[FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY OF WASHINGTON UNIVERSITY MEDICAL SCHOOL.]

"SUGAR SAND" FROM MAPLE SAP; A SOURCE OF MALIC ACID.

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When the sap of the sugar-maple tree (*Acer saccharum*) is evaporated, there always appears a variable quantity of a sandy, insoluble substance called "sugar sand" and "niter," the main constituent of which is a calcium salt of malic acid. Some of this material is deposited on the pan, as the sap approaches the sirup-stage, but more of it is in suspension in the sirup from which it is removed by straining. A considerable quantity of *sugar sand* may be collected in this way. Apparently the only analysis of this material thus far published is that which appeared in the "Indiana Station Report for 1899." As this analysis shows a calcium malate content of only 33 per cent., and was clearly made with material which cannot be regarded as typical, it has seemed worth while to make a further examination of this substance.

Collection of Sugar Sand.

This material is not deposited until the sap approaches the sirup-stage when the temperature is 102–103°. It is advisable to reject the deposit on the pan and take only what is suspended in the sirup. As the sirup cools, the *sugar sand* settles to the bottom of the container. The sirup should be drawn from the sediment which should then be mixed with water to dilute adhering sirup and poured upon a flannel strainer. It should be washed until free from sugar and then spread out in a moderately warm place to dry. When reasonable care is taken to collect as much as possible, from 16–25 pounds may be obtained from about one thousand maple trees in the course of the sugar season. The material used for analysis came from Vermont.

Properties and Analysis of Sugar Sand.

General Properties.—When practically free from sugar, this material forms a coarse powder which appears crystalline under the microscope. It has a light gray color, is tasteless and has a slight odor. It is slightly soluble in water, for repeated treatment with this solvent always gives a filtrate containing calcium. Some samples treated with water give solutions having an acid reaction, whereas other samples give neutral solutions. I have never had a sample which gave an alkaline reaction. Treated with dilute hydrochloric acid, *sugar sand* passes into solution except most of the silica and a small amount of organic matter of unknown composition.

Moisture.—Dried at 110° to constant weight *sugar sand* loses 2.6 per cent. of water.

Loss on Ignition and Ash.—Two determinations of ash after ignition in the blast-lamp flame to constant weight gave a loss of 67.74 per cent. and 32.26 per cent. of ash; and a loss of 67.81 per cent. and 32.19 per cent. of ash.

Analysis of Ash.—A qualitative examination of ash showed that it consisted mainly of calcium with small quantities of magnesium and iron. The only acids were silica and a very small quantity of phosphoric acid. Two quantitative analyses were made of the ash obtained in the preceding ash determinations. The usual analytical methods required for the quantitative separation of the bases and acids mentioned were used and gave the following results:

	1.	2.
Calcium (CaO)	24.03	23.87
Iron (Fe_2O_3)	0.39	0.46
Magnesium (MgO)	0.05	0.07
Silica (SiO_2)	7.74	7.76
Phosphoric acid (P_2O_5)	0.05	0.09
	32.26	32.25
Percentage of ash	32.26	32.19

Organic Acids.—The value of *sugar sand* is wholly dependent upon its malic acid content, the only organic acid present in any quantity. In using this material to prepare pure malic acid and its salts, it is necessary to have some means of determining the exact amount of this acid in the crude product. For this purpose, I have used the following simple method of analysis which is fairly rapid and gives closely agreeing results.

Heat about a gram of material on the water bath for an hour with 25 cc. of normal oxalic acid. Filter into a 250 cc. graduated flask, first decanting the clear supernatant solution upon the filter, then adding the precipitate and washing the latter thoroughly with hot water. Cool the filtrate and bring its volume to 250 cc. with distilled water. The final solution is nearly colorless.

Measure from a buret, or pipet, two separate 50 cc. portions of this solution. Determine in one portion the total acidity by titration with decinormal sodium hydroxide solution. This gives not only the acidity caused by the organic acids in the material, but also that due to the excess of oxalic acid used to decompose the calcium salts. To determin the excess of oxalic acid, acidify the second portion of solution with acetic acid and precipitate calcium oxalate by means of calcium chloride. Collect the precipitated calcium oxalate upon a filter, ignite and weigh as calcium oxide. Since 0.0028 gram of calcium oxide are equivalent to 1 cc. of decinormal sodium hydroxide solution, the quantity of decinormal alkali required to neutralize the organic acids in *sugar sand* can be determined. Three determinations of organic acids (calculated as malic acid) in the same sample showed that it contained 52.33, 52.25, and 52.19 per cent.

Therefore, calculated as malic acid, the average percentage of organic acid in *sugar sand* by this method of analysis is 52.26. It would be natural to expect material of such origin to contain more than one organic acid. But, although many experiments have been made for the purpose of isolating other organic acids, I have succeeded thus far in finding only a mere trace of oxalic acid. This latter acid was recognized by the characteristic envelope-shaped crystals of calcium oxalate seen under the microscope. The amount, however, was so small and the quantity of *sugar sand* used in the experiment so large that I think not much importance should be attached to the result.

Sugar.—The fact that *sugar sand* is precipitated from a concentrated sugar solution would lead one to expect the presence of sugar in this material. The quantity of free sugars, however, can be greatly reduced by thorough washing, but sugar combined with lime might not be removed so easily. The "Indiana Station Report for 1899" records an analysis of "niter" or "sugar sand" which gave 12.74 per cent. of reducing sugars and 26.88 per cent. of non-reducing sugars (cane sugar). Unless these sugars were combined with calcium oxide, which could hardly have been the case, their presence in such quantities could have been detected by tasting. Sy[1] remarks concerning this analysis that it was evidently a "niter" which contained considerable sugar or sirup. Such undoubtedly was the case and for that reason an analysis of typical *sugar sand* was not made in that instance.

The presence of sugar in the material I have examined cannot be detected by tasting, as it is tasteless. Yet a small quantity of sugar might be present without being detected in this way. Commenting further upon the Indiana analysis, Sy says that part of the sucrose is probably present as calcium sucrate. Therefore, in examining this material for sugar, I had three things in mind, namely: reducing sugar (invert sugar), non-reducing sugar (cane sugar), and cane sugar combined with calcium oxide (calcium sucrate). The presence of any appreciable quantity of calcium sucrate does not seem very probable for the reason that *sugar sand* treated with water gives a neutral or acid solution. As previously stated, I have never had a sample which gave an alkaline reaction, and aqueous solutions of the three compounds, which cane sugar forms with calcium oxide, are alkaline.

As a preliminary test for sugar, a weighed quantity of material was mixed with water and allowed to stand for an hour at room temperature. The slightly colored neutral solution was filtered from insoluble matter.

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[1] *J. Franklin Institute*, Oct., Nov., and Dec., 1908.

The filtrate, freed from calcium by means of potassium oxalate solution and filtered from calcium oxalate, gave an immediate reduction when added to boiling diluted Fehling's solution, and consequently contained reducing sugar. Under the conditions an inversion of cane sugar was not possible. Another portion of the same solution was warmed with phenylhydrazine acetate and crystals of an osazone appeared. These crystals under the microscope had the sheaf-like form characteristic of phenylglucosazone and melted at 204°.

Quantitative Determination of Sugars.—The quantity of invert sugar and cane sugar was determined as follows: A weighed amount of *sugar sand* (about 10 grams) was mixed with water and allowed to stand at room temperature with the calculated quantity of oxalic acid required to convert all the calcium into oxalate. After three hours the solution was filtered from insoluble matter which was washed until free from acid. Filtrate and wash water, after neutralization with dry sodium carbonate and filtration, were brought to a volume of 250 cc. The quantity of invert sugar in this solution was determined by titration with a diluted Fehling solution (50 cc. = 0.05 gram of glucose). An aliquot part of this solution, after inversion with hydrochloric acid and neutralization, was again titrated with Fehling's solution. As the result of several determinations, *sugar sand* was found to contain 1.96 per cent. of invert sugar, calculated as glucose; and 3.45 per cent. of non-reducing sugar, calculated as cane sugar.

Examination of Sugar Sand for Sucrate.—Invert sugar and cane sugar are somewhat soluble in 80 per cent. alcohol, whereas compounds of cane sugar with calcium oxide (sucrates) are practically insoluble. A weighed quantity of *sugar sand* (about 100 grams) was extracted three times with 200 cc. portions of boiling 80 per cent. alcohol. The filtered alcoholic extracts were distilled and the thick, sirupy residue treated with cold water and filtered. After removal of some calcium by means of potassium oxalate and filtration, the solution, titrated with Fehling's solution before and after inversion, was found to contain 2.31 per cent. of invert sugar and 3.46 per cent. of cane sugar.

The *sugar sand* extracted three times with 80 per cent. alcohol was tested further for sugar. In one instance it was suspended in water and carbon dioxide was passed through the mixture for several hours. The solution was filtered, freed from calcium, heated with dilute hydrochloric acid on the water bath and brought to a volume of 250 cc. Titration with Fehling's solution gave an entirely negative result. Other tests also failed to show the presence of sugar. These experiments, therefore, show that *sugar sand* contains both invert sugar and cane sugar, but do not indicate the presence of any appreciable amount of sucrate, unless calcium sucrate is slightly soluble in 80 per cent. alcohol.

A considerable quantity of alcohol was used and it may be that some sugar as calcium sucrate was dissolved. The alcoholic extracts always contained calcium, but I have observed that solutions of the residues from these extracts, when treated cautiously with just enough oxalic acid to precipitate all the calcium, become strongly acid. This would seem to indicate that some calcium malate is dissolved, though of course calcium could be there as sucrate in small quantity.

Ether-Soluble Matter.—A portion of the residue from the alcoholic extract is fatty in character and insoluble in water. This result led to a separate ether extraction of *sugar sand*, 100 grams being treated three times with 200 cc. portions of this solvent. The combined ether extracts had a yellowish color. After distillation of ether, the residue dried to constant weight at 100° amounted to 0.37 per cent.

This fatty material had a yellow color and resembled butter in appearance and odor. Unquestionably it has no connection with *sugar sand* itself. It is the custom among manufacturers of maple products to use a small quantity of some fatty substance, such as butter or pork fat, to prevent the boiling sap from frothing over, and some of this fat, if not all, is carried down by the precipitated *sugar sand.* This fact probably accounts for the presence of this ether-soluble matter.

Carbon Dioxide.—Finely powdered *sugar sand* was treated with dilute sulfuric acid in a Mohr alkalimeter, the evolved gas being displaced by dry air and the apparatus being heated on the water bath during the process. The loss in weight, due to carbon dioxide, was 0.66 per cent.

Extraneous Organic Matter.—*Sugar sand* contains more or less organic matter, which finds its way into the sap during collection and evaporation. The nature of these substances has not been determined. They are colored and, though probably not wholly insoluble in water and dilute acids, are largely so. The clogging of filters, when solutions of *sugar sand* in dilute acids are separated from insoluble matter, may be caused in part by these organic substances.

To determin extraneous organic matter *sugar sand* was treated with sufficient dilute hydrochloric acid to dissolve calcium salts and the insoluble matter, collected on a Gooch crucible and washed with a small quantity of alcohol and ether, was dried at 110°. The residue, which was mainly silica, lost 2.35 per cent. upon ignition.

Summary.

Samples of *sugar sand*, collected in different localities, or in the same locality even under the conditions described, will probably vary somewhat in composition from year to year. But it is believed that this analysis, the individual results of which are here summarized, gives within rather narrow limits the quantities of the several constituents which this material may be expected to contain.

ANALYSIS.

Silica (SiO_2)	7.74
Phosphoric acid (P_2O_5)	0.05
Ferric oxide (Fe_2O_3)	0.39
Calcium (Ca)	17.16
Magnesium (Mg)	0.03
Moisture	2.60
Malic acid ($C_4H_4O_5$)	51.48
Invert sugar	2.31
Cane sugar	3.46
Ether soluble matter	0.37
Carbon dioxide	0.66
Extraneous organic matter	2.35
Undetermined (by difference)	11.40
	100.00

Isolation of Malic Acid.

To obtain malic acid from *sugar sand*, it is advisable first to prepare acid calcium malate, $Ca(C_4H_5O_5)_2.6H_2O$. On the basis of a calcium content of about 17 per cent. in *sugar sand*, dissolve 27 grams of crystallized oxalic acid in 300 cc. of water and bring the temperature of the solution to 60° on the water bath but not higher, since acid calcium malate at higher temperatures is said to be decomposed into malic acid and neutral calcium malate. Then add 100 grams of *sugar sand* in small portions at a time and heat at 60° on the water bath for an hour. Half of the calcium will be precipitated as calcium oxalate and the other half will pass into solution as acid calcium malate. Filter on a Büchner funnel and wash the precipitate until free from acid. Concentrate filtrate and washings on the water bath, until crystallization begins, and then cool the solution in ice water, stirring constantly to get small crystals. If these crystals are carefully freed from mother liquor by suction, or better by centrifugalization, and washed with a small quantity of cold water, the product will be snow-white. This salt is sufficiently pure for most purposes, but a second crystallization will give a still better product. In one instance, where a special effort was made to determine how much acid calcium malate could be isolated, about 96 per cent. of the theoretical amount was obtained. Obviously in such a case the entire quantity of material was not pure enough for most purposes unless recrystallized.

In preparing malic acid it is advizable to use acid calcium malate which has been at least once recrystallized, and then thoroughly air-dried. A determination of calcium should then be made. Pure acid calcium malate contains 9.66 per cent. of calcium. Dissolve 30 grams of pure oxalic acid in water, heat the solution on the water bath and while stirring pour in gradually a solution of 100 grams of acid calcium malate heated to 60°. Heat on the water bath for some time and test the clear solution both for calcium and oxalic acid. If either is in excess, carefully add a small quantity of the solution required to remove it. In this way a solution, free from oxalic acid and calcium, will be obtained. Filter and concentrate the filtrate first on the water bath and then for several days in an air-closet at 100°. The temperature should not go much above that point. Set the vessel containing the fused acid in a vacuum desiccator and upon standing it will gradually solidify to a snow-white, crystallin mass.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MICHIGAN.]

THE ACTION OF HALOGEN ACIDS UPON THE OXYARYL-XANTHENOLS.

[PRELIMINARY PAPER.]

BY M. GOMBERG AND C. J. WEST.

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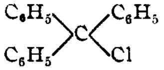
It has been demonstrated[1] that triphenylchloromethane and all its analogues exists in two tautomeric forms: the benzenoid (I) when colorless, and the quinoid (II) when colored. The quinoid compounds behave like salts. In definit solvents they obey the general laws of molecular conductivity; they possess, like all other salts, the capacity to form double salts, to yield perhalides, etc. The salt-like character of these "quinocarbonium" salts was attributed to the carbon atom C*, in so far as it was at all possible to localize upon a single atom in any complex molecule the cause of basicity and of consequent salt-formation.



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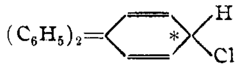
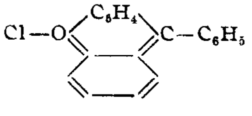
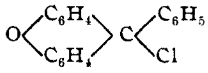


II.

The idea of the quinocarbonium constitution was afterwards extended to the so-called "carboxonium" and "carbothionium" salts—colored compounds obtainable by the action of acids upon xanthenols and thioxanthenols, and to which the constitution (III) had been generally assigned by various investigators, without, however, any direct evidence of an experimental nature. Gomberg and Cone[2] have shown that these xanthenols yield, on proper treatment, absolutely colorless halides. These



III.



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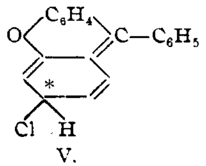

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[1] Gomberg, *Ber.*, 40, 1847 (1907); 42, 406 (1909).

[2] *Ann.*, **370**, 142 (1909); **376**, 183 (1910).