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Novel pyrroloquinoline ribosides from the South African latrunculid sponge *Strongylodesma aliwaliensis*

Robert A. Keyzers,[a] Toufiek Samaai[b] and Michael T. Davies-Coleman[a,*]

[a]*Chemistry Department, Rhodes University, PO Box 94, Grahamstown 6140, South Africa*
[b]*Zoology Department, University of KwaZulu-Natal, Durban 4000, South Africa*

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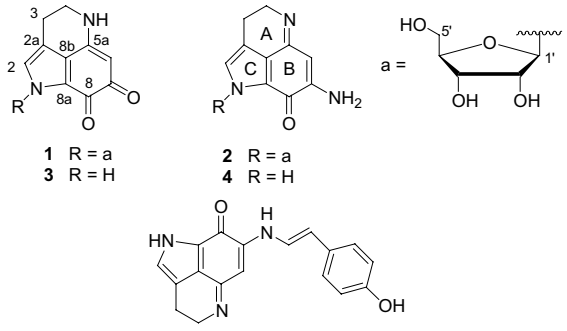
Abstract—Two novel pyrroloquinoline ribosides, *N*-1-β-D-ribofuranosyldamirone C (**1**), and *N*-1-β-D-ribofuranosylmakaluvamine I (**2**) were isolated from a new species of South African latrunculid sponge, *Strongylodesma aliwaliensis*. Standard spectroscopic techniques were used to determine the structures of **1** and **2**. Molecular modeling studies and NOESY data of **1** and **2**, in combination with chiral GC analysis of their derivatized acid hydrolysis products, established the β-D-configuration of the ribofuranose moieties.

Latrunculid sponges are abundant in the sub-tidal zone along the temperate and sub-tropical coast of South Africa[1,2] and are a rich source of alkaloid secondary metabolites containing a 1,3,4,5-tetrahydropyrrolo-[4,3,2-*de*]quinoline structural motif.[3,4] Tricyclic members of this class of bioactive alkaloids, for example, batzellines, isobatzellines, damirones, and makaluvamines, have routinely been isolated from sponges of the genera *Batzella*,[5–7] *Damiria*,[8] *Histodermella*,[9] *Latrunculia*,[4] and *Zyzzya*.[10–12] Tricyclic pyrroloquinolines are not confined to marine sponges and have also been isolated from the terrestrial myxomycete *Didymium bahiense*.[13] In continuation of our search for novel alkaloids from South African latrunculid sponges,[3,4] we have examined an extract of the recently described sponge *Strongylodesma aliwaliensis*, collected with SCUBA from the Aliwal Shoal off the coast of KwaZulu-Natal, South Africa.[14]

Freeze-dried specimens of *S. aliwalensis* (180 g dry wt.) were extracted with MeOH and the MeOH extract concentrated and partitioned between EtOAc and water. The ^{1}H NMR spectrum of the dark brown residue (20.1 g), obtained from lyophilization of the aqueous partition fraction, revealed a plethora of deshielded resonances suggesting the presence of pyrroloquinoline metabolites in this residue. A portion (2 g) of the residue was adsorbed onto HP-20 polystyrene beads and the polar, semi-polar, and non-polar organic metabolites sequentially eluted with increasing concentrations of aqueous acetone. Both the 20% and 30% aqueous acetone fractions eluted from the HP-20 column were concentrated under reduced pressure and chromatographed on a C-18 Sep-Pak® (MeOH/H_2O/0.5%TFA). Gradient reversed phase HPLC (MeOH/H_2O/2% $NH_{3(aq)}$) of selected Sep-Pak® fractions yielded *N*-1-β-D-ribofuranosyldamirone C (**1**, 5.8 mg),[15] *N*-1-β-D-ribofuranosylmakaluvamine I (**2**, 1.9 mg),[16] damirone C (**3**, 5.8 mg),[10] makaluvamine I (**4**, 3.0 mg),[10] and makaluvamine M (**5**, 6.9 mg).[10]



Keywords: *Strongylodesma aliwaliensis*; Sponge; Pyrroloquinoline; D-Ribofuranose; *N*-1-β-D-Ribofuranosyldamirone C; *N*-1-β-D-Ribofuranosylmakaluvamine I.
* Corresponding author. Tel.: +27 46 603 8264; fax: +27 46 622 5109; e-mail: m.davies-coleman@ru.ac.za

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for compounds **1** and **2** (DMSO-d_6)

Position	Compound **1**				Compound **2**		
	δ_C	δ_H	J (mult., Hz)	$^{1}J_{C,H}$ (Hz)	δ_C	δ_H	J (mult., Hz)
2	124.3	7.55	s	192	123.8	7.45	s
2a	117.0	—	—	—	116.8	—	—
3	19.1	2.73	br t (6.8), 2H	132	18.2	2.61	br t (6.8), 2H
4	41.1	3.50	br t (7.0), 2H	141	49.3	3.92	br t (7.0), 2H
NH-5	—	8.31	br s	—	—	[a]	—
5a	153.8	—	—	—	155.7	—	—
6	92.4	5.07	s	161	99.0	5.72	s
7	171.3	—	—	—	[b]	—	—
8	177.4	—	—	—	171.9	—	—
8a	124.8	—	—	—	122.2	—	—
8b	124.4	—	—	—	121.8	—	—
1′	89.6	6.23	d (5.2)	169	89.4	6.29	d (5.3)
2′	75.8	4.11	br t (4.6)	149	75.7	4.13	br t (4.7)
3′	70.7	4.03	br t (4.3)	149	70.3	4.03	br t (4.5)
4′	85.1	3.89	br q (3.7)	148	85.0	3.88	Mult.
5′	61.2	3.55	br d (11.6)	140	61.3	3.57	Mult.
		3.67	br d (11.7)	141		3.63	Mult.
OH-2′	—	5.38	br s	—	—	[a]	—
OH-3′	—	5.14	br s	—	—	[a]	—
OH-5′	—	5.03	br s	—	—	[a]	—

[a] Not observed.
[b] Not observed in DMSO-d_6 but present in CD_3OD.[16]

The molecular formula of **1**, established as $C_{15}H_{16}N_2O_6$ from HRFABMS data,[15] was supported by the 15 ^{13}C and 16 ^{1}H resonances observed in the respective ^{13}C and ^{1}H NMR spectra of **1** (Table 1). The presence of six methines, three methylenes, and six quaternary carbons in **1** was evident from analysis of the ^{13}C and DEPT-135 data while further examination of correlations in the gHSQC spectrum of **1** tentatively identified the protonated carbons as an allylic methylene (δ_C 19.1, δ_H 2.73, 2H); an amino methylene (δ_C 41.1, δ_H 3.50, 2H); an oxymethylene (δ_C 61.2, δ_H 3.67, 3.55), three oxymethines (δ_C 85.1, δ_H 3.89; δ_C 75.8, δ_H 4.11; δ_C 70.7, δ_H 4.03), a glycosidic anomeric methine (δ_C 89.6, δ_H 6.23), a conjugated olefinic methine (δ_C 92.4, δ_H 5.07), and one aromatic methine (δ_C 124.3, δ_H 7.55). Four exchangeable protons were also observed (δ_H 8.31, 5.38, 5.14, 5.03) (Table 1). Two αβ-unsaturated carbonyl resonances (δ_C 177.4, 171.3) and the six olefinic carbon resonances (δ_C 153.8, 124.8, 124.4, 124.3, 117.0, 92.4) accounted for five of the nine degrees of unsaturation implied by the molecular formula of **1** and thus required **1** to be tetracyclic.

The co-occurrence of the known pyrroloquinoline metabolites **3**–**5** in the *S. aliwaliensis* extract suggested that a tricyclic pyrroliquinoline structural motif accounted for three of the rings in the tetracyclic structure of **1**. The definitive HMBC correlations illustrated in Figure 1 and comparison of the ^{1}H and ^{13}C chemical shifts of **1** with those of **3**[10] confirmed the inclusion of a damirone C nucleus in the structure of **1**. A contiguous coupling sequence involving the three oxymethine and the oxymethylene protons, evident in the COSY spectrum of **1**, suggested that a pentose ring was the fourth ring in the tetracyclic structure of this compound. The ^{5}J W-coupling between H-1′ (δ_H 6.23) and H-4′ (δ_H 3.89) observed in the COSY spectrum and reciprocal HMBC correlations between H-1′ and C-4′ (δ_C 85.1), and H-4′ and C-1′ (δ_C 89.6), enabled the assignment of a furanose structure to the pentose sugar moiety (Fig. 1). Additional ^{5}J COSY and ^{3}J HMBC correlations between the anomeric proton (δ_H 6.23) of the pentofuranose ring and H-2 (δ_H 7.55) and C-2 (δ_C 124.3) respectively, of the damirone C nucleus unequivocally positioned the pentafuranose ring at N-1 (Fig. 1).

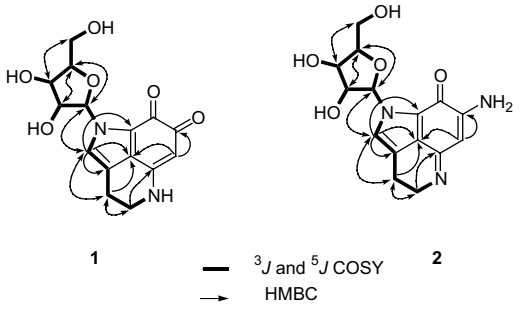

Figure 1. Key COSY and HMBC correlations observed for compounds **1** and **2**.

The molecular formula of **2** ($C_{15}H_{17}N_3O_5$) established from HRFABMS data also implied nine degrees of unsaturation for this compound.[16] A comparison of the ^{1}H and ^{13}C data of **2** with those of **1** limited the major difference between these two compounds to rings A and B, suggestive of the replacement of the damirone C skeleton with a makaluvamine I skeleton. The HMBC and COSY data for this compound (Fig. 1) not only confirmed the presence of a makaluvamine I skeleton in **2**, but also positioned the pentose moiety at N-1 in this compound as described previously for **1**.

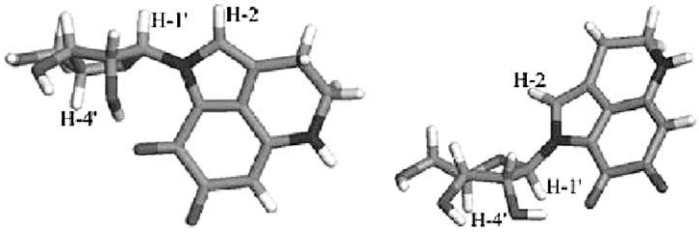

Figure 2. The molecular dynamics minimized conformations of *N*-1-α-D-ribofuranosyldamirone C (left) and *N*-1-β-D-ribofuranosyldamirone C (right).[21]

The conformational flexibility of the furanose ring and extensive ^{1}H NMR signal overlap within the oxymethine envelope frequently hampers the use of $^{3}J_{H,H}$ coupling constants as a means of identifying individual pentose sugars.[17] During the isolation of **1** and **2** we observed that these two compounds underwent significant hydrolysis on prolonged exposure to mildly acidic (0.5% TFA) chromatography solvents. Consequently, acid hydrolysis (1 M TFA) of each compound yielded a mixture of products that, from ^{1}H NMR analysis, included the free pentose sugar. Using McGinnis' GC method[18] we were able to separate the peracetylated aldononitrile derivatives of authentic samples of each of the four aldopentoses; ribose (t_R 4.53 min), xylose (t_R 5.87 min), lyxose (t_R 4.91 min), and arabinose (t_R 5.30 min) on a DB-225 capillary column (225 °C). Similar derivatization and GC analysis of the hydrolysates of **1** and **2** both yielded peaks with a retention time of 4.47 min, thus confirming the ribose identity of the pentose moiety in both these compounds. Separation of the peracetylated aldononitrile derivatives of authentic D- and L-ribose was achieved on a Chirasil-VaL chiral GC column (t_R 19.56 and 19.93 min, respectively).[19] The retention times of the derivatized ribose from hydrolysis of **1** and **2** (19.59 and 19.67 min, respectively) thus established a D-stereochemistry for the ribofuranose moiety in **1** and **2**. The identities of the peaks attributed to the peracetylated aldononitrile derivatives of D-ribose in the hydrosylates were further confirmed by GC–MS.[20]

NOE data have routinely been used to establish the orientation of the glycosidic bond in marine metabolites containing a ribofuranose unit.[22] The β-glycosidic linkage of the D-ribofuranose to the pyrroloquinoline skeleton in **1** and **2** was accordingly determined from the NOESY correlation observed between H-1′ and H-4′ in the NOESY spectra of both compounds. These NOESY data implied a *cis* relationship between H-1′ and H-4′ and required a β-orientation of the pyrroquinoline substituent at the anomeric carbon in **1** and **2**. The *trans* and *cis* orientation of H-1′ and H-4′ are clearly evident in the respective molecular dynamics minimized conformations of *N*-1-α-D-ribofuranosyldamirone C and *N*-1-β-D-ribofuranosyldamirone C presented in Figure 2.

Compounds **1** and **2** are the first known examples of pyrroloquinoline *N*-glycosides. The reversed phase chromatography of pyrroloquinolines is often enhanced by the addition of small amounts of TFA to the aqueous chromatography solvent and it is possible that the presence of these compounds in other sponge extracts may have been missed because of facile acid hydrolysis during chromatographic workup. The biological activity of **1** and **2** is currently under investigation.

Acknowledgements

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15. *N*-1-β-D-Ribofuranosyldamirone C (**1**), scarlet/red amorphous solid; $[\alpha]_D^{14}$ +5.0 (*c* 0.058, MeOH); UV (MeOH) λ_{max} 526 (ε 650), 334 (ε 7990), 246 (ε 14,100) nm; ^{13}C NMR (100 MHz, CD_3OD): 179.6 (C-7), 173.1 (C-8), 158.3 (C-5a), 126.3 (C-8b), 126.1 (C-2), 125.6 (C-8a), 118.9 (C-2a), 93.6 (C-6), 92.5 (C-1′), 86.2 (C-4′), 77.1 (C-2′), 71.1 (C-3′), 62.4 (C-5′), 43.1 (C-4), 20.2 (C-3); ^1H NMR (400 MHz, CD_3OD): 7.55 (s, H-2), 6.30 (dd 7.6, 4.2, H-1′), 5.27 (s, H-6), 4.20 (mult., H-3′), 4.19 (mult., H-2′), 4.05 (mult., H-4′), 3.88 (dd, 12.3, 3.0, H-5′a), 3.77 (dd, 12.3, 3.8, H-5′b), 3.64 (t, 7.1, 2H, H-4), 2.83 (t, 7.0, 2H, H-3); HRFABMS $[M+1]^+$ 321.1087 (calcd for $C_{15}H_{17}N_2O_6$ 321.1087).
16. *N*-1-β-D-Ribofuranosylmakaluvamine I (**2**), orange/brown amorphous solid; $[\alpha]_D^{14}$ +5.3 (*c* 0.19, MeOH); UV (MeOH) λ_{max} 532 (ε 190), 342 (ε 4110), 242 (ε 7815) nm; ^{13}C NMR (100 MHz, CD_3OD): 169.5 (C-8), 159.6 (C-5a), 157.8 (C-7), 127.7 (C-2), 124.5 (C-8a), 120.5 (C-8b), 120.1 (C-2a), 92.6 (C-1′), 87.9 (C-6), 86.7 (C-4′), 78.0 (C-2′), 71.3 (C-3′), 62.6 (C-5′), 43.6 (C-4), 19.5 (C-3); ^1H NMR (400 MHz, CD_3OD): 7.72 (s, H-2), 6.32 (d, 3.5, H-1′), 5.67 (s, H-6), 4.33 (mult., 2H, H-2′ and H-3′), 4.08 (br q, 4.0, H-4′), 3.88 (d, 12.8, H-5′a), 3.83 (mult., 2H, H-4), 3.78 (d, 12.8, H-5′b), 2.93 (t, 7.2, 2H, H-3); HRFABMS $[M+1]^+$ 320.1247 (calcd for $C_{15}H_{18}N_3O_5$ 320.1248).
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19. Temperature held at 100 °C for 1 min, ramped to 180 °C at 4 °C/min, held at 180 °C for 2 min.
20. *m/z* M^+ (%): 316 (3.0), 242 (42), 217 (7.3), 200 (17.7), 140 (24.3), 115 (100.0), 43 (73.6).
21. Molecular dynamics calculations were performed using Accelrys *Cerius*^{2} software from Molecular Simulations Inc., San Diego CA, 1999, results of the calculations were visualized using Accelrys Materials Studio.
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