

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY.]

## THE OCCURRENCE OF RAFFINOSE IN AMERICAN SUGAR BEETS.

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THE sugars first noticed in the Australian Eucalyptus manna by Mudie<sup>1</sup> and by Johnston<sup>2</sup> and called melitose<sup>3</sup> by Berthelot; later identified in the cotton seed as gossypose by Boehm<sup>4</sup>, and finally in molasses and refinery products of the sugar beet and called raffinose by Loiseau<sup>5</sup>, have been shown to be identical with each other. Raffinose has also been found in barley,<sup>6</sup> and in wheat,<sup>7</sup> and is thought to be present in many other plants.<sup>8</sup>

Raffinose, as a distinct kind of sugar, belongs to the not numerous class of tri-saccharides with the formula  $C_{18}H_{32}O_{16}$ . By hydrolysis, its molecule becomes decomposed, forming one molecule each of dextrose, levulose, and galactose.

Its properties have been thoroughly studied and are probably as well understood as those of most of the more common sugars.

Of particular interest is its occurrence in the residual and secondary products resulting in the manufacture of beet sugar. Here it crystallizes with cane sugar, modifying the crystalline form of the latter and increasing the specific rotation markedly. These two properties actually led to the discovery of raffinose in beet sugar products, where its presence for a long time introduced serious and unexplainable errors into the analytical operations of the sugar factory and refinery.

The frequent occurrence of raffinose in the refinery products from beet sugar, and especially those which have been subjected to any of the methods involving the use of strontia, baryta, or lime for the separation of the sucrose from the uncrystallizable molasses, led to the belief that raffinose was a secondary product derived from sucrose or some other sugar in the course of these

<sup>1</sup> *J. de Pharm.*, II, 18, 705.

<sup>2</sup> *J. prakt. Chem.*, I, 29, 485.

<sup>3</sup> *Ann. chim. phys.*, III, 46, 66.

<sup>4</sup> *J. prakt. Chem.*, II, 30, 37.

<sup>5</sup> *Journal des Fabricants de Sucre*, 24, 52; 26, 22, etc.

<sup>6</sup> O'Sullivan: *Chem. News*, 52, 393.

<sup>7</sup> Richardson and Crampton: *Ber. d. chem. Ges.*, 19, 1180.

<sup>8</sup> Scheibler: *Neue Ztschr. für Rübenzuckerindustrie*, 23, 237.

operations and that it was not originally present in the beet juices.

It has, however, been conclusively shown that raffinose is a natural constituent of the sugar beet, from which it has been separated directly by Von Lippmann,<sup>1</sup> and the impossibility of any transformation of sucrose into raffinose by heating with alkalies, has been shown by the investigations of Tollens<sup>2</sup> and others.

The quantity of raffinose in beet juice is, however, very small, amounting to from 0.01 to 0.02 per cent. In beet molasses two to three per cent. of raffinose has been observed, and in various concentrated products of molasses refineries, as high as sixteen per cent. has been noted.

Attention has been called to the fact that the amount of raffinose found in sugar beets, is dependent on climate, soil, seed, conditions of growth, etc., and varies between wide extremes. It might, therefore, reasonably be expected that under some conditions this minor constituent of the sugar beet would be lacking entirely.

The conditions under which sugar beets are grown in America must differ in many respects from those of Northern Europe and many of the observations recorded in the latter country may well be subjected to verification here.

On this account it seemed desirable to ascertain definitely whether raffinose occurs in the products of the American beet.

The attention of one of us was called to certain phenomena observed in the works of the Norfolk Beet Sugar Co., at Norfolk, Neb., and an investigation of the same has furnished the data for this paper.

The process of making sugar from beet juices, as carried on at the Norfolk works, involves the following special operations :

After the clarification, concentration, and crystallization of the beet juices, the uncrystallizable residue is returned to the beginning stages of the process and boiled again with fresh beet juice constituting what is known as a "mixed pan."

This is boiled "to grain," *i. e.*, to such concentration that crystals begin to form, and is sent to the "crystallizer," where,

<sup>1</sup> *Ztschr. des Vereins f. Rübenzuckerindustrie*, 39, 880.

<sup>2</sup> *Ztschr. des Vereins f. Rübenzuckerindustrie*, 39, 921.

being slowly agitated under carefully controlled temperature for about twenty-four hours, an increased crop of crystals is obtained. The molasses remaining at this state is treated by the Steffen's process,<sup>1</sup> which consists essentially in precipitating the sugar from a diluted solution of the molasses, by adding finely powdered quicklime at a temperature of 8° to 12° C.

This product consists in the main of mono- and dicalcium saccharates,  $C_{12}H_{22}O_{11}CaO$  and  $C_{12}H_{22}O_{11}2CaO$ .

These saccharates are mixed with fresh beet juices, and boiled in the "clarifiers" and by subsequent carbonation are decomposed. The sugars thus liberated become mingled with a new portion, fresh from the beets. It will be observed that the same particles of sugar, if they fail to crystallize, may repeatedly pass through this cycle of operations, being recovered finally in the form of saccharates and returned to the beginning of the process.

If now we bear in mind that raffinose behaves like sucrose in many respects, especially toward the alkaline earths, with which it forms insoluble compounds, both in hot and cold solutions, we shall readily see how the small amount originally present in the beet juice may accumulate in the molasses and secondary products of the factory. This tendency is increased by the fact that this sugar would, to a large extent, remain uncrystallized in the presence of a much larger proportion of sucrose, and so at the close of the campaign, practically all of the raffinose present in the entire amount of beets which have been handled during the season may be found in the molasses and second grade sugars which are being repeatedly passed through the cycle of operations described.

If, as has been noted, the amount of accumulated raffinose reaches eight to twelve per cent., or even less, it not only destroys the accuracy of all polarimetric determinations of the purity and concentration of the various secondary products of the factory, but also seriously retards the crystallization of the sucrose itself.

For instance, the presence of even two per cent. of raffinose causes an apparent difference in the purity of the molasses sufficient to determine whether it is to be returned to the pan or be

<sup>1</sup>Stammer: Zucker Fabrikation 1887, 1,047.

sent to the Steffen's process. It is evident, therefore, that successful factory practice may be seriously interfered with if this source of error is ignored.

During the campaign of 1894 and 1895 at the Norfolk factory, it was noticed that the purity coefficient of syrups from the crystallizers, as determined by polarimetric operations, gradually increased, while crystallization seemed to be more and more retarded.

These phenomena could be reconciled with each other only on the assumption of the presence and constant increase of raffinose. Similar observations followed in 1895 and 1896 and it was also observed that the sugar from the crystallizer frequently contained ragged and elongated crystals of abnormal appearance. Frequent inversion tests, according to Creydt's<sup>1</sup> method, indicated the presence of about three per cent. of raffinose, which gradually increased toward the end of the season. On the other hand, attempts to produce mucic acid from the syrups by oxidation with nitric acid, according to Tollen's method, repeatedly failed.

These results, indicating in part the presence of raffinose, seem to render it desirable to undertake the systematic examination of molasses from the "crystallizer," with the object of separating, purifying, and identifying the raffinose, if it should prove to be present. At this time the investigation was transferred to the chemical laboratory of Purdue University.

Renewed attempts to detect raffinose by means of the mucic acid tests were made as follows :

Several ten-gram portions of syrups were oxidized with nitric acid of specific gravity 1.15, according to prescribed methods. Oxalic acid crystallized abundantly from these solutions but no mucic acid could be obtained under these conditions.

Again, to a similar portion of syrup was added so much milk sugar as would yield an amount of galactose equivalent to three per cent. raffinose and this mixture was oxidized in the usual way. This also yielded no mucic acid. The same amount of milk sugar, oxidized in aqueous solution under the same conditions, gave an abundance of mucic acid.

These results illustrate well the unreliability of the mucic acid

<sup>1</sup> *Deutsche Zuckerindustrie*, 11, 757, and 13, 582, etc.

test for raffinose when applied to complex mixtures of salts and organic matters, of which the beet molasses furnishes a good example. Mucic acid was undoubtedly present, as subsequently shown, but was probably held in solution in the form of soluble salts.

Accordingly, steps were taken to separate the sugars in the molasses from other materials with a view of applying the mucic acid test to them directly. This was accomplished by treating about 200 grams of the syrup with an excess of basic lead acetate by which a dense precipitate was thrown down. The liquid was filtered and treated with ammonia until it was strongly alkaline. This produced a second precipitate consisting of lead compounds of sugars. This was filtered, suspended in water, the lead removed with hydrogen sulphide, and the filtrate evaporated to a syrup. To this syrup was added nitric acid of specific gravity 1.15 and the mixture heated in the usual way prescribed in making mucic acid tests.

After three days an abundance of a white crystalline powder separated from the solution, which, after washing and drying, was found to have a melting-point of  $213^{\circ}$  C., showing it to be mucic acid. This may be regarded as a fairly conclusive qualitative proof of the presence of raffinose in the material studied. Nothing short of the actual separation of sugar in pure form and the determination of its constants, can be accepted, however, as conclusive proof of its presence. Numerous methods have been proposed for the isolation of raffinose from mixtures with other sugars and substances.

Most of these methods are based on the insolubility of raffinose in combination with certain metallic oxides as well as its unusual solubility in absolute methyl alcohol. Following any of these methods, however, its separation is acknowledged to be a tedious operation. Our experience in this attempt included many separate methods and operations, from which it will suffice to mention the following:

We first followed the directions of Kodyl,<sup>1</sup> by which the impurities of the syrup are precipitated with basic lead acetate and from the resulting filtrate, the sugars are thrown down by the addition of ammonia.

<sup>1</sup> Oesterreichsch-Ungarische Zeitschrift f. Zuckerindustrie u. Landwirtschaft, 20, 700, and 21, 92.

From this compound of lead and sugars, the latter are liberated by treatment with carbon dioxide or with hydrogen sulphide, and the solution thus obtained may be concentrated to a point of crystallization. Pursuing this method, we were able to obtain a small amount of sugar crystals which had the appearance of raffinose but which were accompanied by other materials of a gum-like nature which prevented their separation in the pure form.

We next employed the method proposed by Scheibler<sup>1</sup> which is based on the principle that strontium oxide throws down sucrose from a cold solution, but in a hot solution will precipitate both sucrose and raffinose. A twelve per cent. solution of about 300 grams of syrup received as much finely powdered strontium oxide as would be dissolved. After standing forty-eight hours, the filtrate received an additional amount of strontium oxide and was boiled for thirty minutes. An abundant precipitate resulted, which was suspended in water and decomposed by means of carbon dioxide. The filtrate from this which was supposed to contain raffinose and sucrose, was evaporated to a syrup, absorbed in dry sand, thoroughly dried and extracted with absolute methyl alcohol, but we were unable to obtain raffinose crystals from the product.

We finally made use of the following method which is a combination of the preceding ones and which proved successful.

2000 grams of syrup were freely diluted with water and treated with basic lead acetate in large excess. Neither sucrose nor raffinose form insoluble compounds under these conditions but a large amount of other material was precipitated from which the solutions were decanted, and after still farther dilution, were treated with ammonia until strongly alkaline. After standing in a warm place some forty-eight hours, a heavy precipitate had settled to the bottom of the vessels. This precipitate is supposed to consist of compounds of one molecule of raffinose or sucrose respectively, with three molecules of lead oxide. This was washed by decantation, suspended in water and treated with sodium carbonate and carbon dioxide until all of the lead had been removed. The filtrate therefrom was concentrated to a thin syrup. It was examined with the polarimeter and estimate made

<sup>1</sup> *Ber. d. chem. Ges.*, 18, 1409.

of the amount of sugar it contained, calculated as sucrose. Strontium oxide was added in the proportion of three molecules to one of sugar and the mixture maintained at a temperature of boiling water for three hours, during which a fine, yellowish white, granular precipitate was thrown down. This presumably consisted of strontium saccharate and raffinosate. It was noted in this and subsequent operations that it was not possible to remove all of the sugar from the solution by this process since the filtrate from these combined strontium compounds, invariably possessed a rotatory power equal to about two to four per cent. of sugar. The strontium compounds thus obtained were decomposed by treatment with carbon dioxide; the solution filtered off and concentrated to a thick syrup. After forty-eight hours, fine crystals could be seen under the microscope, and after a week, the mass had become solid. These crystals were long and needle-like when seen under the microscope. To the naked eye they presented a silky, shimmering appearance, often gathered in bunches radiating from a common center. No crystals resembling sucrose crystals could be observed. After being thoroughly dried, these crystals showed a specific rotation of  $74^{\circ}$ . That of pure raffinose is  $104^{\circ}$  to  $105^{\circ}$ . Of pure sucrose  $66.5^{\circ}$ . These results indicated a mixture of which the greater portion was probably sucrose, although the increased specific rotation pointed to the presence of a small amount of raffinose. Attempts were made to purify this product by extracting with absolute methyl alcohol by which about half the entire mass was dissolved. This solution, on being brought to crystallization, had every appearance of pure raffinose while the insoluble residue gave a specific rotation of pure sucrose.

This process as described was repeated several times until we had obtained what appeared to be several grams of fairly pure raffinose. But while these crystals were invariably of the general superficial character peculiar to raffinose, it was found that the specific rotation of all the different preparations was very far from being that of the sugar sought, varying from  $72^{\circ}$  to  $85^{\circ}$ . The different portions were therefore combined and subjected to repeated extraction with boiling methyl alcohol. These extracts, on being brought to crystallization showed, however, that the separation of raffinose and sucrose could not be accomplished in

this way, since none of the products show a specific rotation greater than  $80^{\circ}$ .

The next step was to employ cold methyl alcohol for such fractional solutions. According to Scheibler,<sup>1</sup> raffinose is readily dissolved in cold methyl alcohol, while sucrose is scarcely affected. Nevertheless, upon treating our products with cold methyl alcohol, we obtained therefrom portions which, after being crystallized, possessed respectively the specific rotation of  $78^{\circ}$ ,  $75^{\circ}$ , and  $71.5^{\circ}$ , showing that no progress had been made in the separation of the two sugars. Finally, all these products were once more combined, dissolved in water, and left to crystallize slowly. As soon as an appreciable amount of crystals had formed in this watery solution, they were removed, dried, and polarized. Five successive fractions thus obtained showed the following specific rotations:

I.  $(\alpha)_D = 86.7^{\circ}$ .

II.  $(\alpha)_D = 103.7^{\circ}$ .

III.  $(\alpha)_D = 99.2^{\circ}$ .

IV.  $(\alpha)_D = 92.0^{\circ}$ .

V.  $(\alpha)_D = 75.0^{\circ}$ .

Of these portions, evidently Nos. II and III were practically pure raffinose. Nos. I and IV were united and subjected to a second fractional crystallization, from which was obtained one small portion which showed a specific rotation of over  $100^{\circ}$ .

From these results we feel justified in concluding that raffinose occurs in the juices of the American sugar beet in appreciable quantities.

Certain peculiarities observed in connection with the crystallization of these mixtures of sucrose and raffinose, seem to be worthy of special mention.

The influence of small amounts of raffinose upon the form of sucrose crystals has been much commented on and investigated. In general, it is well understood that the presence of raffinose in cane sugar tends to produce elongated, sharp-pointed abnormal crystals. But the microscopic aspect of the crystals of such mixtures has been only rarely referred to and, so far as we know, not figured.

In the course of our investigation we had frequent occasion

<sup>1</sup> *Ber. d. chem. Ges.*, 19, 2868.



to examine preparations of our products under the microscope, and were repeatedly surprised to observe that solutions containing apparently but a very small amount of raffinose, would completely crystallize under the microscope into forms which could scarcely be distinguished from pure raffinose.

The accompanying illustrations demonstrate this tendency far better than any mere description.

No. 1 shows the normal form of the crystals of pure sucrose seen under the microscope.

No. 2. The crystals of pure raffinose.

No. 3. The crystal form of a mixture of the two with a specific rotation of  $76^{\circ}$ .

It is to be noted that the influence of the raffinose is manifested not in the appearance of isolated raffinose crystals but in a modified and abnormal appearance of all of the crystals, which are all similar to each other but entirely distinct from those of sucrose.

This tendency on the part of raffinose to so distinctly modify the crystals of sucrose, would seem to afford a means of readily identifying the presence of small amounts of the former in mixture with sucrose.

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## OUR PRESENT KNOWLEDGE OF ARGON, WITH A PARTIAL BIBLIOGRAPHY.

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SINCE the appearance of the account by Lord Rayleigh and Prof. Ramsay, of their joint discovery, there has been evident, in this country and abroad, a general air of expectation, which even the remarkable results that have been obtained have not been sufficient to remove. In fact, instead of allaying this expectancy it has rather been increased, by the accounts of researches thus far made, so that now, even more than at first, the scientific world is looking forward to the solution of the remaining problems concerning argon, with an interest, rendered particularly intense by the commonly accepted conviction that, upon the nature of the solution will depend, in a great measure, our views of certain chemical theories which we have heretofore regarded as firmly established. While the results of the inves-