Novel Oxylipin Metabolites from the Brown Alga *Eisenia bicyclis*

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Nine novel oxylipin metabolites together with several known ones were isolated from the brown alga *Eisenia bicyclis*. Five (**1**−**5**) of them are ecklonialactone derivatives containing a chlorine or an iodine atom, and two (**6** and **7**) are cymathere type oxylipins with a lactone ring or a chlorine atom. The structures of these oxylipin metabolites were confirmed by NMR and mass spectroscopy and compared with spectral data in the literature. The postulated biosynthetic pathway of these metabolites is discussed.

Polyunsaturated fatty acids (PUFAs) from marine algae have played important roles in the pharmaceutical and biochemical fields because of their biological properties. These PUFAs are metabolized by a variety of oxidative enzymes to form many oxylipins containing cyclopropyl and cyclopentyl rings.[1−3] The prostaglandin derivatives formed by the lipoxygenase pathway from C_{20} fatty acids are especially well known in the field of medicine.[4,5] In addition, a few halogenated marine prostanoids with antitumor or antiproliferative activity have been reported.[6−8]

Other researchers have investigated the brown alga *Eisenia bicyclis* (Kjellman) Setchell, often visible around the coast of Japan, for the presence of bioactive compounds as polysaccharides, pyropheophytin, tripeptides, and phlorotannins.[9−12] In our investigation of bioactive compounds from this alga, we have isolated nine novel oxylipins, as well as several known ones, including cyclic oxylipins (ecklonialactones) as described below. In this study we elucidate the structures and discuss the plausible pathways for formation of these oxylipins.

Results and Discussion

Air-dried algae were extracted in MeOH for 1 day. The MeOH extract was partitioned between $CHCl_3$ and H_2O. The organic layer showed inhibition of *Bacillus subtilis* and *Staphylococcus aureus* and was subjected to ODS flash chromatography using a stepwise gradient of CH_3CN/H_2O. A bioassay-guided fraction of the CH_3CN/H_2O extracts using these bacteria resulted in the isolation of many oxylipin metabolites. Thus, the 80% (v/v) CH_3CN fraction was subjected to repeated ODS−HPLC with 60% CH_3CN giving nine novel oxylipins (**1**−**9**) together with known oxylipins, including a diastereomer of a hydroxy epoxide,[13,14] a keto acid,[15] four ecklonialactones (A, C, D, and F),[16−18] and five hydroxy fatty acids.[19] Six (**1**−**5** and **7**) of the novel oxylipins contain a chlorine or an iodine atom; the only chlorinated C_{18} oxylipins hitherto known from algae are egregiachlorides A−C. From this alga we also obtained egregiachloride A, which was identified by comparison with NMR spectra in the literature.[20]

The FABMS spectrum of **1** showed characteristic peaks for $[M + H]^+$ and $[M + H + 2]^+$, which indicate a chlorine atom. The molecular formula, $C_{18}H_{27}{}^{35}ClO_3$, was determined by the HRFABMS spectrum ($[M + H]^+$ *m/z* 327.1726, Δ −0.1 mmu). The ^1H and ^{13}C NMR spectra of **1** were very similar to the corresponding spectra of ecklonialactone C, except that the C-13 field shift in **1** was 14 ppm higher than that of ecklonialactone C (Table 1).[17] The $^1H-^1H$ COSY and HMQC data revealed replacement of a hydroxy group by a chlorine atom at C-13. Similarly, the spectra of **2**, which also contains a chlorine atom, resembled those of ecklonialactone D, indicating that the chlorine atom is attached to the C-13 position, as for **1**.

The stereochemistry of **1** and **2** was determined by their coupling constants and by NOE measurements. The coupling constants of the pentane rings in **1** and **2** corresponded with those of ecklonialactone C and D (see Experimental Section). Irradiation at δ 3.83 (H-12) in **1** increased the peak intensity at δ 5.35 (H-10) and 2.32 (H-15). Also, irradiation at δ 2.97 (H-11) in **1** increased the peak intensity at δ 4.04 (H-13). NOE measurements of **2** were the same as those of **1**; hence, the configurations of C-12 and C-13 in **1** and **2** are *R** and *R**, respectively. The double bonds in **1** and **2** are both *cis* configurations because the coupling constants are about 11 Hz. We have named **1** eiseniachloride A and **2** eiseniachloride B.

Compound **3** gave the molecular formula $C_{20}H_{30}{}^{35}ClO_3$ ($[M + H]^+$ *m/z* 353.1898, Δ +1.4 mmu) by HRFABMS, which indicated the addition of an ethylene group versus **1**. Also, various NMR spectra of **3** revealed replacement of a hydroxy group at C-15 of ecklonialactone F by a chlorine atom analogous to **1** and **2**. Thus, we have named **3** eiseniachloride C.

The molecular formulas of **4**, $C_{18}H_{27}IO_3$ (eiseniaiodide A $[M + H^+]$, *m/z* 419.1105, Δ +2.2 mmu), and **5**, $C_{18}H_{29}IO_3$ (eiseniaiodide B $[M + H]^+$ *m/z* 421.1219, Δ −2.0 mmu, $[M + H - H_2O]^+$ *m/z* 403.1122, Δ −1.3 mmu), were determined by HRFABMS. The presence of an iodine atom was indicated by a MS fragment ion at *m/z* 273 $[M - I]^+$. Also, the negative FABMS of **5** showed a strong peak at *m/z* 127, resulting from dissociation of an iodine atom, and showed a peak at *m/z* 547 formed from the addition of an iodine atom $[M + I]^-$. The ^1H and ^{13}C NMR spectra of **4** and **5** were very similar to those corresponding to **1** and **2** with the exception of δ_C 28.7 and 28.3 in **4** and **5** instead of δ_C 62.7 and 62.5 as in **1** and **2**. The ^{13}C spectrum of position 13 was far upfield due to the effect of the iodine atom, suggesting that **4** and **5** were the iodide analogues of **1** and **2** (Table 1).

The stereochemistry of **4** and **5** was also determined by coupling constants and NOE measurements. It was demonstrated that **4** and **5** have the same configurations as **1** and **2**, respectively.

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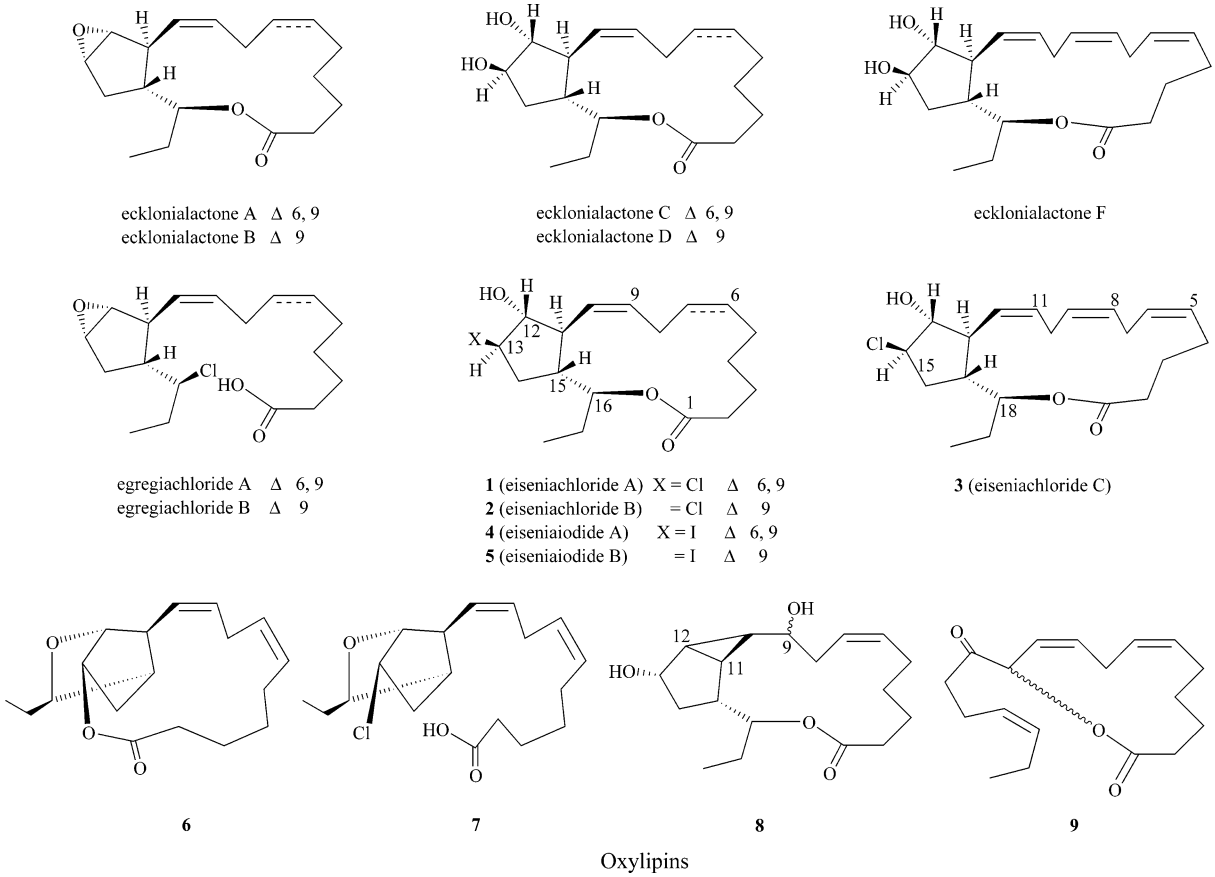
Chart 1



Oxylipins

Table 1. NMR Data for Compounds **1**, **2**, **4**, and **5** in $CDCl_3$

	1			**2**			**4**			**5**		
#	δ_C	δ_H[a]		δ_C	δ_H[a]		δ_C	δ_H[a]		δ_C	δ_H[a]	
1	173.8			173.7			173.8			173.6		
2	32.8	2.50	m	33.3	2.53	m	32.8	2.48	m	33.3	2.49	m
		2.35	m		2.35	m		2.30	m		2.31	m
3	24.5	1.80	m	24.2	1.70	m	24.5	1.75	m	24.2	1.70	m
		1.25	m					1.25	m			
4	27.7	1.75	m	26.2	1.35	m	27.7	1.72	m	26.0	1.35	m
		1.58	m					1.59	m			
5	25.6	2.45	m	25.0	1.60	m	25.6	2.40	m	25.0	1.56	m
		1.80	m		1.20	m		1.78	m		1.20	m
6	126.8	5.40	m	26.9	1.60	m	126.8	5.41	m	26.9	1.56	m
					1.35	m					1.35	m
7	130.0	5.45	m	26.5	1.45	m	130.0	5.43	m	26.5	1.42	m
8	26.7	3.41	m	26.2	2.46	m	26.7	3.42	m	26.2	2.49	m
		2.48	m		1.88	m		2.48	m		1.88	m
9	130.9	5.58	dt	133.2	5.60	dt	131.1	5.59	dt	133.5	5.61	dt
10	130.2	5.35	dd	130.9	5.37	dd	130.4	5.35	dd	130.7	5.37	dd
11	45.4	2.97	ddd	45.0	2.96	ddd	45.1	2.90	ddd	44.8	2.89	ddd
12	85.5	3.83	dd	85.6	3.82	dd	87.0	3.98	dd	87.0	3.97	dd
13	62.7	4.04	ddd	62.5	4.04	ddd	28.7	4.03	ddd	28.3	4.03	ddd
14	36.5	2.13	ddd	36.6	2.12	ddd	38.7	2.30	ddd	38.9	2.28	ddd
		2.05	ddd		2.04	ddd		2.18	ddd		2.19	ddd
15	41.8	2.32	m	41.4	2.34	m	43.3	2.30	m	42.9	2.30	m
16	77.4	4.73	dt	77.4	4.70	dt	77.2	4.72	dt	77.5	4.69	dt
17	25.6	1.58	m	25.5	1.70	m	25.6	1.59	m	25.5	1.70	m
18	9.9	0.82	t	10.0	0.87	t	9.9	0.83	t	10.0	0.89	t

[a] The coupling constants are described in the Experimental Section.

The molecular formula, $C_{18}H_{26}O_3$, of **6** was determined by HREIMS ([M + H]$^+$ *m/z* 291.1932, Δ −2.8 mmu). The ^{1}H and ^{13}C NMR spectra showed characteristic signals at δ 4.36 (δ_C 78.6, C-12), 4.69 (74.5, C-13), and 3.76 (82.1, C-16), which were all attached to carbons bearing oxygen. As two of the three oxygens were accounted for by an ester linkage based on δ_C 174.3 (C-1), the third was bonded at C-12 (δ_C 78.6) and C-16 (82.1) in an ether linkage. The ^{1}H−^{1}H COSY spectrum revealed three spin systems as CH_2 (δ 3.08, 2.58)−CH (5.42)−CH (5.42)−CH (3.02)−, CH_2 (2.18, 1.83)−CH (4.69)−, and CH_3 (δ 0.89)−CH_2 (1.60, 1.36)−CH (3.76)−CH (2.35)−. HMBC correlations between the methine signal at δ 4.36 (H-12) and carbon signals δ_C 82.1 (C-16), 42.5 (C-15), and 27.9 (C-14) were observed. Also,

Table 2. NMR Data for Compounds **6** and **7** in $CDCl_3$

	6					**7**				
#	δ_C	δ_H		*J* (Hz)	HMBC (C)	δ_C	δ_H		*J* (Hz)	HMBC (C)
1	174.3					177.6				
2	34.4	2.35	m			34.0	2.37	t	7.3	
		2.11	ddd	14.7, 11.7, 2.7	1, 3, 4					
3	24.8	1.83	m			24.9	1.69	quint	7.5	1, 2, 4, 5
		1.60	m							
4	29.7	1.46	m			29.4	1.44	quint	7.5	2, 3, 5, 6
		1.25	m							
5	27.6	1.92	m		4, 6, 7	27.4	2.11	m		4, 6, 7
6	133.9	5.42	m			130.2	5.40	ddd	10.4, 10.2, 6.3	5, 8
7	126.4	5.55	m		5, 8, 9	128.3	5.39	ddd	10.4, 10.2, 6.3	5, 8
8	27.4	3.08	m			26.5	2.89	m		6
		2.58	m							
9	132.6	5.42	m			131.5	5.50	m		
10	124.3	5.42	m			124.8	5.77	ddt	10.9, 9.0, 1.7	
11	47.8	3.02	m		10, 13	48.5	3.16	dd	9.0, 1.1	14
12	78.6	4.36	br s		14, 15, 16	83.7	4.13	br s		13, 14, 15, 16
13	74.5	4.69	br dd	7.7, 3.9	11	56.9	3.92	br t	6.4	
14	27.9	2.18	ddd	14.0, 7.7, 1.1	11,12, 15, 16	33.4	2.47	dd	14.2, 7.8	11, 12, 15, 16
		1.83	m				2.02	dt	14.2, 4.4	15
15	42.5	2.35	m			45.3	2.38	m		
16	82.1	3.76	ddd	7.0, 6.8, 1.5	14	82.6	3.86	dt	7.5, 1.5	14
17	25.1	1.60	m			25.2	1.58	ddq	14.1, 7.5, 7.3	15, 16, 18
		1.36	dq	14.1, 7.3			1.33	ddq	14.1, 7.5, 7.3	16, 18
18	10.7	0.89	t	7.3	16, 17	10.7	0.89	t	7.3	16, 17

correlations between signals at δ 3.76 (H-16) and δ_C 27.9 (C-14) and between those at δ 4.69 (H-13) and δ_C 47.8 (C-11) were detected. These results indicate that **6** is not an ecklonialactone derivative but rather a tricyclic compound which is the cyclization product from the C-18 derivative of cymathere ether B.[21]

The molecular formula of **7** was determined to be $C_{18}H_{27}{}^{35}ClO_3$ ([M − H]$^-$ *m*/*z* 325.1581, Δ +1.1 mmu), containing a chlorine atom by HRFABMS. The ^1H and ^{13}C NMR spectra of **7** were similar to those of **6** with the exception of signals at δ 3.92 and δ_C 56.9 (C-13) in **7** instead of δ 4.69 and δ_C 74.5 in **6** (Table 2). Detailed observations of the NMR spectra of **7** revealed replacement of the ester bond in **6** by a chloride ion at C-13.

The stereochemistry of **6** and **7** was considered as follows. The H-13 in **6** exhibits broad double doublets having J = 7.7 and 3.9 Hz. Also, the H-13 in **7** indicates an apparent broad triplet having J = 6.4 Hz. The H-12 in **6** and **7** appeared as a broad singlet because $J_{12,13}$ < 1 Hz. These coupling constants might be explained as the relationship between H-13$_{endo}$−H-14$_{endo}$ and H-13$_{endo}$−H-14$_{exo}$.[22] Thus, it can be seen that H-13 in both **6** and **7** was in the *endo* position as in cymathere ethers. Irradiation at δ 3.76 (H-16) in **6** increased the peak intensity at δ 3.02 (H-11) and 2.35 (H-15). Also, irradiation at δ 4.69 (H-13) in **6** increased the peak intensity at δ 4.36 (H-12) and 2.18 (H-14$_{endo}$). Similarly, irradiation at δ 3.16 (H-11) in **7** increased the peak intensity at δ 3.86 (H-16) and 2.38 (H-15), and with irradiation at δ 5.77 (H-10) increased peak intensity was observed at δ 2.02 (H-14$_{exo}$). Also, irradiation at δ 3.92 (H-13) in **7** increased the peak intensity at δ 4.13 (H-12) and 2.47 (H-14$_{endo}$) as shown Figure 1 (Table 2). The stereochemistry of C-13 in **6** and **7** was the same, so **7** was not formed by the attack of a chloride ion on **6**. The geometry of at least one double bond in **7** was the *cis* configuration because the coupling constant of $J_{9,10}$ was 10.9 Hz. The geometries in **6** were ambiguous, however, because of overlap, but they are thought to have *cis* configurations from the formation mechanism described later.

Compound **8** provided a molecular formula of $C_{18}H_{28}O_4$, and ^1H NMR showed an extraordinarily shielded signal at δ 0.79 for a methine proton. Other characteristic signals

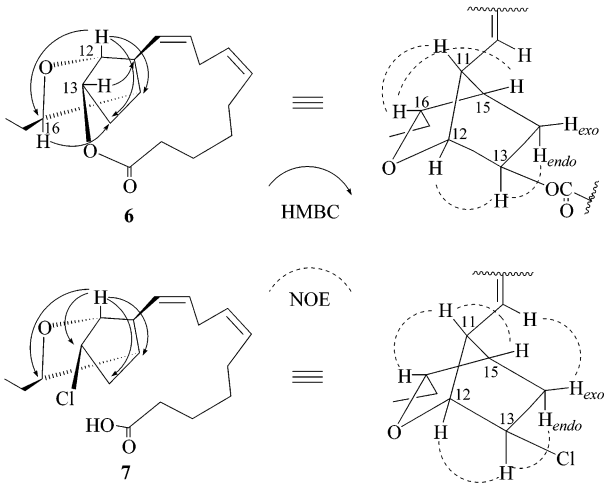


Figure 1. HMBC and NOE correlations of **6** and **7**.

were three methine protons neighboring a cyclic ester and two hydroxy groups at δ 4.85 (δ_C 78.2), 4.22 (74.8), and 4.18 (68.3). The COSY spectrum indicated two spin systems: −CH_2 (δ 2.58, 2.33)−CH_2 (1.80, 1.72)−CH_2 (1.60)−CH_2 (2.08, 1.95)−CH (5.55)−CH (5.32)−CH_2 (2.15)−CH (4.18)−CH (0.79)−CH (1.53)− and CH_3 (δ 0.86)−CH_2 (1.73, 1.48)−CH (4.85)−CH (2.05)−CH_2 (1.64, 1.50)−CH (4.22)−. HMBC correlations between the methine signal at δ 0.79 (H-10) and carbon signals δ_C 74.8 (C-13), 43.8 (C-15), 32.2 (C-8), and 24.4 (C-11) were observed. By HMBC correlation, in combination with COSY spectra, **8** was shown to be a lactone compound in which a three-membered ring was combined with a five-membered ring.

The absolute stereochemistry of C-9 and C-13 was not determined, but the relative stereochemistry was considered as follows. As H-13 at δ 4.22 showed a doublet with $J_{13,14a}$ = 5.0 Hz, $J_{12,13}$ and $J_{13,14b}$ were proven to be near zero. Thus, the dihedral angles of H-12−H-13 and H-13−H-14$_b$ were about 90°. The signal of H-10 showed double triplets, and the coupling constants of $J_{10,11}$ and $J_{10,12}$ were 3.6 Hz, showing that the relationships of H-10−H-11 and H-10−H-12 in the three-membered ring were *trans* geom-

Table 3. NMR Data for Compounds **8** and **9** in $CDCl_3$

	8					**9**				
#	δ_C	δ_H		*J* (Hz)	HMBC (C)	δ_C	δ_H		*J* (Hz)	HMBC (C)
1	174.3					174.2				
2	34.5	2.58	ddd	14.4, 7.8, 3.7	3, 4, 16	35.2	2.59	ddd	13.3, 9.1, 4.0	
		2.33	dd	14.4, 10.1, 4.1			2.34	m		
3	24.0	1.80	m			23.3	1.74	m		2, 5
		1.72	ddd	14.7, 7.8, 3.7	2					
4	27.4	1.60	m			29.2	1.54	m		2, 3, 5, 6
5	26.1	2.08	m			25.7	2.13	m		4, 6, 7
		1.95	ddd	13.5, 8.2, 8.2						
6	130.3	5.55	m		5, 8	131.2	5.33	dddt	10.7, 9.5, 5.4, 1.2	
7	126.6	5.32	dt	10.5, 6.8	5, 8, 9	126.3	5.44	m		
8	32.2	2.15	m		6, 7, 9, 10	28.0	2.97	dt	13.7, 9.5	
							2.78	m		7, 9
9	68.3	4.18	ddd	8.2, 4.8, 4.7	7, 12	139.0	5.93	ddt	10.6, 6.8, 1.0	
10	26.1	0.79	dt	4.7, 3.6	8, 11, 13, 15	120.5	5.57	ddt	10.6, 8.1, 1.4	
11	24.4	1.53	dd	6.0, 3.6	10, 13	74.2	5.82	d	8.1	1, 9, 10, 12
12	27.2	1.60	m		9	205.2				
13	74.8	4.22	d	5.0	10, 11, 14, 15	39.4	2.52	m		12, 14, 15
14a	37.1	1.64	dd	7.8, 5.0	13	21.6	2.34	m		
b		1.50	m		13					
15	43.8	2.05	m			127.2	5.26	dtt	10.7, 7.3, 1.6	
16	78.2	4.85	ddd	9.9, 8.5, 3.2	1, 14, 15, 18	133.7	5.40	dtt	10.7, 7.3, 1.4	
17	26.6	1.73	ddq	14.2, 7.3, 3.2		21.0	2.08	m		15, 18
		1.48	m		16, 18					
18	9.6	0.86	t	7.3	16, 17	14.7	0.95	t	7.5	

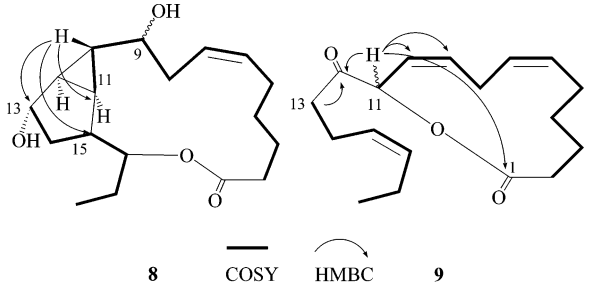


Figure 2. COSY and HMBC correlations of **8** and **9**.

Table 4. Relative Inhibition against Two Bacterial Strains

compound	*Bacillus subtilis*	*Staphylococcus aureus*
1 (eiseniachloride A)	0.36	0.38
2 (eiseniachloride B)	0.31	0.53
3 (eiseniachloride C)	0.29	0.34
5 (eiseniaiodide B)	0.28	0.47
6	none	none
7	0.43	0.40
9	none	none
egregiachloride A	0.35	0.47
ecklonialactone A	0.30	0.41
vancomycin	1.00 (26.8)[a]	1.00 (34.6)[a]

[a] The inhibition diameter (mm).

etry. Irradiation at δ 4.22 (H-13) increased the peak intensity at 0.79 (H-10). Therefore, the stereochemistry of C-13 was *S** configuration, but that of C-9 could not be clarified.

Compound **9** displayed a molecular mass of $C_{18}H_{27}O_3$ ([M + H]$^+$, *m*/*z* 291.1922, Δ −3.8 mmu) by HRFABMS spectra. The ^{1}H and ^{13}C NMR spectra of **9** were analogous to those of PUFAs except for a methine signal at δ 5.82 (d) and a carbonyl signal at δ_C 205.2 (s), respectively. From detailed COSY and HMBC correlations, it was shown to be a lactone compound having a carbonyl group. Thus, connecting COSY cross-peaks led to two spin systems: −CH_2 (δ 2.59, 2.34)−CH_2 (1.74)−CH_2 (1.54)−CH_2 (2.13)−CH (5.33)−CH (5.44)−CH_2 (2.97, 2.78)−CH (5.93)−CH (5.57)−CH (5.82)− and CH_3 (δ 0.95)−CH_2 (2.08)−CH (5.40)−CH (5.26)−CH_2 (2.34)−CH_2 (2.52)−. HMBC correlations between the methine signal at δ 5.82 (H-11) and carbon signals at δ_C 205.2 (C-12), 174.2 (C-1), 139.0 (C-9), and 120.5 (C-10) and between the methylene signal at δ 2.52 (H-13) and the carbon signal at δ_C 205.2 (C-12) confirmed the connectivity of the partial fragments as shown in Figure 2. The geometries of the three double bonds in **9** were shown to be *cis* configurations by their coupling constants being about 10.5 Hz (Table 3).

The biogenesis of the C_{18} oxylipins **1**−**9** (except **3**) might be postulated as follows. Oxidation of 6,9,12,15-octadecatetraenoic acid by lipoxygenase (13-LO) forms a 13-hydroperoxy compound (13-HPOTE), and subsequently, the 15-double bond is added to the 11-position, expelling the hydroxy group to form a cyclopentyloxirane (**X**; 11*S**, 15*R**). Intermediate **X** leads to ecklonialactone A by carboxylate attack to C-16 cation (path A, Figure 3) or to egregiachloride A with a chloride ion (path B). Subsequently ecklonialactone A is cleaved by a chloride or an iodide ion to give **1** and **4** (path a). Similarly, **2** and **5** are formed via ecklonialactone B from octadecatrienoic acid.

In contrast, the carboxylate in **X** attacks the C-13 epoxide from the β face, leading formally to alkoxide at C-12 in the α configuration (path C). This C-12 alkoxide could quench the cation at C-16, forming **6**. Compound **7** could be formed similarly, except a chloride ion would attack the C-13 epoxide in **X** (path D). Thus, both 13 positions in **6** and **7** are of the same configuration.[23]

Compound **8** could be formed by the attack of a hydroxy ion on C-9 in ecklonialactone A (path b).

It seems that formation of **9**, having a carbonyl group in C-12, occurs by lactonization and subsequent isomerization from 13-HPOTE.

Unfortunately as shown in Table 4, these halogenated oxylipins have less inhibition against *Bacillus subtilis* and *Staphylococcus aureus* than vancomycin, which is a widely used antibacterial compound.

The brown alga *Eisenia bicyclis* has a multitude of oxylipin metabolites, and an investigation of other bioactivities of these compounds and of other oxylipins from this alga is now in progress.

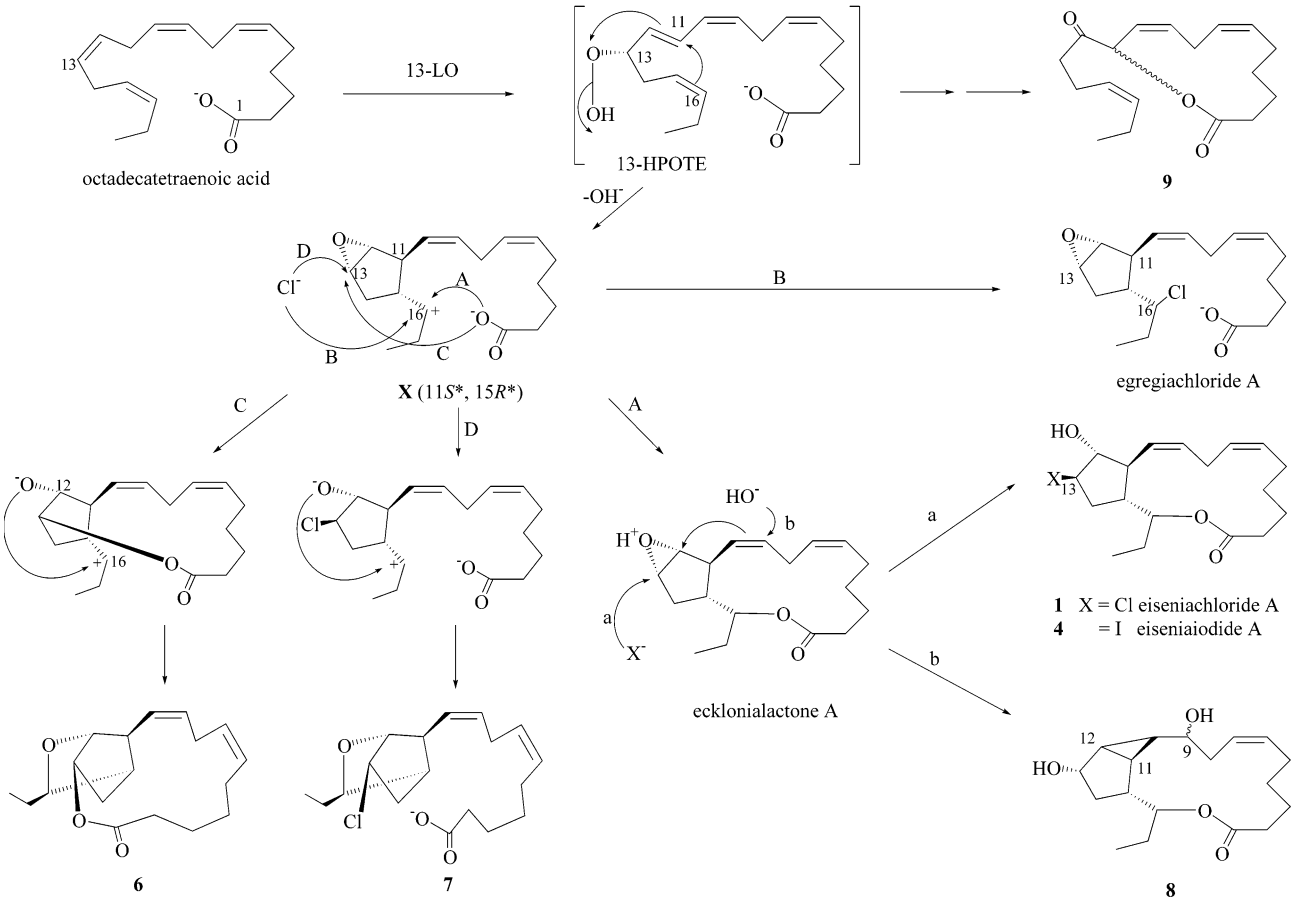


Figure 3. Postulated pathway of isolated oxylipin metabolites.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer 341 digital polarimeter. NMR spectra were measured in $CDCl_3$ on a JEOL ECP-500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Mass spectra were obtained with a JEOL SX-102 mass spectrometer. HPLC was performed on a Shimadzu LC-10 equipped with a RI or a UV detector using a GL Science column (Inertsil prep-SIL, Inertsil prep-ODS, 10 × 250 mm or 20 × 250 mm). The solvents were distilled prior to use.

Plant Material. *Eisenia bicyclis* (Kjellman) Setchell was collected in April 1999 and 2000 at Johgashima Island in Kanagawa prefecture. The alga is often seen on the coast of Japan and was identified by a fishermen's cooperative association of Johgashima. A voucher specimen has been deposited at the Department of Chemistry, Aoyama Gakuin University.

Extraction and Separation. An air-dried sample (300 g) was soaked in MeOH (2 L) for 1 day; after solvent removal, the residue (20 g) was obtained. The extract was partitioned between $CHCl_3$ and H_2O (each 400 mL). The $CHCl_3$ extract (4.0 g) was subjected to ODS flash chromatography (Wakosil 25C18, 9.0 × 7.0 cm) using a stepwise gradient of H_2O/CH_3CN (fraction 1; 100:0), (fraction 2; 60:40), (fraction 3; 40:60), then (fraction 4; 20:80). Fraction 3 (54 mg) was subjected to repeated ODS−HPLC using H_2O/CH_3CN (2:3) to afford five known hydroxy acids. Fraction 4 (45 mg) was also subjected to repeated ODS−HPLC using H_2O/CH_3OH (3:7) or H_2O/CH_3CN (2:3), affording new oxylipins **1** (4.3 mg, 1.4 × 10^{-3} %), **2** (1.2 mg, 0.4 × 10^{-3} %), **3** (1.1 mg, 0.4 × 10^{-3} %), **4** (0.4 mg, 0.1 × 10^{-3} %), **5** (1.6 mg, 0.5 × 10^{-3} %), **6** (0.4 mg, 0.1 × 10^{-3} %), **7** (0.6 mg, 0.2 × 10^{-3} %), **8** (0.9 mg, 0.3 × 10^{-3} %), and **9** (2.0 mg, 0.7 × 10^{-3} %) together with known ecklonialactones A, C, D, and F.

Eiseniachloride A (1): colorless oil $[\alpha]^{20}_D$ −149° (*c* 0.070, $CHCl_3$); IR (KBr disk) ν_{max} 3450, 1732, 1713, 1456, 1227, 1150, 737, 711 cm^{-1}; ^1H and ^{13}C NMR are shown in Table 1. $J_{H-9,H-10}$ = 10.5, $J_{10,11}$ = 9.6, $J_{11,12}$ = 9.1, $J_{12,13}$ = 6.4, $J_{13,14a}$ = 8.7, $J_{13,14b}$ = 6.4, $J_{14a,14b}$ = 14.2, $J_{14,15}$ = 5.4, $J_{15,16}$ = 4.1 Hz, and $J_{6,7}$ could not be determined because of overlap; HRFABMS (glycerol matrix) *m/z* 327.1726 $[M + H]^+$ (calcd for $C_{18}H_{28}{}^{35}ClO_3$, 327.1727).

Eiseniachloride B (2): colorless oil $[\alpha]^{20}_D$ −126° (*c* 0.065, $CHCl_3$); IR (KBr disk) ν_{max} 3447, 1732, 1718, 1456, 1241, 1146, 737 cm^{-1}; ^1H and ^{13}C NMR spectra are shown in Table 1. $J_{H-9,H-10}$ = 10.6, $J_{10,11}$ = 9.6, $J_{11,12}$ = 9.4, $J_{12,13}$ = 6.4, $J_{13,14a}$ = 8.7, $J_{13,14b}$ = 6.0, $J_{14a,14b}$ =14.2, $J_{14,15}$ = 8.7, $J_{15,16}$ = 3.2 Hz; LREIMS (relative int %) *m/z* 310 [100, (M: $C_{18}H_{29}{}^{35}ClO_3$ − $H_2O)^+$]; 275 [90.4, (M − H_2O − Cl) $^+$]; HRFABMS (glycerol matrix) *m/z* 329.1898 $[M + H]^+$ (calcd for $C_{18}H_{28}{}^{35}ClO_3$, 329.1886).

Eiseniachloride C (3): colorless oil $[\alpha]^{20}_D$ −128° (*c* 0.075, $CHCl_3$,); IR (KBr disk) ν_{max} 3420, 1732, 1715, 1456, 1214, 1158, 725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.58, 5.53 (m, 2H, H-11, H-12), 5.41 (m, 2H, H-5, H-6), 5.21 (m, 2H, H-8, H-9), 4.84 (dt, 1H, *J* = 7.3, 4.1 Hz, H-18), 3.98 (dt, 1H, *J* = 8.1, 8.1 Hz, H-15), 3.78 (dd, 1H, *J* = 8.1, 7.3 Hz, H-14), 3.14, 2.73 (m, 2H, H-10), 2.92 (m, 1H, H-13), 2.90, 2.84 (m, 2H, H-7), 2.39 (m, 2H, H-2), 2.30 (m, 1H, H-17), 2.19 (dt, 1H, *J* = 7.3, 7.3 Hz, H-4), 2.06 (dd, 2H, *J* = 8.3, 8.1 Hz, H-16), 1.79, 1.71 (m, 2H, H-3), 1.62, 1.54 (dq, 2H, *J* = 7.3, 7.3 Hz, H-19), 0.85 (t, 3H, *J* = 7.3 Hz, H-20); ^{13}C NMR δ_C 173.5 (C-1), 131.7 (C-12), 130.5 (C-11), 129.2 (C-6), 128.8 (C-5), 128.1 (2C, C-8, -9), 85.5 (C-14), 78.0 (C-18), 62.8 (C-15), 45.1 (C-13), 42.4 (C-17), 36.1 (C-16), 33.6 (C-2), 26.9 (C-4), 26.3 (C-10), 26.0 (C-7), 25.7 (C-19), 24.8 (C-3), 10.0 (C-20); HRFABMS (m-NBA matrix) *m/z* 353.1898 $[M + H]^+$ (calcd for $C_{20}H_{30}{}^{35}ClO_3$, 353.1833).

Eiseniaiodide A (4): colorless oil; chemical shifts of ^1H and ^{13}C NMR are shown in Table 1. $J_{H-9,H-10}$ = 10.8, $J_{10,11}$ = 10.2, $J_{11,12}$ = 9.1, $J_{12,13}$ = 8.1, $J_{13,14a}$ = 7.5, $J_{15,16}$ = 3.4 Hz; LREIMS *m/z* 273 $[M - I]^+$, 261 $[M - I - H_2O]$; HRFABMS (glycerol matrix) *m/z* 419.1105 $[M + H]^+$ (calcd for $C_{18}H_{28}IO_3$,

419.1083). The yield of **4** was a trace amount, so optical rotation and infrared could not be measured.

Eiseniaiodide B (5): colorless oil $[\alpha]^{20}_D$ −193° (*c* 0.078, $CHCl_3$); IR (KBr disk) ν_{max} 3442, 1732, 1458, 1236, 737 cm^{-1}; ^1H and ^{13}C NMR spectra are shown in Table 1. $J_{H-9,H-10}$ = 10.5, $J_{10,11}$ = 9.6, $J_{11,12}$ = 9.2, $J_{12,13}$ = 8.2, $J_{13,14}$ = 7.8, $J_{14,15}$ = 4.4, $J_{15,16}$ = 3.2 Hz; HRFABMS (glycerol matrix) *m*/*z* 421.1219 $[M + H]^+$ (calcd for $C_{18}H_{30}IO_3$, 421.1239), *m*/*z* 403.1122 $[M + H - H_2O]^+$ (calcd for $C_{18}H_{28}IO_2$ 403.1135).

Compound 6: colorless oil $[\alpha]^{20}_D$ +74° (*c*, 0.062, $CHCl_3$); ^1H and ^{13}C NMR, see Table 2; HRFABMS (glycerol matrix) *m*/*z* 291.1932 $[M + H]^+$ (calcd for $C_{18}H_{27}O_3$, 291.1950).

Compound 7: colorless oil $[\alpha]^{20}_D$ −12° (*c* 0.025, $CHCl_3$); ^1H and ^{13}C NMR, see Table 2; HRFABMS (glycerol matrix) *m*/*z* 325.1581 $[M - H]^-$ (calcd for $C_{18}H_{26}{}^{35}ClO_3$, 325.1570), *m*/*z* 327.1540 $[M - H + 2]^-$ (calcd for $C_{18}H_{26}{}^{37}ClO_3$, 327.1541).

Compound 8: colorless oil $[\alpha]^{20}_D$ +12° (*c* 0.078, $CHCl_3$); IR (KBr disk) ν_{max} 3450, 1732, 1716, 1558, 1541, 1508, 1456, 1263, 1150, 1059, 1002, 941, 712 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; LREIMS (relative int %) *m*/*z* 308 [59, (M^+: $C_{18}H_{28}O_4$)] 290 [6, $(M - H_2O)^+$].

Compound 9: colorless oil $[\alpha]^{20}_D$ +11° (*c* 0.060, $CHCl_3$); ^1H and ^{13}C NMR, see Table 3; HRFABMS (glycerol matrix) *m*/*z* 291.1922 $[M + H]^+$ (calcd for $C_{18}H_{27}O_3$, 291.1960).

Bioassay. *Bacillus subtilis* and *Staphylococcus aureus* were purchased from the Japan collection of microorganisms at the Institute of Physical and Chemical Research (RIKEN). Sample (0.1 mg) was added to a paper disk and incubated in the bouillon culture medium at 37 °C. After 12 h, the diameter of the inhibition zone was measured. Table 4 shows the relative activities based on vancomycin as a standard sample (0.1 mg).

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