

aldopentose in 5 ml. of ethanol containing 0.1 g. of freshly distilled aniline was boiled under reflux for 3 hr. Thereafter the solution was partially decolorized with carbon and concentrated to 1 ml.; 2-deoxy-D-erythro-aldopentose anilide crystallized slowly; yield 20 mg. The crude material was recrystallized from ethanol; m.p. 163–164° unchanged on

admixture with an authentic specimen of 2-deoxy-D-erythro-aldopentose anilide^{24,25} of m.p. 165–167°.

(25) W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1358 (1949).

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[CONTRIBUTION FROM THE INSTITUTE OF ORGANIC CHEMISTRY, TECHNICAL UNIVERSITY, BUDAPEST]

Identification of Aldoses in the Form of Formazans via their Phenylhydrazones

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Aldoses have been identified by the use of the formazan reaction. By coupling with diazonium solutions, crystalline sugar formazans with characteristic physical properties have been prepared from the reaction of the corresponding aldose and phenylhydrazine without isolation of the intermediate phenylhydrazone. The optimal conditions for formazan formation have been established.

In earlier communications^{1,2} we have reported that when allowed to react in pyridine solution with cold solutions of diazonium compounds, aldose phenylhydrazones yielded brilliant red, readily crystallized sugar formazans. Owing to their melting point and characteristic crystalline forms, these new nitrogenous sugar derivatives can be used to advantage in identifying aldoses.

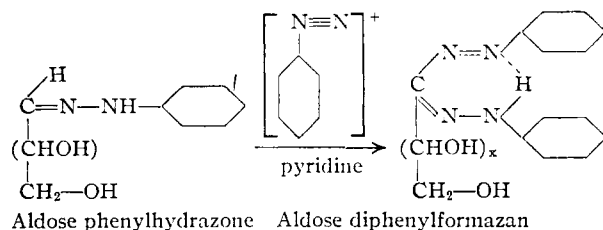


TABLE I

	M.p., °C.	Crystalline form
D-Glucose diphenylformazan	177–178	Red ndls., freq. rosettes
D-Galactose diphenylformazan	167–168	Bronze-red tablets
D-Mannose diphenylformazan	174–175	Bunches of microscopic rus- set ndls.
L-Arabinose diphenylformazan	173–174	Bunches of fine br.-red ndls.
L-Rhamnose diphenylformazan	175–176	Brill. red ndl. cryst.
D-Xylose diphenylformazan	123–124	Lanceolate red ndls.

Because they contain no active methine groups^{3,4} the ketose phenylhydrazones do not yield formazans; this reaction is therefore suitable for distinguishing aldoses from ketoses. It is more specific than the osazone test, frequently used in the identification of sugars, for it yields a different formazan for each aldose, while the latter does not differentiate between epimers.

For the purposes of identifying aldoses, we have simplified this reaction. The sugar phenylhydrazones derived from the reaction mixtures of the sugar and phenylhydrazine are not isolated but, after addition of pyridine, are immediately coupled to give the formazan.

In a previous paper² it has been reported that on standing sugar phenylhydrazone solutions suffer a

considerable diminution of their capacity for coupling, a phenomenon connected with optically observable structural changes. Obviously, two processes are taking place in the reaction of the sugar and phenylhydrazine, which from the point of view of formazan formation have opposing effects: (i) the formation of phenylhydrazone; (ii) the transformation of this into a form unsuitable for coupling.

To decide on the usefulness of the simplified reaction in aldose identification, formazan formation from the corresponding aldoses has been studied as a function of time. For each sugar the reaction with phenylhydrazine has been examined in three different solvents, *viz.*, (i) water, (ii) 50% alcohol-water, (iii) pyridine. After identical periods of time, all three solutions were coupled to yield the formazan in a pyridine medium by the addition of diazotized aniline. The data in the tables reveal that on the whole the yield was highest in pyridine solution, between the 24th and 48th hour. With mannose the nearly insoluble phenylhydrazone separates very soon; therefore in this case the formazan reaction was done with the separated phenylhydrazone and, of course, showed no deviation as a function of time.

Experiments on the separation of mixtures of sugar formazans by means of chromatography are in progress.

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Experimental

Reagents.—D-Glucose puriss. "Gyogyert"; D-galactose puriss. "Riedel"; D-mannose "Hoffmann-LaRoche"; L-arabinose p. u. sci. "Riedel"; L-rhamnose "B.D.H."; D-xylose puriss. "Fluka"; L-sorbose pur. "Merck"; D-fructose p. u. sci. "Schuchardt."

Melting Points.—All m.p.'s were determined in capillary tubes and are uncorrected. As the m.p. changes considerably with the manner of heating,⁵ our bath was heated rapidly to within about 10° of the m.p. Thereafter its temperature was raised slowly, so that the mercury column was unchanging at the moment of melting.

Preparation of Diazonium Solution.—A solution of 9.3 g. of aniline in a mixture of 25 ml. of concd. hydrochloric acid and an equal amount of water was diazotized at 0–5° with

(1) G. Zemplén and L. Mester, *Acta Chim. Acad. Sci. Hung.*, **2**, 9 (1952).

(2) L. Mester and Á. Major, *THIS JOURNAL*, **77**, 4297 (1955).

(3) H. v. Pechmann, *Ber.*, **25**, 3181 (1892).

(4) S. Hünig and O. Boes, *Ann.*, **579**, 28 (1953).

(5) H. v. Pechmann, *Ber.*, **27**, 1682 (1894).

TABLE II

	Hours	Water			Solvent			Pyridine		
		3	24	48	3	50% Alcohol	48	3	24	48
D-Galactose	20.5	64.5	73.0	18.5	56.5	67.0	28.2	60.5	71.0	
D-Glucose	0.0	8.4	0.0	7.9	14.3	18.0	3.4	38.2	64.0	
D-Xylose	30.4	38.5	51.3	23.9	46.4	48.7	29.8	50.5	54.5	
L-Arabinose	38.8	41.5	51.1	46.0	36.4	43.5	48.0	41.3	41.3	
L-Rhamnose	17.5	42.9	13.7	25.5	22.0	12.7	32.4	45.0	24.0	

a solution of 7.5 g. of sodium nitrite in 15 ml. of water. This was diluted to 93 ml.

Preparation of Formazans.—A mixture of 0.45 g. of aldose (corresponding to 0.0025 mole of hexose, or 0.0030 mole of pentose or 0.00275 mole of 6-deoxyhexose) and 0.45 g. of phenylhydrazine (0.00418 mole) was dissolved (i) in 2.5 ml. of water, (ii) in 2.5 ml. of 50% alcohol, (iii) in 2.5 ml. of pyridine⁶ and allowed to stand at room temperature ($20 \pm 2^\circ$).

After the desired period of time, 2.5 ml. of pyridine was added to the aqueous and 50% alcohol-water solution, respectively, and 2.5 ml. of absolute alcohol to the pyridine solution. They were refrigerated and then coupled by dropwise addition of the diazonium solution prepared from 0.24 g. of aniline (1 mole + 7.5%) and allowed to stand for 10 minutes. After this time, the solutions were poured into ice-water five times their volume, and kept in the ice-box overnight. The precipitated, bright-red, mostly crystalline formazans were then collected, dried in air at room temperature, and weighed. In the case of mannose, from all three solvents the precipitated phenylhydrazone was collected, washed with alcohol, water and ether, then dissolved in 68 ml. of pyridine. On adding 7 ml. of ethanol,

the solution was finally coupled with diazo solution prepared from 0.24 g. of aniline; yields 0.63, 0.43, 0.48 g. of formazan (67.5, 45.7, 51.4%).

No formazan could be observed from L-sorbose or D-fructose in any of the media; merely a little of a yellowish-pinky, oleaginous, sticky substance precipitated.

Yields, in per cent., obtained in aqueous solution, in 50% alcohol, and in pyridine are given in Table II.

For identification, the crude products were crystallized to constant m.p. from hot butanol, with the exception of the formazan obtained from D-xylose, which was crystallized by washing the crude product with abs. ether, dissolving it in hot alcohol, and adding hot water to it (alcohol to water 3:2).

Anal. Calcd. for $C_{17}H_{20}O_4N_4$: N, 16.27. Found for D-xylose: N, 16.12, 16.06; for L-arabinose: N, 16.12, 16.03.⁷

Solubility of the resulting formazans was determined with various methods. In distilled water at 20° it was found to be 5–10 mg./100 ml.

(7) For the analyses of the other sugar formazans cf. our previous papers.^{1,2}

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(6) With galactose 90% aqueous pyridine was used.

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Acetolysis of the Glucomannan of Iles Mannan¹

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The glucomannan of Iles mannan, the polysaccharide extracted from the tubers of *Amorphophallus* plants gives on acetolysis followed by deacetylation a mixture of oligosaccharides in addition to D-glucose and D-mannose. Three of the oligosaccharides have been obtained in a crystalline form and have been shown to be 4-O-β-D-glucopyranosyl-α-D-mannopyranose, 4-O-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose) and 4-O-β-D-mannopyranosyl-α-D-glucopyranose. The structure of the last-named hitherto unknown disaccharide has been proved by methylation studies. The structural pattern of the glucomannan polysaccharide is discussed.

Iles mannan obtained from the tubers of the *Amorphophallus oncophyllus* and *A. variabilis* has been shown² to be a mixture of two polysaccharides, a glucomannan and an amylose-like polyglucosan, in the ratio of approximately 6:1. Methylation studies² showed that the glucomannan component was composed of D-glucopyranose and D-mannopyranose units linked together by 1,4'-β-linkages, the ratio of mannose to glucose being approximately 2:1.

Information concerning the sequence of the sugar residues in the glucomannan may be obtained by stepwise degradation and characterization of oligosaccharides of varying degree of polymerization. This paper is concerned with the partial degradation of the glucomannan component of Iles mannan by acetolysis and the isolation and characterization

of three oligosaccharides, namely, 4-O-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose), 4-O-β-D-glucopyranosyl-α-D-mannopyranose and 4-O-β-D-mannopyranosyl-α-D-glucopyranose.

The glucomannan was separated from Iles mannan meal by precipitation as a copper complex and subsequent decomposition of the latter with dilute acid as previously described.² Preliminary experiments were carried out in order to establish the conditions under which acetolysis produced the largest amounts of oligosaccharides. The maximum yield of the oligosaccharide acetates was obtained when the acetolysis³ was conducted initially at 0° and after 24 hours completed by heating. The nature and relative amounts of the products formed by acetolysis was determined by deacetylation of the mixture of acetates followed by qualitative paper chromatography. Acetolysis was found to give several oligosaccharides as well as D-glucose and D-mannose. The mixture of mono- and oligosac-

(1) Paper No. 3459, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper will form part of a thesis to be submitted by H. C. Srivastava to the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D.

(2) P. A. Rebers and F. Smith, *THIS JOURNAL*, **76**, 6097 (1954).

(3) Cf., K. S. Barclay, E. J. Bourne, M. Stacey and M. Webb, *J. Chem. Soc.*, 1501 (1954).