[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ARIZONA]

THE COMPOSITION OF AN ALDOBIONIC ACID FROM FLAXSEED MUCILAGE

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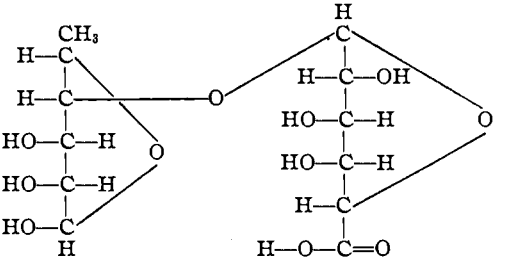
In 1903 Hilger[1] hydrolyzed flaxseed mucilage and found in the hydrolytic products the sugars *d*-glucose, *d*-galactose, *l*-xylose and *l*-arabinose, together with an acid by-product. In 1913 Neville[2] verified the work of Hilger on this mucilage. Abderhalden[3] states that this mucilage yields in addition to sugars, an acid complex which contains pentoses and hexoses.

The investigation reported in this paper deals with the aldobionic acid formed during the partial hydrolysis of flaxseed mucilage. This acid is composed of one molecule of *d*-galacturonic acid joined through its aldehyde group to a molecule of *l*-rhamnose. While the position of the oxide ring in the galacturonic acid and of the glucosidic union on the *l*-rhamnose have not been established, the following structure is suggested.



Experimental Part

Preparation of the Calcium Salt of the Aldobionic Acid.—Flaxseed mucilage was prepared according to the directions of Neville.[2,4] One kilogram of the air-dry mucilage was dissolved in 6 liters of 4% sulfuric acid and heated in a boiling water-bath for twenty hours. The solution was cooled, neutralized by excess calcium carbonate, heated in a boiling water-bath to complete the neutralization and filtered from the insoluble material. The filtrate was concentrated *in vacuo* to a volume of 1.5 liters, decolorized by norit and the salts precipitated in a large dish by addition of 3 volumes of 95% ethanol. After standing overnight with fresh 95% ethanol the salts were triturated with a pestle until granular, filtered, washed with alcohol and ether and dried on a porous plate. The yield was 340 g.

Purification and Analysis of the Calcium Salts.—The crude calcium salts were purified by the method of Levene.[5] For this purpose 340 g. of salts was dissolved in

[1] Hilger, *Ber.*, **36**, 3197 (1903).

[2] Neville, *J. Ag. Sci.*, **5**, 113 (1913).

[3] "Biochemische Handlexikon," Julius Springer, Berlin, **1911**, Band 2, p. 78.

[4] C. A. Morrow, "Biochemical Laboratory Methods," John Wiley and Sons, New York, **1927**.

[5] Levene, *J. Biol. Chem.*, **71**, 471 (1927); Goebel, *ibid.*, **72**, 813 (1927).

900 cc. of water, 1800 cc. of 95% ethanol added, the mixture heated in a boiling water-bath and then allowed to stand overnight. The clear supernatant liquid was decanted, concentrated *in vacuo* to a volume of 200 cc. and the salts precipitated by addition of 4 volumes of 95% ethanol. This was called salt "A." It weighed 24 g. Proceeding in the same general way, the solid residue was separated into fraction "B," weight 65 g., fraction "C," weight 60 g., and fraction "D," weight 75 g.

Samples of each of the above fractions were ground until they would pass an 80-mesh sieve and the analytical determinations described below then made.

Moisture.—Moisture was determined by drying 0.25-g. samples to constant weight in an Abderhalden vacuum drier using phosphorus pentoxide and a bath of boiling toluene.

Ash.—Calcium salts were burned at a low temperature and finally heated to constant weight over a blast lamp. Barium salts were burned at a low temperature but not heated over the blast. This gave barium carbonate. Barium was also determined as the sulfate in the regular way.

Uronic Acid.—The hexose uronic acid was determined by the method of Lefèvre.[6] This is an accurate determination. It is shown later that *d*-galacturonic acid is the uronic acid present. This can be determined approximately by oxidation to mucic acid by means of nitric acid but the method is not quantitative.[7]

Methyl Pentose.—It is shown later that *l*-rhamnose is present in the aldobionic acid; hence the percentage of this sugar is calculated from the pentosan determination. This determination was made according to the A. O. A. C.[8] Since the uronic acid yields furfural, the phloroglucide precipitate resulting from the decomposition of the uronic acid must be calculated, using the factor determined by Lefèvre.[9] When this calculated weight of phloroglucide is deducted from the total weight of phloroglucide, the remainder is the phloroglucide corresponding to the *l*-rhamnose present. From this weight the percentage of *l*-rhamnose hydrate is calculated using the table of Ellett.[10]

Since methyl furfural phloroglucide is soluble in alcohol, the percentage of *l*-rhamnose can also be determined by dissolving out the methyl furfural phloroglucide according to the method of Ellett and Tollens,[11] and Haywood.[12] It is well known that the methyl pentose determination is not accurate but merely an approximation.

The Aldehyde Group.—The percentage of aldehyde present, calculated as CHO, was determined by the method of Cajori.[13] This method was devised for the aldehyde group in mono and disaccharoses. Cajori states that the oxidation of glucose by iodine in sodium carbonate solution is complete in twenty-five minutes. Some sugars, however, require a longer time. The oxidation of the aldehyde group in the aldobionic acid under investigation required three hours for completion.

The purified calcium and barium salts of the aldobionic acid reduce Fehling's solution strongly. On the other hand, calcium and barium salts of the dibasic acid obtained by oxidation of the aldobionic acid with barium hypoiodite show no reduction of Fehling's solution.

[6] A. W. van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, **1920**, pp. 71–76; Lefèvre and Tollens, *Ber.*, **40**, 4513 (1907).

[7] Schorger, *J. Ind. Eng. Chem.*, **8**, 498 (1916).

[8] "Methods of Analysis," A. O. A. C., **1925**.

[9] Van der Haar, Ref. 6, p. 75.

[10] Van der Haar, Ref. 6, p. 82.

[11] Ellett and Tollens, *Z. deut. Zuckerind.*, **42**, 19 (1905).

[12] Haywood, U. S. Bureau of Chemistry, Bull. 105, **1907**, p. 112.

[13] Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

TABLE I

THE ANALYSIS OF CALCIUM SALT OF THE ALDOBIONIC ACID

Fraction	Ash (CaO), %	CO_2, %	Galacturonic acid as mucic acid, %	Methyl pentose, %	Reducing sugar as CHO, %	$[\alpha]_D^{20}$
A	13.23	11.01	41.4	..	5.7	+92.0
B	12.07	11.27	39.45	35.3	5.57	+95.5
C	10.61	11.05	42.6	41.3	4.83	+94.4
D	10.16	11.27	48.7	37.2	4.70	+92.8

An inspection of the above table indicates little difference in the composition of the various fractions. Deviations in the analyses of the various fractions, particularly in the ash, were probably due to the presence of calcium sulfate as an impurity. The data approximate the theoretical for the calcium salt of an aldobionic acid consisting of one hexose uronic acid molecule and one molecule of a methyl pentose, but the results are not conclusive.

Identification of the Constituents of the Aldobionic Acid.—The naphthoresorcinol test and the yield of carbon dioxide by the method of Lefèvre proved the presence of a hexose uronic acid. This was identified as *d*-galacturonic acid by the method of Heidelberger and Goebel.[14] Twenty grams of fraction D salts was dissolved in 200 cc. of 6% hydrobromic acid containing 10 cc. of bromine. This mixture was heated under a reflux condenser in a boiling water-bath. After four hours a white crystalline precipitate began to form. After heating for ten hours the precipitate was filtered off and found to weigh 6.4 g. It was purified by dissolving in the calculated amount of dilute sodium hydroxide and reprecipitating with acid, and identified as mucic acid, melting point 210°.

The sugar present in the aldobionic acid would be oxidized in the above process to a monobasic acid and remain in the filtrate from the mucic acid. This filtrate was freed of excess bromine and hydrobromic acid and concentrated to a volume of 15 cc. To this solution was added 1 g. of phenylhydrazine hydrochloride. After twelve hours a light brown crystalline needle-like solid separated out. After recrystallization from hot ethanol the product melted at 193° and was identical with *l*-rhamnonic hydrazide.

To further establish the presence of *l*-rhamnose in the aldobionic acid, 20 g. of calcium salts D was dissolved in six times its weight of 4% sulfuric acid and hydrolyzed in the autoclave at a gage pressure of one atmosphere for six hours. The acids were removed from this solution by neutralizing with calcium carbonate, filtering, concentrating and precipitating the salts with alcohol. The alcohol solution of the sugars was concentrated *in vacuo* to a sirup. A portion of this sirup in water solution was not fermented by yeast, though when glucose was added it readily fermented. This proves the absence of glucose, mannose and fructose. The remainder of the sirup was oxidized by bromine to the monobasic acid and this acid converted to the phenylhydrazide, which did not crystallize until seeded with pure rhamnonic hydrazide. This on re-

[14] Heidelberger and Goebel, *J. Biol. Chem.*, **74**, 616 (1927).

crystallization from 95% ethanol gave crystalline needles melting at 193° and identical with *l*-rhamnonic phenylhydrazide.

Finally, crystalline *l*-rhamnose hydrate was isolated by hydrolysis of the acid, as follows. Fifty grams of mixed calcium and barium salts of the aldobionic acid was hydrolyzed in the autoclave and the sugar sirup isolated as already described. This sirup was decolorized by norit, all salts removed by solution in absolute alcohol and filtering, the solvent distilled off *in vacuo*, the gum dissolved in half its weight of water and an equal volume of glacial acetic acid added.[15] On seeding with *l*-rhamnose hydrate the mass became solid with crystals. The yield was 7 g. The melting point was 92–94° and the specific rotation was −7.8, constant after ten minutes.

Later experiments on the isolation of *l*-rhamnose hydrate from flaxseed mucilage indicate that the sirup must be fairly pure and contain small amounts of water. Salts and other impurities interfere with the crystallization.

Preparation and Analysis of the Barium Salt of the Aldobionic Acid.—Considerable difficulty was met in purifying the calcium salt of the aldobionic acid. Some calcium sulfate remained in all the fractions even after repeated precipitation of the salts. On the other hand, the barium salt in a fairly pure state can be more readily prepared. For this purpose 200 g. of the mucilage was mixed with six times its weight of 4% sulfuric acid and heated in a boiling water-bath for twenty hours. The acid was neutralized by the careful addition of barium hydroxide solution and an excess of barium carbonate. The barium sulfate was filtered off, the solution concentrated *in vacuo* and the barium salt isolated by precipitation with alcohol. The yield of salt was 40 g. This salt was purified by solution in water, a small amount of alcohol was added to precipitate a trace of gum and any suspended barium sulfate, the solution filtered and the pure barium salt precipitated by alcohol and analyzed.

Analysis of the Barium Salt of the Aldobionic Acid.—Barium determined as $BaCO_3$: found, 17.2%; calcd., 16.85%. Galacturonic acid from the CO_2 determination: found, 48.06%; calcd., 47.60%. Galacturonic acid from mucic acid determination: found, 41.92%; calcd., 47.60%. Aldehyde group: found, 7.09%; calcd., 7.11%. *l*-Rhamnose hydrate: found, 34.9%; calcd., 44.6%. Specific rotation, +79°.

The calculated percentages given above are for the barium salt of an aldobionic acid composed of one molecule of *d*-galacturonic acid and one molecule of *l*-rhamnose with the loss of one molecule of water. The experimental results check very closely with the theoretical values, except in the case of the mucic acid and methyl pentose determinations, both of which are known to be inaccurate methods.

Oxidation of the Aldobionic Acid to a Dibasic Acid.—Fifteen grams of the barium salt of the aldobionic acid was oxidized to the dibasic acid by means of barium hypoiodite.[5] The yield of the barium salt of the dibasic acid was 6.5 g. This salt did not reduce Fehling's solution but gave a positive naphthoresorcinol test and the following results on analysis: Ba as $BaSO_4$, found 24.8%; calcd., 26.00% galacturonic acid from CO_2 determination, found, 39.02; calcd., 39.46%—galacturonic acid from the mucic acid determination, found, 36.10%; calcd., 39.46%—specific rotation, +72°.

The Structure of the Aldobionic Acid.—The experimental work proves conclusively that the aldobionic acid consists of one molecule of *d*-galacturonic acid combined with one molecule of *l*-rhamnose. In this compound one aldehyde group is free and the other combined. If the aldehyde group of *l*-rhamnose is free and that of *d*-galacturonic acid is combined, then the dibasic acid formed by oxidation of the aldobionic acid with barium hypo-

[15] Sands and Klaas, THIS JOURNAL, **51**, 3441 (1929).

iodite would consist of *d*-galacturonic acid combined with *l*-rhamnonic acid. Such a compound would give a high percentage of carbon dioxide when heated with 12% hydrochloric acid, a low percentage of furfural and a positive naphthoresorcinol test. These are the results obtained. On the other hand, if in the aldobionic acid the aldehyde group of the *d*-galacturonic acid is free while the aldehyde group of the *l*-rhamnose is combined, then the dibasic acid formed by oxidation would consist of mucic acid combined with *l*-rhamnose. Such a compound would give no carbon dioxide, a high percentage of furfural and a negative naphthoresorcinol test. These results were not obtained. These facts prove conclusively that in the aldobionic acid the linkage is from the aldehyde group of the *d*-galacturonic acid to one of the secondary alcohol group of the *l*-rhamnose.

Summary

Flaxseed mucilage yields on hydrolysis an aldobionic acid consisting of one molecule of *d*-galacturonic acid and one molecule of *l*-rhamnose. The molecule is joined together by a glucosidic linkage involving the aldehyde group of the *d*-galacturonic acid and an alcohol group of the *l*-rhamnose. This mucilage is similar in composition and structure to some of the plant gums.

Further work on the structure of the mucilage is in progress.

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PARA-PHENYLPHENACYL BROMIDE, A REAGENT FOR IDENTIFYING ORGANIC ACIDS[1]

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Phenacyl bromide and the *p*-halogen substituted phenacyl bromides have been used with great success by Reid and his co-workers[2] for preparing esters which are excellently adapted for characterizing the organic acids therein combined. Such an ester may be prepared easily by the action of the bromide on the sodium salt of an acid in aqueous alcoholic solution; as a rule the ester crystallizes well and may be purified easily by a few crystallizations from aqueous alcohol. The reaction involved may be represented

$$p\text{-}XC_6H_4COCH_2Br + NaOCOR = p\text{-}XC_6H_4COCH_2OCOR + NaBr$$

[1] From a thesis submitted to the Graduate School of the University of Maryland by Jack Bronitsky in partial fulfilment of the requirements for the degree of Master of Science.

[2] Reid and co-workers, THIS JOURNAL, **41**, 75 (1919); **42**, 1043 (1920); **52**, 818 (1930).