Pantherinine, a Cytotoxic Aromatic Alkaloid, and 7-Deazainosine from the Ascidian Aplidium pantherinum

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PANTHERININE, A CYTOTOXIC AROMATIC ALKALOID, AND 7-DEAZAINOSINE FROM THE ASCIDIAN *APLIDIUM PANTHERINUM*

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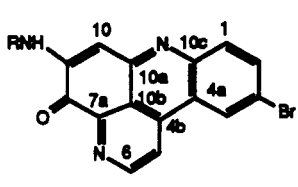
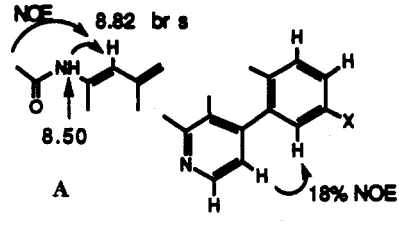
ABSTRACT.—A new cytotoxic aromatic alkaloid, pantherinine [**1**], and a mixture of nucleosides including 7-deazainosine [**3**] have been isolated from the ascidian *Aplidium pantherinum* collected at Stenhouse Bay, South Australia. The structures were determined primarily from ^{1}H- and ^{13}C-nmr data, especially one-bond and multiple-bond proton-carbon correlations. Although 7-deazainosine [**3**] has been known as a synthetic compound for several decades, this appears to be the first report of its isolation as a natural product.

Fused tetracyclic and pentacyclic alkaloids constitute a relatively new class of natural products isolated mostly from ascidians and sponges (1,2). Cytotoxic, antimicrobial, and antiviral activities have been reported for many of these compounds. In our continuing search for bioactive compounds, we have isolated a new cytotoxic polycyclic aromatic alkaloid, designated pantherinine [**1**], and a group of nucleosides, including 7-deazainosine [**3**], from the ascidian *Aplidium pantherinum* (Sluiter), family Polyclinidae, collected at Stenhouse Bay, South Australia.

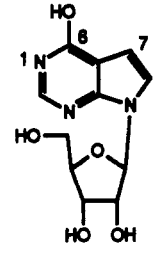
MeOH extracts of freshly thawed *A. pantherinum* were triturated with MeOH. This MeOH-soluble fraction and additional material obtained by $CHCl_3$/MeOH extraction of the specimens were chromatographed separately over LH-20 in MeOH. In each case the early eluting fractions contained mixtures of nucleosides while a purple band eluting later consisted primarily of pantherinine [**1**]. Reversed-phase hplc chromatography of the nucleoside fraction using MeOH-H_2O (90:10) as eluent yielded 7-deazainosine [**3**] and several common nucleosides. Further purification of the purple band with C_{18} hplc using MeOH-H_2O (70:30) as solvent yielded pure pantherinine [**1**].



1 R=H
2 R=Ac



B



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Pantherinine [**1**], a purple powder, was shown by hrfabms to have the mo-

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lecular formula $C_{15}H_8N_3OBr$, corresponding to 13 degrees of unsaturation. An intense ir absorption at 1630 and a ^{13}C-nmr resonance at 179 ppm indicated the presence of one carbonyl group. Because all the ^{13}C-nmr signals appeared between 105 and 179 ppm, it was clear that pantherinine was a polycyclic aromatic alkaloid. The ^1H-nmr spectrum, recorded in DMSO-d_6, revealed signals indicative of a pyridine ring bearing protons on the 2 and 3 positions [δ 9.18, 8.97 (d's, 5.55 Hz)], and a 1,2,4-trisubstituted benzene ring [δ 8.99 (d, 1.8 Hz), 7.96 (dd, 8.8, 1.8 Hz), and 7.87 (d, 8.7 Hz)]. In addition, there was a sharp singlet at δ 6.58 and two broad, exchangeable, singlets at δ 6.60 and 8.46 attributable to NH signals. The chemical shift and coupling data were reminiscent of other marine aromatic alkaloids, such as 2-bromoleptoclinidinone (3,4), the cystodytins (5), and the shermilamines (6,7).

Because of decomposition noted during long standing in solution, and also poor solubility in organic solvents, pantherinine was converted to the monoacetate **2** (red glass) by treatment with Ac_2O/pyridine for further spectral analyses. Mass spectral and ^1H-nmr analyses confirmed the addition of one acetate group. The ^1H-nmr chemical shifts and coupling patterns (Table 1) were consistent with partial structures **A** and **B**. The spatial proximity between the β proton on the pyridine moiety and the isolated proton on the aromatic ring was confirmed by observation of a large nOe (18%) between these signals, when each was irradiated. [Irradiation of H-5 (8.55 ppm) caused some simultaneous irradiation of the amide H (8.50), but this did not cause ambiguity because the location of the acetamide group was independently proven by irradiation of the acetamide methyl resonance.] Cross peaks between these resonances in a NOESY spectrum were also observed. The acetamide group was determined to be proximate to the isolated aromatic proton by the observation of a small nOe enhancement of both the amide proton and the aromatic proton upon irradiation of the acetamide methyl signal.

TABLE 1. Nmr Data for **1** and **2**.

Position	Compound				
	1[a]		**2**[b]		
	^1H(DMSO-d_6)	^{13}C($CDCl_3$/CD_3OD)	^1H($CDCl_3$)	^{13}C($CDCl_3$)	HMBC Correlations
1	7.87 d, 8.7	7.82 d, 8.7	8.12 d, 8.5	132.21	C-3, C-4a, C-10c
2	7.96 dd, 8.8,1.8	7.89 dd, 8.7,2.1	7.98 dd, 8.5,2	135.17	C-4, C-10c
3				122.31	
4	8.99 d, 1.8	8.72 d, 2.1	8.66 d, 2	125.91	C-2, C-4a, C-4b,C-10c
4a				123.37	
4b				136.01	
5	8.97 d, 5.5	8.69 d, 5.7	8.55 d, 5.5	120.75	C-4a, C-6, C-10b
6	9.18 d, 5.5	9.10 d, 5.7	9.29 d, 5.5	150.03	C-4b, C-5, C-7a
7a				144.79	
8				178.32	
9				151.34	
10	6.58 s	6.64 s	8.82	121.78	C-8, C-10b, C-9[c], C-10a[c]
10a				137.06	
10b				116.33	
10c				144.91	
-NH	6.60, 8.46		8.50		
-Me			2.38		
Ac				170.38	

[a]300 MHz.
[b]500 MHz, protonated carbon assignments by HMQC.
[c]Determined by a SINEPT experiment, J=4 Hz.

The remainder of the skeleton was deduced from H/C COSY data (8), obtained from several experiments using different J values (6,8,10 Hz) (Table 1). Two crucial H/C couplings were finally observed only from a selective INEPT (9) experiment using $J=4$ Hz with single-frequency irradiation of H-10 (8.82 ppm). These last correlations confirmed structure **1** and eliminated two alternate structures in which either C-8 or C-9 was protonated. In both the latter cases a correlation between the 8.82 proton and C-7a would have been expected, and in the case of C-8 protonation the observed correlation between this proton and the δ 137.05 carbon (C-10a) would not have been expected.

Although pantherinine [**1**] is structurally similar to a number of other marine aromatic alkaloids, it differs from other brominated members of this series (3,4,6,10) in that bromine is at the 3 rather than at the 2 position. Pantherinine shows mild cytotoxic activity, $ED_{50}=4.5$ μg/ml against P388 murine leukemia cells.

The *A. pantherinum* extracts also yielded a mixture of nucleosides from which a number of common ones, namely 2′-deoxythymidine, uridine, 2′-deoxyuridine, inosine, and 2′-deoxyinosine, were isolated and identified by ^{1}H-nmr analyses. In addition, one novel nucleoside was isolated which exhibited ^{1}H- and ^{13}C-nmr signals related to inosine except that it contained an additional signal for a protonated aromatic carbon. One-bond and multiple-bond H/C COSY experiments confirmed that this nucleoside contained a 7-deazapurine ring. An lrms analysis showed an $[M]^+$ at *m/z* 267, corresponding to the formula $C_{11}H_{13}N_3O_5$, when taking into account the H and C count from nmr data. Since the ^{1}H-nmr spectrum indicated that the sugar moiety was a normal ribose unit, it was concluded that the NH_2 group of the common adenine moiety had been replaced by an OH group in this metabolite, and that the nucleoside was 7-deazainosine [**3**]. This analogue of the naturally occurring inosine has been synthesized (11) and studied for potential biological activity. Comparison of the nmr spectral data of the natural product with that of synthetic material (provided by Dr. Alexander Block, Rosewell Park Cancer Institute, Buffalo, NY) and with literature values (12,13) confirmed that the tunicate nucleoside was indeed 7-deazainosine. This appears to be the first report of **3** as a natural product. The closely related 7-deazapurine nucleoside tubercidin (= 7-deazaadenosine) has been isolated from both a blue-green alga (14) and a *Streptomyces* sp. (15,16). Although tubercidin was found to exhibit cytotoxic activity, 7-deazainosine did not significantly inhibit the growth of murine leukemia cells (P388). Other nucleosides from marine sources that have a substituted pyrrolo[2,3-*d*]pyrimidine base are 5-iodo-5′-deoxytubercidin (17), 5-bromotubercidin aglycone (17), the mycalisines (18), toyocamycin (19), and 5-methoxycarbonyl tubercidin (19).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr data were obtained at 300 and 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C in the solvents specified, using Varian XL 300 and VMR 500 instruments. Ir spectra were obtained on a Bio-Rad 3240-SPC FT instrument and uv spectra on a Perkin-Elmer Lambda 3 uv-vis spectrophotometer. Mass spectra were obtained on a VG ZAB E instrument. Alltech Econosphere 5 μ 10 mm×25 cm columns were used in the hplc separations.

ISOLATION.—*A. pantherinum* was collected at Stenhouse Bay, South Australia, and stored frozen. A voucher specimen, 125-AU-83, is retained at the University of Oklahoma. The specimens were cut and soaked sequentially in MeOH and MeOH-$CHCl_3$ (1:1) to give, after evaporation, two extracts, 100 g (largely salts) and 4 g, respectively. Dry wt of the specimen after extraction was ca. 300 g. The MeOH extract was triturated with MeOH, and the soluble portion of this extract and the MeOH/$CHCl_3$ extract were chromatographed separately over LH-20 using MeOH as eluent. A number of fractions containing nucleosides were obtained, followed by a slow moving, purple fraction. The nucleoside fractions were further re-

solved on C_{18} hplc using MeOH-H_2O (90:10) to give 7-deazainosine [**3**], 2′-deoxythymidine, uridine, 2′-deoxyuridine, inosine, and 2′-deoxyinosine, which were identified by interpretation of ^{1}H-nmr and mass spectra data.

The purple-colored fraction was purified further using C_{18} hplc with MeOH-H_2O (70:30) to give pantherinine [**1**], ca. 4 mg. Pantherinine (ca. 2 mg) was treated with Ac_2O-pyridine (1:1) overnight at room temperature to give pure **2**, after chromatography over Si gel.

Pantherinine [**1**].—Purple powder: ir (film) ca. 3810 (br), 3720 (br), 1665 (w), 1630 (s) cm^{-1}; uv (MeOH) 254 (ε 16,110), 316 (3950), 472 (br, 1620) nm; ^{1}H nmr see Table 1; ^{13}C nmr (DMSO-d_6) (assigned carbons based on analogies with those of **2**) δ 105.6 (C-10), 119.7, 121.2 (C-5), 121.8, 126.5 (C-4), 131.0 (C-1), 134.3 (C-2), 135.3, 135.8, 145.4 (these 3 signals are questionable due to poor S/N), 146.5, 149.8 (C-6), 153.2 (C-9), 179.4; hrms found *m/z* 326.9828 (calcd for $C_{15}H_8N_3OBr^{81}$ $[M]^+$ 326.9831), 324.9831 (calcd for $C_{15}H_8N_3OBr^{79}$ $[M]^+$ 324.9851).

Pantherinine acetate [**2**].—Red glass: ir (film) 3340, 1700 (w), 1655 (s), 1521 (s) cm^{-1}; uv (MeOH) 255 (5794), 293 (2811), 432 (1369) nm; ^{1}H and ^{13}C nmr see Table 1; lrms *m/z* (%) 369 (25), 367 (24), 327 (96), 325 (100), 300 (63), 298 (67).

7-Deazainosine [**3**].—^{1}H nmr (DMSO-d_6) 3.53, 3.61 (AB q, J=3.9, 12, H-5′, H-5′), 3.90 (1H, dd, J=3.6, 3.9, H-4′), 4.10 (1H, br t, J=3.6, H-3′), 4.34 (1H, br t, J=3.6, H-2′), 4.35 (1H, br s, OH-5′), 4.45 (1H, br s, OH-3′), 4.65 (1H, br s, OH-2′), 6.03 (1H, d, J=6.1, H-1′), 6.55 (1H, d, J=3.7, H-7), 7.39 (1H, d, J=3.7, H-8), 7.94 (1H, s, H-2); ^{13}C nmr (DMSO-d_6) 61.88 (br t, J=136, C-5′), 70.86 (br d, J=149.8, C-3′), 74.60 (br d, J=149.2, C-2′), 85.37 (br d, J=132.5, C-4′), 87.21 (d, J=152.7, C-1′) 102.79 (dd, J=175.6, 7.5, C-7), 108.65 (dd, C-5), 121.46 (ddd, J=188.3, 4.5, 7.7, C-8), 144.10 (d, J=203.9, C-2), 148.09 (m, C-4), 158.57 (d, J=6.8, C-6); selective INEPT correlations: proton irradiated [carbon(s) obs.] 7.94 (C-4, C-6), 7.39 (C-4, C-5, C-7), 6.54 (C-4, C-5, C-7), 6.03 (C-4, C-8, C-2′); lrms *m/z* $[M]^+$ 267; thermospray ms *m/z* $[M+H]^+$ 268.

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