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A 4-decade-long (and still ongoing) hunt for palytoxins chemical architecture

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ABSTRACT

Since its isolation dated back to as far as 1971, palytoxin has all along drawn scientists' attention from across the world because of its high toxicity and fascinating chemical architecture. Commitment of the international scientific community to the study of this extremely potent non-proteic toxin has led to discover quite a number of palytoxin analogues. Once confined only to tropical and subtropical areas, palytoxins have recently spread also to more temperate regions, such as the Mediterranean Sea where they have caused severe human intoxications. Studies on the Mediterranean toxic outbreaks brought to light the existence of further palytoxin-like compounds, ovatoxins, never reported elsewhere in the world.

1. How palytoxin was introduced to the scientific world

The lethal potency of palytoxin was well known to ancient Hawaiians who used to smear a moss containing such toxin on their spear points to make them fatal. According to an old legend, the lethal moss – known to native Hawaiians as *limu-make-o-Hana* – started lining the walls of a tidepool near the harbor of Hana, after the ashes of an evil Shark God killed by some fishermen had been thrown into it (Malo, 1951). Locating the only fabled pool where the *limu-make-o-Hana* grew was not an easy task. In fact, over the centuries that place had become taboo to Hawaiians convinced that an ill curse would haunt all those attempting to collect the deadly moss. Nonetheless, in the early '60s thanks to some local informers P. Helfrich and J. Shupe from the Hawaii Institute of Marine Biology succeeded in individuating the tidepool, from which they collected some samples of the toxic moss, thus introducing palytoxin to the scientific world in the first place (Moore and Scheuer, 1971). Some 10 years later, Paul Scheuer from the University of Hawaii came across a reference to the famous cursed moss and decided to scientifically substantiate the ancient legend. First of all, it was ascertained that the sample collected from the Hana tidepool was not a seaweed, as commonly believed, but an animal belonging to the phylum Coelenterata assigned to the genus *Palythoa*, after which the bioactive molecule was termed palytoxin (**1**, Fig. 1) (Moore and Scheuer, 1971). Such a toxin, purified through a series of chromatographic separations monitored by ultraviolet absorption at 263 nm and mouse bioassay alike, appeared as a white, amorphous solid insoluble in chloroform, only sparingly soluble in methanol and ethanol, while totally soluble in water, pyridine, and dimethyl sulfoxide. Moreover, Scheuer was able to provide also some insights into palytoxin molecular formula that was suggested to be $C_{145}H_{264}N_4O_{78}$ with an error of no more than 10% (Moore and Scheuer, 1971).

2. The biological sources of palytoxin

Studies carried out by Walsh and Bowers identified the marine coelenterate associated to the legendary *limu-*

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make-o-Hana, from which Scheuer had isolated palytoxin, as *Palythoa toxica*, a rare species sparingly detected across the Hawaiian Islands (Walsh and Bowers, 1971). Even though palytoxin-like compounds have since been isolated from a number of zoanthids belonging to the genus *Palythoa* (Moore et al., 1975), the real biological origin of this class of toxins remains controversial. Following the observation that palytoxin content in *Palythoa* spp. significantly varied among species, populations of the same species and even seasonally (Moore et al., 1982a), and that sporadic occurrence of palytoxins had been detected in algae (Maeda et al., 1985), crabs (Yasumoto et al., 1986), and fish (Fukui et al., 1987), many have defended the assumption that palytoxins are indeed produced by microorganisms. This has been recently supported by the discovery that dinoflagellates belonging to the genus *Ostreopsis* seem to be the most probable biogenetic originators of palytoxins (Usami et al., 1995). In 1995, in fact, Yasumoto isolated and structurally characterized some palytoxin analogues from extracts of the dinoflagellate *Ostreopsis siamensis*, whose analysis he had undertaken for its strict taxonomical relationship with another toxic dinoflagellate, *Gambierdiscus toxicus*. Successively, further palytoxin-like compounds, termed mascarenotoxins, were extracted from *Ostreopsis mascarenensis* (Lenoir et al., 2004). Finally, palytoxin and some of its analogues, named ovatoxins, have been detected in the Mediterranean *Ostreopsis ovata* (Ciminiello et al., 2008, 2010). Occurrence of palytoxins in such a large number of even biogenetically distant marine organisms could reasonably imply a symbiotic relationship of the above organisms with bacteria (Katikou, 2008). This is also consistent with experimental observations that i) some *Pseudomonas* (Carballeira et al., 1998), *Brevibacterium, Acinetobacter* and *Bacillus cereus* (Seemann et al., 2009) extracts have shown palytoxin haemolytic activity and ii) *Vibrio* spp. and *Aeromonas* spp. were proven producers of molecules antigenically related to palytoxin (Frolova et al., 2000).

3. The monumental structural analysis that disclosed the complex architecture of palytoxin

After its isolation in 1971, it took nearly 11 years before the correct chemical structure of palytoxin (**1**) was disclosed. The challenging and complicated structural investigation of palytoxin was performed by two research groups – one at the University of Hawaii and one at Nagoya University, Japan. Such a study, compounded by a large amount of setbacks related to the high complexity of the molecule as well as to its instability, was carried out through careful spectral analysis of structural fragments deriving from cleavage of palytoxin by sodium periodate or ozonolysis. One of the first paper on the topic was published in 1980, when relying on plasma desorption mass spectrometry with californium (^{252}Cf) the Japanese group exactly established as 2680 the molecular weight of a palytoxin isolated from Okinawan *P. tuberculosa* (Macfarlane et al., 1980). But it was not until 1981 that, independently and almost contemporaneously, both the Hawaiian and the Japanese groups came up with a planar structure of palytoxin. The structure the two groups had assigned to palytoxin was not exactly coincident. The Nagoya University group, which had worked on a palytoxin isolated from Okinawan *P. tuberculosa*, located a hydroxyl group at position C44 and a hemiketal functionality at position C47 (Uemura et al., 1981) as opposed to the Hawaiian group who had proposed a ketal bond connecting C44 with C47 in a sample of palytoxin isolated from a Tahitian *Palythoa* spp. (Moore and Bartolini, 1981) (Fig. 2). This latter hypothesis, though, has never been supported by experimental evidence afterwards (Nakamura et al., 2009). However, it is worthwhile underlining that the Hawaiian group had noted that palytoxin isolated from the Hawaiian *P. toxica* contained some structural modifications in comparison to palytoxin isolated from Tahitian *Palythoa* spp., and suggested that the subtle differences in palytoxins isolated from different *Palythoa* species might have been due to structural differences of the hemiketal functionality at position C47 (Moore and Bartolini, 1981).

In 1982, Kishi (Cha et al., 1982) assigned the absolute stereochemistry – encompassing as many as 64 stereogenic centers in addition to eight double bonds – of the natural isomer of palytoxin, isolated from Okinawan *P. tuberculosa*. This challenging task was successfully accomplished through degradation reactions and chemical synthesis-based approach (Cha et al., 1982; Moore et al., 1982b) (Fig. 1).

4. Identification of further palytoxin analogues

Following the first report on palytoxin in 1971, many research groups from across the world have undertaken scientific studies with the purpose of investigating this fascinating molecule. Efforts carried out by proficient chemists have significantly contributed to identify quite a number of palytoxin analogues isolated from both *Palythoa* spp. and *Ostreopsis* spp., as detailed below.

4.1. *Palytoxin analogues isolated from* Palythoa *spp.*

In 1985, the above mentioned Japanese group at Nagoya University further investigated the toxin content of Okinawan *P. tuberculosa*. The outcome of this study, carried out by extensive NMR analysis of degradation and/or derivatization palytoxin products, was presented on a paper describing the isolation and chemical characterization of four minor palytoxin-like compounds: homopalytoxin (**2**), bishomopalytoxin (**3**), neopalytoxin (**4**), and deoxypalytoxin (**5**) (Uemura et al., 1985). With regard to compound **5**, NMR-based evidence suggested the absence of a hydroxyl group at position C73 compared to palytoxin (Fig. 1). Homopalytoxin and bishomopalytoxin structures were studied by using a 1:1 mixture of them, as chromatographic separation of the two components was not feasible. Nonetheless, structural differences between the two new palytoxins – basically residing in proximity of position Cf (Fig. 1) – were confirmed by periodate oxidation followed by investigation of the deriving fragments. As for neopalytoxin, its NMR spectrum turned out indistinguishable from that of palytoxin and its structure was elucidated through combination of High Resolution (HR) Mass Spectrometry (MS) and NMR spectral analysis of fragments deriving from periodate oxidation followed by acetylation (Fig. 1).