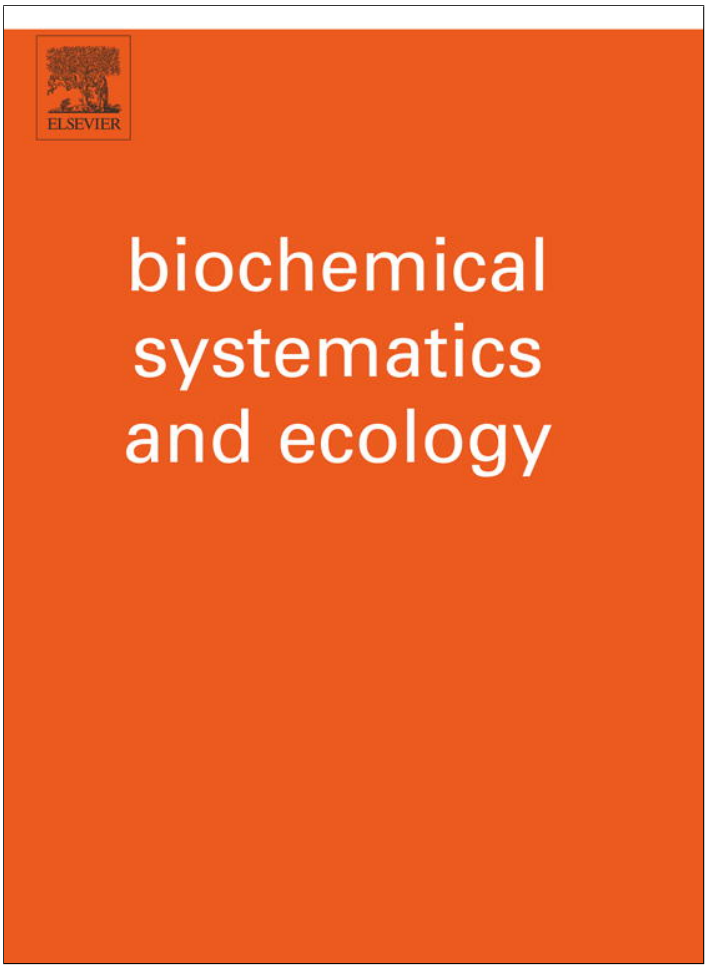

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First chemical study of the sacoglossan *Elysia patagonica*: Isolation of a γ-pyrone propionate hydroperoxide

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1. Subject and source

Sacoglossan sea slugs are a group of opisthobranch herbivorous mollusks primarily associated to siphonalean green algae. They appear to be one of the few metazoan groups to retain functional chloroplasts as intracellular organelles. Species belonging to *Elysia* genus are characterized for large parapodia, which contain the digestive diverticulum where the chloroplasts sequestered are photosynthetically functional for many days to months, useful for the survival of the mollusks.

Elysia patagonica Muniain and Ortea (1997) (Plakobranchidae, Sacoglossa) is the first sacoglossan described from Argentina, and intact chloroplasts of the green algae *Bryopsis* cf. *plumosa* (Chlorophyta: Bryopsidales) were observed in the digestive cells of benthic adults starved for many days (Muniain and Ortea, 1997; Muniain et al., 2001).

2. Previous work

A certain number of chemical studies on elysioidean species collected from distinct geographical areas from Indo-Pacific Ocean to Mediterranean Sea have been appeared in the literature to date (Cimino et al., 1999; Cimino and Ghiselin, 2001, 2009). An aspect of the chemistry of these sacoglossans regards dietary algal metabolites including depsipeptides – i.e. kahalalides from *Elysia rufescens* (Hamann and Scheuer, 1993; Hamann et al., 1996); sesquiterpenoids – i.e. crispatenine from *Elysia (=Tridachia) crispata* (Gavagnin et al., 1997, 2000); diterpenoids – i.e. udoteal from *Elysia translucens* (Gavagnin

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et al., 1994a). The ability of elysioidean mollusks of chemically modifying ingested algal terpenoids has been also reported in some cases – i.e. halimedatetracetate alcohol in *Elysia halimedae* (Paul and Van Alstyne, 1988). However, the most interesting aspect of the chemistry of elysioideans is represented by the occurrence of polypropionates – i.e. elysione from *Elysia chlorotica* (Dawe and Wright, 1986) – which are typically complex cyclic systems constituted by a γ-pyrone moiety bearing an α-methoxy group and either a cyclohexadiene or a bicyclohexene ring at various levels of oxidation (Cimino et al., 1999). The photochemical relationship between these two latter structural architectures has been demonstrated by photoconversion of cyclohexadiene-containing into bicyclohexene-containing molecules (Ireland and Scheuer, 1979; Ireland and Faulkner, 1981). Several pairs of such γ-pyrone polypropionates have been reported from elysioidean sacoglossans from different geographical areas in the world in agreement with the suggestion that these metabolites are synthesized *de novo* rather than simply deriving from dietary sources (Ireland and Scheuer, 1979; Ireland and Faulkner, 1981; Gavagnin et al., 1994b; Díaz-Marrero et al., 2008). The biosynthesis of polypropionates in elysioidean sacoglossans has been rigorously proven in some species by *in vivo* feeding experiments (Ireland and Scheuer, 1979; Gavagnin et al., 1994a; Cutignano et al., 2009).

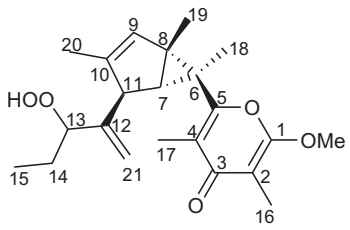
3. Present study

In continuing our research on the chemistry of opisthobranch mollusks, we have recently investigated the chemical components of a population of the sacoglossan *E. patagonica*, collected off San Jorge Gulf (Patagonia, Argentina), during the month of February 2008. The mollusks (129 individuals) were collected on the alga *Bryopsis* cf. *plumosa* in intertidal tide pools during low tide at Punta Marqués (45°57′S, 67°34′W), and immediately frozen at −20 °C. Both species were identified by Dr. Muniain and a voucher sample deposited in the Museo Argentino de Ciencias Naturales (MACN-in: 36350).

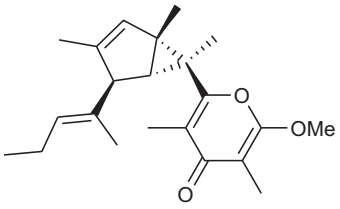
Subsequently, the biological material was lyophilized and transferred to ICB laboratory for the chemical analysis. Both the mollusk (2.0 g) and the alga (1.0 g) were extracted repeatedly with $CHCl_3$ (100 mL × 3) to obtain two lipophilic extracts (310 mg for *E. patagonica*, 222.1 mg for *B.* cf. *plumosa*) that were compared by TLC chromatography (Merck Silica Gel 60 F254 plates). Usual fatty acid and sterol components were present in both organisms extracts whereas an UV–visible band (R_f 0.35, light petroleum ether/diethyl ether, 1:1) was observed selectively in the extract of the mollusk. This extract (310 mg) was fractionated by SiO_2 column chromatography (Merck Kieselgel 60 powder) using a light petroleum ether/diethyl ether gradient as eluent. The fractions containing the UV–visible spot were recovered, combined (26.2 mg) and analyzed by ^{1}H NMR revealing the presence of a mixture of pyrone polypropionate compounds. In fact, signals attributable to an α-methoxy-β,β-dimethyl pyrone moiety as well as to a series of singlet methyl groups that were typical of elysioidean polypropionate skeletons were observed in the proton spectrum. Subsequently, the fraction of interest was submitted to reverse-phase HPLC purification [Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector; C_{18} Supelco column (5 μm; 250 × 10 mm); CH_3CN/H_2O (1:1); flow 3 mL/min; UV 254 nm] to obtain a main pure compound, phototridachiapyrone J (**1**, 1.5 mg).

The remaining related components of the mixture were recovered in too small amount, not enough for the chemical characterization. However, a substantial degradation of these metabolites occurred during the purification work-up. In particular, the relative concentration of compound **1** in the HPLC profile was observed to increase with respect to the other components under the purification conditions strongly suggesting that it derived by conversion of a related component of the mixture.

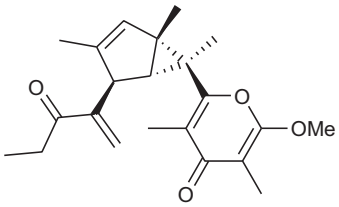
Phototridachiapyrone J (**1**) had the molecular formula $C_{22}H_{30}O_5$ as deduced by the sodiatum molecular ion at *m/z* 397.2243 $[M + Na]^+$ (Micromass Q-TOF MicroTM coupled with a HPLC Waters Alliance 2695), indicating eight unsaturation degrees. Four of them were saturated by a substituted γ-pyrone moiety with typical signals at δ 181.1 (C-3), 162.3 (C-1), 159.6 (C-5), 120.1 (C-4), and 98.9 (C-2) in the ^{13}C NMR spectrum. Intense IR bands at 1608 and 1650 cm^{-1} (Biorad FTS 155 FTIR spectrophotometer) as well as the strong UV absorbance at 256 nm (ε 8480) (Agilent 8453 spectrophotometer) were consistent with this assignment. In the carbon spectrum of **1**, were also present four sp^2 carbon resonances [δ 148.7 (s, C-12), 143.2 (s, C-10), 129.6 (d, C-9), and 114.7 (t, C-21)], which were due to one trisubstituted and one disubstituted double bonds, and thirteen sp^3 carbon signals (7 CH_3, 1 CH_2, 3 CH, and 2 qC). Thus, the remaining two unsaturation degrees required by the molecular formula had to be attributed to two rings, suggesting the presence of a photodeoxytridachione-like skeleton. The ^{1}H NMR spectrum of phototridachiapyrone J (**1**) contained seven 3H signals that were assigned to the β,β′-methyl groups [δ 1.97 (s, H_3-17), and 1.84 (s, H_3-16)] and the methoxy [δ 3.94 (s, –OMe)] located on the γ-pyrone ring, a vinyl methyl [δ 1.68 (bs, H_3-20], two tertiary methyls [δ 1.14 (s, H_3-18), and 1.25 (s, H_3-19)], and a terminal methyl [δ 0.88 (t, J = 7, H_3-15)] in the chain. Analysis of the ^{1}H–^{1}H COSY spectrum allowed to identify two distinct sequences located in the bicyclic moiety and in the lateral chain according to photodeoxytridachione (**2**) (Ireland and Scheuer, 1979; Gavagnin et al., 1994b) and related polypropionates (Ireland and Faulkner, 1981; Ksebati and Schmitz, 1985; Gavagnin et al., 1994b) framework. In addition, a hydroperoxyl function [δ_H 4.31 (t, J = 7 Hz, H-13), δ_C 89.9 (C-13)] was positioned in the lateral chain in α to the exomethylene residue [δ_H 5.26 (bs, H_2-21a), and 5.05 (bs, H_2-21b), δ_C 114.7 (t, C-21)] by diagnostic HMBC correlations between H_2-21 and both C-13 and C-11. All NMR proton and carbon values (Table 1) were in agreement with the proposed structure **1**. Phototridachiapyrone J is closely related to tridachiapyrone E (**3**), previously described from a Caribbean *Elysia crispata* population (Ksebati and Schmitz, 1985) and exhibiting a carbonyl function at C-13 instead of the hydroperoxyl function.



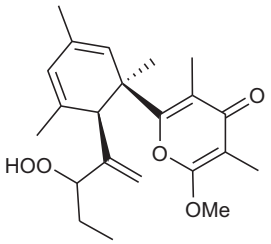
phototridachiapyrone J (**1**)



photodeoxytridachione (**2**)



tridachiapyrone E (**3**)



tridachiapyrone J (**4**)

Related polypropionate hydroperoxyl derivatives have been reported to occur in two sacoglossan species, *Placida dendritica* from Mediterranean Sea (Cutignano et al., 2003) and *Placobranchus ocellatus* from Indo-Pacific Ocean (Fu et al., 2000). In both cases, the origin of these molecules was ascribed to a photochemical oxidation of a co-occurring suitable precursor. This reaction was subsequently confirmed to proceed *via* singlet oxygenation suggesting that sacoglossan polypropionates are responsible for generating singlet oxygen themselves (Zuidema and Jones, 2005, 2006).

Phototridachiapyrone J (**1**) is the 13-hydroperoxyl derivative of photodeoxytridachione (**2**). Analogously with other hydroperoxyl polypropionates, **1** could be formed in the mollusk by photooxidation at C-13 of **2**. On the other side, phototridachiapyrone J (**1**) is the bicyclohexene-containing derivative of tridachiapyrone J (**4**), which was previously reported from the Indo-Pacific sacoglossan *P. ocellatus* (Fu et al., 2000). Alternatively, **1** could be originated from **4** by the same photoconversion mechanism as that reported for the production of photodeoxytridachione (**2**) (Ireland and Scheuer, 1979). However, neither **2** nor **4** were detected in the extract of *E. patagonica* thus preventing any suggestion about the possible origin of **1**.

Table 1
NMR data[a] [b] of phototridachiapyrone J (**1**).

C	δ_C	m[c]	δ_H	m, *J*, Hz	Significant HMBC correlations
1	162.3	s	–	–	H_3-OMe, H_3-16
2	98.9	s	–	–	H_3-16
3	181.1	s	–	–	H_3-16, H_3-17
4	120.1	s	–	–	H_3-17
5	159.6	s	–	–	H_3-17
6	41.5	s	–	–	H_3-18
7	12.8	d	1.24	s	–
8	32.3	s	–	–	H_3-18
9	129.6	d	5.45	s	H_3-19, H_3-20
10	143.2	s	–	–	H_3-20
11	50.1	d	2.83	br. s	H_3-20, H_2-21
12	148.7	s	–	–	H-11
13	89.9	d	4.31	t, 7	H_3-15, H_3-21
14	24.8	t	1.62–1.73	m	–
15	10.4	q	0.88	t, 7	–
16	6.8	q	1.84	s	–
17	10.8	q	1.97	s	–
18	13.0	q	1.14	s	–
19	17.5	q	1.25	s	–
20	13.7	q	1.68	s	–
21	114.7	t	5.26	s	–
			5.05	s	
OMe	54.9	q	3.94	s	–

[a] DRX 600, Avance 400, and DPX 300 MHz Bruker spectrometers; chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) for proton and to $CDCl_3$ (δ 77.0) for carbon.
[b] Assignments made by HSQC and HMBC ($J = 10$ Hz) experiments.
[c] By DEPT sequence.

4. Chemotaxonomic significance

The occurrence in *E. patagonica* of a molecule belonging to deoxytridachione/photodeoxytridachione structural family is in agreement with the chemical scenario depicted for elysioidean sacoglossan opisthobranchs (Cimino et al., 1999; Cimino and Ghiselin, 1998, 2009). These γ-pyrone polypropionates could be considered chemical markers for a selected group of *Elysia* sacoglossans including *E. crispata* (Ireland et al., 1979; Ireland and Faulkner, 1981; Ksebati and Schmitz, 1985), *Elysia diomedea* (Ireland et al., 1978; Ireland and Faulkner, 1981; Cueto et al., 2005; Díaz-Marrero et al., 2008), *Elysia timida* (Gavagnin et al., 1994b), *E. chlorotica* (Dawe and Wright, 1986), *Elysia viridis* (Gavagnin et al., 1994a; Cutignano et al., 2009) and *E. patagonica* (this paper). Interestingly, the sacoglossan *P. ocellatus* is also featured by the same chemistry (Ireland and Scheuer, 1979; Fu et al., 2000; Manzo et al., 2005).

It is retained that elysioidean mollusks employ polypropionates as sunscreen in heavily photolytic habitat and that the biosynthesis of these molecules is influenced by light irradiation (Ireland and Scheuer, 1979; Ireland and Faulkner, 1981). In particular, the strict dependence of the structural polypropionate arrangement on the light conditions has been recently demonstrated for *E. viridis* (Cutignano et al., 2009). Similar mechanisms could be assumed to occur in other elysioidean sacoglossans thus determining the chemodiversity that is found in the nature.

Acknowledgements

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