The Purines, Pyrimidines and Nucleosides in Beet Diffusion Juice and Molasses

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As a continuation of our work on composition of sugar beets and sugar beet liquors (1)[2] the presence of purines, pyrimidines, and nucleosides was investigated and in some cases the quantities determined.

Purines, pyrimidines, and nucleosides are nitrogen-containing compounds that fall in the class of "harmful nitrogen" compounds. Purines contain 3 nitrogen atoms in a fused 5 and 6 member ring structure. Pyrimidines contain 2 nitrogen atoms in a 6 member ring. There may also be amino groups, methyl groups, etc. attached to the ring. Nucleosides are compounds of purines or pyrimidines and a sugar such as ribose. These various compounds are of interest because they are the basic units of nucleotides which in turn form nucleic acids and nucleoproteins. Nucleoproteins, which are present in all cells, are distinctive for different species of plants, but are subject to minor modification. It may be the minor modifications in the nucleoproteins that make the difference between resistance and nonresistance to virus attacks. The purines and pyrimidines are also of interest since nucleoside derivatives such as uridine diphosphate glucose may assist in the synthesis of sucrose in the beet (2).

Increased knowledge of these compounds and methods of separating them may be important in breeding studies, in processing, in improving the storage quality of beets, and possibly, as the source of by-products.

Some work has been done on the purines and pyrimidine composition of European beets. Early work is reviewed by Janacek (3). He lists adenine, guanine, hypoxanthine, xanthine, 7-methylxanthine, and carnine present in beet diffusion juice. Carnine is an indefinite compound and may be a mixture or a compound of hypoxanthine and inosine (hypoxanthine nucleoside). Vavruch (4), using paper chromatography with only slight purification of the sugar beet juice, identified adenine, guanine, hypoxanthine, xanthine, 7-methylxanthine, and guanosine (guanine nucleoside). Uracil was thought to be present. No work on the purine-pyrimidine composition of molasses is known to the authors.

Materials and Methods.

Molasses was obtained from Manteca and Betteravia, California. Diffusion juice was obtained from Manteca and beets for press juice near Davis, California. Ion exchange resins used were Dowex-50, a cation exchanger and Dowex-1, an anion exchanger, (Dow Chemical Company) and Permutit A, an anion exchanger, (The Permutit Company). Chromatographic paper 589, Blue Ribbon, Schleicher and Schuell, was used for paper chromatography with the following solvents: For two-dimensional chromatography, isopropyl alcohol, 170 ml., concentrated hydrochloric acid, 41 ml., water to make

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[2] Numbers in parentheses refer to literature cited.

250 ml. (5) followed by *n*-butyl alcohol saturated with water, 100 ml., concentrated ammonium hydroxide, 1 ml. (6). For one-dimensional chromatography, water-saturated *n*-butyl alcohol (7) and *n*-butyl alcohol, 77 percent water, 13 percent formic acid, 10 percent by volume (7), were used in addition to the other solvents. A Cary Model 11 Spectrophotometer (Applied Physics Corporation) was used to measure the absorbance of solutions in the ultraviolet. A Mineralight Model V-11 (Ultra Violet Products, Incorporated) was used as a source of shortwave ultraviolet light to detect the purine or pyrimidine spots on paper.

Both ion exchange and paper chromatography were used in this Laboratory for the separation and estimation of the purine-pyrimidine type of compounds. Advantage was taken of the strong absorption of ultraviolet light in the region of 260 millimicrons to follow the course of elution and to detect the presence of these compounds on paper. Preliminary work used the general method described by Wall (8). The solution containing purines, etc. was loaded on a column of cation exchange resin (D-50, 60-100 mesh) and eluted with an increasing concentration of hydrochloric acid. To simplify our problem of detection we passed the column eluate through a cell in the Cary Spectrophotometer and obtained a continuous record of the light adsorption at 260 millimicrons. This method was relatively satisfactory for some constituents but compounds that were eluted early were obscured by the presence of large amounts of colored compounds. A different method was necessary to separate the mixture of colored materials from the purines and pyrimidines.

Most of the purines and pyrimidines are adsorbed by either cation or anion exchange resins. A few, such as orotic acid, uracil, and uridine, are too acidic to be adsorbed by cation exchangers and are removed from solution only by anion exchangers. The following method was devised to give a considerable degree of purification prior to paper chromatography. A solution of dilute molasses was passed through a column of cation exchanger (Dowex-50, hydrogen form) and washed with water. The effluent contained acids, sugars, certain purines and pyrimidines such as uracil and some colored materials. This solution was passed through a column of anion exchanger (Permutit A, hydroxide form). The column was washed with water. Sugars and other neutral materials passed through. Acids, pyrimidines, purines, and most colored materials were retained. The column was eluted with saturated aqueous carbon dioxide. The purines or pyrimidines were eluted while the stronger acids and most of the colored material remained on the resin. The solution was concentrated under vacuum. The residue was dissolved in water or dilute (0.1—.5N) hydrochloric acid and chromatographed with a suitable solvent.

Most of the purines remained on the cation resin. These were eluted with 0.4N ammonium hydroxide. The ammonia was removed by vacuum concentration. This solution contained betaine and the amino acids in addition to a number of purines such as adenine and guanine. The solution was passed through a column of anion exchanger (Dowex-1, carbonic acid form). To obtain the resin in this state a suspension of the hydroxide form of the resin was treated with carbon dioxide gas until the pH of the

supernatant was less than 5. With the resin in this form amino acids and betaine pass through and the purines remain and were eluted with aqueous carbon dioxide solution. The resin does not need to be regenerated after each batch and may be used several times. Glutamic and aspartic acids were retained on the resin but were not eluted with carbon dioxide solution. This will give a gradual build-up of these compounds. The purine-containing solution was concentrated under vacuum and chromatographed in a manner similar to the effluent from the Dowex-50.

The purine and pyrimidine spots are located on paper by using a Mineralight having a high light emission at 253.7 millimicrons. Near this wavelength purines and pyrimidines have a high absorbance and show up as purple spots on the paper against a fluorescent background.

For quantitative determinations the original solution of molasses, e.g., 400 ml. of 10 brix molasses, was treated by the ion exchange procedure and concentrated to a small volume. Final concentration and drying may be done in a vacuum desiccator over calcium chloride. There were two samples. The first, called the uracil fraction, is that portion which is not retained on the cation resin. The second, called the adenine fraction, contained those materials that are retained by the cation exchanger. Each is made to a definite volume (10-25 ml.). The samples should be stored in the refrigerator with a small amount of chloroform in the flask. The solutions are applied to the paper with a 0.01-ml. self-filling pipette to a total volume of 0.04 to 0.08 ml. The paper should be dried after each addition. The paper was chromatographed with a suitable solvent and then air dried for several hours. The spots were located with the Mineralight and circled lightly in pencil. They were cut out and extracted for two or more hours in a 10-ml. beaker with 6.00 ml. of 0.1N hydrochloric acid. Approximately 5 ml. of the solution was placed in a 10-cm. micro tube and the absorbance measured in the Cary spectrophotometer. Actually the curve for the absorbance was run from 400 to 220 millimicrons and recorded on the chart. The curve was an additional aid in identification. A blank curve was also run on the paper. In addition to aiding in identification the curves also enabled a more accurate estimation of the blank. The quantity of the purine was calculated with the values for molar absorbance in 0.1N hydrochloric acid (9, 10, 11). A flow diagram is presented in Figure 1.

Results:

Using the method of Wall (8) separations were made of Manteca molasses and Manteca diffusion juice. Because of the colored materials eluted near the beginning of the fractionation, uracil and other substances acting like uracil could not be detected. With this method, adenine appeared to be the principal constituent in molasses with a considerably lesser quantity of guanine and hypoxanthine. Xanthine was not detected. Xanthine and hypoxanthine were found to be present in the diffusion juice but guanine and adenine were absent, or nearly so. This is different from the work reported by Janacek (3) and Vavruch (4). The former reported guanine as the principal constituent and the latter reported adenine as the principal purine with a lesser quantity of guanine. The amino acid tryptophan

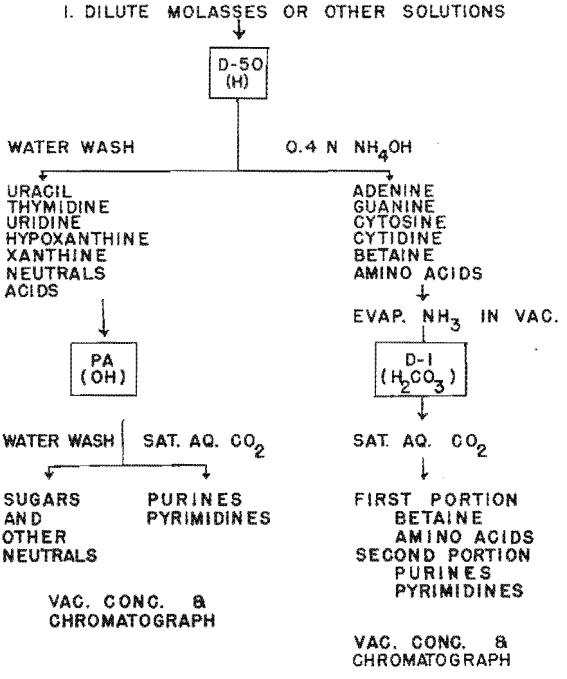

Figure 1.—Ion exchange separation of purine-type compounds.

previously unreported was also found in molasses by the method of Wall (8). Tryptophan was eluted after adenine by the use of 8N hydrochloric acid.

Manteca molasses was treated by the ion-exchange procedure previously described. The fraction not held by the cation resin was treated with the anion resin (Permutit A) and the product chromatographed.

The following compounds were identified from this fraction by the crystallographic properties of their derivatives: uracil, uridine, and hypoxanthine. Thymidine was identified by its chromatographic behavior and ultraviolet absorbance curves in alkaline, acid, and neutral solution. Guanine and adenine were found in the portion eluted from the cation resin and identified by the crystallographic properties of their derivatives.

Table 1.—Purines, Pyrimidines, and Nucleosides in Beet Molasses.

Compound	mg./100 grams non-sugars	mg./l. molasses
Thymidine	15	45
Uridine	210	630
Uracil	7	21
Adenine	150	450
Guanine	100	300
Hypoxanthine	10	30
Unknowns (est.)	15	45

Quantitative data for molasses are given in Table 1. The results are in milligrams per 100 grams of non-sugars and are estimated for a liter of molasses.

Press juice was obtained from a lot of well-mixed cossettes that had been frozen and then thawed with a small amount of water before pressing. The juice was filtered and then treated by the ion-exchange procedure described earlier in this paper. In addition to the compounds found in molasses cytosine and cytidine were identified by their chromatographic behavior and ultraviolet absorption curves. The quantitative data are given in Table 2 and compared with earlier reports on European beets.

Table 2.—Purines, Pyrimidines, and Nucleosides in Sugar Beet Juice (mg./l.)

Compound	Janeck (3)	Vavruch (4)	WURB
Adenine	28	1	40
Guanine	80	3	25
Hypoxanthine	52	4	35
Xanthine	50	5	?
7—Methylxanthine	28	6	?
Uracil		probably	60
Cytosine			5
Guanosine		2	?
Uridine			135
Thymidine			20
Cytidine			10

Several of the compounds in molasses have a high retail value. The retail price of adenine is $2.20 per gram, uridine $4.50 per gram, and thymidine $29.00 per gram. On this basis a liter of molasses has a retail by-product value of $5.00. Probably the compounds could be recovered economically at a price very much lower than this. If a sufficient market could be developed the worth of molasses would be greatly increased.

Summary.

1. Some of the purines, pyrimidines, and nucleosides present in beet molasses have been identified and the quantities determined.

2. A number of the purines, pyrimidines, and nucleosides present in beet juice have been identified and the quantities determined and compared with work on European beets.

3. An ion-exchange method has been developed for separating these compounds from the bulk of the impurities.

4. Uridine, previously unreported, has been found to be the principal constituent of this class of compounds in beet juice and in molasses.

5. Thymidine, cytosine, and cytidine have been found in beet juice.

6. The presence of uracil, reported by Vavruch (4) has been confirmed.

7. Molasses is an attractive commercial source of uridine, adenine, and tymidine.

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