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Shaping the Polypropionate Biosynthesis in the Solar-Powered Mollusc *Elysia viridis*

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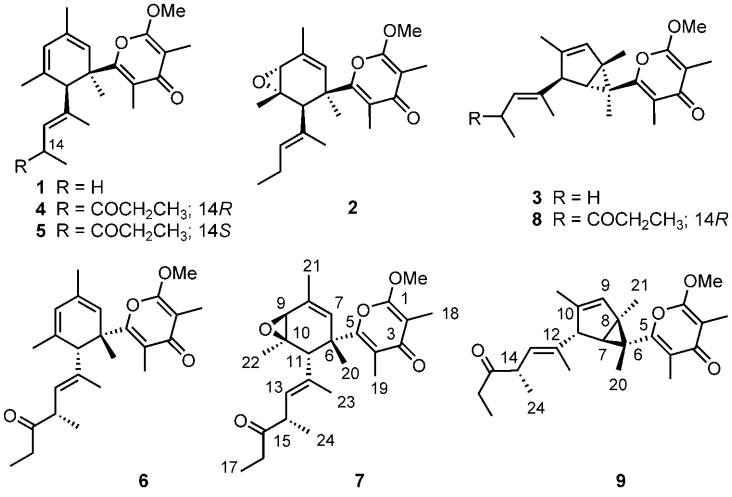
Dedicated to the memory of Prof. Rodolfo Alessandro Nicolaus.

Polypropionates that incorporate pyrones are a family of polyketides featuring the chemistry of a few marine molluscs capable of phototrophic CO_2 fixation as a result of storing viable symbiotic chloroplasts in their bodies. The role and origin of these molecules is poorly investigated, although the unusual biological activities and chemistry of these natural products have recently received renewed interest. Here, we report the results of in vivo studies on production of γ-pyrone-containing polypropionates in the Mediterranean mollusc Elysia viridis. *Biosynthesis of the metabolites in the sacoglossan is shown to proceed through condensation of eight intact C_3 units by polyketide synthase assembly. LC–MS and NMR spectroscopic studies demonstrate that the process involves a pyrone tetraene (**10**) as key intermediate, whereas the levels of the final polypropionates (**6**, **7** and **9**) are related to each other and show a significant dependence upon light conditions.*

Introduction

Gastropod molluscs of the family Elysiidae in the order Sacoglossa have acquired the ability to assimilate algal chloroplasts that can remain photosynthetically active for several months in the mollusc body.[1–3] Persistence of endosymbiont activy requires synthesis of alga proteins in the cytoplasm of the molluscs, thus suggesting that the sacoglossans are able to acquire algal genes in addition to viable plastids upon grazing.[4] With brilliant intuition, Rumpho named these unusual forms of life "solar-powered molluscs".[1]



Chloroplasts acquired by Elysiids could be also involved in the de novo biosynthesis of γ-pyrone-containing polypropionates, a family of polyketides that are found in a few bacteria and fungi, which the molluscs employ as sunscreen in heavily photophilic habitats.[5] Since the first finding in 1978,[6] the intriguing structures and biological functions of elysiid polypropionates have attracted the attention of chemists. The molecular network that is found in the sacoglossans is prototyped by (−)-9,10-deoxytridachione (**1**), (−)-tridachione (**2**) and photodeoxytridachione (**3**), which were first described in the late 1970s from the Pacific Elysiids *Tridachiella diomedea* and *Tridachia crispata*.[6,7] Higher homologues with eight propionate units or acetate/propionate derivatives have been also reported in this family of molluscs.[8–10] In addition, it is not uncommon for the stereochemistry of these molecules to also vary in relation to the animal from which the polypropionate was isolated. Hence, the regular octapropionate skeleton of tridachiapyrone A (**4**) from *Tridachia crispata*[8] is suggested to be an epimer at C14 of isotridachiapyrone A (**5**) from the same animal, and an enantiomer to (+)-elysione (**6**), which was found in *Elysia chlorotica*[9] and *Elysia viridis*.[11] Finally, the in vivo[5] and in vitro[7] light-dependent conversion of the regular heptapropionate **1** into **3** has been described. The reaction has been extensively studied,[12–14] and recently Jones and co-workers[15] proved an unusual diradical mechanism that is triggered by the photo-sensitizing activity of the γ-pyrone moiety (Scheme 1 A). More generally, several authors have suggested that light irradiation can play a role in the formation of Elysiid polypropionates and determines the chemodiversity that is found in nature.

In the present paper, we study the factors that affect the biosynthesis of elysione (**6**) and the novel epoxyelysione (**7**) in *Elysia viridis*. Feeding experiments with ^{13}C-labelled precursors

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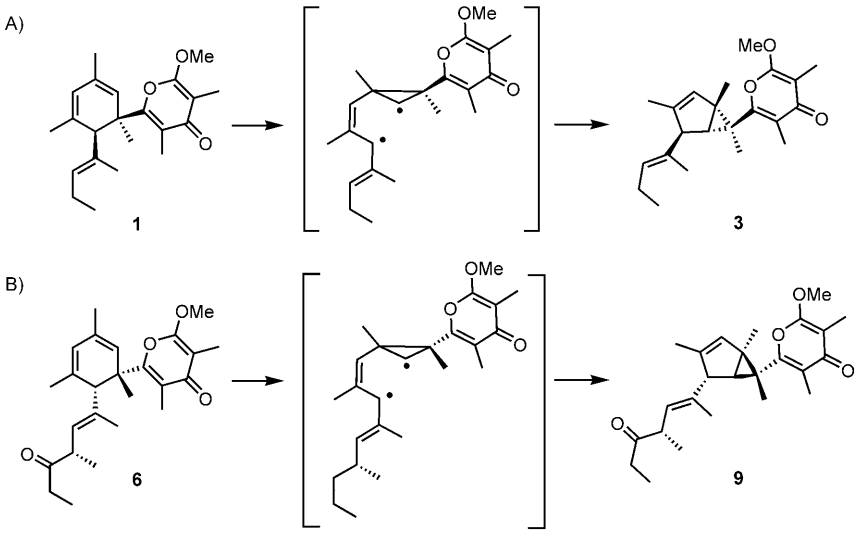


Scheme 1. Proposed diradical mechanism for the skeleton rearrangement of cyclohexadiene into bicyclo-[3.1.0]hexene in propionates from marine sacoglossans (according to ref. [15]).

have been used to unambiguously determine the biosynthesis of the polypropionate skeleton. Furthermore, we describe the effect of light on the polyketide synthesis with the aim to provide a functional rationale for polypropionate production in the invertebrate.

Results

Isolation and characterisation of mollusc metabolites.

E. viridis is a Mediterranean shell-less mollusc that lives in photophilic habitats and feeds on the algae *Codium vermilara* and *Bryopsis plumosa*. The biosynthetic study was complemented by a complete characterisation of the mollusc polypropionates. In particular, 2D NMR spectroscopic experiments on (+)-elysione (**6**) led to the first full NMR spectroscopic assignment of the molecule that was isolated from Dawe and Wright in 1986 (Table 1).[9] As a result of the recent synthesis of (−)-crispatene (**8**),[16] the absolute stereochemistry of the cyclo-

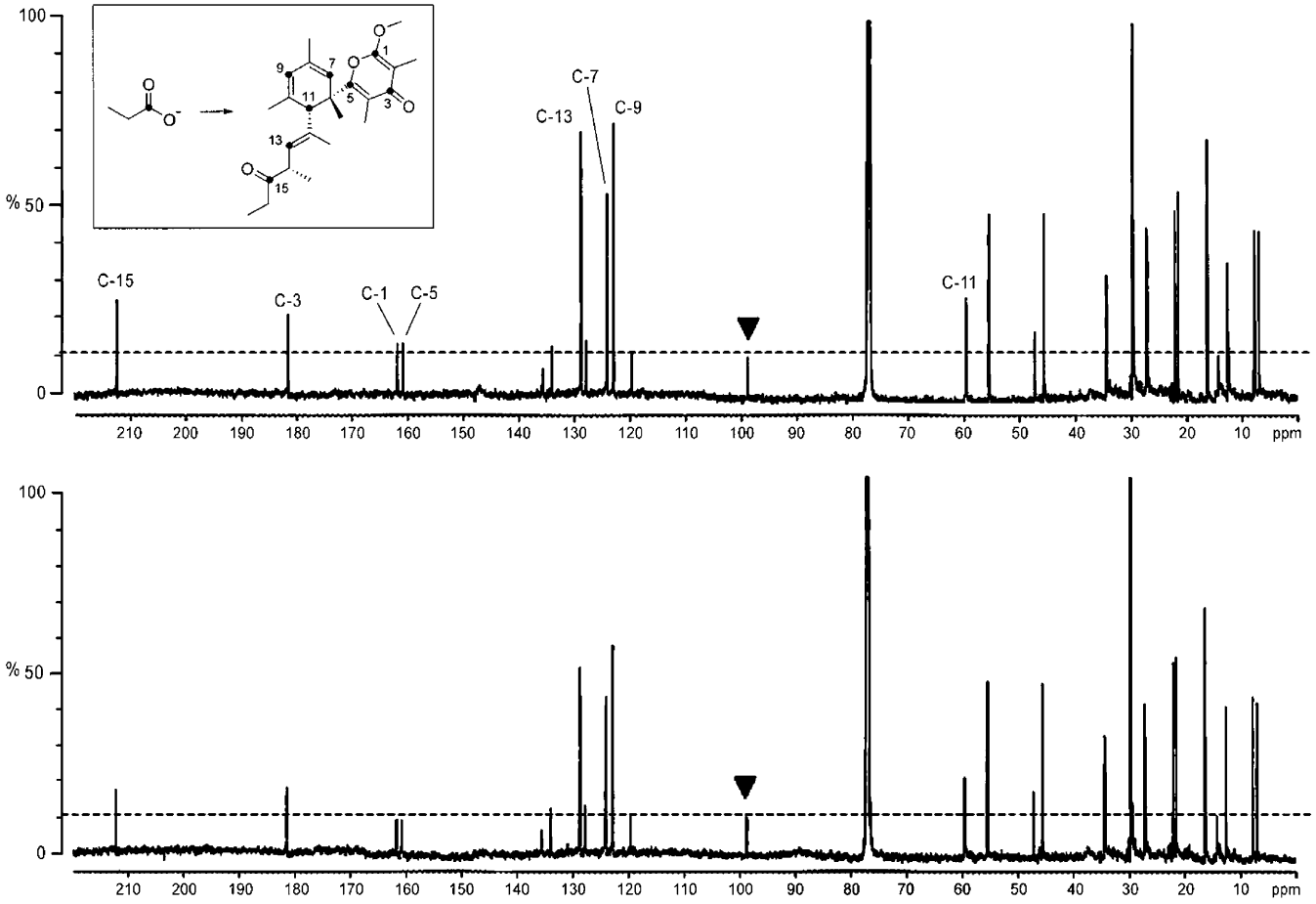


Figure 1. Observed labelling of elysione (**6**) after feeding experiments with sodium [1-^{13}C]propionate. ^{13}C NMR spectra of natural (bottom) and ^{13}C-enriched (top) samples. The insert shows the incorporation pattern of the mollusc polypropionate (numbers indicate the labelled carbons). The dark triangle shows the unlabelled signal (C2) that was used as an internal reference for calculating the ^{13}C-enrichment yield.

Table 1. NMR spectroscopic data of major polypropionates of *Elysia viridis* and ^{13}C-incorporation of elysione (**6**) after feeding experiments with sodium [1-^{13}C]propionate.

	6			**7**	
	^{1}H δ[b], *m*	^{13}C (ppm)	Area increase[a] [%]	^{1}H δ, *m*	^{13}C (ppm)
1		161.7	38.8		161.6
2		98.8	Int. Std		98.2
3		181.5	54.5		181.2
4		119.6	–		118.5
5		160.7	44.9		159.7
6		47.1	–		46.7
7	5.61, s	124.0	12.1	5.99, s	129.2
8		127.8	–		128.5
9	5.70, s	122.8	25.7	3.10, s	57.9
10		133.9	–		60.7
11	2.79, s	59.6	13.8	2.95, s	56.2
12		135.6	–		136.0
13	5.06, d, J=9.4 Hz	128.7	20.5	5.22, d, J=9.0 Hz	129.5
14	3.19, m	45.5	–	3.19, m	44.8
15		212.2	30.5		212.0
16	2.09, m	34.3	–	2.01, bq, J=7.1 Hz	34.6
17	0.88, t, J=7.0 Hz	7.6	–	0.88, t, J=7.1 Hz	7.3
18	1.81	6.9	–	1.78, s	6.6
19	2.09	12.4	–	2.07, s	12.0
20	1.43	27.1	–	1.58, s	31.9
21	1.80	21.5	–	2.02, s	21.8
22	1.71	22.1	–	1.31, s	22.1
23	1.46	14.1	–	1.47, s	12.5
24	1.02, d, J=6.8 Hz	16.2	–	1.04, d, J=6.8 Hz	16.0
OMe	3.97	55.4	–		55.0

[a] Calculated on peak area as follows: Increase = (labelled signal − natural signal)/natural signal. [b] In agreement with ref. [11]. Int. Std = signal used to normalise spectra of natural and labelled **6**.

hexadiene ring in (−)-9,10-deoxytridachione (**1**), tridachiapyrone A (**4**) and its enantiomer, (+)-elysione (**6**), is predictable as depicted. Furthermore, following the arguments of Ksebati and Schmitz,[8] the configuration at C14 of **6** is suggested to be *S*.

Along with (+)-elysione (**6**), the lipid extracts of the mollusc also contained a novel minor metabolite, which was characterised as 9,10-epoxyelysione (**7**, 0.04 mg/specimen) [HRMS (ESI$^+$): *m/z* calcd for $C_{25}H_{34}O_5Na^+$: 437.2304 [*M*+Na]$^+$; found: 437.2285]. For the most part, the two regular polypropionates **6** and **7** have the same NMR spectroscopic signals, except in **7** the double bond C9/C10 has been replaced with a trisubstituted oxirane (Table 1). Accordingly, the NMR spectra of **7** shows an oxygen-bearing carbon atom at 57.9 ppm (C9) that is linked to a ^{1}H singlet at 3.10 ppm (H9). This latter signal had long-range hetero-correlation with the quaternary carbons at 60.7 (C10) and 128.9 ppm (C8). Careful study of NOEs indicates that the oxygen of the epoxide ring and H11 of **7** are *cis*, as are the largest substituents at C6 and C11 (see the Supporting Information); this suggests a relative configuration that is identical to that previously described for tridachione (**2**).[6] Likewise, ^{1}H and ^{13}C NMR spectroscopic data of the cyclohexene core in **7** and **2** were almost identical, thus corroborating the above assignment. On the other hand, (+)-epoxyelysione (**7**) ($[\alpha]_D$ = + 54.3, $CHCl_3$) and (−)-tridachione (**2**) ($[\alpha]_D$ = - 113.3, $CHCl_3$) are presumably enantiomeric at C6, C9, C10 and C11, as are (+)-elysione (**6**) and (−)-deoxytridachione (**1**).[9] The similarity in chemical shifts for H_3-24 in **6** and **7** (1.02 and 1.04 ppm, respectively) argues for the same absolute stereochemistry of *S* at C14.

In vivo feeding experiments with labelled probes

To address the biosynthesis of polypropionates in *Elysia*, live molluscs were injected with sodium [1-^{13}C]propionate (6 mg in 40 μL distilled water per specimen). Incorporation of the labelled material was determined by comparing the peak area of corresponding signals (normalised to C2, 98.8 ppm) in the spectra of isotopically labelled and natural-abundance elysione (**6**; Figure 1). Signals of carbon atoms C1 (161.7 ppm), C3 (181.5 ppm), 5 (160.7 ppm), C7 (124.0 ppm), C9 (122.8 ppm), C11 (59.6 ppm), C13 (128.7 ppm) and C15 (212.2 ppm) manifested an average increase of 30% (Table 1). As showed in the insert of Figure 1, the enriched positions correspond to C1 of propionate and prove the specific incorporation of exogenous intact C_3 units in the skeleton of the mollusc metabolite.

Photoreactivity of Elysia *propionates*: The photochemical rearrangement of marine propionates containing the 1,3-cyclohexadiene moiety to the corresponding bicyclo[3.1.0]hexene skeleton was first described by Ireland and Faulkner, who photolysed deoxytridachione (**1**) to photodeoxytridachione (**3**) by using artificial illumination.[7] Similar skeleton rearrangements are rather common, and in the last years several additional examples have been described in the sacoglossan chemistry.[10,17,18] To test the photochemical stability of *Elysia* propionates, elysione (**6**; 2.1 mg) and epoxyelysione (**7**; 0.3 mg) were

dissolved in benzene and exposed to direct sunlight (800–1200 $\mu E\,m^{-2}\,s^{-1}$) at 4 °C for 3 h. Under these conditions, elysione gave a plethora of photoproducts, the major ones of which were attributable to a family of compounds with a molecular weight of *m/z* 421 ($[M+Na]^+$) and a UV maximum at 264 nm by LC–MS (Figure 2). Epoxyelysione (**7**), on the other hand, was recovered unaffected.

Extensive NMR spectroscopic analysis of the mixture of products that were generated by photorearrangement of elysione led to the identification of a major compound (**9**), which shows ^1H and ^{13}C spectroscopic resonances that are remarkably similar to those of (−)-crispatene (**8**).[16] In particular, the typical bicyclo[3.1.0]cyclohexene core was unequivocally characterised by HSQC and HMBC ($J=7$ and 10 Hz) spectroscopy, which showed the correlation of the quaternary cyclopropyl carbon atoms at 40.7 ppm (C6) and 31.8 ppm (C8) with the angular methyl groups H_3-21 (1.19 ppm) and H_3-20 (1.10 ppm).[10,16] This latter signal also had a very diagnostic correlation with C5 (160.9 ppm) of the γ-pyrone moiety. According to these data, the olefinic carbons (143.3 and 128.9 ppm,) of the cyclopentene ring revealed the typical downfield shift compared to those in cyclohexene or cyclohexadiene systems.[19] Except for the vinyl singlet at 5.37 ppm (H9) and the broad doublet at 5.25 ppm ($J=9.4$; H13), the other signals are identical to that of **6**. It was not possible to isolate the novel product, but in consideration of the similarity of the NMR spectroscopic data with (−)-crispatene (**8**), combined with the origin of this molecule from photorearrangement of the cyclohexadiene system of (+)-**6** (Scheme 1 B), we suggest that **9** might be the enantiomer of the product that was isolated from *Tridachia crispata*[7] and synthesised by Trauner.[16] The remaining LC–MS peaks with retention times ranging from 16 min and 22 min (Figure 2 A) included unreacted elysione (**6**, $t_R=19.36$ min) together with minor, uncharacterised isomers that are probably derived from the diradical intermediate that was suggested by Trauner and Jones (Scheme 1).[15]

Light-induced effect on living animals

Elysia viridis was collected by scuba diving off the Gulf of Naples. The animals were transferred to our institute in an aerated tank and then kept in an aquarium for three weeks with

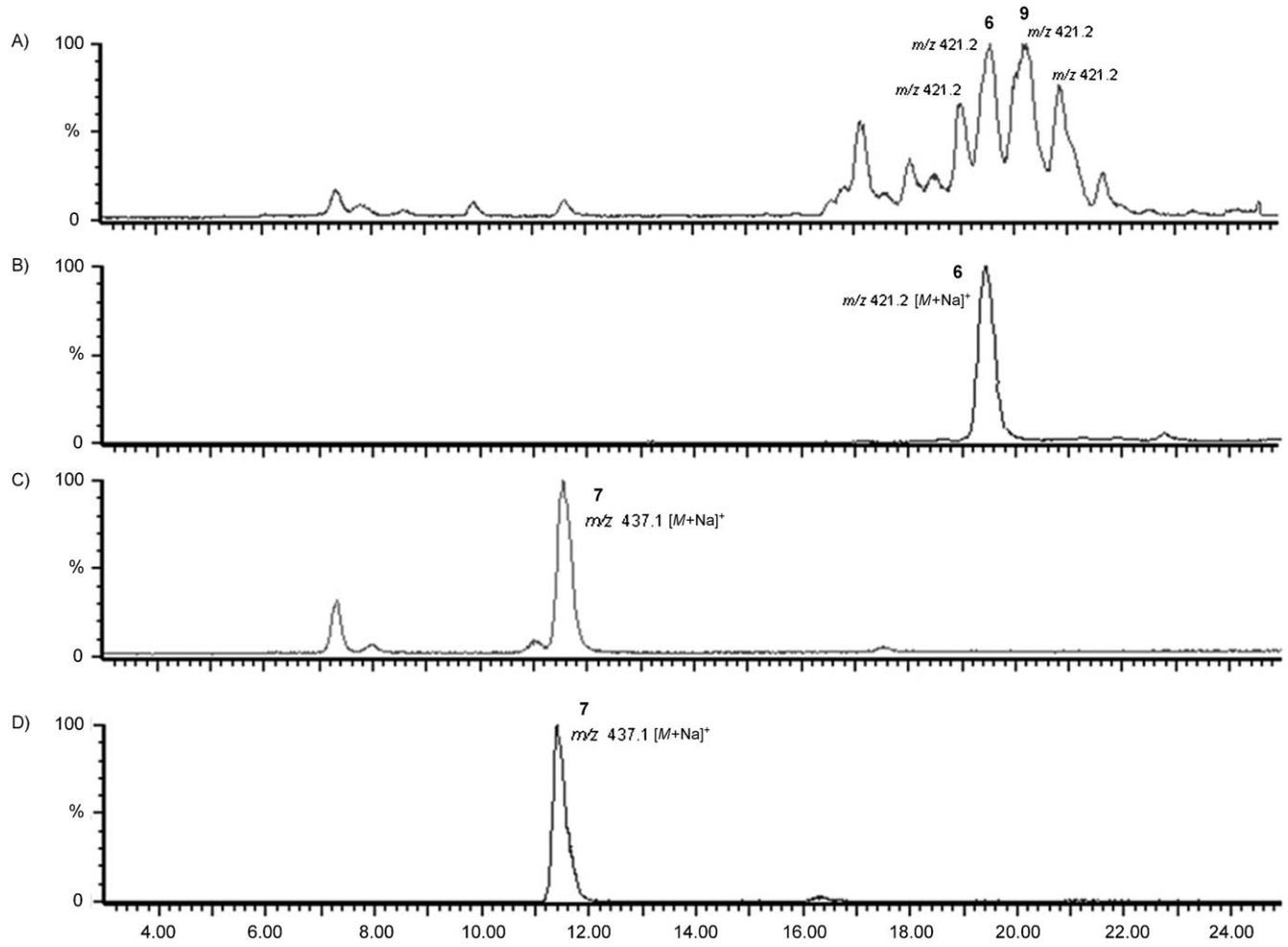


Figure 2. Effect of light exposure on sacoglossan polypropionates. RP-ESI$^+$ LC–MS analysis of pure natural products that were exposed to direct sunlight (800–1200 $\mu E\,m^{-2}\,s^{-1}$) at 4 °C: elysione (**6**) A) after and B) before light exposure; epoxyelysione (**7**) C) after and D) before light exposure. The family of isomeric products that is centred around 18.5 min in (A) is associated with mass peaks *m/z* 421 ($[M+Na]^+$) and UV $\lambda_{max}=264$ nm. Minor peaks (5 min $< t_R <$ 15 min) in profiles (A) and (C) have MS and UV data that do not correlate with polypropionate structures.

cycles of 14 hours of light and ten hours of dark to allow acclimation before experiments started. A group of four specimens was then kept in darkness for 60 hours, whereas a second group of five animals was acclimated to continuous light (UV-B plus visible light, 350 $\mu E\,m^{-2}\,s^{-1}$) for 60 hours. Figure 3 shows the chromatographic profiles of lipid extracts from "dark" and "light" molluscs. Levels of elysione (**6**) and epoxyelysione (**7**) were significantly different under the two experimental conditions, as the former compound was much more abundant in light-acclimated animals. In addition to elysione ($t_R = 19.5$ min) and epoxyelysione ($t_R = 12.2$ min), the extracts of the animals that were kept in the dark showed a peak that eluted later under reverse-phase conditions ($t_R = 22.2$ min). This peak, which is absent in control animals (not shown) was associated with a molecular ion at *m/z* 399 ($[M+H]^+$) and exhibited UV maxima at 277 and 312 nm, which is consistent with the presence of the γ-pyrone ring and octatetraene system, respectively (Figure 4). The two chromophores are almost independent, as has been reported with other polypropionates with a similar molecular arrangement.[20] The data presented above are consistent with a structure of the type **10**, which is a polyunsaturated product that has already been suggested to be a putative precursor of propionate skeletons in sacoglossans.[12,14,21]

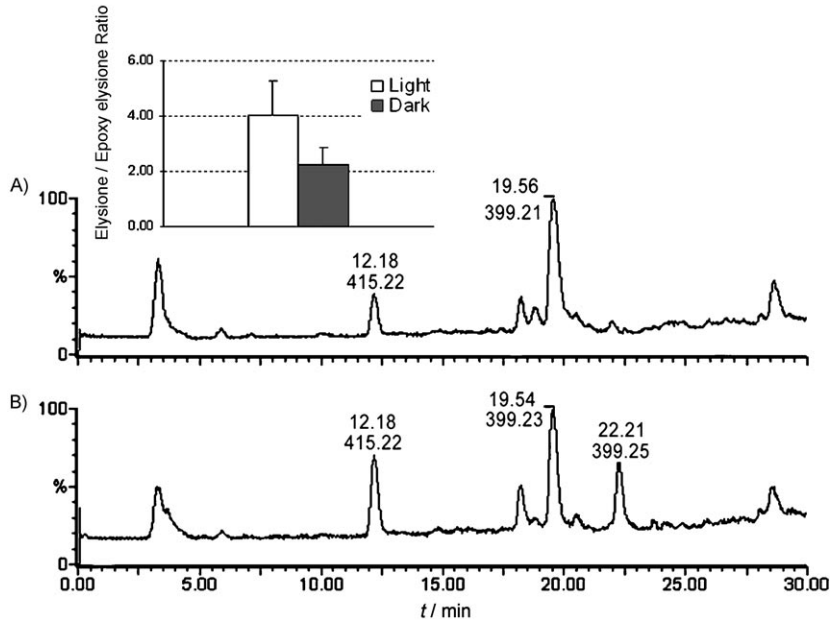


Figure 3. ESI^+ LC–MS on reverse-phase column of extracts from animals that were kept for 60 h under UV-B and visible light (350 $\mu E\,m^{-2}\,s^{-1}$) (A) ($n = 5$) and in the dark (B) ($n = 4$). The insert shows the levels of elysione (**6**) and epoxyelysione (**7**), expressed as ponderal ratio, under the two experimental conditions. Epoxyelysione (**7**), $t_R = 12.1$ min; elysione (**6**), $t_R = 19.5$ min; compound **10**, $t_R = 22.2$ min.

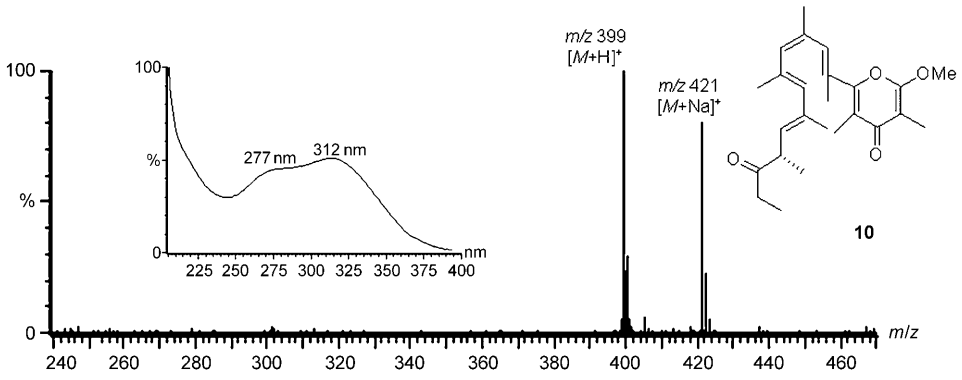
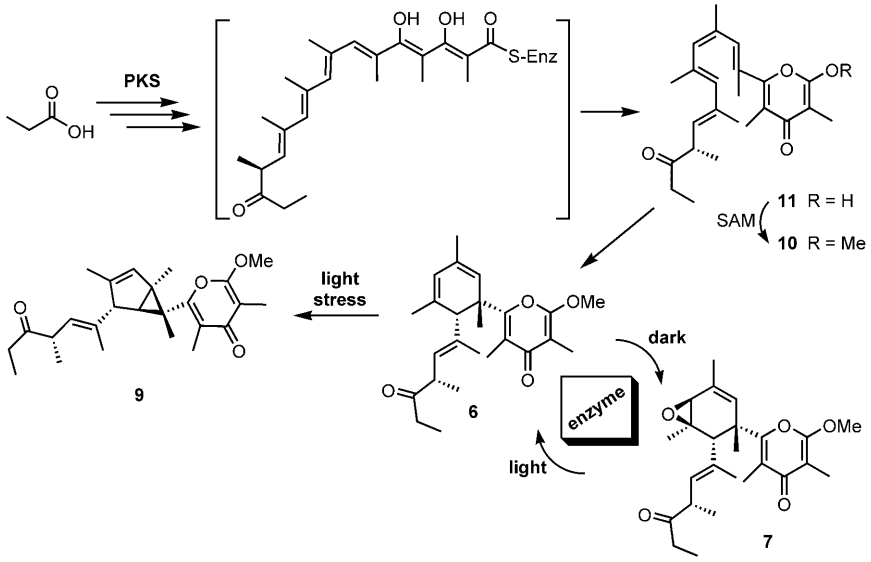


Figure 4. Characterisation of the chromatographic peak ($t_R = 22.2$ min) that was detected in specimens of *Elysia viridis* that were kept in the dark for 60 h (see Figure 3B). The ESI^+ MS spectrum shows a pseudomolecular ion at *m/z* 399 ($[M+H]^+$) in complete agreement with the molecular formula of $C_{25}H_{34}O_4$, which was calculated for **10.** The insert reports the UV spectrum that is associated with the chromatographic peak.

Discussion

Some molluscs of the order Sacoglossa have acquired the ability of phototrophic CO_2 fixation as a result of kleptoplasty.[3] Ireland and Scheuer[5] suggested that a significant part of the fixed carbon could be directed to the biosynthesis of pyrone-containing propionates. The present data demonstrate that *E. viridis* employs propionate, probably as methyl malonate-CoA, for the construction of the regular polyketide skeleton of elysione (**6**) (Figure 1). Formally, the molecule arises from seven rounds of elongation by starting from a propionic unit (Scheme 2). This result rigorously confirms the involvement of intact C_3 unit in polypropionate biosynthesis in marine molluscs,[10,21] thus ruling out the pathway that proceeds through acetate and *S*-adenosyl methionine that operates in fungi.[22]

Active biosynthesis of pyrone-containing propionates in sacoglossans has been related to a photoprotective role.[5,15,21] Following these arguments, we have tested the influence of light on the propionate levels of *E. viridis*. Irradiation of elysione (**6**) with direct sunlight (800–1200 $\mu E\,m^{-2}\,s^{-1}$) induces a photorearrangement of the propionate skeleton, which leads to a complex family of isomeric compounds that have *m/z* 421.2 ($[M+Na]^+$ for $C_{25}H_{34}O_4$; Figure 2). In addition to a residual amount of **6**, the mixture is dominated by a bicyclo[3.1.0]hexene skeleton that can be attributed to *ent*-crispatene (**9**) on the basis of the radical mechanism that is



Scheme 2. Proposal of biosynthesis and photoprotective mechanism of elysione (**6**) and other structurally related propionates of *Elysia viridis*. SAM = *S*-adenosyl methionine. PKS = polyketide synthase.

proposed for rearrangement of sacoglossan polypropionates (Scheme 1 B).[15] Unlike the results that were obtained by Ireland and Faulkner with **1**,[7] the presence of several isomers (Figure 2 A) suggests that photolysis of **6** does not proceed in a specific manner.

In contrast, epoxyelysione (**7**) was practically unaffected under the same light conditions (Figure 2 C, D) as a result of the absence of the cyclohexadiene moiety. The stability of **7** also suggests that this molecule does not have a direct role in limiting the adverse effects that are due to light overexposure. Nevertheless, biosynthesis of epoxyelysione (**7**), as much as that of elysione (**6**), seems to be strongly affected by light (Figure 3). In fact, exposure of live molluscs to non-stressing levels of light (350 $\mu E\,m^{-2}\,s^{-1}$) does not induce detectable rearrangement of the cyclohexadiene skeleton but changes significantly the weight ratio between the two molecules, with an increase of the levels of elysione (**6**) relative to that of epoxyelysione (**7**). Assuming the occurrence of a single biosynthetic pathway (Scheme 2), it is possible that the physiological levels of the two molecules depends on regulatory mechanisms that operate on the final products of the polyketide synthesis, after their release from the putative polyketide synthase. Structural analysis of **6** and **7** reveals that stereochemistry at C6 and C11 is conserved, as it is in the metabolically equivalent pair that is formed by 9,10-deoxytridachione (**1**) and tridachione (**2**) of *P. ocellatus*[5] and *T. diomedea*.[7] Such stereochemical traits suggest that the interconversion of **6** to **7**, as well as **1** to **2**, might occur by enzymatic epoxidation or de-epoxidation of the double bond at C9/C10 in response to light (Scheme 2). Following this line of reasoning, the equilibrium between **6** and **7** might represent a physiological control of the photoprotectant pool (i.e., elysione, **6**) through a process that resembles epoxidation and de-epoxidation of xanthophylls in higher plants, chlorophyta and phaeophyceae.[23]

In dark-acclimated molluscs, we observed the presence of a new compound with structural characteristics that agree with one of the *cis*/*trans* isomers of **10** (Figure 4). Electrocyclic reactions of labile tetraenes have been used for the organic synthesis of complex polycyclic analogues of mollusc cyclohexadiene propionates;[12–14,17] this suggests that the polyketide chemodiversity that is found in worldwide opisthobranch molluscs[24] stems from a common polyene–pyrone precursor.[25] On the other hand, recent genetic studies have proven that formation of the pyrone ring is due to spontaneous cyclisation that occurs after release of the polyketide chain from the thiotemplate.[26a] On these grounds, it is likely that non-methylated precursor of **10**, such as **11** or a diasteromeric analogue, is the direct product of the putative PKS that presides in the polypropionate biosynthesis in the Elysiid mollusc (Scheme 2). Because a similar mechanism should also take place for the synthesis of 9,10-deoxytridachione (**1**) from a chiral polyene, it is worth noting that the electrocyclisation reaction that generates the chemodiversity of Elysiid polypropionates must involve an enzymatic catalysis, as has been already suggested by Darias and co-workers.[17]

Conclusions

Our data suggest a clear picture for the biosynthesis of polypropionates in *E. viridis* (Scheme 2). That is, the release of pyrone–polyene such as **11** from a putative PKS is followed by methylation of the pyrone ring[26] with formation of the unstable intermediate **10**. Enzymatic cyclisation of this last product leads to the hexadiene core of elysione (**6**) that is in equilibrium with the photochemically inactive epoxide **7**. The equilibrium has a key role in regulating the physiological availability of **6**, which is the compound that is responsible for the photoprotective activity as suggested by several authors. In this view, elysione (**6**), epoxyelysione (**7**) and the intermediate **10** form a light-dependent reaction network that regulates the synthesis of the polypropionates and permits a quick activation of the protective process. The polypropionate cycle might then complement the photoprotective role of algal chloroplast pigments, thus justifying the preservation of the committed biosynthetic pathways in the Elysiid mollusc.

Experimental Section

General. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were obtained on an Agilent 8453 spec-

trophotometer. NMR spectra were recorded on a Bruker Avance DRX 600 that was equipped with a cryoprobe operating at 600 MHz for proton and $CHCl_3$ ($\delta_H = 7.26$, $\delta_C = 77.0$ ppm) or C_6HD_5 ($\delta_H = 7.16$, $\delta_C = 128.0$ ppm) were used as reference. Mass spectra were acquired on a *micro*Q-Tof mass spectrometer (Waters) that was equipped with an ESI source and a lock–spray apparatus for accurate mass measurements. Solvents were distilled prior to use.

Collection of the sacoglossans. *E. viridis* (25 specimens) was collected by scuba diving at depths of 2–4 m at Lucrino lake, a salt lagoon in northern area of the Gulf of Naples. 18 specimens were divided into groups (see below) and kept alive in aquaria (300 L of marine water) for 2 weeks with regular cycles of light and dark (14 L:10 D) to allow acclimatisation. The remaining molluscs were treated as controls (7 specimens) and not subjected to any experiment. These animals were frozen in liquid N_2 and kept at $-80\,^{\circ}C$ until analysis.

Extraction and isolation of polypropionates. Frozen molluscs were extracted with acetone (3×20 mL). The organic solvent was removed under reduced pressure and the aqueous residue was partitioned with Et_2O (5×15 mL). The resulting extract was dried (2.2 mg per specimen) and purified by silica gel chromatography by using increasing amounts of Et_2O in petroleum ether. Fractions that eluted with petroleum ether/Et_2O 7:3 contained elysione (**6**; 300 μg per specimen), whereas 9,10-epoxyelysione (**7**; 40 μg per specimen) was eluted with petroleum ether/Et_2O 50:50.

Elysione (6): Colorless oil. $C_{25}H_{34}O_4$. $[\alpha]_D = +156.3$ ($c = 0.29$, $CHCl_3$); for ^1H and ^{13}C NMR spectroscopy data ($CDCl_3$) see Table 1.

9,10-Epoxyelysione (7). Colorless oil. $C_{25}H_{34}O_5$. $[\alpha]_D = +54.3$ ($c = 0.03$, $CHCl_3$); for ^1H and ^{13}C NMR spectroscopy data ($CDCl_3$) see Table 1. ^1H NMR (C_6D_6, 290 K): $\delta = 5.71$ (H7), 5.08 (H13), 3.31 (OMe), 2.85 (H14), 2.74 (H11), 2.72 (H9), 2.15 (H_3-19), 2.12 (H_3-18), 1.91, 1.83 (H_2-16), 1.64 (H_3-21), 1.52 (H_3-20), 1.26 (H_3-23), 1.16 (H_3-22), 0.94 (H_3-17), 0.92 ppm (H_3-24); ^{13}C NMR (C_6D_6, 290 K): $\delta = 210.2$ (C15), 180.7 (C3), 161.6 (C1), 161.0 (C5), 136.0 (C8), 133.6 (C12), 129.8 (C13), 129.4 (C7), 119.7 (C4), 98.5 (C2), 60.7 (C10), 57.6 (C9), 56.9 (C11), 54.5 (OMe), 47.0 (C6), 44.6 (C14), 33.7 (C16), 31.8 (C20), 22.4 (C22), 21.8 (C21), 16.6 (C24), 12.4 (C23), 7.7 (C19), 7.4 (C17), 7.2 ppm (C18); UV(MeOH) $\lambda_{max} = (\varepsilon)$: 253 (12750); HRMS (ESI): calcd *m/z* for $C_{25}H_{34}O_5Na^+$: 437.2304 $[M+Na]^+$; found: 437.2285.

Feeding Experiment. Nine specimens of *E. viridis* were acclimated in an aquarium with marine water for 10 days. Then, the animals were injected by syringe with 6 mg sodium [1-^{13}C]propionate (50 μL of a solution of 240 mg labelled propionate in 2 mL distilled water) every second day for 1 week (about 24 mg per specimen of labelled precursor). On day 8, the animals were frozen and extracted as described above. The ^{13}C NMR spectrum of labelled elysione (**6**; 250 μg per specimen) was recorded at 150 MHz in $CDCl_3$ (0.7 mL) with an interval delay of 3 s (ns = 40 000).

Photo-reactivity of *Elysia* propionates. Elysione (**6**) (2.1 mg) was dissolved in C_6D_6 (0.7 mL) in an end-capped NMR tube under an argon atmosphere. Following Ireland and Faulkner,[7] the solution was exposed to direct sun light at $4\,^{\circ}C$ for 3 h. The reaction mixture was directly analysed by NMR spectroscopy in the same solvent, then dried under N_2 and re-analysed in $CDCl_3$ by an entire set of 2D NMR spectroscopic experiments, including COSY, TOCSY, HSQC and HMBC ($J = 7$ and 10 Hz). Afterwards, the sample was transferred to a vial and dried under reduced pressure. The residue was dissolved in MeOH to a concentration of 0.2 μg μL^{-1} and analysed by LC–MS (ESI^+) by using a linear gradient from MeOH/H_2O 7:3 to 100% MeOH in 30 min. Epoxyelysione (**7**; 0.3 mg in 0.7 mL C_6D_6) was subjected to the same procedure and analysed under the same LC–MS conditions. Compound **9** ^1H NMR ($CDCl_3$, 290 K): $\delta = 5.37$ (H9), 5.25 (H13), 2.73 (H11), 1.19 (H_3-21), 1.10 ppm (H_3-20); ^{13}C NMR: $\delta = 160.9$ (C5), 143.3 (C10),128.9 (C9), 58.9 (C11) 40.7 (C6), 31.8 ppm (C8). The remaining signals of **9** were not diagnostic because of the overlapping with the resonances of elysione (**6**).

Light-induced effect on living animals A group of 5 animals was transferred to a 5 L aerated tank and exposed to artificial light (UV-B plus visible light, 350 μE m^{-2} s^{-1}) at $21\,^{\circ}C$ for 60 h, whereas another group of 4 specimens was kept in the dark under the same experimental conditions. Both groups were then frozen and each specimen was separately extracted as described above. The organic extracts were dissolved in MeOH to a final concentration of 0.2 μg μL^{-1} and analysed by LC–MS(ESI^+) as reported above.

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