

AN INVESTIGATION OF THE SUGARS PRESENT IN THE NECTAR OF FLOWERS OF VARIOUS SPECIES

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INTRODUCTION

Several investigations of variations in the quantity and sugar concentration of nectar secreted by the flowers of different plant species have been carried out; but, except for the work of Beutler (1930), few determinations of the constituent sugars have been made. From the flowers of many species it has been difficult to obtain adequate samples of nectar for qualitative analysis by the available chemical methods. However, application of the micro-analytical technique of paper-partition chromatography developed by Consden, Gordon & Martin (1944) has now made possible the separation and identification of sugars present in extremely small quantities of nectar.

From observations of the foraging behaviour of honeybees, Vansell (1934, 1942), Kleber (1935) and Butler (1945) concluded that the selective visits of nectar-gathering bees to various species growing in any area are influenced by both the quantity and sugar concentration of nectar present in the different flowers. Wykes (1950) also found that, when offered equal amounts of sugar solutions of the same total concentration but consisting of different sugars, honeybees exhibit decided preferences for solutions containing a mixture of sucrose, glucose and fructose. Thus it appears probable that the individual sugars present, in addition to the amount and sugar concentration, may contribute to the relative attractiveness of specific nectars to honeybees.

It was therefore decided to determine the constituent sugars present in nectar secreted by flowers of a wide range of species and, by examining samples of nectar collected at different times during the flowering period, to establish whether the individual sugars in nectar from any one species remain constant.

METHODS

The samples of nectar were collected at random from flowers of plants growing in the field by direct withdrawal in clean, fine glass capillary pipettes. Shortly after collection, the samples were introduced on sheets of Whatman no. 1 filter-paper, and qualitative determinations of the constituent sugars were carried out according to the general method developed by Partridge (1948) for one-dimensional paper-partition chromatography of sugar solutions. By a series of pilot experiments, solvent and spraying reagents suitable for the separation and identification of the principal nectar sugars were selected. The solvent chosen, the *n*-butanol-acetic acid mixture described by Partridge, gave adequate separation of the constituent sugars after irrigation of the filter-paper sheets for approximately 48 hr. The spray used for detecting the position of the sugars was modified by Bacon & Edelman (1951) from that used by Horrocks (1949) and consisted of 0.5 g. benzidine (A.R.), 10 ml. glacial acetic acid, 10 ml. 40% trichloroacetic acid, 80 ml. ethanol. After

development of the chromatograms, the unknown sugars were identified by comparison of the positions of the spots with those given by the known reference sugars introduced in a standard mixture (1 % w/v with respect to each sugar) on every chromatogram. Though there were consistent differences in size and density of the spots between samples of nectar from different species, the area of the spots developed on these chromatograms did not appear to give an accurate quantitative estimate of the amount of sugar present. Attempts to carry out such determinations by this method, suggested by Fisher, Parsons & Morrison (1948) were therefore not made in this series of analyses. However, until quantitative determinations could be carried out at a later date, these differences were not neglected entirely, but were recorded as shown in Table 3.

Table 1. *Constant sugars present in nectar samples collected at different periods from the same species*

Species	Date of sampling	Sugars identified				
		F	G	S	M	MI
<i>Epilobium angustifolium</i> L.	23. vi. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
	11. vii. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
	13. vii. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
	20. vii. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
<i>Tilia vulgaris</i> Hayne	21. ix. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
	2. x. 50	+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
		+	+	+	+	+
	4. vii. 50	+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
	7. vii. 50	+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
	11. vii. 50	+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
	13. vii. 50	+	+	+	.	+

In all tables representing the sugars identified in nectar, F = fructose, G = glucose, S = sucrose, M = maltose, MI = melibiose, R = raffinose.

RESULTS

The sugars identified in samples of nectar collected on different dates from flowers of willow-herb (*Epilobium angustifolium* L.) and lime (*Tilia vulgaris* Hayne) are given in Table 1. It may be seen that the sugars present in the nectar of both these species remained constant throughout the period of investigation.

However, some variations occurred in the sugars present in samples of nectar collected at different times from flowers of blackberry (*Rubus fruticosus* L.) and red clover (*Trifolium pratense* L.) as shown in Table 2.

It is possible that the variation in the occurrence of maltose and melibiose, shown in Table 2, may be due to the variation in the total sugar concentration of the nectar samples collected at different periods from any one species. When the total sugar concentration was low, sugars which occur in relatively low proportions may be present in quantities too small, less than 5 μ g. with certain sugars, to be detected on the chromatograms.

Table 2. *Varying sugars present in nectar samples collected at different periods from the same species*

Species	Date of sampling	Sugars identified				
		F	G	S	M	MI
<i>Rubus fruticosus</i> L.	15. vi. 50	+	+	+	+	.
	16. vi. 50	+	+	+	+	+
		+	+	+	+	+
	18. vi. 50	+	+	+	+	+
		+	+	+	+	+
	23. vi. 50	+	+	+	+	.
	4. vii. 50	+	+	+	.	.
	11. vii. 50	+	+	+	+	.
		+	+	+	+	.
	20. vii. 50	+	+	+	.	.
		+	+	+	.	.
	2. x. 50	+	+	+	.	.
		+	+	+	.	.
		+	+	+	.	.
<i>Trifolium pratense</i> L.	26. vi. 50	+	+	+	.	+
	4. vii. 50	+	+	+	.	.
		+	+	+	.	.
	25. vii. 50	+	+	+	.	.
		+	+	+	.	.
	5. viii. 50	+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
		+	+	+	.	+
	8. viii. 50	+	+	+	.	+
		+	+	+	.	+
	21. ix. 50	+	+	+	.	+
	2. x. 50	+	+	+	.	.
	6. x. 50	+	+	+	.	+

Table 3 shows the general results of analyses of several samples of nectar withdrawn from flowers of sixty species at varying stages during the flowering period.

Fructose, glucose and sucrose occurred in nectar from every species tested except crown imperial (*Fritillaria imperialis* L.). The absence of sucrose in this species confirms the earlier work of Beutler (1930). The total sugar concentration of nectar from *F. imperialis* was found to be extremely low (9.4% mean of eight determinations, 1951), and this species did not appear to be visited by nectar-gathering honeybees.

The data given in Table 3 suggest that the relative proportions of fructose, glucose and sucrose varied in nectar from different species, and that constant differences in the relative proportions of the sugars appear to be characteristic of nectar of any one species, and of certain families, e.g. Cruciferae in which the proportion of sucrose was low, and Labiatae, in certain species of which the proportion of sucrose was high.

Traces of maltose, melibiose and raffinose occurred also in nectar from flowers of some species. It should be noted, particularly for the rarer sugars, that all sugars were identified on the basis of equal R_F values only, and further identification tests were not carried out in this investigation.

Table 3. *Sugars present in samples of nectar from different species*

Family	Species	No. samples	Sugars identified					
			F	G	S	M	MI	R
Liliaceae	<i>Allium cepa</i> L.	11	++	++	++	.	.	.
	<i>Fritillaria imperialis</i> L.	15	++	++	.	.	.	.
	<i>Scilla nonscripta</i> L.	10	++	++	++	+	.	?
Amaryllidaceae	<i>Galanthus nivalis</i> L.	4	++	+	++++	.	.	.
Polygonaceae	<i>Fagopyrum esculentum</i> Moench.	10	+++	+++	++	.	.	.
Ranunculaceae	<i>Eranthis hyemalis</i> Salis.	9	++	++	+++	.	.	.
Cruciferae	<i>Brassica alba</i> Boiss.	10	+++	+++	?	?	.	.
	<i>B. arvensis</i> L.	6	+++	+++	?	.	.	.
	<i>B. napus</i> L.	6	+++	+++	?	.	.	.
	<i>B. rapa</i> L.	5	+++	+++	+	.	.	.
	<i>B. oleracea</i> L.	7	+++	+++	+	+	.	.
	<i>Cheiranthus cheiri</i> L.	6	+++	+++	+	+	.	.
	<i>Aubrietia deltoidea</i> D.C.	5	+++	+++	+	.	.	.
	<i>Raphanus raphanistrum</i> L.	8	+++	+++	?	?	.	.
Saxifragaceae	<i>Ribes nigrum</i> L.	7	++	++	++	.	.	.
	<i>R. grossularia</i> L.	7	++	+	++	.	.	.
Rosaceae	<i>Crataegus monogyna</i> Jacq.	4	++	++	++	.	.	.
	<i>Pyrus malus</i> L.	6	++	++	++	.	.	.
	<i>P. communis</i> L.	4	+++	+++	+	.	.	.
	<i>P. cydonia</i> L.	4	++	++	++	.	.	.
	<i>Mespilus germanica</i> L.	6	+++	+++	++	.	.	.
	<i>Rubus idaeus</i> L.	9	+++	+++	+	?	.	.
	<i>R. fruticosus</i> L.	13	++	++	++	?	?	.
	<i>R. loganobaccus</i> Hort. hybrid	17	+++	+++	+	?	.	.
	<i>Prunus domestica</i> L.	10	+++	+++	+	.	.	.
	<i>P. cerasus</i> L.	6	++	++	++	.	.	.
	<i>P. laurocerasus</i> L.	5	+++	+++	+	.	.	.
Leguminosae	<i>Robinia pseudacacia</i> L.	4	++	++	++	.	.	.
	<i>Medicago sativa</i> L.	6	+++	+++	++	.	.	.
	<i>Melilotus albus</i> Desr.	3	++	++	++	.	.	.
	<i>Trifolium repens</i> L.	7	++	++	++	.	.	.
	<i>T. pratense</i> L.	16	++	++	+++	.	?	.
	<i>T. ambiguum</i> Bieb.	7	++	++	++	.	?	.
	<i>Onobrychis sativa</i> Lmcf.	6	++	++	++	.	.	.
	<i>Vicia faba</i> L.	4	++	++	++	.	.	.
Tropaeolaceae	<i>Tropaeolum majus</i> L.	9	++	++	+++	?	?	?
Sapindaceae	<i>Aesculus hippocastanum</i> L.	9	+	+	+++	.	?	?
Tiliaceae	<i>Tilia vulgaris</i> Hayne	14	++	++	++	.	+	.
Onagraceae	<i>Epilobium angustifolium</i> L.	21	++	++	++	+	+	.
Araliaceae	<i>Hedera helix</i> L.	10	+++	+++	++	.	.	.
Boraginaceae	<i>Borago officinalis</i> L.	36	++	++	++	?	?	.
	<i>Anchusa italica</i> L.	6	++	++	++	+	+	.
Labiateae	<i>Lamium album</i> L.	28	++	++	+++	+	+	.
	<i>L. purpureum</i> L.	5	++	++	+++	.	.	.
	<i>Lavandula spica</i> L.	7	++	++	+++	.	.	.
	<i>Agastache foeniculum</i> Kuntze	9	++	++	++	.	+	.
	<i>Thymus vulgaris</i> L.	4	++	++	++	.	.	.
	<i>Mentha viridis</i> L.	5	++	++	+	.	.	.
	<i>Blephila ciliata</i> Rafin.	12	+	?	++	.	.	.
	<i>Pycnanthemum pilosum</i> Nutt.	8	+	?	+++	.	.	.
Scrophulariaceae	<i>Antirrhinum majus</i> L.	2	++	++	++	.	.	.
	<i>Digitalis purpurea</i> L.	6	++	++	++	.	?	.
	<i>Pentstemon barbatus</i> Torreyi	24	++	++	++	+	?	.
Hydrophyllaceae	<i>Phacelia tanacetifolia</i> Benth.	6	++	++	++	.	.	.
Caprifoliaceae	<i>Symphoricarpus albus</i> (L.) Blake	10	++	++	+++	.	?	.
	<i>Lonicera periclymenum</i> L.	9	++	++	+++	.	.	.
Compositae	<i>Helianthus annuus</i> L.	16	+++	+++	++	.	.	.
	<i>Centaurea montana</i> L.	9	++	++	+++	.	.	.
	<i>Chichorium intybus</i> L.	12	++	++	++	.	.	.
	<i>Taraxacum vulgare</i> Juss.	3	+++	+++	++	.	.	.

Key to symbols used to represent varying relative proportions of sugars:

+ sugar present in markedly low relative proportions.

++ sugar present.

+++ sugar present in markedly high relative proportions.

? sugar present in some samples, absent in others.

The recording in Table 3 of the constituents detected in nectar samples from *Pentstemon* (*Pentstemon barbatus* Torreyi) is incomplete, for in every sample examined, an additional substance of R_F value higher than that of fructose appeared on the chromatograms in a position intermediate between two reference pentose sugars, xylose and ribose. The identity of this unknown substance has not yet been determined.

DISCUSSION

The possible biological significance of the existence of sugars in nectar was discussed by von Frisch in 1934. By offering honeybees solutions of sugars and sugar derivatives including melibiose and raffinose, he found that only fructose, glucose, sucrose, maltose, trehalose, melezitose and α -methyl-glucoside were highly attractive to them; while Vogel (1931) showed that these seven substances, and also raffinose but not melibiose, were all of nutritive value to bees. At the time of these investigations, the only sugars which had been identified in nectar secreted by flowers of normal healthy plants (Beutler, 1930) were sucrose, glucose and fructose. Assuming that nectar secretion has resulted from adaptation to insect visits, von Frisch suggested that it is reasonable to suppose that sugars occurring in nectar would always be attractive to honeybees. However, as sucrose, glucose, fructose and maltose are probably the most commonly occurring plant sugars and are presumably those normally available for the process of nectar secretion, this hypothesis appears to be of little value.

In the absence of further knowledge, the possible significance of the occurrence in nectar of melibiose and raffinose, two rarer sugars, is not clear. From a survey of the results of the chromatographic determinations of the individual sugars present in nectar of all the species examined, the relative importance of the existence of raffinose and melibiose appears to be slight; for it was found that these two sugars occurred in nectar of certain species only, in small proportions and always together with fructose, glucose, sucrose and occasionally maltose.

To extend this work, quantitative determinations of nectar sugars are in progress, and it is hoped that it may later be possible to relate the sugar composition of nectar secreted by different plants with the nectar-gathering activity of honeybees in the field.

SUMMARY

Fructose, glucose and sucrose, sugars which are highly attractive to honeybees, were found by paper-partition chromatography to occur in samples of nectar from flowers of every species examined, except one. In samples of nectar from several species, maltose and in addition two substances of low R_F value which appeared to be melibiose and raffinose, sugars which are not attractive to honeybees, were also detected in relatively small quantities. The constituent sugars and their relative proportions in nectar from any one species tended to remain constant, while their occurrence appeared to be characteristic for certain families.

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