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New Alkaloids from a South African Latrunculid Sponge

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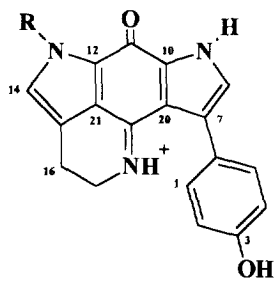
Abstract: The structures of two new bispyrroloiminoquinone alkaloids, tsitsikammamine A (**1**) and tsitsikammamine B (**2**) and two new pyrroloiminoquinone alkaloids 14-bromodiscorhabdin C (**3**) and 14-bromodihydrodiscorhabdin C (**4**) were determined spectroscopically. Compounds **1** and **2** are the first bispyrroloiminoquinone alkaloids to be isolated from a marine sponge while compounds **3** and **4** are the first reported discorhabdin metabolites with a C-14 substituent. All four compounds exhibit antimicrobial activity.

Ongoing chemotaxonomic and morphological studies of the diverse sponge family Latrunculiidae have established a clear correlation between the occurrence of certain secondary metabolites and specific genera within this family. For example, discorhabdin and makaluvamine natural products which share a similar basic pyrroloiminoquinone skeleton, are indicative of the genera *Latrunculia* and *Zyzzya* respectively.[1] The undescribed latrunculid sponge collected recently by SCUBA in the Tsitsikamma Marine Reserve, off the south-eastern South African coast, was found to be a morphological intermediate between these two genera, and a natural product composition incorporating the structural features of both the discorhabdins and makaluvamines was thus predicted for this new genus of sponge. The isolation of tsitsikammamines A and B (**1** and **2**) together with the brominated discorhabdin C derivatives (**3** and **4**) from the latrunculid sponge is consistent with this chemotaxonomic prediction.

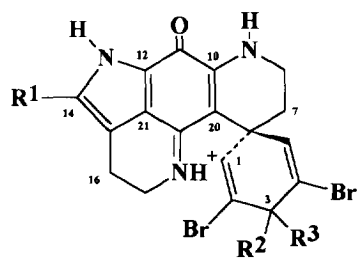
Strong antimicrobial activity is a characteristic of discorhabdin and makaluvamine alkaloids[2] and a combination of antibiotic bioassays and ^{1}H NMR spectroscopy was used to monitor the extraction and subsequent chromatography of the South African latrunculid sponge. Initial separation of the alkaloids from a MeOH:$CHCl_3$ (1:1) extract of this sponge was achieved using a C-18 Sep-Pak® cartridge with a solvent elution gradient from water to methanol. Further chromatography of the mixture of bispyrrolo- and pyrroloiminoquinone compounds on a variety of reverse phase HPLC columns (C-18, C-8 and CN) yielded tsitsikammamine A (**1**, 0.04% dry wt.), tsitsikammamine B (**2**, 0.045% dry wt.), 14-bromodiscorhabdin C (**3** 0.056% dry wt.), and 14-bromodihydrodiscorhabdin C (**4**, 0.019% dry wt.) as dark green oils.

HRFABMS of **1** gave a protonated molecular ion at m/z 304.1087 (Δ -0.1 mDa) thus establishing a molecular formula of $C_{18}H_{14}N_3O_2$ for this compound. Tsitsikammamine A displayed UV (242, 317 and 377 nm) and IR (1660, 1440 and 780 cm^{-1}) absorbances consistent with a pyrroloiminiquinone substructure and a

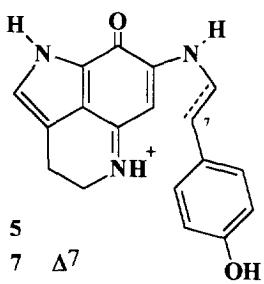
disubstituted aromatic ring.[2,3] The simplicity of the ^{1}H NMR spectra of pyrrroloiminoquinone compounds belies their structural complexity and only four coupled spins systems were evident in the COSY NMR spectrum of **1**. Two of these spin systems revealed coupling from deshielded amino protons at δ 13.0, (br s, NH-13) and δ 13.28 (br s, NH-9) to olefinic proton doublets centred at δ 7.10 (J = 1.8Hz, H-14) and δ 7.16 (J = 2.5Hz, H-8) respectively. The coupling observed firstly between two mutually coupled methylene triplets at δ 3.85 (J = 7.8 Hz, 2H-17) and δ 2.93 (J = 7.8 Hz, 2H-16) and secondly between four deshielded aromatic ring protons resonating at δ7.37 (d, J = 8.4 Hz, H-2, H-4) and δ 6.87 (d, J = 8.4 Hz, H-1, H-5), accounted for the remaining two COSY correlations. These data together with the ^{13}C NMR resonances at δ 166 (C-11), 157 (C-19), 127 (C-12), 123 (C-14), 121 (C-21), 119 (C-15), 45 (C-17) and 17 (C-16) ppm, reminicent of a tricyclic pyrroloiminoquinone moeity,[2] and at δ 158 (C-3), 129 (C-2, C-4), 127 (C-6) and 116 (C-1, C-5) suggestive of a *para* substituted benzene ring,[4] justified twelve of the fourteen degrees of unsaturation required by the molecular formula. Additional olefinic methine (δ 125) and quaternary olefinic (δ 122) ^{13}C NMR signals accounted for a further unsaturation thus necessitating a tetracyclic, and not a tricyclic, skeleton for **1**.



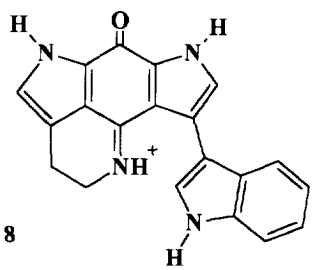
1 R = H
2 R = CH_3



3 R^1 = Br, $R^2 + R^3$ = O
4 R^1 = Br, R^2 = OH, R^3 = H
6 R^1 = H, $R^2 + R^3$ = O



5
7 Δ^7



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Evidence for both the tetracyclic bispyrroloiminoquinone substructure of **1** and the point of attachment of the aromatic substituent to this substructure, followed from HMBC data in which prominent ^{3}J correlations were observed from H-8 (δ 7.16) to C-10, C-20 and C-6. Further ^{3}J HMBC correlations from the aromatic protons H-1 and H-5 (δ 6.87) to the C- 7 quaternary carbon resonance (δ 122) supported the positioning of the aromatic sustituent at C-7. A phenolic IR absorbance at 3150 cm^{-1} and an exchangeable broad hydroxyl proton singlet (δ 10.6) in the ^{1}H NMR spectrum of **1** defined the *para* substituent on the aromatic ring and completed the structural assignment.

Tsitsikammamine B gave a protonated molecular ion in its HRFAB mass spectrum at m/z 318.1252 (Δ -0.9

mDa) consistent with a molecular fomula of $C_{19}H_{16}N_3O_2$. Close similarities in the UV (242, 317, and 374 nm) and NMR data (Table 1) of compounds **1** and **2** pointed to a homologous relationship between these two compounds. The disappearance of the deshielded NH-13 resonance, the collapse of the H-14 olefinic proton doublet to a singlet (δ 7.11) and the appearance of a methyl singlet at δ 3.92 in the ^{1}H NMR spectrum of **2** insinuated that the difference between tsitsikammamines A and B was restricted to a methyl substituent at N-13 in the latter compound. The position of the methyl substituent was further supported by ^{3}J HMBC correlations from the methyl protons to C-12 (δ 126) and C-14 (δ 128).

Munro *et al.* have proposed a biosynthesis of makaluvamine D (**5**) from the amino acid precursors tryptophan and phenylalanine and have also suggested that **5** is, in turn, a precursor of discorhabdin C (**6**).[5] Cyclization at the benzylic position in makaluvamine D, with a concomitant loss of four protons, would yield tsitsikammamine A. Although there was no evidence of **5** or its unsaturated analog, makaluvamine E (**7**), in the crude extract of the South African latrunculid sponge, a similar biosynthetic route is proposed for compounds **1 - 4**.

Table 1. NMR data for compounds **1 - 4**[a].

	1		**2**		**3**		**4**	
Position	^{1}H[b]	^{13}C	^{1}H[b]	^{13}C	^{1}H[b]	^{13}C	^{1}H[b]	^{13}C
1	6.87 d (8.4)	116.2 d	6.86 d (8.1)	116.3 d	7.70 s	150.9 d	6.36 s	134.0 d
2	7.37 d (8.4)	128.9 d	7.36 d (8.1)	129.0 d	-	122.6 s	-	124.4 s
3	-	157.6 s	-	157.6 s	-	171.2 s	4.67 d (8.2)	70.7 d
4	7.37 d (8.4)	128.9 d	7.36 d (8.1)	129.0 d	-	122.6 d	-	124.4 s
5	6.87 d (8.4)	116.2 d	6.86 d (8.1)	116.3 d	7.70 s	150.9 d	6.36 s	134.0 d
6	-	127.2 s	-	127.1 s	-	44.5 s	-	41.9 s
7	-	122.4 s	-	122.4 s	2.02 br s	33.5 t	1.87 br s	34.7 t
8	7.16 d (2.5)	125.0 d	7.16 br s	125.2 d	3.63 br s	38.3 t	3.51 br s	38.1 t
NH-9	13.28 br s	-	13.28 br s	-	10.27 br s	-	10.07 br s	-
10	-	134.6 s	-	134.6 s	-	151.5 s	-	151.5 s
11	-	166.3 s	-	166.8 s	-	164.3 s	-	164.3 s
12	-	127.8 s	-	126.4 s	-	124.1 s	-	124.1 s
NH-13	13.01 br s	-	-	-	13.0 br s	-	13.0 br s	-
14	7.10 d (1.8)	123.1 d	7.11 s	128.0 d	-	112.5 s	-	112.5 s
15	-	119.2 s	-	118.7 s	-	119.8 s	-	119.8 s
16	2.93 t (7.8)	17.6 t	2.91 t (7.8)	17.6 t	2.71 t (7.4)	17.5 t	2.71 t (7.4)	17.7 t
17	3.85 t (7.8)	45.0 t	3.82 t (7.8)	44.7 t	3.71 t (7.4)	43.4 t	3.78 t (7.4)	43.6 t
NH-18	9.75 br s	-	9.80 br s	-	8.22 br s	-	7.76 br s	-
19	-	157.6 s	-	156.2 s	-	152.0 s	-	152.0 s
20	-	113.5 s	-	113.3 s	-	91.7 s	-	96.0 s
21	-	120.7 s	-	120.7 s	-	124.2 s	-	124.2 s
22	-	-	3.92 s	35.8 q	-	-	-	-
OH	10.60 br s	-	10.60 br s	-	-	-	6.20 d (8.2)	-

[a]Spectra were recorded at 400MHz for ^{1}H and 100 MHz for ^{13}C (DMSO-d_6) with chemical shifts(δ) in ppm. [b]Coupling constants (Hz) in parentheses.

Protonated molecular ions in the HRFAB mass spectra of compounds **3** and **4** at m/z 539.8544 (Δ +1.4 mDa) and m/z 541. 8710 (Δ +0.4 mDa) established molecular formulae of $C_{18}H_{13}N_3O_2Br_3$ and $C_{18}H_{15}N_3O_2Br_3$ respectively for these two compounds. The NMR spectral data of **3**, assigned from a combination of COSY, HMQC and HMBC

NMR experiments (Table 1), were in accordance with the published spectral data for discorhabdin C (**6**) isolated from several latrunculid sponges.[2] Comparison of the HRFABMS data of **3** and **6** limited the difference between these two compounds to a single bromine substituent. The absence of a typical H-14 olefinic proton signal at δ 7.22 and an upfield chemical shift (Δ 15ppm) of the C-14 carbon resonance in the ^{1}H and ^{13}C NMR spectra of **3** *cf* **6** placed the bromine substituent at C-14 in the former compound.[2] Analogous carbon chemical shifts in the ^{13}C NMR spectra of **3** and **4** confirmed the same bromine substitution pattern in these two compounds while a difference of two mass units between the molecular masses of **3** and **4**, implied that **4** was the reduction product of **3**. The disappearance of a carbonyl signal (δ 171) and the presence of an oxymethine carbon resonance (δ 71) in the ^{13}C NMR spectrum of **4** suggested that reduction had occurred at C-3. The position of the secondary alcohol functionality was supported by HMBC correlations from the oxymethine proton to C-2 and C-4. Although the plethora of discorhabdins isolated thus far from marine sponges are highly functionalised,[2,5] **3** and **4** represent the first examples from this class of compounds with a C-14 substituent.

The isolation of wakayin (**8**), from an ascidian *Clavelina sp.*, has shown that bispyrroloiminoquinones are not confined to the Phylum Porifera.[3] Compounds **1** - **4** exhibited a level of antimicrobial activity against *Bacillus subtilis* comparable with that of **8**, which has also been reported to have a diverse array of bioactivities including murine cell line cytotoxicity, antifungal and topoisomerase II inhibition. Surprisingly, preliminary studies have shown that while the tsitsikammamines are cytotoxic and exhibit anti-fungal activity they do not inhibit either topoisomerase I or II. This apparent disparity in topoisomerase inhibition by structurally related compounds warrants further investigation.

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