which on recrystallization from 15 parts of hot water melted at 183–185° and showed $[\alpha]^{20}D$ −94.0° in water (*c* 0.27).

Anal. Calcd. for $C_{12}H_{16}O_6$: C, 60.00; H, 6.71. Found: C, 60.07; H, 6.84.

1,6-Anhydro-3-deoxy-di-*O*-acetyl-β-D-*ribo*-hexopyranose.—A suspension of 6.9 g. of phenyl 3-deoxy-β-D-*ribo*-hexopyranoside (V) in 200 ml. of 2.6 *N* aqueous sodium hydroxide was heated for 19 hours at 100° in a silver flask. The clear, pale-yellow solution was neutralized with 3 *N* sulfuric acid, concentrated *in vacuo* to a paste of sirup and crystals, and the slurry extracted thrice with 150-ml. portions of absolute ethanol. The extract was filtered, and the solvent removed *in vacuo* leaving 4.3 g. of sirup which failed to crystallize. Acetylation with acetic anhydride and sodium acetate resulted in the production of 4 g. (45% based on the phenyl glycoside) of crystalline material which on recrystallization from absolute ethanol melted at 114–116° and showed $[\alpha]^{20}D$ −74.0° in chloroform (*c* 0.7).

Anal. Calcd. for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13; CH_3CO, 37.4. Found: C, 52.00; H, 6.13; CH_3CO, 37.0.

Optical Activity of 1,6-Anhydro-3-deoxy-β-D-*ribo*-hexopyranose (VI).—The anhydride diacetate (0.2475 g.) was saponified in *N* sodium hydroxide at room temperature. After 3 hours an equivalent amount of *N* sulfuric acid was added, the volume made to 25.0 ml., and the rotation observed. By this procedure the anhydride VI was found to show $[\alpha]^{20}D$ −79.9°.

Attempted Periodate Oxidation of 1,6-Anhydro-3-deoxy-β-D-*ribo*-hexopyranose (VI).—To 20.0 ml. of the solution referred to in the paragraph above was added 5 ml. of a 0.45 *M* solution of sodium metaperiodate and the volume was made to 50.0 ml. After 20 hours in the dark at 20°, the solution was found to show $[\alpha]^{20}D$ −83.4°; after 9 days the specific rotation was −78.5°. These values do not differ significantly from that established for the anhydride. Periodate consumption in 20 hours was found to be 0.1 mole/mole.

Equilibrium in Acid Solution between 3-Deoxy-D-*ribo*-hexose (VII) and its 1,6-Anhydride (VI).—To 1.3953 g. of anhydride diacetate in a 50.0-ml. volumetric flask was added 6 ml. of 5 *N* sodium hydroxide solution. After 2 hours at room temperature, 10 ml. of 5 *N* hydrochloric acid was added, the volume made to the mark with water, and the solution placed in an oven at 60–62°. In five days the rotation became constant. The aldose content of the equilibrated solution, estimated by the alkali-iodine method,[15] was found to be 89.8% (molar basis). By the method given above for the epimeric system it can be shown that the specific rotation of 3-deoxy-D-*ribo*-hexose (VII) is $[\alpha]^{20}D$ +31.8°.

3-Deoxy-D-*ribo*-hexose.—Tetra-*O*-acetyl-3-deoxy-D-*ribo*-hexose was prepared from the sirupy product of the hydrolysis of methyl 3-deoxy-α-D-*ribo*-hexopyranoside (IV) using the sodium acetate in hot acetic anhydride procedure employed by Černý and Pacák.[14] After recrystallization from absolute ethanol our product melted at 129–130° in agreement with the literature value but showed $[\alpha]^{20}D$ −14° in chloroform (*c* 1) in contrast with the value −20° reported by the earlier authors.

Anal. Calcd. for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 50.45; H, 6.06.

The acetate (1.0 g.) under 10 ml. of methanol was cooled to 3° and 0.2 ml. of *N* sodium methoxide in methanol was added. Solution was effected in four hours at that temperature. After two additional hours the base was destroyed by the addition of 2 drops of glacial acetic acid and the solvents were removed *in vacuo*. The sirupy product, taken up in absolute ethanol, was induced to crystallize and yielded 0.36 g. of material in the form of clusters of very small needles. Deposition of material was remarkably slow. On recrystallization from absolute ethanol the 3-deoxy-D-*ribo*-hexose melted at 105.5–107° and showed in water (*c* 1) $[\alpha]^{20}D$ +102° (initial, extrapolated) → +97.0° (2.5 min.) → +32.2° (3 hours; constant).

Anal. Calcd. for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.79; H, 7.24.

Phenyl 3-Deoxy-α-D-*ribo*-hexopyranoside (VIII).—An attempt was made to prepare the 1,6-anhydro-3-deoxy-β-D-*ribo*-hexopyranose by the alkaline hydrolysis of a mixture of the anomeric phenyl glycopyranosides. A crystalline product was obtained which on recrystallization from ethanol–ether melted at 136–138° and showed $[\alpha]^{20}D$ +172° in water. Rotational and analytical data as well as the method of preparation indicate that the compound is phenyl 3-deoxy-α-D-*ribo*-hexopyranoside which, as might be expected, was not affected by hot, aqueous alkali. The substance, unlike its anomer, is very soluble in water and alcohol.

Anal. Calcd. for $C_{12}H_{16}O_5$: C, 60.00; H, 6.71. Found: C, 60.33; H, 6.88.

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION, ARGONNE NATIONAL LABORATORY]

The Absence of an Amadori Rearrangement in Glucosazone Formation[1]

BY FELIX FRIEDBERG[2] AND LOUIS KAPLAN

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Glucose-1-*t* is converted to its phenylosazone without loss of tritium. This observation invalidates any mechanism of osazone formation requiring an intermediate with two hydrogen atoms on carbon-1, including the Amadori rearrangement proposed by Weygand.

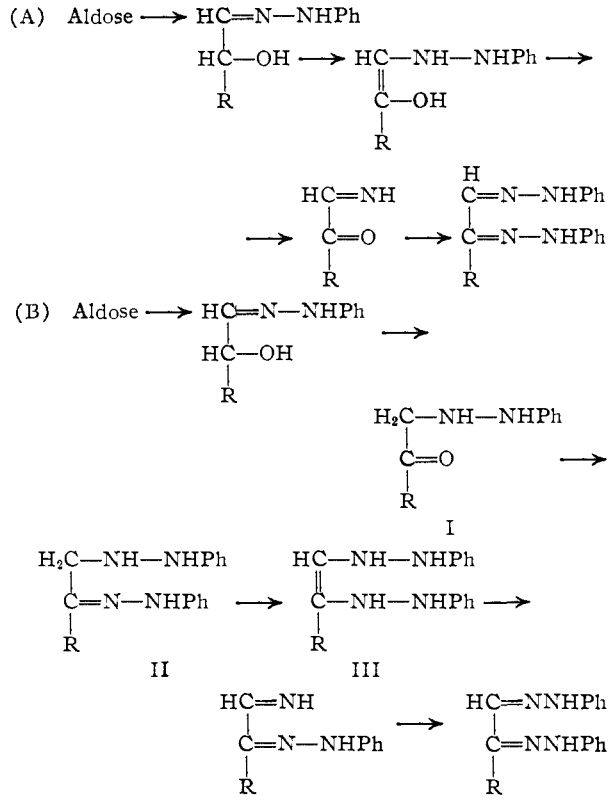
The classical scheme of Emil Fischer for osazone formation suffers from the difficulty of accounting for the oxidizing action of phenylhydrazine. In order to overcome this difficulty, Weygand[3] has proposed that the phenylhydrazone first formed rearranges, producing a carbonyl group at an adjacent carbon which then condenses with more phenylhydrazine. He has suggested two paths for the reaction, as shown by (A) and (B) in the chart. Path B, which involves an Amadori rearrangement,[4]

(1) Work performed under the auspices of the U. S. Atomic Energy Commission.

(2) College of Medicine, Howard University. Resident Research Associate, Argonne National Laboratory, 1956.

(3) F. Weygand, *Ber.*, **73**, 1284 (1940).

(4) Although the enolization involved in path A is sometimes referred to as an Amadori rearrangement (W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 405), J. E. Hodge (*Advances in Carbohydrate Chem.*, **10**, 169 (1955)) recommends that this designation be reserved for the complete conversion of a N-substituted aldosylamine to a N-substituted 1-amino-1-deoxy-2-ketose.



is favored by Weygand,[5] and this mechanism is cited by several reviewers.[6,7]

The preparation of glucose-1-*t* in connection with another problem[8] has permitted a test of Weygand's mechanism B. In this scheme the hypothetical isoglucosamine intermediate I contains two hydrogen atoms on carbon atom 1, one of which is subsequently lost. Any osazone formed by way of this intermediate from glucose-1-*t* would therefore contain less tritium than the original glucose. Since, in fact, the phenylosazone had a molar activity identical with that of the glucose, it must be concluded that I is not an intermediate in glucosazone formation.

Experimental

D-Glucose-1-*t* was prepared by reduction[9] of δ-gluconolactone with sodium borohydride-*t*.[10] The details of the preparation and purification will be described elsewhere.[8] After crystallization in the α-form,[11] the material melted at 146° and had an initial specific rotation of +103°. A portion of the glucose was converted[12] to the β-pentaacetate, m.p. 129–131°. Another portion was heated with phenylhydrazine, acetic acid and sodium acetate to form[13] the phenylosazone, m.p. 202° dec., some of which was oxidized[13] with copper sulfate to the osotriazole, m.p. 195°. The derivatives were recrystallized to constant specific activity as listed in Table I. More than 99% of the tritium in the glucose was shown, by oxidation to calcium gluconate, to be on carbon atom 1.

Tritium assays were performed by ion-current measurement[14] on the gas obtained by the zinc fusion procedure.[15] Replicate determinations on each compound had an average deviation of less than 1%.

Discussion

The results in Table I show that, within an uncertainty of about 1%, there is no loss of tritium during the formation of the phenylosazone from glucose-1-*t*.[16] In the absence of an isotope effect, one-half of the tritium should have been lost if the phenylosazone had been formed from the isoglucosamine derivative I. It is extremely unlikely that the practically quantitative retention of tritium can be explained on the basis of an isotope effect, since an isotopic rate ratio, k_H/k_T, of at least fifty would have to be postulated for the hydrogen migration II → III. It must, therefore, be concluded that Weygand's path B cannot be the principal mechanism of osazone formation, at least in the case of glucose.

TABLE I

Compound	Activity μc./g.	Activity μc./mmole
α-D-Glucose	39.7	7.15
β-D-Glucose pentaacetate	18.0	7.03
Phenyl-D-glucosazone	19.9	7.13
Phenyl-D-glucosotriazole	26.7	7.08

Of the other mechanisms which have been proposed for osazone formation, Weygand's path A is not affected by the isotopic results reported here. It has, however, been challenged[17] (along with path B) on the ground that it does not explain why osazone formation is favored by an electron-attracting group on the phenylhydrazine. The mechanism of Braude and Forbes,[18] patterned after that of Kenner and Knight,[19] overcomes this objection and is consistent with the isotopic results. The alternative proposed[17] by Bloink and Pausacker for osazone formation from benzoin would, if applied to glucose, involve as an intermediate the isoglucosamine derivative I of Weygand's path B and cannot, therefore, be correct for this case.

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