Tetrahedron Letters, Vol. 34, No. 48, pp. 7689-7692, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Egregiachlorides A-C: New Chlorinated Oxylipins from the Marine Brown Alga *Egregia menziesii*

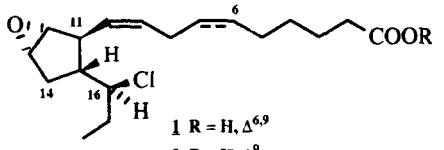
James S. Todd,[1] Philip J. Proteau, and William H. Gerwick*

College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

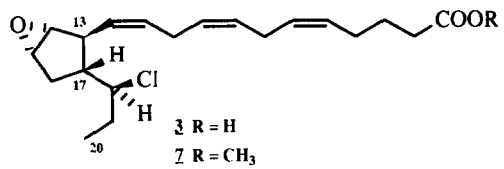
Abstract: Three new chlorinated and carbocyclic oxylipins as well as one olefin analog were isolated from the temperate brown alga *Egregia menziesii* along with the previously reported lactones, ecklonialactones A, B and E. The structures of the new compounds were determined by spectroscopic and mass spectrometric techniques. A biogenesis of the egregiachlorides is proposed which is initiated by lipoxygenation followed by cyclization and termination by chloride trapping of an ω3 carbocation.

Marine organisms are rich in their diversity of carbocyclic oxylipins.[2] Not only are a variety of positions involved in the carbocyclization of polyunsaturated fatty acids, but the types of chemical reactions leading to these products are also diverse. For example, prostaglandin biosynthesis in corals is believed to be initiated by a 8-lipoxygenase to produce 8-HPETE which is transformed to cyclopentanoids via allene oxide intermediates.[3] By contrast, the red alga *Constantinea simplex*,[4] forms cyclopropane-containing oxylipins via a proposed 12-lipoxygenase introduced hydroperoxide which rearranges to a reactive epoxy-cation intermediate. In some brown and red algae, such as *Ecklonia stolonifera*[5] and *Laurencia hybrida*,[6] similar epoxy-cation intermediates are proposed which lead to cyclopentane containing oxylipins. Recently we have found the Oregon kelp, *Egregia menziesii*, is a source of the previously reported carbocyclic oxylipins ecklonialactones A, B and E,[5,7] and we have clarified several stereochemical elements in these molecules with our isolates.[8] Here, we report on the isolation and structure elucidation of a series of co-occurring oxylipins from this same brown alga which are intriguing for their incorporation of a chlorine atom and the resultant biosynthetic implications.

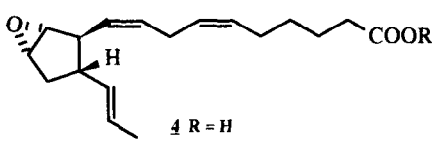
Egregia menziesii (Turn.) Aresc. was collected at Seal Rock State Beach, Oregon and repetitively extracted for its lipid constituents (RT, CH_2Cl_2/MeOH, 2:1, 1 kg dry weight, 37 gm oil). Fractionation of one half of the crude extract using Si gel (EtOAc/hex gradient) in the vacuum mode led to a fraction (955 mg) enriched in the egregiachlorides as well as an olefin analog. Metabolites **1-4** were converted to methyl esters (**5-8**, CH_2N_2) and purified to homogeneity using centrifugal tlc and normal and reverse phase hplc.[9] The planar structures of these derivatized egregia acids (**5**, 4.0 mg; **6**, 7.8 mg; **7**, 1.4 mg; **8**, 0.6 mg) were deduced from spectroscopic data (tables 1,2)[10] and by spectral comparisons with the co-metabolites, ecklonialactones A (**9**), B and E. Initially, nmr analysis suggested these metabolites to simply represent opened lactone analogs of the ecklonialactones as they showed highly comparable spectroscopic features to these lactones but were reactive to CH_2N_2 to form methyl ester derivatives. However, methyl egregiachloride A (**5**) analyzed for $C_{19}H_{29}ClO_3$ by HR CIMS[11] and showed ester carbonyl (ν_{max} = 1739 cm^{-1}) but no hydroxyl stretches by IR.[10] The sequence of methylenes, olefins and deshielded methines in derivative **5** was



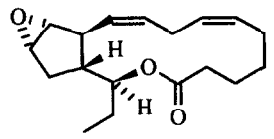
1 R = H, $\Delta^{6,9}$
2 R = H, Δ^{9}
5 R = CH_3, $\Delta^{6,9}$
6 R = CH_3, Δ^{9}



3 R = H
7 R = CH_3



4 R = H
8 R = CH_3



9

determined by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR experiments and found to be highly analogous to ecklonialactone A (**9**). The principle points of difference between ecklonialactone A and egregiachloride A were: 1) egregiachloride A was a free acid, 2) derivative **5** possessed one fewer degree of unsaturation as well as the incorporation of one chlorine atom, and 3) the ^{13}C nmr shift for C16 in **5** was 8.0 ppm higher field than in ecklonialactone A (**9**).[5] The ^{1}H-^{1}H COSY data in combination with ^{1}H-^{13}C HETCOR data were sufficient to unequivocally place a chlorine atom at C-16. This was further substantiated by MS fragmentations in **6** which resulted from cleavage of C15-C16 with consequent loss of Cl (*m/z* 265, 20%).[12] Methyl egregiachlorides B (**6**) and C (**7**) possessed very similar nmr data sets to methyl egregiachloride A (**5**), but exhibited molecular formulae of $C_{19}H_{31}ClO_3$ and $C_{21}H_{31}ClO_3$, respectively.[11] In methyl egregiachloride B (**6**), the point of difference from methyl egregiachloride A was readily apparent from analysis of the ^{1}H-^{1}H COSY (table 1), defining this metabolite to be the C6-7 dihydro homolog of egregiachloride A. Positions of unsaturation and location of chlorine and cyclopentyl and epoxide rings in methyl egregiachloride C (**7**), which derived from a C20 fatty acid, were also readily apparent from ^{1}H-^{1}H COSY and highly analogous to that in derivative **5** (table 1). The structure of the olefin-containing derivative **8** was deduced by a similar comparison of spectroscopic features with compounds **5-7** and **9** and observation in its ^{13}C NMR spectrum of two additional olefinic signals (δ138.3, 123.3) as well as the absence of the δ70.6 signal. Irradiation of H-18 collapsed H-17 to a doublet with a large coupling constant (J = 14.8 Hz), demonstrating a *trans* geometry for the C16-C17 olefin.

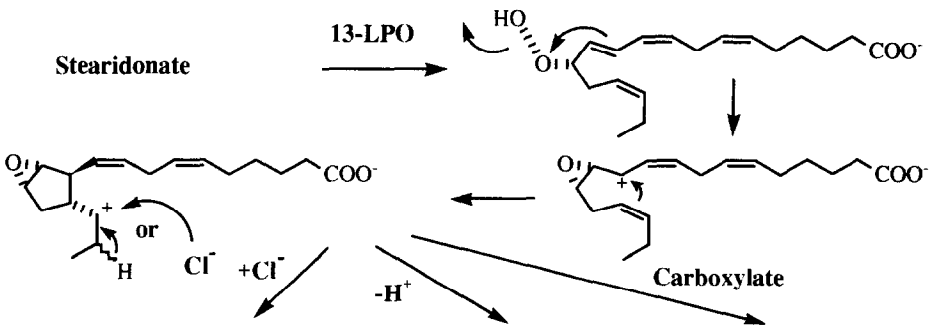
Except for the C16-C17 olefin in derivative **8**, all of the olefinic bonds in derivatives **5-8** were determined to be *cis* by the combined analysis of coupling constants and ^{13}C nmr chemical shifts of adjacent methylene groups (tables 1,2).[13] Detailed features of the relative stereochemistry in this series of metabolites was explored with methyl egregiachloride B (**6**). NOe enhancements of H-11 and H-10 upon irradiation of H-16 and H-12, respectively, demonstrated the *trans* nature of the alkyl substituents on the cyclopentyl ring and α-orientation of the epoxide. A NOESY experiment confirmed the orientation of side chains by showing a correlation between H-10 and H-15. Stereochemistry at C-16 was deduced as *S*[*] by observing nOe between H-17 (δ1.64) and H-14 (δ1.55) in the well dispersed bz-d_6 ^{1}H NMR spectrum ($\theta_{H15\text{-}H16}$ ~ 180°, determined by irradiation of H-16 and observation of H-15 changing from *bdd*, J=9.2, 10.7 Hz to a *bd*, J=9.2 Hz). The same relative stereochemistry at comparable stereocenters in derivatives **5**, **7**, and **8** was shown by

essentially identical ^{13}C nmr shifts at all asymmetric carbons (except of course at C-16 in **8**). Furthermore, derivatives **5-8** are likely of the same enantiomeric series as they all show highly comparable negative optical rotations.[10] It is probable, given their co-occurrence in *E. menziesii* with the ecklonialactones and their likely biogenesis, that they are of the same absolute stereochemistry as the ecklonialactones at comparable centers.[8]

Table 1. ^1H NMR data ($CDCl_3$) for methyl egregiachlorides A-C (**5-7**) and olefin analog **8** (Bruker AC-300 and AM-400 spectrometers; data presented as δ, multiplicity, J in Hz).

Position	Compound 5	Compound 6	Compound 7	Compound 8
(H2)			(2.33 t 7.5)	
(H3)			(1.67-1.76 m)	
H2(H4)	2.32 t 7.4	2.30 t 7.4	(2.12 m)	2.32 t 7.5
H3(H5)	1.62-1.70 m	1.62 bt 7.0	(5.35-5.48 m)	1.66 m
H4(H6)	1.37-1.45 m	1.28-1.44 m	(5.35-5.48 m)	1.40 m
H5(H7)	2.09-2.14 m	1.28-1.44 m	(2.84 bdd 5.5,5.7)	2.09 m
H6(H8)	5.36-5.47 m	1.28-1.44 m	(5.35-5.48 m)	5.34-5.49 m
H7(H9)	5.36-5.47 m	1.28-1.44 m	(5.35-5.48 m)	5.34-5.49 m
H8(H10)	2.96 bdd 6.0,7.0	2.19 m	(2.99 m)	2.84 bdd 7.1,6.4
H9(H11)	5.36-5.47 m	5.47 ddd 10.6,7.4,7.4	(5.35-5.48 m)	5.34-5.49 m
H10(H12)	5.14 dddd 10.7,10.4,1.5,1.4	5.11 bdd 10.4,10.3	(5.16 bdd 10.8,10.4)	5.18 bdd 10.4,10.3
H11(H13)	3.50 bd 10.5	3.47 bd 10.2	(3.51 bd 9.6)	2.95 bd 10.2
H12(H14)	3.26 d 2.6	3.25 d 2.6	(3.27 d 2.6)	3.25 d 2.6
H13(H15)	3.50 bd 10.5	3.51 d 2.0	(3.51 bd 9.6)	3.52 bs
H14(H16)	1.87-2.04 m	1.98 m	(1.94-2.05 m)	2.03 m
				1.83 dd 14.6,1.3
H15(H17)	1.87-2.04 m	1.98 m	(1.94-2.05 m)	2.38 bd 8.7
H16(H18)	3.88 ddd 10.7,8.0,2.3	3.87 ddd 10.4,8.1,2.5	(3.88 ddd 10.6,8.1,2.3)	5.34-5.49
H17(H19)	1.87-2.04 m	1.90 m	(1.87-1.94 m)	5.26 m
	1.51-1.61 m	1.54 m	(1.49-1.62 m)	
H18(H20)	1.01 t 7.1	1.01 t 7.2	1.01 t 7.3	1.61 dd 6.3,1.4
$COO\underline{CH_3}$	3.67 s	3.67 s	(3.67 s)	3.67 s

It is intriguing to speculate on the biogenesis of these chlorine containing prostaglandin-like molecules (**1-3**) as well as the olefin analog (scheme 1). The co-isolation of C18 and C20 hydroxy acids oxidized at the ω6 position suggest initiation by a lipoxygenase showing ω6 specificity. We envision subsequent formation of a pivotal epoxy cation intermediate with subsequent transformation to a cyclopentyl cation. Trapping of this cation could occur either with chloride addition or loss of a proton from C-17.



Scheme 1. Proposed Biogenesis of egregiachloride A (**1**).

Table 2. ^{13}C NMR data for egregiachlorides A-C (5-7) and olefin analog 8.[a]

Carbon #	Compound 5	Compound 6	Compound 7	Compound 8[b]
(C1)			(174.1)	
(C2)			(33.5)	
C1(C3)	174.1	174.3	(24.8)	—
C2(C4)	34.0	34.1	(26.6)	34.0
C3(C5)	24.6	24.9	(129.0)	24.6
C4(C6)	29.1	29.1	(130.1[c])	29.1
C5(C7)	26.9	29.1[c]	(26.1)	26.9
C6(C8)	130.3[c]	29.1[c]	(128.8[c])	130.0[c]
C7(C9)	130.1[c]	29.5[c]	(128.7[c])	129.7[c]
C8(C10)	26.1	27.6	(25.7)	26.2
C9(C11)	127.7[c]	132.3	(127.7[c])	128.5[c]
C10(C12)	127.9	127.6	(128.0)	127.7[c]
C11(C13)	42.2	42.1	(42.3)	45.3
C12(C14)	61.1	61.2	(61.1)	61.3
C13(C15)	58.0	58.0	(58.0)	58.4
C14(C16)	29.8	29.8	(29.8)	33.4
C15(C17)	50.8	50.8	(50.8)	46.6
C16(C18)	70.6	70.6	(70.6)	138.3
C17(C19)	28.8	28.8	(28.8)	123.3
C18(C20)	10.2	10.2	(10.2)	17.6
COOCH3	51.4	51.4	(51.4)	51.5

a) All spectra were recorded in $CDCl_3$ (shifts in ppm) on Bruker AC-300 and AM-400 spectrometers with assignments based on ^1H-^1H-COSY and ^1H-^{13}C HETCOR (for **5**) experiments and comparison to model compounds (references 5, 7, 8); b) carbon values from a DEPT 135 experiment; c) assignments within a column interchangable.

Acknowledgements: We thank Brian Arbogast, Don Griffin and Elizabeth Barofsky of the OSU College of Agricultural Chemistry for the CI and EI mass spectrometry. P.J.P. thanks the NSF for a predoctoral fellowship. This work was supported by the Oregon Sea Grant Program (R/SH-5).

References and Notes

1. On sabbatical leave from Whitman College, Walla Walla, WA 99362.
2. Gerwick, W.H. *Chem. Rev.* **1993**, *93*, 1807-1823.
3. Song, W.-C. and Brash, A.R. *Arch. Biochem. Biophys.* **1991**, *290*, 427-435.
4. Nagle, D.G. and Gerwick, W.H. *Tetrahedron Lett.* **1990**, *31*, 2995-2998.
5. Kurata, K., Shiraishi, K., Takato, T., Taniguchi, K. and Suzuki, M. *Chem. Lett.* **1989**, 267-270.
6. Corey, E.J., De., B., Ponder, J.W., and Berg, J.M. *Tetrahedron Lett.* **1984**, *25*, 1015-1018.
7. Kurata, K., Taniguchi, K., Shiraishi, K., and Suzuki, M. *Phytochemistry* **1993**, *33*, 155-159.
8. Todd, J.S., Proteau, P.J. and Gerwick, W.H. *J. Nat. Prod.* (in press).
9. Centrifugal tlc (30% EtOAc/hex); NP hplc (Phenomenex Maxsil 10Si, 10μ, 500 x 10 mm, 15% EtOAc/hex); RP hplc (Hibar Li Chrosorb RP-18, 7μ, 250 x 10 mm, 80% MeOH/H_2O).
10. FTIR (ν_{max}, cm^{-1}, film): for **5**: 3008, 2946, 2862, 1739,1436,1199,1172,843; for **6**: 3003, 2931, 2855, 1739, 1462, 1437, 1247, 1197, 1172, 843; for **7**: 3012, 2933, 2854, 1738, 1436, 1199, 1170, 843; for **8**: 3010, 2928, 2856, 1739, 1438, 1376, 1199, 1175, 1084, 974, 842. Optical rotations $[\alpha]_D^{27}$ for **5**: -12° (c = 0.44); for **6**: -12° (c = 0.87); for **7**: -13° (c = 0.14); for **8**: -10° (c = 0.09).
11. For **5**: HR CIMS (CH_4) *m/z* $[M+1]^+$ = 341.1883, $C_{19}H_{30}ClO_3$ (0.0 mamu dev.); for **6**: HR CIMS (CH_4) *m/z* $[M+1]^+$ = 343.2040, $C_{19}H_{32}ClO_3$ (0.0 mamu dev.); for **7**: HR EIMS (70 eV) *m/z* $[M]^+$ = 366.1961, $C_{21}H_{31}ClO_3$ (0.0 mamu dev.); for **8**: HR EIMS (70 eV) *m/z* $[M]^+$ = 304.2038 $C_{19}H_{28}O_3$ (0.0 mamu dev.).
12. For unknown reasons, cleavage of C15-C16 in **5** is less favorable (*m/z* 263 = 2%) than in **6**.
13. Rakoff, H. and Emken, E.A. *J. Am. Oil Chem. Soc.* **1983**, *60*, 546-552.

(Received in USA 23 July 1993; accepted 23 September 1993)