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NAUCLEFOLININE: A NEW ALKALOID FROM THE ROOTS OF NAUCLEA LATIFOLIA

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ABSTRACT. A novel indole alkaloid, nauclefolinine (1) and five known triterpenic compounds, rotundic acid (2), α -L-rhamnoquinovic acid (3), 3-O- β -D-glucopyranosyl- β -sitosterol (4), squalene (5) and sitosterol-3-O-6'-stearoyl- β -D-glucopyranoside (6) have been isolated from the roots of *Nauclea latifolia*.

KEY WORDS: Nauclea latifolia, Rubiaceae, Indole alkaloid, Triterpenic acids, Rotundic acid, Rhamnoquinovic acid

INTRODUCTION

Nauclea latifolia Smith is a large evergreen tree abundant in the rain forests of West and Central Africa. The bark finds some local use in the treatment of stomach pains, fever and sometimes diarrhoea [1].

Previous work on *Nauclea latifolia* has yielded a number of alkaloids [2, 3]. We here present the isolation and the structural elucidation of a new alkaloid (1), together with five previously known triterpene derivatives identified as rotundic acid (2) [4], α -L-Rhamnoquinovic acid (3) [5], 3-*O*- β -D-glucopyranosyl- β sitosterol (4) [6], squalene (5) [7] and sitosterol-3-*O*-6'-stearoyl- β -D-glucopyranoside (6) [8].

RESULTS AND DISCUSSION

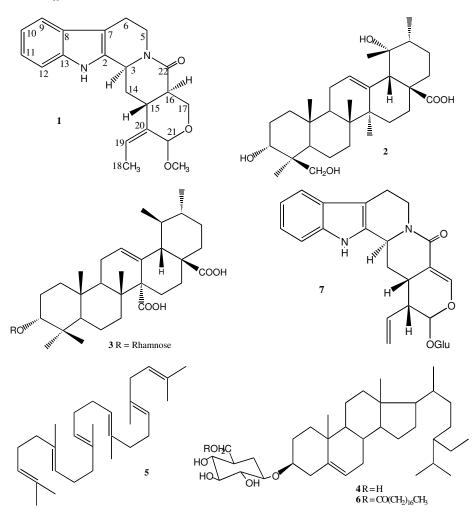
Nauclefolinine **1** was isolated as a dark brown powder m.p. 115-116 °C, $[\alpha]^{25}_{D}$ +3 (CH₂Cl₂, *c* 0.6). Its UV spectrum showed two absorption maxima at 224 and 282 nm characteristic of indole alkaloids [9]. The study of its IR spectrum indicated the presence of a lactam group (1660 cm⁻¹), monoalkylated external exocyclic carbon-carbon double bond (1736 cm⁻¹) [10] and a secondary amine group (3298 cm⁻¹).

The mass spectrum agreed with the $C_{21}H_{24}N_2O_3$ formula for compound **1**. Observation in its mass spectrum of fragments ions at m/z 169 (52%), 156 (27%), 144 (40%) and 143 (72%) suggests the presence of tetrahydro β -carboline ring. The ¹³C NMR of **1** displayed signals for 21 carbon atoms: one vinyl methyl and one OMe ether, four methylenes (sp³), nine methines including five olefinic, three sp³ carbons and one sp³ bearing oxygen, six quaternary sp² carbons including one carbonyl group and two carbon atoms bearing nitrogen. This was confirmed by the ¹H NMR spectrum in which two methyls were observed as singlet and doublet. The signal which appears at δ 3.40 is attributed to the OMe group due to the downfield shift. The proton geminal to this group appears as a doublet at 5.30. This value of the chemical shift indicated

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that, this methine group bears two oxygen atoms. RCH₂OR' protons appear at 3.75 and 4.20, the aromatic protons together appear between 7.34 and 7.50, and the exchangeable amine proton (RNHR') at 8.21 ppm. The comparison of the ¹³C NMR spectrum data (see Table 1) of 1 and those of strictosamide (7) [5] allowed to suggest that, those compounds would have the same carbon skeleton. The carbon-carbon double bond C_{16} - C_{17} of strictosamide has been hydrogenated, and the terminal double bond C_{18} - C_{19} does not exist in compound 1. This terminal double bond C_{18} - C_{19} does not exist in compound 1. This terminal double bond C_{18} - C_{19} does not exist in compound 1. This terminal double bond C_{18} - C_{19} and C_{20} . This suggestion has been confirmed by the Cosy spectrum which showed correlations between H_{18} and H_{19} , H_{16} and H_{15} , H_{19} and H_{21} on the one hand H_{16} and the RCH₂OR' protons on the other. Coupling between H_3 and H_{14} , H_{14} and H_{15} were also observed and the coupling constant value (11.4 Hz) between H_{15} and H_{16} allowed to suggest that, the latter proton is fixed in α position of carbon C_{16} .



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| С | 1 | 7 | Н | 1 | | HMBC Correlations of 1: H _i correlates with C _i |
|------------------|-------|-------|----|----------------------------|-----------------------------------|--|
| 1 | | | 1 | 8.20, s | | |
| 2 | 132.7 | 134.3 | | | | |
| 3 | 53.6 | 52.6 | 3 | H ₃ 5.10 broad | $J_{3-14b} = 6.7 \text{ Hz}$ | H ₃ with C ₁₄ , C ₂ , C ₁₅ |
| | | | | doublet | $J_{3-14a} = 2 Hz$ | |
| 5 | 42.8 | 42.7 | 5 | H _{5a} 4.95, m | H _{5b} 2.95, m | H ₅ with C ₆ , C ₃ , C ₇ , C=O |
| 6 | 20.7 | 20.5 | 6 | H _{6a} 3.05, m | H _{6b} 2.70, m | H ₆ with C ₅ , C ₇ |
| 7 | 111.1 | 107.5 | | | | |
| 8 | 127.3 | 126.9 | | | | |
| 9 | 118.1 | 117.4 | 9 | H ₉ , 7.50, d | $J_{9-10} = 7.7 \text{ Hz}$ | H ₉ with C ₇ , C ₁₁ , C ₁₃ |
| 10 | 119.8 | 118.6 | 10 | H ₁₀ , 7.15, m | | H ₁₀ with C ₁₂ , C ₈ |
| 11 | 122.2 | 120.8 | 11 | H ₁₁ , 7.21, m | | H ₁₁ with C ₉ , C ₁₃ |
| 12 | 111.1 | 111.1 | 12 | H ₁₂ , 7.34, d | $J_{12-11} = 8.0 \text{ Hz}$ | H ₁₂ with C ₈ , C ₁₀ |
| 13 | 135.7 | 135.7 | | | | |
| | | | | H _{14b} , 2.15, m | $J_{14b-3} = 6.7 \text{ Hz}$ | |
| 14 | 27.6 | 25.6 | 14 | H _{14a} , 2.48 | $J_{14a-14b} = 13.6 \text{ Hz}$ | H ₁₄ with C ₁₅ , C ₁₆ , C ₃ |
| | | | | broad doublet | $J_{14a-15} = 11.4$ Hz | |
| | | | | | $J_{14a-3} = 2 Hz$ | |
| 15 | 32.7 | 23.4 | 15 | H ₁₅ , 2.55, m | $J_{15-14a} = 11.4 \text{ Hz}$ | |
| | | | | | $J_{15-16} = 11.4 \text{ Hz}$ | |
| | | | | | $J_{15-14b} = 0.5 \text{ Hz}$ | |
| 16 | 46.2 | 108.7 | 16 | H ₁₆ , 2.38, m | $J_{16-17a} = 5.8 \text{ Hz}$ | H_{16} with C_{14} , C_{15} , C_{17} , C=O |
| | | | | | $J_{16-17b} = 11.4 \text{ Hz}$ | |
| 17 | 59.7 | 146.5 | 17 | H _{17a} , 4.20 dd | $J_{17a-16} = 5.8 \text{ Hz}$ | |
| | | | | H _{17b} , 3.75 t | $J_{17a-17b} = 11.4 \text{ Hz} =$ | H ₁₇ with C ₁₅ , C ₁₆ , C ₁₈ |
| | | | | | J _{17b-16} | |
| 18 | 12.7 | 119.7 | 18 | H ₁₈ , 1.5, d | $J_{18-19} = 6.9 \text{ Hz}$ | H ₁₈ with C ₂₀ , C ₁₉ |
| 19 | 118.4 | 133.1 | 19 | H ₁₉ , 5.42, m | $J_{19-21} = 1.8 \text{ Hz}$ | H ₁₉ with C ₁₈ , C ₁₅ |
| | | | | | $J_{18-19} = 6.9 \text{ Hz}$ | |
| 20 | 135.6 | 42.2 | | | | |
| 21 | 94.9 | 95.9 | 21 | H ₂₁ , 5.30, d | $J_{21-19} = 1.8 \text{ Hz}$ | H ₂₁ with C ₁₇ , OCH ₃ |
| 22 | 168.7 | | | | | |
| OCH ₃ | 54.4 | | | 3.40, s | | CH ₃ with C ₂₁ |

Table 1. ¹H and ¹³C NMR data of 1 and 7, and HMBC correlations of 1.

HMBC correlations (see Table 1) between the methyl of the methoxyl group and C_{21} , vinyl methyl and neighbouring carbon atoms, the other protons and neighbouring carbon atoms allowed to establish connectivities among fragments of the molecule and confirm the structure **1**. More ever the presence of an OMe in C_{21} induced a characteristics ¹³C and ¹H NMR signals around δ 94.9 and δ 5.30, respectively.

Structures (2-6) were determined by means of spectroscopic data and by comparative analysis of physical and spectral data with those in the literature.

EXPERIMENTAL

General. M.p. uncorr. IR: NaCl. NMR spectra were recorded at 125 MHz for ¹³C and 500 MHz for ¹H. Chemical shifts are given in δ value (ppm) with TMS as internal standard. EI-MS was measured at 70 eV. TLC was carried out on silica gel. The alkaloid was detected by their intense

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yellow-white fluorescence in UV light (365 nm), by the yellow colour obtained on spraying with Dragendorff's reagent. The triterpenoid compounds were detected by spraying with 50% solution of H_2SO_4 in H_2O following by heating. UV spectra were determined as methanol solutions.

Plant material. The roots of *Nauclea latifolia* SM were collected on February 1998 in Foumban (Western Province of Cameroon). A voucher specimen has been deposited at the National Herbarium, Yaounde.

Extraction and isolation. The air dried powdered material (3 kg) was extracted in the mixture of methanol and methylene chloride (1:1) (10 L) and the extract was divided in to chloroform and methanol portions. The chloroform soluble fraction (40 g) was subjected to a silica gel dry flash chromatography, elution performed with a mixture of hexane and ethyl acetate of increasing polarity followed by the mixture of ethyl acetate and methanol. Fractions eluted with a mixture of hexane and ethyl acetate (60:40) were rechromatographed over silica gel to yield compound **1** (15 mg).

Fractions obtained with a mixture of hexane and ethyl acetate (20:80) were purified over silica gel to yield 2 (35 mg), 3 (25 mg), 4 (60 mg) and 6 (20 mg) while 5 (22 mg) was obtained from fractions eluted with a mixture of hexane and ethyl acetate (9.5:0.5). TLC data indicated the following polarity from the less to the more polar compound: 5, 1, 6, 2, 3 and 4 with the retention factor 1, 0.86, 0.63, 0.51, 0.43 and 0.2, respectively in the mixture $CH_2Cl_2/MeOH$ (95:5).

Nauclefolinine (1). Dark brown powder. M.p. 115-116 °C. $[\alpha]_{D}^{25}$ +3 (CH₂Cl₂. *c* 0.6). UV: λ_{max} (MeOH) nm: 224, 282. IR ν_{max} (NaCl) cm⁻¹: 3298, 1736, 1660, 1614. ¹H and ¹³C NMR (CDCl₃): see Table 1. EI-MS *m/z* (rel. int.): 352 (100), 320 (60), 307 (25), 169 (52), 156 (27), 144 (40), 143 (72).

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REFERENCES

- 1. Kerharo, J.; Adam, J.G. La Pharmacopée Sénégalaise Traditionnelle, Vigot: Paris; 1974.
- 2. Hotellier, F.; Delaveau, P.; Pousset, J.L. J. Med. Plant Res. 1979, 35, 242.
- 3. Hotellier, F.; Delaveau, P.; Pousset, J.L. C.R. Acad. Sci. Paris 1981, Serie II, 293, 577.
- 4. Nakatani, M.; Miyazaki, Y.; Iwashitaa, T.; Naokia, H.; Hase, T. *Phytochemistry* **1989**, 28, 1479.
- 5. Zeches, M.; Richard, B.; Gueye-M'Bahia, L.; Le Men, L.O. J. Nat. Prod. 1985, 48, 42.
- 6. Ngnokam, D.; Massiot, G.; Nuzillard, J.M.; Tsamo, E. Bull. Chem. Soc. Ethiop. 1994, 8, 15.
- Ngnokam, D.; Massiot, G.; Nuzillard, J.M.; Connolly, J.D.; Tsamo, E.; Morin, C. *Phytochemistry* 1993, 34, 1603.
- 8. Pei-Wu, G.; Fukuyama, Y.; Rei, W.; Jinxian, B.; Nakagawa, K. Phytochemistry 1988, 27, 1895.
- 9. Hotellier, F.; Delaveau, P.; Pousset, J.L. Phytochemistry 1980, 19, 1884.
- 10. Silverstein, R.M.; Webster, F.X. Spectrometric Identification of Organic Compounds, 6th ed., John Wiley: New York; **1998**.

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