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Cyanogenesis in Plants. Part II. The Great Millet, Sorghum vulgare

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VII. *Cyanogenesis in Plants.*—Part II. *The Great Millet*, Sorghum vulgare.

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In a previous paper, our first communication on this subject ('Phil. Trans.,' B, vol. 194, 1901, p. 515), we have shown that the poisonous effects produced by the young plants of *Lotus arabicus* are due to prussic acid, which is not present in the plant as such, but originates in the hydrolytic action of an enzyme, lotase, on a glucoside, lotusin. Recently we have examined a large number of plants which, like this Egyptian vetch, appear, under certain conditions, to possess poisonous properties, and at other times to be innocuous and often valuable as fodder plants or food stuffs, with the view of ascertaining to what extent they contain glucosides furnishing prussic acid.

Among the first of these plants we examined was the Great Millet, *Sorghum vulgare*, a plant widely cultivated in tropical countries for the sake of its nutritious grain, which in many districts of India is the staple food, known as "Juar," of the natives. In the West Indies what is apparently the same plant yields the important "Guinea Corn" and in South Africa "Kaffir Corn."

We were informed by Mr. E. A. Floyer, of Cairo, that in Egypt it is well known to the Arabs that the green portions of the young plant—the vernacular name of which is "Dhurra Shirshabi"—are poisonous, and that during this period the plantations are protected in various ways in order to prevent cattle from feeding on the immature growth. It is to be noted that in Egypt the name "dhurra" is also applied to a variety of maize which is largely cultivated.

Mr. Floyer has given us the following account of the plant in Egypt. "Dhurra shirshabi" is not grown in Egypt as a crop, the yield of corn being too small. It is planted chiefly in order to shade the "Arachis" (ground nut), to which it also affords protection in forming a poisonous hedge. The "thinnings" of the young Millet are often strewn around a cultivated crop, and the neighbours are warned to keep their cattle off. The poison is most intense when young plants, 1 foot high or less, are kept without water for a long time, and such unwatered young plant is highly toxic to cows. The plant appears to have been brought to Egypt from Syria, and is now grown chiefly at Bir Abu Bala, near Ismailia. The "fellaheen" do not plant it.

Cases of poisoning by young *Sorghum* have been also recorded in America and in Australia, where the plant is grown for forage purposes.

In India the poisonous properties of the plant—which bears the vernacular name "juar" or "jowar"—do not appear to be so generally known, although several well authenticated cases of the poisoning of cattle by it, especially during drought, have been recorded, and much has been written on the subject by veterinary surgeons and others, who have, as a rule, assumed that the toxicity is due to the presence of a poisonous fungus or insect upon the plant, or that the Great Millet is not naturally poisonous, and that the deaths of cattle as the result of eating it are due to immoderate consumption, which causes a kind of suffocation from indigestion, technically known as "hoven." The symptoms of "hoven" are not unlike those of prussic acid poisoning, and it is possible that the various leguminous fodders which are known to be particularly liable to produce these effects may, at any rate in some cases, prove, like *Lotus arabicus*, and, as will be shown in the present paper, *Sorghum vulgare*, to furnish prussic acid.

For the material we have employed in the course of this investigation we are indebted to Mr. E. A. Floyer, who was good enough to undertake its collection in Egypt at different stages of growth.

Considerable confusion exists as to the identity of the "Great Millets" grown in different tropical countries. Thus in India the plant is cultivated both as a spring and an autumn crop. The varieties ripening in the spring are probably originally derived from *Sorghum halapense*, a species indigenous to India, whilst the autumn crops are generally referred to *Sorghum vulgare*, yet both spring and autumn crops are called "juar" or "jowar," and are used by the natives indiscriminately. Again, in India a plant with an inflorescence more branched than that of *Sorghum vulgare* has been regarded as a distinct species, and named *Sorghum saccharatum*; this name is however given in the 'Index Kewensis' as a synonym for *Sorghum vulgare*, of which the plant is probably merely a variety.

The plant we have examined has been identified for us by Dr. Schweinfurth as undoubtedly true *S. vulgare*.

Preliminary Experiments.

It was observed that the young plant when crushed and moistened with cold water soon acquired a strong odour of hydrocyanic acid. The production of this acid was confirmed by pressing out a little of the liquid from the moist plant, and distilling it, when a liquid was obtained which gave the characteristic reactions of hydrogen cyanide.

A few grammes of the plant were next exhausted by hot methylated alcohol in a Soxhlet extractor. The solvent was distilled from the solution and the residue boiled with water until nothing more dissolved. The aqueous liquid was then

distilled at first alone, and afterwards with the addition of dilute hydrochloric acid; in the former case none, but in the second, where hydrolysis had occurred, considerable quantities of hydrocyanic acid were found in the distillate.

These observations led us to conclude that *Sorghum vulgare* contains a glucoside which under the influence of some hydrolytic agent simultaneously present undergoes hydrolysis, furnishing as one product hydrocyanic acid, to which the observed toxicity of the young plants must be ascribed.

A determination of the amount of acid which the air-dried plant is capable of producing at different stages of growth was made by leaving a weighed quantity in contact with water for 12 hours, and distilling off the acid formed in a slow current of steam, the liquid being titrated by LIEBIG's method.

The following results were obtained:—

(a) From bright green plants about 12 inches in height;

20 grammes gave a distillate requiring 7·45 cub. centims. $\frac{N}{10}$ silver nitrate, equivalent to ·201 per cent. HCN.

20 grammes gave a distillate requiring 7·8 cub. centims. $\frac{N}{10}$ silver nitrate, equivalent to ·216 per cent. HCN.

(b) From plants about 3 feet high, yellowish-green and ripe; 20 grammes of these mature plants gave no indication of prussic acid, and larger quantities on distillation with water gave amounts too small to be satisfactorily estimated. No prussic acid was obtained from the seeds of the Millet.

It has been asserted by GRESHOFF and TREUB that in many tropical plants hydrocyanic acid occurs as such, that is, in the free state. The existence of the free acid was demonstrated by these observers by immersing a thin section of the plant first in alkali, then in a mixture of ferrous and ferric chlorides, and finally in strong hydrochloric acid. If the plant tissue was stained blue, it was concluded that prussic acid in the free state was present. This test, however, appears to us to be quite inconclusive, as the mere moistening of plant tissue containing both a glucoside capable of furnishing prussic acid on hydrolysis and a hydrolytic enzyme, leads to the immediate production of free acid, which by GRESHOFF and TREUB's method would be regarded as occurring pre-formed in the plant. We have carefully examined various specimens of dhurra for free prussic acid by the following methods.

About 20 grammes of the finely-powdered plant were placed in a distilling flask attached by its branch tube to a long condenser. Into the closed flask a rapid current of steam was passed, which served the double purpose of immediately destroying any enzyme, and of carrying through the condenser any volatile product present in the plant. In the distillate of the plant thus obtained we never found prussic acid, either with young *Sorghum vulgare* or *Lotus arabicus*.

It therefore appears that, like *Lotus arabicus*, the poisonous effects of the young

dhurra are due to the presence of a glucoside, which yields prussic acid under the influence of an enzyme also present in the plant.

Extraction of the Glucoside (Dhurrin).

The finely-powered plant was extracted with alcohol, the solvent distilled off and the residue warmed with water until nothing more dissolved.

To this liquid aqueous lead acetate was added so long as a precipitate formed. The precipitate (lead tannate, &c.) was removed. The filtrate, which was now bright yellow, was treated with sulphuretted hydrogen, care being taken to avoid a large excess, and the lead sulphide was removed by filtration. A stream of air was then drawn through the liquid to remove hydrogen sulphide, and the solution evaporated in a vacuum. After several weeks the syrup deposited a small quantity of a crystalline substance, and more was obtained by adding small quantities of alcohol and dissolving the mixture of precipitated sugar and glucoside in a little water, and setting aside to crystallise as before. This process was very tedious, and the two following methods have been since found to yield the glucoside much more rapidly.

A. The liquid, after the hydrogen sulphide treatment, is evaporated in a vacuum to a convenient volume, and the amount of free sugar determined with FEHLING'S solution. A little more than the calculated quantity of phenylhydrazine necessary to convert this amount of sugar into the osazone is then added, and the mixture heated for 30 minutes at 100° C., filtered, and the filtrate shaken with ether to remove any excess of phenylhydrazine. On evaporation in a vacuum the residue generally solidified to a mass of crystals, which were easily purified by recrystallisation from alcohol. The method always involves the loss of some of the glucoside, and cannot be employed in the isolation of small quantities.

B. The second method, which is the more effective, consists in evaporating in a vacuum the extract left after the lead acetate and hydrogen sulphide treatment with sufficient purified animal charcoal to convert the whole into a powder, which is then exposed in a vacuous desiccator until quite dry, when it is extracted in a Soxhlet apparatus with dry acetic ether. This solvent slowly removes the glucoside, leaving behind nearly all dextrose and brown extractive matter. On distilling off the solvent a syrupy residue is left, which if necessary is again treated in the same manner; usually, however, it crystallises after standing in a vacuum over sulphuric acid for a few days. The substance may be recrystallised from hot alcohol or boiling water.

The glucoside crystallises from water in brilliant leaflets, and from alcohol in small, transparent, rectangular prisms. It has no definite melting point, becoming brown when heated much beyond 100°, decomposing completely at 200°. It is easily soluble in hot alcohol, hot acetic ether and boiling water, separating in crystals on

cooling. It is however retained in solution by aqueous solutions of dextrose, a peculiarity which accounts for the great difficulty we at first experienced in isolating it from the plant.

It appears to contain water of crystallisation, since it loses weight when heated for some time in a water oven, but the amount cannot be accurately determined owing to the decomposition which occurs when the substance is heated near 100°.

Some trouble was met with in obtaining the material in a satisfactory state for analysis owing to the difficulty of removing the water of crystallisation without causing decomposition.

The following combustions were made :---

A. Material recrystallised from alcohol and dried until of constant weight in a vacuous desiccator over sulphuric acid.

·0961 gramme gave	·1887 gramme	CO_2	C 53·6	per cent.
	·0572	,,	H_2O	H 6·5	,,
·1385 ,, ,,	·2698	,,	CO_2	C 53·1	,,
	·0885	,,	H_2O	H 7·07	,,

B. Material recrystallised from water and dried at the ordinary atmospheric temperature on filter paper.

·1260 gramme gave	·2323 gramme	CO_2	C 50·29	per cent.
	·0736	,,	H_2O	H 6·42	,,

C. Material recrystallised from alcohol and dried in a current of warm air at 80° to 90°C.

·1021 gramme gave	·2051 gramme	CO_2	C 54·7	per cent.
	·0452	,,	H_2O	H 4·9	,,

$C_{14}H_{17}O_7N.C_2H_5OH$	requires	C 53·7	H 6·44	per cent.
$C_{14}H_{17}O_7N.H_2O$	,,	C 51·1	H 5·8	,,
$C_{14}H_{17}O_7N$	,,	C 54·0	H 5·5	,,
$C_{20}H_{27}O_{12}N$	,,	C 50·74	H 5·7	,,

The glucoside therefore has the composition represented by the formula $C_{14}H_{17}O_7N$, but when crystallised from alcohol or water the crystals which separate contain one molecular proportion of these solvents.

For the glucoside thus isolated from Egyptian Dhurra we propose the name *dhurrin*.

Hydrolysis of Dhurrin by Acids. Formation of Prussic Acid, Parahydroxybenzaldehyde and Dextrose.

When an aqueous solution of dhurrin is warmed on the water-bath with dilute hydrochloric acid, hydrocyanic acid is almost immediately evolved. If the heating is

continued for some time, the liquid becomes considerably discoloured owing to the further action of the acid upon the products of hydrolysis. In addition to prussic acid, a sugar and a substance soluble in ether are produced.

Parahydroxybenzaldehyde.

About 2 grammes of the dhurrin were dissolved in 50 cub. centims. of distilled water, and to the solution 10 cub. centims. of dilute hydrochloric acid were added, and the mixture heated on the water-bath for 5 minutes. The liquid was then extracted with ether and the ethereal solution dried and distilled. The residue was a brownish oil which, on standing, solidified to a mass of rosettes of needles. The crystals were dissolved in a small quantity of hot water, the solution filtered to remove resin, and cooled, when the substance separated in almost colourless needles, which could be picked out from a small quantity of the brown resin still adhering to them. After a second recrystallisation the melting-point remained unchanged at 118°. The substance is soluble in hot water, alcohol and ether. In aqueous solutions ferric chloride produces a purple coloration, and bromine water a white precipitate, which becomes crystalline on standing; phenylhydrazine produces an immediate crystalline precipitate. When heated in a dry test-tube the substance melts and sublimes in needles on the cooler parts of the tube; the vapour has a pleasant aromatic odour.

A combustion of the purified material, dried at 100°, gave the following results:—

·1267 gramme gave ·3196 gramme CO_2, 68·7 per cent. carbon.
·0529 ,, H_2O, 5·13 ,, hydrogen.
$C_7H_6O_2$ requires C 68·8, H 4·91.

The substance has therefore the composition of parahydroxybenzaldehyde.

Owing to the small amount of material available, the action of bromine on this compound could only be studied by the addition of excess of bromine water to dilute solutions of the substance, a method of investigation which, as the sequel shows, gave rise to rather unexpected results. Under these conditions an amorphous precipitate is formed which soon crystallises in colourless needles, forming after recrystallisation from alcohol felted masses of needles melting at 92°, and having all the properties of tribromphenol.

When a saturated aqueous solution of phenylhydrazine is added to a similar solution of the substance, a crystalline hydrazone is immediately formed, which is insoluble in ether and chloroform but soluble in hot alcohol. By operating in dilute solutions, a well-crystallised product is obtained, melting at 178°. It crystallises from hot alcohol in white needles which, on drying at 100°, become slightly green.

A combustion of the hydrazone gave the following result:—

·1117 gramme gave CO_2, ·3018 gramme 73·6 per cent. carbon.
H_2O, ·0613 ,, 6·07 ,, hydrogen.
$C_6H_4(OH)CH{:}N{\cdot}NHC_6H_5$ requires C 73·52 H 5·6.

The ether soluble hydrolytic product of dhurrin is therefore undoubtedly para-hydroxybenzaldehyde, which melts at 118°, gives a purple colour with ferric chloride, and forms a colourless hydrazone melting at 178°.

The occurrence of tribromphenol amongst the products obtained by brominating parahydroxybenzaldehyde has been observed by WERNER ('Bull.,' 46, 278), but it does not appear to have been previously noticed that, by using bromine water and dilute aqueous solutions of the aldehyde, the latter is converted almost exclusively into tribromphenol. This result was confirmed with a specimen of the aldehyde prepared from phenol and carefully purified from all traces of the latter.

$$C_6H_2(OH)CHO + H_2O + 4Br_2 = C_6H_2Br_3OH + 5HBr + CO_2.$$

Dextrose.

The acid liquid, after removal of the parahydroxybenzaldehyde, was mixed with powdered animal charcoal, warmed for several hours, and filtered. It was slightly yellow, but was sufficiently transparent for observation in a polarimeter, when it showed a marked dextro-rotation. It was next heated with phenylhydrazine for an hour on the water-bath, and the separated osazone collected and recrystallised from hot alcohol, when the characteristic bright yellow needles of glucosazone melting at 204° were obtained. The sugar produced is therefore d-glucose, that is, ordinary dextrose.

The Hydrolysis of Dhurrin and its Chemical Constitution.

Hydrolysis of Dhurrin by Emulsin.—About 1 gramme of the glucoside was dissolved in cold water, and a filtered extract of sweet almonds added, the mixture being then set aside for 12 hours at the ordinary temperature. After a few minutes the odour of hydrocyanic acid was perceptible, and at the end of the experiment over 90 per cent. of the possible quantity of parahydroxybenzaldehyde was obtained. This method of hydrolysing the glucoside is to be preferred to that involving the use of acids, since the aldehyde produced is more easily purified.

The quantitative determination of the acid hydrolytic products of dhurrin appeared to afford a method of confirming the formula assigned to this glucoside from the results of combustion, which are perhaps not completely satisfactory owing to the difficulty of obtaining the substance anhydrous without decomposing it. Attempts were therefore made to determine the amounts of hydrocyanic acid and dextrose produced on hydrolysis. For this purpose a weighed quantity of dhurrin dissolved in water was placed in a small Jena flask, and sufficient dilute (10 per cent.) hydrochloric acid added.

The flask was then corked and secured by wire and heated in a water-bath for 5 minutes. In this way complete hydrolysis is secured without much secondary decomposition. The prussic acid formed was distilled off in a gentle current of steam,

collected in alkali and titrated. The sugar in the residue was estimated gravimetrically by reduction of FEHLING'S solution.

·156 gramme gave a distillate requiring 2·58 cub. centims. $\frac{N}{10}$ silver nitrate = 8·9 per cent. HCN.

·1702 gramme cuprous oxide = ·085 gramme dextrose = 54·5 per cent.

$C_{14}H_{17}O_7N$ requires 8·6 per cent. HCN and 57·1 per cent. dextrose.

$C_{20}H_{27}O_{12}N$,, 6·01 ,, HCN and 80·1 ,, ,,

The formula $C_{14}H_{17}O_7N$ for dhurrin is therefore confirmed, and the hydrolysis of dhurrin by emulsin, or by dilute acid, may be expressed by the equation $C_{14}H_{17}O_7N + H_2O = C_7H_6O_2 + C_6H_{12}O_6 + HCN$.

Alkaline Hydrolysis of Dhurrin, Dhurrinic Acid.—When the glucoside is warmed with aqueous alkalis, it dissolves, with the evolution of ammonia, but no dextrose is formed. On evaporation the solution leaves a sticky hygroscopic residue which cannot be induced to crystallise.

When the hydrolysis is carried out in alcoholic solution by adding a solution of sodium in absolute alcohol to a similar solution of the glucoside, a precipitate forms after a few minutes, consisting of the sodium salt of the acid corresponding to dhurrin, which is its nitrile. This acid may therefore be called *dhurrinic acid*. The sodium salt is highly hygroscopic, it absorbs moisture and becomes gummy when removed from the dry alcohol, and is therefore difficult to free completely from the accompanying sodium carbonate. The free acid is almost more intractable than the sodium salt, and, so far, has only been obtained as a syrup containing sodium chloride. Recourse was therefore had to an examination of its decomposition products in order to establish its constitution.

Hydrolysis of Dhurrinic Acid.—A quantity of the crude sodium salt, prepared as above described, was dissolved in water and dilute hydrochloric acid added. The mixture was heated on the water-bath for an hour, and when cold extracted several times with ether. The ethereal solution was dried and the solvent removed by distillation, leaving a brown oil, which after several days deposited minute transparent needles. These were dried by absorption of the viscous mother liquid in a porous tile.

The substance thus obtained is at first colourless, but in a few days becomes slightly brown. It is soluble in boiling water, alcohol, and ether, and after recrystallisation melts at 180°. With ferric chloride in aqueous solution it gives a slight brown coloration, and with bromine water a precipitate, which after recrystallisation from alcohol melts at 185°.

The acid liquid after extraction with ether strongly reduces FEHLING'S solution, and therefore probably contains dextrose.

The yield of the crystalline hydrolytic product furnished by the hydrolysis of

dhurrinic acid is so small that sufficient material for analysis and identification could not be obtained. A small quantity of the acid was converted into silver salt, and a weighed quantity of the latter ignited, with the following result :—

·1058 gramme gave ·0399 Ag = 38·65 per cent., $C_8H_7O_4Ag$ requires 39·05 per cent.

It seemed highly probable that the alkaline hydrolysis of dhurrin with the formation of dhurrinic acid, and the decomposition of the latter by dilute acids, might be strictly comparable with the similar reactions of amygdalin, which, when hydrolysed by alkalis, furnishes amygdalic acid, this acid by heating with dilute acids being hydrolysed into mandelic acid and dextrose.

Amygdalin.	Amygdalic acid and ammonia.	Mandelic acid + 2 mols. dextrose.
$C_{20}H_{27}NO_{11}$ . . .	$C_{20}H_{28}O_{13} + NH_3$ . . .	$C_8H_8O_3 + 2C_6H_{12}O_6$.
Dhurrin.	**Dhurrinic acid and ammonia.**	**Parahydroxymandelic acid + 1 mol. dextrose.**
$C_{14}H_{17}O_7N$ . . .	$C_{14}H_{18}O_9 + NH_3$ . . .	$C_8H_8O_4 + C_6H_{12}O_6$.

On this analogy the crystalline hydrolytic product of dhurrinic acid would be parahydroxymandelic acid. We have established the identity of the two substances by comparing the hydrolytic product with parahydroxymandelic acid prepared by the hydrolysis of the cyanhydrin of parahydroxybenzaldehyde.

As parahydroxymandelic acid is now prepared for the first time, the following outline of the process employed may be given.

Preparation of Parahydroxymandelic Acid.—Ten grammes of parahydroxybenzaldehyde were dissolved in 50 cub. centims. of boiling water, and 30 grammes of potassium cyanide added to the solution, which was then cooled in a freezing mixture and 50 cub. centims. of strong hydrochloric acid gradually added, the whole being set aside for about 12 hours. The mixture was extracted with ether, the latter being allowed to spontaneously evaporate, leaving an oily residue, which was mixed with 20 cub. centims. of strong hydrochloric acid and sufficient alcohol to keep it in solution. This mixture was boiled for 3 hours, neutralized with sodium carbonate, filtered from the large quantity of resin formed, and extracted with ether in order to remove unaltered aldehyde. The residual liquid was then made acid with dilute sulphuric acid and extracted with ether until exhausted. The solvent was then distilled off, the oily residue boiled with water, to which a little animal charcoal had been added, and the filtered solution evaporated in a vacuum. After several days rosettes of needles appeared in the oily residue, and these after recrystallisation from alcohol melted at 180°, and further resembled the acid obtained from dhurrin in giving a brown coloration with ferric chloride, and a crystalline bromine derivative melting at 185°.

The yield of parahydroxymandelic acid furnished by the process described above is

only about 1 per cent., as this acid is readily converted by hydrochloric acid into a resin dissolving in alcohol with a fine purple colour. The cyanhydrin of parahydroxybenzaldehyde is also very unstable, being easily hydrolysed by water into prussic acid and the aldehyde, so that in each experiment about 50 per cent. of the latter is regenerated. Attempts were made to utilise anisaldehyde cyanhydrin for the preparation of the acid, but although this substance is somewhat more stable than its lower homologue, a considerable loss occurs in decomposing the methoxy-mandelic acid first formed.

A small quantity of the silver salt prepared from the acid obtained as described above gave the following results on analysis :—

·1286 gramme gave ·1642 gramme CO_2, C 34·83 per cent.
·0308 ,, H_2O, H 2·67 ,,

·1286 gramme gave a residue of silver weighing ·0506 gramme = 39·35 per cent.
$C_8H_7O_4Ag$ requires C 34·9, H 2·48, Ag 39·09 per cent.

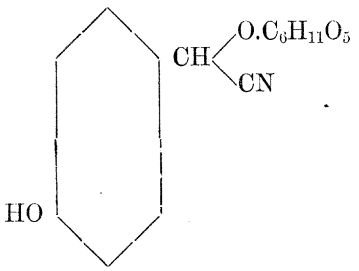
The properties and reactions of dhurrin, as described in the foregoing paragraphs, may for convenience be summarised as follows :—

(1) The glucoside is hydrolysed by emulsin and dilute acids into parahydroxybenzaldehyde, hydrocyanic acid, and dextrose.

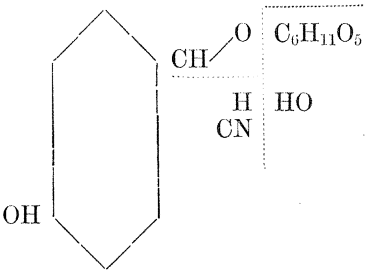
(2) It is decomposed hydrolytically by alkalis into dhurrinic acid and ammonia.

(3) Dhurrinic acid is hydrolysed by dilute acids into parahydroxymandelic acid and dextrose.

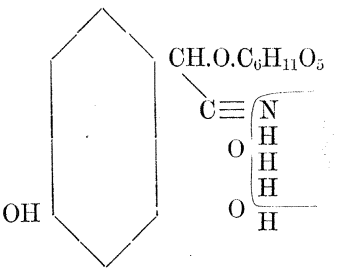
These reactions we believe are fully accounted for by assigning to dhurrin the constitution of a dextrose ether of the cyanhydrin of parahydroxybenzaldehyde, which may be represented by the formula given below



Dhurrin



Hydrolysis by acids and emulsin (prussic acid, dextrose, parahydroxybenzaldehyde).



Hydrolysis by alkalis (dhurrinic acid and ammonia).

Dhurrin is therefore the parahydroxy-derivative of the glucoside of mandelic nitrile which was prepared by FISCHER by the partial hydrolysis of amygdalin with invertase, and resembles this glucoside in the ease with which it undergoes

hydrolysis by emulsin. It is the first member of the class of dextrose ethers (glucosides) of cyanhydrins which has so far been found in nature, amygdalin and lotusin being maltose derivatives.

The Enzyme of Sorghum vulgare.

In the introduction to this paper attention has been drawn to the fact that the plant when moistened with cold water evolves hydrocyanic acid, whilst it no longer does so after exposure to a temperature of 100°, nor is the acid formed when the plant is placed in boiling water. These results point to the presence in the plant of an enzyme, destroyed by heat, which has the power of hydrolysing dhurrin. This enzyme was isolated by extracting the finely-ground plant with cold water, and evaporating the extract so obtained in a vacuous desiccator over quicklime to remove as much hydrocyanic acid as possible. The activity of this extract was then tested by the addition of small quantities to solutions of amygdalin, salicin and dhurrin, these experiments being controlled by the addition of boiled and filtered dhurra extract to similar solutions of these glucosides.

In all three cases the glucoside was quickly hydrolysed, the formation of benzaldehyde, saligenin, and parahydroxybenzaldehyde respectively being recognized by the usual tests for these substances. Comparative experiments in which the action of an extract of sweet almonds was tried side by side with the dhurra enzyme on the same glucosides, showed that the two extracts behaved in precisely the same way. Similar preparations made by precipitating aqueous extracts of sweet almonds and dhurra with alcohol and by precipitating calcium phosphate in such extracts, showed no difference of activity in effecting the hydrolysis of salicin. The glucosidolytic enzyme of *Sorghum vulgare* therefore performs the same functions as the enzyme emulsin which occurs in sweet almonds, and in the present state of our knowledge of the chemistry of enzymes, the two substances may provisionally be regarded as identical.

The Cyanogenetic Constituents of Plants.

Besides lotusin and dhurrin, the glucosides we have isolated from young plants of *Lotus arabicus* and *Sorghum vulgare* respectively, only one other cyanogenetic glucoside is definitely known, that is, the amygdalin derived from bitter almonds, which, however, is found in the seeds of the plant.

The results of our investigations have rendered it probable that the production of prussic acid in a number of other plants may be associated with the presence of cyanogenetic glucosides. Moreover, the question of the occurrence of prussic acid, and the part played by it in vegetable metabolism, involves problems of the first importance in vegetable physiology, with which we intend to deal when we have

obtained a further insight into the nature of other cyanogenetic glucosides now under investigation. So far as *Lotus arabicus* and *Sorghum vulgare* are concerned, it would appear that the existence of a cyanogenetic glucoside in the young plant up to the period when the seeds ripen at any rate may serve as an important protection to the plant from the attacks of animals. It appears that animals, indigenous to the countries in which these plants are native, refuse to eat them in the earlier and poisonous stages of growth. The part played by the glucoside in the general metabolism of these plants and the origin and fate of the cyanogenetic group still remain to be ascertained. The temporary presence in a plant of a considerable quantity of a cyanogenetic glucoside, together with an enzyme capable of decomposing it, appears to us to be a fact which must have an important biological meaning.

As so much interest attaches to the subject from several points of view, we are engaged in investigating the constituents of other plants which furnish prussic acid. Among them we may mention *Phaseolus lunatus* (seeds), *Lotus australis*, *Manihot utilissima*, and *Linum usitatissmum*, as well as a number of little known plants derived from the Colonies which have proved to be poisonous to cattle, some of which may contain cyanogenetic glucosides. From the chemical point of view it is important, in the first instance, to isolate these glucosides and to ascertain their properties, composition, and molecular structure. This work we have now accomplished with the glucosides of *Lotus arabicus* and *Sorghum vulgare*, which are shown to be radically different in chemical constitution, whilst each belongs to a type chemically distinct from that of amygdalin, the only naturally occurring cyanogenetic glucoside hitherto definitely known.

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