DIPLAMINE, A CYTOTOXIC POLYAROMATIC ALKALOID FROM THE TUNICATE DIPLOSOMA SP.

Geeta A. Charyulu, Tawnya C. McKee and Chris M. Ireland²
Department of Medicinal Chemistry, College of Pharmacy
University of Utah, Salt Lake City, Utah 84112

Summary: A novel cytotoxic alkaloid, diplamine (3) was isolated from the tunicate *Diplosoma* sp. The structure was established by interpretation of spectral data and chemical analysis.

A wide variety of highly colored alkaloids have recently been reported from invertebrate marine animals, the large majority of which possess a fused tetracyclic heteroaromatic skeleton. Examples include the varamines (1),3 and the cystodytins (2).4 We now wish to report the structure of an additional member of this family, diplamine (3) from a *Diplosoma* sp.5 tunicate collected in the Fiji Islands. Diplamine is cytotoxic towards L1210 murine leukemia cells with an IC_{50} of 2 x $IO^2 \mu g/ml$ and antimicrobial against *E. coli* and *S. aureus*.

A solvent partition of freeze dried tunicate followed by silica gel column flash chromatography (CHCl₃-MeOH; 90/10) yielded diplamine (3, 0.08%) as a burnt orange solid.⁶ The Fast Atom Bomdardment Mass Spectrum (FABMS) of 3 exhibited MH⁺ and MH⁺+2H ions at m/z 366 and 368, indicative of a quinone moiety. The molecular formula $C_{20}H_{19}N_3O_2S$ was assigned by mass measurement of the MH⁺ ion (366.1279 Δ +.3 mmu). Thorough analysis of ¹H and ¹³C NMR data (Table 1)

Table 1 NMR assignments for Diplamine (3) δ CDCl, (relative to CHCl, int. stand.)

atom #	H^{2}	"C	LR ¹ H to ¹³ C corr.
1	8.46 (dd 8.0, 1.4)	122.76	C3, C11a
2	7.92 (ddd 8.0, 8.0, 1.2)	129.63	C11a
3	7.80 (ddd 8.0, 8.0, 1.4)	131.93	C1, C4a, C5
4	8.27 (dd 8.0, 1.2)	118.94	C2, C5
4a	, , ,	121.28	02, 00
4h	• •	136.59	
4b 5	8.35 (d 5.6)	131.75	C4, C10b
3			
6 7a	9.06 (d 5.6)	149.71	C4, C4b, C6, C7a
/a		146.39	
8 9		179.43	
9		143.39	
10		149.51*	
10a		151.70*	
10b		116.90	
11a		145.49	
12	3.73 (br. s.)	30.02	C9, C10, C10a, C12
13	3.73 (br. s.)		
		39.75	C15
14	6.45		
15		170.56	
16	1.91	23.32	C15, C16
S-Me	2.62	17.86	C9

including one bond and multiple bond (J = 10 Hz) ¹H and ¹³C correlation experiments (Table 1) combined with comparison of similar data for 1 and 2 allowed unequivocal assignment of structure 3 to diplamine. This structural assignment was confirmed by oxidative demethylation of varamine A (1, where R=Me) with ceric ammonium nitrate, ⁷ to obtain a product that was identical in every respect to the natural product.

Diplamine is a member of a growing class of alkaloid pigments reported in the last year from both sponges and tunicates. All of these compounds have planar structures and reported cytotoxicity, suggesting they may intercalate into DNA. The varamines and diplamine, which have the same carbon skeleton as the cystodytins, are an order of magnitude more cytotoxic than the cystodytins, indicating that the thio-methyl may also be important in cytotoxicity.

Acknowledgment. This research was supported by NIH grants CA 36622 and CA 01179. We thank the Ministry of Home Affairs, Fiji Islands, and the crew of the Mollie Dean for assistance in obtaining biological specimens; and Dr. Lee K. Roberts for cytotoxicity data. NMR studies were performed on an IBM AF200 spectrometer purchased with funds from the NSF (PCM 8400801). The operation of the spectrometer is supported in part by funds from the University of Utah Cancer Center Core Grant (5P30 CA042014).

References

- 1. Department of Medicinal Chemistry undergraduate research fellow.
- NIH Career Development Awardee, 1987-1992.
- 3. Molinski, T.F.; Ireland, C.M. J. Org. Chem. 1989, in press.
- Kobayashi, J.; Cheng, J.; Walchli, M.K.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. J. Org. Chem. 1988, 53, 1800.
- The tunicate was identified by Dr. Franscoise Monniot, Museum National d'Histoire Naturelle, Paris, France.
- 6. mp. 202-204°C (dec.); IR (neat) v_{max} 3323, 3066, 2984, 2923, 1651, 1600, 1533 cm⁻¹: UV λ_{max} 377 nm (ϵ 5,984), 300 (ϵ 14,722), 263 (ϵ 24,874).
- Jacob, P. III; Callery, P.S.; Shulgin, A.T.; Castagnoli, N. Jr. J. Org. Chem. 1976, 41, 3627.
- Gunawardana, G.P.; Kohomoto, S.; Gunasekera, S.P.;McConnell, O.J.; Koehn, F.E. J. Amer. Chem. Soc. 1988, 110, 4856: Molinski, T.F.; Fahy, E.; Faulkner, D.J.; van Duyne, G.D.; Clardy, J. J. Org. Chem. 1988, 53, 1340: Roll, D.M.; Ireland, C.M.; Lu, H.S.M.; Clardy, J. J. Org. Chem. 1988, 53, 3276.
- Cooray, N.M.; Scheuer, P.J.; Parkanyi, L.; Clardy, J. J. Org. Chem. 1988, 53, 4619: Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y. Tetrahedron Lett. 1988, 29, 3861: Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y. Sasaki, T.; Ohta, T.; Nozoe, S. Tetrahedron Lett. 1988, 29, 1177.

(Received in USA 19 April 1989)