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NEW CYTOTOXIC ACRIDINE ALKALOIDS FROM TWO DEEP WATER MARINE SPONGES OF THE FAMILY *Pachastrellidae*

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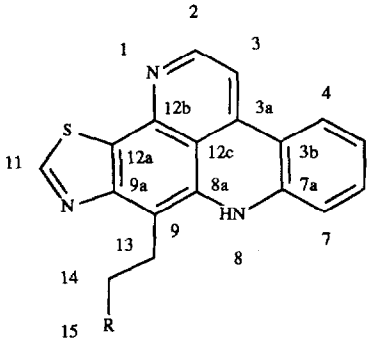
Fused ring alkaloids having the pyrido[4,3,2-mn]acridine skeleton comprise a new class of marine natural products.[1a-h] Their biological activity[1e] and challenging structure elucidation make them an interesting group of compounds. In this paper we report the identification of four new cytotoxic and structurally related alkaloids: nordercitin (1), dercitamine (2), dercitamide (3), and cyclodercitin (4).

The extracts of two sponges, a deep violet *Dercitus sp.* and a red *Stelletta sp.*, collected by manned submersible at depths of 152m and 70m respectively, in the Bahamas,[2] inhibited growth of murine P388 leukemia cells when screened on shipboard at the site of collection. The organisms were kept frozen until extraction with MeOH:toluene (3:1) and 100% MeOH. Fractionation of the *Dercitus* extract by centrifugal countercurrent chromatography using MeOH:CH_2Cl_2:H_2O(5:5:3) gave the previously described dercitin (**5**) as the major antitumor alkaloid.[1e] Further purification of the fractions containing minor metabolites by HPLC (NH_2 stationary phase, solvent system - CH_3CN: MeOH: CH_2Cl_2(4:4:1) containing 1% NH_3OH) gave cyclodercitin (**4**), and two N-oxides of dercitin(N-1 and N-15). Using similar chromatographic procedures, the extract from *Stelleta sp.* gave nordercitin (**1**), dercitamine (**2**), and dercitamide (**3**).

The pyrido[4,3,2-*mn*]thiazolo[3,2-*b*]acridinium-9-ethyl skeleton (Figure 1) in each compound was suggested by the characteristic UV absorption pattern, (λ_{max}(MeOH) 245 (ϵ 13,800), 307 (ϵ 16,900), and 361 nm (ϵ 3900)), which was highly sensitive to the pH of the medium. Further, the aromatic region of the ^1H nmr spectra of each compound consisted of seven signals: a downfield one proton singlet for the thiazole proton (H-11), two vicinal olefin proton signals (H-2 and H-3) and signals for four protons on an ortho disubstituted aromatic ring (H-4 - H-7). In the aliphatic region signals for two vicinal methylene groups were observed. Three bond CH connectivities for all the carbons except for C-12c could be observed in spectra from COLOC[3] and HMBC[4] experiments (Table 1). Comparison of the ^1H and ^{13}C spectra (Tables 1 & 2) of the four compounds showed that they differed from dercitin (**5**) in the substituents on N-1 and/or on the ethyl side chain.

The presence of a methyl function on N-1 in **4 - 6** could be readily detected by a 3 proton singlet observed at 3.5 to 3.9 ppm in CD_3OD or between 4 to 5.6 ppm in TFA-d and a corresponding carbon signal observed between 44 to 52 ppm in either solvent. The N-1 methyl protons also showed 3-bond connectivity to C-2 and C-12b. Further, the presence of a methyl group on N-1 could be deduced from the color of the compound in solution: both types turn deep red to violet(480-510 nm) in acidic medium, but the N-methylated compounds turn blue (590 nm) in basic medium while the others turn yellow (430nm).



1 R = $N(CH_3)_2$

2 R = $NHCH_3$

3 R = $NHCOC_2H_5$

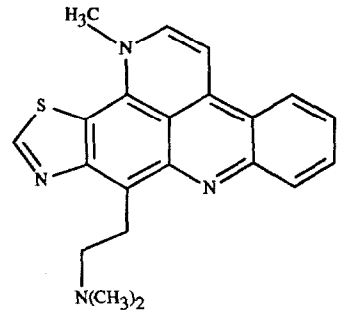


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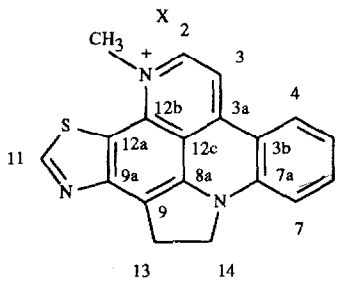
Figure 1



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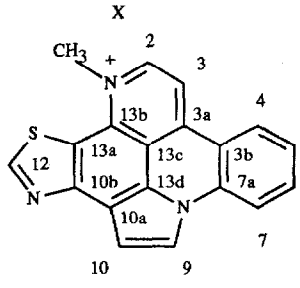

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Figure 2

Nordercitin (**1**) yellow solid, m.p. 176°C, HRFABMS 347.1316, $C_{20}H_{19}N_4S$ (MH^+,Δ+1.4mmu). A ^1H nmr singlet observed at 3.14 ppm (6H, s, $N(CH_3)_2$) and a ^{13}C nmr signal (2C by inverse gated experiment) at 44.75 ppm indicated an N,N-dimethyl function on the ethyl chain giving the structure **1** for this compound. The exchangeable NH proton signal which appears at 12.6 ppm in d_6-pyridine showed nOe to the proton signal at 7.01 ppm(H-7) suggesting that the tautomeric form shown in figure 1 is preferred in solution.

Dercitamine (**2**) orange solid, m.p. 135°C, HRFABMS 333.1189, $C_{19}H_{17}N_4S$ (MH^+,Δ+ 1.6mmu), Comparison of the spectral data of **1** and **2** showed that the latter had an N-methyl (δ 3.39 (3H, s, NCH_3) and δ 45.26 (NCH_3)) instead of an N,N-dimethyl function on N-15. The proposed structure for **2** was further supported by conversion of dercitamine (**2**) to nordercitin upon methylation using $HCHO/HCO_2H$.[5]

Dercitamide (**3**) yellow solid, m.p. 192°C, HRFABMS 375.1285, $C_{21}H_{19}N_4SO$ (Δ-0.5mmu). This compound has a propionamide moiety on the ethyl chain as determined by the ^1H nmr signals observed at δ 2.79

Table - 1 δ ^{1}H (360 MHz, TFA-d)

Proton	1	2	3	4[a,b]	6[b]	1-bond[c]	3-bond[d]
2H	8.58	8.72	8.66	8.39	9.14	*C2*	*C3a,C12b*
3H	8.04	8.19	8.12	7.67	8.87	*C3*	*C3b*
4H	8.32	8.46	8.41	8.26	9.03	*C4*	*C3a,C6*
5H	7.56	7.71	7.66	7.35	8.10	*C5*	*C3b,C7*
6H	7.92	8.06	7.98	7.82	8.41	*C6*	*C4,C7a*
7H	7.73	7.89	7.84	7.32	8.61	*C7*	*C3b,C5*
11H	9.58	9.72	9.88	9.18	9.69	*C11*	*C9a,C12a*
13H	3.77	4.02	3.86	3.76	7.93	*C13*	*C8a,C9a*
14H	3.58	3.82	3.71	4.40	8.91	*C14*	*C9*
1NCH$_3$	-	-	-	4.32	5.56	*1NCH$_3$*	*C2,C12b*
15NCH$_3$	3.14	3.39	-	-	-	*15NCH$_3$*	*C14*

a. spectra were recorded in CD_3OD and CH correlations were observed by HMBC experiments.
b. the numbering shown in Figure 2, **4** is used for both **4** and **6** in these tables. The correct numbering for this skeleton is shown in Figure 2, **6**.
c. one bond CH correlations were observed by HETCOR experiment.
d. three bond CH correlations were observed by COLOC and HMBC experiments.

Table - 2 δ ^{13}C (90.5 MHz, TFA-d)

Carbon	1	2	3	4[a,b]	6[b]
2	143.74	143.39	144.07	150.49	145.15
3	111.05	110.69	111.32	108.49	114.07
3a	153.48	153.13	153.70	140.97	143.41
3b	117.24	116.98	117.57	117.44	116.64
4	127.18	127.42	127.51	127.71	129.18
5	127.78	126.82	128.06	124.24	129.13
6	139.71	139.34	139.95	137.45	138.31
7	120.44	119.91	120.53	116.03	118.22
7a	142.21	141.85	142.60	149.97	141.86
8a	137.06	136.70	137.63	141.06	124.59
9	106.55	106.18	110.43	116.25	117.48
9a	145.27	144.91	144.60	140.81	143.41
11	158.44	158.05	159.88	151.47	154.15
12a	130.60	130.96	128.31	136.65	139.88
12b	134.48	134.13	133.46	134.05	133.97
12c	121.00	121.00	121.89	118.88	120.27
13	32.01	27.84	30.93	29.09	110.73
14	58.49	56.56	39.69	49.02	124.48
1NCH$_3$	-	-	-	51.87	51.91
15NCH$_3$	44.75	45.26	-	-	-

(2H, q, J=7.5 Hz) and 1.39 ppm (3H, t, J=7.5 Hz) and ^{13}C signals observed at δ 184.2 (s), 22.64 (t), and 10.83 ppm (q). The amide proton signal could be observed in $CDCl_3$ as a broad signal at 6.6 ppm coupled to the methylene signal at 3.43 ppm. The aromatic NH proton signal appeared as two singlets at 10.9 and 9.98 ppm in the ratio 1:3 indicating the presence of both tautomers in $CDCl_3$. However, the very low solubility of this compound in $CDCl_3$ did not permit the confirmation of this observation by ^{13}C nmr spectroscopy.

Cyclodercitin (**4**) Blue powder[6], m.p. 298°C, HRFABMS 316.0916, $C_{19}H_{14}N_3S$ (Δ-0.8mmu). The molecular formula and the lack of evidence for functionality on the ethyl chain suggested the presence of an additional ring in the skeleton. However, due to low solubility and the low sensitivity of methylene signals in an HMBC experiment using CD_3OD as the nmr solvent, long range CH correlations were not observed for H-13 or H-14 and the position of the new ring could therefore not be determined. The compound dissolved readily in TFA-d giving a violet solution, but satisfactory spectra could not be obtained due to line broadening. Within 2 hrs the TFA-d solution turned fluorescent yellow and sharp lines in the nmr spectrum were observed. Both nmr and TLC analysis showed the quantitative transformation of the blue compound **4** into a slightly more polar single product **6**. In the ^{1}H nmr spectrum of the yellow product (**6**), the two vicinal methylene proton signals had been replaced with two aromatic proton signals observed at 7.93 (1H, d, J=5.2 Hz, H-10) and 8.91 ppm (1H, d, J=5.2 Hz, H-9) while in the ^{13}C spectrum two new signals appeared at 110.73 (d, J=180.2 Hz) and 124.48 ppm (d, J=187 Hz) in place of the methylene carbon signals. The molecular ion at 314 in both FAB and FD spectra suggested that the blue compound had oxidized. The long range CH correlations confirmed that the pyrido[4,3,2-*mn*]thiazolo[3,2-*b*]acridine skeleton remained unchanged and the additional correlations between the proton signal observed at 8.91 ppm (H-9) and C-7b and C-13d (Figure 2) indicated the presence of the depicted five membered heterocycle in **6**. This allowed the assignment of the novel structure,

9,10-dihydro-1-methylpyrido[4,3,2-*mn*]pyrrolo[3,2,1-*de*]thiazolo[5,4-*b*] acridinium ion, to cyclodercitin (**4**) and the correct numbering of this ring system is shown in Figure 2, **6**.

All compounds inhibited proliferation of P388 murine leukemia cells in vitro and compounds **1**, **2**, and **3** exhibited immunosuppressant activity.[7]

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References and Notes:

1. a) F. J. Schmitz, S. K. Agrawal, S. P. Gunasekera, P. G. Schmidt, and J. N. Shoolary, *J. Am. Chem. Soc. 105*, 6134, **1983**, b) G. Cimino, A. Crispino, S. De Rosa, S. De Stefano, M. Gavagnin, and G. Sodano, *Tetrahedron Lett., 43*, 4023, **1987**, c) S. J. Bloor and F. J. Schmitz, *J. Am. Chem. Soc. 109*, 6134, **1987**, d) T. F. Molinski, E. Fahy, D. J. Faulkner, G. D. Van Duyne, J. Clardy, *J. Org. Chem. 53*, 1341, **1988**, e) G. P. Gunawardana, S. Kohmoto, S. P. Gunasekera, O. J. McConnell, and F. E. Koehn, *J. Am. Chem. Soc. 110*, 4856, **1988**, f) J. Kobayashi, J. Cheng, M. R. Walchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Org. Chem. 53*, 1800, **1988**, g) J. Kobayashi, J. Cheng, H. Nakamura, Y. Ohizumi, Y. Hirata, T, Sasaki, T. Ohta, and S. Nozoe, *Tetrahedron Lett. 29*, 1177, **1988**, h) A. Rudi, Y. Benayahu, I. Goldberget, and Y. Kashman, *Tetrahedron Lett. 29*, 3861, **1988**, i) N. M. Cooray, P. J. Scheuer, L. Parkanyi, and J. Clardy, *J. Org. Chem. 53*, 4619, **1988**, j) A. Rudi, Y. Benayahu, I. Goldbergt, and Y. Kashman, *Tetrahedron Lett. 29*, 6655, **1988**, h) F. S. de Guzman and F. J. Schmitz, *Tetrahedron Lett. 30*, 1069, **1989**.

2. The organisms were collected using HBOI *Johnson-Sea-Link* submersibles and voucher specimens are deposited in the Indian River Coastal Zone Museum (IRCZM). *Dercitus sp.* HB/DBMR # 20-III-87-1-2, IRCZM # 003:00041; *Stelletta sp.* HB/DBMR # 22-III-87-1-7, IRCZM # 003:00042.

3. Y. Sato, M. Geckle, S. J. Gould, *Tetrahedron Lett. 26*, 4019, **1985**.

4. A. Bax, M. F. Summers, *J. Am. Chem. Soc. 108*, 2093, **1986**.

5. R. N. Icke and B. B. Wisegarver, *Org. Syn., Coll. Vol., 3*, 723, **1955.**

6. The counter ion was exchanged to Cl^- by passing through an ion exchange resin. The natural counter ion is not known.

7. Following IC_{50} values were obtained for these compounds in P388 in vitro assays: **1** 4.79, **2** 26.7, **3** 12.0, **4** 1.9, **5** 0.08, and **6** 9.89 μM. The details of immunosuppressent activity will be reported elsewhere.

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