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A STRUCTURE FOR **261C**, A NOVEL TRICYCLIC ALKALOID FROM THE MADAGASCAN POISON FROG, *MANTELLA BETSILEO*

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Abstract-Based upon ^{1}H-NMR, MS and IR spectra, a perhydro 2-allyl-5-ethyl-7-*n*-propylpyrrolo[2,1,5-*cd*]indolizine structure **(1**) is proposed for a major tricyclic alkaloid **261C** isolated from skin extracts of a Madagascan poison frog of the mantellid genus *Mantella.*

Brightly colored poisonous frogs of the mantellid genus *Mantella* are found only in Madagascar. We published a survey in 1993 [1] of alkaloids in seven species and this included many of the pumiliotoxin and homopumiliotoxin classes, many bicyclics of the "izidine" class and several decahydroquinolines. Most have been reported in New World dendrobatid frogs although others, as yet, have been found only in the Madagascan frogs. One quinolizidine alkaloid, **217A**, was isolated in sufficient amounts for a complete structure determination by ^{1}H-NMR spectrometry. [2] We also published in 1996 [3] a tabulation of alkaloids present in skins extracts from various populations of nine *Mantella* species but characterization details of a number of previously unreported alkaloids were not presented.

Recent gas chromatographic-EI-MS spectral studies of skin extracts from a population of the Madagascan frog *Mantella betsileo* indicated the presence of significant amounts of a tricyclic alkaloid of molecular weight 261 with the following EIMS spectrum: m/z 261 (19%) with major fragments at 260 (27), 232 (77), 220 (64), 218 (100), *i.e.* ions consistent with losses of hydrogen, ethyl, allyl and propyl radicals from the molecular ion. The code designation **261C** had been assigned to this alkaloid, when it was first detected as a major alkaloid in skin extracts

of another mantellid frog, *Mantella expectata*. At that time it was designated as having an unclassified structure.[3] Both species containing **261C** were from near the Massif Isahlo Reserve in an arid region of southwestern Madagascar. An HRMS spectrum indicated a molecular formula $C_{18}H_{31}N$ and MS measurements of the above

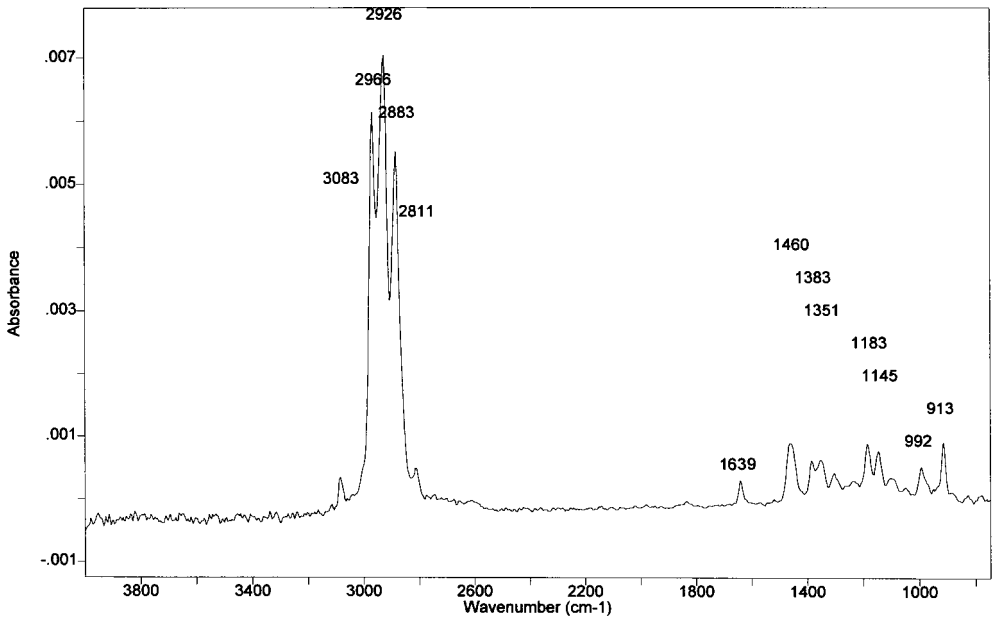
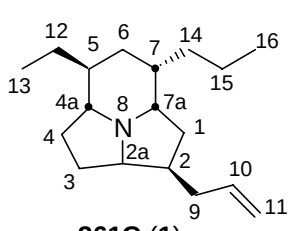
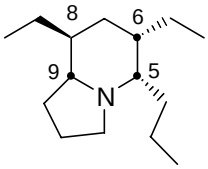


Fig. 1. GC-FTIR spectrum of **261C** (**1**).

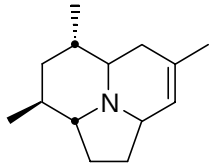
fragment ions were consistent with the proposed fragmetations (see EXPERIMENTAL). A CIMS spectrum with ammonia confirmed 261 as the molecular ion mass and a CIMS spectrum with ND_3 indicated no exchangeable hydrogens. A gas chromatography-Fourier transform infrared (GC-FTIR) spectrum (Figure 1) indicated the presence of a terminal olefin (913, 992, 1639, 3083 cm^{-1}) and only a very weak Bohlmann band was observed at 2811 $cm^{-1.}$ The molecular formula indicated four rings or unsaturations. One double bond was indicated by hydrogenation. A 2.8:1 mixture of two compounds of molecular weight 263 was detected by GC-MS spectral analysis after hydrogenation. Both compounds showed only fragmentations for loss of ethyl and propyl groups (EXPERIMENTAL). Consequently **261C** [1] has a tricyclic structure with ethyl, propyl and allyl substituents. A minor dihydro compound of mol. wt. 263 was present in the original extract where it had cochromatographed with **261C**. It is given a code designation of **263G**.



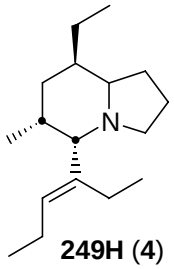
261C (**1**)



223A (**2**)



205B (**3**)



249H (**4**)

Approximately 0.9 mg of **261C** was purified from pooled skin extracts of a population of *M. betsileo* [4] using flash chromatography and HPLC. Various ^{1}H NMR experiments provided data shown in Table 1 and discussed below. Unfortunately the sample was contaminated by some 28 % of the naturally occurring dihydro congener assigned code designation **263G**, which could not be separated from **261C** under all HPLC conditions tried. Alkaloid **263G** must have a different stereochemistry than **261C** since it separated on GC-MS analysis from dihydro-**261C**. This contamination, as well as the overlapping of many signals, even at 800 MHz, made analysis difficult and the proposed structure (**1)** has some inherent ambiguities and must be regarded as provisional. An impurity triplet is seen at δ 1.09 overlapping the triplet assigned to the methyl of an *n*-propyl group. In addition, the multiplets at δ 2.27 and 1.89-1.92 contain impurity peaks. Any attempt to collect more of these frogs from their original habitat proved impossible, as spraying to eradicate locusts with insecticides had killed the frogs and presumably most of the insects upon which they were feeding. Stomach contents of certain species of *Mantella* frogs of Madagascar have been recently studied and are found to be comprised chiefly of small ants [5]. However, as yet no tricyclic alkaloids, similar to **261C**, have been described from Madagascan ants.

Table 1. NMR Data for 261C (1) as DCl salt.

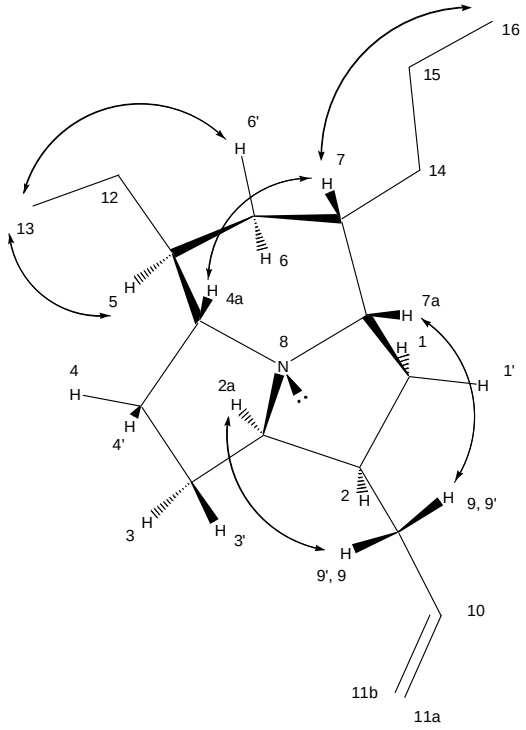
δ_C[a]	H[b]	δ_H[c]	Multiplicity (J, Hz)	Vicinal Hs dihedral angles (calcd, θ, °)[d]	Effect on neighboring H after decoupling irradiation at δ_H[e]
n.d.	1	1.74	dd (4, 14)	1-2 (30)	1' (L, td→dd), 2, 7a (M)
	1'	2.10	td (4.8, 14.4)	1'-2 (89)	1 (L), 2, 7a (L)
n.d.	2	2.27[f]	m	2-2a (43)	1 (S), 1' (M), 2a (S), 3 (S), 3' (M), 4a (M), 9, 9' (t→d); cross peaks[f] 1', 9, 9'
63.24	2a	3.99	ddd (5.7, 6.6, 11.4)	2a-3 (41), 2a-3' (163)	2, 3, 3' (L)
36.49	3	1.89	m	3-4 (10), 3-4' (109)	2a (M)
	3'	1.92	dq (4.4, 11.5-12.9)[e]	3'-4 (132), 3'-4' (14)	2a (L), 4, 4'
36.59	4	2.04	dq	4-4a (106)	4a (S), 4, 4'
	4'	2.29[f]	m	4'-4a (13)	1', 2a, 3, 3', 4a, 9, 9'; cross peaks[f] 3, 3', 4
65.17	4a	4.12	ddd (3.5, 7.4, 11.3)	4a-5 (145)	4, 4' (L), 5
36.59	5	2.22	m	5-6 (27), 5-6' (88)	1, 4a (S), 6 (td→t), 6' (S), 12, 12'
n.d.	6	1.66	dt (4.3, 14)	6-7 (178)	5 (S), 6' (L), 7 (L)
	6'	2.01	dd (2.4, 14.7)	6'-7 (68)	7a (M), 6 (2 L), 14, 14'
41.34	7	2.01	m (see 6')	7-7a (42)	7a (M), 6 (2 L), 14, 14'
60.63	7a	3.89	td (4.3, 13.4)	7a-1 (140), 7a-1' (20)	1 (M), 1' (L), 7 (M)
41.94	9, 9'	2.44	t (7.2)		2[g]
143.3	10	6.04	td (7.0, 17.0)		9[g], 11, 11'
123.4	11a	5.53	dd (1.6, 9.43)		10[g]
	11b	5.50	dd (1.6, 18.0)		10[g]
24.18	12	1.49	m		5
	12'	1.74	m		12, 13, 14, 14'
18.04	13	1.12	t (7.4)		12, 12' (S)
36.34	14	1.46	m (7.4)		5
	14'	1.49	m (7.4)		12'
39.0	15, 15'	1.46	m (7.4)		5[h], 7, 12, 12', 13, 14, 14', 16
17.48	16	1.08	t (7.5)		15, 15'

[a]Carbon frequencies (in ppm) were determined using an HMQC experiment.
[b]A primed hydrogen of a pair of methylene hydrogens is downfield of the unprimed hydrogen.
[c]Relative to HOD as internal standard at 4.78 ppm in a 500 MHz spectrum in D_2O.
[d]Calculated using Chem-3D (CambridgeSoft).
[e]L, M, S, indicate removal of large, medium or small *J*.
[f]Obtained from an 800 MHz 1D or H,H-COSY spectrum.
[g]Coupling determined from cross peak in 500 MHz H,H-COSY.
[h]Underlined signals are artifacts due to proximity of the irradiation to the 1.46 and 1.49 signals.
n.d. = not detected

An allyl group was clearly indicated in a 500 MHz spectrum of **1**·DCl in D_2O by two dd signals at δ 5.53 and 5.50 (one proton each) and a one-proton td signal at δ 6.04 all strongly coupled to one another in an H,H-COSY spectrum. These signals (H-10, H-11a, H-11b) were coupled in turn to a methylene triplet (H-9) at δ 2.44. The signals for the terminal protons (H-11a, H-11b) of the allyl moiety also indicated some contamination by another isomer, perhaps an allyl epimer (est. 10%). Three well-separated one-proton multiplets were observed at δ 4.12 (H-4a, ddd); 3.99 (H-2a, ddd) and 3.89 (H-7a, td), each showing two small or medium couplings and one large coupling. These were assigned to C*H*N hydrogens, each flanked by a methylene and one C*H*R group (R = ethyl, *n*-propyl or allyl, respectively). An HMQC experiment confirmed that each C*H*N signal was correlated with a single carbon only. An H,H-COSY spectrum indicated a similarity in the chemical shifts of the three hydrogens coupled to the H-2a and H-4a signals. Two methyl triplets were seen, clearly separated at δ 1.08 and 1.12 belonging to *n*-propyl and ethyl side chain methyls, respectively. The remaining ten carbons fit most reasonably with a structure having one six-membered ring (ring A) and two five-membered rings (B, C) with a tertiary nitrogen shared by the three rings. A combination of 1D and 2D NMR spectral studies permitted the assignment of all the protons, although it was necessary to rely on 1D decoupling experiments to more definitively assign some signals since 5 hydrogens overlapped (see Table 1). Note that the calculated dihedral angle between H-1' and H-2 is 89° so no cross peak would be expected and indeed none is seen in 2D spectra. An 800 MHz spectrum indicated better separations of H-2 and H-4' as well as H-3' and H-4. The latter two are seen in a 1D spectrum as dq signals, each having three large and one small coupling.

A NOESY spectrum unfortunately allowed only a few non-scalar steric interactions to be seen, chiefly the allylic methylene (H-9/9') with each of the C*H*N signals at δ 3.99 (calcd distance=3.73 Å) and 3.89 (d=3.01 Å). The latter stronger cross peak (H-9/9' with H-7a) may indicate the allyl group and H-7a to be on the same molecular face,

while the weaker cross peak (H-9/9' with H-2a) may indicate that H-2a lies on the opposite face. It does infer, but not conclusively, that the allyl group is attached to the pyrrolidine ring having those two hydrogens. Stronger evidence that the allyl group is at C-2 comes from observing that the signals assigned to H-1 (δ 1.74) and H-1' (δ 2.10) were coupled both to H-7a and H-2. No nOe cross peak was seen with-9/9' and H-4a. A weak nOe cross peak between H-4a and H-7 was also seen. Molecular modeling (see model **1 (3D)**) and an analysis of the coupling constants for the three C*H*N signals put H-4a and H-7a on the same face and H-2a on the opposite face of the molecule. Coupling constants (*J*) from the Karplus relationship for the three dihedral angles (θ) (determined from molecular modeling) of H-2a are: 6, 6 and 11.5 Hz; for those of H-4a: 1, 8 and 11 Hz and for H-7a: 5, 6 and 10 Hz. The calculated dihedral angles (θ) for H-2a and H-7a are in excellent agreement with the observed *J* values; those for H-4a are in fair agreement also (see Table 1).



1 (3D)

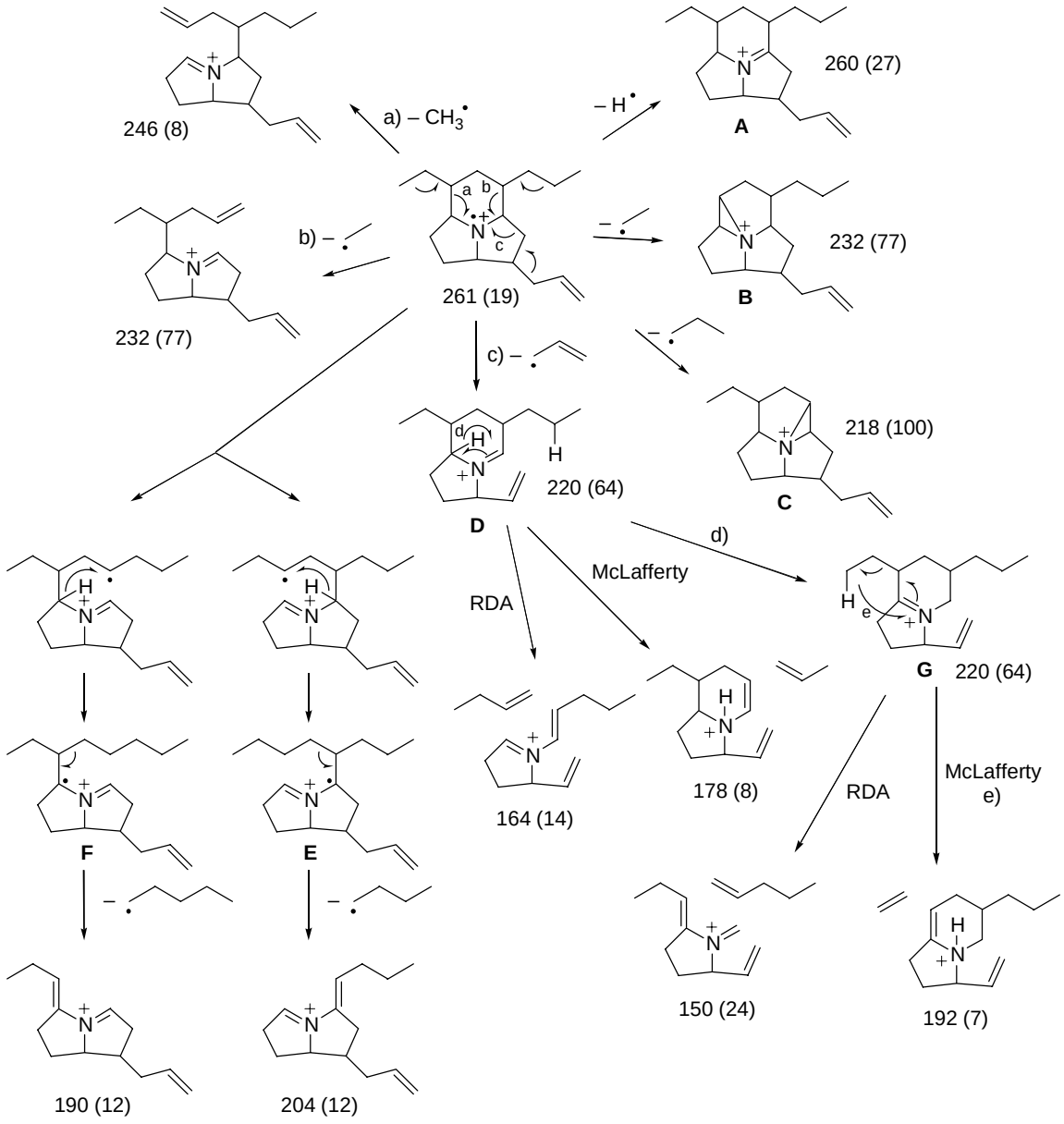
Two additional nOe crosspeaks were observed with the CH_3-13 triplet and the H-5 and H-6' protons (the latter cross peak is weaker), whereas CH_3-16 showed an nOe only with the H-7 (or H-6') signal, providing evidence that the *n*-propyl group is attached to the ring at the C-7 position and the ethyl group at the C-5 position. An analysis of the couplings of H-5 and H-6' indicates the ethyl group is on the same face as the H-4a and H-7a C*H*N protons, while

the *n*-propyl group is on the opposite face. The relative stereochemistry of the six chiral centers is then 2S*, 2aR*, 4aR*, 5S*, 7S*, 7aS*. The two saturated substituents are both on the six-membered ring, since it was observed that H-6 and H-6' are each coupled to H-7 and H-5, which were in turn coupled to the C*H*N signals H-7a and H-4a, respectively. Proton H-7 was also coupled to signals at δ 1.49, assigned to the side-chain *n*-propyl β-hydrogens (H-14, H-14'), and the δ 2.22 signal (H-5) was coupled with protons at δ 1.49 and 1.74 assigned to the diastereotopic ethyl methylene hydrogens (H-12, H-12'). In addition, the six-spin pattern for H-2a, H-3, H-3', H-4, H-4' and H-4a indicated that the ring with those protons had no attached alkyl substituent. A TOCSY spectrum indicated connectivity between H-2a, H-7a and H-9/9' and also H-7 and CH_3-16 as well as H-5 and CH_3-13. Additional TOCSY cross peaks are seen between H-2 and H-7a, H-2a and both H-4 protons, H-3' and H-4a, H-7 and H-15/15' and finally H-7 and H-16.

It will also be noted in **1** that the following methylene hydrogens, 1', 3', 4' and 6' are downfield of their geminal partners and on the same face where they all experience the deshielding effect of the N-lone pair. [6] Molecular modeling (see **1-(3D)**) indicates the six-membered ring (A) is in a skewed boat conformation with a pseudo-axial ethyl (note the expected upfield ^{13}C-NMR signal for C-12, Table 1) and a pseudo equatorial *n*-propyl substitutent. The five-membered rings (B-C), being in a *trans*-fused pyrrrolizidine structure, are almost planar and form an angle of approximately 90º relative to the 6-membered ring. The N-lone pair is on the same face as H-4a and H-7a and **261C** would be expected to have weak or absent Bohlmann bands in its FTIR spectrum as observed. Calculated dihedral angles between those hydrogens and the N-lone pair are 47 and 18º, respectively. Only one hydrogen, H-2a, has close to a *trans*-anti-parallel orientation (calculated $\theta_{2a\text{-}N:} = 174^{o}$). Thus, the minimum requirement of two such hydrogens before Bohmann bands can be observed is not met. We recently reported [7] the structure of an unusual *cis*-fused indolizidine **249H** (**4)** that had a very weak Bohlmann band pattern in its vapor phase FTIR spectrum, a weak pattern nearly identical to that seen with **261C**. Either of the indolizidine substructures in **1** (rings A-B or A-C) will have the N-lone pair and either H-4a or H-7a on the same face, *i.e*. both comprise similar *cis*-fused indolizidines.

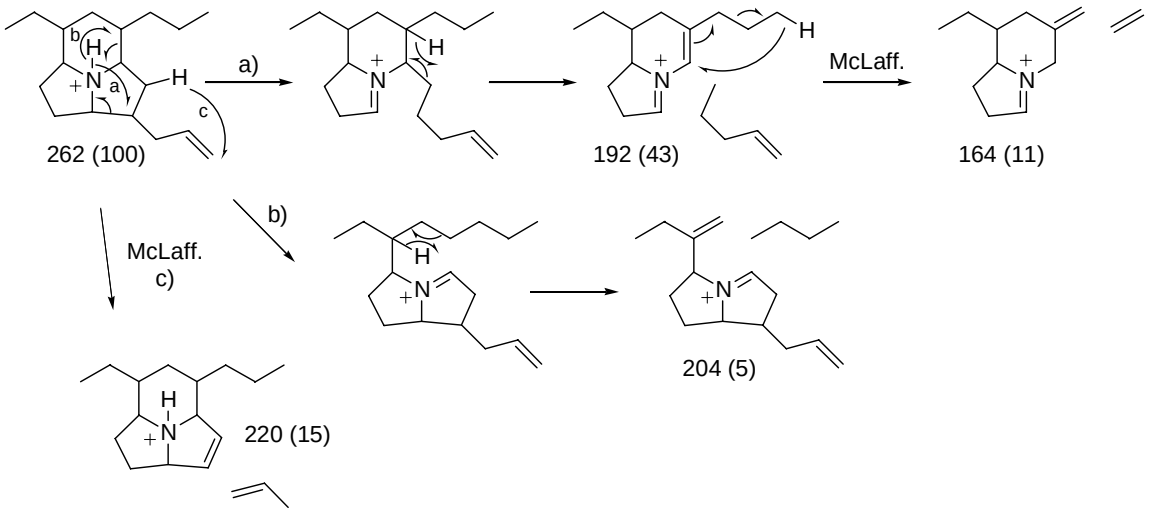
Fragmentation pathways rationalizing the electron impact mass spectrum of **261C** are presented in Scheme 1. The parent radical ion is postulated to lose a hydrogen to yield ion **A** or ethyl or propyl radicals to yield the aziridinium ions **B** and **C**. A concerted fragmentation (pathway c) with loss of an allyl radical would produce the m/z 220 fragment **D** which can undergo a retro-Diels-Alder (RDA) process to produce the m/z 164 ion or a McLafferty

rearrangement to produce the m/z 178 ion. An EI-MS/MS spectrum on the m/z 260 ion, such as represented by **A,** did not show ions at m/z 204 or m/z 190. This tends to rule out an otherwise attractive RDA process yielding directly butene and an m/z 204 ion as well as a similar process whereby an isomer of **A** might produce pentene and an m/z 190 ion. Instead we postulate that these ions are derived from the parent molecular ion by α-cleavages and 1,4 shifts of hydrogen followed by β-elimination from ions **E** and **F**. The parent radical ion can fragment (pathways a, b) with loss of methyl or ethyl radicals to produce the m/z 246 and 232 ions, respectively. Another rearrangement (pathway d) from fragment **D** might yield a second m/z 220 ion **G**, which in turn could produce ions at m/z 150 and m/z 192 by RDA or McLafferty (e) rearrangements, respectively.



Scheme 1. Proposed EIMS fragmentations for **261C** (**1**).

A CI-MS/MS spectrum with ammonia reagent gas and helium gas collision-induced decomposition on protonated **1** (m/z 262) gave m/z 220, 204, 192, 164 as major ions, evidently by cleavage yielding neutral saturated or unsaturated fragments (Scheme 2).



Scheme 2. Proposed CI-MS/MS fragmentations for protonated **261C** (**1**).

We propose two 1,4-H transfers (pathway a) and the cleavage of 1-pentene to give the major m/z 192 ion observed. A 1,6 hydrogen shift of the McLafferty-type would generate the m/z 164 ion and ethylene. The butane loss is rationalized by pathway b and a similar mechanism (not shown) with rupture of the 7-7a bond and extrusion of carbons 6, 7, 14-16 would yield pentane and m/z 190 (weak). A 1,6-hydrogen shift in another McLafferty-type rearrangement (pathway c) would yield propene and the m/z 220 ion. Supporting mechanisms a and c is the observation that CI-MS/MS on either dihydro diastereomer of **1** (see EXPERIMENTAL) no longer gives any significant pentene or propene losses indicating the allyl group has to be present for these losses to occur. Another technique to accomplish CI-MS/MS used the Finnigan LCQ in the APCI mode. MS/MS on the 262 ion with energetic helium (only ca. 5% $(M+H)^+$ remains unfragmented) gave 192 as the base peak.

The structure (**1)** which we propose for **261C** is compatible with all the data we have so far compiled. Such a structure evidently has not been reported to occur in Nature. It might be derived biosynthetically from an additional ring closure from a 5,6,8-trisubstituted indolizidine related to **223A** (**2**). The structure shown for **223A**, as recently revised [8], shares with **1** the same relative configuration at all centers. The C-5 substituent in such a putative indolizidine precursor might be a penta-2,4-diene; such substitutents are often seen in frog skin alkaloids of the izidine, decahydroquinoline and histrionicotoxin classes. [9]

Presumably, **261C** and the structurally related coccinellines and **205B** (**3**) are sequestered into frog skin from dietary beetles. [10] A synthesis of the parent ring system of **261C** has been reported [11] and monomeric, dimeric and trimeric structures containing that 6, 5, 5 system have been reported in an African ant of the genus *Myrmicaria*. [12] Structure **1** proposed for **261C** has no side-chain substituents α to nitrogen. Nevertheless major cleavages of ring substituents in positions β or γ to nitrogens are still possible as seen in the facile loss of methyl from alkaloid **205B,** isolated from the frog *Dendrobates pumilio* and characterized as the tricyclic structure **3** [13]. Saunders and Williams [14] report 59 and 44% losses of methyl substitutents from 3- and 4-methylpiperidines, respectively. Hwang and Fowler report a 55% loss of methyl from a 1,7-dimethylquinolizidine [15], an alkaloid that likewise has no α-methyl substituents.

EXPERIMENTAL

Instrumentation: A Hewlett-Packard model 5890 gas chromatograph having a 25 m × 0.32 mm i.d. HP-5 fused silica-bonded capillary column programmed from 100° to 280° at the rate of 10°/ min, interfaced with a Hewlett-Packard model 5971 Mass Selective Detector and a Hewlett-Packard model 5965B IR instrument with a narrow band (4000-750 cm^{-1}) detector and a Hewlett-Packard ChemStation (DOS based) were used to generate the chromatograms, EIMS, and FTIR spectra of **261C**. A Finnigan 4500 mass spectrometer with a 25 m × 0.25 mm i.d. OV-17 fused silica-bonded column (Supelco) with a 60°-280° program (10°/min or 5°/min) and an INCOS data system was also used to obtain EIMS. High resolution mass spectrometry peaks (HRMS) were measured with a JEOL SX 102 instrument fitted with a 15 m × 0.20 mm i.d. HP-5 column. EI-MS/MS, CIMS and CI-MS/MS studies used a Finnigan GCQ instrument fitted with a Restek RTX-5MS column (30 m, 0.25 mm i.d). CIMS and CI-MS/MS used NH_3 reagent gas. A Finnigan-Thermoquest LCQ mass spectrometer in the APCI mode interfaced with a Hewlett-Packard model 1100 LC and a Phenomenex 25cm x 4.6 mm "Aqua" C-18 column was used to demonstrate homogeneity of **261C** and obtain the MS-MS spectrum of the protonated molecular ion. Deuterium exchange CIMS used ND_3. The 1D- or 2D-(COSY) ^{1}H-NMR spectra in D_2O were measured with a Varian VXR-500S spectrometer (Table 1).

Isolation:

Mantella betsileo: Acid-base partitioning of a methanol extract (see ref. 1) from 43 frogs of *M. betsileo* (collected December 1997 into January 1998 near Massif Isahlo Reserve of Madagascar; est. 3.5 g wet weight of skins) and

evaporation of solvent gave 3.0 mg of an alkaloid mixture. Almost one half of this mixture (1.5 mg), chiefly **261C,** was purified by flash chromatography using a Flash Elute LLC (Elution Solutions) and a 50 mm x 1.5 mm column of 40 μm silica gel eluted with $CHCl_3$-CH_3OH (40:1). Fractions of 4 mL were collected. Alkaloid **261C** (0.9 mg) appeared in fractions 15-24 and was used directly for the NMR experiments. The *M. betsileo* extract contained, in addition to **261C** as the major alkaloid, minor amounts of the following 5,8-disubstituted (disubst.) indolizidines: **195I**, **217B**, **223J.** It also contained minor amounts of a previously undetected 5,6,8-trisubstituted (trisubst.) indolizidine to be designated **195K**, a 1,4 disubst. quinolizidine **207I** and a pumiliotoxin **307A**. Trace alkaloids were the following: A 5,8-disubst. indolizidine **193A**, an unclassified **211D**, a 2,5-disubst. pyrrolidine **225B**, a 3,5-disubst. pyrrolizidine **251K**, pumiliotoxins **251D**, **277B** and either **305D** or **307H** (CIMS is ambiguous), **263G** (a dihydro congener of **261C)**, a tricyclic **259D** alkaloid, possibly a dehydro-**261C**, and two tricyclic alkaloids, **277F** and **277G**, probably hydroxylated analogs of **261C**.

<u>*M. expectata*</u>: Another mantellid species from Massif Isahlo, Madagascar, also contained **261C** but in lesser amounts. It contained mainly pumiliotoxin **251D**, with a 1,4-disubst. quinolizidine **207I** and a 5,8-disubst. indolizidine **219F** in amounts similar to **261C.** Minor alkaloids were a 5,8-disubst. indolizidine **193A**, pumiliotoxins **237A**, **307A** and an unclassified **323D** alkaloid, $C_{19}H_{33}NO_3$, that shows on EIMS a major loss of C_6H_{13} giving an m/z 238 ion. Trace alkaloids were pumiliotoxins **267C** (2 isomers) and **305B**, allopumiliotoxin **323B**, 3,5-disubst. indolizidines **223AB** and **275C**, 5,8-disubst. indolizidines **197C**, **217B**, **219J** and **241F**, 4,6-disubst. quinolizidine **195C** and two unclassified alkaloids **233I** and **235O** (see ref. 9 for code designations and structures of known frog skin alkaloids).

***Catalytic Hydrogenation*:** Small amounts of the remaining crude alkaloid fraction from *M. betsileo* were used for MS and a catalytic hydrogenation. The latter was performed in methanol using an electrolytic hydrogen generator (Whatman, 2 atm.) for 20 min with rapid stirring in a 1 mL vial using traces of a 5% Pd/C (Degussa) catalyst. After filtration, the filtrate by GC-MS showed two diastereomers with protonated molecular ions of m/z 264 (CIMS (NH_3)): 2.75 parts (retention time=10.16 min.):1 part (rt=11.22 min.). The isomer of longer retention time cochromatographed with **261C** on silica, GC and HPLC columns.

***Characterization of* 1**: NMR, (see Table 1)

EIMS: m/z 261 (19), 260 (27), 246 (8), 232 (77), 220 (64), 218 (100), 204 (12), 192 (7), 190 (12), 178 (8), 164 (14), 162 (8), 150 (24).

CI-MS/MS on m/z 262 (100): m/z 220 (26), 204 (9), 192 (47), 190 (5), 164 (13), 136 (7).

CI-MS/MS with the LCQ in APCI mode: m/z 262 (50), 220 (45), 204 (14), 192 (100), 164 (7).

EI-MS/MS on m/z 260 (100): m/z 242, 214, 188, 186, 178, 174, 160, 147, 134 (intensities all < 15%)

EI-MS/MS on m/z 232 (100): m/z 202, 190, 188, 176, 174, 162 (15), 164, 150 (unless indicated, intensities are < 15%).

EI-MS/MS on m/z 220 (100): m/z 192(10), 190 (20), 178, 176, 164(40), 162 (80), 150, 135 (unless indicated, intensities are < 8%).

EI-MS/MS on m/z 218 (100): m/z 190 (17), 189 (82), 174 (10), 162 (25), 160 (82).

HRMS: calcd for $C_{18}H_{31}N$, 261.2452 a.m.u., observed, 261.2457; calcd for $C_{16}H_{26}N$, 232.2075, observed, 232.2065; calcd for $C_{15}H_{26}N$, 220.2052, observed, 220.2065; calcd for $C_{15}H_{24}N$, 218.1903, observed, 218.1909; calcd for $C_{10}H_{16}N$, 150.1278, observed, 150.1283.

Vapor-phase FTIR spectrum, (see Figure 1).

***Characterization of Analogues of* 1** (from *M. betsileo):*

259D: EIMS: m/z 259 (27), 244 (100), 230 (13), 216 (22), 202 (11), 188 (10), 174 (5), 160 (5), 100 (2), 58 (2). Possibly a dehydro-**261C**.

277G (isomer of shorter GC rt): EIMS: m/z 277 (10), 276 (8), 248 (50), 236 (82), 234 (100), 220 (10), 219 (10), 218 (12), 206 (14), 192 (27), 178 (47), 166 (13), 164 (17), 150 (13), 148 (16), 136 (10), 122 (10), 108 (7). Possibly a hydroxy-**261C**.

277H (isomer of longer GC rt): EIMS: m/z 277 (8), 276 (6), 262 (3), 248 (48), 236 (26), 234 (100), 220 (9), 218 (13), 206 (41), 204 (37), 193 (94), 176 (48), 166 (47), 153 (67), 150 (26), 136 (17), 134 (15), 122 (14), 110 (28), 96 (18), 70 (64). Possibly a hydroxy-**261C**.

Dihydro-1 (the unnatural diastereomer observed after reduction of **261C**): EIMS: m/z 263 (47), 262 (100), 248 (10), 235 (22), 234 (93), 221 (13), 220 (53), 207 (20), 206 (47), 193 (27), 192 (72), 179 (36), 178 (32), 165 (35), 164 (54), 150 (48), 136 (18), 122(18), 110 (8).

CI-MS/MS on m/z 264 (100): 248, 234 (24), 220 (28), 206 (37), 194, 192 (57), 180, 178 (29), 164 (40), 152 (38), 150 (21), 138, 136 (17), 122 (15), 108, 96 (unless indicated, intensity < 15%).

GC-FTIR: Similar to Figure 1 except missing 3083, 1639, 992, 914 cm^{-1}. Weak Bohlmann band at 2811 cm^{-1} is unchanged.

263G (a minor diastereomer of dihydro **261C,** present naturally): EIMS: m/z 263 (40), 262 (77), 234 (78), 220 (63), 206 (24), 199 (27), 192 (63), 185 (33), 164 (52), 157 (33), 150 (100), 143 (55), 135 (21), 129 (33), 122 (21), 115 (21), 101 (33). Likely admixed with traces of unreduced **261C** (m/z 248 is not reported above) and with methyl palmitate as indicated by 270, 87 and 74 ions present.

CI-MS/MS on m/z 264 (100): 248, 236, 234 (22), 220 (13), 206 (52), 192 (32), 180, 178, 166, 164 (28), 152 (12), 150 (15), 138, 136 (10), 124 (13), 122 (20), 108 (25), 95 (20), 81 (14) (unless indicated, intensity < 10%).

EI-MS/MS on m/z 262 (100): m/z 233 (9), 206 (20), 204 (18), 192 (35), 190 (5).

EI-MS/MS on m/z 234 (100): m/z 206, 204, 192, 190, 178, 176, 164 (47), 162 (24), 150 (36), 148 (unless indicated, all intensities < 17%).

EI-MS/MS on m/z 220 (100): m/z 218, 192, 190, 178, 176 (20), 164 (50), 162 (36), 160, 150 (33) (unless indicated, all intensities < 17%).

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