[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Preparation of *d*-Galacturonic Acid from *d*-Galactose[1]

BY HAROLD M. SELL AND KARL PAUL LINK

The synthesis of *d*-galacturonic acid from the parent sugar α-*d*-galactose involves the following transformations: I, the acetonation of *d*-galactose to 1,2,3,4-diacetone-*d*-galactose; II, the oxidation of diacetone-*d*-galactose to diacetone-*d*-galacturonic acid with alkaline potassium permanganate; III, the conversion of the potassium salt of diacetone-*d*-galacturonic acid to diacetone-*d*-galacturonic acid; IV, the hydrolysis of diacetone-*d*-galacturonic acid to *d*-galacturonic acid and subsequent crystallization of the free acid.

Ohle and Berend,[2] the originators of this method of synthesizing *d*-galacturonic acid, did not obtain the acid in a crystalline condition and resorted to the use of derivatives (brucine and osazone phenylhydrazine salts). In 1934 Niemann and Link[3] were able to put the method on a more practical basis in that they were able to realize the acid in a crystalline condition, with a yield of 30% of the theoretical. Reichstein and coworkers[4] introduced an improvement in step IV by eliminating the use of a mineral acid in the hydrolysis of the acetone residues.

For most laboratories the logical starting product for the synthesis of *d*-galacturonic acid is the parent sugar α-*d*-galactose. Consequently, we have made a careful study of each stage of the synthesis and have worked out the conditions necessary for success under average conditions.

The major change made in step I is the elimination of the necessity for the purification of the diacetone-*d*-galactose by a high vacuum distillation. The yield of diacetone-*d*-galactose has been increased from 60 to 92%.[2,5] In step II the oxidation has been placed on a firmer basis by increasing the ratio of potassium permanganate from 3 to 5 moles per mole of diacetone-*d*-galactose to be oxidized. By employing anhydrous acetone as the means of inducing the crystallization of the potassium diacetone-*d*-galacturonate this intermediate can be obtained in an analytically pure condition free from the troublesome hygroscopic properties that are usually encountered when the procedure of Ohle and Berend is used.[2] Reichstein and co-workers[4] had placed step IV on such a basis that further improvement was not necessary.

If the procedure outlined below is followed carefully the preparation of pure *d*-galacturonic acid can be realized with ease.

Experimental

Acetonation of α-*d*-Galactose.[2,3]—In a 6-liter wide-necked bottle with ground-glass stopper are placed 90 g. (0.5 mole) of finely pulverized α-*d*-galactose (m. p. 170°; $[\alpha]^{20}\text{D}$ +80.3°; dried for ten hours at 90° over phosphorus pentoxide in a vacuum oven), 200 g. (1.25 moles) of powdered anhydrous copper sulfate,[6] 10 cc. of sulfuric acid (sp. gr. 1.84), and 2 liters (27.4 moles) of dry alcohol-free acetone (dried over anhydrous calcium chloride). After the mixture has been shaken on a mechanical shaker for twenty-four hours it is filtered and the filter cake washed with dry acetone. To the filtrate is added 25 g. of powdered calcium hydroxide and the mixture is shaken for about two hours. The addition of 25-g. portions and the shaking are continued until the solution gives a neutral reaction to Congo red paper. A total of approximately 100 g. (1.35 moles) of calcium hydroxide is required to neutralize the sulfuric acid. The acid must be removed completely to avoid the hydrolysis of the acetone residues during the concentration of the solution.

After the unreacted calcium hydroxide and the calcium sulfate have been removed by filtration, the filter cake is washed with dry acetone. The filtrate is concentrated, first to a thin sirup at atmospheric pressure, then at 50° and 15 mm. (water pump), and, finally, to remove the last traces of acetone, at 100° and 6 mm. (oil pump). The yield of crude diacetone-*d*-galactose, as a thick sirup, is 100–120 g. (76–92%). Pure diacetone-*d*-galactose distils at 130–140° under 0.01–0.001 mm. pressure; $[\alpha]^{20}\text{D}$ -54.7 (c, 3.565; $CHCl_3$); negative Fehling's test.

Oxidation of Diacetone-*d*-galactose.[2]—To a solution of 100 g. (0.38 mole) of crude diacetone-*d*-galactose in water (4.5 liters) and potassium hydroxide (104 cc., 7.5 N) contained in a six-liter jar is added 300 g. (1.9 moles) of powdered potassium permanganate in 60-g. portions over a period of five hours. The solution is stirred mechanically throughout the oxidation, which is usually complete in twenty-four hours. If an excess of potassium permanganate is present, it is destroyed by the addition of hydrogen peroxide. The manganese dioxide is removed by filtration on an asbestos mat on a Büchner funnel and washed thoroughly with water. The filtrate is treated with car-

---

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] H. Ohle and G. Berend, *Ber.*, **58**, 2585 (1925).

[3] C. Niemann and K. P. Link, *J. Biol. Chem.*, **104**, 195 (1934).

[4] T. Reichstein, and co-workers, *Helv. Chim. Acta*, **16**, 1033 (1933).

[5] P. Levene and G. M. Meyer, *J. Biol. Chem.*, **92**, 257 (1931).

[6] The anhydrous copper sulfate is best prepared by drying the pentahydrate in an oven at 110° for several days and then heating in a casserole over a free flame until the powder is white. It should be used immediately after dehydration.

bon dioxide gas until the solution gives no color with phenolphthalein.[7] Concentration of the aqueous solution at 6 mm. and not over 65° leaves a dry residue which is extracted twice with 300-cc. portions of boiling ethanol, the insoluble portions remaining on the filter. Removal of the alcohol (15 mm. and 50°) and addition of 300 cc. of dry acetone (in which monoacetone-*d*-galactose and the unreacted diacetone-*d*-galactose are soluble) yields the crystalline potassium salt of diacetone-*d*-galacturonic acid. Addition of dry acetone to the concentrated mother liquors gives a second crop of the potassium salt, which is recrystallized by dissolving in a small volume of ethanol and then adding acetone. The yield of the potassium diacetone-*d*-galacturonate is 60-80 g. (49–65%); m. p. 200-205° (dec.); $[\alpha]^{20}\text{D}$ −61.1 (*c*, 2.046; H_2O).

Conversion of Diacetone-*d*-galacturonic Acid Potassium Salt to Diacetone-*d*-galacturonic Acid.[2]—A solution of 60 g. (0.19 mole) of the *carbonate-free* potassium salt ($C_{12}H_{17}O_7K \cdot 0.5H_2O$) in sulfuric acid (376 cc. of 0.5 *N*) is extracted four times with 250-cc. portions of ether. The combined ethereal extractions, dried over anhydrous sodium sulfate, are concentrated to dryness at 15 mm. and 50°. The residue is dissolved in 50 cc. of warm benzene. Crystallization is induced by adding 200 cc. of petroleum ether. The yield of diacetone-*d*-galacturonic acid is 40-45 g. (78–88%); m. p. 157°; $[\alpha]^{20}\text{D}$ −84.0°.

Hydrolysis of Diacetone-*d*-galacturonic Acid to *d*-Galacturonic Acid.[4]—A solution of 40 g. (0.15 mole) of diacetone-*d*-galacturonic acid (m. p. 157°) in water (120 cc.) is heated on a water-bath at 95 to 100° for two hours. After decolorizing with 5 g. of activated carbon the clarified solution should be concentrated *immediately* as *rapidly* as possible to a mobile sirup (50°, 6 mm.). The sirup is transferred to an evaporating dish and upon vigorous scratching it will set to a solid crystalline cake.[8] The product is collected on a Büchner funnel, washed with ethanol, dry ether, and dried over phosphorus pentoxide at 25° and 12 mm. pressure. The yield of *d*-galacturonic acid is 20–25 g. (65–81%). The product, being a mixture of the α- and β-forms, will show an initial rotation of $+60° \longrightarrow +80°$ depending on the time involved in concentrating the final solution. Pure α-*d*-galacturonic acid sinters at 110–111° and decomposes at 159–160°. The $[\alpha]^{20}\text{D}$ is +98° initial, final value +50.9° (*c*, 2.1; H_2O).

Summary

An improved method for the synthesis of *d*(+)-galacturonic acid has been described. The yield is 50 g. of *d*(+)-galacturonic acid from 100 g. α-*d*-galactose.

---

[7] It is convenient to invert a glass funnel in a jar whose diameter is slightly greater than that of the funnel. The carbon dioxide gas is then introduced into the solution through the stem of the funnel.

[8] If the crystallization is not spontaneous it can be induced by the addition of absolute alcohol.

MADISON, WISCONSIN RECEIVED JUNE 4, 1938

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 644]

The Molecular Structure of Arsenious Oxide, As_4O_6, Phosphorus Trioxide, P_4O_6, Phosphorus Pentoxide, P_4O_{10}, and Hexamethylenetetramine, $(CH_2)_6N_4$, by Electron Diffraction

BY G. C. HAMPSON AND A. J. STOSICK

In a recent paper,[1] which was published while this work was in progress, there were reported the results of an electron diffraction investigation of the structures of phosphorus trioxide, phosphorus pentoxide and arsenious oxide. The values which we have obtained for P_4O_6 and As_4O_6 are in good agreement with those of Maxwell, Hendricks and Deming. On the other hand, the latter authors were unable to deduce a structure for P_4O_{10} and came to the conclusion that the molecule probably has lower symmetry than that of the point group T_d. The reason for their failure probably lies in the fact that the molecule has an abnormally short P–O distance and we were led to our final structure, which gives an excellent fit with the photographs, only after very many models had been shown to be wrong.

The visual method of measurement was used, the results being compared in the usual way with the approximate scattering formula

$$I = \Sigma_{ij} Z_i Z_j \frac{\sin sr_{ij}}{sr_{ij}}$$

in which r_{ij} is the distance between the ith and jth atoms, Z_iZ_j their atomic numbers and $s = (4\pi \sin \theta/2)/\lambda$, where θ is the scattering angle and λ the wave length of the electrons. Radial distribution curves[2] were also calculated and interatomic distances deduced from them. When a molecule contains several approximately equal distances, the radial distribution method fails to resolve the closely spaced maxima and very little information can be obtained from the curve. A modification of the method, suggested by Dr. V. Schomaker of these Laboratories, in which the estimated intensities are multiplied by a factor

---

(1) Maxwell, Hendricks and Deming, *J. Chem. Phys.*, **5**, 626 (1937).

(2) Pauling and Brockway, THIS JOURNAL, **57**, 2684 (1935).