Acta Chemica Scandinavica, 1995: 49: 540–542
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Short Communication

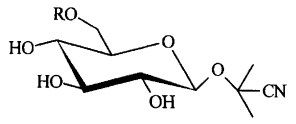
On the Absence of 2-(2′-Cyclopentenyl)glycine-Derived Cyanogenic Glycosides in Cassava, *Manihot esculenta* Crantz

Jens Lykkesfeldt† and Birger Lindberg Møller*

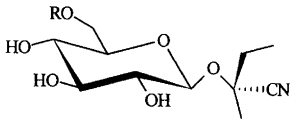
Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark

Lykkesfeldt, J. and Møller, B. L., 1995. On the Absence of 2-(2′-Cyclopentenyl)glycine-Derived Cyanogenic Glycosides in Cassava, *Manihot esculenta* Crantz. – Acta Chem. Scand. 49: 540–542 © Acta Chemica Scandinavica 1995.

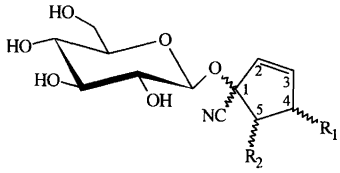
Cassava, *Manihot esculenta* Crantz, accumulates the cyanogenic glycosides linamarin [2-β-D-glucopyranosyloxy-2-methylpropiononitrile] **1** and lotaustralin [(*R*)-2-β-D-glucopyranosyloxy-2-methylbutyronitrile] **2** in roots and leaves.[1] Recently, a microsomal enzyme system has been isolated from cassava, catalyzing the *in vitro* conversion of L-valine into acetone cyanohydrin and of L-isoleucine into 2-butanone cyanohydrin in the biosynthesis of **1** and **2**, respectively.[2] Investigation of the substrate specificity of the microsomal system disclosed its ability to catalyze the conversion of 2-(2′-cyclopentenyl)glycine to the corresponding cyanohydrin at a rate equivalent to 7 % of the rate observed with L-valine, the best substrate found.[2] 2-(2′-Cyclopentenyl)glycine derived cyanogenic glycosides, i.e., tetraphyllin A [(*R*)-1-β-D-glucopyranosyloxy-2-cyclopentenecarbonitrile] **3**, deidaclin [(*S*)-1-β-D-glucopyranosyloxy-2-cyclopentenecarbonitrile] **3a**, epivolkenin [(1*R*,4*S*)-1-β-D-glucopyranosyloxy-4-hydroxy-2-cyclopentenecarbonitrile] **4**, taraktophyllin [1*S*,4*R*)-1-β-D-glucopyranosyloxy-4-hydroxy-2-cyclopentenecarbonitrile] **4a**, tetraphyllin B [(1*R*,4*R*)-1-β-D-glucopyranosyloxy-4-hydroxy-2-cyclopentenecarbonitrile] **5**, volkenin [(1*S*,4*S*)-1-β-D-glucopyranosyloxy-4-hydroxy-2-cyclopentenecarbonitrile] **5a**, and gynocardin [(1*R*,4*S*,5*S*)-4,5-dihydroxy-1-β-D-glucopyranosyloxy-2-cyclopentenecarbonitrile] **6**, have been found exclusively within five closely related tropical and subtropical families: Passifloraceae, Turneraceae, Flacourtiaceae, Malesherbiaceae, and Achariaceae, all belonging to the order Violales.[3–5] Cassava be-



	R
1	H
1a	6′-*O*-β-D-glucopyranosyl



	R
2	H
2a	6′-*O*-β-D-glucopyranosyl



	R_1	R_2		
3	H	H	(*R*)	Tetraphyllin A
3a	H	H	(*S*)	Deidaclin
4	OH	H	(1*R*,4*S*)	Epivolkenin
4a	OH	H	(1*S*,4*R*)	Taraktophyllin
5	OH	H	(1*R*,4*R*)	Tetraphyllin B
5a	OH	H	(1*S*,4*S*)	Volkenin
6	OH	OH	(1*R*,4*S*,5*S*)	Gynocardin

† Present address: Department of Pharmacology, University of Copenhagen, The Panum Institute, 3 Blegdamsvej, DK-2200 Copenhagen N, Denmark.
* Author to whom correspondence should be addressed.

longs to the family Euphorbiaceae, but the result of the biosynthetic *in vitro* studies reported above prompted us to investigate whether 2-(2′-cyclopentenyl)glycine derived cyanogenic glycosides are present in cassava.

In cassava, **1** and **2** are found in ratios about 93:7.[1] Linustatin [2-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-2-methylpropiononitrile] **1a** and neolinustatin [(*R*)-2-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-2-methylbutyronitrile] **2a**, have also been identified in trace amounts.[6]

1, **1a**, **2**, **2a**, and **3–6** can be separated by reversed phase HPLC (Fig. 1). However, HPLC analysis of cassava extracts shows the presence of only **1** and **2** (Fig. 2). The total effluent from the HPLC column was subsequently collected and its cyanide content determined upon treatment with β-D-glucuronidase. High amounts of HCN were released from the fractions known to contain **1** and **2**. No cyanide was generated in the remaining fractions. The lower detection limit of the cyanide assay is 1.0 nmol HCN[7] corresponding to a cyanogenic glycoside content of approximately 3 nmol g^{-1} plant material. The level of **1** and **2** in cassava seedlings is about 10 μmol g^{-1} and 0.7 μmol g^{-1}, respectively.[1] The failure to detect cyanide release from the fractions corresponding to authentic standards of **3–6** demonstrates that if these compounds are present in cassava, the molar amounts of **3–6** are less than 0.03 % compared with **1**.

Consequently, since no 2-(2′-cyclopentenyl)glycine-derived cyanogenic glycosides are detectable in cassava seedlings, the observed metabolism of 2-(2′-cyclopentenyl)glycine reported by Koch *et al.*[2] must reflect the inability of the otherwise highly specific enzyme system fully to discriminate between 2-(2′-cyclopentenyl)glycine and the structurally related amino acids valine and isoleucine.[8,9] The consistently observed co-occurrence of **1** and **2** indicates that a single enzyme system is responsible for their biosynthesis. Small variations at the substrate binding site of this enzyme from one plant species to another or the presence of different free amino acid levels of valine and isoleucine determines whether **1** or **2** is the main cyanogenic glucoside produced. Accordingly, the lack of 2-(2′-cyclopentenyl)glycine-derived cyanogenic glycosides in cassava most likely reflects the inability of the cassava plant to produce the parent amino acid 2-(2′-cyclopentenyl)glycine rather than inadequate enzymatic capabilities. Consequently, feeding experiments or other biosynthetic experiments based on an exogenous supply of this rare amino acid could well give rise to the formation of 2-(2′-cyclopentenyl)glycine-derived cyanogenic glycosides. However, these compounds would be artefacts in the sense that the cassava plant would not produce these *in vivo*. Thus, linamarin, lotaustralin, linustatin and neolinustatin remain the sole identified cyanogenic glycosides of cassava.

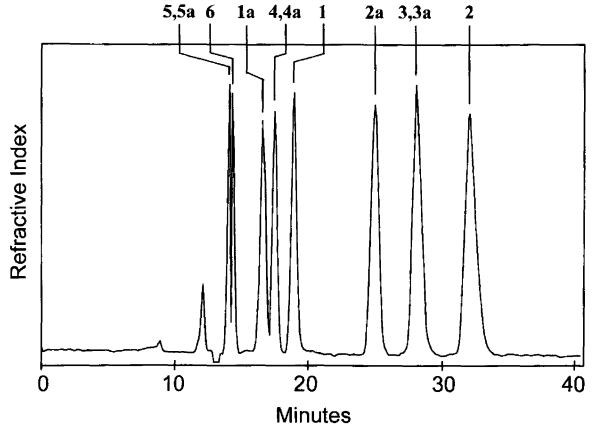


Fig. 1. Separation of **1**, **1a**, **2**, **2a**, and **3–6** by reversed phase HPLC. In the analytical system used, the stereoisomeric pairs **3/3a**, **4/4a**, and **5/5a** co-eluted.

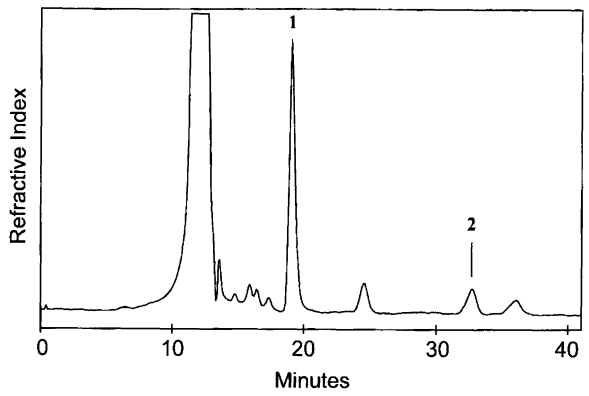


Fig. 2. The cyanogenic glycoside content as analyzed by HPLC. Analysis of the effluent showed no 2-(2′-cyclopentenyl)glycine-derived cyanogenic glycosides. The peak between **1** and **2** has recently been identified as the non-cyanogenic compound isopropyl-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside on the basis of its ^{1}H and ^{13}C NMR data (J.H. Bradbury, personal communication).

Experimental

Plant material. Cassava seeds, *M. esculenta* Crantz, (cross-code SM1234 derived from the female parent CM2967-8) were obtained from Dr. C.H. Hershey, *Centro Internacional de Agricultura Tropical*, Cali, Columbia. Cassava plants were cultivated in moss peat in a greenhouse at 30°C.

Analysis. HPLC was performed at ambient temperature on a column of Lichrosorb RP18 (5 μm, 250 mm × 16 mm i.d.) operated at 3.0 ml 20 % MeOH per min. The column was coupled to a Gilson 131 refractive index detector. Linamarin was purchased from Sigma.

Isolation of cyanogenic glycosides. The plant material was boiled in 80 % MeOH for 5 min, homogenized using a mortar and pestle, and boiled for an additional 5 min.

The suspension was filtered and the combined extracts were concentrated *in vacuo*. The residue was dissolved in 20 % MeOH (20 ml) and subjected to HPLC analysis. A 500 µl aliquot of the concentrated extract was injected on HPLC, and the effluent was collected in 6 ml fractions. The fractions were lyophilized and redissolved in 500 µl H_2O. An aliquot (100 µl) of each was added to test tubes containing 500 µl β-D-glucuronidase (Sigma, type H2, 6000 units ml^{-1}) and incubated at 37°C for 2 h. Subsequent cyanide assays as described in Ref.10 showed the absence of cyanogenic glycosides in all fractions except those known to contain linamarin and lotaustralin.

Acknowledgements. Dr. Jerzy Jaroszewski, Department of Organic Chemistry, Royal Danish School of Pharmacy, is thanked for authentic samples of 2-(2′-cyclopentenyl)glycine derived cyanogenic glycosides. Professor Eric E. Conn, University of California, Davis, USA, is thanked for an authentic sample of lotaustralin. This work was supported in part by the Danish International Developmental Agency (DANIDA), the Center of Plant Biotechnology, The Rockefeller Foundation, and the Commission of the European Communities program Science and Technology for Development.

References

1. Nartey, F. *Phytochemistry 7* (1968) 1307.
2. Koch, B., Nielsen, V. S., Halkier, B. A., Olsen, C. E. and Møller, B. L., *Arch. Biochem. Biophys. 292* (1992) 141.
3. Olafsdottir, E. S., Jaroszewski, J. W. and Arbo, M. M. *Biochem. Syst. Ecol. 18* (1990) 435.
4. Olafsdottir, E. S., Andersen, J. V. and Jaroszewski, J. W. *Phytochemistry 28* (1989) 127.
5. Spencer, K. C. and Seigler, D. S. *Biochem. Syst. Ecol. 13* (1985) 23.
6. Lykkesfeldt, J. and Møller B. L. *Acta Chem. Scand. 48* (1994) 178.
7. Halkier, B. A. and Møller, B. L. *Plant Physiol. 96* (1991) 10.
8. Jaroszewski, J. W., Jensen, P. S., Cornett, C. and Byberg, J. R. *Biochem. Syst. Ecol. 16* (1988) 23.
9. Olafsdottir, E. S., Jørgensen, L. B. and Jaroszewski, J. W. *Phytochemistry 31* (1992) 4129.
10. Halkier, B. A. and Møller, B. L. *Plant Physiol. 90* (1989) 1552.

Received February 13, 1995.