Unprecedented Oxylipins from the Marine Green Alga Acrosiphonia coalita

Matthew W. Bernart, George G. Whatley, and William H. Gerwick

J. Nat. Prod., **1993**, 56 (2), 245-259• DOI:
10.1021/np50092a010 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 2, pp. 245-259, February 1993

UNPRECEDENTED OXYLIPINS FROM THE MARINE GREEN ALGA *ACROSIPHONIA COALITA*

MATTHEW W. BERNART,[1] GEORGE G. WHATLEY,[2] and WILLIAM H. GERWICK*

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331-3507

ABSTRACT.—The Oregon marine chlorophyte *Acrosiphonia coalita* produces an assortment of oxidized polyunsaturated fatty acids, or oxylipins. The smallest of these was a 10-carbon conjugated trienal **1** with antimicrobial properties. Related to **1** were three novel branched-chain conjugated trienals **4**, **5**, and **9** in which the aldehyde was present as a branch on a 17-carbon fatty acid chain. Additionally, two novel conjugated unbranched trienone octadecanoids **8** and **10** were also isolated and characterized. Finally, a family of related epoxy-alcohols (**11**, **12**, **15**, and **17**) was obtained from *A. coalita*. Structures were determined by spectroscopic methods in combination with formation of various degradation products and derivatives. The absolute stereochemistry of several of these metabolites was determined by application of exciton chirality circular dichroic spectroscopy on benzoate derivatives.

Acrosiphonia coalita (Rupr.) Scagel, Garbary, Holden, et Hawkes (Acrosiphoniaceae) (1,2), previously *Spongomorpha coalita* (3), grows commonly in the low- to mid-intertidal region of Oregon. In an ongoing survey of the biomedicinal potential of marine algae from the west coast of the United States, the lipid extract of *A. coalita* was identified as containing metabolites which inhibit the growth of several microorganisms (*Bacillus subtilis, Staphylococcus aureus,* and *Candida albicans*). Large-scale extraction and chromatography led to the isolation of a novel fatty acid derived substance which was responsible for this activity. Furthermore, a number of biogenetically related substances of unique structure were also isolated which demonstrate this alga's capacity for lipoxygenase and subsequent hydroperoxide metabolism.

Oxylipins are becoming a well recognized class of natural products from red algae (4) as well as from other types of seaweeds (5). [Oxylipin was proposed (38) as an encompassing term for polyunsaturated fatty acid metabolites formed by reaction(s) involving one or more steps of mono- or di-oxygenase catalyzed oxygenation, thus including the eicosanoids as well as metabolites of different chain length.] Although the importance of lipoxygenase-derived fatty acid metabolites, such as the leukotrienes (LTs), to human health and disease is well-recognized (6,7), this report is the first to find this structure class in a marine green alga. However, the terrestrial acidophilic green alga *Dunaliella acidophila* was recently described to contain, following methylation of the acidic lipid algal extract, methyl (12*R*)-hydroxy-(9*Z*,13*E*,15*Z*)-octadecatrienoate, methyl (9*S*)-hydroxy-(10*E*,12*Z*,15*Z*)-octadecatrienoate, and methyl ricinoleate [methyl (12*R*)-hydroxy-(9*Z*)-octadecenoate] (8). *Chlorella pyrenoidosa*, another freshwater green alga, is a source of 9- and 13-lipoxygenase (9), as well as hydroperoxide lyase activity (10). The work described herein shows that *A. coalita*, a marine macrophytic green alga, produces oxylipins deriving from similar initial biosynthetic pathways as found in these other green algae but surpasses both in the diversity of their metabolic fates.

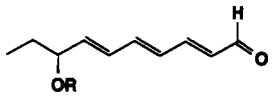
RESULTS AND DISCUSSION

Fresh-frozen *A. coalita* from the central Oregon Coast was lipid-extracted and sub-

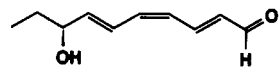
[1]Present address: National Cancer Institute, Laboratory of Drug Discovery, Research and Development, Bldg. 560-15, Rm. 3260, Frederick, MD 21702-1201.

[2]Undergraduate researcher 1985–1986.

jected to vacuum chromatography (vc) (11,12). Fractions eluting in 25–30% EtOAc in cyclohexane were purified by a second vc that yielded two uv-active but CH_2N_2-unreactive compounds **1** and **3**, which could be separated by hplc.



1 R=H
2 R=COAr-*p*-OMe



3

The more polar of these, termed coalital [**1**], was optically active, contained an alcohol functionality by ir, and exhibited a uv spectrum suggestive of a conjugated trienone (λ max 312 nm, $\epsilon = 43{,}000$). Coalital possessed a molecular formula of $C_{10}H_{14}O_2$ by hrms (4° of unsaturation). The ^{13}C-nmr spectrum (Table 1) of **1** contained one carbonyl and six olefinic resonances, accounting for all of the degrees of unsaturation and indicating that **1** was an acyclic molecule.

The ^{1}H-nmr spectrum (Table 1) was well dispersed and interpretable by ^{1}H-^{1}H COSY analysis. This analysis showed that **1** possessed an aldehyde functionality in conjugation with an all trans triene. This spin system extended to a deshielded methine at δ 4.18 which was bordered on the other side by an ethyl grouping. By HETCOR, all of the ^{13}C and ^{1}H resonances in **1** were assigned. The C-8 methine carbon resonated at δ 73.38, indicating it possessed a secondary alcohol.

The absolute stereochemistry of the C-8 secondary alcohol in coalital was determined for the 4-methoxybenzoate derivative **2** by application of the exciton chirality method (13,14). Using the preferred solution conformation of derivative **2** in which H-8 eclipses the C-6–C-7 bond (Figure 1a), as indicated by $J_{7\text{-}8} = 6.4$ Hz ($\theta_{7\text{-}8} \approx 180°$), cd ($\Delta\epsilon_{284}$ max + 6.7, $\Delta\epsilon_{253}$ max − 6.6) indicated a clockwise relationship between the two chromophores. This clockwise relationship assigned the C-8 stereochemistry in derivative **2** as *S*, thus yielding the total structure of coalital [**1**] as (8*S*)-hydroxy-(2*E*,4*E*,6*E*)-decatrienal.

TABLE 1. Nmr Data for Two Isomeric Trienals from *Acrosiphonia coalita*.[a]

Position	*EEE* Trienal **1**[b]				*EZE* Trienal **3**[c]			
	δ ^{13}C	δ ^{1}H	m	*J* (Hz)	δ ^{13}C	δ ^{1}H	m	*J* (Hz)
1	193.68	9.56	d	7.9	193.74	9.63	d	8.0
2	131.35	6.17	dd	15.3, 7.9	132.00	6.17	dd	15.2, 8.0
3	151.89	7.14	dd	15.3, 11	146.07	7.61	dd	15.2, 11.5
4	129.82	6.44	dd	14.5, 11	126.43	6.23	dd	11.5, 11.5
5	142.01	6.67	dd	14.5, 11	138.34	6.44	dd	11.5, 11.5
6	129.06	6.36	dd	15, 11	124.16	6.85	dd	14.8, 11.5
7	142.59	6.02	dd	15, 6.2	142.55	6.01	dd	14.8, 6.1
8	73.38	4.18	dt	6.7, 6.2	73.42	4.23	dt	6.7, 6.1
9	30.04	1.61	dq	7.3, 6.7	30.14	1.63	dq	7.4, 6.7
10	9.60	0.95	t	7.3	9.61	0.97	t	7.4

[a]All spectra were obtained in $CDCl_3$. Chemical shifts are expressed in ppm relative to TMS internal standard.

[b]Spectra were obtained at 9.398 T. Assignments based on COSY and HETCOR experiments.

[c]Spectra were obtained at 7.047 T. Assignments based on COSY and HETCOR experiments.

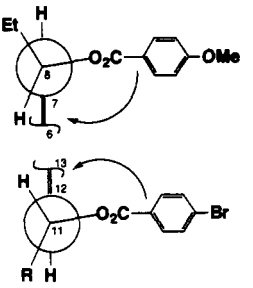


FIGURE 1. Newman projections of predicted favored rotamers of benzoate derivatives of coalital [**1**] and epoxyalcohol **15** used in cd analysis for absolute stereochemistry determination: (a) coalital benzoate derivative **2**; (b) *p*-bromobenzoate derivative **16**.

A second natural product **3** of *A. coalita* was isolated from fractions containing coalital [**1**] and showed optical rotation, uv, ir, and ^{1}H-nmr characteristics quite similar to those obtained for coalital (Table 1). By ^{1}H-^{1}H COSY, the connectivities of trienals **1** and **3** were identical. In **3**, however, a cis olefin was indicated by $J_{4\text{-}5} = 11.5$ Hz. Additionally, comparison of ^{13}C-nmr shifts for the C-3 through C-6 olefinic carbon atoms in **1** and **3**, assigned by HETCOR, substantiated that **3** contained a *Z* C-4–C-5 olefin (15).

Because the *EZE* double bond configuration is the pattern predicted to result from two separate lipoxygenase reactions acting at either end of a homoconjugated triene (16,17) (Scheme 1), we suspected that isomer **3** may represent the true natural product while isomer **1** may be an isolation artifact. We noticed that following procedures which exposed isomer **3** to strong light, formation of the thermodynamically more stable *EEE* isomer **1** appeared to occur. To prove this, isomer **3** was purified under low light conditions using ri detection on hplc and then rapidly analyzed by ^{1}H nmr. This sample was then irradiated in the nmr tube for 1 h with 254 nm light, after which a second ^{1}H-nmr spectrum was recorded. The characteristic signals for both isomers **1** and **3** were present following irradiation. By integration, approximately 75% of isomer **3** had been converted to isomer **1** during the uv treatment. Exposure to high light levels, either in the wild before collection or in the laboratory during workup, may enhance conversion of natural product **3** to the artifactual isomer **1**.

More polar fractions were investigated in an attempt to locate longer-chain potential precursors of compounds **1** and **3**. Vc fractions eluting with 40% EtOAc/cyclohexane were methylated (CH_2N_2) and subjected to additional vc followed by hplc to yield a uv-active oil **4**. Because the uv and ir [$\nu = 3500$ (-OH) and 1671, 1607 cm^{-1} (conjugated trienal)] spectra for compound **4** were similar to those of trienals **1** and **3**, we suspected that compound **4** also contained a conjugated trienone. In compound **4**, however, an additional absorption at $\nu = 1734$ cm^{-1} was attributed to a methyl-esterified carboxyl. By hrms, the observed $[M]^+$ at *m/z* 320.1987 gave a molecular formula of $C_{19}H_{28}O_4$ (6° of unsaturation) for a methyl esterified 18-carbon fatty acid. Analysis of the ^{13}C-nmr spectrum of **4**, which contained 19 carbon resonances, attributed these six degrees of unsaturation to four olefins, one ester carbonyl (δ 174.31), and one aldehyde (δ 193.91); hence, compound **4** was acyclic. The occurrence of an aldehyde functionality in **4** was confirmed by formation of methoxamine derivative **5**,

which showed $[M]^+$ *m/z* 421 and a major fragment ion at *m/z* 390 $[M-OMe]^+$ in eims. As the ^{1}H-nmr spectrum showed that compound **4** possessed three termini [an aldehyde (δ 9.43, s), a methyl ester (δ 3.67), and an aliphatic methyl group (δ 0.96, t)], this trienal was branched.

The ^{1}H-^{1}H COSY of compound **4** was interpreted as two separate spin systems. A C-2 through C-8 spin system of a Δ^6 cis (J_{6-7} = 10.6 Hz) fatty acid was evidenced through sequential correlations between these protons (Table 2). The second spin system began with the methyl triplet at δ 0.96, which was coupled to a methylene doublet of quartets at δ 1.64. This methylene was further coupled to a proton at δ 4.19 (dt), which by chemical shift in the ^{1}H and ^{13}C nmr was a methine proton of a carbon atom bearing a singly bound oxygen. In the ms of the TMSi-ether of Me-ester **4**, fragmentation to give *m/z* 131 $[TMSiOCHEt]^+$ provided additional evidence that compound **4** was hydroxylated at the ω-3 position. The carbinol proton was coupled to an olefinic methinyl doublet of doublets at δ 6.02, which showed 14.9 Hz trans coupling to a complex multiplet methine at δ 6.43. This latter signal coupled to a 2H multiplet at δ 6.66 which was in turn coupled to a one-proton multiplet at δ 6.85, marking the end of the second spin system. By HETCOR, the two overlapping olefinic protons at δ 6.66 were attached to two distinct carbon resonances (δ 141.15 and 127.05).

A quaternary olefin carbon at δ 140.55 must bridge these two spin systems, connecting the bis-allylic methylene with the δ 6.85 olefin. Further, in order to be quaternary, this carbon must be the site of branching in the molecule. By consideration of these partial structures and the molecular formula, the branch must be the aldehyde.

To prove this relationship of atoms in **4**, a series of NOEDS experiments was performed. The aldehyde was shown as being in conjugation with the triene as well as being a substituent of the lone quaternary olefin, as enhancement occurred only in the olefin signal at δ 6.85 following irradiation of the aldehyde proton. Conversely, when the multiplet at δ 6.85 was irradiated, only the aldehyde singlet was enhanced. These results were interpreted as evidence that these two substituents were cis oriented on the C-9–C-10 trisubstituted olefin. Furthermore, irradiation of the bis-allylic methylene at δ 3.11 enhanced the multiplet at δ 6.66, showing that the C-8 methylene and the C-11–C-12 olefin were also cis oriented substituents of the C-9–C-10 *E* olefin. The C-11–C-12 olefin geometry was assigned by comparison of nmr shifts of branched-chain trienal **4** with that of *EEE* trienal **1** and *EZE* trienal **3** (Tables 1 and 2).

The optical rotations of compound **4** were of extremely low magnitude compared to trienals **1** and **3**, suggesting that compound **4** was racemic. Derivatization of methoxamine **5** to the (−)-menthoxycarbonyl derivative (18) at the ω-3 alcohol followed by ozonolysis and methylation gave fragment **6**. Gc and gc-ms analysis of fragment **6** versus standards revealed that the secondary alcohol was racemic, defining **4** as methyl 9-formyl-15(*R,S*)-hydroxy-(6*Z*,9*E*,11*E*,13*E*)-heptadecatetraenoate. Considering that trienals **1** and **3** and the trienones described below are all optically active, the racemic nature of compound **4** may reflect the ease of epimerization of this ω-3 position, or that **4** is the non-stereospecific reduction product of metabolite **9** (see below). Alternatively, it is conceivable that metabolite **4** may be formed non-enzymatically from an unstable intermediate which has not yet been isolated.

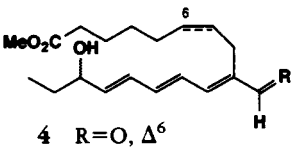
In oxylipin biosynthesis, ω-3 oxygenation is relatively uncommon. Powell and Gravelle (19) reported that aortal enzyme preparations convert EPA to 18-hydroxyeicosapentaenoate. More recently, Oliw (20) reported 18-*R* hydroxylation of arachidonate by monkey seminal vesicles. It has also been reported that LTB_4 may be hydroxylated at C-18 during non-lipoxygenase metabolism by neutrophils (21). A Korean group determined that when α-linolenate was incubated with soybean lipoxygenase, four isomers of 9,16-dihydroperoxy-10,12,14-octadecatrienoic acids could be isolated

TABLE 2. Nmr Data for Three Novel Octadecanoid Trienals Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **4**, **7**, and **9**.[a]

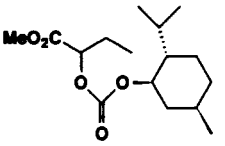
Position	Branched-chain trienal **4**[b]				6,7-Dihydro trienal **7**[c]			Keto-trienal **9**[d]		
	δ ^{13}C	δ ^{1}H	m	*J* (Hz)	δ ^{III}	m	*J* (Hz)	δ ^{1}H	m	*J* (Hz)
1	174.31	—	—	—	—	—	—	—	—	—
2	34.02	2.34	t	7.6	2.30	t	7.3	2.35	t	7.4
3	24.67	1.67	m	—	1.63	tt	7.3, 7.0	1.68	tt	7.6, 7.4
4	29.01	1.43	tt	7.8, 7.4	1.30	m	—	1.44	tt	7.6, 7.6
5	29.98	2.19	dt	7.4, 7.4	1.30	m	—	2.20	dt	7.6, 6.4
6	130.37	5.38	bdt	10.6, 7.4	1.30	m	—	5.42	bdt	10.5, 6.4
7	126.43	5.25	bdd	10.6, 7.2	1.30	m	—	5.23	bdd	10.5, 6.8
8	22.49	3.11	d	7.2	2.34	t	7.2	3.16	d	6.8
9	140.55	—	—	—	—	—	—	—	—	—
10	148.50	6.85	m	—	6.84	m	—	6.89	d	11.6
11	127.05	6.66	m	—	6.65	m	—	7.03	dd	14.5, 11.6
12	141.15	6.66	m	—	6.65	m	—	6.72	dd	14.5, 11.2
13	129.36	6.43	m	—	6.43	m	—	7.27	dd	15.6, 11.2
14	142.10	6.02	dd	14.9, 6.0	5.99	dd	15, 6	6.36	d	15.6
15	73.31	4.19	dt	6.4, 6.0	4.19	dt	7, 6	—	—	—
16	30.02	1.64	dq	7.5, 6.4	1.63	dq	7.5, 7	2.64	q	7.3
17	9.61	0.96	t	7.5	0.96	t	7.5	1.14	t	7.3
9′	193.91	9.43	s	—	9.42	s	—	9.49	s	—
1′	51.53	3.67	s	—	3.66	s	—	3.67	s	—

[a]Chemical shifts ($CDCl_3$) are expressed in ppm relative to TMS internal standard.
[b]Spectra obtained at 7.047 T. Assignments based on COSY and HETCOR experiments.
[c]Spectrum obtained at 7.047 T. Assignments based on comparison to compound **4**.
[d]Spectra obtained at 9.398 T. Assignments based on a COSY experiment and comparison to compound **4**.

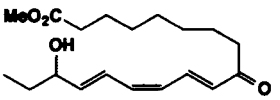
(22). Based on uv and ms data, these were the 9*S EZE* and *EEE* isomers of both diastereomers at C-16. Recently, a Russian group, using a potato lipoxygenase preparation and α-linolenate, was also able to isolate (9*S*),16-dihydroxy-(10*E*,12*Z*,14*E*)-octadecatrienoate (23). An unidentified compound with a uv λ max 309 nm, like those of trienals **1** and **3** and the *A. coalita* trienones discussed below, was also reported in this latter work. Branched, α,β-unsaturaterd aldehydes, such as compound **4**, are unprecedented among fatty acids.



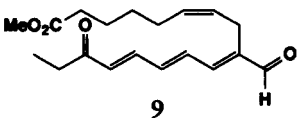
4 R=O, Δ^6
5 R=NOMe
7 R=O; 6,7 dihydro



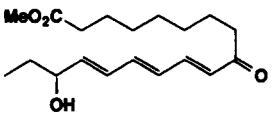
6



8



9



10

During the hplc of compound **4**, two less polar compounds were collected as oils (**7** and **8**). Uv and ^{1}H nmr (Table 2) showed that compound **7** was the 6,7-dihydro-homologue of compound **4**, which would result from the substitution of α-linolenate for stearidonate in the proposed biosynthetic manifold (Scheme 1). Proton assignments for compound **7** followed by analogy to trienal **4**, and defined compound **7** as methyl 9-formyl-15-hydroxy-(9*E*,11*E*,13*E*)-heptadecatrienoate. Stereochemistry at C-16 was precluded given the small amount of compound (0.4 mg).

While the uv spectrum of compound **8** resembled that of trienals **1** and **3** rather than that of branched-chain trienal **4**, its ^{1}H-nmr spectrum (Table 3) lacked any aldehyde resonance. The observed $[M+H]^+$ at *m/z* 395 of the TMSi-ether derivative (cims) corresponded to a molecular formula of $C_{19}H_{30}O_4$ for non-silylated compound **8**. Correlations in the ^{1}H-^{1}H COSY showed two separate spin systems, in similarity to compound **4**. Hydroxylation at the ω-3 position was shown by ^{1}H-^{1}H COSY to be adjacent to a conjugated triene. However, in compound **8** the central olefin showed a cis coupling ($J_{12\text{-}13}$ = 11.3 Hz) while the two flanking olefins were both trans ($J_{10\text{-}11}$ = 15.3, $J_{14\text{-}15}$ = 15.3 Hz) as observed in compound **3**. The other spin system in compound **8** consisted of five aliphatic methylenes as multiplets which were flanked at each end by methylenes appearing as triplets and at shifts compatible with their placement adjacent to carbonyls (δ 2.30, 2.57). One of these carbonyls was the carbomethoxy ester while the other was a ketone in conjugation with the triene. Fragmentation was observed in the lrcims between the C-9 ketone and the olefin at C-10 (*m/z* 185 $[MeO_2C(CH_2)_7CO]^+$) as has been observed in the mass spectrum of methyl 9-oxo-

TABLE 3. Nmr Data for Two Novel Octadecanoid Trienones Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **8** and **10**.[a]

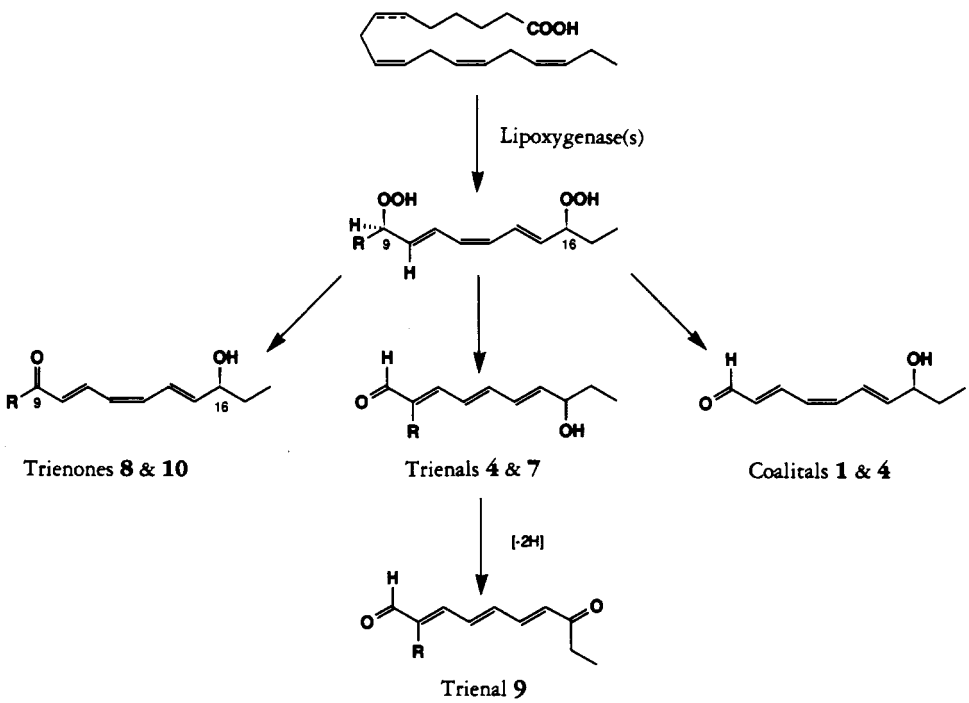
Position	*EZE* Trienone **8**[b]			*EEE* Trienone **10**[c]			
	δ ^{1}H	m	*J* (Hz)	δ ^{13}C	δ ^{1}H	m	*J* (Hz)
1	—	—	—	174.28	—	—	—
2	2.30	t	7.6	34.04	2.30	t	7.5
3	1.63	m	—	24.28[d]	1.62	m	—
4	1.32	m	—	29.07[e]	1.32	m	—
5	1.32	m	—	29.07[e]	1.32	m	—
6	1.32	m	—	28.94[e]	1.32	m	—
7	1.63	m	—	24.86[d]	1.62	m	—
8	2.57	t	7.4	40.72	2.54	t	7.5
9	—	—	—	200.67	—	—	—
10	6.18	d	15.3	129.44	6.17	d	15.4
11	7.64	dd	15.3, 11.3	142.01	7.18	dd	15.4, 10.8
12	6.10	dd	11.3, 11.3	130.54	6.31	dd	14.5, 10.8
13	6.36	dd	11.3, 11.3	140.61	6.60	dd	14.5, 10.6
14	6.84	dd	15, 11.3	129.50	6.36	dd	15.3, 10.6
15	5.92	dd	15, 6	140.74	5.93	dd	15.3, 6.4
16	4.21	dt	6, 5	73.58	4.16	dt	6.4, 5.1
17	1.63	m	—	30.08	1.62	m	—
18	0.96	t	7.5	9.60	0.95	t	7.5
1′	3.67	s	—	51.47	3.66	s	—

[a]Chemical shifts ($CDCl_3$, 7.047 T) are expressed in ppm relative to TMS internal standard.
[b]Assignments based on comparison to trienone **8** and a COSY experiment.
[c]Assignments based on COSY and HETCOR experiments.
[d,e]Assignments may be interchanged within a given letter.

10,12,15-octadecatrienoate (24). Consideration of the above spin systems and diagnostic ms fragmentations defined **8** as the unbranched compound methyl 16-hydroxy-9-oxo-(10*E*,12*Z*,14*E*)-octadecatrienoate.

Another chromatography fraction was also methylated (CH_2N_2) and rechromatographed using vc followed by hplc to give compound **9**. This optically inactive oil analyzed for $C_{19}H_{26}O_4$ ($[M]^+$ *m/z* 318.183) by hrms, while ir showed that **9** contained an ester and a conjugated trienone, but no hydroxyls. The uv spectrum was similar to that of the branched chain-trienals **4** and **7**, and eims displayed a significant fragment at *m/z* 261 $[M - COCH_2CH_3]^+$. The ^{1}H-nmr spectrum of compound **9** (Table 2) was similar to that for trienal **4** but lacked an α-hydroxyl resonance. The entire spectrum was well dispersed, first-order, and dissected by ^{1}H-^{1}H COSY into three distinct spin systems. The first of these was an ethyl group adjacent to a carbonyl, as suggested by the lreims. A second spin system was nearly identical to that of C-2 through C-8 in trienal **4**. The remaining proton signals were part of a conjugated triene in which both disubstituted olefins were of *E* geometry based on coupling constants. Given the close agreement of spectroscopic data between compounds **4** and **9**, the formyl group was placed as a substituent of C-9 trans to C-11, thus defining compound **9** as methyl 9-formyl-15-oxo-(6*Z*,9*E*,11*E*,13*E*)-heptadecatetraenoate.

The last conjugated triene from *A. coalita* was a more polar metabolite, also purified as its semi-synthetic methyl ester **10**. In the hrms, compound **10** showed an $[M]^+$ at *m/z* 322.214 for a molecular formula of $C_{19}H_{30}O_4$ (5° of unsaturation). The ^{13}C-nmr spectrum of **10** showed that the unsaturation resided in three olefins, an ester carbonyl,



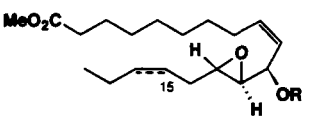
SCHEME 1. Proposed pathways of oxylipin metabolism in *Acrosiphonia coalita*.

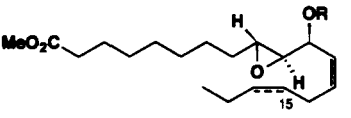
and a conjugated ketone (δ 200.67). The ir displayed an ester, a conjugated carbonyl, and a broad OH stretch. The uv of **10** resembled a conjugated trienal **1** or **3** or a trienone **8**. The ^{1}H-nmr spectrum of **10** was similar to that of trienone **8**, except that coupling constant analysis indicated an *EEE* rather than *EZE* triene. In the eims of **10** a diagnostic cleavage between C-9 and C-10 was observed (*m/z* 185), as seen for trienone **8**. ^{1}H-^{1}H COSY and HETCOR gave all of the proton and carbon assignments (Table 3) and provided additional support for a methyl 16-hydroxy-9-oxo-(10*E*,12*E*,14*E*)-octadecatrienoate structure for compound **10**. Optical rotations for compound **10** were of the same sign and magnitude as those of coalital [**1**], assigning an *S* configuration to C-16. Trienone **10** was thus defined as methyl (16*S*)-hydroxy-9-oxo-(10*E*,12*Z*,14*EZ*)-octadecatrienoate.

Based on our previous experience with isomerization of *EZE* trienal **3** to *EEE* trienal **1**, a similar experiment was conducted with *EZE* trienone **8**. ^{1}H-nmr spectra of pure **8** taken in $CDCl_3$ before and after a 1 h irradiation with 254 nm light showed major proton resonances of trienone **10** appeared (80%) while those of isomer **8** diminished (20%).

While it appears reasonable that the oxidation of C-9 derives from an initial lipoxygenase step utilizing molecular oxygen in the above *A. coalita* trienone metabolites, the origin of the C-16 oxygen is far less certain (Scheme 1). Reasonable pathways can be formulated that involve lipoxygenase oxidation at both C-9 and C-16, leading to metabolites **3** [hydroperoxide lyase reaction to cleave C-8–C-9 (10,25), reduction of C-16 hydroperoxide] and **8** (dehydration of C-9 hydroperoxide, reduction of C-16 hydroperoxide). Isomerizations of the labile cis olefins in **3** and **8** lead to metabolites **1** and **10**, respectively. The biogenesis of the branched chain trienals **4**, **7**, and **9** is a subject of speculation at this point, and may be formed via rearrangement of (a) an allene oxide (26–29), (b) an oxetane involving C-9 and C-11, or (c) an epoxy-triene analogous to LTA_4. Possibilities a and b predict that the C-15 hydroxyl would derive from molecular oxygen, while possibility c predicts it could derive non-stereospecifically from H_2O.

During purification of some of the above uv-active metabolites, several uv-inactive compounds were observed to char blue with aqueous acidic cupric acetate. ^{1}H-nmr analysis of these impure fractions displayed signals typical for epoxy-hydroxy fatty acids (30). Repeated hplc gave an inseparable mixture of two closely related epoxy-hydroxy fatty acids, **11** and **12**, in a 4:1 ratio by ^{1}H-nmr analysis. The ^{1}H and ^{13}C data for the major compound **11** closely matched those reported for methyl (12*S**,13*S**)-epoxy-(11*R**)-hydroxy-(9*Z*,15*Z*)-octadecadienoate (31). The position of the hydroxyl group and overall structure of **11** were verified by the close correspondence of ms obtained for the TMSi derivative of **11** and literature values for the TMSi derivative of methyl 12,13-epoxy-11-hydroxy-9-octadecenoate (32–34). Benzoylation of the 4:1 mixture of **11** and **12** yielded bromobenzoate derivatives **13** and **14** which were readily separable by hplc. Ester **13** was dextrorotatory ([α]D +41°) in agreement with the literature value (+26°) for methyl (12*S*,13*S*)-epoxy-(11*R*)-4-bromobenzoyloxy-(9*Z*,15*Z*)-octadecadienoate (31). Cd analysis of derivative **13** also gave values ($\Delta\epsilon_{245}$ max +7.7) in close agreement with literature values for the same derivative as above ($\Delta\epsilon_{244}$ max +5.9).





11 R=H, ω-3
12 R=H, ω-6
13 R=COAr-*p*-Br, ω-3
14 R=COAr-*p*-Br, ω-6

15 R=H, ω-6
16 R=COAr-*p*-Br, ω-6
17 R=H, ω-3

Eims of derivative **14** gave an $[M]^+$ cluster at *m/z* 508/510 (1:1) indicating a molecular formula of $C_{19}H_{34}O_4$ for the non-benzoylated methyl ester derivative (3° of unsaturation). Fragmentations at *m/z* 439 and 437 $[M-(CH_2)_4CH_3]^+$ were consistent with oxidation at the ω-6 position in compound **14**. The ^{1}H-nmr spectrum of derivative **14**, which was similar to that of derivative **13** except for having two fewer olefinic protons, showed a pair of trans-coupled epoxide protons (δ 3.01, 2.90) and two cis-coupled olefin protons (δ 5.71, 5.49). These data, in combination with ir data which showed the presence of an ester in **14**, accounted for all three degrees of unsaturation attributable to the fatty acid portion of the molecule. The 6.1 Hz coupling constant between the proton at δ 5.58 (H-11) and the epoxide proton at δ 3.01 (H-12) indicated a threo relationship between these substituents (34,35). The optical rotation, uv, and cd data for derivative **14** were also highly comparable to those of derivative **13**, thus defining compound **12** as the 15,16-dihydro homologue of compound **11**, or (12*S*,13*S*)-epoxy-(11*R*)-hydroxy-(9*Z*)-octadecenoate. This pair of linolenic (ω-3) and linoleic (ω-6) derived epoxy-alcohols have been previously found to co-occur in nature (31).

Another optically active methyl ester, **15**, was isolated from less polar chromatography fractions. Its ^{1}H- and ^{13}C-nmr spectra (Table 4) were again indicative of a monounsaturated fatty acid containing a vicinal epoxy-alcohol and were quite similar to those of compounds **11** and **12**. Placement of these functional groups in the carbon chain could not be deduced by nmr due to degeneracy in the aliphatic resonances. However, gc-ms of the TMSi-ether of compound **15** showed a key fragmentation at *m/z* 199 $[TMSiOCHCH=CH(CH_2)_4CH_3]^+$, localizing the hydroxyl group at C-11 (33,34). Therefore, the epoxide was positioned at C-9–C-10. Coupling constant analysis indicated that the epoxide was disposed threo to the allylic hydroxyl. Further, from cou-

TABLE 4. Nmr Data for Three Hydroxy-epoxy Octadecanoids Isolated from *Acrosiphonia coalita* as Methyl Ester Derivatives **11**, **15**, and **17**.[a]

Position	Compound **11**[b]				Compound **15**[c]				Compound **17**[d]		
	δ ^{13}C	δ ^{1}H	m	*J* (Hz)	δ ^{13}C	δ ^{1}H	m	*J* (Hz)	δ ^{1}H	m	*J* (Hz)
1	174.29	—	—	—	174.27	—	—	—	—	—	—
2	34.03	2.30	t	7.5	34.05	2.31	t	7.5	2.30	t	7.6
3	24.87	1.62	m	—	24.87	1.64	tt	7.5, 7.5	1.63	m	—
4	29.47[e]	1.30	m	—	29.26[f]	1.35	m	—	1.31	m	—
5	29.01[e]	1.30	m	—	29.11[e]	1.30	m	—	1.31	m	—
6	29.01[e]	1.30	m	—	29.11[e]	1.30	m	—	1.31	m	—
7	31.49[e]	1.30	m	—	29.00[e]	1.50	m	—	1.42	m	—
8	27.94	2.08	m	—	31.44	1.54	m	—	1.54	m	—
9	145.42	5.61	bdt	11.1,7.4	56.94	2.92	dt	6.7, 2.3	2.93	dt	5.6,2.3
10	127.49	5.50	bdd	11.1,9	61.41	2.78	dd	5.2,2.3	2.80	dd	5.1,2.3
11	67.68	4.29	ddd	9,5.1,4.9	67.89	4.29	ddd	9,5.2,4.9	4.33	ddd	8.6,5.1,4.9
12	60.91	2.83	dd	5.1,2.2	127.43	5.47	bdd	9,9	5.50	bdd	10.8,8.6
13	56.14	2.97	dt	5.4,2.2	134.55	5.61	bdt	9,7	5.61	bdt	10.8,7.4
14	29.18	2.33	bm	—	27.96	2.09	bdt	7,7	2.85	bdd	7.4,7.2
15	122.16	5.33	bdt	10.8,7.4	25.85	1.38	m	—	5.28	bdt	10.7,7.2
16	134.94	5.52	m	—	31.49	1.30	m	—	5.41	bdt	10.7,7.2
17	20.66	2.04	m	—	22.50	1.30	m	—	2.06	dq	7.4,7.2
18	14.17	0.97	t	7.5	14.02	0.89	t	6.7	0.98	t	7.4
1′	51.47	3.67	s	—	51.47	3.67	s	—	3.67	s	—
OH	—	1.92	d	4.9	—	1.85	d	4.9	1.88	d	4.9

[a]All spectra obtained at 7.047 T in $CDCl_3$ and are expressed in ppm relative to TMS internal standard.
[b]Assignments based on a HETCOR experiment and comparison to literature values (31).
[c]Assignments based on COSY and HETCOR experiments.
[d]Assignments based on a COSY experiment and comparison to compound 15.
[e]Assignments in the same column may be interchanged.

pling constant analysis ($J_{11\text{-}12} = 9.3$ Hz), *p*-bromobenzoate derivative **16** existed in the preferred eclipsed rotamer (Figure 1b). A negative first Cotton effect in its cd spectrum indicated negative chirality between the benzoate and C-12 olefin, yielding *S* stereochemistry at C-11. Therefore, the structure of compound **15** was deduced as methyl (9*R*,10*R*)-epoxy-(11*S*)-hydroxy-(12*Z*)-octadecenoate.

The most polar *A. coalita* methyl ester (**17**, $[M - Me]^+$ at *m/z* 381 for TMSi-ether derivative) in the epoxy-alcohol structure class was isolated by hplc. Its ^{1}H-nmr spectrum (Table 4) was again very similar to those of compounds **11**, **14**, and **15**. In this derivative, however, discrete ^{1}H resonances allowed deduction of the C-18 through C-9 spin system. The methyl group at δ 0.98 was coupled to an allylic methylene (δ 2.06) which was further coupled to a cis olefin ($J = 10.7$ Hz). The C-15 olefin proton was also coupled to a bis-allylic methylene (δ 2.85) which was in turn coupled to another cis olefin. The C-12 proton was additionally coupled to an α-hydroxyl methine (δ 4.33) which showed a threo coupling to an adjacent α-epoxy proton (δ 2.80). A trans coupling (2.3 Hz) was measured between the two epoxide protons. H-9 was coupled to overlapped aliphatic multiplets and could not be traced further. However, ms of the TMSi-ether of compound **17** showed a significant fragment at *m/z* 197 for the cleavage alpha to the -OTMSi position, between C-10 and C-11, in direct analogy to fragments seen for the TMSi-ether of **15**. Hence, compound **17** was the ω-3 analogue of epoxy-alcohol **15**. Further, as derivative **17** has the same relative stereochemistry as **15** and showed nearly identical optical rotations at two wavelengths, it must also possess the same absolute stereochemistry. Thus, compound **17** was defined as methyl (9*R*,10*R*)-epoxy-(11*S*)-hydroxy-(12*Z*,15*Z*)-octadecadienoate.

The biosynthesis of epoxy-hydroxy metabolites in *A. coalita* likely follows that deduced in other systems. Oxidation of linoleic or linolenic acids at either C-9 or C-13 by a lipoxygenase, as has been demonstrated with soybean lipoxygenase (36), is followed by an oxygen-rebound mechanism for formation of the epoxy-alcohols (33,37). As only threo isomers were recovered from *A. coalita*, it appears reasonable that the transformations of the hydroperoxide intermediates to the epoxy-hydroxy metabolites may be catalyzed by the lipoxygenase, as has been shown for soybean lipoxygenase 1 (34). Antimicrobial bioassays using the sensitivity disk method showed that coalital [**1**], the only compound tested, was effective at inhibiting the growth of the pathogenic yeast *Candida albicans* at doses as low as 100 μg/disk.

EXPERIMENTAL

COLLECTION, EXTRACTION, AND CHROMATOGRAPHY.—Thalli of *A. coalita* (682 g dry wt) were collected intertidally in July 1988 at Boiler Bay, Oregon, and immediately frozen in dry ice. A voucher specimen has been deposited at the Department of Botany and Plant Pathology Herbarium at Oregon State University. The collection was defrosted overnight in distilled H_2O at 4°, after which the H_2O was decanted and the alga extracted 2× with $CHCl_3$-MeOH (2:1), yielding 6.89 g of extract. This extract was subjected to vc using EtOAc in cyclohexane. Fractions which eluted in 25–30% EtOAc/cyclohexane were further purified via a second vc using the same solvents. Fractions eluting from this column in 15% EtOAc/cyclohexane were purified on hplc (300 nm detection, Alltech Rsil 10 μm, 1 × 50 cm, 5% iPrOH/hexanes). The major uv-active compound (13.4 mg, 0.19% yield) was collected and characterized.

(8S)-Hydroxy-(2E,4E,6E)-decatrienal [**1**].—Unstable oil: ir ν max (film) cm^{-1} 3400 (br OH), 1673, 1612, 996; uv λ max (MeOH) nm 312 (ε = 43,000); $[\alpha]^{25}$ λ nm +21 (589), +22 (578), +25 (546) (*c* = 0.63, Me_2CO); eims (probe) 70 eV *m/z* (rel. int.) $[M]^+$ 166 (31), 137 (22), 109 (100), 94 (58), 81 (85), 79 (55), 57 (48); hreims *m/z* $[M]^+$ 166.1001 ($C_{10}H_{14}O_2$, 0.7 mmu deviation); ^{1}H and ^{13}C nmr see Table 1.

(8S)-(4-Methoxybenzoyloxy)-(2E,4E,6E)-decatrienal [**2**].—Trienal **1** (8.4 mg) in 2 ml CH_2Cl_2, 2 ml Et_3N, 44 μl 4-methoxybenzoyl chloride, and catalytic DMAP were refluxed 4 h, after which the solvents were removed in vacuo. The dried reaction product was triturated in cyclohexane and applied to a vc column, from which nonpolar fractions were injected on hplc [257 nm detection, Waters μPorasil 10 μm, 2× (3.9 × 300 mm), 7% EtOAc/hexanes]. This was followed by hplc (311 nm detection, columns as pre-

vious, 2% iPrOH/hexanes) to yield pure benzoate derivative **2** (1.4 mg, 9% yield): ir ν max (film) cm^{-1} 1708, 1679, 1607, 1511; uv λ max (MeOH) nm 209, 258, 310 (ϵ = 17,000, 26,000, 43,000); cd $\Delta\epsilon_{284}$ max +6.7, $\Delta\epsilon_{253}$ max −6.6, MeOH; $[\alpha]^{23}D$ +81 (c = 0.13, Me_2CO); ^1H nmr (300 MHz, $CDCl_3$) 9.56 (1H, d, J = 7.9 Hz, H-1), 8.02 (2H, d, J = 9.0 Hz), 7.12 (1H, dd, J = 15.2, 11.2 Hz, H-3), 6.94 (2H, d, J = 9.0 Hz), 6.66 (1H, dd, J = 14.9, 10.7 Hz, H-5), 6.46 (1H, dd, J = 14.9, 11.2 Hz, H-4), 6.41 (1H, dd, J = 15.2, 10.7 Hz, H-6), 6.16 (1H, dd, J = 15.2, 7.9 Hz, H-2), 6.02 (1H, dd, J = 15.2, 6.4 Hz, H-7), 5.51 (1H, dt, J = 6.4, 5.9 Hz, H-8), 3.87 (3H, s, -OMe), 1.82 (2H, m, H-9), 1.00 (3H, t, J = 7.6 Hz, H-10); eims (probe) 70 eV *m/z* (rel. int.) $[M]^+$ 300 (7), 148 (3), 135 (100), 107 (6), 94 (20), 77 (25).

(8S)-Hydroxy-(2E,4Z,6E)-decatrienal [**3**].—Compound **3** was purified using the same conditions as for **1**, but in darkened lab with ri detection: ir ν max (film) cm^{-1} 3400 (br -OH), 1673, 1609, 999, 971; uv λ max (MeOH) nm 313 (ϵ = 31,000); $[\alpha]^{25}\lambda$ nm +51 (589), +57 (546), (c = 0.38, Me_2CO); ^1H and ^{13}C nmr see Table 1.

PHOTOISOMERIZATION OF TRIENAL **3** TO TRIENAL **1**.—Trienal **3** was dissolved in 400 µl $CDCl_3$ in a 5-mm nmr tube, protected from light, and characterized by 400 MHz ^1H nmr. The nmr tube containing trienal **3** in solution was then placed ca. 3 cm from a 254 nm light source (Mineralight UVSL 0.12 Amp) for 1 h. Another nmr spectrum of the sample was immediately recorded on the same instrument. By integration, ca. 75% of trienal **3** isomerized to trienal **1** during the 1 h exposure to uv radiation.

Methyl 9-formyl-(15R,S)-hydroxy-(6Z,9E,11E,13E)-heptadecatetraenoate [**4**].—From the original vc, the fraction that eluted in 40% EtOAc/cyclohexane was treated with ethereal CH_2N_2. A second vc yielded a fraction eluting in 20% EtOAc/cyclohexane which was purified on hplc [254 nm detection, Waters µPorasil 10 µm, 2× (300 × 3.9 mm), 4% iPrOH in hexanes] to yield a light oil (14.5 mg, 0.2% yield): ir ν max (film) cm^{-1} 3500 (br -OH), 1734, 1671, 1607, 994, 870, 673; uv λ max (MeOH) nm 320 (ϵ = 37,000); $[\alpha]^{28}\lambda$ nm −1 (589), −1 (578), −2 (546), −3 (436) (c = 0.46, Me_2CO); ^1H and ^{13}C nmr see Table 2. NOe difference spectroscopy: irradiate δ 9.43 (H-9′), enhance δ 6.85 (H-10) 7%; irradiate δ 6.85 (H-10), enhance δ 9.43 (H-9′) 23%; irradiate δ 3.11 (H-8), enhance δ 6.66 (H-11, -12) 8%. Eims (probe) 70 eV *m/z* (rel. int.) $[M]^+$ 320 (4), $[M-OH]^+$ 303 (2), 263 (5), 248 (7), 231 (10), 213 (5), 133 (18), 105 (22), 91 (38), 57 (100); hreims *m/z* $[M]^+$ 320.1987 ($C_{19}H_{28}O_4$, −0.1 ppm deviation). Gc eims of TMSi-ether of **4** (70 eV) *m/z* (rel. int.) $[M]^+$ 392 (20), 367 (7), 335 (37), 245 (9), 237 (11), 179 (15), 131 (20), 91 (23), 73 (100).

FORMATION OF METHOXAMINE DERIVATIVE **5**.—Methyl ester **4** (1.5 mg) was dissolved in 100 µl of a 10 mg/ml methoxime HCl in pyridine solution and left at room temperature for 15 h. After solvent evaporation in vacuo, the reaction mixture was dissolved in Et_2O and applied to tlc. Upon development in 25% EtOAc/hexane, the product was visualized with 2′,7′-dichlorofluoroscein spray reagent under 254 nm light. The band at R_f 0.3 was scraped from the plate and eluted with Et_2O followed by EtOAc. An aliquot was silylated for structure verification: gc eims (70 eV) *m/z* (rel. int.) $[M]^+$ 421 (4), $[M-OMe]^+$ 390 (34), 368 (12), $[M-OMe-TMSiOH]^+$ 300 (16), 264 (19), 131 (30), 73 (100).

STERIC ANALYSIS OF METHOXAMINE DERIVATIVE **5** BY FORMATION OF FRAGMENT **6**.—Derivative **5** (1.5 mg) was ozonized for 12 min in 1 ml $CHCl_3$ at −20°, then reduced in volume under N_2. The menthoxycarbonyl derivative was formed in 50 µl toluene, 50 µl menthoxychlorocarbonate solution, and 10 µl pyridine for 30 min at room temperature. The reaction mixture was partitioned 3× between hexane and H_2O. The hexane solubles were treated with peracetic acid at 50° overnight, reduced under Ar, then dissolved in MeOH and treated with CH_2N_2. The methylated material was purified by preparative tlc (25% EtOAc/hexane), eluted with Et_2O, and analyzed by gc and gc eims versus standards, revealing that fragment **6** was racemic (50% *R* and 50% *S*).

Methyl 9-formyl-15-hydroxy-(9E,11E,13E)-heptadecatrienoate [**7**].—A less polar uv-active fraction was collected during hplc of trienal **4**. Further hplc [ri detection, Versapack 10 µm, 2× (4.1 × 300 mm), 4% iPrOH in hexanes] yielded an oil (0.4 mg): uv λ max (MeOH) nm 318 (ϵ = 57,000); ^1H nmr see Table 2.

Methyl (16)-hydroxy-9-oxo-(10E,12Z,14E)-octadecatrienoate [**8**].—During the hplc of trienal **7**, a less polar, unstable uv-active oil (0.6 mg) was isolated: uv λ max (MeOH) nm 314 (ϵ = 36,000). Positive ci (CH_4) gcms of TMSi-ether of **8**: $[M+H]^+$ 395 (25), $[M-Me]^+$ 379 (11), 351 (10), 323 (22), $[M-OTMSi]^+$ 305 (100), 185 (34), 131 (7), 91 (35), 75 (71), 57 (48). ^1H nmr see Table 3.

Methyl 9-formyl-15-oxo-(6Z,9E,11E,13E)-heptadecatetraenoate [**9**].—The original vc fraction that eluted in 33% EtOAc/cyclohexane was treated with CH_2N_2 and repurified by a second vc. The fraction that eluted in 15% EtOAc/cyclohexane contained a uv-active compound that was purified by hplc [270 nm detection, Waters µPorasil 10 µm, 2× (3.9 × 300 mm), 4% iPrOH/hexanes]. Further hplc [ri detection, Versapack 10 µm, 2× (4.1 × 300 mm), 15% EtOAc/hexanes] yielded an oil (0.7 mg), which at times ap-

peared to crystallize: ir ν max (film) cm^{-1} 1736, 1672, 1615, 1006, 865; uv λ max (MeOH) nm 324 ($\epsilon = 53{,}000$); eims (probe, 70 eV) *m/z* (rel. int.) $[M]^+$ 318 (55), 300 (10), $[M-OMe]^+$ 287 (18), $[M-C_2H_5C=O]^+$ 261 (12), 229 (12), 199 (16), 131 (37), 109 (64), 91 (100), 77 (64); hreims *m/z* $[M]^+$ 318.183 ($C_{19}H_{26}O_4$, −0.3 ppm deviation); ^1H nmr see Table 2.

Methyl (16S)-hydroxy-9-oxo-(10E,12E,14E)-octadecatrienoate [**10**].—From the crude vc, fractions eluting from 45–100% EtOAc were combined, treated with CH_2N_2, and purified via a second vc. Fractions eluting in 25–30% EtOAc/cyclohexane contained an Et_2O-insoluble gray substance, which was removed by filtration. Et_2O solubles were injected on hplc (300 nm detection, Rsil 10 μm, 50 × 1 cm, 40% EtOAc/hexanes), yielding a uv-active substance which charred yellow to purple on tlc upon heating with acidic cupric acetate solution. This fraction was injected on hplc [330 nm detection, Versapack 10 μm, 2× (4.1 × 300 mm), 3% iPrOH/hexanes, followed by 335 nm detection, Nucleosil 100 5 μm, 4.6 × 250 mm, 15% EtOAc/hexanes] to give a single peak. This pure oil (2 mg) was analyzed using Bakerbond Pirkle-type chiral (*R*)-*N*-3,5-dinitrobenzoylphenylglycine (ionic) 5 μm, 4.6 × 250 mm column (3% iPrOH/hexanes), from which compound **10** eluted as a single peak: ir ν max (film) cm^{-1} 3340 (br -OH), 1742, 1720, 1678, 1597, 1577, 1029; uv λ max (MeOH) nm 312 ($\epsilon = 35{,}000$); $[\alpha]^{25}$ λ nm +19 (589), +18 (578), +23 (546), +40 (436) ($c = 0.25$, Me_2CO); eims (probe, 70 eV) *m/z* (rel. int.) $[M]^+$ 322 (10), $[M-H_2O]^+$ 304 (11), $[M-OMe]^+$ 291 (9), 265 (15), 261 (11), 237 (25), 233 (85), 185 (32), 147 (39), 145 (33), 137 (23), 125 (25), 121 (37), 107 (100), 104 (64), 91 (65), 79 (65); hreims $[M]^+$ *m/z* 322.214 ($C_{19}H_{30}O_4$, −1 ppm deviation); ^1H and ^{13}C nmr see Table 3.

PHOTOISOMERIZATION OF TRIENONE **8** TO TRIENONE **10**.—In the same manner as the isomerization of trienal **3** to trienal **1**, trienone **8** was irradiated with 254 nm uv light for 1 h in $CDCl_3$ solution in a 5-mm nmr tube. Nmr spectra recorded before and after irradiation showed that ca. 80% of trienone **8** had isomerized to trienone **10**.

HYDROXY-EPOXY OCTADECANOIDS.—From the original 40% EtOAc/cyclohexane vc fraction which was subsequently methylated, the subfractions from the second vc eluting in 10–15% EtOAc were pooled and further purified on hplc (ri detection, Versapack 10 μm, 2× (4.1 × 300 mm), 3% iPrOH/hexanes]. Additional hplc was attempted (ri detection, Nucleosil 100 5 μm, 4.6 × 250 mm, 1% iPrOH/hexanes + 0.01% HOAc), but the major compound was still accompanied by shouldering peaks. Even utilizing the same conditions above except for the solvent (0.4% iPrOH/hexanes), there remained a ca. 4:1 mixture (4.3 mg) of two related compounds **11** and **12**.

Methyl (12S,13S)-epoxy-(11R)-hydroxy-(9Z,15Z)-octadecadienoate [**11**].—$[\alpha]^{25}$ λ nm −48 (589), −54 (578), −60 (546), −100 (436), −155 (365) ($c = 0.63$, Me_2CO). Gc-ms of the TMSi ether of **11** was comparable to the same derivative of methyl 12,13-epoxy-11-hydroxy-9-octadecenoate (32,33). For ^1H and ^{13}C nmr data, see Table 4.

Methyl (12S,13S)-epoxy-(11R)-(4-bromobenzoyloxy)-(9Z,15Z)-octadecadienoate [**13**].—The 4:1 mixture of **11** and **12** (3.5 mg) was treated as in the production of derivative **3**, except that 25 mg of 4-bromobenzoyl chloride was used and the reaction proceeded at room temperature for 25 h prior to application to a vc column. Fractions eluting in 4–8% EtOAc/hexanes were injected onto hplc [245 nm detection, Versapack 10 μm, 2× (4.4 × 300 mm), 4% EtOAc/hexanes], yielding 1.9 mg (40% yield) of derivative **13**: ir ν max (film) cm^{-1} 1723, 1591, 848, 757; $[\alpha]^{24}D$ +41 ($c = 0.22$, $CHCl_3$) [lit. (31) +26 ($c = 0.49$, $CHCl_3$)]; uv λ max (MeOH) nm 204, 246 ($\epsilon = 25{,}000$, 21,000); cd $\Delta\epsilon_{245}$ max +7.7 [lit. (31) $\Delta\epsilon_{244}$ max +5.9, EtOH]; ^1H nmr (300 MHz, $CDCl_3$) δ 7.91 (2H, d, $J = 8.5$ Hz), 7.57 (2H, d, $J = 8.5$ Hz), 5.71 (1H, dt, $J = 10.6$, 7.5 Hz, H-9), 5.61 (1H, dd, $J = 9.5$, 6.0 Hz, H-11), 5.50 (2H, m, H-10, 16), 5.31 (1H, dt, $J = 10.7$, 7.4 Hz, H-15), 3.67 (3H, s, Me-ester), 3.06 (1H, dd, $J = 6.0$, 2.1 Hz, H-12), 2.95 (1H, dt, $J = 5.4$, 2.1 Hz), 2.41 (1H, ddd, $J = 14$, 7.4, 5.4 Hz, H_a-14), 2.29 (2H, t, $J = 7.6$ Hz, H-2), 2.28 (1H, m, H_b-14), 2.20 (2H, m, H-8), 2.04 (2H, tt, $J = 7.5$, 7.3 Hz, H-17), 1.60 (2H, m, H-3), 1.29 (8H, m, H-4, -5, -6, -7), 0.96 (3H, t, $J = 7.5$ Hz, H-18); eims (probe) 70 eV *m/z* (rel. int.) 439 (1), $[M-C_5H_9]^+$ 437 (1), 275 (1), 237 (5), 208 (2), 185 (66), 183 (67), 157 (7), 95 (10), 81 (32), 67 (33), 55 (56).

Methyl (12S,13S)-epoxy-(11R)-(4-bromobenzoyloxy)-(9Z)-octadecadienoate [**14**].—During hplc of derivative **13**, a less polar derivative (1.1 mg) was isolated: ir ν max (film) cm^{-1} 1723, 1591, 757; $[\alpha]^{24}D$ +29 ($c = 0.12$, $CHCl_3$); uv λ max (MeOH) nm 204, 246 ($\epsilon = 20{,}000$, 17,000); cd $\Delta\epsilon_{244}$ max +6.2, MeOH; ^1H nmr (300 MHz, $CDCl_3$) δ 7.91 (2H, d, $J = 8.6$ Hz), 7.57 (2H, d, $J = 8.6$ Hz), 5.71 (1H, dt, $J = 10.7$, 7.5 Hz, H-9), 5.58 (1H, dd, $J = 9.3$, 6.1 Hz, H-11), 5.49 (1H, dd, $J = 10.7$, 9.3 Hz, H-10), 3.67 (3H, s, Me-ester), 3.01 (1H, dd, $J = 6.1$, 2.1 Hz, H-12), 2.90 (1H, dd, $J = 5.6$, 2.1 Hz, H-13), 2.29 (2H, t, $J = 7.5$ Hz, H-2), 2.21 (2H, dt, $J = 7.5$, 6.6 Hz, H-8), 1.60 (2H, m, H-3), 1.40 (2H, m, H-14), 1.30 (14H, m, H-4, -5, -6, -7, -15, -16, -17), 0.89 (3H, t, $J = 7.0$ Hz, H-18); eims (probe, 70

eV) *m/z* (rel. int.) 510 (0.005), $[M]^+$ 508 (0.004), 439 (1), $[M-C_5H_{11}]^+$ 437 (1), 208 (8), 185 (100), 183 (96), 157 (9), 155 (9), 151 (9), 95 (9), 81 (21), 67 (17), 55 (46).

Methyl (9R,10R)-epoxy-(11S)-hydroxy-(12Z)-octadecenoate [**15**].—From the original 33% EtOAc/cyclohexane vc fraction which was methylated, the fraction eluting from the second vc in 12% EtOAc/cyclohexane was applied to hplc [ri detection, Versapack 10 μm, 2× (4.1×300 mm), 15% EtOAc/hexanes]. The major peak was injected on hplc (ri detection, Nucleosil 100 5 μm, 4.6×250 mm, 0.5% iPrOH/hexanes) to yield a clear oil (2.8 mg): ir ν max (film) cm^{-1} 3420 (br -OH), 1740, 1026, 903; $[\alpha]^{23}$ λ nm +46 (589), +48 (578), +54 (546), +90 (436), +150 (365) ($c=0.28$, Me_2CO). Gc-ms of the TMSi ether derivative matched literature data (33,34). ^1H and ^{13}C nmr data see Table 4.

Methyl (9R,10R)-epoxy-(11S)-(4-bromobenzoyloxy)-(12Z)-octadecenoate [**16**].—This derivative was prepared from compound **15** (2.2 mg) in the same manner as the preparation of derivative **13**, except that the reaction proceeded for 51 h. The fraction eluting from preparative vc in 3% EtOAc/cyclohexane was purified by hplc using the same conditions as for benzoates **12** and **13**. Additional hplc with 3% EtOAc/hexanes yielded a clear, pleasant-smelling oil (0.6 mg): ir ν max (film) cm^{-1} 1723, 1590, 1012, 848, 757; $[\alpha]^{18}D$ −36° ($c=0.07$, $CHCl_3$); uv λ max (MeOH) nm 204, 246 ($\epsilon=24{,}000$, 22,000); cd $\Delta\epsilon_{244}$ max −8.2 (MeOH); ^1H nmr (300 MHz, $CDCl_3$) δ 7.91 (2H, d, $J=8.5$ Hz), 7.57 (2H, d, $J=8.5$ Hz), 5.73 (1H, dt, $J=10.5$, 7.5 Hz, H-13), 5.59 (1H, dd, $J=9.3$, 6.2 Hz, H-11), 5.49 (1H, dd, $J=10.5$, 9.3 Hz, H-12), 3.66 (3H, s, Me-ester), 3.01 (1H, dd, $J=6.2$, 2.1 Hz, H-10), 2.90 (1H, dt, $J=5.5$, 2.1 Hz, H-9), 2.30 (2H, t, $J=7.5$ Hz, H-2), 2.20 (2H, bdt, $J=7.5$, 7.5 Hz, H-14), 1.58 (4H, m, H-3, -8), 1.41 (2H, m), 1.30 (14H, m), 0.88 (3H, t, $J=6.8$ Hz, H-20); eims (probe, 70 eV) *m/z* (rel. int.) 510 (0.016), $[M]^+$ 508 (0.013), 353 (1), 351 (1), 324 (2), 322 (1), 237 (4), 185 (100), 183 (96), 157 (11), 155 (17), 151 (15), 104 (10), 95 (11), 81 (25), 67 (27), 55 (76).

Methyl (9R,10R)-epoxy-(11S)-hydroxy-(12Z,15Z)-octadecadienoate [**17**].—A nonpolar, uv-inactive fraction was recovered from the initial hplc of ketotrienal **9** and purified by hplc in the same manner as compound **14** to yield a light yellow oil (2.1 mg): ir ν max (film) cm^{-1} 3430 (br -OH), 1738, 1037, 882, 721; $[\alpha]^{25}$ λ nm +43 (589), +47 (578) ($c=0.18$, Me_2CO). Gc eims of TMSi-ether derivative (70 eV) *m/z* (rel. int.) $[M-Me]^+$ 381 (0.01), $[M-TMSiOH]^+$ 306 (0.17), 257 (1), 209 (1), 197 (15), 131 (15), 107 (29), 73 (100), 55 (30). ^1H-nmr data are in Table 4.

ACKNOWLEDGMENTS

We thank Mr. Rodger Kohnert for help in obtaining nmr data on the OSU Department of Chemistry's Bruker AM 400 [National Science Foundation (CHE-8216190) and M.J. Murdock Charitable Trust] and Bruker ACP 300 spectrometers (NIH RR 04039 and NSF CHE-8712343). Mr. Brian Arbogast and Mr. Don Griffin helped with low and high resolution (NIH-DRR 1S10RR01409) mass spectra at the mass spectral facility in the OSU College of Agricultural Chemistry. We appreciate Dr. W.C. Johnson and laboratory at OSU Department of Biochemistry and Biophysics for help with CD measurements. WHG gratefully acknowledges financial support by the Fogarty Senior International Fellowship Program (NIH TW01616-01) and mass spectral work at the Karolinska Institutet, Stockholm. Summer funding for MWB was provided by the N.L. Tartar Research Fellowship. Financial support at Oregon State University was from the Oregon Sea Grant Program (R/SH-1).

LITERATURE CITED

1. P.W. Gabrielson, R.F. Scagel, and T.B. Widdowson, "Keys to the Benthic Marine Algae and Seagrasses of British Columbia, Southeast Alaska, Washington, and Oregon," Department of Botany, The University of British Columbia, Vancouver, 1989, pp. 16–23.
2. J.R. Waaland, "Common Seaweeds of the Pacific Coast," Pacific Search Press, Seattle, 1977, pp. 41–42.
3. H.K. Phinney, in: "The Marine Biomass of the Pacific Northwest Coast." Ed. by R. Krauss, OSU Press, Corvallis, 1978, pp. 93–115.
4. W.H. Gerwick, M.W. Bernart, M.F. Moghaddam, Z.D. Jiang, M.L. Solem, and D.G. Nagle, *Hydrobiologia*, **204/205**, 621 (1990).
5. W.H. Gerwick and M.W. Bernart, in: "Advances in Marine Biotechnology: Pharmaceutical and Bioactive Natural Products." Ed. by O.R. Zaborsky and D.H. Attaway, Plenum Press, New York, 1992, pp. 101–152.
6. B. Samuelsson and C.D. Funk, *J. Biol. Chem.*, **264**, 19469 (1989).
7. N. Touchette, *J. NIH Res.*, **4**, 70 (1992).
8. A. Pollio, M. Della Greca, P. Monaco, G. Pinto, and L. Previtera, *Biochim. Biophys. Acta*, **963**, 53 (1988).
9. D.C. Zimmerman and B.A. Vick, *Lipids*, **8**, 264 (1973).

10. B.A. Vick and D.C. Zimmerman, *Plant Physiol.*, **90**, 125 (1989).
11. S.W. Pelletier, H.P. Chokshi, and H.K. Desai, *J. Nat. Prod.*, **49**, 892 (1986).
12. J.C. Coll and B.F. Bowden, *J. Nat. Prod.*, **49**, 934 (1986).
13. N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry," University Science Books, Mill City, 1983.
14. N.C. Gonella, K. Nakanishi, V.S. Martin, and K.B. Sharpless, *J. Am. Chem. Soc.*, **104**, 3775 (1982).
15. E. Breitmaier and W. Voelter, "Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry," 3rd ed., VCH Verlagsgesellschaft, Weinheim, 1987, p. 116.
16. P. Borgeat, S. Picard, P. Vallerand, and P. Sirois, *Prostaglandins Med.*, **6**, 557 (1981).
17. T.H. Lee, J.-M. Mencia-Huerta, C. Shih, E.J. Corey, R.A. Lewis, and K.F. Austen, *J. Biol. Chem.*, **259**, 2383 (1984).
18. M. Hamberg, *Anal. Biochem.*, **43**, 515 (1971).
19. W.S. Powell and F. Gravelle, *Biochem. Biophys. Acta*, **835**, 201 (1985).
20. E.H. Oliw, *J. Biol. Chem.*, **264**, 17845 (1989).
21. W.S. Powell and F. Gravelle, *J. Biol. Chem.*, **265**, 9131 (1990).
22. D.-E. Sok and M.-R. Kim, *Arch. Biochem. Biophys.*, **277**, 86 (1990).
23. A.N. Grechkin, R.A. Kuramshin, E.Y. Safonova, Y.J. Yefremov, S.K. Latypov, A.V. Ilyasov, and I.A. Tarchevsky, *Biochim. Biophys. Acta*, **1081**, 79 (1991).
24. H. Kühn, R. Weisner, L. Alder, and T. Schewe, *Eur. J. Biochem.*, **186**, 155 (1989).
25. M. Luckner, "Secondary Metabolism in Microorganisms, Plants, and Animals," 2nd ed. Springer-Verlag, Berlin, 1984, p. 153.
26. E.J. Corey and S.P.T. Matsuda, *Tetrahedron Lett.*, **28**, 4247 (1987).
27. A.R. Brash, S.W. Baertschi, and T.M. Harris, *J. Biol. Chem.*, **265**, 6705 (1990).
28. M. Hamberg, *Biochem. Biophys. Acta*, **920**, 76 (1987).
29. W.-C. Song and A.R. Brash, *Science*, **253**, 781 (1991).
30. M. Hamberg, R.P. Herman, and U. Jacobsson, *Biochim. Biophys. Acta*, **879**, 410 (1986).
31. T. Kato, Y. Yamaguchi, S. Ohnuma, T. Uyehara, T. Namai, M. Kodama, and Y. Shiobara, *J. Chem. Soc., Chem. Commun.*, 743 (1986).
32. M. Hamberg and B. Gotthammar, *Lipids*, **8**, 737 (1973).
33. T.A. Dix and L.J. Marnett, *J. Biol. Chem.*, **260**, 5351 (1985).
34. G.J. Garssen, G.A. Veldink, J.F.G. Vliegenthart, and J. Boldingh, *Eur. J. Biochem.*, **62**, 33 (1976).
35. E.D. Mihelich, *Tetrahedron Lett.*, **20**, 4729 (1979).
36. H.W. Gardner, *Biochem. Biophys. Acta*, **1001**, 274 (1989).
37. C.R. Pace-Asciak, *J. Biol. Chem.*, **259**, 8332 (1984).
38. W.H. Gerwick, M.F. Moghaddam, and M. Hamberg, *Arch. Biochem. Biophys.*, **290**, 436 (1991).

Received 19 June 1992