

New γ -pyrone propionates from the Indian Ocean sacoglossan *Placobranchus ocellatus*

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Abstract—A new γ -pyrone propionate, compound **9**, and its peroxy derivative **10** have been isolated from the sacoglossan *Placobranchus ocellatus*. The structure and the relative stereochemistry of the new molecules, which displayed an unprecedented carbon skeleton characterised by a bicyclo [4.2.0] octane, have been determined by both spectroscopic methods and comparison with model compounds. By analogy with photodeoxytridachione (**2**), a sunscreen protective role could be also suggested for compound **9** in living *P. ocellatus*.

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Sacoglossans are marine molluscs specialised as algal suctorial feeders. Species belonging to the superfamily Elysioidea are characterised by two large dorso-lateral appendages where they transfer algal chloroplasts which continue to be active for many days. Chemical studies carried out on this superfamily^{1–5} resulted in the isolation of both dietary diterpenoids sequestered from algae and polypropionates. The latter compounds are probably biosynthesised de novo by the mollusc. It has been rigorously proved, after the pioneering work of Ireland and Scheuer,⁶ for the polypropionates possessed by *Elysia viridis*.²

In the course of our investigation on Indian Ocean opisthobranchs we analysed the elysioidean *Placobranchus ocellatus*.[†] A previous study on the same mollusc collected off Hawaii⁶ led to the polypropionate 9,10-deoxytridachione (**1**) that was demonstrated by in vivo experiment to undergo a photorearrangement giving photodeoxytridachione (**2**). More recently, six related propionates (**3–8**) have been reported along with 9,10-deoxytridachione (**1**) from a Philippine population of *P. ocellatus*.⁷

In this paper we describe the structure elucidation of two related γ -pyrone propionates (**9** and **10**), displaying a new bicyclic carbon skeleton, isolated together with known 9,10-deoxytridachione (**1**),⁸ photodeoxytridachione (**2**),⁶ tridachiahydropyrone B (**7**),⁷ tridachiahydropyrone C (**8**)⁷ and *iso*-9,10-deoxytridachione (**11**).⁹

Specimens of *P. ocellatus* were collected off Mandapam (Crusidali Island, India) in shallow water during April 2004. They were immediately frozen and kept at –20 °C until analysis. The mollusc (25 individuals) was extracted with acetone and the diethyl ether soluble fraction (140 mg) of the acetone extract was fractionated on a Sephadex column LH-20 in CHCl₃/CH₃OH, 1:1. The fractions containing the main UV visible metabolites were further purified, [silica-gel column (petroleum ether/diethyl ether gradient) and subsequent reverse-phase HPLC (MeOH/H₂O, 9:1, Kromasil 5 μ , 1 mL/min flow)], to give the known compounds **1** (0.9 mg), **2** (0.7 mg), **7** (1.0 mg), **8** (0.7 mg), **11** (0.8 mg), and the novel molecules **9** (0.6 mg) and **10** (0.8 mg).

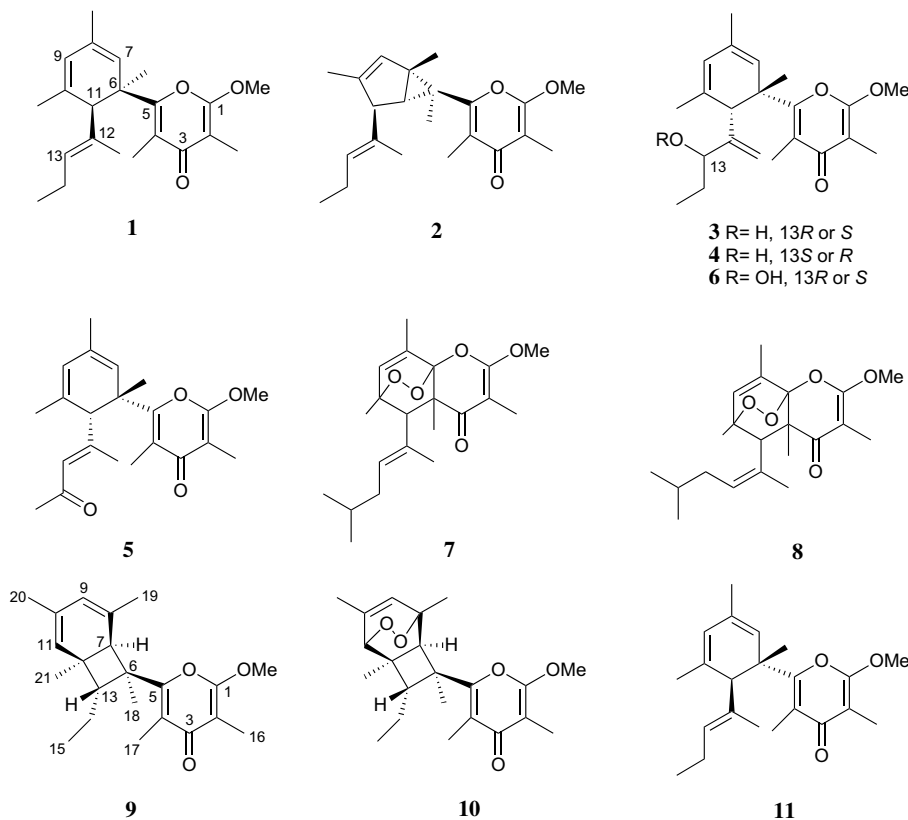
The molecular formula of compound **9**[‡] was deduced by HRESIMS of the sodiated molecular ion observed at *m/z* 365.2097 [M+Na]⁺ indicating the molecular formula

Keywords: γ -Pyrone propionates; Sacoglossan; *Placobranchus ocellatus*; Bicyclo octane.

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[†]A voucher (I-82) specimen of *P. ocellatus* is stored at ICB for inspection.

[‡]Compound **9** [α]_D +9.5° (c 0.06, CHCl₃); IR (liquid film) ν_{\max} 2913, 1651, 1541, 806 cm^{–1}; UV (MeOH) λ_{\max} 260 nm (ϵ 12,100); HRESIMS *m/z* 365.2097 (M+Na), calcd for C₂₂H₃₀O₃ + Na 365.2093.



$C_{22}H_{30}O_3$. The 1H NMR spectrum (Table 1) immediately suggested a polypropionate skeleton. In fact, it exhibited signals for eight methyl groups [3H singlet at δ 4.01, four 3H vinyl singlets at δ 1.75, 1.78, 1.88 and 1.98, two 3H singlets at δ 1.14 and 1.25 and a 3H triplet at δ 0.89], two olefinic protons (two singlets at δ 5.07 and 5.62), two methine protons (a singlet at δ 3.12 and a double doublet at δ 2.42) and finally a methylene group resonating at δ 1.60 and 1.72.

The presence of an α -methoxy- β -methyl γ -pyrone system (partial structure **a**, Fig. 1) was indicated by some typical ^{13}C NMR resonances (Table 1) according to the presence of a conjugated carbonyl group (C-3, δ 182.0), four quaternary carbons (C-1, δ 162.5; C-2, δ 100.9; C-4, δ 116.5; C-5, δ 164.6) and three methyl signals (MeO-, δ 56.4; C-16, δ 7.2; C-17, δ 9.8), attributable, respectively, to the methoxy group and to the β -vinyl methyls of the γ -pyrone system. A strong IR band at 1651 cm^{-1} and an UV absorption at 260 nm ($\epsilon = 12,100$) further supported this hypothesis. Analysis of 1H - 1H COSY experiment led to the definition of the partial structures **b** and **c** (Fig. 1). In particular, the methine at δ 2.42 (H-13) was coupled with the methylene at δ 1.60–1.72 (H₂-14) which was in turn coupled with the methyl triplet at δ 0.89 (H₃-15), according to the residue **b**, whereas the olefinic proton at δ 5.62 (H-9) showed allylic correlations with both a methyl singlet at δ 1.78 (H₃-19) and an olefinic proton at δ 5.07 (H-11) which was in turn coupled with the methyl singlet at δ 1.75 (H₃-20), suggesting a disubstituted diene residue, as depicted in partial structure **c**. Furthermore the sing-

let at δ 3.12 (H-7) showed allylic couplings with both the methyl at δ 1.78 (H₃-19) and the methine at δ 5.62 (H-9), supporting the connection of C-7 to the diene group. Diagnostic long-range 1H - ^{13}C couplings in HMBC experiment aided us to link the partial structures **a**–**c**. In fact, the quaternary carbon at δ 38.4 (C-12) showed cross-peaks with the methine at δ 3.12 (H-7) and also with the methyl singlet at δ 1.14 (H₃-21), while the quaternary carbon at δ 47.4 (C-6) showed diagnostic correlations with the methine at δ 3.12 (H-7) and the methyl at δ 1.25 (H₃-18). Furthermore the proton at δ 2.42 (H-13) showed HMBC connectivities with both methyls at δ 1.14 (H₃-21) and δ 1.25 (H₃-18) allowing us to link partial structure **b** with **c** through two quaternary carbons C-6 and C-12, both leading a methyl substituent. Finally long-range couplings between C-5 (δ 164.6) and both the methine signals at δ 3.12 (H-7) and 2.42 (H-13) and the methyl at δ 1.25 (H₃-18) connected groups **a** and **c**, leading to a carbon framework as depicted in formula **9**.

The proposed structure was further supported by the analysis of compound **10**,[§] exhibiting a molecular formula $C_{22}H_{30}O_5$ with additional 32 u.m.a. with respect to **9** [HRESIMS: m/z 397.1995 (M+Na)⁺]. The 1D and 2D NMR spectra of **10** revealed strong structural analogies with compound **9**, suggesting the presence of the

[§]Compound **10** [α_D +5.0° (c 0.08, $CHCl_3$); IR (liquid film) ν_{\max} 2929, 1652, 1541, 807 cm^{-1} ; UV (MeOH) λ_{\max} 275 nm (ϵ 11,600); HRESIMS m/z 397.1995 (M+Na), calcd for $C_{22}H_{30}O_5$ + Na 397.1991.

Table 1. NMR data^{a,b} for compound **9** and **10**

Position	δ_{H} m (J) ^c	Compound 9		δ_{H} m (J) ^c	Compound 10	
		δ_{C} m ^d	HMBC ^e with H		δ_{C} m ^d	HMBC ^e with H
1	—	162.5 s	OCH ₃ , H ₃ -16	—	161.3 s	OCH ₃ , H ₃ -16
2	—	100.9 s	H ₃ -16	—	100.0 s	H ₃ -16
3	—	182.0 s	H ₃ -16, H ₃ -17	—	181.7 s	H ₃ -16, H ₃ -17
4	—	116.5 s	H ₃ -17	—	119.3 s	H ₃ -17
5	—	164.6 s	H ₃ -17, H ₃ -18, H-7, H-13	—	160.2 s	H ₃ -17, H ₃ -18
6	—	47.4 s	H ₃ -18, H-7	—	41.9 s	H ₃ -18, H-7, H-13
7	3.12 s	49.4 d	H ₃ -18, H ₃ -19, H ₃ -21, H-9, H-11	2.19 s	57.5 d	H ₃ -18, H ₃ -19, H ₃ -21
8	—	129.8 s	H ₃ -19, H-7	—	78.1 s	—
9	5.62 s	125.4 d	H ₃ -19, H-7	5.70 s	126.1 d	H ₃ -19, H ₃ -20, H-11
10	—	130.1 s	H ₃ -20	—	143.6 s	H ₃ -20, H-11
11	5.07 s	122.9 d	H ₃ -20, H ₃ -21, H-7	3.95 bs	83.4 d	H ₃ -20, H ₃ -21
12	—	38.4 s	H ₃ -21, H-7	—	36.7 s	H ₃ -21
13	2.42 dd (2.9, 11.0)	57.3 d	H ₃ -15, H ₃ -18, H ₃ -21	2.68 dd (3.8, 10.8)	43.6 d	H ₃ -15, H ₃ -18, H ₃ -21
14	1.60 m 1.72 m	18.9 t	H ₃ -15	1.34 m 1.60 m	20.3 t	H ₃ -15
15	0.89 t (7.6)	13.1 q	H ₂ -14, H-13	0.87 t (7.6)	12.9 q	H ₂ -14, H-13
16	1.88 s	7.2 q	—	1.84 s	7.0 q	—
17	1.98 s	9.8 q	—	1.94 s	12.9 q	—
18	1.25 s	15.5 q	H-7, H-13	1.51 s	18.9 q	H-7, H-13
19	1.78 s	23.4 q	H-7	1.20 s	21.6 q	H-7, H-9
20	1.75 s	22.5 q	H-11	2.03 s	20.6 q	H-9, H-11
21	1.14 s	32.5 q	H-11, H-13	1.54 s	23.3 q	H-7, H-11, H-13
OCH ₃	4.01 s	56.4 q	—	3.93 s	55.3 q	—

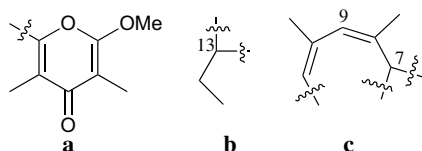
^a Bruker DPX Avance 400 MHz and DPX 300 MHz spectrometers, CDCl₃, chemical shifts (ppm) referred to CHCl₃ (δ 7.26) and to CDCl₃ (δ 77.0).

^b Assignments determined by ¹H–¹H COSY, HSQC and HMBC.

^c Multiplicity given in Hz.

^d By DEPT sequence.

^e J = 10 Hz.

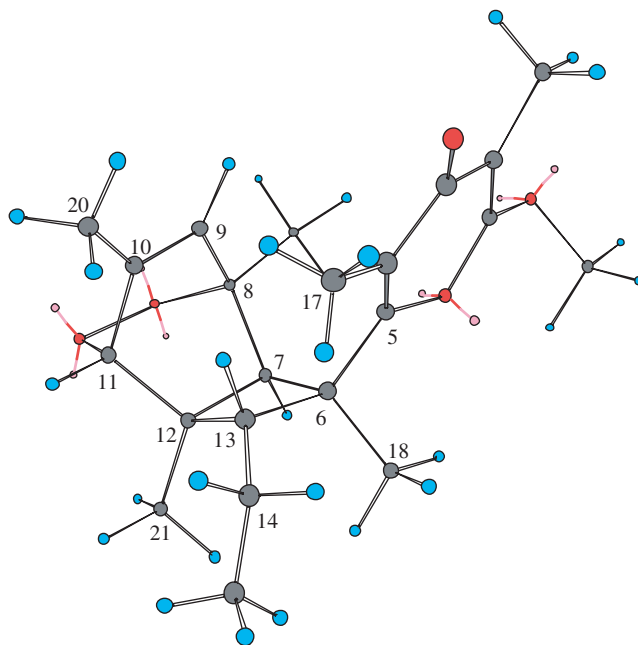
**Figure 1.** Partial structures **a–c** assigned for compound **9**.

same carbon skeleton. In the place of diene system, compound **10** was characterised by a methine linked to an oxygen atom (δ_{H} 3.95, H-11; δ_{C} 83.4, C-11), a oxygenated tertiary sp³ carbon at δ 78.1 (C-8), a trisubstituted double bond [δ 5.70 (H-9), δ 126.1, C-9; δ 143.6, C-10]. According to these data, a peroxide bridge made up from the diene system of **9** was suggested for compound **10**. The remaining part of the molecule was the same as **9**. All proton and carbon resonances were attributed as reported in Table 1 by detailed analysis of NMR (¹H–¹H COSY, HSQC and HMBC).

The relative stereochemistry of compounds **9** and **10**, suggested by comparison of their proton and carbon chemical shifts, was mainly determined on the basis of NOE difference and NOESY experiments recorded on the peroxide derivative **10**.

First of all, the expected *cis* geometry of 7,12 junction was suggested by a diagnostic NOE effect between the angular methine H-7 and the methyl at C-12. The peroxide bridge was α -oriented with respect to the plane of the

bicyclo octane, as depicted in Figure 2, by the upfield shift of methyl C-21 that in compound **9** resonated at δ 32.5 versus δ 23.3 of **10**, suggesting that both the peroxide unit and the angular methyl at C-12 were on the same side of the molecule. Furthermore, the signal at

**Figure 2.** Dreiding model stereoview of compound **10**.

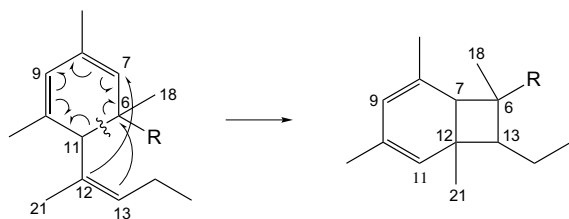


Figure 3. Suggested mechanism for the formation of the new bicyclo octane ring in compound **9** starting from 9,10-deoxytridachione (**1**).

δ 2.68 (H-13) showed strong steric interactions with the olefinic proton at δ 5.70 (H-9), the methyl group at δ 2.03 (H₃-20) and the proton at δ 3.95 (H-11) confirming the suggested orientation.

In addition, steric proximities were observed between H-7 and H₃-18, as well as between H-13 and H₃-17 of the γ -pyrone residue, indicating the relative stereochemistry depicted in Figure 2, with γ -pyrone and H-13 both oriented in the opposite side with respect to H₃-21 and H-7.

The carbon skeleton of compounds **9** and **10** displayed some interesting structural features. By analogy with photodeoxytridachione (**2**), derived from 9,10-deoxytridachione (**1**) by a photochemical rearrangement,³ the formation of bicyclo octane ring system in compounds **9** could be hypothesised in the same manner (Fig. 3). The key step is the homolytic cleavage of the bond between C-6 and C-11, then the subsequent electron rearrangement leads to the formation of two new linkages C-7/C-12 and C-6/C-13.

A sunscreen protective role in the mollusc *P. ocellatus* was proposed for photodeoxytridachione (**2**), by Ireland and Scheuer.⁶ Compound **9**, isolated from the same

sacoglossan, could play an analogous role in the living mollusc, being an additional example of photochemical product deriving from 9,10-deoxytridachione (**1**). On the other side, the peroxy derivative **10** should be considered a work-up-derived compound, most likely formed by 1,4-addition of molecular oxygen to the diene system of compound **9**.

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