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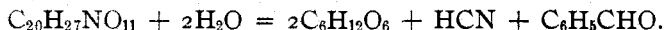
## THE COLORIMETRIC METHOD FOR DETERMINING HYDROCYANIC ACID IN PLANTS WITH SPECIAL REFERENCE TO KAFIR CORN.

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Received August 19, 1913.

This work on a method for the determination of hydrocyanic acid was prompted by the need for an accurate knowledge of the amount of hydrocyanic acid, or glucosides containing hydrocyanic acid, that might be found in Kafir corn, milo maize, Johnson grass and other plants. In work done relative to the amounts of hydrocyanic acid in the saccharin and non-saccharin sorghums, it appeared that there existed no satisfactory method for the estimation of the small percentages of hydrocyanic acid found in plants. These percentages, as shown later, may vary between 0.0018% and 0.03%.

It was observed by Bohm at the beginning of the 19th century that hydrocyanic acid, or a glucoside capable of being broken down into hydrocyanic acid, is present in such plants as bitter almonds, peach kernels, and other fruit seeds.<sup>2</sup> The theory of the action of enzymes on the glucoside amygdalin was announced by Dunstan and Henry.<sup>3</sup> Price<sup>4</sup> working on the enzymes of Indian corn, found that no glucoside capable of furnishing hydrocyanic acid was present in corn stalks. In his experiments, use was made of the ferro-ferricyanide test and the lead acetate method. Price obtained very good results with the ferro-ferricyanide method, detecting 0.0044% hydrocyanic acid in linseed. He gives the following reaction, originated by Liebig and Wöhler in 1837,<sup>5</sup> illustrative of the breaking down of the amygdalin:



Brunnich,<sup>6</sup> in 1903, experimented in one of the British Colonies on sorghum and advised that the plants should not be fed when immature on account of their poisonous characteristics at that period.

Offner<sup>7</sup> used Grignard's sodium picrate paper as a test for hydrocyanic acid in some of the higher fungi, such as *Marasmius oreades* and *Clitocybe infundibuliformis*.

<sup>1</sup> The authors are indebted to Mr. A. A. Jones for many suggestions and for checking the analyses.

<sup>2</sup> Armstrong, "The Simple Carbohydrates and the Glucosides," p. 82.

<sup>3</sup> *Roy. Soc. Proc.*, **67**, 224; **68**, 374.

<sup>4</sup> *Bur. Animal Ind., Circ.* **84**, 74.

<sup>5</sup> Armstrong, *loc. cit.*

<sup>6</sup> *J. Chem. Soc.*, **16**, 788 (1903).

<sup>7</sup> *Bull. Soc. Mycol. de France*, **127**, 342.

Schröder<sup>1</sup> and Dammann in their work on hydrocyanic acid in the Uruguayan millets, such as *Andropogon sorghum* found that the maximum amount, 0.03% HCN, was contained in *saccharatum vulgare*, and 0.02% to 0.014% in *halpensis*. They stated that the dried material contained less hydrocyanic acid than the green plant, due to the loss of the glucoside. The presence of hydrocyanic acid in sorghum and Kafir corn was reported also by Alway, Peters and Slade.<sup>2</sup>

### Experimental.

The following methods for the estimation of hydrocyanic acid were investigated before we decided to select the colorimetric method for special study.

*Silver Nitrate Method.*—To the cold solution of the cyanide add an excess of 10% silver nitrate, acidify with dilute nitric acid and allow to stand over night before filtering. Filter through a porcelain Gooch crucible, prepared with asbestos; wash and weigh as silver cyanide.

The halogens interfere with this test, and it should not be used unless all of them are absent. This test is not applicable where the cyanide may be obtained in small amounts, such as one milligram.

*Ferro-ferricyanide Method.*—To 5 cc. of alkaline potassium cyanide solution, add 15 drops of ferrous sulfate solution, then acidify drop by drop with dilute hydrochloric acid. Add 10 drops of 5% ferric chloride solution. A blue, not green, coloration indicates the presence of cyanides. This test is rather variable, giving in some cases positive results, and in others, negative. The difficulty lies in being unable to keep the solution of ferrous sulfate free from ferric compounds. Care should also be taken that an excess of hydrochloric acid or ferrous sulfate does not spoil the reaction.

*Ammonia-Alum Method.*<sup>3</sup>—Extract the glucoside with dilute alcohol, saturated alum solution and ammonium hydroxide, then filter. Wash the precipitate three or four times with 2.5% ammonia water. Remove the precipitate from the filter and distil, with dilute sulfuric acid, into silver nitrate solution.

This method is open to the same objections mentioned under the lead acetate method below.

*Lead Acetate Method.*<sup>4</sup>—Extract the glucoside with dilute alcohol. Clarify with basic lead acetate. Remove the excess of lead with hydrogen sulfide. Filter and evaporate filtrate to dryness. Take up the residue with hot water, add dilute sulfuric acid, and distil into silver nitrate.

This method is only applicable when hydrocyanic acid is present in

<sup>1</sup> *Chem. Ztg.*, 35, 1436.

<sup>2</sup> *Nebraska Agr. Expt. Sta., Bull.* 77, and *Press Bull.* 27.

<sup>3</sup> *Allen's Organic Analysis*, 3, 92.

<sup>4</sup> *Ibid.*

relatively large quantities, 0.1% or over; when present in smaller amounts only a cloudiness is produced in the silver nitrate solution.

*Mercurous Nitrate Method.*<sup>1</sup>—Extract the glucoside with dilute alcohol. Make alkaline with sodium hydroxide and add three to five cc. of mercurous nitrate solution from a pipet. At the juncture of the two liquids, a black ring will form. Upon shaking the tube, a portion of the ring will dissolve while the remainder becomes light gray. The objection to this method is that it is qualitative only. The delicate color reaction is also very undesirable.

*Thiocyanate Colorimetric Method.*—After considerable experimenting it appeared that the thiocyanate method, with some modifications, would probably be the most satisfactory for determining the very small quantities of cyanides found in such plants as Kafir corn.

Unsuccessful attempts were made to remove the excess of sulfur with bromine or barium chloride. Several sulfides were used in place of ammonium sulfide, but the results were not satisfactory except with potassium sulfide. Five cc. of a solution, 40 grams of potassium sulfide in one liter, may be used in place of the 1 cc. of ammonium sulfide.

In order to render the hydrocyanic acid in the sorghum available, triturate 50 grams of the finely chopped material with 100 cc. of water; wash into a one liter distilling flask with 100 cc. of water. Acidify with 50 cc. of concentrated sulfuric acid and distil about 150 cc. into 50 cc. of a 4% solution of potassium hydroxide. Care should be taken that the end of the condenser dips into the potash solution at all times, or a loss of hydrocyanic acid may result. Make the potash solution to a volume of 250 cc., and use an aliquot equal to one-fifth the entire solution. To this aliquot add 1 cc. of yellow ammonium sulfide and evaporate to dryness on a water bath. Take up with 10 to 15 cc. hot water and barely acidify with dilute hydrochloric acid. Filter through quantitative filter paper until free sulfur is removed. Add one-half cc. dilute hydrochloric acid and boil 5 minutes. Filter to remove free sulfur. Repeat the last operation of boiling and filtering until the solution is absolutely clear.

Make up nearly to the mark in a 50 cc. Nessler tube and add 15 drops of 5% ferric chloride solution. The presence of cyanide in the sample is indicated by a bright cherry-red coloration. If too much acid is present, the solution will be lemon-yellow. If alkaline, the iron will be precipitated, but this condition may be corrected by the addition of a few drops of acid. The exact quantity of potassium cyanide present is determined by matching the color with standards in a colorimeter.

The standard solution is made to contain 15 grams of potassium thiocyanate in a liter of water. This is standardized gravimetrically with

<sup>1</sup> *Am. Chem. J.*, **32**, 480 (1904).

silver until 1 cc. = 14.92 mg. KSCN, which is equivalent to 10 mg. KCN. Fifty cc. of this solution is then diluted for use in the Nessler tubes. Thus 1 cc. of the diluted thiocyanate solution is equivalent to 1 mg. of KCN.

TABLE I.—SHOWING PERCENTAGES OF KCN OBTAINED BY THE SILVER NITRATE METHOD AND WITH THE COLORIMETRIC KSCN METHOD.

Sample.	Calculated per cent. KCN.	AgNO <sub>3</sub> method per cent. KCN.	KSCN method per cent. KCN.
KCN No. 1.....	0.650	0.530	0.555
KCN No. 2.....	0.095	0.098	0.100
KCN No. 3.....	0.980	...	1.000
KCN No. 4.....	0.010	...	0.012
KCN No. 5.....	0.026	...	0.026

All of the above solutions were prepared from Merck's potassium cyanide, which contained 99.68% potassium cyanide. It appears that the colorimetric thiocyanate method gives results which compare well with those obtained by the silver nitrate gravimetric method. There is a tendency toward high results whenever colors are used as standards and it appears to be true in this case. The percentage of potassium cyanide contained in solution No. 3 indicates the high limit of the method. The process gives very satisfactory results for determinations of potassium cyanide in amounts under one-half of 1%. Experiments proved that chlorides, bromides and iodides do not interfere with the reaction.

Chloroform<sup>1</sup> was added to one of the samples to determine if it were thermolyzed during the distilling process. The data obtained indicated that such was not the case when the flame was kept low and not allowed to touch the sides of the flask near the surface of the liquid.

The analyses of several samples of leaves from the Kafir corn plant, collected under varying conditions during the months indicated, are shown in Table II.

TABLE II.—HYDROCYANIC ACID IN THE KAFIR CORN PLANT.

Sample.	Condition of sample.	Colorimetric method.	
		Per cent. KCN.	Per cent. HCN.
No. 1.—Leaves. Plant stunted and frost bitten.			
Collected in November.....	Air dry	0.024	0.0100
No. 2.—Stalk and leaves. Plant in good condition.			
Collected in August.....	Moist	0.0018	0.0007
No. 3.—Leaves, frost bitten. Collected in November.....	Moist	0.0037	0.0015
No. 4.—Entire plant ex roots. Collected in July from field in which stock was poisoned.....	Air dry	0.030	0.0124

The amounts shown in Table II compare with the quantity found by Schröder and Dammann.<sup>2</sup> It appears from the table that Kafir corn which

<sup>1</sup> Crawford, U. S. Bur. Plant Ind., *Bull.* 90.

<sup>2</sup> *Loc. cit.*

is frost bitten, contains more potassium cyanide than the normal plant (No. 2). The plants taken from the field in which the poisoning of stock occurred contained the maximum amount. These plants were very much stunted and almost dry from the lack of water. Although they had been up about six weeks none was over 15 inches high.

The amounts of hydrocyanic acid found in cane and Kafir corn are very small and it is difficult to determine, with certainty, that the quantity is sufficient to kill an animal.

A simple calculation will indicate the amount of the poison that may be present in a cow's stomach. Assuming the capacity of the rumen to be 275 liters, the weight of the contents would be approximately, let us say, 200 kilos. Of this amount at least 50 kilos would be dry matter, and, on a basis of 0.01% of hydrocyanic acid being present in the feed, there would be a total of 5 grams in the stomach. This quantity would, very likely, be sufficient to kill a cow. However, no cases are on record of a beef animal eating 200 kilos (nearly 450 lbs.) at one feeding. Besides, the rumen is not emptied at any time but remains in a well-fed animal at about the same weight, the undigestible matter being removed gradually. For these reasons it is not probable that there would be so large an amount of the hydrocyanic acid found in the stomach as has been mentioned in the calculation outlined.

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## THE DETERMINATION OF SULFUR AND CHLORINE IN THE RICE PLANT.<sup>1</sup>

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In so complex an organism as one of the higher plants it is difficult to determine the true functions of the various elements found in it. Loew<sup>2</sup> has defined the uses of sulfur and chlorine in the plant.

Sulfur is found in plants in definite chemical organic compounds, such as edestin, which contains 0.884% SO<sub>3</sub>, legumin 0.385%, and gliadin of wheat 1.027%.<sup>3</sup>

Sulfur also occurs as mustard oils in plants; for example, the mustard seed and onion.

Sulfur in mineral form as sulfates occurs usually in smaller quantities in plants. Arendt, Ulrich, E. Schulze, Berthelot and Andre, and Fraps,<sup>4</sup> have investigated this form in plants and found it to vary in parts of plants

<sup>1</sup> Published with the permission of the Secretary of Agriculture.

<sup>2</sup> U. S. Dept. of Agr., Div. Veg. Physiology and Path., *Bull.* 18.

<sup>3</sup> THIS JOURNAL, 24, 140-67 (1902).

<sup>4</sup> Abstract from Fraps (Det. of Sulfates in Plants); Tamman, *Ber.*, 19, 261 (1886); ref. 26th Ann. Rept., 1903, N. Car., p. 67.