Chandrasena Memorial Award - 2006

Discovery of bio-active natural products with special reference to fertility regulating compounds from marine sources.

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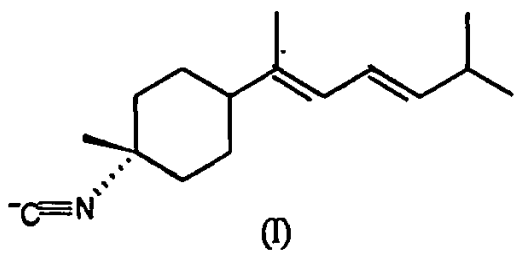
Marine ecosystems cover about 70% of the earth surface & represent > 95% of the biosphere. Oceans contain about 200,000 species of fauna & flora which is about 2-4% of earth's 5-10 million species. 90% of these marine species are confined only to a very small part of the ocean and are mostly restricted to tropical coral and rocky reefs[1]. These exceptional concentrations of marine species to tropical shore lines and coral reefs renders inter and intra species competition for food and space giving specialist life styles to these organisms[2]. The specialist life style of an organism is often expressed through natural compounds of many unique forms and these natural compounds are used for offence, defence, chemical communication & an exceptional degree of qualitative diversity. The taxonomic diversity, genetic variability, intense competition for food and space together with a conducive environment for chemical diversity of these marine species provides an abundance of highly specific more potent and promising esoteric materials for biomedical applications,[2,3]. To date about 10,000 novel marine natural products have been isolated. Nearly 600 new compounds are reported each year and over 90% of all structures are from marine algae & invertebrates.

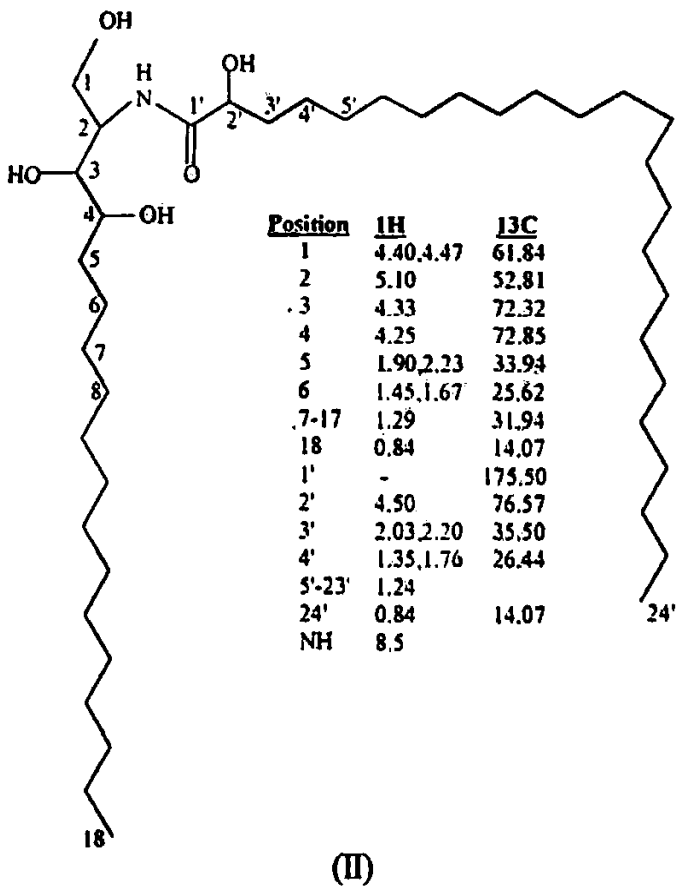
Sri Lanka has a sea area of about eight times the size of the 65,000 sq km land area around the country with about a resourceful 3000 km length of coastal reef harbouring an enormous number of marine plants & animal species that could provide a bulk of bio-active molecules for various bio-medical and agricultural applications. Nevertheless, not much research and development has been focused to uncover the chemistry and biological activities of this remarkable resource.



(I)

In 1988, I started R&D on marine natural products, screening Nudibranch for sedative and analgesic activity under the supervision of Dr. E.D. De Silva and Dr. W.D. Ratnsooriya at the University of Colombo. Dichloromethane/methanol extract of the Nudibranch, *Phyllidia varicosa* which had sedative activity[4] was subjected to activity directed isolation using Rat hole board technique and the active compound was separated and identified as bisabolane isonitrile (1) which was reported earlier as a new compound by Dr. E.D. de Silva[5].

With this experience I started extensive work on bio-activity of marine red algae in 1989 and got myself registered for a M.Phil. at the University of Colombo under the supervision of Dr. L.M.V.Tillekeratne and Dr. W.D. Ratnsooriya. I screened seven different red algal species collected from the southern coast for a range of biological activities: anti-bacterial, anti-hypertensive, anti-fertility, spermicidal, gastro-protective, sedative and analgesic activity and reported anti-hypertensive activity from *Gracilaria corticata*[6], immediate post-implantation contragestative activity from *Gelidiella acerosa*[7] and gastro protective activity from a *Jania* species[8].



Position	1H	13C
1	4.40,4.47	61.84
2	5.10	52.81
3	4.33	72.32
4	4.25	72.85
5	1.90,2.23	33.94
6	1.45,1.67	25.62
7-17	1.29	31.94
18	0.84	14.07
1'	-	175.50
2'	4.50	76.57
3'	2.03,2.20	35.50
4'	1.35,1.76	26.44
5'-23'	1.24	
24'	0.84	14.07
NH	8.5	

(II)

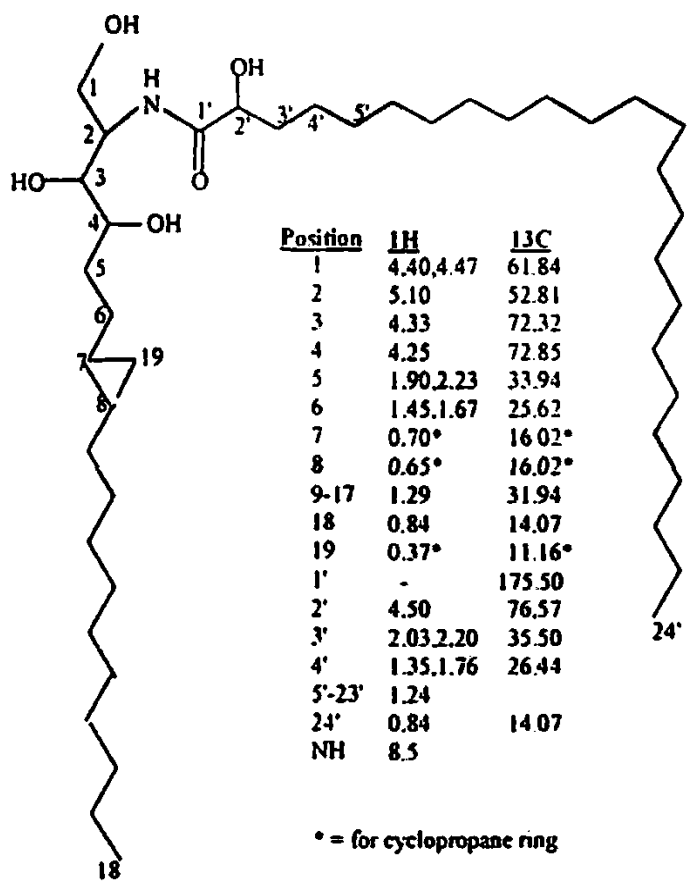
My post graduate work was then focused on the contraceptive effect demonstrated in CH_2Cl_2: MeOH (1:1) extract of red alga *Gelidiella acerosa* as it had 100% post-implantation loss in pregnant rats at a dose of 1000 mg/kg, in dose dependant manner, without overt signs of clinical toxicity[7].

It is noteworthy that this report was the first report of contraceptive activity from marine algae[7,9].

I focused my research on studying the mechanism of contraceptive effect in one line and activity directed isolation & characterization of active compound/s on the other line. After conducting various biochemical, physiological & pharmacological experiments related to reproduction, it was revealed that the observed contragestative effect is mediating by affecting the immediate post-implantation critical progesterone window in pregnant rats. Supply of exogenous

progesterone at this important juncture could maintain the pregnancy until parturition confirming the finding and indicating absence of embryo-toxicity in the extract[10]. These two important findings gave much excitement to the research as at the time the contraceptive research was very interested in compounds with non-steroidal immediate post-implantation contragestative effect mediating *via* an anti-progesterone mechanism.

Activity directed separation through initial modified Kupchan's scheme followed by various chromatographic separations including size exclusion through Sephadex, gradient elusion in silica & VLC, all of which guided by TLC, a white amorphous solid was separated as the active fraction from hexane solubles of CH_2Cl_2: MeOH (1:1) extract with ~100% activity at a concentration as small as 6 mg/kg suggesting a highly potent natural contraceptive[9,11].



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7	0.70*	16.02*
8	0.65*	16.02*
9-17	1.29	31.94
18	0.84	14.07
19	0.37*	11.16*
1'	-	175.50
2'	4.50	76.57
3'	2.03,2.20	35.50
4'	1.35,1.76	26.44
5'-23'	1.24	
24'	0.84	14.07
NH	8.5	

* = for cyclopropane ring

(III)

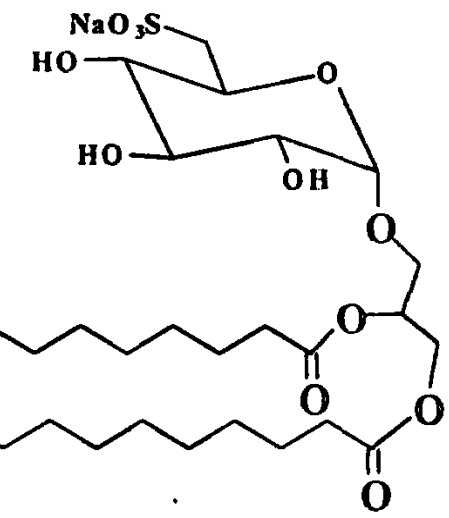
During the course of activity directed isolation of the contragestative compound another white amorphous solid was separated from the more polar fraction of the hexane solubles in considerable purity as evident by HPTLC. This compound was tested for its *in vitro* effect on human spermatazoa. The compound showed remarkable motility and velocity increment at a concentration of 100 µg/ml (see Figure 1)[12]. This finding gave further excitement to the project.

The compounds were then subjected to spectroscopic analysis: ^1H NMR, ^{13}C NMR, DEPT, HMBC, HMQC, HOHAHA, EIMS, FDMS, FAB at HEJ Research Institute of Chemistry under the supervision of Prof. Atta-Ur-Rahman.

The contragestative fraction resulted in two new compounds after extensive spectral analysis. The major compound was identified as N-2'-hydroxy-lignoceroyl-2-amino-1,3,4-trihydroxy-octadecane (**II**) (S-PC-1)[9,13] and the minor compound as N-2'-hydroxy-lignoceroyl-2-amino-1,3,4-trihydroxy-(7-8)-19-cyclo-octadecane (**III**) (S-PC-2)[9,14].

The structure of the human sperm motility stimulant was established as 2',3'-di-*O*-(pentadecanoyl)-glyceryl-6-sulfo-α-D quinovopyranoside (**IV**) (S-ACT-1)[9,12,15] which is also a new compound.

In addition to the novelty of the chemical structures of these compounds this study was the first report of anti-fertility effect from algae, contragestative effect from sphengosines and first natural non-steroidal immediate post-implantation contragestative agent known[9].



(IV)

Report of sperm motility stimulation in this study is the first report of sperm motility stimulation from red algae, from quinovose glycosides and first report of human sperm motility stimulation from a glycolipid[9]. Moreover, S-ACT-1 is the eighth known human sperm motility stimulant to date and the only motility stimulant that has shown the capacity to induce viable immotile sperms motile[12].

During the evaluation of the mechanism of contragestative activity, a range of assays were performed for activities such as oestrogenic/anti-oestrogenic progestational / anti-progestational, anti-implantation, abortfacient, foetal-resorption and also PAF induced human platelet aggregation assay and brine-shrimp toxicity assay[9]. For sperm stimulant S-ACT-1, in addition to testing in a range of concentrations and incubation times, tests such as Hypo-osmotic Swelling Test for membrane integrity and Nigrosin-Eosin test for vitality was performed in support of the finding[9,12].

This complete piece of multidisciplinary work on natural products chemistry and reproductive physiology & pharmacology done by me was then submitted to the University of Colombo for a Ph.D. in 1995.

After receiving the Ph.D. in 1996, I joined the Ceylon Institute of Scientific & Industrial Research (CISIR) in 1997 and resumed my work on marine natural products studying further on the sperm stimulant S-ACT-1 with the objective of continuing this work to develop S-ACT-1 to a potential clinical human sperm motility stimulant for assisted

reproductive programmes with financial assistance from the National Research Council & National Science Foundation. This marked the initiation of marine natural products research at the Natural Products Development Group of the CISIR (now ITI).

This work was started at ITI with re-extraction and separation of S-ACT-1 from the same algae following the same separation procedure. In this work S-ACT-1 was critically analysed for a series of tests related to sperm functions: Fertilizability using Hamster Egg Penetration assay (This assay was for the first time established in Sri Lanka under this project), Functional motility, Hypo-Osmotic Swelling, vitality and a series of tests related to sperm processing in assisted reproductive techniques: Effect on swim-up yield in swim-up technique, effect on sperm separation in filtration techniques and effect as a cryo-protectant in cryo-preservation for sperm banking. All these were carried out for normal & sub-normal sperms in comparison to clinical sperm motility stimulant pentoxifyllin.

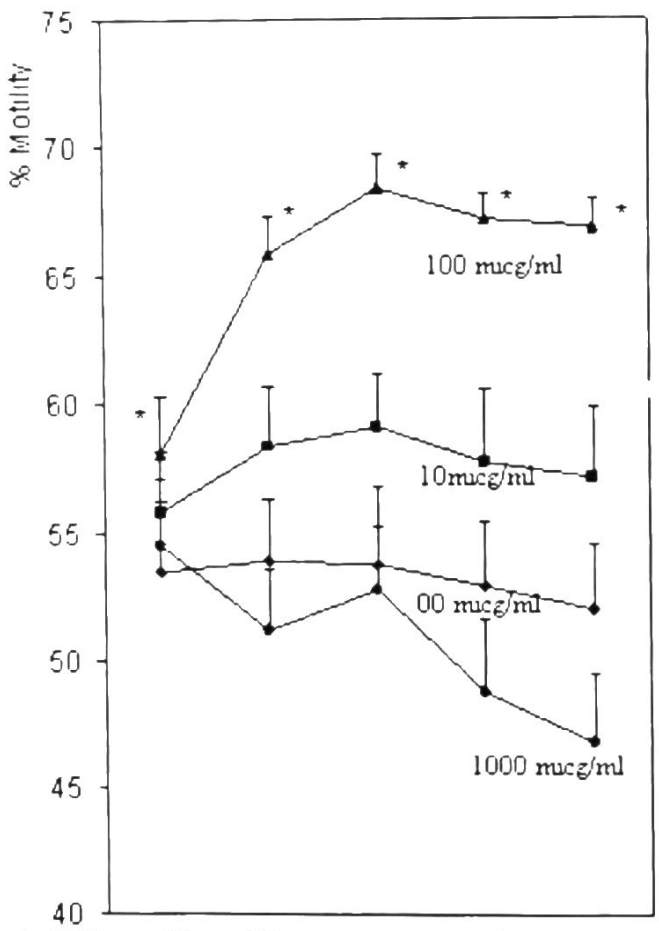


Fig 1: Effect of S-ACT on %motility of Human Sperm

The results were very promising as a sperm stimulant, especially in the swim-up technique[16], since the yield can be increased significantly (Figure 2) by addition of S-ACT-1 to semen and as a cryo-protectant[17] in the cryo-preservation of sperm (Figure 3), effects which clinical stimulant pentoxifylline failed to demonstrate.

Comparison of motility stimulating concentrations of S-ACT-1 with that of caffeine & pentoxifylline (two current clinical motility stimulants) revealed that S-ACT-1 is 20 times more potent than caffeine and 28 times more potent than pentoxifylline[12]. Further, in a separate study Dr. Nimal Chandrasiri of the Veterinary Research Institute, Peradeniya, demonstrated that S-ACT-1 is effective in sheep *in vitro* fertilization over heparin & caffeine as motility stimulants[18].

In summary, human sperm motility stimulant S-ACT-1 isolated from Sri Lankan marine red algae *Gelidiella acerosa* improves, motility both in normal & sub-normal sperm, swim-up yield, filtration yields, quality of cryo-preserved sperm, hamster egg penetration, without deleterious effects on the integrity of sperm DNA. Therefore S-ACT-1 is a potential candidate for a clinical motility stimulant in assisted reproduction. However, it is important to know the effect of S-ACT-1 on ultra structure of sperm and needs SAR studies and the mechanism of action. Extensive studies on SPC-1 & SPC-2 may yield a potential non-steroidal anti-progesterone contraceptive that could be developed into a once a month pill.

This pioneering work on marine algae in fertility research has definitely opened a new area of research and thinking line in fertility management and contributed to the knowledge of natural products chemistry and reproductive physiology and pharmacology. Extensive work on marine natural products would yield potentially useful bioactive molecules and therefore marine natural products research should be encouraged.

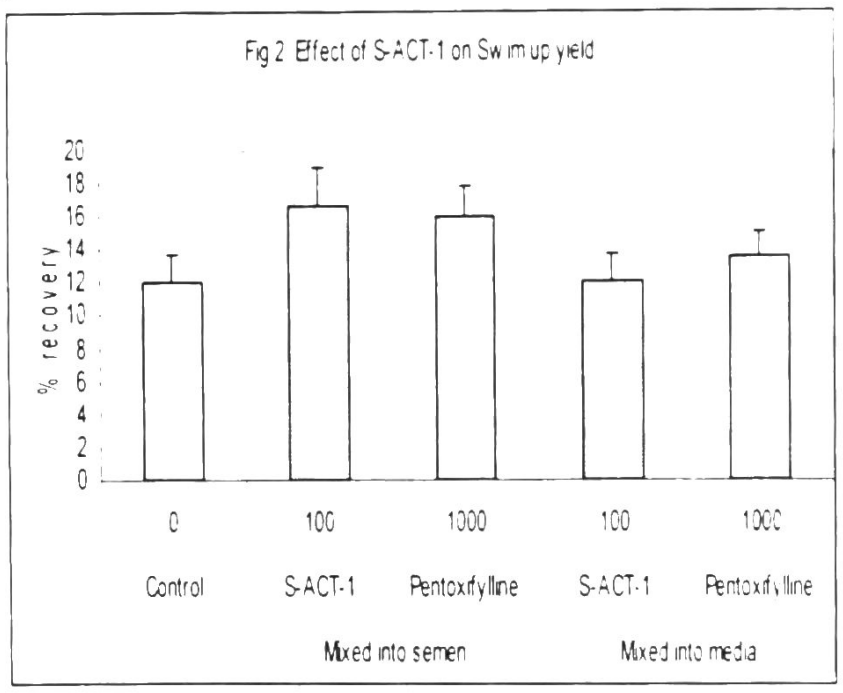
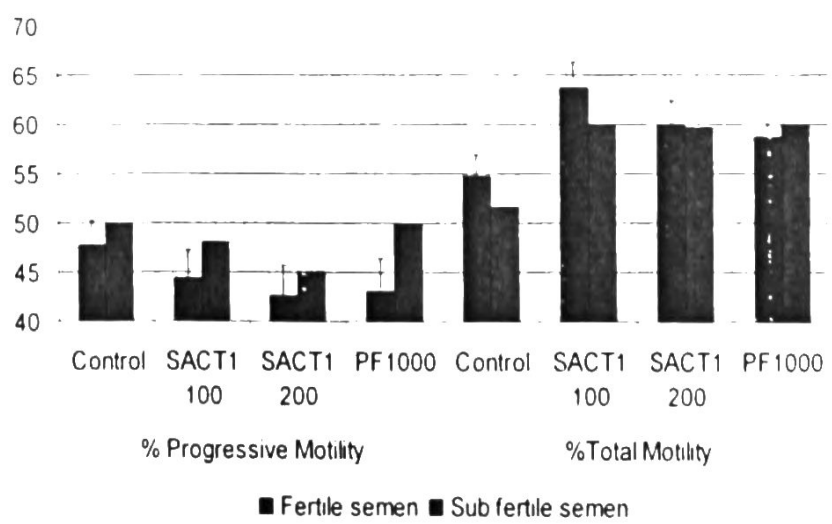


Fig.3: Effect of S-ACT-1 on Cryo-preservation of human sperms



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Prof. E.D. De Silva, Prof. A.S. Amarasekare, Prof. Atta-Ur-Rahman and Prof. H.R. Seneviratne. Research Assistant: Mr. K.G.N.M. Gunaratne

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All Island Interschool Chemistry Quiz Contest 2006/2007

Preliminary written paper of the All Island Interschool Chemistry Quiz contest 2006/2007 will be held on the 11th November 2006. Students from 123 schools from all districts in Sri Lanka will participate in the contest.