

and 28.30 cc. of thiosulphate. The lowest and highest figures give respectively 27.76 and 27.88 per cent. of lead.

The difference between the Guess method of solution of lead chromate and the one which I adopted was tested by sprinkling basic lead chromate upon two filters and dissolving one quantity with nearly boiling 1 : 1 acid and the other in the manner I have described. In the first case 1 cc. thiosulphate = 0.01516 gram chromate; in the second 1 cc. thiosulphate = 0.01446 gram chromate.

Assuming that the second determination was correct, the 0.4336 gram of chromate used in the first case should have required 29.99 cc. of thiosulphate instead of 28.60 cc., which was actually used. With starch iodide paper, chlorine can be readily detected when hot 1 : 1 hydrochloric acid is put upon lead chromate.

In the presence of large quantities of iron, say 20 per cent., the tendency to form basic acetate gives difficulty in dissolving the lead. Of course, any ferric acetate precipitated along with lead chromate is fatal. Guess points out that the presence of antimony, bismuth, silver or gelatinous silica interferes with the process, but says that if the lead is first changed to sulphate and, after separation from the soluble sulphates, dissolved in ammonium acetate as for the molybdate determination, the rest of the process may be carried out as before. In this case the only advantage in the chromate method is its delicacy; it takes a longer time, but duplicate determinations should check within a tenth of one per cent., and often do check within two or three hundredths.

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NOTES ON THE DETERMINATION OF ACIDS IN SUGAR CANE JUICE.

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In a previous report² reference was made to an investigation of the acid constituents in sugar cane juice and its products, which I commenced at the Louisiana Sugar Experiment Station, and I mentioned some of the analytical difficulties encountered in determining certain of these constituents, reporting in detail the development and application of a polariscopic method for the determination of malic acid (and incidentally also of tartaric acid). While work upon the general problem of the acid constituents was interrupted before it was sufficiently rounded off to admit of a report being made on the subject as a whole, yet, besides the work on malic acid already reported, there are other results among those thus far obtained, both on analytical methods and in the

¹ This paper is an abridgement of a paper by the author on the same subject presented at the 42nd General Meeting of the American Chemical Society, July 12, 1910.

² THIS JOURNAL, 3, 563-574 (1911).

examination of cane products, which I deem should be made available to others who may be working on the same or on similar problems. I shall present, therefore, in the following some notes on analytical operations and some determinations and approximate estimations of acid constituents in the cane juice, without presuming in this to report a satisfactory scheme of analysis or the results of ultimate determinations of all the acids present.

In the early stages of the work, after finding the usual methods of determining and identifying commonly occurring organic acids not to work satisfactorily, a number of other methods described in textbooks and the periodical literature and modifications and combinations of these methods were tried. In these preliminary tests of methods, I was assisted by Mr. W. G. Taggart, assistant chemist, who, under my direction, applied them variously—to cane juice, to solutions of molasses or molasses and sugar, to such solutions with added known amounts of certain acids, to solution of definite quantities of sucrose, dextrose, and levulose with or without additions of the acids in question, and to solutions of one or more of the acids alone in water.³

With the exception of a method of Behr⁴ for the separation of the aconitic acid as its acid ammonium salt, practically all the methods described in the literature which we tried were worked out by their authors primarily for the analysis of wines and other fermentation products and fruit juices, and none of them take into consideration the possible presence of

¹ Among the methods receiving special consideration in our experiments are the following:

1. The method of G. Jørgensen (*Z. Unters. Nahr.-Genussm.*, 13, 241-257 (1907); *Chem. Abstr.*, 1, 1448-52 (1907)), for tartaric, succinic, citric, and malic acids, in which, after separating the acids from some of the other matters by means of the lead salt, the tartaric acid is separated as the acid potassium tartrate, the succinic then shaken out with ether and subsequently the citric and the malic acids converted to the barium salts and their separation effected by a procedure based on a difference in their solubility in 50 per cent. alcohol.

2. The method of R. Kunz (*Z. Unters. Nahr.-Genussm.*, 6, 721 (1903); 12, 641-5 (1906); *Chem. Zentr.*, II, 854 (1903); I, 418 (1907)), for the succinic acid, involving the extraction with ether by an extraction apparatus according to Schacherl, and the subsequent oxidation by potassium permanganate of other acids than succinic.

3. The method of Möslinger (*Z. Unters. Nahr.-Genussm.*, 4, 1120-30 (1901); *Chem. Zentr.*, I, 231 (1902); II, 1386 (1903)), for lactic acid, in which its separation from malic and tartaric acids is based upon the solubility of its barium salt in 75 per cent. alcohol.

4. The method of C. Schmidt and C. Heipe (*Z. anal. Chem.*, 21, 534 (1882)), for tartaric, succinic, and malic acids, making use of the calcium salts to separate the tartaric acid from the others, and of the barium salts to separate the succinic from the malic acids. Also a modification of this method ("Official and Provisional Methods of Analysis," U. S. Dept. of Agriculture, Bureau of Chemistry, *Bull.* 107 (revised), p. 80) in which succinic acid is disregarded and citric acid is separated from the malic acid by heating the water solution of the calcium salts of the two.

5. The method of F. Mutellet (*Annales des Falsifications*, 2, 383-6), for tartaric, citric and malic acids, in which the tartaric acid is separated as the acid potassium salt and the other two are separated from each other as the barium salts, by a half volume of alcohol.

6. The method for tartaric acid by J. von Ferentzy (*Chem. Ztg.*, 31, 1118 (1907)), as applied by L. Gowing-Scopes (*Analyst*, 33, 315-9 (1908)), in which the tartaric acid is precipitated as a basic salt by the magnesium mixture and thus separated from the malic or succinic acids.

7. The method for separating aconitic acid by A. Behr (*Ber. d. chem. Ges.*, 10, 351 (1877)), in which the acids are first separated in a free state by means of the lead salts and hydrogen sulphide, then the acid ammonium aconitate formed, which proved to be a suitable compound for its further purification, liberating the acid finally with sulphuric acid and shaking out with ether.

² *Loc. cit.*

aconitic acid. The aconitic acid, however, has been found present in cane juice and its products by Behr,¹ in sugar beet products by v. Lippmann,² and in sorghum by Parsons.³ My own investigations, as will be shown further on, proved this acid to be not only present in Louisiana cane juice and molasses, but to be the predominating one of the acids. As aconitic acid and its salts are, in most of their properties which are involved in the analytical operations, very similar to some of the other acids commonly occurring in plant juices, its presence in the cane products especially complicates the problem of determining which of the other acids are present and in what quantities. The methods available, even in the absence of aconitic acid, are sadly deficient as means of sharp-cut separations. These circumstances led me to make many preliminary experiments on the properties of some of the salts, and the behavior of the acids under various possible treatments for their separation. The results from some of these lines of preliminary experiments are submitted in the following:

I. SOME RESULTS FROM PRELIMINARY TESTS.

1. *Extractibility of the Free Acids with Ether.*—In the method of Jørgensen⁴ in its original form, which method first of all I attempted to apply, we are directed to shake out with ether, repeating two or three times. This proved to be insufficient to extract even 90 per cent. of the succinic acid, as may also be calculated from theoretical grounds, basing the calculations on the relative solubilities of the acid in water and ether. By trial with a solution of succinic acid, making three extractions with equal volumes of ether each time, we recovered less than half the acid. More recently Jørgensen has published a modification of his method⁴ in which he requires more thorough extraction. As aconitic acid is also readily extracted with ether, it is evident that with the cane products it would not suffice simply to titrate this extract with a standard alkali to determine the succinic acid in it. Kunz⁵ offers a procedure with the ether extract to get the true amount of succinic acid, involving the oxidation of the acids other than succinic acid with potassium permanganate in an acid solution, then precipitating the succinic acid with standard silver nitrate solution and titrating back the excess of silver nitrate. He also suggests a more satisfactory way of extracting the solution with ether, using a form of what some German writers⁶ have chosen to call "perforation apparatus." Kunz used the Schacherl⁶ apparatus. Not having this apparatus on hand, I improvised a substitute which was more efficient and at the same time very simple in construction, using it on an ordinary ether extraction flask, and with a tin spiral condenser (see Fig. 1).

The tube *B* to hold the liquid to be extracted may

¹ *Loc. cit.*

² *Ber. d. chem. Ges.*, **12**, 1649 (1879).

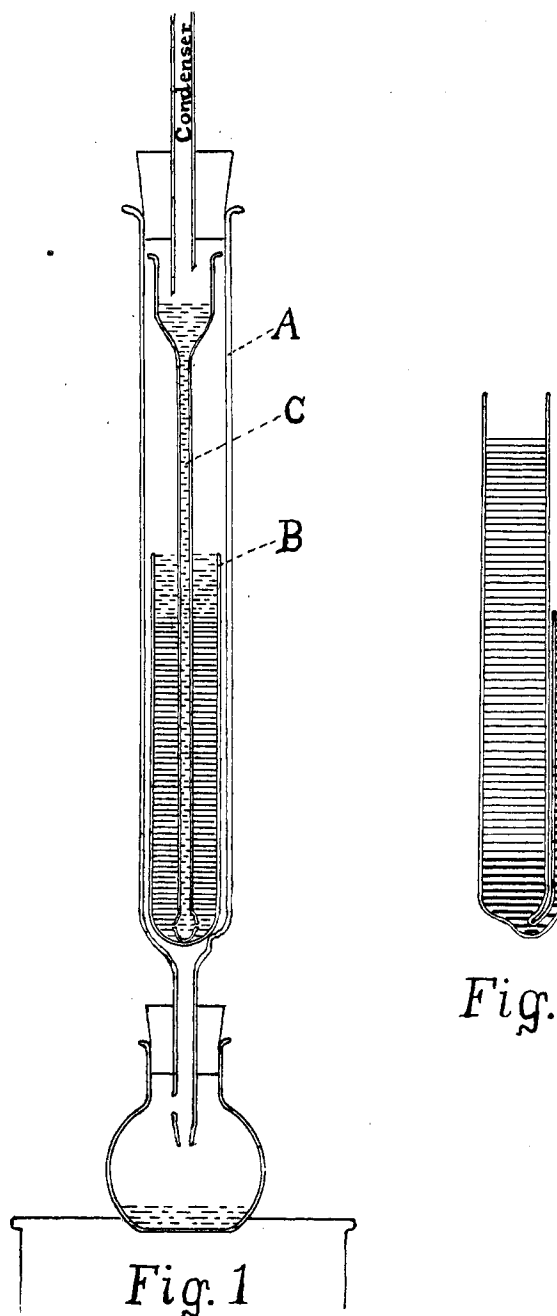
³ *Am. Chem. J.*, **4**, 39; *Ber. d. chem. Ges.*, **15**, 1763 (1882).

⁴ *Z. Unters. Nahr.-Genussm.*, **17**, 396 (1909). *Chem. Abstr.*, **3**, 1781 (1909).

⁵ C. v. d. Heide and H. Steiner, *Z. Unters. Nahr.-Genussm.*, **4**, 114-6 (1902).

⁶ *Ibid.*, **4**, 174-6 (1902).

be chosen of a size to suit the volume, increasing also the size of the outside tube *A*, if necessary. Tube *A* has three indentations around the conical part at the bottom to prevent tube *B* from sealing off the communication with the ether flask below. To avoid too rapid a delivery of ether while filling or under irregular returning from the condenser, so as to cause some of the water solution to be carried over, the openings



at the bottom of the delivery tube *C* should be suitably constricted. Evidently a similar apparatus could be used for extraction (or "perforation") with a heavier liquid like chloroform. For *B* we could substitute one like that represented in Fig. 2 and tube *C* can be omitted or else shortened and suspended with its lower end at the surface of the liquid in *B*. The

solutions with which I had occasion to deal showed little or no tendency to emulsify, therefore I could hurry the extraction as much as the efficiency of my condenser allowed. A tin spiral condenser with an inside diameter of only $\frac{1}{4}$ inch proved too small for the return flow during rapid boiling, yet even with such and with the cooling water at 20 to 23°, 10 hours sufficed to extract practically all the succinic acid. With $\frac{3}{8}$ -inch tin tubes for condensers to this apparatus, the time could doubtless be reduced to 5 or 6 hours. C. v. d. Heide and H. Steiner² have recently subjected the Kunz method for determining the succinic acid to a critical study, and the former of these describes a more complicated apparatus which should be more efficient provided the condenser has sufficient capacity. They report 9 hours, when boiling as fast as they did, usually required for complete extraction, recommending 12 hours for certainty in all cases. They also conclude that the oxidation of other substances accompanying the succinic acid should be carried out in the neutral solution instead of the acid solution.

A number of extraction tests were made with other organic acids under similar conditions to determine approximately to what extent these would accompany succinic acid, to disturb the acidimetric titration of the extract as a measure of the succinic acid, or to occasion losses in the water solution if that is to be used for the determination of other acids, *e. g.*, citric and malic.

Aconitic acid in tests made with solutions of the acids derived from cane juice seemed to be extracted about as rapidly as succinic acid from solutions to which the latter had been added.

In tests with 0.1 *N* citric, tartaric, or malic acids, the same rate and time of boiling which would completely extract the succinic acid, sufficed to extract 37, 23 and 53 per cent., respectively, of these acids, and with mixtures of any two of these approximately the same proportions of those present were extracted, as when each was present alone. In mixtures containing malic and tartaric acids, the amount of each was determined by the polariscopic method reported in a previous paper.² With lactic acid, about twice the time required for succinic acid sufficed for complete extraction of the lactic acid. The extract, after dissolving in water, had practically the same proportion of lactic acid to actual lactic acid as the original solution, as may be expected from equilibrium considerations.

From the foregoing it is evident that the succinic, aconitic, and lactic acids can readily be extracted quantitatively with ether by use of a suitable apparatus, but that it is hardly practicable to extract tartaric, citric, or malic acids quantitatively from their solutions in water. The tests show, also, that the first three acids, if extracted from a solution containing also one or more of the last three, will not thereby be separated free of the last three, and that the residual water solution will not contain the total

of the last three. The separation may, of course, be accomplished more nearly quantitatively by redissolving the ether extract in water and reextracting, and repeating this as often as necessary to attain the degree of accuracy desired.

2. *Solubility or Precipitability of the Acids as Calcium Salts.*—In the first series of tests under this heading, the salts were formed by the addition of calcium acetate in excess to solutions of the free acids. In these, of course, acetic acid is a by-product. In later tests with a few of the acids, the salts were formed by the addition of milk of lime and lime water to the acid, in some cases just to neutrality and in others in excess. In the first series only broad approximations were aimed at, no very definite conditions as to temperature, agitation, and time of standing being maintained. The results from either series have no value as accurate physical constants, but can serve the purpose of suggesting methods for the separation of the acids, allowing, of course, also for the possible modifying influence of other substances present in natural products upon the solubility of these salts. The tests were made by trying different concentrations until a strength of the acid was reached which would just suffice to call forth a precipitate with the excess of calcium acetate, or calcium acetate and 95 per cent. alcohol sufficient to make it 50 per cent. alcohol or 85 per cent. alcohol. Unless otherwise stated, the results given in the accompanying table represent upper limits in strength (in grams per 100 cc. before the addition of alcohol) which do not suffice to give precipitates immediately or very soon, and without rubbing the sides of the tube or inoculating with crystals of the same salts.¹

TABLE I.—UPPER LIMITS, IN GRAMS ACID IN 100 CC. WATER WITH EXCESS OF CALCIUM ACETATE, WHICH DOES NOT CAUSE A PRECIPITATE.

Acids used.	In water alone.	With addition of alcohol to 50 per cent.	With addition of alcohol to 85 per cent.
Tartaric acid	Less than 0.1 if stirred for some time	0.13 for immediate precipitation	0.01
Citric acid	More than 0.32 if cold Less than 0.32 if hot	0.08	0.008
Aconitic acid	More than 5.00 cold or hot ²		
Malic acid	More than 5.00	0.14, less if heated	0.0025
Succinic acid	More than 5.00	0.88	0.03
		2.5, but after starting much ppt. formed	0.05

Calcium acetate alone in water solution, if too concentrated, will gelatinize or form a jelly-like precipitate on addition of alcohol. Between 5 and 6 $\frac{2}{3}$ per cent. was found to be the upper limit in concentration of the water solution which will not give a precipitate when 95 per cent. alcohol is added until an alcoholic concentration of 85 volume per cent. is reached.

In a series of tests in which the calcium salts of citric, aconitic, and malic acids were formed by the addition of calcium hydroxide to the free acid, and

¹ The supply of alcohol from which I used was later discovered to have a considerable acidity, due to volatile acids, which may have exerted some influence in keeping more of the salts in solution.

² See, however, the discussion of aconitic acid below for the behavior of its calcium salt in a neutral solution when heated.

¹ *Z. Unters. Nahr.-Genussm.*, **17**, 291-307, 315-320 (1909).

² *THIS JOURNAL*, **3**, 563-574 (1911).

in which the alcohol used was absolute alcohol, results were obtained differing in some particulars from those given above, and showing also, in the cases tried, a marked difference in the solubility of the salt in a solution exactly neutral (to phenolphthalein) and one having an excess of calcium hydroxide.

With tartaric acid: The concentration in a neutral water solution which, without addition of alcohol, fails to give a precipitate within 20 hours, lies between 0.025 and 0.05 per cent. With the addition of an equal volume of alcohol, the limit is between 0.025 and 0.033 per cent. for immediate precipitation and between 0.0033 and 0.0045 per cent. for precipitation on standing 20 hours. The mother liquor, from which calcium tartrate was precipitated by addition of an equal volume of alcohol and standing 20 hours, contained in two trials the equivalent of 0.0021 per cent. and 0.0030 per cent., respectively, of tartaric acid, computed on the volume of water exclusive of the alcohol.

With citric acid: The limit in water solution with calcium hydroxide to exact neutrality was about 1.42 per cent. in the cold and between 0.25 and 0.3 per cent. in the boiling hot solution, and in each case it was less with an excess of calcium hydroxide.

With aconitic acid: A 10 per cent. solution of the acid in water, neutralized with dry calcium hydroxide, was insufficient to give a precipitate in the cold. Heated to boiling, a 1 per cent. solution, calculated as free acid, sufficed to call forth a precipitate of the calcium salt. In other experiments it developed that the water adhering to the precipitate of (impure) calcium aconitate, on decanting from the precipitate formed on boiling, sufficed to dissolve the precipitate on cooling.

With malic acid: 0.25 per cent. of the acid, exactly neutralized with calcium hydroxide just sufficed to bring forth a precipitate upon addition of an equal volume of absolute alcohol. With an excess of calcium hydroxide, considerably less sufficed.

With lactic acid: A 10 per cent. solution, neutralized with calcium hydroxide, did not give a precipitate, even when an equal volume or when 8 volumes of absolute alcohol were added.

From the foregoing notes on aconitic acid, we can correct the impression that is current and is frequently reproduced in text-books,¹ that the citric acid is the only one of the natural organic plant acids whose calcium salt is less soluble in hot than in cold water. The difference between the solubility of the calcium salt in cold and in hot water is in fact greater in the case of the aconitic acid than in the case of the citric acid.

With lactic acid: A solution of the acid, neu-

tralized with calcium hydroxide and made up to a 15 per cent. solution, gave no precipitate when an equal volume of 95 per cent. alcohol nor any immediate precipitate when 14 volumes of 95 per cent. alcohol were added.

The solubility of the malate of lime in 50 per cent. alcohol is so much greater than that of the lime salts of the other acids mentioned, excepting lactic acid, and so much less soluble in 85 or 90 per cent. alcohol than the calcium lactate, that this affords us a means of removing from malic acid the main bulk of these other acids, if present in sufficient quantity to interfere with my polariscopic method for estimating malic acid, as suggested in my report on that method.²

3. *Solubility or Precipitability of the Acids as Barium Salts.*—The properties of the barium salts of these acids are in many respects known to be analogous to those of the calcium salts. With lactic acid direct tests were made on the solubility of the barium salt with results same as noted above for the calcium lactate. In the application to the acids of cane juice of a method of separation by means of the barium salts, depending upon their different solubilities in water and in alcoholic mixtures of different strength, as in Jörgensen's² method above referred to, the aconitic acid, if present, may be precipitated partly with citric acid and partly with malic acid. Thus in an attempted determination of the cane juice acids by Jörgensen's method, before this fact was taken into consideration, we obtained results indicating from 0 to 0.033 per cent. citric acid and from 0.059 to 0.094 per cent. malic acid from the same sample, according to the number of times we would redissolve and reprecipitate, when in fact, as afterward proved, the sample had but traces or none of these two acids present.

4. *The Acid Ammonium Salts of Aconitic, Citric, and Succinic Acids.*—Behr³ made use of an acid ammonium salt for the purification of aconitic acid, forming it by saturating a portion of the aconitic acid with ammonia, boiling to drive off the excess of ammonia, then adding an equal amount of the free acid. In my work on cane juice acids this method also served this purpose admirably. However, in parallel tests with solutions of succinic acid (which, if present, might accompany aconitic acid in an ether extract) the results were so nearly analogous that this acid ammonium salt could hardly serve as a means for the quantitative separation of these two.

5. *Von Stahre's⁴ Pentabrom Acetone Reaction and Denige's⁵ Acetone Dicarboxylic Acid Reaction as Means of Detecting and Identifying Citric Acid.*—The former of these reactions did not, in our hands, prove a very delicate test for citric acid. The latter reaction, however, is very delicate. Denige's reagent is 5 g. mercuric oxide in 20 cc. concentrated sulphuric acid and 100 cc. water. To 4–5 cc. of the solution (which

¹ Fresenius, "Anleitung zur qualitativen chemischen Analyse," 16th Ed., p. 437; Allen, "Commercial Organic Analysis," 3rd Ed., Vol. I, p. 530.

(Into the latter treatise has crept the misleading statement: "Aconitate of calcium, $\text{Ca}_2(\text{C}_6\text{H}_5\text{O}_6)_2 + \text{H}_2\text{O}$, forms small crystals which require 100 parts of cold water for solution, but are much more soluble in boiling water. Hence aconitic acid gives no precipitate with lime water either in the cold or on boiling, a behavior which distinguishes it from citric acid." The latter statement may be true for so dilute a solution as lime water, but could not be said to apply to a much stronger solution, such as might be obtained by adding milk of lime to aconitic acid.)

² THIS JOURNAL, 3, 563–574 (1911).

³ Z. Unters. Nahr.-Genussm., 13, 241–257 (1907); Chem. Abstr., 1, 1448–52 (1907).

⁴ Ber. d. chem. Ges., 10, 351 (1877).

⁵ Chem. Zentr., II, 418 (1895).

⁶ J. Soc. Chem. Ind., 1898, 802; Chem. Zentr., II, 134 (1898).

should be very dilute in citric acid) is added 1 cc. of Denige's reagent and heated to boiling, then 5 to 6 drops of a 2 per cent. potassium permanganate solution are added. With citric acid in quantities down to mere traces, the first drop of potassium permanganate solution would always call forth a cloudiness due to a very finely divided, readily subsiding precipitate. The test was tried upon the following other substances: aspartic, malic, oxalic, tartaric, lactic, glycolic, fumaric, and succinic acids, and upon pure sucrose. None of these gave a reaction likely to be mistaken for that from citric acid. Oxalic acid gave a white precipitate with the mercury reagent alone, but when this was filtered off, and the potassium permanganate added to the filtrate, no additional precipitate was formed. The test revealed citric acid also in the presence of any of these substances.

When, however, the test was applied to a sample of aconitic acid, which we had on hand, it gave apparently the same reaction as citric acid, differing only in that it was usually not until the second or third drop of potassium permanganate solution was added that the fine pulverulent precipitate formed. Another preparation of aconitic acid, supplied by Messrs. Eimer and Amend, likewise gave the reaction. Whether this was due to contaminations with citric acid, from which the aconitic acid was presumably prepared, remains undetermined. Considering, however, the close relation in constitution, of the aconitic to the citric acid, on the one hand, and to the acetone dicarboxylic acid on the other, I think there is but little doubt that the aconitic acid itself is capable of giving this reaction. We may likewise expect the tricarballic acid to give this reaction. Since aconitic acid and possibly also tricarballic acid are present in the cane juice, the fact that we got the reaction in cane juice with Denige's reagent does not prove either the presence or the absence of citric acid. The fact that here also not the first drop of potassium permanganate called forth the precipitate is some evidence that it is not present.

II. EXAMINATION OF SAMPLES OF CANE JUICE.

From the many samples of juice, molasses, and unrefined sugars examined by one or another course of procedure, I shall here report merely a few of the more definite or conclusive findings from one of the samples, with a few references to results from other samples for comparison.

The sample consisted of 6 liters of juice pressed from cane harvested at the beginning of the season, about Oct. 1, a mixture of the four varieties, D74, D95, La. Purple, and La. Striped.

The initial operations were as follows: The juice was neutralized with sodium hydroxide, precipitated with neutral lead acetate and an equal volume of 95 per cent. alcohol, filtered next day and the precipitate washed with 50 per cent. alcohol, then suspended in hot water, decomposed with hydrogen sulphide, filtered, and the filtrate concentrated upon the water-bath to a small volume. A small portion of this was titrated with normal potassium hydroxide. It indicated acidity in the total residue equivalent to 125.1

cc. normal acid. The titrated portion was united with the rest, the whole diluted with water and precipitated with an excess of calcium acetate and alcohol to an alcohol concentration of 85 per cent. After standing a day it was filtered. The precipitate of calcium salts was treated three times with water, twice with 200 cc. and once with 50 cc. The water solutions were each treated first with alcohol to 50 per cent., filtering after one or more days, then the filtrates further treated with alcohol to 85 per cent., filtering after one or more days. The insoluble residue (which I shall designate A), the precipitates from 50 per cent. alcohol (B, B', and B''), and those from 85 per cent. alcohol (C, C', and C'') were each investigated further by a different course of procedure. Many different operations were applied to these residues and products from them, some of which will be mentioned in the following paragraphs in connection with individual acids reported upon.

1. *Oxalic Acid.*—This acid, if present, should have been in the residue A, of calcium salts which failed to dissolve in water. This residue was treated with acetic acid; the portion not dissolved in acetic acid was dissolved in hydrochloric acid, then reprecipitated with ammonium hydroxide and calcium acetate, acidifying again with acetic acid. A small precipitate was hereby obtained, equivalent to 0.0027 gram oxalic acid. With manganese dioxide and sulphuric acid this residue evolved carbon dioxide, thus verifying the conclusion that it is an oxalate.

2. *Phosphoric Acid.*—This made its appearance in three different residues, two of them from residue A above, which had failed to dissolve in water, and one from a combination of B and B' precipitates from 50 per cent. alcohol. Residue A was treated with acetic acid, the solution in acetic acid was treated with alcohol to 50 per cent., and both precipitate and filtrate examined for phosphoric acid. The precipitate had 0.1586 gram, computed as P_2O_5 . The acidified alcoholic filtrate, after conversion by means of potassium carbonate to the potassium salts and examining for tartaric acid, proved to contain 0.0241 gram phosphoric acid as P_2O_5 . From the residue B and B', obtained by dissolving in water and reprecipitating from 50 per cent. alcohol, I separated 0.0060 gram as P_2O_5 . Thus a total of 0.1887 g. phosphoric acid as P_2O_5 was found.

3. *Sulphuric Acid.*—No effort was made to trace this out quantitatively in all the residues in which it might occur, *viz.*, A, B, B' and B''. In a product from C', the precipitate from 85 per cent. alcohol, I also found a trace of sulphuric acid. From residues B and B', after further operations that might have occasioned small losses, I found 0.0305 g. sulphuric acid as SO_3 .

4. *Tartaric Acid.*—The calcium tartrate, if present in large quantity, should have appeared in A; or if present in small quantity, it should have appeared only in residues B, B', and B'', or possibly in small traces in residues C, C', and C''. Products from A, B, and B', tested in small volume for tartaric acid by conversion to the acid potassium salt, gave nega-

tive results, hence tartaric acid cannot be present in more than mere traces. In other more direct tests made upon cane syrup it also proved to be absent there, in that the acids from 100 grams syrup, separated by use of the lead salts, gave no precipitate from 50 cc. solution in water after neutralizing with calcium hydroxide. This was further verified by the polariscopic method with the uranium compound, in that the uranium produced little or no change in the optical activity.

5. *Malic Acid*.—This, if present, should have appeared in residues C, C', and C'', precipitated from the 85 per cent. alcohol, but principally in C. From this residue the acids were set free by converting them into the lead salts and decomposing the lead salts with hydrogen sulphide as in the initial operations with this sample of juice. The acids thus liberated were examined by the polariscopic method with the uranium salt¹ and showed a mere trace of malic acid present, *viz.*, 0.0170 gram. C' and C'' similarly yielded 0.0101 and 0.0040 gram, respectively. From B and B' combined, there were recovered additional traces, 0.0095 and 0.0054 gram. The total in these five portions is 0.0461 gram. Tests with cane syrup, as stated in the former report, gave results indicating about 0.01 to 0.03 per cent. malic acid in the syrup.

6. *Succinic Acid*.—This should be found principally in residue B, B' and B''. B and B', combined and dissolved in water, was divided into two portions. The first portion of $\frac{1}{3}$ was acidified and extracted with ether by use of the apparatus previously described. In the first five hours, with slow boiling, 0.7438 gram was extracted; in the next 15 hours, 0.1489; and in the next 12 hours, 0.0212 gram. Each residue was dissolved in 25 cc. ether and $\frac{1}{3}$ of each taken. These portions were combined, the ether evaporated, the residues dissolved in water, acidified with sulphuric acid, oxidized with an excess of potassium permanganate, again extracted, the extract residue neutralized and tested with silver nitrate according to Kunz.² It gave no precipitate, hence there was no succinic acid present. The other $\frac{2}{3}$ of the above extracts, after first separating the main bulk of the aconitic acid by heating the solution of the calcium salts, was also tested by Kunz's method for succinic acid and none found.

7. *Aconitic Acid*.—This acid should be mainly in residues B, B', and B'' with smaller amounts also in C, C', and C''. Being readily soluble in ether when free, it should appear along with succinic acid, if the latter were present, in the ether extracts. By conversion of a portion of these residues into the acid-ammonium salt, the crystallization took place so readily and so completely as to make it evident that it was almost pure aconitic acid. The first crystals of brownish color were recrystallized several times from 50 per cent. alcohol to purify a portion, then, when quite colorless, were decomposed with sulphuric acid, extracted with ether, and a melting point determination made. The purified specimen melted at

183–184°, commencing to decompose at the same time. Because of incipient decomposition, and possibly incomplete purification of so small a sample, the melting point was not very sharp, but was near enough to that found by Behr, *viz.*, 187–188°, that this taken in connection with the other properties makes the conclusion that it is aconitic acid a safe one. The first and second residues, mentioned in the preceding paragraphs on succinic acid, extracted by ether from $\frac{1}{3}$ of the solution from B and B' combined, proved on titration to have total acids equivalent to 14 cc. normal acid, which, computed as aconitic acid, amounts to 0.8123 gram (out of 0.8927 gram air-dried residue) or for the total from residues B and B', 2.437 g. No further tests were made to determine the degree of purity of the aconitic acid in these extracts than the indications furnished by the crystallization of the acid ammonium salt above referred to, nor were the smaller residues B'', C, C', and C'' further examined for aconitic acid. Each of these was, in all probability, mainly aconitic acid, except for the small traces of malic acid found in C, C', and C''. Acidimetric titrations of the several acid residues examined from malic acid showed a total acidity, not accounted for as malic acid, equivalent to 0.625 gram aconitic acid, computed upon the whole of the sample. The indications from all these crude acid residues point to about 3 grams, or 0.05 per cent., as the total of aconitic acid in the sample.

In another sample of 5 liters of juice from the same cane, the acids were similarly separated in the free state by means of the lead salts. This acid residue was subjected to ether extraction more directly than was the case in the sample above reported. The total extraction residues after the evaporation of the ether were 2.1542 grams from the first 9 hours, 0.4082 gram from the next 11 hours, and 0.0785 gram from the next 6 hours, a total of 2.6409 grams, or 0.05 per cent. These residues gave the same evidence of being almost wholly aconitic acid, when a pure specimen was prepared from them by means of the acid ammonium salt. The indication as to quantity of aconitic acid in the juice is therefore about the same in this as in the other sample.

8. *Citric Acid*.—This acid, if present, should have appeared in residues B, B', and B'', precipitated as calcium salts from 50 per cent. alcohol. Some products from B and B' which should have contained accumulations of citric acid, if present, could not be made to yield the characteristic crystals of the barium salt. From a new sample of 200 grams cane syrup the acids were separated by means of the lead salts and hydrogen sulphide as in this sample. The acids were converted to the calcium salts, and the solution treated with an equal volume of alcohol. The precipitate thus formed, dissolved in 450 cc. cold water, was heated to boiling and filtered hot. The precipitate thus separated was converted to the barium salt, but did not yield the characteristic barium citrate crystals. I conclude that the citric acid is not present in the juice nor in the syrup used in these tests.

¹ THIS JOURNAL, 3, 563–574 (1911).

² *Loc. cit.*

TABLE II.—SUMMARY OF CONCLUSIONS ON ACIDS IN CANE JUICE ANALYZED.

Names of acids.	Amount from the 6 liters of juice. Grams.	Gram acid per 100 cc. juice.
Sulphuric acid (as SO ₂), total not determined.....	0.0305	0.00051
Phosphoric acid (as P ₂ O ₅).....	0.1887	0.00314
Oxalic acid.....	0.0027	0.00004
Tartaric acid.....	none	none
Malic acid.....	0.0461	0.00077
Succinic acid.....	none	none
Aconitic acid, not determined but indications of about.....	3.0	0.05
Citric acid.....	none	none
Total determined or indicated.....	3.2680	0.05447

Remarks.—If we compare the total of acids accounted for in this summary, equivalent to 60.2 cc. normal solution, with the amount of acidity found in the residue of acids after their separation from the juice by means of the lead salts and hydrogen sulphide, *viz.*, 125.1 cc. normal solution, we find that about half of the acids there present are unaccounted for. Doubtless part of this was acetic acid from lead acetate not completely washed out of the precipitate. Lactic acid was not looked for. If present it would have been left as a calcium salt in the 85 per cent. alcohol which was discarded. Nor was any account taken of tricarballic acid, which v. Lippmann¹ found in sugar beets, along with citric acid and aconitic acid. Some work started on the volatile acids was interrupted before it was finished and no report of interest can at present be made on these nor on such other acids as may be present which are not precipitated with lead acetate. It seems clear from the work thus far accomplished that aconitic acid is the predominating one among those precipitated with lead salts in a neutral 50 per cent. alcohol mixture. The only others thus far identified that are present in significant amounts are phosphoric and sulphuric acids. Oxalic, tartaric, succinic, citric, and malic acids are either completely absent or are present in such small quantities as to have no practical significance to the manufacturer. These facts may have important significance to the sugar chemist and manufacturer, who is seeking to improve the sugar house and refinery operations so as to secure more complete exhaustion of the molasses or to prevent the formation of scale in the evaporation apparatus. This relatively high percentage of aconitic acid is a special characteristic of sugar cane juice and its unpurified products. The suggestion that it occasions the peculiar cane flavor in these products, however, finds no support in the taste of the purified acid, nor in odor or taste developed in heating aconitic acid or its salts, or mixtures of these, with sugar. The free acid facilitates the browning of sugar upon heating, as do also other acids, *e. g.*, tartaric, citric or malic. The phosphoric acid is doubtless removed in part in the clarification process if the juice is limed to neutrality. Such of the phosphate and the sulphate of lime as remains in the clarified juice may become the cause of the formation of scale. With reference to the extraction of sucrose from the molasses, it would

¹ *Ber. d. chem. Ges.*, **11**, 707 (1878); **12**, 1649 (1879).

be interesting to know what are the "molassigenic values" of the aconitic acid and its several salts, *i. e.*, their power of preventing or facilitating the crystallization of sucrose. The high solubility of the lime salt of aconitic acid, even in the hot solution, makes it improbable that any notable quantity of the aconitic acid is removed in the clarification process in which lime is used, or that it is deposited as a scale or sediment during the evaporation in the "effects" or the "pan," unless the presence of sugars to the point of saturation materially reduces the solubility of the calcium aconitate. The aconitic acid therefore accumulates in the molasses, where it is usually present in extraordinary large amounts.

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THE OCCURRENCE OF LEVAN IN SUGAR.

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Greig Smith and Thomas Steele¹ obtained from sucrose solutions inoculated with *Bac. levaniformans* a new gum which they named levan, and of which they made a complete chemical study.

In conjunction with W. L. Owen, bacteriologist for the Louisiana Sugar Experiment Station, in his work on "The Bacterial Deterioration of Sugars"² it became necessary to study this same gum. The results agree with Smith and Steele in that levan hydrolyzes quantitatively into levulose according to the reaction $(C_6H_{10}O_5)_n + n(H_2O) = n(C_6H_{12}O_6)$; that its specific rotary power is about $[\alpha]_D = -40$; that it melts at 200° C.; does not reduce Fehling's solution; and is not precipitated by lead subacetate. Although Smith and Steele found the gum-forming organism to be present in nearly pure cultures and believed that the organism is responsible for the greater part of the deterioration of sugars in storage, they were unable to show by analysis that levan is formed in sugars.

Comparison of results obtained by analyses made monthly of a large number of raw sugars proved that the relation between the fall in single polarization and in sucrose (Clerget) could not be accounted for by inversion. In some cases, while the single polarization was found to decrease the sucrose (Clerget) would slightly increase. Bacteriological examination showed all of these sugars to contain large numbers of the gum-forming organism, and it may readily be seen that should levan be present in a sugar the single polarization would be decreased 0.6° v. for every 1 per cent. gum, and at the same time the sucrose (Clerget) would be increased 0.67° v. by the levan hydrolyzing to levulose during inversion.

To prove that levan was responsible for the unusual changes found to take place in these sugars, attempts were made to separate the gum from solutions of these sugars by precipitating it with alcohol made slightly alkaline with sodium hydroxide. This method, however, brought down so much of the other gummy matter that it was found impossible to separate small

¹ *J. Soc. Chem. Ind.*, Nov. 29, 1902.

² *Louisiana Bulletin*, No. 125.