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Fusaripyrones, novel polypropionates from the Mediterranean mollusc *Haminoea fusari*

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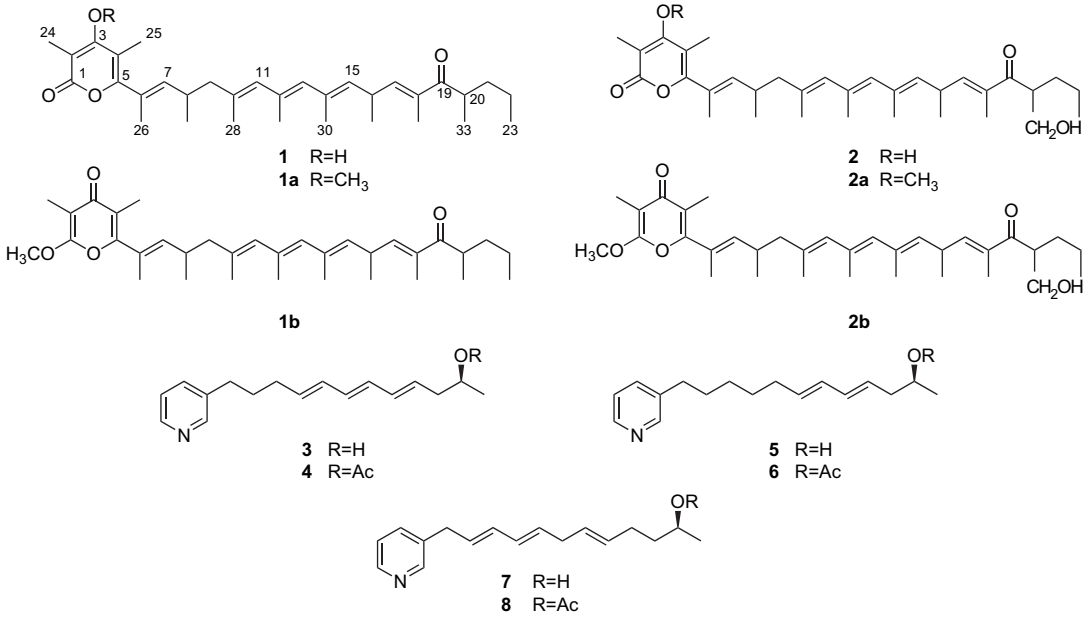
Abstract—A novel regular polypropionate skeleton has been characterized in two new pyrones, named fusaripyrone A (**1**) and fusaripyrone B (**2**), from the lipid extracts of the mollusc *Haminoea fusari*. Here we report elucidation of the polyketide structures of **1** and **2**, isolated as either natural products or isomeric pairs of α-/γ-pyrone methyl derivatives.

1. Introduction

Polypropionates are secondary metabolites common in a few species of marine molluscs of the subclasses Pulmonata and Opisthobranchia.[1,2] Within this latter group of invertebrates, compounds with acyclic and pyrone-based polypropionate skeletons have been mainly reported from sacoglossans, where they may exert a photoprotective role,[3] and from cephalaspideans, in which a defensive function has been invoked.[4] In this paper, we describe a further chemical study of the Mediterranean mollusc *Haminoea fusari* (Cephalaspidea, Gastropoda, Opisthobranchia), which has led to the characterization of two new polypropionates, fusaripyrones A and B (**1** and **2**) together with the previously reported haminols **3**–**8**.[5] The two compounds show an unprecedented skeleton based on regular condensation of 11 propionate units.



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2. Results and discussion

The presence of polypropionates in the ether extract of both mantle and viscera of the mollusc *H. fusari* was previously suggested[5,6] and now confirmed by ^{1}H NMR spectra of the crude extracts, where several methyl signals appeared in the region between δ 0.75 and 2.0. However, in the natural form, isolation of these products was difficult due to quick degradation of the polyketide skeletons. To prevent such a problem and allow structural elucidation, the raw extracts were first methylated with ethereal diazomethane and successively fractionated by radial chromatography (Chromatotron®). The procedure successfully led to two isomeric pairs of methyl derivatives **1a/1b** and **2a/2b** together with the known haminols **3**–**8**[5] and a mixture, hitherto uncharacterized, of minor polyketides.

HR-ESI$^+$-MS of the less polar compound **1a** gave a pseudomolecular ion at *m/z* 545.3604 [M+Na$^+$] due to $C_{34}H_{50}O_4$ (calcd 545.3607). The pyrone-containing skeleton was inferred on the basis of 11 resonances of aliphatic methyl groups, and one methyl singlet at δ 3.10 attributable to a γ-methoxy group on a fully substituted α-pyrone ring. This last assignment was confirmed by the diagnostic ^{13}C resonances at 167.7 (C-3), 165.8 (C-1), 158.4 (C-5), 110.0 (C-2), and 108.1 (C-4) ppm and by the typical lactone IR band at 1724 cm^{-1}. The complete assignment of the acyclic chain was easily accomplished by combined homo- and heteronuclear 2D NMR data. In fact, key HMBC correlations of the downshifted proton at δ 5.33 (C-7, 141.5 ppm) with the quaternary sp^2 carbon at 126.4 ppm (C-6) and the vinyl methyl at 15.1 ppm (C-26) along that with the pyrone carbon at 158.4 ppm (C-5) placed a tri-substituted double bond at C-5 α to the pyrone ring. According to COSY and TOCSY data, the olefinic proton H-7 was further connected through the aliphatic methine at δ 2.60 (H-8) to both the methyl at δ 0.89 (H_3-27) and the methylene at δ 1.96 (H_2-9). The ^{13}C NMR spectrum contained a resonance at 204.8 ppm that, in accordance with the UV absorbance at 230 nm, was assigned to a tri-substituted α,β-unsaturated keto group at C-19 (C-17: 144.0 ppm; H-17: δ 6.41; C-18: 135.7 ppm; C-32: 11.5 ppm; H_3-32: δ 1.94). C-19 also showed clear correlation with the well-defined aliphatic spin system due to the terminal *sec*-pentyl residue (Table 1). In agreement with the polyketide biogenesis, the remaining three formal degrees of unsaturation were assigned to a substituted triene moiety whose protons appeared as two singlets at δ 5.74 (H-11) and 5.88 (H-13) and a doublet at δ 5.34 (H-15). The three vinyl methyls at δ 1.71 (H_3-30), 1.75 (H_3-28), and 1.91 (H_3-29) were unambiguously assigned on the basis of ^2J and ^3J HMBC correlations. Finally, scalar couplings between the bis-allylic methine at δ 3.45 (H-16) with the olefinic H-15 and H-17 completed the structural elucidation of **1a**. The geometry of all the double bonds was inferred to be *E* on the basis of the ^{13}C chemical shifts of the vinyl methyls, all falling below 20 ppm.[7] On the contrary, the stereochemistry at the chiral centers C-8, C-16, and C-20 remains to be determined.

NMR signals assigned to the polyketide chain of **1b** ($C_{34}H_{50}O_4$ deduced by HR-ESI$^+$-MS on the pseudomolecular ion [M+Na]$^+$ at *m/z* 545.3606) were superimposable to those of **1a** except for the resonances of the pyrone ring. Compound **1b**, indeed, displayed one diagnostic carbon at 182 ppm (C-3), suggestive of a conjugated-keto moiety and two downfield shifted methyl groups at δ 2.16 and 2.19 (H_3-24 and H_3-25, respectively). Such signals were consistent with the replacement of the α-pyrone ring of **1a** with a γ-pyrone ring in **1b**, as also confirmed by the typical IR band at 1651 cm^{-1}.

^{1}H NMR spectra of **2a** and **2b** contained 11 methyl resonances instead of 12 as a consequence of oxidation of the methyl group at C-33. An intense broad IR band around 3400 cm^{-1} was consistent with the presence of one hydroxy group in both compounds. This suggestion was confirmed by HSQC data of **2a** showing correlation of the oxygen-bearing C-33 (63.5 ppm) with the two protons at δ 3.58 and 3.78 (H_2-33). A similar correlation pattern was also discernible in the NMR spectra of compound **2b**, which revealed direct coupling of the signal at 64.0 ppm (C-33) with the AB proton system at δ 3.60 and 3.81 (H_2-33). The presence of the hydroxy substituents at C-33 was proved by key HMBC correlations H_2-33/C-19, H_2-33/C-20, H_2-21/C-33 observed and confirmed by comparison of the NMR data of **2a** and **2b** with those of **1a** and **1b** (Table 1). According to this assignment, **2a** and **2b** showed an isobaric pseudomolecular ion at *m/z* 561, differing by 16 amu from that of the isomeric pair **1a** and **1b**.

Fusaripyrones occur as very unstable hydroxy-pyrone derivatives in the ethereal extracts of the mollusc. In agreement with the literature,[8] methylation with CH_2N_2 stabilized the structures allowing purification of the chemically stable pairs **1a/1b** and **2a/2b**. Only after elucidation of these methyl derivatives, we succeeded in the isolation of the natural forms of both compounds. The NMR data of fusaripyrone B (**2**) in C_6D_6 showed ^{1}H and ^{13}C signals of the linear chain almost identical to those described above for **2a**. The only differences arose from the absence of the methoxy group signal and the chemical shift values of both the quaternary carbon C-2 (98.8 ppm) and the methyl groups of the pyrone ring. In particular, the structure of **2** was inferred on the basis of chemical shifts of the methyls at C-24 and C-25 (δ 1.78 and 1.86, respectively), which were closer to the values of α-isomer (δ 1.98 and 1.80) than γ-isomer (δ 2.15 and 2.18) in the corresponding methyl derivatives **2a** and **2b**. On the other hand, a transient set of signals co-occurred with **2** in a freshly prepared NMR sample. We were not able to fully assign these signals although we suggest that they likely correspond to the γ-form in a tautomeric equilibrium with **2**. Analogous results were obtained by the analysis of fusaripyrone A (**1**), the ^{1}H NMR spectrum of which differed from that of **2** substantially for the absence of the hydroxyl methylene protons at δ 3.58 and 3.79 and the presence of an additional methyl doublet at δ 0.88, in agreement with the depicted structure. The structural relationship between hydroxy and methoxy pyrones was unambiguously proved by conversion of pure **2** into a 65:35 mixture of **2a** and **2b** after methylation with ethereal diazomethane.

3. Conclusion

Fusaripyrones, which show a mild antifouling activity (EC_{50}=9.5 ppm) but not general toxicity,[9] are featured by

Table 1. NMR data for compounds **1a**, **1b**, **2a**, and **2b** (C_6D_6, 600 MHz)

C	**1a**		**1b**		**2a**		**2b**	
	^{1}H, m, *J (Hz)*	^{13}C (ppm)	^{1}H, m, *J (Hz)*	^{13}C (ppm)	^{1}H, m, *J (Hz)*	^{13}C (ppm)	^{1}H, m, *J (Hz)*	^{13}C (ppm)
1		165.8		160.5		164.3		161.7
2		110.0		99.6		110.0		99.6
3		167.7		182.0		167.7		180.6
4		108.1		118.5		107.8		118.4
5		158.4		158.7		158.6		157.9
6		126.4		127.3		126.7		126.9
7	5.33, d, *9.6*	141.5	5.35, d, *9.9*	142.8	5.30, d, *10.2*	141.6	5.35, d, *9.7*	141.7
8	2.60, m	31.8	2.60, m	31.6	2.60, m	31.6	2.61, m	31.3
9	1.96, m	48.5	1.95, m	48.2	1.95, m	48.4	1.94, m	47.9
10		132.7[a]		133.6		132.7[a]		133.4
11	5.74, s	132.2	5.72, s	131.6	5.72, s	131.9	5.71, s	131.7
12		132.7[a]		133.1		134.5		133.6
13	5.88, s	132.9	5.85, s	132.9	5.88, s	132.8	5.83, s	133.1
14		132.7[a]		133.2		132.6		133.0
15	5.34, d, *8.9*	132.3	5.29, d, *8.9*	132.3	5.33, d, *8.7*	132.5	5.27, d, *8.7*	132.3
16	3.45, m	33.0	3.42, m	33.4	3.41, m	33.5	3.41, m	33.1
17	6.41, d, *9.2*	144.0	6.38, d, *9.2*	143.9	6.45, d, *9.2*	146.0	6.44, d, *9.2*	145.7
18		135.7		134.7		132.7[a]		135.9
19		204.8		204.7		205.4		205.4
20	3.12, m	39.0	3.09, m	37.0	3.22, m	46.8	3.26, m	46.6
21	1.75, m	37.0	1.75, m	36.7	1.41; 1.58, m	32.3	1.43; 1.56, m	32.2
22	1.18, m	21.2	1.19, m	21.1	1.16, m	20.9	1.17, m	21.2
23	0.78, t, *6.8*	14.4	0.80, t, *7.2*	14.5	0.74, t, *7.2*	14.3	0.75, t, *7.2*	14.0
24	1.99, s	10.5	2.16, s	7.6	1.98, s	10.5	2.15, s	12.3
25	1.80, s	12.1	2.19, s	12.3	1.80, s	11.9	2.18, s	7.4
26	1.76, s	15.1	1.64, s	14.2	1.76, s	14.8	1.64, s	15.4
27	0.89, d, *6.4*	20.4	0.88, d, *6.6*	20.2	0.89, d, *6.7*	20.3	0.90, d, *6.6*	20.2
28	1.75, s	18.3	1.75, s	18.0	1.75, s	18.0	1.75, s	18.0
29	1.91, s	19.1	1.89, s	19.0	1.91, s	19.1	1.88, s	18.8
30	1.71, s	17.7	1.66, s	17.5	1.68, s	17.4	1.65, s	17.1
31	1.0, d, *6.6*	20.8	1.0, d, *6.6*	20.7	0.99, d, *7.2*	20.5	1.0, d, *6.7*	20.5
32	1.94, s	11.5	1.93, s	12.1	1.91, s	11.7	1.92, s	11.5
33	1.08, d, *6.6*	17.9	1.08, d, *6.6*	18.0	3.58, dd, *4.1*, *10.8* 3.78, dd, *7.2*, *10.8*	63.5	3.60; 3.81, m	64.0
OCH_3	3.10, s	59.7	3.19, s	54.5	3.08, s	58.9	3.17, s	54.5

[a] Overlapping signals.

a regular polypropionate skeleton of unusual length, being derived by condensation of a number of units (11 propionates) higher than that described in compounds from taxonomically related cephalaspideans, such as *Smaragdinella calyculata* (**9**, nalodionol, containing 10 units)[10] and *Bulla* species (e.g., **10**, aglajne-3, from *Bulla striata* or **11**, dehydroaglajne-3, from *Bulla gouldiana* containing nine units) (Fig. 1).[8,11,12] Despite this, a comparative analysis of these molecules reveals significant analogies within the structures of cephalaspidean polypropionates, suggesting high homology in the PKS modules underlying the synthesis of these compounds.[13,14] In fact, the C12–C20 fragment of **1** is a recurrent motif in the polypropionates so far described from cephalaspideans. Considering the processive biosynthesis of PKS-products,[15,16] the regular presence of this motif is suggestive of preserved domains in the putative genes within the taxonomic group. De novo biosynthesis of polypropionates has been reported in few species of opisthobranchs,[2,13] including *B. striata*.[17] Feeding experiments have also proved the occurrence of a PKS-like process in *Haminoea* molluscs[18,19] and PKS-genes highly similar to type I of eubacteria and distinct from FAS-genes have been recently reported in sea urchins.[20] Nevertheless, our attempts to trace the origin of PKS-domains in marine molluscs have been unsuccessful so far (unpublished results). In this view, the structural analogies of polyketide metabolites from molluscs could be considered as a piece of information in the still incomplete knowledge on the evolutionary origin of animal PKS. It is worth noting that the metabolic relationships between the three genera are also in agreement with a recent phylogenetic analysis that would encompass the genera

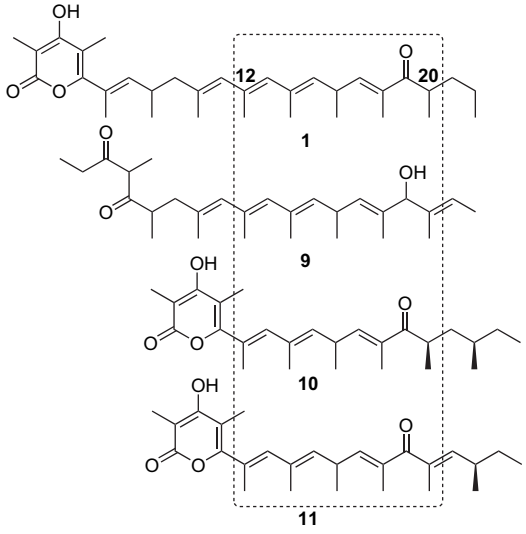

Figure 1. Biosynthetic relationships among polypropionates from cephalaspideans: the substructures in the dot box evidence the structural similarities in fusaripyrone A (**1**, *H. fusari*), nalodionol (**9**, *S. calyculata*), aglajne-3 (**10**, *B. striata*), and dehydroaglajne-3 (**11**, *B. gouldiana*).

Haminoea, *Bulla*, and *Smaragdinella* in the same monophyletic group of Bulloidea.[21]

4. Experimental

4.1. General

Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were obtained on an Agilent 8453 spectrophotometer. IR spectra were recorded on a BioRad FT-IR spectrometer. NMR spectra were recorded on a Bruker Avance DRX 600 equipped with a cryoprobe operating at 600 MHz for proton. Spectra were referenced to C_6D_6 (^{1}H δ 7.15 and ^{13}C 128 ppm) as internal standard. Mass spectra were acquired on a Q-Tof*micro* mass spectrometer (Waters) equipped with an ESI source and a Lock–Spray apparatus for accurate mass measurements. Solvents were distilled prior to use.

4.2. Extraction and isolation of metabolites

The mollusc *H. fusari* (600 specimens) was collected in February–March 2006 at Miseno Lake (Naples) at 0–3 m depth and kept frozen until analyses. According to our standard procedures, the frozen animals were extracted with acetone by sonication (mantle extract) and then by grinding of the tissues (viscera extract). Acetone extracts were concentrated under reduced pressure and the water residue was partitioned with diethyl ether (3×100 mL) and then with *n*-butanol (3×50 mL).

For the isolation of **1a**, **1b**, **2a**, and **2b**, the combined ether extracts of the mantle (0.86 mg/animal) were dried under vacuum, methylated with ethereal diazomethane, and then subjected to radial chromatography on silica plate (Chromatotron®) with *n*-hexane/EtOAc gradient as eluent. The fraction eluting with 80:20 contained methylated α- and γ-pyrone derivatives; haminols 2 (**4**), 4 (**6**), and 6 (**8**) were eluted with 60:40 while haminols 1 (**3**), 3 (**5**), and 5 (**7**) were eluted with EtOAc 100%. Fusaripyrones were also present in viscera extracts (1.7 mg/animal), which were methylated and fractionated as described above. Methylated polypropionates were further purified by reverse phase HPLC (Phenomenex C-18, 100 Å 5 μm MeOH/H_2O 90:10) or by analytical TLC developed in light petroleum/diethyl ether 60:40. Natural extracts were purified on SiO_2 gel column using increasing content of diethyl ether in light petroleum followed by a $CHCl_3$/MeOH gradient elution. Fraction eluting with diethyl ether 100% and $CHCl_3$/MeOH (90:10) were further purified on SiO_2 using a $CHCl_3$/MeOH gradient to give fusaripyrones A (**1**) and B (**2**).

Fusaripyrone A (**1**). ^{1}H NMR δ (C_6D_6) 6.41 (d, J=9.2 Hz), 5.87 (s), 5.72 (s), 5.33 (d, J=9.8 Hz), 5.29 (d, J=9.8 Hz), 3.42 (m), 3.10 (s), 2.57 (m), 1.96 (s), 1.95 (m), 1.94 (s), 1.93 (s), 1.91 (s), 1.77 (s), 1.74 (s), 1.70 (s), 1.45 (m), 1.18 (m), 1.08 (d, J=6.5 Hz), 1.02 (d, J=6.8 Hz), 0.88 (d, J=6.8 Hz), 0.79 (t, J=7.0 Hz).

Compound **1a**. Colorless amorphous solid (0.6 mg). $C_{34}H_{50}O_4$, HR-ESIMS m/z 545.3604 (M+Na$^+$, calcd 545.3607); $[\alpha]_D$ +14.5 (c 0.06, CH_2Cl_2); UV (MeOH) λ_{max} (ε): 284 (6368); 230 (10,075) nm. IR (film KBr) ν_{max} at 2928, 1724, and 1664 cm^{-1}. NMR data: see Table 1.

Compound **1b**. Colorless amorphous solid (1.0 mg). $C_{34}H_{50}O_4$, HR-ESIMS m/z 545.3606 (M+Na$^+$, calcd 545.3607); $[\alpha]_D$ +16.4 (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (ε): 273 (7230); 227 (14,240) nm. IR (film KBr) ν_{max} at 2930, 1600, and 1580 cm^{-1}. NMR data: see Table 1.

Fusaripyrone B (**2**). ^{1}H NMR δ (C_6D_6) 6.49 (H-17, d, J=9.2 Hz), 5.86 (H-13, s), 5.70 (H-11, s), 5.34 (H-15, d, J=8.9 Hz), 5.25 (H-7, d, J=9.5 Hz), 3.79 (H_{2a}-33, dd, J=6.5, 10.6 Hz), 3.58, (H_{2b}-33, dd, J=4.8, 10.6 Hz), 3.41 (H-16, m), 3.29 (H-20, s), 2.57 (H-8, m), 1.78 (H_3-24, s), 1.86 (H_3-25, s), 1.91 (H_3-32, s), 1.90 (H_3-29, s), 1.75 (H_3-26, s), 1.73 (H_3-28, s), 1.68 (H_3-30, s), 1.01 (H_3-31, d, J=6.8 Hz), 0.87 (H_3-27, d, J=6.8 Hz), 0.75 (H_3-23, t, J=7.5 Hz). ^{13}C δ (ppm) (C_6D_6) 205.6 (C-19), 163.8 (C-3), 159.2 (C-5), 146.2 (C-17), 141.2 (C-7), 133.2 (C-13), 132.7 (C-15), 131.7 (C-11), 127.3 (C-6), 105.5 (C-4), 98.8 (C-2), 64.0 (C-33), 48.5 (C-9), 46.7 (C-20), 33.5 (C-16), 32.4 (C-21), 31.6 (C-8), 20.8 (C-22), 20.6 (C-27), 20.2 (C-31), 19.3 (C-29), 17.5 (C-28), 17.3 (C-30), 15.0 (C-26), 14.8 (C-23), 12.0 (C-32).

Compound **2a**. Colorless amorphous solid (0.9 mg). $C_{34}H_{50}O_5$, HR-ESIMS m/z 561.3553 (M+Na$^+$, calcd 561.3556); $[\alpha]_D$ −2.30 (c 0.09, CH_2Cl_2); UV (MeOH) λ_{max} (ε): 266 (11,580); 231 (23,267) nm. IR (film KBr) ν_{max} at 3461, 1732, and 1665 cm^{-1}. NMR data: see Table 1.

Compound **2b**. Colorless amorphous solid (1.1 mg). $C_{34}H_{50}O_5$, HR-ESIMS m/z 561.3539 (M+Na$^+$, calcd 561.3556); $[\alpha]_D$ −9.88 (c 0.11, CH_2Cl_2); UV (MeOH) λ_{max} (ε): 257 (24,850); 226 (31,900) nm. IR (film KBr) ν_{max} at 3400, 1651, and 1584 cm^{-1}. NMR data: see Table 1.

Spectral data for haminols **3**–**8** were identical to those reported in the literature.

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