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The Isolation and Characterization of Agardhilactone, a Novel Oxylipin from the Marine Red Alga *Agardhiella subulata*

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Abstract: A novel tricyclic oxylipin containing δ-lactone, cyclopentane and epoxide rings and a conjugated diene, has been isolated from the marine red alga *Agardhiella subulata*. The structure, including partial stereochemistry, was determined by NMR and GC-MS analysis of menthoxycarbonyl derivatives. Copyright © 1996 Elsevier Science Ltd

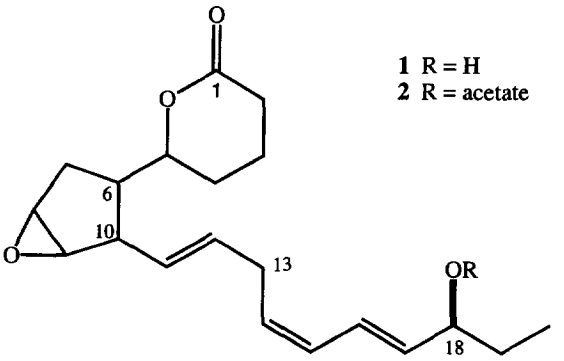
Although relatively few carbocyclic oxylipins have been isolated from marine algae, they exemplify a tremendous diversity in basic structure type.[1] For example, the constanolactones from *Constantinea* possess cyclopropyl rings[2] while those from other algae contain cyclopentyl rings, such as jasmonic acid from *Gelidium*,[3] prostaglandin E_2 from *Gracilaria*,[4] the cymathere ethers from *Cymathere*,[5] and fatty acids containing a terminal cyclopentyl ring from several members of the red algal family Solieriaceae.[6] Further variation in oxylipin structure type is observed in the epoxycyclopentane derivative hybridalactone from *Laurencia*[7] as well as the ecklonialactones from *Ecklonia*.[8,9] In our continuing efforts to describe the organic chemistry of algal-derived oxylipins, we have now isolated a novel epoxycyclopentane derivative, agardhilactone (**1**) from the red alga *Agardhiella subulata* (Solieriaceace). Agardhilactone is the first of a new oxylipin structure class, distinctive by the unprecedented position of the cyclopentyl ring, C6 to C10, within a 20-carbon chain framework.

A collection of *Agardhiella subulata* was made in Massachusetts, September 1994. The alga was immediately frozen, stored at -20 °C, and repeatedly extracted for its lipid metabolites (CH_2Cl_2/MeOH, 2:1, 380 g dry weight, 18.6 g dark oil). Vacuum chromatography of 8.6 g (Hex/EtOAc/MeOH gradient) yielded several polar fractions (66.5 mg) containing blue-charring compounds (50% H_2SO_4) by TLC, indicative of oxylipins. These fractions were combined and further fractionated over reverse phase C-18 silica (H_2O/MeOH gradient), followed by normal phase prep TLC (10% MeOH/$CHCl_3$). After acetylation, 0.6 mg of agardhilactone acetate (**2**) was isolated by NP-HPLC (50% EtOAc/Hex), along with an intractable minor component (ca. 5%). Vacuum liquid chromatography, C-18 silica (H_2O/MeOH gradient), NP-HPLC (70% and 40% EtOAc/Hex), and TLC (5%MeOH/$CHCl_3$) of the additional 10 g of extract gave 0.6 mg of agardhilactone (**1**) along with the same intractable minor isomer; this latter sample was used for stereochemical analysis.

Agardhilactone acetate (**2**) yielded a molecular formula of $C_{22}H_{30}O_5$ $[M+H]^+$ by HRCIMS (375.2170; 0.1 mmu dev.). Of the eight degrees of unsaturation inherent in this formula, five were defined by ^{13}C NMR

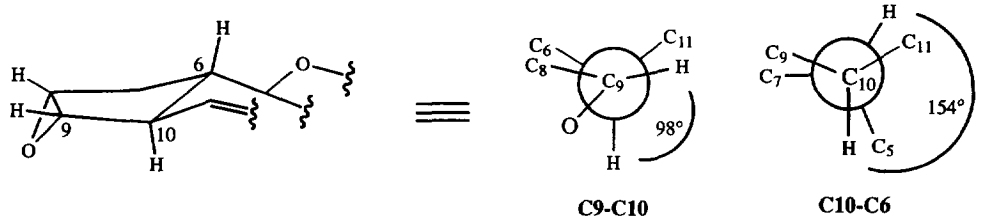
and IR data as two esters ($\upsilon_{C=O}$ 1732, 1738; δ171.8, δ170.1) and six olefinic carbons, thereby indicating three additional rings. One of the rings was present as an epoxide (δ55.7, 60.2) and two of the olefinic bonds were in conjugation (UV λ_{max} = 236 nm, ε = ca. 39,000, MeOH).

Although overlap in the ^{1}H NMR of the δ1.0-1.9 region (H3, H4a, H4b, H6, H7a, and H19) limited the utility of ^{1}H-^{1}H COSY, DQF ^{1}H-^{1}H COSY revealed one extended spin system, H2-H20 and an isolated acetate methyl group (H22). Correlations from a ^{1}H-^{13}C XHCORR showed the protons at δ4.24 (H5), δ5.22 (H18), δ3.46 (H8), and δ3.40 (H9) were attached to carbons bearing oxygen, the latter two as an epoxide. Complete analysis of the ^{1}H-^{13}C XHCORR in concert with the DQF ^{1}H-^{1}H COSY defined a disubstituted cyclopentyl epoxide ring connected to a δ-lactone, fulfilling the remaining two degrees of ring unsaturation, and a 10-carbon chain which contained three double bonds and an acetylated hydroxyl group. The acetate ester was confidently placed at C18 by observing a δ1.11 upfield shift in H18 in the ^{1}H NMR spectrum of underivatized agardhilactone (**1**, table 1). The double bonds at C11-C12 (15.6 Hz) and C16-C17 (15.3 Hz) were defined as *trans* while the C14-C15 double bond was *cis* based on $^3J_{HH}$ (11.0 Hz) and NOESY correlations between H13 and H16 (Table 1), completing the planar structure of **2**.[10]



1 R = H
2 R = acetate

NOESY analysis combined with ^{1}H-^{1}H coupling constants supported a relative stereochemistry around the five membered ring as 6*S**, 8*R**, 9*S**, 10*R**.[11] Correlations between H9 and H6, and H8 and H6 indicated that the epoxide and C1-C5 chain were *cis* to each other. Small coupling constants between H9 and H10 suggested they occupy a *trans* relationship (figure 1) while the cyclopentyl epoxide must be *cis* due to steric constraints. The lactone and C11-C20 substituents were defined as *trans* based on coupling constants (9.0 Hz, figure 1) and ^{13}C NMR similarities to the ecklonialactones,[8,9] hybridalactones,[7] and cymathere lactone.[12]



C9-C10 **C10-C6**

Figure 1. Depiction of selected H-C-C-H dihedral angles of energy minimized (Chem 3D) agardhilactone acetate fragment.

The absolute stereochemistry at C18 was determined by converting agardhilactone (**1**) (0.2 mg) to its corresponding (-)-menthoxycarbonyl (MC) derivative, oxidative ozonolysis to release the C17-C20 fragment and derivation of this fragment to the corresponding methyl ester.[13] GC standards for this same MC derivative gave baseline separation under optimized conditions.[14] With these conditions, the agardhilactone MC derivative was determined as 80% *S*, 20% *R*.[15]

Table 1. ^{1}H and ^{13}C NMR Data for Agardhilactone (**1**) and its Acetate Derivative (**2**).[a]

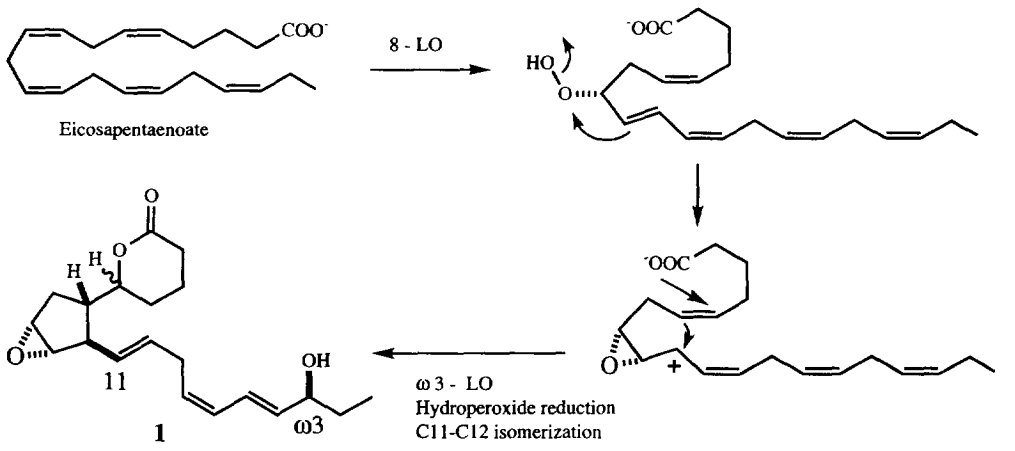
	Compound 1[b]		Compound 2			
#C	^{13}C	^{1}H	^{13}C	^{1}H	NOESY Correlations	HMBC Correlations C ⇒ H
1	171.5	-	171.8	-	-	2a, 2b
2 a	29.5	2.42	29.5	2.42 dt 17.7, 7.5	2b	-
b		2.61		2.61 dt 17.7, 6.5	2a	
3	18.8	1.85	18.8	1.85 m	4b	2a, 2b
4 a	27.2	1.51	27.2	1.51 m	4b	2b
b		1.79		1.78 m	3, 4a	
5	78.5	4.24	78.5	4.24 ddd 11.7, 2.4, 2.4	-	4, 10
6	43.2	1.61	43.2	1.62 m	7b, 9	7, 8, 10
7 a	27.5	1.74	27.6	1.74 m	7b, 8	6, 8
b		2.12		2.12 dd 13.6, 7.5	6, 7a	
8	55.6	3.46	55.7	3.46 bd 7.5	6, 7a	7
9	60.2	3.40	60.2	3.40 dd 2.7, 1.4	6, 10	7
10	44.6	2.72	44.7	2.71 bdd 9.0, 9.0	9	7, 9, 10
11	129.3	5.49	130.3[c]	5.49 m	12	-
12	131.3	5.64	131.2	5.63 dt 15.6, 6.0	11	14, 18
13	30.9	2.95	30.9	2.94 dd 6.8, 6.8	14, 16	13
14	129.0	5.45	129.1[c]	5.47 m	13, 15	-
15	128.7	6.07	128.4	6.01 dd 11.0	14, 16	13, 17
16	125.5	6.50	127.7	6.50 dd 15.4, 11.0	13, 15, 17	15, 17, 18
17	136.4	5.71	131.6	5.59 dd 15.4, 6.8	16, 18	15, 19
18	74.0	4.11	75.9	5.22 dd 13.8, 6.8	17	19, 20
19	29.7	1.57	27.6	1.65 m	-	20
20	9.7	0.94	9.5	0.90 t 7.4	-	19
21	-	-	170.1	-	-	22
22	-	-	21.3	2.06 s	-	-

[a] All spectra recorded on a Bruker AM-400 spectrometer in $CDCl_3$ (^{1}H spectra referenced to TMS at 0.0 ppm; ^{13}C spectra referenced to the centerline of $CDCl_3$ at 77.0 ppm; data presented as δ, multiplicity in Hz).

[b] Assigned by comparison to 2 and model compounds.[16] Multiplicities and J_{HH} similar to those reported for **2**.

[c] Assignment may be interchanged.

We propose the biogenesis of **1** to involve an 8-lipoxygenase (LO)-initiated oxidation of eicosapentaenoic acid. Reaction of the hydroperoxide with the 9,10-olefin with consequent loss of OH^- would form an epoxy carbonium ion which could induce cyclopentyl and lactone ring formation. Completion of the agardhilactone structure could be accomplished by an ω-3 LO (Scheme 1), reduction of the resulting hydroperoxide, and isomerization of the 11,12-olefin from *cis* to *trans*. Several members of the same order, Gigartinales, contain high levels of the putative precursor EPA as well as arachidonic acid.[17] While ω-3 oxidation is relatively uncommon in algae, it was recently proposed for a series of green algal metabolites.[18] Precedence for an 8-LO has been shown in another red alga of the same family (Solieriaceae, *Sarcodiotheca*).[12]



Scheme 1. Proposed biogenesis of agardhilactone (**1**). Absolute stereochemistry at C6, C8, C9, and C10 is arbitrary, although consistent with hypothesized 8*R*-LO-initiated pathway.[12] Relative stereochemistry at C5 is unknown.

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References and Notes

1. Gerwick, W. H. *Chem. Rev.* **1993**, *93*, 1807-1823.
2. Nagle, D. G.; Gerwick, W. H. *J. Org. Chem.* **1994**, *59*, 7227-7237.
3. Krupina, M. V.; Dathe, W. Z. *Naturforsch.* **1991**, *46C*, 1127-1129.
4. Gregson, R. P.; Marwood, J. F.; Quinn, R. J. *Tetrahedron Lett.* **1979**, *20*, 4505-4506.
5. Proteau, P. J.; Gerwick, W. H. *Tetrahedron Lett.* **1992**, *33*, 4393-4396.
6. Miralles, J.; Aknin, M.; Micouin L.; Gaydoou, E.- M.; Kornprobst, J.-M. *Phytochemistry* **1990**, *29*, 2161-2163.
7. Higgs, M. D.; Mulheirn, L. J. *Tetrahedron* **1981**, *24*, 4259-4262.
8. Kurata, K.; Taniguchi, K.; Shiraishi, K.; Hayama, N.; Tanaka, I.; Suzuki, M. *Chemistry Lett.* **1989**, 267-270.
9. Kurata, K.; Taniguchi, K.; Shiraishi, K.; Suzuki, M. *Phytochemistry* **1993**, *33*,155-159.
10. Although not completely characterized, the minor component was structurally similar from C1 to C10 but differed in the location of oxidation between C11 to C20.
11. Lack of nOe correlations between protons of the cyclopentane and lactone rings precluded stereochemical assignment at C5.
12. Proteau, P. J. *Oxylipins from Temperate Marine Algae and a Photoprotective Sheath Pigment from Blue-Green Algae*, Oregon State University, 1993.
13. Hamburg, M. *Anal. Biochem.* **1971**, *43*, 515-526.
14. MC derivatives were detected by GC-MS (11.5 m of HP Ultra-1, 100-210°C at 3.0° C per min., then isothermal for 15 min.), and eluted at 17.03 min. *(S)* and 17.27 min. *(R)*.
15. Although the scalemic nature of C18 could be due to extraction or derivatization procedures, it should be noted that plant lipoxygenases are known to produce *S/R* mixtures exceeding the 80:20 ratio observed herein. [Gardner, H. W. *Biochim. Biophys. Acta* **1991**, *1084*, 221-239.]
16. Bernart, M.; Gerwick, W. H. *Tetrahedron Lett.* **1988**, *29*, 2015-2018.
17. Khotimchenkco, S. V.; Vaskovsky, V. E. *Botanica Marina* **1990**, *33*, 525-528.
18. Bernart, M. W.; Whatley, G. G.; Gerwick, W. H. *J. Nat. Prod.* **1993**, *56*, 245-259.

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