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.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

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

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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.

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[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).

In the present preparation, 2-deoxy-D-ribose is isolated conveniently as the crystalline benzylphenylhydrazone or anilide. Conditions are reported for converting the anilide to the free, crystalline sugar in high yield.

Experimental

Crude "D-Dextro-*meta*-saccharins."—To a warm (50°) solution of 500 g. of sodium hydroxide in 830 ml. of water, over whose surface was directed a stream of nitrogen, was added a solution of 200 g. of anhydrous D-glucose in 500 ml. of water at room temperature. The solution, whose temperature rose spontaneously to about 85°, then was heated on the steam-bath for 8–10 hours under a slow stream of nitrogen. After cooling to 0°, the mixture was acidified by the addition, with cooling and stirring, of 1060 ml. of concentrated hydrochloric acid. Following decolorization with carbon, the solution was concentrated at reduced pressure on the water-pump, finally at 100° for two hours. The residue was triturated several times with a total of 1500 ml. of absolute ethanol, and the extracts were separated from the salt by gravity filtration. The combined alcoholic extracts were concentrated at reduced pressure to a thin sirup (210–225 g.) which then was diluted gradually with 1 lb. of anhydrous ether. After cooling to 0°, the clear, supernatant liquid was decanted and discarded. The residual sirup, containing the bulk of the *meta*-saccharinic lactones, weighed 75–95 g. after being rinsed with ether and dried briefly at 100° on the water-pump.

Crude 2-Deoxy-D-ribose.—The above crude *meta*-saccharinic lactones were converted to the calcium salts by dissolution in 500 ml. of water and treatment, finally with boiling, with a slight excess (30–35 g.) of solid calcium hydroxide. The cooled solution was neutralized with solid carbon dioxide, then was heated to boiling and filtered.

A solution of ferric acetate, prepared by mixing solutions containing, respectively, 16.0 g. of barium acetate monohydrate and 10.0 g. of ferric sulfate + aq. (Mallinckrodt Chemical Works; approx. $7H_2O$) and filtering, was mixed with the solution of calcium saccharinates, and the volume was increased to approximately 2 l. with water. The solution, in a 4-l. beaker, was treated at 40–45° with 100 ml. of 30% hydrogen peroxide. The resulting reaction had subsided in about 1 hour, and an additional 100 ml. of 30% hydrogen peroxide then was added. After another hour, the mixture was decolorized with carbon and was deionized[11] by successive passage over 800–900 ml. each of Amberlite IR-100[12] and Duolite A-4.[13] The colorless effluent was concentrated at reduced pressure to a thin sirup weighing 85–120 g.

2-Deoxy-D-ribose Benzylphenylhydrazone.—The sugar sirup (85 g. from 200 g. of D-glucose) was diluted with 175 ml. of methanol and 70 g. of benzylphenylhydrazine was added. The solution was warmed for 15 minutes on the steam-bath, allowed to stand at room temperature for 18 hours, and then concentrated at reduced pressure. Crystallization was induced in the resulting sirup by seeding with 2-deoxy-D-ribose benzylphenylhydrazone, and the product (32.5 g., m.p. 117–122°) was isolated by triturating with ether and filtering. The hydrazone was purified by recrystallization from methanol with the addition of water; yield 22.9 g., m.p. 126–127°.[8]

Cleavage of the benzylphenylhydrazone to the free sugar may be accomplished with benzaldehyde in the presence of benzoic acid.[14]

2-Deoxy-D-ribose Anilide.[15]—The crude 2-deoxy-D-ribose sirup (120 g. from 200 g. of D-glucose) was diluted with 100 ml. of methanol and 40 ml. of freshly distilled aniline was added. Precipitation of the crystalline anilide began immediately at room temperature. After 20 hours, the solution was cooled to 0°, filtered, and the product washed successively with cold 50% methanol and ether. The anilide[16] (14.5 g., m.p. 172–173°, $[\alpha]^{25}\text{D}$ 46° equil. in pyridine[17]) was sufficiently pure at this stage for cleavage to the sugar. An additional 2.5 g. of the anilide was obtained by concentration of the filtration liquors at reduced pressure.

2-Deoxy-D-ribose from the Anilide.—Cleavage of the anilide to the free sugar with aqueous oxalic acid has been described by Overend, Stacey and Wiggins.[16] However, cleavage with benzaldehyde in the presence of benzoic acid appears to be simpler and more productive.

A mixture of 5 g. of 2-deoxy-D-ribose anilide, 5 ml. of benzaldehyde, 0.5 g. of benzoic acid and 150 ml. of water was shaken at room temperature for 22 hours. The aqueous phase was extracted three times with ether, decolorized with carbon and concentrated at reduced pressure, finally in high vacuum over phosphorus pentoxide. The resulting colorless sirup (3.15 g., 98%) crystallized readily and completely when seeded with 2-deoxy-D-ribose. After being washed by trituration with ethyl acetate containing 5% of methanol, the crystals of α,β-2-deoxy-D-ribose (3.02 g., 94%) showed m.p. 78–82° and $[\alpha]^{25}\text{D}$ −55° in water, *c* 1.

When the shaking at room temperature was replaced by ten minutes of refluxing, the yield of crystalline sugar amounted to only 56%.

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[11] H. G. Fletcher, Jr., H. W. Diehl and C. S. Hudson, THIS JOURNAL, **72**, 4546 (1950).

[12] A product of Rohm and Haas Co., Philadelphia, Penna.

[13] A product of Chemical Process Co., Redwood City, Calif.

[14] J. Meisenheimer and H. Jung, *Ber.*, **60**, 1462 (1927); J. C. Sowden, THIS JOURNAL, **72**, 808 (1950).

[15] 2-Deoxy-D-ribose anilide now is commercially available from The California Foundation for Biochemical Research, 3408 Fowler Street, Los Angeles 63, Calif.

[16] P. W. Kent, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1232 (1949); W. G. Overend, M. Stacey and L. F. Wiggins, *ibid.*, 1358 (1949).

[17] P. A. K. Gorin and J. K. N. Jones, *Nature*, **172**, 1051 (1953).