to rapid crystallization as needles, and after further crystallization overnight at 0°, a yield of 0.8 g. of hydrochloride was obtained. An additional 0.2 g. was obtained from the mother liquor. The pure compound dec. at (gas) 192° (preheated to 175°).

Anal. Calcd. for $C_{18}H_{20}NO_5Cl$: N, 4.58; Cl, 11.60. Found: N, 4.60; Cl, 11.6.

Specific rotation was $[\alpha]^{26}D$ +78.5° (0) → +66.6 (2 hours) in 0.1 *N* hydrochloric acid (*l* 2, *c* 0.9).

The variation in initial extrapolated values for the specific rotation was within 1° for samples crystallized rapidly or slowly and for samples obtained as second crops from the mother liquor. This narrow range suggests that the compound is a pure α-form.

2-Benzylamino-2-deoxy-D-glucose Pentabenzoate.—2-Benzylamino-2-deoxy-β-D-glucose, 1.3 g., was suspended in 70 ml. of pyridine, previously cooled to 0°. The suspension then was cooled rapidly in an acetone–Dry Ice bath, 10 g. of benzoyl chloride was added, and the mixture was kept at −20° for 4 days. The deep red mush was then poured into a mixture of 1500 g. of chopped ice and 25 g. of sodium bicarbonate and allowed to stand overnight. The pale yellow, partially granular product was filtered and washed repeatedly with saturated sodium bicarbonate solution followed by distilled water. Yield after drying *in vacuo* was 4 g. The compound was crystallized by adding 125 cc. of ethanol to a solution of the compound in 50 cc. of hot ethyl acetate and concentrating by boiling to 75 cc. The pentabenzoate rapidly crystallized on cooling to yield 1.49 g. (39%) of fine white prisms, m.p. 238–239.5°.

Anal. Calcd. for $C_{48}H_{39}NO_{10}$: C, 72.99; H, 4.98; N, 1.77. Found: C, 72.8; H, 5.03; N, 1.76; $[\alpha]^{25}D$ +70.0° (in chloroform, *l* 2, *c* 1.02).

2-Benzylamino-2-deoxy-D-gluconic Acid.—A suspension of 1.3 g. of 2-benzylamino-2-deoxy-D-glucose and 6.5 g. of red mercuric oxide in 150 ml. of water was heated in a boiling water-bath for 30 minutes and refluxed for 5 minutes. The amino sugar dissolved without formation of color and with no observable precipitation of mercury. The suspension was filtered hot. The filtrate was treated with hydrogen sulfide and a heavy precipitate of mercuric sulfide formed. After aeration and filtration with carbon, the clear colorless filtrate was concentrated *in vacuo* to 15 ml. with slight crystallization. The aqueous solution was heated to dissolve the precipitate, an equal volume of ethanol was added, and the solution was allowed to crystallize at 0° for 3 days. A yield of 545 mg. (39%) was obtained. Recrystallization from 50% ethanol yielded the pure acid as needles, dec. (gas) 221° (preheated to 200°)

Anal. Calcd. for $C_{18}H_{19}NO_6$: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.7; H, 6.61; N, 4.90.

Specific rotation was $[\alpha]^{26}D$ +50° in 0.1 *N* sodium hydroxide (*l* 2, *c* 1.03). Twenty ml. of this solution was diluted with 5 ml. of 1.0 *N* hydrochloric acid, $[\alpha]^{26}D$ +14.4° (7.5 min.) → +16.7° (94 hr.).

Hydrogenolysis of 2-Benzylamino-2-deoxyglucose.—A solution of 1.1 g. of benzylaminoglucose in 50 cc. of absolute methanol containing 735 mg. of palladium-on-carbon (5%) was shaken with hydrogen at atmospheric pressure and 27° for 4 hours, about 90% of the absorption occurring during the first 2 hours. A net total of 118 cc. (S.T.P.) of hydrogen was absorbed equivalent to 1.29 moles H_2 per mole of compound. After filtration, 3 cc. of 5 *N* hydrochloric acid was added dropwise to the solution. After storage for several hours at 0°, 470 mg. of D-glucosamine hydrochloride was obtained. An additional 100 mg. was obtained from the mother liquor to give a combined yield of 65%. The identity of the compound as α-D-glucosamine hydrochloride was established by comparison of X-ray powder diagrams[11] with authentic material.

Reaction of 2-Alkylamino-2-deoxyhexoses with Phenyl Isothiocyanate.—In a typical procedure, 1.0 g. of 2-*n*-butylamino-2-deoxyglucose was dissolved in 25 cc. of water to which was added 3.0 g. of phenyl isothiocyanate, followed by 100 cc. of acetone. The clear colorless solution turned a pale green in a few minutes but gradually faded to its original color. After standing for 40 hours at room temperature, the solution was concentrated *in vacuo* to a white solid which was extracted once with 25 cc. of chloroform and washed 4 times with 20-cc. portions of benzene. Crystallization of the residue from ethanol yielded 925 mg. (66%) of 5-(D-*arabo*-tetrahydroxybutyl)-3-phenyl-1-*n*-butyl-2-imidazolidinethione as white fibrous crystals. The corresponding ethyl, isopropyl and *n*-propyl derivatives were recrystallized from ethyl acetate, and the benzyl derivative was recrystallized by addition of petroleum ether (60–70°) to an ethyl acetate solution. Yields were generally 50–60%.

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(11) I am indebted to K. J. Palmer of this Laboratory for X-ray measurements.

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[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Effect of Aqueous Sulfuric Acid on Sugars. II. Spectrophotometric Studies on the Hexoses; Identification of the Ether-soluble Products Formed[1]

BY F. A. H. RICE AND LAWRENCE FISHBEIN

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When a number of reducing hexoses were each heated under the same conditions with aqueous sulfuric acid, the ultraviolet absorption spectrum which developed was the same for each of the hexoses studied. However, the rate at which the ultraviolet spectrum developed and the steady state, as measured by the ultraviolet absorption spectra, were characteristic of the individual hexose. The hexoses were found to react at 100° in 4 *N* sulfuric acid in the following order: D-gulose > D-talose > D-galactose > D-altrose > D-mannose > D-glucose. In 20 *N* sulfuric acid at room temperature, however, the order was D-talose > D-mannose > D-galactose > D-glucose > D-altrose. The pentoses under the same conditions in 20 *N* sulfuric acid reacted in the order: D-xylose > D-ribose > D-lyxose > D-arabinose. The formation of the ultraviolet spectrum was found to be primarily due to the production of compounds which could be extracted from aqueous sulfuric acid by means of ether. These compounds were separated by the chromatography of their 2,4-dinitrophenylhydrazones on silicic acid and identified as acetaldehyde, propionaldehyde, formaldehyde and 5-(hydroxymethyl)-2-furaldehyde. Another compound as yet unidentified also was isolated.

In previous studies on the effect of aqueous sulfuric acid on the aldopentoses,[2] it was found that each of the aldopentoses developed the same ultraviolet spectrum under all the conditions of temperature and concentrations studied. The rate at which the spectrum developed and the final steady state, however, were found to be characteristic of the individual pentose. The apparent differences in the ultraviolet spectrum of individual pentoses,[3,4] when examined after identical treatment for a speci-

(1) Published with permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

(2) F. A. H. Rice and L. Fishbein, THIS JOURNAL, **78**, 1005 (1956).

(3) R. M. Love, *Biochem. J.*, **55**, 126 (1953).

(4) Miyoshi Ikawa and C. Niemann, *Arch. Biochem. and Biophys.*, **31**, 62 (1951); *J. Biol. Chem.*, **180**, 923 (1949).

TABLE I

RATIOS OF THE ABSORPTION MAXIMA OF SOME D-ALDOHEXOSES IN ACID SOLUTION

Temp., °C., and concn. of acid	Time	OD_{237}/OD_{290} D-Gulose	D-Talose	D-Galactose	D-Altrose	D-Mannose	D-Glucose
100°	90 min.	0.519	0.500	0.519	0.498	0.508	0.500
(4 N H_2SO_4)	140 min.	.520	.490	.500	0.510	.499	0.500
23°	21 hr.	.522	.443	.700	1.55	.301	1.00
(20 N H_2SO_4)	25 hr.	.524	.448	.698	1.55	.312	1.00

fied interval of time, are attributable to this difference in the rate of formation of the substances responsible for the ultraviolet spectrum and the final steady state and not to the formation of compounds peculiar to the particular pentose. To account for the observed ultraviolet spectrum of a pentose in acid solution, formation of more than one product has been suggested.[3,5] We have found that[2] the compounds primarily responsible for the development of the ultraviolet spectrum can be extracted from aqueous acid by means of ether. These ether-soluble compounds were separated by the chromatography of their 2,4-dinitrophenylhydrazones on silicic acid and identified as 2-furaldehyde, crotonaldehyde, acetaldehyde and formaldehyde. Formaldehyde shows no ultraviolet absorption[6] and hence would probably not contribute to the characteristic ultraviolet spectrum.

It was considered of interest to investigate in the same manner the action of aqueous sulfuric acid on the reducing hexoses and attempt to isolate and identify the compounds responsible for the development of their ultraviolet spectrum.

Results and Discussion

When aldohexoses were heated in aqueous sulfuric acid, the shape of the ultraviolet spectrum which developed did not appear to depend on the configuration of the hexose. The shapes of the curves of a series of ultraviolet spectra of any one aldohexose, taken over a period of time, were essentially the same as the shapes of the curves obtained from any other aldohexose. The rate of development of the ultraviolet spectra depended on the configuration of the hexose. Figure 1 shows the relationship between the optical density or absorbance (O.D.) at 290 mμ and the time of heating. The aldohexoses were dissolved in 4 N sulfuric acid and heated at the temperature of the boiling water-bath. The concentration of each hexose was the same. Completely analogous series of curves were obtained at other concentrations of either hexose or acid. However, the O.D. observed, on reaching a steady state could be increased by raising either the concentrations of the sugar or the acid. The development of the ultraviolet absorption followed essentially the same pattern found in the pentose series,[2] and hence the curves will not be reproduced here.

In 20 N sulfuric acid at room temperature, the hexoses developed ultraviolet absorption spectra at a rate characteristic of the individual hexose and in general showed the same characteristics that were shown by the hexose when heated in less concentrated aqueous acid solution (Fig. 1). On the other hand, the order of reactivity, as measured by the absorbance at the steady state, changed. It was found that, in 20 N acid at room temperature, the final steady state was such that: talose > mannose > galactose > glucose > altrose.

Since a similar change had not been noticed in our studies on the aldopentoses, the four D-aldopentoses were run under the same conditions as the hexoses in 20 N acid at room temperature. The order of activity, as measured by the absorbance at the steady state, changed from: lyxose > ribose > xylose > arabinose to: xylose > ribose > lyxose > arabinose.

The ultraviolet spectrum of any one hexose (in 20 N acid at room temperature), after the elapse of a given time, was not superimposable, until the steady state had been reached, on the spectrum obtained after a longer interval of time. Table I shows the ratio of the two absorption maxima at 237 and 290 mμ for a series of hexoses after they had reached a steady state in 4 N sulfuric acid at 100°. The corresponding ratios in 20 N acid at room temperature also are given. It will be observed that, although the ratios in hot 4 N acid are the same within the limit of our experimental error, the ratios found after treatment with 20 N acid at room temperature depend upon the configuration of the hexose.

After the aqueous acid solution of the hexose was extracted with ether, the aqueous phase showed essentially no ultraviolet absorption. The ether extract, on the other hand, showed the ultraviolet absorption characteristics of the solution obtained by dissolving the hexose in aqueous acid, with absorption maxima at 237 and 290 mμ. The ratio of the O.D. at 237 and 290 mμ was greater in ether than in aqueous acid solution. This may be attributed to the effect of the solvent.[7] When the solution obtained by dissolving a hexose (*e.g.*, D-glucose) in aqueous acid was continuously extracted with ether at room temperature and comparisons were made between that of the ether extract and the ultraviolet absorption of an aqueous acid solution of the same hexose under the same conditions (except for the absence of ether), no differences in the development of the ultraviolet absorption spectra could be found, apart from the ratio of the ultraviolet absorption maxima (OD_{237}/OD_{290}, which was higher for the ether extract). Figure 2 shows no change in the ratio of the absorption maxima in the ether phase during the continuous ether extraction of the hexose in aqueous acid solution. This is to be compared with the change in the ratio of the absorption maxima of the hexose in aqueous acid of the same concentration and temperatures. The two curves are superimposable, as would be ex-

(5) F. Bandow, *Biochem. Z.*, **294**, 124 (1937).

(6) J. E. Purvis, *J. Chem. Soc.*, **127**, 9 (1925).

(7) R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); A. Burawoy, *J. Chem. Soc.*, 1177 (1939); E. C. C. Baly, *Phil. Mag.*, **S.6**, **27**, 632 (1914).

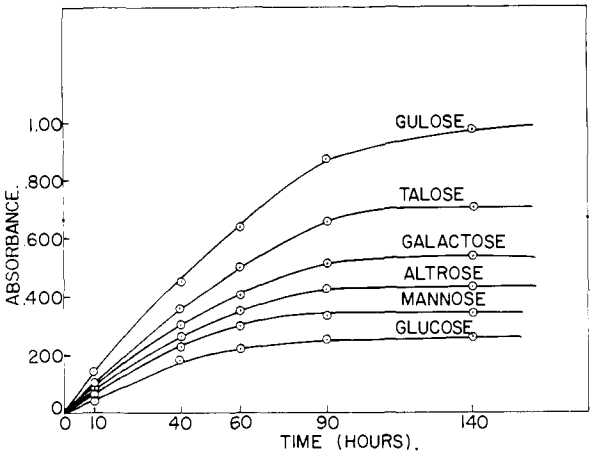

Fig. 1.—Development of the steady state of a number of aldohexoses in 4 *N* sulfuric acid, at 100°; initial concentration of aldohexoses, 40 mg. per 100 ml. of solution.

pected if the observed difference in the ratio of the absorption maxima is due to the effect of solvent on the absorption maxima. Additional weight is given to this explanation by the observation that extraction of the hexose in aqueous acid with ether at any particular time reduces the ultraviolet absorption of the aqueous phase to a negligible value while the characteristic ultraviolet absorption is found in the ether extract.

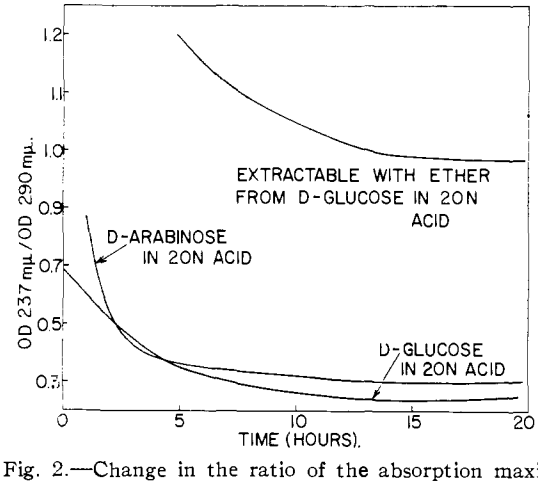

Fig. 2.—Change in the ratio of the absorption maxima (237 mμ/290 mμ) found in the ether phase, as D-glucose (dissolved in aqueous sulfuric acid) is continuously extracted with ether, compared, over a period of time, with the corresponding ratios of the absorption maxima of the unextracted aqueous acid solution of D-glucose. D-Arabinose in aqueous acid solution is shown for comparison: concentration of D-glucose, 40 mg. per 100 ml. of solution; initial concentration of D-arabinose, 40 mg. per 100 ml. of solution; concentration of sulfuric acid, 20 *N*; temperature 23°.

When a solution of a hexose in aqueous sulfuric acid was continuously extracted with ether, the concentration of ether-extractable compounds, as shown by the O.D. at the ultraviolet maxima, increased in the ether phase, even after the point at which a steady state would have been reached by the hexose in aqueous acid solution. Figure 3 shows the increase in O.D. at 290 mμ of the ether extract plotted as a function of time. The corresponding development of the ultraviolet maxima of the sugar in aqueous acid is given for comparison. The O.D. does not reach a steady state, in contrast to the O.D. of the unextracted aqueous acid solution of the hexose. The probable reason for this is that the equilibrium in the aqueous phase is continually shifted by extraction of the aldehydes.

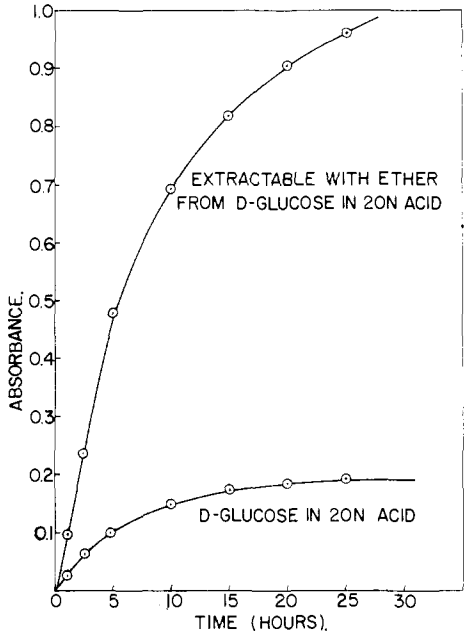

Fig. 3.—Development of ultraviolet absorption at 290 mμ in the ether phase of an aqueous sulfuric acid solution of D-glucose as it is continuously extracted with ether, as compared to the development of the ultraviolet absorption in the unextracted aqueous acid solution: concentration of aldohexoses, 40 mg. per 100 ml. of solution; concentration of acid, 20 *N*; time 30 hr.; temperature, 23°.

In order to isolate and identify the compounds responsible for the development of the ultraviolet absorption spectra from a hexose in acid solution, the hexose (25 g. of D-glucose) was dissolved in 20 *N* sulfuric acid and continuously extracted with ether. The ether extract was then shaken with Brady reagent[8] (a solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid), and the ether was removed at room temperature under an air jet. The insoluble precipitate (approx. 500 mg.) was collected by filtration and chromatographed on a 43 mm. × 250 mm. column, using a 3:1 (by weight) mixture of silicic acid[9]–Celite[10] as the adsorbent.

The column was developed with benzene containing 0.5 ml. of *t*-butyl alcohol per 100 ml. Five zones were observed. The column was then extruded and the zones cut out and their contents eluted with ethanol.

The material from each zone was crystallized from ethanol and the products identified as the 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, formaldehyde and 5-(hydroxy-

(8) O. L. Brady and G. V. Elsmie, *Analyst*, **51**, 77 (1926).

(9) Reagent Grade obtained from Merck and Co., Rahway, N. J.

(10) No. 535 obtained from the Johns-Manville Corp., New York, N. Y.

Table II

Properties of the Chromatographically Separated 2,4-Dinitrophenylhydrazones Formed on Treatment of D-Glucose with Acid

Zone	Weight, mg.	M.p., °C.	Carbon, % Found	Carbon, % Calcd.	Hydrogen, % Found	Hydrogen, % Calcd.	Nitrogen, % Found	Nitrogen, % Calcd.
A (formaldehyde)	55	162–164	40.37	40.0	3.15	2.89	26.70	26.5
B (propionadehyde)	50	154–156	45.53	45.37	4.40	4.20	23.50	23.53
C (acetaldehyde)	45	164–166	42.88	42.85	3.53	3.56	24.72	25.00
D (5-(hydroxymethyl)-2-furaldehyde)	255	198	47.06	47.55	3.26	3.00	18.30	18.00
E ($C_{19}H_{16}N_8O_9$)	10	130–131	45.16	45.6	3.36	3.23	22.08	22.4

methyl)-2-furaldehyde and one compound which has not yet been identified. Table II shows the characteristics of the several 2,4-dinitrophenylhydrazones isolated. D-Glucose, D-mannose and D-galactose gave the same results.

Experimental

The ultraviolet absorption spectra were measured at room temperature by means of a Beckman DU quartz spectrophotometer with matched 1-cm. cells.

A sufficient quantity of the hexose to give the desired concentration was weighed out and dissolved in 100 ml. of aqueous sulfuric acid, previously standardized to the desired concentration. When samples were heated, the volume was kept constant by the addition from time to time of small amounts of water. At intervals, aliquots were withdrawn and cooled at once to 0° in an ice–salt-bath. The appropriate dilutions were made in volumetric flasks with acid of the same concentration and the absorption spectra were recorded at room temperature.

Reaction of D-Glucose with 20 *N* Sulfuric Acid.—Twenty-five grams of D-glucose was dissolved in 50 ml. of 20 *N* sulfuric acid. The solution was immediately extracted with approximately 500 ml. of ether in a continuous liquid–liquid extraction apparatus. At intervals, over a period of 24 hr., aliquots of 2.5 ml. of the ether solution were withdrawn, made up to 10 ml. in a volumetric flask and the ultraviolet absorption of the solution was determined. At the end of 26 hr., the unused ether extract was cooled and shaken with Brady reagent[8] (0.3 g., of 2,4-dinitrophenylhydrazine in 100 ml. of 2 *N* hydrochloric acid) for 10 minutes. The ether was removed at room temperature under an air jet, and the insoluble 2,4-dinitrophenylhydrazones were removed by filtration on a fritted-glass filter, yield 500 mg.

Chromatography of the Mixed 2,4-Dinitrophenylhydrazones. Method 1.—The mixture of 2,4-dinitrophenylhydrazones (500 mg.) was extracted at room temperature with 40 ml. of benzene. Some 250 mg. of the mixture was insoluble and was removed by filtration on a fritted-glass filter. The material which was soluble in benzene was chromatographed on a 3:1 (by weight) mixture of silicic acid[9]–Celite[10] on a 43 mm. × 250 mm. column. The method of Roberts and Green[11] with certain modifications was used. The column was first washed with 1 liter of benzene and then developed with 1500 ml. of benzene containing 0.5 ml. of *t*-butyl alcohol per 100 ml. Three zones were observed: A, light yellow in color, approximately 1 cm. in width at the top of the column; B, orange in color, approximately 1 cm. in width at a distance of approximately 3 cm. from the top of the column; C, orange-brown in color, approximately 1 cm. in width, at a distance of 6 cm. from the top of the column. The zones were cut and their contents eluted with ethanol. The alcoholic solutions, on concentration, yielded crystalline products which were identified as the 2,4-dinitrophenylhydrazones of formaldehyde (A), propionaldehyde (B) and acetaldehyde (C) (Table II).

The fraction of the mixed hydrazones which was insoluble in benzene (approximately 250 mg.) was dissolved with the aid of heat in 1 ml. of *t*-butyl alcohol, and 200 ml. of benzene was added. No precipitation occurred and the clear solution was chromatographed as above on a silicic acid–Celite column. The column was developed with 1500 ml. of benzene containing 1 ml. of *t*-butyl alcohol per 100 ml. of benzene. Two zones were observed: D, orange-red in color, approximately 2 cm. in width at the top of the column; E, light orange-yellow in color, approximately 1 cm. in width at a distance of approximately 7 cm. from the top of the column. The zones were cut and eluted with ethanol. On concentration of the ethanolic solutions, crystalline products were obtained which were identified as the 2,4-dinitrophenylhydrazones of 5-(hydroxymethyl)-2-furaldehyde (D) and E which has not so far been identified. The analyses on the material recovered from zone E indicated a compound of molecular formula $C_{19}H_{16}N_8O_9$ (Table II). The compound gave a blue color when an ethanolic solution was treated with dilute aqueous sodium hydroxide.

Method 2.—The mixed 2,4-dinitrophenylhydrazones were not extracted with benzene but dissolved at once in 1 ml. of *t*-butyl alcohol which was then diluted to 300 ml. with benzene. No precipitation took place. The solution was chromatographed as in method 1, using 1500 ml. of benzene containing 0.5 ml. of *t*-butyl alcohol per 100 ml. to develop the column. Three zones were observed. These were cut out and eluted with ethanol in the usual manner. The zone at the top of the column, orange-red in color and approximately 2 cm. in width, did not yield a crystalline product; the zone approximately 9 cm. from the top of the column, approximately 1 cm. in width, was found to contain material which was identical with the material from E above; the zone 15 cm. from the top of the column, orange-yellow in color and approximately 1 cm. in width, was found to contain material which was identical with that obtained from B above.

The material eluted from the zone found at the top of column was dissolved in 1 ml. of *t*-butyl alcohol, and the solution was then diluted to 300 ml. with benzene. This solution was rechromatographed on silicic acid–Celite, using 1500 ml. of benzene containing 1 ml. of *t*-butyl alcohol per 100 ml. of benzene to develop the column. Three zones were observed. These were cut out and eluted with ethanol in the usual manner. The contents of one zone at the top of the column, orange-red in color and approximately 2 cm. in width, crystallized on concentration of the ethanol solution and was found to be identical with that obtained from D. The zone, light-yellow in color, approximately 1 cm. in width and approximately 4 cm. from the top of the column, yielded material which crystallized on concentration of the ethanol solution and was found to be identical with that obtained from zone A. The zone, orange-yellow in color, approximately 1 cm. in width and approximately 8 cm. from the top of the column, yielded material which crystallized on concentration of the ethanol solution and was found to be identical with that obtained from zone C.

The same compounds were isolated both from D-mannose and D-galactose when they were treated as above.

Acknowledgment.—We wish to express our thanks to Dr. N. K. Richtmyer of the National Institutes of Health for the samples of D-talose and D-altrose.

Indian Head, Md.

(11) J. D. Roberts and Charlotte Green, *Ind. Eng. Chem., Anal. Ed.*, **18**, 335 (1946).