Structure Determination of Sucrose by Acetylation and Acid Hydrolysis¹

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ABSTRACT

For the structure determination of D-(+)-sucrose, which consists of α -D-(+)-glucose and β -D-(+)-fructose, it was acetylated with acetic anhydride and triethyl amine, pyridine, zinc chloride, and sodium acetate as catalysts. The acetylated D-(+)-sucrose was acid-hydrolyzed using sulfuric acid and sodium chloride in methanolic solution. The structures of the reaction products were determined by ¹H-NMR and ¹³C-NMR spectra. The yield of the acetylation indicated the high value in zinc chloride as 70% in zinc chloride catalyst. The acid-hydrolyzed product of the acetylated D-(+)-sucrose, 2,3,4,6,1',3',4',6'-octa-O-acetyl-D-(+)-sucrose, gave 2,3,4,6-tetra-O-acetyl- β -D-(+)-glucose and it suggests that the acetylated D-(+)-sucrose was rearranged through the formation of oxonium ion by mutarotation in the 2,3,4,6-tetra-O-acetyl- α -D-(+)-glucose moiety and through the ring opening in the 1',3',4',6'-tetra-O-acetyl- β -D-(+)-fructose moiety.

Keywords : Carbohydrate, Structure determination, Sucrose, Acetylation, Acid hydrolysis

1. INTRODUCTION

Carbohydrates are organic compounds which are composed of carbon, oxygen and hydrogen, and are biosynthesized by photosynthesis in plants, including tree species. Especially most of chemical constituents of tree species are carbohydrates, except for lignin and aromatic compounds. In addition to the water soluble polysaccharides, celluloses are entirely composed of β -D-(+)-glucose and hemicelluloses are complexes which are combined with glucose, mannose and galactose as a hexose monomer and

arabinose, xylose and fructose as a pentose monomer.

However, celluloses have been studied on the structure and the utilization for a long time, but it is very little to study on hemicelluloses and water soluble sugars. The chemical structures of carbohydrates have long been studied in USA, Japan, and European countries, but there have been many difficulties in the structure determination of hemicellulose and water soluble sugars due to the macromolecules and the complexity of constitutional sugars, and there are very little studies on the structure of carbohydrate in do-

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Fig. 1. Comparison of ¹H-NMR spectra of methylated and acetylated carbohydrates.

mestic (Ma Y. 2011).

A disaccharide which two monomeric sugar units are combined together by dehydration reaction is acid hydrolysable. One of the most abundant disaccharides in nature is sucrose which composes the fruit sugar among the water soluble sugars of plants and tree species. It is connected to 1,2-glycosidic linkage and one of major components of sugar cane and sugar beet.

1D-NMR, ¹H-NMR and ¹³C-NMR, and 2D-NMR, COSY and HSQC, are power tools for the study of carbohydrate structure that can be used to determine the skeleton of sugar molecules and to identify carbons and hydrogens in any special position. In the structural study of carbohydrates, the derivatization such as methylation and acetylation on the hydroxyl groups of carbohydrates is widely used to identify the bonding position of sugars and the presence of

the substituents in the main chain.

In the methylated sugars, the proton signals of the methyl groups are shown at δ 3~4 in the ¹H-NMR spectrum and overlapped with the proton signals of the sugar moiety. This means that there can be any troubles in the analysis of the ¹H-NMR signals of a sugar moiety. However, the proton signals of the acetyl groups are shown at δ 2~3 in the ¹H-NMR spectrum in the acetylated sugar and do not overlapped with those of sugar moiety and acetylation is useful in the structure analysis of sugar (Fig. 1).

D-(+)-sucrose was acetylated, acid hydrolyzed, and then analyzed by NMR spectroscopy to get the basic information for the structure analysis of carbohydrates.

2. MATERIALS AND METHODS

2.1. Experimental Materials

D-(+)-sucrose (25 g, Wako, GR) and acetic anhydride (Daejung, 1 kg, GR) were used for the acetylation in this experiment. Triethylamine (Daejung, 1 kg, EP), pyridine (Daejung, 1 kg, EP), zinc chloride (Daejung, 1 kg, EP) and sodium acetate (Daejung, 1 kg, EP) were also used as catalysts to get the acetylated D-(+)-sucrose. Methylene chloride (Daejung, 4 L, GR), sodium bicarbonate (Daejung, 1 kg, GR), sodium sulfate (Wako, 1 kg, GR) and 6 M hydrogen chloride (Wako, 500 ml, GR) were used to purified the acetylated D-(+)-sucrose. 98% of sulfuric acid (Wako, 500 ml, GR) and NaCl were applied to the methanolic acetylated D-(+)-sucrose solution to get the hydrolyzed D-(+)-sucrose products.

2.2. Acetylation

2.2.1. Acetylation with Triethylamine and Pyridine

Acetic anhydride (7 ml) and pyridine (or triethylamine) (20 ml) were combined together and cooled to 0°C, and D-(+)-sucrose 0.5 g) was added into the solution. The suspension was stirred at 0°C until the sugar is all dissolved. The solution is allowed to stand 18 hr. at room temperature and was then poured with stirring into 20 ml of ice water. The reaction mixture was filtered with suction, washed with cold water, and then dried at room temperature (Peter M. 1995).

2.2.2. Acetylation with Sodium Acetate Anhydrous

The suspension of anhydrous sodium acetate (2 g) and acetic anhydride (15 ml) in a round-

bottomed flask was heated to the boiling point in an efficient fume hood. Then D-(+)-sucrose (1 g) was added into the flask. Initiation of the reaction was indicated by the continuous boiling even after the stopping of the heating ; the flask was placed on a cork ring, and the heating was extinguished. After the reaction was subsided, the solution was brought to a full boil. It was then cooled and poured into 20 ml of ice water. After standing 3 hr. with stirring at room temperature, the reaction product was filtered with suction, washed with cold water, and then dried (Peter M. 1995).

2.2.3. Acetylation with Zinc Chloride

D-(+)-sucrose (0.5 g) was added to the solution of zinc chloride (3.3 g) and acetic anhydride (10 ml) at 0°C, then the mixture was stirred vigorously until the sugar was all dissolved for about 4~5 hrs. The temperature was kept at 20~25°C for 1 hr and then at 50°C for 2 hrs. The cooled solution was stirred for 2 hr. with 20 ml of ice water and then neutralized with an excess amount of sodium hydrogen carbonate. The mixture was extracted with 15 ml, of chloroform; the chloroform solution is washed with water, dried with anhydrous sodium sulfate, and evaporated under the reduced pressure to get a syrup, compound **2**, to be freeze dried (Peter M. 1995).

2.3. Acid Hydrolysis

The acetylated sucrose (0.5 g) and 20 ml of methanol were completely dissolved in a round-bottomed flask at room temperature. Some amount of 98% sulfuric acid was poured in a separatory pressure dropping funnel, some of NaCl powder was poured in a round-bottom flask, and then

two devices were connected together. One end of a tube was connected to the funnel outlet and the other end was connected to a glass pipet to be dipped into the methanolic acetylated sucrose solution. Then sulfuric acid was slowly fell in 4 ~ 5 drops over NaCl powder to creat HCl gas to be injected into the methanolic solution for 5 min. The flask was sealed, stirred to get the acid-hydrolyzed product, compound **3**, for 24 hrs, and then evaporated to be freeze dried.

2.4. Structure Determination of the Reaction Products

¹H- and ¹³C-NMR spectra were recorded on a Bruker Avance DPX 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard and chemical shifts are given in δ (ppm). NMR solvents were DMSO, D₂O and CDCl₃.

2.4.1. Compound 1 (D-(+)-sucrose)

¹H-NMR(400 MHz, δ , CDCl₃) : 3.47 (1H, t, H-4), 3.56 (1H, t, H-2), 3.68 (2H, t, H-1'), 3.76 (1H, t, H-3), 3.81 (2H, m, H-6), 3.82 (2H, m, H-6'), 3.86 (1H, m, H-5), 3.89 (1H, t, H-5'), 4.06 (1H, t, H-4'), 4.22 (1H, t, H-3'), 5.42 (1H, d, J=3.53Hz, H-1).

¹³C-NMR(100 MHz, δ , CDCl₃) : 60.93 (C-6), 62.48 (C-1'), 62.60 (C-6'), 70.28 (C-4), 72.09 (C-2), 73.28 (C-5), 73.31 (C-3), 74.72 (C-4'), 77.44 (C-3'), 83.02 (C-5'), 92.20 (C-1), 104.492 (C-2')

2.4.2. Compound 2 (acetylated D-(+)-sucrose)

¹H-NMR (400 MHz, δ , CDCl₃) : 2.02~2.12 (24H, m, H-acetyl) 4.16 (2H, m, H-1'), 4.17 (2H, m, H-6), 4.22 (1H, m, H-5'), 4.30 (1H, m, H-5), 4.33 (2H, m, H-6'), 4.87 (1H, dd, J=3.6Hz

and J=3.81Hz, H-2), 5.08 (1H, t, H-4), 5.37, (1H, t, H-4'), 5.44 (1H, t, H-3), 5.45 (1H, d, J=4.4Hz, H-3'), 5.69 (1H, d, J=3.69Hz, H-1).

¹³C-NMR(100 MHz, δ , CDCl₃) : 20.60 (acetyl CH₃), 61.86 (C-1'), 63.00 (C-6), 63.71 (C-6'), 68.40 (C-4), 68.62 (C-5), 69.76 (C-3'), 70.40 (C-2), 75.16 (C-4'), 75.87 (C-3), 79.23 (C-5'), 90.08 (C-1), 104.09 (C-2'), 167.47 ~ 169.98 (acetyl C=O).

2.4.3. Compound 3 (acetylated β -D-(+)-glucopyranose)

¹H-NMR(400 MHz, δ , CDCl₃) : 2.01~2.12 (16H, m, H-acetyl), 3.85 (1H, m, H-5), 4.12 (1H, dd, J=2.13Hz and J=2.14Hz, H-6), 4.29 (1H, dd, J=4.52Hz and J=4.52Hz, H-6), 5.12 (1H, dd, J=3.69Hz and J=3.74, H-3), 5.15 (1H, dd, J=2.33Hz and J= 2.71Hz, H-2), 5.26 (1H, t, H-4), 5.72 (1H, d, J=8.16Hz, H-1).

¹³C-NMR(100 MHz, δ , CDCl₃) : 20.60 (acetyl CH₃), 61.85 (C-6), 68.14 (C-4), 70.62 (C-5), 73.11 (C-2), 75.19 (C-3), 90.08 (C-1), 170.06~170.64 (acetyl C=O).

3. RESULTS AND DISCUSSION

D-(+)-sucrose (compound **1**) was acetylated by triethylamine, pyridine, sodium acetate anhydrous, and zinc chloride as catalysts. The acetylated D-(+)-sucrose (compound **2**) was treated using sulfuric acid and NaCl to get the acid-hydrolyzed product and gave the compound **3**, 2,3,4,6-tetra-O-acetyl- β -D-(+)-glucopyranose (Fig. 3).

In the ¹H-NMR spectrum, H-1 gave a doublet at δ 5.42 and the coupling constant was 3.52 Hz indicating α -D-(+)-glucose. H-2, H-3 and H-4 indicated triplet signals at δ 3.56, δ 3.76, and δ 3.47, respectively. H-5 and H-6 showed

Fig. 2. NMR spectrum of Acetylated D-(+)-sucrose.

two multiplet signals at δ 3.86 and δ 3.81, respectively. H-1' of the β -D-(+)-fructose moiety gave a triplet at δ 3.68. H-3', H-4' and H'-5 also gave three triplets at δ 4.22, δ 4.06 and δ 3.89, respectively. Two protons of H-6' showed a multiplet signal at δ 3.82. In the ^{13}C -NMR spectrum, C-1 of the α -D-(+)-glucose moiety gave a signal at δ 92.90. C-2, C-3, C-4 and C-5 indicated the signals at δ 72.09, δ

73.31, δ 70.28 and δ 73.28, respectively. C-6 gave a most-upfielded signal at δ 60.93. C-1' of the β -D-(+)-fructose moiety gave a signal at δ 62.48. C-2', C-3' and C-4' indicated signals at δ 72.09, δ 77.44 and δ 74.72, respectively. C'-5 gave a downfielded signal at δ 83.02 in the spectrum. C-6' showed a signal at δ 62.60. These NMR data were identical to the literature (Mehta B.K. 2008).

Fig. 3. Chemical structures of the compound 1~3.

Compound **2** gave free dried dark brown powder. In the $^1\text{H-NMR}$ of compound **2** (Fig. 2), H-1 of the glucose moiety showed a doublet at δ 5.69 with the coupling constant of 3.69 Hz indicating α -anomer. H-2 gave a doublet signal at δ 4.87. H-3 and H-4 were resonated at δ 5.44 and δ 5.08, respectively, with two triplet signals. H-5 and H-6 gave two multiplets at δ 4.30 and δ 4.17, respectively. H-1' of the fructose moiety gave a multiplet at δ 4.16. H-3' and H-4' showed a doublet and a triplet signals at δ 5.45 and δ 5.37, respec-

tively. H-5' and H-6' indicated two multiplets at δ 4.22 and δ 4.33, respectively. In the $^1\text{H-NMR}$ of compound **2**, the chemical shifts of most protons were downfield shifted about 0.5~1 ppm compare to the compound **1** due to the conjugation effect from the substituted acetyl groups. The methyl protons of the acetyl groups gave eight signals at δ 2.02~2.12. These $^1\text{H-NMR}$ signals were similar to those of the literature (Chandrika B. 2005).

In the $^{13}\text{C-NMR}$ spectrum (Fig. 2), C-1 of the glucose moiety showed a signal at δ 90.08.

Fig. 4. Rearrangement of the acetylated α -D-(+)-glucose through the formation of oxonium ion.

C-2 and C-3 gave signals at δ 70.4 and δ 75.87, respectively. Also C-4 and C-5 resonated at δ 68.40 and δ 68.62, respectively. C-6 gave a signal at δ 63.0. C-1' of the fructose moiety gave a signal at δ 61.86 and C-2' showed a signal at δ 104.09 which was downfield shifted about 30 ppm compare to the signal of compound **2**. It may be the conjugation effect from the substituted acetyl group. C-3' and C-4' gave signals at δ 77.44 and δ 74.72, respectively. Also C-5' and C-6' indicated signals at δ 83.02 and δ 62.60, respectively. The carbonyls of the acetyl group gave eight signals at δ 167.47~169.98 and the methyls of the acetyl groups indicated the signals at δ 20.60~20.80. These NMR data were identical to the acetylated D-(+)-sucrose, 2,3,4,6,1',3',4',6'-octa-O-acetyl-D-(+)-sucrose (Jung M.H. 2004.).

Compound **3** was freeze dried to dark brown

powder. In the ^1H -NMR spectrum, the four methyl protons from acetyl groups gave the multiple signals at δ 2.02~2.12. H-1 gave the doublet signals at δ 5.72 and the coupling constant was 8.16 Hz indicating the structure of β -D-(+)-glucopyranose. H-2 and H-3 showed double doublet signals at δ 5.15 and δ 5.12, respectively. H-4 indicated a triplet signal at δ 5.26 and H-5 gave a multiplet signal at δ 3.85. Two protons of H-6 showed two double doublet signals at δ 4.12 and δ 4.29. According to the above ^1H -NMR data, compound **3** was identical to tetra-O-acetyl- β -D-(+)-glucopyranose and to the known data (Ma Y., 2001; Yang Z., 2000). In the ^{13}C -NMR spectrum, four carbonyl carbons of the acetyl groups gave the four signals at δ 170.06~170.64. Four methyl carbons of the acetyl groups also indicated the signals at δ 20.4~20.6. C-1 gave a signal at 90.8 indica-

Fig. 5. Rearrangement of the acetylated β -D-(+)-fructose through ring opening.

ting β -D-(+)-glucose and C-2 and C-3 were shown the signals at δ 73.11 and δ 75.19, respectively. The other C-4, C-5 and C-6 indicated the signals at δ 68.14, δ 70.62, and δ 61.85, respectively. The above ^1H - and ^{13}C -NMR data were identical to 2,3,4,6-tetra-O-ace-

tyl- β -D-(+)-glucopyranose (Ma Y., 2001; Yang Z., 2000).

The formation of 2,3,4,6-tetra-O-acetyl- β -D-(+)-glucopyranose acid hydrolysis of the acetylated D-(+)-sucrose suggests that 2,3,4,6-tetra-O-acetyl- α -D-(+)-glucopyranose moiety of

the acetylated D-(+)-sucrose was rearranged through the formation of oxonium ion by mutarotation (Fig. 4) (8, 10,) and that 1',3',4',6'-tetra-O-acetyl- β -D-(+)-fructose moiety was also rearranged after the ring opening (Fig. 5) (Hasan S., 1998; Jone F., 1998; Sokolov V.M., 2002.).

The acetylation yield of D-(+)-sucrose by catalysts indicated the highest as 69% in ZnCl_2 , 51% in pyridine, and 49% in both of triethylamine and sodium acetate. However, these yields may be changed according to molecular sizes of carbohydrates.

4. CONCLUSION

D-(+)-sucrose, a disaccharide, was acetylated with acetic anhydride, and triethylamine, pyridine, sodium acetate anhydrous and zinc chloride as reaction catalysts. Then the acetylated D-(+)-sucrose was acid hydrolyzed by HCl gas from 98% sulfuric acid and NaCl to give the hydrolyzed product, 2,3,4,6-tetra-O-acetyl- β -D-(+)-glucopyranose.

The formation of 2,3,4,6-tetra-O-acetyl- β -D-(+)-glucopyranose from the acetylated D-(+)-sucrose by acid hydrolysis means that 2,3,4,6-tetra-O-acetyl- α -D-(+)-glucose moiety of the acetylated D-(+)-sucrose was rearranged through the formation of oxonium ion and that 1',3',4',6'-tetra-O-acetyl- β -D-(+)-fructose moiety was also rearranged after the ring opening.

In the catalytic reaction of D-(+)-sucrose, ZnCl_2 was more effective as the yield of 69% and the rests were around 50%. However, the catalytic yield can be different from the kind of carbohydrate and will need further study in the future.

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