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Novel nauclequiniine from the root extract of *Nauclea pobequinii* (Pob. & Pellegr.) Petit (Rubiaceae)

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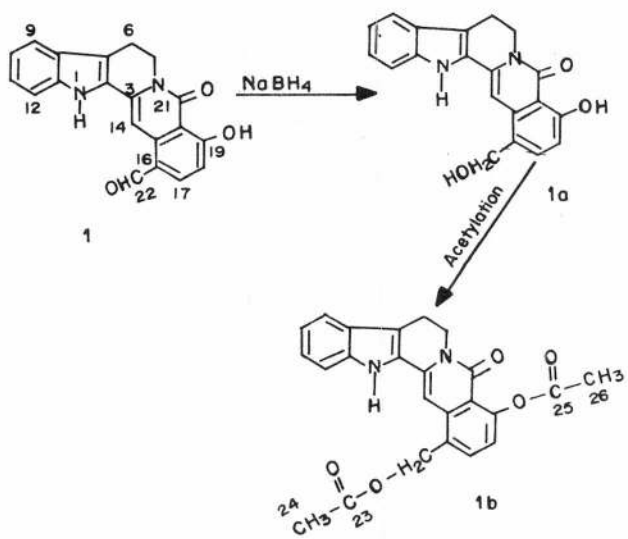
Novel nauclequiniine **1** and two known alkaloids nauclefoline and nauclefidine have been isolated from the root extract of *Nauclea pobequinii*.

Nauclea pobequinii (Rubiaceae) grows in the bushes in Calabar, Cross River State, Nigeria and various parts of it are used as antipyretic agents and in sequels of encephalitis in folk medicine by the local people. Naucleficine, nauclefidine, nauclefoline, 1-acetyl-β-carboline and naucleidinal have been isolated[1] from other species of Rubiaceae family. The isolation and structure determination of a new alkaloid **1** are reported in this paper.

Compound **1** was obtained as orange-red crystals with greenish-yellow fluorescence. High resolution mass spectrum of this compound showed the molecular weight 330.3280 corresponding to the molecular formula $C_{20}H_{14}N_2O_3$. The UV spectrum showed the absorption bands at 260, 290, 366 and 375 nm ($\log \varepsilon$ 4.25, 3.92, 4.39 and 4.39) in 95% EtOH. The UV data and the fluorescence suggested the presence of a highly conjugated system. The imino function conjugated with aldehyde amide carbonyl group can be inferred from the IR spectrum (3350, 1680 and 1640 cm^{-1}), and the IR band at 3150 cm^{-1} can be attributed to hydroxyl group which forms a hydrogen bonding with the carbonyl group.

The EIMS showed fragments at m/z 301 ($M^+ - CHO$) and 284 ($M^+ - CHO\text{-}OH$) confirming the presence of aldehyde and hydroxy groups.

The ^1H NMR spectrum of **1** showed two symmetrical triplets of two protons each centred at δ 3.22 and 4.57 (J=6.5 Hz) attributed to the presence of the sequence $=C\text{-}CH_2\text{-}CH_2\text{-}N<$; a singlet at 8.19 due to C_{14}-proton shifted downfield by the aldehyde group in the periposition, a one-proton signal at 8.11 (dd, J=8 Hz and 2 Hz) attributable to C_{17}-proton because of the deshielding by the aldehyde group. A broad peak appearing at δ 11.80 indicated the presence of a chelated hydroxyl group (C_{19}-OH). Signals due to C_{18}-H were hidden in the multiplet of the indolic protons and appeared between δ 7.15 and 7.44. Two doublets at δ 7.62 and 7.55 were attributed to the protons at C-9 and C-12 respectively. The remaining two signals could be assigned to the NH (δ 8.68) and CHO (δ 10.16) protons. Location of the aldehyde group at C-16 was apparent from biogenetic considerations and was deduced from the features of ^1H NMR spectra of **1** and **1a** (NBH_4 reduction product of **1**).



In order to confirm the structure, **1** was reduced by $NaBH_4$ to give a yellow crystalline compound **1a** (Scheme I) which exhibited UV absorption bands at 345, 362 and 381 nm. The MS of **1a** showed the molecular ion peak at m/z 332 compatible with the molecular formula $C_{20}H_{16}N_2O_3$. The absence of the band at 1680 cm^{-1} in the IR spectrum indicated that the aldehyde group in **1** had been reduced to the carbinol. Its m.p., UV and MS were identical with those of a known reduction product of oxogam-

birtannine[2]. The MS of the acetyl derivative **1b** showed a molecular ion peak at m/z 416 compatible with $C_{24}H_{20}N_2O_5$.

The expected upfield shifts of C_{17}-H (δ 7.41) and C_{14}-H (δ 6.82) as compared with **1** were observed. These data supported the structure **1** for nauclequiniiine which has the framework of benzo[g]indolo[2,3-*a*]quinolizine.

Nauclefidine was obtained as orange yellow crystals which were purified by the centrifugal TLC instrument with 1% MeOH-CH_2Cl_2 as eluant, and nauclefoline, obtained as pale yellow crystals from fractions 13-15 of column II, was recrystallized from acetone. These are known compounds.

Experimental Section

General. The plant material was collected in May 1995 from Calabar Urban, CRS, Nigeria and was authenticated by the Botany Division of the Department of Biological Sciences, University of Calabar, Calabar. A voucher specimen documenting the collection is deposited in the herbarium of the University of Calabar, Calabar.

M.ps were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were run on a Perkin-Elmer double beam spectrometer 580B in KBr pellets. UV spectra were measured on UV-300 spectrophotometer. Mass spectra were taken on a ZAB-2F instrument. ^{1}H NMR spectra were determined on a WH-90 spectrometer using $CDCl_3$ as solvent and TMS as internal reference. A polyvinylsulphonic ion exchange resin (H-form) with cross linking 1 × 1.1 from Aldrich Chemicals, USA was used for the extraction of total alkaloids. Silica gel type HF_{254} was used for TLC while silica gel 200-300 mesh was used for column chromatography. Dragendorff's reagent was used as developing agent.

Extraction and isolation of alkaloids. The powdered roots (90 kg) of *Nauclea pobequinii* were extracted five times with 95% EtOH. The concentrated extract yielded 12.50 g of a gum which was treated with 0.5% HCl until the reaction of alkaloid was weak. A column of 1 kg (dry weight) exchange resin was used to treat the acidic solution. After alkalization with 5% Na_2CO_3, the resin was separately extracted with ether and MeOH in a specially designed extractor to give ethereal extract-A (35.5 g) and methanol extract-B (104 g). Extract-A was fractionated on a silica gel column using chloroform and methanol as eluants

Table I — ^{1}H NMR spectral data of **1**

Proton	Chemical shift δ, ppm	Mult.	Integration	Coupling constant (J) Hz
1	8.68	s	1H (br)	—
5	4.57	t	2H	6.5
6	3.22	t	2H	6.5
9	7.62	d	1H	8.0
10,11,18	7.15-7.44	m	3H	—
12	7.55	d	1H	8.0
14	8.19	s	1H	—
17	8.11	dd	1H	8.0,2.0
19	11.80	s	1H	—
22	10.16	s	1H	—
24	—	—	—	—

Table II — ^{1}H NMR spectral data of **1b**

Proton	Chemical shift δ, ppm	Mult.	Integration	Coupling constant (J) Hz
1	8.61	s	1H	—
5	4.55	t	2H	6.5
6	3.14	t	2H	6.5
9	7.64	d	1H	8.0
10,11,18	7.14-7.40	m	3H	—
12	7.57	d	1H	8.0
14	6.82	s	1H	—
17	7.41	d	1H	8.0
19	—	—	—	—
22	5.44	s	2H	—
24	2.13	s	3H	—
26	2.15	s	3H	—

to give chloroform fraction C (19 g) and methanol fraction D (6.05 g). The fraction-C was continuously fractionated on another silica gel column with CH_2Cl_2-CH_3OH in different ratios giving three fractions: (i) 10.5 g, (ii) 2.93 g and (iii) 1.6 g. Fraction (i) was chromatographed over a silica gel column (1 kg), column I, eluting successively with *n*-hexane-dichloromethane (1 : 1), dichloromethane, and dichloromethane-methanol with differing ratios giving nauclequiniiine (110 mg), nauclefidine (160 mg) and fraction-3 (350 mg). Nauclequiniiine **1** on recrystallization from CH_2Cl_2-MeOH yielded orange-red crystals (90 mg), m.p. 291-92°; UV (EtOH) nm (log ε: 260, 290, 366, 375, 408 (4.25, 3.92, 4.39, 4.39, 4.18);

IR (KBr) cm^{-1}: 3350 (N-H), 1680 (—CHO), 1640 (N−C=O), 3150 (OH); MS (%): m/z 330 (M^+, 100), 302 ($M^+ - CO$, 50), 301 ($M^+ - CHO$, 7) and 284 ($M^+ - CHO\text{-}OH$, 10). High resolution MS: 330.3280 ($C_{20}H_{14}N_2O_3$), requires 330.3430. The ^1H NMR spectral data of **1** are given in Table I.

$NaBH_4$ reduction of 1. To 20 mg of **1** dissolved in 10 mL of MeOH, 20 mg of $NaBH_4$ was added and the mixture refluxed for 1 hr. After acidification, yellow crystals deposited were filtered, dried and recrystallised from MeOH-H_2O to give compound **1a** (12 mg) as crystals, m.p. 281-83°; IR (KBr) cm^{-1}: 3600, 3150, 1640; UV (EtOH) nm (log ε): 345, 362, 381 (3.93, 3.96, 3.86); MS (%): m/z 332 (M^+, 100), 315(3); high resolution MS: 332.3598 ($C_{20}H_{16}N_2O_3$) requires 332.3630.

Acetylation of 1a. Compound **1a** (10 mg) in pyridine (3 mL) was acetylated with Ac_2O for 24 hr. Ice flakes were added and crystals deposited were filtered, washed several times, dried and weighed; the acetylated product **1b**, yield 12 mg, m.p. 128-30°, MS (%): m/z 416 ($M^{+\cdot}$, 100), 330 ($M^+ - 86$, 10); high resolution MS: 416.4130 ($C_{24}H_{20}N_2O_5$) requires 416.4330. The ^1H NMR spectral data of **1b** are given in Table II.

Acknowledgement

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References

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