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OCCURRENCE OF THE CYANOGEN LINUSTATIN IN *HEVEA BRASILIENSIS*

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Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; rubber tree; cyanogenic glucosides; cyanogenesis; linustatin; HCN-metabolism.

Abstract—During seedling development of *Hevea brasiliensis* the cyanogenic diglucoside linustatin is exuded from the endosperm. These data support the hypothesis, that the stored cyanogenic monoglucoside linamarin is glucosylated to linustatin during mobilization of the cyanogenic glucosides.

INTRODUCTION

Studies on cyanogenic glycosides in *Hevea brasiliensis* [1–3] have shown that the cyanogenic glucoside linamarin acts as nitrogen source during seedling development. During mobilization the cyanogenic monoglucoside linamarin is converted to the diglucoside linustatin, which acts as a transport form. In contrast to linamarin, linustatin is not hydrolysed by the linamarase [4] of *Hevea brasiliensis* [5], which shows that linustatin is a suitable transport form of nitrogen. This can, in turn, explain how the cyanogenic material can be transported without loss of hydrogen cyanide (HCN) due to hydrolysis.

This paper presents data on the occurrence of linustatin during germination and seedling development of *Hevea brasiliensis*.

RESULTS AND DISCUSSION

Using the standard method of ref. [6] for detection of cyanogenic glycosides in methanolic extracts, linustatin was not detectable in *Hevea brasiliensis*, even when large amounts (up to 500 g) of leaf or stem material were used. As the main storage tissue for linamarin in *Hevea* seeds is the endosperm (in ungerminated seeds more than 90% of the HCN-potential is localized in the endosperm [7]) and utilization of this substance takes place at least partially in young leaves, a method was devised to collect the substance(s) which are transported from the endosperm tissue to the cotyledons. Using this seed-drainage technique (Fig. 1) it was shown by gas chromatography that the cyanogenic diglucoside linustatin is present in *H. brasiliensis* and occurs during seedling development in endosperm exudates. The liquids obtained by seed-drainage were purified by high pressure liquid chromatography collecting the linustatin fraction. This was used for ^{1}H NMR, the chemical shifts and coupling constants being identical to published data [8]. Additionally, acidic hydrolysis of the linustatin fraction revealed glucose as the only sugar.

Based on these results methanolic extracts of *H. brasiliensis* seeds, representing different developmental stages, were analysed for linustatin: stored seeds contained a considerable amount of linustatin, but freshly collected, unstored and non-germinated seeds were free of, or contained only very low amounts of linustatin.

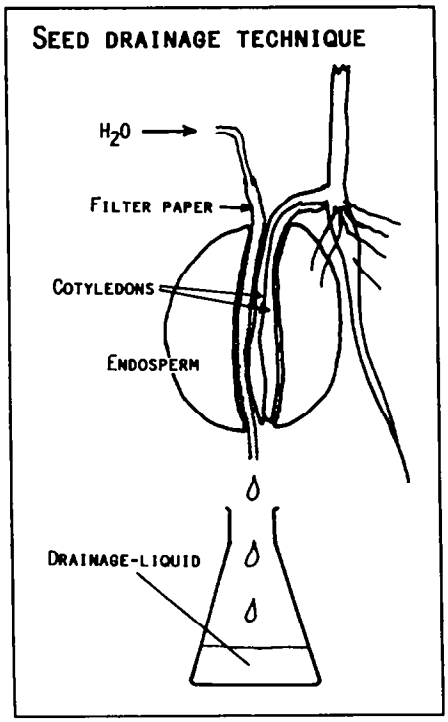

Fig. 1. The seed of seven-day-old *H. brasiliensis* seedlings were drained six days under 90% relative humidity and light–dark ratio of 14/8 hours. Details are given in the Experimental.

It is known that during seedling development of *H. brasiliensis* the content of cyanogenic glucosides decreases to 15% of the original cyanogen content of the seeds [1]. During this period HCN is not liberated, although any metabolism of cyanogens takes place via HCN. Thus, the cleavage of cyanogenic glycosides resulting in HCN production has to be located in close proximity to the enzymes responsible for the assimilation of HCN. As the majority of the cyanogen is stored in the endosperm and as the enzyme for re-fixing HCN (β-cyanoalanine synthase) is located in cotyledons, young leaves and roots of the seedling [2], linustatin must be transported from the storage tissue to these other organs. The linamarin-cleaving β-glucosidase is present intra- and extracellularly in all tissues of *H. brasiliensis* [4], including the space between endosperm and the cotyledons. This excludes transport of the intact cyanogenic monoglucoside. In contrast to linamarin, linustatin cannot be split by the *Hevea* β-glycosidase. Therefore, this diglucoside may function as a hydrolysis-protected transport molecule for linamarin.

EXPERIMENTAL

Seed drainage. The hard pericarp of surface sterilized seeds was removed. The cotyledons, to which half of the large endosperm was attached, were separated carefully. One cotyledon was then separated from the corresponding endosperm part. Two layers of filter paper were placed between those two tissues. A string was used to hold the drained seed in its original position (Fig. 1). During germination, a continuous waterflow (3 ml/hr) was used to washed out the substances exudated from the endosperm. The drainage liquid was collected and freeze-dried.

Purification and identification of linustatin. In order to isolate linustatin from the seed-drainage liquids methanolic extracts of freeze-dried material were chromatographed using HPLC on RP-18 (1.6 × 25 cm) with H_2O–MeCN (97/3, 6 ml/min) with a RI-detector. The linustatin fraction (R_t *ca* 29 min) was chromatographically pure and was used for ^{1}H NMR spectroscopy in D_2O–Me_2CO-d_6 (9:1), (400 MHz, Bruker WM-400).

Gas chromatography. Aliquots of different extracts were dried, dissolved in 20 μl pyridine and silylated with 50 μl *N,N*-bistrimethylsilyltrifluoroacetamide and 20 μl trimethylchlorosilane. Several μl of the solutions were injected into a capillary GLC system using a DB-5 column (15 m × 0.32 mm), He (1 ml/min) as carrier gas, injector 260°; FID 270°.

Hydrolysis. An liquot of the linustatin fraction was hydrolysed with 0.5 N NCl for 90 min at 95°; the sample was then lyophilized, silylated as described above and gas-chromatographed on a DB-1 column (15 m × 0.53 mm) at 170° with N_2 (5 ml/min) as carrier gas; injector 195°; FID 200°.

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