Tubercidin Analogs from the Ascidian *Didemnum voeltzkowi*

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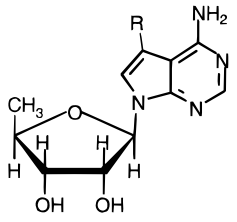
Two new 5′-deoxypyrrolo[2,3-*d*]pyrimidine (7-deazapurine) nucleosides, 5′-deoxytubercidin and 5′-deoxy-3-bromotubercidin, were identified from the ascidian *Didemnum voeltzkowi*. Two known anomers of 5′-deoxy-3-iodotubercidin were also purified from the extract. Assignments were made on the basis of ^{1}H and ^{13}C chemical shifts as well as HPLC−MS experiments.

Marine organisms have proven to be a rich source for a wide variety of modified nucleosides.[1,2] Pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) nucleosides such as the natural product tubercidin have been reported from microorganisms,[3] marine algae, and a marine sponge.[4] Kazlauskas *et al.*, Davies *et al.*, and Phillis *et al.* described the isolation and bioactivity of 5′-deoxy-3-iodotubercidin (**1**) from the red algae *Hypnea valendiae*.[4−6] In the same series of publications, the isolation of the nucleoside base 4-amino-3-bromopyrrolo-[2,3-*d*]pyrimidine from the marine sponge *Echinodictyum* sp. was reported. We report here the isolation of three tubercidin analogs; **1**, 5′-deoxy-3-bromotubercidin (**2**), and 5′-deoxytubercidin (**3**) from the ascidian *Didemnum voeltzkowi* (Savigny, 1816, family *Didemnidae*). To the best of our knowledge, both compounds **2** and **3** are novel compounds.



1 R=I
2 R=Br
3 R=H

The frozen ascidian (400 g wet weight) was extracted with MeOH and the resulting crude extract partitioned according to a modified Kupchan fractionation protocol.[7] The $CHCl_3$-soluble material and the aqueous MeOH were then subjected to C18 VLC (vacuum liquid chromatography) and HPLC. Compounds **1** and **2** were purified from the $CHCl_3$-soluble Kupchan fraction while **3** was purified from the aqueous MeOH soluble fraction. The nucleosides thymidine and uracil were also identified as components of the aqueous MeOH Kupchan fraction.

The presence of pyrrolo[2,3-*d*]pyrimidine deoxyribose nucleosides was initially recognized by a fragment in the FABMS at *m*/*z* 251 in all three tubercidin analogs. The molecular ion of the brominated compound showed the expected M + 2 isotope pattern for a singly brominated molecule, while the iodinated compound contained an M − 126 ion suggesting halogen matrix exchange. The ribose sugar moiety was recognizable in **1** and **3** by characteristic anomeric proton doublets at 5.99 and 6.12 ppm, respectively, in the ^{1}H NMR spectra of the compounds (Table 1). The absence of geminal proton coupling expected for 5′ protons of nucleosides in the ^{1}H NMR of **1** and **3** suggested modification at this site. Doublets at 1.26 and 1.39 ppm that integrate to three protons in the ^{1}H NMR of **1** and **3**, respectively, were consistent with 5′ deoxy sugars.

Table 1. ^{1}H and ^{13}C Assignments of Compounds **1** and **3**

	compd			
	1		**3**	
position	^{13}C[a,b]	^{1}H	^{13}C	^{1}H
2	102.9	7.75, s	123.2	7.23, d (3.7)
3	49.9		101.2	6.64, d (3.7)
4	157.2		158.9	
6	151.9	8.09, s	152.3	8.08, s
8	127.9		104.0	
9	149.5		151.2	
1′	89.3	5.99, d (5.0)	89.7	6.12, d (4.6)
2′	77.9	4.37, dd (5.0,5.2)	75.8	4.42, dd (4.6,5.5)
3′	76.4	3.83, dd (5.3,5.3)	76.5	3.96, dd (5.5,5.5)
4′	81.3	3.87, dd (6.5,5.3)	80.8	4.06, dq (6.4,5.8)
5′	13.2	1.26, d (6.5)	19.2	1.39, d (6.4)

[a] Chemical shift values from spectra collected in CD_3OD. [b] Coupling constants reported in Hz.

The previous publication of **1** from the red algae *Hypnea valendiae* reported the purification of two isomers of the compound. On the basis of J_{H1-H2} coupling constants Wells proposed that the two compounds were anomers. We also have evidence for the presence of two chromatographically distinct isomers of **1** from *Didemnum voeltzkowi*. C18 VLC of the $CHCl_3$ Kupchan fraction yielded a mixture of nucleosides that eluted using CH_3CN/H_2O (30:70). When this material was subjected to C18 HPLC using CH_3CN/0.1 M aqueous NH_4OAc (20:80) two distinct peaks eluted at 16 min and 21 min. The first peak appeared pure by NMR spectroscopy and contained one molecular ion at *m*/*z* 377 in the FABMS. The ^{1}H NMR spectrum of material isolated as the second peak showed slight doubling of all of the signals in the ^{1}H NMR spectrum, while the FABMS showed peaks at *m*/*z* 377 and two peaks of equal intensity at *m*/*z* 327 and 329. This suggested that the sample contained a second isomer of 3-iodo-5′-deoxytubercidin as well as a brominated analog.

HPLC−MS was then employed to further investigate the structure of the brominated compound. Using a modified Buck gradient[9] two major peaks were clearly resolved by HPLC. The thermospray mass spectrum

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of the first HPLC peak contained ions at *m/z* 331, 329, 251, 215, 213, and 134. The *m/z* 331 and 329 peaks corresponded to the molecular ion, and the 251 ion represented loss of a bromine atom with subsequent replacement by a proton. The 215 and 213 pair of ions arose from the loss of a deoxyribose sugar, demonstrating that the molecule is brominated somewhere on the nucleoside base. The mass spectrum of the second HPLC peak contained a molecular ion at *m/z* 377 representing **1** as well as ions corresponding to the same fragmentation pattern as the brominated compound. The ^{1}H NMR spectrum of the mixture contained singlets of different intensities at 7.78 and 7.71 ppm, assignable to C-2 of the pyrrolopyrimidine base. The observation that these signals were singlets was consistent with halogenation on C-3.

Further attempts to separate the compounds involved C18 HPLC with a solvent system of CH_3CN/0.05% aqueous trifluoroacetic acid (20:80) resulted in degradation of the compounds. We were unable to further characterize the brominated analog at this point as no more sample was available.

5′-Deoxytubercidin was initially detected in the aqueous MeOH-soluble Kupchan fraction after it eluted from a C18 VLC column using MeOH/H_2O (40/60). This material was further purified by using C18 HPLC with a solvent system of CH_3CN/0.1 M aqueous NH_4OAc adjusted to pH 5 to yield pure compound **3** (10.2 mg). FABMS gave a molecular ion of *m/z* 251. ^{1}H coupling constants of 3.7 Hz for protons assigned to position 2 and 3 are expected values for the 5-membered aromatic ring. Chemical shifts for ^{1}H and ^{13}C NMR data agree with expected values based on the halogenated analogs.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR experiments were performed on a Varian Unity 500 MHz spectrometer. Spectra were referenced to residual undeuterated solvent peaks or solvent ^{13}C solvent signals. High- and low-resolution FABMS mass measurements were performed on a Finnegan MAT 95 high-resolution gas chromatograph/mass spectrometer. The HPLC thermospray−MS was perfomed on an instrument of in-house design, the details of which have been previously published.[8] The HPLC gradient used in the experiment is a slight modification of the gradient developed by Buck, using procedures that have been previously published.[9,10]

Extraction and Characterization of Nucleosides. The ascidian was collected from isolated rocks scattered throughout shallow tide pools on Apo Reef, Philippines. A voucher specimen has been submitted to the University of the Philippines labeled as GC95-94-10. The frozen sample was ground using a blender and repeatedly extracted with MeOH. The dried extract was resuspended in 200 mL of 90% MeOH−10% H_2O. This solution was extracted with hexane, and then an additional 20 mL of H_2O was added to the aqueous MeOH fraction. The resulting 30% aqueous MeOH fraction was then extracted with $CHCl_3$. The $CHCl_3$-soluble material was then subjected to a C18 VLC column using 100 cm^3 LiChroprep RP18 in a 10 cm diameter column. Fractions were eluted using a step gradient using increasing amounts of CH_3CN. All of the halogenated tubercidin analogs eluted in the CH_3CN/H_2O (70:30) wash.

Reversed-phase HPLC (Rainin Dynamax Microsorb 4.6 × 250 mm 5 μm column) of this fraction using CH_3CN/0.1 M aqueous NH_4OAc adjusted to pH 5 using acetic acid (15:85) yielded pure **1** (2.5 mg) and a mixture of the second anomer of **1** and compound **2** (2.3 mg).

A step gradient C18 VLC column using MeOH/H_2O solutions was performed on the aqueous MeOH Kupchan fraction. 5′-Deoxytubercidin eluted in the 60% MeOH−40% H_2O wash. This material was further purified by C18 HPLC using 15% CH_3CN−85% 0.1 M NH_4OAc adjusted to pH 5 to yield compound **3** (10.2 mg).

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