ference number data are from a table published by Longsworth.[11]

Λ_V is the equivalent conductance of a mixture calculated by the Van Rysselberghe and Nutting equation, Λ_R the equivalent conductance of a mixture calculated by the mixture rule, Λ_0 the equivalent conductance of a mixture observed at 25°, and % signifies the percentage deviation of the calculated from the observed equivalent conductance.

The results show that the mixture rule and the equation of Van Rysselberghe and Nutting give the same results when applied to these solutions. The deviation of the calculated from the experimental equivalent conductance of the mixture is in each case within the aggregate experimental error involved in the measurements of electrical conductances and transference numbers.

Lack of reliable electrical conductance and transference number data for appropriate pure salt solutions prevents further application of these rules to the data on mixed salt solutions.

Summary

1. The specific and equivalent conductances of various solutions of salts occurring in sea water have been measured at 5° intervals from 0 to 25° inclusive. The data for pure sodium chloride and pure potassium chloride solutions at 25° have been shown to be in close agreement with the measurements of Shedlovsky.

2. Certain electrical conductance data in the "International Critical Tables" are not in good agreement.

3. Temperature coefficients of equivalent conductance have been calculated for the twenty solutions at each of the six temperatures used. Although the coefficients for magnesium sulfate solutions are quite different from those of sodium chloride, the addition of magnesium sulfate to sodium chloride solutions in the ratio used has no detectable effect upon the coefficients of sodium chloride. The addition of potassium chloride to sodium chloride solutions in the ratio used has no detectable effect upon the temperature coefficients of sodium chloride.

4. The mixture rule and the equation proposed by Van Rysselberghe and Nutting give the same results when applied to part of the data on sodium chloride + potassium chloride solutions at 25°. These results agree with the measured values within the experimental error.

(11) L. G. Longsworth, THIS JOURNAL, **57**, 1185–1191 (1935).

SEATTLE, WASH. RECEIVED JANUARY 24, 1939

[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

A Water-Soluble Glucosan from Barley Roots

BY W. Z. HASSID

In the study of respiration of excised barley roots by Hoagland and Broyer[1] it was necessary to identify and determine the soluble carbohydrates. Since Yemm[2] and later Archbold and Barter[3] reported fructose anhydrides in barley leaves the identification of this polysaccharide was attempted in the roots. Upon investigation, however, no fructose anhydride could be found in any considerable quantity but instead a water-soluble glucose anhydride was isolated.

Fructosans have been known to be distributed widely in the Gramineae.[4] Their constitution was studied by Challinor, Haworth and Hirst,[5] Haworth, Hirst and Lyne[6] and also by Schlubach and his co-workers.[7] A water-soluble glucosan or glucose anhydride such as that which is now shown to exist in barley roots has not previously been reported in any plant.

Experimental

The glucosan was isolated as follows from barley roots grown in Hoagland's culture solution for about three weeks. The roots were placed into boiling 95% alcohol in such quantity that the final alcohol concentration was 75 to 80%. After extracting under a reflux condenser for six hours the alcoholic extract was poured off and the

(1) D. R. Hoagland and T. C. Broyer, *Plant Physiol.*, **11**, 471 (1936).

(2) E. W. Yemm, *Proc. Roy. Soc.* (London), **B117**, 483 (1935).

(3) H. K. Archbold and A. M. Barter, *Biochem. J.*, **29**, 2689 (1935).

(4) A. de Cugnac, *Bull. soc. chim. biol.*, **13**, 125 (1931).

(5) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1560 (1934).

(6) W. N. Haworth, E. L. Hirst and R. R. Lyne, *Biochem. J.*, **31**, 786 (1937).

(7) H. H. Schlubach, H. Knoop and M. Y. Liu, *Ann.*, **504**, 30 (1933), also **511**, 140 (1934); H. H. Schlubach and K. Koenig, *ibid.*, **514**, 182 (1934); H. H. Schlubach, *ibid.*, **523**, 130 (1936); H. H. Schlubach and H. Peitzner, *ibid.*, **530**, 120 (1937); H. H. Schlubach and H. Böe, *ibid.*, **532**, 191 (1937); H. H. Schlubach and H. Lendzian, *ibid.*, **532**, 200 (1937).

residual material dried *in vacuo* at 80°. It was then ground to a powder, again extracted with 95% alcohol for four hours and dried. Fifty-gram lots of the dried root material mixed with 1.5 liters of water were shaken with a mechanical shaker for twelve hours and then filtered by suction on a Büchner funnel. The extract was evaporated under reduced pressure at 50° to about 50 cc. and poured into an excess of alcohol, whereupon a white precipitate formed. The precipitate was centrifuged off, dissolved in water and the inorganic salts removed by electrodialysis. The dialyzed solution was concentrated to a small volume, precipitated with 95% alcohol and dried *in vacuo* at 70°. About 0.20 g. of the material was obtained from 50 g. of dry roots.

Properties of the Polysaccharide.—The polysaccharide was a white water-soluble powder, did not reduce Fehling's solution and was devoid of nitrogen. It did not give a coloration with iodine and was not affected by barley β-malt amylase, prepared according to Hanes,[8] or invertase (commercial Wallerstein invertase scales). It contained 3% moisture and 0.6% ash. Its specific rotation (*c*, 0.25) in water was $[\alpha]_D$ +201. The elementary analysis for carbon and hydrogen showed it to have the empirical formula of $(C_6H_{10}O_5)_n$: C, 44.3%; H, 6.4% (calculated on a dry basis). The theoretical C and H content for this compound: C, 44.4%; H, 6.2%.

Hydrolysis of the Polysaccharide and Identification of Glucose.—Heating with 1 *N* hydrochloric acid for thirty minutes on a steam-bath proved to be the most satisfactory condition for the hydrolysis of the polysaccharide. The reducing value determined on the neutralized hydrolyzate by oxidation with ferricyanide and titration with ceric sulfate[9] was 92%, calculated as glucose. The aldose value determined iodometrically according to the method of Yemm[2] was 95%. The Seliwanoff reaction carried out on the hydrolyzate as described by Roe[10] was negative for six lots (about 0.2 g. each) of the polysaccharide. In two other cases, however, the preparations gave a faintly positive Seliwanoff reaction. When analyzed according to the method of Roe[10] 2 to 5% of fructose was found. The orcinol test on the hydrolyzate was negative. When the polysaccharide was distilled with 12% hydrochloric acid and the distillate treated with thiobarbituric acid no precipitate was formed. This showed the absence of pentose or uronic acid units in the polysaccharide. When the hydrolyzate was treated with phenylhydrazine hydrochloride and sodium acetate according to Mulliken[11] a yellow precipitate was formed when heated in a boiling water-bath for five minutes. It was identified by its melting point and by the shape of its crystals under the microscope as that of glucose. Under these conditions a nearly white precipitate of mannose phenylhydrazone is formed within less than half a minute. To show that the osazone was not mannose, the following additional experiment was performed: 0.2 g. of the polysaccharide was hydrolyzed with 1 *N* sulfuric acid, neutralized with barium hydroxide, filtered, evaporated to 20 cc. and cooled. To 10 cc. of this solution 0.5 cc. of glacial acetic acid and 1 cc. of phenylhydrazine were added. The test-tube containing the mixture was allowed to stand for three hours. No precipitate was formed. When 0.1 g. of mannose was heated with 1 *N* sulfuric acid, neutralized with barium hydroxide and then treated with glacial acetic acid and phenylhydrazine as before, a nearly white precipitate, which was later identified as mannose phenylhydrazone, formed within ten minutes. Upon concentration of the rest of the filtrate (the remaining 10 cc. of the hydrolyzate from 0.2 g. of the polysaccharide) and heating with phenylhydrazine hydrochloride and sodium acetate, the yellow glucose phenylosazone separated out within about five minutes. These analyses show that the polysaccharide isolated from the roots is composed mainly of glucose units and in some cases mixed with a small amount of fructose.

Acetylation of the Polysaccharide.—The polysaccharide was acetylated according to the method used by Haworth, Hirst and Lyne.[6] One gram of the polysaccharide was dissolved in 2 cc. of water. Twenty cc. of ice-cold pyridine was added and shaken for a few minutes. The solution was then treated (in several portions) with 20 cc. of acetic anhydride at 0°. The mixture was allowed to stand overnight at room temperature, then poured into an excess of cold water. The acetylated polysaccharide, which precipitated in the form of a white powder, was filtered, washed free of acid with water and dried *in vacuo* at 70°. The acetylated polysaccharide was readily soluble in chloroform and acetone. Its specific rotation (*c*, 0.4) in chloroform was $[\alpha]_D$ +112°. Its acetyl content was 44.3% (calculated $COCH_3$ content for the triacetate, $C_6H_7O_5(CH_3CO)_3$, 44.8%). The specific viscosity of a 1% solution of the triacetate in *m*-cresol was 0.11. This corresponds to a molecular weight of 3160, determined by Staudinger's formula with $K_m = 10^{-3}$.[12] and shows the polysaccharide to consist of 11 glucose residues. The molecular weight of the triacetate determined by the method of Rast[13] was 2130, corresponding to about 7 residues in the polysaccharide molecule. However, since it is not certain that these methods can be applied for the molecular weight determination of this polysaccharide, the values obtained should be regarded only as indicating that the polysaccharide is of a relatively low molecular weight, perhaps not more than about 10 glucose units.

Methylation and Hydrolysis of the Polysaccharide.—Two and one-half grams of the acetylated polysaccharide was dissolved in 40 cc. of acetone and treated at 55° with 20 cc. of methyl sulfate and 50 cc. of 30% sodium hydroxide over the course of three hours. The solution was then boiled, neutralized with dilute sulfuric acid, concentrated to a small volume and precipitated by the addition of petroleum ether. About 1.2 g. of methylated product was obtained. The methoxyl content of the methylated polysaccharide was 43.4% (calculated for $C_6H_7O_2(OCH_3)_3$, 45.6%). The specific rotation $[\alpha]_D$ in chloroform (*c*, 0.2) was +204°.

The methylated polysaccharide was permeated with 10

(8) C. S. Hanes, *Biochem. J.*, **30**, 168 (1936).

(9) W. Z. Hassid, *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).

(10) J. H. Roe, *J. Biol. Chem.*, **107**, 15 (1934).

(11) S. P. Mulliken, "Determination of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y. 1911, Vol. I, pp. 29–33.

(12) H. Staudinger, "Die hochmolekülaren organischen Verbindungen Kautschuk und Cellulose," Verlag von Julius Springer, Berlin, 1932, pp. 56–75.

(13) F. Pregl, "Quantitative Organic Microanalysis" (English translation by E. Fyleman), 2nd ed., P. Blakistons' Sons & Co., Philadelphia, Pa., 1930.

cc. of glacial acetic acid in a flask for an hour under reduced pressure. Twenty cc. of 5% hydrochloric acid was added and the mixture hydrolyzed at 100° for twelve hours. Barium carbonate, 10% in excess of the hydrochloric acid used, was then added to the solution and the mixture evaporated to dryness under reduced pressure, water being added from time to time to remove the acetic acid. The residue was dried by a mixture of alcohol and benzene and then exhaustively extracted with benzene. The extract containing the trimethyl- and tetramethylglucose was separated according to Macdonald.[14] The aqueous solution was extracted five times with 15-cc. portions of chloroform and the combined chloroform extracts washed with 25 cc. of water. This chloroform extract, which is supposed to contain tetramethylglucose, was evaporated to dryness. The small amount of residue left was not sufficient for analysis. The aqueous solution was evaporated to dryness and extracted with a mixture of benzene and ether. The sirup (about 0.8 g.) left upon evaporation did not crystallize on standing after inoculation with a crystal of 2,3,6-trimethylglucose. The methoxyl content of this sirup was 41.5% (calculated OCH_3 content for trimethylglucose, $C_6H_9O_3(OCH_3)_3$, 41.9%). Since 2,3,6-trimethylglucose crystallizes readily, there was reason to believe that the methylated sirup was some other trimethylglucose derivative which had no tendency for crystallization. That this was the case was shown in the following work.

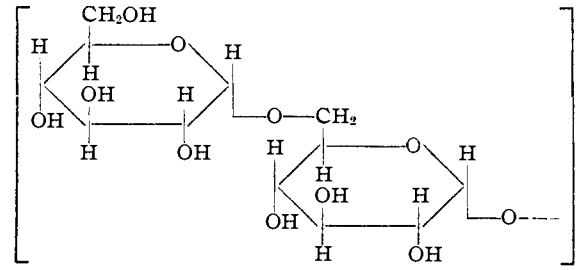
About 1 g. of the methylated polysaccharide was prepared and then hydrolyzed to the sirupy trimethylglucose as before. This fraction was combined with the previously obtained trimethylglucose fraction and boiled under a reflux condenser for twelve hours with 75 cc. of methyl alcohol containing 1.5% hydrogen chloride. The solution was neutralized with silver carbonate and filtered. The filtrate was clarified with charcoal, filtered on a Büchner funnel containing a thin layer of talc and evaporated under reduced pressure to a sirup. The methoxyl content of this sirup was 51.8%, which corresponded to that of trimethylmethylglucoside (calculated for $C_6H_8O_2(OCH_3)_4$, 52.6%). The sirup was distilled *in vacuo* (0.1 mm. pressure). The first few drops distilled over at 125–135°. Then the distillate, constituting the main portion, became more viscous and distilled at 145–160°. The sirup, on standing for several days, began to crystallize. The semisolid mass was then pressed on a porous glass plate and the crystals recrystallized from petroleum ether. The specific rotation of the trimethylmethylglucoside (0.1 g. dissolved in 10 cc. of chloroform) $[\alpha]_D$ was approximately −10°. Reported $[\alpha]_D$ values for 2,3,4-trimethyl-β-methylglucoside are −11.9°[15] and −20.57°.[16] The 2,3,6- and 2,4,6-trimethylglucoses are crystalline, but their methyl glucosides are liquids.[17,18] Since trimethylmethylglucoside having a negative rotation could be obtained in crystalline form and the crystalline trimethylglucose could not, it seems probable that this was the 2,3,4-trimethyl-β-methylglucoside. This being the case, the hydroxyls of the fifth and sixth carbon atoms of the glucose units constituting the polysaccharide are evidently engaged in linkage. Since, from stereochemical considerations, the possibility of a 1,6-internal ring structure for glucose appears to be unlikely, the glucose units that constitute the polysaccharide may therefore be considered as linked together by bonds each of which engages the reducing group of one glucopyranose unit and the hydroxyl of the sixth carbon atom of a contiguous glucopyranose unit.

The writer is indebted to Professor D. R. Hoagland and Mr. W. H. Dore for their interest and valuable suggestions and to Mr. L. W. Tuttle for assisting with the analytical work.

Summary

A water-soluble glucose anhydride was isolated from barley roots which yielded glucose on hydrolysis with acid. This polysaccharide could not be hydrolyzed with invertase or β-amylase.

On methylation and subsequent hydrolysis a trimethylmethylglucoside was obtained which appeared to be the 2,3,4-trimethyl-β-methylglucoside. On this basis a 1,6-linkage between individual glucose units in the polysaccharide is suggested. The arrangement shown below indicating the linkage between two units is tentatively proposed.



BERKELEY, CALIF. RECEIVED FEBRUARY 13, 1939

(14) J. Y. Macdonald, THIS JOURNAL, **57**, 771 (1935).

(15) J. C. Irvine and J. W. H. Oldham, *J. Chem. Soc.*, 2729 (1925).

(16) F. L. Fowler, I. K. Buckland, F. Brauns and H. Hibbert, *Can. J. Research*, **B15**, 486 (1937).

(17) W. N. Haworth, E. L. Hirst and J. I. Webb, *J. Chem. Soc.*, 2681 (1928).

(18) W. N. Haworth and W. G. Sedgwick, *ibid.*, 2573 (1926).