STUDIES ON HONEY II. ISOLATION OF KOJIBIOSE, NIGEROSE, MALTOSE AND ISOMALTOSE FROM HONEY

STUDIES ON HONEY

II. ISOLATION OF KOJIBIOSE, NIGEROSE, MALTOSE AND ISOMALTOSE FROM HONEY

By

Toshiyuki WATANABE and Kiyoshi ASO

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received January 25, 1960*)

Previously, we reported (1) that 15 honey samples produced mainly in the Tohoku region of Japan were analysed and the sugar components of one kind of honey (*Lespedeza bicolor*) were fractionated by a carbon-Celite column chromatography and that the sugars in each fraction were estimated. Glucose, fructose, sucrose, maltose, melezitose, eight di- or tri-saccharides and nine higher oligosaccharides were detected by paper chromatography and 15 spots of them contained ketose.

On the other hand, White *et al.* (2) isolated nigerose from honey and Malyoth (3), Vavruch (4), White *et al.* (5), Goldschmidt *et al.* (6), Galanti *et al.* (7) detected maltose in honey by paper chromatography and Goldschmidt *et al.* (6) detected isomaltose in honey by paper chromatography. Recently, White *et al.* (8) isolated nigerose, maltose, isomaltose, sucrose, turanose and maltulose from honey and identified them by their infrared spectras. But m.p. and $[\alpha]_D$ were not described.

We now report on the isolation and identification of kojibiose, nigerose, maltose and isomaltose from honey as crystalline octaacetates by carbon-Celite column and Magnesol-Celite column chromatographic procedures.

Experimental

I. Fractionation of sugars in honey by carbon-Celite column chromatography.

Three hundred g of honey (*Lespedeza bicolor*) was dissolved in 2 *l* of distilled water and neutralized with NaOH, the pH adjusted to 6.2. It was poured on a column (550×125 mm) composed of the same amount of carbon

The original Japanese report was published in the Nippon Nogei-Kagaku Kaishi, 33, 1054 (1959).

(Takeda, 800 g) and Celite (No. 545, 800g) and eluted with water (22 *l*), 2.5% (22 *l*), 5% (14 *l*), 10% (18 *l*), 15% (18 *l*), 20% (14 *l*), 25% (12 *l*) and 30% (30 *l*) ethanol successively. The eluates were concentrated and examined by paper chromatography as shown in Fig. 1.

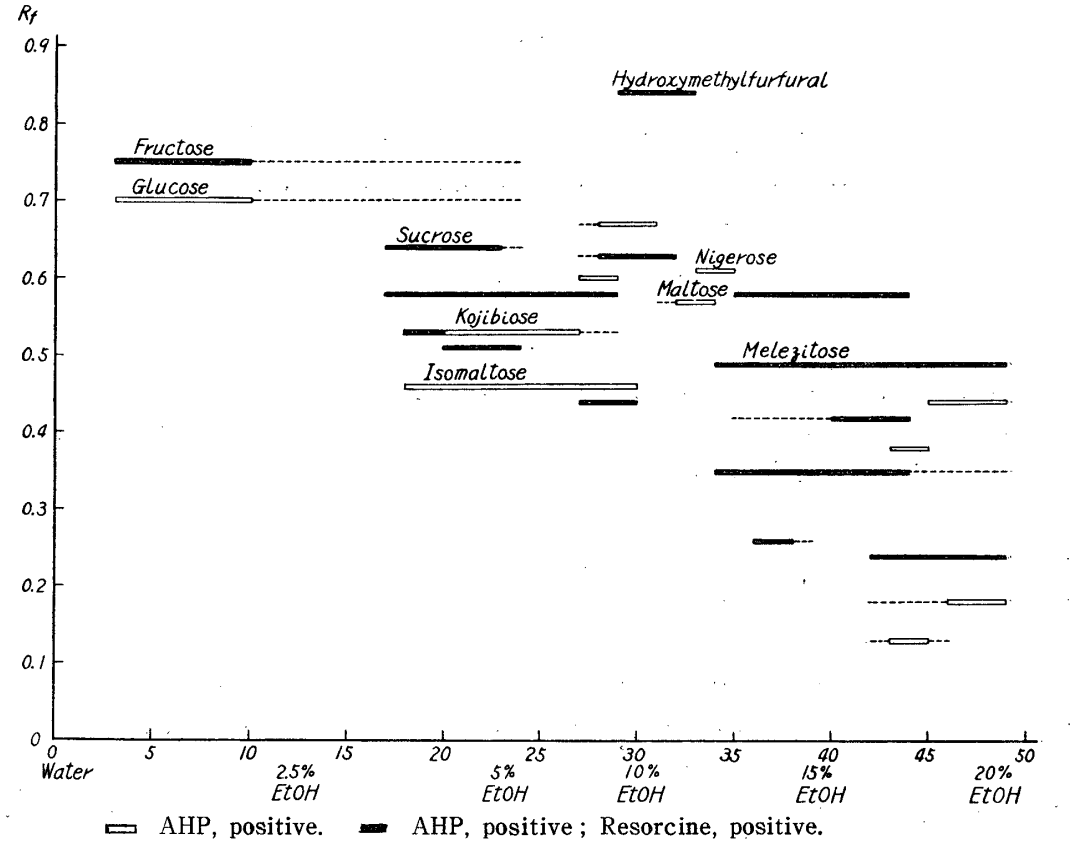

Fig 1. Carbon column chromatographic behavior of honey (*Lespedeza bicolor*).

Mixed chromatograms were run with the test sample (honey) and known sugars using pyridine : butanol : water (4 : 6 : 3) as the developing solvent (triple ascending development). The sugars were located by spraying with aniline hydrogen phthalate and a resorcine reagent. The number in parentheses represents the Rf values. Twenty-six spots corresponding to glucose, fructose, kojibiose, nigerose, maltose, isomaltose, sucrose, melezitose and 16 oligosaccharides were detected on the paper chromatogram of sugars in honey fractionated by a carbon-Celite column chromatography. Sixteen spots of them contained ketose. The water fractions contained glucose and fructose, and the 2.5–5 per cent ethanol fractions were composed of kojibiose, isomaltose and sucrose, 10 per cent ethanol fractions contained nigerose and maltose. Melezitose appeared in the 10–20 per cent ethanol fractions. These glucobioses were eluted in turn to 1, 6–, 1, 2–, 1, 4–, 1, 3– linkage just as in the same behavior as reported on Rice-koji (9), Hydrol (10) and Mirin (Sweet saké) (11).

II. Paper ionophoresis (12) of the sugars in honey.

The solution of glucose, isomaltose, leucrose, kojibiose, trehalose and 2, 3, 4, 6-tetramethyl glucose were spotted as the control. The water fractions and 2.5–5 per cent ethanol fractions (kojibiose-isomaltose fraction) of honey were spotted on filter paper. This filter paper was wetted by a borate buffer solution. The buffer solution consists of 0.05N borax and 0.1N NaOH solution (6 : 4) and its pH is 9.8. The 600V.D.C. of constant voltage is charged. This ionophoresis requires about three hours and the electric currents are 20 to 30 mA. After the ionophoresis was finished, the filter paper was dried at 60°C. As the spraying reagents, $KMnO_4$–$AgNO_3$ reagent was used. The results are shown in Fig. 2.

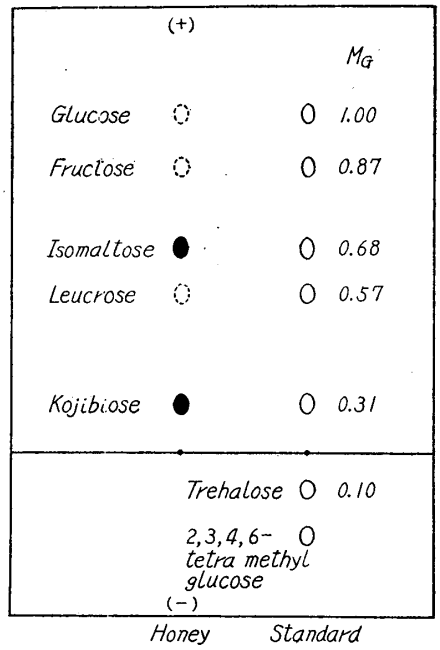

Fig 2. Paper ionogram of kojibiose-isomaltose fraction of honey.

Five spots corresponding to glucose, fructose, isomaltose, kojibiose and leucrose were detected on the paper ionophoresis of the sugars in honey (kojibiose-isomaltose fraction). But trehalose was not detected.

III. Fractionation of kojibiose-isomaltose fraction by carbon-Celite column chromatography in the presence of borate buffer (pH 10.0) (13).

The effluent portions (Fr. 21–28, 2.5–5 per cent ethanol fractions), containing kojibiose and isomaltose from three columns (900g of honey), were combined (18.5 g of dried material). The portion was contaminated with considerable amounts of two oligosaccharides containing ketose, glucose, fructose, sucrose and a trace of leucrose.

The sugar mixture was then rechromatographed on carbon (250 g)-Celite (250 g) column (530×80 mm) using the gradient elution method with 0–3.0 per cent aqueous ethanol containing borate buffer (pH 10.0). The eluates were passed through a column of Amberlite IR-120 (H^+ form). After removal of the borate ion from the sugar complex by repeated distillation with methanol, the sugar solution was concentrated under reduced pressure and examined by paper chromatography. The results are shown in Table 1.

After irrigating the chromatogram with pyridine : butanol : water (4 : 6 : 3), the sugars were located by spraying with aniline hydrogen phthalate and resorcine reagent. The number in parentheses represents the Rf values.

The 0 per cent ethanol fractions contained glucose and fructose, and 0.5–1.5 per cent ethanol fractions were composed of isomaltose and several

sugars containing ketose. Kojibiose appeared in the 2.0–3.0 per cent ethanol fractions. Leucrose (14) was detected in honey by mixed paper chromatography with a known sugar kindly supplied by Dr. F. H. Stodola.

Table 1. Fractionation of kojibiose-isomaltose fraction by carbon-Celite column chromatography in the presence of borate buffer (pH 10.0).

Fraction No.	Volume of effluent (*l*)	Solvent used for elution		Sugar components by PPC	Yield* (g)
1- 2	1	Borate buffer	EtOH 0%	No sugar	...
3- 6	2	〃	〃	Fructose, Glucose	
7- 8	1	〃	〃	HMF, Fructose, Glucose	1.5
9-10	1	〃	0.5%	HMF, Fructose, Glucose	
11	0.5	〃	〃	Fructose, Glucose	
12	0.5	〃	〃	Fructose, Glucose, Oligo. (0.24), Oligo. (0.20), Isomaltose	0.3
13-16	2	〃	〃	Oligo. (0.24), Oligo. (0.20), Isomaltose	7.2
17-20	2	〃	1.0%	Oligo. (0.24), Oligo. (0.20), Isomaltose	
21-24	2	〃	〃	Oligo. (0.24), Isomaltose	3.5
25-30	3	〃	1.5%	Oligo. (0.24), Isomaltose	
31-32	1	〃	〃	Oligo. (0.36), Oligo. (0.30), Leucrose, Isomaltose	1.2
33-35	1.5	〃	2.0%	Oligo. (0.36), Oligo. (0.30), Leucrose, Isomaltose	
36-40	2.5	〃	〃	Oligo. (0.36), Oligo. (0.30), Isomaltose	0.8
41-45	2.5	〃	〃	Oligo. (0.36), Sucrose, Kojibiose	0.6
46-52	3.5	〃	〃	Sucrose, Kojibiose	1.9
53-60	4	〃	2.5%	Sucrose, Kojibiose	1.3
61-65	2.5	〃	〃	Kojibiose	
66	0.5	〃	〃	Kojibiose, Oligo. (0.15)	
67-68	1	〃	3.0%	Kojibiose, Oligo. (0.15)	0.2
69-74	3	〃	〃	Kojibiose	

* An amorphous dry substance was weighed.

IV. Isolation of Kojibiose.

The fractions containing kojibiose (Fr. 51–63) were united and concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 1.3 g white amorphous powder. This powder of 1.3 g was acetylated with 0.7 g of sodium acetate and 6.5 *ml* of acetic anhydride at 100–110°C for two hours and then poured into 150 *ml* of water and after one day it was extracted with chloroform. The chloroform layer was dried and evaporated to give 1.9 g of syrup, which on addition of hot ethanol, was left to stand overnight at room temperature. But,

since direct crystallization of the acetylated sugar was not successful, Magnesol-Celite column chromatography of the acetate was carried out.

One and a ninth g of this crude acetate was dissolved in 10 *ml* of benzene and poured on a column (300×40 mm) of Magnesol-Celite (5 : 1) and developed with 1000 *ml* of benzene : t-butanol (100 : 1, by volume). A zone that appeared at 165–217 mm from the top of the column by means of potassium permanganate streak indicator was sectioned from the column and eluted with acetone. Removal of the solvent left 0.5 g of syrup, from which 208 mg of fine prisms were obtained upon crystallization from ethanol. After recrystallization, it had m.p. 117–118°C, $[\alpha]_{18}^{D}$ + 113.8 (c, 1.2; chloroform).

The upper portion (10–165 mm) of the column was eluted with acetone, and removal of the solvent left 0.7 g of syrup. This syrup was rechromatographed on a Magnesol-Celite column. A zone that appeared at the bottom (140–185 mm from the top) of the column by means of potassium permanganate streak indicator was sectioned from the column and eluted with acetone. Removal of the solvent left 0.1 g of syrup, from which 11 mg of fine prisms were obtained upon crystallization from ethanol. It had m.p. 166°C. Neither of the acetates showed depression of melting points on admixture with α- and β-kojibiose octaacetate (166°C and 117°C) synthesized chemically by Matsuda (15).

V. Isolation of Isomaltose.

The fractions containing isomaltose (Fr. 13–20) were concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 7.2 g of white amorphous powder. This powder of 2.1 g was acetylated with 1.2 g of sodium acetate and 10.5 *ml* of acetic anhydride at 100–110°C for two hours, poured into 300 *ml* of water and after one day it was extracted with chloroform. The chloroform layer was dried and evaporated to give 3.1 g of syrup, which on addition of hot ethanol, was left to stand overnight at room temperature. But, since direct crystallization of the acetylated sugar was not successful, Magnesol-Celite column chromatography of the acetate was carried out.

One and a half g of this crude acetate was dissolved in 10 *ml* of benzene and poured on a column (300×40 mm) of Magnesol-Celite (5 : 1) and developed with 1000 *ml* of benzene : t-butanol (100 : 1, by volume). Three zones were located on the extruded column by alkaline potassium permanganate. The upper zone is 58–93 mm and the middle zone is 122–174 mm and the lower zone is 174–205 mm from the top. Each zone was sectioned and eluted with acetone and evaporated. From the upper zone, 0.15 g of syrup was obtained, but crystallization of this acetate is not successful. From the middle zone, removal of the solvent left 0.3 g of syrup, from which 37 mg of fine prisms were

obtained upon crystallization from ethanol. After recrystallization, it had m.p. 144–145°C undepressed on admixture with authentic β-isomaltose octaacetate. From the lower zone, 0.1 g of syrup was obtained but crystallization of this acetate was not successful. The effluent from the column was evaporated. 0.4 g of syrupy acetate was obtained which was crystallized from ethanol, yield 138 mg. After recrystallization, it had m.p. 145–146°C, $[\alpha]_D^{19}$ + 97.8 (c, 1.6; chloroform) unchanged on admixture with known β-isomaltose octaacetate.

VI. Isolation of Nigerose and Maltose.

The fractions containing nigerose and maltose (Fr. 32–35) were concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 5.0 g of white amorphous powder. This white powder of 2.5 g was acetylated with 1.5 g of sodium acetate and 12.5 *ml* of acetic anhydride at 100–110°C for two hours, poured into 300 *ml* of water and after one day, it was extracted with chloroform, the chloroform layer was dried and evaporated to give 3.7 g of syrup, which on addition of hot ethanol, was left to stand overnight at room temperature. But, since direct crystallization of the acetylated sugar was not successful, Magnesol-Celite column chromatography was carried out.

One and a seventh g of this crude acetate was dissolved in 10 *ml* of benzene and poured on a column (300×40 mm) of Magnesol-Celite (5 : 1) and developed with 1000 *ml* of benzene : t-butanol (100 : 1, by volume). Three zones were located on the extruded column by alkaline potassium permanganate. The upper zone is 25–70 mm and the middle zone is 70–128 mm and the lower zone is 210–223 mm from the top. Each zone was sectioned and eluted with acetone and evaporated. From the upper zone, 0.2 g of syrup was obtained which was crystallized from ethanol, yield 3 mg. It had m.p. 185–187°C. This acetate was not identified.

From the middle zone, removal of the solvent left 0.4 g of syrup, from which 107 mg of fine prisms were obtained upon crystallization from ethanol. After several recrystallizations, it had m.p. 150°C, undepressed on admixture with authentic β-nigerose octaacetate. From the lower zone, 0.1 g of syrup was obtained but crystallization of this acetate was not successful. The effluent from the column was evaporated. 0.7 g of syrupy acetate was obtained which was crystallized from ethanol, yield 375 mg. After recrystallization, it had m.p. 159–160°C unchanged on admixture with authentic β-maltose octaacetate.

Summary

Honey (*Lespedeza bicolor*) was fractionated by a carbon-Celite column

using water and 2.5–30 per cent ethanol as successive elution solvents. The effluent portions containing kojibiose and isomaltose (2.5–5.0 per cent ethanol fraction) were then rechromatographed on a carbon-Celite column using the gradient elution method with 0–3.0 per cent aqueous ethanol containing borate buffer (pH 10.0). Kojibiose fraction (2.0–3.0 per cent ethanol) and isomaltose fraction (0.5–1.0 per cent ethanol) were acetylated as usual. These sugars were isolated as their crystalline octaacetates by Magnesol-Celite column chromatography. Nigerose and maltose were also isolated as their crystalline octaacetates by a carbon-Celite and Magnesol-Celite column chromatographic procedures. Leucrose was detected in honey by paper chromatography.

Acknowledgement

The authors gratefully acknowledge for the gift of leucrose from Dr. F. H. Stodola, Northern Utilization Research Branch, U.S.A., and wish to acknowledge their indebtedness to Dr. K. Matsuda for his kind advice.

References

1) Aso, K., T. Watanabe and K. Yamao; (1958) Hakko-Kogaku Zasshi **36**, 39.
2) White, J. W., Jr., C. R. Eddy, J. Petty and N. Hoban: (1958) Anal. Chem., **30**, 506.
3) Malyoth, E.: (1951) Naturwissenschaften **38**, 473.
4) Vavruch, I.: (1952) Chem. Listy 46, 116.
5) White, J. W., Jr., and J. Mahr: (1954) A.O.A.C., **37**, 478.
6) Goldschmidt, S. and H. Burkert: (1955) Hoppe-Seyler's Z. Physiol. Chem., **300**, 188.
7) Galanti, M. et J. Wernimont: (1958) Rev. Fermentations et Inds Aliment **8-2**, 55.
8) White, J. W., Jr., and N. Hoban: (1959) Arch. Biochem. Biophys., **80** 386.
9) Matsuda, K. and K. Aso: (1954) Hakko-Kogaku Zasshi **32**, 399.
10) Aso, K. and K. Shibasaki: (1955) Nippon Nogei-Kagaku Kaishi **29**, 856.
11) Aso, K., T. Watanabe and A. Hayasaka: (1959) Hakko-Kogaku Zasshi **37**, 189.
12) Aso, K. and S. Hamada: (1955) Hakko-Kogaku Zasshi **33**, 45.
13) Barker, S.A., E. J. Bourne and O. Theander: J. Chem. Soc., **1955**, 4276.
14) Stodola, F. H., E. S. Sharpe and H. J. Koepsell: (1956) J. Amer. Chem., Soc., **78**, 2514.
15) Matsuda, K.: (1957) Nature **180**, 985.