[Contribution from the Soil Fertility Laboratory, Bureau of Chemistry and Soils, U. S. Department of Agriculture]

THE PRESENCE OF URONIC ACIDS IN SOILS

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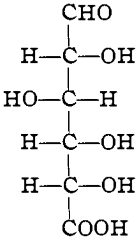
Uronic acids, represented by glucuronic and the isomeric galacturonic acids, while not often found free in natural products, are abundant in plant and animal tissues as part of complex bodies, frequently spoken of as polyuronides. As such they are found in some hemicelluloses, in pectin, and in the glycoproteins of animal tissues and apparently in some of the "slime" products of micro-organisms.

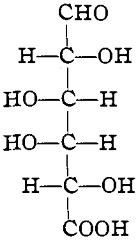
Such plant and animal tissues, of course, find their way into soils and complex compounds containing uronic acid nuclei might be expected to form some part of the organic matter of soils.

Uronic acids are represented by the formula $C_6H_{10}O_7$ and structurally



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H-C-OH} \\ | \\ \mathrm{HO-C-H} \\ | \\ \mathrm{H-C-OH} \\ | \\ \mathrm{H-C-OH} \\ | \\ \mathrm{COOH} \end{array}$$

d-Glucuronic



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H-C-OH} \\ | \\ \mathrm{HO-C-H} \\ | \\ \mathrm{HO-C-H} \\ | \\ \mathrm{H-C-OH} \\ | \\ \mathrm{COOH} \end{array}$$

d-Galacturonic

These acids are characterized by being rather easily decarboxylated by heating with strong acid, each molecule losing one molecule of carbon dioxide, being thereby converted into the corresponding pentose sugar

$$C_6H_{10}O_7 = C_5H_{10}O_5 + CO_2$$

d-Glucuronic acid by this process—boiling with 12% hydrochloric acid—gives *d*-xylose, while *d*-galacturonic acid gives *l*-arabinose.

Each of these sugars, when boiled with 12% hydrochloric acid for liberation of carbon dioxide, breaks down further to give furfural and water

$$C_5H_{10}O_5 = C_5H_4O_2 + 3H_2O$$

so that under this treatment there is evolution of carbon dioxide and formation of furfural at the same time.

It has been known for some time that practically all soils when boiled with 12% hydrochloric acid give furfural, the quantity of which can be determined in the usual way by precipitation as the phloroglucide. Shorey and Lathrop[1] showed that in ten soils of quite widely differing types the furfural obtained calculated to pentosan, varied from 0.055 to 2.75%—in one case representing 28.5% of the total carbon in the soil. Lathrop[2]

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[1] Shorey and Lathrop, This Journal, **32**, 1680 (1910).

[2] Lathrop, unpublished data.

found in nine soils—clays and loams—the furfural calculated to pentosan varied from 0.181 to 0.598% and in sixteen peats varied from 0.50% in the peat from Lake Mattamuskete, North Carolina, to 5.25% on a brown fibrous peat from the Florida Everglades (mile post 39).

Heretofore the formation of furfural when a soil is boiled with 12% hydrochloric acid, has been ascribed to the presence of pentosans, represented in plant tissues by that somewhat indefinite group of compounds known as hemicelluloses, and assumed to be in soils as plant residues. In fact, such a pentosan was isolated from a sample of Marshall loam from North Dakota, a soil containing 6.97% organic carbon.[3]

However, it may be assumed that furfural from soils might be derived from any plant or animal constituent known to give rise to furfural and not necessarily from a pentosan.

A method for determining uronic acids based on a measurement of the carbon dioxide evolved was first proposed by Lefèvre and Tollens[4] and later modified by Dore, McKinnis, Nanji, Paton and Ling, and Dickson, Otterson and Link.[5]

This method depends on heating the material with an excess of boiling 12% hydrochloric acid until there is no further evolution of carbon dioxide, this dioxide being absorbed in a suitable apparatus for determination.

When this method is applied to soils containing carbonates the inorganic carbon dioxide must first be determined by treatment with weak acid in such a way that decarboxylation of the uronic acid is not brought about.

The method used in the work reported here is, briefly, as follows. A three-necked 500-cc. flask was used for the reaction. Into one side neck a stream of carbon dioxide-free air was passed. Into the center neck an upright reflux condenser was fitted, the other side neck being used for the introduction of acid through a dropping funnel. The outlet at the top of the condenser was connected with a carbon dioxide absorber which consisted of a Folin–Denis bell fitted into a wide-necked Erlenmeyer flask through a two-holed stopper. The outlet from this was connected to another Erlenmeyer flask similarly equipped with a Folin–Denis bell. The outlet of this was connected to a source of suction. The incoming air was freed of carbon dioxide by passing through a soda lime tower, next through a Milliken wash bottle charged with a concentrated solution of potassium hydroxide, then through a small wash bottle detector charged with barium hydroxide solution.

The absorbers were charged with a half-saturated solution of barium hydroxide.

Twenty-five grams of soil was used, except that in the case of peat 10 g. was taken. These were placed in the flask, 200 cc. of water and enough hydrochloric acid to make a 1% solution added, and the flask heated to boiling for one hour, the carbon dioxide evolved being absorbed in barium hydroxide solution and determined as described later.

The carbon dioxide given off by this treatment for one hour was, as the work was

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[3] Schreiner and Shorey, Bureau of Soils Bulletin No. 74 (1910).

[4] Lefèvre and Tollens, *Ber.*, **25**, 2569 (1892); *ibid.*, **40**, 4153 (1907).

[5] Dore, THIS JOURNAL, **48**, 232 (1926); McKinnis, *ibid.*, **50**, 1911 (1928); Nanji, Paton and Ling, *J. Soc. Chem. Ind.*, **44**, 2537 (1925); Dickson, Otterson and Link, THIS JOURNAL, **52**, 775 (1930).

originally planned, assumed to be derived from carbonates (inorganic carbon). The absorbers were then changed or recharged, enough hydrochloric acid added to the reaction flask to make a 12% solution, the flask placed in an oil-bath and the bath heated so that the contents were kept boiling. This usually required a bath temperature of 140–145°.

This was continued for five hours, the carbon dioxide being absorbed in barium hydroxide.

In no case was there any precipitate formed in the second absorber, even with a very rapid current of air passing through the apparatus.

At the conclusion of the operation the absorber was disconnected and the precipitated barium carbonate filtered off rapidly by suction on a small Büchner funnel. The bell and flask were rapidly washed into the funnel and the washing continued until all barium hydroxide was removed. Barium carbonate adhering to the bell and flask was then dissolved with dilute hydrochloric acid and after changing the receiver this solution was poured on the funnel, more acid being used if necessary to dissolve all the barium carbonate and the filter thoroughly washed. To the solution of barium chloride thus obtained dilute sulfuric acid was added to precipitate the barium as sulfate. This was determined in the usual way and calculated to the equivalent of carbon dioxide.

This method has a disadvantage in the danger of carbonation of the barium hydroxide solution in the process of handling and filtering, but a little experience will enable one to overcome this. It has been found that when this operation was carried out carefully and expeditiously, the maximum error was 0.5 mg. of carbon dioxide, which, when working with 25 g. of soil, is negligible. The method has an advantage in that errors introduced by the carrying over of hydrochloric acid or other volatile products are eliminated.

The results of the examination of eleven soils are reported in this paper. The soils were as follows.

No. 1, Houston clay No. 1. No. 2, Houston clay No. 2. No. 3, Houston clay No. 3. These three were soils from experimental fields near Austin, Texas, Nos. 2 and 3 are calcareous, that is, contain enough calcium carbonate to effervesce on the addition of acid. No. 4, Chester loam from Virginia. No. 5, Norfolk fine sandy loam from North Carolina. No. 6, Washburn loam from Aroostook County, Maine. No. 7, Caribou loam from the same location. No. 8, Greenville sandy loam from DeWitt, Georgia. No. 9, Portsmouth fine sandy loam from New Bern, North Carolina. No. 10, Muck from near North Liberty, Indiana. No. 11, Everglades peat from Belle Glade, Florida. In the tables following these soils will be referred to briefly by name.

The general organic characteristics of the soils examined, together with their reaction, are stated in Table I. All determinations were made on air-dried samples and the results calculated to the oven-dried basis.

With the exception of the Houston soils, the soils have a neutral or acid reaction but appreciable quantities of carbon dioxide were given off by the treatment for one hour with 1% hydrochloric acid.

Whether this carbon dioxide is derived from inorganic carbonate, oc-

TABLE I

ORGANIC CARBON, NITROGEN REACTION

Soil	PH	Organic C	Total N	Soil	PH	Organic C	Total N
Houston No. 1	7.6	1.43	0.08	Caribou	4.8	3.00	0.24
Houston No. 2	8.3	0.95	.06	Greenville	5.4	0.96	.04
Houston No. 3	7.8	1.47	.16	Portsmouth	6.6	.88	.08
Chester	5.8	1.50	.17	Muck	6.8	30.9	3.85
Norfolk	4.7	0.82	.05	Peat	7.0	53.3	3.19
Washburn	5.0	4.90	.35				

cluded carbon dioxide or from the first stages of the decarboxylation of uronic acids, is under investigation.

In Table II are given the quantities of uronic acid carbon dioxide obtained after the preliminary treatment for one hour with 1% hydrochloric acid, together with the calculated equivalent of uronic acid, both being stated as percentage of the soil.

Since uronic acids are frequently a part of the more complex polyuronides, the total quantity of organic matter represented by the uronic acid carbon dioxide may be much larger than the calculated uronic acid shown in this table.

TABLE II

URONIC ACID CARBON DIOXIDE AND THE CALCULATED EQUIVALENT OF URONIC ACID PERCENTAGE OF SOIL

Soil	Uronic acid CO_2	Uronic acid	Soil	Uronic acid CO_2	Uronic acid
Houston No. 1	0.14	0.617	Caribou	0.36	1.587
Houston No. 2	.11	.484	Greenville	0.08	.353
Houston No. 3	.13	.512	Portsmouth	.07	.308
Chester	.24	1.058	Muck	1.80	7.938
Norfolk	.08	0.352	Peat	1.69	7.462
Washburn	.41	1.808			

Uronic acid ($C_6H_{10}O_7$) contains 37% carbon and some idea of the proportion of uronic acid to the total organic matter of the soil may be had by comparing the uronic acid carbon with the total organic carbon. Such a comparison is made in Table III.

The theory of the evolution of furfural to the process of decarboxylation of uronic acids has already been pointed out. Carbon dioxide is first split off, leaving a pentose sugar which further breaks down to furfural and water. In other words, the decarboxylation must be complete or the formation of furfural will not be complete.

It has been contended that complete decarboxylation of uronic acids can be brought about only by heating with 12% hydrochloric acid to 135–140° for four or preferably five hours,[6] and with this in mind the determinations of uronic acid carbon dioxide in soils reported here were made by heating at that temperature for five hours.

[6] Dickson Otterson and Link, THIS JOURNAL, **52**, 775 (1930).

TABLE III

COMPARISON OF URONIC ACID CARBON WITH TOTAL ORGANIC CARBON IN SOIL

Soil	Organic carbon in soil	Uronic acid carbon	Uronic acid carbon, percentage of total organic carbon
Houston No. 1	1.43	0.228	15.9
Houston No. 2	0.95	.179	18.8
Houston No. 3	1.47	.216	14.7
Chester	1.50	.427	28.4
Norfolk	0.82	.130	15.8
Washburn	4.90	.667	13.7
Caribou	3.00	.586	18.8
Greenville	0.96	.130	13.5
Portsmouth	.88	.113	12.8
Muck	30.9	2.86	9.25
Peat	53.3	2.75	5.15

Determinations of furfural in soils made in this Laboratory heretofore were made according to the method of the Association of Official Agricultural Chemists adopted for pentosans in feeding stuffs, and in order to have figures comparable with those previously obtained, this method was applied to the soils under discussion.

This method, which is essentially that of Kröber,[7] calls for heating to boiling with 12% hydrochloric acid at a rate such that 30 cc. distils over in ten minutes and this continued until 360 cc. has collected, which entails heating for only a little more than two hours.

It seemed that this method might give less than the theoretical quantity of furfural due to incomplete decarboxylation of the uronic acid. As a matter of fact, the quantities obtained by this method and presented in Table IV are much less than the theory calls for, and except for the peat are less than 50% of the theoretical.

TABLE IV

FURFURAL OBTAINED FROM SOILS COMPARED WITH THE CALCULATED QUANTITY

Soil	Furfural, percentage of soil	Furfural equivalent to uronic acid content	Furfural, % of calcd.
Houston No. 1	0.050	0.299	16.7
Houston No. 2	.030	.234	12.8
Houston No. 3	.037	.277	13.7
Chester	.113	.512	22.0
Norfolk	.046	.181	25.4
Washburn	.272	.881	30.8
Caribou	.141	.757	18.6
Greenville	.020	.160	12.5
Portsmouth	.050	.143	35.0
Muck	1.880	3.840	49.0
Peat	2.070	3.600	57.5

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[7] Kröber, *J. Landw.*, **48**, 379 (1900).

To determine whether or not this was due to the shortness of time of heating, the determinations were repeated, heating for five hours, but in no case was more furfural obtained, and in most cases slightly less.

It has been generally observed that even when working with pure uronic acids the quantity of furfural obtained is less than the theoretical, whereas the theoretical quantity of carbon dioxide is obtained. This is no doubt due to the reactivity of furfural and its tendency to form reversion products or furan derivatives. It is quite likely that in a complex mixture of organic and inorganic material, such as a soil, this tendency is accentuated with the consequent greater loss of furfural.

That this is so is indicated by the following experiment.

	Phloroglucide
0.5 g. of pectin gave	0.1284 g.
10 g. of Portsmouth sandy loam	.0140 g.
Total when determined separately	.1424
When mixed and furfural determination made the mixture gave	.1402
0.25 g. of pectin gave	.0642
10 g. of Washburn loam gave	.0490
Total determined separately	.1137
When mixed and furfural determined the mixture gave	.1020

The pectin used was the commercial "lemon pectin."

The simultaneous evolution of carbon dioxide and formation of furfural on heating with strong acid being characteristic of uronic acids and the polyuronides present in plant tissues, it seems fair to assume when soils behave in the same way that they contain uronic acids or complex substances of which such acids are a part.

The identity of such compounds is part of an investigation not yet completed, but the following data are submitted in substantiation of the foregoing conclusion.

A hot water extract of one of the soils (Houston No. 3) on evaporation to dryness yielded a gray powder that amounted to 0.07% of the soil and contained 61% organic matter. Another portion of the same soil heated with water in an autoclave at 30 pounds pressure yielded a similar extract, 0.11% of the soil and containing 76% organic matter.

Both these extracts were readily soluble in water again with the exception of a small portion (about 5%) that was inorganic in nature. Such a water solution gave the following reactions. It gave a strong Molisch reaction. It did not reduce Fehling's solution but did so after heating for a few minutes with hydrochloric acid. This reducing property, however, soon disappeared on continued heating with acid. Heated with hydrochloric acid and a small portion of orcin a green color was formed. Heated with hydrochloric acid and naphthoresorcinol, it gave a dark colored precipitate which was soluble in ether with a reddish-violet color, and in warm benzene with a blue color. These color reactions, with the exception of that with naphthoresorcinol, are given by pentoses or pentose yielding material.

When the water solution is subjected to dialysis there is left a somewhat opalescent viscous solution or dispersion from which several volumes (four or five) of 95% alcohol throw down a gelatinous precipitate which readily can be filtered off. This, when treated with water, forms again an opalescent dispersion which gives all the color reactions mentioned much more strongly than the original solution of the extract.

The residue from hot water extraction on heating with 12% hydrochloric acid gave 2.5% of furfural.

The residue from the water extract made under pressure when heated with 1% hydrochloric acid for one hour gave 0.5% of carbon dioxide and heating further for five hours with 12% hydrochloric acid gave 3.24% of carbon dioxide.

It is evident that water extraction under pressure as in this case extracts but a small portion of the uronic acid constituents. This soil (Houston No. 3) contained uronic acid carbon dioxide 0.13%, whereas calculating the 3.24% of the water extract back to the original soil gives but 0.0035% of uronic acid carbon dioxide.

A further separation of uronic acid material from soil was effected in the following manner. The soil was treated with 2% sodium hydroxide until the extract gave a strong Molisch reaction—this sometimes required several days. The supernatant liquor was decanted from the soil, made slightly acid with acetic acid, excess of barium carbonate added and the liquor concentrated to about one-quarter of its volume, filtered and further concentrated until it became sirupy.

This sirup, when poured into four volumes of 95% alcohol, formed a gelatinous precipitate which was readily filtered off and washed with alcohol. This precipitate dries to a horny mass which, when pulverized, forms a light gray powder. The precipitate or the dry powder when treated with water forms an opalescent solution or dispersion—more readily if a small quantity of barium acetate be added. This solution gives a precipitate of barium sulfate on addition of sulfuric acid and is evidently a barium salt or contains a barium salt of some uronic acid complex. The solution obtained in this way gave all the color reactions noted in connection with the water extract. The dry powder when heated with 12% hydrochloric acid gave off both carbon dioxide and furfural.

Separations of this kind were made from two of the soils (Houston No. 3 and Chester loam) and no differences have been noted in the character of the material so obtained. A larger quantity could be obtained from the Chester loam than from the Houston soil and larger quantities are obtainable by extraction with alkali than by water extraction.

The demonstration of the presence of uronic acid or polyuronides in soils is, in itself, the establishment of a new fact in regard to the character of the organic matter of soils, and further interest immediately connected with this fact lies in the identity of these uronic acid compounds, their origin and fate in the soil. These, naturally, are under investigation.

There are at least two points of a more or less analytical nature raised by the work here reported. One is the determination of inorganic carbon (carbonates) in soils. It is quite evident that heating soil with 1% hydrochloric acid gives rise to an evolution of carbon dioxide that, in some cases, one can scarcely conceive of as being present as inorganic carbonates. For instance, of the soils discussed in this paper five have a strong acid reaction and on heating for one hour with 1% hydrochloric acid, gave the following quantities of carbon dioxide.

These quantities of carbon dioxide were not included in stating the quantity of uronic acid carbon dioxide in Table II, but it is likely that they arise from the initial decarboxylation of uronic acids and should be added

TABLE V

CARBON DIOXIDE OBTAINED BY HEATING FOR ONE HOUR WITH 1% HYDROCHLORIC ACID

Soil	PH	Carbon dioxide, per cent. of soil
Chester	5.8	0.124
Norfolk	4.7	.044
Washburn	5.0	.204
Caribou	4.8	.220
Greenville	5.4	.075

to the figures stated there, but in view of the uncertainty as to their origin they were ignored. It seems quite probable that many of the discrepancies observed in determinations of carbon dioxide in soils have been due to variations in temperature and strength of acid used.

The other point is in connection with what may be called proximate analysis of soil organic matter. The most recent and probably at present the best known of such methods is that proposed by Waksman and Stevens.[8]

By this method the organic matter is separated into fractions: ether soluble, water soluble, alcohol soluble, hemicelluloses, cellulose, lignin, etc.

Apart from certain fundamental concepts that lead to erroneous conclusions in the application of this method, the most serious defect lies in that no place is provided for classes of organic soil constituents that from time to time may emerge from the obscurity of humus investigations. Uronic acids or polyuronides furnish an example of this. These are not soluble in ether or alcohol and are only very slightly extracted by hot water. On heating with acid, as the method calls for in the determination of hemicelluloses, they disappear as carbon dioxide and furfural, and such reducing sugar as might be formed from polyuronides completely disappears under the treatment.

Link and Niemann[9] have recently contributed to our knowledge on this point, showing that when polyuronides are hydrolyzed with weak acids the uronic acids split off and are destroyed as soon as formed.

We find, then, that a class of organic compounds that occur in soils that may represent from 5 to 25% or more of the organic carbon is, by this method, not only ignored, but so far as determination is concerned, is almost completely eliminated.

Summary

In this paper there are presented results of the examination of eleven samples of soil for the presence of uronic acid, according to methods adopted and in use for the determination of these acids in vegetable products.

The soils are from eight locations and represent types varying from sandy loams containing 0.82, 0.88 and 0.96% of organic carbon, to loams contain-

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[8] Waksman and Stevens, *Soil Science*, **26**, 113 (1928).

[9] Link and Niemann, THIS JOURNAL, **52**, 2474 (1930).

ing 3.00 and 4.90% of organic carbon, a muck containing 30% and a peat containing 53% organic carbon. These soils varied in reaction from P_H 4.7 to 8.3.

The uronic acid carbon dioxide varied from 0.07 to 1.80% of the soil, and this, when calculated to the equivalent uronic acid, varied from 0.308 to 7.94% of the soil.

The uronic acid carbon calculated from these figures varied from 0.113 to 2.86% of the soil and this uronic acid carbon varied from 5.15 to 28.4% of the total organic carbon.

The presence of uronic acids or complex substances containing them (polyuronides) was confirmed by the separation from two of the soils of colloidal material having all the properties of such uronic acid complexes.

This separation was made both by hot water extraction and extraction with dilute sodium hydroxide.

The bearing of the presence of uronic acids in soils on certain analytical operations has been pointed out. These are the determination of carbonates in soils, and methods proposed for the proximate analysis of the organic matter of soils.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BRYN MAWR COLLEGE]

THE POTENTIALS OF SOME UNSTABLE OXIDATION-REDUCTION SYSTEMS

BY LOUIS F. FIESER

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Although a considerable amount of data concerning the oxidation-reduction potentials of well-defined organic systems has accumulated during the past ten years, relatively few efforts have been made to study by direct potentiometric measurement those systems which are alterable and with which the potential of a solution is subject to a definite drift with time as the result of some reaction which consumes either the oxidant or the reductant, or both. Such systems, to be sure, have been studied by colorimetric and electrometric indicator methods, but these are to be classed as indirect methods inasmuch as they involve the observation of the effect of the unstable system upon the color or the potential of an oxidation-reduction system of unalterable components, and it is the potential of the latter, rather than that of the former, which actually is measured.

The potential of the alterable system has been the basis of observation in only a few investigations. Biilmann and Blom[1] have recorded the equilibrium potentials, together with the velocity constants, for the disappearance of the reductant, for two azo-hydrazo systems. They followed the

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[1] Biilmann and Blom, *J. Chem. Soc.*, **125**, 1719 (1924).