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Three New Alkaloids from the Marine Tunicate *Cystodytes violatinctus*

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Abstract: Three novel alkaloids, shermilamine D and E (**1** and **2**) and tintamine (**3**), two pyridoacridines (**1** and **2**), and one alkaloid possessing a new heterocyclic system have been isolated from the Indian Ocean tunicate *Cystodytes violatinctus* collected at the Mayotte Lagoon, Comoros Islands, northwest of Madagascar. The structures of all compounds were elucidated on the basis of spectroscopic data, mainly 1D and 2D NMR data.

Unique antiviral and antitumor bioactive alkaloids from tunicates have recently attracted considerable attention to these sessile marine invertebrates.[1,2] The marine tunicate family Polycitoridae is well-known for its contents of a variety of alkaloids from different groups.[3] Several years ago, we isolated debromoshermilamine (shermilamine B) from a *Eudistoma* sp., family Polycitoridae, from the Red Sea,[4] also reported by Scheuer from a *Tridiemnum* sp.,[5] and five other new alkaloids, i.e., the segolins and eilatin—all novel pyridoacridines.[6,7]

A whole variety of other pyridoacridines have also been isolated from the genera *cystodytes*, of the same family.[3]

In the course of a survey of chemical constituents of marine organisms from the lagoon of Mayotte, Comoros Islands, northwest of Madagascar, we have investigated the purple tunicate *Cystodytes violatinctus*, of the family Polycitoridae (Monniot 1988). From this organism, we have isolated six compounds, i.e., the three earlier reported segolins[4] and three new alkaloids, compounds **1**−**3**, designated shermilamine D and E and tintamine. While shermilamine D and E are pyridoacridines, tintamine possesses a new unprecedented skeleton, i.e., tropono-1,2-dihydro-3,6-phenantroline. The six compounds were isolated from the MeOH−$CHCl_3$ extract by intensive chromatographies.

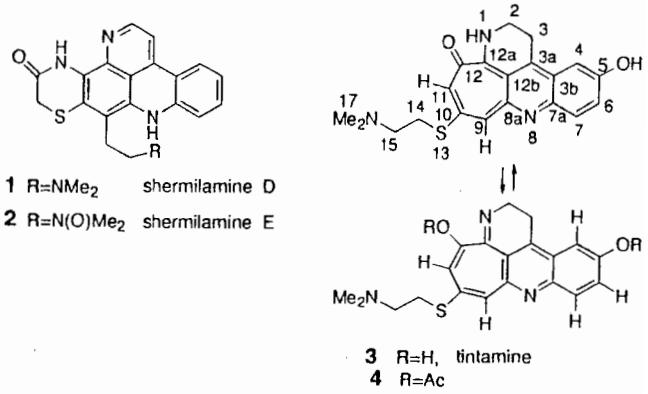

Figure 1.

The major alkaloid, shermilamine D (**1**), obtained as an amorphous orange powder, analyzed for $C_{21}H_{20}N_4OS$ by HREIMS, *m/z* 376.1356 [M^+, Δmmu = +0.1] and ^{13}C NMR data, implying 14 degrees of unsaturation. Compound **1** inhibited division of fertilized sea urchin eggs at a concentration of 0.063 mg/mL.[8] Characteristic, in the spectral data of **1** (see the Experimental Section), were an amide group (δ_H 9.05 brs, NH; δ_C 163.5s and ν 1623 cm^{-1}), two coupled pyridine protons [δ 8.40 (d, J = 4.8 Hz) and 7.20 (d, J = 4.8 Hz)], a 1,2-disubtituted benzene ring [δ 7.80 (brd, J = 7.9 Hz), 6.98 (brt, J = 7.5 Hz), 7.33 (brt, J = 7.5 Hz), and 6.84 (brd, J = 8.0 Hz)], and a downfield resonating isolated methylene group (δ 3.50 brs, 2H). CH cross-peaks (Experimental Section, HMBC) and comparison of the NMR data of 1 with literature values[4,5] pointed clearly to a pyridoacridine, namely, the pentacyclic ring system of the shermilamines.[4,5] Also evident from the NMR spectra was a (dimethylamino)ethyl group (δ_C 27.4 t, 58.7 t and 44.9 q × 2, and δ_H 2.96 brt (2H), 2.62 brt (2H) and 2.50 s $2CH_3$'s, respectively), which was further supported from the *m/z* 58 (CH_2=NMe_2^+, 100) peak in the mass spectrum. COSY and HMBC correlations confirmed this assignment and established the connectivity of the latter group to C-9 (Figure 1). Shermilamine D is therefore deacetyl-*N*,*N*-dimethyl shermilamine B.

Shermilamine E (**2**), obtained as an amorphous brownish powder, analyzed for $C_{21}H_{20}N_4O_2S$—one additional

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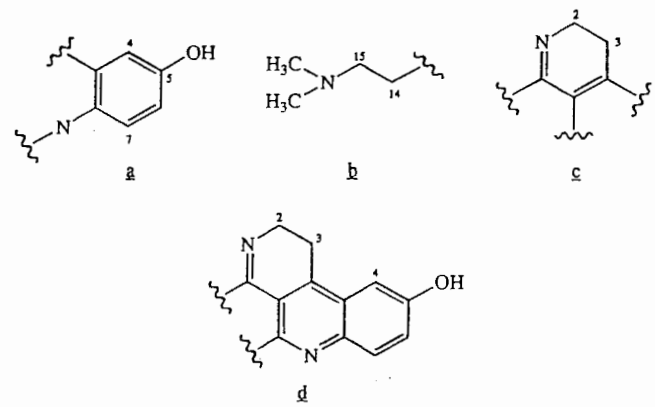
Table 1. NMR Data of Tintamine (3) (500 MHz, in $CDCl_3$ + CD_3OD 4:1)

C no.	δ_C (m)	δ_H (m, J in Hz)	COSY + TOCSY	HMBC(H→C)	NOESY
2	47.4 (t)	3.75 (t, 7.5)	3, 4	3, 3b, 7a, 12, 12a	3
3	26.3 (t)	2.66 (t, 7.5)	2, 4	3b, 4, 7a, 12a	2, 4
3a	136.1 (s)				
3b	119.1 (s)				
4	114.3 (d)	6.65 (d, 1.9)	2, 3, 7	3, 5, 6, 7a	3
5	160.0 (s)				
6	113.5 (d)	6.72 (dd, 8.5, 1.9)	4, 7	4, 5, 7a	7
7	130.6 (d)	8.12 (d, 8.5)	4, 6	3b, 4, 5	6
7a	140.7 (s)				
8a	151.6 (s)				
9	111.8 (d)	6.78 (d, 1.9)	11	8a, 11, 12b, 14	15
10	137.2 (s)				
11	112.3 (d)	7.18 (brs)	9	8a, 9, 12b, 14	15
12	167.0 (s)				
12a	159.6 (s)				
12b	142.4 (s)				
14	31.3 (t)	3.08 (m)	15	9, 10, 15	9, 11
15	59.0 (t)	3.19 (m)	14	10, 14, 17	9, 11
17	43.1 (q)	2.76 (s)		15, 17	

oxygen over what was found for compound **1**. The NMR data of **2** (see the Experimental Section) pointed to a close relationship between **1** and **2**. Furthermore, it was clear from these data that the difference between **1** and **2** was in the aliphatic appendage of the molecule (δ_C 58.5 q and δ_H 3.29 s (6H) ($-NOMe_2$); δ_C 22.3 t, 68.2 t and δ_H 3.40 and 3.42 t ($-NOCH_2CH_2-$), vide supra).

Comparison of the $(CH_3)_2NCH_2CH_2-$ NMR data of **1** and the corresponding data of **2** with $(CH_3)_3N$ and $(CH_3)_3NO$[9] established the *N*-oxide structure of **2**.[10] The latter assignment was also in full agreement with the *m/z* 332-[$M^+ - (CH_3)_2NO$, 53%] fragment in the mass spectrum. Compound **2** is less active and inhibited division of fertilized sea urchin eggs at concentration of 0.13 mg/mL only, vide supra.

Tintamine (**3**), an amorphous yellow powder, the third new compound, was obtained in minute amounts only, and its molecular formula was determined as $C_{20}H_{22}N_3O_2S$ by HRFABMS (*m/z* 368.1390, MH^+). The proposed molecular formula was corroborated by the ^{13}C NMR spectrum (Table 1), showing 20 distinct resonance lines; six sp^3 and 14 sp^2 C-atoms, whose multiplicity (2 × CH_3, 4 × CH_2, 5 × CH, and 9 × quaternary C, determined by a DEPT experiment) accounted for 19 out of the 21 protons, leaving two hydrogens to be linked to heteroatoms. The ^1H NMR spectrum (Table 1) paralleled the ^{13}C spectrum in showing 14 protons linked to sp^3 C-atoms and five aromatic protons. COSY and HMBC cross-peaks as well as the ^1H and ^{13}C chemical shifts suggested that three out of the five aromatic protons (δ 8.12 d, 6.72 dd and 6.65 d, Table 1) are accommodated in partial structure **a**.[11] Ten of the aliphatic protons showed a typical pattern compatible with a (dimethylamino)ethyl functionality (**b**)—similar to the appendage of compounds **1** and **2**. The remaining two aliphatic methylenes [δ_H 3.75 (t, J = 7.5 Hz, 2H) and 2.66 (t, J = 7.5 Hz, 2H), δ_C 47.4 and 26.3 t, respectively] suggested a $=NCH_2CH_2C=$



moiety (**c**). HMBC cross-peaks, to and from the latter two methylenes (Table 1), suggested them to be condensed to an aromatic ring system.[11,12] Most important were correlations from H_2-2 and H_2-3 to C-3b, -4, and -7a (as given in Table 1; up to five bond correlations). Further proof for the spatial vicinity of H-3 and H-4 came from a NOE measured between the two in a d-NOE experiment (3%). Additional CH− correlations from H-4, -6, and -7 to C-3 to -7a (as specified in Table 1) and the C-chemical shifts of C-3a, -8a, and -12b suggested the partial tricyclic structure **d**.[12] The remaining two aromatic protons [δ 7.18 (brs) and 6.78(d, J = 1.9 Hz)] were determined to be meta to each other according to their mutual 1.9 Hz coupling constant[13] and H,H COSY correlation peak (Table 1). Further support for the latter suggestion came from similar CH-correlations of each one of these two protons to the C-atoms of the seven-membered ring (Table 1, HMBC). In addition to C-9 and -11 carrying the latter two protons, two other C-atoms (δ_C 137.2 s and 167.0 s) had to be accounted for. On the basis of the chemical shifts[14] and CH-correlations (e.g., from H-2 to C-12 and C-12a, Table 1) the structure of **3** was completed by a tropone ring. The latter 2,4,6-cycloheptatrieneone ring has to carry the (dimethylamino)ethyl group (moiety **b**), which is suggested to be linked to C-10, of the tropone, through a sulfur atom. The latter suggested sulfide was fully supported by CH-correlations from and to this thio amino side chain (Table 1). A 2-aminotropone is expected to exist in two amino−ketone and imino−enol tautomers, thus lowering the δ_C value of the C=O group. Indeed, acetylation of **3** gave the expected diacetate (**4**) in which one acetylation took place on the 5-phenolic OH group and the other on the amino tropone C-12 hydroxyl group. That both esterifications took place on phenolic groups was evident from the single IR absorption, of **4**, at 1762 cm^{-1} and the two 3H-signals at 2.34 and 2.43 ppm. The proton chemical shifts of H-4, -6, -9 and -11 in **4** [$\Delta\delta_H$ (OAc−OH) values of 0.39, 0.37, 0.35, and 0.54, respectively] and the same for the ^{13}C chemical shift values [$\Delta\delta_c$ (OAc−OH) values of 5.0, 5.9, 7.8, and 7.4, respectively] were also in full agreement

(9) $(CH_3)_3N$: δ_C 47.6q; δ_H 2.31s. $(CH_3)_3NO$: δ_C 59.7q, δ_H 3.2s.

(10) The following *N*-oxides of pyridoacridine alkaloids have previously been reported. *N*-Oxides of dercitin: Gunawardana, G. P.; Kohmoto, S.; Burres, N. S. *Tetrahedron Lett.* **1989**, *30*, 4359. That compound **2** is a natural compound was evident from the ^1H NMR of the crude extract against the absence of *N*-oxides following the same workup in *Eudistoma* sp.[4,7]

(11) Both the ^1H and ^{13}C chemical shifts agreed well with a 3-alkyl-4-aminophenol; i.e.: Cimino, G.; Crispino, A.; De Rosa, S.; De Stefano, S.; Gavagnin, M.; Sodano, G. *Tetrahedron* **1987**, *43*, 4023.

(12) The ^{13}C chemical shifts of partial structure **c** were in good agreement with this part in eudistone A. He, H.; Faulkner, D. J. *J. Org. Chem.* **1991**, *56*, 5369.

(13) H-11, being most likely further coupled to the vicinal acidic OH(12) proton, in the enol-tautomeric structure, gives rise to a broadened signal.

(14) δ_C 176.2 (s, C-1), 157.4 (s, C-2), 112.7 (d, C-3), 129.9 (d, C-4), 123.5 (d, C-5), 136.2 (d, C-6), 136.7 (d, C-7) were measured for 2-aminotropones: Bagli, L. F.; St-Jacques, M. *Can. J. Chem.* **1978**, *56*, 578.

(15) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. In *Spectrometric Identification of Organic Compounds*, 5th ed.; Wiley-Interscience: Singapore, 1991; p 240.

with the location of the two OH groups.[15] Besides the CH-correlations of the amino side chain to the tropone, NOE enhancements between H-14 and H-15 to both H-9 and H-11 were in full agreement with the suggested structure. The $^1J_{CH}$ coupling constants of C-9 and -11, 157 and 162 Hz, respectively, are in full agreement with reported values for tropone.[16] Tintamine (**3**) therefore possesses the unprecedented tropono-1,2-dihydro-3,6-phenantroline ring system.

Previously, it was suggested by us[17] and also demonstrated synthetically that the biogenesis of pyridoacridines involves a reaction between kynuramine, an oxidation metabolite of tryptophan, and benzoquinone (or its precursor). Similarly, it can be suggested that tintamine (**3**) is obtained from kynuramine and tropolone (or its precursor), which is a natural product too. Synthetic efforts aimed to support this biogenesis are in progress.

Experimental Section

General Experimental Procedures. NMR, IR, MS, and UV spectrometer data have been previously published.[4,18]

Collection, Extraction, and Isolation Procedures. The dark purple tunicate, voucher no. AM-35, *C. violatinctus* (Monniot 1988), order Aplousobranchia, family Polycitoridae, was collected by scuba (−10 m) at the Mayotte lagoon, Comoros Islands, northwest of Madagascar in April 1996. After collection, the tunicate was immediately frozen down to −25 °C. The frozen tunicate was then extracted with a mixture of MeOH−$CHCl_3$ (1:2) × 3 to give a dark brown gum (0.96 g). Solvent partition between aqueous MeOH and CCl_4, $CHCl_3$, and *n*-butanol resulted in three fractions (166, 148, and 32 mg, respectively). The fractions were separately chromatographed, first on Sephadex LH-20 eluted with MeOH−$CHCl_3$−hexane (1:1:2), and then several times on silica gel columns eluted with $CHCl_3$ and $CHCl_3$ with increasing percentages of MeOH, up to 30%. From the repeated chromatographies, we have obtained [percent of the crude extract; R_f on silica gel plates (the solvent system); spots color] the following: a mixture of segolins [4.5; 0.28−0.34 (5% MeOH, 95% $CHCl_3$); brown to purple spots without spraying], **1** [10; 0.2 (10%MeOH, 90% $CHCl_3$); purple spot with H_2SO_4−vaniline[19] spraying], **2** [0.4; 0.1 (10% MeOH, 90% $CHCl_3$); brownish spot after spraying], **3** [0.4; 0.4 (50% MeOH, 50% $CHCl_3$); yellow spot after spraying]—all as amorphous powders.

Shermilamine D (1): orange powder; IR (neat) ν 3500, 3326, 1623 cm^{-1}; UV (MeOH) λ_{max} (nm) (log ϵ) 232 (4.35), 281 (4.25), 297 (4.18), 348 (3.74), 392 (3.51), 468 (3.58); UV (MeOH, H^+) λ_{max} (nm) (log ϵ) 230 (4.33), 281 (4.23), 302 (4.32), 316 (4.42), 363 (3.50), 382 (3.49), 530 (3.60); ^{1}H NMR ($CDCl_3$) δ 8.40 (d, J = 4.8 Hz, H-2), 7.20 (d, J = 4.8 Hz, H-3), 7.80 (brd, J = 7.9 Hz, H-4), 6.98 (brt, J = 7.5 Hz, H-5), 7.33 (brt, J = 7.5 Hz, H-6), 6.84 (brd, J = 8.0 Hz, H-7), 3.50 (brs, H-11), 2.96 (brt, J = 4.0 Hz, H-14), 2.62 (brt, J = 4.8 Hz, H-15), 2.50 (S, Me's-17), 10.20 (brs, NH-8), 9.05 (brs, NH-13); ^{13}C NMR ($CDCl_3$) δ 150.7 (d, C-2), 106.8 (d, C-3), 140.2 (s, C-3a), 116.3 (s, C-3b), 123.7 (d, C-4), 120.6 (d, C-5), 131.7 (d, C-6), 116.4 (d, C-7), 140.7 (s, C-7a), 132.9 (s, C-8a), 116.7 (s, C-8b), 109.6 (s, C-9), 121.4 (s, C-9a), 29.7 (t, C-11), 163.5 (s, C-12), 121.3 (s, C-13a), 137.3 (s, C-13b), 27.4 (t, C-14), 58.7 (t, C-15), 44.9 (2q, C-17); COSY 2/3; 3/2; 4/5; 5/4, 6; 6/5, 7; 7/6; 14/15; 15/14; HMBC (H to C) 2/3, 13a, 13b; 3/2, 8b; 4/3b, 6, 7a; 5/3a, 3b, 6, 7; 6/4, 7a; 7/3b, 5; 11/9a, 12; 14/8a, 9a, 15, 17; 17/15; NH-13/9a, 11, 13b; HREIMS *m/z* 376.1356 (Δmmu +0.1, 30), 58 ($C_3H_8N^+$, 100).

Shermilamine E (2): brown amorphous powder; IR (neat) ν 3500, 3326, 1623 cm^{-1}; UV (MeOH) λ_{max} (nm) (log ϵ) 232 (4.29), 296 (4.10), 349 (3.70), 391 (3.51), 464 (3.49); UV (MeOH, H^+) λ_{max} (nm) (log ϵ) 230 (4.25), 281 (4.18), 300 (4.21), 315 (4.25), 361 (3.53), 382 (3.50), 526 (3.45); ^{1}H NMR ($CDCl_3$, CD_3OD 4:1) δ 8.44 (d, J = 5.0 Hz, H-2), 7.20 (d, J = 5.0 Hz, H-3), 7.78 (brd, J = 8.0 Hz, H-4), 6.99 (brt, J = 7.5 Hz, H-5), 7.34 (brt, J = 7.5 Hz, H-6), 7.10 (brd, J = 8.2 Hz, H-7), 3.50 (brs, H-11), 3.40 (brt, H-14), 3.42 (brt, H-15), 3.29 (s, Me's 17), 11.80 (brs, NH-8), 9.10 (brs, NH-13); ^{13}C NMR ($CDCl_3/CD_3OD$ 4:1) δ 150.8 (d, C-2), 107.1 (d, C-3), 140.1 (s, C-3a), 115.9 (s, C-3b), 123.5 (d, C-4), 121.1 (d, C-5), 131.9 (d, C-6), 116.8 (d, C-7), 140.4 (s, C-7a), 132.2 (s, C-8a), 116.9 (s, C-8b), 106.0 (s, C-9), 120.7 (s, C-9a), 29.9 (t, C-11), 163.6 (s, C-12), 121.2 (s, C-13a), 137.7 (s, C-13b), 22.3 (t, C-14), 68.2 (t, C-15), 58.5 (2 × q, C-17); HMBC the same as for 1; HRFABMS *m/z* 393.1402 (Δmmu −1.7), 332 ($M^+ - C_2H_6NO$, 53).

Tintamine (3): yellow powder; IR (KBr) ν 3492, 3436, 3270 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRFABMS *m/z* 368.1390 (calcd for $C_{20}H_{22}N_3O_2S$ [MH^+] *m/z* 368.1432); *m/z* 327 (33), 223 (21).

Tintamine Diacetate (4). Tintamine (5 mg) was left overnight at rt in a mixture of Ac_2O−pyridine 1:1 (1 mL). Evaporation of the solution afforded compound **4** (5 mg): amorphous powder; IR (neat) ν 3500, 1762, 1201 cm^{-1}; UV (MeOH) λ_{max} (nm) (log ϵ) 265 (3.68), 308 (3.67); UV (MeOH, H^+) λ_{max} (nm) (log ϵ) 280 (3.52), 340 (3.58), 371 (3.65); ^{1}H NMR ($CDCl_3$) δ 3.95 (t, J = 7.5 Hz, H-2), 2.75 (d, J = 7.5 Hz, H-3), 7.04 (d, J = 1.9 Hz, H-4), 7.09 (dd, J = 8.5, 1.9 Hz, H-6), 8.55 (d, J = 8.5 Hz, H-7), 7.14 (d, J = 1.9 Hz, H-9), 7.72 (d, J = 1.9 Hz, H-11), 3.25 (brt, H-14), 3.38 (brt, H-15), 2.84 (s, Me's-17); 2.43 (s, Me-Ac); 2.34 (s, Me-Ac); ^{13}C NMR ($CDCl_3$) δ 47.7 (t, C-2), 26.1 (t, C-3), 119.3 (d, C-4), 119.4 (d, C-6), 130.3 (d, C-7), 119.6 (d, C-9), 119.7 (d, C-11), 31.2 (t, C-14), 59.2 (t, C-15), 43.3 (q, C-17), 20.1 (q, Ac), 20.8 (q, Ac), 169.1 (s, 2Ac) (because of the small amount of compound **4** the quaternary carbon atoms were indistinguishable).

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Supporting Information Available: ^{1}H NMR spectra of shermilamine D and E and tintamine (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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