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ISOLATION AND STRUCTURE OF CONSTANOLACTONES A AND B, NEW CYCLOPROPYL HYDROXY-EICOSANOIDS FROM THE TEMPERATE RED ALGA *CONSTANTINEA SIMPLEX*

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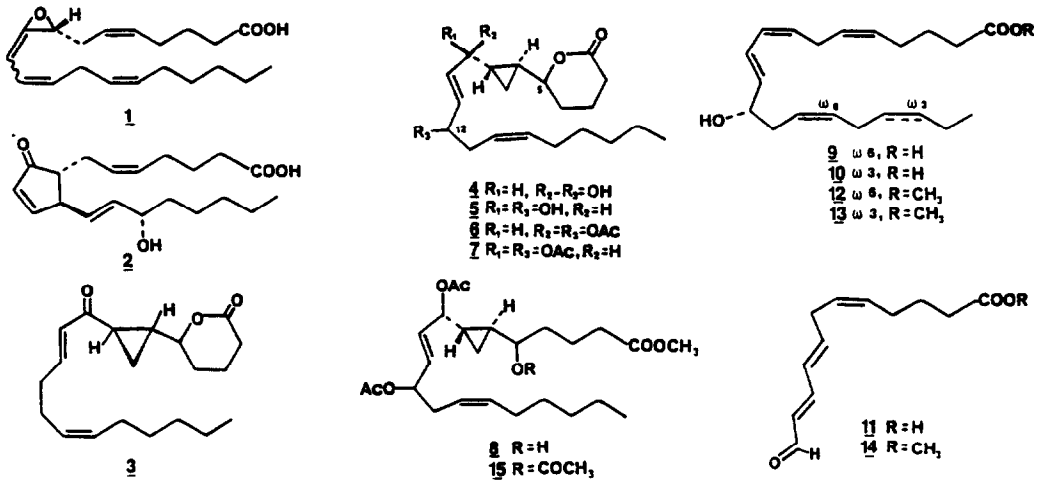
Summary: Two new cyclopropyl and lactone containing eicosanoids, constanolactone A and B, were isolated as synthetic diacetate derivatives from the temperate red alga *Constantinea simplex* and their structures determined by spectroscopic means. A hydrolysis product of one of these helped to define the lactone size in these new eicosanoids. The co-occurrence of three known 12-lipoxygenase metabolites, 12-S-HETE, 12-S-HEPE, and 12-oxododeca-5(Z), 8(E), 10(E)-trienoic acid, supports a 12-lipoxygenase origin for the new compounds.

The metabolism of arachidonic acid (AA) by marine organisms is unique in that lipoxygenase pathways are widely used to produce a diversity of novel products, including oxidized AA derivatives from red algae[2-7] and carbocyclized AA derivatives from marine corals and algae.[8-11] Examination of the mechanistic details of prostaglandin biosynthesis in one coral, *Plexaura homomalla*,[11] has been shown to involve the intermediacy a novel 8-lipoxygenase product, allene oxide 1.[12] Further, both boiled and unboiled acetone powders from *P. homomalla* catalyze the carbocyclization of allene oxide 1 to cyclopentyl (2) and cyclopropyl (3) containing products.[13] The significance of the production of cyclopropanoid 3 to normal physiological pathways of arachidonic acid metabolism in the coral is uncertain given that it is a very minor product of these incubation experiments and apparently not an enzymatic product of 1.[13] Hence, our isolation of similar cyclopropyl lactones, constanolactones A (4) and B (5), as major natural products (3-4% of the extractable lipids) from the temperate red alga *Constantinea simplex*, suggests that such products are of physiological relevance, at least in marine plants.[14]

As part of an extensive survey of the biomedicinal potential of seaweed extracts from the Pacific Northwest of the United States, the crude lipid extract (CH_2Cl_2/MeOH 2:1) of *C. simplex* (Seal Rock, Oregon) was found by TLC to contain a diversity of likely eicosanoid-type natural products giving colorful (blue, yellow, red) $Cu(OAc)_2$ or H_2SO_4 staining reactions. Vacuum chromatography of a June collection (2.0 g oil/620 g dry wt. of alga) yielded fractions that were mixtures of related substances. Following derivatization (CH_2N_2) and NP-HPLC (5% EtOAc/hex) of one chromatographic fraction, minor amounts of three known eicosanoids, 12-(S)-HETE (9 - 2.0 mg, 0.1%), 12-(S)-HEPE (10 - 0.3 mg, 0.015%) and 12-oxo-5(Z), 8(E), 10(E) dodecatrienoic acid (11 - 0.9 mg, 0.044%), were all isolated as synthetic methyl ester derivatives (12-14). Another vacuum chromatography fraction (146 mg, 80% EtOAc/hex) was treated with Ac_2O/pyr followed by CH_2N_2/Et_2O and NP-HPLC (35% EtOAc/hex) to yield three closely related and new compounds (6 - 32.7 mg, 1.6%; 7 - 39.9 mg, 2.0%; 8 - 2.3 mg, 0.11%), the structure elucidation and biosynthetic significance of which are presented.

Constanolactone A diacetate (6), $[a]_D^{23^0}$ = -5.4° (c = 3.05, $CHCl_3$), was a colorless oil which gave a prominent $(M + H - 2HOAc)^+$ by HR CIMS (CH_4) at 301.2165 (0.3 mamu dev.) analyzing for $C_{20}H_{29}O_2$. The diacetate character of this derivative was readily apparent from NMR and



LR CIMS (obs. m/z 421 $[(M+H)^+$, 6%], 361 $[(M+H-HOAc)^+$, 50%], 301 $[(M+H-2HOAc)^+$, 100%]). Five of its seven degrees of unsaturation were accounted for by four olefinic carbons and 3 ester carbonyls (table 1), and thus, **6** was bicyclic. As derivative **6** was unreactive to CH_2N_2 and showed the C-2 protons as a complex second order pattern, one of the cycles involved lactonization of the carboxylic acid. The final degree of unsaturation was present as a cyclopropyl ring as revealed by characteristic ^1H NMR bands at 0.625, 0.726, 1.03 and 1.2 ppm. Hence, **6** was a 20-carbon eicosanoid with 1 lactone, 2 secondary acetates, 2 olefins, and a cyclopropyl ring.

The location of these functional groups followed from analysis of the 1H-1H COSY in $CDCl_3$ (table 1) in which it was possible to define the spin system throughout the entire carbon skeleton. Coupling constants for the C10-C11 (15.5 Hz) and C14-C15 (10.9 Hz) olefins defined them as E and Z, respectively. Further, couplings between the C6-C8 cyclopropyl protons defined a trans geometry ($J_{6\text{-}7a}$=5.2 Hz, $J_{6\text{-}7b}$=8.4 Hz, $J_{7a\text{-}7b}$=5.2 Hz, $J_{7a\text{-}8}$=8.8 Hz, $J_{7b\text{-}8}$=5.2 Hz). Relative stereochemistry at C5, C9 and C12 remain uncertain, however, that at C12 is probably 'S' based on the co-occurrence of other 12-S eicosanoids in this alga.

Derivative **7** of constanolactone B (**5**), $[\alpha]_D^{23^0}$ = -4.8^0 (c = 2.08, $CHCl_3$) gave a small $(M+H)^+$ at 421.2590 and a large $(M + H - 2 \times HOAc)^+$ fragment ion at 301.2168 (+ CI, CH_4) which analyzed for $C_{24}H_{37}O_6$ (0.0 mamu dev.) and $C_{20}H_{29}O_2$ (0.0 mamu dev.) by HR mass measurement, respectively. Again, the diacetate character of this derivative was readily confirmed by NMR (table 1). In fact, the spectral data for **7** were nearly identical to that obtained for derivative **6**, and extensive ^1H-^1H COSY defined the location of all functional groups in **7** just as in **6**. Differences between the two compounds in their a) coupling constants to H9 (**6**, $J_{8\text{-}9}$=6.9 Hz, $J_{9\text{-}10}$=6.2 Hz; **7**, $J_{8\text{-}9}$=7.9 Hz, $J_{9\text{-}10}$=2.7 Hz), b) ^{13}C NMR chemical shifts at C5, C7, and C9, and c) ^1H nmr shifts at H6 through H11 (table 1) indicated that constanolactone B diacetate (**7**) was the C9 epimer of constanolactone A diacetate (**6**).

Further insight as to the position of lactonization in constanolactone A, and hence of B as well, was afforded by isolation of the ring opened hydrolysis product (**8**) as an artifact (table 1). This was apparently formed during workup of the acetylation reaction since acetates were present at C9 and C12 but not at C5. Further, since **8** contained a methyl

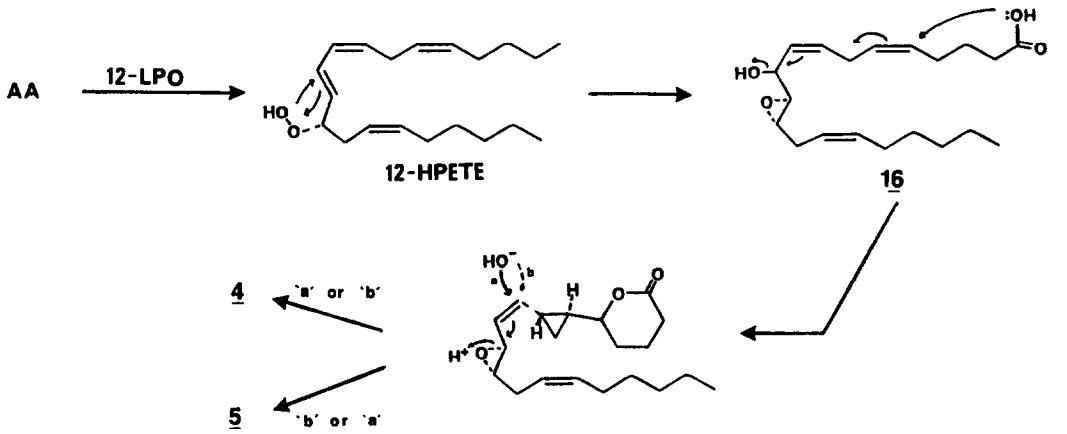
Table 1. 1H and 13C NMR Data for Diacetates of Constanolactones A (6), B (7) and hydrolysis product 8.[a]

	Derivative 6		Derivative 7		Derivative 8
#C	$^{13}C(d_6\text{-Bz})^c$	^1H ($CDCl_3$)[b]	$^{13}C(d_6\text{-Bz})^c$	^1H ($CDCl_3$)[b]	$^1H(d_6\text{-bz})^b$
1	169.78[f]	---------	169.97[f]	-------	--------
2	29.65	a)2.56 dt (J=11.6,5.6) b)2.4-2.5 m	29.47	a)2.54 m b)2.49 m	2.15 t (J=7)
3	18.38	a)1.80 m b)1.9-2.05 m	18.27	a)1.80 m b)1.97 m	a)1.75 m b)1.68 m
4	27.68[h]	a)1.98 m b)1.6-1.7 m	27.56	a)1.98 m b)1.65 m	1.45 m
5	82.07	3.80 ddd (J=10.7,7.4,3.0)	81.53	3.82 ddd (J=10.0,6.6,3.1)	2.68 dt (J=7,7)
6	22.90[d]	1.03 m	22.01[d]	1.2 m	0.55-0.68 m
7	8.1	a)0.625 ddd (J=8.8,5.2,5.2) b)0.726 ddd (J=8.4,5.2,5.2)	6.646	a)0.68 ddd (J=8.5,5.2,5.2) b)0.60 ddd (J=8.5,5.2,5.2)	a)0.3 ddd (J=8,5,5) b)0.55-0.68 m
8	21.2[d]	1.2-1.3 m	21.04[d]	1.2 m	1.0 m
9	75.91	4.90 ddd (J=6.9,6.2,1.1)	75.47	4.85 bdd (J=7.9,2.7)	4.84 dd (J=7,5,7)
10	131.45[e]	5.68 dd (J=15.5,6.2)	130.78[e]	5.72 m	5.9 dd (J=15,7)
11	129.73[e]	5.81 ddd (J=15.5,6.2,1.1)	130.28[e]	5.70 m	5.75 dd (J=15,7)
12	73.41	5.2-5.33 m	73.34	5.2 m	5.39 ddd (J=7,7,7)
13	32.72	a)2.35 m b)2.45 m	32.76	a)2.35 m b)2.4 m	a)2.35 ddd (J=14,7,7) b)2.45 ddd (J=14,7,7)
14	124.0	5.2-5.33 m	124.01	5.2 m	5.45 m
15	133.23	5.5 bdt (J=10.9,7,3)	133.33	5.5 bdt (J=10.8,7.1)	5.5 bdt (J=10,7)
16	27.69[h]	1.95-2.05 m	27.69	2.02 m	2.0 bdt (J=7,7)
17	29.48	1.21-1.36 m	29.58	1.35 m	1.3 m
18	31.78	1.21-1.36 m	31.76	1.35 m	1.3 m
19	22.93[d]	1.21-1.36 m	22.90	1.30 m	1.25 m
20	14.26	0.886 t (J=6.7)	14.24	0.885 t (J=6.7)	0.90 t (J=7)
OAc	169.50[f]	-------	169.49[f]	-------	
	20.82[g]	2.08[i]	20.89[g]	2.06s[h]	1.76 s
	169.00[f]	-------	169.45[f]	-------	
	20.71[g]	2.07[i]	20.76[g]	2.1s[h]	1.70 s
OMe					3.35

(a) Assignments are based on ^1H-^1H COSY and ^1H-^{13}C Hetcor experiments and comparisons to model compounds.[3-6] All spectra run in the solvent indicated with TMS as an internal chemical shift reference; (b) Obtained on a Bruker AM 400; (c) Obtained on a Bruker AC 300; (d-i) Assignments may be interchanged.

ester, opening of the lactone must have occurred prior to treatment with CH_2N_2. Proof that it was the C5 alcohol which was free in **8** followed from ^1H NMR analysis of acetylation product **15** in which the C5 methine shifted from 2.65 to 4.35 ppm.[15]

While we have observed a widespread 12-lipoxygenase-type metabolism in many marine algae,[7] we have never found a marine plant metabolite which logically derives from the 8-lipoxygenase manifold as proposed for *P. homomalla*. Moreover, in our evaluation of the metabolites of *C. simplex*, we find strong structural evidence for an active 12-lipoxygenase system in this alga (metabolites **9-11**). These observations lead us to propose that these cyclopropyl lactones are formed via a 12-lipoxygenase pathway in this marine plant (scheme 1). Further, hepoxilin B_3 (**16**), a rearrangement product of 12-HPETE[16] which we have recently isolated from several different red algae,[6,17] seems a rationale potential intermediate in the biosynthesis of the constanolactones. Loss of water from C-10 in **16** would form an epoxy cation (alternately produced directly from 12-HPETE) which could readily rearrange to a cyclopropyl-lactone. Non-stereospecific 1,4 addition of water to the allylic epoxide would generate constanolactones A (**4**) and B (**5**). Isolation of these novel cyclopropyl-containing eicosanoids from *C. simplex* further points out the generality of unusual lipoxygenase biosynthetic pathways in marine organisms.



Scheme 1. Proposed biogenesis of constanolactones A (4) and B (5) from 12-lipoxygenase initiated metabolism of arachidonic acid.

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