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The Sugar Content of Nectars

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Observations of the selection by honeybees of solutions of sugars which occur in nectar showed that sucrose, glucose and fructose were not equally attractive to bees, which appeared to prefer a mixture of the three sugars (Wykes, 1952*a*). Few determinations of individual sugars present in different nectars have been carried out, and relatively large volumes were necessary for the analytical methods used by Beutler (1930), Vansell (1944) and others. However, as the flowers of most plants secrete nectar sparingly, such methods cannot be applied to investigations of nectar available from a wide range of honey-producing species. By using the technique of paper partition chromatography it has been possible to carry out qualitative determinations of sugars present in nectar from many species (Wykes, 1952*b*). These analyses confirmed the earlier work of Beutler (1930), showing that sucrose, glucose and fructose, sugars readily accepted by honeybees (von Frisch, 1934), are normal constituents of nectar; they also showed that other sugars may occur, which is in agreement with the work of Täufel & Reiss (1952). During the summer of 1951 this technique was applied to quantitative investigations of nectar sugars secreted by flowers of some important British honey-producing species and others which appeared from the earlier qualitative work to secrete nectar of unusual sugar composition.

METHODS

At intervals during the flowering period of species studied, samples of nectar were gathered at random from plants in the field. Shortly after collection determinations of the sugar content of each nectar sample were carried out as follows:

(*a*) *Glucose and fructose.* Individual sugars were separated on paper chromatograms according to the general method of Partridge (1948), using modified methods adopted by Jermyn & Isherwood (1949) for quantitative analyses.

Six spots of every nectar sample, each 2-4 μ l., were introduced with a micropipette (Hawthorne, 1947) 5.5 cm. apart on a sheet of Whatman no. 1 filter paper. The solvent was the *n*-butanol-acetic acid-water mixture described by Partridge (1948). After 72-96 hr. the chromatograms were removed from the tank and air-dried. Two vertical strips, each containing the separated component sugars of the two outside spots, were cut from the sides of the papers and sprayed with either a benzidine-trichloroacetic acid reagent (Bacon & Edelman, 1951), or a *p*-anisidine hydrochloride reagent (Hough, Jones & Wadman, 1950) with a few crystals of SnCl₂ added. By reference to the two guide strips, the four areas of paper each containing one of the separated sugars (glucose and fructose) were cut from the chromatogram. The quadruplicate papers for each sugar and a corresponding paper blank were refluxed (Flood, Hirst & Jones, 1947) and the reagents of Somogyi (1945) and Nelson (1944) were used for quantitative determinations of glucose and fructose. Because of practical limitations of time and equipment, no sucrose estimations were carried out.

(*b*) *Total sugars.* Measurements of total sugars present in each sample were made with a refractometer giving sugar concentration, expressed as percentage sucrose, on a direct-reading scale. As the work of Schneller (1926), Zerban & Martin (1944) and others suggested that a close agreement exists between the refractive indices of the main constituent sugars of nectar, no corrections were applied to the readings.

RESULTS

It will be seen from Table 1 that the proportions of the constituent sugars varied markedly in nectar secreted by different species. For any one species, however, the figures for the ratio of fructose/glucose and of (total sugars)/(fructose + glucose) were reasonably constant. The greatest variation in the fructose/glucose ratio was shown in nectar from mountain mint (*Pycnanthemum pilosum* Nutt.), but for this species glucose was present in amounts which were too small to be estimated reliably by the methods used.

Table 1. *Sugar content of nectars*

Species	Date of collection	Sugar determinations				
		Glucose (G) (g./100 ml.)	Fructose (F) (g./100 ml.)	Total sugars (g./100 ml.)	Ratio: F/G	Ratio: Total/F + G
<i>Sinapis alba</i> L. (white mustard)	27. x. 51	9.2	8.3	17.1	0.90	0.98
	7. xi. 51	6.3	5.2	11.5	0.83	1.00
	10. xi. 51	5.2	4.3	9.3	0.83	0.98
		9.5	8.3	17.6	0.87	0.99
<i>Rubus fruticosus</i> L. agg. (blackberry)	3. viii. 51	13.5	14.2	45.4	1.05	1.64
	7. viii. 51	5.3	5.2	15.4	0.98	1.47
	8. viii. 51	5.9	5.8	16.8	0.98	1.43
	15. viii. 51	9.2	9.3	25.2	1.01	1.36
<i>R. loganobaccus</i> L. H. Bailey (loganberry)	3. vii. 51	10.8	10.8	21.7	1.00	1.00
	5. vii. 51	25.2	25.8	51.4	1.02	1.00
	9. vii. 51	8.3	8.3	17.0	1.00	1.02
<i>Trifolium repens</i> L. (white clover)	12. vii. 51	12.2	14.2	39.3	1.17	1.49
	18. vii. 51	9.6	11.1	32.6	1.16	1.53
	2. viii. 51	7.7	9.6	25.4	1.25	1.47
<i>T. pratense</i> L. (red clover)	23. viii. 51	2.2	8.0	29.6	3.64	2.89
	15. ix. 51	0.7	2.3	8.3	3.29	2.75
	16. x. 51	1.5	5.1	15.9	3.40	2.41
<i>Tilia × vulgaris</i> Hayne (common lime)	16. vii. 51	8.3	8.3	35.0	1.00	2.11
	19. vii. 51	9.8	10.0	42.2	1.02	2.13
	22. vii. 51	8.9	8.8	31.0	0.99	1.75
	25. vii. 51	5.3	5.4	21.0	1.02	1.96
<i>Chamaenerion angustifolium</i> (L.) Scop. (rosebay willow-herb)	23. vii. 51	5.5	7.9	20.5	1.44	1.53
	30. vii. 51	5.9	9.3	24.6	1.59	1.62
	13. viii. 51	11.3	16.3	41.4	1.44	1.50
	23. viii. 51	5.7	8.5	18.4	1.49	1.30
<i>Borago officinalis</i> L. (borage)	19. ix. 51	6.9	6.3	28.8	0.91	2.18
	20. ix. 51	6.9	6.8	27.7	0.99	2.03
	23. ix. 51	5.7	5.3	26.1	0.93	2.37
	10. x. 51	4.0	3.8	21.6	0.95	2.77
<i>Lavandula spica</i> L. (lavender)	11. viii. 51	3.2	4.4	32.4	1.38	4.26
	26. viii. 51	2.0	2.8	24.0	1.40	5.00
<i>Pycnanthemum pilosum</i> Nutt. (mountain mint)	28. ix. 51	0.6	9.4	26.4	15.67	2.64
	1. x. 51	0.3	6.8	21.9	22.67	3.07
	3. x. 51	0.4	6.4	16.81	18.00	2.55
	5. x. 51	0.2	4.6	14.01	23.01	2.80
<i>Hedera helix</i> L. (ivy)	14. x. 51	8.7	2.6	11.53	0.30	1.01
	18. x. 51	7.6	2.5	10.4	0.33	1.03

DISCUSSION

The wide variation in the ratio of fructose to glucose in nectar secreted by flowers of the different species examined is of particular interest, as Dyce (1931) and others showed that this ratio influenced the tendency of honeys to granulate. Nectar from mustard (*Sinapis alba* L.) gave one of the lowest observed ratios, and honey prepared from the nectar of this and other Crucifers is known to granulate readily. Crystallization of nectar on the receptacle of flowers of ivy (*Hedera helix* L.) has often been attributed, after Müller's observations (1878), to a richness of the secretion. However, nectar secreted by this late-flowering species is often dilute; the high glucose content shown by the present analyses may be the factor critical for rapid granulation.

The ratios of (total sugars)/(fructose + glucose) show fairly close agreement with the results of other workers who have examined nectar secreted by the same or allied species, e.g. *Tilia cordata* Mill., *Borago officinalis* L. (Beutler, 1930), *Brassica napus* L. (Beutler, 1930; Hasler & Maurizio, 1949). Considerable variation in the ratio of these sugars, however, was found in nectar from *Eucalyptus globulus* Labill. by Vansell (1941); and Bonnier (1878) had earlier stated that the proportions change with the age of the flower.

The question of whether the proportions of the three major sugars normally remain constant in nectar secreted by any one species, as is suggested by the present exploratory work, remains the subject for further investigations.

SUMMARY

1. Sugars present in nectar secreted by twelve species were separated on paper chromatograms, and quantitative determinations of the amounts of glucose and fructose were made. The total sugar content of each sample was measured with a refractometer.

2. The proportions of glucose and fructose varied greatly in nectar from different species.

3. For any one species, the proportions of the different sugars appeared to remain relatively constant.

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The Metabolism of Pyruvate in Bull Spermatozoa

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Although the metabolism of semen is considered to be predominantly glycolytic (see Mann, 1949), many studies of its respiratory metabolism have been made, some with a view to finding a correlation between respiratory activity and fertilizing capacity (Walton & Edwards, 1938; Gosh, Casida & Lardy, 1949). In such studies much attention was given to the magnitude of the endogenous respiration of washed spermatozoa, and where the metabolism of added substrates was studied conclusions regarding their fate were in most cases drawn from respiration measurements. In the present paper an investigation into the metabolism of pyruvate in bull spermatozoa is reported. It was suggested by observations made in a previous study (Terner, 1951) that in an actively synthesizing tissue, such as the lactating mammary gland, pyruvate is incompletely oxidized and that its complete oxidation is brought about by 2:4-dinitrophenol (DNP). Since the latter is known to inhibit synthetic reactions

(see Clifton, 1946, 1952) it was believed that the magnitude of the effect of DNP on the respiration of spermatozoa metabolizing pyruvate might provide a measure of their metabolic vigour based on their synthetic activity. The results of the studies now reported suggest that it may be possible to assess the quality of spermatozoa by manometric measurements under certain experimental conditions. A preliminary account of the work described in this paper has been published (Melrose & Terner, 1951, 1952).

EXPERIMENTAL

Preparation of sperm suspensions. The semen of bulls of various dairy breeds, kept for the purpose of artificial insemination, was collected by the usual short pattern artificial vagina (Walton, 1945). All ejaculates were examined immediately after collection for motility and sperm density. For the purpose of this paper no attempt was made to distinguish between grades of motility other than 'good' and 'poor'. Samples were considered to be of good motility