Placidenes C−F, Novel α-Pyrone Propionates from the Mediterranean Sacoglossan *Placida dendritica*

Adele Cutignano, Angelo Fontana,* Licia Renzulli, and Guido Cimino

Istituto di Chimica Biomolecolare (ICB) del CNR, Via Campi Flegrei 34, 80078, Pozzuoli, Napoli, Italy

Received January 17, 2003

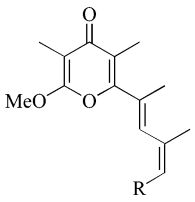
Four new α-pyrone-containing propionates (**5**−**8**) and an unprecedented hydroperoxide **9** have been isolated from the mantle extract of *Placida dendritica*, a Mediterranean sacoglossan that lives upon the green alga *Bryopsis plumosa*. The new metabolites co-occur with the related compounds **1**−**4**, which have been described in previous studies of the mollusc. The presence of **9** opens intriguing perspectives on the ecological role of placidenes. This paper reports the isolation and structural elucidation of the new compounds **5**−**9**.

Pyrone-containing compounds constitute a characteristic trait of a restricted group of marine molluscs of the order Sacoglossa.[1] These compounds, which are formally derived from a polyketide pathway, are associated with specific ecophysiological functions of the molluscs, since they may act as mediators in tissue regeneration and chemical defense.[1] In agreement with this view, it is generally accepted that most of these propionates are biosynthesized de novo by the sacoglossans, although such aptitude has been rigorously proved only in a few species (*Placobranchus ocellatus*,[2] *Cyerce cristallina*,[3,4] *Ercolania funerea*,[5] and *Elysia viridis*[6]).

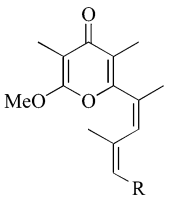
In the aim of studying the biosynthetic origin of the sacoglossan polypropionates, we have recently reinvestigated the lipid extract of *Placida dendritica*,[7] a Mediterranean mollusc that is known to possess a number of regular and irregular propionates named placidenes A and B (**1** and **2**) and isoplacidenes A and B (**3** and **4**). The chemical reanalysis of the lipid extracts of the mollusc led to the isolation of four novel α-pyrone-containing propionates (**5**−**8**), together with the unusual hydroperoxide **9**, formally derived by oxidation of **1**. In this paper, we describe the isolation, characterization, and chemical reactivity of these metabolites under biomimetic conditions.

Following our standard procedures, a mantle extract of *P. dendritica* was prepared by soaking the whole animals in acetone bath. The resulting material was chromatographed on a silica gel column by eluting with a gradient system of petroleum ether/Et_2O. On the other hand, homogeneous fractions obtained with 80% and 70% petroleum ether were further purified on RP-HPLC to give the known γ-pyrones **1**−**4**,[7] together with the four novel polyketides **5**−**8**, here named placidenes C−F, and the hydroperoxide **9**.

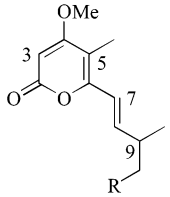
APCI-MS spectra of **5** (placidene C) showed a pseudomolecular ion at *m*/*z* 223 $(M + H)^+$ that, in agreement with ^{13}C NMR data, suggested the molecular formula $C_{13}H_{18}O_3$. An intense IR band at 1714 cm^{-1} together with a ^{13}C NMR signal at 163.9 ppm indicated the presence of a conjugated ester with a UV maximum at 319 nm. In agreement with the literature data,[3,5] the α-pyrone ring of **5** was characterized by three ^1H NMR signals at δ 5.48 (H-3), 3.82 (Me-O), and 1.94 (Me-5), as well as by the diagnostic ^{13}C NMR resonances of C-3 (88.4 ppm), C-4 (170.9 ppm), and C-6 (154.3 ppm).[8] The ^1H NMR spectrum of **5** also showed two



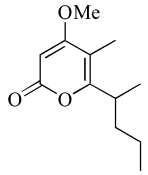
1 R = CH_2CH_3
2 R = CH_3



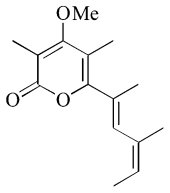
3 R = CH_2CH_3
4 R = CH_3



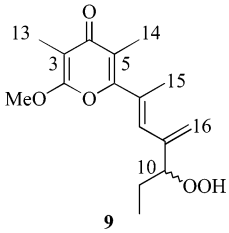
5 R = CH_3
6 R = CH_2CH_3



7



8



9

downshifted signals (δ 6.20 and 6.61, H-7 and H-8) that were attributed to the protons of an exocyclic double bond, whose geometry was established to be *E* according to the coupling constant value of 15.4 Hz. The remaining part of the alkyl chain was inferred on the basis of COSY correlations of the methine proton at δ 2.21 (H-9) with the signals at δ 6.61 (H-8), 1.06 (Me-9), and 1.41 (H_2-10). The NMR data were completed by a terminal methyl group (δ 0.88) coupled to the protons at C-10. The above assignments were confirmed by HMBC data that allowed unambiguous identification of the atoms in the pyrone ring and the joining of the unsaturated alkyl chain to C-6 of the cycle (Table 1).

The structure of placidene D (**6**) (IR max at 1722 cm^{-1} and UV max at 319 nm) is rather similar to that of **5**. The spectroscopic analogies between **5** and **6**, in fact, suggested that these compounds belong to a homologous series, with placidene D (**6**) differing from placidene C (**5**) only in the

* Corresponding author. Tel: +39 081 8675096. Fax: +39 081 8041770. E-mail: afontana@icmib.na.cnr.it.

10.1021/np0300176 CCC: $25.00
Published on Web 10/08/2003

Table 1. NMR Data ($CDCl_3$, 400 MHz) of Placidenes C−F (**5**−**8**)[a,b]

	5		**6**		**7**		**8**	
	δ_H, m, *J* (Hz)	δ_C	δ_H, m, *J* (Hz)	δ_C	δ_H, m, *J* (Hz)	δ_C	δ_H, m, *J* (Hz)	δ_C
2		163.9		164.0		164.8		165.3
3	5.48, s	88.4	5.48, s	88.4	5.42, s	87.5		110.2
4		170.9		170.9		171.0		168.4
5		106.3		106.3		106.2		109.1
6		154.3		154.3		164.1		158.7
7	6.20, d, 15.4	117.0	6.20, d, 15.4	116.8	2.90, dt, 8.9;6.9	34.5		128.8
8	6.61, dd, 15.4, 8.2	145.3	6.61, dd, 15.4, 8.3	145.6	1.48, m	36.6	6.06, bs	134.3
9	2.21, m	39.2	2.32, m	37.3	1.24, m	20.7		132.1
10	1.41, q, 7.3	29.3	1.32, m	38.8	0.88, t, 7.35	14.0	5.46, bq, 7.0, 0.9	124.1
11	0.88, t, 7.3	11.7	1.27, m	20.4			1.58, bd, 7.0	15.1
12			0.89, t, 7.0	14.1				
Me-3							2.06, s	10.3
Me-5	1.94, s	8.7	1.94, s	8.7	1.89, s	9.0	2.04, s	12.0
Me-7					1.19, d, 6.9	18.3	1.87, d, *1.6*	16.3
Me-9	1.06, d, 7.6	19.7	1.06, d, 6.7	20.1			1.81, bs	23.2
OMe	3.82, s	56.0	3.82, s	56.1	3.81, s	56.0	3.84, s	60.2

[a] Numbering is in agreement with ref 7. [b] Assignments are supported by DEPT, HSQC, and HMBC experiments.

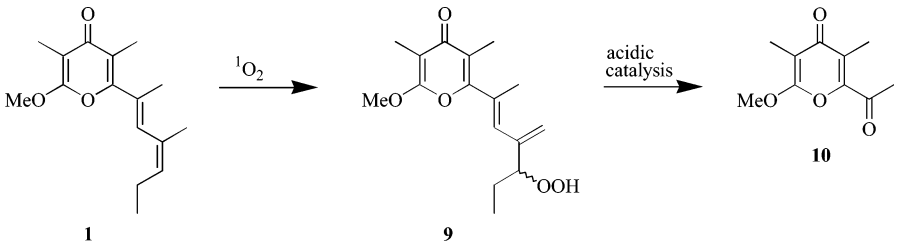


Figure 1. Formation and decomposition of 10-hydroperoxyl placidene A (**9**).

presence of an additional methylene group in the alkyl chain. Accordingly, the ^{1}H NMR spectrum of **6** contained the same resonances observed for **5** with the exception of a signal at δ 1.27 (H_2-11), which showed coupling with the terminal methyl moiety at δ 0.89 (H_3-12). The MS pseudomolecular ion at 237 *m*/*z* $(M + H)^+$, 14 amu greater than placidene C (**5**), supported the molecular formula $C_{14}H_{20}O_3$, thus confirming the depicted structure of **6**.

Placidene E (**7**) (MW = 210, $C_{12}H_{18}O_3$) showed the same NMR data for the substituted α-pyrone ring of **5** and **6** (Table 1) but also a significant blue-shift of the UV absoption, with a λ_{max} centered at 286 nm. These data were in agreement with a reduced extension of the chromophore due to the presence of a saturated alkyl chain, the structure of which was easily inferred by 2D NMR experiments (Table 1). HMBC connectivities (H-7/C-5 and Me-7/C-6) secured the linkage of the alkyl chain to the pyrone ring through C-6.

Placidene F (**8**) showed a structure slightly differing from **5**−**7** for the presence of a pentasubstituted pyrone moiety. In fact, the ^{1}H NMR spectrum of **8** ($C_{15}H_{20}O_3$) exhibited four vinyl methyl groups falling in the region between δ 1.81 and 2.06. Two of these signals were unambiguously assigned to Me-3 (δ 2.06) and Me-5 (δ 2.04) on the basis of HMBC correlations with C-4 (168.4 ppm) and C-6 (158.7 ppm). The remaining part of **8** was identified as a 1,3-dimethyl pentadienyl residue for the presence of clear correlations between the other two methyl groups (δ 1.87 and 1.81) and the protons at δ 6.06 (H-8) and 5.46 (H-10), as well as between this latter signal and the terminal methyl at δ 1.58 (H_3-11). The depicted structure was in agreement with the UV maximum at 312 nm and with the HMBC correlations H-8/C-6 and Me-7/C-6. The *E* and *Z* geometry of double bonds in **7** and **9** is suggested on the basis of ^{13}C chemical shifts of the methyl signals of Me-7 (16.3 ppm) and Me-9 (23.2 ppm).[9]

The hydroperoxide **9** showed a typical signal due to the α-methoxy (δ 3.97) and β,β'-methyl groups (δ 2.12 and 2.03) of a tetrasubstituted γ-pyrone ring. The NMR data (Experimental Section) suggested close analogies with the structure of placidene A (**1**), even if there was evidence for only one vinyl methyl group (δ 1.87). The ^{1}H NMR spectrum also supported the presence of an exomethylene double bond (δ 5.50 and 5.35, H_2) and showed a downshifted proton at δ 4.36 (H-10). This latter signal was coupled to the methine carbon at 90.5 ppm (C-10) in the HSQC experiment, thus supporting the occurrence of a partial substructure featured by a secondary hydroperoxyl group in α-position to a terminal methylene moiety.[10] These data were confirmed by HMBC experiments that showed connectivities between C-10 (90.5 ppm) and the exomethylene protons, as well as from the C9 (δ 142.8 ppm) to H-9 (δ 6.21), H-10, and the exomethylene hydrogens. In agreement with the structure of a C10 oxygenated derivative of placidene A (**1**), COSY and TOCSY experiments confirmed the coupling of the secondary hydroperoxyl residue with the methylene protons at δ 1.58 and 1.51 (H_2-11), as well as the correlation of this latter system with the terminal methyl moiety at δ 0.95. Compound **9** is very likely formed by a singlet oxygen ene reaction of placidene A (**1**) (Figure 1). The hydroperoxide was unusually stable, although it gave **10** after standing a few hours in $CHCl_3$.[11] The formation of this latter product is quite interesting and may involve acid-catalyzed decomposition of a cyclic peroxide followed by a retro-aldol reaction (Figure 1).

In conclusion, the Mediterranean sacoglossan *P. dendritica* contains a pool of regular (**1** and **3**) and irregular (**2**, **4**−**8**) polypropionates featured by γ- and α-pyrone moieties. The new metabolites **5**−**8** appeared structurally related to α-pyrones isolated from *E. funerea*[5] and *C. cristallina*.[3] Although no feeding experiments were carried out for testing the biosynthesis of **5**−**8** in *P. dendritica*, the de novo origin of these metabolites still remains the most plausible hypothesis. In this regard, it is interesting to note that the irregular skeletons of some placidenes (e.g., **2** or **5**) may derive from methylation or demethylation of a

regular acetate or propionate skeleton, as already suggested for the cyercenes of *C. cristallina*,[3] but it may also arise from a mixed biosynthesis that involves simultaneously acetate and propionate units.[12] However, the cooccurrence of compounds containing both α- and γ-pyrone rings in the same organism might imply the conversion of one form into the other during the chemical workup. To test this hypothesis, we examined the stability of the carbon skeletons of **1** and **5** under different acidic conditions ($1 < pH < 5$), but no conversion was observed with both compounds. Both α- and γ-pyrone rings derive from acyclic precursors, but these experiments suggest that the cyclization process leads irreversibly to the two different series of products. Finally, the hydoperoxypolypropionate **9** is very likely produced by photooxidation of the main mollusc metabolite, placidene A (**1**). We have evidence for the presence of other hydroperoxides probably derived by oxidation of the polypropionate pool. Although these metabolites may be produced during the chemical analysis of the mollusc extracts, we cannot rule out that the polypropionate mixture plays a photoprotective role in the living mollusc. In this regard, it is interesting to note that placidenes (compounds **1** and **5**−**8**) were not toxic to either *Gambusia affinis* or tumor cells.

Experimental Section

General Experimental Procedures. 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 by a Bruker Avance DRX 400 operating at 400 MHz for protons. Spectra were referenced to $CHCl_3$ (δ 7.26) as the internal standard. MS spectra were run on a Shimadzu LCMS 2010 apparatus equipped with an APCI source. Solvents were distilled prior to use.

Extraction and Isolation of Placidenes. A total of 65 specimens of *Placida dendritica* collected at Fusaro Lake (Gulf of Naples) in March 2001 were extracted with acetone (3 × 100 mL). The combined extracts were evaporated under reduced pressure, and the aqueous residue was partitioned with diethyl ether (3 × 100 mL) and then with *n*-butanol (3 × 100 mL). The ether-soluble material was concentrated, giving a brown-green oil (87 mg), and fractionated on a silica gel column using a gradient elution from 10% to 50% diethyl ether in petroleum ether. Fractions eluted with 80:20 and 70:30 EP/EE were further purified by RP-HPLC (ODS-2 column) with a MeOH/H_2O gradient elution system from 67% to 100% of MeOH, monitoring UV absorbance at 250 nm, affording known placidenes **1**−**4**, novel metabolites **5**−**8**, and the hydroperoxide **9** as pure compounds. Spectral data of compounds **1**−**4** were identical to those reported in the literature.[7]

Placidene C (5): colorless amorphous solid (1.2 mg); $C_{13}H_{18}O_3$; $[\alpha]_D$ +22.8 (*c* 0.012, MeOH); UV (MeOH) λ_{max} (ϵ) 226 (21200), 319 (6400) nm; IR (film KBr) ν_{max} 1714 cm^{-1}; NMR data, see Table 1; APCI-MS, *m*/*z* (%) 223 $[M + H]^+$ (58) and 255 $[M + MeOH + H]^+$ (100).

Placidene D (6): colorless amorphous solid (1.3 mg); $C_{14}H_{20}O_3$; $[\alpha]_D$ +30.0 (*c* 0.013, MeOH); UV (MeOH) λ_{max} (ϵ) 226 (15900), 319 (4500) nm; IR (film KBr) ν_{max} at 1722 cm^{-1}; NMR data, see Table 1; APCI-MS (MeOH), *m*/*z* (%) 237 $[M + H]^+$ (77) and 269 $[M + MeOH + H]^+$ (100).

Placidene E (7): colorless amorphous solid (2.5 mg); $C_{12}H_{18}O_3$; $[\alpha]_D$ −56.3 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (ϵ), 286 (4200) nm; IR (film KBr) ν_{max} at 1706 cm^{-1}; NMR data, see Table 1; APCI-MS (MeOH), *m*/*z* (%) 211 $[M + H]^+$ (50) and 243 $[M + MeOH + H]^+$ (100).

Placidene F (8): colorless amorphous solid (1.0 mg); $C_{15}H_{20}O_3$; UV (MeOH) λ_{max} (ϵ) 203 (9200), 230 (5000), 312 (5600) nm; IR (film KBr) ν_{max} at 1706 cm^{-1}; NMR data, see Table 1; APCI-MS (MeOH), *m*/*z* (%) 249 $[M + H]^+$ (82) and 271 $[M + MeOH + H]^+$ (100).

Compound 9: colorless amorphous solid (1.5 mg); ^1H NMR ($CDCl_3$) δ 6.21 (1H, bs, H-8), 5.49 (1H, bs, H-16a), 5.35 (1H, bs, H-16b), 4.36 (1H, t, $J = 6.2$ Hz, H-10), 3.97 (3H, s, OMe), 2.12 (3H, s, H_3-15), 2.03 (3H, s, H_3-14), 1.87 (3H, s, H_3-13), 1.58 (1H, m, H-11a), 1.51 (1H, m, H-11b), 0.95 (3H, t, $J = 6.7$ Hz, H_3-12); ^{13}C NMR ($CDCl_3$) δ 181.3 (C-4), 161.6 (C-2), 158.1 (C-6), 131.5 (C-8), 131.2 (C-7), 118.6 (C-16), 118.2 (C-5), 99.6 (C-3), 90.6 (C-10), 55.3 (OMe), 24.7 (C-12), 16.5 (Me-7), 11.8 (Me-5), 10.0 (C-12), 6.91 (Me-3).

Compound 10: ^1H NMR ($CDCl_3$) δ 4.08 (OCH_3), 2.54 (Me-7) 2.32 (Me-5), 1.91 (Me-3); ^{13}C NMR ($CDCl_3$) δ 193.1 (C-7), 180.1 (C-4), 161.8 (C-2), 148.4 (C-6), 126.4 (C-5), 101.7 (C-3), 28.1 (Me-7), 10.1 (Me-5), 7.25 (Me-3).

Acknowledgment. This research was partially supported by a PharmaMar grant. The authors are grateful to the personnel of the "Servizio NMR dell'Istituto di Chimica Biomolecolare" for their technical assistance.

References and Notes

(1) Cimino, G.; Ciavatta, M. L.; Fontana, A.; Gavagnin, M. In *Bioactive Compounds from Natural Sources*; Corrado Tringali, Ed.; Taylor and Francis: London, 2001; Chapter 15, pp 577−637.
(2) Ireland, C.; Scheuer, P. J. *Science* **1979**, *205*, 922−923.
(3) Vardaro, R. R.; Di Marzo, V.; Crispino, A.; Cimino, G. *Tetrahedron* **1991**, *47*, 5569−5576.
(4) Di Marzo, V.; Vardaro, R. R.; De Petrocellis, L.; Villani, G.; Minei, R.; Cimino, G. *Experientia* **1991**, *47*, 1221−1227.
(5) Vardaro, R. R.; Di Marzo, V.; Marin, A.; Cimino, G. *Tetrahedron* **1992**, *48*, 9561−9566.
(6) Gavagnin, M.; Marin, A.; Mollo, E.; Crispino, A.; Villani, G.; Cimino, G. *Comp. Biochem. Physiol.* **1994**, *108B*, 107−115.
(7) Vardaro, R. R.; Di Marzo, V.; Cimino, G. *Tetrahedron Lett.* **1992**, *33*, 2875−2878.
(8) Turner, W. V.; Pirkle, W. L. *J. Org. Chem.* **1974**, *39*, 1935−1937.
(9) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972.
(10) Kitagawa, I.; Cui, Z.; Son, B. W.; Kobayashi, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1987**, *35*, 124−134.
(11) The presence of the keto-derivative **10** was already evident after the acquisition of the NMR spectra needed for the elucidation of **9** in $CDCl_3$ (14 h). However, the two compounds co-occurred for several days even keeping the sample in $CHCl_3$ on the bench at room temperature.
(12) Staunton, J.; Wilkinson, B. In *Topics in Current Chemistry*: *Biosynthesis of Aliphatic Polyketides*; Leeper, F. J., Vederas, J. C., Eds.; Springer-Verlag: Berlin, 1998; Vol. 195, pp 49−52.

NP0300176