U. S. ARMY NATICK LABORATORIES
PIONEERING RESEARCH DIVISION

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Cyanogenetic Glycosides and Manioc

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This report represents a survey of the literature on cyanogenetic glycosides and manioc.

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Cyanogenetic Glycosides and Manioc

I. Introduction

This report is based on a literature survey made as a preliminary to the projected investigation of the organic compounds of manioc by the Organic Chemistry Laboratory. It is intended to include material that seems likely to be of particular interest or significance for this investigation, rather than to give a complete review of the topics considered.

The section on manioc of the report prepared by the Tropical Products Institute,[1] various reviews on cyanogenetic glycosides, and appropriate references from these sources were consulted in the literature survey. In addition, Chemical Abstracts was systematically searched under the following headings: cyanogenetic glycosides, linamarin (phaseolunatin), and manioc (cassava). The report will be divided into sections on cyanogenetic glycosides, linamarin, and manioc.

II. Cyanogenetic Glycosides

A number of general articles on cyanogenetic glycosides are included in the list of references (refs. 2-11). In several of these the emphasis is on the use of the cyanogenetic property in chemotaxonomy rather than on the chemistry of the compounds. The best review is that by Georges Dillemann in Volume VIII of the "Encyclopedia of Plant Physiology" (1958).

A. Occurrence

Although the first crystalline cyanogenetic glycoside

was isolated in 1830, only about a dozen true cyanogenetic glycosides have been characterized up to the present time. The identity of the particular cyanogenetic glycoside present in the plant has been demonstrated for about a hundred species. About ten times that number of plants are known to contain cyanogenetic substances, however. Undoubtedly many of the latter are glycosides of known structure, but, as Dillemann[2] points out, it seems likely that new compounds are also present. Although the first crystalline cyanogenetic glycoside was obtained from the seeds of Rosaceae and although cyanophoric substances are of frequent occurrence in this family, they also occur in Leguminosae, Gramineae, and numerous other families.

At least until recently, definite identification of the cyanogenetic glycoside in a particular plant has depended upon its isolation in the pure state. Dillemann[2] suggests that the difficulties generally encountered in the isolation account for the relatively small number of species in which identity has been established. It appears also that only in a few instances, perhaps because of these difficulties of isolation, have more than the principal cyanogenetic glycoside of a plant been identified. Recent attempts to apply chromatographic procedures in such work will be considered later. Alston and Turner[4] feel that "in recent years cyanogenetic compounds have been rather neglected."

B. Classification

Dillemann[2] defines "true cyanogenetic glycosides" as those which contain a nitrile group and which yield HCN on enzymatic hydrolysis. He lists thirteen (or twelve; two may be identical) such substances. In one of these the nitrile group is located in the sugar portion of the molecule, and it is in the aglycone portion in the remaining cases. The structure of the aglycone moiety in several of the glycosides is in doubt.

Dillemann also lists three "pseudo-cyanogenetic" glycosides, which do not contain a nitrile group but which can liberate HCN under appropriate chemical treatment. Recently a group of three or four related compounds (cycasins) that also appear to be pseudo-cyanogenetic glycosides has been reported.

C. Methods of Analysis

A red color (isopurpuric acid) with sodium picrate paper has been frequently used as a simple qualitative test for the liberation of HCN from a sample of plant material. The method he has used for screening a large number of samples is described by Gibbs (ref. 5, p. 12). Other methods for the detection of HCN, such as the Prussian blue test, have the advantages of specificity or sensitivity. A summary of the methods used is given by Seifert.[7] A test-paper modification of the Prussian blue method has been described.[12]

The methods used for the quantitative determination of HCN in cyanogenetic substances have been reviewed by

Seifert,[7] and a variety of procedures have been employed in practice. Titration with $AgNO_3$ in alkaline solution in the presence of KI is a method[13] that has apparently been frequently used. A spectroscopic method for the determination of HCN released from a cyanogenetic glycoside has recently been described by Gander.[14,15] Methods for the analysis of HCN released from lima bean samples have been compared by Viehoever.[16] A micro method for the determination of cyanide, in which 0.2 microgram samples can be determined with reasonable accuracy, has been reported.[17]

Procedures to be employed for the hydrolytic liberation of HCN from the cyanophoric material have received considerable attention.[7] There is evidence[13,18] that in the case of the manioc plant auto-enzymatic hydrolysis and acid hydrolysis must be combined for satisfactory results.

D. Chromatographic Methods

In order to permit rapid screening of cyanogenetic plants, particularly as a tool in chemotaxonomy, a chromatographic method that is specific for cyanogenetic glycosides would be highly desirable. Dillemann[19] has experimented with a paper chromatographic method in which the chromatogram is sprayed with an enzyme. The chromatogram is then placed in contact with a paper treated with sodium picrate, and the liberated HCN produces a red spot. This method was somewhat successful, although it is of course necessary to employ the proper enzyme. With an emulsin preparation as a spray

reagent, for example, linamarin gave no spot.

Dillemann[19] has also reported the use of lead tetraacetate as a visualizing agent in the paper chromatography of several known cyanogenetic glycosides. He suggests that the sodium picrate method be used for the specific identification of cyanogenetic glycosides after initial location of the glycosides by a reagent such as lead tetraacetate.

The successful separation of linamarin and the closely related lotaustralin by paper chromatography has recently been reported in a biosynthetic study by Butler and Butler.[20] These cyanogenetic glycosides were satisfactorily visualized in this work by spraying the paper with linamarase solution and placing it in contact with a sheet of paper treated with sodium picrate. In addition, after the areas containing the glycosides had been located, the corresponding areas from chromatograms on aliquots of sample were cut out, eluted with water, and analyzed for radioactivity.

In similar work,[15] dhurrin, the glucoside of p-hydroxymandelonitrile, was eluted from a paper chromatogram with water and hydrolyzed with emulsin. The quantity of liberated HCN was determined spectroscopically by the method referred to above.[14] In such chromatographic work on cyanogenetic glycosides a general glycosidic spray reagent could be used for the location of the spots, and the analysis for HCN by spectroscopic or colorimetric techniques could then provide evidence of cyanogenetic character.[7]

E. General Methods of Isolation

Since no new glycoside of the class of true cyanogenetic glycosides has been isolated since 1938, the recorded methods for their isolation are generally classical in nature. Rosenthaler in "Handbuch der Pflanzenanalyse"[10] gives the experimental details of the isolation of about ten cyanogenetic glycosides, and he has also briefly described a general method of isolation.[21] The glycoside has usually been extracted from the plant material with alcohol. After removal of the alcohol, and, occasionally, after clarification of an aqueous solution, the glycoside has been purified by standard methods of solvent precipitation and crystallization.

Seifert[7] states that all known cyanogenetic glycosides are soluble in water and alcohol. Their insolubility in ether and petroleum ether provides a method of separation from fats and lipids. Extraction with ethyl acetate has served as a method of purification in some cases, and water, alcohol, ethyl acetate, and chloroform have been used as crystallizing solvents.

F. Variations in Plants

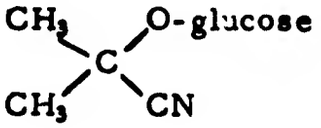
The quantity of cyanogenetic material in plants appears to be remarkably susceptible to variation. Wide variations exist among different species, and differences are also found for plants of the same species. Variations in cyanogenetic content result from differences in soil or climatic conditions.

An additional factor is the age of the plant; the quantity of cyanophoric material generally decreases as the plant matures.

Variations are also observed for different parts of the plant. In the case of manioc, for example, the content of the rind is generally higher.

III. Linamarin

Linamarin, or phaseolunatin, the cyanogenetic glycoside of manioc, has been shown to be the glucoside of acetone



cyanohydrin.[22,23]

In addition to its occurrence in Manihot utilissima, it has been found in seedlings of Linum usitatissimum (flax), in the seeds of Phaseolus lunatus (lima beans), in Lotus australis and Trifolium repens (white clover), in the seeds of Hevea brasiliensis, and in a number of other species. Because of the economic importance of some of these plants, particularly in human and animal nutrition, and because of its toxic properties, linamarin and its detection have received considerable attention.

A. Question of α- or β-Glucoside

Dunstan, et al.[24] considered that linamarin was an α-glucoside. Their evidence has been shown to be inconclusive,[25] however, and the β-configuration for the glucoside has become generally accepted in the literature. The synthesis by Fischer and Anger[26] of linamarin, with properties comparable to those

of the natural substance, under conditions that generally afford β-glucosides would seem to be strong evidence for this configuration. Fischer and Anger found that the behavior of the synthetic glucoside on enzymatic hydrolysis was similar to that of the natural linamarin; it was only broken down slowly on treatment with emulsin.

The conclusiveness of the evidence for the β-configuration is variously regarded in the literature. Robinson[11] states that the synthesis by Fischer and Anger definitely showed that the α-configuration was incorrect. In his review, however, Dillemann[8] considers that although the β-configuration is very probable, it has not been rigorously demonstrated. Conclusive evidence, such as a comparison with both α- and β-forms, would seem to be of interest.

Although the melting point of synthetic linamarin was reported as 142-143°(corr.)[26] and the glycoside isolated from cassava by Dunstan, et al.[27] melted at 138°(corr.), it seems unlikely that the difference is of any particular significance. Melting points ranging from 132 to 141° have been reported for linamarin isolated from natural sources, and the variations probably merely reflect differences in purity or in the technique of melting point determination. Finnemore and Cooper[28] have suggested that the lower melting point may result from contamination of linamarin by lotaustralin, the glucoside of methyl ethyl ketone cyanohydrin. Finnemore and Cooper obtained evidence that linamarin was present in

Lotus australis together with lotaustralin. Interestingly in this connection, Dillemann[19] observed that a sample of linamarin obtained from flax seedlings gave two spots, rather close together, on a paper chromatogram.

IV. Manioc (Cassava)

Investigations of the amino acids, vitamins, and other nutritional factors in manioc tubers are summarized in the report of the Tropical Products Institute.[3] As noted in that report, aside from the isolation of linamarin and studies on the nutritional factors, it appears that the organic chemistry of manioc has received little attention.

A. Organic Compounds of Manioc

Although linamarin is the only cyanogenetic glycoside that has been obtained from manioc, the work of Vuaflart[29] suggests that more than one type of cyanophoric substance is present. The results of Vuaflart are rather inconclusive and difficult to interpret, but they indicate that manioc meal contains material that yields HCN on maceration with water and additional material that requires acid hydrolysis for release of HCN. Generally similar observations have been made by others.[13,18] A complete examination of manioc for cyanogenetic substances by a chromatographic method is obviously desirable.

Peckolt[30] in 1886 isolated a crystalline substance (manihotin) from manioc tubers. It melted at 160°, did not contain nitrogen, and may be a hexitol. In this connection it is interesting to note that Finnemore and Cooper[28] isolated

pinitol (5-O-methyl-D-inositol) together with the glucoside of the cyanohydrin of methyl ethyl ketone (lotaustralin) from Lotus australis, and they point out that pinitol occurs in a number of other plants that contain cyanogenetic glycosides.

Freise[31] has reported a study on some organic compounds obtained from the juice expressed from manioc tubers, though with little or no description of the isolation procedures employed. This juice is apparently used medicinally in some areas in the treatment of various conditions, such as eczema and scabies. Freise isolated a glycoside that was hydrolyzed enzymatically to glucose, hydrogen cyanide, and a crystalline substance. The latter melted at 144-146° and had an indicated formula of $C_{15}H_{10}O_6$. A sulfur-containing essential oil, which is described as a moderately strong diuretic, and a saponin are also reported.

In addition to the work on the organic compounds of manioc described above, two early studies on the chemistry of manioc were carried out by Henry and Boutron-Charlard in 1833 (summarized in ref. 27) and by Peckolt[30] in 1886. Aside from manihotoxin (linamarin) and the manihotin described above, no definitely characterized substances are reported by these workers.

The cyanogenetic material contained in the leaves of manioc has apparently not been identified, although it seems likely that linamarin is present. It has been reported (summary in ref. 1; apparently early work of Peckolt) that

crystalline substances with properties suggestive of cyanogenetic glycosides have been obtained from the leaves, but they were not identified.

B. Hydrogen Cyanide Content of Manioc

Analyses of about a hundred varieties of manioc for available HCN by Raymond, et al.[32] gave an average value of 15.8 mg. from 100 g. of whole root, with a maximum value of 43.4 mg. As noted above, the content of the peel is generally higher, and quantities over 100 mg./100 g. have been recorded.[18] Similarly to other cyanogenetic plants, manioc shows considerable variation in the quantity of liberated HCN with variety, growing conditions, etc. Even tubers from the same clump can reportedly exhibit appreciable differences.[18] It is thus probably rather difficult to predict reliably the content of any particular batch.

The quantity of HCN liberated from the edible portion of fresh manioc leaves has been found to be in the range 18.6-24.5 mg./100 g. by Raymond, et al.[32]

C. Question of Collection of Manioc

A number of references were noted that may have some bearing on the problem of acquiring a satisfactory supply of the manioc tubers. Dunstan, et al.[27] in their work on manioc in 1906, found that they could not successfully import fresh roots from the West Indies. They discovered, however, that roots of bitter manioc that had been sliced when fresh and dried in the sun were "fairly rich" in cyanogenetic material, and they

eventually used rind of bitter manioc that had been dried in the sun. The plant undoubtedly undergoes enzymatic hydrolysis and loses significant quantities of HCN in this process, however. Razafimahery[33] showed that the HCN content of a fresh sample decreased from 26 mg./100 g. to 21 mg./100 g. after 24 hours and to 10 mg./100 g. after 174 hours on drying in the sun.

Studies of the effects of various methods of drying and storage on the HCN content of manioc tubers have been reported by Joachim and Panditteseker[18] and by Charavanapavan.[34] They have found that there is less loss of HCN when drying is carried out at temperatures above 72°C, as a result of inactivation of the enzyme above that temperature. Thus, while HCN losses of about 50% can occur on drying at 60°C, losses of only 16% are reported on drying at 90-100°. At the latter temperature dried samples with as much as 25 mg./100 g. of bound HCN may be obtained.

Charavanapavan states that the tubers can be kept fresh even up to as long as a week in an atmosphere of carbon dioxide, but the effect of such treatment on the liberation of HCN is not clear.

Aside from their susceptibility to enzymatic hydrolysis, cyanogenetic glycosides, at least in some cases, can apparently be quite stable. In his screening of a large number of plants for cyanogenesis, Gibbs[5] has occasionally used dried or herbarium specimens when fresh samples were not obtainable. Lüdtke[35] has reported that the linamarin-splitting enzyme was

completely destroyed by boiling for one hour at pH 5 but that the glucoside was unaffected by this treatment.

D. J. Rogers and M. Milner[36], of the New York Botanical Garden and the United Nations, respectively, have recently published a paper on the amino acid profile of manioc leaves. Samples of the leaves were shipped in plastic bags with dry ice from Jamaica to New York by air freight or transported from Brazil in the cold storage unit of a ship. On hydrolysis with the linamarin enzyme and with acid the Jamaican samples gave an average value for cyanide of 46 p. p. m. (wet) and the Brazilian samples an average value of 56. 5 p. p. m. Thus, in the case of leaves, transportation with preservation of cyanogenetic material appeared to be at least somewhat successful under these conditions

Although frozen samples were used in the work above, initiation of the enzymatic release of HCN from plants by cooling has been noted in the literature (ref. 2, p. 1051; ref. 33, p. 71). If this is a factor to be considered, it might also be of significance in the preparation of samples for shipment by freeze-drying. In any case, it should be possible to make a rather rapid quantitative assay of any particular sample for cyanogenetic material. It might be worthwhile initially to ship and assay trial samples, in order to evaluate methods of transport.

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