SYNTHESIS OF THE HEXURONIC ACIDS

I. THE SYNTHESIS OF \textit{dl}-GALACTURONIC ACID FROM MUCIC ACID*

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(Received for publication, December 8, 1931)

INTRODUCTION

In the course of Fischer's classical researches in the sugar group, the oxidation of the aldoses to their dicarboxylic acids and reduction of these acids to the corresponding aldonic acids constituted significant steps in the elucidation of the stereochemical relationships of the sugars.

While developing a proof for the stereochemical configuration of mucic acid, which was regarded correctly by van't Hoff as one of the inactive systems of the ten theoretically possible dicarboxylic 6-carbon sugar acids, Fischer (1, 2) noted that when the lactone of mucic acid was reduced to \textit{dl}-galaetonic acid, an intermediate acid was formed in the course of the reduction which he called "Aldehydeschleimsäure."

Fischer pointed out that since complete reduction of the lactone of mucic acid yields \textit{d}- and \textit{l}-galaetonic acid, the intermediate "Aldehydeschleimsäure" should also consist of a \textit{d} and an \textit{l} component.

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These relationships are illustrated structurally by the following formulae:

Mucic acid  d-Galacturonic  l-Galacturonic  d-Galactonic  l-Galactonic  
(inactive)  acid  acid  acid  acid

Fischer's "Aldehydeschleimsäure"

The existence of the racemic "Aldehydeschleimsäure," as an intermediate acid formed in the course of the complete reduction of mucic acid lactone to dl-galactonic acid was inferred by Fischer on the basis of the following. He observed that, whereas the lactone of mucic acid did not reduce Fehling's solution, as it was being reduced, the reducing value of the reaction mixture increased to a maximum and then decreased.

Although Fischer stated that he intended to isolate the acid and characterize it more definitely, he did not report further work on it in the course of his extensive studies in the carbohydrate group. In 1908, Mandel and Neuberg (3) reported that the Fischer "Aldehydeschleimsäure" gave the naphthoresorcinol test developed by Tollens for aldehyde and ketonic acids.

Apparently no one has attempted to isolate the intermediate

1 Fischer (1) states, the lactone of mucic acid, in contrast to mucic acid, can be reduced readily, to form an aldehydic acid, which can be detected by its behavior toward Fehling's solution. The experimental studies involved are in progress. Later (2) Fischer states, the first phase of the reduction of the lactone of mucic acid yields, as has been mentioned before, an aldehydic acid whose presence can be detected with Fehling's solution.
sugar acid formed in the course of the reduction of the lactone of mucic acid, for this gap in the sugar group was regarded of sufficient importance to warrant a special comment (4) and furthermore, the comprehensive supplementary volume of Abderhalden (5) makes no reference to this acid.

On the other hand, the d form of galacturonic acid, one of the components of Fischer's "Aldehydeschleimsäure" was isolated in the pure state for the first time by Ehrlich, who found it to be one of the chief components of the pectin in the root of the sugar beet (6). Since then Ehrlich and his coworkers have isolated it from the pectin of other plant tissue (7). In 1923, Kiliani reported that he had isolated the l form of galacturonic acid by the oxidation of d-galactonic acid with nitric acid (8), but subsequently had the grace to refute this work and admit that an error had been made (9). Ohle and Berend reported the synthesis of d-galacturonic acid by the oxidation of diacetone d-galactose with potassium permanganate, but they were unable to isolate the free acid in a crystalline form (10).

Recently the aldehydic sugar acids have attained a preeminent position in biochemical and physiological researches due to their almost universal occurrence in both the plant and animal organism (5). Because of the great demand for relatively large quantities of these acids we have been engaged in the difficult task of developing satisfactory procedures for the preparation of the naturally occurring uronic acids (11) and also in a study of the properties of these interesting and important members of the sugar group (12). Since an inevitable destruction of the uronic acids occurs in the course of their preparation from natural sources (12) it was deemed advisable to explore the possibility of synthesizing them from the corresponding dicarboxylic sugar acids. The success that we have had with the synthesis of dl-galacturonic acid has led us to attempt the synthesis of other members of the group. In a forthcoming publication we will report on the synthesis of dl-alluronic acid from allomucic acid.

**EXPERIMENTAL**

The method of synthesizing dl-galacturonic acid reported in this paper was formulated along the lines originally laid down by Fischer (1, 2) in his experiments on the reduction of mucic acid.
lactone to \textit{dl}-galactonic acid. However, it was found advisable to introduce certain important modifications in the experimental procedure. We were able to establish a definite intermediate end-point in the course of the reaction, so that a good yield of the pure \textit{dl}-aldehydic acid could be obtained.

\textit{Preparation of Mucic Acid—}The mucic acid was prepared by the oxidation of lactose under the conditions of Kent and Tollens (13) and then carefully washed with distilled water to remove all traces of nitric acid. The acid employed in this investigation had a melting point of 220°. It was completely soluble in 60 parts of boiling water, and entered into solution without discoloration.

\textit{Preparation of Mucic Acid Lactone (1, 2)—}50 gm. of mucic acid obtained by the above procedure were dissolved in 3 liters of boiling water contained in a large porcelain evaporating dish. The solution was then concentrated rapidly over a free flame to approximately 700 cc., whereupon it was cooled rapidly to 5° and filtered on a Buchner funnel. The clear filtrate contained the monolactone of mucic acid and mucic acid in the ratio of 10 parts of lactone to 1 part of acid. This ratio was determined by an alkalimetric titration. The actual isolation of the lactone is not performed. This is not a hindrance for the objective in view, since the solution thus obtained can be reduced directly.

\textit{Reduction of the Monolactone of Mucic Acid (1, 2, 14)—}The aqueous solution of the lactone\(^2\) was introduced into a 1 liter beaker, surrounded by an ice bath, and cooled to 5°. While the solution was being stirred vigorously, 300 gm. of 2.5 per cent sodium amalgam\(^3\) were added during the course of 20 minutes. Dilute sulfuric acid (0.2 N) was added to the lactone solution throughout the entire course of the reduction, as recommended by Fischer and Kiliani in their procedure for the reduction of saccharic acid lactone to \textit{d}-glucuronic acid (15). After the reaction

\(^2\) From 50 gm. of mucic acid, about 10 gm. of the acid precipitates out in the concentration process. Of the remaining 40 gm. 5 gm. of mucic acid are in solution and the rest is present as the lactone.

\(^3\) The ratio of sodium, in the form of amalgam, to lactone was 1.8 mols to 1.0 mol, for this ratio, under the conditions used, gave to the reaction product a maximum reducing power as determined by its action on Fehling’s solution. This ratio may be increased in favor of the sodium but this involves the accompanying danger of carrying the reduction to the aldonic acids which are difficult to separate from the aldehydic acids.
had ceased (10 to 15 hours) the solution was decanted from the residual mercury. The filtrate was then cooled to 5° and basic lead acetate added carefully until no further precipitation took place. The precipitate was then filtered off rapidly on a Buchner funnel and washed with 500 cc. of ice-cold water. It was then suspended in 400 cc. of ice-cold water and again filtered off at the pump. The precipitate, considerably freed from water-soluble salts, was suspended in a finely divided form in 1 liter of water and decomposed with hydrogen sulfide. The lead sulfide was removed by filtering on an asbestos mat and the filtrate aerated to eliminate the residual hydrogen sulfide remaining in solution. At this stage it is of importance to have the solution free from lead. It was noticed in a number of trials that lead is often present in the filtrate and that it must be removed by additional treatments with hydrogen sulfide.

The aerated filtrate was subsequently neutralized with an excess of barium carbonate (100 gm.), heated on a water bath at 70° for 30 minutes, and filtered. The filtrate was concentrated at 40° and 15 mm. pressure to a volume of 75 cc. Carbon dioxide was then passed through the solution for 5 hours. After this treatment the solution was placed in an ice chest for 2 to 3 days. This procedure eliminates any barium combined with the sugar acid in the form of the so called metal saccharates. The solution was centrifuged to remove the precipitated barium carbonate, and then poured into 800 cc. of 95 per cent ethyl alcohol, while the latter was being stirred vigorously. After standing for 1 hour the precipitated barium salt was filtered on a Buchner funnel, sucked dry, and redissolved in 100 cc. of water. It was then reprecipitated with 4 volumes of 95 per cent alcohol, filtered, and washed successively with hot 95 per cent ethyl alcohol, hot absolute alcohol, and finally with absolute ethyl ether. The barium salt obtained was partially dried over calcium chloride in a vacuum desiccator at room temperature and then completely dried in a vacuum oven under 4 mm. pressure and a temperature of 50°. Numerous trials indi-

4 This requires from 700 to 800 cc. of a basic lead acetate solution prepared according to the Association of Official Agricultural Chemists as given by Browne (16).

5 Reprecipitation of the barium salt is necessary to remove any barium acetate that may be present.
cated that a yield of 15 to 20 gm. of \textit{dl}-barium galacturonate are obtained from 50 gm. of mucic acid. This yield, calculated on the basis of mucic acid used, no recovery being assumed, is from 24 to 32 per cent of the theoretical. Allowing for a 20 per cent recovery of mucic acid during the preparation of the lactone, the yield is from 35 to 40 per cent of the theoretical.

\textit{Constants}—Theory demands for \((\text{CsH}_9\text{O}_7)_2\text{ Ba}\), 26.3 per cent barium. Found, 26.7 to 27.0 per cent Ba. Optical rotation in water \([\alpha]_b^{25} = 0.0^\circ\). The \textit{dl}-barium galacturonate does not have a sharp melting point, even when pure, but begins to decompose at 180\(^\circ\). In this respect it is comparable to the pure \textit{d}-barium salt prepared from citrus pectin (11).

\textit{Isolation of \textit{dl}-Galacturonic Acid from Barium Salt}—22 gm. of \textit{dl}-barium galacturonate were dissolved in 500 cc. of water and subjected to an additional purification by heating at 50\(^\circ\) in the presence of 5 gm. of blood charcoal and 5 gm. of kieselguhr. After filtering, the solution was cooled to 20\(^\circ\). Ethyl alcohol (95 per cent) was then added to produce a permanent turbidity, 100 cc. being necessary. While the solution was being vigorously agitated, 425 cc. of 0.2 \(\text{n}\) sulfuric acid were added during the course of 3 hours. 10 gm. of blood charcoal and 5 gm. of kieselguhr were added to the solution, still neutral to Congo red, which was then heated to 50\(^\circ\) and filtered through an asbestos mat at the pump. The clear colorless solution was concentrated at 40\(^\circ\) and 15 mm. pressure to a volume of 170 cc. The concentrate was then poured slowly into 400 cc. of 95 per cent ethyl alcohol to precipitate the undecomposed barium salt. It is advisable to use slightly less than the theoretical quantity of acid required so as to minimize lactone formation. The unchanged barium salt was filtered off, dried in a vacuum, and weighed. The clear filtrate was again concentrated at 35\(^\circ\) under 15 mm. pressure to a volume of 75 cc. The concentrate was then filtered into a crystallizing dish through a mat of asbestos. After seeding with a crystal of the free \textit{d} acid, which is desirable but not necessary, the dish was placed in a vacuum desiccator. After exhausting the desiccator for 15 minutes it was sealed off and the contents of the dish allowed to crystalize. Crystallization, if the experiment is successful, is usually complete in 2 to 3 days. The crystalline mass obtained was suspended in 30 cc. of 95 per cent ethyl alcohol and filtered
off on a hardened filter. The crystals were then washed with small successive portions of 95 per cent ethyl alcohol, absolute alcohol, and finally anhydrous ethyl ether. The crystalline \(d\)-galacturonic acid was dried to a constant weight at room temperature in a vacuum desiccator containing phosphorous pentoxide. The \(d\)-galacturonic acid crystallizes as the monohydrate in small microscopic needles. Yield, 4.5 gm. from 20 gm. of barium salt or 30 per cent of the theoretical. The yield can be increased to 50 per cent of the theory by reworking the mother liquor.

**Melting Point**—The substance sinters at 110°, turns red at 135°, and melts at about 156° with effervescence and decomposition. The melting point of the mixture of the pure \(d\) acid with an authentic specimen of the \(d\) component prepared from citrus pectin showed no depression.

**Neutralization Equivalent**—1.0 gm. required 46.98 cc. of 0.1 N NaOH, whereas theoretically 1.0 gm. of \(C_5H_{11}O_5(COOH)\) requires 47.20 cc. of 0.1 N NaOH.

**Optical Rotation**—\([\alpha]_D^{25} = 0.0^\circ\) (in water).

**DISCUSSION**

The successful reduction of mucic acid lactone to \(d\)-galacturonic acid depends greatly on the purity of the mucic acid used. Fischer (1, 2) repeatedly emphasized the necessity for pure starting materials in the course of researches in the sugar group, and it was found that success in the final crystallization of the free \(d\) acid is only accomplished if this precaution is strictly observed. Consequently it is advisable to prepare the mucic acid from pure lactose or galactose rather than from the galactans obtained from the Western larch (Larix americana), for when prepared from this source, preparations are obtained that are difficult to purify.

In the reduction of the lactone with sodium amalgam it has been found more practicable to reduce the aqueous solution of the lactone rather than to attempt to isolate this intermediate product. The lactone has been isolated by Fischer (2) but the yield is so unsatisfactory as to make this step inadvisable.

Sodium amalgam was chosen as the reducing agent, because it is the only one of those that were tried which satisfied the needs of the reaction in question. Metallic calcium and catalytic re-
duction with the Adams-Voorhees catalyst at 2 atmospheres of hydrogen pressure gave no apparent reduction.

As a result of experience based upon thirty-two preparations of the $dl$-barium galacturonate from 50 gm. portions of mucic acid, we have found the following points of manipulative technique important enough to warrant mention. (1) Better yields are obtained if the reduction of the lactone is carried out in the presence of sulfuric acid, as Kiliani found with the lactone of saccharic acid (15). (2) Attempts to remove the unchanged mucic acid from the reaction mixture by forming the lead salt with neutral lead acetate led to appreciable losses of the aldehydeic acid. (3) Barium carbonate in excess has proved more desirable than stoichiometrical amounts of barium hydroxide, for decomposition due to alkalinity is thereby reduced to a minimum. Furthermore the solution can be heated safely in the presence of an excess of barium carbonate to effect complete conversion of the lactone of the aldehydeic acid to the barium salt. (4) The use of carbon dioxide to remove the barium bound as saccharate is necessary, for if this treatment is eliminated the barium salt may contain as high as 40 per cent barium, whereas theory demands 26.3 per cent.

The yield of the free $dl$-galacturonic acid from its barium salt corresponds to that reported by Link and Nedden (11) for the $d$-barium salt prepared from the polygalacturonide of citrus pectin. The resolution of the racemic galacturonic acid into the $d$ and $l$ components over the brucine salts will form the subject of a separate communication.

SUMMARY

Crystalline $dl$-galacturonic acid has been prepared by the reduction of mucic acid with sodium amalgam in a slightly acid solution. This synthesis was effected by conversion of the mucic acid into its monolactone which, upon reduction, yielded the racemic ($dl$-) aldehydeic acid. This was isolated in the form of its barium salt, which was converted into the crystalline free acid by decomposition with sulfuric acid.

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J. Biol. Chem. 1932, 95:203-211.

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