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Cylindricines A and B, Novel Alkaloids from the Ascidian *Clavelina cylindrica*

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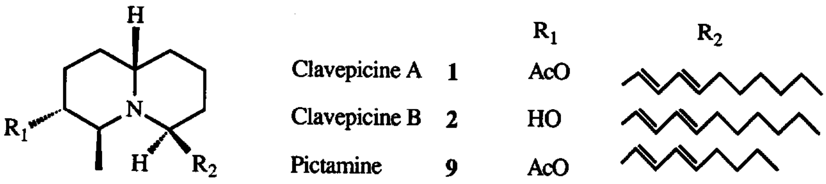
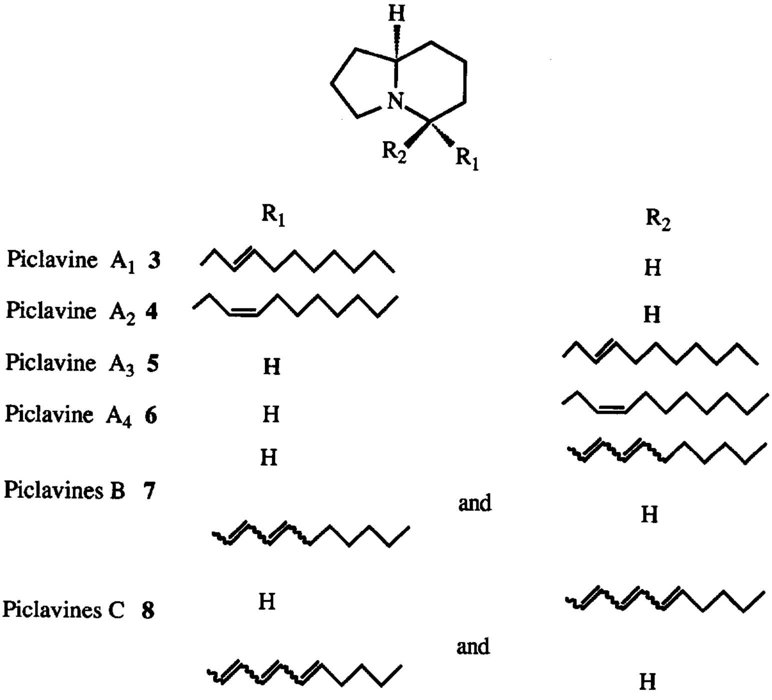
Abstract: The ascidian *Clavelina cylindrica*, which was collected in Tasmania, has yielded two novel alkaloids, cylindricine A, *rel*-(3*R*,4*R*,5*S*,7a*R*,11a*R*)-3-chloromethyl-5-hexyl-perhydro-pyrrolo-[2,1-j]quinolin-7-one, (**12**), and cylindricine B, *rel*-(3*S*,5*R*,6*S*,8a*R*,12a*R*)-3-chloro-6-hexyl-perhydropyrido[2,1-j]quinoline-8-one, (**13**). Cylindricine B is the first example of the new pyrido-[2,1-j]-quinoline ring system while cylindricine A is the first pyrrolo[2,1-j]quinoline known from nature. Single crystal X-ray studies support the assignment of the two alkaloids.

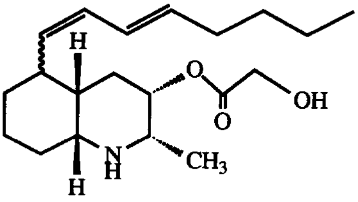
INTRODUCTION

Ascidians are marine benthic invertebrates from which a large number of secondary metabolites have been isolated with a high proportion of the compounds containing nitrogen and being biologically active.[1] The ascidian *Clavelina cylindrica* has not been previously investigated for secondary metabolites although the chemistry of some other members of the genus has been reported in the literature. *C. picta* obtained in Bermuda gave the quinolizidine alkaloids clavepictine A, (1) and clavepictine B, (2)[2] together with the indolizidine alkaloids piclavines A_1-A_4, (3)-(6), piclavines B, (7) and piclavines C, (8)[3]. The same ascidian obtained from Venezuela gave pictamine, (9).[4] Clavepictine A and pictamine differ only by the length of the side chain.

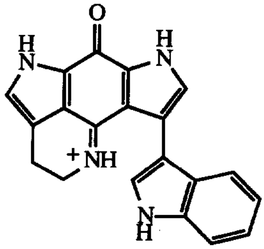
C. lepadiformis has yielded the new decahydroquinoline alkaloid lepadin A, (**10**).[5] An unidentified member of the *Clavelina* genus has given wakayin, (**11**),[6] as a novel pyrroloiminoquinone alkaloid.







Lepadin A **10**



Wakayine **11**

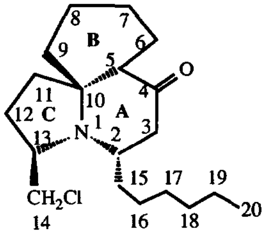
Clavelina cylindrica (Quoy and Gaimard) Suborder Aplousobranchia, forms colonies which are translucent and grey to blue in colour. A collection of the ascidian was made by scuba diving in Deep Glen Bay, East Coast, Tasmania. Crude extracts were active by the brine shrimp bioassay and gave a positive Mayer's test for alkaloids. In this paper we wish to report the structures of the two main alkaloids that were present. The two compounds have novel tricyclic structures and were readily interconvertible.

RESULTS AND DISCUSSION

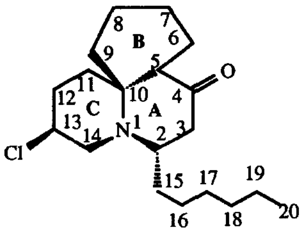
Isolation and structural determination

Repeated silica gel chromatography of the dichloromethane extract of freeze dried *Clavelina cylindrica* followed by reverse phase HPLC separation yielded cylindricine A and cylindricine B as two light yellow coloured oils in 0.039% and 0.034% isolated yield respectively.

High resolution EIMS of cylindricine A indicated a molecular formula of $C_{19}H_{32}ClNO$, having an index of hydrogen deficiency of four. Cylindricine A was a tertiary aliphatic amine as revealed by its basic nature together with its ^1H NMR spectrum which showed no aromatic protons nor any exchangeable proton signals. The ^{13}C NMR and DEPT spectra indicated the presence of a carbonyl group at 210.7 ppm, one CH_2Cl group at 50.1 ppm, one quaternary carbon, three CH carbons, one CH_3 group and 12 CH_2 groups in the range 43.9-23.0 ppm. These results indicated that the compound was tricyclic with the nitrogen at a bridgehead. Since many of the NMR signals from the methylenes were not well resolved, the sizes of the rings and the length of the saturated side chain which was also present could not be determined unambigously so that X-ray diffraction studies seemed well suited to this structural problem. To get good single crystals, cylindricine A was converted into a picrate. The X-ray crystallographic data are consistent with the formulation of cylindricine A as the novel compound *rel*-(3*R*,4*R*,5*S*,7a*R*,11a*R*)-3-chloromethyl-5-hexyl-perhydro-pyrrolo[2,1-j]-quinolin-7-one (**12**). This information as well as some two-dimensional NMR spectral data allowed assignment of the NMR spectra (Table 1).



Cylindricine A **12**



Cylidricine B **13**

The drawings of (**12**) and (**13**) show the non-systematic atom numbering system which has been used for both the NMR and crystallographic data. No absolute configuration is implied.

Another, somewhat less abundant alkaloid, cylindricine B was also obtained from *C. cyclindrica.* High resolution EIMS indicated that the molecular formula was $C_{19}H_{32}ClNO$, that is cylindricine B was an isomer of cylindricine A. Its NMR spectral data showed that cylindricine B was also a tricyclic tertiary aliphatic amine. Comparison of the NMR spectral data of cylindricine A and cylindricine B revealed that cylindricine B did not possess a CH_2Cl group, rather the chlorine was attached to a methine carbon which gave a signal at 4.18 ppm in the ^1H NMR spectrum. Another noticeable difference was found in the ^{13}C NMR spectrum where the quaternary carbon in cylindricine A was at 70.9 ppm while the quaternary carbon in cylindricine A was shifted to higher field at 59.8ppm. To unambigously determine the final details of the structure, a single crystal X-ray structure was determined of the picrate of cylindricine B. The X-ray study was consistent with the formulation of cylindricine B as *rel*-(3*S*,5*R*,6*S*,8a*R*,12a*R*)-3-chloro-6-hexyl-perhydropyrido-[2,1-j]quinoline-8-one (**13**). The NMR spectral data of cylindricine B are given in Table 1.

Solutions of either cylindricine A or cylindricine B both gave, after 6 days, the same equilibrium mixture of 3:2 of cylindricine A and cylindricine B. This process could be followed easily by NMR spectroscopy. The interconversion between cylindricine A and cylindricine B only occurred when the compounds were present as free bases. Their salts, for example the picrates, were quite stable in solution at room temperature for several weeks. The interconversion involves the chlorine and the nitrogen participating in a stereospecific ring-opening ring-closing reaction. The reaction, which involves inversion of the nitrogen, may be concerted or involve an aziridinium ion intermediate—Scheme 1.

Cylindricine A, with its pyrrolo[2,1-j]quinoline ring system, is closely related to the indolizidine system of the piclavines by having an extra six-membered carbocylic fused ring (ring B). In the same way, cylindricine B, with its pyrido[2,1-j]quinoline system is related to the quinolizidine system of the clavepicines and pictamine. This is the first reported occurence of the novel pyrido[2,1-j]quinoline ring system, either in a natural product or a synthetic compound. Cylindricine A is the first natural product with the pyrrolo[2,1-j]-quinoline ring system although the system has been reported once in the literature in connection with synthesis.[7]

The equilibrium mixture of alkaloids cylindricine A and cylindricine B exhibited bioactivity. It was active in the brine shrimp bioassay causing significant mortality at a level of 3×10^{-2} mmol.

Single crystal X-ray structure determinations

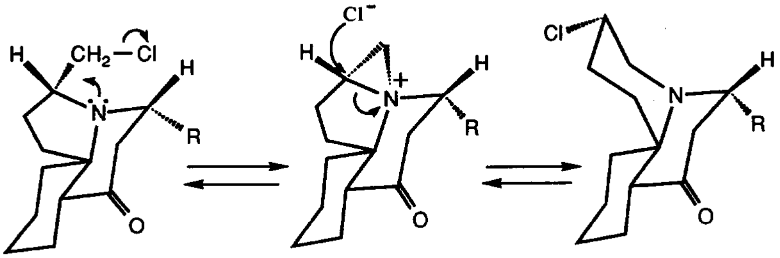
In the case of (**12**) and (**13**), structural assignment is more than usually heavily dependent on the X-ray experiments, and results of these are recorded in appropriate detail; absolute configurations are not assigned.

Unique diffractometer data sets ($2\theta/\theta$ scan mode; monochromatic Mo Kα radiation, $\lambda = 0.71073$Å) were measured at room temperature (~295K) within the specified $2\theta_{max}$ limit

Table 1. NMR Data for Cylindricine A and Cylindricine B

Atom No.	H (12) δ	C (12) δ	H (13) δ	C(13) δ
2	3.32	55.7	2.99	54.9
3	2.11, 2.36	43.7	2.34, 2.45	46.3
4	-	208.9	-	208.4
5	2.08	51.4	2.17	54.6
6	1.28, 2.61	23.9*	2.57	22.6*
7	1.46, 1.82	25.3*	1.26, 1.60	23.1*
8	1.75, 1.90	23.0*	1.69, 1.89	22.9*
9	1.41, 1.65	35.9*	1.25, 2.17	33.7*
10	-	70.9	-	59.8
11	1.44, 1.75	36.8*	1.44	34.8*
12	1.66, 1.99	35.4*	1.81, 1.96	31.3*
13	3.50	58.9	4.18	57.9
14	3.32, 3.68	50.1	2.65, 3.26	50.7
15	2.08	27.8*	1.54	23.7*
16	1.40	30.2*	1.44	32.8*
17	1.40	27.8*	1.44	24.5*
18	1.20, 1.54	23.6*	1.44	22.2*
19	1.51	32.9*	1.44	30.6*
20	1.24	15.0	1.23	15.0

*These assignments may be interchangeable. Assignments are supported by $^1H/^1H$ and $^1H/^{13}C$ two-dimensional NMR spectra, chemical shifts are given in ppm relative to the solvent peak of benzene-d_6 at 7.4 and 128.7ppm respectively.



an aziridinium ion intermediate

12

13

Scheme 1

yielding N independent reflections; N_o of these with $I > 3\sigma(I)$ were considered 'observed' and used in the large block least squares refinements without absorption correction. Anisotropic thermal parameters were refined for the non-hydrogen atoms; $(x, y, z, U_{iso})_H$ were included constrained at estimated values. Nitrogen atom assignment was based on refinement behaviour; the ring skeleton was initially assigned a full carbon complement, the nitrogen atom sites being indicated by thermal parameter behaviour and significant lowering of residuals on substitution, also by the hydrogen bonding pattern (see below). Conventional residuals R, R_w on $|F|$ are quoted, statistical weights being derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\sigma^4(I_{diff})$. Computation used the XTAL 3.0 program system[8] implemented by S.R. Hall. Pertinent results are given in the Figures and Tables.

Crystal/refinement data. (**12**). $C_{25}H_{35}ClN_4O_8$, $M = 555.0$. Triclinic, space group $P1$ (C_1^1, No. 1), $a = 12.651(4)$, $b = 12.523(5)$, $c = 9.617(4)$Å, $\alpha = 90.43(3)$, $\beta = 103.67(3)$, $\gamma = 110.89(3)$°, $V = 1376$Å^3. D_c $(Z = 2) = 1.34$ g.cm.$^{-3}$; $F(000) = 588$. $\mu_{Mo} = 1.9$ cm.$^{-1}$; specimen: cuboid, ~ 0.4 mm. $2\theta_{max} = 47.5$°; $N = 4179$, $N_o = 2545$; $R = 0.065$, $R_w = 0.064$.

(**13**). $C_{25}H_{35}ClN_4O_8$, $M = 555.0$. Triclinic, space group $P1$, $a = 14.022(5)$, $b = 12.250(2)$, $c = 9.077(4)$Å, $\alpha = 109.10(2)$, $\beta = 94.03(3)$, $\gamma = 109.22(2)$°, $V = 1363$ Å^3. D_c $(Z = 2) = 1.35$ g.cm.$^{-3}$; $F(000) = 588$. $\mu_{Mo} = 1.9$ cm.$^{-1}$; specimen: 0.42 x 0.08 x 0.35 mm. $2\theta_{max} = 45$°; $N = 4651$, $N_o = 3012$; $R = 0.053$, $R_w = 0.053$.

Comment. Interestingly, both structures crystallise in the unusual space group $P1$, totally devoid of symmetry, with two formula units in the asymmetric unit. Despite high thermal motion at the nitro substituents of the picrate groups and the cation tails in particular, the polycyclic cation cores are well defined, permitting assignment of the nitrogen atom on the basis of refinement with some confidence, together with the constitution of the cation tails; the latter have different dispositions within the two independent cations of each structure, with the large ellipsoids towards the end possibly a foil for unresolved disorder. All six-membered rings of all cations adopt 'chair' dispositions. Remarkably, nitrogen atom assignment is assisted by a consistent aggregation of alkaloid and picrate moieties into pairs, consistent with interaction of the picrate phenolic oxygen atoms with the putative NH functionalities of the alkaloids. N(1)...O(111) distances for the 8A, 8B; 9A, 9B ion pairs are 2.70(1), 2.67(1); 2.71(1), 2.70(1)Å; regrettably, refinement precision does not permit precise location of the associated hydrogen atoms, and assessment of the completeness of their transfer from phenolic to base functionalities.

Figure 1. The four independent cations of the two structures, 20% thermal ellipsoids are shown for the non-hydrogen atoms and hydrogen atoms have arbitrary radii of 0.1Å.

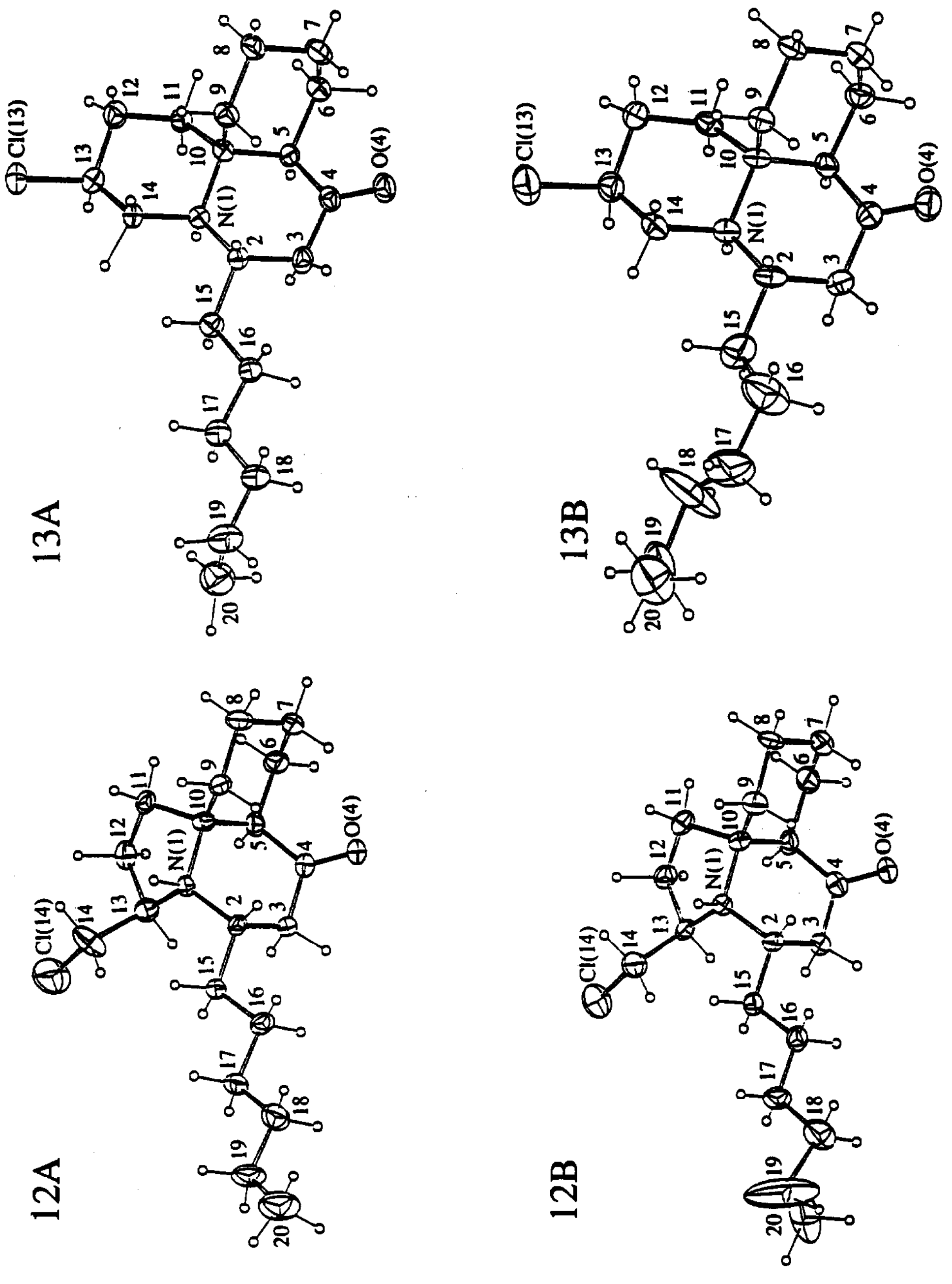


Figure 2. The four alkaloid cation/picrate ion-pair aggregates of the two structures. Putative hydrogen bonds are dotted.

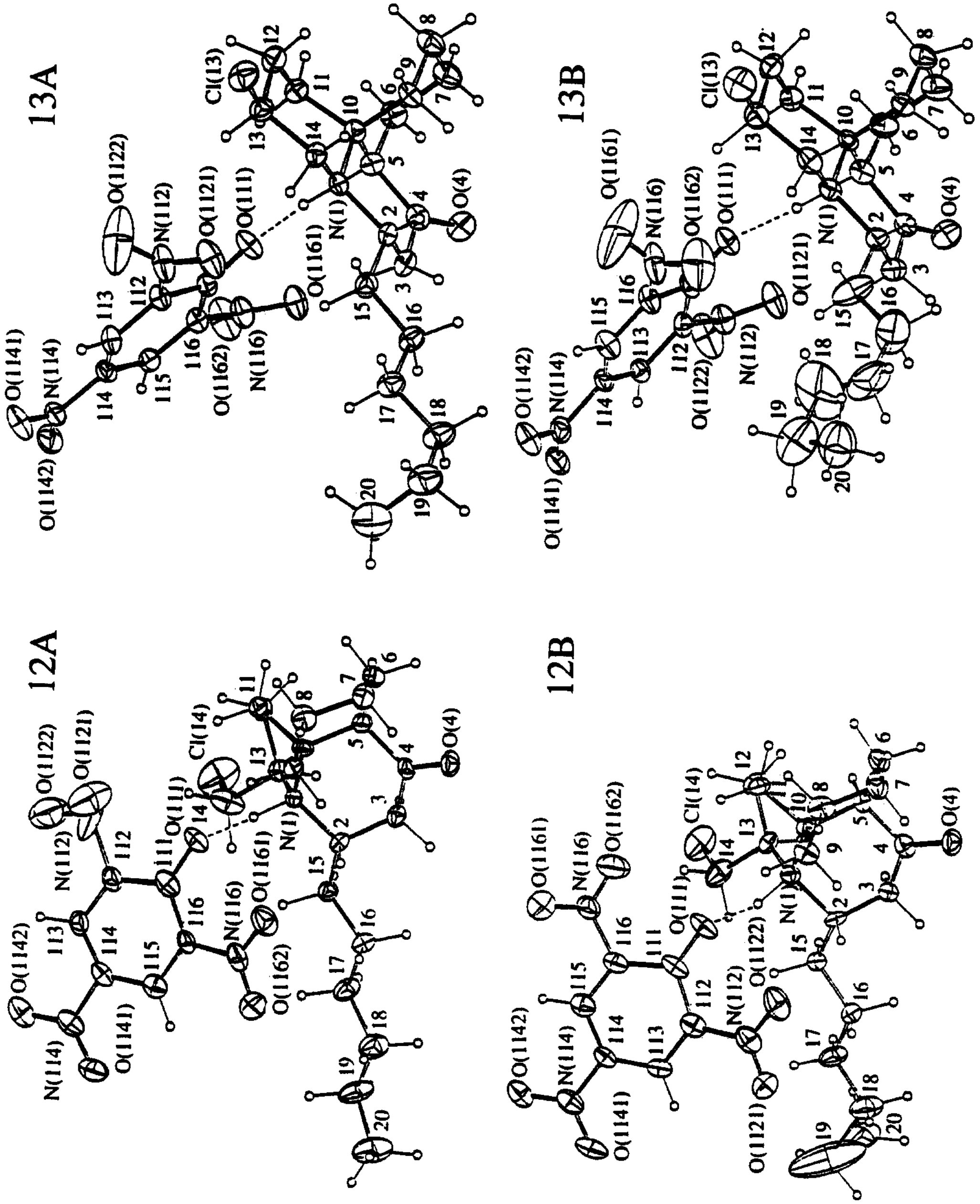


Table 2. Non-hydrogen atom coordinates and equivalent isotropic thermal parameters, (**12**, **13**)

	atom	x	y	z	U(eq) Å^2	x	y	z	U(eq) Å^2
		Molecule A				Molecule B			
12	N(1)	0.0590(6)	-0.0381(6)	0.6129(7)	0.045(3)	0.4153(6)	-0.2943(6)	0.2303(7)	0.051(3)
	C(2)	0.1858(7)	0.0080(7)	0.6083(9)	0.050(4)	0.2895(7)	-0.3341(7)	0.1402(9)	0.051(4)
	C(3)	0.2347(8)	-0.0876(8)	0.6235(9)	0.058(5)	0.2465(8)	-0.2358(7)	0.120(1)	0.055(4)
	C(4)	0.1616(8)	-0.1891(7)	0.5183(9)	0.053(4)	0.3257(9)	-0.1353(8)	0.068(1)	0.066(5)
	O(4)	0.2039(5)	-0.2371(6)	0.4501(7)	0.073(3)	0.2895(6)	-0.0859(6)	-0.0298(7)	0.080(4)
	C(5)	0.0333(8)	-0.2365(7)	0.515(1)	0.059(5)	0.4549(8)	-0.0945(8)	0.145(1)	0.061(5)
	C(6)	-0.0366(9)	-0.3365(8)	0.403(1)	0.081(5)	0.532(1)	-0.0009(9)	0.077(1)	0.089(6)
	C(7)	-0.0551(9)	-0.2974(9)	0.249(1)	0.085(6)	0.554(1)	-0.0459(9)	-0.057(1)	0.086(6)
	C(8)	-0.1125(9)	-0.2102(9)	0.240(1)	0.084(6)	0.6038(9)	-0.1383(9)	-0.024(1)	0.077(5)
	C(9)	-0.0363(8)	-0.1056(8)	0.353(1)	0.062(5)	0.5198(9)	-0.2348(8)	0.036(1)	0.073(5)
	C(10)	-0.0200(7)	-0.1436(7)	0.501(1)	0.053(4)	0.5006(7)	-0.1920(7)	0.171(1)	0.055(4)
	C(11)	-0.1296(8)	-0.1803(8)	0.558(1)	0.066(5)	0.6069(8)	-0.1597(8)	0.301(1)	0.064(5)
	C(12)	-0.0884(9)	-0.1727(8)	0.718(1)	0.075(6)	0.5579(8)	-0.1621(9)	0.429(1)	0.069(5)
	C(13)	0.0296(8)	-0.0714(8)	0.756(1)	0.065(5)	0.4395(7)	-0.2582(8)	0.391(1)	0.057(4)
	C(14)	0.013(1)	0.0328(9)	0.831(1)	0.105(7)	0.4356(9)	-0.360(1)	0.477(1)	0.092(6)
	Cl(14)*	0.0(-)	0.0(-)	1.0(-)	0.178(3)	0.4439(4)	-0.3255(4)	0.6528(3)	0.133(2)
	C(15)	0.2585(8)	0.1107(8)	0.724(1)	0.053(4)	0.2117(8)	-0.4335(7)	0.205(1)	0.055(4)
	C(16)	0.3779(8)	0.1784(8)	0.701(1)	0.068(5)	0.0932(8)	-0.4967(8)	0.106(1)	0.070(5)
	C(17)	0.4405(9)	0.2837(9)	0.811(1)	0.082(5)	0.0176(9)	-0.5947(9)	0.169(1)	0.092(6)
	C(18)	0.553(1)	0.366(1)	0.783(1)	0.105(7)	-0.102(1)	-0.662(1)	0.071(1)	0.134(8)
	C(19)	0.613(1)	0.471(1)	0.890(1)	0.118(7)	-0.161(2)	-0.796(3)	0.143(3)	0.42(3)
	C(20)	0.711(1)	0.565(1)	0.855(2)	0.17(1)	-0.207(2)	-0.725(2)	0.191(3)	0.34(2)
	O(111)	-0.0413(7)	0.1155(5)	0.5244(8)	0.085(4)	0.4925(7)	-0.4649(5)	0.2089(8)	0.090(4)
	C(111)	-0.017(1)	0.2199(9)	0.561(1)	0.076(6)	0.461(1)	-0.5684(8)	0.226(1)	0.075(6)
	C(112)	-0.0916(9)	0.2581(8)	0.616(1)	0.078(6)	0.354(1)	-0.6582(9)	0.146(1)	0.072(5)
	N(112)	-0.205(1)	0.158(2)	0.639(2)	0.24(1)	0.2807(8)	-0.6324(7)	0.0220(9)	0.087(5)
	O(1121)	-0.253(1)	0.124(1)	0.519(2)	0.25(1)	0.1854(8)	-0.7047(7)	-0.031(1)	0.134(5)
	O(1122)	-0.261(1)	0.190(1)	0.673(2)	0.23(1)	0.3178(8)	-0.5404(7)	-0.0255(9)	0.117(5)
	C(113)	-0.0664(9)	0.3681(9)	0.673(1)	0.071(5)	0.3178(8)	-0.7708(8)	0.183(1)	0.066(5)
	C(114)	0.0388(9)	0.4459(8)	0.676(1)	0.066(5)	0.3910(9)	-0.8015(8)	0.287(1)	0.063(5)
	N(114)	0.0746(8)	0.5639(7)	0.7432(8)	0.081(5)	0.3507(8)	-0.9191(7)	0.325(1)	0.083(5)
	O(1141)	0.1746(7)	0.6338(6)	0.7566(9)	0.100(4)	0.2520(7)	-0.9851(6)	0.2650(9)	0.111(5)
	O(1142)	-0.0021(8)	0.5847(6)	0.7871(8)	0.102(5)	0.4182(7)	-0.9490(6)	0.4139(9)	0.101(4)
	C(115)	0.1191(9)	0.4232(8)	0.618(1)	0.070(5)	0.4956(9)	-0.7261(8)	0.361(1)	0.071(5)
	C(116)	0.0906(8)	0.3125(8)	0.557(1)	0.059(5)	0.5288(9)	-0.6172(8)	0.333(1)	0.071(5)
	N(116)	0.1708(8)	0.2962(7)	0.4849(9)	0.085(5)	0.6442(7)	-0.5377(7)	0.424(1)	0.096(5)
	O(1161)	0.1452(8)	0.2024(7)	0.4192(9)	0.112(5)	0.7148(9)	-0.5752(8)	0.470(1)	0.169(7)
	O(1162)	0.2631(7)	0.3744(7)	0.493(1)	0.124(5)	0.6625(8)	-0.4405(7)	0.449(1)	0.165(6)
13	N(1)	0.1318(5)	-0.0592(6)	1.3649(7)	0.048(3)	-0.3522(5)	-0.0040(6)	0.9575(8)	0.054(3)
	C(2)	0.0872(6)	-0.0862(7)	1.5017(9)	0.051(4)	-0.3145(7)	0.0160(8)	1.128(1)	0.071(4)
	C(3)	0.1660(6)	-0.1091(8)	1.5999(9)	0.064(4)	-0.3960(7)	0.0280(8)	1.227(1)	0.073(5)
	C(4)	0.2749(6)	-0.0150(7)	1.643(1)	0.061(4)	-0.4996(7)	-0.0713(8)	1.150(1)	0.063(4)
	O(4)	0.3261(5)	0.0213(6)	1.7761(8)	0.078(3)	-0.5533(5)	-0.1164(7)	1.2294(8)	0.093(4)
	C(5)	0.3151(6)	0.0201(7)	1.5104(9)	0.052(4)	-0.5383(6)	-0.1010(7)	0.976(1)	0.059(4)
	C(6)	0.4216(7)	0.1289(9)	1.572(1)	0.074(5)	-0.6396(7)	-0.2148(9)	0.908(1)	0.078(5)
	C(7)	0.4130(7)	0.2526(9)	1.667(1)	0.082(5)	-0.6207(7)	-0.3351(9)	0.888(1)	0.091(5)
	C(8)	0.3340(7)	0.2812(8)	1.569(1)	0.078(5)	-0.5412(7)	-0.3523(8)	0.792(1)	0.075(4)
	C(9)	0.2287(6)	0.1692(7)	1.513(1)	0.064(4)	-0.4396(6)	-0.2371(7)	0.8683(9)	0.060(4)
	C(10)	0.2382(6)	0.0485(7)	1.4109(9)	0.052(4)	-0.4552(6)	-0.1165(7)	0.8784(9)	0.050(3)
	C(11)	0.2723(6)	0.0492(7)	1.257(1)	0.059(4)	-0.4841(6)	-0.1124(7)	0.715(1)	0.063(4)
	C(12)	0.2008(7)	0.0721(8)	1.148(1)	0.072(5)	-0.4063(8)	-0.1238(9)	0.608(1)	0.075(5)
	C(13)	0.0957(7)	-0.0286(8)	1.1105(9)	0.062(4)	-0.3077(7)	-0.0141(8)	0.694(1)	0.074(5)
	Cl(13)*	0.0(-)	0.0(-)	1.0(-)	0.097(1)	-0.2043(3)	-0.0242(4)	0.5903(5)	0.123(2)
	C(14)	0.0552(6)	-0.0416(7)	1.2571(9)	0.053(4)	-0.2717(6)	-0.0086(8)	0.859(1)	0.068(4)
	C(15)	-0.0144(6)	-0.1977(8)	1.443(1)	0.063(4)	-0.2139(8)	0.140(1)	1.203(1)	0.130(8)
	C(16)	-0.0607(7)	-0.2345(9)	1.574(1)	0.071(4)	-0.164(1)	0.160(2)	1.345(2)	0.20(1)
	C(17)	-0.1689(7)	-0.3361(9)	1.511(1)	0.080(5)	-0.058(2)	0.281(2)	1.416(2)	0.22(1)
	C(18)	-0.2167(7)	-0.367(1)	1.644(1)	0.094(6)	-0.026(2)	0.362(2)	1.373(4)	0.33(2)
	C(19)	-0.327(1)	-0.458(1)	1.596(2)	0.123(8)	0.074(1)	0.483(2)	1.451(2)	0.19(2)
	C(20)	-0.333(1)	-0.582(1)	1.494(2)	0.139(9)	0.142(2)	0.449(2)	1.503(3)	0.20(2)
	O(111)	0.1886(4)	-0.2529(5)	1.2223(7)	0.072(3)	-0.3902(5)	0.1956(6)	0.9464(8)	0.079(3)
	C(111)	0.1747(6)	-0.3647(7)	1.1448(9)	0.049(4)	-0.3717(6)	0.3107(8)	0.995(1)	0.059(4)
	C(112)	0.0944(6)	-0.4420(7)	1.0064(9)	0.054(4)	-0.4354(6)	0.3690(8)	1.0823(9)	0.057(4)
	N(112)	0.0255(7)	-0.3873(8)	0.960(1)	0.094(5)	-0.5220(6)	0.2960(8)	1.129(1)	0.078(4)
	O(1121)	-0.0092(5)	-0.3294(7)	1.0545(9)	0.105(4)	-0.5196(5)	0.2033(7)	1.152(1)	0.107(4)
	O(1122)	0.009(1)	-0.401(2)	0.826(1)	0.28(1)	-0.5932(6)	0.3296(8)	1.153(1)	0.134(6)
	C(113)	0.0781(6)	-0.5637(8)	0.9152(9)	0.060(4)	-0.4155(6)	0.4950(8)	1.1296(9)	0.053(4)
	C(114)	0.1439(6)	-0.6152(7)	0.9552(9)	0.051(4)	-0.3280(7)	0.5701(7)	1.098(1)	0.058(4)
	N(114)	0.1293(7)	-0.7417(7)	0.8608(9)	0.065(4)	-0.3660(6)	0.7437(6)	1.2253(8)	0.089(4)
	O(1141)	0.0559(5)	-0.7990(6)	0.7492(9)	0.097(4)	-0.2280(5)	0.7700(6)	1.1244(9)	0.093(4)
	O(1142)	0.1904(6)	-0.7848(6)	0.8934(8)	0.087(4)	-0.3053(7)	0.7028(7)	1.1530(9)	0.068(4)
	C(115)	0.2253(6)	-0.5456(8)	1.085(1)	0.058(4)	-0.2635(6)	0.5222(8)	1.016(1)	0.072(5)
	C(116)	0.2374(6)	-0.4289(7)	1.1754(9)	0.051(4)	-0.2876(7)	0.3982(9)	0.970(1)	0.073(5)
	N(116)	0.3228(6)	-0.3609(8)	1.315(1)	0.075(4)	-0.2135(9)	0.349(1)	0.888(2)	0.122(7)
	O(1161)	0.3116(5)	-0.2867(7)	1.4306(8)	0.089(3)	-0.204(2)	0.354(2)	0.773(2)	0.40(3)
	O(1162)	0.3988(6)	-0.3867(7)	1.3112(9)	0.120(4)	-0.1685(8)	0.304(1)	0.943(1)	0.181(9)

*defines origin

Table 3. Ring torsion angles, degrees (**12**, **13**)

Cpd/molecule	(**12**) A,B	(**13**) A,B
Ring A		
10 - 1 - 2 - 3	-54.2(9), -53.7(10)	-54.9(9), -54.8(9)
1 - 2 - 3 - 4	52.7(10, 51.4(10)	47.9(9), 48.2(12)
2 - 3 - 4 - 5	-52.3(11), -48.0(12)	-46.0(11), -46.4(14)
3 - 4 - 5 - 10	50.6(11), 45.5(13)	46.2(10), 47.7(12)
4 - 5 - 10 - 1	-48.4(10), -45.7(10)	-49.3(8), -50.7(10)
5 - 10 - 1 - 2	52.4(9), 51.2(10)	56.2(9), 56.7(10)
Ring B		
10 - 5 - 6 - 7	53.5(12), 52.4(11)	55.3(10), 53.0(11)
5 - 6 - 7 - 8	-56.3(11), -56.0(11)	-53.8(11), -54.4(10)
6 - 7 - 8 - 9	58.0(10), 58.1(10)	54.4(10), 56.1(11)
7 - 8 - 9 - 10	-58.8(12), -59.5(10)	-58.7(10), -58.2(11)
8 - 9 - 10 - 5	56.3(10), 57.2(10)	59.8(10, 58.0(8)
9 - 10 - 5 - 6	-54.5(10), -52.9(9)	-57.1(9), -55.5(10)
Ring C		
13,14 - 1 - 10 - 11	36.4(9), 39.0(10)	-58.1(9), -56.2(9)
1 - 10 - 11 - 12	-43.1(9), -44.2(9)	61.2(9), 60.3(9)
10 - 11 - 12 - 13	33.2(11), 35.0(11)	58.8(9), -61.1(11)
11 - 12 - 13 - 1,14	-8.7(12), -9.7(11)	53.3(11), 56.1(12)
12 - 13 - 14 - 1		-54.0(10), -57.2(11)
12 - 13 - 1 - 10	-18.0(11), -18.3(10)	
13 - 14 - 1 - 10		55.7(8), 57.3(10)
Crossovers		
4 - 5 - 10 - 9	73.8(8), 77.0(10)	69.3(8), 69.9(7)
6 - 5 - 10 - 1	-176.7(8), -175.5(7)	-175.7(6), -176.1(8)
4 - 5 - 10 - 11	-156.7(7), -154.3(9)	-165.1(6), -165.4(7)
6 - 5 - 10 - 11	75.0(10), 75.0(10)	68.5(8), 69.3(10)
13,14 - 1 - 10 - 5	-79.7(9), -79.1(8)	-175.8(6), -174.4(8)
13,14 - 1 - 10 - 9	156.8(9), 157.2(8)	66.0(9), 65.1(9)
2 - 1 - 10 - 9	-71.1(10), -72.4(10)	-62.0(10), -63.8(9)
2 - 1 - 10 - 11	168.5(7), 168.3(8)	174.0(8), 174.8(7)

Atoms are denoted by number only

EXPERIMENTAL

General

All NMR data were obtained on a Brucker AM-300 spectrometer. Solution spectra were measured in benzene-d_6. Mass spectra were determined with a Vacuum Generator micromass 9090 instrument. Analytical thin-layer chromatography was performed using Merck 60 F_{254} silica gel aluminum-backed plates and the alkaloids were visualised by spraying with iodoplatinate reagent while Merck 230-400 mesh silica gel 60 was used for flash chromatography. A preparative Rainin C_{18} reverse phase column was used for HPLC. All solvents were redistilled or HPLC grade and petroleum bp 60-80 °C was used.

Collection, extraction and isolation

The *Clavelina cylindrica* was collected in January 1991 at 10m depths from Deep Glen Bay, East Coast, Tasmania (147° 59' E, 42° 58' S). A reference sample is deposited in the Tasmanian Museum and Art Gallery, Hobart (TMHD 2235). The ascidian was frozen immediately after collection and then freeze dried. The dried material (327g) was exhaustively extracted with dichloromethane to give a brown tar (9.1g). This extract was purified by flash chromatography, first using 75% petroleum /25% ethyl acetate and then 95% petroleum /5% ethyl acetate.The two alkaloids were then separated and purified by using HPLC with acetonitrile to give pure cylindricine A (123mg) and cylindricine B (113mg).

Cylindricine A. The alkaloid cylindricine A was obtained as a light yellow oil. ^{1}H and ^{13}C NMR solution spectra see Table 1. EIMS: M^+ 325 (4.4%) 327(M^++2, 1.1%) 282 (45.9%), 276 (19%), 240 (100%). HREI m/z 325.219 ($C_{19}H_{32}ClNO$ requires 325.219).

Cylindricine B. The alkaloid cylindricine B was isolated as a light yellow oil, ^{1}H and ^{13}C NMR spectra see Table 1. EIMS: M^+ 325 (4.4%), 327 (M^++2, 1.1%), 282 (45.9%), 276 (19%), 240 (100%). HREI mass spectrum m/z 325.219 ($C_{19}H_{32}ClNO$ requires 325.219).

Bioassay

Brine shrimp (*Artemia salina*) larvae were exposed to the equilibrium mixture of cylindricine A and cylindricine B in seawater by the standard method.[9] With a concentration of 3×10^{-2} mmol the % deaths after 8 and 24 hours were 14.3 and 29.9 while with a concentration of 0.3 mmol the % deaths after 8 and24 hours were 24.5 and 42.6 and with a concentration of 3 mmol the % deaths after 8 and 24 hours were 46.9 and 92.3 respectively.

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