Structure and Stereochemistry of Constanolactones A–G, Lactonized Cyclopropyl Oxylipins from the Red Marine Alga *Constantinea simplex*

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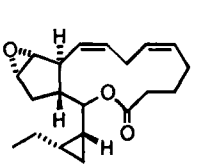
Extracts of the Oregon marine alga *Constantinea simplex* were found to contain a mixture of ω6 and ω3 unsaturated constanolactones, lactonized cyclopropyl-containing metabolites that logically derive from arachidonic and eicosapentaenoic acids, respectively. Detailed spectroscopic analysis of the isolated compounds, as natural products and various ester derivatives, afforded the planar structures of seven structurally related constanolactones. Constanolactones A–D possess 1,4-diol functionalities while constanolactones E–G contain a vicinal diol functionality. The absolute stereochemistry at all stereocenters in constanolactones A–D and at two stereocenters in constanolactones E and F were determined by chiral chromatography of fragments and chiroptical measurements of various mono- and dibenzoate derivatives and by comparable rotations within the two series (A–D and E–G). Isolation of these various diastereomeric diols, as well as of two presumed methanol adducts from $CHCl_3$/MeOH extraction of *C. simplex*, leads us to speculate on the occurrence of highly unstable allylic epoxides in this red alga.

Introduction

Marine invertebrates[1,2] and algae[3,4,5] are a rich source of oxidized, often carbocyclic,[6] fatty acid metabolites which have recently become known as "oxylipins".[7] The isolation of hybridalactone (**1**) from the marine red alga *Laurencia hybrida* represented the first example of a cyclopropyl and lactone-containing oxylipin.[8] Recently, however, cyclopropyl- and lactone-containing eicosanoids have been isolated from a wide variety of unrelated marine organisms: halicholactone (**2**) and neohalicholactone (**3**) from the sponge *Halichondria okadai*[9,10] and the brown alga *Laminaria sinclairii*,[11] aplydilactone (**4**) from the mollusk *Aplysia kurodai*,[12] and (**5**) from the soft coral *Plexaura homomalla*.[13]

Constantinea simplex is a small (2–10 cm dia.) "mushroom-shaped" red alga which grows attached to low intertidal and subtidal rocks from California to Alaska.



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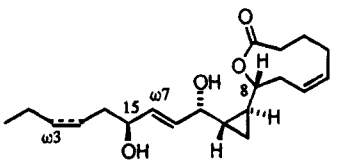
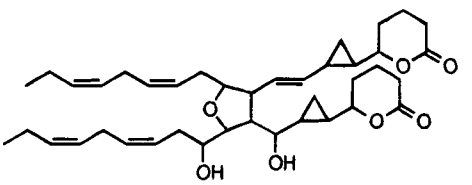


2 ω7
3 ω3



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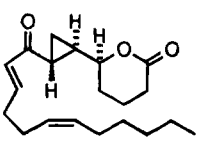

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Collections of *C. simplex* obtained from Seal Rock, OR were the source of two new lactonized cyclopropyl oxylipins which we named constanolactone A (**6**) and B (**7**).[14] Originally, **6** and **7** were isolated as synthetic diacetate derivatives (**8** and **9**) and their planar structures determined by spectroscopic means. Our continued investigation of *C. simplex* chemistry has lead to the discovery of several new constanolactones (**17, 18, 24–26**) which, in combination with stereochemical analysis and examination of key biosynthetic processes,[15] provides important clues to the unique mechanism of cyclopropyl–lactone formation in this alga. Herein, we report the isolation and structure elucidation of these new oxylipin metabolites (constanolactones C–G) and complete the structure

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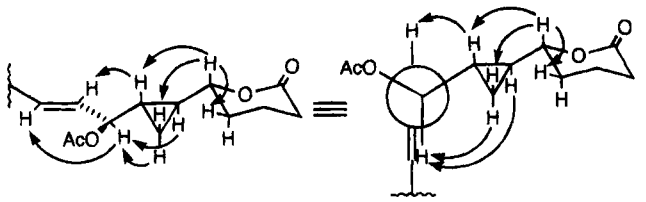


Figure 1. Depiction of selected NOESY correlations for peracetate derivative of constanolactone A (**8**).

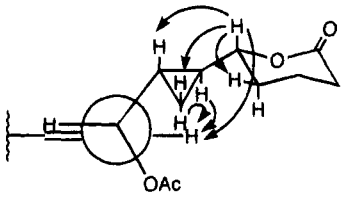
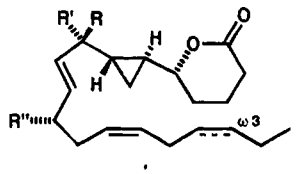


Figure 2. Depiction of selected NOESY correlations for peracetate derivative of constanolactone B (**9**).

elucidation of constanolactones A and B (**6** and **7**) with assignment of absolute stereochemistry.



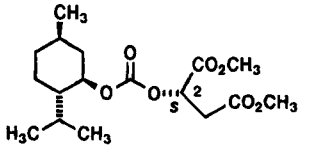
6 constanolactone A: R=H, R'=R"=OH
7 constanolactone B: R=R"=OH, R'=H
8 constanolactone A-diacetate: R=H, R'=R"=OAc
9 constanolactone B-diacetate: R=R"=OAc, R'=H
10 constanolactone A-MC: R=H, R'=R"=OMC
13 constanolactone A-BrBz: R=H, R'=R"=$OCOC_6H_5Br$
14 constanolactone B-BrBz: R=R"=$OCOC_6H_5Br$, R'=H
15 constanolactone A-mixed ester: R=H, R'=$OCOC_6H_5Br$, R"=OAc
16 constanolactone A-BrBz: R=H, R'=$OCOC_6H_5Br$, R"=OH
17 constanolactone C: (ω3) R=H, R'=R"=OH
18 constanolactone D: (ω3) R=R"=OH, R'=H
19 constanolactone C-diacetate: (ω3) R=H, R'=R"=OAc
20 constanolactone D-diacetate: (ω3) R=R"=OAc, R'=H
21 constanolactone D-BrBz: (ω3) R=R"=$OCOC_6H_5Br$, R'=H
22 constanolactone A 9-OMe: R=H, R'=OCH_3, R"=OH
23 constanolactone B 9-OMe: R=OCH_3, R'=H, R"=OH

Results and Discussion

Intertidal collections of *C. simplex* collected between 1989 and 1993 from the Oregon coast were similarly extracted and analyzed for oxylipins. Constanolactones A (**6**) and B (**7**) were isolated as natural products by a combination of normal and reversed phase HPLC and their stereochemistries deduced by various spectrochemical techniques. The identities of **6** and **7** were assigned spectroscopically (Experimental Section) and confirmed by acetylation and comparison by ^{1}H-NMR with authentic samples of **8** and **9**.

The relative stereochemistry of C-5 to C-9 in constanolactone A and B was spectroscopically deduced using the diacetate derivatives **8** and **9.** Coupling constants between the C-6−7−8 protons defined the *trans* (*S*,S**) geometry of the cyclopropyl group in **8** (J_{6-7a} = 5.2 Hz, J_{6-7b} = 8.4 Hz, J_{7a-7b} = 5.2 Hz, J_{7a-8} = 8.8 Hz, J_{7b-8} = 5.2 Hz) and **9** (J_{6-7a} = 5.2 Hz, J_{6-7b} = 8.5 Hz, J_{7a-7b} = 5.2 Hz, J_{7a-8} = 8.5 Hz, J_{7b-8} = 5.2 Hz).[13] Further, in both **8** and **9**, significant NOE interactions were observed from H-5 to H-7a and H-8, as well as from H-5 to H-3b and H-4a. However, the NOE profile of **8** and **9** were significantly different in the region proximate to C-9. NOE interactions in **8** were observed from H-8 to H-7a and H-10, and from H-9 to H-6 and H-7b (Figure 1). Compound **9**, the C-9 epimer of **8**, exhibited NOE interactions between H-5, H-6 and H-7b and the overlapping resonance observed for the olefinic protons H-10 and H-11 (Figure 2). The structurally related metabolite **5** has recently been synthesized and its relative configuration assigned.[16] A 5*R**,6*S**,8*S** configuration in **8** and **9** was shown by direct spectroscopic comparison of the C-5 proton resonance of **8** (δ 3.80 ddd, 10.7, 7.4, 3.0 Hz) and **9** (δ 3.82 ddd, 10.0, 6.6, 3.1 Hz) with synthetic **5** (δ 3.88 ddd, 10.2, 7.5, 3.3 Hz) as well as with the 5*R**,6*S**,8*S** (δ 3.90 ddd, 10.6, 7.7, 3.2 Hz) and 5*S**,6*S**,8*S** (δ 4.06 ddd, 10.2, 6.8, 3.2 Hz) 2,4-dinitrophenylhydrazine derivatives of **5**, prepared as intermediates in the synthesis of **5**.[16] We therefore deduce a relative configuration of 5*R**,6*S**,8*S**,9*S** in **8** and 5*R**,6*S**,8*S**,9*R** in **9**.

The stereochemistry at C-12 in both **6** and **7** was determined by forming semisynthetic (−)-menthoxycarbonyl (MC) derivatives followed by ozonolysis and comparison with standards using ^{1}H NMR and GC-MS. The bis-MC derivative **10** was prepared by treatment of **6** with an excess of (−)-menthoxycarbonyl chloride and isolated by elution over silica gel. The presence of two MC-esters in **10** was confirmed by ^{1}H NMR shifts for H-9 and H-12 at δ 4.83 and 5.10, respectively, a downfield shift of 1 ppm compared with those of **6**, clearly indicating esterification of both free hydroxyl groups. Ozonolysis of the bis-MC derivative **10**, followed by an oxidative workup with peracetic acid, methylation, and preparative TLC purification resulted in a 30% yield of dimethyl-MC-malate derivative **11**. The ^{1}H NMR spectra of the synthetic standards, 2*S* (**11**) and 2*R* (**12**) dimethyl-MC-malate, showed them to be differentiated by the chemical shift dispersion of the ester methyl groups [δ 3.778 and 3.722 (Δδ = 0.056) in **11**; δ 3.792 and 3.720 (Δδ = 0.072) in **12**]. As obtaining baseline separation of these malate derivatives by GC is sometimes problematic, this ^{1}H NMR method is a useful alternative when sample size permits (we estimate a reliable ^{1}H NMR determination can be made on as little as 50 μg of malate derivative). Comparison of the ^{1}H-NMR spectra of the two standard dimethyl-MC-malates with that produced from constanolactone A (**6**) showed the latter to be identical to that prepared from L-malate, thereby establishing the C-12 stereochemistry in **6** as *S*. These results were confirmed by GC retention times as synthetic *S*- and constanolactone A-derived dimethyl-MC-malates (**11**) coeluted (t_R = 26.34 min) while the *R* derivative **12** had a longer retention time (t_R = 26.46 min).



11
12 C-2 "*R*"

Constanolactone B diacetate (**9**), obtained from earlier work,[14] was saponified and then converted to the MC derivative, ozonized, and methylated to yield the C-11 to C-14 fragment as a dimethyl-MC-malate derivative. This derivative was analyzed by GC using conditions giving baseline separation of the two malate enantiomers and showed a peak only at the retention time corresponding to the *S*-malate (**11**).

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Table 1. ^1H and ^{13}C NMR Data for Peracetates of Constanolactones C (19) and D (20)

	constanolactone C peracetate (**19**)		constanolactone D peracetate (**20**)	
position	^1H ($CDCl_3$)	$^{13}C^a$	^1H ($CDCl_3$)	$^{13}C^a$
1		NA		NA
2a	2.40 ddd (17.7, 8.5, 6.8)	29.52	2.44 ddd (17.5, 8.4, 6.8)	29.52
2b	2.56 bdt (17.7, 6.6)		2.55 bdt (17.5, 7.4)	
3a	1.80 m	18.42	1.80 m	18.28
3b	1.96 m		1.94 m	
4a	1.60 m	27.87	1.65 m	27.79
4b	2.0 m		2.0 m	
5	3.80 ddd (10.5, 7.4, 3.1)	82.30	3.82 ddd (10.0, 7.2, 3.1)	81.91
6	1.2–1.3 m	20.75[b]	1.20 m	21.71[c]
7a	0.62 dt (8.6, 5.3)	7.62	0.59 dt (8.6, 5.5)	6.73
7b	0.73 dt (8.8, 5.3)		0.68 dt (8.5, 5.5)	
8	1.04 m	20.75[b]	1.2 m	21.22[c]
9	4.91 dd (7.2, 5.6)	75.30	4.85 bdd (7.9, 2.6)	75.49
10	5.69 ddd (15.7, 5.6, 0.9)	130.89	5.71 m	130.42
11	5.82 ddd (15.7, 6.0, 1.0)	129.16	5.71 m	129.45
12	5.29 m	73.06	5.3 m	73.01
13	2.4 m	32.22	2.4 m	32.31
14	5.26–5.4 m	123.78	5.2–5.4 m	123.63
15	5.47 dtt (10.8, 7.3, 1.5)	131.29	5.49 dtt (10.7, 7.2, 1.5)	131.38
16	2.78 t (7.1)	25.64	2.78 t (7.2)	25.64
17	5.26–5.4 m	126.79	5.2–5.4 m	126.79
18	5.26–5.4 m	132.17	5.2–5.4 m	132.16
19	2.0 m	20.54	2.0 m	20.10
20	0.98 t (7.5)	15.24	0.98 t (7.5)	14.23
		Acetate Esters		
	2.07 s	20.09[d]	2.06 s	20.30[e]
	2.08 s	20.09[d]	2.09 s	21.22[e]

[a] ^{13}C NMR data from ^{13}C DEPT (135°). [b–e] Assignments may be interchanged.

The absolute stereochemistry at C-9 in constanolactone A (**6**) was determined by circular dichroic (CD) analysis of mono- and bis-*p*-bromobenzoate derivatives of **6**. Treatment of **6** with 4-bromobenzoyl chloride and a catalytic quantity of 4-(dimethylamino)pyridine yielded the bis(*p*-bromobenzoate) **13** and the mono-*p*-bromobenzoate monoacetate derivative **15**, presumably a transesterification product of mono-*p*-bromobenzoate **16** and EtOAc during workup. The C-12 acetate and C-9 *p*-bromobenzoate substitutions were assigned based upon a comparison of the α-ester ^1H NMR resonances in **15** with those of the diacetate derivative **8**. In both, H-12 resonated at δ 5.3 while in mixed ester **15**, H-9 was downfield (δ 5.17) compared to diacetate **8** (δ 4.90).[14] While the CD spectrum of bis(*p*-bromobenzoate) **13** was not reliably interpretable because of free rotation in centers separating the various chromophores, the C-9 allylic *p*-bromobenzoate chromophore in mixed ester **15** was ideally suited for determination of the C-9 stereochemistry by exciton chirality.[17] The combination of a negative nondegenerate *p*-bromobenzoate Cotton effect ($\Delta\epsilon$ −7.3) at 244.5 nm in **15**, and a preferred rotomer conformation for C-9–C-10 in which the C–H and C=C bonds eclipse as indicated by a $^3J_{H9-H10}$ = 5.3 Hz (typically 5.2–9.2 Hz),[18,19] indicated a left-handed helicity between these groups and defined the stereochemistry at C-9 as *S* (Figure 3).

The uncertain origin of the mixed ester **15** prompted us to repeat the bromobenzoylation of **6** in the absence of 4-(dimethylamino)pyridine catalyst with intent of producing the C-9 mono-*p*-bromobenzoate **16**. Following workup, derivative **16** was isolated by silica gel flash chromatography and HPLC. Its identity as the C-9 mono-*p*-bromobenzoate was effectively revealed by the chemical shifts of the C-9 (δ 5.07) and C-12 (δ 4.20) proton bands in comparison with the natural product **6** (C-12 = δ 4.17) and bis(*p*-bromobenzoate) **13** (C-9 = δ 5.17). A relatively large C-9 to C-10 $^3J_{HH}$ = 6.2 Hz and similar CD spectrum to that of **15** ($\Delta\epsilon$ −4.6 at 242 nm) confirmed the 9*S* stereochemistry of **16**, as above. Therefore, taking into consideration the relative configuration as assigned by NOESY and coupling constants, the C-12 stereochemistry from analysis of the chiral MC-derivative **11**, and the above CD analysis, the absolute configuration of **6** is 5*R*,6*S*,8*S*,9*S*,12*S*. Since constanolactone B (**7**) is the C-9 epimer of **6** and yields the same chiral MC-derivative (**11**) upon reaction with (−)-menthoxycarbonyl chloride and ozonolysis, the absolute configuration of **7** may be assigned as 5*R*,6*S*,8*S*,9*R*,12*S*.

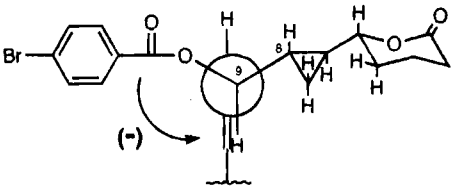

Figure 3. Newman projection of predicted favored rotamer of 9-(*p*-bromobenzoate) derivatives **15** and **16** used in CD analysis for determination of absolute stereochemistry.

Further investigation of minor compounds obtained from acetylation of a crude mixture of constanolactone natural products resulted in the isolation and identification of two new constanolactone analogs (**17** and **18**) as synthetic diacetate derivatives (**19** and **20**). The new derivatives **19** and **20** possessed nearly identical ^1H and ^{13}C NMR features to the diacetates of constanolactone A (**8**) and constanolactone B (**9**). Further, by MS, these diacetate derivatives were 2 mass units smaller than constanolactone A diacetate (**8**) and constanolactone B diacetate (**9**), and hence, each possessed one additional olefinic bond. The location of the new olefin was revealed

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(19) Gung, B. W.; Karipides, A.; Wolf, M. A. *Tetrahedron Lett.* **1992**, *33*, 713–6.

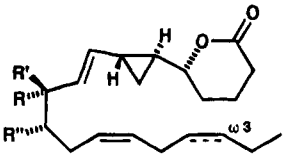
Table 2. ^1H and ^{13}C NMR Data for C-9 Methyl Ethers of Constanolactones A (22) and B (23) in $CDCl_3$

	derivative 22		derivative 23
position	^1H	$^{13}C^a$	^1H
1		NA	
2a	2.44 ddd (17.6, 8.5, 6.8)	29.01	2.44 ddd (17.7, 8.3, 6.8)
2b	2.55 dddd (17.6, 7.0, 5.3, 1.3)		2.55 ddd (17.7, 6.9, 6.7)
3a	1.80 m	18.16	1.80 m
3b	1.96 m		1.98 m
4a	1.67 m	27.46	1.68 m
4b	2.0 m		2.04 m
5	3.82 ddd (10.2, 7.2, 3.1)	82.96	3.70 td (9.5, 3.3)
6	1.11 m	20.68	1.09 m
7a	0.52 dt (8.4, 5.4)	5.24	0.65 dt (8.5, 5.2)
7b	0.58 dt (8.6, 5.4)		0.79 dt (8.7, 5.3)
8	1.6 m	20.69	1.65 m
9	3.42 bt (6.9, 5.7)	81.69	3.12 bt (7.0)
10	5.57 ddd (16.2, 6.9, 1.1)	135.19	5.66 m
11	5.72 ddd (16.2, 5.7, 0.6)	124.01	5.66 m
12	4.18 bq (6)	71.39	4.19 bq (6.1)
13	2.32 bq (6.2)	35.10	2.34 m (9 lines)
14	5.37 dtt (10.9, 6–7.5, 1.6)	129.05	5.39 bdt (10.9, 7.1)
15	5.55 m	133.50	5.54 bdt (10.9, 7.3)
16	2.0 m	27.24	2.0 m
17	1.35 m	29.35	1.35 m
18	1.3 m	31.31	1.3 m
19	1.3 m	22.34	1.3 m
20	0.89 t (6.8)	13.83	0.89 t (6.8)
OMe	3.32 s (3H)	56.09	3.27 s (3H)

[a] ^{13}C NMR data from ^{13}C DEPT (135°).

through signals for an additional two proton bis-allylic triplet at 2.78 ppm and a downfield and sharp C-20 methyl triplet (Table 1), thus defining **19** and **20** as ω-3 unsaturated analogs of **8** and **9**, respectively. Relative stereochemistry in diacetate **19** and **20** was assigned the same as in **8** and **9** based on the near superimposability of ^1H and ^{13}C NMR shifts and coupling constants for all pertinent regions of the spectra.[14] Additionally, the CD spectrum for **21**, the synthetic bis(*p*-bromobenzoate) derivative of **18**, was nearly identical to that of **14**, the bis(*p*-bromobenzoate) derivative of constanolactone B (**7**), indicating that they possess the same overall absolute stereochemistry.

A subsequent collection of *C. simplex* yielded, following extraction with warm $CHCl_3$/MeOH, silica gel chromatography, and HPLC, two additional constanolactone-related products, **22** (0.02%) and **23** (0.049%). Their structures were elucidated principally by comparison of NMR data with that of constanolactone A (**6**) and B (**7**). The only significant differences between the NMR spectra of these new compounds and the natural products **6** and **7** were a new three proton methyl singlet resonance at 3.32 ppm in **22** and at 3.27 ppm in **23**, and an upfield shifted C-9 proton ($\Delta\delta$ = 0.3 ppm in **22** and $\Delta\delta$ = 0.53 in **23**, Table 2). Hence, these new compounds were the C-9 methyl ether analogs of **6** and **7**, respectively, and presumably arise as solvolysis artifacts produced during extraction with MeOH. The significance of this finding is discussed in more detail below. Three additional metabolites were isolated from the same collection of *C. simplex* which yielded the original peracetate derivatives of constanolactone A (**8**) and B (**9**).[14] A silica gel vacuum chromatographic fraction eluting with 45% EtOAc in hexanes was acetylated to give, following additional purification by normal-phase HPLC, three new constanolactone derivatives, E diacetate (**27**), F diacetate (**28**), and G diacetate (**29**). Since both the proton and carbon count and mass spectrum for **27** and **28** were nearly identical with those of the peracetate constanolactone derivatives **8** and **9** ($C_{24}H_{36}O_6$), it was apparent that **27** and **28** were either regio- or stereochemical isomers of **8** and **9**. By ^1H and ^{13}C NMR, the C-1 to C-8 portion of both **27** and **28** were the same as in **8** and **9** (Table 3).[14] However, from COSY analysis, the new compounds differed from **8** and **9** in the C-9 to C-12 region. This was reflected by a downfield shift of the C-8 methine proton (δ 1.6 and 1.54 in **27** and **28**, respectively, versus δ 1.2–1.3 in **8** and **9**), C-9 and C-10 protons occurring at shifts typical for an olefin, and the C-11 and C-12 protons occurring at shifts consistent with these positions bearing acetoxy groups (Table 1). The C-9–C-10 olefin in both **27** and **28** was of a *trans* geometry as revealed by a J_{H9-H10} = 15 Hz coupling constant. The ^1H NMR spectrum of derivative **29**, constanolactone G diacetate, was highly analogous to that of **28**, differing only in the absence of the methylene band at δ 1.30–1.35 and the presence of additional olefinic and bis-allylic resonances, thus defining derivative **29** as the ω-3 analog of constanolactone F diacetate (**28**).



24 constanolactone E: R=R"=OH, R'=H
25 constanolactone F: R=H, R'=R"=OH
26 constanolactone G: (ω3) R=H, R'=R"=OH
27 constanolactone E-diacetate: R=R"=OAc, R'=H
28 constanolactone F-diacetate: R=H, R'=R"=OAc
29 constanolactone G-diacetate: (ω3) R=H, R'=R"=OAc
32 constanolactone E-BrBz: R=R"=$OCOC_6H_5Br$, R'=H
33 constanolactone F-BrBz: R=H, R'=R"=$OCOC_6H_5Br$

Constanolactones E (**24**) and F (**25**) were subsequently isolated as natural products from another extract of frozen *C. simplex* (May 1992). The diols **24** and **25** were slightly less polar than constanolactone A (**6**) and B (**7**), eluting with 50% EtOAc in hexanes from silica gel and were readily purified by normal-phase HPLC. Overlapping olefinic/α-acetoxyl regions in derivatives **27** and **28** were resolved in the ^1H NMR spectra of natural products

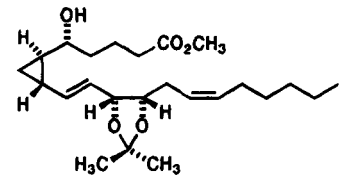
Table 3. ^1H and ^{13}C NMR Data for Constanolactones E (24) and F (25) and Diacetate Derivatives (27) and (28) in $CDCl_3$

position	constanolactone E (24)		constanolactone E diacetate (27)		constanolactone F (25)		constanolactone F diacetate (28)	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		171.50		171.32		171.50		171.40
2a	2.46 ddd (17.6, 8.5, 7.0)	29.26	2.46 ddd (17.7, 8.7, 7.0)	29.16	2.46 ddd (17.8, 8.6, 6.9)	29.29	2.5–2.6 m	29.16
2b	2.57 bdt (17.6, 6.3)		2.57 dt (17.7, 6.6)		2.57 ddd (17.8, 7.9, 6.5)			
3a	1.8 m	18.40	1.8 m	18.45	1.8–2.0 m	18.45	1.8–2.0 m	18.43
3b	1.94 m		1.94 m					
4a	1.7 m	27.74	1.66 td	27.81	1.66 m	27.79	1.66 m	27.78
4b	2.0 m		2.0 m		1.99 m		1.99 m	
5	3.79 ddd (10.2, 7.8, 3.1)	83.25	3.78 ddd (9.6, 7.7, 3.1)	82.99	3.78 ddd (10.2, 7.8, 3.1)	83.29	3.74 ddd (10.8, 7.4, 3.1)	83.15
6	1.12 m (7 lines)		1.12 m	24.87	1.11 m (7 lines)	24.95	1.12 m (7 lines)	24.94
7a	0.72 dt (8.8, 5.3)	10.64	0.71 dt (8.7, 5.2)	10.75	0.71 dt (8.7, 5.2)	10.50	0.68 dt (8.6, 5.2)	10.59
7b	0.77 dt (8.4, 5.3)		0.79 dt (8.6, 5.2)		0.76 dt (8.5, 5.3)		0.75 dt (8.6, 5.2)	
8	1.59 m (7 lines)	19.32	1.6 m	19.25	1.57 m	19.35	1.54 m	19.37
9	5.4 bdd (15.7, 8.1)	135.64	5.39 dd (15.4, 8.2)	138.65	5.39 dd (15.5, 8.2)	135.51	5.4 m	138.16
10	5.63 dd (15.7, 6.8)	124.74	5.55 dd (15.4, 7.6)	122.16	5.57 dd (15.5, 6.8)	127.96	5.3–5.5 m	123.01
11	4.10 dd (6.8, 3.8)	74.84	5.29 m	74.81	3.91 t (6.3)	75.13	5.31 m	74.19
12	3.66 m (5 lines)	73.90	5.03 td (6.0, 3.7)	73.70	3.48 dd (6.3, 5.7)	74.20	4.99 q (6.2)	73.71
13	2.25 m	29.93	2.28 m	28.05	2.25 m	31.05	2.30 bt (6.5)	28.54
14	5.37 m	126.37	5.27 m	123.37	5.4 m	124.54	5.3 m	123.01
15	5.5 m	133.54	5.49 bdt (10.8, 6.8, 1.5)	133.26	5.55 m	133.53	5.48 m	133.46
16	2.0 m	27.39	2.0 m	27.37	1.98 m	27.39	1.98 m	27.29
17	1.35 m	29.50	1.35 m	29.51	1.35 m	29.53	1.35 m	29.51
18	1.3 m	31.47	1.3 m	31.47	1.3 m	31.52	1.3 m	31.49
19	1.3 m	22.53	1.3 m	22.55	1.3 m	22.55	1.3 m	22.54
20	0.89 t (6.8)	14.03	0.89 t (6.8)	14.04	0.89 t (6.7)	14.06	0.89 t (6.7)	14.04
				Acetates				
		Me	2.04 s	21.04		Me	2.06 s	21.14
			2.05 s	21.17			2.07 s	20.95
		C=O		170.45		C=O		170.38
				169.93				169.94

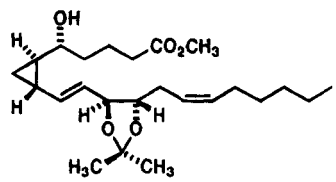
24 and **25** (Table 3), allowing for straight forward deduction of the C-8 to C-12 region of these molecules. The difference in $^1H-^1H$ coupling constants between H-11 and H-12 in the vicinal diols **24** ($^3J_{11-12}$ = 3.8 Hz) and **25** ($^3J_{11-12}$ = 6.3 Hz) suggested an *erythro/threo* relationship for these two compounds. The relative configurations at C-5, C-6, and C-8 in vicinal diols **24–26** were deduced to be identical to that in constanolactones A–D (5*R**,6*S**,8*S**) by comparison of the ^1H and ^{13}C NMR of authentic **6–9** with the C-5 to C-8 region in **24** and **25** and the peracetate derivatives **27–29**.

A series of derivatives of **24** and **25** were produced in order to further investigate the stereochemistry at C-11 and C-12. Treatment of **24** or **25** with 2,2-dimethoxypropane gave the methyl ester acetonide derivatives of constanolactone E (**30**) and F (**31**) (Experimental Section). A relatively small $^3J_{H11-H12}$ value (6.2 Hz) and dissimilar magnetic environment of acetonide methyl groups ($\Delta\delta$ = 0.14 ppm) were indicative of an *erythro* configuration in derivative **30**, while a relatively large H-11 to H-12 $^3J_{HH}$ value (8.1 Hz) and similar magnetic environment of acetonide methyl groups ($\Delta\delta$ = 0.007 ppm) established the diol configuration in derivative **31** as *threo*.[20]

The absolute stereochemistry of C-11 and C-12 in constanolactones E (**24**) and F (**25**) were determined by CD analysis of the corresponding bis(*p*-bromobenzoate) derivatives, **32** and **33**. The structures of the bis(*p*-bromobenzoate) derivatives **32** and **33** were confirmed by UV, ^1H-NMR, and CIMS. However, for both derivatives, the CD spectra showed a weak positive homochromophoric exciton coupling. Apparently, the two bis(*p*-bromobenzoate) derivatives adopt different conformations so as to both give weakly positive split Cotton effects. However, in derivative **33**, a pronounced bathochromic



30 constanolactone E-acetonide methyl ester

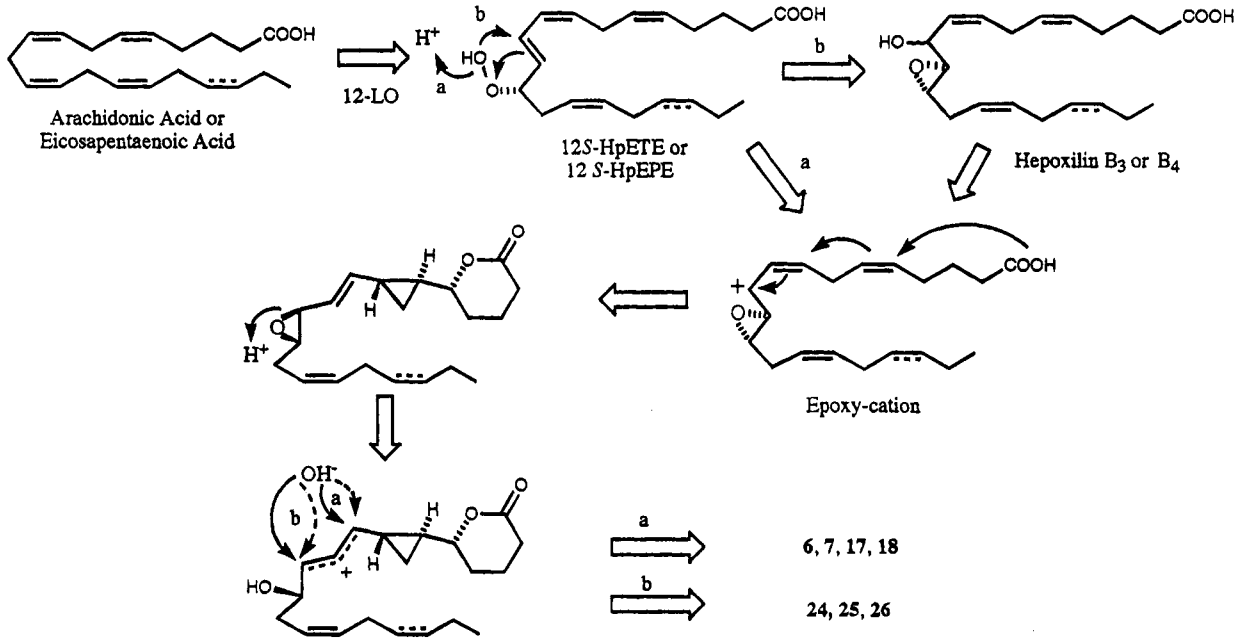


31 constanolactone F-acetonide methyl ester

shift in the CD maxima (λ_{max} = 255, 240 nm) was observed. We interpret this to signify that the C-11 benzoate shows exciton coupling both to the C-12 benzoate (positive) and C-9–C-10 olefin (negative, $\Delta\epsilon$ + 5.4, −11.8; λ_{max} = 255, 240 nm).[17] A computerized simulation in which the negative C-9 benzoate to C-10–C-11 olefin coupling observed for compound **16** was mathematically subtracted from the curve obtained for **33** produced a symmetrical CD curve with maxima more typical of benzoate to benzoate couplings (λ_{max} = 238, 253 nm). Similarly, bis(*p*-bromobenzoate) derivative **32** gave anomalous intensities in its CD spectrum ($\Delta\epsilon$ +9.1; λ_{max} = 252.5 nm) which we interpret to be due to a positive C-11 benzoate to olefin coupling which overlaps a positive benzoate–benzoate coupling. In both derivatives **32** and **33** relatively large C-9 to C-10 proton coupling constants of 8 and 7 Hz, respectively (typically 5.2–9.2 Hz),[18,19] established the preferred rotomer conformations, in each case, with eclipsed C–H and C=C bonds. Thus, in derivative **32**, the positive C-11 *p*-bromobenzoate to olefin

(20) Chuche, J.; Dana, G., and Monot, M. R. *Bull. Soc. Chim. Fr.* **1967**, *9*, 3300–7.

Scheme 1. Biosynthetic Proposal for Origin of *C. simplex* Cyclopropyl–Lactones



coupling indicates a right-handed helicity between these groups and defines the stereochemistry at C-11 as *R* while the negative C-11 *p*-bromobenzoate to olefin coupling in **33** indicates a left handed helicity or 11*S* stereochemistry (Figure 4). As the relationship between alcohols in **24** and **25** was shown to be *erythro* and *threo*, respectively, from analysis of the acetonide derivatives **30** and **31**, the absolute stereochemistry at C-11 and C-12 is given as 11*R*,12*S* for constanolactone E (**24**) and 11*S*,12*S* for constanolactone F (**25**). Thus, the overall stereochemistry in constanolactone E (**24**) is defined as 5*R**,6*S**,8*S**,11*R*,12*S*, and constanolactone F (**25**) as 5*R**,6*S**,8*S**,11*S*,12*S*.

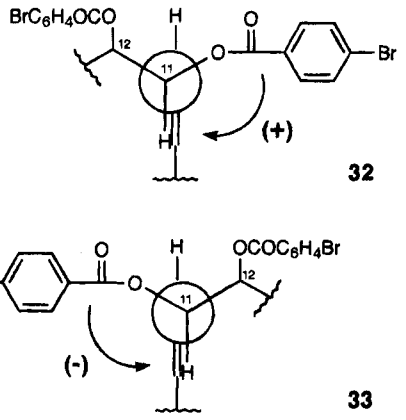


Figure 4. Newman projections of predicted favored rotamers of bis(*p*-bromobenzoate) derivatives **32** and **33** used in CD analysis for determination of absolute stereochemistry.

Conclusions

The red marine alga *Constantinea simplex* utilizes arachidonic and eicosapentaenoic acids to produce both simple (i.e. 12(*S*)-hydroxyeicosatetraenoic acid, 12(*S*)-hydroxyeicosapentaenoic acid)[14] as well as highly functionalized oxylipins (e.g. constanolactones A–G). We have hypothesized that these metabolites biogenetically derive from lipoxygenase-initiated oxidation of polyunsaturated fatty acid precursors.[4,5,14] Key to this hypothesis (Scheme 1), and in common to additional suspected routes of oxylipin metabolism in other algae,[21] is the formation of an epoxy-cation intermediate in which the cation induces cyclopropyl and lactone ring formation. This leads in turn to the formation of an allylic epoxide, a potential end product of the enzymatic pathway. As both epimers at C-9 in the 1,4-diols (constanolactones A–D) and C-11 in the 1,2 diols (constanolactone E, F) were isolated, it is possible that these diol products result from non-enzymatic hydrolysis (1,2 or 1,4) of the α,β-unsaturated epoxide intermediate. This hypothesis is substantiated by our isolation of the two epimeric C-9 methyl ether derivatives (**22** and **23**), presumed MeOH solvolysis products of this unstable unsaturated epoxide that form during the extraction process.

An analogous biogenetic hypothesis has been proposed[1,11] for the formation of the sponge metabolites halicholactone (**2**) and neohalicholactone (**3**).[9,10] However, the sponge metabolites are proposed to derive from the 15-lipoxygenase metabolites 15-hydroperoxyeicosatetraenoic acid (15-HpETE) and 15-hydroperoxyeicosapentaenoic acid (15-HpEPE) rather than from 12-hydroperoxyeicosatetraenoic acid (12-HpETE) and 12-hydroperoxyeicosapentaenoic acid (12-HpEPE) as in *C. simplex*. Further, isolation of the ω-3 unsaturated constanolactones C (**17**) and D (**18**) from this red alga is informative as these were proposed as biogenetic precursors to aplydilactone (**4**),[1] a nonsymmetrical oxylipin dimer isolated from the herbivorous mollusc *Aplysia kurodai*.[12]

It is interesting to note that oxylipins containing cyclopropyl and lactone rings are a growing class of marine-derived natural product, having now been isolated from red[6,14] and brown algae,[11] sponges,[9,10] opisthobranch molluscs,[12] and corals.[13] If our findings parallel other cases of discovery of novel oxylipins first in primitive creatures and later in mammalian systems,[22,23] then we can perhaps anticipate isolation of this structure class from yet more complex life forms in the future.

(21) Todd, J. S.; Proteau, P. J.; Gerwick, W. H. *Tetrahedron Lett.* **1993**, *34*, 7689–92.
(22) Hamberg, M.; Gerwick, W. H.; Asen, P. A. *Lipids* **1992**, *27*, 487–93.
(23) Oliw, E. H.; Brodowsky, I. D.; Hornsten, L.; Hamberg, M. *Arch. Biochem. Biophys.* **1993**, *300*, 434–9.

Experimental Section

General Methods. Spring and early Summer collections of small (blade diameter typically 2–5 cm) *C. simplex* plants were obtained from exposed low-intertidal locations (−0.3 to −0.8 M) at Seal Rock and Boiler Bay, OR. These were frozen on site with CO_2(s) and stored frozen prior to extraction ($CHCl_3$/MeOH 2:1). Extracts of large "older" individuals (diameter > 6 cm) collected from relatively nonexposed sites contained an insignificant oxylipin content. UV spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer and IR spectra were recorded on a Nicolet 510 FT-IR spectrometer. CD measurements were obtained on a Jasco 41A spectropolarimeter. Low resolution mass spectra were obtained on either a Varian MAT CH7 spectrometer or by GC-MS using a Hewlett Packard 5890 Series II gas chromatograph and a 5971 mass selective detector. HRMS were obtained on a Kratos MS 50 TC. HPLC was performed using a M-6000 pump, U6K injector, and either a R401 differential refractometer or a lambda-Max 480 lc spectrophotometer. NMR data were obtained on either Bruker AC 300 or Bruker AM 400 spectrometers. ^{1}H NMR spectra were acquired with tetramethylsilane (TMS) as an internal chemical shift reference and ^{13}C spectra were referenced to the center line of $CDCl_3$ at 77.0 ppm. ^{13}C assignments are based on ^{1}H–^{13}C HETCOR, DEPT multiplicity data, and comparison with previously identified constanolactone derivatives.[14] Coupling constants are reported in hertz. TLC-grade (10–40 μm) silica gel was used for vacuum chromatography, and Kieselgel 60 silica (40–63 μm) was used for flash chromatography . Aluminum-backed thin-layer chromatography sheets were used for TLC, and all solvents were distilled prior to use.

Isolation of Constanolactones A (6) and B (7). Approximately 8 L of frozen *C. simplex* (400 g extracted dry weight) was repetitively extracted (3×, as above), to yield 12.49 g of a dark green oil. The extract was subjected to silica gel vacuum chromatography, using a stepwise gradient from 0 to 100% (v/v) EtOAc in hexanes. Fractions eluting with EtOAc concentrations greater than 50% were determined by TLC to be of similar composition and were pooled. This combined fraction was further purified by silica gel flash chromatography, using a stepwise gradient from 10 to 100% (v/v) MeOH in $CHCl_3$. Reversed-phase chromatographic separation (Sep-Pak C_{18} cartridge, 85% (v/v) MeOH in H_2O) proved necessary in order to remove significant amounts of coeluting glycolipid impurities. The natural products were then isolated by normal phase (NP) HPLC (10-μm Phenomenex Maxsil Si column; 500 × 10 mm; 20% (v/v) 2-propanol in hexanes; differential refractometer detection; flow rate at 6.0 mL/min) to yield constanolactone A (**6** 12.4 mg, 0.10%) and B (**7** 15.8 mg, 0.13%, contained some ω3 unsaturated analog).

Constanolactone A (6): oil; $[\alpha]_D$ +1.4° (*c* 1.00, MeOH); $[\alpha]_D$ −3.8° (*c* 1.31, $CHCl_3$); IR (neat) 3320, 2958, 2924, 1712, 1258, 1248, 1099, 1045, 1031 cm^{-1}; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 5.78 (m, 2H), 5.54 (dt, 1H, *J* = 10.8, 7.3), 5.39 (dt, 1H, *J* = 10.8, 7.2), 4.17 (dt, 1H, *J* = 4.6, 6.1), 3.72 (m, 2H), 2.56 (dt, 1H, *J* = 17.8, 6.4), 2.44 (ddd, 1H, *J* = 17.8, 8.5, 6.9), 2.32 (bq, 2H, *J* = 6.7), 2.05 (m, 2H), 1.99 (m, 1H), 1.96 (m, 1H), 1.80 (m, 1H), 1.68 (ddd, 1H, *J* = 13.2, 9.9, 4.5), 1.35 (m, 2H), 1.3 (m, 4H), 1.20 (m, 1H), 1.02 (m, 1H), 0.88 (t, 3H, *J* = 6.7), 0.75 (dt, 1H, *J* = 8.7, 5.3), 0.61 (dt, 1H, *J* = 8.5, 5.3); (300 MHz, C_6D_6) δ 5.97 (m, 2H), 5.67 (dt, 1H, *J* = 10.9, 6.8), 5.56 (dt, 1H, *J* = 10.9, 7.0), 4.30 (dt, 1H, *J* = 4.3, 6.4), 3.75 (dd, 1H, *J* = 6.9, 4.3), 3.12 (td, 1H, *J* = 8.9, 3.4), 2.5 (m, 2H), 1.98–2.13 (m, 8H), 1.32 (m, 2H), 1.25 (m, 4H), 1.1 (m, 1H), 0.88 (t, 3H, *J* = 6.9), 0.81 (m, 1H), 0.62 (dt, 1H, *J* = 8.7, 5.2), 0.32 (dt, 1H, *J* = 8.5, 5.2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.70, 133.65, 133.17, 131.74, 124.48, 83.77, 74.07, 71.59, 34.95, 31.46, 29.48, 29.25, 27.71, 27.38, 23.36, 22.52, 20.31, 18.29, 14.03, 7.50; (75 MHz, C_6D_6) δ 171.06, 134.03, 132.38, 132.13, 126.0, 83.52, 74.00, 71.91, 35.86, 31.86, 29.78, 29.49, 27.84, 27.68, 23.70, 22.98, 20.43, 18.32, 14.32, 7.61; CIMS (CH_4, positive ion) *m*/*z* [M + H − $H_2$0]$^+$ 319 ((73), [M + H − 2($H_2$0)]$^+$ 301 (100), 283 (11), [M + H − C_8H_{15}]$^+$ 225 (11), 217 (10), 207 (12), 163 (23), 111 (10); FABMS (glycerol, negative ion) *m*/*z* [2(M) − H]$^-$ 671.4 (8), 519.3 (12), [M − H + glycerol]$^-$ 427.3 (100), [M − H + H_2O]$^-$ 353.3 (18), [M − H]$^-$ 335.2 (78), [M − H − C_8H_{15}]$^-$ 223.1 (21); HR-FABMS (glycerol, negative ion), obs 335.2221 calc for $C_{20}H_{31}O_4$ 335.2220 [M − H]$^-$.

Constanolactone B (7): oil; $[\alpha]_D$ +10.2° (*c* 1.00, MeOH); IR (neat) 3390, 2955, 2925, 1727, 1725, 1716, 1243, 1035, 972 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (m, 2H), 5.54 (dt, 1H, *J* = 10.9, 7.2), 5.38 (dt, 1H, *J* = 10.9, 7.2), 4.15 (m, 1H), 3.73 (ddd, 1H, *J* = 10.0, 7.8, 2.9), 3.65 (m, 1H), 2.56 (dt, 1H, *J* = 17.7, 6.6), 2.45 (ddd, 1H, *J* = 17.7, 8.5, 6.9), 2.3 (m, 2H), 2.04 (m, 2H), 2.0 (m, 1H), 1.93 (m, 1H), 1.82 (m, 1H), 1.7 (ddd, 1H), 1.35 (m, 2H), 1.3 (m, 4H), 1.13 (m, 2H), 0.89 (t, 3H, *J* = 6.7), 0.61 (dt, 1H, *J* = 8.7, 5.3), 0.56 (dt, 1H, *J* = 8.7, 5.3); (300 MHz, C_6D_6) δ 5.96 (m, 2H), 5.65 (dt, 1H, *J* = 11.0, 7.0), 5.56 (dt, 1H, *J* = 11.0, 7.0), 4.30 (dt, 1H, *J* = 5.6, 6.3), 3.70 (dd, 1H, *J* = 6.9, 5.3), 3.21 (td, 1H, *J* = 9, 3.3), 2.56 (dt, 1H, *J* = 14.4, 6.7), 2.45 (dt, 1H, *J* = 14.4, 6.5), 2.1 (m, 4H), 1.34 (m, 2H), 1.1–1.4 (m, 4H), 1.26 (m, 4H), 1.1 (m, 2H), 0.88 (t, 3H, *J* = 6.9), 0.47 (dt, 1H, *J* = 8.7, 5.1), 0.27 (dt, 1H, *J* = 8.7, 5.1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.78, 133.23, 133.23, 131.68, 124.43, 83.63, 74.01, 71.66, 35.10, 31.44, 29.45, 29.23, 27.73, 27.37, 23.34, 22.50, 21.23, 18.31, 14.02, 6.62; (75 MHz, C_6D_6) δ 171.38, 133.90, 132.74, 132.29, 126.00, 83.56, 74.20, 72.26, 35.83, 31.87, 29.78, 29.54, 27.85, 27.66, 23.83, 22.99, 21.32, 18.33, 14.34, 6.77; CIMS (CH_4, positive ion) [M + H − $H_2$0]$^+$ *m*/*z* 319 (56), [M + H − 2($H_2$0)]$^+$ 301 (100), 283 (10), [M + H − C_8H_{15}]$^+$ 225 (9), 217 (8), 207 (14), 163 (18), 111 (10). (Some ω3 unsaturated analog impurity.)

Isolation of Constanolactones A and B Peracetate Derivatives (8 and 9) and C and D Peracetate Derivatives (19 and 20). The isolation of the peracetate derivatives of constanolactones A (**8** and **9**) was previously reported.[14] Further investigation of less abundant acetylated derivatives isolated from the same fractions containing **8** and **9** (NP-HPLC, 10-μm Alltech RSIL silica column; 500 × 10 mm; refractive index detection; 35% (v/v) EtOAc in hexanes; flow rate 9.0 mL/min) yielded two new constanolactone peracetate derivatives, **19** (4.2 mg, 0.17%) and **20** (5.7 mg, 0.23%).

Constanolactone A Peracetate (8). The identity of **8** was established previously in ref 14. Additionally, the following correlations were observed by ^{1}H–^{1}H NOESY (400 MHz, degassed $CDCl_3$) H-2a (H-2b), H-3a (H-3b), H-3b (H-4, 5, 8), H-5 (H-7a, 8, 12 or 14), H-6 (H-7b, 9), H-7a (H-8), H-7b (H-9), H-8 (H-9, 11), H-9 (H-10, 11), H-10 (H-12 or 14), H-11 (H-12 or 14), H-12 (H-13a, 15, 16, H_6-17–19, -OAc), 13a (H-16), 13b (H-16, -OAc), H-14 (H-15, 16, 17–19, -OAc), H-15 (H-16), H-16 (H_6-17–19, -OAc), H_6-17–19 (H-20).

Constanolactone B Peracetate (9). Correlations observed by ^{1}H–^{1}H NOESY (400 MHz, degassed $CDCl_3$) H-2a (H-2b), H-3 (H-4a), H-4a (H-4b, 5), H-5 (H-6* or 8, 7a*, 10 or 11, 12), H-6 or 8 (H-7a, 7b*, 9, 10 or 11), H-7a (H-7b*), H-7b (H-9*, 10 or 11), H-9 (H-10 or 11), H-10 or 11 (H-12, 13a, 13b, 15, 16), H-12 or 14 (H-13a, 13b, 15, 16), 13a (H-14, 16), 13b (H-14), H-15 (H-16), H-16 (H-17 or 18, -OAc), H_6-17–19 (H-20, -OAc); *confirmed by NOE difference spectroscopy.

Bis(menthoxycarbonyl)constanolactone A (10). Constanolactone A (**6**, 2.6 mg, 7.7 μmol) was dissolved in a toluene/pyridine mixture (5:1, 120 μL), 100 μL of $CHCl_3$ was added to prevent precipitation of solids in flask, an excess of (−)-menthoxycarbonyl chloride (50 μL of a 1.0 mM solution in toluene) was added, and the flask was purged with N_2, sealed (flask occasionally opened to monitor reaction by TLC), and stirred (1.5 h, 23°). The reaction was stopped with the addition of 3.0 mL of MeOH and evaporated under vacuum. The product was dissolved in 100% hexanes and applied to a small silica column. Components eluting in both 100% hexanes and 3% (v/v) EtOAc in hexanes were removed. The following fraction eluting with 10% (v/v) EtOAc in hexanes was evaporated to yield **10** (4.9 mg, 7.0 μmol, 90.4% yield): oil; IR (neat) 2956, 2929, 2871, 1737, 1458, 1369, 1285, 1254, 1037, 956 cm^{-1}; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 5.87 (m, 1H, *J* = 15.8, 6.2), 5.75 (dd, 1H, *J* = 15.8, 5.8), 5.51 (bdt, 1H, *J* = 10.9, 7.2), 5.32 (bdt, 1H, *J* = 10.9, 7.2), 5.10 (q, 1H, *J* = 6.2), 4.83 (bt, 1H, *J* = 6.6), 4.50 (m, 2H, 7 lines), 3.93 (ddd, 1H, *J* = 10.5, 6.4, 3.0), 2.55 (dt, 1H, *J* = 17.8, 6.2), 2.42 (ddd, 1H, *J* = 17.8, 8.3, 6.9), 1.9–2.1 (m, 6H), 1.96 (m, 1H), 1.8 (m, 1H), 1.68 (m, 4H), 1.35 (m, 2H), 1.2–1.5 (m, 8H), 0.99–1.1 (m, 2H), 0.90 (m, 15H, 7 lines), 0.84–0.9 (m, 1H), 0.79 (m, 6H, 3 lines), 0.77 (m, 1H), 0.68 (dt, 1H, *J* = 8.6, 5.4).

Formation of Bis(menthoxycarbonyl)constanolactone B from Constanolactone B Peracetate (9) and Ozonolysis To Form Dimethyl (Menthoxycarbonyl)malate (11). Treatment of constanolactone B diacetate (**9**, 5 mg) with 0.4 mL of 10% NaOH and 1.6 mL MeOH for 18.5 h at rt was followed by acidification to pH 3 (pH paper) and extraction with EtOAc. The reduced EtOAc-soluble product was redissolved in MeOH (0.5 mL) and treated with excess ethereal CH_2N_2 for 1 min. Excess reagent was removed under a stream of N_2 and applied as a band in Et_2O to a TLC plate and developed in 100% EtOAc. The major product, R_f = 0.07–0.17, was removed and extracted with EtOAc. A portion of the product (1% by volume) was treated with equal volumes (3 drops) of pyridine, 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane for 20 min. Excess solvent and reagents were removed *in vacuo*, and the products were dissolved in hexane and analyzed by GC-MS (120° to 220°, 10° min, 18.05 min retention) obs *m/z* 405 (1), 384 (8), 383 (27), 293 (9), 257 (57), 243 (10), 203 (6), 167 (47), 129 (25), 103 (13), 73 (100). Hydrogenation of this TMS ether derivative with H_2/Pd on $CaCO_3$ in MeOH for 30 min followed by retrimethylsilyation as detailed above, gave a derivative with clearer MS cleavage patterns: (120°–220°, 10° min, 19.80 min retention) obs. *m/z* 446 (2), 408 (1), 397 (19), 385 (8), 345 (28), 307 (8), 295 (14), 289 (14), 255 (50), 229 (17), 215 (19), 191 (14), 129 (96), 73 (100). One-third of the remainder of the triol methyl ester was treated with 50 μL of toluene, 10 μL of pyridine, and 50 μL of (−) menthoxycarbonyl chloride for 30 min at rt. TLC purification of the resulting derivative (10% EtOAc/hex) was followed by ozonolysis for 2 min at −20° followed by 10 min at rt. Excess O_3 was removed under a stream of N_2 and the product treated overnight with 0.3 mL of peracetic acid at 50°. Again, reagents were removed under a stream of N_2, methylated with CH_2N_2 as above for 1 min, solvents removed under N_2, dissolved in hexane and analyzed by GC versus standards: (170°, obs 14.869 min retention (standard *S*-malate = 14.893 min, *R*-malate = 15.011 min). The constanolactone B-derived malate derivative did not show a detectable peak at the *R*-malate retention time, and is therefore, essentially "100%" *S*.

Ozonolysis of Bis(menthoxycarbonyl)constanolactone A (10) To Form Dimethyl (Menthoxycarbonyl)malate (11). The bis(menthoxycarbonyl) derivative of constanolactone A (**10**, 3.4 mg, 4.9 μmol) was added to 1.0 mL $CHCl_3$, the solution was cooled to −11 °C (ethylene glycol and solid CO_2), and O_3 was bubbled through the solution (2 min). The reaction flask was removed from the bath and allowed to reach rt (10 min) and then evaporated under vacuum. Concentrated acetic acid (1.0 mL) and 30% H_2O_2 (250 μL) were added and the flask sealed in a 48° water bath overnight (17.5 h). The products were dried under N_2, dissolved in MeOH (1.0 mL), and treated with CH_2N_2/EtOH (1.0 mL, 2 min, 23°). The product was evaporated under vacuum, dissolved in Et_2O, and isolated by prep-TLC (10% (v/v) EtOAc in hexanes) to yield **11** (0.5 mg, 1.5 μmol, 30% recovery): oil; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 5.40 (dd, 1H, *J* = 6.7, 5.7), 4.57 (td, 1H, *J* = 11.0, 4.4), 3.778* (s, 3H), 3.722* (s, 3H), 2.92 (m, 3 lines, 2H), 1.98–2.1 (m, 2H), 1.68 (bd, 2H, *J* = 12), 1.39–1.53 (m, 2H), 1.0–1.13 (m, 2H), 0.92 (m, 4 lines, 6H), 0.83–0.9 (m, 1H), 0.81 (d, 3H, *J* = 6.9); GC-EIMS (70 eV) *m/z* 206 (18), 138 (78), 123 (42), 113 (33), 95 (68), 81 (100), 71 (28), 56 (42), 44 (60), 41 (22). *Data is reported to three decimal points in order to clearly differentiate **11** and **12**.

Formation of Authentic Dimethyl (Menthoxycarbonyl)-*S*-malate (11). L-Malate (39.8 mg, 0.294 mmol, 99%) in 400 μL of MeOH was treated with an excess of CH_2N_2/Et_2O (3 min) in a 10 mL recovery flask and dried under N_2. The methylated L-malate (48.4 mg, 0.29 mmol) was dissolved in a toluene/pyridine mixture (4:1, 250 μL), an excess of (−)-menthoxycarbonyl chloride (600 μL of a 1.0 mM solution in toluene) was added, and the flask was purged with N_2, sealed, and stirred overnight (24 h, 23°). The product was dissolved in 1% (v/v) EtOAc in hexanes and fractionated over a small silica flash column. The fraction eluting in 3% (v/v) EtOAc in hexanes was evaporated to yield **11** (61.5 mg, 0.179 mmol, 60.9% yield): IR (neat) 2956, 2933, 1747, 1292, 1264, 1218, 1197, 1173, 1038, 956 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (dd, 1H, *J* = 6.7, 5.7), 4.57 (td, 1H, *J* = 11.0, 4.4), 3.778* (s, 3H), 3.722* (s, 3H), 2.92 (m, 3 lines, 2H), 1.98–2.1 (m, 2H), 1.68 (bd, 2H, *J* = 12), 1.39–1.53 (m, 2H), 1.0–1.13 (m, 2H), 0.92 (m, 4 lines, 6H), 0.83–0.9 (m, 1H), 0.81 (d, 3H, *J* = 6.9); GC-EIMS (70 eV) *m/z* 207 (4), 138 (100), 123 (40), 113 (29), 95 (78), 81 (74), 55 (31). *Data is reported to three decimal points in order to clearly differentiate **11** and **12**.

Formation of Authentic Dimethyl (Menthoxycarbonyl)-*R*-malate (12). D-Malate (40.3 mg, 0.30 mmol, 99%) in 1.0 mL of MeOH was treated with an excess of CH_2N_2/Et_2O (3 min) in a 10 mL recovery flask and dried under N_2. The methylated L-malate was dissolved in a toluene/pyridine mixture (4:1, 250 μL), an excess of (−)-menthoxycarbonyl chloride (600 μL of a 1.0 mM solution in toluene) was added, and the flask was purged with N_2, sealed, and stirred overnight (24 h, 23°). The product was dissolved in 1% (v/v) EtOAc in hexanes and fractionated over a small silica flash column. Components eluting in both 1 and 3% (v/v) EtOAc in hexanes were discarded. The following fraction eluting with 100% EtOAc was evaporated to yield **12** (106.2 mg, 0.30 mmol, 100% yield): IR (neat) 2957, 2933, 1747, 1439, 1371, 1292, 1265, 1219, 1198, 1174, 1039, 956 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.39 (dd, 1H, *J* = 6.6, 5.6), 4.54 (td, 1H, *J* = 10.9, 4.4), 3.792* (s, 3H), 3.720* (s, 3H), 2.93 (m, 3 lines, 2H), 2.1 (bd, 1H, *J* = 11.8), 1.94 (pd, 1H, *J* = 7.0, 2.6), 1.69 (bd, 2H, *J* = 11.4), 1.38–1.5 (m, 2H), 1.10 (q, 1H, *J* = 11.7), 0.97–1.11 (m, 1H), 0.91 (m, 3 lines, 6H), 0.83–0.9 (m, 1H), 0.79 (d, 3H, *J* = 6.8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.17, 169.06, 153.75, 79.296, 70.88, 52.55, 51.99, 46.76, 40.39, 35.80, 33.90, 31.26, 25.93, 23.22, 21.78, 20.49, 16.13; GC-EIMS (70 eV) *m/z* 207 (3), 138 (100), 123 (44), 113 (29), 95 (74), 81 (68), 55 (27). *Data is reported to three decimal points in order to clearly differentiate **11** and **12**.

Formation of Bis(*p*-bromobenzoyl)constanolactone A (13) and 12-Acetoxy-9-(*p*-bromobenzoyl)constanolactone A (15). To 7.7 mg of **6** (0.023 mmol) were added 102 mg of 4-bromobenzoyl chloride (98%, 0.466 mmol) and a catalytic amount of 4-(dimethylamino)pyridine in dry CH_2Cl_2/triethylamine (3:1, 10 mL). The solution was purged with N_2 and stirred at rt (23°) for 22 h. The solvents were evaporated under vacuum and the products dissolved in hexanes and applied to a small silica flash column. The fractions eluting with 20 and 50% (v/v) EtOAc in hexanes were evaporated under vacuum and further purified by NP-HPLC (dual 10-μm Alltech Versapak Si columns; 300 × 4.1 mm; 30% (v/v) EtOAc in hexanes; UV detection at 254 nm; flow rate 2.0 mL/min) to give 1.2 mg of pure bis(*p*-bromobenzoate) constanolactone A (**13**, 0.002 mmol) and 0.7 mg of 12-acetoxy-9-(*p*-bromobenzoyl) constanolactone A (**15**) (0.0013 mmol, 5.4% yield).

Bis(*p*-bromobenzoyl)constanolactone A (13): oil; IR (neat) 2955, 2928, 1719, 1590, 1398, 1267, 1237 cm^{-1}; UV (MeOH) λ_{max} 246 nm (log ε 4.54); CD (MeOH) Δε +1.4, −1.7 (λ_{max} 252, 238 nm); ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.91 (2 broad lines, 4H, -OCOBzBr), 7.59 (4 broad lines, 4H, -OCOBzBr), 5.99 (ddd, 1H, *J* = 15.7, 6.1, 0.9), 5.86 (ddd, 1H, *J* = 15.7, 5.6, 0.7), 5.54 (q, 1H, *J* = 6.1–6.3), 5.49 (dtt, 1H, 10.8, 7.2, 3.3), 5.36 (dtt, 1H, 10.8, 7.1, 3.3), 5.17 (dd, 1H, *J* = 7.7, 5.8), 3.87 (ddd, 1H, 10.4, 7.0, 3.1), 2.50 (dt, 1H, *J* = 17.7, 6.1), 2.43 (ddd, 1H, *J* = 17.7, 8.7, 7.0), 2.02 (bt, 2H, 7.4), 2.0 (m, 2H), 1.6–1.9 (m, 4H), 1.2–1.3 (m, 8H), 1.11 (m, 1H), 0.86 (t, 3H, *J* = 6.7), 0.80 (dt, 1H, *J* = 8.8, 5.3), 0.70 (dt, 1H, *J* = 8.6, 5.4).

Formation of Bis(*p*-bromobenzoyl)constanolactone B (14) and Bis(*p*-bromobenzoyl)constanolactone D (21). A 85:15 mixture of **7** and **18** (11.2 mg, *ca.* 33 μmol) was treated with 103 mg of 4-bromobenzoyl chloride (98%, 0.460 mmol) and a catalytic amount of 4-(dimethylamino)pyridine in dry CH_2Cl_2/triethylamine (5:1, 12 mL). The solution was stirred under N_2 at rt for 22 h. The solvents were evaporated under vacuum and the products partitioned (4×) between Et_2O and dil $NaHCO_3$ (pH *ca.* 10 by paper). The dried Et_2O extract was triturated with hexanes and purified by preparative TLC (1:1, Et_2O/Bz). The major UV-active band was removed and eluted with EtOAc, and the solvent was evaporated *in vacuo* and further purified by NP-HPLC (Versapack Si, 2 × 300 mm × 4.1 mm, 30% EtOAc/hexanes, 2.0 mL/min) to yield 8.6 mg of bis(*p*-bromobenzoyl)constanolactone B (**14**, 12.3 μmol, 44% yield) and 1.5 mg bis(*p*-bromobenzoyl)constanolactone D (**21**,

2.2 μmol, 44% yield). Pure bis(*p*-bromobenzoyl)constanolactone B (**14**) was an oil: IR (neat) 2955, 2928, 1720, 1590, 1397, 1267, 1236 cm^{-1}; UV (MeOH) λ_{max} 246 nm (log ε 4.58); CD (MeOH) Δε +6.3 (λ_{max} 250 nm); ^1H NMR: (300 MHz, $CDCl_3$) δ 7.92 (3 broad lines, 4H), 7.59 (4 broad lines, 4H), 5.91 (m, 2H, *J* = 15.7, 6.1, 0.9), 5.53 (m, 1H), 5.50 (dt, 1H), 5.35 (dt, 1H), 5.07 (dd, 1H, *J* = 8.4, 2.5), 3.90 (ddd, 1H), 2.4–2.6 (m, 4H), 2.0 (q, 2H), 1.6–1.9 (m, 4H), 1.37 (td, 1H), 1.2–1.3 (m, 7H), 0.86 (t, 3H, *J* = 7.0), 0.78 (dt, 1H, *J* = 8.8, 5.3), 0.68 (dt, 1H, *J* = 8.6, 5.4).

12-Acetoxy-9-(*p*-bromobenzoyl)constanolactone ([illegible]**):** oil; IR (neat) 2955, 2926, 1736, 1725, 1519, 1268, 1237 cm^{-1}; UV (MeOH) λ_{max} 246 nm (log ε 4.47); CD (EtOH) Δε −7.3 (λ_{max} 244.5 nm); ^1H NMR: (300 MHz, $CDCl_3$) δ 7.92 (dt, 2H, *J* = 8.6, 2.1), 7.59 (dt, 2H, *J* = 8.6, 2.1), 5.88 (bdd, 1H, *J* = 16.0, 5.8), 5.78 (bdd, 1H, *J* = 16.0, 5.3), 5.48 (bdt, 1H, *J* = 10.9, 7), 5.30 (m, 1H), 5.30 (m, 1H), 5.17 (dd, 1H, *J* = 7.7, 5.3), 3.85 (m, 1H), 2.57 (bdt, 1H, *J* = 18, 6.8), 2.45 (ddd, 1H, *J* = 18, 8.6, 6.8), 2.39 (m, 2H), 2.07 (s, 3H), 1.99 (m, 2H), 1.95 (m, 1H), 1.95 (m, 1H), 1.84 (m, 1H), 1.65 (m, 1H), 1.25–1.35 (m, 8H), 1.12 (ddd, 1H, *J* = 11.4, 9.6, 5.1), 0.87 (t, 3H, *J* = 6.9), 0.81 (dt, 1H, *J* = 8.8, 5.3), 0.68 (dt, 1H, *J* = 8.5, 5.4); CIMS (CH_4, positive ion) *m*/*z* $[M + H]^+$ 561 (8), $[M + H - AcOH]^+$ 501 (17), $[M + H - (BrC_6H_4CO_2H)]^+$ 361 (35), $[M + H - (BrC_6H_4CO_2H) - AcOH]^+$ 301 (100), $[BrC_6H_4CO_2H + H]^+$ 201 (11).

Formation of 9-(*p*-bromobenzoyl)constanolactone A (16). Constanolactone A (**6**) (2.5 mg, 7.9 μmol) was dissolved in dry CH_2Cl_2/triethylamine (5:1, 12 mL) and small portions of 4-bromobenzoyl chloride (98%) (typically 3 to 10 mg) were added regularly over a period of 5 days while the reaction was stirred at rt (23°). The solvents were evaporated under vacuum and the products were dissolved in hexanes and applied to a small silica flash column. The fraction eluting with 100% Et_2O was evaporated *in vacuo* and further purified by NP-HPLC (10-μm Phenomenex Maxsil Si column; 500 × 10.0 mm; 50% (v/v) EtOAc in hexanes; UV detection at 254 nm; flow rate at 8.0 mL/min) to give *ca.* 0.5 mg of pure 9-(*p*-bromobenzoyl)constanolactone A (**16**, 1.0 μmol, *ca.* 13% yield): oil; IR (neat) 3400, 2956, 2926, 1716, 1589, 1268, 1241 cm^{-1}; UV (MeOH) λ_{max} 245 nm (log ε 4.34); CD (EtOH) Δε −4.6 (λ_{max} 242.0 nm); ^1H NMR: (300 MHz, $CDCl_3$) δ 7.92 (bd, 2H, *J* = 8.6), 7.59 (bd, 2H, *J* = 8.6), 5.95 (bdd, 1H, *J* = 15.7, 5.7), 5.83 (bdd, 1H, *J* = 15.7, 6.4), 5.55 (bdt, 1H, *J* = 10.9, 7.4), 5.38 (bdt, 1H, *J* = 10.9, 7.2), 5.07 (dd, 1H, *J* = 8.3, 6.2), 4.20 (bq, 1H, *J* = 6.1), 3.78 (m, 1H), 2.57 (m, 1H), 2.45 (m, 1H), 2.33 (m, 2H, *J* = 6), 1.99 (m, 2H), 1.95 (m, 1H), 1.95 (m, 1H), 1.84 (m, 1H), 1.65 (m, 1H), 1.25–1.35 (m, 8H), 1.09 (m, 1H), 0.87 (t, 3H, *J* = 6.9), 0.9 (m, 1H), 0.69 (dt, 1H, *J* = 8.5, 5.3); CIMS (CH_4, positive ion) *m*/*z* $[M + H]^+$ 519 (5), $[M + H - H_2O]^+$ 501 (11), 347 (27), $[M + H - (BrC_6H_4CO_2H)]^+$ 319 (92), $[M + H - (BrC_6H_4CO_2H) - H_2O]^+$ 301 (100), 282 (11), $[BrC_6H_4CO_2H + H]^+$ 201 (97), 163 (43).

Constanolactone C Peracetate (19): oil; $[\alpha]_D$ 0° (*c* 0.62, MeOH); IR (neat) 3446, 2960, 2931, 2859, 1737, 1733, 1662, 1646, 1372, 1235, 1037, 1023 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data in Table 1; ^1H NMR (400 MHz, C_6D_6) δ 6.13 (ddd, 1H, *J* = 15.3, 5.9, 1.3), 5.92 (ddd, 1H, *J* = 15.3, 5.0, 1.0), 5.58 (m, 2H), 5.5 (m), 5.43 (m, 2H), 4.99 (dd, 1H, *J* = 8.7, 5.0), 2.86 (m, 3H), 2.62 (dt, 1H, *J* = 14.6, 6.5), 2.52 (dt, 1H, *J* = 14.6, 6.4), 2.04 (m, 2H), 1.96 (m, 1H), 1.80 (s, 3H), 1.76 (s, 3H), 1.32 (m, 1H), 1.27 (m, 1H), 1.17 (m, 1H), 1.01 (m, 1H), 0.92 (t, 3H, *J* = 7.5), 0.89 (m, 1H), 0.61 (m, 2H), 0.20 (dd, 1H, *J* = 8.3, 1.4); GC-EIMS (70 eV) *m*/*z* $[M + H_2O]^+$ 436 (1), $[M + H - AcOH]^+$ 359 (1), 309 (10), $[M - 2(AcOH)]^+$ 298 (12), 267 (11), 231 (28), 207 (63), $[M - 2(AcOH) - C_8H_{13}]^+$ 189 (100), 171 (40), 129 (44), 99 (55), 91 (61); CIMS (CH_4, positive ion)$[M + H]^+$ *m*/*z* 419 (10), $[M + H - AcOH]^+$ 359 (35), $[M + H - 2(AcOH)]^+$ 299 (100); HRCIMS (CH_4, positive ion), obs $[M + H]^+$ 419.2433, calc for $C_{24}H_{35}O_6$ 419.2433.

Constanolactone D Peracetate (20): oil; $[\alpha]_D$ −4.7° (*c* 0.26, $CHCl_3$), $[\alpha]_D$ +0.2° (*c* 0.88, MeOH); IR (neat) 2960, 2931, 1737, 1372, 1237, 1037, 1021, 969 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data in Table 1; ^1H NMR (400 MHz, C_6D_6) δ 5.85 (m, 2H), 5.5 (m, 2H), 5.4 (m, 2H), 4.99 (dd, 1H, *J* = 7, 5), 2.93 (m, 1H), 2.80 (t, 2H, *J* = 7), 2.50 (dt, 1H, *J* = 15, 7), 2.35 (dt, 1H, *J* = 15, 7), 2.0 (m, 4H), 1.87 (s, 3H), 1.72 (s, 3H), 1.3 (m, 1H), 1.25 (m, 1H), 1.05 (m, 4H), 0.92 (t, 3H, *J* = 7.5), 0.9 (m, 1H), 0.28 (dt, 1H, *J* = 8.5, 5.0), 0.20 (dt, 1H, *J* = 8.5, 5.0); GC-EIMS (70 eV)$[M + H_2O]^+$ *m*/*z* 436 (1), $[M + H - AcOH]^+$ 359 (1), 341 (3), 309 (8), $[M - 2(AcOH)]^+$ 298 (15), 281 (9), 267 (9), 231 (24), 207 (93), $[M - 2(AcOH) - C_8H_{13}]^+$ 189 (90), 171 (41), 129 (43), 117 (47), 109 (52), 107 (53), 99 (69), 91 (68), 81 (100); FAB-MS (glycerol, positive ion) *m*/*z* 461 (2), $[M + H]^+$ 419 (2), $[M + H - AcOH]^+$ 359 (1), $[M - 2(AcOH)]^+$ 299 (2), 185 (40), 93 (90), 79 (76), 67 (100), 55 (75); FABMS (glycerol, positive ion), obs 419.2434, calc for $C_{24}H_{35}O_6$ 419.2434 $[M + H]^+$.

Bis(*p*-bromobenzoyl)constanolactone D (21). Formed as described above during formation of derivative **14**. Pure derivative **21** was an oil: IR (neat) 3450, 2959, 2931, 1719, 1590, 1484, 1398, 1267, 1238, 1102, 1012, 969, 757 cm^{-1}; UV (MeOH) λ_{max} 246 nm (log ε 4.55); CD (MeOH) Δε +6.7 (λ_{max} 272 nm); ^1H NMR (300 MHz, $CDCl_3$) δ 7.92 (3 broad lines, 4H), 7.59 (4 broad lines, 4H), 5.89 (m, 2H), 5.55 (m, 1H), 5.5 (dt, 1H), 5.35 (dt, 1H), 5.30 (m, 1H), 5.25 (dtt, 1H, *J* = 10.7, 7.0), 5.07 (bdd, 1H, *J* = 8.4, 2.5), 3.89 (ddd, 1H), 2.77 (bt, 2H, *J* = 6.9), 2.4–2.6 (m, 4H), 2.02 (m, 2H), 1.6–1.9 (m, 4H), 1.35 (m, 1H), 1.23 (m, 1H), 0.94 (t, 3H, *J* = 7.5), 0.79 (dt, 1H, *J* = 8.7, 5.4, 5.4), 0.68 (dt, 1H, *J* = 8.9, 5.4, 5.3); ^{13}C NMR (75 MHz, ^{13}C DEPT 135°, $CDCl_3$) δ 132.18 (CH), 131.69 $(CH)_2$, 131.62 $(CH)_2$, 131.15 $(CH)_2$, 131.09 $(CH)_2$, 130.48 (CH), 129.51 (CH), 126.45 (CH), 123.32 (CH), 122.80 (CH), 81.29 (CH), 76.49 (CH), 73.78 (CH), 32.30 (CH_2), 29.42 (CH_2), 27.83 (CH_2), 25.56 (CH_2), 21.60 (CH), 20.46 (CH_2), 19.72 (CH), 18.25 (CH_2), 14.14 (CH_3), 6.98 (CH); FABMS (3-nitrobenzyl alcohol, negative ion) $[M - H + 3\text{-NBA}]^-$ *m*/*z* 851/853/855 (1:2:1), 778/780/782 (2:2:1), 744/746/ 748 (0.5:1:0.5), 713/715/717 (0.5:1:0.5), 325.2 (2), 311.1 (1), 198.9/201 (100:93), 79/81 (69:64).

Isolation of MeOH Solvolysis Products 22 and 23. Frozen *C. simplex*, collected May 5, 1992 (250 g dry weight), was repetitively extracted (3×) with warm $CHCl_3$/MeOH (2:1) to yield 2.26 g of dark green oil. The extract was subjected to silica gel vacuum chromatography, using a stepwise gradient from 0 to 100% (v/v) EtOAc in hexanes. Fractions eluting with 20 to 30% (v/v) EtOAc in hexanes were determined by TLC to be of similar composition and were pooled. This combined fraction was further purified by silica gel flash chromatography, using a stepwise gradient from 1.5 to 50% (v/v) 2-propanol (IPA) in hexanes, MeOH flush. The fractions eluting with IPA concentrations greater than 20% were subjected to preparative NP-HPLC (10-μm Phenomenex Maxsil Si column; 500 × 10 mm; 20% (v/v) IPA in H_2O; differential refractometer detection; flow rate at 3.0 mL/min) followed by an analytical HPLC separation (dual 10-μm Alltech Versapak Si columns; 300 × 4.1 mm; 15% (v/v) IPA in hexanes; differential refractometer detection; flow rate 4.0 mL/min) to yield two minor epimeric compounds (22 0.5 mg, 0.02% and 23 1.1 mg, 0.05%).

9-*O*-Methylconstanolactone A (22): oil; $[\alpha]_D$ +7.3° (*c* 0.21, MeOH); IR (neat) 3421, 2955, 2925, 1733, 1724, 1718, 1242, 1080, 1047, 1034, 973 cm^{-1}; ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data in Table 3; GC-EIMS (70 eV) *m*/*z* $[M - MeOH - H_2O]^+$ 300 (0.7), $[M - C_8H_{15}]^+$ 239 (22), $[M - H_2O - C_8H_{15}]^+$ 221 (4), $[M - C_8H_{15} - MeOH]^+$ 207 (99), $[M - C_8H_{15} - MeOH - H_2O]^+$ 189 (24), 161 (24), 113 (100), 109 (64), 99 (64); FABMS (sulfolane, negative ion), obsd 349.2379 calc for $C_{21}H_{33}O_4$ 349.2379 $[M - H]^-$.

9-*O*-Methylconstanolactone B (23): oil; IR (neat) 3421, 2958, 2927, 2857, 1728, 1724, 1287, 1275, 1080, 1074 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) data in Table 2; GC-EIMS (70 eV)-$[M - AcOH]^+$ *m*/*z* 332 (2), $[M - MeOH - AcOH]^+$ 300 (4), $[M - C_8H_{15}]^+$ 281 (5), 249 (4), 239 (24), $[M - AcOH - C_8H_{15}]^+$ 221 (32), $[M - C_8H_{15} - MeOH - AcOH]^+$ 207 (27), 205 (29), $[M - C_8H_{15} - MeOH - AcOH]^+$ 189 (16), 183 (15), 161 (12), 155 (19), 127 (19), 113 (100), 109 (27), 99 (24).

Isolation of Constanolactones E (24) and F (25). As above, **24** and **25**, were reisolated as natural products for further investigation. Frozen *C. simplex* (790 g of dry weight) was repetitively extracted (3×, as above). The extract was subjected to silica gel vacuum chromatography, using a stepwise gradient from 10 to 100% (v/v) EtOAc in hexanes. The fraction eluting with 50% (v/v) EtOAc in hexanes (60.3 mg) was subjected to NP-HPLC (10-μm Phenomenex Maxsil

Si column; 500 × 10 mm; 20% (v/v) 2-propanol in hexanes; differential refractometer detection; flow rate at 6.0 mL/min). A second NP-HPLC (dual 10-μm Alltech Versapak Si columns; 300 × 4.1 mm; 10% (v/v) EtOAc in hexanes; differential refractometer detection; flow rate 3.0 mL/min) was necessary to separate constanolactone F (**24** 9.0 mg) from G (**25** 3.2 mg).

Constanolactone E (24): oil; $[\alpha]_D$ +33° (*c* 0.22, MeOH); IR (neat) 3427, 2956, 2927, 1732, 1244, 1048, 1038, 966 cm^{-1}; ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data in Table 3; CIMS (CH_4, positive ion) $[M + H]^+$ *m/z* 337 (14), $[M + H - H_2O]^+$ 319 (100), $[M + H - 2(H_2O)]^+$ 301 (96), 197 (46), 179 (66), 161 (40), 151 (18), 123 (18), 111 (14); HRCIMS (CH_4, positive ion), obs $[M + H]^+$ 337.2377, calc for $C_{20}H_{33}O_4$ 337.2375, obs $[M + H - H_2O]^+$ 319.2273, calc for $C_{20}H_{31}O_3$ 319.2273.

Constanolactone F (25): oil; IR (neat) 3422, 2956, 2925, 1731, 1243, 1080, 1044, 1038, 963 cm^{-1}; ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data in Table 3; CIMS (CH_4, positive ion) $[M + H - H_2O]^+$ *m/z* 319 (96), $[M + H - 2(H_2O)]^+$ 301 (100), 254 (14), $[M + H - C_8H_{15}]^+$ 225 (7), 197 (26), 179 (78), 161 (40), 147 (31), 129 (27), 123 (62), 111 (20).

Isolation of Constanolactones E–G Peracetate Derivatives (27–29). A silica gel vacuum chromatographic fraction (459 mg, 50% (v/v) EtOAc in hexanes) from the same collection of *C. simplex* which yielded **8** and **9** was subjected to a second silica gel vacuum chromatography, using a stepwise gradient from 0 to 100% (v/v) EtOAc in hexanes. The fraction eluting with 45% (v/v) EtOAc in hexanes (26.9 mg) was dissolved in 0.5 mL of pyridine and stirred with 0.5 mL of Ac_2O at rt overnight (27.5 h). Three new peracetate derivatives **27** (6.8 mg, 0.27%), **28** (3.2 mg, 0.13%), and **29** (0.5 mg, 0.02%) were subsequently isolated by NP-HPLC (dual 10-μm Alltech Versapak Si columns; 300 × 4.1 mm; 35% (v/v) EtOAc in hexanes; differential refractometer detection; flow rate 5.0 mL/min).

Constanolactone E Peracetate (27): oil; $[\alpha]_D$ −17.4° (*c* 0.41, MeOH); IR (neat) 3378, 2955, 2928, 2855, 1736, 1370, 1226, 1037, 963 cm^{-1}; ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data in Table 3; ^1H NMR (300 MHz, C_6D_6) δ 5.70 (dd, 1H, *J* = 14.8, 8.3), 5.62 (dd, 1H, *J* = 8.3, 6.9), 5.5 (dd, 1H, *J* = 10, 7), 5.45 (m, 1H), 5.4 (m, 1H), 5.29 (dd, 1H, *J* = 14.8, 8.7), 2.96 (ddd, 1H, *J* = 9.8, 7.1, 2.7), 2.45 (dt, 1H, *J* = 14.8, 7.8), 2.33 (dt, 1H, *J* = 14.8, 5.8), 2.02 (m, 2H), 1.97 (m, 2H), 1.84 (s, 3H), 1.72 (s, 3H), 1.3 (m, 3H), 1.25 (m, 4H), 1.1 (m, 2H), 0.94 (m, 2H), 0.88 (t, 3H, *J* = 6.7), 0.63 (m, 1H), 0.32 (m, 2H); ^{13}C NMR (75 MHz, C_6D_6) δ 169.88, 169.37, 169.24, 139.32, 133.38, 124.11, 122.82, 81.49, 75.23, 73.85, 31.76, 29.56, 29.47, 28.60, 27.72, 27.62, 25.08, 22.94, 20.79, 19.34, 18.40, 10.47; GC-EIMS (70 eV) $[M - AcOH]^+$ *m/z* 360 (1.4), 318 (10), $[M - C_8H_{15}]^+$ 309 (4), $[M - 2(AcOH)]^+$ 300 (18), 281 (14), 267 (15), 207 (33), $[M - 2(AcOH) - C_8H_{13}]^+$ 191 (30), 178 (56), 161 (19), 149 (35), 131 (26), 117 (37), 94 (100).

Constanolactone F Peracetate (28): oil; $[\alpha]_D$ +55° (*c* 0.19, MeOH); IR (neat) 3390, 2955, 2930, 2857, 1739, 1372, 1229, 1037, 958 cm^{-1}; ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) data in Table 3; ^1H NMR (300 MHz, C_6D_6) δ 5.62 (dd, 1H, *J* = 7.5, 5.5), 5.52 (m, 1H), 5.27 (dd, 1H, *J* = 12.6, 5.5), 5.49 (m, 2H), 5.34 (dd, 1H, *J* = 15.2, 8.5), 3.01 (m, 1H), 2.40 (m, 2H), 2.00 (m, 2H), 1.97 (m, 2H), 1.79 (s, 3H), 1.70 (s, 3H), 1.3 (m, 3H), 1.24 (m, 4H), 1.10 (m, 2H), 0.92 (m, 2H), 0.87 (t, 3H, *J* = 6.8), 0.66 (m, 1H), 0.36 (dt, 1H, *J* = 8.4, 5.2), 0.29 (dt, 1H, *J* = 8.8, 5.0); ^{13}C NMR (75 MHz, DEPT (135°, C_6D_6) δ 138.18 (CH), 133.27 (CH), 125.39 (CH), 123.39 (CH), 81.02 (CH), 74.06 (CH), 73.68 (CH), 31.51 (CH_2), 29.28 (CH_2), 28.78 (CH_2), 27.39 (CH_2), 27.39 (CH_2), 24.74 (CH), 20.40 (CH_3), 20.40 (CH_3), 18.79 (CH), 18.12 (CH_2), 13.99 (CH_3), 10.16 (CH_2); CIMS (CH_4, positive ion) $[M + H]^+$ *m/z* 421 (13), $[M + H - AcOH]^+$ 361 (38), 329 (13), 319 (15), $[M + H - 2(AcOH)]^+$ 301 (100), 283 (8); HRCIMS (CH_4, positive ion), obs $[M + H]^+$ 421.2590, calc for $C_{24}H_{37}O_6$ 421.2590.

Constanolactone G Peracetate (29): oil; IR (neat) 3433, 2958, 2918, 2850, 1736, 1712, 1650, 1646, 1366, 1225, 1039 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.45 (m, 1H), 5.40 (dd, 1H, *J* = 10.9, 7.4), 5.4 (m, 2H), 5.25–5.45 (m, 2H), 5.3 (m, 1H), 5.02 (dt, 1H, *J* = 6.7, 5.9), 3.75 (ddd, 1H, *J* = 10.8, 7.4, 3.1), 2.75 (bt, 2H, *J* = 7.2), 2.57 (ddd, 1H, *J* = 17.8, 7.5, 6.5), 2.45 (ddd, 1H, *J* = 17.8, 8.6, 6.9), 2.33 (bt, 2H, *J* = 6.9), 2.07 (s, 3H), 2.06 (m, 2H), 2.05 (s, 3H), 2.0 (m, 1H), 1.95 (m, 1H), 1.8 (m, 1H), 1.7 (m, 1H), 1.54 (m, 1H), 1.13 (m, 1H), 0.97 (t, 3H, *J* = 7.6), 0.76 (dt, 1H, *J* = 8.6, 5.2), 0.69 (dt, 1H, *J* = 8.7, 5.2); (400 MHz, C_6D_6) δ 5.63 (dd, 1H, *J* = 7.4, 5.8), 5.5 (m, 3H), 5.4 (m, H), 5.36 (dd, 1H, *J* = 16.3, 6.9), 5.28 (dt, 1H, *J* = 6.9, 5.8), 3.00 (m, 1H), 2.80 (m, 2H, *J* = 6.2), 2.4 (m, 2H), 2.0 (m, 6H), 1.78 (s, 3H), 1.69 (s, 3H), 1.2–1.37 (m, 1H), 1.06 (m, 1H), 0.91 (t, 3H, *J* = 7.6), 0.9 (m, 1H), 0.66 (m, 1H), 0.37 (dt, 1H, *J* = 8.6, 5.2), 0.29 (dt, 1H, *J* = 8.7, 5.2); ^{13}C NMR (100 MHz, C_6D_6) δ 169.71, 169.24, 168.64, 138.50, 132.31, 131.71, 124.15, 123.67, 81.20, 74.38, 73.82, 30.14, 29.50, 29.05, 27.60, 25.98, 25.04, 21.50, 20.88, 20.63, 19.04, 18.40, 14.41, 10.43; FABMS (2:1 thioglycerol:glycerol, positive ion) $[M + H]^+$ *m/z* 419.2 (34), $[M + H - AcOH]^+$ 391.3 (22), 359.2 (14), 317.2 (20), $[M + H - 2(AcOH)]^+$ 299.2 (82), 281.1 (14), 215.1 (21), 207.1 (24), 149.1 (20), 121.1 (23), 109.1 (37), 95.1 (48), 81.1 (65), 69.1 (79), 55.1 (100); HRFABMS obs $[M + H - 2(AcOH)]^+$ at 299.2012, calc for $C_{20}H_{27}O_2$ 299.2011.

Formation of the Acetonide of Methyl Constanolactone E (30). A catalytic amount of *p*-toluenesulfonic acid was added to 1.1 mg of **24** (3.3 μmol) dissolved in 2,2-dimethoxypropane (0.5 mL). The solution was stirred at rt for 105 min. Triethylamine (50 μL) was then added to increase the pH prior to evaporation under vacuum. The products were dissolved in hexanes and kept basic with the addition of triethylamine (10 mL) and applied to a small silica flash column. The fraction eluting with 10% (v/v) EtOAc in hexanes was further purified by NP-HPLC (10-μm Alltech Versapak, 2 × 300 mm × 4.1 mm; 25% (v/v) EtOAc in hexanes, 2.0 mL/min) to give *ca.* 1 mg of pure methylated acetonide product **30** (2.5 μmol, 75% yield) as an oil: IR (neat) 3485, 2986, 2955, 2931, 2859, 1740, 1457, 1437, 1379, 1368, 1246, 1216, 1166, 1080, 1056, 1012, 967 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.54 (dd, 1H, *J* = 15.2, 8.2), 5.49 (m, 1H), 5.37 (dd, 1H, *J* = 15.2, 8.4), 5.35 (m, 1H), 4.48 (dd, 1H, *J* = 8.2, 6.2), 4.10 (dt, 1H, *J* = 8.0, 6.2), 3.67 (s, 3H), 3.06 (m, 1H), 2.35 (t, 2H, *J* = 7.3), 2.28 (dt, 1H, *J* = 14.6, 8.0), 2.15 (dt, 1H, *J* = 14.6, 6.2), 2.04 (bq, 2H, *J* = 7.2), 1.77 (m, 2H), 1.60 (m, 2H), 1.48 (s, 3H), 1.44 (m, 1H), 1.35 (m, 2H), 1.34 (s, 3H), 1.3 (m, 4H), 1.00 (7 lines, 1H), 0.89 (t, 3H, *J* = 6.8), 0.70 (dt, 1H), 0.67 (dt, 1H); GC-EIMS (70 eV) $[M - CH_3]^+$ *m/z* 393 (1.0), $[M - H_2O]^+$ 390 (1.5), 361 (2), $[M - CH_3 - (CH_3)_2CO_2]^+$ 334 (1.5), $[M - CH_3 - (CH_3)_2CO_2 - H_2O]^+$ 301 (4), 250 (7), 236 (7), 221 (9), 207 (15), 189 (23), 178 (19), 161 (23), 147 (27), 131 (55), 119 (25), 105 (28), 99 (100).

Formation of Acetonide of Methyl Constanolactone F (31). A catalytic amount of *p*-toluenesulfonic acid was added to 4.2 mg of **25** were dissolved in 2,2-dimethoxypropane (0.5 mL). The solution was stirred at rt (23°) for 105 min. Triethylamine (50 μL) was then added to increase the pH prior to evaporation under vacuum. The products were dissolved in hexanes and kept basic with the addition of triethylamine (10 μL) and fractionated over a small silica flash column. A fraction eluting with 10–100% (v/v) EtOAc in hexanes was further purified by NP-HPLC (10-μm Alltech Versapak, 2 × 300 × 4.1 mm, 25% (v/v) EtOAc in hexanes, 2.0 mL/min) to give 2.0 mg of pure methylated acetonide product **31** (5 μmol, 40% yield) as an oil: IR (neat) 3485, 2985, 2955, 2930, 2859, 1740, 1719, 1457, 1437, 1377, 1370, 1241, 1230, 1222, 1171, 1073, 1054, 1023, 967 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.55 (m, 1H), 5.49 (bdd, 1H, *J* = 15.3, 7.8), 5.4 (m, 1H), 5.35 (dd, 1H, *J* = 15.3, 8.5), 3.98 (t, 1H, *J* = 8.1), 3.70 (dt, 1H, *J* = 8.1, 5.7), 3.67 (s, 3H), 3.03 (m, 1H), 2.35 (t, 2H, *J* = 7.3), 2.32 (m, 2H), 2.01 (bq, 1H, *J* = 6.9), 1.77 (m, 2H), 1.60 (m, 2H), 1.403 (s, 3H), 1.396 (s, 3H), 1.35 (m, 2H), 1.3 (m, 4H), 1.0 (7 lines, 1H), 0.89 (t, 3H, *J* = 6.8), 0.71 (dt, 1H, *J* = 8.6, 5.2), 0.65 (dt, 1H, *J* = 8.6, 5.2); GC-EIMS (70 eV) $[M]^+$ *m/z* 408 (1), $[M - H_2O]^+$ 390 (1), 361 (1), $[M - CH_3 - (CH_3)_2CO_2]^+$ 334 (2), $[M - CH_3 - (CH_3)_2CO_2]^+$ 319 (2), $[M - CH_3 - (CH_3)_2CO_2 - H_2O]^+$ 301 (4), 250 (9), 236 (8), 221 (10), 207 (15), 189 (27), 178 (38), 161 (26), 147 (29), 131 (58), 119 (30), 109 (46), 105 (29), 99 (100).

Formation of Bis(*p*-bromobenzoyl)constanolactone E (32). Constanolactone E (**24**, 9.9 mg, 29 μmol), 100.7 mg of 4-bromobenzoyl chloride (98%, 0.45 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in dry CH_2Cl_2/triethylamine (5:1, 12.0 mL). The solution was purged with N_2 and stirred at rt (23°) for 18 h. The solvents were

evaporated under vacuum and the products were dissolved in hexanes and fractionated over a small silica gel flash column. The fractions eluting with Et_2O were further purified by NP-HPLC (10-μm Phenomenex Maxsil Si column, 500 × 10.0 mm, 30% (v/v) EtOAc in hexanes, UV detection at 254 nm, flow rate 8.0 mL/min) to give 10.8 mg of the pure bis(*p*-bromobenzoate) **32** (15 μmol, 53% yield) as an oil: UV (EtOH) λ_{max} 246 nm (log ϵ 4.56); CD (EtOH) $\Delta\epsilon$ +9.1 (λ_{max} 252.5 nm); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (bd, 2H, J = 8.5, 1.7), 7.82 (bd, 2H, J = 8.5, 1.7), 7.59 (bd, 2H, J = 8.5, 1.7), 7.55 (bd, 2H, J = 8.5, 1.7), 5.71 (dd, 1H, J = 15.0, 8.0), 5.63 (dd, 1H, J = 8.0, 3.7), 5.50 (dd, 1H, J = 15.0, 8.4), 5.40 (m, 2H), 5.35 (m, 1H), 3.77 (ddd, 1H, J = 10.4, 7.7, 3.0), 2.56 (dt, 1H, J = 17.6, 6.7), 2.5 (m, 2H), 2.45 (ddd, 1H, J = 17.6, 8.6, 6.8), 2.0 (m, 3H), 1.9 (m, 1H), 1.8 (m, 1H), 1.6 (m, 2H), 1.35 (m, 2H), 1.3 (m, 4H), 1.09 (7 lines, 1H), 0.85 (t, 3H, J = 6.7), 0.79 (dt, 1H, J = 8.6, 5.2), 0.71 (dt, 1H, J = 8.6, 5.2); ^{1}H NMR (300 MHz, C_6D_6) δ 7.91 (d, 2H, J = 8.4), 7.83 (d, 2H, J = 8.2), 7.29 (d, 2H, J = 8.2), 7.16 (d, 2H, J = 8.4), 5.97 (dd, 1H, J = 8.0, 4.5), 5.85 (dd, 1H, J = 15.0, 8.3), 5.76 (dt, 1H, J = 7.6, 4.5), 5.53 (m, 2H), 5.45 (dd, 1H, J = 15.0, 8.8), 3.02 (m, 1H), 2.65 (dt, 1H, J = 14.0, 7.4), 2.53 (m, 1H), 2.06 (bq, 2H, J = 6.9), 2.00 (bq, 2H, J = 6.8), 1.42 (m, 1H), 1.32 (m, 2H), 1.25 (m, 4H), 1.12 (m, 2H), 0.97 (m, 2H), 0.91 (t, 3H, J = 6.8), 0.67 (m, 1H), 0.39 (m, 2H); CIMS (CH_4, positive ion) $[M]^+$ m/z 700 (3), 623 (2), $[M + H - BrC_6H_4CO_2H]^+$ 501 (15), $[M + H - 2(BrC_6H_4CO_2H)]^+$ 301 (72), $[M + H - 2(BrC_6H_4CO_2H) - H_2O]^+$ 283 (8), 229 (8), $[BrC_6H_4CO_2H+H]^+$ 201 (82), 183 (15), 157 (19), 123 (100).

Formation of Bis(*p*-bromobenzoyl)constanolactone F (33). Constanolactone F (**25**) (1.4 mg, 0.0042 mmol), 23.8 mg of 4-bromobenzoyl chloride (98%, 0.106 mmol), and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in dry CH_2Cl_2/triethylamine (5:1, 6.0 mL). The solution was purged with N_2 and stirred at rt (23°) for 17 h. The solvents were evaporated under vacuum and the products were dissolved in hexanes and fractionated over a small silica flash column. The fractions eluting with 36–80% Et_2O in hexanes were further purified by NP-HPLC (10-μm Alltech Versapak, 2 × 300 × 4.1 mm, 30% (v/v) EtOAc in hexanes; 254 nm detection, 2.0 mL/min) to give *ca.* 0.5 mg pure bis(*p*-bromobenzoate) product **33** as an oil: UV (EtOH) λ_{max} 246 nm (log ϵ 4.29); CD (EtOH) $\Delta\epsilon$ +5.4, −11.8 (λ_{max} 255, 240 nm); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (bd, 4H, J = 8.4), 7.51 (bd, 4H, J = 8.4, 1.4), 5.62 (dd, 1H, J = 15, 7), 5.56 (bd, 1H, J = 7), 5.5 (m, 2H), 5.4 (m, 1H), 5.35 (m, 1H), 3.73 (m, 1H), 2.5 (m, 3H), 2.4 (m, 1H), 1.99 (m, 1H), 1.95 (m, 2H), 1.8–2.0 (m, 2H), 1.66 (m, 1H), 1.54 (m, 1H), 1.3 (m, 6H), 1.12 (m, 1H), 0.81 (t, 3H, J = 6.8), 0.76 (m, 1H), 0.67 (m, 1H); CIMS (CH_4, positive ion) m/z $[M]^+$ 700 (0.5), 623 (0.5), $[M + H - BrC_6H_4CO_2H]^+$ 501 (9), $[M + H - 2(BrC_6H_4CO_2H)]^+$ 301 (48), $[M + H - 2(BrC_6H_4CO_2H) - H_2O]^+$ 283 (6), 229 (9), $[BrC_6H_4CO_2H + H]^+$ 201 (92), 183 (16), 157 (22), 123 (100), 105 (19).

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Supplementary Material Available: Spectral data for all new compounds (^{1}H, ^{13}C NMR, NOEDS and NOESY spectra for **8** and **9**, and other miscellaneous NMR experiments), CD spectra of *p*-bromobenzoate derivatives **14**, **15**, **21**, **32**, and **33**, and GC-EIMS data for (−)-menthoxycarbonyl derivatives **11** and **12** (91 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.