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PRIANOSIN A, A NOVEL ANTILEUKEMIC ALKALOID FROM THE OKINAWAN MARINE SPONGE *Prianos melanos*

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Summary: A novel alkaloid, prianosin A (**1**), with potent antineoplastic activity has been isolated from the Okinawan marine sponge *Prianos melanos*. Its absolute stereostructure was determined by single crystal x-ray diffraction analysis.

Marine organisms have been a rich source of compounds with antitumor activity.[1] During our survey of bioactive compounds from Okinawan marine organisms,[2] powerful antileukemia activities were detected in extracts of some sponges. We report here the isolation and structure determination of prianosin A (**1**), a novel alkaloid from the Okinawan marine sponge *Prianos melanos*. Prianosin A (**1**) was active in assays for antileukemia activity and for release of Ca^{2+} from the sarcoplasmic reticulum.[3]

The green sponge *P. melanos* was collected at Motobu Peninsula (-2 to 3 m) of Okinawa Island in June 1986. The methanol-toluene (3/1) extract was partitioned with toluene and water. The chloroform soluble portion of the aqueous phase was partially purified by silica gel column chromatography ($CHCl_3$/MeOH, 95/5) followed by LH-20 ($CHCl_3$/MeOH, 1/1) and silica gel (pet. ether/$CHCl_3$/MeOH, 20/5/1) column chromatographies to give prianosin A (**1**) (0.02%, wet weight). Compound **1** was characterized as its free base: mp>300° C; $[\alpha]_D^{24}$ +248° (c 0.19, $CHCl_3$); IR (KBr) 3700-2500, 1680, 1645, 1610, 1520, 1400, 1330, 1120, 810, and 710 cm^{-1}; UV (MeOH) λ_{max} 248 (ε 17600), 355 (11500), and 430 (sh) nm.

The molecular formula $C_{18}H_{14}O_2N_3BrS$ for **1** was determined by HRFABMS (m/z 416.0053, M^++H, Δ -1.5 mmu). The ^1H NMR spectrum showed 12 nonexchangeable and two exchangeable protons. The ^{13}C NMR spectrum showed 18 carbons and a DEPT experiment confirmed the pres-

ence of 12 protons attached to carbons. The ^{1}H and ^{13}C signals for **1** were assigned by two-dimensional ^{1}H-^{1}H shift correlation and ^{1}H selective decoupling experiments as shown in Table 1. The quaternary carbon chemical shifts for C3, C6, C10, C11, and C19 were assigned by analogy to those (δ 188.6, 44.8, 150.4, 165.5, and 145.1) of compounds[4-7] related to **1**. The reaction of **1** with diazomethane gave the N13-methyl derivative (**2**) [FABMS, m/z 430 (M^{+}+H)]. The ^{1}H NMR spectrum[8] of **2** differed from that of **1** by the addition of the N(13)-methyl group (δ 3.90) and the shift of H14 (δ 6.88 → 6.64) to higher field.

The structure and absolute configuration of prianosin A were established unequivocally by a single crystal x-ray analysis. Prianosin A crystallized from methanol-ethyl acetate (1/2) in space group $P2_12_12_1$ with **a**=7.112(1), **b**=14.404(2), and **c**=20.621(4) Å. All unique diffraction maxima with $2\theta < 114^{o}$ were collected on a computer controlled diffractometer using Cu Kα radiation. A phasing model for the 1535 (92%) observed reflections was found by deconvoluting the Patterson synthesis to locate heavy atoms and Fourier recycling of increasingly larger molecular fragments. The crystallographic residual with anisotropic heavy atoms, fixed hydrogens, and anomalous dispersion corrections for Br and S is 0.039.[9] The enantiomer refined to 0.046. A computer generated perspective view of **1** is given in Figure 1. The molecular parameters are fully consistent with the tautomeric form shown as **1**.[9, 10]

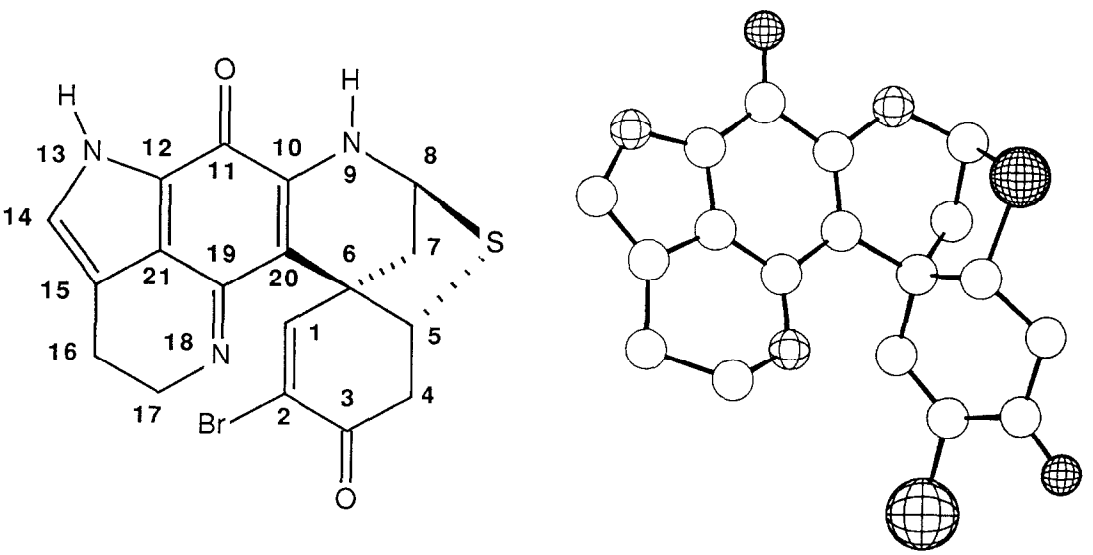

Figure 1. Various structural drawings of prianosin (**1**). On the left is a standard chemical drawing which also has the atomic numbering scheme. On the right is a computer generated perspective drawing of the final x-ray model with absolute configuration. Hydrogens are omitted from the x-ray drawing for clarity.

A plausible biosynthesis of prianosin A (**1**) could involve the α-amino acids tyrosine (C1 to N9) and tryptophan (C10 to C21). The structure of prianosin A (**1**) is closely related to discorhabdin C[5], isolated from a sponge of the genus *Latrunculia*. Prianosin A (**1**) was cytotoxic with IC_{50} values of 37 and 14 ng/mL against L1210 and L5178Y murine leukemia cells *in vitro*. In addition, prianosin A induced Ca^{2+} release from sarcoplasmic reticulum[3] and was 10 times more potent than caffeine in this assay.[11]

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8. **2:** ^{1}H NMR (500 MHz, CDCl3) δ 7.55 (s, 1H, H1), 6.64 (s, 1H, H14), 5.90 (bs, 1H, H9), 5.33 (bs, 1H, H8), 4.68 (dd, 7.5 and 12.2 Hz, 1H, H5), 4.18 (m, 1H, H17), 3.92 (m, 1H, H17), 3.90 (s, 3H, N13-CH3), 2.88 (dd, 12.2 and 17.0, 1H, H4), 2.82 (dd, 7.5 and 17.0, 1H, H4), 2.75 (dd, 3.9 and 11.8, H7), and 2.65 (m, 3H, H7 and H16).

9. All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: FOBS86, by G. Van Duyne, Cornell University, 1986; MULTAN 80 and RANTAN 80, by P. Main, S.E. Hull, L. Lessinger, G. Germain, J.P. Declercq, and M.M. Woolfson, University of York, England, 1980; BLS78A, by K. Hirotsu, and E. Arnold, Cornell University, 1980; PL1PLOT, by G. Van Duyne, Cornell University, 1984; TABLES, by G. Van Duyne, Cornell University, 1986.

10. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. They can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. Please give a complete literature citation when ordering.

11. The minimum effective concentrations of prianosin A and caffeine were 30 and 300 μM respectively.

Table 1. ^{1}H and ^{13}C NMR Data for Prianosin A (**1**). The chemical shifts are on the δ scale. The proton spectra were recorded at 500 MHz in d_6-DMSO. The values in parentheses were recorded at 500 MHz in $CDCl_3$. The carbon spectra were recorded at 22.5 MHz in d_6-DMSO. Carbon chemical shift values marked with an asterisk may be exchanged.

Atom	H Shift	Mult.	J values	C Shift	Mult.
1	7.55 (7.55)	s		156.8	d
2				122.0*	s
3				187.9	s
4	3.00 (2.86)	dd	12.5, 16.4	50.4	t
	2.65 (2.74)	m	6.3, 16.4		
5	4.50 (4.70)	dd	6.3, 12.5	55.5	d
6				49.6	s
7	2.85 (2.86)	dd	3.9, 11.8	39	t
	2.53 (2.74)	d	11.8		
8	5.20 (5.34)	bs	3.9	60.8	d
9-NH	7.30 (5.92)	bs			
10				153.8	s
11				169.5	s
12				121.1*	s
13-NH	12.20 (9.19)	bs			
14	7.00 (6.88)	s		124	d
15				118.6*	s
16 (2H)	2.65 (2.74)	m		17.9	t
17	4.10 (4.25)	dt	6.5, 17.5	45	t
	3.85 (3.97)	dt	9.0, 17.5		
19				141.7	s
20				114.3*	s
21				117.3*	s

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