STUDIES ON BEER II. GLUCO-BIOSES IN BEERS

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By

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Previously, we have reported (1) that the general analyses of bottled beers of the trade mark "Asahi Gold," "Sapporo," "Kirin", "Takara," "Dortmunder Actien (DA)," "Löwenbräu München (LM)," "San Miguel (SM)"; "Lavie" and "Liner"* and canned beers of the first three were carried out and especially their sugar components were analyzed by the paper chromatographic method.

There were two types of beers, a) KN-type which contains kojibiose and nigerose but almost no maltose as in Asahi Gold, Sapporo, DA and SM and b) M-type which contains maltose and a small amount of nigerose but no kojibiose as in Kirin, Takara and LM.

On the gluco-bioses in beers, Gjertson (2) has reported that the sugar components of beers were fractionated by a carbon-Celite column chromatography (Carbon CC) and, maltose and isomaltose were detected by paper chromatography (PPC). Stöckli (3, 4) has detected maltose and isomaltose by PPC. Montreuil *et al.* (5) have reported that the dialysable constituents of six kinds of beers were concentrated and the identifications and determinations were then carried out by PPC. Maltose, isomaltose and two unidentified gluco-bioses were detected. MacWilliam *et al.* (6) have detected nigerose in beers by PPC, isolated them by cellulose column chromatography, and identified as turanosazone.

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

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* The first four are produced in Japan, next two (DA and LM) are in Germany and SM in Philippine. Lavie and Liner are both imitation beers of Japan.

The original Japanese report was published in Nippon Nogei-Kagaku Kaishi. **35**, 1078 (1961).

Experimental

I. Fractionation of sugars in beers by Carbon CC.

Beers of KN-type (20 bottles, 12.66 *l*) and M-type (6 bottles, 3.8 *l*) were concentrated to 2 *l* (KN-type) and 0.5 *l* (M-type) under reduced pressure, respectively. Twice volumes of 95 per cent ethanol were added into the concentrated beer. After removal of the precipitate by filtration, the filtrate was concentrated and passed through a column of Amberlite IR-120 and IRA-410. The deionized solutions were again concentrated to 1 *l* (KN-type, total sugar 130.54g/*l*, as glucose) and 0.5 *l* (M-type, total sugar 49.59g/0.5 *l*, as glucose), respectively, followed by neutralization with NaOH, pH adjusted to 6.2.

The concentrated KN-type beer was poured on a column (550×125mm) composed of the same amount of carbon (Takeda, 800g) and Celite (No. 545, 800g) and eluted with water (20 *l*), 2.5 per cent (20 *l*), 5 per cent (20 *l*), 10 per cent (24 *l*), 15 per cent (32 *l*), 20 per cent (24 *l*), 25 per cent (20 *l*), 30 per cent (40 *l*) and 50 per cent (52 *l*) ethanol successively. The concentrate of M-type beer was eluted with water (20 *l*), 2.5 per cent (12 *l*), 5 per cent (24 *l*), 10 per cent (28 *l*), 15 per cent (24 *l*), 20 per cent (24 *l*), 25 per cent (28 *l*), 30 per cent (46 *l*) and 50 per cent (38 *l*) ethanol successively.

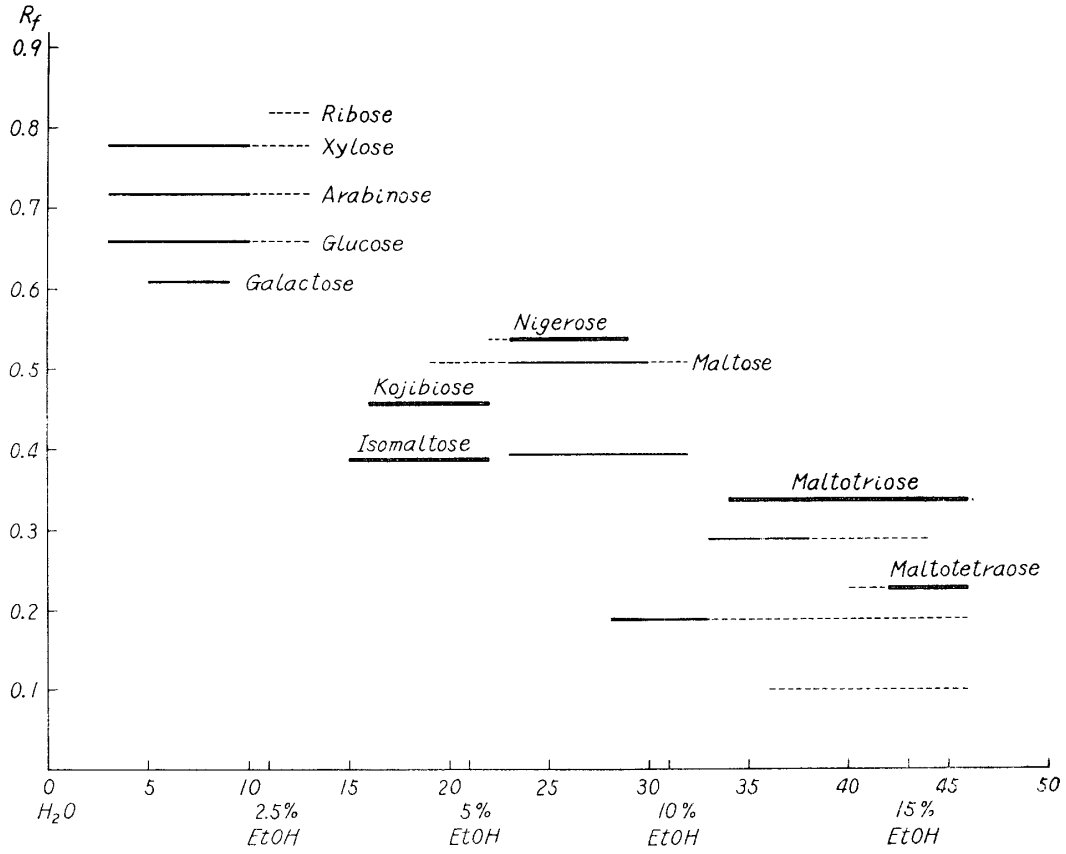

Fig. 1. Carbon column chromatographic behavior of sugars in beers of **KN-type**.

Each eluate was concentrated to 10*ml* under reduced pressure and examined by PPC. The results are shown in Fig. 1 and Fig. 2.

PPC was carried out with Toyo filter paper No. 2. The chromatograms were developed by the ascending technique with the mixture of pyridine : butanol : water (4:6:3). The sugars were located by spraying aniline hydrogen phthalate.

From the results of Figs. 1 and 2, 15 spots were detected in the beers of KN-type and 14 spots in the beers of M-type. Ribose, xylose, arabinose, glucose and galactose were eluted in water and the first half of 2.5 per cent ethanol fractions of KN-type and eluted in water and 2.5 per cent ethanol fractions of M-type. In the case of KN-type, isomaltose and kojibiose were eluted in 2.5 per cent and the first half of 5 per cent ethanol fractions, nigerose in 5 per cent ethanol fraction and a small amount of maltose in the latter half of the 2.5 per cent, 5 per cent and the first half of 10 per cent ethanol fractions. In the case of M-type, isomaltose was eluted in 5 per cent ethanol fraction, a large amount of maltose in 5 and 10 per cent ethanol fractions and a small amount of nigerose in 5—10 per cent ethanol fractions.

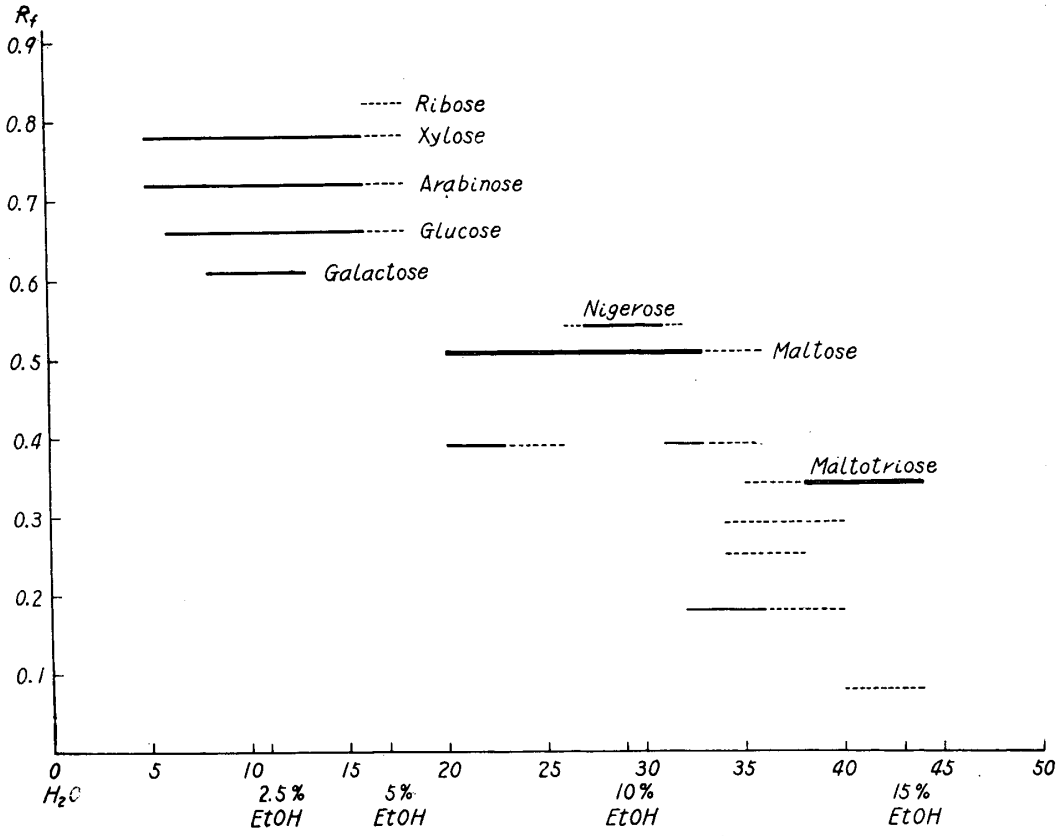

Fig. 2. Carbon column chromatographic behavior of sugars in beers of **M-type.**

II. Fractionation of kojibiose-isomaltose fraction by Carbon CC in the presence of borate buffer (pH 10.0).

The effluent portions (No. 16-21, 2.5-5 per cent ethanol fractions) containing kojibiose and isomaltose in the beers of KN-type was concentrated to give 7.8g of syrup. The sugar mixture was dissolved in 80*ml* of the borate buffer solution and was then rechromatographed on carbon (80g)-Celite (80g) column (400×50mm) using the gradient elution method with 0-2.0 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 to 20*ml* under reduced pressure and examined by PPC. The results are shown in Table 1.

Table 1. Fractionation of kojibiose-isomaltose fraction by Carbon CC 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	0.5	Borate buffer	EtOH 0 %	—	—
2— 4	1.5	〃	〃	Isomaltose	2.2
5— 8	2.0	〃	〃	Isomaltose	2.3
9—13	2.5	〃	0.5 %	Isomaltose	0.5
14—15	1.0	〃	〃	Isomaltose, Kojibiose	0.1
16	0.5	〃	〃	Kojibiose	0.1
17—24	4.0	〃	1.0 %	Kojibiose	0.9
25—31	3.5	〃	1.5 %	Kojibiose	0.5
32	0.5	〃	〃	—	—
33—38	3.0	〃	2.0 %	—	—

After irrigating the chromatogram with the mixture of pyridine: butanol: water (4 : 6 : 3), the sugars were located by spraying with aniline hydrogen phthalate reagent.

The 0 per cent ethanol fractions contained only isomaltose and 0.5 per cent ethanol fractions were composed of isomaltose and a small amount of kojibiose. Only kojibiose appeared in the 1.0-1.5 per cent ethanol fractions.

III. Identification of Kojibiose.

The fractions containing kojibiose (No. 17-24) were united and concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 0.9g of white amorphous powder. The white powder was dissolved in about 3*ml* of methanol and seeded with crystalline α-kojibiose. After being allowed to stand overnight, 0.67g of fine prisms were

obtained. After recrystallization from methanol, it had m.p. 186-187° undepressed on admixture with authentic α-kojibiose (7).

0.2g of α-kojibiose was acetylated with a mixture of 1.3g of pyridine and 1g of acetic anhydride and 0.26g of α-kojibiose octaacetate was obtained. After recrystallization from ethanol, it had m.p. 166° unchanged on admixture with authentic α-kojibiose octaacetate.

IV. Identification of Isomaltose.

The fractions containing isomaltose (No. 5-8) were concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 2.3g of white amorphous powder.

This powder was acetylated with 1.3g 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 being kept one day, it was extracted with chloroform. The chloroform layer was dried and evaporated to give 3.5g of syrup, which on addition of hot ethanol, was left to stand overnight at room temperature. However, direct crystallization of the acetylated sugar was not successful. One and a half g of this crude acetate was dissolved in 10*ml* of benzene and poured on a column (300×40mm) of Magnesol-Celite (5 : 1) and developed with 1000*ml* of benzene : t-butanol (100 : 1, by volume). Two zones were located on the extruded column by alkaline potassium permanganate. The upper zone was 35-61mm and middle zone was 61-110 mm from the top. Each zone was sectioned and eluted with acetone and evaporated. From the upper zone, 0.2g of syrup was obtained. But crystallization of this acetate was not successful. From the middle zone, removal of the solvent left 0.4g of syrup, from which 22mg of fine prisms were obtained upon crystallization from ethanol. After recrystallization, it had m.p. 144-145° undepressed on admixture with authentic β-isomaltose octaacetate. From the rest of the column, 0.4g of syrup was obtained. But crystallization of this acetate was not successful. The effluent from the column was evaporated. 0.1g of syrup was obtained but crystallization was not successful.

V. Identification of Maltose

The fractions containing maltose and a small amount of nigerose (No. 27-30) from the beers of M-type were concentrated under reduced pressure to give a syrup which upon treatment with hot methanol followed by concentration, gave 2.8g of white amorphous powder.

This white powder was acetylated with 1.7g of sodium acetate and 14*ml* of acetic anhydride at 100-110°C for two hours, poured into 300*ml* of water and after being kept for one day, it was extracted with chloroform, the chloroform layer was dried and evaporated to give 5.4g of syrup, which on addition of hot ethanol, was left to stand overnight at room temperature. 3.24g of fine prisms

were obtained. After recrystallization, it had m.p. 159-160° unchanged on admixture with authentic β-maltose octaacetate.

VI. Identification of Nigerose.

The concentrated mother liquor separated from the crystallines of β-maltose octaacetate gave 1.5g of syrup. The crude acetate was dissolved in 10*ml* of benzene and poured on a column (300×40mm) 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 was 13-52mm and the middle zone was 105-145mm and the lower zone was 145-188mm from the top. From the upper zone, removal of the solvent left 0.3g of syrup, from which 97mg 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, removal of the solvent left 0.4g of syrup, from which 10mg of fine prisms were obtained upon crystallization from ethanol. After recrystallization, it had m.p. 159-160° unchanged on admixture with known β-maltose octaacetate. The effluent from the column was evaporated. 0.1g of syrupy acetate was obtained. But crystallization of this acetate was not successful.

Discussion

Kojibiose, nigerose, maltose and isomaltose were isolated as their crystalline free sugar or crystalline octaacetates from beers. It is not certain whether these sugars were originally contained in the malt, or formed during the process of saccharification, or by the transglucosylation of enzyme in the malt or yeast.

Gunja *et al.* (8) have reported that isomaltose, panose and maltotriose were synthesized from maltose and glucose by *Saccharomyces cerevisiae*, but kojibiose and nigerose were not detected. Shibasaki *et al.* (9) have investigated the action of *Schizosaccharomyces pombe* on maltose. Kojibiose, nigerose, isomaltose, panose and isomaltotriose were detected. However, no study was hitherto carried out on the formation of kojibiose and nigerose by the transglucosylation of *Saccharomyces cerevisiae.*

Summary

Beers were fractionated by a carbon-Celite column using water and 2.5-50 per cent ethanol as successive elution solvents. The effluent portions containing kojibiose and isomaltose (2.5-5.0 per cent ethanol fraction of beers of KN-type) were then rechromatographed on a carbon-Celite column using the gradient elution method with 0-2.0 per cent aqueous ethanol containing borate buffer (pH 10.0).

From the kojibiose fraction, crystalline α-kojibiose was obtained and the acetylation of this crystalline α-kojibiose with a mixture of pyridine and acetic anhydride gave α-kojibiose octaacetate.

By the acetylation of isomaltose fraction followed by Magnesol-Celite column chromatography, β-isomaltose octaacetate was isolated.

Nigerose and maltose were isolated as their crystalline octaacetates from beers of M-type by a carbon-Celite and Magnesol-Celite column chromatographic procedures.

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