[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Rotatory Dispersion of Amylaceous Polysaccharides and Their Triesters

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Measurements of rotatory dispersions in the visible region have been made for corn amylose and amylopectin in alkali, for corn starch, corn amylose and amylopectin triacetates and tricarbanilates in chloroform and pyridine, respectively, and for dextran tricarbanilate in morpholine. The rotatory dispersions were all simple and normal. One-term Drude equations were calculated for the dispersions. Effects of the phenyl group in determining the optical rotations of the carbanilates have been indicated.

In a previous publication[2] the optical rotations of polysaccharide tricarbanilates were discussed in relation to the structures of these carbohydrate materials. Unexpectedly, the tricarbanilates of starch and its components were found to have negative optical rotations in pyridine. Similar reversals of rotation have been noted for the carbanilates of α-methyl-D-mannoside[3] and of arbutin and esculin.[4] It was of interest to measure the rotatory dispersion of the polysaccharide tricarbanilates to find whether (a) the carbanilino groups caused anomalies in the rotatory dispersion, and (b) the effect of molecular structural factors on the optical rotation might be greater at wave lengths other than that of sodium light.

Rotatory dispersion measurements have been reported on a variety of carbohydrates such as the simple sugars and sugar acetates,[5] the aldehydo sugars and their acetates,[6] the glycosides,[5c,7] sugar acids and their lactones,[8] and many others. However, very few references[9] are found on the rotatory dispersion of polysaccharides or their derivatives and none of these references include studies on starch or its components. It was therefore decided to include amylose, amylopectin, and their acetates among the compounds to be studied.

This paper reports the rotatory dispersion in the visible region (Na lines 5893 Å. and Hg lines 4339, 4358, 4916, 5461, 5770, 5791, 6073 and 6234) of amylose and amylopectin in potassium hydroxide solution, of corn starch, corn amylose and amylopectin triacetates, and tricarbanilates in chloroform and pyridine, respectively, and of dextran tricarbanilate in morpholine.

Experimental

Materials.—Corn amylose and amylopectin were separated by the butanol precipitation procedure.[10] The amylose was recrystallized until it sorbed at least 190 mg. of iodine per gram.[11] The fractions were acetylated at 100° (6 hours) with pyridine and acetic anhydride, and gave products with the acetyl content required for a triacetate. The various carbanilates used for rotation were those previously described.[2]

Apparatus.—The optical rotations were measured on a Schmidt and Haensch polarimeter. Either a General Electric sodium vapor lamp or a G–E H-4 mercury arc served as the source of illumination. This light was passed through a Bausch and Lomb large spectrometer (catalog No. 33-82-09) which was used as a monochromator. The light from the source was focused on the slit of the monochromator by a quartz lens. This adjustable slit, which controls the spectrum line width, was set at approximately 0.54 mm. A half-shadow angle of 4° was generally used, but this was increased for certain readings when greater light intensity was required.

Standardization.—The rotatory power of a 26% solution of Bureau of Standards sucrose was $[\alpha]^{25}D$ +66.2°. $[\alpha]^{25}_{5461}$ 78.1°, $[\alpha]^{25}_{4358}$ 128.3°, compared with accepted values[12] of 66.5, 78.3 and 128.5, respectively, all measured at 20°. The standardization of the instrument was also verified by finding an exact agreement between observed and standard values on a quartz plate calibrated by the National Bureau of Standards.

Results and Discussion

The rotatory dispersions of all of the polysaccharides measured, and of their esters, were normal and simple (or pseudo-simple[13]). That is, the specific optical rotation increased in numerical value with decrease in wave length of the light used. When the reciprocal of the specific rotation was plotted against the square of the wave length, the points lay on a straight line (*cf.* Figs. 1 and 2). Hence the variation in rotation could be expressed approximately by a one-term Drude equation

$$[\alpha]\frac{t}{\lambda} = \frac{A}{\lambda^2 - \lambda_0^2}$$

where A and λ_0 are empirically determined constants. Specific rotations obtained for the various substances at 1% concentration, and the constants derived for calculating rotation values at different wave lengths, are found in Table I.

Agreement between observed and calculated values was usually within 1.5% although occasional deviations of greater magnitude were encountered.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

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(12) "International Critical Tables," E. W. Washburn, ed., McGraw-Hill Book Co., Inc., New York, N. Y., Vol. 2, 1927, p. 336.

(13) T. M. Lowry, *Chemistry and Industry*, **54**, 477 (1935).

TABLE I

ROTATORY DISPERSIONS OF POLYSACCHARIDES AND THEIR ESTERS

λ, A.	Corn amylose in 1 *N* KOH	Corn amylopectin in 1 *N* KOH	Corn starch triacetate in $CHCl_3$	Corn amylose triacetate in $CHCl_3$	Corn amylopectin triacetate in $CHCl_3$	Corn starch tricarbanilate in pyridine	Corn amylose tricarbanilate in pyridine	Corn amylopectin tricarbanilate in pyridine	Dextran tricarbanilate in morpholine
				$[\alpha]^{25}\lambda$, observed					
4339	+301.7°	Unreadable	+328.2°	+336.8°	+317.9°	−217.8°	−225.7°	−194.3°	+771.9
4358	302.0	+304.9°	330.6	329.0	314.0	213.1	219.4	192.7	758.5
4916	233.0	236.7	255.0	260.6	239.9	128.4	150.2	111.1	546.6
5461	182.7	190.0	198.3	199.1	197.7	88.8	103.0	83.8	410.5
5770	161.8	170.3	176.8	182.5	175.0	77.1	91.0	69.3	360.6
5791	162.1	170.9	177.4	178.6	172.6	68.6	88.0	68.3	360.1
5893	156.1	164.8	170.6	175.4	170.1	66.0	82.5	62.0	343.0
6073	146.0	150.3	157.5	155.2	160.6	70.8	79.8	62.2	324.0
6234	139.7	140.0	155.5	151.3	152.0	62.4	73.0	56.1	309.7
				Drude equation constants of above compounds					
$A.\rightarrow$	50.86	54.63	56.48	55.86	56.31	−16.49	−21.60	−15.81	99.61
λ_0^2 (sq. microns)	0.0215	0.0107	0.0162	0.0224	0.0111	0.1125	0.0926	0.1069	0.0586
				Wave lengths used to obtain constants					
	4358 and 5893	4358 and 5461	4359 and 5893	4339 and 5791	4339 and 5770	4358 and 5461	4339 and 6234	4339 and 6234	4358 and 5791

This is considered satisfactory in view of the fact that we were dealing with macromolecular materials of unknown homogeneity, with solutions that were quite dilute, and that many solutions possessed a turbidity which made it necessary to use short polarimeter tubes which resulted in a low observed rotation.

The wave lengths denoted by λ_0 are usually considered to represent the absorption bands in the ultraviolet which are associated with the optical activity. Our values of λ_0 averaged 1250, 1275 and 3225 Å. for the starch polysaccharides, their acetates and their carbanilates, respectively. λ_0 for the dextran carbanilate was 2425 Å. These λ_0 values are not strictly comparable since different solvents were used. However, it is of interest that the phenyl group always caused the position of these bands to be closer to the visible region than is true for the unsubstituted or acetylated polysaccharides. This is in accord with the well-known absorption of ultraviolet light by benzenoid derivatives in this region of the ultraviolet, whereas hydroxylic compounds and their acetates absorb only at much lower wave lengths.[6b,14]

It seems that the rotations of the carbanilates are greatly influenced by the aromatic ring, operating on the asymmetric centers through the —NH-CO— grouping. Expressed otherwise, the phenyl groups acquire induced dissymmetry and give rise to a partial rotation dependent on the absorption band of the aromatic nucleus. In fact, most of the rotatory power of the carbanilates in the visible region appears to be due to this induced dissymmetry of the phenyl group. Pigman[7c,d] has discussed the operation of similar phenomena in the case of various types of glucosides. Lowry,[13,15] too, has pointed out the influence of chromophoric groups on the optical rotation of various portions of the molecule of which they are a part. The sign of the induced dissymetry term is positive for dextran tricarbanilate and negative for the other polysaccharide carbanilates measured. Complex differences of this type cannot be explained at this time. Suitably transparent solvents were not

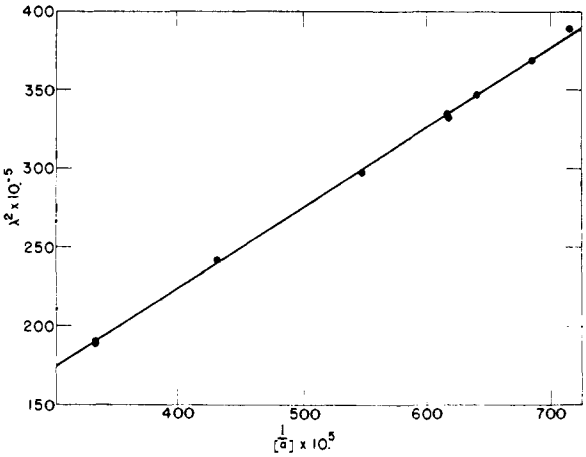

Fig. 1.—Rotatory dispersion of corn amylose in 1 *N* KOH.

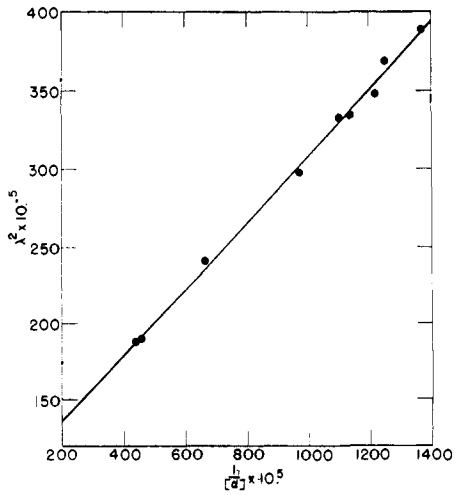

Fig. 2.—Rotatory dispersion of corn amylose tricarbanilate in pyridine.

(14) J. W. Rowen, C. M. Hunt and E. K. Plyler, *J. Research Natl. Bur. Standards*, **39**, 133 (1947).

(15) T. M. Lowry, "Optical Rotatory Power," Longmans, Green and Co., London, 1935, p. 408.

found for carrying out absorption measurements on solutions of the carbanilates in the ultraviolet.

Amylose, amylopectin, and starch carbanilates have similar dispersions; approximately parallel curves are obtained if wave length is plotted *versus* specific rotation of the various compounds. This is also true for the polysaccharides and their acetates. Molecular structural factors do not enhance the differences among the optical rotations of the various polysaccharides at any particular wave length, to an extent that advantage accrues from the use of light other than that of the sodium D-line.

The use of trade names in this publication does not necessarily constitute endorsement of these products nor of the manufacturers thereof.

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PEORIA, ILLINOIS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, DEPARTMENT OF SURGERY OF BETH ISRAEL HOSPITAL AND HARVARD MEDICAL SCHOOL]

Preparation of Naphthyl β-D-Glycopyranosides as Chromogenic Substrates for β-D-Glucopyranosidase[1]

BY KWAN-CHUNG TSOU AND ARNOLD M. SELIGMAN

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For the study of β-D-glucopyranosidase activity colorimetrically, 2-naphthyl β-D-glucopyranoside, 1-bromo-2-naphthyl β-D-glucopyranoside, 4-chloro-1-naphthyl β-D-glucopyranoside, 2-naphthyl β-D-galactopyranoside and 6-bromo-2-naphthyl β-D-ribopyranoside were synthesized and subjected to enzymatic hydrolysis by mammalian tissues. Their rates of hydrolysis were found to be slower than those of 6-bromo-2-naphthyl β-D-glucopyranoside and 6-bromo-2-naphthyl β-D-galactopyranoside. The riboside was not susceptible to hydrolysis by any organ including those which were able to hydrolyze 6-bromo-2-naphthyl β-D-glucopyranoside. Not only was the absence of β-D-ribopyranosidase demonstrated in these organs but evidence was thereby provided that β-D-glucopyranosidase requires a hydroxyl group at C_3 *cis* to the glucosidic oxygen for attachment to the substrate.

Synthetic substrates which yield naphthols after enzymatic hydrolysis have been employed in both histochemical and colorimetric methods for the demonstration of a variety of hydrolytic enzymes. The liberated naphthol was converted to an insoluble azo dye by coupling with an appropriate diazonium salt. In this way enzymatic activity has been localized histochemically within sections of tissue[2-7] or measured quantitatively in homogenates by extraction of the azo dye with an organic solvent for measurement of the color density.[7-11]

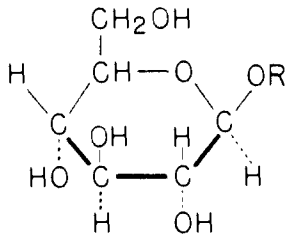

Fig. 1.

Based on these principles, the synthesis of 6-bromo-2-naphthyl β-D-galactopyranoside provided both a colorimetric and histochemical method for demonstrating β-D-galactopyranosidase activity in mammalian tissue.[12] The synthesis of 6-bromo-2-naphthyl β-D-glucopyranoside provided a colorimetric method for measuring β-D-glucopyranosidase activity in mammalian tissue.[13] In the course of this study, 1-bromo-2-naphthyl β-D-glucopyranoside (I), 4-chloro-1-naphthyl β-D-glucopyranoside (II), 2-naphthyl β-D-galactopyranoside (III) and 6-bromo-2-naphthyl β-D-ribopyranoside (IV) were also synthesized. Their preparation and susceptibility to enzymatic hydrolysis by mammalian tissues is reported below.

Helferich[14] and Pigman[15] have shown that the specificity of β-D-glucopyranosidase for various glucosidic substrates resides in the sugar moiety of the substrates. They have also shown that formation of an enzyme–substrate complex requires the hydroxyl groups at both C_2 and C_4 to be beneath the plane of the ring (Fig. 1). The configurational requirements of C_3 for enzyme–substrate complex formation are less well understood.[16] Since there is evidence that the pentosides are hydrolyzed by the same enzymes that hydrolyze corresponding hexosides,[17] and since the hydroxyl group at C_3 in D-ribopyranose is on the lower side of the plane

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, Federal Security Agency.

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(9) A. M. Seligman and M. M. Nachlas, *J. Clin. Invest.*, **29**, 31 (1950).

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(12) R. B. Cohen, K.-C. Tsou, S. H. Rutenburg and A. M. Seligman, *ibid.*, **195**, 239 (1952).

(13) R. B. Cohen, S. H. Rutenburg, K.-C. Tsou, M. Woodbury and A. M. Seligman, *ibid.*, **195**, 607 (1952).

(14) B. Helferich, *Ergeb. Enzymforsch.*, **7**, 83 (1938).

(15) W. W. Pigman, *Advances in Enzymol.*, **4**, 41 (1944).

(16) Gottschalk in the chapter on "The Specificity of Glycosides" in *Adv. in Carbohydrate Chem.*, **5**, (1951), postulated without experimental proof that "the action of glycosidase is initiated by chemisorption, at the enzyme surface, of substrate molecule, with the glycosidic oxygen contacting the attacking group of the enzyme and with hydroxyl groups *cis*-disposed to the glycosidic oxygen, in juxtaposition to hydrogen bond forming groups of the enzyme." Such a hydroxyl group occurs at C_3 in β-D-glucopyranosides.

(17) Reference 14, p. 46.