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PICRATIDINE, A NEW INDOLE ALKALOID FROM *PICRALIMA NITIDA* SEEDS

REGINALD ANSA-ASAMOAH, *,[1]

Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana

GOVIND J. KAPADIA,*

College of Pharmacy and Pharmacal Sciences, Howard University, Washington, D.C. 20059

HELEN A. LLOYD, and EDWARD A. SOKOLOSKI

Laboratory of Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT.—The seeds of *Picralima nitida* collected in Ghana afforded a new indole alkaloid, picratidine [**1**], whose structure was deduced as *N*-methylpicraline through interpretation of spectral data. Five other alkaloids, akuammine, akuammmidine, akuammicine, akuammigine, and pseudoakuammigine, were also isolated.

Picralima nitida (Stapf.) Th. & H. Durand (syn. *Picralima klaineana*, Pierre) is a West African plant belonging to the Apocynaceae family. The seeds are commonly used in the Ivory Coast, Ghana, and Nigeria as an antimalarial and antipyretic (1). A previous study showed that the plant was without an effect on avian malaria (2). The crude alkaloidal extract produced analgesia in the rat (3) and local anesthesia in the guinea pig.[2]

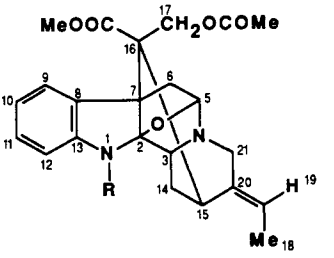
Several indole alkaloids have been previously isolated from the seeds of *P. nitida* (4–9). Among the pharmacologically active *Picralima* alkaloids are akuammine (10), akuammidine (11), and pseudoakuammigine (12).

The purpose of the present investigation was to undertake the re-isolation of the seed alkaloids for their pharmacological evaluation. During the course of the study a new indole alkaloid, picratidine [**1**], was isolated. Its structure was deduced as *N*-methylpicraline through interpretation of spectral data. Re-isolation of akuammine, akuammidine, akuammicine, akuammigine, and pseudoakuammigine from the seeds of *P. nitida* was also realized.

RESULTS AND DISCUSSION

The dried, powdered seeds were extracted with petroleum ether (30–60°) and 6% aqueous HOAc. After the aqueous acidic extract was basified with NH_4OH, alkaloids were extracted with EtOAc and $CHCl_3$. The EtOAc and $CHCl_3$ were combined and chromatographed over Al_2O_3, and the alkaloids were isolated by elution with solvents of increasing polarity. The known alkaloids were identified by comparison of their spectral data with those reported in the literature (13–16) or by mmp, co-tlc, and superimposable ir and nmr spectra with authentic samples.

Picratidine [**1**] was obtained as a colorless crystalline product, mp 185–



1 R=Me
2 R=H

[1]Visiting Senior Fulbright Research Scholar, 1988–89, from Ghana at Howard University, College of Pharmacy and Pharmacal Sciences, Washington, D.C. 20059.

[2]Ansa-Asamoah, unpublished results.

187°, and displayed an $[M]^+$ at *m/z* 424 ($C_{24}H_{28}O_5N_4$) and significant peaks at *m/z* 409 $[M-Me]^+$, 365 $[M-COOMe]^+$, 351 $[M-CH_2OAc]^+$, and 253 with a metastable ion at 150.

The ^{1}H-nmr spectrum was found to be identical to that of picraline [**2**] reported by Durham *et al.* (17), except for a singlet at 2.93 ppm which we assigned as an *N*-methyl at position 1. Table 1 gives the chemical shifts, coupling constants, and multiplicities. Homonuclear correlation spectroscopy (COSY) was used to obtain the connectivities of the ^{1}H-nmr resonances. The aromatic resonances (δ 6.5 to 7.5) could be recognized as an ABCD system found in ortho-substituted aromatic ring systems. The existence of an exocyclic double bond was verified by the single proton quartet at δ 5.38 with coupling to the methyl at δ 1.6. The large geminal coupling of the protons on C-21 suggests that these protons are forced into a diaxial configuration.

Although the occurrence of picraline [**2**] in *P. nitida* had been reported earlier (6), picraline was not detected (gc-ms) in the present study in any of the extracts or chromatography fractions.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Thomas Hoover mp apparatus and are uncorrected. The uv spectra were recorded on a Perkin-Elmer 552 spectrophotometer. Ir spectra were determined on a Perkin-Elmer 1420 Infracord spectrophotometer. Ms were measured on an *LKB 9000* spectrometer. The nmr spectra were obtained on a standard Varian XL-200 spectrometer. The sample was dissolved in $CDCl_3$ (ISOTEC) in a 5-mm tube. The ^{1}H-nmr spectrum was obtained with 16K data points and exponential apodization giving a 0.2 Hz broadening factor. The COSY (18) data were obtained using a 512 × 512 data table with zero filling in both dimensions to 1024 × 1024 data points. The final data were symmetrized to remove artifacts. Specific rotations were recorded on a Perkin-Elmer 241 MC Polarimeter.

PLANT MATERIAL.—The ripe fruits of *P. nitida* were collected in January–March 1988 from trees at the Botanic Garden on the campus of the University of Science and Technology in Kumasi, Ghana. A voucher specimen is deposited in the herbarium of the Faculty of Pharmacy at the University in Kumasi. The seeds were dried, after removing the testa, and powdered.

PREPARATION OF ALKALOID FRACTION.—The dry seed powder (1.5 kg) was extracted by

TABLE 1. Chemical Shifts, Multiplicities and Couplings of Picratidine [**1**].

Proton	Shift (ppm)	Mult.[a]	Coupling (Hz)	Coupling B
H-3	3.8	br, d of d	2.8	3.8
H-5	4.73	d	2.3	
H-6α	2.4	d of d	13.9	2.59
H-6β	3.2	d	13.9	
H-9	7.42	d	7.7	
H-10	6.81	d of d	7.8	7.7
H-11	7.13	d of d	7.7	7.54
H-12	6.62	d	7.54	
H-14α	1.96	d of d	13.8	3.8
H-14β	1.94	d of d	13.8	1.5
H-15	3.3	br, s	1.5	
H-17α	4.52	d	11.03	
H-17β	3.88	d	11.03	
H-18Me	1.6	br, s	7.08	1.9
H-19	5.38	q	7.07	1.3
H-21α	3.15	d	17.6	
H-21β	3.82	d	17.6	
N-Me	2.93	s		
COOMe	3.68	s		
OAc	1.53	s		

[a]br = Broadened, s = singlet, d = doublet.

percolation with 2 liters of petroleum ether (30–60°). The process was repeated twice. Evaporation of the solvent under reduced pressure afforded an oily residue (10 g). The marc was percolated with 6% aqueous HOAc (4 liters) for 24 h and filtered. The process was repeated five times; the filtrates were combined (20 liters) and basified to pH 9 with concentrated NH_4OH. Each 1-liter portion of the alkaline filtrate was extracted with three portions of EtOAc (300 ml) followed by two two portions of $CHCl_3$ (200 ml). The combined EtOAc extract was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The combined $CHCl_3$ extract was similarly treated, and the two extracts were pooled to afford the alkaloid fraction (45 g).

ISOLATION OF ALKALOIDS.—The alkaloid fraction (40 g) was adsorbed on to Al_2O_3 (100 g) (Spence Type H) and chromatographed over a column of Al_2O_3 (600 g) using *n*-hexane, *n*-hexane–Et_2O (1:1), Et_2O, and $CHCl_3$-Et_2O (1:4) in the order listed.

Elution of the column with *n*-hexane–Et_2O (1:1) afforded three major pools. Fractions 1–12 gave one alkaloidal positive spot on tlc, which was oily and did not crystallize. Fractions 13–30 afforded on concentration and fractional crystallization picratidine [**1**] (0.049 g), mp 185–187°, and akuammigine (0.23 g). Picratidine displayed uv λ max EtOH at 233 and 293 nm (ε 8620, 3430), and its ir showed carbonyl stretching (γ max $CHCl_3$) at 1736 cm^{-1}; specific rotation $[\alpha]^{22}D -100° \pm 2°$ (EtOH, $c = 0.07$). Combined fractions (31–114) on concentration and fractional crystallization furnished akuammicine (0.61 g) and pseudoakuammigine (0.41 g).

Elution of the column with anhydrous Et_2O provided fractions 115–192 which afforded on concentration akuammidine (0.47 g).

Elution of the column with $CHCl_3$-Et_2O (20:80) afforded a mixture of akuammidine and akuammine in the fractions 193–200. Fractions 221–285 afforded akuammine (1.87 g), the major alkaloid of *P. nitida*.

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