Uoamines A and B, Piperidine Alkaloids from the Ascidian *Aplidium uouo*

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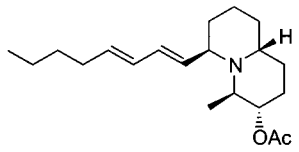
A specimen of the ascidian *Aplidium uouo* from Maui contained two piperidine alkaloids, uoamines A and B, that differed only in the geometry of a 3-thiomethylacrylate ester group. The alkaloids exhibit conformational mobility in the NMR time frame, which complicated the elucidation of their structures by interpretation of spectroscopic data.

Alkaloids based on a 2-amino-3-hydroxyoctadecane moiety, sometimes referred to as lysine-derived metabolites,[1] have been found in several genera of ascidians.[2] The bi- and tricyclic members of this family, exemplified by pictamine (**1**),[2] are most often found in *Clavelina* spp.,[3,4] while acyclic and monocyclic examples are metabolites of *Pseudodistoma* spp.[5] In this paper we describe the isolation from *Aplidium uouo* and structural elucidation of uomines A (**2**) and B (**3**), which are monocyclic piperidine alkaloids in which the 3-hydroxyl group is esterified by (*E*)- or (*Z*)-3-thiomethylacrylic acid, respectively.



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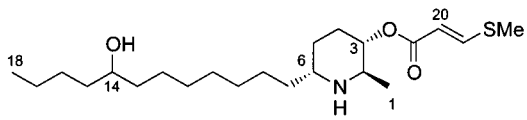

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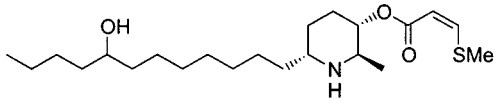

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Specimens of the ascidian *Aplidium uouo*[6] (collection # 97-204) were collected by hand using scuba at Hawea Point, Maui, in October 1997. The methanol-soluble material from the lyophilized specimen (21.5 g dry wt) was defatted and desalted using centrifugal countercurrent chromatography (CCCC). Acid/base partition gave a crude alkaloid fraction (23 mg), a portion (13 mg) of which was further purified by CCCC to obtain samples of uoamine A (**2**, 7.6 mg, 0.035% dry wt) and uoamine B (**3**, 3.1 mg, 0.014% dry wt).

Uoamine A (**2**), $[\alpha]_D$ +5° (*c* 0.16, $CHCl_3$), was obtained as a colorless oil. The molecular formula, $C_{22}H_{41}NO_3S$, was deduced from the $[M + H]^+$ peak at *m/z* 400.2883 (Δ −0.3 mmu). The IR spectrum contained bands at 3400 (OH, NH) and 1705 cm^{-1}, the latter being assigned to an α,β-unsaturated ester. On examination of the ^1H and ^{13}C NMR spectra, it was immediately obvious that the spectra were abnormal, with key signals being much broader than expected. We obtained the sharpest ^1H NMR spectra at 500 MHz and 55 °C, but under those conditions, key signals in the ^{13}C NMR spectrum (125 MHz) were slightly broader than those recorded at 100 MHz and 25 °C and occurred at slightly different chemical shift values. To our surprise, the best inverse-detected spectra (HSQC, HMBC, HSQC-TOCSY) were obtained at 300 MHz. The conformational mobility implied by these data made it difficult to define the stereochemistry of the molecule using standard methods.

The (*E*)-3-thiomethylacrylate ester was assigned using a combination of spectral data. The IR band at 1705 cm^{-1} and UV absorption at 275 nm are in good agreement with literature values.[7] The HMBC experiment showed a correlation from the thiomethyl signal (Me-22) at δ 2.23 to the olefinic carbon signal at δ 147.0 (C-21), which was correlated to the H-21 signal at 7.84 in the HSQC spectrum. Further HMBC correlations from the H-21 proton signal to signals at δ 113.5 (C-20) and 164.7 (C-19) completed the assignment. The $J_{20,21}$ = 15 Hz coupling constant and the ROESY correlation from Me-22 to H-20 defined the *E*-geometry. The C-19 signal showed a further HMBC correlation to a signal at δ 4.96 (δ_C 72.2, C-3), which was coupled to a broad signal at 3.35 (δ_C 48.7, C-2), which was in turn coupled to a methyl signal at 1.18 (δ_C 15.4, C-1): these data allowed a nitrogen to be placed at C-2. HMBC correlations between H-2 and C-6 (δ 49.6), H-2 and C-4 (δ 24.8), H-6 (δ 2.97) and C-2, and H-6 and C-4 required the presence of a piperidine ring. The remainder of the molecule must therefore consist of a 12-carbon *n*-alkyl chain containing an alcohol group. The position of the hydroxyl group was reliably defined by a combination of HMBC and carbon chemical shift data. The terminal methyl signal at δ 0.91 (Me-18) showed correlations to C-17 (δ 23.1) and C-16 (δ 28.2), the chemical shift of which is shifted upfield from that expected in a normal alkyl chain (ca. 32 ppm) by the γ-effect of the hydroxyl group. This assignment was confirmed by the observation of a HMBC correlation from the H-14 signal at δ 3.58 to C-16.

The relative stereochemistry about the piperidine ring was determined by careful interpretation of the ROESY data, while taking into account the conformational mobility indicated by the broadening of signals in the NMR spectra. The key correlations are from Me-1 to H-3 and H-6, which requires that all three are on the same face of the piperidine ring. With the piperidine ring in a chair conformation, this would imply that the alkyl chain is equatorial, while both the methyl group and the ester are axial. Molecular modeling indicates that in this conformation the ester group is located under the piperidine ring so that C-21, which gives rise to a broadened ^{13}C NMR signal, approaches the piperidine nitrogen. The H-21 signal is

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broadened but also undergoes slow deuterium exchange, possibly due to transfer of deuterium from the ring nitrogen, during NMR studies at 55 °C in $CDCl_3$. We realize that this analysis is quite speculative because we have been unable to determine the exact nature of the conformational mobility, but it is worth noting that there is a weak ROESY correlation from the H-2 signal to the alkyl chain signal at δ 1.29, which might be expected in the alternate chair conformation of the piperidine ring in which the alkyl group is axial and both the methyl and ester groups are equatorial. Due to the conformational mobility, we have made no attempt to determine the relative configuration at C-14 because this requires that the absolute configurations of C-14 and the piperidine ring be determined independently.

Uoamine B (**3**), $[\alpha]_D$ +8° (*c* 0.15, $CHCl_3$), is an isomer of uoamine A, in which the 3-thiomethylacrylate ester has the *Z*-configuration. The IR spectrum contained bands at 3430 and 1700 cm^{-1}, while the UV spectrum revealed an absorption at 283 nm. In the ^1H NMR spectrum, the signals at δ 7.09 (d, 1 H, J = 10 Hz), 6.02 (d, 1 H, J = 10 Hz), and 2.40 (s, 3 H) are clearly indicative of a (*Z*)-3-thiomethylacrylate. The corresponding ^{13}C NMR signals are at δ 19.4 (C-22), 112.6 (C-20), 153.2 (C-21), and 165.0 (C-19). All other signals in the ^1H and ^{13}C NMR spectra are shifted very slightly with respect to the comparable signals in the spectra of uoamine A. Once again, the ^1H NMR signals associated with the piperidine ring are broadened due to conformational mobility, but the ^{13}C NMR signals are relatively sharp. Examination of the ^1H NMR spectra of uoamine B (**3**) recorded over a period of about 6 weeks revealed a very slow isomerization to uoamine A (**2**). The reverse isomerization was not observed.

Experimental Section

General Experimental Procedures. IR and UV spectra were recorded on Perkin-Elmer 1600 FT-IR and Bio 20 spectrophotometers, respectively. ^1H, COSY, HMBC, HMQC, GHSQC-TOCSY, and ROESY NMR spectra were measured on Varian Unity 500 MHz and Inova 300 MHz spectrometers at 25 and 55 °C. ^{13}C and DEPT spectra were measured on a Varian Gemini 400 MHz spectrometer. Optical rotations were measured on a Rudolf Autopol III polarimeter (*c* g/100 mL) at 589 nm. High-resolution FABMS data were obtained on a VG ZAB mass spectrometer at the U. C. Riverside Regional Facility. All solvents were distilled prior to use.

Animal Material. Specimens of *Aplidium uouo* Monniot and Monniot 1987 (Polyclididae) were collected by hand using scuba at Hawea Point, Maui, in 1997 and were kept frozen until extraction. The voucher specimen was identified by Françoise Monniot, who had previously described this ascidian from French Polynesia.[6]

Extraction and Isolation. The lyophilized ascidian (21.5 g dry wt) was diced and extracted with methanol (3 × 300 mL). The combined MeOH extracts were evaporated to obtain an oil (600 mg) that was fractionated by using centrifugal countercurrent chromatography using a 2:3:3:2 H_2O−MeOH−EtOAc−hexane solvent system with the aqueous phase as the mobile phase. The stationary phase was evaporated to obtain an oil (180 mg). The oil was partitioned between 1 M KOH and Et_2O (3 × 150 mL), and the combined organic fractions were partitioned against 1 M HCl (3 × 150 mL). The aqueous phase was made basic with 6 M KOH and extracted with an equal volume of Et_2O, which was dried over Na_2SO_4, and the solvent was evaporated to obtain a 7:3 mixture (23 mg, 0.107% dry wt) of uoamines A (**2**) and B (**3**). The isomers could by separated by submitting a portion (13 mg) of the mixture to CCCC using 1:3:3:3 H_2O−MeOH−EtOAc−hexane solvent system, adjusted to pH 8 prior to degassing, with the organic phase as the mobile to obtain pure uoamines A (3.5 mg, 0.033% dry wt) and B (1.5 mg, 0.014% dry wt) and a mixed fraction (8 mg, 7:3 ratio) that could be recycled.

Table 1. ^{13}C (100 MHz, $CDCl_3$) and ^1H (500 MHz, $CDCl_3$) NMR Data for Uoamide A (**2**)

C no.	δ_C	δ_H	mult, *J*(Hz)	HMBC	ROESY
1	15.4[a]	1.18	d, 3 H, 7	C-2, C-3	H-2, H-3, H-6
2	48.7[b]	3.35	qd, 7, 4	C-1, C-3, C-4, C-6	H-1, H-3
3	72.2[a]	4.96	ddd, 8, 4, 3.5	C-1, C-2, C-4, C-5, C-19	H-1, H-2, H-4
4	24.8[a]	~1.78	m		
5	28.2[a]	~1.30	m		
6	49.6[a]	2.97	m	C-2, C-4, C-8	H-1
7	34.4[a]	1.29	br s		
8	26.9[b]	1.29	br s		
9	29.9	1.29	br s		
10	29.9	1.29	br s		
11	30.0	1.29	br s		
12	25.9	1.29	br s		
13	37.8	1.40	m		
14	72.2	3.58	m	C-16	
15	37.5	1.40	m		
16	28.2	1.29	br s		
17	23.1	1.29	br s		
18	14.4	0.91	t, 3 H, 7	C-17, C-16	
19	164.7				
20	113.5	5.72	d, 15	C-19, C-21	H-22
21	147.0[a]	7.84	d, 15	C-19, C-20, C-22	
22	14.7	2.33	s, 3 H	C-21	H-20
NH		1.80	br s		

[a] Broader and shifted at 125 MHz. [b] Slightly broader at 125 MHz.

Uoamine A (2): colorless oil; $[\alpha]_D$ +5.0 (*c* 0.16, $CHCl_3$); UV (95% EtOH) λ_{max} 275 nm (ϵ 10 740); IR (film) 3390, 2930, 1705, 1580, 1465, 1255 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table; HRFABMS obsd *m/z* 400.2883 $(M + H)^+$, $C_{22}H_{42}NO_3S$ requires 400.2885.

Uoamine B (3): colorless oil; $[\alpha]_D$ +8.0 (*c* 0.15, $CHCl_3$); UV (95% EtOH) λ_{max} 283 nm (ϵ 1372); IR (film) 3430, 2950, 1700, 1580, 1455, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, H-18), 1.25 (d, 3 H, J = 7 Hz, H-1), 2.40 (s, 3 H, H-22), 3.25 (br m, 1 H, H-6), 3.47 (br s, 1 H, H-2), 3.57 (m, 1 H, H-16), 5.06 (br s, 1 H, H-3), 6.02 (d, 1 H, J = 10 Hz, H-20), 7.09 (d, 1 H, J = 10 Hz, H-21); ^{13}C NMR ($CDCl_3$) δ 13.7 (C-18), 14.2 (C-1), 19.4 (C-22), 22.9 (C-4, C-17), 25.7 (2C), 26.3, 28.0 (C-16), 29.0, 29.2, 29.4, 29.6, 37.3 (C-15), 37.5 (C-13), 49.2 (C-2), 51.9 (C-6), 67.6 (C-3), 71.9 (C-14), 112.6 (C-20), 153.2 (C-21), 165.0 (C-19); HRMALDIMS obsd *m/z* 400.2886 $(M + H)^+$, $C_{22}H_{42}NO_3S$ requires 400.2885.

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Supporting Information Available: Copies of 300 and 500 MHz ^1H NMR spectra, 125 and 100 MHz ^{13}C NMR spectra, gHSQC, HMBC, HSQC-TOCSY, and ROESY spectra of uoamine A, and ^1H and ^{13}C NMR spectra of uoamine B. This material is available free of charge via the Internet at http://pubs.acs.org.

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