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# THE CYANOGENIC GLUCOSIDES AND GLUCOSIDASES OF RUBBER SEED KERNEL

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**Abstract:** In previous studies, linamarin was identified as the only cyanogenic glucoside of rubber seed kernel. This study indicates that small amounts of lotaustralin are also present. Behaviour of linamarase (EC 3.2.1.21) from rubber seed kernel indicated the presence of multiple enzymes similar to that of the linamarses of manioc rind. The pH optimum and activation energy of the enzyme was found to be approximately 6.2 (with a shoulder at 5.4) and 10.1 - 10.3 Kcal, mole$^{-1}$ respectively. Activity appeared to decline on dilution.

## 1. Introduction

The cyanogenic glucoside linamarin ($\alpha-\beta-$D-glucopyranosyloxy-2-methyl, propionitrile) has been reported in many plants including cassava, rubber, flax, white clover, lima bean, etc.[2] Although it is found together with lotaustralin (2-$\beta$-D-glucopyranosyloxy-2-methyl butyronitrile) in most plant sources, the leaves and seeds of *Hevea brasiliensis* (rubber) have been reported to contain only linamarin[2] despite the fact that these two glucosides appear to be generally synthesized by the same set of enzymes.[3,6]

In this study indirect evidence has been provided to show that lotaustralin occurs in small amounts in rubber seed kernel.

The non-specific $\beta-$glucosidase, linamarase (EC 3.2.1.21) from cassava and other sources has been extensively studied.[1,4,5,7-10,14] We showed the presence of multiple linamarases in cassava and that treatment of the major linamarase (linamarase B) with 4 M Urea produced a linamarase A.[8,9] Selmar et al. ,[11] showed that the linamarase of *Hevea brasiliensis* leaf was a homo-oligomer with monomeric units of 64 K Daltons, which could undergo association. In the present study, evidence has been provided to show that the enzymes of cassava tuber and rubber seed kernel are similar to each other. Further, that linamarases of cassava and rubber seed on passage through a Sephadex G-200 column tend to associate. The pH activity curve and Arrhenius activation energy of rubber seed kernel linamarase has been compared with the available data on other linamarases.

## 2. Experimental

### 2.1 Samples

Rubber seeds (one day old) were collected from the field and decorticated. Cassava tubers were collected from the market and the rind (peel) was separated from the tuber for experimental work.

### 2.2 Studies on Cyanogenic Glucosides

Decorticated kernels of rubber seed (10g) were homogenized in cold water (5-10°C, 200 ml) and subjected to autolysis for 24 h. at room temperature. On steam distilling the autolysate, 50 ml of distillate was collected. An aliquot was subjected to gas liquid chromatographic analysis using a Shimadzu GC-9A instrument equipped with a flame ionisation detector.

Conditions of analysis were as follows. Injection volume, 2 $\mu$l; column, glass 1.7 x 5 mm (OD), packed with 10% Carbowax 20M; carrier gas, $N_2$ (30ml $min^{-1}$), column temperature, 80°C (isothermal); injector temperature, 200°C.

### 2.3 Isolation of Linamarin

Linamarin was isolated from cassava rind and rubber seed kernel by the method of Wood[13].

### 2.4 Preparation of Linamarase

Linamarase was isolated by acetone precipitation of an extract of cassava rind and rubber seed kernel and assayed (Wood)[13] using the alkaline-picrate spectrophotometric method (Pieris *et al.*).[8] It was purified on Sephadex G-200 (Pieris *et al.*).[8]

### 2.5 Linamarase Activity

The effect of temperature was measured using 0.1M citrate buffer pH 6.0 linamarin, 14.4 $\mu$ moles; linamarase, 0.13 $\mu$ moles-$CN^-$ $min^{-1}$ ; incubation time, 10 min and temperature of activity measurement, 10°, 20°, 30° and 40°C. The effect of pH was measured between pH 4.6 and 6.8 (0.1M citrate buffer) (Pieris et al.).[8] Incubation time, 15 min; temperature, 30°C; linamarin, 14.4 $\mu$ moles; linamarase, 0.13 $\mu$ moles $CN^-$ $min^{-1}$.

## 3. Results

### 3.1 Cyanogenic Glucosides

Incubation of purified glucosides with linamarase produced only acetone on GC analysis showing the presence of linamarin. However, the autolysate of rubber seed, leaf and kernel produced acetone and ethyl methyl ketone in the ratio of 97:3, indicating that small amounts of lotaustralin was also present. The GC chromatogram

of the leaf autolysate contained 3 other small peaks which were also present in leaves of other non-cyanogenetic plants (eg. *Cocos nucifera* and *Artocarpus nobilis*).

### 3.2 Sephadex Separation of Crude Linamarase

Acetone precipitated linamarase of rubber seed kernel on passing through a column of Sephadex G-200 yielded a peak with a pronounced shoulder at the lower eluent volume side of the peak (Figure 1). On mixing the crude enzyme of manioc rind and rubber seed kernel (a total of 55 $\mu$ g $CN^-$ $min^{-1}$) the peak was symmetric (Figure 2). The activity recovered from the column was 151 $\mu$g $CN^-$ $min^{-1}$.

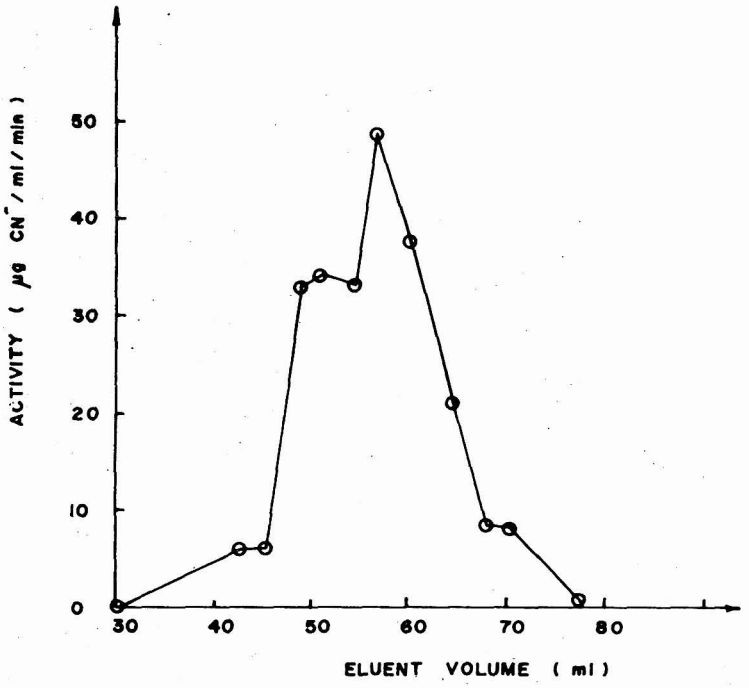


**Figure 1 : Gel chromatography of crude extract of rubber seed kernel linamarase (Sephadex G-200).**

The figure shows the main peak and a pronounced shoulder at lower eluent volume. The latter suggesting the associated form of Selmar.[11]

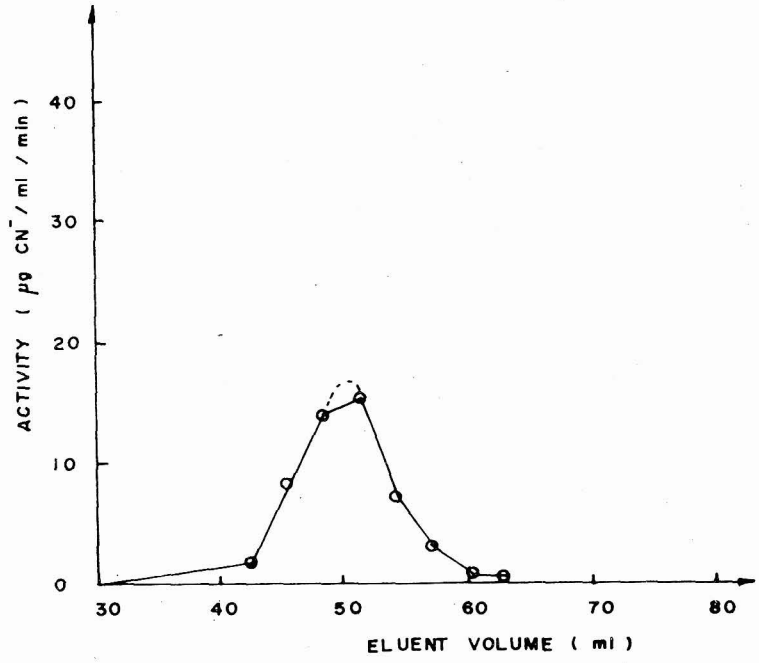


**Figure 2 : Gel chromatography of a mixture of crude rubber seed kernel and cassava linamarases.**

Conditions of chromatography identical to Figure 1. The main peak of Figure 1 is no longer evident. New peak position coincides with original shoulder.

### 3.3 Arrhenius Activation Energy

Neglecting the 40°C point the Arrhenius activation energy of the major peak of rubber seed kernel was found to be 10.1 to 10.3 Kcal. $mole^{-1}$ (Figure 3).

### 3.4 Effect of pH

A pH optimum at 6.2 with a shoulder at pH 5.4 was observed (Figure 4).

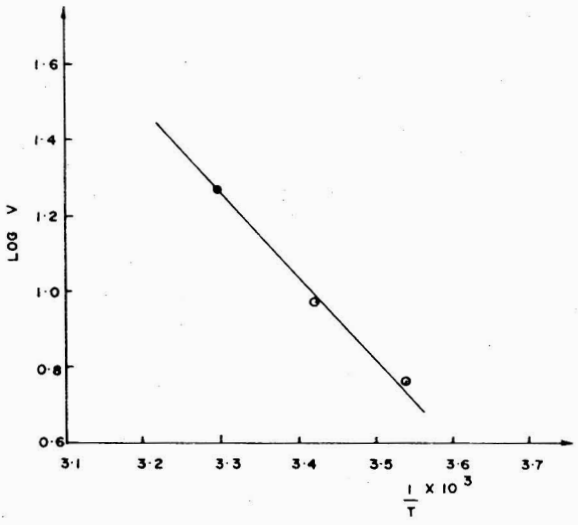


**Figure 3 : Arrhenius plot for activation energy.**
$40^0$C point has been neglected assuming enzyme has begun to denature.

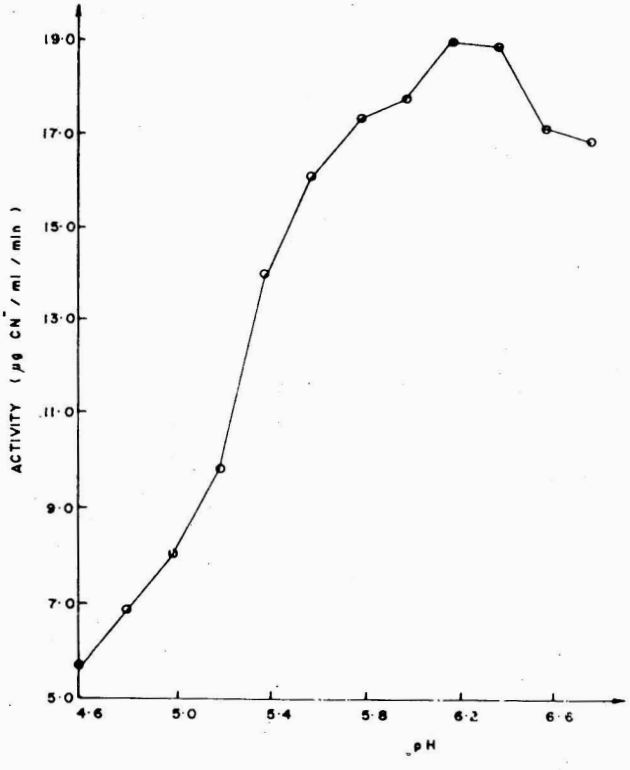


**Figure 4 : The pH activity curve**

## 4. Discussion

It appears that rubber seed kernel and leaf contains small amounts of lotaustralin. This is consistent with theories of biosynthesis of the two glucosides which suggest that they are synthesized by the same set of biosynthetic enzymes with a different initial substrate.[3,6]

Gel filtration studies showed that: (a) a mixture of cassava rind and rubber seed linamarases ran in the same position with a symmetric peak - indicating that the eluted enzymes had approximately the same molecular weight, (b) the peak of the mixture had moved to a higher molecular weight position compared to the rubber seed kernel linamarase and that the peak eluent volume was identical to that of the original shoulder, (c) the activity of the mixture arising from the column was 2.7 fold the activity of the enzyme added to the column.

The above is consistent with association as proposed by Selmar *et al.*,[11] provided the higher molecular weight form has increased activity. This also pre-supposes that the cassava and rubber seed linamarases are very similar, if not identical, to allow them to associate.

The study of the effect of pH on enzyme activity showed that the curve was very similar to that of linamarase B of cassava.[8] A survey of the literature (Table 1) indicates that many of the linamarases have similar pH optima and therefore probably a similar mechanism of action.

**Table 1: Comparison of pH optima of linamarases**

| Study | Source | pH Optima |
|---|---|---|
| Present work | Rubber Seed Kernel | 6.2 |
| Pieris *et al.*[8] | Peel from Cassava tubers | 6.0 - 6.6 (linamarase A) |
| Pieris *et al.*[9] | Peel from Cassava tubers | 6.2 (linamarase B) |
| Bileski *et al.*[1] | Cassava tubers | 5.5 |
| Cooke *et al.*[4] | Parenchymal tissue and the peel of Cassava | 6.0 |
| Schewale *et al.*[10] | Sclerotium rolfsi | 4.2 |
| Gonde *et al.*[5] | Candida molishiana (Exocellular) | 4.0 - 4.5 |
| Ikediobi *et al.*[7] | Fusarium equiseti | 6.0 |
| Yeoh and Sia[14] | Cassava leaf | 6.5 |

Although the Arrhenius activation energy was higher than that reported previously (Table 2), it is possible that this is due to activation energy being measured in earlier studies at temperatures above 37°C, the point at which linamarase is reported to begin to get deactivated.[1] It was also noticed that enzyme activity

appeared to decline on dilution. It is uncertain whether this is due to denaturation or reversible dissociation to a less active form.

**Table 2 : Comparison of activation energies of linamarases**

| Study | | Temperature °C | Parameter used | Source | Activation energy Kcal.mol$^{-1}$ |
|---|---|---|---|---|---|
| Present work | | 10 - 30°C | V | Rubber seed kernel | 10.1 |
| | | 10 - 30°C | K | " | 10.3 |
| | | 10 - 40°C | V | " | 4.3 |
| | | 10 - 40°C | log V vs $\frac{1}{T}$ | " | 8.4 |
| | | 10 - 30°C | log V vs $\frac{1}{T}$ | " | 10.3 |
| | | 10 - 40°C | log K vs $\frac{1}{T}$ | " | 8.5 |
| | | 10 - 30°C | log K vs $\frac{1}{T}$ | " | 10.3 |
| Pieris[8,9] | | | | | |
| | A | 30 - 50°C | log K vs $\frac{1}{T}$ | Cassava peel | 5.7 |
| | B | 30 - 50°C | log K vs $\frac{1}{T}$ | " | 3.3 |
| | C | 30 - 50°C | log K vs $\frac{1}{T}$ | " | 5.2 |
| | D | 30 - 50°C | log K vs $\frac{1}{T}$ | " | 5.4 |
| Umezurike[12] | | - | - | Botryod-plodia theobromae | 4.5 - 6.0 |
| Ikediobi *et al.*[7] | | - | - | Fusarium equiseti | 8.21 |

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