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LINAMARIN, LOTAUSTRALIN, LINUSTATIN AND NEOLINUSTATIN FROM *PASSIFLORA* SPECIES

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Key Word Index—*Passiflora pendens*; *P. warmingii*; *P. adenopoda*; Passifloraceae; cyanogenic glycosides; linamarin; lotaustralin; linustatin; neolinustatin.

Abstract—Linamarin, lotaustralin, linustatin, and neolinustatin were isolated from *Passiflora pendens* (subgenus *Plectostemma*, section *Cieca*). Linamarin and lotaustralin were isolated from *P. adenopoda* (subgenus *Plectostemma* section *Pseudodysosmia*), and linamarin (possibly accompanied by linustatin) was isolated from *P. warmingii* (section *Cieca*). These data suggest that several members of the subgenus *Plectostemma* of *Passiflora* are capable of elaborating valine/isoleucine-derived cyanogenic glycosides instead of or in addition to those which contain cyclopentenoid structures.

INTRODUCTION

Linamarin and lotaustralin are valine/isoleucine-derived cyanogenic glycosides which are widely distributed in the Linaceae, Fabaceae, Euphorbiaceae and Asteraceae [1–6]. Linamarin (2-β-D-glucopyranosyloxy-2-methylpropionitrile) [2, 3, 7–11] and lotaustralin (*R*)2-β-D-glucopyranosyloxy-2-methylbutyronitrile) [11, 12] usually occur together in a given plant as one set of enzymes is responsible for the biosynthesis of both [9, 13–15]. Linustatin and neolinustatin, the 6′-*O*-glucosides of linamarin and lotaustralin respectively, have been reported only from seeds of *Linum usitatissimum* L. (Linaceae) [16].

The immature fruit of *Passiflora adenopoda* has been reported poisonous to humans. All parts of the plant which were tested contained cyanogenic materials except the tendrils and completely ripe fruit [17]. Members of the genus *Passiflora* typically produce cyanogenic glycosides with cyclopentenoid ring structures [18–21], although one species, *Passiflora edulis* contains prunasin [22]. Representatives of *Passiflora* subgenus *Plectostemma* section *Cieca* contain several cyclopentenoid cyanogenic glycosides such as passicoriacin and epipassicoriacin [Spencer and Seigler, submitted for publication], and passisuberosin and epipassisuberosin [23]. The presence of linamarin has previously been reported from *P. warmingii* [18, 19]. To date there are no reports of structures of cyanogenic compounds from plants of section *Pseudodysomia*.

As a part of a chemosystematic study of cyanogenesis in the genus *Passiflora*, we undertook to determine the extent of distribution of aliphatic cyanogenic glycosides in species of these two sections.

RESULTS AND DISCUSSION

Linamarin, lotaustralin, linustatin and neolinustatin were isolated from *P. pendens*. Examination of the ^{13}C NMR spectra of the cyanogenic glycosides of this species showed peaks identical with those reported [16, 24, 25] for a mixture of the above four compounds. Isolated fractions gave peaks identical to those reported for each compound, except in the case of lotaustralin where the small amount present precluded isolation and complete characterization of the compound. In this instance, GC of the sample (after silylation) on OV-101 [26] indicated the presence of linamarin and epilotaustralin/lotaustralin, while GC of the peracetylated sample on Silar-10C and ECNSS-M [27], indicated that the latter fraction consisted of 13% (*S*)-epilotaustralin and 87% (*R*)-lotaustralin. ^{1}H NMR spectra were also consistent with a mixture of these four cyanogens [25]. The ^{1}H NMR spectra of the TMS derivatives of compounds isolated from the mixture were obtained for linamarin, linustatin and neolinustatin. Linamarin was obtained from *P. warmingii* as previously reported [18]. A second cyanogen gave spectral data that suggested the presence of linustatin, but due to the small quantity present, these results need to be reconfirmed. Linamarin and lotaustralin were also isolated from *P. adenopoda*.

The presence of all four known valine/isoleucine derived compounds distributed among several species of *Passiflora* subgenus *Plectostemma* sections *Cieca* and *Pseudodysosmia* suggests the pathway is common among members of these groups. Further, cyanogenic diglycosides have only rarely been found in vegetative tissues of plants.

EXPERIMENTAL

Plant material. Living material of *P. pendens* J. MacDougal, sp. nov. in ed. (J. M. 570, 571, Duke University Herbarium, DUKE) was a gift of J. MacDougal, Department of Botany, Duke University. *P. adenopoda* D.C. material was obtained from the Missouri Botanical Garden (*C. Nelson and A. Clewell* 730, 1972, Honduras, MO 2112554). Seeds of *P. warmingii* Mast. were

obtained from the Royal Botanic Gardens, Kew and from the Botanic Garden of the University, Leiden, The Netherlands. Voucher specimens of each were deposited in the University of Illinois Herbarium (ILL).

Isolation of the glycosides. P. pendens: fresh leaf material (240 g) was ground with 80% MeOH in a blender. The suspension was then filtered and concd under vacuum at 40° to a syrup. This material was then partitioned between $CHCl_3$ and H_2O. The aq. phase was placed on a cellulose column (microcrystalline cellulose: Whatman CF1: Whatman CF11, 1:1:1) and eluted with Me_2CO–H_2O (5:1). Fractions were collected (15 ml) and the cyanogenic material was found in fractions 19–30 by placing small aliquots of each in a vial, evaporating the Me_2CO, adding linamarase (see below) and testing with Feigl–Anger strips [28]. The cyanogenic fractions were concd and chromatographed on Whatman 3MM paper in Me_2CO–H_2O (5:1). The cyanogenic compounds were located by cutting a 1 cm strip from the centre of the chromatogram and testing 1 cm^2 sections from this strip as above. The cyanogenic band (R_f 0.75) was desorbed with H_2O, concd under vacuum and rechromatographed on paper with MeCOEt–Me_2CO–H_2O (15:5:3). The cyanogenic material (R_f 0.65, very broad) was desorbed with H_2O concd under vacuum and recrystallized from EtOH (yield 0.3%).

P. warmingii (Leiden): fresh leaf material (100 g) was ground, extracted with 80% MeOH, concd and extracted with $CHCl_3$ as above. The aqueous phase was concd under vacuum and placed on a silica gel column (58 micron) and eluted with $CHCl_3$–MeOH (17:3). Fractions (10 ml) 36–67 were found to contain cyanogenic glycosides when tested as above. These were pooled and concd under vacuum; the final product was recrystallized from EtOH (yield 0.4%).

P. warmingii (Kew): fresh leaf material (219 g) was extracted as above. The aq. extract was placed on a cellulose column ane eluted as above. The cyanogenic material was located in fractions (20 ml) 20–50. These were combined and concd under vacuum and the resulting syrup chromatographed on Whatman 3MM paper in MeCOEt–Me_2CO–H_2O (15:5:3). The cyanogenic band (R_f 0.4) was desorbed with H_2O and recrystallized from EtOH (yield 0.4%).

P. adenopoda: dried herbarium material (3 g) was extracted as above. The aq. extract was chromatographed on Whatman 3MM paper in Me_2CO–H_2O (5:1). The cyanogenic material (R_f 0.75) was desorbed in H_2O and recrystallized from EtOH.

HPLC analysis of cyanogens. All samples were separated on an amine column (Alltech) in 80% MeCN (flow rate 1.0 ml/min) and detected with a refractive index detector. Linamarin eluted at 5.2 min, lotaustralin at 4.9 min, linustatin at 11.8 min and neolinustatin at 10.5 min. These values were identical to those obtained for commercially available linamarin (Calbiochem) and linustatin and neolinustatin isolated from *Linum usitatissimum* (flax seed) [16].

Preparation of linamarase. Fresh flax seed (50 g) was ground in a blender with cold Me_2CO (1 l.). The resulting suspension was filtered under vacuum and rinsed with additional cold Me_2CO (1 l.). The solid material was dried under vacuum and resuspended in phosphate buffer (pH 6.8, 500 ml), stirred in an ice bath for 1 hr and filtered. This filtrate was dialysed against three changes of phosphate buffer (pH 6.8) for 12 hr each. The resulting linamarase enzyme preparation was tested to insure the absence of cyanide and then its hydrolytic activity was confirmed by testing against commercially available linamarin (Calbiochem) with the Feigl–Anger method.

Identification of sugars. All materials were assayed for glucose by the glucose oxidase method of ref. [29]. Combined glucose oxidase and quantitative cyanide assay by the Lambert method [30] revealed glucose to cyanide molar ratios of 1:1 for unknown linamarin and lotaustralin samples and 2:1 for unknown linustatin and neolinustatin samples.

Spectral determination. ^{13}C NMR spectra of samples obtained by HPLC analysis were measured on a Nicolet NT-360 (360 MHz) and a Bruker WM-500 (500 MHz) spectrometer in D_2O with TSP as reference. Some spectra were run again with dioxane as reference to clarify low-field signals. ^1H NMR spectra of samples from HPLC were recorded as TMS derivatives in $CDCl_3$. These were prepared as previously described [20].

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