4-(1-Methyltetrahydropyridyl)-diphenylcarbinol (XI).—To an ethereal solution of 0.4 mole of phenyl lithium, prepared from 6.28 g. of bromobenzene and 0.56 g. of lithium, was added 3.17 g. of the mixture of methyl 1-methyltetrahydroisonicotinate and methyl 1-methylisonipecotate (IX) from the low pressure hydrogenation of methyl isonicotinate methiodide. The mixture was heated under reflux for 3 hr. and, after cooling, poured into ice and water. The water and ether insoluble solid was removed by filtration giving 1.58 g. of XI, m.p. 179.0–179.8, after recrystallization from ethanol.

Anal. Calcd. for $C_{19}H_{21}NO$: C, 81.68; H, 7.58. Found: C, 80.93; H, 7.53.

The layers in the filtrate were separated and the water layer extracted with 50 ml. of ether. The dried extracts were distilled leaving 1.72 g. of X, m.p. 130–132°, as residue.

Catalytic Reduction of 4-(1-Methyltetrahydropyridyl)-diphenylcarbinol (XI).—A solution of 2.0 g. of XI in 100 ml. of absolute methanol was shaken for one hour at room temperature with 0.2 g. of platinum oxide catalyst under 60 atm. pressure of hydrogen. Removal of the catalyst and solvent gave 1.95 g. (96.5%) of 1-methyl-4-piperidyldiphenylcarbinol (X), m.p. 125–130°. After recrystallization from methanol, the solid melted at 127.5–130.0° and showed no depression of melting point on mixing with an authentic sample of X.

1-Methyl-4-piperidylidenediphenylmethane (XII).—A solution of 4.0 g. of 1-methyl-4-piperidyldiphenylcarbinol (X) in 30 ml. of 1:1 sulfuric acid–water was heated on a steam-bath for 1.5 hr., poured into water and neutralized. The basic solution was extracted with ether, and the combined, dried ether extracts were fractionally distilled. The fraction, b.p. 205–208° at 12 mm., lit.[8] b.p. 145–150° at 1 mm., unlike the previously reported cases, crystallized on standing or seeding to give 3.09 g. (82.6%) of 1-methyl-4-piperidylidenediphenylmethane (XII), m.p. 55.0–56.3°.

The hydrobromide of XII, after recrystallization from ethanol, melted at 264–266°. XII hydrobromide is only sparingly soluble in water.

Anal. Calcd. for $C_{19}H_{22}BrN$: Br, 23.2. Found: Br, 23.4.

The Reaction of 1-Methyl-4-piperidylidenediphenylmethane (XII) with Bromine Water. (a) At 100°.—To a suspension of 4.0 g. of XII hydrobromide in 50 ml. of water, heated on a steam-bath, was added in 5-ml. portions 62 ml. of saturated (0°) bromine water. Heating was continued for 0.5 hr. or until all the solid was in solution and the reaction mixture was allowed to stand for 2 hr. The precipitated solid redissolved on heating and the solution was neutralized with potassium hydroxide. The amines precipitated immediately and, after coagulation, were isolated by filtration to give 3.34 g. (air dried) of the mixture. Trituration of the amine mixture with petroleum ether (b.p. 30–60°) gave, as the soluble component, 1.53 g. of 1-methyl-4-phenyl-4-piperidyl phenyl ketone (VII), m.p. 77.0–78.5°. The insoluble residue consisted of 1.44 g. of 4-hydroxy-1-methyl-4-piperidyldiphenylcarbinol (V), m.p. 158.0–160.8°. These products account for 89% of the starting material.

(b) At 40–50°.—A suspension of 4.0 g. of 1-methyl-4-piperidylidenediphenylmethane (XII) in 250 ml. of water was treated with 7 ml. of 48% hydrobromic acid at 40–50° and 100 ml. of bromine water was added in portions. A large amount of undissolved solid remained even after the addition of 250 ml. of water. The mixture was cooled and the solid was removed by filtration and triturated with acetone. The acetone insoluble portion, 0.85 g., was found to be unreacted XII hydrobromide, m.p. 263–265°. Concentration of the acetone solution gave 1.51 g. of a hydrobromide, m.p. 150° dec., which gave evidence of being 4-bromo-1-methyl-4-piperidyldiphenylcarbinol hydrobromide (XIII).

Anal. Calcd. for $C_{19}H_{23}Br_2NO$: 1Br, 18.1; 2Br, 36.2. Found: Br (Volhard), 18.5; Br (Stepanow), 33.4.[12]

The acid filtrate from the removal of XIII and XII hydrobromide was neutralized with potassium hydroxide and the solid which separated was triturated with petroleum ether (b.p. 30–60°). From the petroleum ether 0.75 g. of 1-methyl-4-phenyl-4-piperidyl phenyl ketone (VII), m.p. 75–77°, was obtained. The petroleum ether-insoluble portion yielded only traces of 4-hydroxy-1-methyl-4-piperidyldiphenylcarbinol (V).

Acknowledgments.—The authors wish to express deep appreciation to Dr. S. M. McElvain for aid in initiating this problem and encouragement and suggestions which promoted the completion of the work. The authors also wish to express appreciation for a sample of 1-methyl-4-phenylisonipeconitrile furnished them by Sterling–Winthrop Research Institute of Rensselaer, N. Y.

(12) The total halogen analysis of XIII was consistently low as compared with the theoretical value; however, it is sufficiently high to indicate the presence of two bromine residues. The low value for the total halogen analysis undoubtedly results from the relative ease of elimination of the bromine on the 4-position. One recrystallization of XIII from acetone lowered the bromine content to 31.3%.

DURHAM, NEW HAMPSHIRE

[CONTRIBUTION FROM THE RADIOCHEMISTRY LABORATORY, DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

Radioisotopic Dilution Analysis for D-Glucose and Gentiobiose in Hydrol

BY JOHN C. SOWDEN AND ALFRED S. SPRIGGS[1]

RECEIVED FEBRUARY 22, 1954

The D-glucose and gentiobiose contents of a representative sample of hydrol have been determined by radioisotopic dilution analysis. The D-glucose content by this method agrees well with the corresponding value obtained elsewhere by direct isolation methods. In contrast, the gentiobiose content as determined by the present method is considerably greater than hitherto reported.

Hydrol is the residual sirup obtained after the commercial crystallization of D-glucose from acid hydrolysates of corn starch. Since mineral acids catalyze not only the hydrolysis of oligo- and polysaccharides but also the condensation of monosaccharides,[2] hydrol is a product both of the acid hydrolysis of starch and of the acid reversion of D-glucose. Oligosaccharides arising solely from the hydrolysis of starch are necessarily limited in structure to moieties of the original starch structure whereas no such limitations can be applied to the structures of those oligosaccharides resulting from acid reversion of D-glucose. Consequently, although D-glucose appears to be the only monosaccharide constituent of hydrol, the oligosaccharide fraction is complex and as yet has not been resolved completely into its component sugars.

The first disaccharide to be identified as a con-

(1) Corn Industries Research Foundation Fellow.

(2) A. Wohl, *Ber.*, **23**, 2084 (1890); E. Fischer, *ibid.*, **23**, 3687 (1890); H. Frahm, *Ann.*, **555**, 187 (1944); E. Pacsu and P. T. Mora, THIS JOURNAL, **72**, 1045 (1950); W. R. Fetzer, E. K. Crosby, C. E. Engel and L. C. Kirst, *Ind. Eng. Chem.*, **45**, 1075 (1953).

stituent of this "corn sugar molasses" was gentiobiose,[3] and compelling evidence that it arises solely as a reversion product from D-glucose has been recently reported.[4] 6-*O*-α-D-Glucopyranosyl-D-glucose (brachiose, isomaltose) also has been identified in hydrol and can be considered partly as a product of starch hydrolysis and partly as a product of D-glucose reversion.[4,5] Other disaccharides that have been recognized positively as constituents of the hydrol mixture are α,α-trehalose[5] and maltose.[5,6] The former can be only a product of reversion while the latter may arise both by the hydrolytic and reversion routes.

Previous analyses of hydrol for specific sugars or classes of sugars have depended upon direct isolation of the sugars[5] or their derivatives[3,5,7] or upon copper reducing methods.[8] In the present work, we have applied radioisotopic dilution analysis to determine the amounts of D-glucose and gentiobiose, respectively, in a typical sample of hydrol. The advantage of this method in the analysis of complex sugar mixtures[9] stems from the fact that it does not rely on the efficiency of isolating the component being determined. Our analytical results for D-glucose agree well with the values obtained by direct isolation of this single monosaccharide constituent of the mixture.[5] In contrast, our value (9%) for the gentiobiose content is considerably greater than values (5–5.7%) reported hitherto for this disaccharide.[3,5] It is believed that the presently reported concentration of gentiobiose in our sample of hydrol is accurate within the small limits of error attending the determinations of specific radioactivity.

Experimental

Hydrol.—The sample of hydrol was kindly furnished by Mr. George T. Peckham, Jr., Research Director, Clinton Foods, Inc., Clinton, Iowa. The manufacturers analysis of this material showed moisture, 25.28%; ash, 8.85%; protein (N × 6.25), 0.19%. The carbohydrate content, calculated as the remainder, was thus 65.68%.

1-C^{14}-D-Glucose.—The labeled sugar (*ca.* 0.05 μc./mg.) was prepared from D-arabinose *via* the nitromethane synthesis with C^{14}-nitromethane.[10]

C^{14}-Gentiobiose.—The labeled disaccharide was synthesized by the Koenigs–Knorr method with the modifications of Reynolds and Evans.[11] Uniformly labeled D-glucose[12] (950 mg., *ca.* 0.085 μc./mg.) was converted to tetra-*O*-acetyl-D-glucopyranosyl bromide (1.13 g.) and the latter was condensed with non-radioactive 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose[13] (0.965 g.) to give β-gentiobiose octaacetate (0.821 g., m.p. 195°, $[\alpha]^{26}D$ −5.1°, *c* 2 in chloroform). The octaacetate (200 mg.) was deacetylated according to the directions of Thompson and Wolfrom[14] to yield anhydrous β-gentiobiose (52 mg., m.p. 193–194°, *ca.* 50,000 cts./min./mg.).

Standard Radioactive Sugar Solutions.—The radioactive sugars were dissolved separately in water to give solutions containing 2–2.5 mg./ml. These solutions were standardized, using 0.05-ml. aliquots, against known D-glucose and gentiobiose, respectively, in the manner described previously.[9] The results of the standardizations are shown in Tables I and II.

TABLE I

STANDARDIZATION OF STOCK 1-C^{14}-D-GLUCOSE SOLUTION

Sample	D-Glucose, mg.	Specific radioactivity, cts./min./mg.	Total radioactivity, cts./min.
1	19.97	375.9	7507
2	33.58	220.8	7414
3	55.35	133.7	7398
4	61.34	120.7	7404
5	77.20	95.3	7357
Check	48.26	154.2	7442
		Average	7420 ± 35

TABLE II

STANDARDIZATION OF STOCK C^{14}-GENTIOBIOSE SOLUTION

Sample	Gentiobiose, mg.	Specific radioactivity, cts./min./mg.	Total radioactivity, cts./min.
1	26.0	215.4	5601
2	34.6	158.9	5498
3	49.3	112.5	5546
4	68.6	82.0	5625
5	80.8	69.1	5583
Check	58.6	95.7	5608
		Average	5576 ± 37

Radioisotopic Dilution Analysis for D-Glucose in Hydrol.—A sample of 372.4 g. of hydrol was diluted to 1 l. with distilled water. To aliquots of 10 ml. each of this solution were added aliquots of 0.50 ml. each of the stock radioactive D-glucose solution. The solutions were deionized by passage through Amberlite I.R.-100[15] and Duolite A-4[16] ion exchange resins, the effluents were concentrated at reduced pressure and the resulting sirups were dried by repeated concentrations at reduced pressure with absolute ethanol. The dried sirups were dissolved in 20-ml. portions of 95% ethanol and seeded with minute traces of D-glucose. After crystallization was completed at 50°, the supernatant liquid was decanted and the sugar washed with cold 95% ethanol. For radioassay, the D-glucose was recrystallized to constant specific radioactivity from 95% ethanol with intermediate drying at 110° in high vacuum over phosphorus pentoxide. The samples of sugar thus purified showed m.p. 145.5–146° and $[\alpha]^{27}D$ 52.1°, equil. in water.

As a final check, the analysis was repeated after the addition of a known amount of D-glucose to an aliquot of the hydrol solution. The results of the analyses for D-glucose are shown in Table III.

Radioisotopic Dilution Analysis for Gentiobiose in Hydrol.—To aliquots of 25 ml. each of a solution containing 332.5 g. of hydrol in 1 l. were added aliquots of 2.00 ml. each of the stock radioactive gentiobiose solution. The solutions then were chromatographed on columns (300 × 80 mm.) of Darco G-60[17]–Celite-535[18] (1:1 by weight),[19] with water, 5% ethanol and 15% ethanol as successive elution solvents.[20] Gentiobiose was isolated from the 15% ethanol effluents-

(3) H. Berlin, THIS JOURNAL, **48**, 1107, 2627 (1926).

(4) A. Thompson, M. L. Wolfrom and E. J. Quinn, *ibid.*, **75**, 3003 (1953).

(5) Edna M. Montgomery and F. B. Weakley, *J. Assoc. Offic. Agr. Chemists*, **36**, 1096 (1953).

(6) Cellobiose recently has been isolated in the form of its crystalline octaacetate from our sample of hydrol. Experiments are currently in progress to determine whether this sugar is formed by reversion during the acid hydrolysis of starch or whether it is an artifact in this case, arising from cellulosic impurities in the original starch.

(7) C. D. Hurd and S. M. Cantor, THIS JOURNAL, **60**, 2677 (1938).

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(9) J. C. Sowden and R. Schaffer, THIS JOURNAL, **74**, 499 (1952).

(10) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(11) D. D. Reynolds and W. L. Evans, THIS JOURNAL, **60**, 2559 (1938).

(12) We are indebted to Professor W. Z. Hassid, University of California, for generously supplying this material.

(13) Prepared according to H. A. Lardy and H. O. L. Fischer, *J. Biol. Chem.*, **164**, 513 (1946).

(14) A. Thompson and M. L. Wolfrom, THIS JOURNAL, **75**, 3605 (1953).

(15) A product of Rohm and Haas Co., Philadelphia, Penna.

(16) A product of Chemical Process Co., Redwood City, Calif.

(17) A product of Atlas Powder Co., New York, N. Y.

(18) A product of Johns–Manville Co., New York, N. Y.

(19) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(20) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, *ibid.*, **74**, 1062 (1952).

TABLE III

D-GLUCOSE AND GENTIOBIOSE CONTENT OF HYDROL

Specific radioactivity of sugar, cts./min./mg.	Total weight of sugar, g.	Sugar in hydrol,[a] %
	D-Glucose	
46.9	1.582	64.7
46.6	1.592	65.1
46.4	1.599	65.4
46.7	1.588	64.9
41.6[b]	1.784	64.8[c]
	Gentiobiose	
451	0.495	9.07
460	.485	8.88
316[b]	.705	9.25[c]

[a] Based upon manufacturers assay of 65.68% ash-free, protein-free dry solids. [b] Artificial hydrol sample made by adding 200 mg. of sugar to aliquot of hydrol solution. [c] After subtracting 200 mg. of known, added sugar. Sample calculation: Weight of hydrol, 3.724 g.; D-glucose, g. = $\frac{0.50}{0.05} \times \frac{7420}{46.9} \times 0.001 = 1.582$; D-glucose in hydrol, % = $\frac{1.582 \times 100}{3.724 \times 0.6568} = 64.7$.

after acetylation, as the β-octaacetate,[20] m.p. after recrystallization from 95% ethanol, 194–195°; yields, 0.35–0.40 g. Deacetylation[14] gave crystalline β-gentiobiose. For radioassay, the sugar was recrystallized[14] to constant specific radioactivity with intermediate drying at 110° in high vacuum over phosphorus pentoxide. A check analysis was performed on an aliquot of the hydrol to which known gentiobiose had been added. The results of the analyses for gentiobiose are shown in Table III.

Acknowledgment.—We are pleased to acknowledge the generous support of this work by the Corn Industries Research Foundation, New York, N. Y.

ST. LOUIS, MISSOURI

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

A Convenient Method of Preparing 2-Deoxy-D-ribose[1]

BY JOHN C. SOWDEN

RECEIVED MARCH 1, 1954

The biochemically important sugar 2-deoxy-D-*erythro*-pentose ("2-deoxy-D-ribose," "thyminose") has been prepared in satisfactory yield from D-glucose in two simple steps. D-Glucose first was isomerized according to the directions of Nef by hot, concentrated alkali to give, among other products, a mixture of 3-deoxy-D-*ribo*-hexonic and 3-deoxy-D-*arabo*-hexonic acids (the "D-dextro-*meta*-saccharinic acids"). The 3-deoxyhexonic acids then were degraded by the method of Ruff with hydrogen peroxide in the presence of ferric acetate to give the deoxypentose.

In spite of intensive efforts,[2] no simple and economical source has been found for 2-deoxy-D-ribose since the discovery of this sugar as a constituent of the nucleic acids.[3] The preparation of the deoxypentose, either from natural sources or through synthesis, has remained sufficiently difficult to hamper seriously any thorough study of its chemical or biochemical behavior. A preparation from D-glucose which makes 2-deoxy-D-ribose readily accessible now has been accomplished.

During their extensive studies of the saccharinic acids that are formed by the action of alkali on reducing sugars, Kiliani and co-workers isolated a crystalline 3-deoxyhexonic lactone through treatment of lactose[4] or galactose[5] with lime-water. The corresponding acid, when subjected to a Ruff[6] degradation, produced a crystalline 2-deoxypentose[7] ("metasaccharopentose"). Kiliani did not assign configuration to the deoxypentose, but it became apparent that the sugar was 2-deoxy-D-*threo*-pentose when Levene and Mori[8] synthesized it from D-xylose by the glycal method. The respective constants recorded for the two preparations were: "metasaccharopentose,"[7] m.p. 95°, $[\alpha]_D$ 0° in water; m.p. of benzylphenylhydrazone, 117–118° and "2-xylodesose,[8]" m.p. 92–96°, $[\alpha]_D$ −2° in water; m.p. of benzylphenylhydrazone, 116–118°. It is interesting that Levene and Mori apparently overlooked the preparation of Kiliani and Naegeli since they state, with reference to their own product, "xylodesose has now been prepared for the first time."

Beginning with D-glucose, Nef isolated an epimeric pair of crystalline 3-deoxyhexonic lactones from the action of aqueous sodium hydroxide on the sugar.[9] He assigned to the acids the D-*ribo* and D-*arabo* configurations on the assumption that they were formed intramolecularly from D-glucose with preservation of the D-*erythro* configuration on carbons 4 and 5. In addition, Nef made the observation that D-glucose, when treated with hot, concentrated alkali, produces these two acids in a combined yield of about 20%. Thus, if the configurations assigned by Nef for these acids were correct, it was apparent that they should be, through application of the Ruff degradation, a fruitful source of 2-deoxy-D-ribose. Such, indeed, is the case.

It has been found that, by combining Nef's conditions for the alkaline isomerization of D-glucose with a subsequent Ruff degradation of the crude 3-deoxyhexonic acids,[10] 2-deoxy-D-ribose can be obtained readily. Approximately 5 g. of the deoxypentose is produced in this way from 100 g. of D-glucose.

(1) A preliminary report of this work appeared in Abstracts Papers, Am. Chem. Soc., **124**, 15D (1953).

(2) W. G. Overend and M. Stacey, *Adv. Carbohydrate Chem.*, **8**, 45 (1953).

(3) P. A. Levene and E. S. London, *J. Biol. Chem.*, **81**, 711 (1929); **83**, 793 (1929).

(4) H. Kiliani, *Ber.*, **16**, 2625 (1883).

(5) H. Kiliani and H. Sanda, *ibid.*, **26**, 1649 (1893).

(6) O. Ruff, *ibid.*, **31**, 1573 (1898); **34**, 1362 (1901).

(7) H. Kiliani and H. Naegeli, *ibid.*, **35**, 3528 (1902); H. Kiliani and P. Loeffler, *ibid.*, **38**, 2667 (1905).

(8) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803 (1929).

(9) J. U. Nef, *Ann.*, **376**, 1 (1910).

(10) Shortly after the initial report of the present work,[1] the degradation of "calcium dextro-*meta*-saccharinate" by the Ruff method to give 2-deoxy-D-ribose was reported by G. N. Richards, *Chem. and Ind.*, **39**, 1035 (1953).