



## ISOLATION OF PERIDININOL, AN ANTI-SPASMODIC CAROTENOID PIGMENT, FROM *ZOANTHUS* SP.

P. S. PARAMESWARAN and C. T. ACHUTHANKUTTY

National Institute of Oceanography, Dona Paula, Goa-403 004.

**A C<sub>37</sub> carotenoid pigment, peridininol, isolated from a marine *Zoanthus* sp. exhibited promising anti-spasmodic activity against nicotine and serotonin in *in vitro* studies using guinea pig ileum. Its purification and structure are presented along with <sup>1</sup>H and <sup>13</sup>C NMR assignments, aided by the 2D experiments.**

### Introduction

The marine invertebrates belonging to the Order Zoanthidae (Phylum: Coelenterata, Class: Anthozoa) are good sources for several unique/novel molecules, exhibiting promising biological and pharmacological properties. A classic example is palytoxin, which is highly-toxic to mice (LD<sub>50</sub> = 0.15 μg/kg) besides being an active cardiovascular agent especially for coronary arteries. Paragrazines, the novel nitrogen-containing pigments with pharmacological properties similar to that of papaverine, zoanthamines (Makoto *et. al.*, 2000), useful for treatment of osteoporosis and different ecdysone analogues are, but, a few examples.

In our preliminary investigation, the acetone extract of *Zoanthus* sp., collected from Goa exhibited promising oxytocic, antifouling and anti-spasmodic activities. The anti-spasmodic activity was observed against nicotine and serotonin-induced spasms in *in vitro* assay using guinea pig ileum (Gonsalves *et. al.*, 2000). The isolation and biological evaluation of the oxytocic agent, 2-deoxy ecdysterone, purified from the chloroform fraction of this extract was reported earlier (Parameswaran *et. al.*, 2001). The 2-deoxy ecdysterone is a known crustacean-moulting hormone and its oxytocic activity is comparable to that of PGF<sub>2</sub> and to oxytocin in *in vitro* assay using guinea pig uterus. The isolation and structure of peridininol, the active anti-spasmodic agent from this animal, are reported in this paper.

### Experimental

Infrared and UV-V is absorption spectra were recorded on SHIMADZU instruments. NMR spectra were recorded BRUKER 400 MHz instrument, at Indian Institute of Science, Bangalore and the mass spectrum was recorded in MICROMASS QUATRO Triple quad MS/MS instrument at Central Drug Research Institute, Lucknow.

The marine carotenoids peridinin, peridininol and fucoxanthin are alkali-labile and hence their extraction and purification were always difficult. For example, the commonly used saponification method is unsuitable as these compounds readily decompose in alkaline medium. Recently, a new method involving acetone-methanol extraction of powdered dry alga followed by chromatography over silica gel column and crystallization have been reported for isolation of these compounds in high yield (Haugan and Jensen, 1989). Extraction of peridininol using acetone, followed by chromatography over sephadex LH-20 (Mobile phase: acetone) and silica gel was found to be very satisfactory (Parameswaran *et al* 2001).

Neoxanthin: R = X  
 Dinoxanthin: R = AC

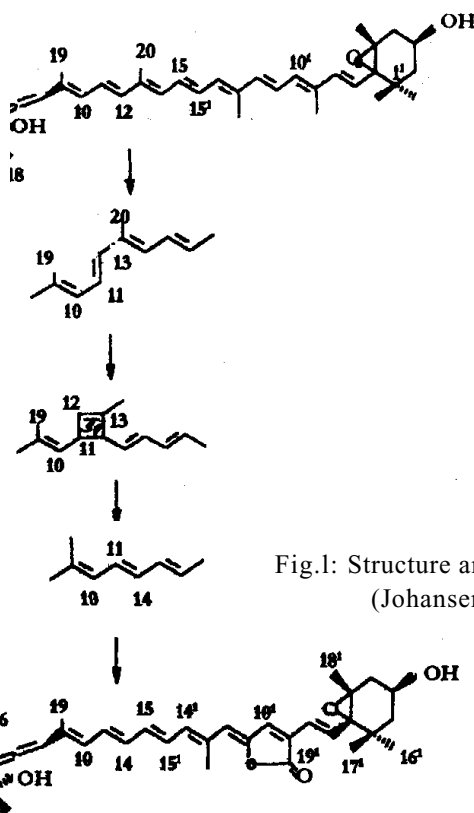


Fig.1: Structure and biogenesis of peridinol (Johansen et. al., 1977, 1980)

Peridinol: R=X  
 Peridinin: R = AC

The petroleum ether soluble fraction (Fr 1), which exhibited anti-nicotinic and anti-serotonin activities, was purified by flash chromatography over silica gel column using gradient acetone- petroleum ether system, yielding 10 fractions (1-1 to 1-10). Bioassay determined fraction 1-9 to contain the active compound(s). Subsequently, this fraction was further purified over sephadex LH-20 using acetone as eluent, yielding subfractions 1-9-1 to 1-9-4. Bioassay of these fractions located the activity in fraction 1-9-3. Final purification of the above fraction was carried out on a silica gel column using gradient acetone-petroleum ether system to yield the active compound, peridinol, as an orange colored amorphous solid, as a single spot on TLC (SiO<sub>2</sub>, acetone - pet. ether=2:3, R<sub>f</sub> = 0.8).

Peridinol: [α]<sub>D</sub><sup>25</sup> = -2.27 (c=2.0, CHCl<sub>3</sub>), IR (KBr): 3410, 2926, 2860, 1928, 1748, 1520, 1454, 1370, 1180, 1148, 1128, 1022, 986, 952, 910, 818, 758 & 640 cm<sup>-1</sup>; UV-Vis (MeOH): 450 nm; EMS (%): 588(M<sup>+</sup>, 85), 571(12), 289(20), 273(25), 165(70), 154(88), 136(92), 115(60), 91(80), 77(100), 63(44), 51(45) and 39(47).

## Results and discussion

Solvent-solvent partitioning followed by bioassay indicated the anti-spasmodic activity in the petroleum ether soluble fraction. Further bioassay-guided purification of this fraction over silica gel (gradient acetone-hexane mixture) and sephadex LH-20 (acetone) columns isolated an orange colored compound, homogenous on thin layer chromatography (R<sub>f</sub> = 0.8, silica gel, acetone: petroleum ether = 40:60) (Gonsalves *et. al.*, 2000).

The infra red absorption bands at 3410, 1928, 1748 and 1520 cm<sup>-1</sup> indicated the presence of hydroxyl, allenic, α-unsaturated γ-lactone, and unsaturations in the molecule. The strong UV-Vis absorption at 450 nm also supported conjugation in the molecule. The

Table 1: NMR assignment of peridininol (400 MHz, CDC13)

Carbon No	<sup>13</sup> CNMR	<sup>1</sup> HNMR	HMBC values
C-1'	35.29s		
C-2'	47.05t	1.64 m, 1.24 m	
C-3'	64.15d	3.90 m	
C-4'	40.88t	1.64 m, 2.33 m	
C-5'	67.5s		
C-6'	71.05s		
C-7'	121.78d	6.37(d,15.6 Hz)	71.05,168.75
C-8'	133.58d	7.17(d,15.6 Hz)	71.05, 124.74,
C-9'	124.74s		
C-10'	136.31d	7.03 s	133.58, 146.74, 168.75
C-11'	146.74s		
C-12'	119.24d	5.74 s	15.36,136.31,138.05,146.74
C-13'	133.9s		
C-14'	138.05d	6.45(d,11.5 Hz)	15.36,119.24,137.25
C-15'	131.54d	6.61 (dd/ or t, 12.9 Hz)	
C-16'	24.87 q	0.98 s (a-Me)	19.85,35.29,47.05,71.05
C-17'	19.85q	1.20 s (-Me)	24.87,35.29,47.05
C-18'	29.29 q	1.26 s	40.88,64.15,67.5,71.05
C-19'	168.75s		
C-20'	15.36q	2.23 s	119.24,133.90,138.05
C-1	35.84s		
C-2	49.37t	1.94 m, 1.3 m	
C-3	64.23	4.3 m	
C-4	48.86t	1.40 m, 2.23 m	
C-5	72.95s	117.80s	
C-6	117.80s		
C-7	Not observed		
C-8	103.14d	6.03 s	13.97,117.8,127.98
C-9	134.0s		
C-10	127.98d	6.10 (d, 11.5 Hz)	13.97,103.14,132.92,134.0
C-11	128.85d	6.62 (t, 12.7 Hz)	
C-14	132.92d	6.40 (dd,8.7 15.6 Hz)	
C-15	137.25d	6.52 (mor dd, 11 and 13.5 Hz)	
C-16	32.11q	1.07 s	35.84,49.37,117.8
C-17	31.32q	1.33 s	
C-18	29.48q	1.34 s	48.86,72.95
C-19	13.97q	1.80s	103.14,117.4,127.98,134.0

<sup>1</sup>H NMR spectrum had 11 vinyl proton signals between 5.70 to 7.30 ppm besides 8 methyl group signals between 0.90 to 1.40 ppm. The <sup>13</sup>C NMR spectrum had 36 carbon signals between 170 and 14 ppm, and their multiplicities were determined as 11 quarternary, 13 methine, 4 methylene and 8 methyl carbons by DEPT 135 experiment. Apparently, the allenic middle carbon signal, which appears at 200 ppm was not observed here. Molecular weight of the compound was determined to be 588 by EIMS, corresponding to the molecular

formula  $C_{37}H_{48}O_6$ , well in agreement with the NMR results. Based on these results, the structure was finalized as peridininol, a carotenoid pigment, known to be produced by marine dinoflagellates (Haugan and Jensen, 1989; Jensen, 1978). The structure was confirmed by extensive 2D NMR experiments, which also facilitated unambiguous assignments of most of the NMR signals of this compound for the first time (Table\*!, Fig 1).

Peridininol co-occurs as a minor metabolite, in almost all dinoflagellates alongwith its 3-acetate, peridinin. Peridinin, apparently, is produced from dinoxanthin, by expulsion of three carbons (Fig 1) ([ohansen *et. al.*, 1977, 1980). Its 3-hydroxy analog, peridininol could be obtained *via* deacetylation of peridinin or directly from neoxanthin (Fig 1).

Though carotenoids are photosynthetic accessory pigments of plant origin, many of them have been detected in different animals of dietary origin also. Thus sulcatoxanthin isolated from the sea anemone, *Ammonia sulcata* (Jensen, 1978) was identical with peridinin (Strain *et al.*, 1976). The same carotenoid was isolated from the gorgonian coral *Isis hippuris* too and its presence ascribed to the zooxanthellae (dinoflagellate symbionts) (Jensen, 1978). Examination of animal guts under microscope revealed the presence of zooxanthellae, indicating possible dinoflagellate origin of the compound.

The  $^1H$  and  $^{13}C$  NMR assignments of peridinin were reported by Krane and Jensen (1992). This compound inhibits proliferation of human neuroblastoma GOTO cells (Nishino *et. al.*, 1991). It is also a benign antifouling agent and is non-toxic to barnacles (Shimizu *et. al.*, 1993). However very little information regarding the biological properties or NMR studies of peridininol are available, probably, due to its low natural abundance as compared to peridinin, its 3-acetate analog. Contrary to this, peridinin could not be isolated from this extract in the present experiment.

## References

- Gonsalves C, Achuthankutty CT, Naik C G and Parameswaran P S**, 2000, A process for the isolation of peridininol from *Zoanthus* sp. Biological Activity: anti-nicotinic and antiserotonin, Indian Patent No. 184974
- Haugan J A and Jensen SL**, 1989, Improved isolation procedure for fucoxanthin and peridinin, *Phytochem*, 28, 2797-98.
- Jensen S L**, 1978, Marine Carotenoids, in: *Marine Natural Products Chemistry, Chemical and Biological Perspectives*, P J Scheuer (Ed), Academic Press, New York, pp 1-73.
- Johansen JE, Jensen JL and Borch G**, 1977, Studies on the Absolute configuration of peridinin and dinoxanthin, in: *Marine Natural Products Chemistry*, D J Faulkner and W H Fenical, (Eds), Plenum Press, New York, pp225-237.
- Johansen J E, Borch G and Jensen J L**, 1980, Algal Carotenoids 25, Chirality of Peridinin and Dinoxanthin, *Phytochem*, 19, 441-444.
- Krane J, Ackermann T and Liaen-Jensen S.**, 1992, Carotenoids 47-NMR study of all transperidinin, including complete  $^1H$  and  $^{13}C$  NMR assignments, *Magn Reson Chem* 30, 12,1169-77.
- Makoto K, Kohji Y, Tomoko T, Daisuke U**, 2000, Zoanthamines, Antiosteoporotic Alkaloids, in: *Drugs from Sea*, Fusetani, N., (Ed), S Karger AG, Basel (Switzerland), pp98-106.
- Nishino A, Iwashima A, Tanaka Y, Yamano Y, Shibata Y and Torihara M**, 1991, Anti-tumor activity of peridinin and its structurally related butenolide compounds, *Kyoto Furitsu Ika Daigaku*, 100, 8, 831-835.
- Parameswaran P S, Naik C G, Gonsalves C and Achuthankutty C T**, 2001, Isolation of 2-deoxy ecdysterone, a novel oxytotic agent from a marine *Zoanthus* sp., *J Indian Inst. Sci.*, 81, 169-173.
- Shimizu N, Konya K and Miki W**, 1993, Japanese patent 05331010 A2, dated 14 Dec.
- Strain HH, Svec W A, Wegfahrt P, Rapoport H, Haxo FT, Norgard S, Kjoson Hand Jensen LS**, 1976, Algal Carotenoids 14, Structural Studies on peridinin, Part 1. Structure elucidation, *Acta Chem. Scand Ser B*, 30, 109-120.