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A FUNDAMENTAL STUDY OF THE PHOTOCHEMISTRY OF CARBOHYDRATES, CELLULOSE, AND RELATED COMPOUNDS

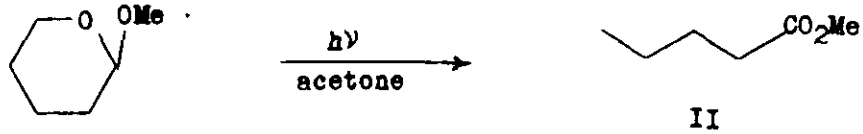
SUMMARY

The photochemical reactions of 2-methoxytetrahydropyran (I) in the presence of two different hydrogen atom abstractors was investigated as a model system for carbohydrate degradation. Irradiation of I in the presence of acetone produced methyl valerate by way of a ring opening reaction. Irradiation of I in the presence of N-bromosuccinimide gave, in addition to the analogous methyl 5-bromovalerate, methyl 2,5-dibromovalerate and methyl 2,2,5-tribromovalerate. Two different pathways for these additional photoproducts are considered. Initial attempts to choose have not eliminated either pathway, but it is hoped that additional experimental work will allow a choice.

RESULTS AND DISCUSSION

The photochemistry of carbohydrates is complicated by the polyfunctionality of the compounds. Almost all of the literature work has involved irradiations in the presence of oxygen, which has led to extensive degradation. Since the carbohydrates have no appreciable chromophore (1), a reasonable explanation for the photochemistry observed is that carbonyl containing impurities function as hydrogen atom abstractors and in the presence of oxygen, extensive oxidation takes place.

In order to examine this reaction in more detail, the photochemistry of a model glycoside system was studied in the presence of hydrogen atom abstractors. Thus, irradiation of a mixture of acetone and 2-methoxytetrahydropyran (I) through pyrex gave methyl valerate (II) as the major product along with smaller amounts of less volatile materials, most of which could be attributed to acetone degradation products. The reaction

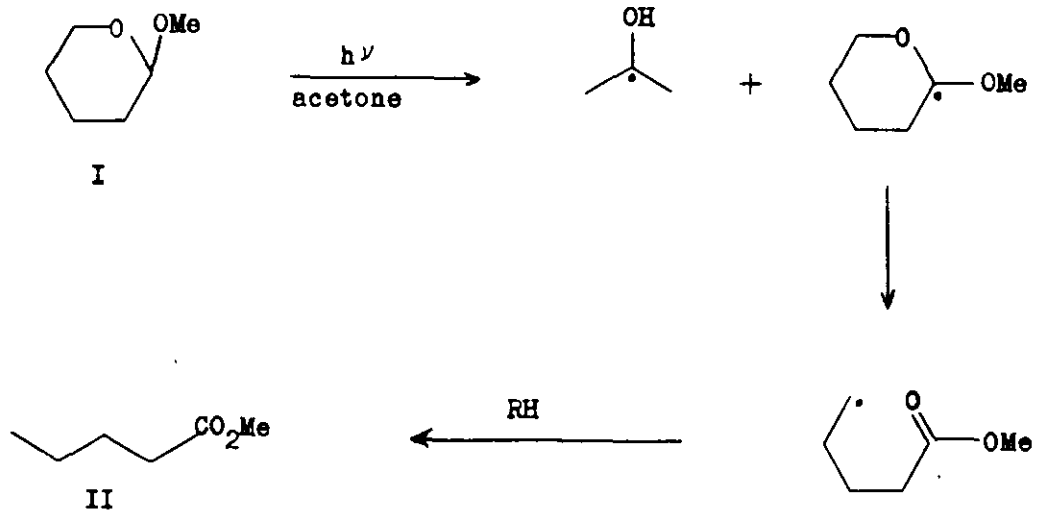


I II

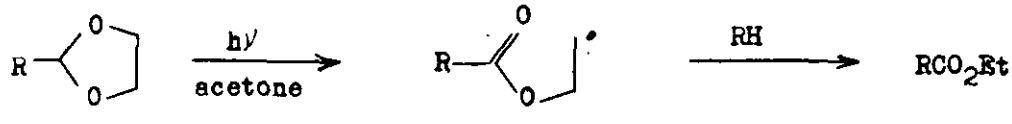
was relatively slow, requiring 22 hours for approximately 30% conversion. The most reasonable mechanism is shown in Scheme I. Hydrogen atom abstraction by excited acetone would be expected to be most efficient at the 2-position since this gives a radical stabilized by two adjacent oxygens. If this radical picks up a hydrogen atom from the system, then starting material is regenerated and no net reaction is observed. If, however, the radical

rearranges by cleavage of the C-6 carbon-oxygen bond, then an ester function is generated with a radical center at the other end of the chain. This radical could then pick up a hydrogen atom from the system and produce the observed product, methyl valerate (II).

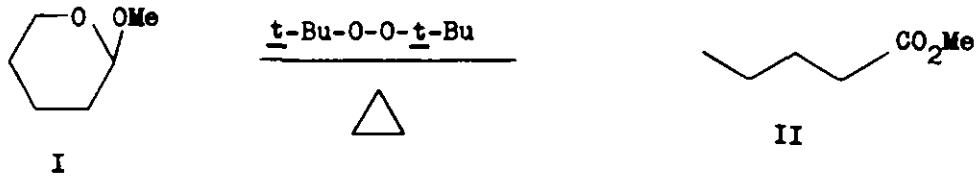
Scheme I. Mechanism for the Formation of Methyl Valerate From 2-Methoxy-tetrahydropyran



Such a process is not without precedent. Elad and Youssefyeh (2) found that irradiation of mixtures of acetone and cyclic ketals, such as III, gave acyclic esters in yields of 14-55%. A similar mechanism was proposed, although in their system the rearranged radical center is formed on the "alcohol" portion of the ester.

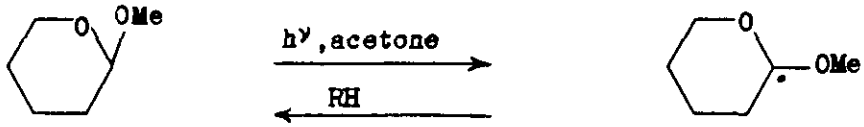


The same process can be promoted thermally. Thus Huyser and Garcia found that treatment of I with di-t-butyl peroxide at 120° also gave methyl valerate (II), presumable by the same mechanism (3).



I II

The initial hydrogen abstraction step is, at least in principle, reversible. Also, the radical formed can abstract a hydrogen atom from some

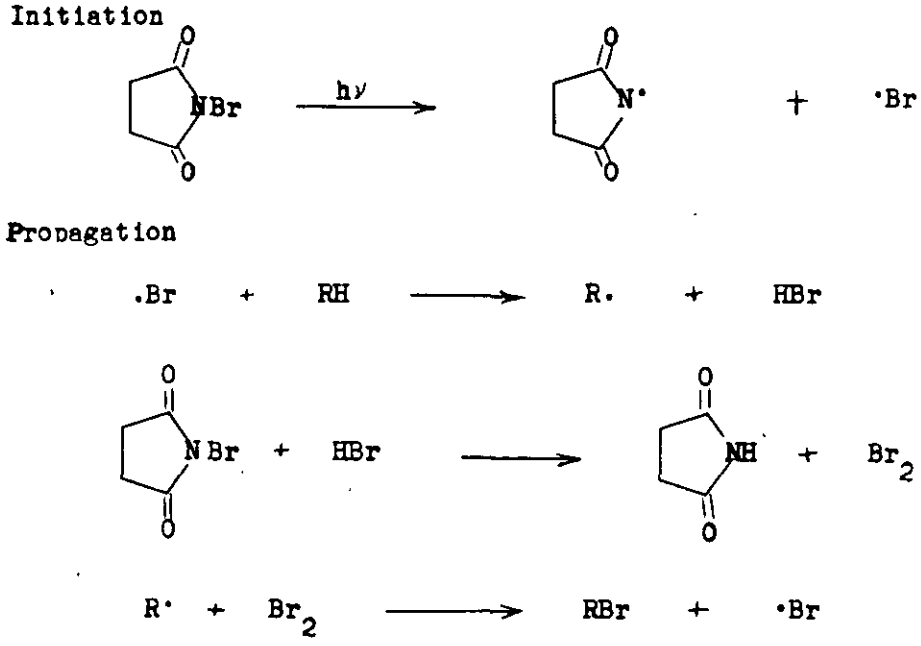


I

other source. If this happens before some other competing process, then starting material is regenerated and no reaction is observed. Thus, it is possible that hydrogen atom abstraction takes place at other positions in I and that it is not detected because either no rearrangement is available, or the rate of rearrangement is slow relative to reversion to starting material. In order to overcome this difficulty, the use of a different hydrogen abstractor which also functions as a radical trap was examined.

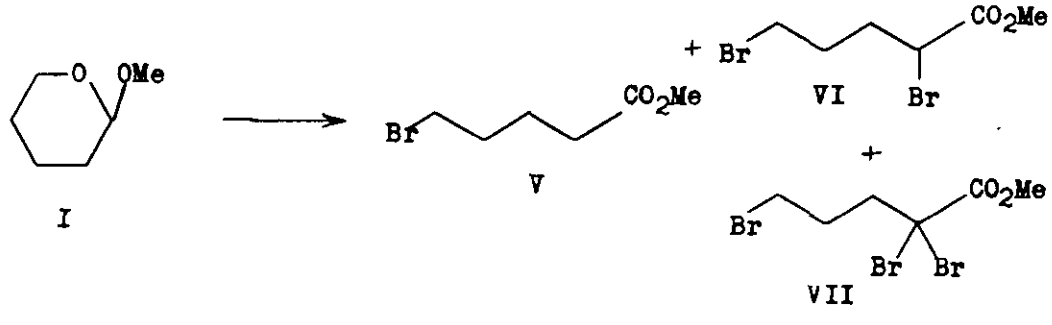
N-Bromosuccinimide (NBS, IV) undergoes homolytic cleavage upon irradiation to give bromine atoms and succinimide radicals. The bromine atoms can then function as hydrogen atom abstractors and a chain reaction such as that shown in Scheme II can occur. In this system radical centers end up substituted

Scheme II. Photochemical bromination with N-bromosuccinimide.

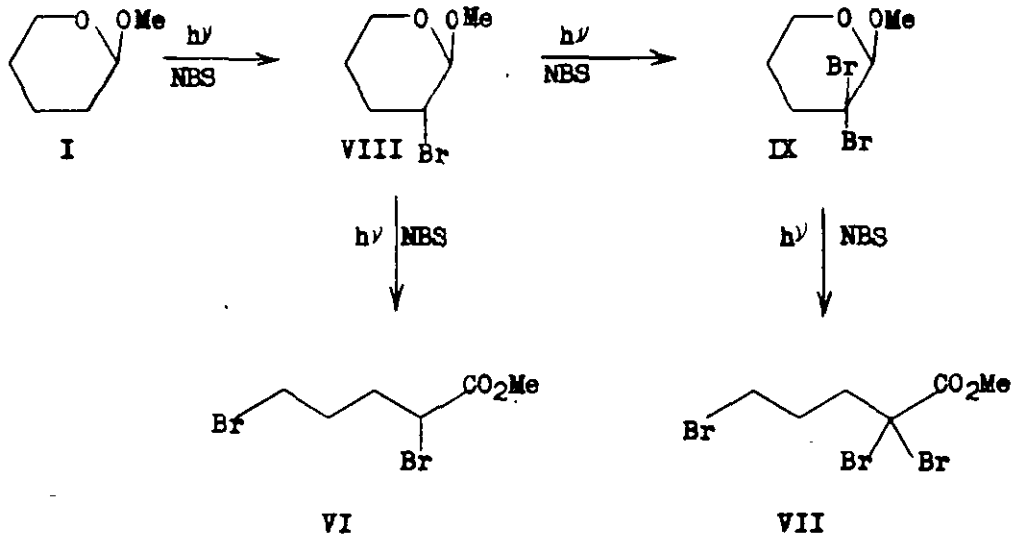


with bromine. Thus, it should be possible to detect hydrogen abstraction at any position, assuming that the reaction products are stable to the reaction conditions.

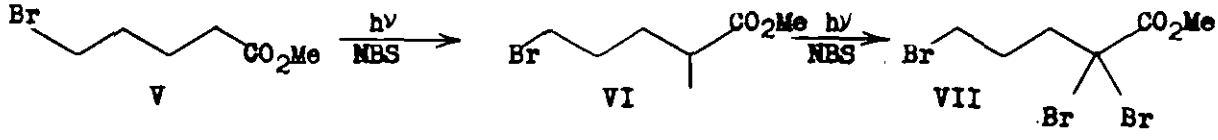
Irradiation of a mixture of N-bromosuccinimide and 2-methoxytetrahydropyran (I) through pyrex gave a mixture of products. The reaction was much faster than the acetone promoted reaction. The major products were isolated and identified as methyl 5-bromovalerate (V), methyl 2,5-dibromovalerate (VI), and methyl 2,2,5-tribromovalerate (VII). Thus, the ring opening process observed



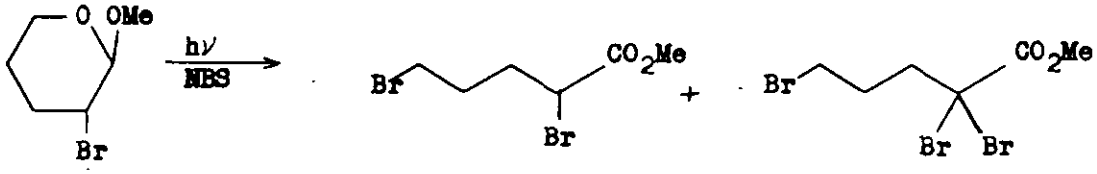
in the acetone promoted reaction also occurs in this system. In addition, bromination occurs on the carbon which was originally the 3-position in the starting material. There are two ways this could occur. Hydrogen abstraction at the 3-position of the starting material would produce 3-bromo-2-methoxytetrahydropyran (VIII). A second such abstraction would give 3,3-dibromo-2-methoxytetrahydropyran (IX). These materials could then undergo the ring opening reaction to give the observed products VI and VII respectively.



Alternatively, methyl 5-bromovalerate (V) could be brominated alpha to the ester function and give the other products VI and VII. Such bromination of esters is not a common reaction with NBS, but there is at least one report in the literature for such a reaction (4).



Irradiation of 3-bromo-2-methoxytetrahydropyran and NBS did indeed give a mixture of VI and VII in what appeared to be a very clean reaction.



VIII VI VII

This is, of course, necessary but insufficient evidence for the first possibility. So far no conclusive evidence for VIII or IX in the original reaction mixture could be found. Small peaks with appropriate retention times were present in the vapor phase chromatography (VPC) traces, but the materials have not been isolated. If the rate constants for destruction of VIII and IX were much greater than those for their formation, then only very small amounts would accumulate.

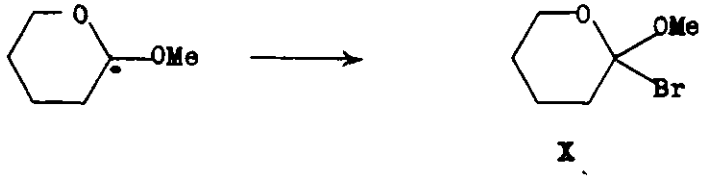
Irradiation of a mixture of methyl 5-bromovalerate and NBS gave, in addition to starting material, two photoproducts. Their retention times were approximately the same as those of VI and VII, but a preparative scale reaction would be needed to confirm these tentative assignments.

At this point it is impossible to say whether the polybrominated products were derived from V or VIII and IX. Hydrogen abstraction at the 3-position does not seem favorable since it generates an unstable radical.* It seems unlikely that such a process could account for the relatively large amounts of VI and VII that are formed even at relatively early stages in the reaction. It is hoped that a few additional experiments could clear up this

*Bromination under ionic conditions would give substitution at the 3-position. This is a standard reaction for the Br_2 bromination of ketals.

uncertainty. This is quite important, since the relative positions of attack are still not well defined in carbohydrate photochemistry. If a radical can be formed at the 3-position in I, where no great stability is associated with the radical, then one couldn't expect much selectivity in the carbohydrates, where essentially all positions are stabilized by oxygen functions and only a small difference in stability would be expected between a radical formed at the anomeric position (C-1) and at other positions in the molecule.

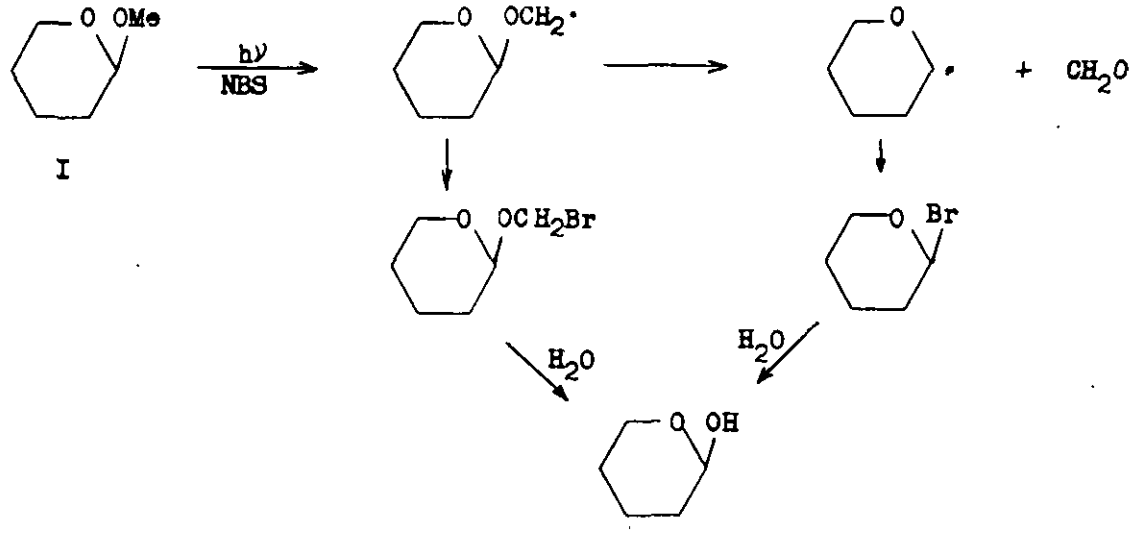
So far not much evidence has been obtained for "quenching" of the initially formed radical to give 2-bromo-2-methoxytetrahydropyran (X). One would not expect X to be very stable and if one simply concentrates the filtered reaction mixture, darkening and the formation of nonvolatile materials results. However, if the filtered reaction mixture is washed with 5% sodium bicarbonate



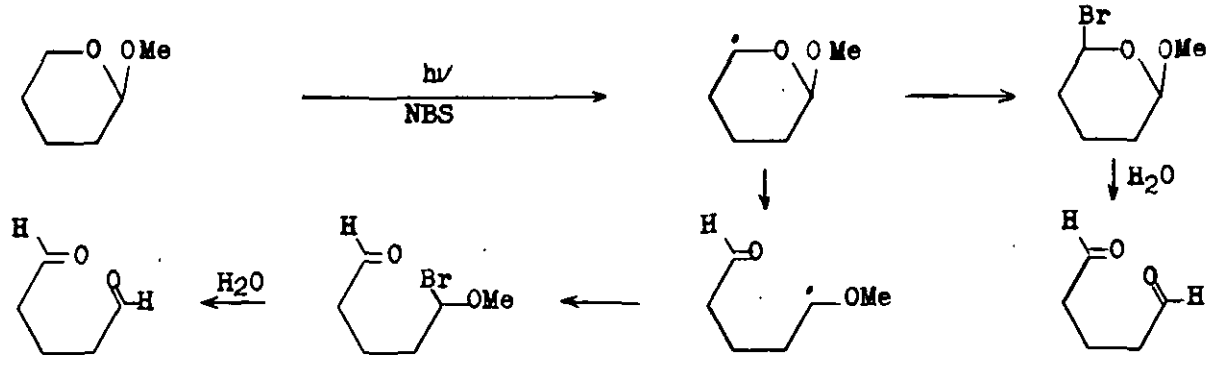
X

before concentrating, then a much more stable residue is isolated. Also, if the filtered reaction mixture is washed with water, the pH of the aqueous layer is less that one. Compound X would be expected to hydrolyze in water to produce HBr. This would account for these observations. However, so far no evidence has been found for X or its other hydrolysis products.

There are also other positions where one might expect hydrogen abstraction. Thus, hydrogen abstraction on the methyl group would give an oxygen stabilized radical. If this radical picked up bromine, or if it lost formaldehyde before picking up bromine, it would give a product which would be expected to be hydrolyzed to 2-hydroxytetrahydropyran (i.e., 5-hydroxyvaleraldehyde) and HBr. This has not been observed.



Similarly, hydrogen abstraction at the 6-position would give a radical which is stabilized by an adjacent oxygen. This radical could pick up bromine or undergo ring opening before picking up bromine. Both of these processes would give an intermediate which might be expected to hydrolyze to glutaraldehyde. This also, has not been observed, although it cannot be ruled out as a minor process.



Thus, since no hydrogen abstraction seems to occur at the methyl group or at the 6-position, which would give stabilized radicals, it seems unlikely that hydrogen abstraction at the 3-position would occur. However, the question still remains. Do VIII and IX form by an ionic mechanism and then give VI and VII, or do VI and VII come from V? It is hoped that the answer will be forthcoming.

EXPERIMENTAL SECTION

Solvents. All solvents were distilled slowly through a 12" vigreux column. Tertiary butyl alcohol was refluxed for at least two hours with calcium hydride before distillation.

Gas Chromatographic Analyses. Photochemical reactions were monitored by VPC. Two sets of conditions were employed. The first utilized a 5% SE 30 on 60/70 mesh Anakrom ABS, 1/4" x 5' column. A thermal conductivity detector was used with a temperature program of 70° plus 4°/min. The second utilized a 10% Carbowax 20M on Chromosorb W, 1/8" x 5' column. A flame ionization detector was used and a temperature program of 72° plus 6°/min. The first set of conditions was used for preparative and analytical work.

Irradiation of 2-Methoxytetrahydropyran and Acetone. A large number of irradiations under a variety of conditions were carried out. Several solvent systems were used. The following conditions, which were similar to those of Elad and Youssefyeh (2), were found to be about the best. A mixture of 0.600 g of 2-methoxytetrahydropyran, 2.0 ml of acetone, and 18 ml of tertiary butyl alcohol was irradiated for 22 hr through pyrex with 16 RPR 3000 lamps in the Rayonet Reactor. Nitrogen was bubbled through the solution for 20 min prior to and during the irradiation. VPC of the photolysate indicated a 65:35 mixture of starting

2-methoxytetrahydropyran and methyl valerate. The methyl valerate had identical retention times on both columns. Preparative VPC gave pure starting material with the same NMR spectrum as authentic material and a small amount of methyl valerate contaminated with starting material which showed the characteristic methyl ester singlet at δ 3.62 at the same position as authentic methyl valerate. A small amount of material gave several small peaks at much longer gas chromatographic retention times. These were also produced, although in slightly different ratios, by irradiation of an acetone-t-butyl alcohol blank.

Irradiation of 2-Methoxytetrahydropyran and N-Bromosuccinimide. A large number of irradiations were carried out with various concentrations and irradiation times. Two irradiations are reported here. In the first a stirred mixture of 1.076 g (9.27 mmol) of 2-methoxytetrahydropyran, 0.274 g (1.54 mmol) of N-bromosuccinimide, and 47 ml of CCl_4 was irradiated through pyrex with RPR 3000 lamps in the Rayonet Reactor. Nitrogen was bubbled through the solution for 30 min prior to and during the 15 min irradiation. The mixture was allowed to cool to room temperature, filtered, washed with 5% sodium bicarbonate, dried (Na_2SO_4), and concentrated. VPC analysis showed two major peaks in addition to starting material, the first of which showed three small shoulders and the second showed one shoulder. The peaks were isolated by preparative VPC. The NMR of the first peak was essentially the same as authentic methyl 5-bromovalerate (see table), as was the VPC retention time. The retention time and NMR of the second peak were essentially the same as methyl 2,5-dibromovalerate (see table). One of the shoulders of the first peak had a retention time approximately the same as 3-bromo-2-methoxytetrahydropyran, but it was present in insufficient amount to isolate.

In the second irradiation, a stirred mixture of 1.292 g (11.1 mmol) of 2-methoxytetrahydropyran, 1.230 g (6.92 mmol) of N-bromosuccinimide, and 50 ml of CCl_4 was irradiated under the same conditions for 1.3 hr. The work-up was designed to remove starting material by hydrolysis and formation of the water soluble product of the aldehyde with Girard's Reagent P. Later it was found that pumping was simpler. Thus, the photolysate was filtered and concentrated. The residue was combined with 1.5 g of Girard's Reagent P, 20 ml of 95% ethanol, and 2.0 ml of acetic acid and refluxed for 1.0 hr. The mixture was poured into dilute brine and extracted twice with ether. The ether extracts were dried (Na_2SO_4) and concentrated to give a yellow oil. VPC analysis showed seven peaks, the largest of which was collected and gave infrared and NMR spectra which suggested methyl 2,2,5-tribromovalerate (see table).

Irradiation of 3-Bromo-2-methoxytetrahydropyran and N-Bromosuccinimide. A stirred mixture of 0.499 g (2.56 mmol) of 3-bromo-2-methoxytetrahydropyran, 0.199 g (1.12 mmol) of N-bromosuccinimide, and 50 ml of CCl_4 was irradiated as above for 42 min. The mixture was filtered, washed with 5% sodium bicarbonate, dried (Na_2SO_4), and concentrated. VPC showed three peaks which were collected. The collected material was analyzed by infrared and NMR spectroscopy. The first peak (ca. 72%) was starting material, the second peak (ca. 21%) was methyl 2,5-dibromovalerate, and the third peak (ca. 7%) spectra identical to the material tentatively identified as methyl 2,2,5-tribromovalerate in the previous irradiation, above.

Irradiation of Methyl 5-Bromovalerate and N-Bromosuccinimide. A stirred mixture of 156 mg (0.80 mmol) of methyl 5-bromovalerate, 155 mg (0.87 mmol) of N-bromosuccinimide, and 18 ml of CCl_4 was irradiated as above for 100 min.

The mixture was filtered and the solution was concentrated to give 205 mg of a yellow oil. VPC analysis showed three peaks in the approximate ratios of 68:30:2. The first of these corresponded in retention time exactly to starting material. The second and third corresponded approximately to methyl 2,5-dibromovalerate and the material tentatively identified as methyl 2,2,5-tribromovalerate, respectively. A preparative run should be made to verify these tentative product assignments.

Methyl 5-Bromovalerate. This material was prepared by the method of Gaudry and Berlinguet (5) starting with dihydropyran. Hydrolysis and oxidation gave 5-hydroxyvaleric acid. Bromination and esterification gave the desired compound. The material is also available commercially.

3-Bromo-2-methoxytetrahydropyran. This material was prepared by the method of Lemieux and Fraser-Reid (6). Thus, 8.40 g (0.100 mol) of dihydropyran was added to an ice cold, stirred slurry of 23.6 g (0.140 mol) of silver acetate in 500 ml of methanol. Bromine (7.2 ml, 22.5 g, 0.140 mol) was then added dropwise over a 10 min period and the mixture was stirred for an additional 20 min. The mixture was filtered through Celite, concentrated *in vacuo*, taken up in chloroform, washed with 5% sodium bicarbonate and 5% sodium thiosulfate, dried (Na_2SO_4), concentrated, and distilled to give 12.23 g (63%) of the desired bromoacetal, bp 95-96° (*ca.* 25 mm) [Lit. (6) 78° (10 mm)]. The NMR spectrum indicated that the product was predominantly trans (*ca.* 90%).

TABLE

NMR Spectral Data

Compound	Source	Chemical Shifts (δ, p.p.m.) CH_3O	Other
2-methoxytetrahydropyran (I)	a	3.30	4.43 (br, 1 H, $CH(OR)_2$), 3.40-3.95 (m, 2 H, CH_2OR), and 1.25-2.00 (br, 6 H, $CH_2CH_2CH_2$)
3-bromo-2-methoxytetrahydropyran (VIII)	b	3.39	4.47 (d, 1 H, J = 4 Hz, $CH(OR)_2$), 3.45-4.10 (m, 3 H, CH_2OR and CHBr), and 1.30-2.60 (m, 4 H, CH_2CH_2)
3,3-dibromo-2-methoxytetrahydropyran (IX)	c	3.55	4.45 (s, 1 H, $CH(OR)_2$), 3.68-4.23 (m, 2 H, CH_2OR, 2.52-3.02 (m, 2 H, CH_2CBr_2), and 1.58-2.08 (m, 2 H, CH_2)
methyl valerate (II)	d	3.62	2.10-2.45 (m, 2 H, CH_2CO_2R) and 0.75-1.90 (m, 7 H, aliph.)
methyl 5-bromovalerate (V)	b	3.64	3.40 (t, 2 H, J = 6 Hz, CH_2Br), 2.32 (t, 2 H, J = 6 Hz, CH_2CO_2R), and 1.35-2.17 (m, 4 H, CH_2CH_2)
methyl 2,5-dibromovalerate (VI)	c	3.80	4.30 (br t, 1 H, J = 6 Hz, CHBr), 3.43 (t, 2 H, J = 6 Hz, CH_2Br), and 1.67-2.50 (m, 4 H, CH_2CH_2)
methyl 2,2,5-tribromovalerate (VII)	e	3.89	3.45 (t, 2 H, J = 6 Hz, CH_2Br), 2.50-2.90 (m, 2 H, CH_2CBr_2), and 1.90-2.50 (m, 2 H, CH_2)

a. McKelvey, R. D., The Institute of Paper Chemistry, Appleton, Wisconsin, Project 3134: Report 3, June 22, 1973.

b. This report.

c. Spectra furnished by R. K. Brown, Department of Chemistry, University of Alberta. See Roff, J. E., and Brown, R. K., Canad. J. Chem., 51:3354(1973).

d. Aldrich Chemical.

e. This report, from the irradiation of VIII.

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6. Lemieux, R. U., and Fraser-Reid, B., Canad. J. Chem., 43:1460 (1965).

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A FUNDAMENTAL STUDY OF THE PHOTOCHEMISTRY OF CARBOHYDRATES, CELLULOSE, AND RELATED COMPOUNDS

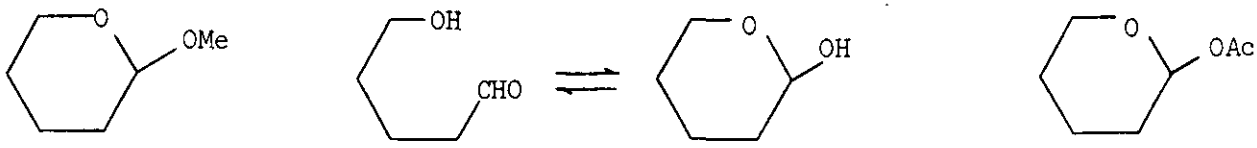
SUMMARY

2-Methoxytetrahydropyran (I), 5-hydroxyvaleraldehyde (II), and 2-acetoxytetrahydropyran (III) were prepared as carbohydrate models for photochemical studies. Irradiation of I in the presence of ketone sensitizers resulted in the recovery of starting material. Irradiation of a mixture of I and N-bromosuccinimide gave at least four photoproducts, the major one of which has tentatively been identified as methyl 2,5-dibromovalerate. Irradiation of II gave starting material. The pH of the solution after irradiation in the presence of oxygen decreased, indicating possible production of carbon dioxide or carboxylic acid. Possible mechanisms for these reactions are discussed in light of the current literature.

INTRODUCTION

In the previous report (1) it was noted that the photochemistry of carbohydrates was complicated by the polyfunctionality of the compounds. It was decided that it would be instructive to study some model systems containing the functional groups found in carbohydrates before returning to the sugars themselves.

The following compounds were prepared for model studies: 2-methoxytetrahydropyran (I), 5-hydroxyvaleraldehyde (II), and 2-acetoxytetrahydropyran (III). These compounds were all prepared by the same general reaction (equation 1) following



I IIa IIb III

literature procedures (see Experimental Section).

(1)

I R = CH_3
II R = H
III R = Ac

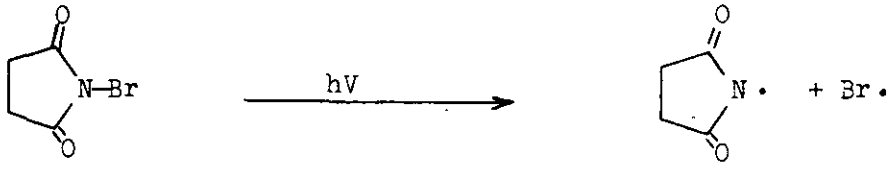
Compound I is a model for a simple glycoside, and as such, might provide useful information concerning cellulose photochemistry. Compound II, which exists to the extent of over 98% as the cyclic, hemiacetal form (IIb), is a very good model for a reducing sugar. It undergoes a dynamic equilibrium reaction between cyclic and acyclic forms analogous to mutarotation in sugars. Both compounds I and II contain the acetal group (or hemiacetal) and should serve as models for the "acetal chromophore" of carbohydrates (2). Compound III is a model for the common acetate derivatives of sugars. It was prepared as a reference compound for v.p.c. analysis of II as the more volatile acetate. However, the photochemistry of III might also prove interesting if time permits.

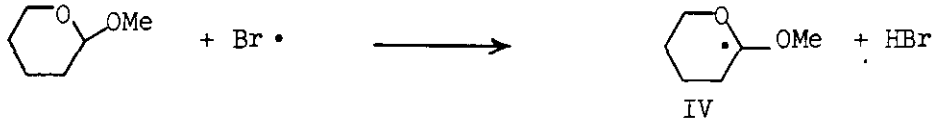
RESULTS AND DISCUSSION

Irradiation of 2-methoxytetrahydropyran (I) under a variety of conditions using ketone sensitizers resulted in no reaction or the isolation of photoproducts

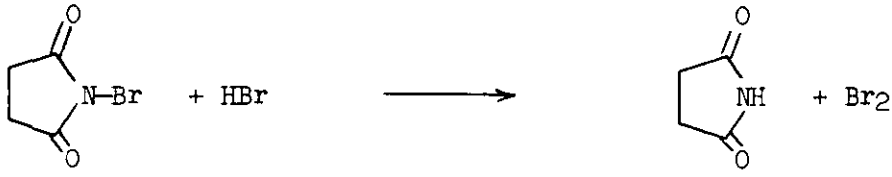
of the sensitizers. Therefore, it was decided to use another photoinitiated hydrogen-abstraction system; N-bromosuccinimide (NBS).

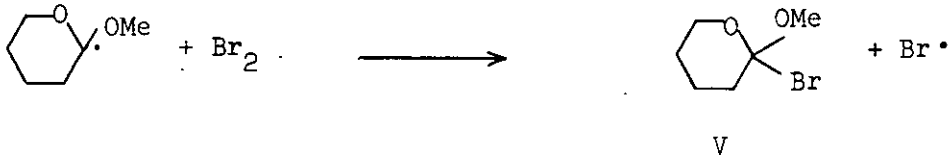
A priori, one might predict the following free radical chain reaction based on relative radical stabilities and the mechanism of NBS reactions (3).

Initiation: 

Propagation: 

IV

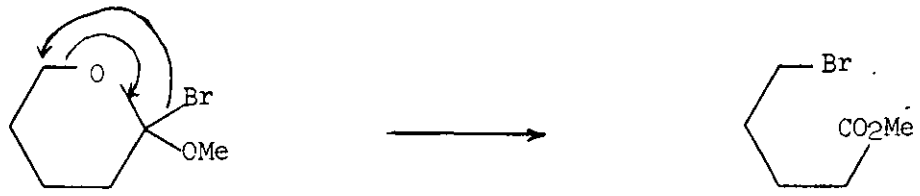




V

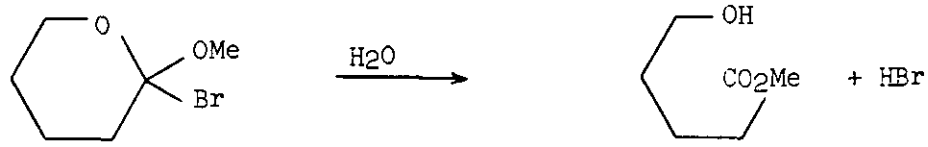
Termination: Radical combination

Compound V would not be expected to be very stable. Therefore, one might expect to isolate methyl 5-bromovalerate (VI) by the intramolecular elimination of alkyl bromide (equation 2) as proposed for a similar reaction by Marvel and Joncich (4). Alternatively, hydrolysis of compound V would be expected to



V VI (2)

give methyl 5-hydroxyvalerate (VII) (equation 3).



V VII (3)

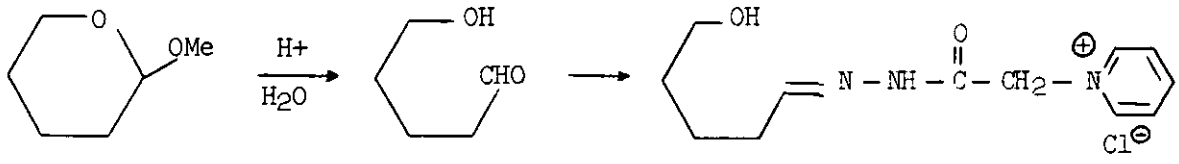
Radical IV could also open up to give radical VIII (equation 4) which

IV VIII (4)

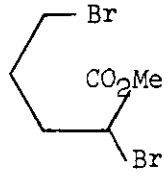
would then yield bromoester VI under the reaction conditions. Such a process was claimed to occur in the t-butyl peroxide catalyzed reaction of I to give methyl valerate (5).

Irradiation of a stirred mixture of NBS and I in carbon tetrachloride through pyrex followed by removal of NBS and succinimide by filtration and starting material by hydrolysis in the presence of Girard's Reagent P (equation 5) gave a mixture of at least 4 photoproducts, as shown by v.p.c.

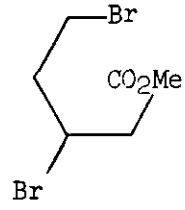
(5)



The NMR spectrum of the mixture of photoproducts showed singlets at δ 3.83 and 3.94, indicative of methyl esters. Preparative v.p.c. resulted in the isolation of the major product. The infrared and NMR spectra showed that it was a methyl ester. The mass spectrum showed that it contained <u>two</u> bromines. The fragmentation pattern in the mass spectrum was consistent with either methyl 2,5-dibromovalerate (IX) or methyl 3,5-dibromovalerate (X).

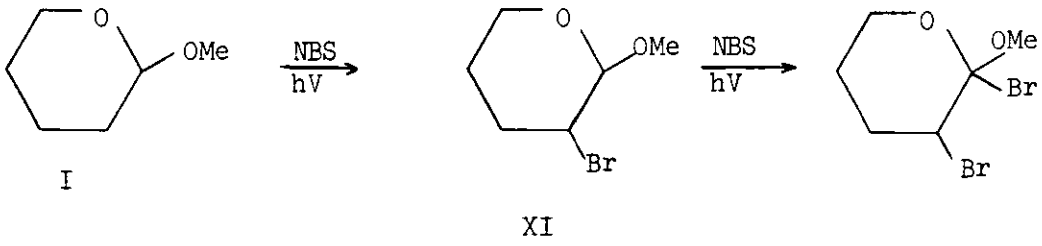


IX



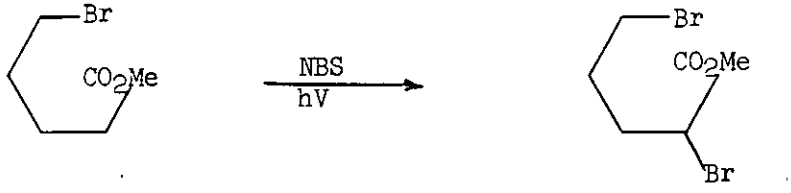
X

On mechanistic grounds, IX is a more reasonable candidate. Acetals are known to brominate α to as well as on the acetal carbon (<u>4</u>). Both processes together would give a dibromoacetal which could rearrange to IX (equation 6).



I XI (6)

An alternative method of obtaining IX would be through NBS bromination of bromoester VI α to the carbonyl group (equation 7). An analogous reaction has



VI IX (7)

reported previously (6).

No evidence has yet been found for bromoacetal XI which has a distinctive NMR spectrum (7). It is possible that the bromoester VI is the methyl ester which showed up in the NMR spectrum at δ 3.82. However, it appears that this ester is formed later in the irradiation than IX. Such behavior would not be consistent with a precursor.

Methyl 2,5-dibromovalerate (IX) is a known compound (8). Complete spectral data were not reported. Therefore, a letter was sent to the author requesting more complete spectral data or a sample. No reply has yet been received.

The other photoproducts are currently under investigation.

Irradiation of 5-hydroxyvaleraldehyde (II) under nitrogen under conditions where 46% of the incident light was absorbed gave only starting material. A similar irradiation in the presence of oxygen resulted in the isolation of starting material. However, it was noted that the pH of the aqueous solution after irradiation was 4, while that of the distilled water was 6. This could be explained by the production of a small amount of carbon dioxide or carboxylic acid. Further work is being done in this area.

FUTURE WORK

An attempt will be made to isolate sufficient amounts of the other photoproducts from the reaction of I and NBS for their identification. The identity of these other products and their relative abundance with irradiation time should provide information as to possible intermediates in this reaction.

Prolonged irradiation of I in the presence of ketone sensitizers might be reinvestigated. The irradiation of these compounds in the presence of oxygen will show differences which should be meaningful with regard to the conditions of cellulose degradation.

Preliminary experiments indicate that 3-bromo-2-methoxytetrahydropyran (XI) can be prepared by the bromination of I. Submitting this compound to the conditions of NBS bromination would provide information as to its possible role as an intermediate in this system.

After these models for the acetal group are thoroughly studied, extension of this work to other functional groups found in carbohydrates should prove meaningful.

EXPERIMENTAL SECTION

Purification of Materials. Dihydropyran, acetone, acetophenone, and carbon tetrachloride were distilled and stored in the dark. N-Bromosuccinimide was recrystallized from water. Girard's Reagent P (Arapahoe Chemical) was dissolved in hot, 95% ethanol, treated with decolorizing carbon, filtered, and cooled slowly to effect recrystallization. Deionized, distilled water was used throughout this work.

2-Methoxytetrahydropyran (I). The method of Woods and Kramer was used (9). Methanol (20 ml., 16 g., 0.5 mol.) was added slowly (10 min.) dropwise to a stirred solution of hydrochloric acid (2 drops) in 45.5 ml. (42 g., 0.5 mol.) of dihydropyran. The exothermic reaction subsided after 45 min. and the mixture was stirred for an additional 1.8 hr. Sodium hydroxide (ca. 300 mg.) was added and after 15 min. the mixture was distilled through a 12 cm. vigreux column to give 35.0 g. (60.3%) of I, b.p. 123-124° (lit. 125°).

5-Hydroxyvaleraldehyde (II). The method of Woods was utilized (10). Dihydropyran (9.8 ml., 9.0 g., 0.107 mol.) was added to a stirred solution of 0.5 ml. of hydrochloric acid in 30 ml. of water and the mixture was allowed to react for 3.5 hr. Aqueous sodium hydroxide solution was added until the mixture was slightly basic, at which time the mixture became quite cloudy. The mixture was extracted with ether for 18 hr. with a continuous extractor. The ether solution was concentrated and the residue vacuum distilled to give 4.6 g. (42%) of II, b.p. 83-84° (ca. 35 mm.) [lit. 62-66° (9-10 mm.)]. Infrared and NMR spectra indicated that the material was predominately in the cyclic hemiacetal form (2-hydroxytetrahydropyran) with no evidence of the acyclic carbonyl form. The ultraviolet spectrum

in water showed λ_{max} 277 nm. (ϵ 0.6) consistent with ca. 1% aldehydo form. In pentane this extinction dropped to 0.15, implying even less open-chain form.

2-Acetoxytetrahydropyran (III). The procedure of Bowman and Fordham was used (11). Acetic acid (27.6 ml., 30 g., 0.50 mol.) was added dropwise over a 20 min. period to a stirred solution of 91 ml. (84 g., 1.0 mol.) of dihydropyran and 6 mg. of toluenesulfonic acid. The mixture was stirred for an additional 50 min. A suspension of 2.0 g. of sodium carbonate in 4.0 ml. of water was added and the mixture was stirred for 10 min. The decanted organic layer was dried (Na_2SO_4) and the low boiling material was removed by atmospheric distillation, b.p. 75-94°. The residue was vacuum distilled to give 14.2 g. (20%) of III, b.p. 78-83° (ca. 9 mm.) [lit. 42-43° (1 mm.)].

Irradiation of 2-methoxytetrahydropyran (I) in the presence of acetone. A solution of 2.301 g. of I in 70 ml. of acetone and 230 ml. of benzene was irradiated through a pyrex filter with a Hanovia 450 watt medium pressure mercury lamp for 2.5 hr. Nitrogen was bubbled through the solution for 30 min. prior to and during the irradiation. A 150 ml. aliquot was concentrated in vacuo to ca. 1 ml. The NMR spectrum showed only starting material and benzene. A 125 ml. aliquot of the solution was diluted with 55 ml. of acetone and 120 ml. of benzene. The solution was irradiated as before for an additional 6.5 hr. After concentration, the NMR again showed no reaction.

Irradiation of 2-methoxytetrahydropyran (I) in the presence of acetophenone. A mixture of 0.835 g. (7.2 mmol.) of I and 0.864 g. (7.2 mmol.) of acetophenone was irradiated through pyrex in a Rayonet Photochemical Reactor with 16 rpr 3000 lamps. Nitrogen was bubbled through the mixture for 20 min. prior to and during irradiation.

After 2.25 hr. the NMR spectrum showed only starting materials. After 17 hr. a small singlet started to show up at δ 7.16. After 40 hr., considerable reaction had taken place. The NMR spectrum showed a doublet at δ 1.45 and a singlet at 7.14. These peaks suggest the possibility of α-phenylethanol, resulting from photoreduction of acetophenone, although a comparison spectrum was not found in the literature.

Irradiation of 2-methoxytetrahydropyran (I) and N-bromosuccinimide. A stirred mixture of 1.292 g. (11.1 mmol.) of I, 1.230 g. (6.92 mmol.) of NBS, and 50 ml. of carbon tetrachloride was irradiated for 1.3 hr. through pyrex in a Rayonet Photochemical Reactor with rpr 3000 lamps. Nitrogen was bubbled through the solution for 20 min. prior to irradiation. The photolysate was filtered and concentrated in vacuo. The residue was combined with 1.5 g. of Girard's Reagent P, 20 ml. of 95% ethanol, and 2.0 ml. of acetic acid and refluxed for 1.0 hr. The mixture was poured into dilute brine and extracted twice with ether. The ether extracts were dried (Na_2SO_4) and concentrated to give a yellow oil. V.p.c. analysis (5% SE-30, 1/4" x 5', 105-150° at 4°/min.) showed at least four products and no starting material. The largest peak was collected and showed the following spectral data: infrared (neat) 2970, 1730 (C=O), 1435, and 1255 $cm.^{-1}$; NMR (CCl_4) δ 3.94 (s, 3H, OCH_3), 3.47 (t, 2H, J=6 Hz., $BrCH_2$), 2.50-2.90 (m, 2H, CH_2), and 2.00-2.50 (m, 2H, CH_2) (no signal at ca. 4.4 for $CHBr-CO_2Me$); mass spectrum m/e 272, 274, 276 ($C_6H_{10}Br_2O_2$ parent), other peaks showed a similar fragmentation pattern as published for methyl 5-bromovalerate (12) except for a discrepancy of two mass units attributed to instrumental instability.

Irradiation of 5-hydroxyvaleraldehyde (II). A solution of 504 mg. of II in 15 ml. of water was irradiated through quartz for 60 min. in a Rayonet Photochemical

Reactor with rpr 2537 lamps. Nitrogen was bubbled through the solution for 15 min. prior to and during irradiation. The solution was diluted to 50 ml. with water and continuously extracted with ether for 17 hr. The ether was concentrated in vacuo to give a yellow oil whose NMR spectrum indicated only starting material.

Irradiation of 5-hydroxyvaleraldehyde (II) in the presence of oxygen. A solution of 501 mg. of II in 15 ml. of water was irradiated as in the previous example except oxygen was used in place of nitrogen. The pH of the solution after irradiation was 4. Based on pK_a values, this would correspond to a concentration of 6×10^{-4} M carboxylic acid or 2×10^{-2} M carbonic acid. The aqueous solution was extracted with ether (2 x 20 ml.) and the ether extracts concentrated to give material whose infrared spectrum was essentially the same as starting material.

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A FUNDAMENTAL STUDY OF THE PHOTOCHEMISTRY OF CARBOHYDRATES, CELLULOSE, AND RELATED COMPOUNDS

SUMMARY

The photochemistry of methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, gulonic lactone, and phenyl β-D-glucopyranoside was investigated. The methyl glycosides do not have an appreciable chromophore and were found to be photochemically inert in the absence of oxygen at the wavelengths studied. Gulonic lactone gave a small amount of a reducing sugar as a photoproduct. Although the structure of this material was not proved, a possible mechanism is offered as rational. Further irradiation gave more extensive degradation and a complex mixture of photoproducts. Irradiation of phenyl β-D-glucopyranoside resulted in the loss of aromatic absorption in the NMR spectrum and the appearance of carbonyl absorption in the infrared. Similar irradiation in the presence of oxygen produced carboxyl groups.

INTRODUCTION

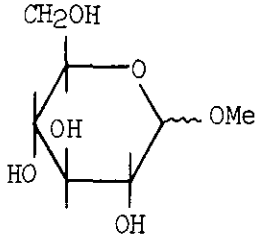
The photochemistry of carbohydrates, including cellulose, has been investigated previously by other workers (2, 9) but there seems to be little agreement as to the processes occurring. A significant portion of the investigators have invoked an "acetal chromophore" (1), which is claimed to have a weak absorption at around 280 nm., to explain their results. Generally these irradiations were carried out in the presence of oxygen and extensive degradation occurred.

In the hope of gaining a better understanding of these systems, particularly with regard to reaction mechanisms, a more detailed study was undertaken. This preliminary report contains some of the early results of the study, which are necessarily incomplete.

Methyl α- and β-D-glucopyranoside (I and II), L-gulonic-γ-lactone (III), and phenyl β-D-glucopyranoside (V) were chosen for a preliminary survey of the carbohydrates. The methyl glucosides I and II were chosen because they have the acetal group and no other chromophore. Gulonic lactone (III) was chosen because it is a weak chromophore and also because it is a reasonable model for an oxidized impurity in a sugar or cellulose. Phenyl glucoside V was selected because it has an appreciable chromophore and also because it might be a good model for the lignin-carbohydrate bond (10).

RESULTS AND DISCUSSION

Methyl α-D-glucopyranoside (I) and methyl β-D-glucopyranoside (II) were irradiated in aqueous solution. Direct irradiation in the absence of oxygen gave no reaction. This is what one would predict on the basis of the ultraviolet spectrum

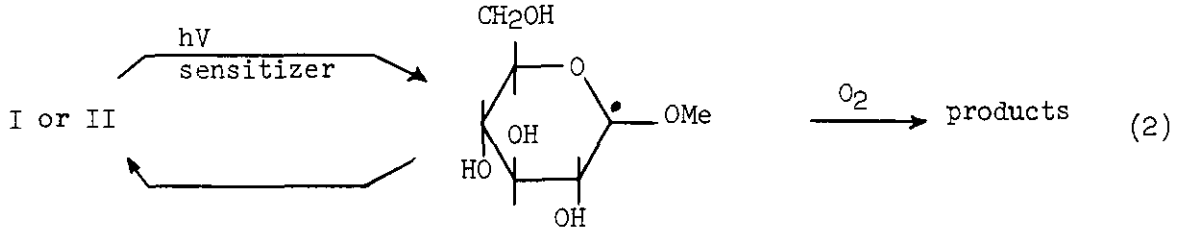


$$\xrightarrow[\text{direct or sensitized}]{h\nu} \text{no reaction} \qquad (1)$$

since only about 1% of the incident light would be absorbed at the concentration used (ca. 0.1 M). Thus, the "acetal chromophore" (1), if it exists, is insufficient to bring about reaction.

Glucosides I and II were also irradiated in the presence of acetone as sensitizer. The carbohydrates were recovered unchanged. A considerable amount of material of low volatility was also produced but this material could be produced by irradiation of an aqueous acetone blank. The identity of this material was not determined but it presumably consisted of acetone photoproducts and/or photoproducts of acetone impurities.

The failure to detect carbohydrate photoproducts from glucosides I and II should not be construed as proof that these glycosides are inert to ultraviolet light or photochemical sensitization. Carbohydrates are generally degraded by ultraviolet light in the presence of oxygen (2). It is not generally agreed whether sensitizers are needed for the reactions. It is quite possible that a reversible photochemical step, such as sensitized hydrogen atom abstraction (eq. 2), does occur. In the presence of oxygen the radical produced could be oxidized to products. In

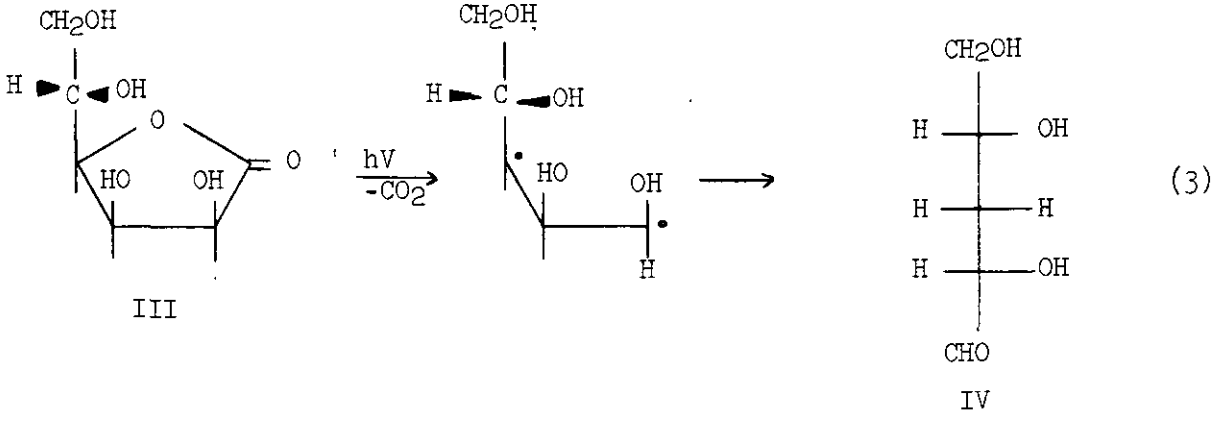


(2)

the absence of oxygen, the radical could return to starting material via hydrogen abstraction. However, hydrogen abstraction at C-1 would produce the same radical from I and II so one would expect a mixture of I and II from such a process. This was not observed. Such a process still could occur at another position in the molecule.

L-Gulonic-γ-lactone (III) has a slightly better chromophore than the methyl glucosides I and II. Irradiation of an aqueous solution of III with mercury resonance lamps (90% 254 nm. light, 3% light absorption) in the absence of oxygen resulted in the production of a small amount of a reducing sugar, as shown by aniline phthalate thin layer chromatography (t.l.c.) indicator. In an attempt to produce enough of this photoproduct for isolation and identification a second sample was irradiated with the medium pressure mercury lamp with vycor filter, conditions where much greater light absorption would be expected. Under these conditions extensive degradation occurred and very little starting material remained. The photolysate was a complex mixture of compounds, none of which have been identified yet.

A reasonable candidate for the reducing sugar is 3-deoxy-L-xylose (IV) (definitive name: 3-deoxy-L-erythro-pentose), resulting from decarboxylation of III, as shown in Equation (3). This material was not available for comparative purposes and might be difficult to obtain.



III IV (3)

Phenyl β-D-glucopyranoside (V), a carbohydrate derivative with a relatively strong chromophore, was irradiated with 254 nm. light under conditions where essentially 100% of the light would be absorbed. In the absence of oxygen there was no

evidence of reaction after 2 hr. After 8.5 hr. the NMR showed a loss of ca. 20% of the aromatic signal relative to the rest of the spectrum. T.l.c. showed at least three products in addition to starting material. A similar sample, irradiated with the more intense medium pressure lamp, showed the buildup of a carbonyl peak at 1708 $cm.^{-1}$. Irradiation in the presence of oxygen gave material whose infrared spectrum showed a carbonyl peak at 1715 $cm.^{-1}$ and a very broad hydroxyl stretch characteristic of a carboxylic acid.

CONCLUSIONS

Although the work described in this report is not complete at this time, certain conclusions are possible and they have been used to guide further work. Irradiation of nonabsorbing carbohydrate derivatives with 254 nm. light in the absence of oxygen results in no measurable product. Incorporation of a chromophore into the system does make the compound more labile. The polyfunctionality of carbohydrates causes complex mixtures to be formed and the polar nature of these products makes them difficult to separate by chromatographic means.

When this work was being done a gas chromatograph was not available on a regular basis. Since then, use of such an instrument on a more or less regular basis was obtained and analysis of volatile derivatives of these carbohydrates might be possible.

In order to simplify the chemical and analytical problems inherent in the carbohydrate systems, it was decided to study the photochemistry of the functional groups present, with the hope of returning to the carbohydrates with a better understanding of the processes. The logical starting point for such a study in view of present controversy is with the "acetal chromophore." The first portion of such a study will be described in the next report.

EXPERIMENTAL SECTION

Purification of Commercial Carbohydrates. Methyl α-D-glucopyranoside (I), methyl β-D-glucopyranoside (II), and gulonic lactone (III) were recrystallized from aqueous alcohol as described in Report 1. Sharp melting points were obtained which agreed within 0.5° of literature values.

Penta-O-acetyl-β-D-glucopyranose. This compound was prepared by the method of Wolfrom and Thompson (3). Thus a slurry of 50.0 g. (0.61 mol.) of anhydrous sodium acetate in 700 ml. of acetic anhydride was heated to boiling with a flame. Anhydrous glucose (100 g., 0.625 mol.) was added in small portions at a rate sufficient to maintain boiling without the flame. After the addition was complete the solution was heated to a vigorous boil for 5 min. and then allowed to cool to room temperature. The mixture was poured onto ca. 2 l. of cracked ice, stirred occasionally for 2.5 hr., and filtered. The air-dried product was dissolved in hot 95% ethanol, treated with decolorizing carbon, and cooled slowly to effect recrystallization. Yield: 129.2 g. (59.6%) in four crops, m.p. 131-132° (lit. 132°).

Phenyl tetra-O-acetyl-β-D-glucopyranoside. The method of Montgomery, Richtmyer, and Hudson was used (4). A mixture of 20.0 g. (0.051 mol.) of penta-O-acetyl-β-D-glucopyranose, 19.3 g. (0.205 mol.) of phenol, and 0.32 g. of toluenesulfonic acid monohydrate was heated on a steam bath for 1.5 hr. while being evacuated with an aspirator. The red oil was cooled somewhat and dissolved in 150 ml. of ethylene dichloride. The solution was washed thoroughly with 2 N sodium hydroxide solution and brine, dried (Na_2SO_4), concentrated, and the residue recrystallized from 95% ethanol to give 10.9 g. (50.2%) of the title compound, m.p. 124-5° (lit. 125-6°).

Phenyl β-D-glucopyranoside (V). The general method of Bates was used (5). A sodium methoxide solution, prepared by adding ca. 25 mg. of sodium to 3 ml. of methanol, was added to a mixture of 4.6 g. of phenyl tetra-O-acetyl-β-D-glucopyranoside in 20 ml. of methanol. The mixture was heated at reflux for 10 min. and allowed to stand at room temperature for 2 hr. Water (ca. 1 ml.) was added and the solution was concentrated to 5 ml. and taken up in 20 ml. of water. The solution was passed through a column of Amberlite MB-3 ion exchange resin (15 ml.) and eluted with 70 ml. of methanol. The solution was concentrated and the residue recrystallized from aqueous ethanol to give 2.22 g. (80%) of V, m.p. 173.5-174.5° (lit. (6) 175-176°).

Ultraviolet Absorption Spectra. The ultraviolet spectra of compounds I, II, and III were given in Report 1 and are included here for convenience. All spectra were run in deionized, distilled water except V, which was run in 95% ethanol.

Compound	λmax (ε)	ε 254
Methyl α-D-glucopyranoside (I)	280 (0.035)	0.03
Methyl β-D-glucopyranoside (II)	E.A.[a]	0.04
Gulonic lactone (III)	216 (84)	0.2
Phenyl β-D-glucopyranoside (V)	216 sh.(6200) 261 (725) 267 (922) 273.5 (781)	450

[a] E.A. = end absorption; no maximum at wavelength greater than 215 nm.

Irradiation of Methyl α- and β-D-glucopyranoside (I) and (II). Aqueous solutions (not degassed, 10 ml.) of I (0.096 M) and II (0.104 M) were irradiated simultaneously in a Rayonet Reactor in quartz tubes with rpr 2537 lamps for 19 hr. The solutions were concentrated in vacuo and dissolved in ca. 1 ml. of deuterium oxide. The NMR spectra indicated no reaction.

Irradiation of Methyl α- and β-D-glucopyranoside (I) and (II) in the presence of acetone. Solutions (not degassed, 10 ml.) of I (0.088 M) and II (0.12 M) in 1:4 (V/V) acetone (C.P. grade):water were irradiated under the conditions of the previous procedure for 20 hr. The NMR spectra showed starting material and two new, broad multiplets centered at δ 1.25 and 2.25. These together integrated to ca. 60% of the area of the starting material. Irradiation of aqueous acetone blanks gave essentially the same broad multiplets using either C.P. or distilled acetone.

Irradiation of L-Gulonic-γ-lactone (III) with mercury resonance lamps. A solution of 0.196 g. of III in 14.0 ml. of water was irradiated in a Rayonet Reactor with rpr 2537 lamps for 45 min. Nitrogen was bubbled through the solution for 10 min. prior to and during irradiation. The solution was concentrated in vacuo. The NMR spectrum of the residue looked very much like starting material with some additional peaks at δ 4.5-4.8 which might be attributed to better resolution. T.l.c. on silica gel G eluted with n-butyl alcohol, ethyl acetate, isopropyl alcohol, acetic acid, water (7:20:12:7:12) (7) showed starting material (R_f 0.59) and a product (R_f 0.80). Visualization with aniline phthalate indicator (8) showed that the product was a reducing sugar. Acetylation of the photolysate gave material

identical by NMR to acetylated starting material, indicating that the product was formed in a minor amount.

Irradiation of L-gulonic-γ-lactone (III) with Hanovia medium pressure lamp. A solution of 1.367 g. of III in 300 ml. of water was irradiated with a Hanovia 450 watt medium pressure mercury lamp through a vycor filter. Nitrogen was bubbled through the solution for 30 min. prior to and during irradiation. After 1.0 hr., t.l.c. indicated that only a small amount of photoproduct had formed. After 3.0 hr. the solution was concentrated in vacuo. NMR of the residue (DMSO-d6) showed that some peaks of the starting material were completely gone and there were some very broad absorptions typical of a mixture.

Irradiation of phenyl β-D-glucopyranoside (V) with mercury resonance lamps. A solution of 197 mg. of V in 135 ml. of water was irradiated in a Rayonet Reactor with rpr 2537 lamps for 2.0 hr. Nitrogen was bubbled through the solution for 20 min. prior to and during irradiation. Foaming was noticed during the last hour. The solution was extracted with ether and concentrated in vacuo. The NMR and t.l.c. indicated only starting material. Concentration of the ether extracts gave only 5 mg. of material. The recovered starting material was dissolved in water and irradiated as before for 6.5 hr. and freeze-dried. The NMR of the residue was quite similar to starting material with some broadening of peaks. The integral for the aromatic protons had decreased by 20% relative to the rest of the spectrum.

Irradiation of phenyl β-D-glucopyranoside (V) with Hanovia medium pressure lamp. A solution of 613 mg. of V in 295 ml. of water was irradiated with a Hanovia 450 watt medium pressure mercury lamp through a vycor filter. Nitrogen was bubbled through the solution for 15 min. prior to and during irradiation. Foaming developed

during irradiation causing an overflow of solution. The photolysate (230 ml.) was concentrated *in vacuo* to give 437 mg. (91% based on volume remaining). The NMR of the residue showed some similarity to starting material, but was considerably broadened, particularly in the C-H region. The infrared spectrum showed a carbonyl peak at 1708 $cm.^{-1}$. A portion of the photolysate was acetylated. T.l.c. of this material showed a spot of the same mobility as phenyl tetra-*O*-acetyl-β-D-glucopyranoside and a streak of less mobility. The NMR of this acetylated photolysate was also similar to acetylated starting material but the aromatic region integrated to somewhat less than 5 protons. Column chromatography failed to yield another compound.

Irradiation of phenyl β-D-glucopyranoside (V) in the presence of oxygen. A solution of 267 mg. of V in 280 ml. of water was irradiated with a Hanovia 450 watt medium pressure lamp through a vycor filter. Oxygen was bubbled through the solution for 5 min. prior to and during irradiation. The NMR spectrum of the residue showed some of the peaks of starting material and some broad peaks difficult to assign. It appeared that considerable degradation had taken place. The infrared spectrum showed a carbonyl peak at 1715 $cm.^{-1}$ and a very broad OH stretch characteristic of a carboxylic acid. T.l.c. on silica gel G eluted with *n*-propyl alcohol, ethyl acetate (1:4) showed starting material, a spot of *ca.* 1/3 the mobility of starting material, and considerable material at the origin. Column chromatography on silica gel eluted with the same solvent gave material whose infrared spectrum showed only very broad, poorly resolved peaks. This material was acetylated and the NMR showed at least seven acetate singlets and no aromatic peaks.

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Editorial

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R. D. McKelvey

A FUNDAMENTAL STUDY OF THE PHOTOCHEMISTRY OF CARBOHYDRATES, CELLULOSE, AND RELATED COMPOUNDS

SUMMARY

The ultraviolet spectra of eleven carbohydrates and derivatives were run. The aldoses and glycosides showed only end absorption. The ketoses, fructose and sorbose, showed an absorption maximum at 277 nm. This is explained in terms of a small amount (ca. 2%) of the keto form in solution. The infrared spectrum of fructose showed a weak band at 1718 $cm.^{-1}$ characteristic of a carbonyl group. Commercial carbohydrates were found to contain significant amounts of ultraviolet absorbing impurities. Changes in pH had little effect on the spectrum of glucose until a pH of 10.85, at which point an absorption at 270 nm. began to gradually build up, presumably due to a chemical reaction. Calcium ion had a small effect on the spectrum of fructose. Ferric ion had no effect.

INTRODUCTION

A study of the ultraviolet (UV) spectra of several sugars and derivatives was undertaken in order to obtain background information for a photochemical study to follow. The purpose of the investigation was to determine the types of chromophores present in sugars in solution. In particular, it was hoped that this study would determine the credibility of the proposed "acetal chromophore" (1) in such systems. The effect of inorganic ions and pH was included in this study.

RESULTS

The UV spectra of several carbohydrates are given in Table I. The compounds are divided into classes according to chemical structure. The compounds were chosen to represent the functional groups present in cellulose and hemicellulose, as well as free sugars. The miscellaneous compounds are included for comparative purposes and as derivatives of possible photochemical interest.

Few of the published papers on carbohydrate photochemistry give experimental details on the purification methods used prior to irradiation. Therefore, to evaluate the chromophoric properties of some of the typical impurities found in carbohydrates and to monitor the effectiveness of the purification methods used in the present study (see Experimental Section), the UV spectra of the commercial compounds, which in most cases were samples which had been on the shelf for an undetermined length of time, were run and are included in Table I.

In order to verify the absorbance of fructose (Figure 1) three different samples from two different suppliers were treated with decolorizing carbon and recrystallized several times. In all cases the molar extinction coefficient obtained was 0.38 ± 0.02. Column chromatography on silica gel gave material which was contaminated with silicates from the column. Some of the silicates could be removed by filtration through a millipore filter. The resultant solution had a much stronger end absorption than aqueous fructose, but the absorption maximum was still present, although shifted to slightly shorter wavelength due to the contribution from the end absorption tail.

TABLE I

UV SPECTRA OF COMMERCIAL AND PURIFIED CARBOHYDRATES[a]

	COMMERCIAL	PURIFIED
Aldoses		
L(+)Arabinose[b]	λ_{max} 272 (ϵ 1.15) sh 230 (ϵ 0.12) sh 225 (ϵ 0.17) sh 275 (ϵ 0.15)	E.A. (ϵ_{250} 0.005)
D(+)Galactose[b]		E.A. (ϵ_{250} 0.015)
D(+)Glucose[c]		E.A. (ϵ_{250} 0.04)
Ketoses		
D(-)Fructose[d]	λ_{max} 277 (ϵ 0.75)	λ_{max} 277 (ϵ 0.39)
L(-)Sorbose[d]	λ_{max} 272 (ϵ 0.64)	λ_{max} 277 (ϵ 0.15)
Glycosides		
α-Methylglucopyranoside	N.D.[e]	λ_{max} 280 (ϵ 0.035)
β-Methylglucopyranoside	N.D.	E.A. (ϵ_{250} 0.04)
Cellobiose[b]	E.A.[f] (ϵ_{250} 0.09)	E.A. (ϵ_{250} 0.05)
Miscellaneous Compounds		
Pentaacetyl-β-d-gluco-pyranose[g]		λ_{max} 208 (ϵ 260)[i]
Gulonic Lactone[d]	λ_{max} 216 (ϵ 84)	λ_{max} 216 (ϵ 84)
Glucose phenylosazone[g,h]	-------	λ_{max} 253 (ϵ 23,000) 307 (ϵ 12,600) 388 (ϵ 25,000)

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[a] All wavelengths are in nanometers with molar extinction coefficients given in parentheses. When only end absorption was observed, the extinction at 250 nm. is given.

[b] Matheson, Coleman, and Bell.

[c] Mallinckrodt.

[d] Phanstiehl.

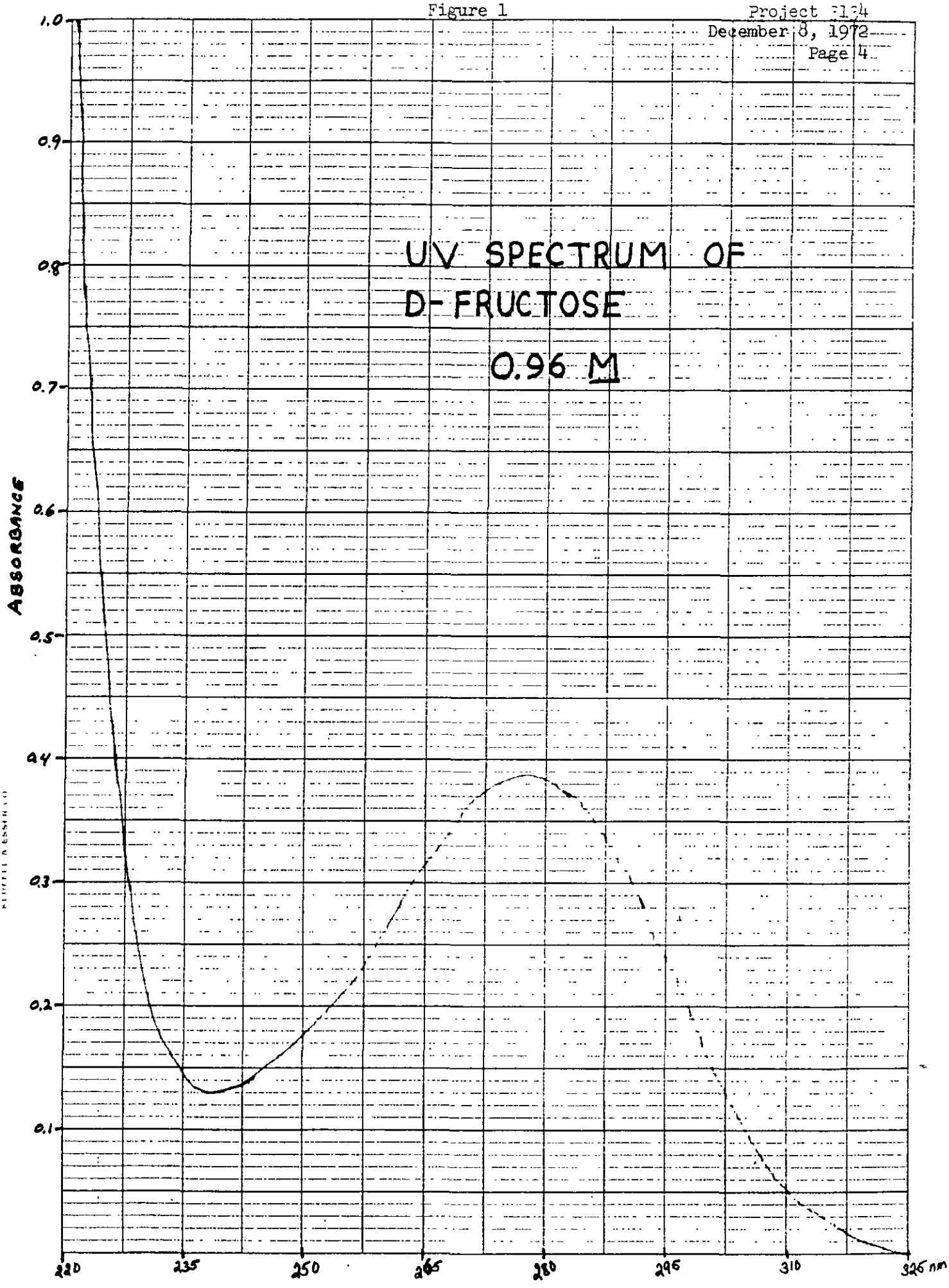
[e] N.D. means not determined.

[f] E.A. means end absorption only, no maximum above 210 nm.

[g] Prepared in this laboratory, see Experimental Section.

[h] Systematic name for this compound is D-arabino-hexulose phenylosazone.

[i] May not be accurate, due to oxygen absorption.


UV SPECTRUM OF
D-FRUCTOSE
0.96 M
ABSORBANCE
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
220
235
250
265
280
295
310
325 nm

Freehand extrapolation of the end absorption tail and subtraction from the peak gave an absorption maximum of 277 nm. and an extinction of 0.35, which is within experimental error of the previously obtained value. Furthermore, recrystallization of this chromatographed material gave pure fructose whose UV spectrum was identical to that given in Table I.

To obtain further evidence that the absorptivity was due to fructose, a sample was reduced with sodium borohydride. The crude product after deionization showed only an end absorption tail, with no hint of a shoulder near 277 nm. The molar extinction at 277 nm. due to the tail was 0.17. Thus, the species absorbing at 277 nm. in the fructose solutions is reduceable with sodium borohydride to a material which does not absorb in this region.

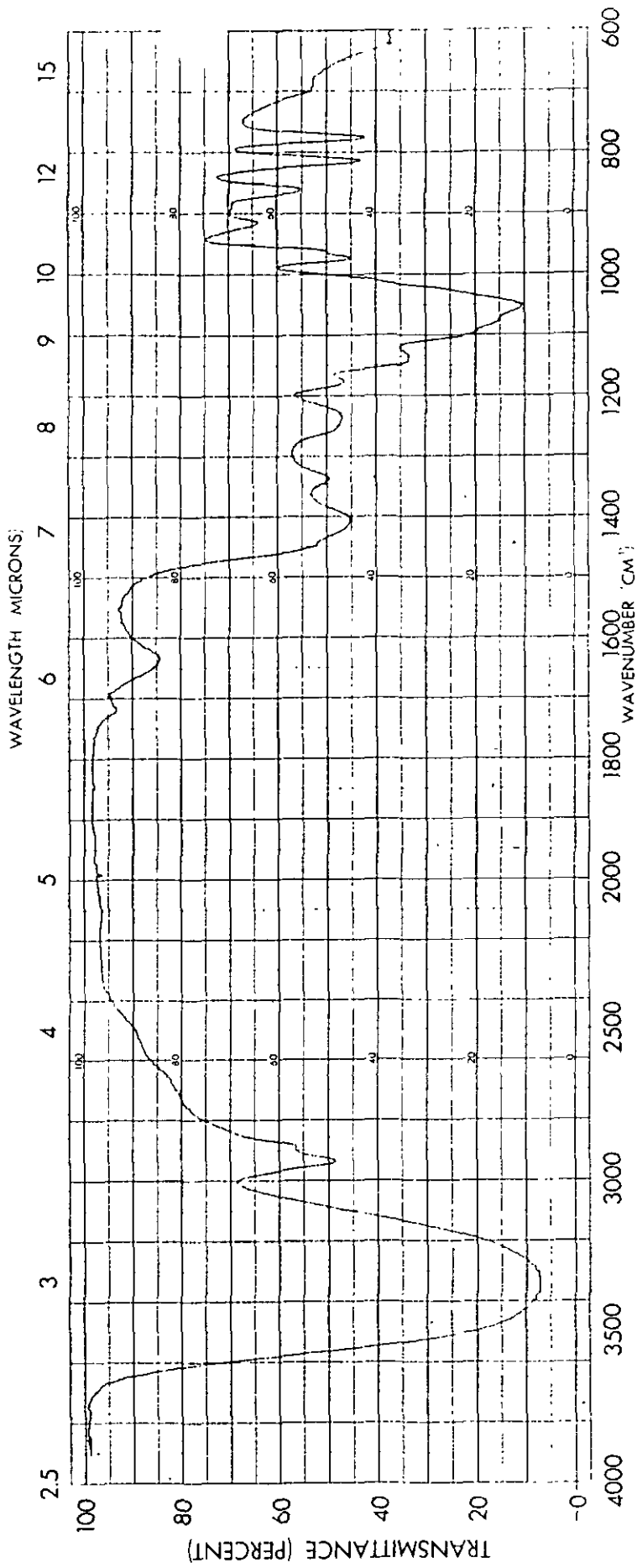
The infrared spectrum (Figure 2) of the sirup obtained by freeze-drying a solution of fructose showed a definite, although weak, absorption at 1718 $cm.^{-1}$, consistent with a small amount of keto form in solution.

The UV spectrum of fructose was also run in the presence of calcium chloride. The calcium chloride was slightly in excess of equimolar with the fructose. The absorption maximum shifted slightly to 273 nm. and the molar extinction coefficient (0.52) was somewhat higher. After 17 hr., the extinction coefficient decreased to 0.44.

In order to determine whether ferric ion could have a strong effect on the UV spectra of carbohydrates, dilute solutions of three such compounds were run in the presence of ferric sulfate. Thus, solutions of ca. 10^{-2} molar in glucose, cellobiose, and methyl β-D-glucoside were run in the presence of 2×10^{-4} normal ferric sulfate. The solutions were run against a blank of

Figure 2

INFRARED SPECTRUM OF FREEZE-DRIED FRUCTOSE



2×10^{-4} normal ferric sulfate and in all three cases, no significant absorption appeared in the UV spectrum. Under these conditions, only complex formation which caused a 50 fold increase in the absorptivity of the carbohydrate would have shown up. The strong UV spectrum of ferric sulfate prevented going to stronger cation concentrations.

The effect of pH on the UV spectrum of glucose was determined by running spectra in phosphate and borate buffers at several pH values. No change in the UV spectrum was observed for freshly dissolved samples over the range of pH 1.35 to 10.85. Upon standing for 26 hours, no change was noted from pH 1.35 to 9.05. However, the pH 10.85 sample showed a new absorption maximum at 270 nm. (ϵ 0.7) and a shoulder at 295 nm. A second run where absorbances were taken at 8 and 32 hours showed that this increase in absorption was a continuous process which was not complete in 32 hours.

DISCUSSION

The UV spectra of the aldoses and glycosides are about what one would expect for these compounds based on their structures. Since they are saturated compounds, they would not be expected to have absorption maxima at wavelengths greater than 210 nm. Most of the evidence indicates that only extremely small amounts of the aldehydo form of aldoses are present in aqueous solution [e.g. glucose, 0.0022 to 0.0031% was found by polarography(2)]. The absorption maximum observed for methyl α-D-gluco-pyranoside is very weak and was less intense after two recrystallizations than after one. Hence, it is probably due to an impurity.

The small molar extinction coefficients for these compounds should not be dismissed as photochemically insignificant. Table II shows the calculated values for the percentage of light absorbed for 1.0 molar solutions and 1.0 cm. path length for various extinction coefficients.

TABLE II

LIGHT ABSORPTION OF 1.0 M SOLUTIONS AND 1.0 CM. PATHLENGTH

MOLAR EXTINCTION COEFFICIENT	PERCENTAGE OF LIGHT ABSORBED
0.005	1.115%
0.010	2.28 %
0.020	4.50 %
0.040	8.80 %
0.100	20.6 %
0.400	60.3 %

Thus, even for a molar extinction coefficient as low as 0.005, which was the lowest found at 250 nm. in this study, sufficient light is absorbed to bring about a photochemical reaction at a reasonable rate.

The UV spectra observed for the miscellaneous compounds were also what one would expect, based on their structures. Esters would be expected to have absorption maxima at fairly short wavelengths(3)while phenylosazones are known to have several very intense maxima (4).

The ketoses showed appreciable absorption. Both had maxima at 277 nm. Considerable effort was made to purify fructose by various methods and in all cases the same UV spectrum was obtained. Furthermore, other workers have reported the same results previously (5,6). Therefore, it would appear that the absorptivity is due to fructose and is not caused by an impurity. Sodium borohydride reduction, under conditions which would reduce fructose to a mixture of glucitol and mannitol, eliminated the absorption maximum at 277 nm. Therefore,

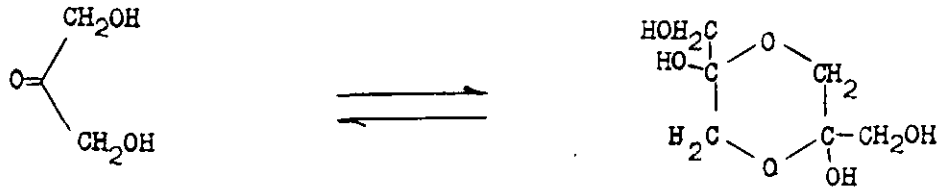
if the absorption was due to an impurity, then the impurity would have to be reduceable by sodium borohydride under these conditions.

The most reasonable explanation for the observed absorption is that there is an appreciable amount of acyclic keto form present in the aqueous solutions of these ketoses. The band at 1718 $cm.^{-1}$ in the infrared spectrum of fructose lends additional credence to this explanation.

There is considerable controversy in the literature concerning the amount of keto form of fructose present in solution. Bayer and Widder (7) methylated fructose and analyzed the mixture by gas chromatography. They found 3.4% of 1, 3, 4, 5, 6-pentamethyl-keto-D-fructose and claimed that this represented the amount of keto form in the solution of the free sugar. This method assumes that either all forms of the equilibrium mixture are methylated at the same rate or that the rate of methylation is very fast relative to the rate of equilibration, although the authors did not point out these assumptions. Considering the drastic conditions used, which included a six hour reflux in methanolic hydrochloric acid, it is doubtful whether the conditions of the assumptions were met. However, it is of interest to note that the values found for the aldehydo forms of galactose (0.7%), arabinose (0.3%), and glucose (0.3%) were considerably less than that found for fructose.

Spectroscopic methods are more desirable for studying equilibria since they do not disturb the systems under investigation. Listowsky and coworkers (5) examined several 2-ketohexoses by UV and circular dichroism. Their results for fructose (λ_{max} 278 nm., ϵ 0.46) and sorbose (λ_{max} 280 nm., ϵ 0.54) are similar to those obtained in the present study. Their extinction

coefficients are somewhat higher, expecially for sorbose, but they do not mention purification of the commercial sugars used. Using 1, 3-dihydroxy-2-propanone as model compound, the authors concluded that these ketoses exist with ca. 2 percent of the keto form present in solution. Their model compound could exist in equilibrium with its cyclic dimer, which would



make their results too high. However, close agreement between the extinction coefficient of the model (19.2) and that of acetone (16) makes this seem unlikely.

Swenson and Barker (6) criticized the use of UV for determining carbonyl forms of sugars as being too nonspecific. They favored the use of the carbonyl stretching band in the infrared spectrum. Their UV spectrum of pruified fructose (λ_{max} 277 nm., ϵ 0.38) is in very close agreement with that found in the present study. However, they did not find any carbonyl peak in the infrared spectrum of 0.3 M fructose in D_2O solution.

Tipson and Isbell (8) studied the infrared spectra of a series of carbohydrates. They recorded the spectra of the pure anomers and freeze-dried equilibrium mixtures in potassium halide pellets. Their spectrum for equilibrated fructose 0.5 $CaCl_2$ matches very closely the spectrum obtained in the present study for fructose, including a weak band at 1712 cm^{-1} (compared to 1718 cm^{-1} in the present study) which they attribute to carbonyl stretching of a small

amount of the keto form of fructose. Apparently the calcium chloride does not perturb the infrared spectrum greatly.

It is difficult to rationalize the difference between the infrared results of Swenson and Barker (6) and those of Tipson and Isbell (8), which are consistent with those of the present study. However, the first authors only showed a small portion of their spectrum and it is difficult to judge the overall intensity. It is possible that their spectrum is too weak to show this weak band.

Therefore, in the lack of further evidence to the contrary, it would seem that the most logical conclusion is that the UV absorption observed for fructose is not due to an impurity, but rather, due to a small amount (ca. 2%) of the keto form in the equilibrium mixture. This keto form will show up in the infrared spectrum if it is run concentrated enough. The UV results for sorbose show that approximately half as much keto form is present in its equilibrium mixture.

The conclusion of Swenson and Barker (6) that the UV absorption of fructose is due to an impurity does not seem valid. However their statement advising caution in the use of ultraviolet spectroscopy for this type of analysis seems warranted in view of the large differences in the UV spectra of commercial and purified sugars found in their work and in the present study. Careful purification of the compounds should be done before running the spectra.

The effect of calcium ion on the UV spectrum of fructose is also of interest. It is well known that sugars form complexes with calcium ion.

Angyal (9) has studied this subject in detail, and in particular, the effect of complex formation on the sugar conformation. Thus, it is possible that the observed change in the UV spectrum is due to a shift in the conformational equilibrium of fructose. The calcium ion undoubtedly also changes the electron distribution and solvation of fructose. At present, one cannot say which, if any, of these is causing the observed change.

Ferric ion had little or no effect on the UV spectrum of glucose under the conditions used. However, ferric sulfate absorbs strongly in the UV and visible regions of the spectrum so it could be a factor in light absorption in carbohydrate systems.

Hydroxide ion concentration had a strong effect on the UV spectrum of glucose when the pH reached ca. 11, although the effect was not instantaneous. Berl and Feazel (10) observed that an absorption maximum at 295 nm. gradually and irreversibly appears from a 3% glucose solution containing 0.01 normal sodium hydroxide (pH ca. 12). Since the reaction is slow and irreversible, it is probably the result of a base catalyzed condensation reaction, and not simply ionization.

The present results do not give a definitive answer to the question of the acetal chromophore. One would not expect such a saturated system to absorb light at wavelengths longer than ca. 220 nm. In the aldoses and glycosides, no maxima occur in the UV spectra, and so the acetal chromophore, if it exists, must have a very small extinction coefficient.

Another approach which might give meaningful information concerning the acetal chromophore is molecular orbital calculations. Several computer

programs are available through the Quantum Chemistry Program Exchange at nominal cost. Such calculations would give an energy difference between the ground and excited states for such a structural unit and thus predict a wavelength of absorption.

EXPERIMENTAL SECTION

PENTAACETYL-β-D-GLUCOPYRANOSE. This material was prepared by the method of Wolfrom and Thompson (11). The crude product (183 g., 84.6%) from 100 g. of D-glucose was treated with decolorizing carbon and recrystallized from 95% ethyl alcohol to give 129.2 g. (59.8%) of pure product.

GLUCOSE PHENYLOSAZONE. A mixture of 4.0 g. (0.022 mole) of glucose, 12.1 g. (0.084 mole) of phenylhydrazine hydrochloride, 12.1 g. (0.148 mole) of sodium acetate, and 80 ml. of water was heated on a steam bath for 1.5 hr., cooled to room temperature, and filtered. The material was recrystallized from a mixture of ethanol, water, and acetone to give 3.0 g. (38%) of yellow crystals.

PURIFICATION OF CARBOHYDRATES. All materials were recrystallized from aqueous ethanol as shown in Table III. Some compounds, where the hot solution showed color, were treated with decolorizing carbon, as indicated in Table III. Samples were stored under vacuum over phosphorus pentoxide for at least 24 hr. before bottling. All melting points were taken in evacuated, sealed capillaries, and are corrected. Good agreement with literature values was obtained in all cases, as shown in Table III.

Table III

RECRYSTALLIZATION SOLVENTS AND MELTING POINTS

Compound	Recry'n Solvent % EtOH	M.P.	Lit. M.P.	Ref.
L-Arabinose	80	157-158	157-160	a
D-Galactose	70	167-167.5	165	a
D-Glucose	80	151-152.5	147[e]	a
D-Fructose[g]	95	109.5-110.5	103-105	a
L-Sorbose[g]	80	163.5-164	165	a
α-Methylglucopyranoside	95	167.5-168	168	a
β-Methylglucopyranoside[g]	95	105-106	105	b
Cellobiose	30	234-235[e]	225[e]	a
Pentaacetyl-β-D-gluco-pyranose	95	131-132	135	b,c
Gulonic lactone	40	185.5-186	185	d
Glucose phenylosazone	f	208-208.5[e]	208	a

[a]Merck Index, Seventh Edition, Merck and Co., 1960. [b]F. J. Bates, Polarimetry, Saccharimetry, and the sugars, U. S. Govt. Printing Office, Washington, D. C., 1942. [c]S. Coffey, ed., Rodd's Chemistry of Carbon Compounds, Vol. I, Part F, Elsevier, London, 1967. [d]Handbook of Chemistry and Physics, 46th Edition, The Chemical Rubber Co., Cleveland, 1965. [e]Melted with decomposition. [f]Recrystallized from a mixture of water, ethanol, and acetone. [g]Treated with decolorizing carbon.

UV SPECTRA. All UV spectra were run on a Carey 15 Ultraviolet Spectrophotometer. All spectra were run in deionized, distilled water except pentaacetyl-β-D-glucopyranose and glucose phenylosazone, which were run in distilled 95% ethyl alcohol. For most of the carbohydrates, which showed low extinctions, concentrations of ca. 1 molar were used. Compounds of greater absorptivity were run at correspondingly lower concentrations.

CHROMATOGRAPHY OF FRUCTOSE. Fructose (1.00 g.) was chromatographed on a 2.8 x 62 cm column of silica gel (Grace Grade 62, 60-200 mesh) which had been slurry packed with chloroform-95% ethanol (2:1) and washed with 300 ml. of the same solvent. Elution with the above solvent (100 ml. fractions) gave 1.14 g. in fractions 5-8. Dissolution of the concentrated fractions in water gave turbid solutions. Most of this turbidity could be removed by filtration through millipore filters (0.45 microns). This was probably due to colloidal silica eluted off the column by the polar solvent system.

The UV spectrum of this material showed strong end absorption, which contributed to the absorption at 277 nm. Freehand extrapolation of the end absorption and subtraction from the absorption maximum gave an average value of 0.35 for the molar extinction of fractions 6 and 7. Recrystallization of these two fractions gave material with an absorption maximum at 277 nm. and an extinction of 0.39.

SODIUM BOROHYDRIDE REDUCTION OF FRUCTOSE. A combination of the general methods from the literature (12,13) was used. Thus, a solution of 181 mg. (4.80 mole) of sodium borohydride in 5.0 ml. of water was added to a solution of 740 mg. (4.11 mole) of recrystallized fructose in 10.0 ml. of water. The solution was

stirred for 18 hours at room temperature. Amberlite IR 120H cation exchange resin (ca. 3 g.) was added, giving a pH of 3 after 10 minutes. The mixture was filtered and concentrated. Five 20 ml. portions of methanol were added to the residue and distilled in vacuo to remove boric acid and give a white solid (645 mg., 86.4%) which was presumable a mixture of glucitol and mannitol.

This crude product showed very strong end absorption which extended to beyond 400 nm. (Such a strong absorption was also obtained by concentration of 80 ml. of the same methanol to 2.5 ml.) The crude product was passed through a 25 ml. of column of Amberlite MB-3, eluting with 75 ml. of water. This purified material showed end absorption with no hint of a bulge in the curve near 277 nm. The molar extinction at 277 nm. was 0.16, due to the end absorption tail.

INFRARED SPECTRUM OF FRUCTOSE. Recrystallized fructose was dissolved in water, allowed to stand at room temperature for 5 hours, and freeze-dried overnight (18 hours) to give a sirup. The infrared spectrum was run as a smear on a sodium chloride plate using a Perkin Elmer 621 infrared spectrophotometer. The spectrum is shown in Figure 2.

EFFECT OF pH ON THE UV SPECTRUM OF GLUCOSE. The following buffer solutions were prepared:

pH 1.35 Hydrochloric acid (0.83 ml.) was diluted to 100 ml. with water.

pH 3.25 Dibasic sodium phosphate dodecahydrate ($Na_2HPO_4 \cdot 12\ H_2O$, 1.43 g.) and 1.68 g. of citric acid monohydrate were dissolved in water and diluted to 100 ml.

pH 3.90 Dibasic sodium phosphate dodecahydrate (3.70 g.) and 2.40 g. of citric acid monohydrate were dissolved in water and diluted to 100 ml.

pH 9.05 Sodium borate decahydrate (1.52 g.) and 0.25 g. of boric acid were dissolved in water and diluted to 100 ml.

pH 10.85 Dibasic sodium phosphate dodecahydrate (4.0 g.) and 0.09 g. of sodium hydroxide were dissolved in water and diluted to 100 ml.

All of these buffers were transparent in the ultraviolet at wavelengths greater than 240 nm. Approximately 900 mg. samples of glucose were dissolved in 5.0 ml. of each buffer solution. UV spectra were run with an aqueous glucose solution in the reference beam. No absorption was observed which was not attributable to the buffer for the freshly dissolved solutions. After standing for 26 hr. the spectra were rerun. All samples were unchanged except the pH 10.85 sample, which showed a maximum at 270 nm. (ϵ 0.7) and a shoulder at 295 nm. Another sample in this buffer showed a similar spectrum with extinctions of 0.21 and 1.13 after 8 and 32 hours, respectively.