STUDIES ON THE UNFERMENTABLE SUGARS (IX) IONOPHORESIS OF SUGARS

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By

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We have hitherto experimented on many paper partition chromatography of sugars, but owing to the contamination of spots of some kind of sugars it was not often easy to resolve and judge them on the paper chromatogram although the developing solvents, spraying reagents and managements were variously changed. As one method to solve it, we tried the filter paper ionophoresis applying the theory of combination of sugars and borates which was reported by Böeseken (1) in 1949.

Since this method requires a shorter time than paper chromatography and is connected with the chemical structure of sugars, we have experimented especially on D-glucopyranosidic-disaccharides (viz. gluco-bioses), including the means of presumption of the structure of sakébiose and kojibiose in saké and koji-extracts.

Experimental

(A) Apparatus of Ionophoresis

We have made the apparatus in accordance with Foster (2). As shown in Fig. 1, A are in general two glass plates, each of which measures 17 cm × 32 cm

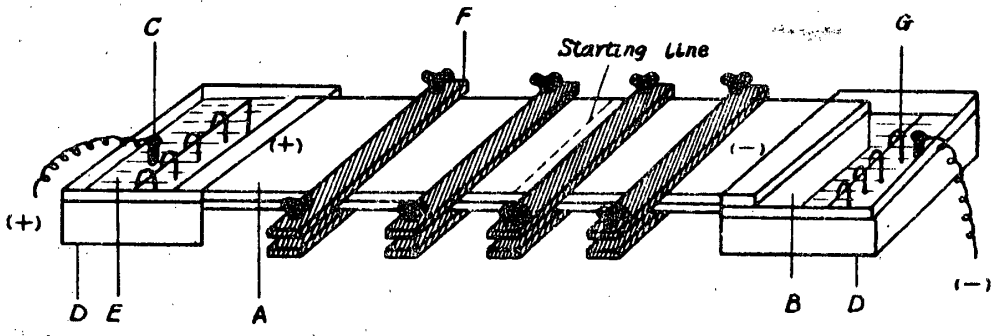

Fig 1. Apparatus of Ionophoresis

× 0.5 cm in size, B is a filter paper (TOYO filter paper No. 2) 40 cm × 16.8 cm in size, C is a carbon electrode (carbon electrode of dry cells were used for the electrode), D are porcelain vessels, which have glass covers and divided two parts, each measureing 20 cm × 12cm × 6 cm in size, E are borate buffer solution, F are wooden clumps to make firm the two glass plates of A, and G are bridges to connect the two parts of a vessel D. The constant voltage supplyer is a home-made-apparatus (Fig. 1) which produces a peak of 1000 V. DC.

(B) Managements of Ionophoresis

As shown in Fig. 2, the origin lines have been made paralel on the position of 14 cm from the one end of the TOYO filter paper No. 2, and the samples are spotted at a suitable interval within 10 cm in the middle parts of this line. The volume of the samples are varied on the sort of samples, but the proper quantities are 50 to 200γ because the developments of the color is more difficult under the influence of pH than that in the paper partition chromatography.

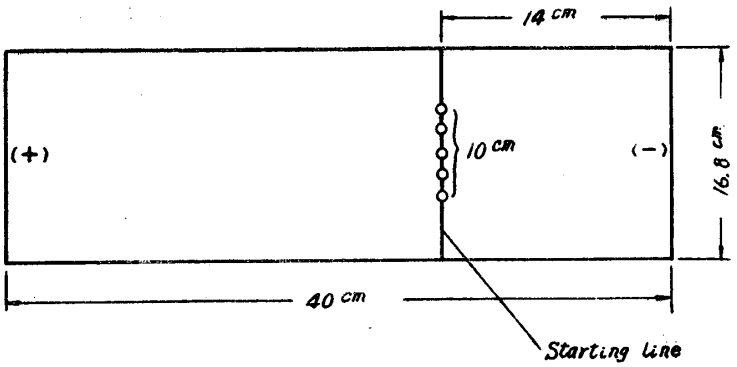

Fig. 2 Filter paper for Ionophoresis

At this time, one per cent solution of glucose and five percent solution of 2,3,4,6–tetramethylglucose were spotted as the control on each sample. After the spotted filter paper has been wet by borate buffer solution from both ends to about 1 cm near the origin lines and its excessing moisture has been absorbed by another dried filter paper, it is held between the glass plates and clumped sufficiently. Both ends of this filter paper are put into the buffer solution which is filled up in the vessels, carbon electrodes are inserteds, and then the vessels are closed up tightly by covers.

In about ten minutes after, the filter paper becomes wet to the origin line and thus it will be in a equilibrium. Then 600 V. DC of constant voltage is charged (the ionophoresis in the earler work was charged with low voltages as 200 to 300 volts, but recently voltages higher than 2000 volts is often used (6)). This ionophoresis requires about three hours and the electric currents are 20 to 27 mA. After the ionophoresis was finished, the filter paper was dried at 60°C. As spraying reagents, the acidic-anilinhydrogen-phthalates have been used chiefly and the color has been developed by heating for ten minutes at 140°C.

The ammoniacal $AgNO_3$ solution (7) was used in cases of trehalose and sucrose, and heated for five minutes at 110°C. The buffer solution consists of 0.05 N borax and 0.1N NaOH solution (6:4) and its pH is 9.8.

(C) MG-Values of Mono-and Di-saccharides

The above experiments showed the MG-values as indicated in Table 1 and 2. Fig. 3 is one example of the filter paper ionograms of gluco-bioses.

Table 1. MG of Monosaccharides

Monosaccharides	MG (Authors)	Consden et al +
D-Glucose	1.00	14.6
D-Xylose	1.00	—
L-Arabinose	0.93	13.9
D-Galactose	0.90	13.1
D-Fructose	0.87	13.1
D-Mannose	0.69	10.0
L-Rhamnose	0.52	7.8

+ Mobilities of sugars (cm^2/v. sec $\times 10^5$) at 20°C in 0.05 N borate buffer solution pH 9.7

Table 2. MG of Disaccharides

Disaccharides	Linkage	MG (Authors)	MG (Fosters)
Gentiobiose	1,6-β	0.71	0.75
Isomaltose	1,6-α	0.68	0.69
Sakébiose	1,3-α	0.67	0.69
Laminaribiose	1.3-β	0.65	0.69
Maltose	1,4-α	0.33	0.32
Kojibiose	1,2-α	0.31	—
Sophorose	1,2-β	0.30	—
Cellobiose	1,4-β	0.27	0.28
Trehalose	1,1-α,α	0.10	—
Lactose	—	0.38	0.38
Sucrose	—	0.12	—

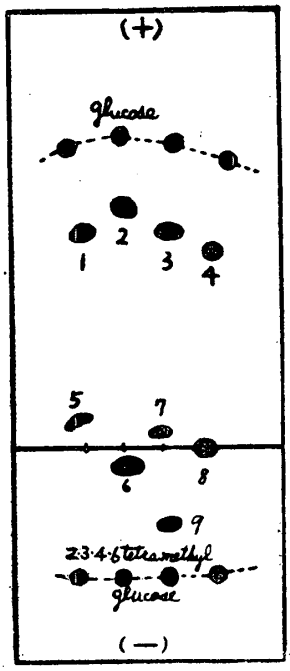

Fig 3. Paper-gram of iono-phoresis of gluco-biose

1. Isomaltose
2. Gentiobiose
3. Sakébiose
4. Laminaribiose
5. Maltose
6. Cellobiose
7. Kojibiose
8. Sophorose
9. Trehalose

Of the standard sugars which have been used in the experiments, maltose and cellobiose were Takeda Chemicals, isomaltose was kindly provided by Dr. Allene Jeanes, laminaribiose by Dr. Bourne and trehalose by Mr. T. Sato. Gentiobiose and sakébiose were prepared as shown in the previous reports (8, 9). Kojibiose was prepared from the koji-extract, and sophorose from hydrol (manufactured by Sanmatsu Kogyo Co. Ltd.) by using the carbon column chromatography and paper partition chromatography (next report), and the latter was identified with the sample which was provided by Dr. Cramer. The technique of filter paper ionophoresis was taken place as described in Foster's report.

Discussion

(1) As shown in Table 2, the MG-values of gluco-bioses are divided into three groups, namely (a) 0.71 to 0.75, (b) 0.30 to 0.27 and (c) 0.10. The gluco-bioses having 1,6–linkage as isomaltose and gentiobiose, and 1,3–linkage such as sakébiose and laminaribiose were contained in (a)-group. Maltose and cellobiose having 1,4–linkage, and kojibiose and sophorose having 1,2-linkage were contained in (b)-group. Trehalose having 1,1-linkage came under (c)-group. Among the same linkage, α-linkage showed higher value of MG than β-linkage, except for 1,6-linkage. To decide this facts, the equilibrium of α–form and β–form, and their molecular stero-structure should be studied.

(2) The MG-values of gluco-bioses are changed greatly by the linkage system, namely it is noteworthy that any OH position in the reducing glucose unit is protected with the non-reducing glucose unit.

Considering the MG-values (Table 3) of methyl-glucoses reported by Foster (5), it is found that the gluco-biose, in which the OH position corresponding to that in methly-glucose is protected, has MG–value of similar level as each methyl-glucose. The above facts and the coloring of the spots on the chromatogram (next report) showed that the previous presumption, that kojibiose and sakè-biose may be 1,2–α– and 1,3–α–linked gluco -bioses, respectively, was not wrong.

Table 3. MG of Methylglucose

Substances	MG
D–Glucose	1.00
6–Methyl–D–Glucose	0.82
3–Methyl–D–Glucose	0.82
4–Methyl–D–Glucose	0.27
2–Methyl–D–Glucose	0.23

(3) The ionogram produced by the apparatus shown in Fig. 1 formed a curve. as shown in Fig. 3. This seems to be due to the presence of the differences between their electric resistances, because the degree of press by glass plates is

different between the middle part and both side parts of the filter paper. There is a tendency of the spots deflecting to the outer side of the filter paper.

(4) The buffer solution having comparatively higher pH-values shows larger MG-values.

(5) As spraying reagents, aniline-hydrogen-phthalate and ammoniacal $AgNO_3$ were used. With the former reagent, acetic acid was added to the reagent until acidic, and 2,3,4,6–tetramethylglucose was developed to a red colored spot by heating the filter paper at 140°C for ten minutes. With the latter, the ionogram was developed by heating at 110°C for five minutes, and the fading of colors of the spots was prevented by washing the paper with water.

Summary

The MG-values of various sugars, especially D-glucopyranosidobioses, were obtained by the filter paper ionophoresis. The results showed that there are three groups in the gluco-bioses according to the size of the MG-values, namely the first group having 1,3– and 1,6-linkages, the second group having 1,2– and 1,4–linkages and the third group having 1,1–linkage. It was also proved that the previous presumption, that kojibiose and sakèbiose may be 1,2-α– and 1,3–α–linked gluco-bioses respectively, was not wrong.

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