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A NEW METHOD FOR THE DETERMINATION OF CYANOGENETIC GLYCOSIDES

T.K. Obidairo

Biochemistry Department, University of Benin, P.M.B. 1154, Benin City, Nigeria

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The prussion blue test for hydrogen cyanide has been combined with the use of Tiron (disodium salt of catechol-3.5 disulphonic acid) to determine quantitatively the hydrogen cyanide from cyanogenetic glycosides. The method is sensitive and colorimetric.

Key words: Tiron, Cyanogenetic glycosides, Colorimetric.

INTRODUCTION

Poisoning by cyanogenetic glycosides is usually due to the ingestion of glycoside-containing plants. The wide occurrence of cyanogenetic glycosides in plants has been known in prechristian Egypt and Rome where they were used for suicide, murder and judicial execution. During the first world war, beans were imported into European countries primarily as livestock feed. These beans occasionally caused the loss of livestock and one such shipment caused trouble in the United States [1]. Mistaken identification of tropic form of "lima beans" for navy beans has resulted in human poisoning [2]. The deaths of children have occurred after eating kernels of apricot and peach [3]. There is also a recorded case of human adult killed following the ingestion of apple pips [4]. Pigs have been poisoned by eating plumstone [5]. In West Africa, where the bitter cassava (*Manihot utilissima*) and (*Manihot esculenta*) are very common, there have been cases of poisoning in human beings. The latter variety is widely used throughout Africa as a staple food [6]. It is also being used as livestock feed and in the starch and alcohol industries. The major disadvantage in the use of these different plant materials is that they contain cyanogenetic glycosides which are capable of releasing hydrogen cyanide [7]. Cyanide, a potent and rapid acting chemical asphyxiant, deprives tissues of necessary oxygen, victims have died within two minutes of ingestion of 300 mg HCN [8]. HCN acts by inhibiting the enzyme cytochrome oxidase which is a terminal respiratory catalyst. Cyanide forms a highly stable complex with ferric iron and by this complexing, the respiratory enzyme ferricytochrome oxidase is converted to ferricytochrome cyanide. The overall effect is the inability of cells to use the oxygen brought to them as oxyhaemoglobin thereby producing death by cellular anoxia [9,10].

Cyanogenetic glycosides are hydrolysed by mineral acids or specific enzymes with the formation of HCN. Many tests for HCN, some of which are specific, are known. The thiocyanate test reported by Anderson and made more delicate by Stubbs and Elsdon [11] would detect not less than 50 mg HCN. The picric acid method modified by Ikediobi [12] and the pyridine-pyrazolone procedure are known and suffer the disadvantage that the cyanogenetic glycosides have to be extracted first before the test is carried out. These two methods have been compared and divergent results were obtained when applied to processed cassava products [13]. The Prussian blue test [14] is not only very sensitive but specific. An attempt has been made by Curry to use the Prussian blue test for the quantitative estimation of HCN by visual comparison with the blue colour produced by standards [15]. Such a method based on visual comparison depends on the visual power of the operator and can only be approximate while the results will vary from one operator to another.

A method which makes use of instrumentation will overcome these difficulties. Once established for HCN, the method could be adopted to the cyanogenetic glycosides. The purpose of this work [15] to determine the amount of iron present in the Turbul blue complex with a view to using this to estimate the cyanide and hence the glycoside concentration by colorimetric measurement.

Tiron, the disodium salt of catechol-3,5 disulphonic acid, has been shown to react with various metallic ions to give highly coloured complexes [16]. Tiron is a white powder which dissolves in water to give a colourless solution which is stable on standing. At pH 9-10 ferric iron gives a red complex while at pH 3.5-4.5 the complex is blue. These colours are specific for iron. The alkaline reaction has been found by the author to be more sensitive.

MATERIALS AND METHODS

Tiron reagent: 2.355 gm Tiron in 1 litre of distilled water. Stock solution of ferric nitrate containing 50 μg ferric iron/ml). Glycine/NaOH buffer prepared as follows:

(A) 0.2 M glycine
(B) 0.2 M NaOH
50 ml of (A) was mixed with 45.5 ml of (B) and diluted to a total volume of 200 ml.

Ferric iron estimation. From the standard stock solution of iron was pipetted into separate test tubes solutions equivalent to 5 μg, 10 μg, 15 μg, 20 . . . 40 μg of ferric iron respectively. The volume of each test tube was made up to 1 ml with distilled water. A blank using 1 ml of distilled water was run at the same time. Two drops of the glycine/NaOH buffer was added to each followed by the addition of 1 ml of the Tiron reagent. 1 ml of distilled water was added to each tube and the tubes allowed to stand at room temperature for 1 hr. The optical density at 480 nm of the resultant red colour was read in the spectrophotometer. A graph of optical density against concentration of ferric iron was plotted. A straight line was obtained.

Estimation of KCN. From a standard stock solution equivalent of 10 μg, 20 μg, 30 μg . . . 50 μg KCN were subjected to the Prussian blue test [14]. Appropriate volume of the KCN standard solution was put in the pear-shaped flask. The test paper was prepared by dipping Whatman No. 50 paper (2.3 cm diameter) in 10 % ferrous sulphate aqueous solution for 5 minutes. It was then removed allowed to dry and the dried paper immersed in 20 % NaOH. It was quickly blotted between two dry filter papers. The test paper was placed in the holder, one end of which was fixed into one of the necks of the pear-shaped flask and the other end connected to a vacuum pump. 5 mls of 10 % trichloroacetic acid (TCA) was added and the flask placed in a hot water bath 37°. Nitrogen was bubbled into the flask through a second neck of the flask. (Fig. 1). After 20 minutes, the test paper was removed and dropped in conc. HCl/water (1/4) mixture, when a Turnbull's blue spot appeared. The ferrous sulphate impregnated paper was stable. Each blue spot was removed and treated as follows:

The blue spot was washed with conc HCl/water (1/1) and then put in a micro Kjeldahl flask, 5 drops of conc. sulphuric acid and 20 ml of conc nitric acid (analar grade) were added and heated on a microflame until no more brown fumes were evolved. Care must be taken to avoid charring. It was allowed to cool and 5 ml of conc. ammonium hydroxide was allowed to trickle down the side to make alkaline. Excess ammonia was boil off until the mixture was almost dry. 1.5 ml glycine/NaOH buffer was added followed by the addition of 0.5 ml Tiron reagent and the volume made up to 3 ml with the buffer. A blank paper was similarly tested. The optical density at 480 nm

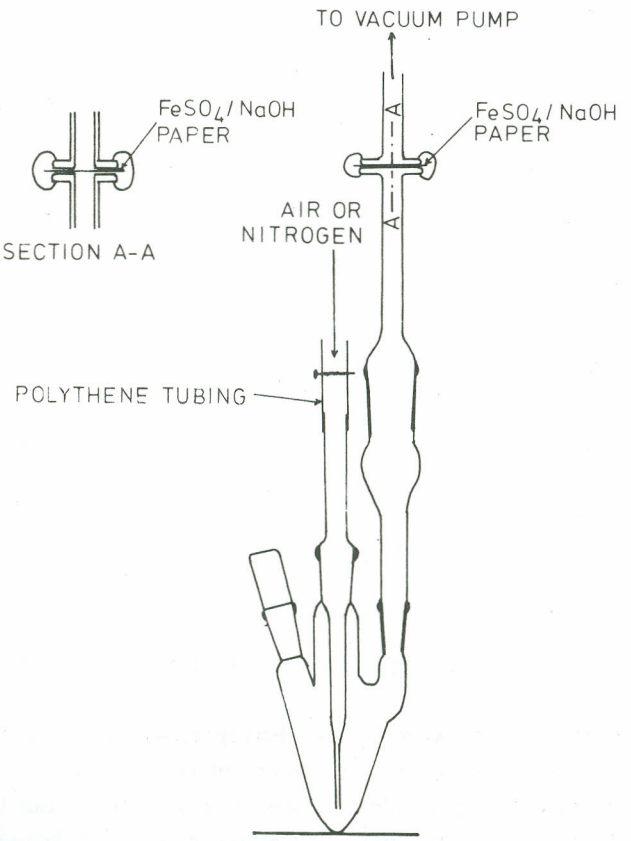

Fig. 1.

was read. A graph of amount of KCN (μg) against O.D was plotted. This gave a straight line.

Application of the Prussian blue test to cyanogenetic glycoside amygdalin. The Prussian blue test was applied to a standard solutions of Amygdalin using the enzyme emulsion for hydrolysis. The hydrolysis was carried out at 37°. Application of Tiron reagent to cyanogenetic glycosides estimation:

The procedure for estimation of HCN using the Tiron reagent as described for KCN above was applied to standard Amygdalin solution.

100 μg, 200 μg, 300 μg . . . 700 μg samples were individually first subjected to the Prussian blue test. The Turbull's blue spots were then subjected to Tiron reagent treatment as described under KCN. A graph of optical density at 480 nm against amygdalin (μg) was plotted. A straight line was obtained (Fig. 2).

The method was similarly applied to Prulaurasin (extracted from cherry-laurel leaves) and Linamarin (extracted from linseed meal). Identical results were obtained.

RESULTS AND DISCUSSION

The Tiron reagent used in the estimation of ferric iron, KCN and cyanogenetic glycosides gave in each case a linear

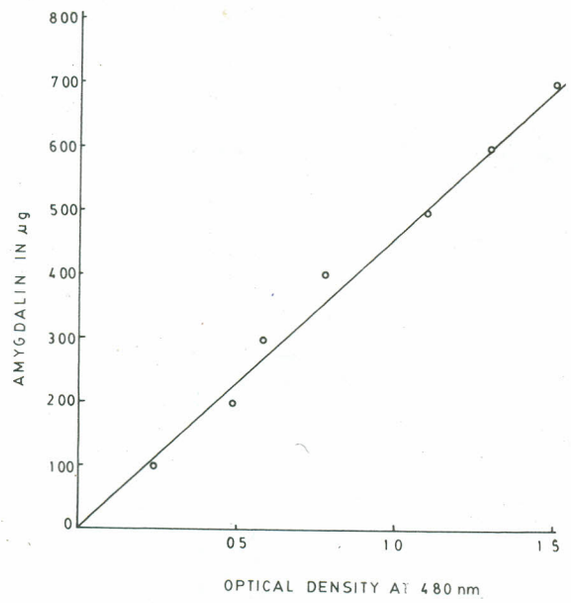

Fig. 2. Optical density at 480 nm.

relationship between optical density at 480 nm and the amount of ferric iron or amount of HCN or amount of cyanogenetic glycosides. As low as 2 µg of KCN can be detected by this method. The method can therefore be used to estimate cyanogenetic glycosides in plants or in toxicological analysis. The medium in which the cyanogenetic glycosides are contained does not affect the determination.

It has the advantage of being sensitive and did not depend on the visual comparison of colours as with the Curry's method [15]. It provides a method for the quantitative estimation of glycosides using instrumentation to measure the colour production. It also has the advantage that extraction of the glycosides is not mandatory as is the case with the pyridine pyrasolone method [17] and the alkaline picrate method [12]

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