[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY AND THE UNIVERSITY OF ILLINOIS]

Carbohydrate Analysis as Applied to Honey

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A new, direct method of analysis of a sugar mixture containing mono, di and trisaccharides was reported in 1941 by Hurd, Liggett and Gordon.[2] This method consisted in conversion of the sugars to the propionic esters followed by vacuum distillation of the propionates under controlled conditions. Accuracy on synthetic mixtures was of the order of 1–2% for the monosaccharide component and 2–4% for the disaccharide.

It was of interest to apply this method to a natural sugar mixture such as honey. For this purpose several samples of honey of certified origin were kindly furnished by Dr. G. P. Walton of the Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

Ordinarily the monosaccharides in honey are fructose and glucose, with the former slightly in excess. To test the distillation method on this kind of mixture a "synthetic honey" was analyzed. In this mixture the ratio of fructose to glucose in the monosaccharide was 40:34. The total composition of the mixture was: monosaccharide 85.3%, sucrose 9.8%, raffinose 4.9%. Results of the analysis revealed: monosaccharide 84.5%, disaccharide 11.1%, trisaccharide 4.4%. Accuracy of this order, therefore, could be expected on the various samples of honey. It was not found possible to estimate the glucose–fructose ratio with any confidence by this distillation procedure, hence these two sugars were combined as monosaccharide in this work. In the original work on mixtures by Hurd and Liggett it was suggested that perhaps a quantitative analysis of fructose and glucose could be developed, since fructose pentapropionate distilled somewhat more readily than glucose pentapropionate, but no such quantitative accuracy has been achieved on known mixtures of fructose and glucose.

In the same year as the work of Hurd, Liggett and Gordon a method was presented by Becker and Englis[3] for the determination of fructose in the presence of glucose and sucrose. This method involved oxidation with potassium ferricyanide in the presence of sodium carbonate (to accelerate the oxidation of fructose) and disodium hydrogen phosphate (to retard the oxidation of glucose) followed by acidification and titration with ceric sulfate.

When the examination of the honey samples was planned it seemed desirable to compare the results of the analysis by the distillation procedure with those obtained by the usual chemical methods and particularly to supplement the distillation method with a method which would aid in further characterization of the components of the monosaccharide group.

So far as the carbohydrates are concerned, the usual examination of honey includes the determination of total reducing sugar (as invert sugar), a direct and invert polarization at 20°, an 87° polarization and determinations of fructose, sucrose and dextrins. Sucrose is determined by change in polarization with inversion or by increase in reducing sugar after controlled hydrolysis. The fructose may be calculated from the double temperature polarization data or determined by a selective oxidation procedure. Glucose is estimated by a subtraction of the percentage of fructose from the total reducing sugar. Dextrin is found by precipitation with alcohol. These and other determinations are of value in the identification of the type of honey and in the detection of adulteration, but it is recognized that they do not give a complete picture of the actual composition of the sample. This admission of lack of full knowledge is shown by the fact that most tables which list the components of honey ordinarily will include an undetermined fraction of a magnitude ranging from zero to seven or more per cent.

In the present paper, under the heading of "Analysis by Oxidation Methods," the usual assumption was made that glucose and fructose were the components of the reducing sugar fraction and that sucrose was the only disaccharide of significance. Because the initial interest was in the establishment of the proportion of glucose and fructose, these sugars were estimated not only by the previously mentioned method of Becker and Englis but also by the Jackson and Mathews[4] modification of the Nyns selective method.

Results are given in Table I of analyses of six different samples of honey by the propionate method (A) and by the oxidation methods (B). As will be seen, the monosaccharides show up somewhat higher in method B than in method A, whereas the disaccharides are considerably lower. Trisaccharides and higher sugars are shown to be present in appreciable amounts by method A. These differences suggest that some reducing disaccharides are present in honey and that these compounds are oxidized along with the monosaccharides in method B. Although reducing disaccharides have not been reported as generally present in honeys, the presence of maltose has

(1) Corn Products Refining Company Fellow at Northwestern University, 1941–1944.

(2) Hurd, Liggett and Gordon, THIS JOURNAL, **63**, 2656, 2657, 2659 (1941).

(3) Becker and Englis, *Ind. Eng. Chem., Anal. Ed.*, **13**, 15 (1941).

(4) Jackson and Mathews, *Bur. Standards J. Research*, **8**, 403 (1932); *J. Assoc. Offic. Agric. Chem.*, **15**, 198 (1932).

TABLE I

COMPARATIVE ANALYSES BY DISTILLATION AND OXIDATION METHODS

Honey used	No.	Method	Monosaccharide, %	Disaccharide, %	Trisaccharide, etc., %
Sweet clover	1	A	72.3	6.1	2.4
(Minnesota)		B	76.6	1.1	...
99% Heartsease	2	A	68.3	10.4	3.9
(Illinois)		B	76.0	1.0	...
Orange blossom	3	A	70.3	10.8	2.7
(California)		B	76.3	4.4	...
Buckwheat	4	A	67.4	6.6	1.8
(New York)		B	73.8	1.2	...
Tupelo	5	A	68.8	11.4	4.1
(Florida)		B	75.2	2.9	...
Cedar honeydew	6	A	56.5	15.6	9.1
(California)		B	63.2	2.7	...

been suggested. Elser[5] in 1924 inferred the presence of maltose in honeys on the basis of characteristic osazone formation. Some preliminary work on the distillation analysis of a sample of white clover honey (Illinois) was performed by Hurd and Liggett[6] and the propionate disaccharide fraction (3.8 g.) was set aside to crystallize. There was no separation of sucrose octapropionate but this substance is known to crystallize with difficulty[2] even when pure. It melts at 45–46°. Instead, a small quantity of crystals melting at 133–135° appeared. This was probably crude maltose octapropionate, because a mixed melting point determination with maltose octapropionate of m. p. 141–142° was 133–136°. Van Voorst[7] has reported small amounts of maltose in each of a series of samples which he examined. His method of analysis for this sugar was a biochemical one in which all other sugars except maltose were fermented away by a maltase-free yeast.

In Table III the fructose found by the selective oxidation method of Jackson and Mathews is consistently lower than that of Becker and Englis. Many previous tests of these methods with pure sugars and some tests with natural products have shown good agreement. The differences in results found here are correlated roughly with the quantity of undetermined material and some explanation of the divergence may be found when further study of this fraction has been made.

Experimental

Analysis by Propionylation.—Approximately 25 g. of honey was weighed with a 300-ml. flask and 175 ml. of methanol added. The flask was stoppered and permitted to stand for forty-eight hours with frequent shaking. The small quantity of precipitated dextrins and gums was separated by decantation through a small Hirsch funnel. The dextrin-free filtrate was freed of solvent on the steam-bath (vacuum), and the residual honey dried by adding 400 ml. of xylene and distilling the latter off under diminished pressure. To the dried honey was added pyridine (200 ml.), and the mixture stirred mechanically until dissolved. Propionic anhydride (125 ml.) was added, and the mixture was stirred for eighteen hours. The mixture was then poured into water and the excess anhydride permitted to hydrolyze for several hours. The sirupy propionates were then extracted into ether and the ether solution washed with water, 4 *N* hydrochloric acid, water, saturated sodium bicarbonate solution (until gas evolution ceased) and again with water. The solution was then dried over anhydrous sodium sulfate and decolorized by filtration through a bed of Norite under which was placed a thin layer of Filter-cel. The ether was removed by distillation (last traces *in vacuo*), and the sirupy propionates transferred to the weighed distillation flask. The analytical distillation was conducted following the general procedure of Hurd, Liggett and Gordon.[2] With the pressure maintained between 10^{-3} and 10^{-5} mm. the monosaccharide fraction was collected at 140–190°, the disaccharide fraction at 230–290°, and the residue was regarded as polysaccharide. Each fraction was weighed and calculated to free sugar. Duplicate runs gave good checks. Results of representative runs are summarized in Table II. The values for water in this table are taken from Table III.

TABLE II

DATA FOR HONEY ANALYSES

Numbering as in Table I	1	2	3	4	5	6
Original honey, (X)	25.47	32.76	26.16	31.41	32.11	32.70
Dextrins, g.	0.06	0.22	[a]	0.76	[a]	0.98
Dry honey + dextrins, g.	20.62	27.26	21.91	24.69	27.06	27.43
Water, g.	4.85	5.50	4.25	6.72	5.05	5.27
Dry, dextrin-free honey, (Y), g.	20.56	27.02	21.91	23.93	27.06	26.45
Propionate of monosaccharide, g.	40.75	44.33	38.61	44.63	41.87	38.33
Propionate of disaccharide, g.	3.13	6.13	5.36	3.96	6.28	9.39
Propionate of polysaccharide, g.	1.18	2.21	1.31	1.02	2.20	5.36
Monosaccharides in (Y), g.	18.40	22.32	18.35	21.16	22.08	18.45
Disaccharides in (Y), g.	1.56	3.42	2.82	2.09	3.66	5.01
Polysaccharides in (Y), g.	0.61	1.29	0.72	0.56	1.33	2.97
Monosaccharide in (X), %	72.3	63.3	70.3	67.4	68.8	56.5
Disaccharide in (X), %	6.1	10.4	10.8	6.6	11.4	15.6
Polysaccharide in (X), %	2.4	3.9	2.7	1.8	4.1	9.1
Water in (X), %	19.0	16.8	16.2	21.4	15.7	16.1
Dextrins in (X), %	0.2	0.6	...	2.4	...	3.0
	100.0	100.0	100.0	99.6	100.0	100.3

[a] Dextrins not removed.

(5) Elser, *Mitt. Lebensm. Hyg.*, **25**, 92 (1924).

(6) Ph.D. Dissertation of R. W. Liggett, Northwestern University, 1941.

(7) Van Voorst, *Chem. Weekblad*, **38**, 522 (1941); *Z. Untersuch. Lebensm.*, **83**, 414 (1942); *C. A.*, **37**, 2092, 4922 (1943).

TABLE III

ANALYSIS OF HONEYS BY OXIDATION METHODS

Honey, numbering as in Table I	1	2	3	4	5	6
Total solids by ref. index, %						
(a) Schönrock table	80.00	82.2	82.3	77.9	83.4	83.8
(b) Marvin table	81.5	83.7	84.0	79.2	85.0	85.5
Total solids by oven drying	81.0	83.2	83.8	78.6	84.3	93.9
Analysis by Jackson and Mathews method						
Fructose	38.3	40.7	38.7	35.8	43.5	28.4
Glucose	38.6	35.2	37.2	37.3	31.5	36.1
Total reducing sugars	77.0	75.9	75.9	73.1	75.0	64.5
Analysis by Becker and Englis method						
Fructose	40.7	45.2	41.3	40.7	49.2	33.5
Glucose	35.9	30.8	35.0	33.1	26.0	29.7
Total reducing sugars	76.6	76.0	76.3	73.8	75.2	63.2
Sucrose	1.1	1.0	4.4	1.2	2.9	2.7
Dextrins	..	2.8	..	..	..	..
Undet. matter	4.4	6.3	3.1	3.6	6.2	18.1

The quantity of dextrin and gum precipitated by methanol was small but its removal was helpful for smooth distillation. With proper care, however, reliable analytical results were obtainable without this step, as in runs 3 and 5.

Analysis by Oxidation Methods.—With the exception of the previously mentioned method for fructose by Becker and Englis,[3] the procedures used are described in detail in the handbook of the A. O. A. C.[8] The total solids were estimated by the oven-drying procedure (*in vacuo* at 70°) after dispersing the sample on quartz sand in an aluminum dish. As a check on the drying method, the refractive value of each sample was taken and the apparent solids found from the sucrose table of Schönrock and a special table for honey proposed by Marvin.[9] This table gives results in better accord with the drying method.

Total reducing sugars were estimated by the method of Lane and Eynon.[10] Sucrose was found from the increase in reducing sugar after hydrolysis with hydrochloric acid at room temperature.

These results are presented in Table III.

(8) "Methods of Analysis," A. O. A. C., 5th ed., 1940.

Summary

Six samples of honey have been analyzed by a distillation method and by oxidation methods. The distillation method gives lower values for the monosaccharides and higher values for the disaccharides than are shown by the oxidation methods when it is assumed that sucrose is the only disaccharide present. The presence of maltose or some other reducing disaccharide is indicated as a general component of the several samples. Some higher saccharides are also indicated. The suggestion is made that the variance in results obtained by two selective oxidation methods for fructose is associated with the reducing disaccharides which are present in honey. The distillation method is a valuable technique for checking and pointing out deficiencies in other processes for carbohydrate estimation.

(9) G. E. Marvin, *American Bee J.*, 212 (1934).

(10) Lane and Eynon, *J. Soc. Chem. Ind.*, **42**, 32T (1923).

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Nitration of 2,3-Dimethylbutane[1]

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The successful vapor-phase nitration of ethane, propane and the two butanes was accomplished by Hass, Hodge and Vanderbilt.[2] The nitrations of *n*-pentane and isopentane were reported by Hass and Patterson[3] and Seigle and Hass,[4] respectively. In every case these investigators found the mono nitration products expected as predicted by the activated complex theory of nitration.[5]

The vapor-phase nitration procedure is now being expanded to the hexanes. This paper deals specifically with the nitration of 2,3-dimethylbutane. This hydrocarbon is of interest not only because it serves as further evidence of the generalizations previously reported regarding the identity of nitroparaffins obtained under these conditions but also because it possesses two tertiary hydrogen atoms. It is important to know whether these tertiary hydrogen atoms are replaced in the nitration process to form the corresponding dinitroparaffin. This compound can be reduced to 2,3-dimethyl-2,3-butanediamine, an interesting synthetic intermediate.

Konovalov[6] has reported the nitration of 2,3-dimethylbutane with nitric acid at 125°[7] to give 2,3-dimethyl-2-nitrobutane and 2,3-dimethyl-2,3-dinitrobutane, the latter is a solid melting at 208°.

Five compounds are to be expected when this hydrocarbon is subjected to vapor-phase nitra-

(1) This article contains material from the doctoral thesis of M. H. Danzig.

(2) H. B. Hass, E. B. Hodge and B. M. Vanderbilt, *Ind. Eng. Chem.*, **28**, 339 (1936).

(3) H. B. Hass and J. A. Patterson, *ibid.*, **30**, 67 (1938).

(4) L. W. Seigle and H. B. Hass, *ibid.*, **31**, 648 (1939).

(5) H. B. Hass and E. F. Riley, *Chem. Rev.*, **32**, 373 (1943).

(6) M. Konovalov, *J. Russ. Phys.-Chem. Soc.*, **37**, 1119–1125 (1905).

(7) The statement in Brooks' "Chemistry of the Non-Benzenoid Hydrocarbons," p. 61, and Ellis' "Chemistry of Petroleum Derivatives," Vol. I, p. 1043, that "diisopropyl reacts readily with nitric acid at 20°" cannot be found in the reference given[8] (M. Konovalov, *J. Russ. Phys.-Chem. Soc.*, **37**, 1119–1125 (1905)). It does occur, however, in an article by Markownikoff (W. B. Markownikoff, *Chem. Zentr.*, **70**, I, 1064 (1899); *Ber.*, **32**, 1441–1445 (1899)), who used fuming nitric acid (specific gravity 1.53). Experiments in this laboratory have shown that while 2,3-dimethylbutane seems to be unattacked by ordinary nitric acid at room temperature, a vigorous exothermic action is observed when fuming nitric acid is employed under these conditions.

(8) I. M. Heilbron, *Dictionary of Organic Compounds*, Vol. 1, p. 570, Oxford University Press, 1934.