[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Effect of Ionizing Radiation on Carbohydrates. The Irradiation of Sucrose and Methyl α-D-Glucopyranoside[1]

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The effects of cathode radiation on 50% aqueous solutions and powdered samples of sucrose and methyl α-D-glucopyranoside are reported. The extent of hydrolysis of aqueous sucrose (based on the amount of invert sugar formed) increased with increasing irradiation dosage and at 104 megareps was 22.2, 27.0 and 37.8% while being cooled with ethanol–(solid carbon dioxide), ice and water, and ambient air, respectively. Evidence was obtained that about 10% of the sucrose (at all three temperatures) was transformed into non-reducing substances. The product of irradiated 50% aqueous sucrose contained D-glucose and D-fructose, the latter being identified by paper chromatography and the former by an isolated derivative. Powdered sucrose was irradiated at ambient air and ice-water temperatures. The extent of hydrolysis of powdered sucrose as a function of dosage reached a maximum at an intermediate dose. This effect was more pronounced in the samples cooled with ambient air. Powdered and 50% aqueous samples of methyl α-D-glucopyranoside, irradiated with 104 megareps at ice-water temperature, hydrolyzed to the extent of 3.3 and 6.3%, respectively (based on conversion to D-glucose). Evidence from paper chromatography indicated the formation of substantial amounts of D-glucose. *G* (invert sugar) values for irradiated sucrose and *G* (glucose) values for irradiated methyl α-D-glucopyranoside were calculated.

In order to obtain further understanding of the processes occurring when organic materials are exposed to ionizing radiations, a number of samples of carbohydrates were irradiated with cathode rays and the products were examined for gross physical and chemical changes.[2] The production of reducing sugars was noted when sucrose was acted on by high-speed electrons (cathode rays). Paper chromatography of the irradiation product indicated that inversion (hydrolysis) had taken place. This is in agreement with early workers[3] who observed that inversion occurred when aqueous sucrose solutions were exposed to X-rays. This preliminary work suggested that the glycosidic bond might be especially sensitive to ionizing radiations. In the work herein reported, a study was made of the effects of cathode rays on powdered and 50% aqueous solutions of sucrose and methyl α-D-glucopyranoside.

Samples of sucrose, as powder and as 50% aqueous solutions, were exposed to cathode rays of varying doses at three different temperatures: ambient air, ice-water and ethanol–(solid carbon dioxide). The results, which are plotted in Figs. 1 and 2, are based on copper-reduction values. In these graphs, the apparent percentage sucrose hydrolyzed against dose and *G* (invert sugar) against dose are delineated. These values may not necessarily indicate hydrolysis for the sucrose irradiated in the solid form. The data for the irradiated aqueous sucrose solutions, given in Table I, are based on the differences in copper-reduction values before and after the hydrolysis of the original, unchanged sucrose. The extent of hydrolysis of 50% aqueous solutions of sucrose (based on the amount of invert sugar formed) was found to increase with increased irradiation (Fig. 1). The greatest hydrolysis was found in samples which were cooled in their frozen condition with an ethanol–(solid carbon dioxide) bath during irradiation. The least hydrolysis was observed in samples irradiated at ambient temperature. Evidence was obtained that about 10% of the original sucrose, at all three temperatures of irradiation, had been altered to non-reducing substances. This can be seen from a summation of values under columns C and D for each temperature of irradiation (Table I); the quantities are very nearly the same for the three temperatures and represent about 90% of the total potential reducing power of the original sucrose. Relative to this, it has been found in our laboratory that D-fructose is much more susceptible to cathode radiation than is D-glucose.[2] Another interesting aspect regarding the (cathode ray)-induced decomposition of sucrose is the fact that the greatest value for the ratio of reducing substance found to altered sucrose also occurred at the temperature of ethanol–(solid carbon dioxide). This can readily be seen by comparing the values under columns C and (100 - D)(Table I) and might be interpreted to mean that the decomposition of the reducing substances formed as a result of glycosidic cleavage took place at the highest rate at ambient temperature.

D-Glucose and D-fructose were the only copper-reducing substances detected in the irradiated aqueous sucrose solutions. The evidence for D-glucose was obtained from its paper chromatographic behavior (Table II) and from a comparison of the melting point and the X-ray powder diffraction pattern of the derived pentaacetate with that of an authentic specimen of β-D-glucopyranose pentaacetate. The pentaacetate was obtained in good yield corresponding to 37% of the monosaccharide fraction of the irradiated product. The evidence for D-fructose is not as definitive and is based on paper chromatography (Table II). The spots coinciding with D-fructose were less intense in color and smaller in area than the corresponding D-glucose spots, supporting the previous findings that fructose is more sensitive to the action of ionizing radiations than is glucose. In consideration of the large amount of D-glucose recovered from the irradiated sucrose samples, it would then appear that these results establish the selective hydrolysis of the glycosidic linkage in the

(1) This paper represents research undertaken in coöperation with the Quartermaster Institute for the Armed Forces under Contracts No. DA44-109-qm-1772 and No. DA-19-QM-515 with The Ohio State University Research Foundation (Projects 597 and 661), and has been assigned number 690 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the view or indorsement of the Department of Defense. Preliminary communication: *Abstracts Papers Am. Chem. Soc.*, **130**, 16A (1956).

(2) M. L. Wolfrom, W. W. Binkley, L. J. McCabe, (Mrs.) T. M. Shen Han and A. M. Michelakis, *Radiation Research*, in press.

(3) M. C. Reinhard and K. L. Tucker, *Radiology*, **12**, 151 (1929).

TABLE I

DETERMINATION OF RESIDUAL SUCROSE AFTER CATHODE-RAY IRRADIATION OF ITS 50% AQUEOUS SOLUTION WITH 104 MEGAREPS[a]

Coolant during irradiation	Copper reducing values of irrad. sucrose, ml. 0.005 *N* Cu/mg. prod. Before acid hydrolysis (A)	After acid hydrolysis (B)	Apparent reducing sugars in irradiated products, % (B − A)	(C)	Sucrose in irradiated products, % (D)	Total sugars in irradiated products, % (C + D)	Sucrose altered, % (100 − D)	Apparent sucrose altered (copper reduction basis), %
Ambient air	1.71	6.86	5.15	23.2	66.4	89.6	33.6	22.3
Ice–water	2.22	6.93	4.71	30.1	60.7	90.8	39.3	29.0
Ethanol–solid CO_2	2.91	6.77	3.86	39.5	49.8	89.3	50.2	38.3

[a] The irradiation was delivered at 8 intermittent doses each of 13 megareps at the rate of 6.5 megareps per min.

TABLE II

PAPER CHROMATOGRAPHIC DATA FROM CATHODE-RAY IRRADIATED SAMPLES OF SUCROSE AND METHYL α-D-GLUCOPYRANOSIDE

Carbohydrate	Irradiation dosage, megareps	State of sample	Coolant	R_g
Sucrose	104	50%[b]	Ethanol–solid CO_2	0.72, 1.00, 1.18
Sucrose	104	50%[b]	Ice–water	.72, 1.00, 1.17
Sucrose	104	50%[b]	Ambient[a]	.72, 1.00, 1.17
Methyl α-D-glucopyranoside	104	50%[b]	Ice–water	1.00
	104	Powder	Ice–water	1.01
Sucrose	0	....	.....	0.72
D-Glucose	0	....	.....	1.00
D-Fructose	0	....	.....	1.17

[a] *Ca.* 27°. [b] Aqueous solution.

irradiation of sucrose with cathode rays. The isolative work was performed in samples of aqueous sucrose exposed to doses much higher than that (about 3 megareps) required for sterilization; however, from the relationship observed on plotting apparent sucrose hydrolyzed against radiation dosage (Fig. 1) an extrapolation to the sterilization range is clearly feasible.

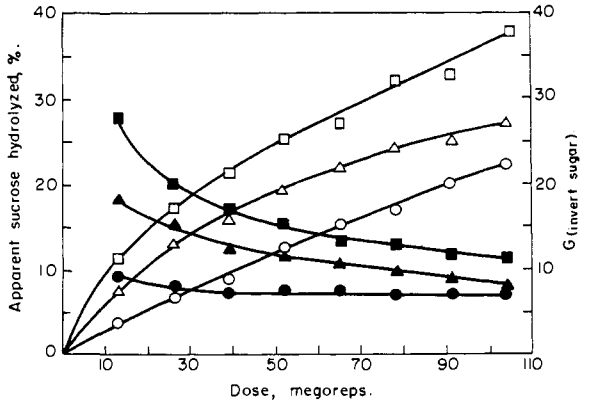

Fig. 1.—Irradiation of sucrose in 50% aqueous solution by cathode rays at the rate of 6.5 megareps per minute. For apparent % sucrose hydrolyzed against dose: □, cooled with ethanol and solid carbon dioxide; △, cooled with ice and water; ○, ambient air temperature (about 27°). For $G_{(\text{invert sugar})}$ against dosage: ■, cooled with ethanol and solid carbon dioxide; ▲, cooled with ice and water; ●, ambient air temperature (about 27°).

The investigation of the effects of cathode rays on powdered sucrose was centered on the determination of copper-reducing values. A gross physical examination of irradiated powdered sucrose was reported previously.[2] In contrast to the results from the irradiation of aqueous sucrose solutions, the percentage hydrolysis of powdered sucrose as a function of radiation dosage increased to a maximum at 26 megareps and then decreased at higher dosages. This effect was found to be more pronounced in the results obtained at ambient temperatures. Since the percentage hydrolysis was based on conversion to reducing products, the high peak at 26 megareps could be indicative of destruction of the radiation products which were

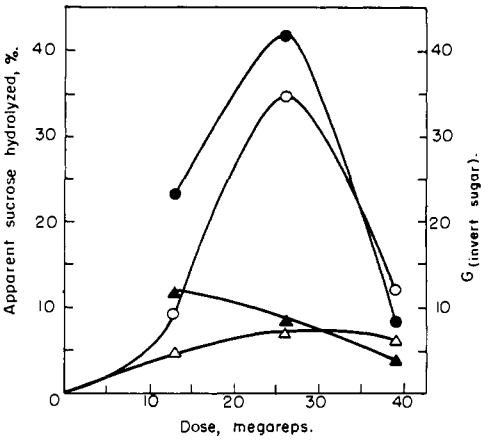

Fig. 2.—Irradiation of powdered sucrose by cathode rays at the rate of 6.5 megareps per minute. For apparent % sucrose hydrolyzed against dose: ○, cooled with ambient air; △, cooled with ice and water. For $G_{(\text{invert sugar})}$ against dose: ●, ambient air temperature; ▲, cooled with ice and water.

reducing. This is quite likely since glucose and fructose (especially), which are probable reducing products in the irradiation of sucrose, are radiation sensitive. An analogy to this can be found in the work of Saeman,[4] who found that a peak in the acid hydrolysis of cellulose was due to the destruction of glucose. Irradiated powdered sucrose was pink in color, and showed a strong signal (as did irradiated methyl α-D-glucopyranoside) when subjected to electron paramagnetic resonance spectroscopy.[5] A recently initiated investigation[6] of irradiated

(4) J. F. Saeman, *Ind. Eng. Chem.*, **37**, 43 (1945); J. F. Saeman, Janet L. Bubl and E. L. Harris, *Ind. Eng. Chem., Anal. Ed.*, **17**, 35 (1945).

(5) This electron paramagnetic resonance spectroscopy was performed by Dr. J. P. O'Meara of the Southwest Research Institute, San Antonio, Texas.

(6) In cooperation with Professor Dudley Williams and Mr. J. E. Geusic, Department of Physics, The Ohio State University; signals were detected at dosages as low as 250,000 reps.; *cf.* D. Williams, J. E. Geusic, M. L. Wolfrom and L. J. McCabe, *Proc. Natl. Acad. Sci. U. S.*, **44**, 1128 (1958).

powdered sucrose by paramagnetic resonance spectroscopy indicates that several radical entities exist in the irradiation product.

Further evidence, which may be taken in support of the selective action of ionizing radiation on glycosidic bonds, can be found in the effects observed with methyl α-D-glucopyranoside. Samples of methyl α-D-glucopyranoside, as powder and as 50% aqueous solutions, were exposed to cathode radiation in doses ranging from 13–104 megareps while being cooled in an ice–water-bath. The results, which are plotted in Fig. 3, are based

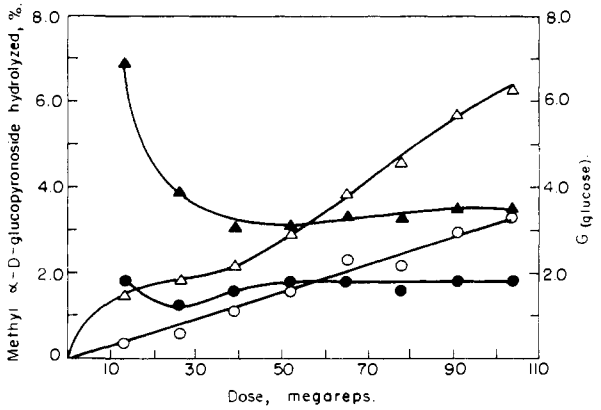

Fig. 3.—Irradiation of methyl α-D-glucopyranoside by cathode rays at the rate of 6.5 megareps per minute. For % methyl α-D-glucopyranoside hydrolyzed against dose: △, 50% aqueous form; ○, powdered form. For $G_{(glucose)}$ against dose: ▲, 50% aqueous form; ●, powdered form.

on copper-reduction values. In this graph the apparent percentage methyl α-D-glucopyranoside hydrolyzed against dose and *G* (glucose) against dose are delineated. At 104 megareps, 3.3 and 6.3% of the powdered and aqueous methyl α-D-glucopyranoside samples, respectively, were hydrolyzed. Evidence from paper chromatography indicated the presence of substantial amounts of D-glucose. In comparison to sucrose, methyl α-D-glucopyranoside was found to be more resistant to hydrolysis under the same experimental conditions. This observation is in accord with results from acid hydrolysis[7,8] and ultraviolet irradiation,[9,10] all of which are in support of a more labile glycosidic bond in sucrose than in methyl α-D-glucopyranoside.

The calculation[11] of the *G*-values was based on the apparent percentage sugar hydrolyzed. In the case of sucrose (*G*(invert sugar)) the percentage hydrolysis was based on the amount of invert sugar formed (copper reduction method), while for methyl α-D-glucopyranoside (*G*(glucose)) the percentage hydrolysis was based on conversion to D-glucose (copper reduction method). In evaluating the significance of the *G*-values it must be remembered that no allowance was made for the non-reducing substances formed. Results given in Table I show that at a dosage of 104 megareps, at three different irradiation temperatures, approximately 10% of sucrose was changed to non-reducing products. Using the data of Saeman, Millett and Lawton[12] and 32 e.v. per ion pair, one obtains a *G*-value of about 9 for the destruction of carbohydrates in the irradiation of cellulose. This would be in reasonable agreement with our values for irradiated aqueous sucrose at the higher doses. The high *G* (invert sugar) values we obtained for aqueous sucrose at the low doses could be due to an oxygen effect or some indirect effect. The *G* (invert sugar) values for the irradiated powdered crystalline sucrose were different from those for the aqueous solutions. A difference would be expected since the primary reaction in the irradiation of aqueous sucrose is probably on the water, while in the case of the powdered sample, attack is probably directly on the sugar. A maximum *G* (invert sugar) value was found at an intermediate dose in the solid sucrose irradiated at room temperature. Since G (invert sugar) is based on the amount of invert sugar formed, the peak at an intermediate dose could be due to destruction of irradiation products, and, as pointed out previously, glucose and fructose, which could be formed in the radiation process, are radiation sensitive. The *G* (glucose) values for irradiated aqueous methyl α-D-glucopyranoside are less than the *G* (invert sugar) found in the sucrose series. This is not unexpected, since the glycosidic bond in sucrose is more labile than the glycosidic bond in methyl α-D-glucopyranoside.[7–10]

Although it is recognized that there are basic differences in the effects of ultraviolet and ionizing radiations, evidence is taken from the literature for an example of an analogous effect. This is the apparent selective hydrolysis of the glycosidic linkage of some carbohydrates by ultraviolet irradiation.[9,10,13,14] The postulation of a mechanism for the (cathode ray)-induced cleavage of the glycosidic bond would hardly be in order from the presented data; however, the similarity in results with those from the ultraviolet irradiation of glycosides, the high yield of D-glucose from the irradiated mixture, the high *G*-values, the observation of stable, free radicals in the irradiated powdered methyl α-D-glucopyranoside and powdered sucrose samples, increased fluorescence in the irradiated powders,[2] and the comparable hydrolysis of samples as powder and as aqueous solutions, are all consistent with the postulation of the formation of activated molecules or stable free radicals, which, on coming in contact with water molecules, split at the most labile bond which appears here to be the glycosidic bond. The rather high degree of hydrolysis might possibly be accounted for by an intramolecular transfer of absorbed energy on the monosaccharide residues to the acetal oxygen bridge. The evidence for the transfer of energy along a carbon chain to ulti-

(7) R. F. Jackson and C. L. Gillis, *Sci. Papers Natl. Bur. Standards*, **16**, 141 (1920).

(8) H. S. Isbell and Harriet L. Frush, *J. Research Natl. Bur. Standards*, **24**, 125 (1940).

(9) A. Guillaume and G. Tanret, *Compt. rend.*, **201**, 1057 (1935).

(10) A. Guillaume and G. Tanret, *Bull. soc. chim. biol.*, **18**, 556 (1936).

(11) M. Burton, *J. Phys. Colloid Chem.*, **51**, 611 (1947).

(12) J. F. Saeman, M. A. Millett and E. J. Lawton, *Ind. Eng. Chem.*, **44**, 2848 (1952).

(13) F. Lieben, L. Lowe and B. Bauminger, *Biochem. Z.*, **271**, 209 (1934).

(14) G. Tanret, *Compt. rend.*, **202**, 881 (1936).

mately effect a reaction further down the chain can be found in the investigations of Carpenter[15,16] and of Mandl and McLaren,[17] who, in order to explain their experimental results on the ultraviolet irradiation of certain peptides, postulated that the energy absorbed by the aromatic nucleus of the amino acid travels along a chain of at least two carbon atoms and one nitrogen atom, or a chain of three carbon atoms, to eventually rupture the molecule at the peptide bond. The same phenomenon has been postulated by Heidt[18] to explain results obtained in the action of ultraviolet light on some glycosides. A more rigorous treatment of the data and the postulation of possible mechanisms for this reaction must await further work.

Experimental

Irradiation Source.—The high-energy electron beam (cathode rays) was supplied by a resonant transformer in conjunction with a cathode ray tube. The source utilized was a 1 mev. peak, 500-μa. beam-out unit.[19] The dose was measured by a specially constructed, air ionization chamber. This unit was located at the General Electric Co., Milwaukee, Wisc. The necessary volume of water was added to each sample just prior to irradiation. In all cases the sample thickness was about 1 mm. The irradiated samples were returned to this Laboratory by air mail where they were placed in a deep-freeze refrigerator (about $-15°$) to await further processing. The samples exposed to cathode rays were irradiated at varying doses. Unless otherwise indicated, the dose rate was 6.5 megareps per min.

The **Irradiation of Sucrose with Cathode Rays.**—Samples of 50% aqueous solutions of sucrose[20] were irradiated in open aluminum containers (5.9 cm. diam.) with high-energy electrons in dosages ranging from 13×10^6 to 104×10^6 reps obtained by 1 to 8 intermittent passes of 13 megareps each. These irradiations were carried out at three different temperatures: ambient, ice-water and ethanol–(solid carbon dioxide). The last-named condition involved the irradiation of a solid solution. The irradiated samples were lyophilized and dried at reduced pressure over phosphorus pentoxide for a period of 1 week. Their degrees of apparent hydrolysis were determined by the copper reduction method of Somogyi[21] (Fig. 1); 1 mg. of invert sugar reduced 7.5 ml. of 0.005 *N* Cu.

Powdered sucrose samples (2 g.) were irradiated in air in open aluminum containers at ambient temperature. These samples were subjected to 1 to 3 intermittent doses, each of 13 megareps. A duplicate series was run at the temperature of an ice–water-bath. The irradiated samples were dried under reduced pressure over phosphorus pentoxide at 25° and their copper-reduction values were determined as before (Fig. 2). Because of the unusual trend noted in the apparent percentage hydrolysis of the samples of powdered sucrose irradiated at ambient air temperature, another series was prepared and irradiated as described above. The apparent percentage hydrolysis of this series (powdered sucrose irradiated at ambient air temperature) was in agreement with that calculated for the initial series, maximum hydrolysis being observed at a dose of 26 megareps.

In order to determine the amount of unaltered sucrose after irradiation, a series of 50% aqueous sucrose solutions were irradiated as described previously. The irradiated samples were lyophilized and subsequently dried at reduced pressure over phosphorus pentoxide at approximately 20°. The copper-reduction values were determined as before and then on the hydrolyzed samples, the hydrolysis being effected by treatment with 0.1 *N* hydrochloric acid for 100 min. at 60° (Table I).

A paper chromatogram was prepared, according to the method of Partridge,[22] of the solids recovered from the 50% aqueous sucrose samples which had been irradiated (104 megareps) at the temperatures of ambient air, ice-water and ethanol–(solid carbon dioxide); 2% aqueous solutions of the recovered solids were prepared, and five applications of each solution were made at designated positions 8 cm. from the unpointed end of a 14×45 cm. sheet of Whatman No. 1 filter paper. The sheet was placed in all-glass chamber equipped for paper chromatography and developed with 1-butanol–ethanol–water (40:11:19 by vol.). The chromatogram was dried in air at 20–25° and sprayed with *p*-anisidine hydrochloride in 1-butanol. The sprayed chromatogram was heated for 10 min. in moist air at 100° in order to produce the brown spots indicative of the sugars. Three spots were observed on the developed paper chromatogram, the mobilities of which were found to coincide with those produced by known samples of D-glucose, D-fructose and sucrose (Table II).

One gram samples of 50% aqueous sucrose which had received 78, 91 and 104 megareps of irradiation at the temperature of ethanol and Dry Ice were blended and dissolved in 12 ml. of absolute methanol. In order to separate unaltered sucrose from the irradiation products, the methanolic solution was nucleated with sucrose and maintained at -5 to 0° for 18 hr. Crystals were obtained which were washed with a cold alcoholic solution (methanol–ethanol, 4:1 by vol.). The crystals were dried at 20–25° over phosphorus pentoxide at reduced pressure; yield 0.48 g. The solvent from the residual material was removed by distillation under reduced pressure and the residue (2.36 g.) was dissolved in 50 ml. of water. The resulting solution was chromatographed, according to the method of Whistler and Durso,[23] on a 170×45 mm. (diam.) carbon column containing 100 g. of an adsorbent mixture composed of equal parts (by wt.) of Darco G-60[24] and Celite.[25] The column was prewetted before use with 1500 ml. of water. The chromatogram was developed with 1600 ml. of water to elute the monosaccharide fraction from the column. Further development with 3000 ml. of 5% ethanol eluted the disaccharide fraction. The monosaccharide and the disaccharide fractions were each concentrated under reduced pressure to 50 ml. The solutions were filtered and the filtrates were lyophilized and subsequently dried at reduced pressure over phosphorus pentoxide at 20–25°. The yields of the monosaccharide and the disaccharide fractions were 0.91 and 1.34 g., respectively.

An amount of 0.69 g. of the above monosaccharide fraction and 0.2 to 0.4 g. of freshly fused zinc chloride were placed in a 100-ml. round-bottomed flask equipped with a mechanical stirrer. The flask and contents were kept at 0 to 2° during the addition of 20 ml. of acetic anhydride and for the following 17 hr. Because the reactants had not dissolved completely, the reaction temperature was increased to 50° at which temperature the acetylation mixture became homogeneous within 1 hr. The acetylation reaction was allowed to proceed for an additional hour at the same temperature. The cooled reaction mixture was poured onto 50 g. of finely crushed ice and stirred until homogeneous. The solution was partially neutralized with sodium bicarbonate and was then diluted with 100 ml. of water to facilitate the subsequent solvent extraction. The acetylated products were recovered from the partially neutralized solution by extraction with one 20-ml. portion and three 15-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure at 50° to a solid; yield 1.31 g.

The acetylated product (0.65 g.) was dissolved in 25 ml. of benzene and chromatographed on a 35 (diam.) $\times$ 180 mm. column of Magnesol[26]–Celite[25] (5:1 by wt.) which had been prewetted with 35 ml. of benzene. The chromatogram was developed with 800 ml. of a benzene–ethanol mix-

(15) D. C. Carpenter, *J. Franklin Inst.*, **232**, 76 (1941).
(16) D. C. Carpenter, THIS JOURNAL, **62**, 289 (1940).
(17) Inez Mandl and A. D. McLaren, *Nature*, **164**, 749 (1949).
(18) L. J. Heidt, *J. Franklin Inst.*, **234**, 473 (1942).
(19) J. A. Knowlton, G. R. Mahn and J. W. Ranftl, *Nucleonics*, **11**, No. 11, 64 (1954).
(20) Recrystallized commercial sucrose.
(21) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).
(22) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).
(23) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).
(24) An activated carbon produced by the Atlas Powder Co., New York, N. Y.
(25) No. 535, a diatomaceous filter-aid produced by the Johns–Manville Co., New York, N. Y.
(26) A synthetic hydrated magnesium acid silicate produced by the Westvaco Chemical Division of the Food Machinery and Chemical Corp., South Charleston, W. Va.

ture (benzene–ethanol, 500:1 by vol.). The extruded column was streaked with a freshly prepared solution of alkaline potassium permanganate (0.1 g. of potassium permanganate, 1.0 g. of sodium hydroxide and 10 ml. of water). Four zones were detected and subsequently eluted from the sectioned adsorbent with acetone. The locations and yields of material from these zones were as follows: top zone, 0–5 mm. (distance from the top of the column), 53 mg.; zone one, 34–42 mm., 93 mg.; zone two, 54–65 mm., 93 mg.; zone three, 83–113 mm., 276 mg.; interzone one, 5–34 mm., 71 mg.; and interzone two, 113–132 mm., 3 mg. The total yield was 589 mg. The material in zone three crystallized. This material (200 mg.) was rechromatographed on a Magnesol column with 100 ml. of benzene–1-butanol (500:1 by vol.) as developer. The column was extruded, streaked, and eluted in the manner described previously. The zone material crystallized; yield 192 mg., m.p. 106–109° uncor. An X-ray powder diffraction pattern of the crystalline derivative was obtained using filtered CuKα radiation. The resulting pattern was compared and found to be identical with that of an authentic sample of α-D-glycopyranose pentaacetate.[27]

The Irradiation of Methyl α-D-Glucopyranoside.—Powdered samples of methyl α-D-glucopyranoside[28] were subjected to 1 to 8 intermittent dosages of irradiation, each of 13 megareps. During irradiation the samples were cooled by an ice–water-bath. The irradiated samples were dried under reduced pressure over phosphorus pentoxide at 20–25° for a period of 2 weeks. The degree of apparent hydrolysis (based on conversion to glucose) was determined by the copper-reduction method of Somogyi[21] (Fig. 3).

(27) M. L. Wolfrom and H. B. Wood, THIS JOURNAL, **71**, 3175 (1949).

(28) A product of the Corn Products Refining Co., Argo, Illinois. This material was recrystallized from ethyl alcohol before use.

Samples of 50% aqueous mixtures of methyl α-D-glucopyranoside were irradiated as described above. Lyophilization of the irradiated samples yielded thin, brittle films which were ground into powders and dried under reduced pressure over phosphorus pentoxide at 20–25°. The degree of apparent hydrolysis was determined as described above and the data plotted in Fig. 3.

A descending paper chromatogram was made of two samples of irradiated methyl α-D-glucopyranoside in order to test for the presence of sugars. One of the two samples had been irradiated as a powder while the other had been irradiated as a 50% aqueous solution. Both samples had received 104 megareps of cathode irradiation while being cooled by an ice–water-bath. Aqueous solutions (2%) were prepared from the dried, irradiated samples and applied to a paper chromatogram according to previous directions. The chromatogram was developed for 40 hr. with 1-butanol–ethanol–water (40:11:19 by vol.), air-dried, and sprayed with *p*-anisidine hydrochloride. An examination of the developed paper chromatogram (Table II) revealed a single spot which was found to have an R_g-value[29] of unity.

Calculation of *G*-Values.—The *G*-values (the number of molecules decomposed per 100 e.v. of energy absorbed) were calculated as

$$G = \frac{(\text{fraction of sugar hydrolyzed})(6.02 \times 10^{23})(100)}{(\text{mol. wt. sugar})(\text{dose in megareps})(56 \times 10^{18}\ \text{e.v./g.-megareps})}$$

Acknowledgment.—The assistance of Dr. D. S. Miyada in preparing this manuscript is acknowledged.

(29) A ratio of the relative mobility of the substance to D-glucose.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

2,4-Dinitrophenyl Ethers of the Alditols[1]

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The facile formation of the fully substituted 2,4-dinitrophenyl ethers of a number of alditols and of *myo*-inositol has been demonstrated. These stable, crystalline derivatives possess sharp, characteristic melting points. Although several alternate methods for their preparation were explored, the method of choice involves the room temperature reaction of 2,4-dinitrofluorobenzene with the polyhydroxy compound in *N*,*N*-dimethylformamide solution in the presence of triethylamine.

Although the 2,4-dinitrophenyl ethers of a great number of monohydric alcohols and phenols have been described,[2,3] very little effort has been directed to the preparation of similar fully substituted derivatives of the polyhydric alcohols. Whalley[3] prepared the bis-(2,4-dinitrophenyl) ether of ethylene glycol, and the mono-(2,4-dinitrophenyl) ethers of ethylene glycol and glyceritol were synthesized by Blanksma and Fohr.[4] The extension of similar techniques to the preparation of fully substituted 2,4-dinitrophenyl ethers of polyhydroxy substances was undertaken in the hope that crystalline derivatives, useful for identification, would result or that the reagent of choice, 2,4-dinitrofluorobenzene, might exhibit selective reactivity toward different types of hydroxyl groups.

The reaction of a number of polyhydric alcohols (Table I) with 2,4-dinitrofluorobenzene dissolved in *N*,*N*-dimethylformamide occurred readily at room temperature. The addition of a base, triethylamine (preferable), alkali carbonate or bicarbonate was necessary. Otherwise, no ether derivative could be detected, even after extended reaction times, and the polyhydric alcohol could be recovered in high yield. The use of a 10% molar excess of 2,4-dinitrofluorobenzene sufficed to produce the fully substituted pure alditol ethers in 20 to 80% yields. Larger molar excesses of reagent would undoubtedly improve these yields but the reagent is relatively expensive. Slightly lower yields were obtained when the inorganic bases were employed. These bases reacted with the 2,4-dinitrofluorobenzene to form the alkali 2,4-dinitrophenoxide and were less effective than the tertiary amine in promoting the dissolution of the alditol. If the relative amount of 2,4-dinitrofluorobenzene and the reaction time were increased, equally good yields resulted on employing the

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