Anal. Calcd. for $C_8H_9NO_5S$: C, 41.55; H, 3.92. Found: C, 41.58, 41.52; H, 3.90, 4.09.

Reaction of 3-Nitro-4-chlorophenyltrimethylammonium Iodide with Sodium Methoxide at 45°.—The purpose was to detect the possible occurrence of demethylation[6,7] as a side reaction. A solution of 7.50×10^{-4} mole of the quaternary ammonium salt and 7.75×10^{-4} mole of sodium methoxide in 50 ml. of methanol was kept overnight at 45°. The reaction was quenched with a measured excess of sulfuric acid and back-titrated with standard sodium hydroxide using a *p*H meter. The curve had only one break, which was very sharp. 6.33×10^{-4} mole of methoxide had been consumed in the reaction. The solution was acidified and halide ion precipitated with silver nitrate; the weight of the precipitate showed that 6.29×10^{-4} mole of chloride ion had been formed. A separate potentiometric titration showed that the expected product of demethylation, 3-nitro-4-chlorodimethylaniline (obtained by synthesis), did not exhibit basic properties under the conditions of this titration.

Rate Measurements.—In each kinetic run the initial concentration of the aromatic compound and of sodium methoxide was 0.015 molar. The necessary amount of aromatic compound was weighed to the nearest tenth of a milligram and dissolved in magnesium-dried methanol in a volumetric flask. Just previous to each run, the required volume of standard sodium methoxide solution was added to the flask contents. In early runs the reaction mixture was made up to the mark with methanol at room temperature, and the temperature corrections for flask and pipet volumes were introduced into the rate calculations. Later reaction mixtures were made up to volume at the reaction temperature after temperature equilibration of both the reactants in methanol and the methanol diluent. After dilution, the flask was thoroughly shaken and placed in the thermostat (constant to ±0.05°).

The time of release of each pipetted sample (always one-tenth of the original reaction mixture) into standard hydrochloric acid was recorded to the nearest second, no zero time for the reactions being noted because of its irrelevance to graphic determination of the value of k. A constant volume of hydrochloric acid (always an excess) was used for the quenching of every sample in each series of runs and the excess was back-titrated with standard sodium hydroxide (*p*H meter). From six to eight samples were withdrawn in each run. Not less than three runs were made on each compound at each temperature (see Table IV).

Rate Calculations.—Calculations were based upon the equation: $1/(a - x) = kt + C$, in which a is the initial concentration of sodium methoxide in moles per liter and x is the moles per liter which have reacted in time t. The slope, calculated by the method of least squares, of the plot of $1/(a - x)$ *vs.* t for each run was taken as the value of k. Our assumption of second-order kinetics was substantiated by the straight line graphs which resulted.

In Table III we present a typical experimental record for the determination of the value of k in a particular run.

The values for the rate coefficients, obtained at 25, 35 and 45°, for the reactions of three 4-substituted-2-nitrochlorobenzenes with sodium methoxide in methanol solution are listed in Table IV.

From the equation[16a]

$$\log k = \log \frac{(ekT)}{h} - \frac{\Delta E}{2.303RT} + \frac{\Delta S^{\ddagger}}{2.303R}$$

values of ΔE were obtained by plotting values of log k against the reciprocal of absolute temperature. The three points in each plot fell virtually on a straight line. The slope, calculated by the method of least squares, was multiplied by $-2.303R$; the product was ΔE, the energy of activation. By substitution of the ΔE values so obtained, and of values of log k and of T for temperature 25° in the equation, and solving arithmetically, values of $\Delta S^{\ddagger}$, the entropy of activation, were found. These values of energy and entropy of activation are displayed in Table I.

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[CONTRIBUTION FROM THE AGRICULTURAL CHEMISTRY DEPARTMENT, PURDUE UNIVERSITY]

Crystalline Xyloheptaose[1]

BY ROY L. WHISTLER AND CHEN-CHUAN TU

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Crystalline xyloheptaose, a linear β-1 → 4 linked oligosaccharide composed of xylopyranose units, has been isolated by charcoal chromatography from among the fragments produced by the partial hydrolysis of corn cob xylan. Xyloheptaose melts at 240–242° and has $[\alpha]^{25}D$ −74°. It is shown to be a member of the xylodextrin series.

Previously there has been described the isolation and characterization of a crystalline polymer-homologous series of oligosaccharides extending without interruption from xylobiose to xylohexaose.[2,3] The series is of particular interest since the members are composed entirely of pentose sugar units and because the members may be compared to the corresponding members of the cellulose series which are identical save for possessing a primary carbinol group attached to carbon atom C-5 of each ring unit.

Members of the xylodextrin series are obtained by chromatographic separation of partially hydrolyzed xylan. By extending these techniques it is now possible to separate, in pure crystalline form, xyloheptaose. This oligosaccharide can be obtained in a state of purity only by repeated purification.

The mixture of products obtained when xylan is partially hydrolyzed is roughly separated by charcoal chromatography[4] and the fraction richest in heptasaccharide is rechromatographed on charcoal. From this second chromatographic column may be selected an eluant fraction consisting of pure xyloheptaose which can be crystallized from 75% ethanol. X-Ray data indicate that the heptasaccharide is crystalline but has a crystal lattice almost identical to that of the hexasaccharide. The substance has a melting point of 240–242° which is only slightly higher than that of xylohexaose. A plot of melting point against degree of polymerization for the known xylodextrins is shown in Fig. 1. As is the case with other polymers the melting point

(1) Journal Paper No. 648 of the Purdue Agricultural Experiment Station.

(2) R. L. Whistler and C.-C. Tu, THIS JOURNAL, **73**, 1389 (1951).

(3) R. L. Whistler and C.-C. Tu, *ibid.*, **74**, 3609 (1952).

(4) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

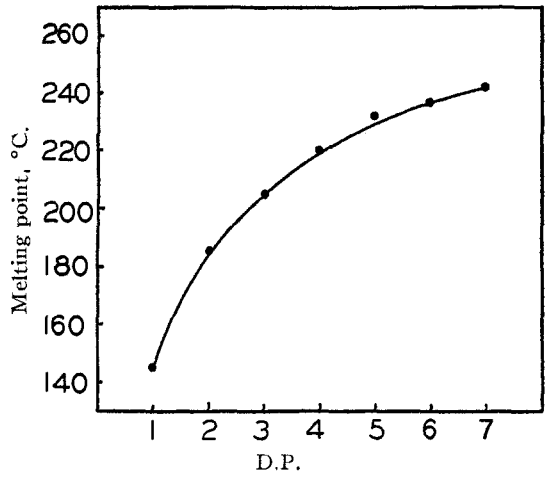

Fig. 1.—Melting point of xylodextrins *versus* degree of polymerization.

differences between members grow successively smaller with increasing D.P. so as to bring about a leveling off. In the xylodextrins the melting point levels off at a value considerably below the decomposition point of xylan. The low melting point of the oligosaccharide of 5, 6 and 7 D.P.'s may be ascribed to their crystallization as complexes with water.

A degree of polymerization of seven for the new oligosaccharide is indicated by oxidation of the reducing group with iodine, by finding the calculated reducing power after complete hydrolysis and by measurement of the formic acid produced when the intact oligosaccharide is oxidized by periodate ion.

That xyloheptaose is a true member of the polymer-homologous series from xylan is indicated by periodate oxidation and by hydrolysis to produce fragments, all of which are lower members of the series. Thus, when the oligosaccharide is slowly hydrolyzed with mineral acid and the mixture examined by paper chromatography, there is found shortly after initiation of the reaction all lower members of the series from D-xylose through to xylohexaose. As hydrolysis continues, oligosaccharides of highest D.P. disappear one after another until only D-xylose remains. For these reasons it is assumed that xyloheptaose is a linear molecule composed only of D-xylopyranose units linked in β-1 $\rightarrow$ 4 fashion.

Experimental

Partial Hydrolysis of Xylan.—Approximately 90 g. of xylan was hydrolyzed in 4.5 l. of fuming hydrochloric acid (d. 1.42) at 0° until the optical rotation indicated that two-thirds of the theoretical amount of D-xylose had been produced. The solution was then poured into a mixture of ice and sodium bicarbonate added to neutrality. The filtered solution was run onto a charcoal–Celite column[3,4] 75 × 850 mm. Some of the sugars were desorbed by washing the column successively with 60 l. of water, 24 l. of 5% ethanol and 24 l. of 15% ethanol. The column was extruded and divided into two equal portions. The lower half was discarded and the upper portion was transferred into another tube to produce a column 54 × 790 mm. This column was washed with 8 l. of water and the effluent discarded. The column was then washed successively with four 2-l. portions of 20% ethanol, four 2-l. portions of 30% ethanol and four 2-l. portions of 40% ethanol. Each effluent portion was separately evaporated to dryness under vacuum at 45°, and a sample of each dried powder was developed on a paper chromatogram. The paper strips were developed downward for 73 hours with a mixture of water–pyridine–butanol-1 in the ratio 3:5:5. The locations of the sugars were identified by spraying the strip with Tollens reagent, drying and developing the color by heating to 105° for 1–2 minutes in an oven. The yield of sugar mixture in each portion of effluent from the charcoal column and the various sugars present as indicated by paper chromatogram are shown in Table I.

TABLE I

SUGAR YIELD AND COMPOSITIONS OF ELUATES FROM A CHARCOAL COLUMN OF XYLAN HYDROLYSATE

Eluate in 2-l. portions		Dry sugar yield, %	Sugars present
20%	1	0.6	. . .
	2	0.6	X_4, X_5
	3	1.6	X_4, X_5
	4	1.2	X_5, X_6
30%	1	1.4	X_5, X_6
	2	4.3	X_5, X_6
	3	2.2	X_6, X_7
	4	1.2	X_7
40%	1	1.3	X_7
	2	1.2	X_7, X_8
	3	0.9	X_8, trace of higher members
	4	0.5	X_8, higher members

Here X_4, X_5, etc., represent xylotetraose, xylopentaose and higher oligosaccharides composed of D-xylose units. The degree of polymerization was indicated for X_4, X_5 and X_6 by comparison of the sugars with known samples.[2,3]

Crystallization of Xyloheptaose.—One gram of xyloheptaose from either the fourth 2-liter portion of 30% ethanol or the first 2-liter portion of 40% ethanol (see Table I) was dissolved in 10 ml. of water. Twenty ml. of absolute alcohol was added to the solution. The solution was warmed and filtered. Upon cooling to room temperature crystallization occurred. The yield was 0.5 g. The crystals melted at 232–234°. After twice recrystallizing from 75% ethanol, the sugar changed to brown at 236° and melted at 240–242°, $[\alpha]^{25}D$ −74° (*c* 1.01, in water).

Anal. Calcd. for $C_{35}H_{58}O_{29}\cdot 2H_2O$: C, 42.95; H, 6.35. Found: C, 42.6; H, 6.4.

Equivalent Weight of Xyloheptaose.—A 0.1066-g. sample of xyloheptaose was weighed into a 250-ml. erlenmeyer flask and dissolved in 1 ml. of water. Seven ml. of I_2 (0.0587 *N*) and 25 ml. of buffer solution (*p*H 11.3) were added simultaneously to the flask. The solution was allowed to stand for 20 minutes and then titrated with standard sodium thiosulfate solution (0.0107 *N*) as described previously.[3] Seventeen and ninety-five hundredths ml. of the thiosulfate solution was required to complete the reaction while 38.38 ml. was used for the blank. The observed equivalent weight was 485 while the theoretical value is 471. Another sample gave an equivalent weight of 480.

Hydrolysis of Xyloheptaose.—A 3% solution of xyloheptaose in 0.05 *N* hydrochloric acid was hydrolyzed at 99°. At intervals 0.1-ml. portions of the solution were neutralized with sodium bicarbonate and placed on a paper strip for chromatographic development with water–pyridine–butanol-1 (3:4:6 ratio).

The first hour sample was developed on paper for 144 hours with the above solvent mixture and showed the presence of only the lower homologs. Only seven spots were present on the paper. The first six corresponded to the series D-xylose through xylohexaose when compared with known specimens. As hydrolysis continued one after another of the oligosaccharides disappeared until finally only a D-xylose spot remained. After hydrolysis was complete the solution was neutralized with 0.05 *N* sodium hydroxide and titrated iodometrically as described before. The equivalent weight of the hydrolysate was 72 while the theoretical value is 75. The final optical rotation of the solution was also that calculated for D-xylose alone.

Periodate Oxidation.—A sample of 0.0810 g. which was sufficient to produce 10 mg. of formic acid, was dissolved in

redistilled water. The oxidation was conducted in the usual manner at 18° in the dark.

Moles of formic acid produced per mole of carbohydrate were 2.4 moles in 46 hr., 2.5 moles in 110 hr., 2.8 moles in 214 hr. and 2.9 moles in 314 hr.; moles of periodate consumed in the same times were 6.8, 7.5, 8.2 and 9.0, respectively. These are consistent with the expected values of 3 moles of formic acid produced and 9 moles of periodic acid consumed.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY COLLEGE, GALWAY, IRELAND]

The Action of Phenylhydrazine on the Periodate Degradation Products of β-D-Glucopyranosyl Sulfones

BY EILIS BLANCHFIELD AND THOMAS DILLON

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When the dialdehyde sirup obtained by the action of periodic acid on β-D-glucopyranosyl sulfone reacts with phenylhydrazine, the products are glyoxal diphenylhydrazone and benzenesulfinic acid. The reaction is an example of the reaction between the periodate oxidation products of glucosides and phenylhydrazine, discovered by Barry and the benzenesulfonic acid found in the products of the reaction is due to disproportionation of the benzenesulfinic acid.

In a recent paper under the above title, Bonner and Drisko[1] state that phenylhydrazine reacts with the periodate degradation products of pyranosyl sulfones to give glyoxal bisphenylhydrazone and benzenesulfonic acid, and to account for the latter product of the reaction they formulate a series of reactions in which phenylhydrazine acts as an oxidizing agent. It seemed to us that the reactions described were merely cases of the reaction discovered by Barry[2] in this Laboratory in 1943, in which phenylhydrazine disintegrates the periodate oxidation products of starch and of other 1,4-linked polysaccharides forming glyoxal bisphenylhydrazone. The reaction appears to be generally applicable to periodate-oxidized glucosides and indeed to all semi-diacetals of glyoxal[3] and, since there is no reason why the sulfones should be an exception, there is no necessity to assume oxidation by phenylhydrazine as part of the mechanism of the change and the presence of benzenesulfonic acid in the products must be otherwise explicable. As a matter of fact, it has long been known[4] that aqueous solutions of benzenesulfinic acid on heating to 130°, change into benzenesulfonic acid and phenylbenzene thiosulfonate, that this disproportionation takes place slowly at ordinary temperatures and that it is much accelerated by the presence of hydrochloric acid. We have actually observed that its *alkaline* solution develops an odor of thiophenol on standing for a few days. Bonner and Drisko appear to have experienced some difficulty in proving the presence of benzenesulfonic acid in their reaction products, and they were able to separate only a small fraction of the yield of this acid to be expected from their theory.

We repeated Bonner and Drisko's experiment with phenyl β-D-glucopyranosyl sulfone and, when we took the necessary precautions to avoid disproportionation, we had no difficulty in identifying benzenesulfinic acid among the reaction products. Without such precautions, however, the latter acid was not found. Like other periodate degradation products of glucosides,[3b] phenyl β-D-glucopyranosyl sulfone also reacted with hydroxylamine to give glyoxime. Here there could be no question of an oxidation.

Experimental

Preparation of Phenyl β-D-Glucopyranosyl Sulfone and Its Oxidation with Periodate.—These experiments were carried out by the methods of Bonner and Drisko,[5] yielding a clear sirup. This sirup dissolved in dilute sulfuric acid, and did not give with ferric chloride the orange precipitate characteristic of benzenesulfinic acid.[6]

Reaction with Phenylhydrazine.—0.03 g. of the above sirup was dissolved in 2.5 cc. of warm water and 0.03 cc. of phenylhydrazine was added, followed by sufficient glacial acetic acid to bring it into solution. The mixture was left aside for 5 days at room temperature. The color of the liquid changed from yellow to red, and crystals of glyoxal bisphenylhydrazone gradually separated. These were filtered off and the filtrate was brought to *p*H 12 and extracted 14 times with ether, to remove excess phenylhydrazine. A portion of the remaining alkaline solution was neutralized with strong hydrochloric acid and then made acid with sulfuric acid. When a concd. soln. of ferric chloride was added, the orange precipitate characteristic of benzenesulfinic acid fell out.

The rest of the alkaline solution was left aside and after about 48 hours it smelled strongly of thiophenol. A little of it acidulated and tested with ferric chloride now gave no precipitate. On further standing on a watch glass, the alkaline solution deposited crystals of sodium salts. These were decomposed with a little sulfuric acid and extracted with cold water, in which benzenesulfinic acid is sparingly soluble. The aqueous solution on evaporation deposited crystals, m.p. 44°, evidently benzenesulfonic acid. The undissolved residue was extracted with ether, in which benzenesulfinic acid is easily soluble. On evaporation, the ether solution left a minute amount of crystalline material.

One gram of the sirup was dissolved in 75 cc. of water and to the solution 1.65 cc. of phenylhydrazine added, followed by sufficient glacial acetic acid to bring it into solution. This mixture was heated on the boiling water-bath for 30 min., as in Bonner and Drisko's experiment. On cooling, crystals of glyoxal bisphenylhydrazone separated and the mixture was extracted 15 times with 20 cc. of ether each time. The extracted liquid was brought to *p*H 12 and again extracted several times with ether. It was then neutralized with hydrochloric acid, acidulated with sulfuric acid and treated with ferric chloride, whereupon a heavy orange precipitate fell out. This was filtered off, treated with excess of ammonia and filtered from ferric hydroxide. When the filtrate was acidulated with hydrochloric acid a

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