THE PREPARATION OF d-GALACTURONIC ACID FROM LEMON PECTIC ACID.*

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INTRODUCTION.

A noteworthy advance in the knowledge of the constitution of the pectic compounds was afforded through the excellent researches of Ehrlich and his coworkers (1), who discovered that d-galacturonic acid, an isomer of d-glucuronic acid, was a constituent of all pectic substances so far examined. Later researches showed that the pectic acid obtainable from sugar beet pectin (2) can be regarded as a triacetyl-arabino-galacto-dimethoxy-tetragalacturonic acid, wherein the galacturonic acid comprises approximately 68 per cent of the molecule. Essentially the same structure was deduced for the pectic acid from flax pectin (3) which is made up of 61 per cent of galacturonic acid, and the pectic acid from orange peelings which is composed of 73.7 per cent of galacturonic acid.

In 1924, the senior writer had occasion to require considerable quantities of galacturonic acid for biochemical studies conducted in conjunction with Dr. James G. Dickson of the Department of Plant Pathology. After considerable experimentation with the various commercial preparations obtainable and several prepared in the laboratory from sugar beet pulp and apple pumice, it was found that a lemon pectin preparation from the California Fruit Growers Exchange Laboratory afforded the most economical and convenient starting material. In view of the fact that the study of the chemistry of the pectin constituents (4) has recently attained a rather preeminent position in organic and plant biochemical

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Researches,¹ which have created a considerable demand for both galacturonic and glucuronic acid, it is deemed advisable to publish the method of isolating galacturonic acid which has been used with remarkable success in our laboratory.

The lemon pectin preparation obtainable from the California Fruit Growers Exchange, a grayish white amorphous powder completely soluble in hot water, is prepared by extracting the lemon pulp with citric acid at a temperature of about 90°. After concentrating, the pectin is precipitated with alcohol, the precipitate pressed and washed with alcohol, again pressed, and finally dried. The amount of impurity present is very small. This pectin preparation is perhaps the purest commercial preparation obtainable. It is supplied at such a nominal cost that the lengthy and tedious process of preparing the pectin on a large scale in the laboratory after the procedure of Ehrlich (2) proves to be a very expensive and time-consuming process.

The lemon pectin preparation which we have used is in reality lemon pectic acid after the nomenclature of Ehrlich (1). It contains approximately 80 per cent of galacturonic anhydride. It is therefore 8 to 10 per cent higher in this constituent than the pectic acids obtained by Ehrlich from other sources. This difference is readily accounted for through the researches of Ehrlich and Kasaury ((5) p. 174, 175). Apparently in the lemon pulp, either by the action of organic acids or through enzymatic action a partial hydrolysis of the complex pectin molecule proceeds before the tissue is killed. This results in the removal of some of the carbohydrate constituents (arabinose and galactose). Or in the process of isolating the pectic acid from the pulp by means of citric acid at 90°, a partial removal of the readily hydrolyzable carbohydrate constituents is brought about. The complex galac-

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turonic acid ester however is so stable that it is unaffected in either one of the processes mentioned. For the preparation of galacturonic acid from lemon pectic acid the high galacturonic acid anhydride content is obviously advantageous since thereby the yield of the free galacturonic acid is appreciably increased.

A survey of the work done in the last decade on the polyuronic acid anhydrides and also the recent work on the aldobionic acids, reveals a significant fact with reference to the properties of the free uronic acids. Whenever the experimental procedure used in the isolation of a uronic acid involves the use of weak mineral acids as the hydrolytic agent, the actual yield of the specific uronic acid isolated is invariably extremely low, in most cases less than 30 per cent of the theoretical quantity obtainable (calculated on the basis of the uronic acid content of the source material).

The complex polyuronides are quite stable towards weak mineral acids, and hydrolyze very slowly. The reverse is the case with the free uronic acids. They are readily decarboxylated by weak mineral acids (2 to 5 per cent strength) resulting in the formation of carbon dioxide, furfuraldehyde, and furan substances. We have found that it is the instability of the freshly liberated uronic acids in weak mineral acid solution that is primarily responsible for the low yields obtained. In this paper we have accounted for the quantity of galacturonic acid destroyed under our experimental conditions. In a forthcoming paper we shall publish detailed results on the stability of $d$-galacturonic acid and $d$-glucuronic acid in weak mineral acid solutions.

The method of preparing $d$-galacturonic acid through its barium salt from lemon pectic acid, which is outlined in detail below, surpasses in expediency and efficiency all methods hitherto published by Ehrlich and other workers, wherein various other fruit and plant tissues serve as the starting material (6). Incidentally, this appears to be the first time that $d$-galacturonic acid is reported to be the basic constituent of lemon pectin, thereby confirming Ehrlich’s view that galacturonic acid is the fundamental constituent of all pectic substances. It should however be stated, that Suarez (7) was the first to isolate $d$-galacturonic acid from lemon pulp, in the form of its barium salt.
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EXPERIMENTAL.

Preparation of the Barium Salt of d-Galacturonic Acid.

The hydrolysis of the pectin is accomplished in 2.5 per cent sulfuric acid (using 15 gm. of the dried pectin in a liter of the acid) by boiling under a reflux condenser for 15 hours. The acid is neutralized with a suspension of barium carbonate in water while the contents of the flask are maintained at 90° by heating on a water bath. After the neutralization is completed the somewhat darkened solution is cleared with blood charcoal by heating for an additional 15 minutes. The barium salts and the charcoal are removed by filtering through a Buchner funnel and washed with hot water. A second clarification is usually necessary and is performed by heating the solution for 15 minutes with more charcoal and some infusorial earth. The solution is then filtered through a Buchner funnel containing a mat of paper pulp on a hardened filter paper. Thereby the fine charcoal which would later be precipitated with the barium galacturonate is completely removed. The solution is then concentrated to 0.1 of its original volume at 50° under 15 mm. pressure. After removing any flocculent matter present, the solution is cooled and poured into 4 volumes of 95 per cent alcohol. This procedure is accompanied with vigorous stirring to prevent the barium galacturonate from settling out in large masses. The salt is filtered off and washed with hot 95 per cent alcohol and ether and is then redissolved in 100 cc. of hot water. It is advisable to clarify the aqueous solution again with a small portion of infusorial earth before reprecipitating it with alcohol, for the purer the barium salt of galacturonic acid is, the more slowly it precipitates from an alcoholic solution. After the second precipitation the barium galacturonate is partially dehydrated by suspending first in 95 per cent and then in 99 per cent alcohol. The alcohol is in turn replaced with ether. The ether is then removed by spreading the salt on a porous plate in a vacuum desiccator over calcium chloride. After the preliminary drying mentioned above the barium galacturonate is completely dehydrated at 80° in a vacuum tube or oven containing P₂O₅. The salt should emerge from this drying process as a snow-white amorphous powder.

A barium determination made by directly ashing a small quan-
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quantity in a crucible with concentrated sulfuric acid serves as a reliable criterion of the purity of the salt. Preparations with a barium content of 26 to 27 per cent are readily obtained. Theory demands for \((C_6H_8O_7)_2\) Ba. 26.3 per cent barium. The yield of the barium salt from 80 gm. of pectin with an initial uronic anhydride content of 80 per cent is approximately 40 gm. This is equivalent to 42.6 per cent of the theoretical quantity obtainable. Approximately 32 per cent of the theoretical amount obtainable as the barium salt is destroyed in the course of the 15 hour hydrolysis by the 2.5 per cent sulfuric acid, a loss that cannot be minimized.\(^2\) The remaining 27 per cent theoretically obtainable as the barium salt is lost in the processes of purification and through the formation of reversion products. When uronic acids are heated in weak mineral acids some condensation and reversion products are formed which in turn are not rehydrolyzed to the free acid.

**Conversion of Barium Galacturonate into \(d\)-Galacturonic Acid.**

10 gm. of the dried barium salt are dissolved in 400 cc. of water and decomposed with 0.1 N sulfuric acid, slightly less acid than is necessary to decompose completely all of the salt being used. It is necessary to prevent an excess of acidity due to sulfuric acid for the production of reversion products and lactone formation is accelerated in acid solution. The decomposition is best brought about by admitting the 0.1 N sulfuric acid from a burette while the solution containing the barium salt is being agitated with a mechanical stirrer. After the acid has been added the solution is

\(^2\) This loss was quantitatively ascertained by the following control experiment. Two 1.0 gm. samples of the pectin were boiled for 15 hours in 2.5 per cent sulfuric acid in the apparatus recently described by us for the determination of uronic acids (see Dickson, A. D., Otterson, H., and Link, K. P., *J. Am. Chem. Soc.*, 52, 775 (1930)). The quantity of carbon dioxide liberated was 0.0673 gm. and 0.0682 gm. respectively, which is equivalent to 6.73 and 6.82 per cent of carbon dioxide. The carbon dioxide values multiplied by the factor 4.4 for the conversion into uronic acid give 29.6 and 30 per cent of galacturonic acid. Calculated on the basis of the actual amount of galacturonic acid that a pectin sample containing 80 per cent of galacturonic anhydride would yield, if the hydrolysis were quantitative, a destruction of 29.6 and 30 per cent of the pectin molecule is equivalent to a destruction of 33.6 and 34.1 per cent of the total amount of galacturonic acid initially present. (We wish to thank Mr. Carl Niemann for conducting the control experiments.)
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warmed to 35° and maintained at this temperature for 30 minutes to
accelerate the settling of the barium sulfate in a form to permit
filtering. The barium sulfate is removed and the solution care-
fully concentrated at 40° under reduced pressure to a syrup of the
consistency of glycerol. The thin syrup is poured into sufficient
95 per cent alcohol to insure a final concentration of above 90 per
cent after dilution by the water in the syrup. The undecomposed
barium galacturonate will fall out as a flocculent precipitate, at the
same time any inorganic barium will also be precipitated. After
these foreign substances are removed, the alcoholic solution is
concentrated to a syrup which is taken up in hot 92 per cent
alcohol, filtered from any flocculent material that exists, and
decolorized with a small amount of blood charcoal and a pinch of
infusorial earth. The alcoholic solution is then finally concen-
trated to a syrup which will still permit an easy transference to a
round bottom crystallizing dish. By keeping the dish in a desic-
cator for 24 hours over calcium chloride, crystallization usually
begins after the addition of a few cc. of absolute alcohol and is
complete after 24 hours. The crystals are filtered, washed with
95 per cent alcohol, then with absolute alcohol, and finally with
ether. The drying should be conducted at room temperature
over P_2O_5 in a vacuum desiccator or vacuum tube. *Heating
discolor the free acid and eventually destroys it.* The galacturonic
acid crystallizes in microscopic needles with a melting point of
159-160°. The acid usually becomes yellow at 115°, red at
about 135°, dark brown above 150°, and melts at 159° with froth-
ing and decomposition. The optical rotation in water after 24
hours is +53.59°.

The carboxyl group of galacturonic acid can be directly titrated
with phenolphthalein as the indicator. The neutralization value
can therefore be used as an accurate criterion of purity. The
direct neutralization of 0.5560 gm. of d-galacturonic acid required
26.10 cc. of 0.1 N NaOH. Upon this basis 1.0 gm. would require
46.95 cc. of 0.1 N NaOH, whereas theoretically 1.0 gm. of C_9H_11O_6-
(COOH) requires 47.20 cc. of 0.1 N NaOH.

The yield of pure d-galacturonic acid readily obtainable from
10.0 gm. of the barium salt is approximately 2.21 gm., equivalent to
30.5 per cent (theoretical from 10.0 gm. of barium salt is 7.44).
By recrystallizing the mother liquors it is possible to increase the
yield to 50 per cent.
A convenient source material for the preparation of crystalline $d$-galacturonic acid is lemon pectic acid which can be obtained commercially. The pectic acid is hydrolyzed in 2.5 per cent sulfuric acid and the galacturonic acid first obtained as its barium salt. The barium galacturonate is converted into the free galacturonic acid by decomposition with 0.1 N sulfuric acid. Precise directions for the preparation of both compounds are given.

In conclusion the writers wish to express their gratitude to Mr. W. E. Baier and Mr. C. O. Wilson of the Research Department of the California Fruit Growers Exchange, Ontario, California, who kindly furnished the lemon pectin preparations used in this research.

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