Crystal Structure of Akuammigine Picrate Hydrate

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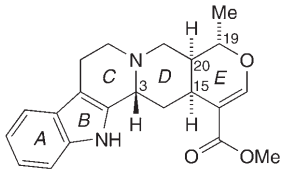
The single-crystal X-ray data of akuammigine picrate hydrate (**1**·Picr·H_2O) confirm the relative configuration of the indole alkaloid akuammigine (**1**) as *epiallo* (*Fig. 1*). With reference to the known (15*S*)-configuration due to biosynthesis, the absolute configuration of the other stereogenic centers is thus given by (3*R*,19*S*,20*S*). Four crystallographically independent molecules are observed in the asymmetric unit (*Fig. 2*). Each of the alkaloid cations forms H-bonds to a H_2O and a picrate anion (*Fig. 3*). The H_2O molecules are further associated by a H-bond as indicated by the short O···O distance (*Table 2*). The conformation in the solid state of the picrate hydrate is now firmly established, and a cute H-bonding motif is observed.

Introduction. – Alkaloids were extracted from seeds of the African species *Picralima nitida* (STAPF) T. DURAND & H. DURAND (syn. *P. klaineana* PIERRE) as early as 1926 [1]. Reportedly, 'akuamma' was the native name of the *P. nitida* tree, which had a reputation as a remedy for malaria in the Gold Coast Colony [2], but later the correct use of the name was disputed [3]. Nevertheless, alkaloids were named akuammine, akuammiline, akuammigine, akuammidine, akuammenine, and akuammicine [4]. UV Spectroscopy and color reactions were employed to classify them as indole alkaloids [5–7]. The constitution of akuammigine (**1**) was first proposed with an additional Me group [8] but was revised shortly afterwards [9]. The position of the C=C bond in ring *E* of the heteroyohimbines was deduced from UV spectra of model compounds [10]. The configuration has been a topic of interest ever since. In the α,β notation [11], the possible stereoisomers are defined on the basis of ring *D* configuration as *allo* (H_α–C(3), H_α–C(20)), *epiallo* (H_β–C(3), H_α–C(20)), *normal* (H_α–C(3), H_β–C(20)), and *pseudo* (H_β–C(3), H_β–C(20) [12]. The presence of distinct peaks in the 2800–2700 cm^{-1} region of IR C–H stretching vibrations, which became known as '*Bohlmann* bands', was found to be useful for the empirical assignment of the configuration of H–C(3) as α (thus *normal* or *allo*) [13]. These peaks were thought to arise from a diaxial interaction between the N-atom lone pair and the vicinal *trans*-oriented C–H bond [14]. Later, the rule was modified to apply more specifically to compounds with at least two vicinal H-atoms in axial positions [15]. Since akuammigine lacked these bands, the H–C(3) orientation was designated β and presumed to be equatorial. The pitfall of this method is that it requires conformational purity which is possible only in rigid *D*/*E* *trans*-fused systems (*normal*, *pseudo*) but obviously not in rings with a flexible *cis*-junction (*allo*, *epiallo*) due to ring inversion and N-atom inversion. In contrast, H–C(15) always has an α-orientation, and the latter was chemically

correlated with *Cinchona* alkaloids of known [16] absolute (*S*)-configuration at the corresponding stereogenic center [17]. It was then attempted to elucidate ring geometries in various indole alkaloids by comparing reaction rates in oxidation–reduction studies [18][19], and akuammigine was assigned the *pseudo* geometry. On the basis of new ^{1}H-NMR data, the configuration of the Me–C(19) group was assigned α and the configuration at C(20) of akuammigine was tacitly revised (thus *epiallo*) [20], and later the perils of assigning configuration of indole alkaloids based on chemical degradation were discussed [21]. Next, an equilibrium of two akuammigine conformers was observed by temperature-dependent ^{13}C-NMR [22] and was corroborated by circular dichroism (CD) [23][24]. A third conformer, where ring *D* adopts a boat conformation, was invoked to give a better explanation of ^{1}H-NMR coupling constants [25]. Within this context, IR spectra are arguably meaningless.



1 Akuammigine

Syntheses of racemic akuammigine [26][27], a biomimetic synthesis from secologanin and tryptamine [28], and an asymmetric formal total synthesis [29] were reported. Furthermore, conversion of the alkaloids isopteropodine [30] and hirsuteine [31] to akuammigine (**1**) established additional stereochemical relationships.

In contrast to other 'akuamma' alkaloids, akuammigine (**1**) showed no efficacy in opioid-receptor bioassays [32]. The compound exhibited only negligible affinity to adrenergic receptors [33][34]. Moreover, the anticipated antimalarial activity could not be substantiated [35].

In the meantime, other sources of akuammigine (**1**) have been discovered such as *Mitragyna parvifolia* (ROXB.) KORTH. from Sri Lanka and India [24][36][37], *Catharanthus roseus* (L.) G. DON. [38] and *C. ovalis* MARKGR. [39] cell cultures, Asian *Uncaria rhynchophylla* MIQ. [23], *U. bernaysii* F. MUELL. [40], and *U. attenuata* KORTH. [41]. More recently, akuammigine (**1**) has been isolated from young leaves of Peruvian *U. tomentosa* (WILLD.) DC. [42]. Herein, we report the crystal structure of its picrate hydrate.

Results and Discussion. – Young leaves of *U. tomentosa* contain, beside oxindole alkaloids, akuammigine (**1**) as the major indole alkaloid [42]. It was identified by comparison of measured and published UV, IR, NMR, and MS data and chromatographic behavior [43]. Single crystals of **1** were difficult to obtain from several solvents and mixtures. Finally, when we resorted to aqueous picric acid (=2,4,6-trinitrophenol), suitable crystals of a picrate, $\mathbf{1}\cdot\mathrm{Picr}\cdot\mathrm{H_2O}$, were grown by slow cooling of a saturated solution. However, solving the structure still remained a challenge due to the rather high number of light atoms in the cell of a chiral space group. Details of the refinement are collected in *Table 1*.

Table 1. *Crystal and Refinement Data of Akuammigine Picrate Hydrate* (**1** · Picr · H_2O)

Formula		$C_{27}H_{29}N_5O_{11}$	θ Range [°]	1.84 – 24.00
M_r [g mol^{-1}]		599.55	Absorption correction	none
Crystal system		monoclinic	Index ranges	$-12 \le h \le 10$, $-24 \le k \le 25$,
Space group		$P2_1$		$-26 \le l \le 26$
Unit cell dimensions	a [Å]	11.0766(2)	Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0696P)^2$
	b [Å]	22.0775(2)		$+ 1.3899P]$ where $P = (F_o^2 + 2F_c^2)/3$
	c [Å]	23.4714(4)	Reflections measured	30308
	β [°]	90.943(1)	Independent reflections	17797 ($R_{int} = 0.021$)
	V [Å^3]	5739.00(15)	Reflections with $I > 2\sigma(I)$	15514
Z		8	Data, restraints, parameters	17797, 1, 1586
D_x [g cm^{-3}]		1.388	R_1, wR_2 ($I > 2\sigma(I)$)	0.0480, 0.1222
μ [mm^{-1}]		0.11	R_1, wR_2 (all data)	0.0578, 0.1284
$F(000)$		2512	$(\Delta/\sigma)_{max}$	0.004
Temperature [K]		233(2)	Goodness-of-fit	1.05
Radiation, wavelength [Å]		MoK_α, 0.71073	$\Delta\rho_{min}$, $\Delta\rho_{max}$ [e Å^{-3}]	– 0.18, 0.40

The X-ray data of **1** · Picr · H_2O confirmed the relative configuration as *epiallo*. With reference to the known (15*S*)-configuration due to biosynthesis, the absolute configuration of the other stereogenic centers of **1** is thus given by (3*R*,19*S*,20*S*). Four crystallographically independent molecules of both the protonated akuammigine and the picrate anion were observed in the asymmetric unit as shown in *Fig. 1*.

Each of the four alkaloid cations adopts the same conformation with only minor differences. As expected, the aromatic rings *A* and *B* are planar. Ring *C* is twisted with N(2) below the *A*/*B* plane by 0.233(4) Å and C(17) above by 0.481(4) Å, respectively, when viewed as in *Fig. 2*. The minor conformational differences are illustrated by the corresponding values of the other independent alkaloid cations, thus N(2) below the plane by 0.301(4), 0.340(4), and 0.185(4) Å, and C(17) above by 0.420(5), 0.411(4), and 0.538(4) Å, respectively.

The *C*/*D* ring junction is *trans*, and ring *D* is a regular chair, with H–C(3) β but axial; the H-atom at the protonated tertiary-amine moiety (at N(2), see *Fig. 2*) is axial. The *D*/*E* rings are *cis*-fused; H–C(15) is always α (in this case equatorial), and H–C(20) is α, therefore necessarily axial. Ring *E* is a twisted half-chair. Interestingly, the Me–C(19) group, which is α, clearly adopts a pseudoaxial position, even more pronounced than in a regular chair, because the half-chair is flatter. This conformation observed in the solid state is in agreement with the one assumed to be predominant in solution [22][25]; however, this is in contrast to a conformation postulated earlier [12] and having a pseudoequatorial Me–C(19) which would require an axial orientation of the bulky indole substituent at C(3) to accommodate the Me–C(19) as an equatorial substituent in the preferred conformation. An ORTEP diagram of **1** · H^+ is shown in *Fig. 2*.

Each of the cationic alkaloid molecules forms H-bonds to a H_2O molecule and a picrate anion, with H–N(1) of the indole and the protonated quinolizidine H–N(2) moieties (see *Fig. 3*) acting as donors, whereas the O-atom of C=O acts as acceptor. Pairs of H_2O molecules are associated by a H-bond as indicated by the short O···O

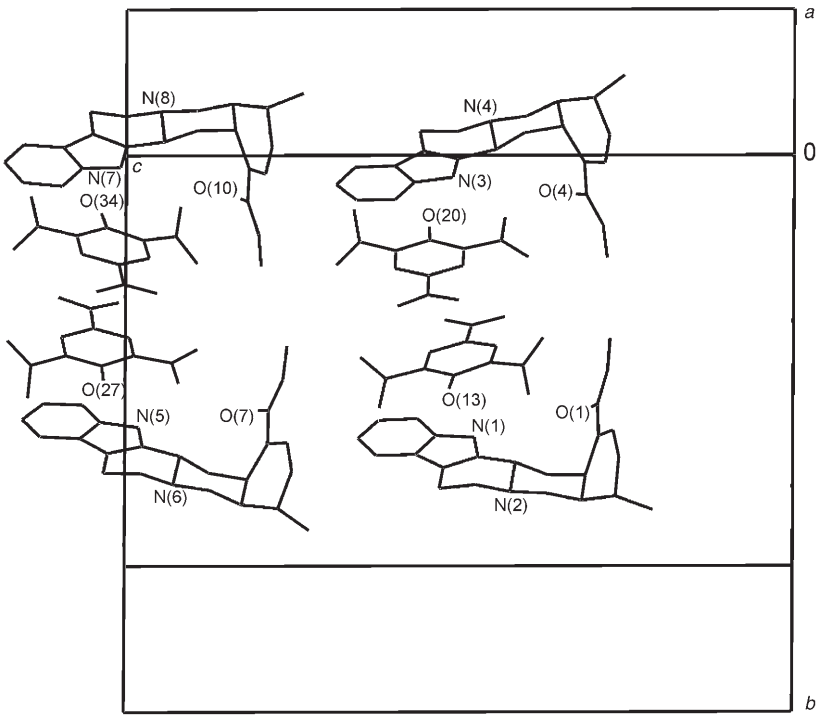


Fig. 1. *Asymmetric unit of akuammigine picrate hydrate* (**1** · Picr · H_2O). H-atoms and H_2O molecules are omitted for clarity.

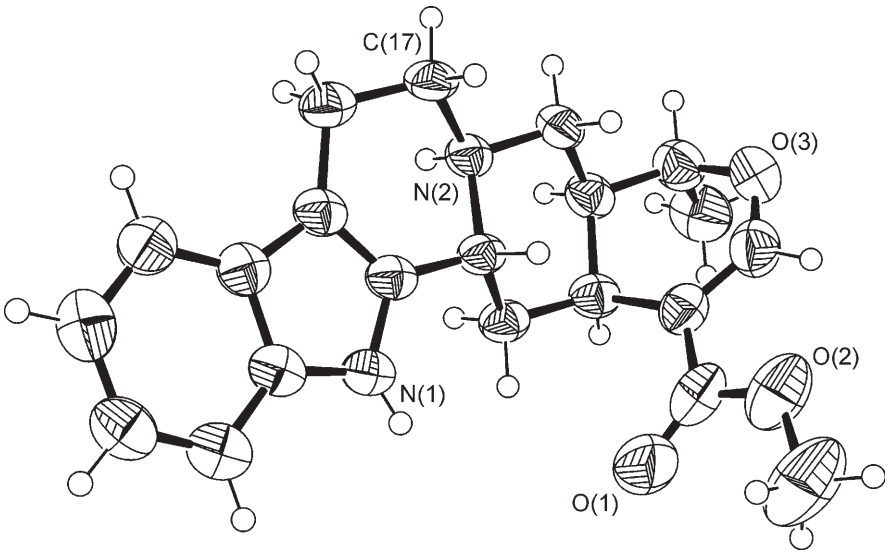


Fig. 2. *ORTEP Plot of one of the four independent protonated akuammigine molecules* (**1** · H^+; 50% probability ellipsoids, arbitrary numbering of the atoms)

distances of 2.854(5) and 2.854(8) Å, although the H-atoms of the H_2O molecules could not be exactly located (*Fig. 3*). The relevant H-bonding parameters are summarized in *Table 2*.

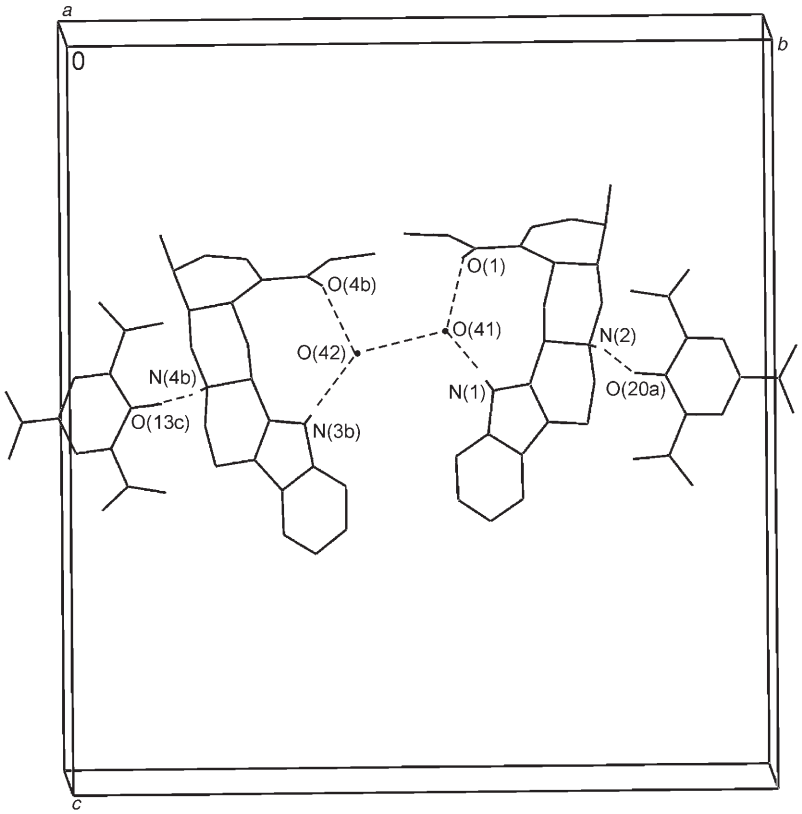


Fig. 3. *Hydrogen-bonding in akuammigine picrate hydrate* (**1**·Picr·H_2O). Symmetry codes: a: $1-x$, $1/2+y$, $1-z$; b: $-1+x$, y, z; c: $1-x$, $-1/2+y$, $1-z$.

In conclusion, the conformation of the *epiallo* indole alkaloid akuammigine (**1**) is now firmly established, at least in the solid state of the picrate hydrate, and a cute H-bonding motif is observed.

Experimental Part

Akuammigine (=*(3β,19α,20α)-16,17-Didehydro-19-methyloxayohimban-16-carboxylic Acid Methyl Ester*; **1**): *Isolation*. Plant material was obtained from the Botanical Garden of the University of Innsbruck, Austria. Dried leaves were pulverized and extracted with 0.1M HCl. The soln. was neutralized, extracted with CH_2Cl_2, and the solvent was evaporated. The residue yielded, after repeated column chromatography (silica gel; AcOEt/hexane and AcOEt/MeOH): pure **1**.

Table 2. *Selected Hydrogen-Bonding Parameters for Akuammigine Picrate Hydrate* (**1** · Picr · H_2O)

D–H···A	H···A [Å]	D···A [Å]	D–H···A [°]
N(1)–H···O(41)	2.00	2.828(5)	164
O(41)···O(1)		2.902(6)	
N(2)–H···O(20)[a])	1.92	2.772(3)	166
O(41)···O(42)		2.854(5)	
N(3)–H···O(42)[b])	2.22	2.940(4)	172
O(42)[b])···O(4)		2.763(4)	
N(4)–H···O(13)[c])	1.98	2.777(3)	174
N(5)–H···O(43)	2.10	2.881(5)	163
O(43)···O(7)		2.979(6)	
N(6)–H···O(34)[d])	1.91	2.773(3)	166
O(43)···O(44)		2.854(8)	
N(7)–H···O(44)[b])	2.04	2.826(5)	168
O(44)[b])···O(10)		3.029(8)	
N(8)–H···O(27)[e])	1.93	2.801(3)	174

[a]) $1-x, 1/2+y, 1-z$. [b]) $1+x, y, z$. [c]) $2-x, -1/2+y, 1-z$. [d]) $1-x, 1/2+y, 2-z$. [e]) $2-x, -1/2+y, 2-z$.

X-Ray Crystal-Structure Analysis[1]). A yellow crystal of the alkaloid picrate **1** · Picr · H_2O ($0.4 \times 0.3 \times 0.25$ mm) was used. X-Ray diffraction data were collected on a *Nonius-Kappa-CCD* diffractometer operated in the ϕ- and ω-scan mode, by using graphite-monochromated MoK_α radiation. The lattice parameters and their estimated standard deviations were determined from least-squares refinement of 65592 reflections in the range of $1 < \theta < 25°$. Data reduction was carried out with programs Denzo and Scalepack [44]. The structure was solved by direct methods and refined with the programs SHELXS97 and SHELXL97 [45] in the SHELXTL software package [46]. The H-atoms of the H_2O molecules could not be exactly located and were omitted. H-Atoms at N-atoms were found and refined isotropically, H-atoms at C-atoms were refined by using a riding model. For all non-H-atoms, anisotropic displacement parameters were refined. The O-atoms in the picrate anion show elongated ellipsoids, probably due to some motion or unresolved disorder. The absolute configuration was set by reference to the known absolute configuration at C(15). *Fig. 2* was drawn with ORTEP [47], and H-bonds and the molecular packing geometry were calculated with Mercury [48].

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[1]) CCDC-664278 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

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