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Styelsamines A−D: New Tetracyclic Pyridoacridine Alkaloids from the Indonesian Ascidian *Eusynstyela latericius*

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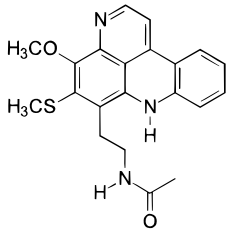
Over the last 15 years, a diverse range of pyridoacridine alkaloids have been reported from the marine environment.[1] These alkaloids are typically tetra- or pentacycles, e.g. varamine B (**1**),[2] usually possessing a functionalized alkylamine side chain. The most recent examples are arnoamine A (**2**) and the corresponding methyl ether arnoamine B, oxygenated pentacyclic pyridoacridines reported from a *Cystodytes* sp. ascidian.[3]



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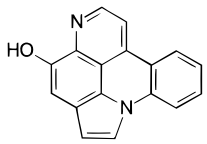

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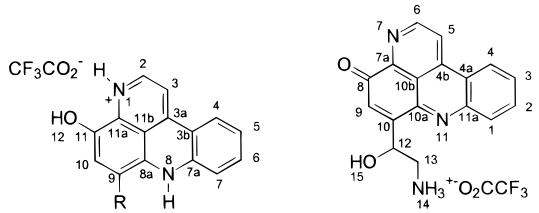
As part of our continuing search for biologically active secondary metabolites from ascidians, we now report the structures of four new tetracyclic pyridoacridine alkaloids (**3**, **5**−**7**), the styelsamines, from an extract of the ascidian *Eusynstyela latericius* (Sluiter, 1904) (Order Pleurogona, Family Styelidae).[4] Partitioning the cytotoxic ethanolic extract by C18 flash chromatography followed by repeated use of Sephadex LH-20 gel filtration using methanol−trifluoroacetic acid (0.1%) eluent afforded a range of novel pyridoacridine alkaloids that were characterized either as their trifluoroacetate salts or as the free base.

A molecular formula of $C_{17}H_{15}N_3O_2$ for **3** was established by HRFAB mass spectroscopy. Several features of the ^{1}H and ^{13}C NMR spectra of **3** were reminiscent of pyridoacridine alkaloids.[1] A combination of COSY and ROESY NMR experiments established the presence of a fused 1,2-disubstituted benzene−2,3,4-trisubstituted pyridine ring system (N-1 to NH-8) common to the pyridoacridine alkaloids, as well as isolated 2,3,4,5-tetrasubstituted phenol (H-10, OH-12) and ethanolamine (C-13 to NH_3^+-15) spin-systems. ^{1}H−^{13}C direct and long-range connectivities were established by HMQC and HMBC NMR experiments, allowing the unequivocal assignment of all carbon resonances in **3**. Crucial HMBC correlations between H-13 (δ 5.62) and carbon resonances at δ 126.6 (C-8a), 118.3 (C-9), and 117.4 (C-10) secured the placement of the ethanolamine side-chain at C-9 and hence completed the structure of styelsamine A (**3**). The unusually shielded chemical shift of the hydroxy-bearing aryl carbon C-11 (δ_C 137.3) is not without precedent in pyridoacridine alkaloids: the corresponding carbon of arnoamine A (**2**) (C-8) has a reported chemical shift of 139.8 ppm.[3] A lack of detectable optical activity in **3** by either ORD or CD indicated that styelsamine A was isolated as a racemic mixture.

Over a period of two weeks for which the NMR data of pure **3** was obtained, it was noticed that a second series of NMR resonances were becoming more intense. After standing at room temperature for 2 months, the sample was composed of a 1:3 mixture (as determined by NMR) of **3** and a closely related new compound, **4**, which was purified from the mixture by a combination of LH-20 and C-18 chromatography. The observation of spectroscopic data indicative of a carbonyl group (δ_C 183.0, ν 1678 cm^{-1}), significant changes in the ^{13}C NMR chemical shifts of ring-A carbon resonances, and the lack of pH dependence of the UV chromophore indicated that compound **4** was a pyridoacridone alkaloid related to the cystodytin family of marine alkaloids.[5] Analogous to the cystodytins, HRFABMS of **4** in a glycerol matrix showed an ion at 294.1245 Da corresponding to matrix reduction of the protonated molecular ion.



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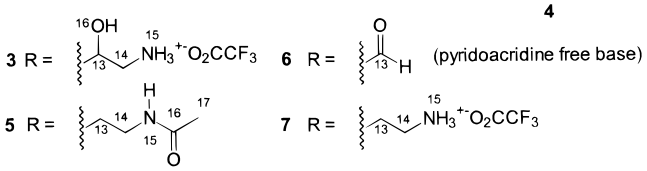

HRFABMS established a molecular formula of $C_{19}H_{17}N_3O_2$ for styelsamine B (**5**). The NMR data for **5** were almost identical to **3**, with the greatest differences centering on the resonances associated with the C-9 alkyl side-chain. In the case of **5**, ^{1}H NMR signals observed at δ 1.91 (3H, s), 2.96 (2H, t, J = 6.5 Hz), 3.24 (2H, dt, J = 6.5, 7.5 Hz) and an exchangeable resonance at δ 8.50 (1H, t, J = 5.5 Hz) suggested the presence of an *N*-acetyl-1,2-disubstituted ethylamine moiety. This was confirmed by the observation of *N*-acetyl resonances in the ^{13}C NMR

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Table 1. ^{1}H NMR Data (DMSO-d_6) (δ, Mult, J) for Styelsamines A (3), B (5), C (6), and D (7)

atom	3	5	6[a]	7
1	13.70 (br s)	13.47 (br s)		13.61 (br s)
2	8.33 (d, 6.0)	8.24 (d, 6.5)	8.80 (d, 5.5)	8.30 (d, 6.5)
3	7.65 (d, 6.0)	7.54 (d, 6.5)	7.93 (d, 5.5)	7.60 (d, 6.5)
4	8.24 (d, 8.5)	8.20 (d, 8.5)	8.33 (d, 8.0)	8.23 (d, 8.5)
5	7.24 (dd, 8.0, 8.0)	7.22 (dd, 7.5, 7.5)	7.28 (m)	7.23 (m)
6	7.69 (dd, 8.0, 8.0)	7.70 (m)	7.61 (m)	7.69 (m)
7	7.99 (d, 8.5)	7.67 (m)	7.61 (m)	7.69 (m)
8	11.13 (br s)	11.50 (br s)	12.13 (br s)	10.84 (br s)
10	7.79 (s)	7.43 (s)	7.38 (br s)	7.47 (s)
12	10.98 (br s)	10.78 (br s)	9.15 (br s)	10.98 (br s)
13	5.62 (br d, 7.0)	2.96 (t, 6.5)	9.93 (s)	3.20 (br t, 7.0)
14	3.07 (m) 2.89 (m)	3.24 (dt, 6.5, 7.5)		3.10 (br m)
15	8.18 (br s)	8.50 (t, 5.5)		7.95 (br s)
16	6.47 (br s)			
17		1.91 (s)		

[a] Data for free base. All other compounds characterized as TFA salts.

Table 2. ^{13}C NMR Data (DMSO-d_6) Observed for Compounds 3, 5, 6, and 7

atom	A (**3**)	B (**5**)	C (**6**)[a]	D (**7**)
2	143.6 (3)[b]	143.3 (3)	151.6 (3)	143.3 (3)
3	105.5 (2)	104.9 (2)	109.3 (2)	105.1 (2)
3a	149.1 (2, 4)	149.2 (2, 4)	140.9 (2, 4)	148.9 (2, 4)
3b	113.9 (3, 5, 7, 8)	113.9 (3, 5, 7, 8)	116.7 (3, 8)	113.8 (3, 5, 7, 8)
4	125.4 (6)	125.4 (6)	124.4 (6)	125.2 (6)
5	122.6 (7)	122.0 (7)	123.0	122.4 (7)
6	134.8 (4)	135.0 (4)	132.6 (4)	134.7 (4)
7	117.9 (5)	117.6 (5)	117.9 (5)	117.6 (5)
7a	140.9 (4, 6)	141.0 (4, 6)	137.1 (4, 6, 8)	141.0 (4, 6)
8a	126.6 (8, 10, 13)	128.3 (8, 10, 13)	134.6 (10, 13)	128.3 (10, 13)
9	118.3 (8, 10, 13)	116.2 (13, 14)	108.4 (8, 13)	113.8 (13)
10	117.4 (13)	121.6 (13)	113.6	121.8 (13)
11	137.3 (10)	136.6 (10)	139.0	137.2 (10)
11a	127.0 (2, 10)	125.9 (2, 10)	142.8 (2, 10)	126.7 (2, 10)
11b	120.3 (3, 8)	120.3 (3, 8)	117.9 (3, 8, 13)	120.4 (3, 8)
13	64.3 (10)	30.3 (10, 14)	191.8 (10)	28.2 (10)
14	44.0 (13)	37.7 (13, 15)		37.9 (13)
16		171.4 (14, 15, 17)		
17		22.4		

[a] Data for free base. All other compounds characterized as TFA salts. [b] Numbers in parentheses are protons to which the carbon correlated to in HMBC NMR experiments.

spectrum (δ 22.4 (q) and 171.4 (s)) and by 2-D HMBC correlations (Table 2).

A molecular formula of $C_{16}H_{10}N_2O_2$ for styelsamine C (**6**) was established by HRFABMS. The NMR data for compound **6** again indicated the presence of a 9-substituted-11-hydroxypyridoacridine substructure. In this case, the observation of a one proton singlet in the ^{1}H NMR spectrum at 9.93 ppm, and lack of any alkyl proton resonances, suggested that the C-9 substituent was an aldehyde functional group. Continued attempts to obtain a pure sample of **6** using acidic solvent systems only resulted in compound degradation. Purification was achieved by chromatography using amino-bonded silica gel, where elution with chloroform−5% methanol afforded the free base of styelsamine C (**6**) as a poorly soluble orange solid. The structure was confirmed by mass spectrometry and by analysis of NMR spectral data (Tables 1 and 2).

Inspection of the ^{1}H and ^{13}C NMR data for compound **7** indicated the presence of a nonacylated ethylamine side-chain. Subsequent analysis of 2-D NMR data acquisitions confirmed the structure of styelsamine D as the deacetyl analogue of styelsamine B.

Compounds **3**, **5**, **6**, and **7** exhibited mild cytotoxicity toward the human colon tumor cell line HCT-116, with IC_{50} values of 33, 89, 2.6, and 1.6 μM, respectively.

Experimental Section

Collection, Extraction, and Isolation Procedures. The purple ascidian was collected using SCUBA (−10 m) from Ujung Pandang, Indonesia, in 1996, and kept frozen until used. A voucher specimen (CI-96-8-1) is held in the Department of Medicinal Chemistry, University of Utah. An ethanolic extract of frozen ascidian tissue (85 g) was partitioned between chloroform and aqueous methanol−trifluoroacetic acid (1%) to afford a purple polar fraction. Purification of the aqueous fraction by LH-20 (methanol−trifluoroacetic acid (0.1%)) and C-18 reverse phase flash and HPLC chromatography (water−methanol−trifluoroacetic acid, 70:30:0.1) afforded styelsamine A (**3**) as the blue bis-trifluoroacetate salt (5.4 mg, 6×10^{-3} % wet weight). Additional minor colored components were visible in other fractions, but lack of material prevented their structures from being determined. A second collection of the ascidian was undertaken at the same location in November 1997. The ethanolic extract of this material (450 g wet weight) was partitioned between chloroform and aqueous methanol−trifluoroacetic acid (1%) to afford a purple polar fraction. Purification of this fraction by column chromatography on C18 reverse phase silica gel using MeOH−aqueous trifluoroacetic acid solvent mixtures eluted a number of blue and red bands over the solvent composition range of 30% to 60% methanol−aqueous trifluoroacetic acid (0.1%). Each of the separately collected colored bands were then subjected to repeated gel filtration chromatography on Sephadex LH-20 using methanol−trfluoroacetic acid (0.1%) eluent, to yield in order of C18 polarity, styelsamines A (**3**, 7.2 mg, 1.6×10^{-3} % wet weight), D (**7**, 25.2 mg, 5.6×10^{-3} % wet weight), C (**6**, 25 mg, impure), and B (**5**, 10.1 mg, 2.2×10^{-3} %

Table 3. NMR Data for Iminoquinone 4

atom	^{13}C	^{1}H (mult, *J*)	HMBC (^{13}C → ^{1}H)
1	130.7	8.23 (d, 7.5)	H-3
2	132.0	8.03 (dd, 7.5, 7.5)	H-4
3	130.0	7.94 (m)	H-1
4	124.1	8.95 (d, 8.0)	H-2
4a	121.7		H-1, H-3, H-5
4b	136.7		H-4, H-6
5	120.4	9.05 (d, 5.0)	H-6
6	150.0	9.26 (d, 5.0)	H-5
7a	146.3		H-6, H-9
8	183.0		
9	130.9	7.11 (s)	
10	149.3		H-9
10a	151.7		H-9
10b	117.8		H-5
11a	144.3		H-2, H-4
12	64.2	5.67 (br m)	H-9
13	44.3	3.18 (m)	
		3.50 (m)	
14		7.97 (br m)	
15		6.40 (br s)	

wet weight). Styelsamine C was further purified by chromatography on amino-bonded silica gel. Elution with chloroform−5% methanol afforded clean styelsamine C (**6**, 4.0 mg, 9×10^{-4} % wet weight) as the free base.

Styelsamine A (3): purple solid; HRFABMS (3-nitrobenzyl alcohol, MH^+) calcd for $C_{17}H_{16}N_3O_2$ 294.1243, found 294.1226; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (rel intensity) 294 (100, MH^+); IR ν_{max} (film) 3102, 2942, 1678, 1584, 1443, 1202, 1143 cm^{-1}; UV (MeOH−TFA) λ_{max} (log ϵ) 222 (sh) (3.86), 244 (3.71), 276 (3.93), 294 (3.96), 386 (3.17), 554 (3.02) nm; UV (MeOH−KOH) λ_{max} (log ϵ) 272 (3.85), 378 (3.39) nm; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 2.

Iminoquinone (4): Styelsamine A (**3**, 4.4 mg) was left at room temperature in DMSO-d_6 for 2 months, after which time iminoquinone **4** (3.3 mg) was isolated by combinations of LH-20 and C-18 chromatography as a brown solid: HRFABMS (glycerol, $MH^+ + 2H$) calcd for $C_{17}H_{16}N_3O_2$ 294.1243, found 294.1245; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (rel intensity) 292 (100, MH^+); IR ν_{max} (film) 3072, 1678, 1202, 1131 cm^{-1}; UV (MeOH−TFA) λ_{max} (log ϵ) 226 (3.97), 272 (3.95), 387 (3.49) nm; UV (MeOH−KOH) λ_{max} (log ϵ) 268 (3.97), 376 (3.49) nm; ^{1}H and ^{13}C NMR (DMSO-d_6) see Table 3.

Styelsamine B (5): purple solid; HRFABMS (3-nitrobenzyl alcohol, MH^+) calcd for $C_{19}H_{18}N_3O_2$ 320.1399, found 320.1397; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (rel intensity) 320 (100, MH^+), 247 (15); IR ν_{max} (film) 3072, 1678, 1660, 1619, 1581, 1367, 1199, 1128 cm^{-1}; UV (MeOH−TFA) λ_{max} (log ϵ) 222 (sh) (4.43), 244 (4.28), 278 (4.55), 294 (4.60), 372 (sh) (3.57), 388 (3.70), 572 (3.72) nm; UV (MeOH−KOH) λ_{max} (log ϵ) 262 (4.38), 378 (3.97) nm; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 2.

Styelsamine C (6): orange solid; HRFABMS (3-nitrobenzyl alcohol, MH^+) calcd for $C_{16}H_{11}N_2O_2$ 263.0821, found 263.0814; MS (FAB, glycerol) *m/z* (rel intensity) 263 (100, MH^+); IR ν_{max} (film) 3283, 1650, 1630, 1515, 1242 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ϵ) 248 (4.90), 284 (4.82), 334 (4.22), 350 (4.27), 418 (4.75), 480 (4.41) nm; UV ($CHCl_3$−TFA) λ_{max} (log ϵ) 282 (4.85), 304 (4.89), 422 (4.69), 510 (4.33) nm; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 2.

Styelsamine D (7): purple solid; HRFABMS (3-nitrobenzyl alcohol, MH^+) *m/z* 278.1280, $C_{17}H_{16}N_3O$ requires 278.1293; MS (FAB, 3-nitrobenzyl alcohol) *m/z* (rel intensity) 278 (100, MH^+), 261 (20), 247 (15); IR ν_{max} (film) 3072, 1672, 1584, 1202, 1131 cm^{-1}; UV (MeOH−TFA) λ_{max} (log ϵ) 222 (4.30), 238 (4.17), 276 (4.37), 294 (4.38), 320 (3.80), 372 (3.54), 386 (3.60), 560 (3.43) nm; UV (MeOH−KOH) λ_{max} (log ϵ) 270 (4.26), 376 (3.95) nm; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 2.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **3**−**7** (10 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.