camphorsulfonic acid (2.2 mg, 0.01 mmol, 0.2 equiv). After 10 h, additional vancosamide glycal **8** (10 mg, 0.6 equiv) was added, and stirring was continued at room temperature for an additional 6 h. The reaction mixture was then diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, water, and brine. The solution was dried over $MgSO_4$, filtered, and concentrated, and the resulting clear colorless oil was chromatographed over silica gel (using 17.5-40% EtOAc-hexanes elution) to provide recovered **3b** (13.5 mg, 48% recovery) and the desired disaccharide **10b** (23.0 mg, 51%): $[\alpha]^{25}_D = -61.73°$ (c 0.81, $CHCl_3$); IR ($CHCl_3$) 3380, 3000, 2940, 1700, 1660, 1600, 1490, 1475, 1300, 1280, 1260, 1120, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (d, 2 H, J = 7.3 Hz, ArH), 7.17-7.60 (m, 23 H, ArH), 7.02 (t, 1 H, J = 8.4 Hz, ArH), 6.78 (br s, 1 H, NHBz), 6.57 (d, 2 H, J = 8.4 Hz, ArH), 5.47 (d, 1 H, J = 3.9 Hz, H1'), 5.15 (d, 1 H, J = 7.7 Hz), 5.02 (s, 1 H, H4'), 4.90 (br s, 2 H), 4.76-4.84 (m, 3 H), 4.59 (d, 1 H, J = 11.0 Hz), 4.47 (m, 2 H), 4.02 (t, 1 H, J = 8.0 Hz), 3.78 (s, 6 H, ArOMe), 3.64-3.82 (m, 3 H), 3.40 (m, 1 H), 2.92 (d, 1 H, J = 13.7 Hz, eq H2'), 2.12 (dd, 1 H, J = 3.9, 13.7 Hz, ax H2'), 2.05 (s, 3 H, C3'-Me), 1.07 (d, 3 H, J = 6.4 Hz, H6'); FABLRMS (NOBA + NaI) m/e (relative intensity) 938 (2.8), 784 (4.6), 353 (27.0), 352 (100), 307 (8.2), 289 (6.0), 231 (17.0), 230 (4.4), 181 (11.5), 167 (4.6), 165 (5.5); FABHRMS calcd for $C_{56}H_{60}NO_{12}$ 938.4117, found 938.4084. Anal. Calcd for $C_{56}H_{59}NO_{12}$: C, 71.70; H, 6.34; N, 1.49. Found: C, 71.43; H, 6.06; N, 1.23.

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Registry No. **1**, 1404-90-6; **1**·xHCl, 1404-93-9; **2**, 55628-54-1; **3a**, 135192-39-1; **3b**, 135192-43-7; **3c**, 138925-03-8; **3d**, 138925-04-9; **4a**, 138925-14-1; **4b**, 138925-15-2; **5a**, 138925-05-0; **5b**, 138925-06-1; **6a**, 138925-07-2; **6b**, 138925-08-3; **7**, 37091-13-7; **7** 1-demethyl derivative, 138925-16-3; **7** 1-demethyl derivative β-isomer, 138925-17-4; **8**, 138925-09-4; **9a** (X = H), 138925-11-8; **10a**, 138925-12-9; **10b**, 138925-13-0; 2,6-$(MeO)_2$PhOH, 91-10-1; phenol, 108-95-2; methyl salicylate, 119-36-8; o-bromophenol, 95-56-7; 3,3-dimethyldioxirane, 74087-85-7.

Epibatidine: A Novel (Chloropyridyl)azabicycloheptane with Potent Analgesic Activity from an Ecuadoran Poison Frog

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Abstract: A potent non-opioid analgesic, epibatidine, has been isolated from skins of the Ecuadoran poison frog, *Epipedobates tricolor*, and its structure determined by MS, IR, and ^1H NMR analyses as *exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane. It represents a unique new class of alkaloids.

Skin extracts from an Ecuadoran poison frog, *Epipedobates tricolor*, of the family Dendrobatidae yielded a number of alkaloids, including the major alkaloid pumiliotoxin **251D**, whose indolizidine structure was revealed by X-ray crystallographic analysis.[1] These frogs had been obtained primarily because of the presence of a trace alkaloid in the skin extract that caused a Straub-tail response[2] when injected in mice.[3,4] The Straub-tail reaction initially served as an assay for this trace alkaloid during purification. Gas chromatographic-mass spectral analysis of fractions obtained by chromatography of alkaloids from *Epipedobates tricolor* on a silica column (see ref 1 for details) indicated that the trace alkaloid causing the Straub-tail reaction had molecular ions (208, 210) and several pairs of fragments all in a 3:1 ratio, indicating the presence of chlorine.[5] High-resolution mass measurements (Table I) established the formula for the m/z 208

Table I. High-Resolution Mass Measurements of Epibatidine

obsd	percent of base peak	calcd for	error (mmu) (calcd - obsd)
210.0764	4.4	$C_{11}H_{13}N_2{}^{37}Cl$	-2.9
208.0769	15.5	$C_{11}H_{13}N_2{}^{35}Cl$	-0.15
181.0259	~1	$C_9H_8N_2{}^{37}Cl$	+8.6
179.0362	2.7	$C_9H_8N_2{}^{35}Cl$	+1.4
150.1289[a]	3.3	$C_{10}H_{16}N$	-3.3
142.0245	2.7	$C_7H_7N^{37}Cl$	-0.9
141.0287	3.4	$C_7H_8N^{35}Cl$	+6.8
140.0263	9.2	$C_7H_7N^{35}Cl$	+0.4
124.1148[a]	3.6	$C_8H_{14}N$	+2.2
83.0749	2.1	C_5H_9N	-1.6
69.0489	100	C_4H_7N	+8.9

[a] Probably due to an impurity. The data indicate a much higher hydrogen to carbon ratio than that found in the parent ions.

(1) Daly, J. W.; Tokuyama, T.; Fujiwara, T.; Highet, R. J.; Karle, I. L. *J. Am. Chem. Soc.* **1980**, *102*, 830-836. From 750 skins, a total of 21 mg of the major alkaloid pumiliotoxin **251D** was isolated. The trace alkaloid that is the subject of the present paper is present at levels of at least 20-fold less than pumiliotoxin **251D**.

(2) A Straub-tail reaction is characteristic of opiate alkaloids and has been used as an assay for opiate agonists and antagonists (Aceto, M. D.; McKean, D. B.; Pearl, J. *Br. J. Pharmacol.* **1969**, *36*, 225-239). Unlike the Straub-tail reaction caused by morphine and other opiates, the reaction caused by the frog alkaloid was not reversed by naloxone (see Table II).

(3) Daly, J. W.; Brown, G. B.; Mensah-Dwumah, M.; Myers, C. W. *Toxicon* **1978**, *16*, 163-188.

(4) Daly, J. W.; Myers, C. W.; Whittaker, N. *Toxicon* **1987**, *25*, 1023-1095.

(5) Since a chloro substituent had not been previously seen in any of the more than 200 dendrobatid alkaloids, we considered it possible that a chlorine had been introduced into the **208/210** alkaloid from HCl during the usual preparation of alkaloid fractions from methanolic skin extracts (partition into $CHCl_3$, extraction into 0.1 N HCl, basification, and re-extraction into $CHCl_3$). Consequently, a separate extract was partitioned using CH_2Br_2 and HBr. The chlorine-containing **208/210** alkaloid was still obtained. It was also present in the methanol extracts of skin; thus, chlorine is not artifactually incorporated during isolation.

Table II. Comparison of Activity of Morphine and Epibatidine (Alkaloid **208/210**)

	dose eliciting marked Straub-tail[a] (mg/kg)	hot plate analgesia[b] ED_{50} (mg/kg)	IC_{50} inhibition [^{3}H]dihydromorphine binding[c] (nM)
morphine	10	1	1.1
epibatidine	0.020	0.005[d]	8800

[a] The Straub-tail reaction (>45° arch) elicited by morphine as blocked by administration of 5 mg/kg of naloxone 20 min prior to 20 mg/kg of morphine. The Straub-tail reaction elicited by 20 μg/kg of epibatidine was reduced only slightly by naloxone. Straub-tail reaction with epibatidine at this dose persists for 1–2 h. [b] Assay as described (Eddy, N. B.; Leimbach, D. *J. Pharmacol. Exp. Therap.* **1953**, *107*, 385–393). [c] Assay with guinea pig brain preparations and 1 nM [^{3}H]-dihydromorphine as described (Pert, C. P.; Snyder, S. *Mol. Pharmacol.* **1974**, *10*, 868–879). [d] Potent analgetic activity also was apparent in the Nilsen assay, but sufficient material was not available for detailed study. Assay as described (Nilsen, P. L. *Acta Pharmacol. Toxicol.* **1961**, *18*, 10–22).

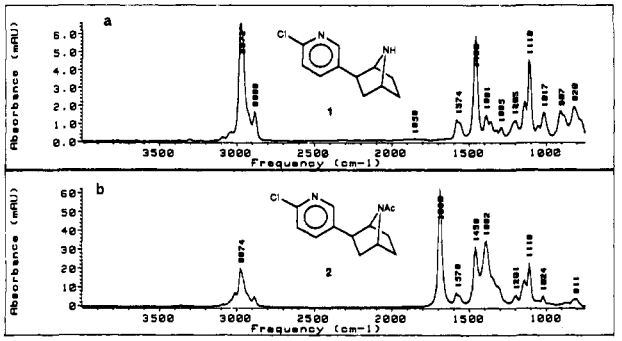


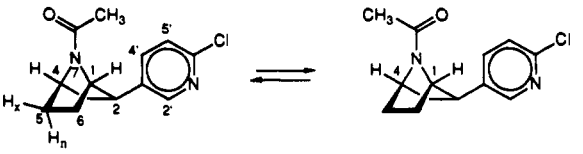
Figure 1. GC–FTIR spectra of epibatidine (a) and *N*-acetylepibatidine (b). Spectra were obtained with a Hewlett-Packard 5965A FTIR instrument with a narrow band (4000–750 cm^{-1}) detector and interfaced with a Hewlett–Packard 5890 gas chromatograph fitted with an HP-5 fused silica column (30 m × 0.32 mm), programmed 100° to 280° at 10°/min.

ion as $C_{11}H_{13}N_2{}^{35}Cl$, indicating the presence of six rings or double bonds. The major ^{35}Cl-containing fragments were $C_9H_8N_2Cl^+$ (*m/z* 179, $M^+ - C_2H_5$) and $C_7H_7NCl^+$ (*m/z* 140, $M^+ - 68$), while the base peak was $C_4H_7N^+$ (*m/z* 69).[6] The **208/210** alkaloid was purified further by HPLC,[7] yielding material for biological evaluation. The name epibatidine, referring to its origin from *Epipedobates tricolor*, is given to this alkaloid. The alkaloid proved to be a potent analgesic with a non-opioid mechanism of action in preliminary tests (Table II). The properties of the **208/210** alkaloid, epibatidine, are as follows: a basic, relatively polar alkaloid[8] with one exchangeable[6] and acetylatable NH; it has a UV spectrum with $\lambda_{max}^{CH_3OH}$ at 217 nm with a broad shoulder at 250–280 nm (absorbance ratio 2:1), a chromophore suggesting a pyridine moiety[9] among other possibilities. It gave a negative Ehrlich test on TLC for pyrroles or indoles.

The GC–FTIR spectrum of epibatidine, shown in Figure 1, has similarities to the IR spectrum of the tobacco alkaloid anabasine, 2-(3-pyridyl)piperidine, which has significant absorbances at 1428 and 1112 cm^{-1},[10] suggesting that epibatidine contains a pyridine moiety and, in particular, a 2-chloropyridine moiety. The IR spectrum of 2-chloropyridine has absorbances at 1423 and 1132 cm^{-1}.[10]

Further purification of epibatidine involved conversion to an *N*-acetyl derivative. A combined fraction containing mainly epibatidine and some pumiliotoxins first was freed of neutral material by partitioning between EtOAc and 0.1 N HCl, followed by alkalinization of the HCl layer and re-extraction of alkaloids into EtOAc and then removal of the EtOAc under a stream of N_2. After acetylation, the material was repartitioned between EtOAc and 0.1 N HCl. The N-acetylated epibatidine was recovered in the EtOAc, while the other dendrobatid alkaloids (allopumiliotoxins **267A** and **323B**), being basic tertiary amines and hence nonacetylated, were again removed into the HCl. The mass spectrum[11] and the GC–FTIR spectrum (new absorptions at 1690 and 1392 cm^{-1}) of *N*-acetylepibatidine indicated one *N*-acetyl unit. A comparison with FTIR spectra for *N*-acetylpyrrolidine (1686, 1408 cm^{-1}) and *N*-acetylpiperidine (1683, 1419, 1250 cm^{-1}) indicated a similarity of the *N*-acetylepibatidine with the former. Hydrolysis of the *N*-acetyl derivative to epibatidine proved very difficult, presumably due to protonation of the pyridine under acidic conditions and steric factors under basic conditions.[12]

Table III. ^{1}H NMR (500 MHz) Parameters for *N*-Acetylepibatidine (**2**)[a]



2A **2B**

proton(s)	δ^A (ppm) (*J*, Hz)	δ^B (*J*, Hz)
CH_3CO	1.88[s]	2.09[s]
1	3.93[d] ($J_{1,6x}$ = 4.0)	4.69[d] (*J* = 4.8)
2n[b]	3.01[dd] ($J_{2,3x}$ = 4.4, $J_{2,3n}$ = 8.8)	2.96[dd] (*J* = 4.8, 8.8)
3x	1.78–1.92[m] (A + B) overlapping 5x (A + B) and 6x (A + B)	
3n	2.02[dd] ($J_{2,3n}$ = 8.8, $J_{3x,3n}$ = 12.5)	2.16[dd] ($J_{2,3n}$ = 9.2, $J_{3x,3n}$ = 12.1)
4	4.85[t] ($J_{3x,4} = J_{4,5x}$ = 4.8)	4.29[t] (*J* = 4.8)
5x	1.78–1.92[m] (A + B)	
5n, 6n	1.50–1.78[m] (A + B, 2 protons)	
6x	1.78–1.92[m] (A + B)	
5′	7.28[d] (J_{ortho} = 8.4)	7.24[d] (*J* = 8.1)
4′	7.50[dd] (J_{meta} = 2.6, J_{ortho} = 8.2)	7.58[dd] (*J* = 2.6, 8.5)
2′	8.26[d] (J_{meta} = 2.6)	8.20[d] (*J* = 2.2)

[a] The ^{1}H NMR and ^{1}H COSY spectra were obtained with a Varian VXR-500S spectrometer with 0.75 mg of *N*-acetylepibatidine (**2**) in $CDCl_3$. The amount of **2** was determined by addition of a known amount of CH_3OH to the sample at the end of the experiment. δ (ppm) values were measured relative to the $CHCl_3$ signal at 7.20 ppm. Some couplings were established with the ^{1}H COSY spectrum. [b] Note: $J_{1,2n} \cong J_{3n,4} \cong J_{4,5n} \cong J_{6n,1} \cong 0$ Hz (see text).

(6) Initially, it was thought[4] that the gas chromatographic peak with apparent parent ions at *m/z* 208/210 (*m/z* 209, 211 on chemical ionization with NH_3 and *m/z* 211, 213 with ND_3) was a pyrolysis product, because the low R_f of the alkaloid on TLC (see ref 8) argued for the presence of polar groups such as hydroxyls.

(7) The epibatidine-containing fractions (108–112) from silica chromatography also contained allopumiliotoxins **267A** and **323B** (see ref 1).

(8) TLC: The R_f of epibatidine is 0.25 on silica gel (CH_3OH–$CHCl_3$ 1:9). The R_f of anabasine is 0.26 in this system.

(9) 2-Chloropyridine has $\lambda_{max}^{heptane}$ 269 nm (ε 5320). Amounts of **1** could not be accurately measured for a meaningful estimation of ϵ_{max}. Our small supply of purified **2** was exhausted in futile hydrolysis attempts. Regrettably, no UV spectrum was recorded with this material.

(10) *Aldrich Library of FTIR spectra, Vapor Phase Spectra*, 1st ed.; Pouchert, C. J. Ed.; Aldrich Chemical Co.: Milwaukee, WI, 1989; Vol. 3; anabasine, spectrum 1537D, 2-chloropyridine, spectrum 1525C. 3-Chloropyridine (spectrum 1526C) has pairs of strong absorptions at 1469, 1410 and 1114, 1023 cm^{-1}.

(11) Mass spectral intensity relative to base peak = 100 in parentheses. Epibatidine: *m/z* 211/209 (3/10) $(M + 1)^+$, 181/179 (<1/1), 142/140 (2/8), 69 (100). *N*-Acetylepibatidine: *m/z* 250 (<1) (252 not detected), (M^+) 191 (3), 164 (3), 142/140 (6/18), 111 (10), 69 (100), 68 (45). These GC–EI mass spectra were obtained with a Finnigan Model 800 ion trap. The mass range 50–350 was scanned once per second. The mass detector was interfaced with a Hitachi oven fitted with the same column and using the same program as the GC–FTIR spectrophotometer (see caption for Figure 1). M + 1 ions are often more prominent than M^+ for alkaloids in the ion trap.

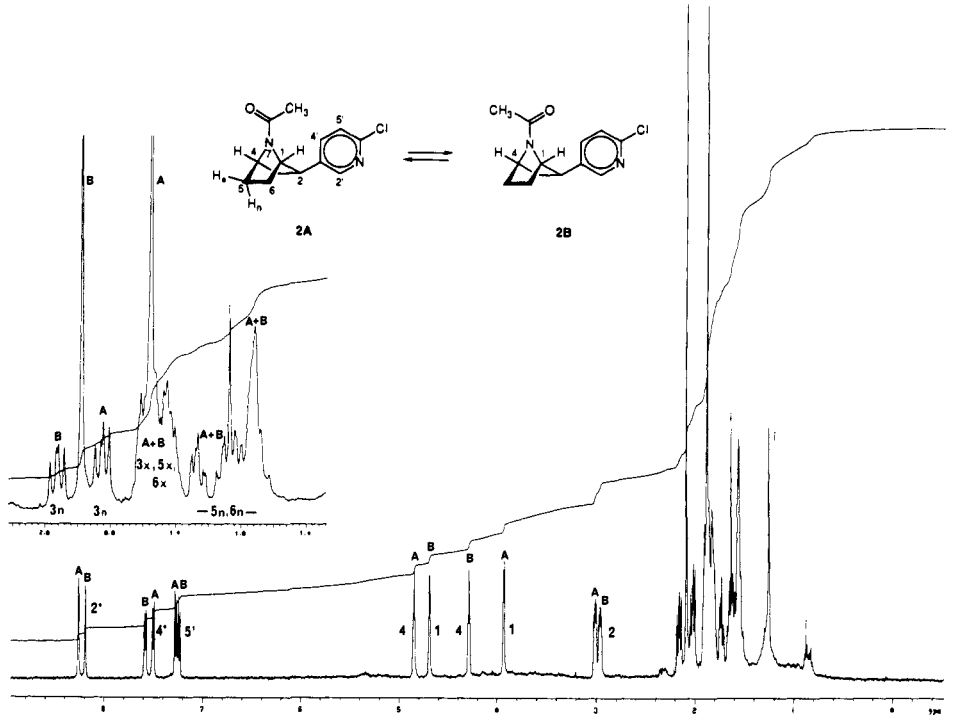
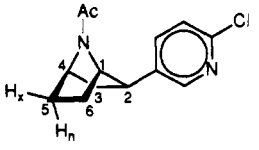


Figure 2. ^{1}H NMR (500 MHz) spectrum of *N*-acetylepibatidine in $CDCl_3$ (see Table III): x = exo, n = endo, i = impurities.

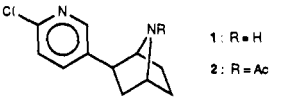
Table IV. Coupling Constants in Rotamers of *N*-Acetylepibatidine and Calculated Dihedral Angles



vicinal hydrogens	ϕ (deg) Chem 3D[20]	ϕ (deg) Quanta[21]	obsd J (Hz) in rotamers A	obsd J (Hz) in rotamers B
1–2n	87	73	0	0
2n–3x	124	105	4.4	4.8
2n–3n	2	13	8.8	9.2
3n–4	90	96	0	0
3x–4	39	21	4.8	4.8
6x–1	40	32	4.8	4.0
6n–1	89	90	0	0
5n–4		76		
5x–4		46	4.8	4.7
5x–6x		10		
5n–6n		10		
5n–6x		112		

Therefore, further structural studies were conducted with *N*-acetylepibatidine.

Analysis of the 500-MHz ^{1}H NMR spectrum of *N*-acetylepibatidine in $CDCl_3$ (Figure 2 and Table III) allowed the unambiguous assignment of the structure and stereochemistry of epibatidine as *exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]-heptane (**1**) and the *N*-acetyl derivative as **2**. Epibatidine is the first member of this class of alkaloids, which represents a previously unknown structural type.[13] It has been detected as a trace alkaloid in only a few species of dendrobatid frogs.[14]



The ^{1}H NMR spectrum of **2** (Figure 2) is recognized as a series of doubled signals, stemming from nearly equimolar *N*-acetyl rotamers, **2A** (52%) and **2B** (48%), in which the amide acetyl group is constrained to planarity with the C(1)–N(7) and C(4)–N(7) bonds. Interconversion between rotamers is very slow due to the partial double-bond character of the *N*-acetyl bond. In rotamer **2A**, we observe a large deshielding of H_4 relative to H_1 by the anisotropy of the acetamido carbonyl group.[15] In rotamer **2B**, H_1 is deshielded relative to H_4. Saturation-transfer experiments confirmed the rotamer hypothesis, e.g., both H_{1A} and

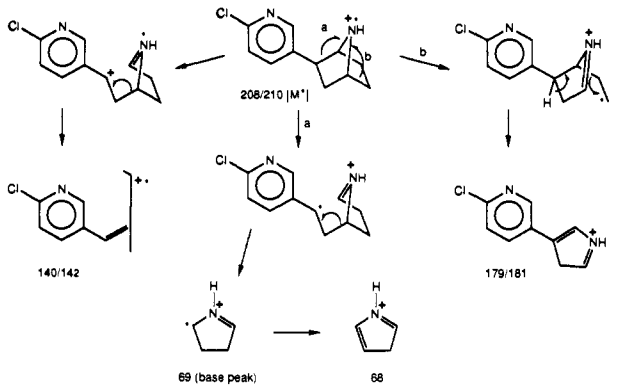
(12) Hydrolysis was finally attained as follows: *N*-Acetylepibatidine (~0.1 mg) was treated in a sealed vial with 100 μL of H_2SO_4 (concentrated)–H_2O (2:1) for 20 h at 135 °C. Neutralization and extraction (EtOAc) provided a trace of 97 parts of **1** and 3 parts of a byproduct isomeric with *N*-acetylepibatidine and having an acetyl group in the pyridyl moiety. The byproduct had the following mass spectrum. EIMS (ion trap): 251 (8), 250 (8), 207 (25), 193 (10), 191 (25), 181 (18), 179 (52), 167 (15), 139 (52), 126 (25), 104 (40), 96 (100). The yield of epibatidine was not determined, but was less than 5%. The following ^{1}H NMR signals for epibatidine (D_2O/DCl) could be detected (NT = 10^4) and some tentative assignments made: δ 8.41^d (2.6 Hz) ($H_{2'}$), 8.08^{dd} (2.7, 8.4) ($H_{4'}$), 7.68^d (8.8) ($H_{5'}$), 4.9^d (*J* not measd) (H_1), 4.23^{dd} (7.0, 13.9) (H_{3x}), 4.28^t (4.6) (H_4), 3.46^{dd} (6.0, 10) (H_{2n}), 2.38^{dd} (9.7, 13.8) (H_{3n}), all other signals 2.10–1.73^m (δ_{HOD} at 4.78 ppm). NMR studies on **2** were not extensive since we hoped to characterize **1** more completely, but failed in this goal due to poor conversions. We did not try higher temperatures on **2** to coalesce the rotamer signals nor was any attempt made to obtain a ^{13}C NMR spectrum of **2**.

(13) A review on nitrogen-bridged six-membered-ring systems states that this ring system has yet to be found in nature (Kricka, L. J.; Vernon, J. M. *Adv. Heterocycl. Chem.* **1974**, *16*, 87). Only one related alkaloid has been detected in frogs. It was present in very small amounts in less polar fractions from *Epipedobates tricolor*. The alkaloid had protonated parent ions on GC–chemical ionization mass spectral analysis at 309/311. The amounts were too small for detailed analysis, but it appeared likely from GC–FTIR (3460, 1690, 1600 cm^{-1}) that it was an *N*-hydroxyacyl derivative of epibatidine. EIMS (direct probe): 310, 308 (<1), 207 (25), 169 (40), 143 (5), 142 (8), 141 (10), 140 (20), 69 (100).

(14) In *Epipedobates tricolor*, the alkaloid **208/210** (epibatidine) occurs in variable amounts in different populations. It also has been found in one population of *Epipedobates anthonyi*, but not in any other populations of that frog, and at very low levels in *Epipedobates espinosai* and *Epipedobates pictus*. About 1 μg per frog occurred in certain populations of *Epipedobates tricolor* and in the one population of *Epipedobates anthonyi*, while lesser amounts occurred in other populations of *Epipedobates tricolor* and in populations of *Epipedobates espinosai* and the one population of *Epipedobates pictus*.

(15) Note that in *N*-isopropyl-*N*-methylacetamide (La Planche, L. A.; Rogers, M. T. *J. Am. Chem. Soc.* **1963**, *85*, 3728–3730) the $CH(CH_3)_2$ is deshielded in the rotamer having the isopropyl and carbonyl group in a cis (*Z*) relationship relative to the trans (*E*) rotamer ($\Delta\delta$ = 0.65 ppm), although the $CH(CH_3)_2$ hydrogens in the *Z* rotamer are slightly shielded with respect to those of the *E* rotamer ($\Delta\delta$ = 0.12 ppm). Model studies to confirm the deshielding of *N*-carbinyl hydrogens cis and in the plane of the carboxamido carbonyl (cf. H_1 and H_4 in **2**) are in progress. Until their completion, we regard our structural assignments for **2A** and **2B** as provisional only.

Scheme I



H_{1B} doublets disappeared simultaneously as did both H_{4A} and H_{4B} triplets on saturation of one of the doublets or one of the triplets. The signals of Table III were assigned to the A or B rotamer by intensity (A > B) and a 2D ^1H COSY correlation spectrum, which was used to assign the 4′ and 5′ signals and those from H_1, H_2, H_4, H_{3x} and H_{3n}.

Signals in the δ 7.2–8.3 region are assigned to a 6-chloropyridine substituted at the 3-position.[16] Only one structure was compatible with the remaining ^1H NMR signals and mass spectral fragmentations, and that structure necessitated a number of vicinal couplings of $J \cong 0$ Hz. This suggests a cyclohexane ring in the boat conformation for a portion of the structure, since it is well known that the coupling between bowsprit hydrogens and adjacent trans axial hydrogens is small or zero. This moiety is implicit in a 7-azabicyclo[2.2.1]heptane. It has been reported[17] in the parent heterobicyclic compound that no coupling at 100 MHz could be detected between bridgehead and adjacent endo hydrogens. The carbocyclic bicyclo[2.2.1]heptane system has received more study. Anet[18] has estimated from Barton models that the equivalent bridgehead–endo coupling in camphane 2,3-diols would be zero for a dihedral angle of 79°. Ramey et al.[19] reported small (0.5–1.1 Hz) bridgehead–endo couplings in a series of bridged bicyclo-[2.2.1]heptanes. We report in Table IV the calculated dihedral angles (ϕ) for **2** using computer modeling programs. These models also indicate that the *N*-Ac group is coplanar with C(1)–N–C(4). The methyl hydrogens of the acetyl group are calculated to be 2.7–4.0 Å from the center of the pyridine ring. The acetyl oxygen is 2.7 Å from H_1 or H_4. The chloropyridyl group must occupy the 2-exo position in the bicyclic compound for the signal of the 2-proton (endo) to show no coupling with the adjacent bridgehead hydrogen.

The electron-impact mass spectral fragmentation pathways in Scheme I are consistent with the structure deduced by ^1H NMR spectroscopy for epibatidine. Note that either the pyridyl or the azabicyclic moiety can carry the positive charge, although the latter is favored.

The potency of epibatidine relative to morphine in eliciting a Straub-tail reaction and in causing hot plate analgesia is remarkable, with epibatidine being many times more potent than morphine (Table II). It would appear that a non-opioid mechanism is involved, since naloxone, a general opioid antagonist, does not reverse the effects of epibatidine.[2] In addition, epibatidine has very low affinity for opioid receptors, since it is nearly 9000-fold less potent than morphine at such receptors (Table II). The structure of epibatidine is unique among analgetic agents, and structure–activity relationships for it and synthetic analogues in which the pyridyl and azabicycloheptyl moieties are varied will be necessary before any correlations to other analgetic structures are warranted. Synthesis of epibatidine and analogues is in progress.

Experimental Section

Instrumentation. Exact mass measurements were performed by Dr. Peter Roller of the National Cancer Institute (Bethesda, MD) using the photoplate technique with a JEOL 01SG-2 mass spectrometer. Initial GC–MS studies used a packed GC column. Recent studies have employed either a Finnigan 4500 mass spectrometer or a Finnigan Model 800 ion trap detector and fused silica capillary columns, the former with a 25 m × 0.25 mm OV-1 (Supelco) column and the latter with a 30 m × 0.20 mm HP-5 (Hewlett-Packard) column and a Hitachi gas chromatograph. Chemical ionization mass spectrometry utilized the former instrument with NH_3 or ND_3 reagent gases, the latter permitting the determination of exchangeable hydrogens. GC–FTIR spectra were obtained using a Hewlett-Packard Model 5965A instrument with a narrow band (4000–750 cm^{-1}) detector and a 59970 IRD ChemStation interfaced with a Hewlett-Packard Model 5890 gas chromatograph having a 30 m × 0.32 mm HP-5 fused silica capillary column. ^1H NMR spectra in $CDCl_3$ were measured with a Varian VXR-500S spectrometer.

Isolation of Epibatidine (1). An alkaloid fraction (60 mg) was prepared from extracts of 750 frogs of the dendrobatid species *Epipedobates tricolor*[1] and was subjected to chromatography on a prepacked silica gel 60 column (Merck 1.0 × 24 cm) with chloroform–methanol–aqueous ammonia (6 N) (500 mL of 800:10:0.1 followed by 1000 mL of 1000:100:0.2) as described.[1] Five-milliliter fractions were collected. Fractions 108–111 contained the bulk of the alkaloid **208/210** (epibatidine) that elicited the Straub-tail reaction in mice. The estimated recovery of Straub-tail equivalents from the column was about 40%. Fraction 108 in methanol was concentrated to 0.4 mL and further purified by HPLC on Partisil PXS 10/25 PAC with a solvent of acetonitrile–0.01 M $(NH_4)_2CO_3$ at 4 mL/min. Fractions of 0.5 mL were extracted with chloroform, and then the chloroform was dried over Na_2SO_4 and evaporated. Fraction 4 contained mainly alkaloid **208/210** on the basis of thin-layer and gas chromatographic analyses and was used for further spectral analysis and biological testing (see Table II). Fraction 3 also contained substantial amounts of alkaloid **208/210**, but contained in addition pumiliotoxins **267A** and **323B**.

Purification of Epibatidine (1) as *N*-Acetylepibatidine (2). Fraction 3 was evaporated to near dryness with nitrogen and then dissolved in 200 μL of EtOAc and extracted with three portions (200 μl) of 0.1 N HCl to separate the desired basic alkaloids from neutral contaminants. The combined aqueous extracts were made alkaline with saturated $NaHCO_3$ and re-extracted with several portions of 200 μL of EtOAc. These were combined, dried (Na_2SO_4), and evaporated under nitrogen. Two drops of acetic anhydride were added, and the solution was allowed to stand for 2 h at room temperature. Saturated $NaHCO_3$ was then added and the aqueous layer extracted with several portions of EtOAc. The EtOAc layer was extracted with three 200-μL portions of 0.1 N HCl to completely remove any contaminating basic tertiary amines, such as **267A** and **323B**, dried (Na_2SO_4), and evaporated to dryness with a nitrogen stream. GC–MS and GC–FTIR analyses revealed that the residue (*N*-acetylepibatidine (**2**)) from the EtOAc layer was virtually homogeneous. Accordingly, it was used directly for the ^1H NMR studies without further purification.

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(16) Chemical shifts ($CDCl_3$) for $H_{2'}$, $H_{4'}$, and $H_{5'}$ of **2** are within 0.06 ppm and *J* values are within 0.2 Hz of those reported for the aromatic hydrogens of 2-chloro-5-picoline, but deviate up to 0.27 ppm for the 3-chloro-6-picoline isomer. Other chloropicolines with a 1,2,4-disposition of hydrogens deviated much more in δ and *J* values from those observed for **2**. Busby, R. E.; Iqbal, M.; Khan, M. A.; Parrick, J.; Shaw, C. J. G. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1578–1582.

(17) Fraser, R. R.; Swingle, R. B. *Can. J. Chem.* **1970**, *48*, 2065–2074. Interestingly, the ^1H NMR spectrum of *exo*-2-chloro-7-(trichloroacetyl)-7-azabicyclo[2.2.1]heptane showed no discrete rotamer signals, although *N*-acetyl-7-azabicyclo[2.2.1]heptane does.

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