Trachycladines A and B: 2′-*C*-Methyl-5′-deoxyribofuranosyl Nucleosides from the Marine Sponge *Trachycladus laevispirulifer*

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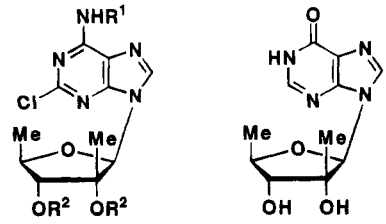
Nucleoside natural products are important chemical models for drug discovery and therapeutic intervention in human diseases including cancer, fungal infections, and viral infections related to human immunodeficiency viruses (HIV's). Recent reviews by Isono[1,2] list more than 200 known naturally occurring nucleoside antibiotics including several highly modified nucleosides isolated from marine invertebrates, such as sponges and algae.[1-3] We now describe two novel cytotoxic nucleosides trachycladine A (9-(2′-*C*-methyl-5′-deoxy-β-D-ribofuranosyl)-2-chloroadenine, **1**) and trachycladine B (9-(2′-*C*-methyl-5′-deoxy-β-D-ribofuranosyl)-2-hypoxanthine, **2**) that occur in the sponge *Trachycladus laevispirulifer* Carter (Order Axinellida, Family Trachycladidae) from Exmouth Gulf, Western Australia. Both nucleosides possess an undescribed branched 5-deoxy-2-*C*-methyl sugar, and **1** is an analog of 2-chloro-2′-deoxyadenosine which has demonstrated remarkable clinical activity against hairy cell leukemia.[4-6] Chloroadenosine analogs are rare in nature, having been found previously only in *Streptomyces* sp.[7,8]

The methanol extract of *T. laevispirulifer* (84.5 g) was separated by modified Kupchan solvent partition,[9] silica gel flash chromatography ($CHCl_3$:MeOH mixtures) and reverse-phase chromatography (C_{18} MeOH/H_2O 1:1) to afford **1** as an amorphous solid (149.9 mg, 0.18%). Other fractions from the silica gel column contained a complex mixture of nucleosides which was separated by chromatography on Sephadex LH-20 and reversed phase HPLC to yield **2** as an amorphous solid (0.3 mg, 0.0004%).

The formula of **1**, $C_{11}H_{14}ClN_5O_3$, was determined from FAB and electrospray mass spectra (MH^+ *m*/*z* 300/302, 3:1 ratio; HRFABMS *m*/*z* 300.0862, Δmmu 0.1) and revealed an unsaturation number of seven. Examination of the ^1H and ^{13}C NMR spectra of **1** in d_6-DMSO (Table 1), together with HETCOR and COLOC experiments, suggested a nucleoside structure. The base was identified as a C-2 substituted adenine by observation of strong UV absorption at 264 nm (ε 14 200), the presence of a broad NH_2 signal (δ 7.83, 2H) and sharp singlet (δ 8.18, s, H-8) in the ^1H NMR spectrum, long-range ^1H–^{13}C COLOC correlations (Table 1) and comparison of ^{13}C NMR chemical shifts with spongosine.[10] The chlorine substituent was located at C-2 of the adenine base in **1** by observation of an intense 2-chloroadenine fragment ion signal in the electrospray mass spectrum (*m*/*z* 170/172, 35%), absence of an H-2 adenine ^1H NMR signal, and comparison of ^{13}C NMR aromatic signals with those of authentic 2-chloroadenosine ($\Delta\delta_C < 0.4$). Exhaustive acetylation (Ac_2O, py, DMAP) of **1** gave a triacetyl derivative **3**, $C_{17}H_{20}ClN_5O_6$ (FABMS MH^+ 426.1196, Δmmu 1.6), confirming the presence of two free hydroxyl groups in the glycoside moiety. The 2-chloroadenine base accounted for all the sp^2 carbons in **1**; hence, the balance of the unsaturation number required a monocyclic sugar.



1 $R^1 = R^2 = H$ **2**

3 $R^1 = R^2 = Ac$

5 $R^1 = H$, $R^2 = C(CH_3)_2$

The structure of the sugar moiety in **1** was shown to be the unprecedented branched chain furanose 2-*C*-methyl-D-5-deoxyribose. The ^1H NMR spectrum (d_6-DMSO) of **1** revealed a linear, coupled four-spin system and an isolated anomeric proton singlet (δ 5.80, s, H-1′). Absence of vicinal coupling to H-1′ suggested that C-2′ was a quaternary sp^3 carbon. A COSY experiment showed a methyl doublet (δ 1.36, d, $J = 6.0$ Hz, 3H, H-5′) coupled only to the first of two mutually coupled vicinal oxymethine protons (δ 3.92, dq, $J = 8.7$, 6.0 Hz, 1H, H-4′; 3.81, dd, $J = 8.7$, 6.8 Hz, 1H, H-3′), the latter of which was further coupled to an exchangeable hydroxyl proton (δ 5.25, d, $J = 6.8$ Hz, 3′-OH). Since only the chemical shift of H-3′ moved significantly downfield (Δδ 1.55 ppm) upon acetylation to give **3**, trachycladine A (**1**) is a vicinal 2′,3′-diol with a C-2′ tertiary hydroxyl group. Long range ^1H–^{13}C correlations (COLOC) were observed from H-1′ to C-2′ and C-3′, thus connecting the two ^1H spin systems. Remaining COLOC correlations were entirely consistent with a 2′-*C*-Me-5-deoxy-ribofuranose.

The relative and absolute stereochemistries of **1** were established as follows. Conversion of **1** to the acetonide **5** (2,2-dimethoxypropane, *p*-TSA, 60 °C, 21 h) established a *cis*-2′,3′-diol while the *cis* relationship of H-1′ and H-4′ was revealed by a 6% NOE of H-4′ upon irradiation of the H-1′ ^1H NMR signal. Two furanose ring twist conformations, $^2T_3$ and $^3T_2$, are commonly observed in furanosyl nucleosides.[11,12] The H-3′, H-4′ coupling constant found in **1** ($J = 8.7$ Hz) is only consistent with *trans* methyl (C-5′) and C-4′ hydroxyl in a $^3T_2$ ring which is further supported by NOE measurements. The 2′-*C*-Me is pseudoequatorial and β relative to the sugar ring as evidenced by its relatively high field ^1H NMR chemical shift (δ 0.79, s, CH_3) due to diatropic shielding of the 2′β-

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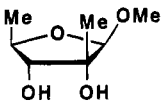
Table 1. ^1H and ^{13}C NMR Data for Trachycladine A (1)[a]

position	^{13}C NMR δ (mult)	^1H NMR δ (mult, J Hz, integ)	COLOC correlations
2	156.8 (s)		
4	149.9 (s)		
5	118.1 (s)		
6	153.0 (s)		
8	139.9 (d)	8.18 (s, 1H)	C-4, C-5
6-NH_2		7.83 (s, 2H)	C-5
1′	91.3 (d)	5.80 (s, 1H)	C-4, C-8, C-2′/C-3′
2′	78.4 (s)		
3′	78.5 (d)	3.81 (dd, 8.7, 6.8, 1H)	
4′	77.6 (d)	3.92 (dq, 8.7, 6.0, 1H)	
5′	17.4 (q)	1.36 (d, 6.0, 3H)	C-4′
2′-CH_3	20.2 (q)	0.79 (s, 3H)	C-1′, C-2′/C-3′
2′-OH		5.16 (s, 1H)	
3′-OH		5.25 (d, 6.8, 1H)	

[a] Recorded in $(CD_3)_2SO$ at 300 MHz (^1H) and 75.4 MHz (^{13}C). COLOC optimized for $^{2-3}J_{CH}$ 10 Hz.

C-methyl group by the *exo* purine ring as also seen in synthetic 2′-*C*-methyl sangivamycin.[13] Pronounced negative Cotton effects seen in the CD spectrum of **1** (λ 229 nm, Δε −0.67; 260, −1.62) are characteristic of β-purin-9-yl-D-ribofuranosides.[14] Since the relative stereochemistry and orientation of the 2-chloroadenine base **1** are established, the CD data places the sugar in the D-ribose series, as depicted.

Methanolysis of **1** (0.5 M HCl, MeOH, sealed tube 60 °C, 24 h) furnished the novel volatile crystalline β-*O*-methyl glycoside **4** ($C_7H_{14}O_4$, $[\alpha]_D$ −127°, sublimes < 40°, *ca.* 12 mmHg). The 1-β configuration at the anomeric carbon of **4** follows from the specific rotation of **4** ($[\alpha]_D$ −127°, $CHCl_3$). Angyal has found, from measurements of many examples, that the anomeric configuration in 1-*O*Me-D-furanosides correlates with the sign of rotation.[15] Empirically, β-OMe-furanosides (1*R*) are levorotatory and α-OMe-furanosides (1*S*) are dextrorotatory in $CHCl_3$. Since we know **4** is a D-sugar and is strongly levorotatory, it follows the anomeric configuration must be β.



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Comparison of the formula of trachycladine B (**2**), $C_{11}H_{14}N_4O_4$, (HRFABMS, MH^+ *m/z* 267.1100, Δmmu 0.7) with that of **1** showed replacement of chlorine by hydrogen and substitution of the elements of NH_2 by OH. Compound **2** retained the 2-*C*-methyl-D-5-deoxyribose sugar as shown by the ^1H NMR spectrum of **2** in d_4-MeOH, however, the base portion now contained two aromatic protons (δ 8.04, s, 1H; 8.05, s, 1H) consistent with hypoxanthine. The presence of hypoxanthine in **2** was supported by the UV spectrum (λ 249 nm), the formula and ^1H NMR data, and confirmed by degradation. Methanolysis of **2** (0.5 M HCl, MeOH, sealed tube 60 °C, 24 h) followed by analytical HPLC gave a single UV active peak identical with hypoxanthine obtained by methanolysis of (−)-inosine (retention time and UV spectrum, diode array detection). The small amount of **2** available precluded further characterization.

Although isolated from a sponge, trachycladine A (**1**) bears structural similarity to natural products from soil-born actinomycetes. The only other known halogenated nucleosides are 2-chloroadenosine[8] and its two 5′-*O*-sulfamoyl derivatives, AT-265[8] and ascamycin (all from *Streptomyces* sp.[7,8]), adechlorin (a 2′-chlororiboside from *Actinomadura* sp.[16]), the 4′-fluororiboside, nucleocidin (a rare fluorinated natural product from *Streptomyces calvus*[17,18]), and an iodopyrrolopyrimidine (from the alga *Hypnea valendiae*[19]). 2-Chloroadenosine is a potent agonist of the A2 adenine receptor[20] while synthetic 2-chloro-2′-deoxyadenosine has been successful in recent clinical trials against hairy cell leukemia, frequently producing lasting remission after a single dose.[4-6] Trachycladine A (**1**) showed *in vitro* cytotoxicity against several human cells lines including leukemia CCRF-CEM (IC_{50} 0.4 μg.mL^{-1}), colon tumor HCT-116 (IC_{50} 0.9 μg mL^{-1}), breast tumors MCF-7 (IC_{50} 0.2 μg mL^{-1}), MDA-MB-435 (IC_{50} 0.25 μg mL^{-1}), and MDA-N (IC_{50} 0.1 μg mL^{-1}), but was inactive against some yeasts (*Candida albicans*, *Saccharomyces carlsbergensis*) and bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) in a disk diffusion assay at 200 μg/disk. Compound **1** also exhibited moderate toxicity to brine shrimp (LD_{50} 0.26 mg.mL^{-1}).[21] Insufficient amounts of **2** were available for biological testing.

Experimental Section

General Procedures. NMR spectra were recorded at 300 MHz for ^1H and 75.4 MHz for ^{13}C. ^1H NMR and ^{13}C NMR spectra are referenced to solvent signals at 7.26 and 77.0 ppm for $CDCl_3$, 3.30 and 49.0 ppm for CD_3OD, and 2.49 and 39.5 ppm for $(CD_3)_2SO$, respectively. Mass spectra were provided by the Facility for Advanced Instrumentation, University of California, Davis, and by the University of Minnesota Chemistry Department Mass Spectrometry Service Laboratory. Other general experimental procedures are listed elsewhere.[22]

Sponge Collection. The brown-orange sponge *T. laevispirulifer* Carter (Order Axinellida, Family Trachycladidae) (93−07−078) was collected in January 1993 by hand using SCUBA at a depth of −10 m at Exmouth Gulf, Western Australia. The animals were immediately frozen at −20 °C until required. The sponge was identified by M. K. Harper (Scripps Institution of Oceanography, University of California, San Diego). A voucher specimen is archived at the Department of Chemistry, University of California, Davis.

Sponge Extraction and Isolation of 1 and 2. Lyophilized animals (84.5 g) were extracted with MeOH (3 × 1 L) and filtered. The extracts were combined, concentrated to approximately 300 mL, and successively extracted using a modified Kupchan partition as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v H_2O), CCl_4 (20%), and $CHCl_3$ (40%). The aqueous phase was concentrated to remove MeOH then extracted with *n*-BuOH. The $CHCl_3$ (771 mg) and *n*-BuOH (1.36 g) extracts were separately fractionated by flash chromatography

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on silica gel (stepwise gradient elution, $CHCl_3$/MeOH 98:2 to 100% MeOH), followed by reversed phase chromatography (C_{18} silica cartridge, MeOH/H_2O 1:1) to afford trachycladine A (**1**) as a pale yellow amorphous solid (149.9 mg, 0.177% dry weight of animal). Fractions from the *n*-BuOH extract not containing **1** were pooled and further purified by gel filtration chromatography (sephadex LH-20, MeOH) and reversed phase HPLC (Microsorb C_{18} column, gradient elution MeOH/H_2O 10:90 to 50:50) to afford trachycladine B (**2**) as a colorless amorphous solid (0.3 mg, 0.0004%).

Trachycladine A (1): $C_{11}H_{14}ClN_5O_3$; $[\alpha]_D$ −19.6° (*c* 0.41 MeOH); UV (MeOH) λ_{max} 264 nm (ϵ 14 200); CD (MeOH) 229 nm ($\Delta\epsilon$ −0.67), 260 (−1.62); IR (film) υ_{max} 3325 cm^{-1}, 3185, 1645, 1595, 1310, 1065; ^1H and ^{13}C NMR (d_6-DMSO) see Table 1; ^1H NMR (CD_3OD) δ 0.93 (s, 3H), 1.47 (d, J = 6.1 Hz, 3H), 3.88 (d, J = 8.7 Hz, 1H), 4.07 (dq, J = 8.7, 6.1 Hz, 1H), 5.93 (s, 1H), 8.07 (s, 1H); MS (electrospray) *m*/*z* 300 (MH^+, 100%), 170 (2-Cl-adenine + H^+, 35); HRMS (FAB) found 300.0862 (MH^+), $C_{11}H_{15}ClN_5O_3$ requires 300.0863.

Trachycladine B (2): $C_{11}H_{14}N_4O_4$; UV (MeOH) λ_{max} 249 nm (ϵ 15 000); ^1H NMR (CD_3OD) δ 0.92 (s, 3H), 1.47 (d, J = 6.2 Hz, 3H), 3.79 (d, J = 8.7 Hz, 1H), 4.08 (dq, J = 8.7, 6.2 Hz, 1H), 6.02 (s, 1H), 8.04 (s, 1H), 8.05 (s, 1H); MS (FAB) *m*/*z* 267 (MH^+, 13%); HRMS (FAB) found 267.1100 (MH^+), $C_{11}H_{15}N_4O_4$ requires 267.1093.

Preparation of Triacetyl Derivative 3. Acetylation of trachycladine A (**1**, 2.4 mg) under standard conditions (pyridine, acetic anhydride, DMAP, 18 h) afforded, after flash chromatography (silica gel, $CHCl_3$/MeOH 95:5), the triacetyl derivative **3** (1.5 mg, 44%), and the diacetyl derivative (1.5 mg).

Triacetyl derivative 3: $C_{17}H_{20}ClN_5O_6$; ^1H NMR ($CDCl_3$) δ 1.38 (s, 3H), 1.55 (d, J = 6.4 Hz, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 2.68 (s, 3H), 4.22 (qd, J = 6.4, 5.3 Hz, 1H), 5.36 (d, J = 5.3 Hz, 1H), 6.38 (s, 1H), 8.09 (s, 1H), 8.50 (bs, 1H); MS (FAB) *m*/*z* 426 (MH^+, 16%), 215 (100); HRMS (FAB) found 426.1196 (MH^+), $C_{17}H_{21}ClN_5O_6$ requires 426.1180.

Diacetyl derivative: $C_{15}H_{18}ClN_5O_5$; ^1H NMR ($CDCl_3$) δ 1.39 (s, 3H), 1.55 (d, J = 6.4 Hz, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 4.20 (qd, J = 6.4, 5.3 Hz, 1H), 5.37 (d, J = 5.3 Hz, 1H), 5.80 (bs, 2H), 6.34 (s, 1H), 7.94 (s, 1H); MS (FAB) *m*/*z* 384 (MH^+, 22%), 215 (100); HRMS (FAB) found 384.1049 (MH^+), $C_{15}H_{19}ClN_5O_5$ requires 384.1075.

Methanolysis of 1: 1β-*O*-Methyl-2β-*C*-methyl-5-deoxy-D-ribofuranose (4). A solution of **1** (10.0 mg, 0.033 mmol) in 0.5 M HCl/MeOH (2.0 mL) was heated at 60 °C in a sealed tube for 24 h. Methanol was removed under a stream of nitrogen and the residue chromatographed on silica gel ($CHCl_3$/MeOH 99:1 then 95:5) to afford 1β-*O*-methyl-2β-*C*-methyl-5-deoxy-D-ribofuranose (**4**, 2.6 mg, 48%): $C_7H_{14}O_4$; $[\alpha]_D$ −127° (*c* 0.15 $CHCl_3$); IR (film) υ_{max} 3395 (br) cm^{-1}, 2970, 2930, 1100, 1060, 1010, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (s, 3H), 1.35 (d, J = 6.3 Hz, 3H), 2.20 (br s, 2H), 3.37 (s, 3H), 3.63 (d, J = 6.8 Hz, 1H), 3.93 (dq, J = 6.8, 6.3 Hz, 1H), 4.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.2 (q), 20.6 (q), 50.0 (q), 79.1 (s), 79.3 (d), 81.0 (d), 108.7 (d); MS (CI) *m*/*z* 180 ($M + NH_4^+$, 100%), 163 (MH^+, 6); HRMS (CI) found *m*/*z* 163.0970 (MH^+), $C_7H_{15}O_4$ requires 163.0970.

Preparation of Acetonide Derivative 5. A suspension of **1** (7.0 mg, 0.023 mmol) in 2,2-dimethoxypropane (2.0 mL) and *p*-TsOH (crystal) were heated to 60 °C for 21 h. The mixture was concentrated under reduced pressure and chromatographed on silica gel (EtOAc) to afford the *acetonide* **5** (2.7 mg, 34%): $C_{14}H_{18}ClN_5O_3$; $[\alpha]_D$ −23° (*c* 0.24 MeOH); UV (MeOH) λ_{max} 264 nm (ϵ 11 800); IR (film) υ_{max} 3360 cm^{-1}, 3310, 3175, 2975, 2930, 1650, 1585, 1575, 1305; ^1H NMR ($CDCl_3$) δ 0.93 (s, 3H), 1.47 (d, J = 6.1 Hz, 3H), 3.88 (d, J = 8.7 Hz, 1H), 4.07 (dq, J = 8.7, 6.1 Hz, 1H), 5.93 (s, 1H), 8.07 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.6 (q), 20.0 (q), 27.2 (q), 28.3 (q), 79.3 (d), 89.3 (s), 90.1 (d), 91.9 (d), 114.7 (s), 118.6 (s), 139.1 (d), 150.8 (s), 154.5 (s), 155.9 (s); MS (FAB) *m*/*z* 340 (MH^+, 10%); HRMS (FAB) found 340.1166 (MH^+), $C_{14}H_{19}{}^{35}ClN_5O_3$ requires 340.1176.

Comparison of Methanolysis Products of 1 and (−)-Inosine. A solution of **2** (ca. 50 μg), in 0.5 M HCl/MeOH (1.2 mL) was heated at 60 °C in a sealed tube for 24 h. The product was examined by analytical HPLC (Microsorb C_{18} 5 μm, 4.7 × 300 mm, 1 mL min^{-1} MeOH/0.1% aq TFA, 5:95) with diode array detection, to reveal a single UV active peak (rt 5.42 min, λ_{max} 249 nm). Methanolysis of (−)-inosine under similar conditions gave a single product (rt 5.45 min, λ_{max} 249 nm) assigned to hypoxanthine.

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Supplementary Material Available: ^1H NMR spectra of **1**–**5**, ^{13}C NMR spectra of **1**, **4**, and **5**, COSY, HETCOR, and COLOC spectra of **1** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS: see any current masthead page for ordering information.

Note Added in Proof: Since submission of this manuscript, the structure of the nucleoside kumusine was presented from unpublished results (Scheuer, P. J. *J. Nat. Prod.* **1995**, *58*(*3*), 335-353; structure 9). Kumusine was isolated from *Theonella* sp. and appears to be the enantiomer of (−)-trachycladine (**1**).

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