



Original Article

Is phototridachiahdropyrone a true natural product?☆



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ABSTRACT

The occurrence of (–)-phototridachiahdropyrone (**5**) in nature has been proven. This compound has been now identified as minor component of the extract of marine sacoglossan mollusk *Elysia crispata* from which the main (–)-tridachiahdropyrone (**4**) was previously described. Synthetic (±)-**5** was formerly obtained by Moses' group by biomimetic photochemical conversion of (±)-tridachiahdropyrone (**4**). The same authors suggested that compound **5** had to be a natural product derived from precursor **4** "yet to be discovered". Comparison of CD profiles of natural (–)-**4** and (–)-**5** indicated the same absolute configuration for both compounds. This evidence is in agreement with the concerted mechanism proposed for the photochemical conversion.

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Introduction

The solar radiation by penetrating the sea surface strongly influences the physical, biological and chemical processes of sea flora and fauna, forcing marine organisms to adopt strategies for defending themselves to harmful UV radiation (Ireland and Scheuer, 1979). This occurs particularly in shallow waters where the exposition to sunlight is intense. Among the organisms living in highly photophilic habitats, a group of herbivorous marine opisthobranch gastropods belonging to the family Plakobranchidae (Mollusca: Gastropoda: Sacoglossa) are known as "solar-powered mollusks" (Rudman, 1998; Rumpho et al., 2000). Actually, these animals assimilate chloroplasts from siphonaceous marine algae and maintain the active organelles for several months in their own tissues where they carry out the photosynthesis (Jensen, 1997; Rumpho et al., 2000, 2008; Evertsen et al., 2007). Natural products from plakobranchids include photo-active γ -pyrone polypropionate-derived compounds that have been suggested to serve as sunscreens to protect the mollusks from damaging UV radiation (Ireland and Scheuer, 1979). The molecular network of these polypropionates displays complex cyclic structures all including a distinctive γ -pyrone moiety bearing an α -methoxy group. Starting from the first report of tridachione in the late 1970s from the Pacific *Elysia* (= *Tridachiella*) *diomedea* (Ireland et al., 1978; Ireland and Faulkner, 1981), a certain number of such

γ -pyrone polypropionates have been described so far from different plakobranchidean species collected in distinct geographical areas (reviewed by Cimino et al., 1999; Cimino and Ghiselin, 2009; recent reports by Díaz-Marrero et al., 2008; Carbone et al., 2013). This is 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 plakobranchidean 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).

Four distinct structural architectures can be recognized in plakobranchidean polypropionates: 1,3-cyclohexadiene derivatives, e.g. 9,10-deoxytridachione (**1**) (Ireland and Faulkner, 1981); bicyclo[3.1.0]hexanes, e.g. photodeoxytridachione (**2**) (Ireland and Scheuer, 1979); bicyclo[4.2.0] hexanes, e.g. ocellapyrone A (**3**) (Manzo et al., 2005; Miller and Trauner, 2005); and fused pyrone-containing bicyclic ring derivatives, e.g. tridachiahdropyrone (**4**) (Gavagnin et al., 1996; Jeffery et al., 2005; Sharma et al., 2008).

The photochemical relationship between cyclohexadiene-containing and bicyclohexene-containing sacoglossan polypropionates was demonstrated by photoconversion of **1** into **2** in both *in vitro* (Ireland and Faulkner, 1981; Zuidema et al., 2005) and *in vivo* (Ireland and Scheuer, 1979). The *in vitro* experiments demonstrated that the conversion of **1** into **2** occurs with retention of optical activity according to the $[\sigma 2_a + \pi 2_a]$ rearrangement mechanism proposed by Ireland and Faulkner (1981). The alternative biradical pathway via a triplet excited state process has been also suggested (Zuidema et al., 2005). The *in vivo* experiments led to the observation that the natural light-dependent process may not be enzymatic and is prompted when the UV radiation penetrating the dorsal surface of

☆ Dedicated to Prof. Rosângela De Almeida Epifanio's memory. She spent one year in our institute giving, with her enthusiasm, culture, will, and rigorous work, relevant contributions to marine chemistry.

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the mollusk exceeds the absorption limits of the γ -pyrone moiety, consistent with the sunscreen protective role suggested for these polypropionates (Ireland and Scheuer, 1979). The photochemistry of plakobranchidean polypropionates has been extensively studied in the last ten years and several syntheses including biomimetic synthesis have been appeared in the literature (reviewed by: Beaudry et al., 2005; Miller and Trauner, 2006; Sharma et al., 2011). Based on these studies, it has been proposed that many complex polypropionate metabolites may be derived biosynthetically from linear polyenes with all *E*-configuration (Moses et al., 2003; Rodriguez et al., 2007). All the core cyclic structures should be formed through mechanisms involving the *E*-*Z* double bond isomerization followed by thermal and/or photochemical electrocyclic cyclization with [4+2] cycloaddition reactions or [2+2] concerted rearrangements. Supporting this hypothesis, a number of diverse polypropionates from plakobranchids have been synthesized starting from a linear tetraene-pyrone precursor (Eade et al., 2008; Sharma et al., 2009).

In this group of complex molecules, tridachiahypopyrones are unique members exhibiting the most interesting and unusual structural motifs with the γ -pyrone forming part of the core framework and the rearrangement of C-12 methyl group shifted to the C-13 position. The prototype (–)-tridachiahypopyrone (**4**) was isolated several years ago by us from a Venezuelan collection of *Elysia crispata* (Gavagnin et al., 1996) and the originally proposed structure (**4a**) was later reassigned as **4** by synthesis (Jeffery et al., 2005; Sharma et al., 2008, 2009). Related oxidized derivatives were also described from *Placobranchus ocellatus* (Fu et al., 2000; Sharma et al., 2009).

Surprisingly, biomimetic photochemical synthesis of (\pm)-tridachiahypopyrone (**4**) performed by Moses's group (Sharma et al., 2009, 2011) led to the additional unprecedented polypropionate (\pm)-**5**, which was obtained by photochemical conversion of **4** and named phototridachiahypopyrone. The authors suggested that compound **5**, the structure of which was secured by X-ray analysis, could be a natural product "yet to be discovered" (Sharma et al., 2009; Sharma and Moses, 2010).

Now, we have re-examined the extract of *E. crispata*, the same as previously investigated (Gavagnin et al., 1996, 1997, 2000), with the aim to verify this hypothesis. A minor metabolite co-occurring with (–)-tridachiahypopyrone (**4**) had been detected at that time but the structure was not determined. We report here the characterization of this compound, just identified as (–)-phototridachiahypopyrone (**5**).

Materials and methods

General procedures

Si-gel chromatography was performed by using precoated Merck F254 plates and Merck Kieselgel 60 powder. Optical rotations were measured on a Jasco DIP370 digital polarimeter. The UV spectra and CD curves were recorded on a Agilent 8453 spectrophotometer and JASCO 710 spectropolarimeter, respectively. The IR spectra were taken on a Bio-Rad FTS 7 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker WM 500 MHz and a Bruker AM 400 MHz spectrometers in CDCl_3 ; chemical shifts are reported in parts per million referenced to CHCl_3 as internal standard (δ 7.26 for proton and δ 77.00 for carbon). EI-MS spectra were measured on a TRIO 2000 VG Carlo Erba spectrometer.

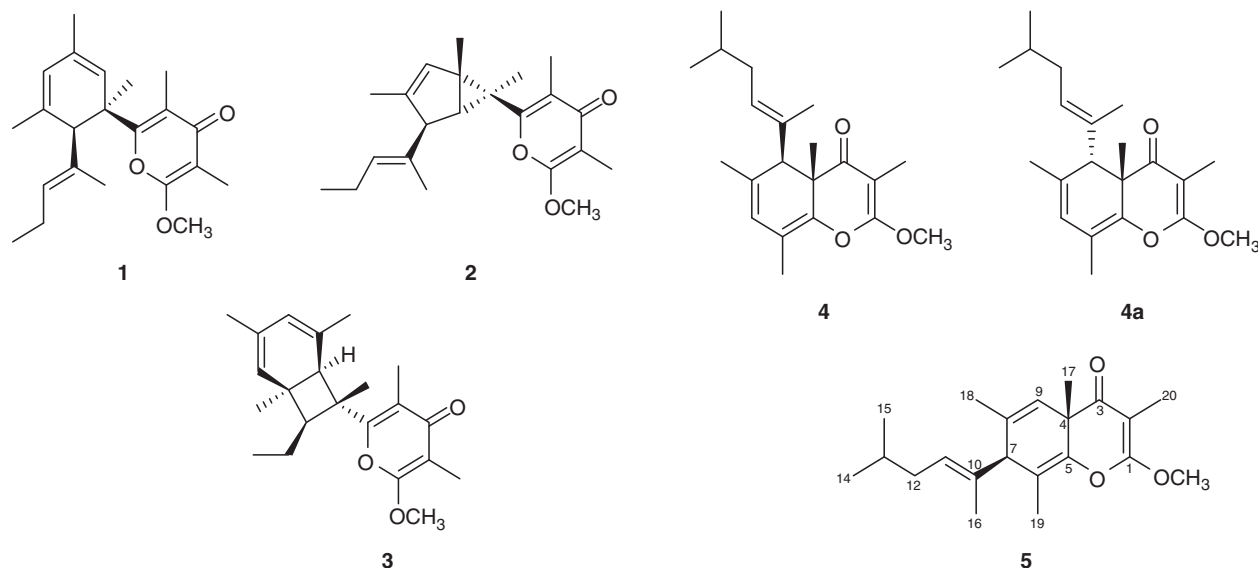
Biological material

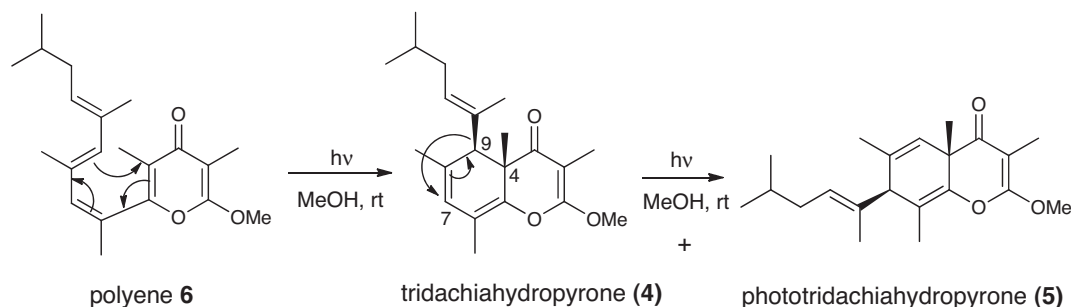
Elysia crispata individuals (25 animals, average size 8 cm) were collected by SCUBA divers off Mochima (Venezuela) at a depth of 3–10 m, in November 1993, as it has been previously described (Gavagnin et al., 1996, 1997). The mollusks were identified by Prof. J. Ortea (Universidad de Oviedo), immediately frozen and subsequently transferred to Istituto di Chimica Biomolecolare laboratory, in Italy.

Purification of phototridachiahypopyrone (**5**) and acquisition of spectroscopic data

As it has been already reported in the previous papers (Gavagnin et al., 1996, 1997), the frozen material was exhaustively extracted with acetone. The diethyl ether-soluble portion (1.16 g) of the acetone extract was analyzed by TLC and then fractionated by Si-gel column chromatography (light petroleum ether/diethyl ether gradient) to give a series of polypropionates (Gavagnin et al., 1996, 1997), including (–)-tridachiahypopyrone (**4**), which was the main component (15.8 mg) of the fractions eluted by light petroleum ether/diethyl ether, 9:1. Additional less polar fractions that were at that time collected have been now combined (10.7 mg) and submitted to Si-gel chromatography (light petroleum ether/ CHCl_3 , 6:4) to give pure compound **5** (3.5 mg).

(–)-Phototridachiahypopyrone (**5**): oil; $[\alpha]_D -46.0^\circ$ (CHCl_3 , $c=0.35$); CD (*n*-hexane, $c=3.9 \times 10^{-5}$) λ_{max} $[\theta]$: 307 (–7353), 270 (+23,320), 220 (+21,530) nm; IR (liquid film) ν_{max} 1585 (shoulder),





Scheme 1. Biomimetic conversion of polyene **6** into **4** and **5**.

From Sharma et al. (2009).

Table 1
NMR data^a of phototridachiahydropyrone (**5**).

Carbon	δ_{H} (mult, <i>J</i>)	δ_{C}	<i>m</i>	HMBC (C→H)
1	–	165.7	<i>s</i>	H ₃ -20; –OMe
2	–	87.9	<i>s</i>	H ₃ -20
3	–	194.8	<i>s</i>	H-9; H ₃ -17; H ₃ -20
4	–	44.6	<i>s</i>	H-7; H-9; H ₃ -17
5	–	145.0	<i>s</i>	H-7; H-9; H ₃ -17; H ₃ -19
6	–	117.4	<i>s</i>	H-7; H ₃ -19
7	3.08 (<i>s</i>)	57.5	<i>d</i>	H-9; H-11; H ₃ -18; H ₃ -19
8	–	131.8	<i>s</i>	H-7; H ₃ -18
9	5.95 (<i>s</i>)	12.8	<i>d</i>	H-7; H ₃ -17; H ₃ -18
10	–	133.4	<i>s</i>	H-7; H ₂ -12; H ₃ -16
11	5.41 (bt, 7.1)	129.2	<i>d</i>	H-7; H ₂ -12; H ₃ -16
12	1.96 (dd, 7.1, 6.9)	37.4	<i>t</i>	H-11; H ₃ -14; H ₃ -15
13	1.65 (<i>m</i>)	28.9	<i>d</i>	H ₂ -12; H ₃ -14; H ₃ -15
14	0.91 (<i>d</i> , 6.6)	22.4	<i>q</i>	H ₂ -12
15	0.91 (<i>d</i> , 6.6)	22.4	<i>q</i>	H ₂ -12
16	1.38 (<i>bs</i>)	11.9	<i>q</i>	H-7; H-11
17	1.34 (<i>s</i>)	27.6	<i>q</i>	H-7
18	1.60 (<i>s</i>)	21.0	<i>q</i>	H-7; H-9
19	1.64 (<i>s</i>)	13.1	<i>q</i>	H-7
20	1.64 (<i>s</i>)	6.7	<i>q</i>	–
–OMe	3.99 (<i>s</i>)	55.0	<i>q</i>	–

^a Bruker WM 500 and AM 400 MHz spectrometers; CDCl₃; assignments made by ¹H–¹H COSY, HSQC and HMBC experiments (*J* = 6 and 10 Hz).

1613 (broad) cm⁻¹; UV (MeOH) λ_{max} 271 (ϵ = 6400) nm; EIMS, *m/z* (%): 330 (*M*⁺, 6), 315 (36), 243 (50), 233 (59), 173 (100); HREIMS, *m/z* 330.2210 (C₂₁H₃₀O₃ requires 330.2195). ¹H and ¹³C NMR in Table 1.

Results and discussion

The extract of the sacoglossan *E. crispata* has been re-considered with the aim to identify compound **5** among the minor metabolites which were not previously described (Gavagnin et al., 1996, 1997). In particular, the less polar fraction obtained by the first chromatographic fractionation of crude diethyl ether extract of *E. crispata* (Gavagnin et al., 1996, 1997) has been re-analyzed. Further purification steps of this fraction had led to the isolation of the main component, (–)-tridachiahydropyrone (**4**), as previously described (Gavagnin et al., 1996). A minor more polar related compound had also been detected in the same fraction at that time (unpublished data) but it was not purified and characterized by spectroscopic analysis. However, a preliminary ¹H NMR analysis of an unpurified sample had showed a structural relationship with compound **4**. The fractions containing this unreported compound have been now combined, checked by ¹H NMR and then submitted to Si-gel purification to give 3.5 mg of pure (–)-phototridachiahydropyrone (**5**).

Compound **5** had the molecular formula C₂₁H₃₀O₃ and the EIMS fragmentation pattern the same as that observed for

tridachiahydropyrone (**4**). Analysis of NMR spectra of **5** confirmed the close structural relationship with **4**, in particular indicating the presence of the bicyclic core including the γ -pyrone ring as well as of the lateral alkyl chain. A check of published data (Supporting information in Sharma et al., 2009) confirmed that natural polypropionate **5** was phototridachiahydropyrone. Full assignment of proton and carbon values that was not previously reported is listed in Table 1.

As it was mentioned before, (±)-phototridachiahydropyrone (**5**) was an unexpected product formed in the course of photochemical electrocyclic conversion of γ -pyrone polyene precursor **6** to (±)-tridachiahydropyrone (**4**) under irradiation with a UV lamp (Scheme 1 according to Sharma et al., 2009). A selective tandem sequence of photochemical transformations was observed: first, the formation of **4** by cyclization of **6** and, subsequently, the conversion of **4** into **5**, under the same reaction conditions. Prolonged irradiation of **4** resulted into the complete and irreversible conversion to **5** suggesting that phototridachiahydropyrone (**5**) is the preferred photochemical product (Sharma et al., 2009).

This conversion was suggested to occur through a photochemical 1,3-sigmatropic migration of lateral alkyl chain from C-9 to C-7 according to retention of the relative configuration of methyl at C-4 and the side chain in **5** with respect to **4**.

Natural (–)-phototridachiahydropyrone (**5**) is optically active and displays the CD profile identical with that of natural (–)-tridachiahydropyrone (**4**) (Fig. 1) implying the same absolute configuration. However, the absolute stereochemistry of tridachiahydropyrones remains to be determined and thus enantiomers drawn in structures **4** and **5** have been chosen arbitrarily.

The isolation of propionate **5** from *E. crispata* supports the ideas that led to predictions of its existence in nature. Natural **5**, in fact, could be generated from **4** through a concerted photochemical mechanism according to synthetic process (Sharma et al., 2009; Sharma and Moses, 2010). In the natural habitat, this transformation appears to be only partial as indicated by the approximate ratio of tridachiahydropyrones (**4**:**5**, 5:1) detected in the *E. crispata* extract. This is most likely due to the attenuation of UV light in the seawater by the masking effect influenced by several factors such as dissolved organic materials, depth of water, temperature, etc. (Sharma and Moses, 2010).

The finding of phototridachiahydropyrone (**5**) as a natural product is in agreement with the photochemical framework of polypropionates from plakobranchids and represents an additional evidence that these molecules may act as sunscreens for the producing organisms by protecting them from harmful radiation and oxidative damage. Recently, the interaction of tridachiahydropyrone (**4**) and the corresponding linear polyene precursors with cell membranes has been provided by biophysical evidences. The phospholipid bilayer of the molluscan cell membrane has been suggested to be the site of localization of these compounds where they serve as natural sunscreens (Powell et al., 2012).

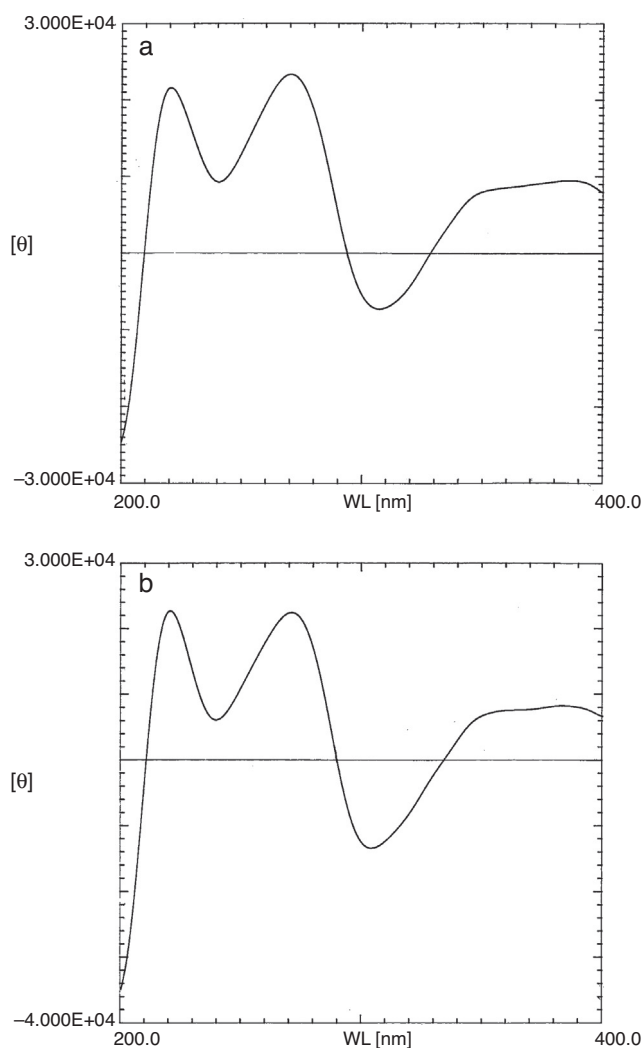


Fig. 1. CD curves $[\theta]$ of (a) (–)-phototridachiahypopyrone (5) and (b) (–)-tridachiahypopyrone (4).

Authors contributions

EM contributed in running the purification work. MG supervised the laboratory work, analyzed spectroscopic data, and drafted the paper. GC contributed to analysis of the data and to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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