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AKUAMMINE AND DIHYDROAKUAMMINE, TWO INDOLOMONOTERPENE ALKALOIDS DISPLAYING AFFINITY FOR OPIOID RECEPTORS

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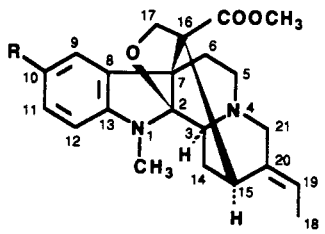
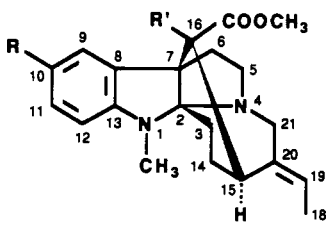
ABSTRACT.—Akuammine [**1**], an indolomonoterpene alkaloid, which is the major component of the seeds of *Picralima nitida*, was reduced to dihydroakuammine [**4**]. This compound has structural analogy with eseroline [**7**], for which affinity for opiate receptors was reported. The present investigation showed that **1** and **4** also bind (with lower affinity however) to μ and κ opiate receptors. ^{1}H- and ^{13}C-nmr spectra of **1** and **4** have been fully assigned by 2D nmr experiments.

Akuammine [**1**] is the major alkaloid of the seeds of *Picralima nitida* Stapf (Apocynaceae) (1,2). Among the other constituents of this plant is pseudoakuammigine or 10-deoxyakuammine [**2**]. When treated in different reducing conditions (2,3), **2** leads by rearrangement to dihydropseudoakuammigine[1] [**3**], a compound with the same tricyclic hexahydropyrroloindole system as physostigmine [**6**]. (−)-Eseroline [**7**], the hydrolyzed compound from **6**, has strong analgesic properties of the morphinic type (4–7), and the structural analogy between **7** and dihydroakuammine [**4**] (phenol and aminal groups, same stereochemistry for the two fused nitrogen rings) prompted us to prepare **4** from its precursor, akuammine [**1**], and to investigate a possible affinity of these two alkaloids for opioid receptors.

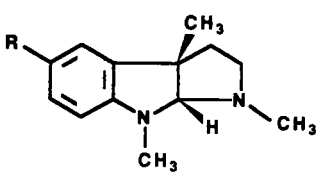
Akuammine [**1**], which was extracted from *P. nitida* seeds according to Lévy (8), was identified by its spectral data and particularly by ^{1}H nmr (Table 1)



1 R=OH
2 R=H



3 R=H, R′=CH_2OH
4 R=OH, R′=CH_2OH
5 R=OMe, R′=H



6 R=OC(=O)NHMe
7 R=OH

[1]This name is used according to J.E. Saxton ("The Alkaloids," Vol. 8, Academic Press, New York, 1965, p. 136), but it does not show skeleton rearrangement from **2** to **3**.

TABLE 1. ^{1}H-nmr Data of Akuammine [**1**] and Dihydroakuammine [**4**] fumarate in DMSO-d_6.

1		**4**	
Proton	δ_H, mult[a], J in Hz	Proton	δ_H, mult[a], J in Hz
H_a-6	1.20, dd 14, 3	H_a-14	1.3–1.5, m
H-18	1.42, dd 7.2, 1.6[b]	H_a-3, H_a-6	1.6–1.8, m
H_a-14	1.93, br d 14, 3, 1[b]	H-18	1.70, d 7
H_b-14	2.13, br d 14, 3, 1[b]	H_b-6, H_b-14	1.8–2.05, m
H_a-5	2.43, dd 11, 5	H_b-3	2.30, t 13, 13
N-Me	2.63, s	N-Me	2.48, s
H_a-21	2.73, d 17	H_a-5	2.68, dd 12, 9
H_b-6	3.05–3.3, m 14, 5[c]	H_b-5	2.95–3.05, m
H_b-5, H-15, H_a-17	3.3–3.45, m	H_a-21	3.05, d 16
COOMe	3.74, s	H_a-17	3.15, d 11
H_b-21	3.76, br d 17, 1.6[b]	H-15	3.62, d 3.5
H_b-17	3.86, d 7	COOMe	3.66, s
H-3	3.94, br s 3, 1[b]	H_b-21	3.86, br d 16
H-19	5.32, q 7.2	H_b-17	3.96, d 11
H-9, -11, -12	6.40–6.45, br s	OH (alcohol)	4.8, br s
OH	8.70, s (exch. D_2O)	H-19	5.30, q 7
		H-12	6.06, d 8
		H-11	6.40, dd 8, 2.3
		fumaric acid	6.58, s
		H-9	7.22, d 2.3
		OH (phenol)	8.3, br s

[a]br = broadened, s = singlet, d = doublet, q = quadruplet, m = multiplet.
[b]J measured after irradiations and enlargements.
[c]The third J is not measurable.

and ^{13}C nmr after comparison with pseudo-akuammigine [**2**] (9,10). Homonuclear and heteronuclear correlations allowed full assignments of signals in ^{1}H and ^{13}C nmr. Chemical shifts are in agreement with those of **2** except for the signals of C-6 and C-14 which have to be inverted (Table 2). The reduction of **1** to **4** is done with zinc in HOAc medium according to Mansour *et al.* (11), and the air-sensitive **4** stabilized and kept easily as a salt (fumarate) (12). The structure of **4** is established by spectral analysis, especially ^{1}H and ^{13}C nmr. The ^{13}C-nmr spectrum of fumarate in DMSO-d_6 (Table 2) corresponds to the summation of the spectra of **4** and fumaric acid. Full and unambiguous assignments in ^{1}H and ^{13}C nmr were established by 2D homo- and heteronuclear experiments (Tables 1 and 2). They are in agreement with those of vincorine [**5**] (13,14) except for the signals of C-3 and C-6 on the one hand and C-9 and C-12 on the other hand, which have to be inverted. The ^{1}H,^{13}C 2D spectrum showed actually that the carbon resonances at δ 115.1 and 105.3 ppm were respectively correlated to H-9 (J = 2.3 Hz) and H-12 (J = 8 Hz); in the same way, the carbon resonance at 21.5 ppm was correlated to a signal at 2.30 ppm unequivocally assigned to one H-3 proton by the ^{1}H,^{1}H-COSY spectrum and also by irradiation experiments. The other differences that were observed are mainly due to the presence of an additional primary alcohol group (shielding of C-8 and C-14, deshielding of C-7 and C-16).

The affinities of three opioid receptor subtypes for the three compounds were investigated. Since the compounds were not available as radioactive labels, displacement studies were performed and relative affinities calculated (Figure 1, Table 3). None of the compounds had

TABLE 2. ^{13}C-nmr Spectral Data of Akuammine [**1**] and Dihydroakuammine [**4**].

Carbon	**1** (DMSO-d_6)		**2**[a] ($CDCl_3$)	**4** (fumarate) (DMSO-d_6)	**5**[b] ($CDCl_3$)
C-2	103.8		103.9	95.2	97.9
C-3	51.8[c]		51.8	21.5	40.6
C-5	49.8		50.7	53.0	56.1[d]
C-6	31.3		28.0	45.2	20.4
C-7	53.1		53.6	59.6	57.3
C-8	143.1[d]	143.7[f]	142.3	131.6	138.2
C-9	112.7	114.6[f]	127.3	115.1	105.5
C-10	150.9	146.7[f]	119.8	148.5	152.3
C-11	110.1[e]	109.7[f]	122.4	113.6	111.7
C-12	110.5[e]	111.2[f]	109.8	105.3	112.1
C-13	144.1[d]	144.4[f]	151.7	142.7	143.6
C-14	27.6		31.3	20.5	26.3
C-15	40.4		41.0	33.3	34.8
C-16	57.8		58.4	55.9	50.7
C-17	73.0		74.3	64.9	
C-18	12.5		12.9	14.6	13.6
C-19	116.9		118.1	121.9	123.2
C-20	140.2		139.2	139.5	138.2
C-21	53.8		54.5	57.4	58.2[d]
N-Me	29.4		28.9	27.7	28.3
COOMe	171.7		172.1	174.6	173.5
COO*Me*	51.9[c]		52.6	51.3	51.7
Fumaric acid				166.4 134.2	

[a]Data for this compound are from Hu *et al.* (10).
[b]Data for this compound are from Das *et al.* (14).
[c,d,e]Interchangeable assignments.
[f]Theoretical values calculated from **2** according to Levy *et al.* (15).

the nanomolar affinity of the enkephalin analogues that were used as the reference compounds. Compound **1** had micromolecular affinities for κ and μ receptors; its affinity for the δ opioid receptor was ten times lower. Compound **4** was more selective than its congener; it displayed micromolecular affinity for the κ receptor; the IC_{50} on the μ receptors was 2×10^{-5} M and it was inactive on the δ

TABLE 3. Relative Affinities for Opioid Receptors.

Compound	IC_{50} values		
	δ receptor	κ receptor	μ receptor
1	2.0×10^{-5} M	3.0×10^{-6} M	4.0×10^{-6} M
4	$>10^{-4}$ M	6.0×10^{-6} M	2.0×10^{-5} M
7	1.0×10^{-5} M	3.0×10^{-7} M	3.0×10^{-7} M
Reference	DPDPE[a]	U 50488H[b]	DAGO[c]
	6.0×10^{-9} M	1.0×10^{-9} M	2.2×10^{-9} M

[a][D-Pen^2, D-Pen^5]enkephalin.
[b]*trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide methane sulfonate hydronate (Upjohn, Kalamazoo, MI).
[c]Tyr-D-Ala-Gly-(Me)Phe-Gly-ol.

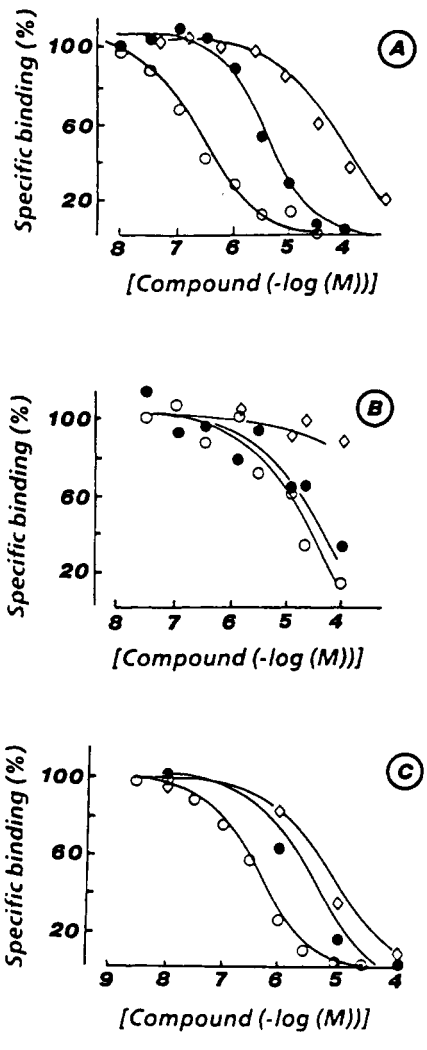


FIGURE 1. Displacement from opioid receptors. Displacement of radioligands from μ-receptors (A), δ-receptors (B) and κ-receptors (C) by compounds **1** (●), **4** (◇), and **7** (○).

receptor. Compounds **1** and **4** had about ten times lower affinity than eseroline [**7**]. As expected, **7** displayed significant affinity for κ and μ receptors (3×10^{-7} M); its affinity is 100-fold lower than the affinity of reference compounds U 50488H and DAGO for the receptors. The tested compounds are devoid of affinity for δ opioid receptors. In conclusion, the preliminary pharmacological data reported here show that κ and μ receptors have affinity for compounds **1** and **4** (however less than for compound **7**). Compound **1** binds with higher affinity than **4** to the μ receptor although its structure is less close to **7**.

To our knowledge, this is the first time that binding of indolomonoterpene alkaloids to opioid receptors was demonstrated. In the case of akuammine, the major alkaloid of *P. nitida* seeds, this affinity could explain the analgesic activity reported for the crude alkaloid extract of this plant (16). The structure-activity relationship within this class of akuammine-like compounds is under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were acquired on a Unicam SP 1800, optical rotation on a Schmidt-Haensch polarimeter; mass spectra were determined with a Nermag R10-10C. Nmr spectra were acquired on a Bruker Ac-200 (200 MHz for ^{1}H, 50.3 MHz for ^{13}C) in DMSO-d_6 using TMS as internal reference and heteronuclear ^1J ^{1}H-^{13}C connectivities performed with standard microprograms Bruker.

Akuammine [**1**], *tartrate*.—Akuammine [**1**] (0.115 g, 0.3 mM) was dissolved by heating in THF-MeOH (1:1) and added to an MeOH solution of tartaric acid (0.045 g, 0.3 mM). Evaporation of the solvent afforded pure amorphous salt as a white powder [**1** and its salt are identical on alumina tlc in CH_2Cl_2-MeOH (97:3)]. $C_{26}H_{32}N_2O_{10}$: found C 58.62, H 6.13, N 5.12; calcd C 58.64, H 6.06, N 5.26.

Reduction of **1** *to dihydroakuammine* [**4**].—Zn powder (6 g) and $CuSO_4$ (0.020 g) were heated in 50 ml HOAc-H_2O (3:2) (100°). A solution of **1** (0.4 g) in 25 ml HOAc-H_2O (3:2) was added, and the mixture was stirred for 2.5 h at 100°. After cooling and filtration, the solution was diluted with H_2O, $NaHCO_3$ was added, and the mixture was extracted at pH 5 and 7 with CH_2Cl_2. The pH 7 extract was dried over anhydrous Na_2SO_4, and the solvent was evaporated (<40°), affording almost pure **4** (0.180 g) as a slightly pink powder. Compound **4** was cold-stored (−15°) under N_2 owing to its oxidizable character.

Dihydroakuammine [**4**], *fumarate*.—Compound **4** (0.153 g, 0.4 mM) was dissolved in MeOH and added to an MeOH solution of fumaric acid (0.058 g, 0.5 mM). The mixture was diluted with EtOAc and concentrated to remove MeOH. Crystallization in the refrigerator overnight afforded 0.140 g pure fumarate as a slightly pink

powder. $C_{26}H_{32}N_2O_8$: found C 62.30, H 6.35, N 5.72; calcd C 62.39, H 6.44, N 5.60; $[\alpha]^{25}D$ $-101.5°$ ($c = 0.68$, MeOH); uv λ max (EtOH) nm (log ε) 248 (4.04), 331 (3.64); uv λ max (H_2O) nm (log ε) 243 (4.00), 322 (3.58); eims *m/z* (%) 384 (100), 369 (9), 116 (7), 98 (19); ^1H and ^{13}C nmr (DMSO-d_6) see Tables 1 and 2.

Eseroline [**7**], *fumarate*.—Compound **7** was obtained from physostigmine according to Yu *et al.* (12).

RECEPTOR BINDING ASSAYS.—Receptor binding assays were performed by incubating membranes prepared from rat central nervous system with 1 nM [^{3}H]-pCl-DPDP-enkephalin for 270 min at 20°, according to Vaughn *et al.* (17), or with 1 nM [^{3}H]-DAGO for 60 min at 20°, according to Borea *et al.* (18), for δ and μ receptors. For κ receptors guinea pig cerebellum homogenate was incubated with 1.5 nM [^{3}H]-U 69593 for 60 min at 20°, according to Nock *et al.* (19). After the incubation period, bound and unbound radioligands were separated by rapid filtration, and radioactivity bound to membranes in the absence and presence of unlabelled compounds was counted in a beta-scintillation counter. Specific binding was determined with 10^{-5} M unlabelled DPDP-enkephalin, DAGO, or U50488H and was greater than 70%. Displacement curves were established and IC_{50} values (molar concentration of unlabelled compound at which half-maximal displacement of radioligand occurred) were calculated by a Lundon program (Lundon Software Inc. 1987, Cleveland, Ohio) on a Vax computer. Each experiment was performed in duplicate; duplicates were the same within the frame of experimental reproducibility. The result of one of the two determinations is given.

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