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PICRANITINE, A NEW INDOLE ALKALOID FROM *PICRALIMA NITIDA* (APOCYNACEAE)

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ABSTRACT. A new indole alkaloid, picranitine, has been isolated from the seeds of *Picralima nitida*, along with five known indole alkaloids picratidine, akuammine, pseudoakuammine, akuammicine and akuammidine previously identified from the same source. Structures were elucidated through exhaustive spectral studies including 1D (^{1}H and ^{13}C NMR) and 2D-NMR (HMQC, HMBC and NOESY).

KEY WORDS: *Picralima nitida*, Indole alkaloids, Picranitine, Picratidine, Akuammine, Pseudoakuammine, Akuammicine, Akuammidine

INTRODUCTION

Picralima nitida (Staf.) Th & H. Durant (Apocynaceae) is a medium size tree growing in the Western and Central zones of Africa and used by local population to treat many ailments [1, 2]. In Cameroon the seeds are employed against malaria and stomach pains. The applications in folk medicine have encouraged researchers to investigate the natural products of this plant, and so far a number of indole alkaloids have been characterized with some displaying affinity for opioid receptors [3, 4]. In continuation of our search for new antiparasitic metabolites from Cameroonian medicinal plants, we here report the isolation and characterization of a new indole alkaloid, picranitine (**1**), obtained from the alkaline fraction of the ethanol extract of the seeds of *P. nitida*. In addition five previously described indole alkaloids, picratidine (**2**) [5], akuammine (**3**) [6], pseudoakuammine (**4**) [7], akuammicine (**5**) [7] and akuammidine (**6**) [8] were also isolated.

RESULTS AND DISCUSSION

Air-dried and ground seeds of *P. nitida* were extracted with ethanol at room temperature. The residue obtained after concentration under reduced pressure was subjected to alkaline fractionation. The alkaloid-containing portion was chromatographed over silica gel to yield six alkaloids (**1-6**).

Picranitine (**1**) was obtained as yellowish crystals in ethyl acetate mp 215-217 °C. The HRMS displayed a molecular ion peak at *m/z* 384 consistent with the formula $C_{21}H_{24}N_2O_5$. This was in full agreement with the 21 carbon signals observed in the ^{13}C NMR spectrum (Table 1), which showed resonances of two carbonyl ester signals (δ 175.1 and 174.8) and two oxygenated carbons at 65.2 and 60.2. Acetylation of **1** yielded a monoacetate derivative indicating the presence of one hydroxyl group in the structure. The IR spectrum revealed absorptions corresponding to hydroxyl (3450 cm^{-1}) and two carbonyl functions at 1735 (ester) and 1745 cm^{-1}

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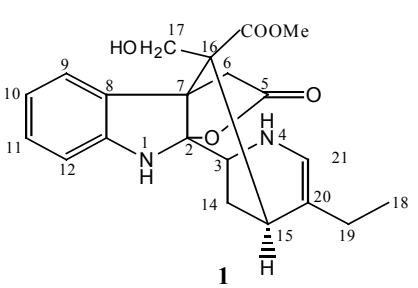
(lactone). The ^{1}H NMR spectrum (Table 1) was found to be close to picratidine (**2**) [5] with four aromatic protons in an ABCD splitting pattern between δ 7.24 and 6.62, but only two methyl functions were observed in **1**. The methyl group obtained as a doublet and the olefinic proton as a doublet in the ^{1}H NMR spectrum of picratidine (**2**) were, respectively, displayed as triplet at δ 0.92 and singlet at δ 6.01 in the ^{1}H NMR spectrum of **1**, suggesting the migration of the double bond.



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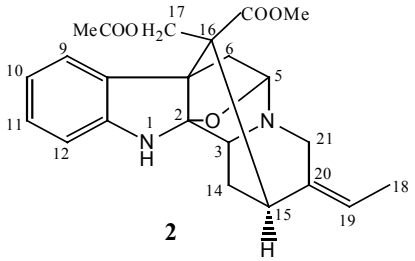

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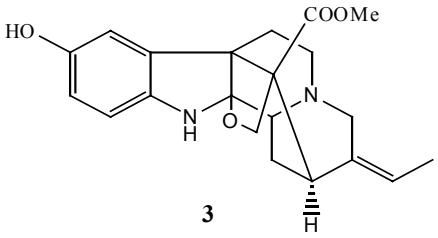

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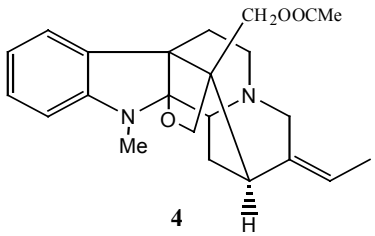

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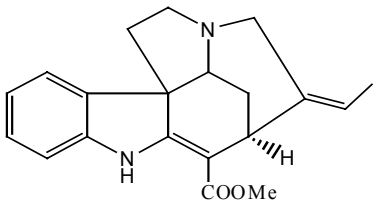

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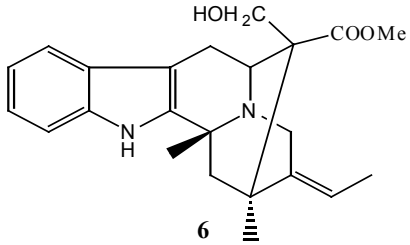

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The proton and carbon spectra were unambiguously assigned by analysis of the 2D-NMR experiment. ^{1}H and ^{13}C chemical shifts were collected in inverse mode two dimensional HMQC and HMBC and proton chemical shifts correlation COSY was recorded. In the HMQC spectrum signal present at δ 65.5 corresponded to a quaternary oxygenated carbon while the one at δ 60.1 was assigned to an oxymethylene carbon. Careful analysis of the COSY and HMBC spectra (summarised in Table 1) led to the overall construction of the skeletal framework of the molecule. In the HMBC spectrum pertinent correlation were observed between the proton H-6 and the carbons C-2, C-5, C-7 and C-16 and between the proton H-3 and the carbons C-2, C-14, C-21.

The relative stereochemistry at C-3 and C-5 of **1** was determined with the help of NOESY experiment and the coupling constants observed in the ^{1}H NMR spectrum. Significant

correlation in the NOESY spectrum between H-3 and H-15 indicated the same orientation for the two protons.

Table 1. ^{1}H and ^{13}C NMR chemical shifts assignment and HMBC correlations for picranitine (**1**).

Position	δ_C	δ_H	HMBC
1			
2	109.8		
3	48.2	3.75 bs	C-2, C-14, C-21
4			
5	175.1 or 174.8		
6	39.3	3.19 d, J 17.0 Hz	C-5, C-7, C-8, C-2
		2.93 d, J 17.0 Hz	C-5, C-7, C-8, C-2
7	53.0		
8	131.5		
9	126.2	7.24 dd, J 7.7 and 1.5 Hz	C-7, C-11, C-13
10	120.0	6.72 td, J 7.7 and 1.1 Hz	C-8, C-12
11	129.2	7.04 dd, J 7.7 and 1.1 Hz	C-9, C-13
12	110.1	6.62 d, J 7.7	C-8, C-10
13	147.2		
14	23.6	1.53 dt, J 13.2 and 3.5 Hz	C-2, C-16, C-20
15	32.1	3.05 m	C-2, C-16, C-20
16	60.2		
17	65.6	3.96 s	C-7, C-15, C-16
18	13.4	0.92 t, J 7.4 Hz	C-19, C-20
19	28.0	2.05 dq, J 14.0 and 7.4 Hz	C –15, C-18,C-20, C-21
20	108.4	1.97 dq, J 14.0 and 7.4 Hz	C-15, C-18, C-20, C-21
21	127.3	6.01 s	C-3, C-15, C-19, C-20
$COOCH_3$	175.1 or 174.8		
OCH_3	51.3	3.00 s	

The structures of the five known derivatives were assigned in a similar manner by spectroscopic methods and the NMR and MS data were all in agreement with those previously reported.

EXPERIMENTAL

General. Mps are uncorrected. ^{1}H NMR (125 MHz) were recorded in $CDCl_3$ or $(CD_3)_2SO$ with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancement using shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^{1}J_{CH}$ = 145 Hz and $^{2}J_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Brüker UXNMR software (rev. 941001). Chemical shifts are given in ppm with solvent signals as reference. EIMS was recorded by Jeol SX102 spectrometer at 70 eV. CC was performed on Merck silica gel 60 and TLC on silica gel GF-254 precoated plates and detection accomplished by spraying with 50% H_2SO_4 followed by heating.

Plant material. Seeds of *P. nitida* were collected at Lomie, Eastern Province of Cameroon in March 1996. Voucher specimens are kept at the National Herbarium, Yaounde.

Extraction and isolation. Dried powder seeds of *P. nitida* (2.0 kg) were extracted sequentially with ethanol, and the extract obtained was concentrated under vacuum to yield 97 g of residue.

This extract was triturated with 0.1 N HCl and the combined acidic solution was exhaustively partitioned with CH_2Cl_2 to give 17 g of CH_2Cl_2 extract. The aqueous layer was adjusted to pH 9 with a solution of 10% NH_3 and the precipitate was filtered to obtain alkaloid that was dissolved in CH_2Cl_2 and washed several times with water. The organic layer was dried with Na_2SO_4 and concentrated to give 54 g of alkaloid, which was subjected to chromatographic separation over a column of Al_2O_3. Elution was performed with a mixture of hexane-ethyl acetate with increasing polarity. Fractions of 250 mL were collected and monitored by TLC with appropriate solvent system to give three main portions. The portion eluted with hexane/ethyl acetate (7/3) contained a mixture of picratidine (**2**) and pseudoakuammine (**4**) purified by a second column chromatography followed by recrystallization. Akuammicine (**5**), which was the main constituent of fractions collected with hexane/ethyl acetate (6/4), was purified by recrystallization to give 33 mg of the product. Portions collected with 50% and 70% of ethyl acetate in hexane contained picranitine (**1**), akuammine (**3**) and akuammidine (**6**) were regrouped and subjected to column chromatography and pure product obtained by fractional recrystallization to yield 60 mg of **1**, 23 mg of **3** and 70 mg of **6**.

Picratine(**1**). Yellowish crystals from ethyl acetate, mp 215-217 ^oC; $[\alpha]_D$ +157^o ($CHCl_3$, c 0.1); IR ν_{max} (cm^{-1}) 3450, 1745, 1735, 1610, 1230, 1100; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 384 (M^+, 94%), 353 (27%), 281 (26%), 246 (39%), 108 (100%); HREIMS $[M]^+$ *m/z* 384.4255 (calcd. for $C_{21}H_{24}N_2O_5$ 384.4257).

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REFERENCES

1. Irvine, F.R. *Woody Plant of Ghana*, Oxford University Press: London; **1961**.
2. Walker, A.; Sillans, R. *Les Plantes Utiles du Gabon*, Lechevalier, P. (Ed.); Paris; **1961**.
3. Menzies, J.R.W.; Paterson, S.J.; Duwiedjua, M.; Corbett, A.D. *Eur. J. Pharm.* **1998**, 350, 101.
4. Corbett, A.D.; Menzies, J.R.W.; MacDonald, A.; Paterson, S.J.; Duwiedjua, M. *Br. J. Pharmacol*. **1996**, (Product. Supplem.), 334P.
5. Ansa-Asamoah, R.; Kapadia, G.J.; Iloyd, H.A.; Sokoloski, E.A. *J. Nat. Prod*. **1990**, 53, 975.
6. Lewin, G.; Le Menez, P.; Roland, Y.; Renouard, A.; E. Giesen-Crouse, E. *J. Nat. Prod*. **1992**, 55, 380.
7. Moeller, B.L.; Seedorff, L.; Nartey, F. *Phytochemistry*, **1972**, 11, 2620.
8. Janot, M.M. *Fr. Farmaco, Ed. Sci*. **1966**, 21 656.