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SYNTHESIS OF VITAMIN C FROM PECTIC SUBSTANCES[1]

By Horace S. Isbell

ABSTRACT

A new process for the preparation of vitamin C from beet pulp and other pectic substances has been developed. The pectic substance is treated with a pectinase, the resulting galacturonic acid is separated in the form of a difficultly soluble salt which is reduced with hydrogen to a salt of L-galactonic acid. The salt is converted to L-galactono-lactone and oxidized to 2-keto-L-galactonic acid, which is lactonized and enolized to yield ascorbic acid (vitamin C). Electronic interpretations are presented for the conversion of methyl 2-keto-L-galactonate to ascorbic acid by basic catalysts, for the lactonization and enolization of 2-keto-hexonic acids by acid catalysts, and for the formation of furfural and reductic acid from pentoses, galacturonic acid, and ascorbic acid.

CONTENTS

I. INTRODUCTION

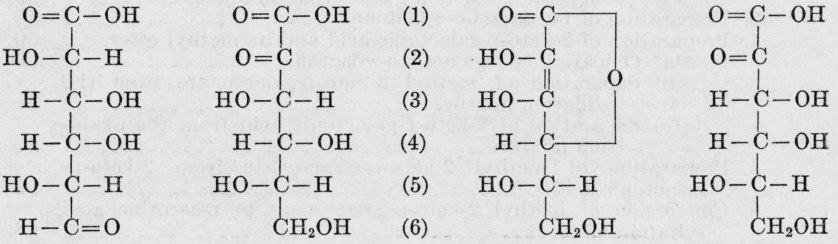
Because of the necessity of an adequate supply of vitamin C for the proper healing of wounds [1][2] an unprecedented demand for this sub-

[1] This paper was presented before the Division of Sugar Chemistry and Technology of the American Chemical Society at Cleveland, Ohio, April 5, 1944.

[2] Figures in brackets indicate the literature references at the end of this paper.

stance has been created by the present national emergency. Part of the requirements of the military and civilian populations is derived from fresh fruits and vegetables but this source is not adequate and must be supplemented by the synthetic vitamin. The process in present use for the synthesis of vitamin C [2] includes reduction of D-glucose to sorbitol, bacterial oxidation of this to L-sorbose, formation of diacetone sorbose, oxidation to diacetone-2-keto-L-gulonic acid, conversion to methyl 2-keto-L-gulonate followed by lactonization with the elimination of methanol, and rearrangement to ascorbic acid (vitamin C). As the process is long and complicated, it seemed desirable to seek a shorter method of synthesis.

To obtain the natural vitamin rather than an isomer thereof, it is necessary to use materials and reactions which lead to the L-*threo* configuration for carbons 4 and 5 of the product. Perhaps the most obvious raw material is galacturonic acid [3] and this was, in fact, used in the first synthesis of vitamin C [4]. The original method involved partial degradation and resynthesis of the molecule. It was not satisfactory for large scale production because it included the production of L-xylosone, a step which can be accomplished only with poor yield, and the preparation of galacturonic acid from D-galactose, a long and expensive process. Galacturonic acid, however, is an abundant natural carbohydrate, and methods have been developed in recent years for its preparation from plant materials. In the form of pectic substances, it comprises a large part of the pulp from citrus fruits and about 25 percent of the solids which remain after extraction of sugar from sugar beets. Since the annual production of sugar beets is approximately 7,000,000 tons, they provide a vast source of raw material which might be used for the production of galacturonic acid.



I. Galacturonic acid. II. 2-Keto-L-gulonic acid. III. L-Ascorbic acid (vitamin C). IV. 2-Keto-L-galactonic acid.

A comparison of the structures of galacturonic acid, I, 2-keto-L-gulonic acid, II, and L-ascorbic acid, III, reveals that galacturonic acid is isomeric with 2-keto-L-gulonic acid, a substance readily converted to ascorbic acid, and that the three compounds have the L-*threo* configuration for carbons 4 and 5. It was recognized early by Reichstein and Grüssner [5] that ascorbic acid usually occurs in plants containing large quantities of galacturonic acid, and attempts were made by them to rearrange galacturonic acid to ascorbic acid. The experiments gave highly reducing substances but did not yield appreciable quantities of ascorbic acid, and subsequently no method has been devised for effecting the conversion.

In the conversion of galacturonic acid to ascorbic acid, it is necessary to reduce the aldehyde group to $-CH_2OH$, and to oxidize either car-

bon 2 or carbon 3 so as to obtain an enediol without disturbing the configurations of carbons 4 and 5. Reduction of the aldehyde group is a simple matter and was part of the process originally used in the first synthesis [4]. The oxidation of either carbon 2 or carbon 3 is more difficult, but recently considerable progress has been made in the development of methods for the production of 2-keto-aldonic acids. Thus 2-keto-gluconic acid is now made by bacterial oxidation [6], and several methods have been devised for the oxidation of aldonic acids, in general, to 2-keto-aldonic acids [7]. Consideration of the probable mechanism for the enolization and lactonization of 2-keto-L-galactonic acid, discussed more fully on page 56, led to the premise that 2-keto-L-galactonic acid, IV, would yield ascorbic acid by an enolization and lactonization reaction, and that a new process for the production of ascorbic acid might include the following steps: Galacturonic

$$\text{acid} \xrightarrow{\text{hydrogenate}} \text{L-galactonic acid} \xrightarrow{\text{oxidize}} \text{2-keto-L-galactonic acid} \xrightarrow{\text{enolize and lactonize}} \text{ascorbic acid (vitamin C).}$$

At the time the investigation was begun it was known that esters of 2-keto-aldonic acids by treatment with sodium methylate undergo enolization and lactonization reactions with the formation of ascorbic acids [8]. However, it was generally believed that an ester of 2-keto-L-gulonic acid was necessary to obtain vitamin C (see p. 171 of [9]). 2-Keto-L-galactonic acid differs from 2-keto-L-gulonic acid only in the configuration of carbon 3, and consequently it was of academic as well as of practical interest to attempt the synthesis by the method outlined above.

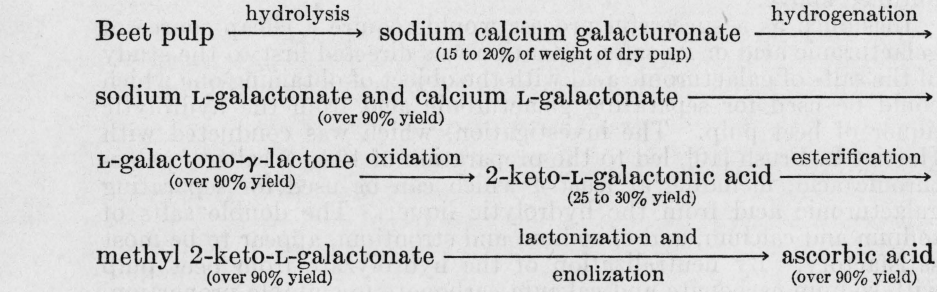
Inasmuch as a successful process would require a cheap source of galacturonic acid or its salts, attention was directed first to the study of the salts of galacturonic acid with the object of obtaining one which could be used for separating galacturonic acid from the hydrolytic liquor of beet pulp. The investigation, which was conducted with Harriet L. Frush [10], led to the preparation of 12 new salts of galacturonic acid, including at least 4 which can be used for separating galacturonic acid from the hydrolytic liquor. The double salts of sodium and calcium, and of sodium and strontium, appear to be most satisfactory. By neutralization of the hydrolyzate from beet pulp with sodium carbonate and calcium carbonate in suitable proportion, followed by concentration of the solution, crystalline sodium calcium galacturonate was obtained in yield corresponding to about 18 percent of the dry pulp, or approximately 70 percent of the galacturonic acid content of the hydrolyzate.

Prior to the present investigation it was known that salts of galacturonic acid on reduction with either sodium amalgam [4, 11] or with hydrogen in the presence of catalysts [12] give salts of L-galactonic acid. Reduction of calcium galacturonate, sodium calcium galacturonate, or sodium strontium galacturonate, with hydrogen, in the presence of Raney nickel, gave salts of L-galactonic acid in nearly quantitative yield. Treatment of the salts with a chemically equivalent quantity of sulfuric acid, and dehydration, gave L-galactono-γ-lactone in good yield. Several of the methods reported in the literature [7] for the oxidation of aldonic acids to the corresponding 2-keto-acids were investigated and the oxidation of galactono-γ-lactone with

sodium chlorate in the presence of vanadium oxide [7c] was found to be the most satisfactory. Improvements were made in the procedure for isolating the product, and 2-keto-L-galactonic acid and 2-keto-D-galactonic acid were obtained in the crystalline state. The acids were converted to the crystalline methyl esters and these substances were lactonized and rearranged by the sodium methylate method [8]. The product from methyl 2-keto-L-galactonate on acidification gave a crystalline acid which proved to be L-ascorbic acid (vitamin C); by similar treatment methyl 2-keto-D-galactonate gave the crystalline enantiomorph, D-ascorbic acid. Thus the entire process was carried through as originally planned.[3]

It will be observed from the accompanying diagram that the process is relatively simple. Theoretically, 0.7 part by weight of ascorbic acid might be derived from 1 part by weight of sodium calcium galacturonate. The yield of ascorbic acid from sodium calcium galacturonate in the present study was approximately 20 percent of the theoretical, or 14 percent by weight, and the yield of sodium calcium galacturonate from beet pulp was 18 percent by weight. Thus 1 ton (2,000 lb) of dry beet pulp would yield approximately 50 pounds of ascorbic acid. The low yield is due in large measure to the lack of a highly efficient method for oxidizing L-galactonic acid to 2-keto-L-galactonic acid. Undoubtedly this step, as well as other steps in the process, will be improved so that ultimately the new process will compare favorably with the sorbose process.[4]

NEW PROCESS FOR THE PREPARATION OF ASCORBIC ACID.



II. EXPERIMENTAL PROCEDURE

1. PREPARATION OF SODIUM CALCIUM GALACTURONATE[5]

(a) FROM BEET PULP

A solution of galacturonic acid was prepared by enzymic hydrolysis of beet pulp in much the same manner as in the preparation of galac-

[3] In July 1942, the author filed patent applications relating to several phases of the problem, including the preparation of sodium calcium galacturonate from beet pulp. However, the same process was the subject of an application filed seven months earlier by Pasternack and Regna. The latter matured into United States Patent 2,338,534, January 4, 1944. A recent publication by Regna and Caldwell [13] reports the preparation of crystalline 2-keto-D-galactonic acid, and its successive conversion to methyl 2-keto-D-galactonate and to D-ascorbic acid. It is therefore apparent that others have been engaged also in a study of the synthesis of vitamin C from pectic substances and have developed independently, essentially the same process as that worked out by the present author.

[4] Possibly an organism will be found for oxidizing L-galactonic acid, in manner analogous to the oxidation of D-gluconic acid to 2-keto-D-gluconic acid.

[5] See footnote 3.

turonic acid from pectic acid [14]. In a typical preparation, 1 kg of dried beet pulp was suspended in 10 liters of water and mixed with 100 g of Pectinol 100 D.[6] The material was kept at 40° C and protected from mold growth by a layer of toluene. After 7 days the mixture was filtered. The residue was washed with hot water and finally discarded. The hydrolyzate (pH 3.4) was neutralized with a mixture of sodium carbonate and calcium carbonate in the proportion of one atom of sodium to one of calcium, and was concentrated in a vacuum still to a convenient volume. The crystals that formed were separated, washed with water, and dried. The yield of sodium calcium galacturonate hexahydrate thus obtained was 180 g. It was recrystallized from hot water and used for the preparation of calcium L-galactonate.

(b) FROM PEELS OF CITRUS FRUIT

Application of the process described in the foregoing paragraph to citrus fruit peels in place of beet pulp gave 55 g of sodium calcium galacturonate from 1 kg of moist orange peel, and 60 g of sodium calcium galacturonate from 1 kg of moist grapefruit peel. The amounts of water and Pectinol used in these experiments were half of those used with dry beet pulp.

2. PREPARATION OF CALCIUM L-GALACTONATE

The reduction of salts of galacturonic acid to salts of L-galactonic acid by the action of sodium amalgam was a step in the original synthesis of vitamin C [4]. Subsequently, reduction with hydrogen and a nickel catalyst has been used by Tipson [15] and others for the preparation of derivatives of L-galactonic acid, and a German patent [12] discloses the catalytic reduction of salts of galacturonic acid. The procedure employed in the present investigation is an adaptation of a method [16] reported to give high yields of sorbitol from glucose.

(a) FROM CALCIUM GALACTURONATE

Seventy grams of calcium galacturonate [10], 400 ml of water, and a nickel catalyst were placed in a bomb and treated with hydrogen at a pressure of 1,000 lb. and a temperature of 80° to 100° C. The catalyst was prepared from 20 g of Raney nickel alloy by digesting it with 200 ml of 25-percent aqueous sodium hydroxide for a period of 2 hours, and washing it with water. After 6 hours of hydrogenation, a negative test for copper-reducing substances indicated complete reduction of the calcium galacturonate. The bomb was emptied and rinsed with hot water, and the solution containing the reduction product was filtered. Evaporation of the filtered solution resulted in the crystallization of calcium L-galactonate. The crystalline salt was collected on a filter, washed with water, and dried at room temperature. The yield was 72 g, corresponding to 85 percent of the theoretical.

(b) FROM SODIUM CALCIUM GALACTURONATE

One hundred fifty grams of sodium calcium galacturonate, 500 ml of water, and a nickel catalyst were placed in a bomb and treated with hydrogen, at a pressure of 1,500 lb and a temperature of 80° C.

[6] A commercial pectinase manufactured by Rohm & Haas, Philadelphia, Pa. See Willaman and Kertesz, United States Patent 1,932,833, Oct. 31, 1933.

The catalyst was prepared from 20 g of Raney nickel alloy. After 6 hours of hydrogenation the solution containing the reduction product was filtered and, after the addition of 25 g of calcium nitrate, concentrated in a vacuum still to about 100 ml. The crystals of calcium L–galactonate which formed were separated, washed with cold water, dried, and weighed. The yield (140 g) was 93 percent of the theoretical.

The hydrogenations described above were conducted at high pressures because suitable equipment was available and convenient. Hydrogenations under conditions similar to those given, but at pressures of 15 to 30 lb, gave equally satisfactory yields of calcium L–galactonate but required more time.

3. PREPARATION OF L-GALACTONO-γ-LACTONE

A dry mixture containing 1 mole of calcium L-galactonate and 1 mole of oxalic acid was added with stirring to 1 liter of hot water. The resulting calcium oxalate was separated by filtration, and the filtrate was evaporated to dryness at 80° C under reduced pressure. The L-galactono-γ-lactone, which crystallized in nearly quantitative yield, was recrystallized from ethyl alcohol. The optical rotation and melting point of the product ($[\alpha]_D^{20}=+78°$ and mp 134° C) are in agreement with the constants reported for L-galactono-γ-lactone by Richtmyer, Hann, and Hudson [17].

4. PREPARATION OF 2-KETO-L-GALACTONIC ACID AND ITS METHYL ESTER

(a) OXIDATION OF L-GALACTONO-γ-LACTONE

One hundred eighty grams of L-galactono-γ-lactone, 1 liter of anhydrous methyl alcohol, 30 g of sodium chlorate, 2 g of vanadium pentoxide, and 3.5 g of phosphoric anhydride were placed in a 2-liter glass-stoppered flask and shaken mechanically for several days. When all of the chlorate had reacted, as shown by a change in color from brown to greenish blue, the solution was filtered and the solid material, largely vanadium pentoxide, was discarded. The procedure followed to this point differs from that described by Pasternack and Regna [7c] only in that phosphoric anhydride was used in place of phosphoric acid. The phosphoric anhydride causes rapid esterification of the lactone, with the result that the galactono-lactone is converted to methyl galactonate prior to oxidation. An analysis of the solution containing the oxidation product revealed the presence of copper-reducing substance corresponding to 54 g of 2-keto-L-galactonic acid. The filtered solution was mixed with 10 g of cadmium carbonate and concentrated under reduced pressure in a vacuum still. As the evaporation proceeded, crystals separated from solution. When the material in the distillation flask reached a pasty consistency it was diluted with 200 ml of isopropyl alcohol and seeded with crystalline methyl L-galactonate.

After 18 hours at 0° C, the solid material was separated from the alcoholic solution by filtration. The solids (110 g) consisted of methyl L-galactonate, L-galactono-γ-lactone and sodium chloride. The alcoholic solution contained methyl 2-keto-L-galactonate with small quantities of L-galactono-γ-lactone, methyl L-galactonate, methyl oxalate, and other esters not yet identified. In a manufacturing process the

residue could be used in place of fresh L-galactono-γ-lactone. In the present case, however, the methyl L-galactonate and the gamma lactone in the solid residue were reclaimed in the form of cadmium L-galactonate. The salt reclaimed corresponded to 60 g of L-galactono-γ-lactone. Inasmuch as the solution contained 54 g of 2-keto-L-galactonic acid, by analysis, the yield of 2-keto-L-galactonic acid in solution was 41 percent of the theoretical amount based on the weight of lactone used and not recovered. The alcoholic solution containing the oxidation product was used for the preparation of methyl 2-keto-L-galactonate, and a similar solution obtained in a duplicate experiment was used for the preparation of 2-keto-L-galactonic acid.

(b) SEPARATION OF METHYL 2-KETO-L-GALACTONATE FROM THE OXIDATION MIXTURE

The isopropyl alcoholic solution obtained by the oxidation of 180 g of L-galactono-γ-lactone described in the preceding section was evaporated under reduced pressure to a thick sirup. This sirup was triturated with ten 100-ml portions of warm acetone, and the residue was discarded. The acetone extracts were combined and concentrated in a vacuum still to remove the acetone. The residual sirup (50 g) was dissolved in 50 ml of isopropyl alcohol, seeded with methyl 2-keto-L-galactonate and placed in a refrigerator. After 18 hours the resulting crystals were separated by filtration, washed with isopropyl alcohol, and dried. A microscopic examination of the crystals showed two substances: One, L-galactono-γ-lactone, was present in chunky truncated crystals; the other, methyl 2-keto-L-galactonate was present in long slender crystals, usually pointed at both ends. The crystalline mixture weighed 25 g and the copper-reducing value indicated that the material contained about 80 percent of methyl 2-keto-L-galactonate. The mixture melted at 125° to 130° C $[\alpha]_D^{20}=+20°$. The mother liquor gave a second crop of crystals (2 g), which melted at 140° C $[\alpha]_D^{20}=+6°$. The material was nearly pure methyl 2-keto-L-galactonate. The lactone-ester mixture obtained in the first crop was fractionally recrystallized from isopropyl alcohol. By alternately seeding with the gamma lactone, removing a crop of crystals rich in the lactone, and then seeding the residual mother liquor with the ester, several small fractions of L-galactono-γ-lactone were obtained and about 5 g of methyl 2-keto-L-galactonate, which melted at 145° to 148° C. $[\alpha]_D^{20}=+4.7°$.

Separation of methyl 2-keto-L-galactonate from the solution obtained by oxidation of L-galactono-γ-lactone provides material which can be converted directly to ascorbic acid. But since the separation of the ester from the accompanying impurities is difficult, and 2-keto-L-galactonic acid is easily purified, it seems more practicable at present to hydrolyze the crude ester, separate the resulting 2-keto-L-galactonic acid in the pure crystalline state, and reconvert it to the methyl ester as described in the next section.

(c) SEPARATION OF 2-KETO-L-GALACTONIC ACID FROM THE OXIDATION MIXTURE

An isopropyl alcoholic solution obtained by the oxidation of 180 g of L-galactono-γ-lactone, as described on page 50, was evaporated under reduced pressure to a sirup, which was dissolved in 2 liters of water containing 2 ml of acetic acid. The solution was heated to boiling in a flask connected with a reflux condenser. From time to

time small samples were taken, and the acidity was determined by titration with standard alkali (bromthymol blue indicator). The titration showed that hydrolysis was complete in 4 hours, at which time the solution was transferred to a vacuum still and concentrated to a thick sirup. During the evaporation, crystals of 2-keto-L-galactonic acid formed. The mixture of sirup and crystals was diluted with an equal volume of acetic acid and placed in a refrigerator for crystallization to take place. After 1 day the crystals were collected on a filter, washed with aqueous acetic acid, and dried. The first crop of crystals (mp 168° C) weighed 30 g. Additional crops obtained from the mother liquor were mixtures of 2-keto-L-galactonic acid and L-galactono-γ-lactone, from which 2 g of pure 2-keto-L-galactonic acid was obtained. Since the first crop was substantially pure 2-keto-L-galactonic acid, the yield of the crystalline acid was 32 g. This is approximately 60 percent of the amount shown by analysis or 24 percent of the theoretical, based on the amount of L-galactono-γ-lactone used and not reclaimed.

The material used for analysis and for the determination of optical rotation and other properties was recrystallized by dissolving the crude acid in 2 parts of hot water, filtering the solution with the aid of a little decolorizing carbon, and evaporating it under reduced pressure in the presence of seed crystals. The crystals of pure 2-keto-L-galactonic acid which formed were collected on a filter, washed with cold water, and dried, at room temperature, over calcium chloride: mp 170° C; $[\alpha]_D^{20}=+5.2°$ (water, c=4). Analysis: Calculated for $C_6H_{10}O_7$: C, 37.1; H, 5.2. Found: C, 37.2; H, 5.5.

2-Keto-L-galactonic acid separates from impure sirups in truncated plates, which, if undisturbed, form in rosette-like clusters. On recrystallization, the crystals become thicker and more prismatic.

5. PREPARATION OF METHYL 2-KETO-L-GALACTONATE FROM 2-KETO-L-GALACTONIC ACID

The esterification of 2-keto-L-galactonic acid can be carried out by the methods developed for the esterification of 2-keto-gulonic acid [18].

Ten grams of 2-keto-L-galactonic acid was refluxed with 100 ml of anhydrous methyl alcohol containing 1.5 g of hydrogen chloride. After 2 hours the solution was cooled and the hydrogen chloride was neutralized by the addition of silver carbonate. The resulting silver chloride was separated by filtration; the filtrate was evaporated under reduced pressure at 35° C to a volume of 12 ml. The concentrated sirup was diluted with 20 ml of acetone, and petroleum ether was added to the point of saturation. The mixture was allowed to stand overnight in the refrigerator. The crystals of methyl 2-keto-L-galactonate which formed were collected on a filter and washed with a mixture of acetone and ligroin in the proportions of 1:2. The air-dried crystals obtained in the first crop weighed 5 g, and in addition, 2 g was separated from the mother liquor. Although the yield was only 70 percent, a higher yield could be obtained by cycling the mother liquor. In a large-scale operation, an azeotropic distillation and dehydration process could be employed in place of the procedure described here.

Seven and one-half grams of the ester was recrystallized by dissolving it in 50 ml of hot anhydrous methyl alcohol, filtering the solution, cooling it to room temperature, and adding about 75 ml of a mixture consisting of 2 parts by volume of acetone and 1 part of ligroin. The crystals which formed in the course of several hours were separated by filtration, washed with a mixture of acetone and ligroin, and dried over calcium chloride and paraffin at room temperature $[\alpha]_D^{20}=+4.7°$ (water, c=4). Analysis: Calculated for $C_7H_{12}O_7$:C, 40.4; H, 5.8. Found: C, 40.2; H, 5.9.

The substance has an agreeable sweet taste. It reduces alkaline copper reagents even at room temperature. The melting point varies widely, according to the method of measurement. In Pyrex tubes the compound melts at 145° to 150° C, depending upon the rate of heating, but in soft-glass tubes the melting point is depressed at least 10 degrees, presumably through the effect of the alkali in the glass in causing rearrangement. The presence of an unaltered 2-keto-L-galactonate structure in the ester was established by regeneration of 2-keto-L-galactonic acid in the following manner: A 0.5-g sample of the ester was dissolved in 10 ml of water containing 1 drop of concentrated hydrochloric acid. The solution was placed in a beaker and allowed to evaporate at room temperature. After 24 hours a crystalline residue remained, which melted at 168° to 170° C, and did not lower the melting point of an authentic sample of 2-keto-L-galactonic acid.

6. CONVERSION OF METHYL 2-KETO-L-GALACTONATE TO L-ASCORBIC ACID (VITAMIN C)

Two grams of crystalline methyl 2-keto-L-galactonate was added at room temperature, and in the absence of oxygen to 15 ml of 0.7 *N* sodium methylate in absolute alcohol. In a few minutes the methyl 2-keto-L-galactonate went into solution and shortly thereafter a light-yellow sodium salt precipitated. After 15 minutes a quantity of 1 *N* sulfuric acid (in 75-percent aqueous isopropyl alcohol), equivalent to the sodium methylate previously used, was added from a burette. The solution was diluted with 10 ml of isopropyl alcohol, and the resulting crystalline sodium sulfate was separated by filtration. The filtrate was concentrated in a vacuum to a sirup, which crystallized. The crystals were collected on a filter, washed with tertiary amyl alcohol, and dried in a vacuum at room temperature. $[\alpha]_D^{20}=23°$ (water, c=4). The product (1.2 g) melted at 190° to 192° C, and a mixed melting-point determination with authentic L-ascorbic acid showed no depression. Hence the material is L-ascorbic acid. Analysis: Calculated for $C_6H_8O_6$:C, 40.9; H, 4.6. Found: C, 40.9; H, 4.6.

7. PREPARATION OF 2-KETO-D-GALACTONIC ACID

The preparation of 2-keto-D-galactonic acid from D-galactosone was reported in 1929, [19], but the crystalline acid was not prepared until recently [13]. The crystalline acid was obtained also in the course of the present investigation. D-galactono-γ-lactone was oxidized and 2-keto-D-galactonic acid was isolated in the manner described in sections 4(a) and 4(c) for the enantiomorphs. The yield of 2-keto-D-galactonic acid from 180-g samples of D-galactono-γ-

lactone varied from 25 to 35 g. The acid was recrystallized from hot water and was dried at room temperature over calcium chloride in a vacuum desiccator. Melting point 170° C. $[\alpha]_D^{20}=-5.2°$ (water, c=5). Analysis: Calculated for $C_6H_{10}O_7$:C, 37.1; H, 5.2. Found: C, 37.1; H, 5.5.

Regna and Caldwell [13] report a melting point of 170° to 171° C and $[\alpha]_D^{20}=-6.7°$ (water, c=1.2).

The acid reduces alkaline copper reagents. The reducing power of the acid by the modified Scales method [20] is about 93 percent of the reducing power of glucose.

8. PREPARATION OF METHYL 2-KETO-D-GALACTONATE

Crystalline methyl 2-keto-D-galactonate was prepared from the oxidation product of D-galactono-γ-lactone in the manner given on page 51 for the preparation of the enantiomorphic substance, methyl 2-keto-L-galactonate. The substance was prepared also by esterification of 2-keto-D-galactonic acid by the method described on page 52. Analysis: Calculated for $C_7H_{12}O_7$:C, 40.4; H, 5.8. Found: C, 40.4; H, 5.9. The melting point, like that of the L isomer, varies widely, according to the method of measurement. In Pyrex tubes, rapidly heated, the compound melts at 145° to 150°C; in soft-glass tubes, purchased from a chemical supply company, it melted at about 135° C. $[\alpha]_D^{20}=-4.6°$ (water, c=1.7).

For methyl 2-keto-D-galactonate, Regna and Caldwell [13] have reported: mp 138° to 139° C; $[\alpha]_D^{20}=-11.3°$ (water, c=1.2). The melting point lies within the range observed in the present study, but the optical rotation differs widely.

The substance has a sweet taste. It is sensitive to alkaline oxidation agents and reduces Fehling's solution slowly at room temperature.

9. CONVERSION OF METHYL 2-KETO-D-GALACTONATE TO D-ASCORBIC ACID

Two grams of crystalline methyl 2-keto-D-galactonate was treated with sodium methylate and the product was worked up in the manner described for the preparation of L-ascorbic acid from methyl 2-keto-L-galactonate. The crude product weighed 1.1 g and had a specific rotation of −25°. After recrystallization from aqueous acetic acid, the substance had a melting point of 191° C. $[\alpha]_D^{20}=-23.8$ (water, c=3), in agreement with the specific rotation of D-ascorbic acid. Analysis: Calculated for $C_6H_8O_6$: C,40.9; H,4.6. Found: C,40.9; H,4.7.

The production of D-ascorbic acid from methyl 2-keto-D-galactonate corroborates the work of Regna and Caldwell [13].

III. ELECTRONIC INTERPRETATIONS

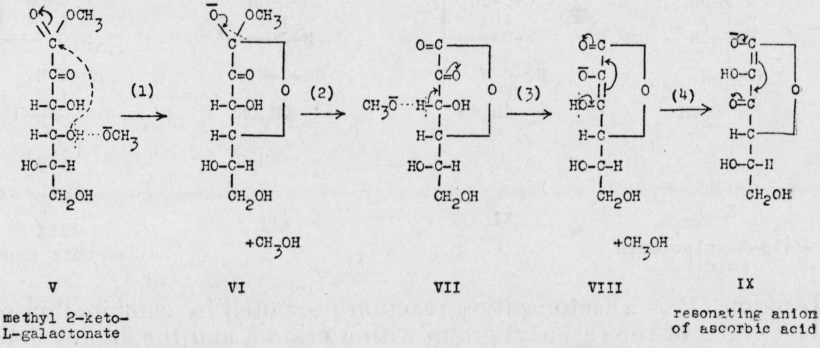
The principle of consecutive electron displacement (previously used by the author to account for numerous reactions in the carbohydrate field [21]) furnishes a satisfactory basis for the interpretation of many reactions related to the synthesis of ascorbic acid. The fundamental concept is, that the peculiar properties of systems involving double bonds may be explained by the migration of electrons from points of high electron density to points of lower electron density, frequently

with the addition and elimination of ions.[7] Nearly all of the reactions are induced by the formation of intermediate complexes with acid catalysts or with basic catalysts. In the equations that follow, dotted lines connecting two groups indicate the formation of intermediate complexes; dotted lines separating groups represent points of cleavage, and curved arrows indicate the displacement of electrons under the influence of the attacking agent. In reactions catalyzed by acids, the proton donor is represented somewhat arbitrarily as H^+.

1. LACTONIZATION AND ENOLIZATION OF METHYL 2-KETO-L-GALACTONATE BY SODIUM METHYLATE

The rearrangement of methyl 2-keto-L-galactonate to the anion of L-ascorbic acid is represented by formulas V to IX. Reaction (1) is promoted by combination of the hydrogen of the hydroxyl on carbon 4 with the methylate ion. This is followed by cleavage of methyl alcohol and the formation of an intermediate VI having an ortho acid structure. This hypothetical substance is similar to intermediates which have been postulated in the hydrolysis of esters (see p. 395 of

FORMATION OF ASCORBIC ACID FROM METHYL 2-KETO-L-GALACTONATE



[23]). Decomposition of the intermediate yields a ketonic lactone VII which gives ascorbic acid by enolization. Each mole of the ester V converted to sodium ascorbate requires 1 mole of sodium methylate. The methylate anion is converted to methyl alcohol, and the sodium ion combines with the anion of ascorbic acid. The formulas arbitrarily represent the lactonization reaction as preceding the enolization reaction. The changes may take place simultaneously, or even in the reverse order; in any case, substantially the same interpretation applies.

At first glance the arrangement of the groups about the double bond of ascorbic acid might be expected to give rise to a *cis* and a *trans* isomer. However, the *trans* isomer is not possible, because a *trans* arrangement of the hydroxyl groups about the double bond places carbons 1 and 4, also, in the *trans* position, and prevents ring closure. In some respects the ring of ascorbic acid is comparable to that of maleic anhydride, whereas a *trans* isomer would be comparable to an anhydride of fumaric acid, a structure incapable of existence. During the conversion of the methyl 2-keto-ester to ascorbic acid, the asymmetry of carbon 3 is

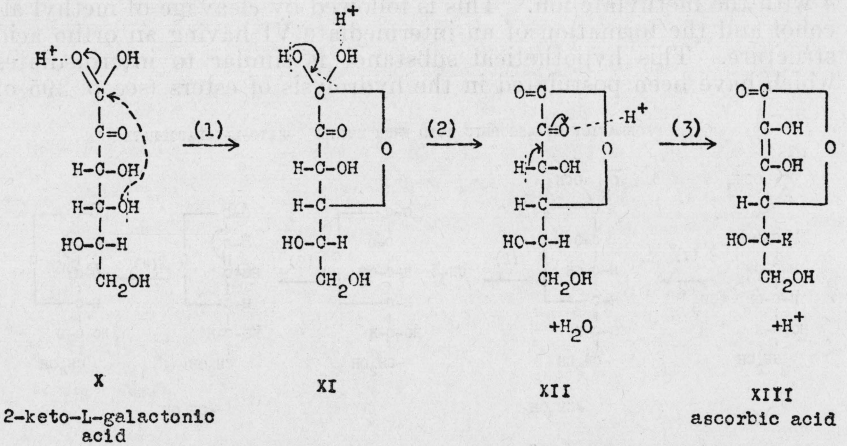
[7] General discussions of this concept may be found in [22, 23, 24, 25].

destroyed through the enolization reaction and the subsequent formation of a resonating structure. Hence substances which differ merely in the configuration of carbon 3 would be expected to yield the same product. The configurations of carbons 4 and 5, however, are retained, and the different configurations for these carbons give rise to four isomers, namely D- and L-ascorbic acid, and D- and L-isoascorbic acid.

2. LACTONIZATION AND ENOLIZATION OF 2-KETO-HEXONIC ACIDS BY THE ACTION OF ACID CATALYSTS

The lactonization and enolization of 2-keto-hexonic acids by treatment with aqueous acid [2, 26] may be represented by formulas X to XIII.

FORMATION OF ASCORBIC ACID FROM 2-KETO-L-GALACTONIC ACID



X
2-keto-L-galactonic acid

XI

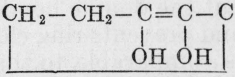
XII

XIII
ascorbic acid

Reaction (1) is a lactonization reaction promoted by combination of the oxygen of the carboxyl group with a proton, and the approach of the oxygen of carbon 4 to carbon 1; reaction (2) depicts a mechanism for the decomposition of the ortho acid intermediate. The electronic changes indicated in formula XI account for the instability of two hydroxyls attached to a single carbon.

3. FORMATION OF FURFURAL AND REDUCTIC ACID FROM PENTOSES, GALACTURONIC ACID, AND ASCORBIC ACID

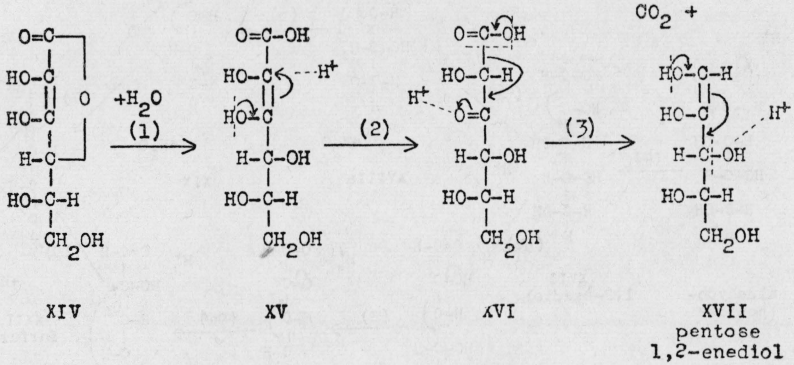
When ascorbic acid is heated with 12-percent hydrochloric acid, furfural is evolved [27]. When pentoses or hexuronic acids are similarly treated, in addition to furfural, small quantities of a strongly reducing, enolic acid are formed. The latter substance was named "reductinsäure" by Reichstein and Oppenauer [28] and was shown to be

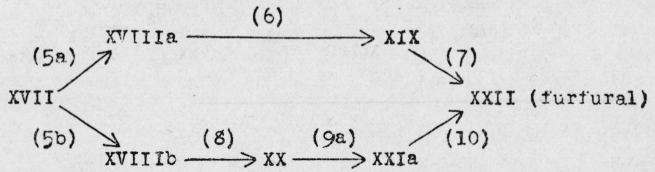


The name was translated in English to "reductic acid." The reactions for the formation of reductic acid and furfural appear to be related, and explicable by means of consecutive electron displacements.

The production of furfural from ascorbic acid is represented by the reactions of formulas XIV to XVII followed by the reactions given for the pentoses in formulas XVII to XXII. According to the hypothetical reactions, furfural is derived from both ascorbic acid and the pentoses through the intermediate production of a pentose 1, 2-enediol, XVII. In the case of the pentoses, the 1, 2-enediol yields reductic acid, presumably by reactions (5b), (8), (9b), (11), (12), (13), and (14). Inasmuch as the 1, 2-enediol appears to be formed from ascorbic acid, as well as from the pentoses, one might expect that a closer study of the reaction of ascorbic acid with aqueous acid would also reveal the formation of small amounts of reductic acid. The 1, 2-enediol of L-xylose is obtained from the beta ketonic acid, XVI, through the cleavage of carbon dioxide, reaction (3). The diagram accounts

FORMATION OF FURFURAL FROM ASCORBIC ACID

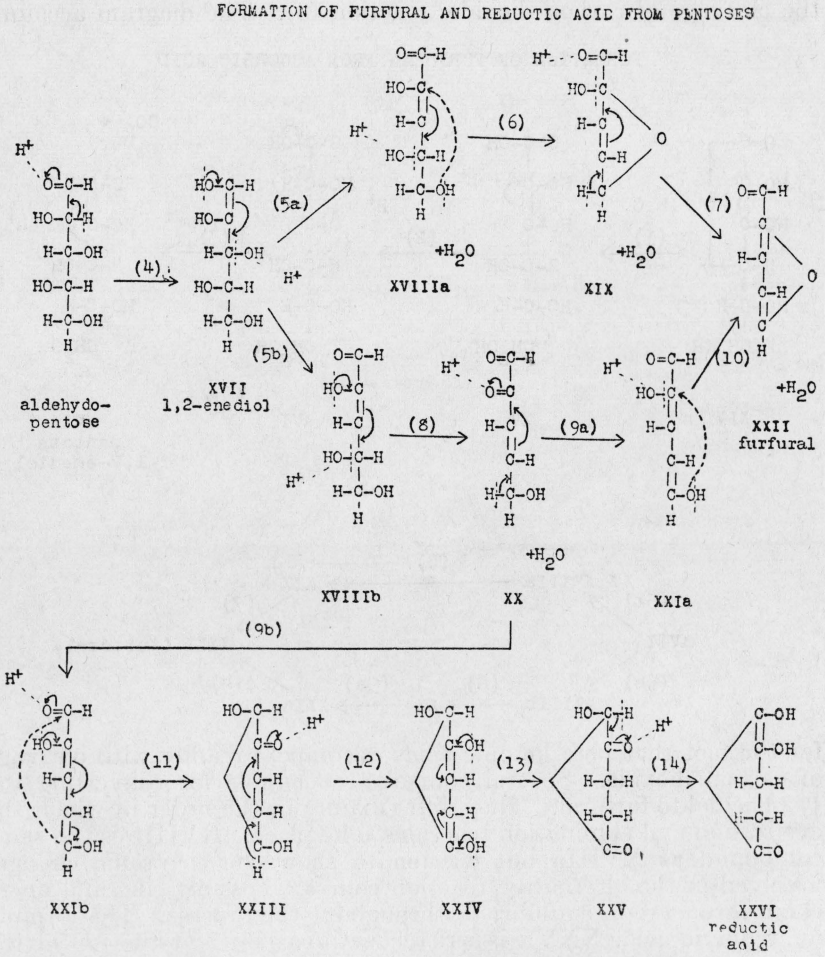




for the fact that beta ketonic acids decompose readily with cleavage of carbon dioxide. Several courses are possible for converting the 1, 2-enediol to furfural. They differ mainly in the order in which the cyclization and elimination reactions take place. XVIIIb is the same compound as XVIIIa but written to show the electronic changes involved in the alternative reaction course. Possibly ring and open-chain forms are in equilibrium throughout the process. The formula for reductic acid, XXVI, is written with carbon 2 at the top of the formula in order to show the relationship to the preceding intermediates. The substance contains an enediol group adjacent to a carbonyl group. Presumably, its anion has a resonating structure analogous to that of the anion of ascorbic acid, IX. The substance of formula XXI was previously suggested by Aso as a possible intermediate in the formation of reductic acid [29]. The mechanism for the forma-

tion of furfural is similar to that suggested by the present author for the formation of furfural from methyl pentoses [21]

The formation of furfural and reductic acid from galacturonic acid by treatment with aqueous acid is explained by the reactions of formulas XXVII to XXXIII. The elimination of carbon dioxide, reaction (18), is similar to the elimination of carbon dioxide from beta ketonic acids, mentioned in connection with formula XVI. According to the suggested mechanism, carbon dioxide is liberated, not from galacturonic acid with the formation of L-arabinose, as frequently assumed, but from an unsaturated intermediate obtained by enolization and de-enolization reactions. Reactions (16a), (17), and (18)

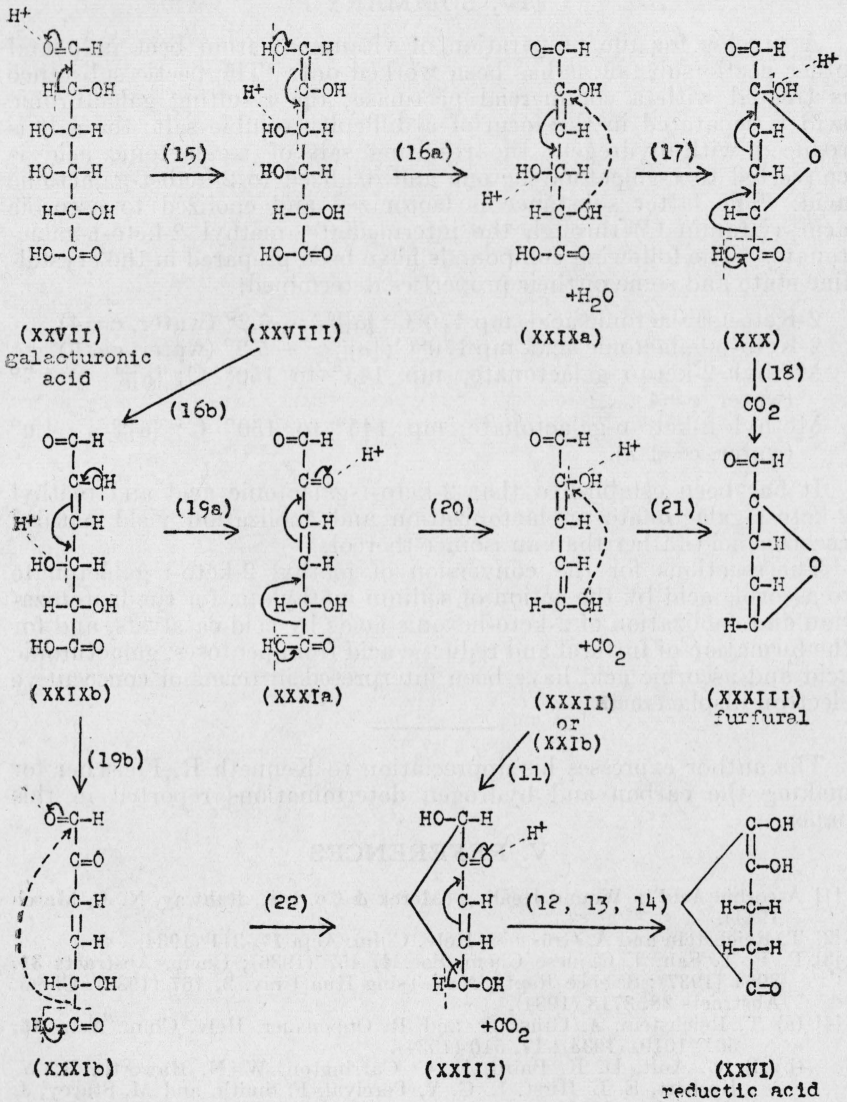


represent the cyclization reaction as preceding the elimination of carbon dioxide, whereas reactions (16b), (19a), (20), and (21) show an alternative course in which the cyclization reaction is last.

In the formation of reductic acid from galacturonic acid, two courses are shown for the conversion of XXXI to XXIII, a substance

which yields reductic acid by enolization and ketonization reactions. In one mechanism, the elimination of CO_2 and cyclization are represented as successive steps, reactions (20) and (11); in the other mechanism, elimination of CO_2 and cyclization occur in one step, reaction (22).

FORMATION OF FURFURAL AND REDUCTIC ACID FROM GALACTURONIC ACID



The possibility of two reaction mechanisms for the formation of reductic acid from galacturonic acid and only one for its formation from the pentoses may account for the higher yield of this substance from galacturonic acid. Supposedly, in both cases, the formation of reductic acid is promoted by combination of the acid catalyst with the

carbonyl group of carbon 1. This leaves a low electron density at carbon 1, a condition which is rectified by a shift of electrons from carbon 5, obtained in the case of XXIb by ketonization of the enolic group at carbon 2 and in the case of XXXIb by elimination of the carboxyl group as carbon dioxide.

IV. SUMMARY

A process for the preparation of vitamin C from beet pulp and other pectic substances has been worked out. The pectic substance is treated with a commercial pectinase; the resulting galacturonic acid is separated in the form of a difficulty soluble salt; the salt is reduced with hydrogen; the resulting salt of L-galactonic acid is converted to L-galactono-lactone and oxidized to 2-keto-L-galactonic acid. The latter substance is lactonized and enolized to ascorbic acid (vitamin C) through the intermediate, methyl 2-keto-L-galactonate. The following compounds have been prepared in the crystalline state and some of their properties determined:

2-Keto-L-galactonic acid, mp 170°C; $[\alpha]_D^{20}=+5.2°$ (water, c=4).
2-Keto-D-galactonic acid, mp 170°C; $[\alpha]_D^{20}=-5.2°$ (water, c=5).
Methyl 2-keto-L-galactonate, mp 145° to 150° C; $[\alpha]_D^{20}=+4.7°$ (water, c=4).
Methyl 2-keto-D-galactonate, mp 145° to 150° C; $[\alpha]_D^{20}=-4.6°$ (water, c=1.7).

It has been established that 2-keto-L-galactonic acid and methyl 2-keto-L-galactonate on lactonization and enolization yield natural ascorbic acid rather than an isomer thereof.

The reactions for the conversion of methyl 2-keto-L-galactonate to ascorbic acid by the action of sodium methylate, for the lactonization and enolization of 2-keto-hexonic acids by acid catalysts, and for the formation of furfural and reductic acid from pentoses, galacturonic acid and ascorbic acid have been interpreted in terms of consecutive electron displacement.

The author expresses his appreciation to Kenneth R. Fleischer for making the carbon and hydrogen determinations reported in this paper.

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