A SPECTROPHOTOMETRIC STUDY OF THE BEHAVIOR OF CARBOHYDRATES IN SEVENTY-NINE PER CENT SULFURIC ACID*

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(Received for publication, May 10, 1949)

Carbohydrates *per se* do not exhibit significant specific absorption in the 210 to 400 mμ region (1, 2), but, when exposed to the action of strong mineral acids, they are transformed into products which do absorb specifically in this region and which will react with a variety of reagents to give colored addition products. This latter fact has been utilized in the development of colorimetric methods suitable for the identification and estimation of mono-, di-, and polysaccharides, as, for example, in the carbazole-sulfuric acid method described by Dische (3, 4). Although a considerable amount of information is available with respect to the spectral characteristics of the various colored addition products (5–7), relatively little is known about the absorption spectra of solutions of carbohydrates in strong sulfuric acid, the medium used in most of these procedures.

Bandow (8) determined the absorption spectra of solutions of a number of sugars in concentrated sulfuric acid after the solutions had been allowed to remain at room temperature for a day and noted that, while the spectra were not superimposable, the absorption curves for the hexoses, pentoses, and glucuronic acid were similar to that of furfural determined under the same conditions. In the course of a systematic study (6) of the variables influencing the Dische carbazole method the spectra of solutions of glucose, galactose, mannose, fructose, and *N*-acetylglucosamine in 79 per cent sulfuric acid were determined, and it was noted (6) that with fructose the strong extinction, observed with solutions that had stood at 25° for 40 minutes, decreased when these solutions were subsequently heated at 100° for 15 minutes. In contrast to the above, solutions of the aldohexoses exhibited no significant specific absorption when allowed to stand at 25° for 40 minutes but did absorb strongly in the 315 mμ region after the solutions had been heated at 100° for 15 minutes.

The above observations suggested the possibility of utilizing the ultra-

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

† Contribution No. 1299.

violet absorption spectra of strong sulfuric acid solutions of the saccharides for analytical purposes and we have now demonstrated that this procedure is a practical one in a number of cases. The desirability of using 79 per cent sulfuric acid as a medium was suggested by earlier studies in these laboratories (6), and it will be noted that sulfuric acid of this concentration is still an excellent solvent for practically all polyhydroxy compounds (9).

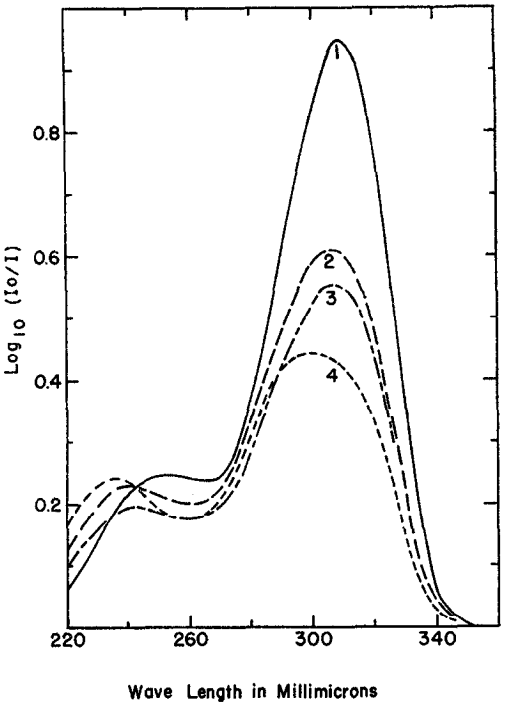

FIG. 1. Spectra of heated solutions of the aldopentoses in 79 per cent sulfuric acid. Curve 1, 100 γ of xylose; Curve 2, 100 γ of ribose; Curve 3, 100 γ of arabinose; Curve 4, 100 γ of lyxose.

EXPERIMENTAL

84 per cent (by weight) sulfuric acid was prepared from reagent grade acid. The carbohydrate solutions contained 100 γ of anhydrous compound per ml., account being taken of water of crystallization whenever necessary. The glucosamine hydrochloride solution contained 100 γ of free base per ml. The identity and purity of the individual compounds were checked by a determination of their optical rotation and their melting points.

9 ml. portions of 84 per cent sulfuric acid contained in 15 $\times$ 150 mm.

test-tubes were thoroughly chilled in an ice bath. 1 ml. portions of the aqueous carbohydrate solutions were then added and the resultant solutions mixed by stirring with a glass rod, the tubes being immersed in the ice bath to avoid a rise in temperature. These solutions, which were uniformly 79 per cent in sulfuric acid, were placed in a bath kept at 25° for 2 hours or in a boiling water bath for 15 minutes. The spectra of these solutions were determined, at room temperature, after cooling in a water bath, with a Beckman model DU spectrophotometer equipped with

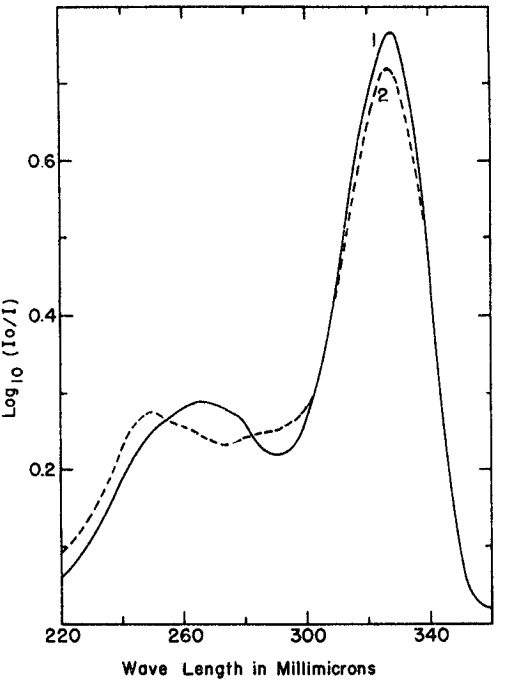

FIG. 2. Spectra of heated solutions of two 6-desoxyaldohexoses in 79 per cent sulfuric acid. Curve 1, 100 γ of rhamnose; Curve 2, 100 γ of fucose.

1 cm. quartz cells. The values reported are averages of two or more separate determinations, with a maximum deviation of ± 5 per cent from the mean value. The curves were constructed from readings taken at 10 mμ intervals except in regions of maxima and minima, where the interval was reduced to 5 and 2.5 mμ.

The absorption curves for solutions of glucose, galactose, mannose, fructose, and *N*-acetylglucosamine in 79 per cent sulfuric acid, after these solutions had been heated at 100° for 15 minutes, have been presented earlier (6). The absorption curves for comparable solutions of xylose,

arabinose, ribose, lyxose, rhamnose, fucose, sorbose, glucosamine, glucurone, and galacturonic acid are given in Figs. 1 to 3, inclusive.

The salient spectral characteristics of 79 per cent sulfuric acid solutions of the carbohydrates investigated are given in Table I. Molecular extinction coefficients were calculated from the relation $\epsilon = 1/cd \log_{10} I_0/I$, where c = the final concentration in moles per liter of the solution and

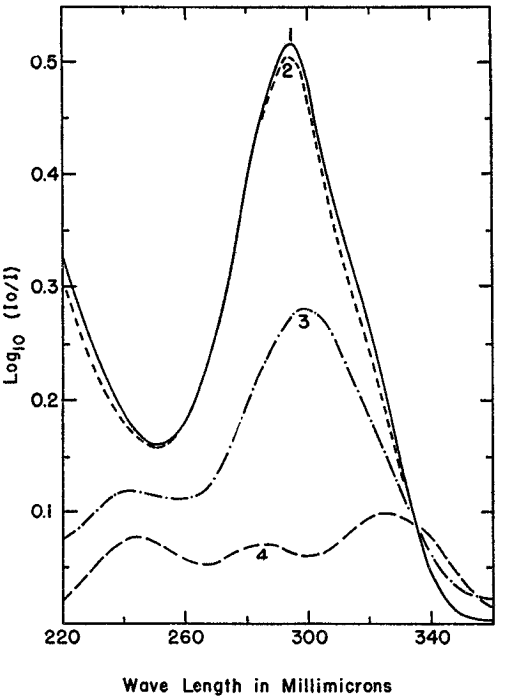

FIG. 3. Spectra of heated solutions of various sugars in 79 per cent sulfuric acid Curve 1, 100 γ of glucurone; Curve 2, 100 γ of galacturonic acid; Curve 3, 100 γ of sorbose; Curve 4, 100 γ of glucosamine.

d = the cell thickness in cm. In Table I values of $\lambda_{max.}$ and $\epsilon_{max.}$ are given for solutions heated at 100° for 15 minutes; values of ϵ_{315} for solutions maintained at 100° for 15 minutes and at 25° for 2 hours, respectively, are recorded. Extinction coefficients were determined at 315 mμ, because practically all of the principal maxima lie in the region of 300 to 325 mμ and values obtained at 315 mμ appear to be generally useful for comparative purposes. In addition, fructose solutions maintained at 25°, which were of particular interest because of the unusual behavior of the ketohexoses, absorb maximally at this wave-length.

The 2 hour period selected for observations at 25° was a compromise.

TABLE I

Spectral Characteristics of Solutions of Various Carbohydrates in 79 Per Cent Sulfuric Acid

Carbohydrate	15 min. at 100°			2 hrs. at 25°
	$\lambda_{max.}$	$\epsilon_{max.} \times 10^{-3}$	$\epsilon_{315\ m\mu} \times 10^{-3}$	
	mμ			
D-Xylose	309	14.3	13.6	2.7
	254	3.8		
D-Arabinose	307	8.3	7.7	0.46
	243	3.0		
D-Ribose	307	9.2	8.5	0.82
	242	3.5		
D-Lyxose	299	6.6	5.7	1.26
	237	3.6		
L-Fucose	327	11.8	8.9	0.98
	250	4.6		
L-Rhamnose	327	12.6	9.5	1.00
	266	4.8		
D-Glucose	308	4.0	3.7	0.09
	250	1.6		
D-Mannose	305	2.3	2.2	0.11
	249	2.9		
D-Galactose	300	4.9*	3.6	0.13
	250	2.2		
D-Fructose	313	3.1	3.0	11.5
	255	1.4		
L-Sorbose	299	5.0	3.9	10.6
	243	2.2		
D-Glucurone	294	9.3	5.5	0.02
D-Galacturonic acid	294	9.8	5.6	0.17
N-Acetyl-D-glucosamine			0.31	0.0
D-Glucosamine hydrochloride	245	1.4	1.5	0.04
	287	1.25		
	325	1.75		
D-Mannitol			0.09	0.0
meso-Inositol			0.02	0.0
Adonitol			0.0	0.0
Sucrose			6.3	12.0
Maltose			6.9	0.17
Trehalose			6.9	0.03
Lactose			7.1	0.24
Melibiose			7.2	0.31
Raffinose			9.8	11.6
Melezitose			10.0	12.8
Starch			3.2†	0.02†
Glycogen			3.1†	0.06†
Inulin			2.6†	9.6†

* This value is about 0.8 higher than that calculated from a previous determination (6).

† Calculated by taking the anhydrohexose unit ($C_6H_{10}O_5$) as the molecular unit.

A period longer than 2 hours at 25° was not judged desirable, because solutions of the pentoses, notably xylose, began to absorb rather strongly and with a period of less than 2 hours the extinction coefficients of the solutions of sorbose and the fructose containing oligosaccharides were increasing too rapidly to permit the attainment of reproducible results. It was noted previously (6) that the extinction coefficients of the bands in the 300 to 325 mμ region decreased slowly when the solutions were maintained at 100° for prolonged periods. It appears that with a number of sugars $\epsilon_{max.}$ is attained in approximately 5 minutes heating at 100°, decreasing 10 to 15 per cent during the next few minutes of heating. 15 minutes at 100° appeared to be a reasonable compromise, considering convenience, reproducibility, and general applicability.

TABLE II

Behavior of Classes of Monosaccharides in 79 Per Cent Sulfuric Acid

Class	At 25°, 315 mμ		At 100°, 315 mμ	
	$\epsilon \times 10^{-3}$	$\text{Log}_{10} I_0/I$*	$\epsilon \times 10^{-3}$	$\text{Log}_{10} I_0/I$*
Aldopentoses and 6-Desoxyaldohexoses	>0.4	>0.025	>5.0	>0.35
Aldohexuronic acids	<0.2	<0.010	>5.0	>0.25
Aldohexoses	<0.2	<0.010	<4.0	<0.25
Ketohexoses	>10.0	>0.550	<4.0	<0.25
Polyols	<0.1	<0.005	<0.1	<0.005

* Of solutions containing 10 γ of anhydrous compound per ml. of acid solution

Results

From the data contained in Table I certain generalizations, summarized in Table II, may be made with respect to the behavior of non-nitrogenous monosaccharides in 79 per cent sulfuric acid. It is evident that the information contained in Table II will permit one to assign a given non-nitrogenous monosaccharide to a particular class and thus facilitate its ultimate characterization either by further spectrophotometric analysis, by means of the data contained in Table I, or by paper chromatography (10).

With binary mixtures of non-nitrogenous monosaccharides it follows from the data given in Table I that those mixtures which contain a ketohexose or a 6-desoxyaldohexose as one component are amenable to analysis by the above spectrophotometric procedure. Binary mixtures of two members of the same class (Table II) in most cases cannot be analyzed satisfactorily, nor can binary mixtures containing only aldopentoses, aldohexoses, or aldohexuronic acids, though in these cases a value for $\log_{10} I_0/I$ greater than 0.25 for a solution containing 10 γ of solute per ml., heated at 100° and observed at 315 mμ, will be indicative of the presence of an appreciable amount of aldopentose or aldohexuronic acid.

A number of color reactions suitable for distinguishing between aldoses and ketoses and based upon treatment of the sugar with sulfuric or hydrochloric acid and a second reagent have been described (11). In view of the fact that adequate spectrophotometers are now generally available, it would appear from the present investigation that direct spectrophotometric examination of the acid solution without the addition of a second reagent is the more satisfactory procedure, especially for quantitative observations.

The applicability of the direct spectrophotometric method to oligosaccharides is illustrated by data contained in Table I. It will be noted that in general there is good agreement between the extinction coefficients

TABLE III

Analysis of Mixtures of Galactose and Fucose

Mixture	Amount		Moles galactose / Moles fucose	
	Added	Found*	Added	Found
	γ	γ		
Galactose	54.9	58	1.00	1.08
Fucose	50.0	49		
Galactose	82.4	84.5	3.00	3.21
Fucose	25.0	24		
Galactose	27.4	27	0.33	0.32
Fucose	75.0	77		
Galactose	54.9	57	2.00	2.16
Fucose	25.0	24		
N-Acetylglucosamine†	33.7			

* Average of a duplicate analysis.

† The absorption at 300 and 327 mμ was corrected for the slight amount due to the *N*-acetylglucosamine added.

of solutions of the oligosaccharides and those expected on the basis of the component monosaccharides. The data clearly show that maltose, trehalose, lactose, and melibiose contain 2 aldohexose residues; sucrose, an aldohexose and a ketohexose residue; and raffinose and melezitose, 1 ketohexose, and 2 aldohexose residues.

Whereas the ketohexoses are distinguishable from the other classes of sugars listed in Table II because of the large extinction coefficients developed in 79 per cent sulfuric acid solutions at 25°, the 6-desoxyaldohexoses are distinguishable from the other classes because of the position of the principal maxima developed in 79 per cent sulfuric acid solutions heated at 100°. It will be noted that the principal maxima of heated solutions of fucose and rhamnose are located at 327 mμ; *i.e.*, at a longer wave-length than the maxima of heated solutions of the other types of

monosaccharides. Fortunately the extinction coefficients of these maxima at 327 mμ are quite large, making it possible to resolve the spectra of solutions containing 6-desoxyaldohexose and other types of sugar into a "6-desoxyaldohexose peak" and a second peak characteristic of the other type of monosaccharide present in the mixture.

Fucose and rhamnose are known to occur in a number of immunologically active polysaccharides (12), and in studies in these laboratories on preparations of blood group A-specific substance from hog gastric mucosa it has been found that the amount of 6-desoxyaldohexose present in these preparations can be determined from the spectra of their solutions in 79 per cent sulfuric acid after the latter have been heated at 100° for 15 minutes. The values so obtained for the fucose and galactose content of preparations of blood group-A specific substance were in good agreement with values noted by other investigators.[1] Several representative analyses of fucose-galactose and fucose-galactose-*N*-acetylglucosamine mixtures are given in Table III. These values were calculated from extinction values found at 300 mμ (galactose) and 327 mμ (fucose) for solutions of the mixtures and of the pure monosaccharides.

The direct spectrophotometric determination of carbohydrates by the above procedure should be used with caution in the investigation of complex natural products until interferences arising from non-carbohydrate components can be evaluated. Although it has been found that the method is applicable for the estimation of carbohydrates in the presence of significant quantities of representative simple proteins,[1] no information is available in regard to possible interference by other classes of organic compounds.

SUMMARY

The spectral characteristics of solutions of eighteen representative monosaccharides in 79 per cent sulfuric acid have been determined for solutions maintained for 2 hours at 25° and 15 minutes at 100°. The use of these data for the identification of ketohexoses and 6-desoxyaldohexoses has been pointed out, as has their application to the quantitative determination of certain monosaccharides when present singly or in admixture with other monosaccharides, either as the monosaccharides or as components of oligosaccharides and polysaccharides.

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