thyme (*Lochnera rosea Reichb.*), phlox (*Phlox paniculata L.*), and touch-me-not (*Impatiens Toxtori Miq.*), were analysed by PPC. The sugars of the nectar from the stomach of honeybees were also analysed, and detection of sugars by PPC, and separative determination by PPC were carried out.

Experimental

I. Analyses of the nectar from the flower of tobacco.

The samples of the nectar from the flower of tobacco were prepared as follows. The flowers of tobacco (*Nicotiana Tabacum L.*) 2483 g were collected on the 19th of July 1960, from the tobacco field in Ohnuki, Tajirimachi, Miyagi Prefecture, Japan, and picked off from the ear of the flower and the corolla of the flower was separated from the calyx. The nectar under the cylindrical part of the corolla (see to Fig 1) was dissolved in 80 per cent

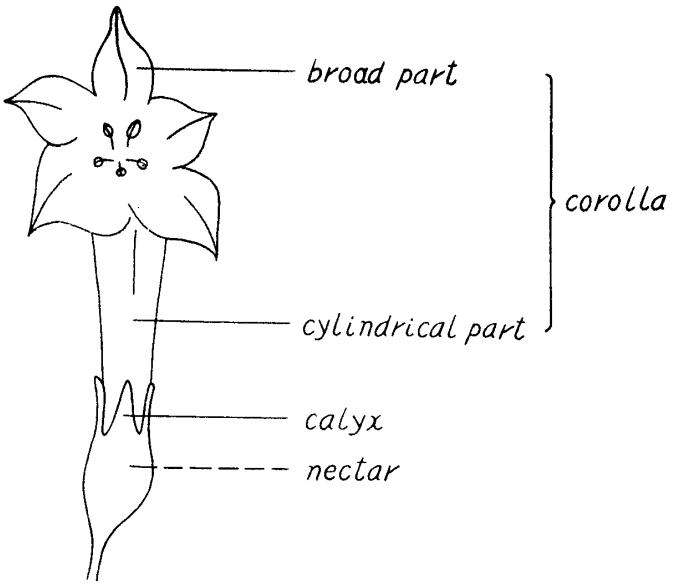

Fig 1. Flower of tobacco.

ethanol, filtered, concentrated under reduced pressure, dissolved in distilled water and filled up to 100 *ml.* The sugar content was determined as follows: total invert sugar 6.95 g/100 *ml*, reducing sugar 3.98 g/100 *ml*, sucrose 2.82 g/100 *ml.*

The methods of analyses were as follows. Total invert sugar was determined by the Somogyi method after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes followed by neutralization with NaOH and reducing sugar was determined by the Somogyi method, and both

results were estimated as glucose. Sucrose was calculated by multiplying 0.95 to the difference between the amounts of the total invert sugar and the reducing sugar.

The reducing sugar content in the nectar from tobacco was 58.5 per cent of total sugar and sucrose content was 41.5 per cent of total sugar.

II. PPC of sugars in nectar.

The samples of nectar were prepared as follows. The nectar from tobacco was dissolved in 80 per cent ethanol, concentrated under reduced pressure. The nectar from evening primrose was collected by sip with the injection syringe from the center of the flower. The flowers of buddeja, wild pink, wild thyme, touch-me-not and phlox were picked off and separated from the calyx. The nectars were collected from the cylindrical part of the flower, spotted on Toyo filter paper No. 2, and developed three times by the ascending method with pyridine: butanol: water (4:6:3) as developing solvent. The sugars were located by spraying with aniline hydrogen phthalate and resorcinol reagent. These results are shown in Table 1.

Table 1. Sugar composition of nectar by PPC

Tobacco	*Nicotiana Tabacum L.*	1960, 7 fru, glu, su
Evening primrose	*Oenothera Lamarckiana Ser.*	1960, 8 su
Buddleja	*Buddleja insignis Carr.*	1960, 8 fru, glu, su
Wild pink	*Dianthus superbus L.*	1960, 8 fru, glu, su, oligo. I, II, III
Wild thyme	*Lochnera rosea Reichb.*	1960, 8 fru, glu, su
Touch-me-not	*Impatiens Toxtori Miq.*	1960, 9 fru, glu, su
Phlox	*Phlox paniculata L.*	1960, 9 fru, glu, su

From the above results, three kinds of sugars (fructose, glucose and sucrose) were detected in the nectar from tobacco, buddleja, wild pink, touch-me-not and phlox. Only sucrose was detected in the nectar from evening primrose. And glucose, fructose, sucrose and three other oligosaccharides were detected in the nectar from wild pink. These oligosaccharides had the *Rf* value of 0.43, 0.32, 0.20 respectively and the oligosaccharide of 0.43 contained ketose.

III. Separative determination of sugars.

The separative determination of sugars in the nectars from tobacco and touch-me-not by PPC was carried out.

The nectar from tobacco was dissolved in 80 per cent ethanol, filtered, concentrated under reduced pressure. In the case of the nectar from touch-me-not, the sample were directly spotted. Sugars were developed three times by the ascending method with phenol: butanol: acetic acid: water (20:20:8:40) as the developing solvent. The sections corresponding to each sugar spot

shown by the guide strip were cut off. These sections were eluted successfully with distilled water and each sample of eluate was determined. Glucose and fructose fractions were determined by the Somogyi method, and sucrose and other oligosaccharides fractions were determined in 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 2.

Table 2. Separative determination of sugars in nectar.

	fructose (%)	glucose (%)	sucrose (%)	oligosaccharides (%)
Tobacco	28.84	28.57	42.59	
Touch-me-not	23.59	18.64	55.09	2.68

From the results, the ratio of sucrose, fructose and glucose showed the same tendency as reported by Bailey *et al.*, although their floral sources were different from each other.

In comparison with the sugar composition of nectar and honey, the amounts of glucose plus fructose in honey was over 90 per cent and sucrose content was about 4 per cent. It may be attributed to the action of enzyme excreted by honeybees in the beehive that the sucrose content of honey is very small as compared with that of nectar.

IV. Fractionation of sugars in nectar from tobacco by Carbon CC.

It was already reported that honey contains more than 30 kinds of sugars and 20 of them contains ketose. Fractionation of sugars in nectar from tobacco by Carbon CC was carried out to examine carefully whether the nectar also contains so many kinds of sugars.

The nectar from tobacco was dissolved in 80 per cent ethanol, filtered, concentrated under reduced pressure to remove ethanol, dissolved in water, filled up to 50 *ml* (contained 6.74 g/50 *ml* as invert sugar, 3.86 g/50 *ml* as reducing sugar) was poured on a column (40×5 cm) composed of the same amounts of active carbon (Takeda 70 g) and Celite (No. 545, 70 g) and eluted with water (3 l), 2.5 per cent (3 l), 5 per cent (3 l), 10 per cent (3 l), 15 per cent (3 l), 20 per cent (3 l) 25 per cent (3 l) and 30 per cent (3 l) ethanol successively. The eluates were concentrated and examined by PPC as shown in Table 3.

The number in parentheses represent the *Rf* values.

From the result of Table 3, fructose, glucose, sucrose and an unidentified oligosaccharide containing ketose were detected in the nectar from tobacco. This oligosaccharide will be studied in the future. In 20 per cent, 25 per cent and 30 per cent ethanol fractions, no sugar was detected.

Table 3. Fractionation of sugars in nectar from tobacco by Carbon CC.

Fraction No.	Volume of effluent (l)	Solvent used for elution	Sugar composition by PPC	Yield (g)	Yield (%)
1	0.5	Water	No sugar	—	—
2-5.	2	〃	Fructose, Glucose	3.69	55.78
6	0.5	〃	Fructose, Glucose, Sucrose	0.04	0.61
7	0.5	2.5%EtOH	Fructose, Glucose, Sucrose	0.17	2.57
8-12	2.5	〃	Sucrose	2.04	31.29
13-18	3	5%EtOH	Sucrose	0.63	9.52
19-20	1	10%EtOH	No sugar	—	—
21-24	2	〃	Oligo. (0.19)	0.009	0.14
25-30	3	15%EtOH	Oligo. (0.19)	0.006	0.09
31-36	3	20%EtOH	No sugar	—	—
37-42	3	25%EtOH	No sugar	—	—
43-48	3	30%EtOH	No sugar	—	—

Analyses of sugars in each fraction were carried out. The fraction eluted with water (glucose and fructose fraction) was determined directly by the Somogyi method, 2.5-15 per cent ethanol fractions (sucrose and an oligosaccharide containing ketose fraction) were determined by the Somogyi method after heating with 0.1 per cent HCl on the boiling water bath for 30 minutes followed by neutralization with NaOH. The results are shown in Table 3.

From the results of the separative determination of sugars by PPC and Carbon CC, it is observed that in the nectar from tobacco, sucrose was about a half of total sugar, glucose and fructose content were almost equal (28.6 per cent and 28.8 per cent of total sugar, respectively) and oligosaccharide content was very small.

From the above results, the sugar composition of nectar was comparatively simple, but in honey, glucose and fructose were the main sugars and many other kinds of oligosaccharides could be detected. It appears that these oligosaccharides might be converted from sucrose, glucose and fructose etc. by the action of enzymes excreted by honeybees or by the effect of temperature in the beehive.

V. Analyses of nectar from the stomach of honeybees.

The nectar was collected from the stomach of about 50 honeybees just returned to the beehive. The collected nectar was dissolved in 80 per cent ethanol, filtered, diluted with distilled water, filled up to 100 *ml.*, and determined as follows. Total invert sugar, 0.147 g /100 *ml*; reducing sugar, 0.090 g/100 *ml*; sucrose, 0.054 g/100 *ml.*

Analyses was carried out by the same method as for the nectar from tobacco. From the above results, the reducing sugar content in the nectar

from the stomach of honeybees was 62.5 per cent of total sugar and sucrose content was 37.5 per cent of total sugar. In comparison with the nectars from the flower, the reducing sugar content in the nectar from the stomach of the honeybees was about 4 per cent, and 20 per cent larger than those of the nectars from tobacco and touch-me-not respectively.

VI. PPC of sugars in nectar from stomach of honeybees.

The nectar from the stomach of honeybees was dissolved in 80 per cent ethanol, filtered and examined by PPC. Fructose, glucose, sucrose and two oligosaccharides were detected and one of the oligosaccharide contains ketose. From these *Rf* values, it appears that the oligosaccharides in nectar from the stomach of honeybees are different from that of nectar from tobacco.

VII. Separative determination of sugars in the nectar from the stomach of honeybees.

The separative determination of sugars in the nectar from the stomach of honeybees were carried out by the same method as for the nectar from tobacco. The results are as follows. Fructose 31.74 per cent, glucose 30.53 per cent, sucrose 36.02 per cent, oligosaccharides 1.71 per cent.

Bailey *et al.* (4) carried out the separative determination of sugars in the nectars from the stomach of honeybees working satsuma orange and white clover. The results are as follows. Satsuma orange: fructose 33.14 per cent, glucose 35.58 per cent, sucrose 31.28 per cent; white clover: fructose 28.86 per cent, glucose 32.67 per cent, sucrose 38.47 per cent.

As compared with the nectar from the flower, the sucrose content in the nectar from the stomach of honeybees is decreased, while glucose and fructose content is increased. It appears that sucrose might be converted to glucose and fructose by the action of enzymes excreted by honeybees during the transportation from flower to beehive.

Summary

In the nectar from tobacco (*Nicotiana Tabacum L.*), *Buddleja insignis Carr.*, *Lochnera rosea Reichb.*, *Impatiens Textori Miq.* and *Phlox paniculata L.*, glucose, fructose and sucrose were detected by PPC. Only sucrose was detected in the nectar from *Oenothera Lamarckiana Ser.* And in the nectar from *Dianthus superbus L.*, three unknown oligosaccharides were detected besides the above three known sugars. In the nectar from the stomach of honeybees, two oligosaccharides were detected besides the above three sugars.

The separative determination of sugars in the nectar from tobacco, *Impatiens Textori Miq.* and in the nectar from the stomach of honeybees were carried out by PPC.

The sugar components in the nectar from tobacco were fractionated by a Carbon CC and the sugars in each fraction were estimated. Glucose, fructose,

sucrose and a very small amount of an oligosaccharide containing ketose were detected.

As compared with the nectar from the flower, the sucrose content in the nectar from the stomach of honeybees is decreased, while glucose and fructose content is increased.

It may be attributed to the action of enzyme excreted by honeybees in the beehive that the sucrose content of honey is very small as compared with that of nectar.

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