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Aplysiopsenes: an additional example of marine polyketides with a mixed acetate/propionate pathway

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ABSTRACT

First chemical study of the Hermaeidae sacoglossan *Aplysiopsis formosa* from Azores led to aplysiopsenes, α-pyrone polyketides with a mixed acetate/propionate structural pathway.

Polypropionates displaying a pyrone ring are quite rare in nature. These are detected in some *Streptomyces* bacteria[1] and fungi of different genera.[2] Surprisingly, the biosynthesis experiments proved two different pathways: a mixed acetate/propionate in *Streptomyces*[3] and methylation of a polyacetate chain in fungi.[4,5] Related compounds were found in some marine organisms and, in particular, in sacoglossan molluscs.[6] Origin and function of these molecules are challenging topics for international debates. Some authors[7] suggested that these are produced by symbiotic microorganisms living in the molluscs, whereas others[8] proved that γ-pyrones in sacoglossan polypropionates can catalyse the production of highly reactive singlet oxygen. Simple α- and γ-pyrone polyketides were found in four sacoglossan species: *Cyerce nigricans*,[9,10] *Cyerce cristallina*,[11–13] *Ercolania funerea*[14] and *Placida dendritica*.[15,16] All these molluscs, belonging to the superfamily Limapontioidea, are characterised by dorso-lateral appendages, named cerata, where the chloroplasts sequestered from the dietary algae can remain active for many months. The cerata can be easily autotomised in case of external stress including the escape from the predators.[6]

This superfamily contains three families: Polybranchiidae, Hermaeidae and Limapontiidae.[17] Chemical studies conducted on several species belonging to Polybranchiidae and Limapontiidae families have shown that, with the exception of *Costasiella* species,[18] they contain α- and γ-pyrone polyketides de novo biosynthetised.[19] The multifunctional biological roles of these molecules include an involvement in the chemical defence as well as in the regeneration of the detached appendages. The individuation of the true source of the carbon skeletons of pyrone-containing propionates has been object of several investigations. However, the de novo origin through a mixed acetate/propionate pathway has been suggested to be the most plausible hypothesis in molluscs.[19] This suggestion has been very recently confirmed by incorporation of both propionate and acetate precursors labelled with stable isotopes in the Mediterranean sacoglossan *P. dendritica*.[20] To date, no chemical study was reported for molluscs belonging to the Hermaeidae family.

We describe here the finding of four α-pyrone polypropionates, named aplysiopsenes A–D (**1**–**4**), in the hermaeidean sacoglossan *Aplysiopsis formosa* Pruvot-Fol. Interestingly, these molecules display a shorter side chain with respect to related pyrone-polypropionates reported from the other limapontioidean sacoglossans.[9–16]

Fourteen *A. formosa* individuals (20–25 mm average size) were collected along the coasts of São Jorge Island in the Azores (northern Atlantic Ocean), immediately frozen and then transferred to ICB in Naples. The frozen material was repeatedly extracted with acetone (50 ml × 3) at room temperature and the aqueous residue, after evaporation of the organic solvent under reduced pressure, was partitioned between water and Et_2O. The Et_2O extract (11.7 mg) was chromatographed by silica gel column using a

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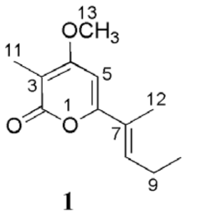

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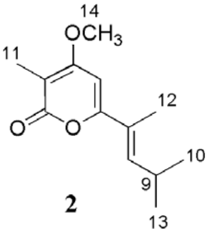

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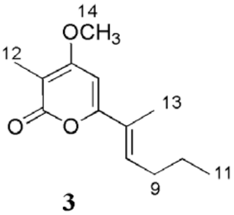

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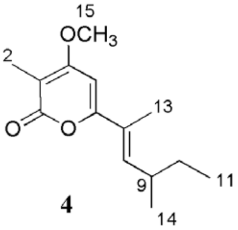

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gradient eluent system from light petroleum to Et_2O. The fractions obtained with light petroleum ether/Et_2O 85:15 to 8:2 were further purified by analytical RP-HPLC (gradient MeOH/H_2O, from 60% to 100% MeOH, UV detector 210 nm) to yield in order of decreasing polarity aplysiopsene A (**1**, Rt 17.3), B (**2**, Rt 24.1), C (**3**, Rt 25.0) and D (**4**, Rt 31.4).

Aplysiopsene A (**1**)[21] was isolated as a colourless oil and showed a sodiated-molecular peak at *m/z* 231.0998 in the HRESIMS spectrum (231.0997 calculated for $C_{12}H_{16}O_3Na$), which indicated the molecular formula $C_{12}H_{16}O_3$. An intense IR band at ν_{max} 1678 cm^{-1} and the presence of signals at δ 6.10 (1H, s, H-5), 3.90 (3H, s, H_3-13) and 1.94 (3H, s, H_3-11) in the ^1H NMR spectrum as well as at δ 165.9 (C-4), 164.4 (C-2), 160.1 (C-6) and 91.4 (C-5) in the ^{13}C NMR spectrum (Table 1) suggested the presence of a 3-methyl-4-methoxy-6-substituted-α-pyrone system. The ^1H NMR spectrum of **1** showed additional signals at δ 6.61 (1H, t, J = 7.3, H-8), 2.24 (2H, quintet, J = 7.3, H_2-9), 1.88 (3H, br s, H_3-12) and 1.08 (3H, t, J = 7.3, H_3-10) that were attributed to a 2-pentenyl alkyl chain linked to C-6 of the α-pyrone ring as showed in structure **1**. The geometry of the double bond at C-7 was inferred as *E* by both the carbon value of C-12 (δ 12.2) and a NOE effect observed between H_3-12 and H_2-9. Comparison of the spectral data of **1** with those reported in the literature for similar polypropionates isolated from both sacoglossans[14] and fungi[22] further supported the structure proposed. In particular, aplysiopsene A was closely related to the fungal metabolite nectriapyrone[23] differing from the latter in the presence of an additional CH_2 unit in the side chain.

The spectroscopic data of aplysiopsene B (**2**)[24] were very similar to those of compound **1**. The sodiated-molecular peak at *m/z* 245.1157 in the HRESIMS spectrum (245.1154 calculated for $C_{13}H_{18}O_3Na$) indicated the molecular formula $C_{13}H_{18}O_3$ with an additional CH_2 moiety with respect to **1**. Analysis of both ^1H and ^{13}C NMR spectra of **2** clearly indicated the presence of the same α-pyrone moiety, whereas the terminal moiety of the alkyl chain was different. In fact, typical signals attributable to an isopropyl residue at δ 2.70 (1H, dq, J = 6.7, 9.5, H-9) and δ 1.05 (6H, d, J = 6.7, H_3-10 and H_3-13) were observed in the ^1H NMR spectrum of **2** in the place of the signals due to the terminal ethyl group in compound **1**. The carbon value of C-12 (δ 12.0) was consistent with the 7*E*-stereochemistry.

The molecular formula $C_{13}H_{18}O_3$ of aplysiopsene C (**3**),[25] which was deduced by the sodiated-molecular peak at *m/z* 245.1149 in the HRESIMS spectrum, was the same (245.1154 calculated for $C_{13}H_{18}O_3Na$) as that of compound **2**. Analysis of both proton and carbon spectra of **3** showed that in this molecule, the alkyl chain linked to the α-pyrone moiety contained a terminal *n*-propyl group. In fact, in the ^1H–^1H COSY spectrum of **3**, the olefinic proton resonating at δ 6.62 (1 H, t, J = 9.5, H-8) was correlated with a methylene at δ 2.20 (2 H, m, H_2-9) which in turn coupled with another methylene resonating at δ 1.50 (2 H, m, H_2-10) finally linked to a terminal methyl at δ 0.94 (3 H, t, J = 7.3, H_3-11). The remaining part of the molecule was confirmed to be the same as that of **1** and **2**.

Aplysiopsene D (**4**)[26] had the molecular formula $C_{14}H_{20}O_3$ as obtained by the HRESIMS sodiated peak at *m/z* 259.1311 (259.1310 calculated for $C_{14}H_{20}O_3Na$). The structural analogies with the co-occurring compounds **1**–**3** were evident by analysis of ^1H and ^{13}C NMR spectra. As for the other molecules, the only difference was in the terminal part of the alkyl chain. In particular, the proton spectrum displayed in the high-field region two methyl signals at δ 1.02 (3H, d, J = 6.7, H_3-14) and δ 0.88 (3H, t, J = 7.5, H_3-11), a methine multiplet at δ 2.45 (1H, m, H-9), and a methylene signal at δ 1.35 (2H, m, H_2-10) according to the presence of a 2-butyl moiety linked to the olefinic proton H-8. Compound **4** showed strong structural similarities with fungal metabolite phomenin B,[22] being the corresponding 9,10 dihydroderivative.

All proton and carbon resonances of aplysiopsenes A–D were assigned by 2D NMR experiments.

Apparently, aplysiopsenes represent an additional example of marine polyketides with a mixed acetate/propionate pathway in

Table 1
^1H and ^{13}C NMR data in $CDC1_3$ of aplysiopsenes (**1**–**4**)

C	**1**		**2**		**3**		**4**	
	δ $^{13}C^a$ m^b	δ $^1H^c$ m J (Hz)	δ $^{13}C^a$ m^b	δ $^1H^c$ m J (Hz)	δ $^{13}C^a$ m^b	δ $^1H^c$ m J (Hz)	δ $^{13}C^a$ m^b	δ $^1H^c$ m J (Hz)
2	164.4 s	–	164.4 s	–	165.2 s	–	164.4 s	–
3	102.2 s	–	102.2 s	–	102.2 s	–	102.1 s	–
4	165.9 s	–	165.7 s	–	165.7 s	–	165.4 s	–
5	91.4 d	6.10 s	91.5 d	6.10 s	91.6 d	6.10 s	91.5 d	6.10 s
6	160.1 s	–	159.5 s	–	160.1 s	–	160.2 s	–
7	125.8 s	–	123.8 s	–	126.0 s	–	124.6 s	–
8	136.8 d	6.61 t 7.3	141.9 d	6.46 d 9.5	135.4 d	6.62 t 7.3	141.1 d	6.42 d 9.5
9	21.6 t	2.24 quin 7.3	28.0 d	2.70 dq 6.7, 9.5	30.7 t	2.20 m	35.2 d	2.45 m
10	13.3 q	1.08 t 7.3	22.3 q	1.05 d 6.7	22 4 t	1.50 m	30.3 t	1.35 m
11	8.5 q	1.94 s	8.1 q	1.92 s	13.9 q	0.94 t 7.5	n.d.	0.88 t 7.5
12	12.2 q	1.88 s	12.0 q	1.88 s	8.6 q	1.94 s	8.0 q	1.94 s
13	55.9 q	3.90 s	22.3 q	1.05 d 6.7	12.4 q	1.89 s	10.8 q	1.90 s
14			55.9 q	3.90 s	56.1 q	3.90 s	19.8 q	1.02 d 6.1
15							55.4 q	3.91 s

[a] Bruker 300 MHz spectrometer, chemical shirts (ppm) referred to $CDC1_3$ (δ 77.0).
[b] Multiplicities deduced by DEPT experiment.
[c] Bruker 400 and 600 MHz, assignments made by ^1H–^1H COSY, HSQC and HMBC (J = 10 Hz) experiments.

agreement with the structure features of secondary metabolites of limapontoidean sacoglossan species. However, this biosynthesis has to be confirmed by rigorous biosynthetic experiments. *A. formosa* is the first limapontioidean sacoglossan possessing only α-pyrone polyketides. In addition, the extra ring conjugation is limited to only one double bond. Probably *A. formosa* and the other studied polyketide-possessing limapontioideans should occupy in a hypothetic evolutionary scenario very near but distinct positions.

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21. Compound **1**. 0.7 mg, 6.2%; UV λ_{max} (EtOH)/nm 228 (13,000), 321 (7500); HRESIMS *m/z* 231.0998 (231.0997 calculated for $C_{12}H_{16}O_3Na$); ^{1}H and ^{13}C NMR data are given in Table 1.
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24. Compound **2**. 0.7 mg, 6.2%, UV λ_{max} (EtOH)/nm 228 (13,000), 321 (7500); HRESIMS *m/z* 245.1157 (245.1154 calculated for $C_{13}H_{18}O_3Na$); ^{1}H and ^{13}C NMR data are given in Table 1.
25. Compound **3**. 1.5 mg, 12.5%, IR ν_{max}/cm^{-1} (film) 1681; UV λ_{max} (EtOH)/nm 228 (10,900), 321 (7000); HRESIMS *m/z* 245.1149 (245.1154 calculated for $C_{13}H_{18}O_3Na$); ^{1}H and ^{13}C NMR data are given in Table 1.
26. Compound **4**. 0.4 mg, 3.4%, $[\alpha]_D^{25}$ -42.4 (*c* 0.04, $CHCl_3$); IR ν_{max}/cm^{-1} (film) 1681; UV λ_{max} (EtOH)/nm 228 (9800), 321 (7000); HRESIMS *m/z* 259.1311 (259.1310 calculated for $C_{14}H_{20}O_3Na$); ^{1}H and ^{13}C NMR data are given in Table 1.