

## Placidenes: Cyercene-like Polypropionate $\gamma$ -Pyrone from the Mediterranean Ascoglossan Mollusc *Placida dendritica*

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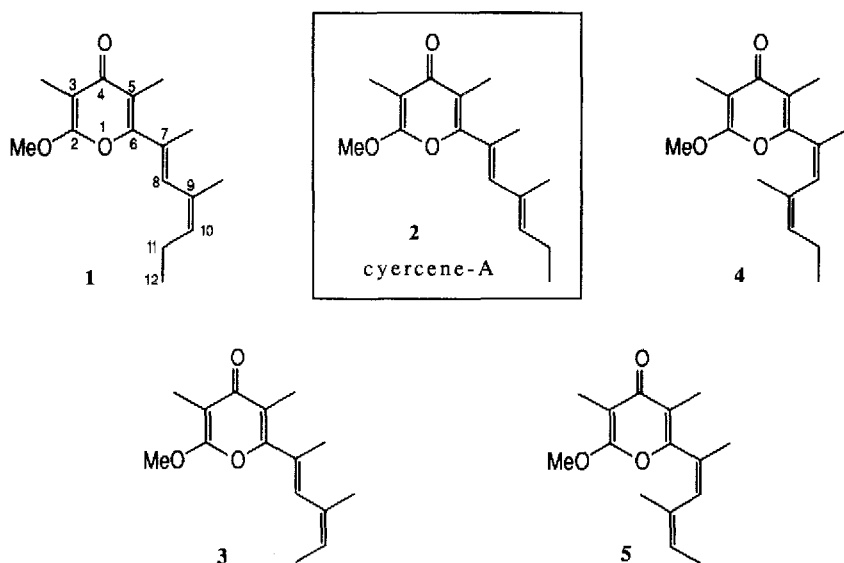
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**Abstract:** Four new 6-substituted-2-methoxy-3,5-dimethyl-4H-pyran-4-ones, closely related to the recently described multifunctional cyercene-A (2), were isolated from *Placida dendritica*. This is the first chemical study on an ascoglossan belonging to the Stiligeridae family.

Continuing our research on the chemical ecology of Mediterranean opisthobranchs<sup>1</sup>, we have recently directed our attention to species belonging to the Ascoglossa order<sup>2,3,4</sup> which includes three superfamilies: Oxynoidea, Elysoidea and Polybranchioidea<sup>5</sup>. Oxynoidea are the most ancestral species from an evolutive point of view. In fact, they retain a relic shell and live almost exclusively on siphonous *Caulerpa* algae. On the contrary, the shell-less molluscs belonging to the superfamily Polybranchioidea are the most evolved species. The loss of the shell is accompanied by a profusion of large easily detachable dorso-lateral appendages (cerata) and by an increased size of the foot which favours the exploitation of new sources of food, such as the filamentous algae *Chaetomorpha* and *Bryopsis*<sup>6</sup>. The first chemical studies of Mediterranean ascoglossans supported these evolutionary lines. The shelled *Oxynoe olivacea* is protected by the sesquiterpenoids oxytoxins -1 and -2<sup>2</sup> obtained by chemical modification of caulerpenyne, the main secondary metabolite from the alga *Caulerpa prolifera*<sup>7</sup>. On the contrary, the Polybranchiidae *Cyerce cristallina* is able to biosynthesize *de novo* a series of polypropionate pyrones, cyercenes<sup>3</sup>, that are likely to play a role either as defence allomones or as chemical mediators of cerata regeneration. Surprisingly, related pyrones virtually deriving from five propionate units have been previously found only in the Australian *Cyerce nigricans*<sup>8,9</sup>.

The superfamily Polybranchioidea includes two families: Polybranchiidae and Stiligeridae, but, to the best of our knowledge, no chemical study has been performed on the Stiligeridae species. In this paper we report the results of a study conducted on the Stiligeridae *Placida dendritica* (Alder and Hancock, 1843) and the chemical characterization of four new  $\gamma$ -pyrones which, analogously to cyercenes, virtually derive from five propionates units.

*Placida dendritica* is a very small shell-less ascoglossan which lives perfectly camouflaged on the green alga *Bryopsis plumosa*. In March 1991 30 specimens of the mollusc (wet weight: 16.78 g) were caught in the Gulf of Naples at a depth ranging from 2 to 6 meters. The chloroform soluble fraction (140 mg) from the acetone extract of the mollusc was analysed by SiO<sub>2</sub>-TLC (petroleum ether:diethyl ether 1:1) revealing a strong UV visible component (R<sub>f</sub>= 0.4) that was recovered by semipreparative SiO<sub>2</sub> plates and submitted to HPLC analysis. Reverse phase HPLC (Spherisorb ODS 2 column, 4.5 x 250 mm, 5 μm; 40 min gradient from 60 to 75% methanol in water; flow rate 0.8 ml/min) led, in order of elution, to three fractions: A, *iso*-placidene-B, 0.5 mg; B, mixture of placidene-B and *iso*-placidene-A, 5mg; C, placidene-A, 4 mg. Fraction B was further purified by straight phase HPLC (SiO<sub>2</sub> Spherisorb S 5W, *n*-hexane:ethyl acetate 9:1, flow rate 1 ml/min) yielding *iso*-placidene-A (1.5 mg) and placidene-B (2 mg). All placidenes exhibited spectral data (IR, UV, NMR, MS) in agreement with the presence of an α-methoxy-β,β'-dimethyl-γ-pyrone moiety linked to a side alkyl chain containing seven or eight carbons.



In particular placidene-A (1), C<sub>16</sub>H<sub>22</sub>O<sub>3</sub> showed the following data: *m/z* (%): 262 (M<sup>+</sup>, 65%), 247 (M<sup>+</sup> - CH<sub>3</sub>, 49%), 233 (M<sup>+</sup> - C<sub>2</sub>H<sub>5</sub>, 100%);  $\nu_{\max}$  (CHCl<sub>3</sub>): 1657, 1614, 1597 cm<sup>-1</sup>;  $\lambda_{\max}$  (CH<sub>3</sub>OH): 262 (ε=9400) nm. All these data were almost identical to those of cyercene-A (2). The similarity was confirmed by the <sup>1</sup>H-NMR spectrum (table 1) of 1 which displayed, analogously to 2, signals easily attributable to the α-methoxy (δ 3.97) and to the β,β' methyls (δ 1.87 and 2.04) on the γ-pyrone ring. The spectrum was completed by the signals, similar but not identical to those of cyercene-A (2), of the protons of the side chain in agreement with the presence of two olefinic protons (δ 6.17, H-8, bs; δ 5.39, H-10, t, J=7.3 Hz), two vinyl methyl (δ 1.91 and 1.85; CH<sub>3</sub>-7 and CH<sub>3</sub>-9, respectively) and, finally, a terminal methyl (δ 0.97, t, J=7.5 Hz) linked to a vinyl methylene (δ 1.98, m) that in turn also exhibited a vicinal coupling with H-10 (J=7.3 Hz), which was allylically coupled with the

**Table 1** -  $^{13}\text{C}$ -NMR (125.8 MHz) and  $^1\text{H}$ -NMR (500 MHz) data<sup>a,b</sup> of placidenes (**1**, **3**, **4**, **5**)

C	<b>1</b> <sup>c</sup>		<b>3</b>		<b>4</b>		<b>5</b>	
	$\delta^{13}\text{C}$	$\delta^1\text{H}$	$\delta^{13}\text{C}$	$\delta^1\text{H}$	$\delta^{13}\text{C}$	$\delta^1\text{H}$	$\delta^{13}\text{C}$	$\delta^1\text{H}$
2	161.9 <i>s</i>		161.9 <i>s</i>		162.4 <i>s</i>		162.0 <i>s</i>	
3	99.3 <i>s</i>		99.4 <i>s</i>		99.8 <i>s</i>		99.8 <i>s</i>	
4	181.4 <i>s</i>		181.5 <i>s</i>		180.9 <i>s</i>		181.0 <i>s</i>	
5	117.9 <i>s</i>		118.0 <i>s</i>		119.5 <i>s</i>		119.0 <i>s</i>	
6	158.3 <i>s</i>		158.3 <i>s</i>		156.3 <i>s</i>		n. d.	
7	127.9 <i>s</i>		128.1 <i>s</i> <sup>d</sup>		126.7 <i>s</i> <sup>e</sup>		n. d.	
8	135.1 <i>d</i>	6.17	134.9 <i>d</i>	6.15	132.1 <i>d</i>	6.41	132.0 <i>d</i>	6.40
9	130.2 <i>s</i>		131.9 <i>s</i> <sup>d</sup>		130.2 <i>s</i> <sup>e</sup>		n. d.	
10	132.3 <i>d</i>	5.39	124.5 <i>d</i>	5.49	134.8 <i>d</i>	5.31	126.8 <i>d</i>	5.40
11	22.6 <i>t</i>	1.98	15.0 <i>q</i>	1.60	21.7 <i>t</i>	2.02	14.1 <i>q</i>	1.61
12	13.9 <i>q</i>	0.97			14.1 <i>q</i>	0.93		
CH <sub>3</sub> -9	23.1 <i>q</i>	1.85	23.1 <i>q</i>	1.84	23.1 <i>q</i> <sup>f</sup>	1.56	23.0 <i>q</i> <sup>h</sup>	1.57
CH <sub>3</sub> -7	16.0 <i>q</i>	1.91	16.0 <i>q</i>	1.90	21.8 <i>q</i> <sup>f</sup>	2.05	21.8 <i>q</i> <sup>h</sup>	2.06
CH <sub>3</sub> -5	11.9 <i>q</i>	2.04*	11.9 <i>q</i>	2.05*	10.8 <i>q</i>	1.83 <sup>g</sup>	10.8 <i>q</i>	1.83 <sup>i</sup>
CH <sub>3</sub> -3	6.9 <i>q</i>	1.87*	6.9 <i>q</i>	1.87*	6.9 <i>q</i>	1.86 <sup>g</sup>	6.9 <i>q</i>	1.86 <sup>i</sup>
-OCH <sub>3</sub>	55.2 <i>q</i>	3.97	55.2 <i>q</i>	3.97	55.4 <i>q</i>	3.94	55.3 <i>q</i>	3.94

a)  $\text{CDCl}_3$ , TMS = O; some  $^{13}\text{C}$  resonances were not detected (n.d.) for scarcity of material.

b)  $^{13}\text{C}$  multiplicities were determined by DEPT sequence experiments.

c) Assignments confirmed by  $^1\text{H}$ - $^{13}\text{C}$  heterocorrelation.

d-i) Assignments with identical superscripts may be interchanged.

\*) Erroneously interchanged for cyercene-A<sup>3</sup> and aglajne-3 methyl ether<sup>10</sup>.

protons of CH<sub>3</sub>-9. In addition, the presence of an allylic coupling between H-8 and CH<sub>3</sub>-7 was secured by  $^1\text{H}$ - $^1\text{H}$  NMR decoupling experiments. On the basis of this evidence **1** must be a geometric stereoisomer of **2**. The  $^{13}\text{C}$ -NMR spectrum (table 1) exhibiting a carbon quartet at  $\delta$  23.1 (CH<sub>3</sub>-9, assigned by  $^1\text{H}$ - $^{13}\text{C}$  heterocorrelation) suggested a Z configuration for the  $\Delta^9$  double bond. A positive nuclear Overhauser enhancement, between H-10 and CH<sub>3</sub>-9, confirmed the proposed stereochemistry.

Placidene-B (**3**),  $\text{C}_{15}\text{H}_{20}\text{O}_3$ ;  $m/z$  (%): 248 ( $\text{M}^+$ , 62%), 233 ( $\text{M}^+ - \text{CH}_3$ , 100%);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ): 1657  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ): 260 ( $\epsilon=11200$ ) nm. The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra of **3** (table 1) were almost identical to those of **1**, differing only for the absence of the CH<sub>3</sub>CH<sub>2</sub>- resonances replaced by those ( $\delta$   $^1\text{H}$ , 1.60, 3 H, d,  $J=6.7$  Hz;  $\delta$   $^{13}\text{C}$ , 15.0, q) assigned to a further vinyl methyl, in agreement with structure **3**. NOE experiments confirmed the Z stereochemistry at the  $\Delta^9$  double bond.

*Iso*-placidene-A (**4**),  $\text{C}_{16}\text{H}_{22}\text{O}_3$ ;  $m/z$  (%): 262 ( $\text{M}^+$ , 26%), 247 ( $\text{M}^+ - \text{CH}_3$ , 52%), 233 ( $\text{M}^+ - \text{C}_2\text{H}_5$ , 100%);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ): 1658, 1611, 1592  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ): 254 ( $\epsilon=10340$ ) nm. The comparison of the  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra (table 1) of **4** with those of **1** and **2** easily led to suggest a stereoisomeric structure characterized by both the double bonds in the side chain having a Z configuration. The

$^{13}\text{C}$ -NMR resonances ( $\delta$  23.1 and 21.8) of both the carbons of the vinyl methyls at C-7 and C-9 strongly supported the suggested stereochemistry. Positive nuclear Overhauser enhancements were recorded between H-8 and  $\text{CH}_3$ -7 and, also, between H-10 and  $\text{CH}_3$ -9.

*Iso*-placidene-B (5),  $\text{C}_{15}\text{H}_{20}\text{O}_3$ ;  $m/z$  (%): 248 ( $\text{M}^+$ , 33%), 233 ( $\text{M}^+ - \text{CH}_3$ , 100%);  $\nu_{\text{max}}(\text{CHCl}_3)$ : 1657, 1614, 1597  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}(\text{CH}_3\text{OH})$ : 251 ( $\epsilon=14000$ ) nm. Analogously to the couple 1-3, 5 displayed NMR resonances (table 1) almost identical to those of 4, the only differences being due to the presence of a vinyl methyl group instead of the terminal ethyl.

All placidenes were completely absent from extracts of the green alga *Bryopsis plumosa*. Most probably placidenes, like cyercenes, are biosynthesized *de novo* by the mollusc. Studies on the distribution, origin and biological properties of placidenes are now in progress. Preliminary results revealed a strong toxicity of the crude extract of *P. dendritica* to *Gambusia affinis*<sup>11,12</sup> and the absence of any activity on the *Hydra vulgaris* regeneration assay<sup>13</sup>.

Polypropionate pyrones are quite unusual among natural products. However, it is interesting to note that more complex polypropionates have been found in some Ascoglossa species belonging to the Elysioidea superfamily<sup>14-17</sup>. A fascinating hypothesis could be that the evolution in the Ascoglossa order is somehow linked to the ability of synthesizing multifunctional polypropionates.

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