Absolute Stereochemistry of Neohalicholactone from the Brown Alga Laminaria sinclairii

Philip J. Proteau, James V. Rossi, and William H. Gerwick

J. Nat. Prod., **1994**, 57 (12), 1717-1719• DOI:
10.1021/np50114a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 12, pp. 1717-1719, December 1994

ABSOLUTE STEREOCHEMISTRY OF NEOHALICHOLACTONE FROM THE BROWN ALGA *LAMINARIA SINCLAIRII*

PHILIP J. PROTEAU,

College of Pharmacy

JAMES V. ROSSI,

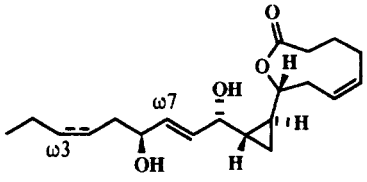
Department of Biochemistry and Biophysics

and WILLIAM H. GERWICK*

College of Pharmacy and Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

ABSTRACT.—Phytochemical analysis of an extract from the brown alga *Laminaria sinclairii* led to the isolation of neohalicholactone, a cyclopropyl-containing oxylipin previously isolated from a marine sponge, *Halichondria okadai*. Unequivocal stereochemical analysis of the C-15 hydroxyl group showed this isolate to be of opposite overall absolute stereochemistry compared to that proposed for halicholactone, a related compound from the sponge, and by our inference, sponge-derived neohalicholactone. Comparison of chiroptical data for all three compounds indicates the absolute stereochemistry of the sponge compounds is most probably opposite to that previously proposed.

Laminaria sinclairii (Harv.) Farl., Anders. & Eaton (Laminariaceae) has recently been shown in our laboratory to produce a variety of interesting oxylipins, including several hydroxy acids and three novel divinyl ethers (1). However, careful scrutiny of several minor fractions obtained during isolation of the above compounds showed one to possess a cyclopropyl-containing oxylipin. Following isolation of this compound by hplc, spectroscopic analysis (Experimental) showed it to be identical in overall structure and relative and absolute stereochemistry to neohalicholactone [**1**][1], an oxylipin originally isolated from the marine sponge *Halichondria okadai* Kadota along with the related metabolite halicholactone [**2**] (2,3). Our proposed biogenesis for halicholactone and neohalicholactone (4) (Figure 1) suggests that the C-15 stereochemistry is introduced via a lipoxygenase in the first step of the biogenetic pathway. We have previously shown that the putative 15-lipoxygenase of *Laminaria* spp., including *L. sinclairii*, introduces a C-15 hydroxyl function of *S* stereochemistry (1). In the original report on oxylipins **1** and **2** from *H. okadai*, a 15*R*-stereochemistry was deduced for halicholactone (2). Hence, it was of interest to unequivocally determine the absolute stereochemistry at C-15 in this algal isolate of neohalicholactone, and to consider our results in the context of the earlier stereochemical studies.



1 ω3
2 ω7

The absolute stereochemistry at C-15 in our isolate of neohalicholactone was determined by converting **1** to its corresponding (−)-menthoxycarbonyl (MC) derivative (5), oxidative ozonolysis to release the C-14 to C-17 fragment, and derivatization of this fragment to the bismethyl ester. Gc standards for this same

[1]Comparison of the authentic ^{1}H- and ^{13}C-nmr spectra for *H. okadai*-derived neohalicholactone and *L. sinclairii*-derived neohalicholactone showed them to be identical.

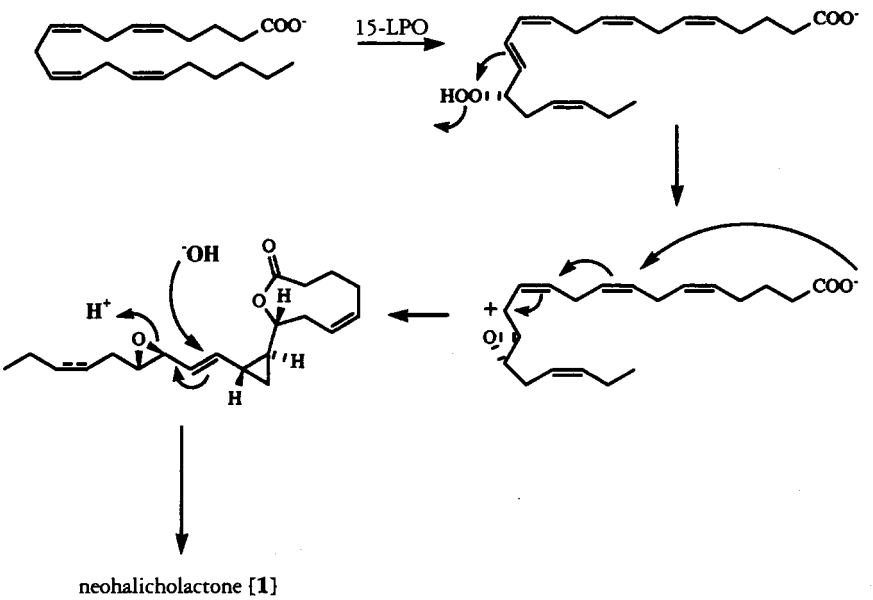


FIGURE 1. Proposed biogenesis of neohalicholactone [**1**].

MC derivative, as obtained from the authentic *R* and *S* malates, gave baseline separation under optimized conditions (Experimental). Under these conditions, the neohalicholactone-derived malate fragment analyzed as essentially 100% *S*, and hence *L. sinclairii* neohalicholactone is overwhelmingly of 15*S* stereoconfiguration, in opposition to that reported for halicholactone (2), and by our inference, that for sponge-derived neohalicholactone (3). Because the optical rotation of our isolate of neohalicholactone ($[\alpha]^{27}D$ −77°), is the same sign and of similar magnitude to that reported (2) for the sponge isolate of neohalicholactone ($[\alpha]^{16}D$ −54.2°), we conclude that the absolute stereochemistry in both the alga-derived and sponge-derived neohalicholactone, and likely in that of halicholactone ($[\alpha]^{23}D$ −85.4°), is 8*R*, 9*S*, 11*S*, 12*S*, 15*S*. This result is opposite that previously proposed for the sponge compounds (2).[2]

[2]Determination of the 15*R* stereochemistry in halicholactone [**2**] was based on measurement of a small optical rotation for a halicholactone-derived fragment (2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr data were collected on a Bruker AC 300 spectrometer at 300 MHz for ^1H and at 75 MHz for ^{13}C DEPT. All shifts were reported with respect to residual solvent (C_6D_6=7.20 ppm; $CDCl_3$=77.0 ppm). Optical rotations were measured on a Perkin Elmer model 141 polarimeter. Ir data were collected on a Nicolet 510 spectrometer. A high-resolution electron impact mass spectrum (hreims) was recorded on a Kratos MS 50 TC. Hplc was run with a Waters M6000 pump, Rheodyne 7010 injector, and a Waters ri detector. Gas chromatography-electron impact mass spectrometry (gc-eims) was performed on a Hewlett-Packard 5890 series II gc connected to a Hewlett-Packard 5971 mass spectrometer. Tlc was run on Merck aluminum-backed normal-phase tlc sheets.

PLANT MATERIAL.—*L. sinclairii* was collected from mid-intertidal rocks in May 1990 at Strawberry Hill on the Oregon coast. A voucher specimen (SH-25May90-3) is on file at the College of Pharmacy, Oregon State University, and is available through W.H.G.

EXTRACTION AND ISOLATION.—The alga (2.5 gallons) was frozen on site in CO_2(s) and later stored at −20°. Blades and thalli of the plant material (640 g dry wt) were allowed to sit overnight in 2:1 $CHCl_3$-MeOH and then filtered through cheesecloth. This initial extraction was followed by two additional extractions during which the algae/solvent mixture was gently heated. All extracts were combined and concentrated *in*

vacuo to yield 7 g of a viscous brown oil. The crude extract was separated using vacuum chromatography on normal-phase tlc grade Si gel. The EtOAc to hexanes ratio was steadily increased and the column was rinsed with MeOH. Of the 14 fractions (200–250 ml each), polar fraction 12 (100% EtOAc) showed on tlc several blue-charring compounds upon heating with H_2SO_4. This fraction was further separated by Sephadex LH-20 chromatography using EtOAc-MeOH (1:1) as eluent. Fractions 9–13 of this latter chromatography (100–140 ml elution volume) contained most of the blue-charring compound and were recombined. ^{1}H-Nmr analysis showed this recombined material to possess a cyclopropyl ring-containing compound and was further separated, following methylation (CH_2N_2, Et_2O) to aid removal of contaminating fatty acids, by repetitive flash chromatography (2×, Merck Kieselgel 60, 230–400 mesh, gradient of 50–100% EtOAc/hexanes). The fractions containing this blue-charring compound were again recombined and further purified by hplc (Maxsil 10 μm, 1×50 cm, 20% *i*-PrOH/hexanes). Hplc fraction 2 contained the majority of the new compound, neohalicholactone **[1]** (1.3 mg), which was characterized by spectroscopic methods.

Determination of Absolute Stereochemistry of Neohalicholatone **[1]**.—An aliquot of the neohalicholactone (200 μg) was reacted with 20 μl (−)-menthyl chloroformate in 50 μl toluene and 10 μl pyridine for 30 min. Solvents were removed *in vacuo* and the residue was resuspended in 10% EtOAc in hexanes. The reaction products were purified from polar materials over a short column of Si gel. The (−)-menthoxycarbonyl (MC) derivative was ozonized for 10 min in CH_2Cl_2 (−10°) followed by treatment with peracetic acid at 50° overnight. The reaction mixture was evaporated and the residue resuspended in MeOH and methylated with ethereal CH_2N_2. The methylated derivative was examined by gc-eims and gc (25 m, HP Ultra-1, 130–190° at 2.0° per min, then isothermal for 15 min) and compared to the corresponding *R*- (39.51 min) and *S*-malate (39.28 min) standards.

Neohalicholactone **[1]**.—Pure **1** showed the following data: $[\alpha]^{27}D$ −77° (*c*=0.14, $CHCl_3$); hreims observed *m/z* $[M-18]^+$ 316.2038 (0.3 mmu error for $C_{20}H_{28}O_3$); gc-eims of trimethylsilyl ether derivative of **1** (method of formation as in Ref. 1) observed *m/z* $[M-69]^+$ 409 (14, $M-C_5H_9$), 319 (8), 309 (15), 307 (10), 279 (5), 243 (18), 129 (18), 73 (100); ir (film) ν max 3409, 1737, 1717 cm^{-1}; ^{1}H nmr (300 MHz, C_6D_6) δ 5.73–5.74 (2H, m, H-13, -14), 5.53 (1H, m, H-18), 5.42 (1H, m, H-17), 5.40–5.46 (2H, m, H-5, -6), 4.38 (1H, ddd, *J*=12, 8.9, and 1.3 Hz, H-8), 4.00 (1H, m, H-15), 3.56 (1H, m, H-12), 2.39 (1H, m, H-4a), 2.34 (1H, m, H-7a), 2.27 (2H, m, H_2-16), 2.12 (2H, m, H_2-2), 1.97 (2H, m, H_2-19), 1.92 (1H, m, H-7b), 1.79 (1H, m, H-4b), 1.59 (2H, m, H_2-3), 1.07 (1H, m, H-11), 0.93 (3H, t, *J*=7.5 Hz, H_3-20), 0.87 (1H, m, H-9), 0.48 (1H, ddd, *J*=8.6, 4.8, and 4.8 Hz, H-10a), 0.30 (1H, ddd, *J*=8.6, 4.8 and 4.8 Hz, H-10b); ^{13}C nmr DEPT 135 (75 MHz, $CDCl_3$) δ 135.4 (C-14, +), 134.7 (C-13, +), 133.3 (C-17, +), 131.9 (C-5, +), 124.6 (C-6, +) 123.6 C-18, +), 76.1 (C-8, +), 74.2 (C-12, +), 71.6 (C-15, +), 35.2 (C-16, −), 33.8 (C-7, −), 33.6 (C-2, −), 26.5 (C-4, −), 25.3 (C-3, −), 23.4 (C-11, +), 20.7 (C-19, −), 19.5 (C-9, +), 14.2 (C-20, +), 8.2 (C-10, −).

ACKNOWLEDGMENTS

We thank Elizabeth Barofsky of the Oregon State University College of Agricultural Chemistry for hrms measurements. We thank Professor K. Yamada, Nagoya University, for providing authentic spectra of neohalicholactone. This work has been supported by the Oregon Sea Grant Program (R/SH-5) and NIH (CA 52955).

LITERATURE CITED

1. P.J. Proteau and W.H. Gerwick, *Lipids*, **28**, 783 (1993).
2. H. Niwa, K. Wakamatsu, and K. Yamada, *Tetrahedron Lett.*, **30**, 4543 (1989).
3. H. Kigoshi, H. Niwa, K. Yamada, T.J. Stout, and J. Clardy, *Tetrahedron Lett.*, **32**, 2427 (1991).
4. W.H. Gerwick, D.G. Nagle, and P.J. Proteau, in: "Topics in Current Chemistry, Vol. 167." Ed. by P.J. Scheuer, Springer-Verlag, Berlin 1993, pp. 117–180.
5. M. Hamburg, *Anal. Biochem.*, **43**, 515 (1971).

Received 26 April 1994