Comparison of HPTLC – Fluorodensitometry and HPLC for the Assay of Strictosamide in the Leaves, Root and Stem Bark of *Nauclea latifolia*

Pierre Duez*, André Milcamp, Marius Lompo, Pierre Guissou, and Michel Hanocq

Key Words:

Preparative extraction
Post-chromatographic derivatization
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Nauclea latifolia
Strictosamide

Summary

Because of its structure and its high concentration in the roots of *Nauclea latifolia*, it is possible that the alkaloid strictosamide is responsible for the plant's pharmaco-toxicological properties; preparative extraction and assay methods have therefore been developed. Strictosamide in methanolic extracts was purified by column chromatography on silica gel. Densitometry on HPTLC silica gel provided evidence that degradation had occurred between spot application and chromatography; this could be prevented by the addition of 2 % α-tocopherol succinate to sampled solutions. Post- chromatographic heating at 105 °C for 24 h generated highly fluorescent degradation products, which increased both the specificity and the sensitivity of the analysis.

Strictosamide was also assayed by reversed phase HPLC on an octadecyl column with an anionic counter ion. Both analytical procedures were successfully applied to extracts of leaves, root, and stem bark, and yielded similar results, suggesting both are suitable for the determination of strictosamide. Strictosamide could be detected in *Nauclea latifolia* leaves.

1 Introduction

The roots of *Nauclea latifolia* Sm. (Rubiaceae) are widely used in West Africa as a traditional medicine notably to regularize gastro-intestinal troubles but also for their antipyretic and anthelminthic properties; the leaves are used as bowel function regulators [1,2]. Several indoloquinolizidine alkaloids and glycoalkaloids have previously been isolated from the roots [3–5] and leaves [6,7], but with extremely low yields. Strictosamide (strictosidine lactam or isovincoside lactam; **Figure 1**), a supposed biogenetic precursor of these other alkaloids, although present in the roots at high concentrations [8], has been overlooked by several authors, probably because of its unusual solubility properties and degradation by alkaline media; this compound is also the major alkaloid of another plant of the African Pharmacopeia, *N. pobeguinii* [10].

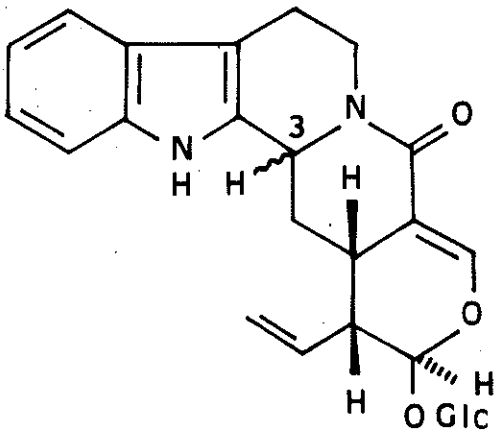

Figure 1
The structure of strictosamide (3α stereochemistry at position 3) [8].

The structure of strictosamide is similar to the basic structure of numerous physiologically active monoterpenoid indole and quinoline alkaloids such as the Rauvolfia or Vinca alkaloids. This, and its occurrence at high concentration in the roots [8] are reasons for suspecting it may be responsible for the demonstrated pharmaco- toxicological [11–13] and antimicrobial [14] properties of *N. latifolia*. In view of further pharmacological tests and in order to study the effects of ecophysiological conditions on the production of alkaloids in *N. latifolia*, preparative extraction and assay methods have been developed.

P. Duez, A. Milcamp, and M. Hanocq, Unité de Chimie Bioanalytique, de Toxicologie et de Chimie Physique Appliquée, Université Libre de Bruxelles, C.P. 205/1, Bd. du Triomphe, 1050 Bruxelles, Belgium; and M. Lompo and P. Guissou, Institut de Recherche sur les Substances Naturelles, C.N.R.S.T., B.P. 7192, Ouagadougou, Burkina Faso.

Strictosamide could be isolated from crude methanolic extracts by preparative low pressure chromatography on a silica gel column and a new HPTLC – fluorodensitometric method has enabled rapid and reliable assay in roots, stem bark, and leaves. Results have been compared with those obtained by a new HPLC method performed on an octadecylsilane column.

2 Experimental

2.1 Plant Material

Leaves, stem bark, root heartwood, and bark of *Nauclea latifolia*, harvested in Burkina Faso from natural stations, Boromo, Moany, Orodara, and Dindéresso (voucher specimen Lejoly 80/023, BRLU Herbarium), were dried for three days at ambient temperature in a shady ventilated room and powdered to pass a 315 μm sieve.

2.2 Preparative Extraction

Root bark powder (20 g) was extracted with methanol (2 × 200 ml) and the extract evaporated under reduced pressure to yield a crude product (4 g). This was dissolved in methanol (10 ml), added to kieselguhr (4 g), re-evaporated to dryness under reduced pressure, applied to a 500 × 15 mm i.d. glass column packed with silica gel 60 (30 g; 230–400 mesh; Merck, FRG; wet packing with chloroform) and eluted with chloroform – methanol (90 + 10). Fractions (5 ml) were collected; elution of strictosamide (0.3 g; elution volume, 360 to 440 ml) could be detected by TLC (mobile phase chloroform – methanol, 90 + 10, *v/v*) of aliquots diluted with an equal volume of methanolic solution of α-tocopherol acid succinate (2 %) which was added to prevent degradation of strictosamide after application on the layer (*cf. infra*). Collected fractions were mixed with kieselguhr (0.3 g), evaporated to dryness, and chromatographed on a 250 × 15 mm i.d. glass column packed as described above. The column was eluted with chloroform – acetone – methanol (65:25:10) and 2 ml fractions collected; strictosamide fractions (0.25 g; elution volume, 40 to 80 ml) were evaporated to dryness under reduced pressure. The product obtained was found to give an homogenous spot by HPTLC and a single peak by HPLC. The identity of the alkaloid was checked by TLC R_f value [15], and by IR [16] and ^{13}C NMR [10] spectroscopy in comparison with an authentic sample of the strictosamide [3–7].

^{13}C NMR spectra in CD_3OD solution with dioxane as internal reference were recorded at 62.9 MHz on a Bruker WP 250 instrument. Chemical shifts [δ ppm] were very similar to those of *Zeches et al.* [10]: C-2 (135.1), C-3 (55.4), C-5 (45.0), C-6 (22.4), C-7 (109.6), C-8 (129.0), C-9 (118.9), C-10 (120.5), C-11 (122.8), C-12 (112.6), C-13 (138.1), C-14 (27.7), C-15 (25.3), C-16 (110.7), C-17 (149.4), C-18 (120.9), C-19(134.7), C-20(45.1), C-21(98.5), C-22(167.4), C-1 (100.9), C-2 (74.7), C-3 (78.5), C-4 (71.7), C-5 (78.3), C-6 (62.9).

2.3 Quantitative Extraction

Nauclea latifolia leaves, root or stem bark powder (20 mg) were weighed into 10 ml glass-stoppered centrifuge tubes and methanol (5 ml) was added. This suspension was shaken for 15 min and centrifuged at 2000 g; these two steps were repeated thrice with further methanol (3 × 5 ml). The supernatant solutions were combined, evaporated to dryness under reduced pressure, and dissolved in methanol.

Standards for HPLC were dilutions in methanol of a stock solution prepared by dissolving strictosamide (5 mg) in methanol (25 ml). All solutions used for HPTLC assays were prepared using a 2 % methanolic solution of α-tocopherol acid succinate instead of methanol.

2.4 HPTLC Assay

HPTLC was performed on 10 × 20 cm plates coated with silica gel 60 F_{254} (Merck, Darmstadt, FRG). Standards and extract solutions (0.5 μl) were applied with a micropipet (Drummond Microcaps, Broomall, USA) 15 mm from the lower edge of plates. Plates were developed with chloroform – acetone – methanol (65 + 25 + 10, *v/v*) in a saturated N tank lined with filter paper (saturation time, 30 min). Ascending chromatography was performed at ambient temperature; the development distance was 60 mm.

After development, plates were left at 105 °C for 24 h in a ventilated oven in order to transform strictosamide into a highly fluorescent derivative. Plates were then left 2 h at ambient temperature in darkness for fluorescence stabilization and the spots were measured with a Shimadzu CS-930 high speed TLC scanner with the settings: zigzag swing width, 8 mm; scan step in the *y* direction, 0.1 mm; beam size, 0.4 × 0.4 mm; fluorescence mode with λ_{exc} = 406 nm, filter 3 (UV cut-off, λ = 480 nm); linearizer off, background correction off, vertical integration. Mean values were calculated by integration of nine spots corresponding to three different standard concentrations, each analyzed twice, and three spots of the solution of unknown concentration.

2.5 HPLC Assay

HPLC was performed with a system comprising a Gilson model 303 pump with 5SC head, a Rheodyne 7125 sample loop, a Gilson model HM UV detector operating at λ = 225 nm, and a 100 × 3 mm i.d. ChromSep cartridge prepacked with ChromSpher 5 μm C_{18} (Chrompack, The Netherlands). The mobile phase was a 55:45 mixture of 0.1 M sodium dihydrogen phosphate containing 5 mM 1-octanesulfonic acid sodium salt and methanol at a flow-rate of 1 ml/min; 20 μl samples were injected.

3 Results and Discussion

3.1 Preparative Extraction

It appears from previous work [8,10,17] that alkali should be avoided because of the possible degradation of the stric-

tosamide lactam function by amino compounds. Extraction solvents of different polarity (*e.g.* dichloromethane, chloroform, methanol) have been used; our trials with low-polarity solvents resulted in poor selectivity and extraction yields. Methanol offered no better selectivity but the yields were considerably higher and this solvent was, therefore, subsequently used as extraction solvent. In view of this, it was quite surprising that during column chromatography on silica it was possible to recover strictosamide among the fractions of lower polarity. This is probably explained by the mixed polarity of the molecule resulting from the sugar moiety. Columns were processed within one day and in darkness in order to minimize silica-catalyzed degradation.

3.2 HPTLC – Densitometry

The elution solvent was derived from experience with the column chromatography system to obtain efficient separation of strictosamide from other compounds in the crude extracts (**Figures 2** and **3**). Degradation of strictosamide was observed on the silica gel layers; this probably resulted from silica-catalyzed oxidation accelerated by elevated temperatures and, to a lesser extent, by exposure to light. This degradation was found to occur:

(i) between spot application and chromatography, as has been previously observed for reserpine and rescinnamine [18]; this could be prevented by addition of 2 % α-tocopherol acid succinate to sample solutions (**Figure 4**);
(ii) after development – the UV absorbance at λ = 225 nm (strictosamide λ_{max}) decreased as a function of time; this led to a shift of λ_{max} from 225 nm to 235 nm, and the appearance of an intense fluorescence signal (Figure 2); post-chromatographic degradation was then deliberately exploited by heating the plate 105 °C for 24 h; the fluorescent degradation products produced could be measured with a substantially lower detection limit.

This greater detection sensitivity resulting from the fluorescence offered even better selectivity; the signals from most of the compounds present in the extract disappeared into the background noise.

The response from the fluorescent derivative was linearly dependent on strictosamide concentrations for amounts between 10 and 125 ng per 0.5 µl spotted. The correlation coefficient, r, was typically greater than 0.995; the absolute detection limit was *ca* 2.5 ng per spot.

3.3 HPLC

Reversed phase HPLC enabled resolution of strictosamide in extracts of *Nauclea latifolia* (**Figure 5**). A counter-ion was added to the mobile phase in order to improve peak shape and prevent band distortion. The purity of peaks was tested by UV spectroscopy (absorbance ratios at different wavelengths, **Table 1**). The linear calibration range was between 20 and 110 ng injected and the absolute detection limit was *ca* 11 ng injected.

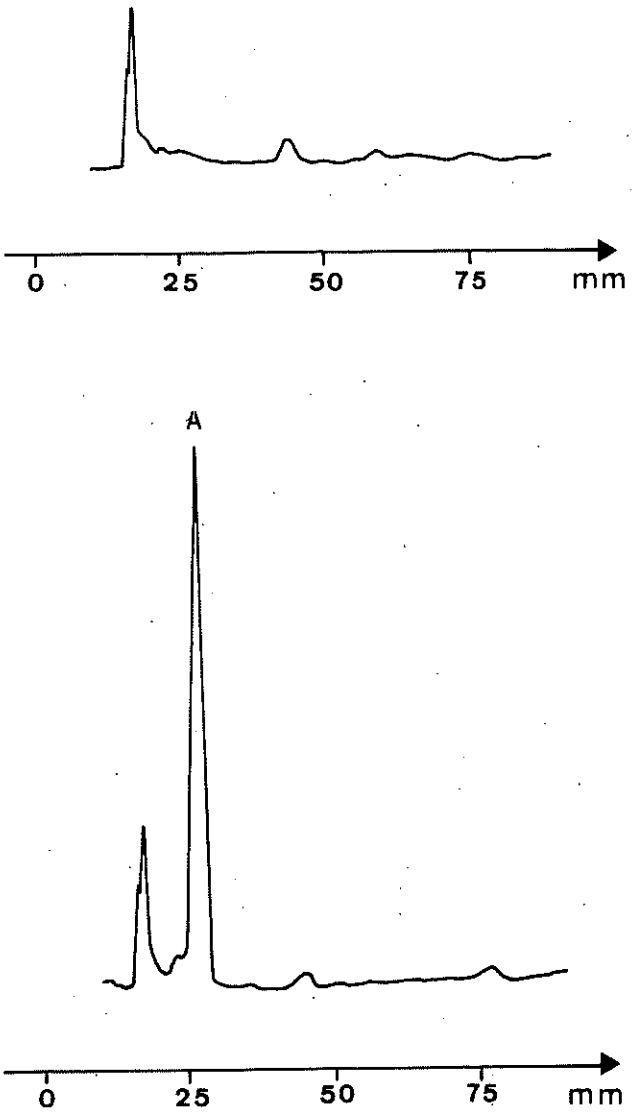

Figure 2

Fluorodensitometric scan of a chromatogram of an extract of *Nauclea latifolia* root bark: upper, scanning without post-chromatographic degradation (plate dried for 5 min at ambient temperature under a stream of nitrogen and immediately scanned); lower, scanning after post-chromatographic degradation (105 °C for 24 h); peak A, strictosamide.

Table 1

UV absorbance ratios of HPLC peaks for strictosamide in a standard solution and in a root extract (stopped-flow scanning; mean values, n = 3).

Wavelength [nm]	Strictosamide absorbance ratio	
	Standard solution	Root extract
A250/A240	0.86	0.87
A260/A240	0.74	0.75
A280/A250	0.64	0.63
A260/A250	0.86	0.86
A220/A260	1.80	1.82

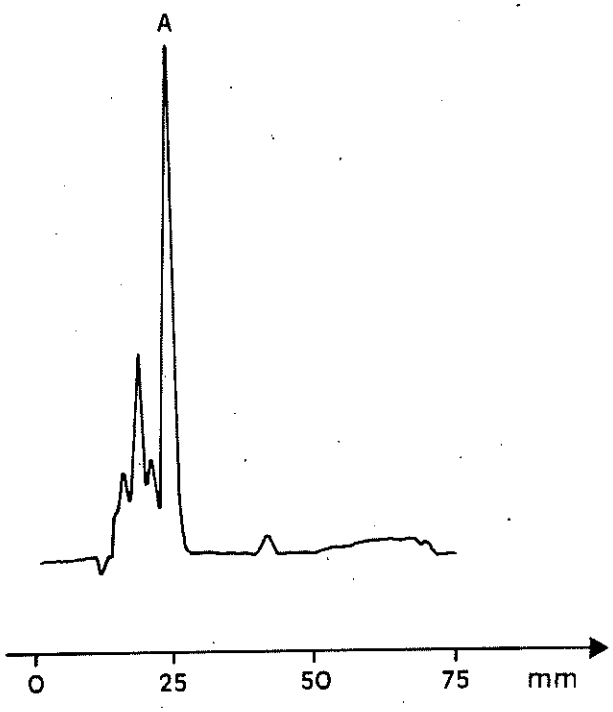

Figure 3

Fluorodensitometric scan of a chromatogram of an extract of *Nauclea latifolia* leaves; conditions and peak identity as for Figure 2.

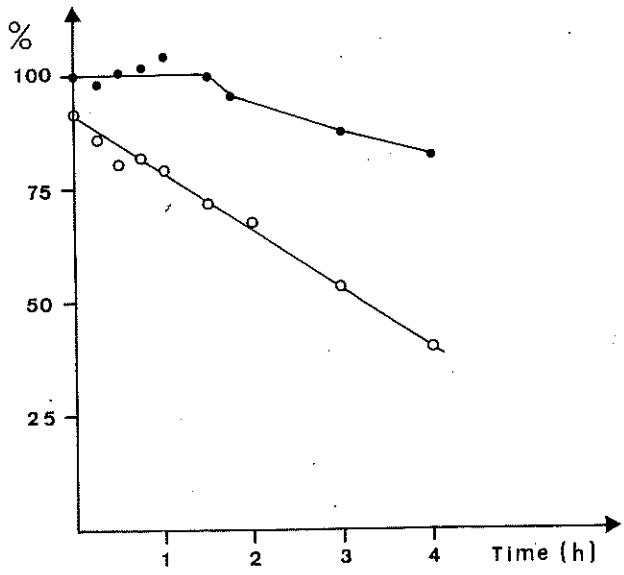

Figure 4

Prechromatographic degradation of strictosamide between the time of application on the silica gel plate and development (time 0). Between each spot application plates were left at ambient temperature in the dark. The ratio of the strictosamide signal at time *t* to that at time 0 (expressed as a percentage) as a function of the time between application and development for a solution of strictosamide with (●—●) and without (○—○) addition of α-tocopherol acid succinate; conditions as for Figure 2.

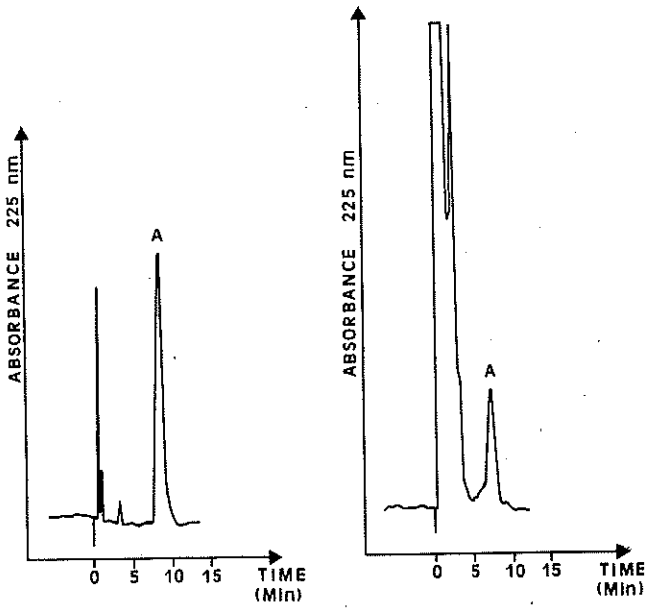

Figure 5

HPLC chromatograms of extracts of *Nauclea latifolia* root bark (left) and leaves; peak A, strictosamide.

3.4 Quantitative Extraction and Comparison of HPTLC – Densitometry with HPLC

A four-step extraction procedure ensured that at least 99 % of the strictosamide was extracted without degradation. The densitometric HPTLC method proposed has ben applied to the determination of strictosamide in the leaves, root, and stem bark of *Nauclea latifolia* from Burkina Faso (Africa); the data obtained were compared with those obtained by HPLC and found to be similar (**Table 2**).

4 Conclusion

To the best of our knowledge, this is the first time that strictosamide has been detected in *Nauclea* leaves by HPLC (retention time confirmed by UV spectroscopy) and HPTLC (R_f confirmed by comparison of pre- and post-chromatographic degradation patterns). HPTLC and HPLC gave similar results but the sensitivity of HPTLC was higher (limits of detection 2.5 and 11 ng, respectively).

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Table 2

Determination of strictosamide in *Nauclea latifolia* extracts; comparison of results from HPTLC fluorodensitometry and HPLC (n = 3).

Sample (harvesting region)	Strictosamide content [% of dry weight]					
	HPTLC			HPLC		
	m[a]	s[b]	s %[c]	m	s	s %
Leaves 1 (Orodara)	0.27	0.001	0.4	0.27	0.01	4
Leaves 2 (Moany)	0.43	0.01	2	0.37	0.02	5
Leaves 3 (Dindéresso)	0.20	0.02	10	0.15	0.02	13
Root bark 1 (Boromo)	3.27	0.05	2	3.02	0.09	3
Root bark 2 (Boromo)	2.55	0.02	0.8	2.40	0.08	3
Root wood (Boromo)	1.41	0.03	2	1.58	0.04	3
Stem bark (Orodara)	0.16	0.03	19	0.18	0.01	6

[a] mean (n = 3)
[b] absolute standard deviation
[c] relative standard deviation

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