2. Preferential hydrolysis of I has been found to yield crystalline 1,2-cyclohexylidene-D-glucofuranose. A crystalline tribenzoate of this substance has also been secured.

3. Proof of structure of all these substances is offered.

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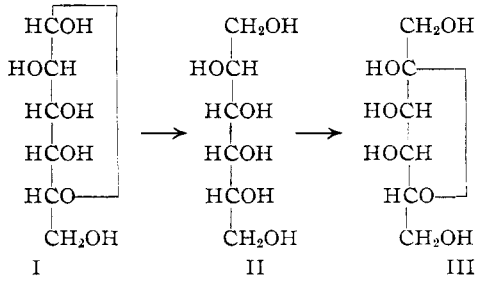
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Synthesis of D-Tagatose by Biochemical Oxidation and by an Improved Chemical Method[1]

BY EZRA L. TOTTON AND HENRY A. LARDY

In connection with work being carried out in this Laboratory on the intermediary metabolism of galactose, it became necessary to prepare D-tagatose (III). Since a wide variety of polyalcohols containing *cis*-hydroxyl groups at one end of the carbon chain had been oxidized to ketoses by *Acetobacter* species,[2] it was decided to test the action of *Acetobacter suboxydans* on D-talitol (II) as a means of preparing D-tagatose (III).



D-Talitol (II) was prepared for this purpose by the catalytic reduction of D-altrose (I). The latter was synthesized by methods developed by Robertson and Griffith[3] and Richtmyer and Hudson.[4]

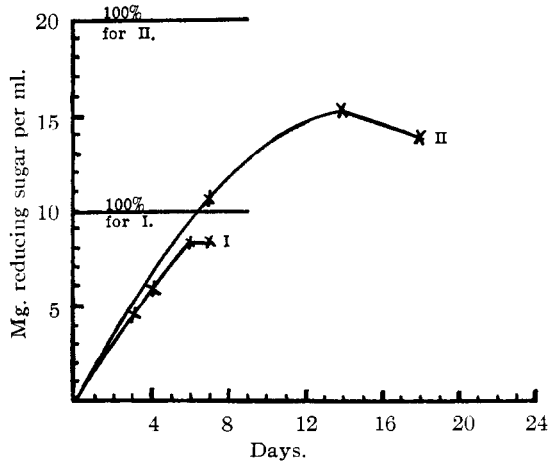


Fig. 1.—Oxidation of 1 and 2% solutions of D-talitol by *A. suboxydans*.

The D-talitol was rapidly oxidized by growing cultures of *A. suboxydans*. At concentrations of talitol below 5%, D-tagatose (III) was produced in yields of 75 to 84%. The rate of oxidation at 30° is shown in Fig. 1. When more concentrated solutions (5 to 16%) of talitol were employed, about 50% of the talitol was converted to tagatose. The yields quoted were based on a copper reduction method[5] standardized against pure D-tagatose. The bacterial oxidation product from D-talitol was demonstrated to be D-tagatose, since it gave a strongly positive test with Selivanov's reagent, was only very slightly oxidized by alkaline iodine, and gave a *p*-bromophenylosazone and a phenylosazone which were identical with those prepared from galactose.

The oxidation of D-talitol (II) to D-tagatose (III) by *A. suboxydans* offers a synthesis of D-tagatose which might be of preparative value should D-talitol become more readily available.

Since the synthesis of sufficient D-talitol to meet our needs for preparing tagatose would have been too laborious, we used the chemical procedure of Reichstein and Bosshard[6] for the preparation of larger quantities of tagatose. However, when using their procedure considerable difficulty was experienced in isolating, consistently, the tagatose from the mixture of isomerized galactose. The method was therefore modified by the use of lead acetate rather than absolute ethanol[6] to precipitate proteins and gums following the fermentation of the remaining galactose. The excess lead ions were then removed with an IR-100 ion exchange resin.[7] This modification eliminated many of the difficulties involved in the isolation of the product, and insured reproducible results.

It was also found that the rate of removal of galactose from the isomerized mixture may be increased by the use of a strain of yeast which rapidly adapts to galactose fermentation. The baker's yeast used by Reichstein and Bosshard required four days to ferment the excess galactose.

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the U. S. Public Health Service (RG: 313).

(2) Bertrand, *Compt. rend.*, **126**, 762 (1898); Hann, Tilden and Hudson, THIS JOURNAL, **60**, 1201 (1938); Anderson and Lardy, *ibid.*, **70**, 594 (1948).

(3) Robertson and Griffith, *J. Chem. Soc.*, 1193 (1935).

(4) Richtmyer and Hudson, THIS JOURNAL, **65**, 740 (1943).

(5) Schaffer and Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

(6) Reichstein and Bosshard, *Helv. Chim. Acta*, **17**, 753 (1934).

(7) From Resinous Products Co., Philadelphia, Pennsylvania.

In this work a culture of *S. cerevisiae* (Y-30)[8] fermented the galactose in fourteen to twenty-four hours.

The D-tagatose obtained by this modification of Reichstein and Bosshard's procedure agreed in m. p. with that reported by them for a product recrystallized several times. It gave the same characteristic tests and derivatives as the D-tagatose produced by the action of *A. suboxydans* on D-talitol. Since the bacterial oxidation product has a higher negative rotation, $[\alpha]^{25}D$ − 5°, than any previously described specimen of D-tagatose, it probably represents a purer compound.

Experimental[9]

D-Talitol (II) was prepared by the catalytic reduction of 18 g. of D-altrose in a 14% aqueous solution with 18 g. Raney nickel[10] under 1500 lb. hydrogen pressure at 100° for five hours.

D-Tagatose (III).—In a typical experiment, a solution of 1 g. of talitol, 0.5 g. of Difco yeast extract and 0.1 g. of glycerol in 100 ml. of water were placed in a cotton-stoppered 2-liter erlenmeyer flask. After autoclaving for fifteen minutes at 15 lb. pressure the cooled solution was inoculated with 1 ml. of a growing culture of *A. suboxydans*.[8] A test for reducing sugar was negative. After incubation at 30° for four days the medium was well covered with pellicle and an aliquot showed a reducing sugar value of 5.8 mg. per ml. calculated as tagatose. Two days later the substrate was 84% oxidized. The value was unchanged on the following day. The bacterial culture was then filtered to remove the cells, decolorized with norite and the proteins were removed from the solution by adding 6 ml. of saturated lead acetate solution. The clear filtrate was passed through a bed (30 cm. × 2.5 cm. diameter) of IR-100 ion exchange resin in the acid phase to remove the lead ions. The solution was concentrated under reduced pressure to a dry sirup. This was then taken up in 6 ml. of hot absolute methanol and 25 ml. of hot absolute ethanol was added with shaking. After 75 ml. of cold absolute ethanol was added, the mixture was allowed to stand one hour before filtering. The filtrate was again evaporated to a dry sirup under reduced pressure. The product was taken up in the smallest possible amount of absolute methanol and hot absolute ethanol was added until the first signs of turbidity appeared. Scratching the flask initiated crystallization. The product was collected after crystallization had proceeded at 5° for twenty-four hours. The dry crystals weighed 0.76 g. and melted at 120°. Recrystallization from 0.5 part water and 3 parts absolute alcohol gave a product which melted at 131–132°; $[\alpha]^{25}D$ − 5° (*c* 1 in water).[10a] Repeated recrystallizations raised the m. p. to 133–134° but did not alter the rotation. Reichstein and Bosshard[6] reported m. p. 134–135° and $[\alpha]^{20}D$ −2.3° (*c* 2.19 in water) for recrystallized tagatose prepared by treating galactose with pyridine.

Reaction with Hypoiodite (Willstätter-Schudel titration).—This reaction was run under conditions described by Hinton and Macara.[11] In a standardizing run 0.080 g. of glucose was quantitatively oxidized by iodine, giving a value of 1.40 g. iodine per g. of glucose. Under these conditions, galactose gave an iodine value of 1.17 g. per g. of galactose; the bacterial oxidation product of D-talitol gave an iodine value of 0.018 g. of iodine per gram of product.

D-Tagatose, reacting for twenty-five minutes with the reagent of Shaffer and Somogyi,[5] gave a copper reduction value 79% as great as an equivalent amount of glucose reacting for fifteen minutes.

The Selivanov Test.—The procedure of Roe[12] was followed in carrying out this test. A quantitative comparison (Evelyn colorimeter, filter 490) of the color formed by Selivanov's reagent and the bacterial oxidation product with the color formed by the reagent and D-fructose showed that the intensity of the colors were practically identical.

D-*lyxo*-Hexose *p*-Bromophenylosazone.—The method of Neuberg[13] was used to prepare this compound from a sample of the bacterial oxidation product. Two recrystallizations from 70% ethanol gave crystals which melted at 180–182° (slow heating). A mixed m. p. with an authentic sample prepared from galactose showed no depression. Neuberg reported a m. p. of 182–183° for D-*lyxo*-hexose *p*-bromophenylosazone prepared from galactose.

Anal. Calcd. for $C_{18}H_{20}O_4N_4Br_2$ (516.2): N, 10.85. Found: N, 10.73.

D-*lyxo*-Hexose Phenylosazone.—The procedure of Fischer[14] was followed for the synthesis of the phenylosazone from a sample of the bacterial oxidation product. Three recrystallizations from ethanol and water gave crystals which melted at 186° (slow heating). A mixed m. p. with a sample prepared from galactose showed no depression.

Anal. Calcd. for $C_{18}H_{22}O_4N_4$ (358.39): N, 15.64. Found: N, 15.55.

The Synthesis of D-Tagatose by an Improved Chemical Method.—One hundred grams of dry galactose in 1 liter of dry pyridine[15] was refluxed for five hours; the reaction mixture was protected from moisture with a calcium chloride tube. The pyridine was removed under reduced pressure. Remaining traces of pyridine were removed by evaporating, successively, two 0.5-liter portions of water under reduced pressure. The solution was concentrated to 125 ml. and 200 ml. of warm absolute ethanol was added in small portions. After twenty-four hours at 5°, the galactose was collected on a filter and washed with methanol. About 75% of the original galactose was recovered. The mother liquor was concentrated under reduced pressure to a thick sirup (30 ml.). A small amount of galactose was removed by filtration. The combined sirup and rinsings (50 ml.) were divided into two equal parts and each portion was added to a 2-liter erlenmeyer flask containing 500 ml. of a 0.5% Difco yeast extract solution. After sterilizing, each solution was inoculated with 25 ml. of a growing culture of *S. cerevisiae* (Y-30) and incubated with shaking at 30° for twenty-four hours. The combined medium was filtered, decolorized with norite, and concentrated under reduced pressure to 70 ml. Five ml. of saturated lead acetate solution was added. After filtering, the solution was passed through an IR-100 exchange resin in the acid phase. Three hundred ml. of warm absolute alcohol was added to the solution. After three hours the mixture was filtered and the mother liquor concentrated to a thin, free flowing sirup. Scratching the flask initiated crystallization, and the flask was allowed to remain at 5° for twenty-four hours. The product weighed 6 g. (6% of theoretical), and melted at 124–125°, $[\alpha]^{25}D$ −0.8° (*c* 5 in water). When recrystallized from ½ part water and 3 parts absolute alcohol, m. p. 131–132°, $[\alpha]^{25}D$ −0.8° (*c* 5 in water). This m. p. agrees with that reported for D-tagatose by Reichstein and Bosshard.[6] The product gave the same characteristic tests and derivatives as the compound produced by the bacterial oxidation of D-talitol.

(8) Thanks are due Professor Elizabeth McCoy, who kindly supplied the cultures of *S. cerevisiae* and *A. suboxydans*.

(9) All melting points were determined in capillaries. Anschütz, NBS calibrated thermometers were used.

(10) Pavlic and Adkins, THIS JOURNAL, **56**, 2463 (1934).

(10a) Final equilibrium value. The compound showed a slight downward mutarotation.

(11) Hinton and Macara, *Analyst*, 1 (1920).

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

(13) Neuberg, *Ber.*, **32**, 3384–3388 (1899).

(14) Fischer, *ibid.*, **17**, 579 (1884).

(15) Dried by refluxing and distilling in the presence of barium oxide

Summary

Acetobacter suboxydans oxidized D-talitol to D-tagatose, m. p. 133–134°, $[\alpha]^{25}D$ −5°, in yields of 75 to 84%.

The isomerization method by which Reichstein and Bosshard prepared tagatose from galactose has been modified to obtain more consistent yields by the use of an ion exchange resin in the purification procedure.

MADISON 6, WISCONSIN RECEIVED MAY 9, 1949

[CONTRIBUTION FROM THE PURDUE RESEARCH FOUNDATION AND DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

An Improved Synthesis of Esters of Nitroacetic Acid[1]

BY H. FEUER, H. B. HASS[2] AND K. S. WARREN[3]

Esters of nitroacetic acid have recently come into prominence as intermediates in the preparation of amino acids.[4] These esters have been prepared by W. Steinkopf[5] who employed two methods. The one which gave a 37% yield of the ethyl ester consisted of suspending dipotassium nitroacetate in absolute ethanol and introducing dry hydrogen chloride.

The second method, which gave a 71% yield based on nitroacetic acid, involved the following three steps

$$2CH_2NO_2 + 2KOH \longrightarrow KO_2N{=}CHCOOK + NH_3 + 2H_2O \quad (1)$$

$$KO_2N{=}CHCOOK + 2HCl \longrightarrow O_2NCH_2COOH + 2KCl \quad (2)$$

$$O_2NCH_2COOH + ROH \xrightarrow{H^+} O_2NCH_2COOR + H_2O \quad (3)$$

The disadvantage of this procedure lies in the second step because it requires the preparation and isolation of nitroacetic acid from the dipotassium salt and this can only be accomplished with a 60–70% yield. Furthermore, nitroacetic acid is very unstable. It decomposes into nitromethane and carbon dioxide, and this further decreases the yield in the esterification step. Steinkopf[5] stated that he was unable to prepare esters directly from the dipotassium salt with concd. sulfuric acid and the desired alcohol. He claimed that the rate of decomposition of the nitroacetic acid liberated from its dipotassium salt was faster than the rate of esterification. This statement seemed, however, contradictory to the fact that he was able to prepare esters by working with the free nitroacetic acid and using an equimolar amount of concd. sulfuric acid at −15 to +3° as indicated in step 3 above.

It seemed therefore advisable to attempt the synthesis of esters of nitroacetic acid directly from the dipotassium salt by acidification with concd. sulfuric acid and by working at low temperatures. As a result, the methyl and ethyl esters have been prepared in a 60% yield.

The results of several experiments are given in the following three tables to illustrate the influence of reaction temperature, reaction time, and amount of sulfuric acid upon the ester formation.

TABLE I

EFFECT OF REACTION TEMPERATURE ON THE YIELD OF METHYL NITROACETATE

In run No. 6 anhydrous sodium sulfate was omitted.

Run	Temp. during first 24 hours, °C.	Temp. during the next 144 hours, °C.	Yield, %
1	0 to +5	23 to 25	12
2	− 2 to 0	23 to 25	28
3	−15 to −10	23 to 25	38
4	−60 to −50	23 to 25	60
5	−60 to −50	1 to 5	60
6	−60 to −50	1 to 5	45

TABLE II

EFFECT OF REACTION TIME ON THE YIELD OF METHYL NITROACETATE

In run No. 9 anhydrous sodium sulfate was omitted. In all ten experiments 90.5 g. (0.5 mole) of dipotassium nitroacetate, 500 ml. (12.38 moles) of methanol, 100 g. (1 mole) of concd. sulfuric acid, and 15 g. (0.1 mole) of anhydrous sodium sulfate (except in 6 and 9), were used.

Run	Time in hr. at −60 to −50°	Yield, %
7	1	25
8	24	40
9	24	29
10	48	45

TABLE III

EFFECT OF THE AMOUNT OF CONCD. SULFURIC ACID ON THE YIELD OF METHYL NITROACETATE

In all these experiments the same conditions and amounts were used as in run No. 4.

Moles concd. H_2SO_4	Yield, %
1	18
1.5	45
2	60
3	60

The data in Table I show that a lower reaction temperature during the first twenty-four hours of

(1) Presented before the Division of Organic Chemistry at the St. Louis Meeting of the American Chemical Society, September 8, 1948.

(2) Present address: General Aniline and Film Corp., New York City, N. Y.

(3) Present address: Picatinny Arsenal, Dover, N. J.

(4) Lyttle and Weisblat, THIS JOURNAL, **69**, 2118 (1947).

(5) Steinkopf, *Ann.*, **434**, 21 (1923).