

# UNITED STATES PATENT OFFICE

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## PROCESS FOR REDUCING ESTERS OF ALDONIC ACIDS

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sec. 266)

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The invention described herein may be manufactured and used by or for the Government of the United States for governmental purposes without the payment to me of any royalty thereon in accordance with the provisions of the Act of March 3, 1883, as amended (45 Stat. 467; 35 U. S. C. 45).

This invention relates to a process for the sodium amalgam reduction of esters of aldonic acids. It is the object of the present invention to simplify the procedure and to make the process more efficient and suitable for the preparation of radioactive sugars at the millimole level.

In the reduction of lactones and esters of aldonic acids to sugars with sodium amalgam by the classical method, it is customary to maintain the mixture slightly acid by the manual addition of sulfuric acid at a rate determined by the use of Congo red, or other test paper. In order to obtain high yields, close control of pH is necessary. Control of pH is difficult under the customary procedure because the rate at which the amalgam reacts depends upon its condition, temperature, rate of stirring and other factors. Furthermore, manual adjustment of pH is not satisfactory for very small quantities of material, or for use in closed systems.

According to the present invention, the acidity of the reaction mixture in the reduction of aldonic acids in ester form with sodium amalgam, is held within a narrow range by conducting the reaction in the presence of a difficultly soluble acid. With vigorous stirring the acid dissolves so as to maintain the solution saturated and thus eliminates the necessity for manual addition of the acid. This eliminates constant attention of the operator during the reaction period, which in some cases amounts to several hours, and also avoids loss of material by testing. The improved process makes possible the reduction of very small quantities of aldonic acids in ester form in a closed system. These advantages are particularly important for the preparation of radioactive sugars. Furthermore the new process is more efficient than the old and gives exceptionally high yields of sugar with a minimum amount of work. The use of oxalic acid to remove sodium salts rather than sulfuric acid is another important feature of the present invention.

The following examples are given by way of illustration:

*Example 1.—Reduction of radioactive D-glucono- $\delta$ -lactone with sodium acid oxalate buffer*

One millimole (178 mg.) of radioactive D-

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glucono- $\delta$ -lactone and 2.3 g. of 5-percent sodium amalgam in the form of pellets are added with vigorous stirring at 0° C. to 20 ml. of water containing 0.7 g. of oxalic acid and 0.8 g. of sodium oxalate. Stirring is continued until the amalgam is spent. The mercury is then separated and the solution is neutralized by the addition of enough sodium hydroxide to give a permanent pink color with phenolphthalein indicator, followed by just sufficient oxalic acid to discharge the color. The solution is then mixed with 50 ml. of methanol and 25 ml. of ethanol to precipitate most of the sodium oxalate. After 20 minutes, the crystalline sodium oxalate is collected on a filter, washed with methanol and discarded. The alcoholic liquor is evaporated under reduced pressure to a volume of 10 ml., and is then passed at the rate of 10 drops per minute through a column (1.4 x 16 cm.) filled with a mixture of equal parts of Amberlite IR100 and Duolite A4 ion exchange resins. The column is washed with 250 ml. of distilled water, and the eluate is concentrated under reduced pressure substantially to dryness. The residue is dissolved in 0.4 ml. of acetic acid and the solution is seeded with  $\alpha$ -D-glucose. After 2 days at room temperature and 1 day at 0° C., the acetic acid is separated with a pipette, and the crystals are washed 3 times with 0.2-ml. portions of acetic acid. Analysis of the reaction mixtures showed that they contained glucose in amounts ranging from 85 to 90 percent of the theoretical yield, but only 135 mg. of crystalline glucose was isolated. This corresponds to a 75 percent yield of crystalline sugar. The difficultly soluble buffer in this case is sodium acid oxalate. This material dissolves as the reaction proceeds so as to maintain the solution saturated and thus eliminate the necessity for the manual addition of acid. The radioactive sugar prepared was D-glucose 1-C<sup>14</sup>, but the process is applicable to any isotopic modification of the sugar.

*Example 1.—Reduction of D-mannonic- $\gamma$ -lactone with a benzoic acid buffer*

One millimole (178 mg.) of radioactive D-mannonic- $\gamma$ -lactone is dissolved in 20 ml. of ice water, 1.5 g. of benzoic acid are added, and the mixture is stirred in an ice bath. Sodium amalgam pellets (4.6 g. of 5-percent amalgam) are added in one batch, and stirring is continued until all of the sodium amalgam has reacted (1 hour). At this point the mercury is removed with a pipette, and another addition of 1.2 g. of benzoic acid and 4.6 g. of amalgam is made.

Stirring is continued until all of the amalgam has reacted. An analysis at this point shows the production of sugar in about 80-percent yield. The mercury is then removed, and oxalic acid (1.26 g.), equivalent to the sodium originally contained in the sodium amalgam is added, and the mixture is extracted with ether to remove the benzoic acid. The sodium oxalate present in the solution is precipitated by the addition of 2 volumes of methanol and 2 of ethanol. The alcoholic liquor is concentrated under reduced pressure to 10 ml. and the solution is then passed over an ion exchange column (1.4 x 26 cms.) containing a mixture of equal parts of Amberlite IR100 and Duolite A4. The aqueous eluate is evaporated to a thick sirup. The sirup is dissolved in about 0.5 ml. of methanol, and isopropyl alcohol is added to the point of incipient turbidity. The solution is seeded with  $\alpha$ -D-mannose and set aside for crystallization to take place. After several days, the mother liquor is withdrawn, and the crystalline radioactive mannose is washed with a 1-1 mixture of isopropyl alcohol and methanol. The product, after drying, weighs 100 mg.

*Example 3.—Process for the preparation of C<sup>14</sup>-labeled lactose*

To a flask, cooled in ice water, and containing 1 millimole of radioactive cyanide and 1 millimole of sodium hydroxide is added 1 millimole of sodium bicarbonate and 1 millimole of 3-( $\beta$ -D-galactopyranosyl)-D-arabinose dissolved in 10 ml. of water. The solution is allowed to stand overnight at 5° C. and then at room temperature for 4 days. Hydrolysis of the resulting nitriles and removal of the ammonia formed is then effected by heating the solution under reduced pressure for 4 hours at 60° C. The material is diluted to 10 ml. with water, and cations are removed by passing the solution through a column of Amberlite IR100 (1.6 x 24 cm.). The eluate and washings are evaporated to 5 ml. under reduced pressure, 5 ml. of methyl Cellosolve are added and evaporation is continued by passing a current of air over the solution. When the solution becomes thick, it is diluted with a mixture of 5 drops of methyl Cellosolve and 1 drop of water. The material is seeded with crystalline lactobionic lactone and stored in a desiccator. Crystallization is allowed to take place over a period of one week, with the addition each day of sufficient methyl Cellosolve to keep the residue moist. When the lactobionic acid is completely lactonized, the flask is placed in an ice bath, a mechanical stirrer is inserted, and 0.4 g. of sodium oxalate, 0.36 g. of oxalic acid, 20 ml. of water and 2.5 of 5-percent sodium amalgam are added with vigorous stirring. Stirring is continued until all of the amalgam has reacted. The mercury is removed, the solution is neutralized with sodium hydroxide, and diluted with 2 volumes of methanol and 2 volumes of ethanol. The precipitated sodium oxalate is removed by filtration, washed, and discarded. The alcoholic solution is evaporated and diluted to 10 ml. with water. The solution is then passed over an ion exchange column (1.6 x 26 cm.) containing equal parts of Amberlite IR100 and Duolite A4. The aqueous eluate and washings are concentrated under reduced pressure to a thick sirup. The sirup is dissolved in a few drops of methanol and the solution is brought to incipient turbidity by the addition of isopropanol. Crystallization is induced by seeding with  $\alpha$ -lactose. In the course of sev-

eral days about 140 mg. of crystalline radioactive lactose separates.

*Example 4.—Reduction of methyl galactonate*

A 0.5-millimole sample of methyl-D-galactonate is dissolved in 10 ml. of ice water, 1.5 g. of benzoic acid is added, and the mixture is stirred in an ice bath. Sodium amalgam pellets (4.6 of 5-percent amalgam) are added in one batch, and stirring is continued until all of the sodium amalgam has reacted. At this point, the mercury is removed, and 1.2 g. of benzoic acid and 4.6 g. of sodium amalgam are added. Stirring is continued until the malgam has reacted. The mercury is again separated, and reduction is continued with the addition of 1.2 g. of benzoic acid and another 4.6-gram portion of sodium amalgam. When this amalgam has reacted, an analysis shows the production of D-galactose in 85-percent yield and crystalline galactose is readily obtained from the solution, after removal of the cations and anions, by the method given under the preparation of mannose.

It will be apparent that the embodiments shown are only exemplary and that various modifications can be made within the scope of my invention as defined in the appended claims.

I claim:

1. The process of making aldose sugar, which comprises reacting an aqueous solution of an aldonic acid in ester form with sodium amalgam in the presence of a difficultly soluble acid selected from a group consisting of benzoic acid and sodium acid oxalate, said difficultly soluble acid being present in an amount sufficient to maintain said solution saturated, and separating the resulting sugar from the reaction mixture.

2. The process as recited in claim 1, wherein the aldonic acid is carbon-14 labeled aldonic acid.

3. The process of making radioactive D-glucose, which comprises reacting carbon-14-labeled D-glucono- $\Delta$ -lactone with sodium amalgam in the presence of a difficultly soluble acid selected from a group consisting of benzoic acid and sodium acid oxalate, said difficultly soluble acid being present in an amount sufficient to maintain said solution saturated, and separating the resulting sugar from the reaction mixture.

4. The process of making radioactive lactose, which comprises reacting carbon-14-labeled lactobionic- $\Delta$ -lactone with sodium amalgam in the presence of a difficultly soluble acid selected from a group consisting of benzoic acid and sodium acid oxalate, said difficultly soluble acid being present in an amount sufficient to maintain said solution saturated, and separating the resulting sugar from the reaction mixture.

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