BIOCHEMISTRY

Non-occurrence of Lotusin in *Lotus arabicus* L.

In 1901 Dunstan and Henry[1] reported the occurrence of lotusin, the maltose cyanohydrin ether of lotoflavin (quercetin[2]), in air-dried samples of *Lotus arabicus* L. collected in Egypt. Nearly forty years later, Henry[3] isolated lotaustralin (α-hydroxy-α-methyl-butyronitrile-β-D-glucose) from the same species.

An interest in this laboratory in studying the biosynthesis of lotusin led to a re-investigation of the cyanogenic glycosides in *L. arabicus*. Plants grown in greenhouses of the University of California were examined at various stages of growth for the presence of cyanogenic substances. Following extraction and chromatographic separation, as described elsewhere[4], the cyanogenic glycosides present in the plants were detected by the action of HCN on picrate paper[5] or the presence of reducing sugars[6] following their release by linamarase prepared from linseed meal by the method of Coop[7]. Evidence was obtained that a compound with the structure of lotusin does not occur in *L. arabicus*, but the presence of still another cyanogenic glucoside, linamarin (α-hydroxy-isobutyronitrile-β-D-glucose), was recorded. The evidence may be summarized as follows:

(1) The R_F values of the cyanogens in *L. arabicus* corresponded with those for linamarin and lotaustralin[4] in the following solvents: Butanol : pyridine : water, 6 : 4 : 3 (v/v); propanol : water, 7 : 3 (v/v); isopropanol : acetic acid : water, 70 : 5 : 25 (v/v); butanol : acetic acid : water, 120 : 30 : 50 (v/v); methylethyl ketone : acetone : water, 30 : 10 : 6 (v/v).

(2) The values for the cyanide content of the plant samples obtained either by hydrolysing the cyanogenic glycosides in the extract with endogenous enzyme(s) or with linamarase are similar to those obtained by eluting the areas on chromatograms which contain linamarin and lotaustralin and hydrolysing with linamarase (Table 1). Because cyanophoric plants normally contain an endogenous enzyme which can degrade the cyanogen found in that plant, the cyanide values obtained by use of the endogenous enzyme would represent a maximum value. Since the action of linamarase on samples eluted from the appropriate chromatographic areas always accounted for all the cyanide, there could be no large amounts of other cyanogens in the plant.

(3) When generally labelled L-valine-^{14}C and L-isoleucine-^{14}C are administered to *L. arabicus* seedlings, radioactivity is rapidly incorporated into the aglycone moieties of linamarin and lotaustralin but not into any other cyanogenic substance.

(4) Finally, analyses of the chromatographically isolated cyanogenic constituents (which fully account for the total cyanide released from the plant) for the cyanide, acetone and sugar moieties of the glycoside give a ratio of 1 : 1 : 1 (see Table 2). There is no corresponding release of quercetin.

Table 1. Cyanide Content of *Lotus arabicus* Plants as Determined by Different Procedures

Age of plants	Procedure employed *A*	*B*	*C*	*D*
	μmoles per g (fresh wt.)			
5 days	5·80	6·20	5·93	5·87
37	9·72	10·32	14·20	13·93
81	7·70	9·95	10·78	11·99
96	4·62	6·40	7·84	8·20
137 (flowering)	1·44	1·56	3·15	2·76

Procedure *A*: Endogenous enzyme. *B*: Endogenous enzyme plus linamarease. *C*: Hydrolysis by linamarase of aqueous extract of residue obtained by extracting plant with 80 per cent ethanol and evaporating. *D*: Hydrolysis by linamarase of area from chromatogram which contains linamarin and lotaustralin.

Table 2. Analysis of Components of the Cyanogenic Glycosides of *Lotus arabicus* L

Age of plants	μmoles per g (fresh weight) Acetone	Cyanide	Glucose
12 days	9·16	9·42	8·94
45	11·34	11·86	12·03
96	7·93	8·42	7·64
137 (flowering)	2·54	2·76	2·86

After elution of the cyanogenic glycoside from the chromatogram the various components were determined as follows: the HCN released after enzyme hydrolysis was trapped in 0·1 N NaOH by overnight aeration and determined by the Aldridge method[8]. The acetone moiety was steam-distilled over into 0·1 per cent 2,4-dinitrophenyl hydrazine in 2 N HCl and spectrophotometrically assayed[9]. The procedure[10] for glucose estimation is based on the phosphorylation of glucose by ATP in the presence of yeast hexokinase and the subsequent oxidation of glucose-6-phosphate by TPN^+ in the presence of the dehydrogenase. The TPNH formed is read at 340 mμ. Quercetin release was checked by the method of Nagshki *et al.*[11].

Dunstan and Henry believed lotusin to be an ether of maltose cyanohydrin and a new flavanoid, lotoflavin. The latter was later shown to be quercetin. Although hydrolysis of plant extracts by HCl results in a release of quercetin, there appears to be no relationship to the cyanogenic glucosides present in this species.

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An Antifungal Triene from a *Streptomyces* sp.

While polyene antifungal antibiotics are of frequent occurrence among *Streptomyces* spp., those so far described contain 4–7 double bonds in conjugation. We wish to report the production of an antifungal conjugated triene, *MM*8, by a *Streptomyces* sp., ACC 1293. The investigation is incomplete; but as no further work is contemplated we felt that a brief account of our findings might be of interest.

Streptomyces sp. ACC 1293 was isolated in 1955 by Dr. Joyce McCloskey from a soil with a high chalk content. It is grown at 25° under stirred, aerated conditions in Raulin–Thom medium containing 3 per cent 'Dextrolact'. The fermentation, the progress of which may be followed by means of the ultra-violet absorption of the product, is usually collected after about 70 h.

*MM*8 (λ_{max} 262, 272 and 283 mμ, $E^{1\%}_{1\,cm}$ = 550, 730 and 535, respectively; ν_{max} 1,745 cm^{-1}) is obtained as a buff amorphous powder from chloroform extracts of culture filtrates. It is light-sensitive, rapidly losing both its ultra-violet absorption and its antifungal activity, but is stable to cold dilute acid in the dark. Addition of 3 N sodium hydroxide to an alcoholic solution of *MM*8 causes replacement of the triene absorption by a broad maximum at 335 mμ. *MM*8 is transformed by shaking in chloroform solution with 0·0001 N sodium hydroxide into a mixture of two compounds detected by thin-layer