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Evolution of the secondary metabolite versus evolution of the species*

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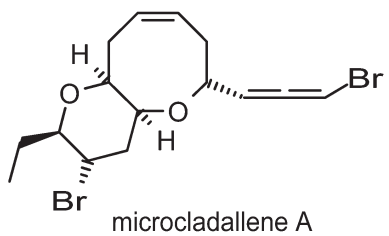
Abstract: Four stereochemical series of diastereomeric polyhalogenated chamigrane sesquiterpenes—headed by obtusol, isoobtusol, rogiolol, and cartilagineol—suggest the existence of four lineages of red seaweeds in the genus *Laurencia*. On another front, concerning marine ciliates, euplotane sesquiterpenes characterize worldwide the morphospecies *Euplotes crassus*, well differentiated from *Euplotes raikovi*, *Euplotes rariseta*, and *Euplotes vannus*, which furnish different-skeleton sesquiterpenoids, and the latter also C_{30}-backbone isoprenoids. The latter three morphospecies, however, show polymorphism in the variability of their isoprenoids.

Unarguably, the modern construction of phylogenetic trees and evolutionary events concerning species is based on molecules that are found in all organisms, such as rRNA coding fractions and highly conserved proteins for long distances, or polymorphic enzymes for population structure and dynamics. However, the correlation between gene and protein expression is low, and that between proteins and secondary metabolites is even lower, which throws much shadow on "lower" species that compete for space and resources, or defend themselves from predators, largely thanks to secondary metabolites. These often constitute their best taxonomic and phylogenetic markers. In this regard, biologists and natural products chemists live largely in separate, artificial worlds—the evolutionary biologist snubbing the secondary metabolite, and the chemotaxonomy of the natural products chemist providing little *trait d'union*, as it is typically concerned with gross comparisons of identities or similarities in molecular structures that ignore deep-seated chemical features.

An example of gross structural comparison concerns a nominal species of red seaweed, *Laurencia microcladia* Kützing, 1865, of Atlantic origin although rather common in infralittoral areas of the Mediterranean (Fig. 1). The story begins with a sample of this seaweed from Cap Ferrat, along the Côte d'Azur, giving new polyhalogenated C_{15} acetogenins, a class of compounds unique to seaweeds in the genus *Laurencia* and opisthobranch mollusks that feed on them. In this case, these are of the most com-



microcladallene A

Fig. 1 Oxepane-type C_{15} acetogenin isolated from *Laurencia microcladia* Kützing 1865 from Cap Ferrat (Côte d'Azur).

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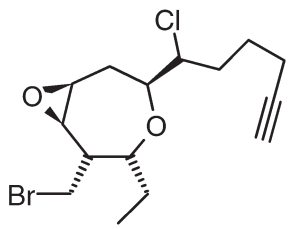
mon type, oxocanes, albeit new like microcladallene A [1]. No other metabolites have been described for this alga.

Oxepane-type C_{15} acetogenins were only known for *Laurencia nipponica* from Japan [2], until they were discovered as the preferred mode of heterocyclization by *L. microcladia* Kützing, 1865 from a very restricted tract of coast called Il Rogiolo—no more than a few hundred meters in length—in the Ligurian Sea, south of Livorno (Fig. 2). Actually, they were first discovered there in a coexisting sponge [3], but the origin was later traced to the seaweed [4]. These metabolites—exemplified here by rogiolenyne A—are not only of the unusual oxepane type, but they bear also C-branching, which has since remained a unique example in the whole history of natural products. The only other secondary metabolites found in this alga were chamigrane sesquiterpenes, acyclic furanoditerpenes, and pimarane diterpenes [5].

Another sample of *L. microcladia* Kützing, 1865 from the Bay of Calenzana in Elba Island (Fig. 3) has recently given a sesquiterpene of a new skeleton—calenzanane—as a major product, besides ordinary fatty compounds [6].

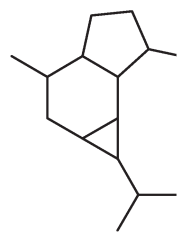
From these families of metabolites of vastly different biogenesis it is apparent that the secondary enzyme systems of these three samples of seaweed, determined microscopically by expert taxonomists to be *L. microcladia* Kützing, 1865 [1,3,4], are largely unrelated to each other. The differences should be traced back to the coding genes.

Now let us move from a gross to a fine comparison, based on chamigrane sesquiterpenes that for *L. microcladia* Kützing, 1865 have only been found in the Rogiolo strain (Fig. 4). In this case, it was the new rogiolol acetate [7], while obstusol had been isolated from a sample of *Laurencia obtusa* from the Canary Islands [8], isoobtusol from another sample of nominally the same seaweed from the same islands, other than from Jamaica and Western Australia [8]. Later, we raised problems of structural assignment for a chamigrane isolated from *Laurencia cartilaginea* from Ohau [9], reassigning and renaming it cartilagineol [9]. This assignment was confirmed by the isolation of cartilagineol from *Laurencia* sp. from the Philippines [10]. From these stereochemical series of chamigranes, we sug-



rogiolenyne A

Fig. 2 Oxepane-type C-branched C_{15} acetogenin isolated from *Laurencia microcladia* Kützing 1865 from Il Rogiolo (Tuscany).



calenzanane

Fig. 3 New skeleton of sesquiterpenes isolated from *Laurencia microcladia* Kützing 1865 from Elba Island.

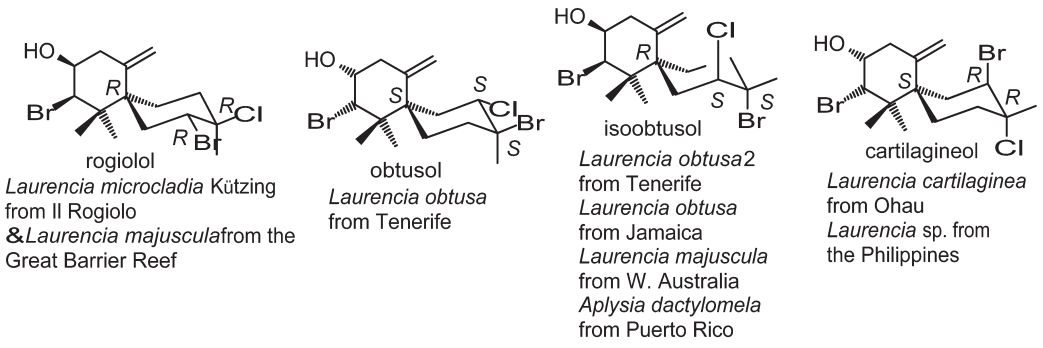


Fig. 4 Diastereomeric polyhalogenated chamigrane sesquiterpenes from *Laurencia* spp.

gested the existence of four *Laurencia* lineages, comprising also co-occurring cuparane sesquiterpenes [9]. A biogenetic scheme from putative bisabolene precursors may be proposed to accommodate both these chamigranes and co-occurring cuparanes [9].

These observations call for a taxonomic revision of this algal genus, which plays a key role in the ecology of temperate and tropical coasts.

On another front, when we began to investigate marine ciliated protozoans in the subclass Hypotrichida, both morphological and molecular biology data proved ambiguous in deciding between separate species of *Euplotes vannus* and *Euplotes crassus* or a complex, and the latter idea prevailed. We started with the most frequently sampled hypotrichid, finding in any sample throughout the seven seas the same pattern of secondary metabolites (euplotins, establishing a new sesquiterpene class, euplotane), and the putative acyclic precursor of them, preuplotin [11]. A simple thin-layer chromatography (TLC) kit was devised to quickly recognize this protozoan as *E. crassus* (Fig. 5) [12]. With such an array of homogeneous specimens, taxonomic keys based on morphological observations could also be refined, and we acquired the skill to distinguish typical specimens of *E. crassus* from *E. vannus*.

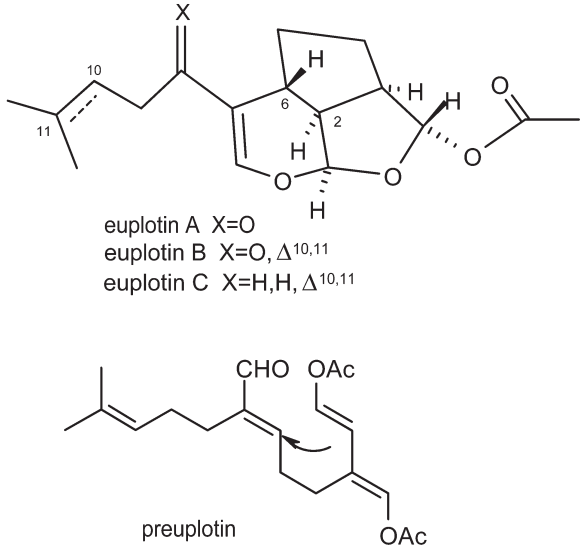


Fig. 5 Sesquiterpenes isolated from *Euplotes crassus.*

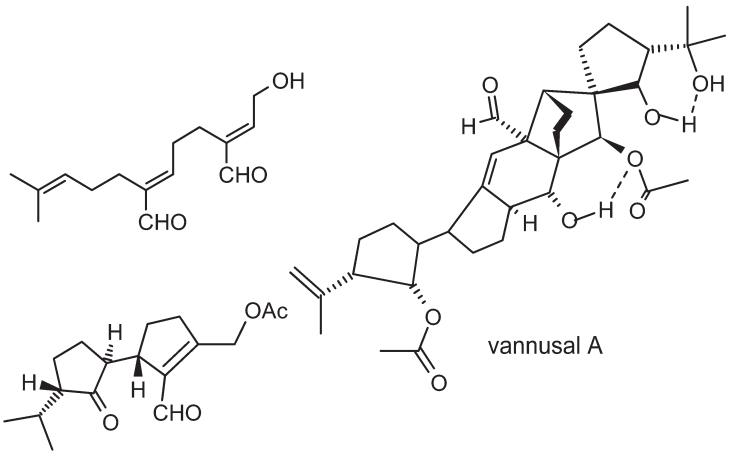


Fig. 6 Isoprenoids isolated from *Euplotes vannus.*

In contrast with the former, *E. vannus* proved polymorphic (Fig. 6); a sample from the Côte d'Azur gave both a reduced form of preuplotin and a cyclized form presumably deriving from it [13]. Sampled in the Celebes Sea, *E. vannus* remarkably gave instead a bisacetylated C_{30} metabolite, vannusal A [14]. This metabolite remains a biogenetic puzzle: it was imagined to arise along a deviating route—against chemical rules—from the squalene pathway to triterpenes [14]. Subsequent preliminary identification of a sesquiterpene isolated from this ciliate that resembles halved vannusal A (Fig. 6) suggests, however, a biogenesis via the coupling of two molecules of sesquiterpenes [15].

Whichever the case, our studies above have relegated to oblivion the idea of a *E. vannus* complex. *E. crassus* is a species that has developed a homogeneous tract of secondary molecular characters, and evolution of this species is destined either to maintain all these characters (euplotins A–C) or to loose all of them. In contrast, the evolution of *E. vannus* is still playing around a set of different secondary molecular characters that perhaps will distinguish different populations.

Our studies of other marine ciliated protists have also contributed to distinguish what appeared to the protistologist as single species. This was the case of *Euplotes raikovi* Amagaliev, 1966 (Fig. 7), until we discovered that a strain from Casablanca produces the sesquiterpene raikovenal and its putative precursor preraikovenal [16], while both a Californian strain and various strains from the Mediterranean produce epiraikovenal. Photocyclization of preraikovenal (the first example of tele[2+2]photocyclization reaction) gave mainly *ent*-epiraikovenal, from which the elusive *ent*-prereikovenal is suggested to be the biogenetic precursor of epiraikovenal [16]. These observations speak clearly for polymorphism.

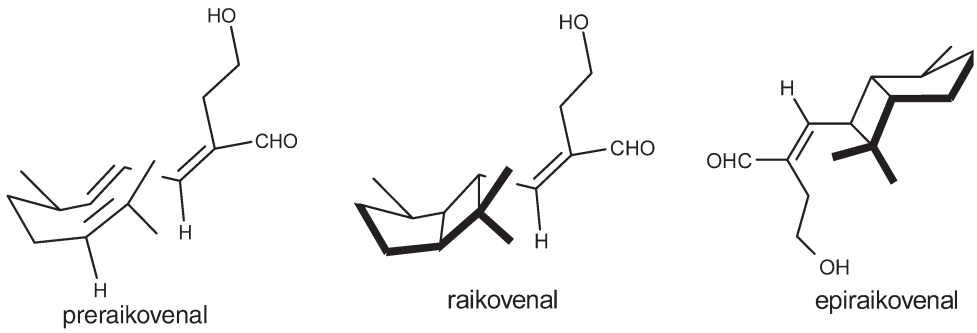


Fig. 7 Sesquiterpenes isolated from *Euplotes raikovi.*

Euplotes rariseta Curds, West et Dorahy, 1974 was also thought by protistologists to be a single species. However, strains from southern Brazil, Tenerife, and northeastern and southeastern Australia produce two sesquiterpenoids, rarisetenolide and its epoxide, while a strain from New Zealand produces epirarisetenolide [17]. Strains that are now recognized within this same morphospecies, collected at the same spot in Brazil or in New Zealand, produce two families of related diterpenoids, in which the isopropylidene group is prenylated [18]. Thus, it is beyond doubt that *E. rariseta* shows polymorphism, perhaps including strains that had been assigned to new species, like *Euplotes focardii* Valbonesi and Luporini, 1990. Production by this strain of focardin, which shows much of the characters of rarisetenolide and epirarisetenolide [19], urges a taxonomic reexamination of this strain.

In conclusion, our studies from one side have indicated a phylogenetic *trait d'union* in the taxonomically most confused area of seaweeds in the genus *Laurencia*, which play a role of protagonists in the ecology of most rocky coasts, and from the other side we have revitalized marine protozoology by posing challenges to ciliatologists in an area that was considered to be acquired knowledge.

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