Micromelones A and B, Noncontiguous Polypropionates from *Micromelo undata*

José G. Napolitano, María L. Souto, José J. Fernández,* and Manuel Norte*

Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain

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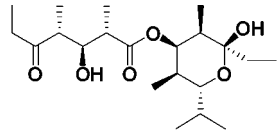
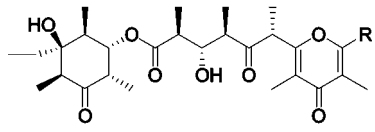
In this paper we report on the isolation and structural elucidation of two new noncontiguous polypropionates, micromelones A (**10**) and B (**11**), that have been isolated from the marine gastropod *Micromelo undata*. Their structures were determined through the interpretation of their spectroscopic data, and a biosynthetic pathway from a common linear precursor with other polypropionate groups has been proposed.

Gastropod mollusks have been a prolific source of structurally novel natural products for over 40 years.[1–4] A wide range of secondary metabolites derived from the iterative condensation of propionate units have been isolated from opisthobranch or pulmonate mollusks,[3] and a few of them do not contain a contiguous carbon skeleton, as would be expected from regular polyketide biosynthesis. Included in this small number of examples of these metabolites are dolabriferol (**1**) isolated from the anaspidean mollusk *Dolabrifera dolabrifera*,[5] baconipyrones A−D (**2**−**5**) isolated from the pulmonate *Siphonaria baconi*,[6] siserrone A (**6**) identified in extracts of *Siphonaria serrata*,[7] membrenones A (**7**) and B (**8**) isolated from the opisthobranch mollusk *Pleurobranchus membranaceus*,[8] and finally, the first acyclic noncontiguous polypropionate ester, **9**, from *Siphonaria australis*.[9,10]

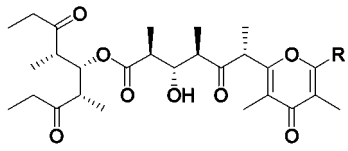


Dolabriferol (**1**)



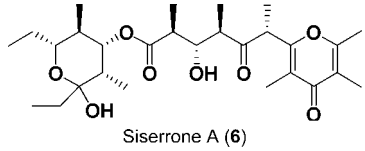
Baconipyrone A (**2**); R=CH_2-CH_3

Baconipyrone B (**3**); R=CH_3



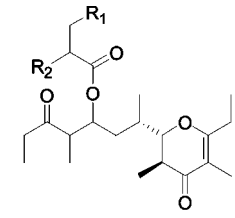
Baconipyrone C (**4**); R=CH_2-CH_3

Baconipyrone D (**5**); R=CH_3



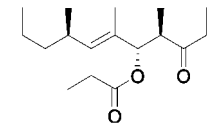
Siserrone A (**6**)

As part of our ongoing program to investigate natural products from diverse marine organisms, specimens of opisthobranch mollusk



Membrenone A (**7**); R_1= CH_3; R_2= αCH_3

Membrenone B (**8**); R_1= R_2= H



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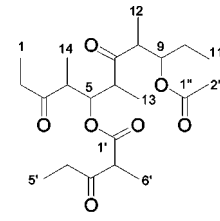
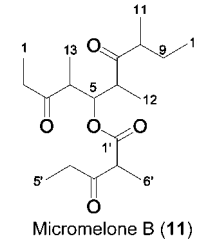
Micromelo undata have been studied. These are small, beautiful creatures with pigmented bodies and delicate blue tones, included in the cephalaspidean group, such as *Philinopsis speciosa*[11] and *Bulla striata*.[12] The specimens of *M. undata* were collected from the intertidal zone along the north coast of Tenerife. From the acetone extract of these specimens, obtained by purification on Sephadex LH20, MPLC, and HPLC chromatographies, we have isolated two new polypropionate metabolites, micromelones A (**10**) and B (**11**), whose structures have been proposed on the basis of the interpretation of their spectroscopic data.



Micromelone A (**10**)



Micromelone B (**11**)

Micromelone A (**10**) was obtained as an optically active, colorless, amorphous solid ($[\alpha]^{25}_D$ +24 (*c* 0.05, $CHCl_3$)). The molecular formula for **10** was determined as $C_{22}H_{36}O_7$ from its

* To whom correspondence should be addressed. Tel: +34 922318586. Fax: +34 922318571. E-mail: jjfercas@ull.es; mnorte@ull.es.

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Table 1. NMR Data ($CDCl_3$) for Micromelones A (**10**) and B (**11**)

	micromelone A (**10**)			micromelone B (**11**)		
position	δ_C	mult.	δ_H (*J* in Hz)	δ_C	mult.	δ_H (*J* in Hz)
1	7.5	CH_3	1.04, t (7.3)	7.9	CH_3	1.02, t (6.7)
2	35.1	CH_2	2.47, m	35.6	CH_2	2.48, m
3	211.4	C		212.0	C	
4	47.4	CH	2.89, dq (4.2, 6.5)	47.7	CH	2.88, dq (4.2, 6.7)
5	74.1	CH	5.45, dd (3.2, 4.2)	74.8	CH	5.47, dd (3.8, 4.2)
6	45.6	CH	3.14, dq (3.2, 7.3)	45.1	CH	2.99, dq (3.8, 7.6)
7	211.8	C		214.6	C	
8	47.9	CH	3.22, dq (6.0, 6.8)	46.6	CH	2.77, dq (6.7, 6.7)
9	75.6	CH	4.91, ddd (6.0, 5.8, 5.8)	26.7	CH_2	1.64, m; 1.36, m
10	23.2	CH_2	1.49, m; 1.56, m	12.0	CH_3	0.83, t (7.3)
11	9.4	CH_3	0.85, t, (7.3)	15.1	CH_3	1.05, d (6.7)
12	10.0	CH_3	1.06, d (6.8)	10.8	CH_3	1.03, d (7.6)
13	9.9	CH_3	1.04, d (7.3)	13.6	CH_3	1.12, d (6.7)
14	13.3	CH_3	1.16, d (6.5)			
1′	168.7	C		169.8	C	
2′	52.5	CH	3.45, q (6.8)	53.0	CH	3.44, q (7.0)
3′	205.8	C		206.5	C	
4′	35.0	CH_2	2.36, m	35.1	CH_2	2.52, m
5′	10.5	CH_3	1.09, t (7.0)	10.7	CH_3	1.01, t (7.3)
6′	29.6	CH_3	1.25, d (6.8)	30.0	CH_3	1.32, d (7.0)
1″	170.6	C				
2″	20.8	CH_3	2.02, s			

HRMS molecular ion observed at *m*/*z* 412.2474. The IR spectrum showed absorbance consistent with carbonyl functions at ν_{max} 1732 and 1718 cm^{-1}. The ^{13}C NMR data for this compound provided 22 carbon signals due to three ketone and two ester carbonyl groups, six sp^3 methines including two oxymethines, three sp^3 methylenes, and eight methyl groups, suggesting a polypropionate skeleton (Table 1). Five isolated spin systems were established from analysis of the $^1H-^1H$ COSY and HSQC experiments, (a) three of them involving two carbons, H_3-1 (δ_H 1.04)/H_2-2 (δ_H 2.47), H_2-4′ (δ_H 2.36)/H_3-5′ (δ_H 1.09), and H-2′ (δ_H 3.45)/H_3-6′ (δ_H 1.25), and (b) two systems connecting five carbons, from methyl H_3-11 (δ_H 0.85) to methyl H_3-12 (δ_H 1.06) and from methyl H_3-13 (δ_H 1.04) to methyl H_3-14 (δ_H 1.16) (Figure 1).

The HMBC data for **10** demonstrated the linkage of these separate proton networks by the carbonyl group correlations. Thus, this experiment showed correlations for H-4 (δ_H 2.89) and the methylene protons H_2-2 with the ketone carbonyl C-3 (δ_C 211.4), which successfully connected the H_3-1/H_2-2 and the H_3-13/H_3-14 spin systems. The chemical shift of the deshielded carbons C-5 (δ_C 74.1) and C-9 (δ_C 75.6) suggested that ester functionalities were attached to those carbons. This was supported by the long-range connectivity between the oxymethine protons H-5 (δ_H 5.45) and H-9 (δ_H 4.91) with the ester carbonyls C-1′ (δ_C 168.7) and C-1″ (δ_C 170.6), respectively. Moreover, the HMBC correlation between methine H-2′ (δ_H 3.45) and the carbonyls C-1′ and C-3′ (δ_C 205.8), which in turn correlated with H_2-4′ and H_3-5′, linked this spin system with the one described above, between H_3-1 and H_3-13. Further HMBC correlations were observed for methines H-6 (δ_H 3.14), H-8 (δ_H 3.22), and H-9 to the ketone carbonyl C-7 (δ_C 211.8), thereby completing the structure elucidation of **10**. This proposed structure was supported by the fragmentations pattern observed in the MS spectrum (Figure 2).

Micromelone B (**11**), $[\alpha]^{25}_D$ +28 (*c* 0.08, $CHCl_3$), was obtained as a colorless, amorphous solid. The molecular formula for compound **11** was established as $C_{19}H_{32}O_5$ by the HRMS ion at *m*/*z* 340.2264. Initial analyses of ^1H NMR, HSQC, and $^1H-^1H$ COSY experiments for **11** indicated that its chemical structure resembled **10**. The main differences observed in the NMR spectra were the absence of oxymethine signals for C-9 at δ_C 75.6 (δ_H 4.91) together with those for the acetyl group present in compound **10**, establishing the lack of the acetate moiety at C-9 in compound **11** (Table 1). Moreover, the $^1H-^1H$ COSY correlations between H-8 (δ_H 2.77) and the diastereotopic protons at δ_H 1.36 and 1.64 (H_2-9) and those with a terminal methyl group centered at δ_H 0.83 (C-10) completed the main polypropionate carbon chain between the methyl groups at C-1 (δ_H 1.02 and δ_C 7.9) and C-10 (δ_H 0.83 and δ_C 12.0). The HMBC connectivities for H-5 (δ_H 5.47 and δ_C 78.4) and H-2′ (δ_H 3.44 and δ_C 53.0) with the carbonyl group at C-1′ (δ_C 169.8) linked this moiety to the main backbone at C-5 in **11**, similar to that previously described for **10** (Figure 1). Attempts to establish the stereochemistry of these compounds were impossible due to their linear nature and their fast decomposition during the acquisition of spectroscopic data.

Although the nutritious habits of this marine mollusk, *Micromelo undata*, have not been reported, phylogenetically related opisthobranchia species[13–15] are specialized worm feeders.[16] Since metabolites containing polypropionate motifs have not yet been found in marine worms, it would thus appear that these characteristic metabolites are likely to be biosynthesized *de novo* by this mollusk.[3,12] These compounds are characterized by the presence

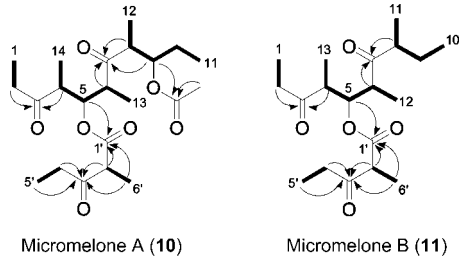

Figure 1. Significant HMBC correlations (arrows) for compounds **10** and **11**. Fragments obtained from COSY/TOCSY (bold lines).

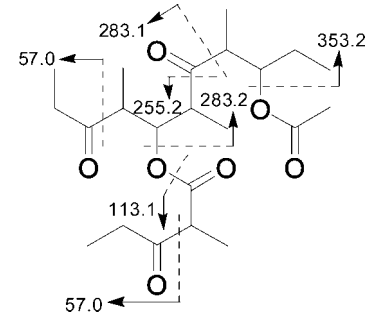

Figure 2. Key fragments of **10** in the EI mass spectrum.

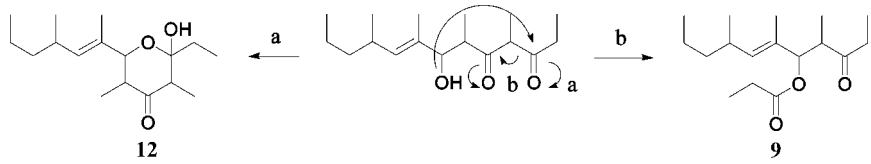

Figure 3. Hypothesis for the formation of the contiguous and noncontiguous polypropionate carbon backbone.

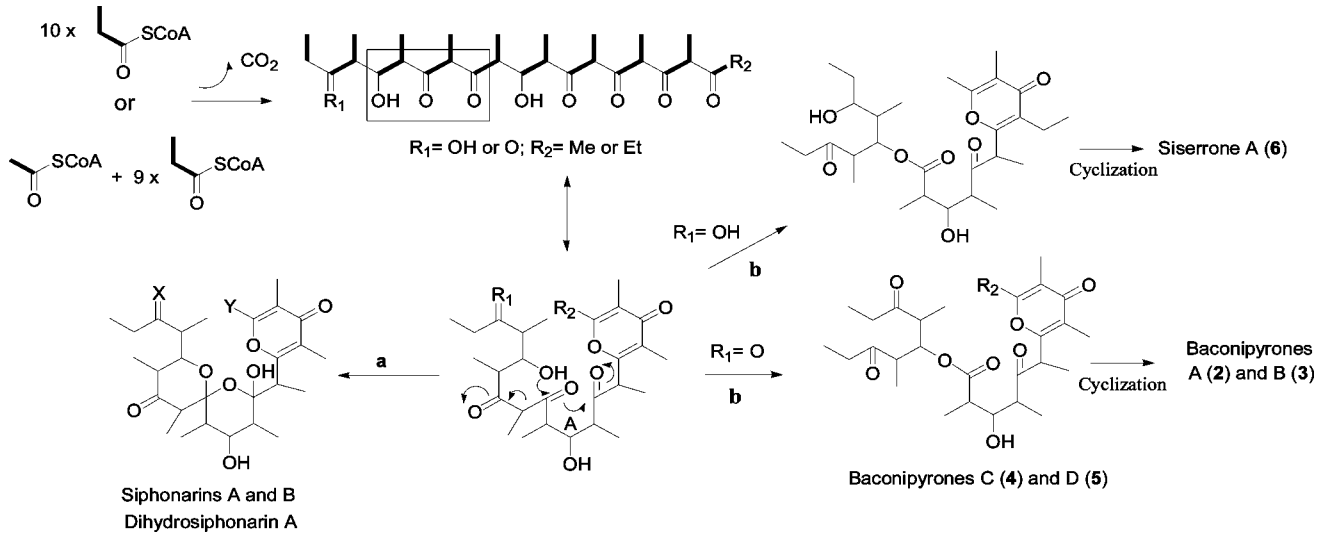

Figure 4. Plausible biosynthetic pathway for siphonarins, siserrone, and baconipyrones.

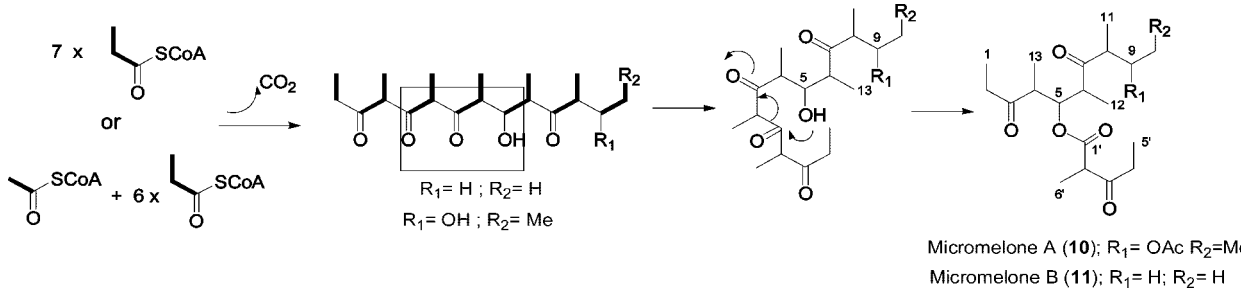

Figure 5. Postulated biosynthetic pathway of the micromelones' skeleton.

of an unusual noncontiguous polypropionate backbone, for which there are only a few examples, such as the baconipyrones A–D (**2**−**5**) and siserrone A (**6**), which are proposed as being rearrangement products generated from the contiguous polypropionate precursors dihydrosiphonarin and siphonarin, respectively.[3,6,7] Nevertheless, a unique biosynthetic pathway could possibly explain the formation of both groups of compounds from a common linear precursor. Developing this hypothesis, the presence of a 1-hydroxy-3,5-diketone system in the linear polypropionate precursor leads us to suggest a 1–5 nucleophilic attack by the hydroxyl group at the ketone. This, in turn leads us to propose two routes, **a** and **b**, that would explain the formation of the contiguous or noncontiguous polypropionate compounds. The simplest example is observed for hemiacetal **12** and the ester **9** isolated from *S. australis,*[9] which are derived from a common linear hexapropionate precursor with the 1-hydroxy-3,5-diketone system, as shown in Figure 3. This hypothesis is likewise applicable to the biosynthesis of the siphonarins and baconipyrones, which result from the condensation of 10 or 9 propionate units plus an acetate unit to give the appropriate linear precursor, which can evolve into the siphonarins (route **a**) or the baconipyrones (route **b**) (Figure 4).

Micromelones A and B possess a noncontiguous heptapropionate skeleton, and although their contiguous common precursor has not yet been isolated, it is possible to suggest a biogenetic pathway as shown in Figure 5.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer 241 polarimeter using a sodium lamp operating at 589 nm. The IR spectrum was measured on a Bruker IFS55 spectrometer. NMR experiments were performed on a Bruker AVANCE 400 MHz and AMX 500 MHz instruments. Chemical shifts are reported relative to TMS, and coupling constants are given in Hz. Mass spectra were recorded on a VG AutoSpec FISON spectrometer. HPLC was carried out with an LKB 2248 system equipped with a photodiode array detector. TLC was performed on AL Si gel Merck 60 F_{254}, and TLC plates were visualized by spraying with phosphomolybdic acid reagent and heating.

Biological Material. A total of 98 specimens of *Micromelo undata* were collected by hand from the intertidal zone at Punta del Hidalgo, in the north of Tenerife (Canary Islands), and stored in acetone until worked up. A voucher of these specimens was deposited at Departamento de Biología Marina in the Universidad de La Laguna (ULL), Tenerife, Spain.

Extraction and Isolation. The biological sample blended with acetone was decanted, and the specimens were re-extracted with the same solvent. The acetone extracts were pooled, concentrated to give 1.07 g of crude extract, and successively chromatographed by gel filtration on a Sephadex LH-20 column eluted with a mixture of $CHCl_3$−MeOH−*n*-Hex (1:1:2). The selected fraction (0.24 g) was subjected to medium-pressure reversed-phase chomatrography using a Lobar LiChroprep RP-8 column with MeOH−H_2O (9:1). Final purification of micromelone A (**10**) was achieved on a μ-Bondapak

C18 HPLC column using an isocratic elution of CH_3CN-H_2O (1:1), yielding 1.6 mg of pure substance. Micromelone B (**11**) (4.2 mg) was purified using the same column with a mixture of $MeOH-H_2O$ (17:3).

Micromelone A (10): amorphous, white powder; $[\alpha]^{25}_D$ +24 (*c* 0.05, $CHCl_3$); IR ν_{max} ($CHCl_3$) 2975, 2920, 2851, 1732, 1718, 1458, 1375, 1237 and 1175 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m*/*z* 412.2474 $[M]^+$ (calcd for $C_{22}H_{36}O_7$, 412.2461).

Micromelone B (11): amorphous, white powder; $[\alpha]^{25}_D$ +28 (*c* 0.08, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2976, 2926, 2863, 1735, 1723, 1462, 1375, 1237 and 1178 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HREIMS *m*/*z* 340.2264 $[M]^+$ (calcd for $C_{19}H_{32}O_5$, 340.2250).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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