The Absolute Configuration of Ecklonialactones A, B, and E, Novel Oxylipins from Brown Algae of the Genera Ecklonia and Egregia

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THE ABSOLUTE CONFIGURATION OF ECKLONIALACTONES A, B, AND E, NOVEL OXYLIPINS FROM BROWN ALGAE OF THE GENERA *ECKLONIA* AND *EGREGIA*

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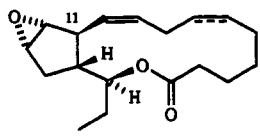
ABSTRACT.—Ecklonialactones A, B, and E, previously isolated from the brown alga *Ecklonia stolonifera*, have been isolated from the Oregon phaeophyte *Egregia menziesii*. The structure and relative stereochemistry of ecklonialactone E were independently determined by various nmr techniques. The absolute stereochemistry of ecklonialactone A was deduced by cd analysis of a dibenzoate derivative, which indicated it possessed a 11*S*, 12*R*, 13*S*, 15*R*, 16*S* stereochemistry. Similar ^{1}H- and ^{13}C-nmr data and optical rotations for all of the ecklonialactones indicate that B and E have the same stereochemistry as A at comparable stereocenters.

During an investigation of the oxylipin chemistry of brown algae (1–3), we isolated ecklonialactones A [**1**], B [**2**] and E [**3**] from the MeOH-CH_2Cl_2 extract of *Egregia menziessi* (Turn.) Aresch. This is a common brown alga from the West Coast of the United States (order, Laminariales; family, Alariaceae), also known by its appearance as the "feather boa kelp." These three metabolites have been previously isolated from the Japanese alga *Ecklonia stolonifera* Okamura (order, Laminariales; family, Laminariaceae), and their planar structures and relative stereochemistry have been described by spectroscopic and X-ray diffraction techniques (4,5). In this paper, we report an independent structure elucidation of ecklonialactone E [**3**], which was only very recently identified from *E. stolonifera* (5), and a determination of the absolute stereochemistry of all three metabolites by cd and optical rotation methodology.

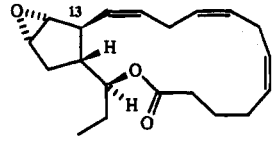
The structure of **3** was determined by comparing its ^{1}H-, ^{13}C- and DEPT nmr data with those given for ecklonialactone A [**1**] (4). The ^{1}H-nmr spectra of **1** and **3** were very similar except that **3** had an extra 2H vinylic proton peak (δ 5.32, m) and two bis-allylic methylene groups (δ 2.70 and 2.94, 1H each, m, H_2-7; δ 2.83 and 3.01, 1H each, m, H_2-10). These data, in combination with mass spectral data, which showed that **3** was 26 mass units larger than ecklonialactone A [**1**], suggested that **3** was a C-20 homologue. Additional nmr experiments (see Experimental) supported this structural assignment. The relative stereochemistry of **3** was established to be the same as in **1** by virtue of their nearly identical ^{13}C-nmr shifts and ^{1}H-^{1}H coupling constants at comparable positions in the ring. A cis configuration of the Δ^5 olefin in **3** was established by measurement of a $J_{5,6}$ = 10.9 Hz upon ^{1}H irradiation of H-4.

Because the relative stereochemistry of ecklonialactone A [**1**] had been unequivocally established by X-ray crystallography (4), compound **1** was selected for efforts aimed at determining absolute stereochemistry in this series. Opening of the epoxide in **1** with 1M $HClO_4$ gave diol **4**, which was fully characterized by ^{1}H-^{1}H homonuclear decoupling, nOe difference spectroscopy, and NOESY nmr experiments. Noteworthy were the nOe enhancements of the H-13,16 signals upon irradiation of H-11 and the NOESY cross-peaks between H-10 and H-12,15; H-13 and H-11; and H-16 and H-11. The relative configuration of the newly formed hydroxyl groups was thus shown to derive from addition of H_2O at C-13, thereby inverting this stereocenter. These data and ^{1}H- and ^{13}C-nmr spectra recorded for this synthetic derivative are in

[1]On sabbatical leave from Whitman College, Walla Walla, Washington.



1 Δ6,9
2 Δ9



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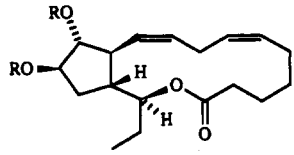

4 R=H
5 R=COC_6H_4Br

close agreement with those reported for a naturally occurring diol from *E. stolonifera* (ecklonialactone C) (5). Treatment of **4** with *p*-bromobenzoyl chloride produced dibenzoate derivative **5**, which was characterized by ^{1}H-, ^{13}C-, and DEPT and ^{1}H-^{1}H COSY nmr experiments. The cd spectrum of **5** displayed negative first and positive second Cotton effects, indicating that the two *p*-bromobenzoate chromophores possessed a negative exciton chirality (6). Hence, the absolute configuration of **5**, and therefore **4** as well, was established as 11*S*, 12*R*, 13*R*, 15*R*, 16*S*. Correspondingly, the absolute stereochemistry in ecklonialactone A [**1**] is 11*S*, 12*R*, 13*S*, 15*R*, 16*S*.[2] Because ecklonialactones B [**2**] and E [**3**] have essentially identical ^{13}C-nmr shifts to ecklonialactone A for carbon atoms at all chiral centers, thus indicating that they possess the same relative stereochemistry, and all three metabolites display similarly negative $[\alpha]_D$ values, the absolute stereochemistry of these other ecklonialactones is likely to be the same as in **1**.

One of the more intriguing aspects of the occurrence of prostaglandin-like metabolites in marine life-forms is that many appear to be formed by pathways very different from those used by mammalian systems (i.e., lipoxygenase- rather than cyclooxygenase-initiated metabolism)(7). Accompanying the report of ecklonialactones C–F from *E. stolonifera* was a proposed biogenesis that involves a diepoxide intermediate (5). However, initiation of concerted ring-forming and lactonization reactions requires, by this proposal, an unprecedented proton abstraction from C-11. We have recently proposed an alternative route to the ecklonialactones (8), which is conceptually similar to that proposed for two analogous metabolites, hybridalactone from the red alga *Laurencia hybrida* (9) and cymathere ether A from the brown alga *Cymathere triplicata* (2). In our proposal the pathway is initiated by an ω6-lipoxygenase, a postulated enzymatic capacity of brown algae (2,3), which oxidizes α-linolenate at C-13 (Figure 1). In support of this pathway in *E. menziesii*, we have characterized from its extract the simple reduction products of these proposed hydroperoxide intermediates, as well as several related hydroxy-acids (13-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid, 13-hydroxy-6*Z*,9*Z*,11*E*,15*Z*-octadecatetraenoic acid and 15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid), by comparing the corresponding methyl ester, trimethylsilyl ether derivatives to

[2]Relative stereochemical descriptors 11*R** and 13*S** given for diol **4** (ecklonialactone C) by Kurata *et al.* (5) are incorrectly applied (ecklonialactones C and D in this reference are pictured with the correct 11*S*, 12*R*, 13*R*, 15*R*, 16*S* stereochemistry).

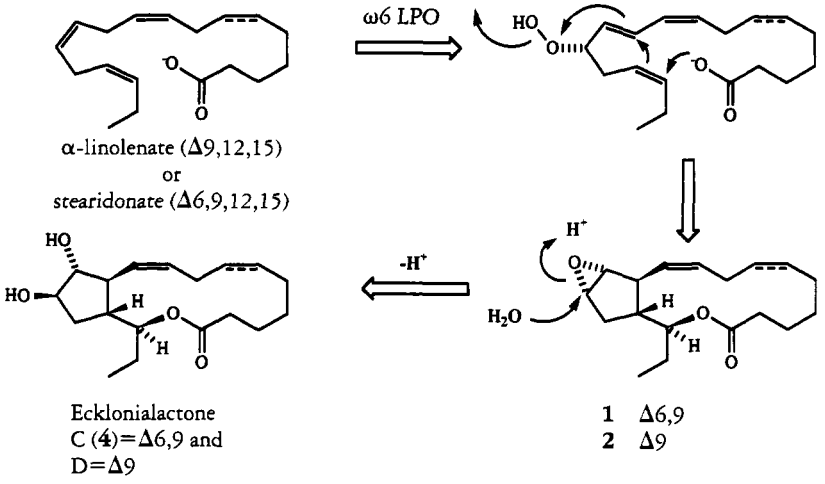

FIGURE 1. Proposed biogenesis of ecklonialactones A–D.

standards by gc-ms (3). By our scheme, formation of these cyclopentyl lactone products occurs via formal loss of HO^- from the hydroperoxide intermediates with subsequent formation of the epoxide, cyclopentyl, and ultimately, lactone rings. Our proposal implies that the stereochemistry at C-13 in ecklonialactone A arises from the initial lipoxygenation event. That we have determined the C-13 stereochemistry in ecklonialactone A as *S* is consistent with the stereospecificity shown by other brown algal lipoxygenase products oxidized at this position (e.g., 13*S*-HOT from *Laminaria* spp.) (3). Furthermore, the red algal oxylipin hydridalactone (10), has been shown to possess the same absolute configuration at centers comparable to the ecklonialactones (9).

EXPERIMENTAL

COLLECTION, EXTRACTION, AND CHROMATOGRAPHY.—Stipes and thalli of *Egregia menziesii* (8 kg wet wt, 1 kg dry wt) were collected at Seal Rock State Beach, Oregon, in September 1992. A voucher specimen is on deposit at the Department of Botany and Plant Pathology Herbarium at Oregon State University. The fresh alga was chopped and extracted with MeOH-CH_2Cl_2 (1:2, 3×7.5 liters). After removal of H_2O by partition, the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to yield a green oil (37 g). Approximately half of the oil was chromatographed over Si gel (50×95 mm i.d., vlc) using a gradient of EtOAc in hexanes. The residue from elution with 20% EtOAc/hexanes (2.79 g) was methylated with CH_2N_2 (3 min, room temperature) and further chromatographed using centrifugal tlc (4 mm Si gel rotor, 10% EtOAc/hexanes). Hplc (Phenomenex Maxsil 10 Si, 10 μm, 500×10 mm i.d., 10% EtOAc/hexanes) of a major yellow fraction obtained by centrifugal tlc (331 mg), followed by passage over a small column of Bakerbond C-18 ODS (13 mm×11 mm i.d., 40 μm, 85% MeOH/H_2O) to remove colored impurities, sequentially gave **1** (39.2 mg, 0.21%), **2** (11.4 mg, 0.062%) and **3** (17.0 mg, 0.092%). Identification of ecklonialactones A [**1**] and B [**2**] was based on a close match of ^{1}H-nmr, ^{13}C-nmr and optical rotation data with those reported (4) (**1** $[\alpha]^{28}D$ −84.8° (*c*=1.59, $CHCl_3$); **2** $[\alpha]^{28}D$ −40.0° (*c*=1.15, $CHCl_3$). For ecklonialactone E [**3**], ^{1}H-nmr, ^{13}C-nmr, ^{1}H-^{1}H COSY, ^{1}H-^{13}C XHCORR, and optical rotation data matched those reported (5) (**3** $[\alpha]^{28}D$ −78.0° (*c*=1.76, $CHCl_3$).

SYNTHESIS OF DIOL **4**.—Ecklonialactone A (**1**, 14.1 mg) was stirred in THF (0.4 ml) containing 1M $HClO_4$ (0.2 ml) at room temperature for 8 h and then neutralized with sat. aq. $NaHCO_3$. Solvents were removed *in vacuo* and the dry residue extracted with Et_2O. Purification of diol **4** from the resulting mixture by hplc (Phenomenex Maxsil 10 Si, 10 μm, 500×10 mm i.d.; 80% EtOAc-hexanes) gave 7.2 mg of a colorless oil (48%) showing: ^{1}H nmr ($CDCl_3$, 300 MHz) δ 5.55 (1H, ddd, *J*=10.8, 10.7, 3.6 Hz, H-9), 5.40–5.46 (2H, m, H-6, H-7), 5.34 (1H, ddd, *J*=10.3, 10.2, 2.2 Hz, H-10), 4.73 (1H, ddd, *J*=6.8, 6.6, 4.1 Hz, H-16), 4.08 (1H, ddd, *J*=8.7, 5.9, 5.9 Hz, H-13), 3.60 (1H, dd, *J*=8.8, 6.2 Hz, H-12), 3.40 (1H, m, H-8), 2.92 (1H, ddd, *J*=9.5, 9.4, 9.4 Hz, H-11), 2.13–2.56 (7H, m, H-2, H-5, H-8, H-15, 2×OH), 1.50–1.96 (8H, m, H-3, H-4, H-5, H-14, H-17),

1.19–1.37 (1H, m, H-3), 0.83 (3H, t, J=7.5 Hz, H-18); ^{13}C-nmr data were essentially identical to those reported (5); structural and stereochemical assignments were determined by DEPT, ^{1}H-^{1}H COSY, nOe difference and NOESY nmr experiments.

CONVERSION OF DIOL **4** TO DIBENZOATE DERIVATIVE **5**.—Diol **4** (7.2 mg) was added to a stirred solution of *p*-bromobenzoyl chloride (52 mg), CH_2Cl_2 (4 ml), Et_3N (0.5 ml) and a catalytic amount of 4-dimethylaminopyridine. After reaction for 22 h at room temperature, the solvent was removed under N_2 and the residue triturated with Et_2O. Purification of the Et_2O-soluble material (20.8 mg) by hplc (Phenomenex Maxsil 10 Si, 10 μm, 500×10 mm i.d., 15% EtOAc-hexanes) gave **5** (4.0 mg, 29%) as a colorless oil: ir ν max (film) cm^{-1} 3009, 2970, 2932, 2877, 2860, 1724, 1685, 1590, 1485, 1398, 1264, 1101, 1012, 755; uv λ max (EtOH) nm 248 (ε 42,000); cd $\Delta\epsilon_{252}$ max −43.0, $\Delta\epsilon_{235}$ max +2.9 (EtOH); ^{1}H nmr (400 MHz, $CDCl_3$) δ 7.885 (2H, d, J=8.4 Hz), 7.881 (2H, d, J=8.4 Hz), 7.58 (2H, d, J=8.4 Hz), 7.57 (2H, d, J=8.4 Hz), 5.35–5.56 (6H, m, H-6, H-7, H-9, H-10, H-12, H-13), 4.86 (1H, m, H-16), 3.34 (1H, m, H-8), 2.56 (1H, m, H-2), 2.15–2.49 (7H, m, H-2, H-5, H-8, H-11, H-14, H-15), 1.98 (1H, m, H-14), 1.72–1.90 (2H, m, H-3), 1.65 (2H, m, H-17), 1.26 (1H, bs, H-4), 0.87 (3H, t, J=7.4 Hz, H-18); ^{13}C nmr (100.6 MHz, $CDCl_3$) δ 173.7 (s), 165.3 (s), 165.0 (s), 131.80 (2C, d), 131.77 (2C, d), 131.25 (2C, d), 131.24 (2C, d), 130.9 (d), 130.1 (d), 128.79 (s), 128.74 (d), 128.65 (s), 128.4 (s), 128.3 (s), 126.8 (d), 82.9 (d), 77.7 (d), 76.8 (d), 43.3 (d), 42.3 (d), 33.6 (t), 32.9 (t), 27.8 (t), 26.4 (t), 25.8 (t), 25.6 (t), 24.7 (t), 9.9 (q); fabms (positive ion, 3-nitrobenzyl alcohol) *m/z* (rel. int.) $[M+1]^+$ 673 (1), 675 (2), 677 (1), 473 (12), 475 (11), 338 (7), 273 (15), 185 (100), 183 (100).

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