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THE ACTION OF WEAK MINERAL ACIDS ON URONIC ACIDS[1,2]

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Introduction

When either *d*-glucuronic or *d*-galacturonic acid is heated with 12.0% hydrochloric acid, decomposition follows with the liberation of carbon dioxide, furfuraldehyde and water, according to the equation

$$C_6H_{10}O_7 = C_5H_4O_2 + 3H_2O + CO_2$$

Conjugated uronic acids, the so-called polyuronides found in pectins, gums, alginic acids, the specific polysaccharide substances of certain microorganisms, and other plant materials also yield carbon dioxide when heated with 12.0% hydrochloric acid. The carbon dioxide liberated according to the above reaction serves as a basis for the accurate determination of these substances by any one of several methods.[3] Before it had been established with certainty that the plant gums contain uronic acids, Zeisel and Konschegg reported that gum acids not only yield large quantities of carbon dioxide when heated with 12.0% hydrochloric acid, but that they also produce considerable amounts of carbon dioxide when the acid is more dilute. This observation of Zeisel and Konschegg was originally published in the first edition of Wiesner, "Die Rohstoffe des Pflanzenreichs," Vol. III, p. 87. Reference is again made to this obscure observation in the latest edition of Wiesner.[4] Apparently they did not publish this observation in a chemical journal; as a result this important fact has been overlooked by many workers even after it had been demonstrated with certainty that plant gums contain uronic acids.[5] Con-

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

[2] Part of this research was done by one of us (K. P. L.) during 1925–1927 while a Fellow of the International Education Board, posted at the time at the University of Zürich, Switzerland. The author wishes to acknowledge his indebtedness to Professor Paul Karrer, Director of the Institute of Chemistry, University of Zürich, for the privilege of extending the research while a student in his laboratory, and for the kindly advice and help received. To the Board of Directors of the International Education Board, New York City, the author wishes to extend his thanks for the Fellowship grant which enabled him to work in the laboratory mentioned.

[3] (a) Tollens and Lefèvre, *Ber.*, **40**, 4513 (1907); also (b) Dickson, Otterson and Link, THIS JOURNAL, **52**, 775 (1930), where reference is made to the various methods in use for the determination of uronic acids.

[4] Wiesner, "Die Rohstoffe des Pflanzenreichs," Engelmann, Leipzig, **1927**, Vol. I, 4th ed., p. 974.

[5] For early literature on this subject see Wiesner "Die Rohstoffe des Pflanzenreichs," and Czapek, "Biochemie der Pflanzen," 3d ed. Vol. I, p. 673, Gustav Fischer, Jena, **1922**.

sequently the many articles which have appeared on uronic acids and polyuronides up to within very recent times reveal the very significant fact that whenever the experimental procedure involves a prolonged weak mineral acid hydrolysis, the subsequent yields of the free acids obtained are invariably either very small or nil.

In general polyuronide substances are hydrolyzed only very slowly by weak mineral acids, consequently there is ample opportunity for the destruction of the free acids liberated during the hydrolysis. Some of the many papers wherein the destructive action of weak mineral acids has caused experimental difficulties, whose actual sources were not recognized, have been cited in an article by one of us in THIS JOURNAL.[3b]

Although most investigators have not been cognizant of the fact that uronic acids are destroyed by weak mineral acids, recently some investigators have reported their observations on this subject. Thus Heidelberger and Goebel in a study of the aldobionic acid obtained from the specific polysaccharide of Type III Pneumococcus stated that when the aldobionic acid was hydrolyzed in 1 *N* sulfuric acid for twenty hours, half of the molecule (the sugar acid) was largely destroyed.[6] Butler and Cretcher in a study of the composition of gum arabic obtained an aldobionic acid which on hydrolysis with 5% sulfuric acid yielded *d*-galactose. However, the acidic fraction was present in too small amounts to be identified. This was due to the fact that under the conditions of the hydrolysis the uronic acid was largely destroyed. Control experiments indicated that the aldobionic acid was decomposed to the extent of about 44%.[7] Heidelberger and Kendall[8] obtained an aldobionic acid from gum arabic which they oxidized to the dicarboxylic acid. When this acid was hydrolyzed in 1 *N* sulfuric acid for eighteen hours, 28.0% of the glucuronic acid formed was decarboxylated. In their study on the alginic acid from *Macrocystis Pyrifera*, Nelson and Cretcher[9] state, "the hydrolysis of alginic acid with dilute sulfuric acid of various concentrations was attempted but soon abandoned because of the simultaneous loss of carbon dioxide." Several years ago, one of us (K. P. L.) devised a method for the preparation of galacturonic acid from lemon pectic acid, and glucuronic acid from cherry gum. While engaged on this study it was necessary to determine what strength of mineral acid would effect a maximum hydrolysis of the source material and at the same time produce a minimum destruction of the liberated uronic acid. At first many experiments were conducted with various concentrations of mineral acids below 1.0%. It was found that the source material hydrolyzed so slowly that most of the liberated uronic

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[6] Heidelberger and Goebel, *J. Biol. Chem.*, **70**, 613 (1926); *ibid.*, **74**, 613 (1927).

[7] Butler and Cretcher, THIS JOURNAL, **51**, 1519 (1929).

[8] Heidelberger and Kendall, *J. Biol. Chem.*, **84**, 641 (1929).

[9] Nelson and Cretcher, THIS JOURNAL, **51**, 1914 (1929).

acid was destroyed as rapidly as it was formed. It was found necessary to use higher concentrations of the mineral acids to hydrolyze the material effectively, thereby causing the ratio of uronic acid liberated to the uronic acid destroyed to approach an optimum value.

In this paper we present the results obtained by the action of mineral acids of concentrations in common use in the hydrolysis of pectins, hemicellulose, gums, gum acids and other plant substances. The effect of various dilute concentrations of hydrochloric acid and sulfuric acid were ascertained on the following substances; (a) crystalline *d*-glucuronic and *d*-galacturonic acid, (b) gum arabic and a gum arabic acid both of which contain a polyglucuronide complex, (c) lemon pectin acid which contains a polygalacturonide, (d) an aldobionic acid from cherry gum wherein one half of the molecule is a uronic acid

Experimentation

The experiments were conducted on the various substances following the procedure recently described.[3b] The heating was conducted for the prescribed periods at a bath temperature of 135–140°, which was found to keep the solution in the reaction flask at constant ebullition, provided boiling chips were used. The hydrolysis was terminated by removing the electric heater and oil-bath. The aspiration of carbon dioxide-free air through the apparatus was continued for an additional thirty minutes so as to remove the carbon dioxide remaining in the reaction flask. After this had been accomplished, the amount of barium hydroxide utilized during the run was determined in the usual manner. All of the experiments reported below represent the average of duplicate analyses that agreed to within 0.50%.

The Decarboxylation of *d*-Galacturonic Acid under Various Conditions.—Pure *d*-galacturonic acid, m. p. 159°, $[\alpha]_D^{20}$ +53.40 in water, prepared from lemon pectic acid by a method soon to be published was used in these experiments.

Action of 2.5% Sulfuric Acid.[10]—Three-tenths g. heated for two hours liberated 0.0088 g. of carbon dioxide which is equivalent to a destruction of 12.9%; 0.30 g. heated for four hours liberated 0.0169 g. of carbon dioxide, which represents a destruction of 24.75%.

Action of 1 *N* Sulfuric Acid.[11]—Three-tenths g. heated for fifteen hours liberated 0.0396 g. of carbon dioxide, equivalent to a destruction of 58.10%.

Action of 2.0% Hydrochloric Acid.[12]—Three-tenths g. heated for one and one-half

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[10] Two and one-half per cent. sulfuric acid is frequently used in the hydrolysis of hemicelluloses, the time interval varying from two to four hours.

[11] 1 *N* sulfuric acid is used most extensively in the hydrolysis of gum acids, the time interval being about fifteen hours.

[12] Whenever hydrochloric acid is used in the hydrolysis of hemicellulose the time of the hydrolysis and the concentration of the acid are usually less than when sulfuric acid is employed. The hydrolytic action of dilute hydrochloric acid is more drastic than that of weak sulfuric acid. Consequently in the hydrolysis of hemicellulose both the concentration and the length of the hydrolysis are decreased.

hours liberated 0.0094 g. of carbon dioxide, equivalent to a destruction of 13.65%; 0.30 g. heated for three hours liberated 0.0197 g. of carbon dioxide, equivalent to a destruction of 29.30%.

The Decarboxylation of *d*-Glucuronic Acid under Various Conditions.—Pure *d*-glucuronic acid, m. p. 156°, $[\alpha]_D^{20}$ +34.0 in water, prepared from gum arabic was used in these experiments.

Action of 2.5% Sulfuric Acid.—One-quarter g. heated for two hours liberated 0.0056 g. of carbon dioxide, equivalent to a destruction of 9.66%; 0.25 g. heated for four hours liberated 0.0096 g. of carbon dioxide, equivalent to a destruction of 18.20%.

Action of 1 *N* Sulfuric Acid.—One-quarter g. heated for two hours liberated 0.0078 g. of carbon dioxide, equivalent to a destruction of 13.72%; 0.25 g. heated for three hours liberated 0.0110 g. of carbon dioxide, equivalent to a destruction of 19.40%; 0.25 g. heated for four hours liberated 0.0146 g. of carbon dioxide, equivalent to a destruction of 24.60%; 0.25 g. heated for fifteen hours liberated 0.0326 g. of carbon dioxide, equivalent to a destruction of 57.20%.

Action of 2.0% Hydrochloric Acid.—Three-tenths g. heated for one and one-half hours liberated 0.0091 g. of carbon dioxide, equivalent to a destruction of 13.35%; 0.30 g. heated for three hours liberated 0.0197 g. of carbon dioxide, equivalent to a destruction of 28.80%.

The Destruction of *d*-Glucuronic Acid in the Course of Its Preparation from Gum Arabic.—Weinmann[13] has recently published the most successful method for the preparation of *d*-glucuronic acid from a naturally occurring plant substance. One gram of gum arabic with a uronic acid content of 17.60% was hydrolyzed in 2.0% hydrochloric acid for one and one-half hours. During this time 0.0048 g. of carbon dioxide was liberated, equivalent to a destruction of 2.11% of the total gum or 12.0% of the total quantity of glucuronic acid present in the original gum.

One gram of gum acid, prepared from the gum arabic employed above according to Weinmann's method (uronic acid content of the gum acid 27.70%) was hydrolyzed in 1 *N* sulfuric acid for fifteen hours, whereupon 0.0349 g. of carbon dioxide was liberated, equivalent to a destruction of 15.28% of the gum acid or 55.05% of the total quantity of glucuronic acid present in the gum acid.[14]

The Destruction of *d*-Galacturonic Acid in the Course of Its Preparation from Lemon Pectic Acid.—One g. of lemon pectic acid (galacturonic acid content 88.0%) was hydrolyzed for fifteen hours with 2.0% sulfuric acid; in the course of the above reaction 0.0677 g. of carbon dioxide was liberated, equivalent to a destruction of 27.80% of the pectic acid molecule or 33.85% of the total amount of the galacturonic acid present.

The Destruction of the Uronic Acid Residue of an Aldobionic Acid in 1 *N* Sulfuric Acid.—The barium salt of an aldobionic acid isolated from cherry gum was used in this experiment. The free acid has not been obtained in a crystalline condition, although the barium salt is apparently pure, since it liberates one molecular equivalent of carbon dioxide when heated with 12.0% hydrochloric acid and has the correct barium content; 0.5958 g. of the barium salt equivalent to 0.50 g. of the free acid was hydrolyzed for fifteen hours, whereupon 0.0330 g. of carbon dioxide was liberated, equivalent to a destruction of 53.00%, since complete decarboxylation gave 0.0627 g. of carbon dioxide.

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[13] Weinmann, *Ber.*, **62**, 1637 (1929).

[14] The yield of *d*-glucuronic acid from gum arabic by Weinmann's method is approximately 50 g. of the acid from 1 kilo of the gum. Calculated on the basis of the uronic acid content of the gum this is equivalent to 28.50% of the total amount of glucuronic acid theoretically available. Accounting for the inevitable hydrolytic destruction, the maximum yield obtainable is 40% of the uronic acid present in the gum.

Discussion

In view of the fact that free uronic acids are destroyed by weak mineral acids, an inevitable destruction takes place when they are prepared from their parent sources, *i. e.*, gum arabic, gum acids, pectic acids, aldobionic acids (of the type recently obtained from gums) and various microörganisms. Polyuronide substances are in general quite resistant to the action of weak mineral acids. Consequently, when the free uronic acids are sought, the hydrolytic procedure should be directed toward the attainment of a rapid hydrolysis of the parent substance in order to minimize the destruction of the free acid. The general practice of using 1 *N* sulfuric acid in preference to 1 *N* hydrochloric acid when a prolonged hydrolytic procedure is involved is to be recommended.

The following factors enter into a consideration of the destruction of uronic acids by weak mineral acids; the strength of the acid, the temperature, the concentration of the uronic acid in the hydrolytic medium and the length of exposure of the free acids to the hydrolytic agent. Concentrations of hydrochloric or sulfuric acid above 5.0% are so effective in their decarboxylating action that the employment of these acids in the hydrolysis of polyuronides is not to be recommended. The concentration of the mineral acid employed should be less than 5%, but more than 2%, since the hydrolysis of most polyuronides proceeds too slowly in concentrations of 2.0% or less with the result that the free uronic acid is destroyed almost as rapidly as it is liberated. At temperatures below 100° the destructive action of weak mineral acids upon uronic acids decreases rapidly (unpublished data). However, it is difficult to effect the hydrolysis of the polyuronide substances at these temperatures. Many experiments were conducted in an attempt to hydrolyze polyuronides below 100°. However, the yields of the free acid were so small that temperatures below 100° proved to be impracticable. For this reason we have not presented the experimental results attained with various concentrations of weak mineral acids at temperatures ranging from 65–100°.

The rate of decarboxylation of a uronic acid by the action of weak mineral acids also varies with the concentration of the uronic acid present. Thus in the case of the decarboxylation of *d*-glucuronic acid in 1 *N* sulfuric acid, 13.72% of an original sample of 0.25 g. in 100 cc. was destroyed in two hours and 24.60% destroyed at the end of four hours. From the rate of decarboxylation during the first four hours, complete decarboxylation of the sample should have been effected in the course of approximately sixteen hours. However, at the end of fifteen hours only 57.20% had been destroyed, based upon the amount of carbon dioxide liberated.

We have observed that reversion products of the uronic acids are readily formed in weak mineral acid solutions. The higher the initial concentration of the uronic acids, the greater and the more rapid is the formation of the reversion products. These reversion products are quite stable toward weak acids, for in an hydrolysis conducted with 1 *N* sulfuric acid for over one hundred hours, all of the carbon dioxide theoretically obtainable from a sample of *d*-galacturonic acid had not been liberated, although free *d*-galacturonic acid was no longer present. The formation of these reversion products helps to explain the fact that the decarboxylation of a given amount of uronic acid in any weak mineral acid is not a linear function referred to time. In addition, the action of weak mineral acids does not appear to proceed quantitatively according to the equation

$$C_6H_{10}O_7 = C_5H_0O_2 + 3H_2O + CO_2$$

as is the case when the concentration of the acid is 12.0%. Apparently some side reactions occur, as the quantity of furfural produced is less than the amount expected on the basis of the carbon dioxide produced. A substance is produced in the course of the reaction which is not furfuraldehyde, but it reduces Fehling's solution readily at room temperature, and gives the various tests for pentose sugars. The nature of this substance is being studied for it appears to be not without significance since it seems to be a furan derivative.

It has been postulated repeatedly by DeChalmont,[15] Spoehr,[16] Haworth,[17] Ehrlich[18] and others that the pentose sugars arise from uronic acids by direct decarboxylation. Thus xylose would be formed from glucose, and arabinose from galactose. The formation of the aforementioned furan product by the action of dilute mineral acids is indicative of the fact that uronic acids can apparently be decarboxylated *in vitro* without the reaction proceeding to the furfuraldehyde stage. When furfuraldehyde is formed from a uronic acid by the action of 12% hydrochloric acid, it is assumed that a pentose sugar is intermediate in this reaction. According to Nelson and Cretcher[9] the pentoses reported by Kylin, Hoagland and Lieb (*loc. cit.*) to be present in the alginic acid from *Macrocystis pyrifera* might be regarded as having been formed by decarboxylation of the uronic acid that comprises this alginic acid.

In concluding, it should be stated that the methods now in use in plant physiological and plant biochemical studies for the determination of pectin substances and hemicelluloses are inaccurate due to the error introduced through the destruction of the uronic acids that comprise part of the molecule of these substances. This is particularly the case in the estimation of the hemicelluloses, which is accomplished by determining the total reducing power after an acid hydrolysis, which is usually done

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[15] DeChalmont, *Am. Chem. J.*, **16**, 610 (1893); *Ber.*, **27**, 2722 (1894).

[16] Spoehr, *Carnegie Inst. Pub.*, No. 387, 42, 75 (1917).

[17] Haworth, *J. Soc. Chem. Ind.*, **28**, 295T (1927); *Helv. Chim. Acta*, **11**, 534 (1928).

[18] Ehrlich and Schubert, *Biochem. Z.*, **203**, 343 (1929).

with 2.5–5% hydrochloric or sulfuric acid.[19] It has been shown by O'Dwyer,[20] Schmidt[21] and co-workers, Schwalbe and Feldtmann[22] and others, that hemicelluloses contain uronic acids. A hemicellulose preparation from the maize plant has been obtained that contains *d*-glucuronic acid.[23]

The authors wish to acknowledge their indebtedness to Dr. Allan Dickson for assistance rendered during the course of this work.

Summary

d-Glucuronic and *d*-galacturonic acids are decarboxylated when heated with either hydrochloric or sulfuric acid of concentrations below 5.0%. In the course of the preparation of these acids from their parent polyuronide substances by hydrolytic procedures, an inevitable destruction occurs. This destruction accounts for the low yields that are usually obtained in the course of the preparation of the uronic acids from their natural source materials, and also contributes to an error in the course of the determination of hemicellulose and pectin in plant physiological studies. Besides carbon dioxide, furfuraldehyde and some reversion products, the action of dilute mineral acids produces a furan derivative whose nature has not been determined.

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ROTENONE. V. THE IDENTITY OF ISOTUBAIC AND ROTENIC ACIDS

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When an alcoholic potassium hydroxide solution of rotenone is refluxed for several hours, an acid is obtained in a yield of about 5% which represents approximately half of the rotenone molecule and which is known as tubaic acid. Kariyone, Kimura and Kondo[1] have established the empirical formula $C_{12}H_{12}O_4$ for tubaic acid and this formula has been confirmed by Takei.[2]

Tubaic acid possesses the properties of a phenolcarboxylic acid. It shows an acid reaction to litmus and requires 1 mole of alkali for its neu-

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[19] Report of Committee on Methods, *Plant Physiology*, **2**, 93–95 (1927).

[20] O'Dwyer, *Biochem. J.*, **17**, 501 (1923); **19**, 694 (1925); **20**, 656 (1926).

[21] Schmidt, Meinel and Zintl, *Ber.*, **60**, 503 (1927).

[22] Schwalbe and Feldtmann, *ibid.*, **58**, 1534 (1925).

[23] Unpublished data of the senior author to which reference has been made in This Journal, **51**, 2506 (1929).

[1] Kariyone, Kimura and Kondo, *J. Pharm. Soc.* (Japan), No. 514, 1094 (1924); *ibid.*, No. **518**, 377 (1925).

[2] Takei, *Ber.*, **61**, 1003 (1928).