Phytochemistry, Vol. 26, No. 8, pp. 2399–2400, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

NEOBETANIN: A NEW NATURAL PLANT CONSTITUENT

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(*Received* 22 *December* 1986)

Key Word Index—*Beta vulgaris*; Chenopodiaceae; *Opuntia ficus-indica*; Cactaceae; betalains; pigments; neobetanin.

Abstract—Neobetanin, recently reported to be present in methanolic extracts from the root of *Beta vulgaris* and petals and fruits of some other members of the Caryophyllales, has been found as the major constituent in the fruit of *Opuntia ficus-indica*. This substantiates the natural occurrence of neobetanin.

In a recent communication, Wyler [1] claimed that neobetanin, i.e. 14,15-dehydrobetanin, characterized by Alard *et al.* [2] as a natural product, was "probably an artifact formed during the isolation process" [1]. Wyler and co-workers frequently found neobetanin in aged betanin samples [3, 4]. We found also artifical neobetanin during procedures of betanin isolation [Strack, unpublished]; however, we wish to point out, that we did not observe an artifical appearance of neobetanin in betanin-containing methanolic (80% aqueous) extracts, stored for several months in the dark at −20°. Thus we were unable to detect this new pigment in most of the stored betanin-containing extracts, which we used in the previous thin-layer electrophoretic survey of members of the Caryophyllales [2].

Since Wyler [1] reported that he could not detect neobetanin in fresh juice of *Beta vulgaris*, we reinvestigated the occurrence of this constituent in the cultivar used in our study as described [2], including chromatography on Sephadex ionexchanger [3, 4] (SP C25, H^+; Sigma, München, F.R.G.) using 9 mM formic acid as a solvent, and quantitative high-performance liquid chromatography (see legend to Fig. 1). We were able to show unambiguously the natural occurrence of neobetanin in both fresh juice of squeezed red beet root tissue (*Beta vulgaris* subsp. *vulgaris* var. *conditiva* Alef. cv "*Rote Kugel*") and in fresh methanolic root extracts, and found a molar betanin–neobetanin ratio (see below) of about 1:0.025. We were unable to prove the occurrence of neobetanin in the red-coloured aerial parts of *B. vulgaris*, e.g. in the leafstalks or leaf veins.

In addition, we quantified some of the other previously investigated [2] and new betalain extracts from other members of the Caryophyllales and found a remarkable high concentration of neobetanin in fruits of *Opuntia ficus-indica* (L.) Mill. (Fig. 1). This neobetanin has been isolated as described [2] and its structure proved by NMR spectroscopy (data not shown; see ref. [2]). When the different log ε values for betanin and neobetanin of 4.75 [5] and 4.26 [3], respectively, are taken into account, fresh extracts from *Opuntia ficus-indica* fruit flesh show a betanin–neobetanin ratio of about 1:2.5. It is interesting to note that two other *Opuntia* species, *O. bergeriana* Web. ex Berger and *O. basilaris* Eng. & Big. var. *ramosa* Parish, lacked neobetanin.

In summary, the present study corroborates our previous study and substantiates the natural occurrence of neobetanin.

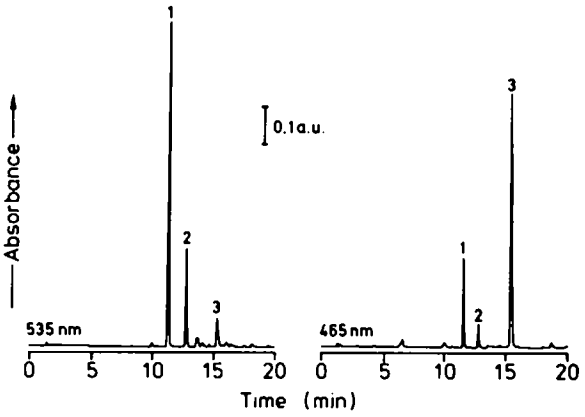

Fig. 1. High-performance liquid chromatographic Analyses of an extract from fruits of *Opuntia ficusindica* on a Nucleosil C_{18} column (5 μm, 250 × 4 mm i.d.; Macherey-Nagel, Düren, F.R.G.). Peak identification: 1, betanin (R_t 11.4 min); 2, isobetanin (R_t 12.9 min); 3, neobetanin (R_t 15.4 min). Development: linear gradient elution within 20 min from solvent A (1% HOAc in H_2O) to 20% solvent B (1% HOAc in MeCN) in (A + B) at a flow rate of 1.5 ml/min. Detection was at 535 nm (left) to favour the absorbance of betanin and isobetanin and at 465 nm (right) to favour the absorbance of neobetanin. Freshly cut fruit flesh from *Opuntia* was crushed in MeOH for 1 min and 20 μl of the filtrate was directly injected onto the chromatographic column.

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Acknowledgements—D.S. gratefully appreciates financial support and a Heisenberg fellowship provided by the Deutsche Forschungsgemeinschaft (DFG), Bonn. We thank Prof. H. Reznik (Cologne) for stimulating discussions.

REFERENCES

1. Wyler, H. (1986) *Phytochemistry* **25**, 2238.
2. Alard, D., Wray, V., Grotjahn, L., Reznik, H. and Strack, D. (1985) *Phytochemistry* **24**, 2383.
3. Wyler, H. and Meuer, U. (1979) *Helv. Chim. Acta* **62**, 1330.
4. Wyler, H., Meuer, U., Bauer, J. and Stravs-Mombelli, L. (1984) *Helv. Chim. Acta* **67**, 1348.
5. Piattelli, M., De Nicola, M. G. and Castrogiovanni, V. (1969) *Phytochemistry* **8**, 731.

Phytochemistry, Vol. 26, No. 8, pp. 2400–2401, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

OCCURRENCE OF THE CYANOGEN LINUSTATIN IN *HEVEA BRASILIENSIS*

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(*Received* 5 *January* 1987)

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.