ACTA CHEMICA SCANDINAVICA 16 (1962) 827–830

The Isolation of a Xylobiose Monoacetate from the Waste Liquor of a Birch Sulphite Cook

INGEMAR CROON

Billeruds Research Laboratory, Säffle, Sweden

A monoacetate of xylobiose has been isolated by chromatographic methods from the spent liquor of a birch sodium sulphite cook. Periodate oxidation and paper electrophoresis studies indicate that the *O*-acetyl group is located in the 2-position of the non-reducing xylose residue.

The isolation of this compound implies the presence also of other acetylated oligosaccharides in the birch sulphite cooking liquor.

The behaviour of the *O*-acetyl groups of birch xylan during different types of sulphite processes has been studied in this laboratory [1,2]. These studies have indicated that acetylated oligosaccharides should be present in the liquor during certain stages of the acid sulphite cook. An investigation was undertaken in order to follow the dissolution of the carbohydrate material during the cook and to attempt to isolate some partially acetylated oligosaccharides.

The sulphite liquor from a birch cook was purged of free sulfur dioxide and added directly to a carbon-Celite column. The column was irrigated with water to remove inorganic salts. The sugars were eluted using a water-ethanol gradient.

The carbohydrate composition of the waste liquor investigated was very complex. The major part (about 70 % of the material eluted with up to 50 % ethanol) consisted of monosaccharides: xylose, glucose, mannose, galactose, arabinose and rhamnose. These monosaccharides, eluted first from the column, were followed by disaccharides, trisaccharides, aldobiouronic acids, etc. At least 25 different components were indicated by paper chromatographic analysis. Fractions which contained approximately the same components were combined and evaporated *in vacuo*. In this way ten fractions were obtained, some of which contained acetyl groups. The fraction with the highest acetyl content (1.6 %) contained a substance with a R_{xylose}-value of 1.31 in solvent A and 1.20 in solvent B. The other substraces in this fraction were an aldotriouronic acid and xylotetraose, both of which have low R_{xylose}-values in solvent B. The substance with the high R_{xylose}-value was easily isolated therefore by cellulose column chromatography using the latter solvent. The compound

crystallized upon evaporation of the solvent and after two recrystallizations from hot ethanol had m.p. 195—196°C and $[\alpha]_D^{20} = -15$ ($c = 2$ in water). The infrared spectrum of the substance showed absorption at 1 745 cm^{-1} and 1 260 cm^{-1}, thereby indicating the presence of ester groups. Treatment with sodium methoxide in methanol gave a substrance with the same paper chromatographic and electrophoretic mobility as xylobiose. It crystallized from methanol and had m.p. 183—185°C [3]. The phenyl osazone hemihydrate decomposed at 204—206°C in good agreement with reported values for xylobiose phenylosazone-hemihydrate [3].

A quantitative acetyl analysis by gas chromatography gave an *O*-acetyl value of 13.20 % in good agreement with the calculated value 13.27 % for a monoacetyl xylobiose. Elemental analysis (C 44.17, H 6.30, O 49.53 %) was also in agreement with that of a monoacetyl derivative of xylobiose (C 44.4, H 6.18, O 49.38 %). The identity of the compound was further established by complete acetylation affording the crystalline hexa-acetate of xylobiose, melting point (155—156°C), undepressed by admixing with authentic xylobiose hexa-acetate [4].

The substance consumed 2.8 moles of periodate with the liberation of 0.92 moles of formic acid. A unique structure can not be assigned to the compound solely on the basis of this periodate oxidation data. (If position 2 in the reducing xylose unit is free, a formic acid ester will be formed. The determination of the formic acid in this ester depends on the method of analysis employed). However, substitution at the 2-position in the reducing unit and the 3-position in the non-reducing unit may be excluded. As the 4-position in the nonreducing unit is bonded in the xylan chain, the most likely position of the acetyl group should be in the 3-position of the reducing unit or the 2-position of the non-reducing unit.

Garegg and Lindberg [5] in studying the paper electrophoresis of carbohydrates in sulphonated phenyl boric acid at pH 6.5, found pronounced differences between various substituted sugar alcohols, depending upon the site of the substitution. Thus xylitol, 4-*O*-methylxylitol and 3,4-di-*O*-methyl-xylitol had the M_{mannitol}-values 0.9, 0.43 and 0.0. Methyl-β-xylopyranoside did not move at all under these conditions. Therefore by studying the electrophoretic mobility of the corresponding mono-*O*-acetyl-xylobiitol, it should be possible to decide whether or not the *O*-acetyl groups are linked to the 3-position in the non-reducing xylose residue. The latter substance was prepared and found to have the M_{mannitol}-value 0.50, slightly higher than xylobiitol 0.44. This result strongly indicates that the 3-position in the reducing xylose residue is not substituted and implies that the *O*-acetyl group is located in the 2-position in the nonreducing residue.

DISCUSSION

In natively acetylated birch xylan, the *O*-acetyl groups seem to be linked mainly (about 75 %) in 3-positions of the xylose residues [6,7]. In the isolated xylobiose monoacetate, however, the *O*-acetyl group is most probably located in the 2-position of the non-reducing xylose residue. That it is found in the non-reducing residue seems natural, as bulky substituents in equatorial posi-

tions should render glycosides more stable to acid hydrolysis [8]. Further, the *O*-acetyl group is an electron-withdrawing substituent, and in the sugar residue, they are known to decrease the rate of acid hydrolysis of a glycoside [9]. A substituent in the 2-position, that is closer to the glycosidic linkage should be more efficient than a substituent in the 3-position.

It is probable that some of the *O*-acetyl groups in 3-positions have also survived the sulphite cook and that a number of acetylated oligosaccharides are present in the spent liquor.

EXPERIMENTAL

Paper chromatography. Paper: Whatman No. 1. Solvents:
Solvent A: Butanol, acetone, water, 4:5:1
Solvent B: Ethylacetate, acetic acid, water, 9:2:2.
Spray reagents: Aniline phthalate and silver nitrate/sodium ethoxide.

The waste liquor was obtained from a laboratory cook on birch (*Betula verrucosa*) chips, performed in a 10 litre tumbling stainless steel digester. The cook employed a cooking acid of 6 % total and 1 % combined sulphur dioxide. Heating time 5 h to 150°C and kept 45 min at this maximum temperature; liquor-to-wood ratio 4:1; maximum pressure 7.0 kp/cm^2. Sodium base was used. Pulp yield was 46 %; roe number 2.7 and pulp viscosity 77 cPT. The acetyl content of the pulp was 1.09 % and the neutral sugar composition: glucose 90.8 %, mannose 0.8 % and xylose 8.4 %.

Fractionation of the mono- and oligosaccharides in the waste liquor. Waste liquor (1 l) was freed from sulphur dioxide by bubbling nitrogen for one hour. The liquor was then added to a carbon-Celite column (3.5 × 55 cm) which was irrigated with water (10 l). The monosaccharides eluted by this treatment were recovered by passage through ion exchange columns and concentration of the solution. A linear gradient from water to 50 % ethanol was then employed and fractions (20 ml) collected, using an automatic fraction collector. Fractions 96—108 contained a component moving faster than xylose. R_{xylose} = 1.31 in solvent A and 1.20 in solvent B. These fractions were combined and taken to dryness by evaporation *in vacuo*.

Isolation of fast moving compound. The latter material (1.24 g), which had an acetyl content of 1.6 %, was dissolved in ethyl acetate-acetic acid-water, 1:1:1 (10 ml), and added to a cellulose column (3 × 30 cm) and eluted with solvent B.

The fractions which contained the component with R_{xylose} = 1.20 were combined and evaporated to dryness whereupon the substance crystallized. After recrystallization from hot ethanol (yield 130 mg) the crystals had m.p. 195—196°C and $[\alpha]_D^{25} = -15°$ (c = 2 in water). The infrared spectrum exhibited absorption maxima at the following frequencies cm^{-1}: 3 350, 2 940, 1 745, 1 650, 1 430, 1 380, 1 260, 1 135, 1 070, 1 030, 990, 970, 940, 890, 860 and 750.

Deacetylation of the substance. The substance (20 mg) was dissolved in sodium methoxide (3 ml) and the solution allowed to stand for 5 h. The sodium ions were removed by cation exchange (IR-120, H$^+$-form). A gas chromatographic run indicated methyl acetate in the solution. On evaporation of the solvent, the deacetylated compound crystallized, m.p. 183—185°C [3]. The substance gave only xylose upon hydrolysis. To a solution of the crystals (10 mg) in water (1.5 ml) phenyl hydrazine (0.175 g), 20 % acetic acid (1.0 ml) and sodium bisulphite (10 mg) were added. The solution was heated for 2 h at 100°C (bath temp.). The crystals which separated were filtered, washed with water and recrystallized from 60 % ethanol, m.p. 204—206°C in good agreement with reported values for xylobiose phenyl osazone hemihydrate [3].

Determination of the acetyl content of the xylobiose acetyl derivative. The quantitative estimation of the acetyl content of the substance was performed with the gas chromatographic method devised by Spingler and Markert [10]. The substance was found to contain 13.20 % acetyl which corresponds to a monoacetyl derivative of xylobiose (13.27 %).

Acetylation of the monoacetyl derivative of xylobiose. The substance, (10 mg) was heated at 100°C for 1 h with a mixture of acetic acid (0.5 ml), acetic anhydride (4.5 ml) and sodium acetate (0.5 g). The solution was poured into a mixture of ice and water. The acetate

crystallized spontaneously and was filtered after decomposition of all of the acetic anhydride. The crude acetate was washed with dilute sodium bicarbonate and recrystallized from alcohol. M.p. 155–156°C alone or on admixture with authentic β-xylobiose hexaacetate [4].

Periodate oxidation of the substance. The compound was dried *in vacuo* at 40°C for 2 h over P_2O_5. 10.0 mg was weighed into a 10 ml volumetric flask and made up to the mark with an ice-cold solutionof metaperiodate (0.4 g/100 ml). The solution was stored in the refrigerator (+ 3°C).

The periodate consumption was determined by taking aliquots (1 ml) and adding to a solution containing 2 ml of 10 % potassium iodide and 2 ml 3 N sulphuric acid. The solution was diluted with a few ml of water. The iodine liberated was titrated with 0.01 N $Na_2S_2O_3$.

The formic acid production was measured as follows: 1 ml aliquots were withdrawn and added to a solution of ethylene glycol (0.5 ml) in water (5 ml) The mixture was allowed to stand in the dark (refrigerator) for 15 min. 10 % potassium iodide (1 ml) was added and the iodine was titrated with 0.01 N $Na_2S_2O_3$. After 24 h the sample had consumed 2.8 moles of periodate with the liberation of 0.92 moles of formic acid.

Reduction of the xylobiose monoacetate. The substance (2 mg) was refluxed together with Raney-nickel (20 mg) in 70 % ethanol (2 ml) for 3 h. After filtration the reduced material was recovered by evaporation to dryness.

Saponification of the xylobiitol monoacetate. Half of the xylobiitol monoacetate was dissolved in methanol (1 ml) and a solution (0.2 ml) consisted of 10 % ammonia in methanol was added. The mixture was allowed to stand overnight after which the xylobiitol was isolated by evaporation to dryness [11].

Paper electrophoresis. Buffer solution: 0.05 M phenyl boric acid orthosodium sulphonate titrated with sodium hydroxide to pH 6.5 and 0.06 M sodium phosphate buffer solution of pH 6.5. Voltage 0.75 kV; Time 2 ½ h; Temperature 40°C.

Acknowledgement. The author is indebted to Drs. E. Goldstein and P. Garegg for their suggestions and experimental assistance. My thanks are also due to Prof. B. Lindberg for valuable discussions and to Billeruds AB for permission to publish this part of the investigation.

REFERENCES

1. Öhrn, O. E. and Croon, I. *Svensk Papperstidn.* **63** (1960) 601.
2. Annergren, G. E. and Croon, I. *Svensk Papperstidn.* **64** (1961) 618.
3. Whistler, R. L., Bachrach, J. and Tu, C. C. *J. Am. Chem. Soc.* **74** (1952) 3059.
4. Srivastava, H. C. and Smith, F. *J. Am. Chem. Soc.* **79** (1957) 982.
5. Garegg, P. J. and Lindberg, B. *Acta Chem. Scand.* **15** (1961) 1913.
6. Bouveng, H. O., Garegg, P. J. and Lindberg, B. *Acta Chem. Scand.* **14** (1960) 742.
7. Bouveng, H. O. *Acta Chem. Scand.* **15** (1961) 96.
8. Shafizadeh, F. and Thompson, J. *J. Org. Chem.* **21** (1956) 1059.
9. Whistler, R. L. and Richard, G. N. *J. Am. Chem. Soc.* **80** (1958) 4888.
10. Spingler, H. and Markert, F. *Microchim. Acta* **1959** 121.
11. Slotta, K. H. and Heller, N. *Ber.* **63** (1930) 1024.

Received October 30, 1961.