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## The Nature and Origin of the Poison of *Lotus arabicus*

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## XI. *The Nature and Origin of the Poison of Lotus arabicus.*

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*Lotus arabicus* is a small herbaceous plant with pink flowers, having the general habit of a vetch. It is indigenous to Egypt and grows abundantly throughout Nubia, and is noticeable as green patches along the bed of the Nile above Luxor.

It has been described by LINNÆUS ('Mantissa Plantarum,' vol. 1, p. 104) in the following terms :—

Leguminibus cylindricis aristatis. Caulibus prostratis, pedunculis trifloris, bracteis monophyllis.

Habitat in Arabia.

Caules.—Plures, sesquipedales, prostrati, vix pubescentes.

Folia.—Ternata, petiolata.

Foliola.—Subpetiolata, obovata, obtusiuscula, subincana, fere glabra.

Stipulæ.—Fere magnitudine figuraque foliorum.

Petioli.—Brevissimi.

Pedunculi—erecti, triflori s. 4-flori, foliis sæpe longiores.

Flores.—Pedicellati nutantes pallide rufescentes; vexillo venis rubris; alis includentibus carinam.

Calycis—Dentes subulati, distantes.

Bractea.—Ad exortum pedunculorum propriorum, monophylla, erecta, foliolis similis.

Noctu dormit floribus arcte pedunculo communi approximatis, super imposita bractea, foliolis, stipulisque conniventibus. Legumina cylindrica aristata.

We have not been able to find any later description of the plant. ASCHERSON and SCHWEINFURTH ('Flora Egyptiaca'), and also BOISSIER, refer to the description given by LINNÆUS.

The plant is known to the Arabs as "Khuther," and when mature and the seeds fully ripe, it is used by them as a fodder for cattle. The Arabs are aware that in the earlier stages of its growth, especially just before the ripening of the seeds, the plant is highly poisonous. This fact is, however, not generally known to Europeans, who have consequently suffered through the loss of horses, donkeys, sheep, and cattle,

to whom this plant is highly attractive, both on account of its appearance and fragrant odour. There is some evidence that certain animals indigenous to Egypt have become immune to the poisonous effects of this plant.

The air-dried plant, which retains to a remarkable degree its bright green colour, has a pleasant herbaceous odour, resembling that of dry hay. The attention of both the military and civil authorities in Egypt having been frequently directed to the toxic properties of this plant, the Director of Kew Gardens was consulted, and Sir W. THISELTON-DYER referred to us the investigation of the matter.

To the interest and energy of Mr. ERNEST FLOYER, of Cairo, Member of the Egyptian Institute, we are very greatly indebted, especially for the trouble he has taken to collect for us in Egypt the abundance of carefully selected material required for this research. The plants collected at different stages of growth were thoroughly dried in the air before their despatch to England.

#### *Preliminary Investigation.*

In the preliminary examination the important observation was made that when the dried unripe plant is crushed with water and allowed to stand for a few hours it gradually develops a strong odour of prussic (hydrocyanic) acid. When this mixture was distilled, a distillate was obtained which exhibited all the chemical reactions of this compound.

The amount of hydrocyanic acid produced has been determined by distilling the finely powdered dried material with water, with which it had been in contact for some hours, the distillate being collected so long as it gave the Prussian-blue reaction for hydrocyanic acid. The entire distillate was made distinctly alkaline, and the hydrocyanic acid estimated volumetrically with silver nitrate.

The determinations with different portions of a consignment of plant with flowers and a few seed pods attached gave 0·304 and 0·345 per cent. of hydrogen cyanide, calculated on the air-dried material. With younger plants, bearing flower buds as well as flowers, 0·263 per cent. was obtained.

This latter result confirmed the impression gained from qualitative examination of plants of various ages, that the poison reaches its maximum at or about the period when flowering ceases and the seeds begin to develop.

When the plant is extracted with alcohol, no prussic acid is found in the extract, and similarly when the plant is covered with boiling water little or no prussic acid is evolved. The production of the acid by cold water was, however, not inhibited by the presence of salicylic acid, boric acid, phenol, or other similar antiseptics, which might be expected to interfere with microbic action.

The various aqueous extracts always exerted a considerable reducing power on alkaline solutions of copper salts. The production of hydrocyanic acid from the plants in the presence of cold water, a result which does not ensue from the action

of either alcohol or boiling water, recalls the very similar behaviour of the seed of the bitter almond tree (*Prunus amygdalus*, var. *amara*), which, as is well known, contains a glucoside amygdalin and an enzyme emulsin, the interaction of which in the presence of cold water results in the hydrolysis of the amygdalin, with the formation of hydrocyanic acid, dextrose, and benzaldehyde. The similarity in the behaviour of *Lotus arabicus* and bitter almonds was highly suggestive of the existence of substances in the former similar to amygdalin and emulsin. This suggestion received further support in the observation that when the dried alcoholic extract of Lotus was warmed with dilute hydrochloric acid, hydrocyanic acid was produced, together with a sugar capable of reducing FEHLING'S solution.

#### *Extraction of the Glucoside.*

The dried plant reduced to a fine powder was extracted in the cold with purified methylated alcohol. The mixed alcoholic liquors were then concentrated to a syrup, which was boiled repeatedly with water in order to separate the glucoside from the chlorophyll and resin. Tannin, gum, and similar substances were removed by precipitation with lead acetate in the usual manner. The purified aqueous solution, after removal of excess of lead, was bright yellow in colour. It was evaporated on the water-bath until of a syrupy consistence, when it was exposed in a vacuum desiccator over quicklime, and as all other methods failed to induce anything to crystallise, it was left in the desiccator and well stirred every day. After some weeks a small quantity of a substance crystallising in minute yellow needles separated. This was removed from the viscid mother liquor by absorption of the latter in a porous tile, which was then broken up and extracted with water. This extract evidently still contained far more of the substance than could be readily separated in a crystalline condition. From nearly a kilogramme of the plant only about .25 gramme of crystalline material was extracted, but by working up about 10 kilogrammes of the plant a quantity sufficient for analysis was eventually obtained.

The substance thus prepared proved to be a glucoside, which it is proposed to name *lotusin*. It was re-crystallised from hot alcohol, and was thus obtained in needles having a very faint yellow colour. It is bitter to the taste, and does not reduce FEHLING'S solution except after long boiling. It has no definite melting point, but decomposes when slowly heated. Unlike quercitrin and other similar glucosides of colouring matters, lotusin is not precipitated by lead acetate, a fact which we have utilised in purifying it.

With the exception of amygdalin, lotusin is the only glucoside definitely known which furnishes prussic acid as a decomposition product.

Combustions of specially purified material, dried at 100° C. until of constant weight, gave the following results:—

·1418	gramme	gave	·2708	gramme	CO <sub>2</sub>	=	52·08	per cent.	C.
			·0618	„	H <sub>2</sub> O	=	4·83	„	H.
·1620	„		·3134	„	CO <sub>2</sub>	=	52·59	„	C.
			·071	„	H <sub>2</sub> O	=	4·8	„	H.

C<sub>28</sub>H<sub>31</sub>O<sub>16</sub>N requires C 52·7 per cent. ; H 4·3 per cent.

In a preliminary account of this enquiry ('Roy. Soc. Proc.' vol. 67, p. 224), we assigned to lotusin the formula C<sub>22</sub>H<sub>19</sub>NO<sub>10</sub> as representing the simplest combination of the decomposition products obtained from it. The new formula deduced from the results of analyses given above and from other data differs from that formerly given by a molecule of dextrose. A quantitative determination of the hydrolytic products has shown that 2 molecules of dextrose are produced by the hydrolysis of a molecule of lotusin, instead of one, as we had assumed in our preliminary communication.

#### *Hydrolysis of Lotusin by Acids.*

When an aqueous solution of lotusin is mixed with dilute hydrochloric acid and the mixture is warmed on the water-bath for a few minutes, a crystalline yellow precipitate forms, and the liquid acquires a strong odour of hydrocyanic acid. In examining the nature of the coloured product, it was found convenient to utilise for its preparation, the mother liquors from which as much lotusin as possible had been crystallised, since, as has been already pointed out, these still contained a large amount of lotusin in solution. The products of hydrolysis were, however, in the first instance prepared from purified lotusin, and their identity with those obtained from the mother liquors definitely established.

#### *Identification of the Sugar.*

About 1 gramme of lotusin was hydrolysed in aqueous solution by the addition of a little hydrochloric acid and subsequent warming. The crystalline precipitate was filtered out, the liquid decolourised by animal charcoal, and examined in the polarimeter, when a distinct dextro-rotation was observed. The liquid was then somewhat evaporated and a solution of phenylhydrazine acetate added, the evaporation being continued until crystals began to separate. These, on re-crystallisation from warm alcohol, melted at 202–203° C., and evidently consisted of phenylglucosasone, which is stated to melt at 204° C. The sugar was in all respects identical with dextrose.

The amount of dextrose produced on hydrolysis of lotusin was determined gravimetrically, through the cuprous oxide reduced from FEHLING'S solution, with the following result :—

·1532 gramme of lotusin, hydrolysed by dilute hydrochloric acid, gave dextrose sufficient to produce an amount of cuprous oxide corresponding to ·1792 gramme  $\text{CuO} = 53\cdot4$  per cent. dextrose.

The equation



requires the formation of  $56\cdot5$  per cent. dextrose.

*Examination of the Yellow Crystalline product of Hydrolysis, Lotoflavin.*

It was found most convenient in preparing quantities of this material to bring about the hydrolysis by the addition of a little strong hydrochloric acid, warming gently for a few minutes, and rapidly filtering. At first there is some resinous matter produced which remains on the filter. The filtrate deposits almost immediately a yellow precipitate which, on examination with a lens, is found to consist of minute needles. The hydrolysis of lotusin is easily effected, even in dilute solution, by hydrochloric acid, but is not readily induced by sulphuric or nitric acids even when the acid solution is warmed for several hours on the water-bath. The insoluble hydrolytic product can be re-crystallised from its solution in hot glacial acetic acid, when it forms bright yellow needles melting indefinitely above  $200^\circ\text{C}$ . As the substance is a yellow colouring matter, the name *lotoflavin* is proposed for it. Lotoflavin dissolves in alcohol and in aqueous alkaline solutions with a bright yellow colour, but it is insoluble in water, chloroform, ether, and light petroleum. Its solutions in alcohol form orange-red precipitates with soluble lead and barium salts.

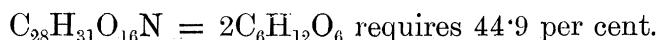
Combustions of lotoflavin, re-crystallised several times from acetic acid and dried at  $100^\circ\text{C}$ ., gave the following results :—

·176	gramme	gave	$\text{CO}_2$	·4041	gramme	=	62·6	per cent.	C.
			$\text{H}_2\text{O}$	·0581	„	=	3·79	„	H.
·0894	„		$\text{CO}_2$	·2067	„	=	63·09	„	C.
			$\text{H}_2\text{O}$	·0232	„	=	2·8	„	H.

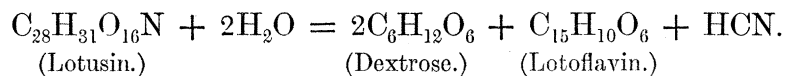
$\text{C}_{15}\text{H}_{10}\text{O}_6$  requires C  $62\cdot9$  per cent. ; H  $3\cdot5$  per cent.

The amount of lotoflavin produced by the hydrolysis of lotusin was determined by hydrolysing a known weight of the latter with hydrochloric acid, collecting, washing, and weighing the lotoflavin precipitated.

·6128 gramme gave ·286 gramme lotoflavin, *i.e.*,  $46\cdot6$  per cent



The hydrolysis of lotusin by hydrochloric acid may, therefore, in the light of the evidence now given, be represented as taking place according to the following equation :—



*Action of Acetic Anhydride on Lotoflavin. Tetracetyl-lotoflavin.*

When the colouring matter is dissolved in acetic anhydride and the solution warmed at 100° C. for about 2 hours a nearly colourless solution is produced. To isolate the products, the mixture was poured into excess of water, and the whole shaken well at intervals until the odour of acetic anhydride had disappeared. The liquid was then poured off and the precipitated acetyl derivative washed, dried, and dissolved in hot alcohol, and the solution set aside. On cooling, it deposited colourless needles, which, after re-crystallisation from alcohol, showed a constant melting point of 176–178° C.

On combustion, the following results were obtained :—

·0739	gramme	gave	·1636	gramme	CO <sub>2</sub>	=	60·29	per cent.	C.
			·0250	„	H <sub>2</sub> O	=	4·34	„	H.
·102	„		·2255	„	CO <sub>2</sub>	=	60·36	„	C.
			·0399	„	H <sub>2</sub> O	=	3·78	„	H.

C<sub>15</sub>H<sub>6</sub>O<sub>2</sub>(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>4</sub> requires C 60·7 per cent. ; H 3·9 per cent.

The amount of lotoflavin produced on hydrolysis of the acetyl derivative was determined and found to be as follows :—

·1185	gramme	gave	·0756	gramme	=	63·8	per cent.
·1014	„		·0641	„	=	63·2	„

C<sub>15</sub>H<sub>6</sub>O<sub>2</sub>(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>4</sub> requires 62·9 per cent.

The substance is therefore *tetracetyl-lotoflavin*.

*Action of Methyl Iodide on Lotoflavin. The Isomeric Trimethyl Ethers of Lotoflavin.*

About 1 gramme of lotoflavin was dissolved in methyl alcohol and a small amount of solid potash added to the solution and then methyl iodide in excess. The mixture became hot and the reaction progressed rapidly. The solution was boiled for an hour to ensure complete methylation. The excess of methyl iodide and the solvent were then distilled off and the residue mixed with water to remove the

potassium iodide formed. The insoluble portion was dissolved in hot methyl alcohol. This solution on cooling furnished yellow crystals melting at 125° C.

These on re-crystallisation from a somewhat dilute solution in methyl alcohol gave a first fraction melting at 125° C. and a second melting at 175° C. As there appeared to be at least two substances present, a larger amount of the methyl ether was prepared and re-crystallised. Working with this new material it was found that two mutually convertible methyl ethers had been produced, one, the  $\alpha$ -form, melting at 125° C., separating from solutions prepared by boiling the methyl ether with methyl alcohol in insufficient quantity for solution, and a second, the  $\beta$ -form, melting at 175° C., and separating always as a second fraction, in largest quantity from more dilute solutions prepared by gently warming the methyl ether with excess of methyl alcohol.

The  $\alpha$ -methyl ether crystallises in rosettes of needles of a bright yellow colour, whilst the  $\beta$ -form crystallises in long needles of an old-gold colour and silky lustre. The  $\beta$ -form is more soluble in methyl alcohol than the  $\alpha$ -form. It is somewhat remarkable that, although the transition point between the two forms must be lower than the boiling point of methyl alcohol (66° C.), since the  $\beta$ -form is converted into the  $\alpha$ -form by boiling with this solvent, yet the  $\beta$ -form can be heated in the solid state without change far beyond the melting point of the  $\alpha$ -methyl ether. During this operation the  $\beta$ -form must be in a condition of labile equilibrium. A similar phenomenon is presented by sulphur, which in the rhombic form can be heated up to its melting point, 114·5° C., although the temperature of transition to monoclinic sulphur is only 95·4° C.

The number of methoxyl groups present in the two methyl ethers was determined in the usual way by ZEISEL'S method.

$\alpha$ -methyl ether	·1196	gramme	gave	·2565	gramme	silver iodide	=	28·3	per cent.
									methoxyl.
$\beta$ -	·1008	„	„	·2139	„	„	=	28·07	per cent.
									methoxyl.

$C_{15}H_7O_3(OCH_3)_3$  requires 28·35 per cent.

The substances are therefore isomeric trimethyl ethers.

*Action of Acetic Anhydride on the Trimethyl Ether of Lotoflavin. Acetyltrimethyl-lotoflavin.*

Since lotoflavin yields a tetracetyl derivative, and therefore probably contains four hydroxyl groups, and yet only yields a trimethyl ether, it was of interest to determine whether the remaining hydroxyl group of the methyl ether could be acetylated. About 2 grammes of the  $\alpha$ -modification of the trimethyl ether was dissolved in excess of acetic anhydride and the mixture warmed on the water-bath



for an hour. Excess of water was then added, and after standing for a day the mixture was filtered and the insoluble precipitate washed with water, dried, and dissolved in boiling alcohol. On cooling the solution, pale yellow needles were obtained of an acetyl derivative melting constantly at  $147^{\circ}$  C.

A determination of the amount of methyl iodide formed by the action of hydriodic acid gave the following result :—

·12 gramme gave ·231 gramme AgI = 25·5 per cent. of methoxyl.

$C_{15}H_6O_2(OCH_3)_3C_2H_3O_2$  requires 25·1 per cent.

By the action of acetic anhydride on the  $\beta$ -modification of the trimethyl ether the same acetyltrimethyl ether was obtained.

#### *Action of Mineral Acids on Lotoflavin.*

It is known that certain of the natural vegetable colouring matters have the property of combining with a molecule of sulphuric acid and other mineral acids. The compounds so formed are stable in the presence of acetic acid but are immediately hydrolysed by water into their constituents. It was of interest to examine the behaviour of lotoflavin in this respect and attempts were therefore made to prepare, by the methods described by PERKIN ('Journ. Chem. Soc.,' 1895), similar acid compounds of lotoflavin. To boiling saturated solutions of the colouring matter in glacial acetic acid a few drops of the following acids were respectively added—sulphuric, hydrochloric, and hydriodic acids. In each case there was a perceptible darkening in colour of the solution, but on cooling no crystalline compound separated and on evaporation in a vacuum desiccator only amorphous residues containing the original colouring matter were obtained. Parallel experiments carried out with quercetin and fisetin, the colouring matters of quercitron bark and of young fustic, gave abundant quantities of the acid compounds of these substances. It has been shown by PERKIN that those colouring matters which have the property of forming acid compounds also possess the property of decomposing alcoholic solutions of the alkaline acetates, with the formation of insoluble metallic derivatives of the colouring matter.

When a saturated alcoholic solution of lotoflavin is added to a saturated alcoholic solution of potassium acetate, no precipitate is formed, even on standing or on boiling for some time. Parallel experiments carried out with quercetin gave an abundance of the potassium derivative of quercetin. It is therefore proved that lotoflavin neither forms compounds with mineral acids nor reacts with potassium acetate to form a potassium salt.

*Action of Fused Potash on Lotoflavin.*

In order to gain further knowledge of the constitution of lotoflavin its decomposition by fused alkalis was examined.

About 2 grammes of the colouring matter were dissolved in 5 cub. centims. of a 20 per cent. solution of potash, and to this mixture about 2 grammes of solid potash were added and the whole kept just fused for about an hour. The product was then cooled, dissolved in water, and the solution made acid, filtered, and repeatedly shaken with ether.

The ethereal liquids were mixed, decolourised with animal charcoal, dried over calcium chloride, and the solvent distilled off. The residue was dissolved in a little water, the solution filtered and concentrated in a vacuum desiccator. After several weeks a small quantity of a crystalline acid substance separated, which after re-crystallisation from hot water, melted at 207° C., exhibited the fluorescein reaction after fusion with phthalic anhydride, gave a pink coloration with aqueous ferric chloride, and possessed the other properties of *β-resorcylic acid*.

The residual liquid, which left a gummy mass when evaporated, contained *phloroglucinol*, as was proved by the power of striking a violet colour with ferric chloride and of staining a magenta colour a pine shaving moistened with hydrochloric acid. Eventually a small amount of phloroglucinol crystallised out from this viscid residue and was identified by its melting point and general properties.

*Alkaline Hydrolysis of Lotusin--Lotusinic Acid.*

When lotusin is warmed with alkaline liquids it passes into solution, ammonia being evolved and the liquid becoming bright yellow. From aqueous solutions it is not easy to isolate the product of hydrolysis, and it was found more convenient to carry out the hydrolysis in alcoholic solution. If lotusin is added to alcoholic potash (20 per cent.) it gradually passes into solution with evolution of ammonia. On standing, an insoluble potassium salt separates in the form of bright yellow needles.

This potassium salt is very soluble in water, almost insoluble in alcohol, ether, and light petroleum.

When aqueous silver nitrate is added to an aqueous solution of the potassium salt, a bright yellow silver salt is precipitated.

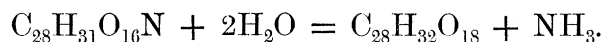
A determination of the amount of silver in this salt was made with the following results:—

·306 gramme gave ·0430 gramme silver = 14·05 per cent. Ag.

$C_{28}H_{31}O_{18}Ag$  requires 14·14 per cent. Ag.

This salt decomposes easily and has not been obtained crystalline.

The production of this acid from lotusin may therefore be represented by the following equation :—



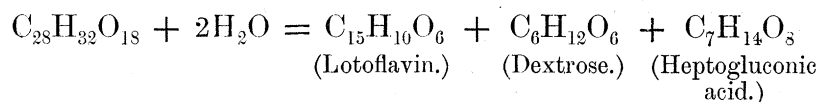
It is proposed to call this acid *lotusinic acid*.

#### *Hydrolysis of Lotusinic Acid.*

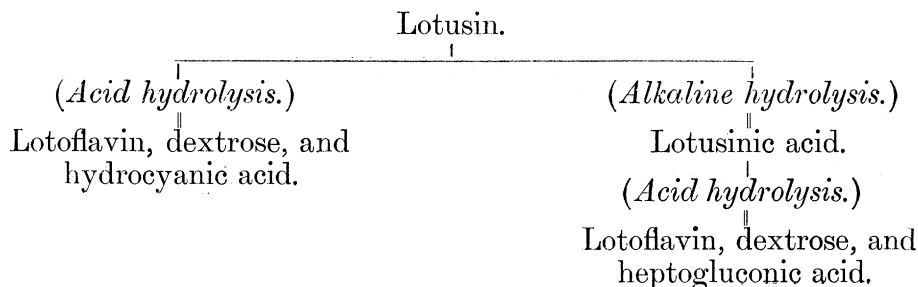
When the potassium salt of this acid is dissolved in water and the solution made acid by the addition of dilute hydrochloric acid, no precipitate is formed, but on attempting to evaporate the mixture, a crystalline yellow substance separates and the liquid acquires the property of reducing FEHLING'S solution. The crystalline yellow precipitate proved to be lotoflavin, since it gave an acetyl derivative melting at 175° C. possessing all the characteristics of tetracetyl lotoflavin.

From the filtered liquid, by addition of phenylhydrazine, phenylglucosazone melting at 204° C. was obtained.

The products of hydrolysis of lotusinic acid are therefore lotoflavin and dextrose. In addition to these substances, an acid is produced which it has so far been found impossible to obtain in a pure condition. It remains in the syrup when the liquid obtained by hydrolysing lotusinic acid in aqueous solutions is evaporated, and does not crystallise when stirred, or after solution in hot alcohol, or when ether is added in small quantities from day to day to its alcoholic solution. There can be little doubt from its properties that this acid is hepto-gluconic acid, and that the decomposition of lotusinic acid may be represented by the following equation, which shows that one of the dextrose residues of lotusin appears as a carboxylic acid.



Before proceeding to discuss the constitutional formula of lotusin and its decomposition products, it will be convenient to summarise in the following manner the relationships which have been found to exist between them.

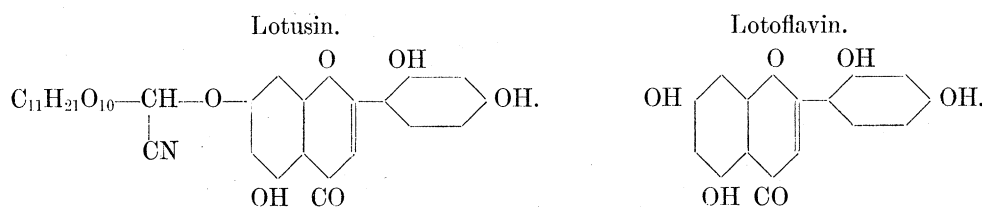


Lotoflavin acted upon by—

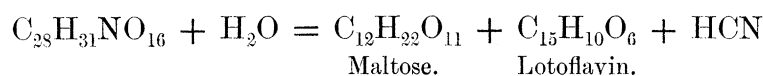
- (a) Acetic anhydride . . . . . gives a *tetracetyl* derivative.  
 (b) Methyl iodide . . . . . „ *trimethyl* ether.  
 (c) Methyl iodide and subse-  
 quently by acetic anhydride „ *mon-acetyl trimethyl* ether  
 (d) Mineral acids . . . . . form no addition compounds.  
 (e) Alcoholic potassium acetate . forms no potassium derivative.  
 (f) Fused potash . . . . . yields phloroglucinol and  $\beta$ -resorcylic acid.

*Constitution of Lotusin and Lotoflavin.*

The experimental results already given in detail and tabulated above, are we think best accounted for by assigning to *lotoflavin* the constitution of a tetrahydroxy phenylated pheno- $\gamma$ -pyrone, and to *lotusin* that of a cyanhydrin of a maltose ether of lotoflavin. These structures may be graphically represented thus :



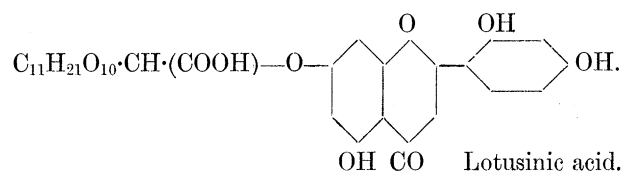
When hydrolysed by acids the initial decomposition of lotusin may be represented by the following equation—



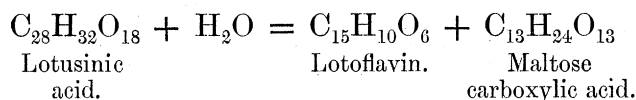
giving maltose  $C_{12}H_{22}O_{11}$ , lotoflavin  $C_{15}H_{10}O_6$ , and hydrocyanic acid HCN.

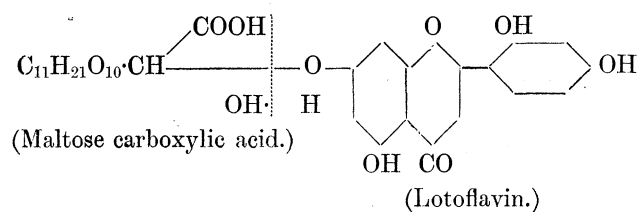
As the result of further action of the acid, the maltose is at once hydrolysed to dextrose.

The conversion of lotusin into lotusinic acid (in alkaline solution) takes place in the manner characteristic of the hydrolysis of nitriles into their corresponding acids :—

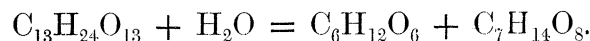


The further hydrolysis of lotusinic acid by acids may be represented thus :

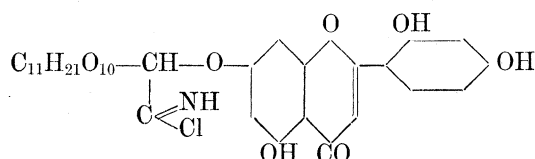




giving maltose-carboxylic acid and lotoflavin, the former breaking up at once by the further action of the acid into dextrose and hepto-gluconic acid—



The fact that lotusin is readily hydrolysed by hydrochloric acid and not by sulphuric acid may perhaps be explained by assuming that an intermediate chlorimide is formed through which the rupture of the molecule is facilitated—



*The Hydrolysis of Cyanhydrins.*—The constitutional formula we propose for lotusin appeared to satisfactorily account for all its reactions with the exception of the production of hydrocyanic acid by action of dilute hydrochloric acid, which required further elucidation. For it is generally stated that cyanhydrins, whether derived from aldehydes or ketones, furnish the corresponding acids when hydrolysed by either acids or alkalis, except in the case of  $\alpha$ -hydroxycyanides, which under the influence of alkalis lose hydrocyanic acid with formation of an aldehyde. Thus the pentacetyl cyanhydrin of arabinose when acted on with alcoholic potash furnishes hydrocyanic acid and arabinose. It seems, however, that the pure cyanhydrins have been isolated and their properties studied only in a very few cases. In general, the effects of hydrolysis by strong acids have been alone ascertained, and little or nothing is known as to the effect of water and of dilute acids on cyanhydrins of known constitution. We therefore investigated the question with typical cyanhydrins.

*Hydrolysis of the Cyanhydrin of Benzaldehyde (Mandelic Nitrile).*—When mandelic nitrile is mixed with water and the mixture shaken vigorously and then allowed to settle, the separated aqueous solution gives all the reactions of hydrocyanic acid, and by long-continued shaking it was found that practically the whole of the hydrocyanic acid could be removed from the cyanhydrin with the formation of benzaldehyde. Thus after shaking 2 grammes of the nitrile about twelve times in succession with an equal volume of water, the oil which finally separated solidified completely to benzaldehyde phenylhydrazone on the addition of phenylhydrazine. It was incidentally found that if phenylhydrazine be added to mandelic nitrile and the mixture allowed to stand for several days, benzaldehyde phenylhydrazone is formed with gradual evolution of hydrocyanic acid.

The splitting off of hydrocyanic acid occurs when mandelic nitrile is allowed to stand in contact with dilute hydrochloric acid or when warmed with it, but with concentrated acid phenylglycollic acid (mandelic acid) is formed, and no hydrocyanic acid is produced.

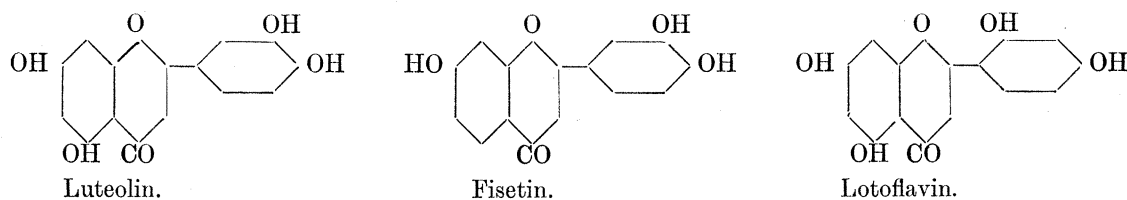
*Hydrolysis of Levulose Cyanhydrin.*—Attempts were made to isolate the cyanhydrins of dextrose and maltose by the addition of 50 per cent. solutions of hydrocyanic acid to aqueous solutions of these sugars, but in both cases the corresponding carboxylic acid is so rapidly formed that it was impossible to obtain the intermediate compound. Levulose cyanhydrin was, however, prepared by the method described by KILLIANI and DULL ('Ber.,' 1890, vol. 23, p. 450). When warmed with dilute hydrochloric acid it gave hydrocyanic acid, but with concentrated acid it passed directly into levulose carboxylic acid.

*Hydrolysis of Pentacetyl Gluconitrile.*—This substance was prepared in crystals melting at 83° by the method described by WOHL ('Ber.,' vol. 26, p. 730), which consists in acting on the oxime of ordinary dextrose with acetic anhydride in presence of sodium acetate. It was found that whilst the substance was readily converted into the corresponding acid by the action of strong hydrochloric acid, the action of very dilute acid led to the separation of hydrocyanic acid.

These results, therefore, made it justifiable to assume that a cyanhydrin of the formula we suggest for lotusin would evolve prussic acid when hydrolysed by hydrochloric acid, as lotusin is found to do.

In determining the constitution of lotoflavin, its great resemblance to colouring matters of the quercetin type, now generally regarded as phenylated phenyl- $\gamma$ -pyrones, must be taken into account.

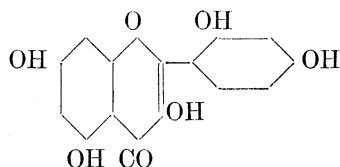
Lotoflavin is isomeric with two yellow colouring matters of this class, viz., fisetin (from young fustic, *Rhus cotinus*) and luteolin (from *Reseda luteola*), and corresponds with them in general behaviour. To these two isomerides the following formulæ have been assigned, whilst the third is that now proposed for lotoflavin :—



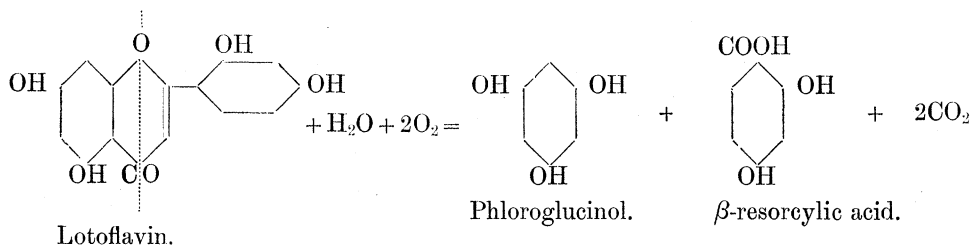
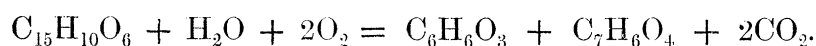
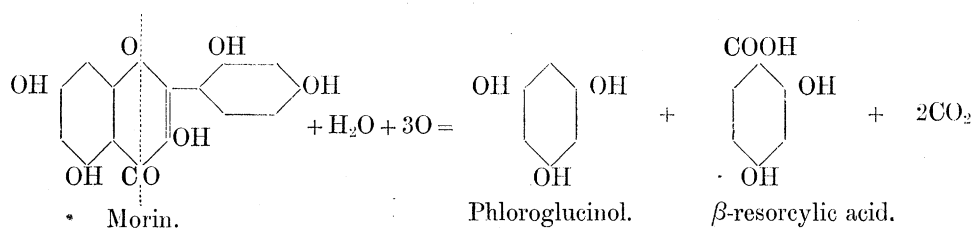
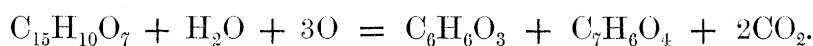
It will be seen that these formulæ represent the isomerides as containing four hydroxyl groups, and in support of this assumption may be brought forward the fact that each forms a tetracetyl derivative. On the other hand, luteolin and lotoflavin form with methyl iodide only trimethyl ethers instead of the tetramethyl ethers which might be expected. This phenomenon of incomplete methylation is also exhibited by quercetin and myricetin, which are other members of this class of colouring matters, and also by various hydroxy-derivatives, such as  $\beta$ -resorcylic acid; the common feature

in these cases being that each of these compounds contains a hydroxyl group in the so-called ortho position to a carbonyl group. This is the case with one hydroxyl group in the formulæ given above for lotoflavin and luteolin.

The decomposition products afforded by lotoflavin when fused with potash are identical with those given by *morin*, a colouring matter contained in *Morus tinctoria*. To the latter BABLICH and PERKIN ('Journ. Chem. Soc.,' vol. 69, 1896, p. 797) have assigned the following formula :—



The similarity of the decomposition products of morin and lotoflavin by fused potash are shown in the following scheme :—



In each case the phloroglucinol carboxylic acid, under the conditions of the experiment, forms phloroglucinol and carbon dioxide, whilst the side chains of the resorecinol compounds simultaneously formed, are oxidised to carboxylic groups producing β-resorecylic acid. Morin is therefore to be regarded as a hydroxy-lotoflavin.

There remains for consideration one point in which lotoflavin differs from most of the members of the quercetin group of colouring matters, viz., in its inability to form addition compounds with mineral acids or to replace acetic acid in the acetates of the alkali metals with the formation of a metallic derivative.

It has, however, been shown by A. G. PERKIN that so far only those members of

this class of colouring matters which possess in their catechol nucleus two hydroxyl groups in the ortho position to each other exhibit this behaviour, so that the formation of such compounds in the case of lotoflavin is not to be expected from the formula we propose for it.

We have assumed in the formula assigned to lotusin that the maltose cyanhydrin residue is attached to one of the hydroxyl residues of the pheno-pyrone ring. The exact point of attachment cannot, however, be decided on the experimental evidence at present available as to the properties of derivatives of this class of compounds.

#### *The Enzymes of Lotus arabicus.*

As has been already pointed out, the method of production of hydrocyanic acid from this plant, by crushing it in contact with cold water, is characteristic of enzymic hydrolysis, and this mode of origin is further supported by the fact that the formation of the acid is prevented by boiling water and by alcohol, but not by antiseptics in cold aqueous solution. As several enzymes are already known which have the power of effecting the hydrolysis of glucosides, it was a matter of considerable interest to ascertain whether the glucoside-destroying enzyme of *Lotus arabicus* was identical with any of these. In making preparations of the enzymes of the plant the latter was extracted with water saturated with chloroform in order to ensure antiseptic conditions. The usual methods of obtaining solid preparations were used, such as precipitation of the aqueous extract by alcohol, precipitation of the enzymes upon inert substances, such as calcium phosphate, and evaporation *in vacuo* of an aqueous extract of the plant.

#### *Preparation of Aqueous Extract.*

About 200 grammes of the finely powdered plant were extracted in the cold with chloroform water. The extract contained much hydrocyanic acid, which was removed by exposure in a vacuous desiccator over solid potash, leaving a brown coloured liquid, which on boiling formed a considerable coagulum of albumen. With this extract preliminary experiments were made to ascertain if it were competent to hydrolyse starch and certain glucosides.

#### *Action of the Aqueous Solution on Starch.*

Starch paste was prepared of such a consistence that when warm and fresh it could just be poured into test-tubes, and, on cooling, the latter could be inverted without any flow occurring. To a series of such tubes the following substances were added :—

- (a.) 1 cub. centim. distilled water.
- (b.) 1       ,,       of fresh Lotus extract.
- (c.) 1       ,,       of boiled Lotus extract.
- (d.) 1       ,,       of a 5 per cent. aqueous solution of malt extract.



In about 3 hours the contents of tubes *b* and *d* had become liquid and could no longer be inverted, whilst in tubes *a* and *c* the paste still remained semi-solid.

*Lotus arabicus* must therefore contain *diastase*.

#### *Action of Aqueous Extract on Proteids.*

It was observed that the clear chloroform-water extract on standing for several days gradually deposited a small amount of a precipitate, and at the same time the extract lost the property of giving a coagulum when boiled. To some of this extract a small quantity of an aqueous extract of the peas of *Lathyrus sativus* was added. This mixture when freshly made coagulated on boiling, but after four days' standing it remained clear after boiling.

A series of twelve test-tubes, each containing 5 cub. centims. of fresh Lotus extract, was prepared, each tube being carefully closed with cotton-wool. The tubes were set aside, and one of them raised to the boiling point on consecutive days. It was found that the amount of albumen coagulated gradually decreased until when the tenth tube was reached no precipitation occurred.

*Lotus arabicus* therefore contains a proteolytic enzyme.

#### *Action of the Aqueous Extract on Glucosides.*

These experiments were carried out in test-tubes, saturated solutions of amygdalin and salicin being placed in these, to which was then added 1 cub. centim. of Lotus extract, and, for the sake of comparison, to another similar set was added 1 cub. centim. of sweet almond extract (emulsin).

The sets of tubes comprised the following :—

<i>aa, a'</i> .	Containing respectively salicin and amygdalin with 1 cub. centim. boiled Lotus extract.
<i>bb, b'</i> .	„ „ „ „ „ „ 1 cub. centim. fresh Lotus extract.
<i>cc, c'</i> .	„ „ „ „ „ „ 1 cub. centim. almond extract.
<i>dd, d'</i> .	„ „ „ „ „ „ 1 cub. centim. distilled water.

Small samples of the tubes *a, b, c, d* were taken every half-hour and tested by means of ferric chloride for the presence of saligenin. Tubes *b* and *c* showed the presence of saligenin at the end of the first half-hour, while *a* and *d* did not show it even at the end of a week.

The decomposition of amygdalin is easily observed owing to the formation of benzaldehyde. Tube *c* had a distinct odour of benzaldehyde at the end of the first half-hour, while tube *b* had only a faint odour at the end of 3 hours, which

became well marked after 24 hours, whilst *a* and *d* showed no such effect. It is therefore proved that one of the enzymes of *Lotus arabicus* is able to decompose both salicin and amygdalin, though slowly.

*Action of Enzymes on Lotusin.*

A series of tubes containing each 5 cub. centims. of an aqueous solution of lotusin were made, and to these were added—

- a.* 1 cub. centim. of sweet almond extract (emulsin).
- b.* 1 cub. centim. of solution of malt extract (diastase).
- c.* 1 cub. centim. of aqueous extract of yeast (invertase).
- d.* 1 cub. centim. of aqueous extract of Lotus.

Only in tube *d* was there a rapid formation of hydrocyanic acid, but after 2 days it was also apparent in tube *a*.

As the glucoside-destroying enzyme of Lotus is thus shown to be different from invertase, diastase, and also from emulsin, it is proposed to call it *Lotase*.

*Separation of the Enzymes of Lotus arabicus.*

*Precipitation by Alcohol.*

A chloroform-water extract of the plant made as already described was poured into three times its volume of 90 per cent. alcohol. The voluminous white precipitate so formed was collected, re-dissolved in water, and re-precipitated in the same manner. It was then dried by exposure to the air on glass plates. It formed translucent scales almost completely soluble in water. Its aqueous solution rapidly liquefied starch paste, and destroyed the coagulable albumen of *Lathyrus sativus*, but was incapable of hydrolysing either salicin or amygdalin. It appears, therefore, that *lotase* is rapidly destroyed by contact with alcohol. This proteid is also unstable in presence of glycerin. In both these respects *lotase* differs from emulsin.

*Precipitation on Calcium Phosphate.*

To a chloroform-water extract of the plant a few cub. centims. at a time of calcium-chloride solution and sodium-phosphate solution were successively added, the whole being well shaken after each addition of the two reagents. When a sufficient quantity of calcium phosphate had been formed the mixture was shaken for about an hour and filtered, the precipitate being drained by the filter-pump, and dried by exposure to the air on glass plates. The preparation so obtained was a white amorphous powder, which charred when heated in a test-tube. It exhibited both proteolytic and amylolytic power, and hydrolysed both salicin and amygdalin. It consisted, therefore, of a mixture of the three enzymes present in this plant.

*Action of Heat on Lotus Extract.*

Test-tubes containing the extract were kept at a temperature varying from 30° to 60° C. for several hours, and their activities on starch, salicin, and albumen afterwards examined. It was found that exposure to a temperature of 50° almost entirely abolished the activity of the extract towards salicin, whilst it left almost unimpaired the power of attacking starch and coagulable proteid.

There is therefore further ground for the conclusion that the plant contains three separate enzymes, one amylolytic, a second proteolytic, and the third glucosidolytic, and that these three functions are not exerted by one and the same enzyme.

*Constituents of the Old Plants.*

It has been already mentioned, as a fact known to the Arabs, that old plants with fully ripe seeds are not poisonous, and we find that old plants furnish little or no prussic acid when crushed with water. Further examination has proved that lotase is still present in the plant after maturity, since aqueous extracts were able to effect the hydrolysis of lotusin and also of amygdalin and salicin. Small quantities of the colouring matter, lotoflavin, were also present, but lotusin was present either in minute quantity or was entirely absent.

It would therefore appear that the glucoside, lotusin, is concerned in the metabolism of the plant after the seeds are ripe, when it gradually disappears and the plant becomes non-poisonous.

The presence of lotoflavin renders it highly probable that the glucoside is hydrolysed in the metabolic process, and that the lotase is concerned in this action. There may be a further biological significance in the simultaneous presence in the plant of the glucoside and its hydrolytic enzyme, in the protection thus afforded to the plant at a critical period in its life-history through the poisonous effects it produces on animals which eat it in the earlier stages of growth, before the seeds are quite ripe and about to fall.

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## ADDENDUM (September, 1901).

*The Constituents of Lotus arabicus grown in England.*

In connection with the question of the effect of climate and soil on the constituents of plants, it became of interest to determine whether lotusin is contained in plants grown in England. Through the kindness of Sir W. THISELTON-DYER a few plants were grown under glass at Kew with seeds sent from Egypt, whilst Sir WILLIAM S. CHURCH, Bart., President of the Royal College of Physicians, was good enough to grow other plants at Hatfield.

The plants grown at Kew did not develop satisfactorily; many of them "damped off," and from the few that reached maturity no definite evidence of prussic acid could be obtained, although traces of lotoflavin were present. The plants grown at Hatfield, however, did remarkably well, and a number of them were examined after flowering. These showed undoubted evidence of the existence of lotusin and the production of prussic acid, from which it may be concluded that under the altered conditions of growth in this country, the constituents of the Arabian plant remain substantially the same.

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