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Short Communications

A new, unexpected marine source of a molting hormone. Isolation of ecdysterone in large amounts from the zoanthid *Gerardia savaglia*[1]

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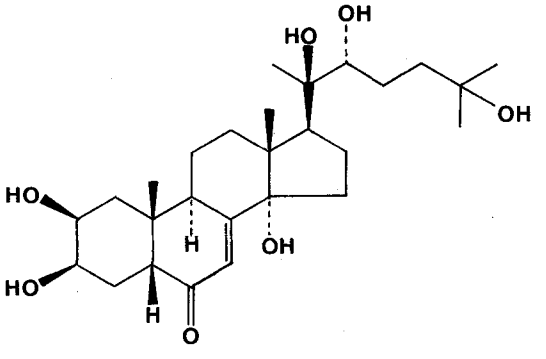
Summary. Ecdysterone was found in large amounts in the Mediterranean zoanthid *Gerardia savaglia* both immediately after its collection and after it had been kept for 15 months in an aquarium. This is the first time that an ecdysteroid has been found in a marine animal which does not belong to the phylum Arthropoda.

Several polyhydroylated steroidal Δ^7-6 ketones have been initially isolated in extremely small amounts from both insects[5a] and crustaceans[5a,b] during ecdysis, and have hence been termed ecdysteroids. These molting hormones have also been found to occur in trace amounts in other terrestrial animals, such as nematodes[5c] and, usually in higher amounts, are also widely distributed in terrestrial plants[5a].

We report here that, during our search for natural antifungals[1], we have isolated ecdysterone (**1**) instead, in large quantities, from the zoanthid *Gerardia savaglia* Bert. (Cnidaria, Anthozoa, Hexacorallia, Zoanthidea).

The zoanthid, about 20 g dry wt, was collected in November 1980 in South Tyrrhenian, at a depth of 45 m, and immediately was steeped in ethanol. A small portion of the colony was maintained alive in an aquarium[6]. The zoanthid kept in ethanol was homogenized and twice extracted with fresh ethanol at room temperature. The combined extracts were evaporated at reduced pressure and the residue was partitioned first between water (100 ml) and ethyl ether (3 × 100 ml), and then between water and n-butanol (2 × 100 ml). The residue (2.2 g) after evaporation, at reduced pressure, of the butanol extract was subjected, one portion at a time, to reverse phase HPLC on a Jobin-Yvon Miniprep (25–40 µm RP-18, 50 g; methanol-water 1:1, 8 ml min^{-1} for 35 min, under 10 at, monitoring at $\lambda = 254$ nm). The central fractions, on evaporation at reduced pressure, gave 0.061 g of colorless crystals which were further purified, practically without weight loss, by reverse phase



HPLC on a Merck 10 × 250 mm LiChrosorb RP-18,7-µm column, methanol-water 1:1. Recrystallization from methanol-ethyl acetate gave colorless crystals, m.p. 234–238 °C.

Spectral studies immediately revealed that the compound is an ecdysteroid and, in fact, both the above recrystallized and non-recrystallized samples were undistinguishable by either TLC or HPLC from commercially available ecdysterone (**1**) (Sigma). Also, our samples of **1** and commercially available **1** gave superimposable mass[7a], ^{1}H-NMR[7c], ^{13}C-NMR- and UV-spectra, as well as the same optical rotation. We report here both UV and optical-rotation data in greater details than was so far available[6b-d] as well as

high field ^{13}C-NMR-data: UV (CH_3OH) λ_{max} (ε) 242(10, 200), 311 nm (158); $[\alpha]_{\lambda}^{20}$ (CH_3OH), c0.014) − 128.3° (λ365), 189.4° (λ435), 84.0° (λ546), 71.2° (λ577), 66.8° (λ589 nm); ^{13}C-NMR[8] (pyridine-d_5) δ(multiplicities and/or assignments are given when unambigously attributable) 203.44 (s, C-6), 166.05 (s, C-8), 121.69 (d, C-7), 84.22 (s, C-14), 77.57 (d, C-22), 76.89 (s, C-20), 69.59 (s, C-25), 68.15 (d, C-2 or C-3), 68.07 (d, C-2 or C-3), 51.40, 50.15, 48.14 (s, C-13), 42.63, 38.69 (s, C-10), 38.02, 34.49, 32.43, 32.04, 31.78, 30.11, 30.01, 27.48, 24.47, 21.68, 21.50, 21.16, 17.90.

These findings pose a number of intriguing questions about the origin and the role of ecdysterone in *G. savaglia* and about the relationship, if any, with the same steroid in marine crustacea. Regarding the origin of the steroid, it is relevant to note that *G. savaglia*, after it had been kept for 15 months in our aquarium[6], still gave ecdysterone in roughly the same large amounts as immediately after its collection.

It may be that part of the ecdysterone found in the zoanthid kept in our aquarium has a dietary origin, e.g. from copepods and plancton[6]. However, we could not detect ecdysterone in the diet[6] by HPLC-UV on examination of diet amounts sufficient to the zoanthid for months. This agrees with the fact that crustaceans may normally contain ecdysterone in amounts not larger than a few mg per ton[5a]. Therefore, unless ecdysterone was either not used, nor given to the surroundings by the zoanthid kept in the aquarium, the above findings show that the zoanthid cannot have received all its ecdysterone from the diet alone. It cannot then be ruled out that ecdysterone is synthetized within the zoanthid from, possibly, dietary cholesterol. These facts urge both the examination of *G. savaglia* from different marine areas and a careful examination of the zoanthid for microbial symbionts, which could well be the producers of ecdysterone. Although the presence of zoochlorellae seems to be excluded owing to the pale yellow color of the zoanthid, which was not altered in the aquarium conditions, nothing is known about other symbionts for *G. savaglia*. Ultimately, if no symbionts are found, a study of the biosynthesis of ecdysterone by the zoanthid could be attempted.

The role of ecdysterone as a hormone in the zoanthid is ruled out because of the high concentration of the steroid. We may consider a defensive role, as has been suggested, but never substantiated, for ecdysteroids accumulated by plants[5]. This stimulates the study of predators of *G. savaglia*, about which nothing is known. Nudibranchs are possible candidates, because they are known to feed on coelenterates (e.g. on hydroids), thereby accumulating their steroids[9]. Crustaceans are not predators of *G. savaglia*, perhaps because crustaceans are affected by ecdysterone[5].

We conclude that it is likely that ecdysteroids will prove to be much more widely distributed in the marine environment than was thought, unless *G. savaglia* possesses it uniquely. The recent finding that pinnasterol, an ecdysteroid-like sterol, is a constituent of the red alga *Laurencia pinnata* Yamada[10] might support this hypothesis.

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Marine diatoms affecting the stability of oil-in-water emulsions and hydrocarbon distribution in sea water

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Summary. The importance of phytoplankton in stabilizing oil-in-water emulsion and affecting hydrocarbon distribution in the sea was studied by using marine diatoms. All microalgae tested increased emulsion stability and favored the presence of polycyclic aromatics in the sea water.

The formation of oil-in-water emulsions in the sea after an oil spill, extensively discussed over the last few years[1,2], has mainly been described as the result of physical and chemical processes[3]. However, laboratory work on hydrocarbon uptake by a marine diatom showed that algal cultures could support higher hydrocarbon concentrations than sterile media[4]. The subsequent aim was therefore to examine by standard emulsification procedures[5] whether marine diatoms, the most important group of a phytoplankton community, could also be considered as factors increasing the stability of oil emulsions. In addition, the distribution of the oil components into the water phase and emulsion was studied fluorimetrically, because of the ecological significance of the aromatic compounds[2].

Experimental part. Four marine diatom species, *Skeletonema costatum* (Grev.) Cleve, *Cyclotella cryptica*, Reinan, J. Lewin and Guillard, *Nitzschia closterium* (Ehr.) W.Sm. and *Chaetoceros affinis* Laud., were used as test organisms. Culture media and conditions have been described elsewhere[6]. The stability of the emulsion was tested by the