Pteroenone: A Defensive Metabolite of the Abducted Antarctic Pteropod *Clione antarctica*†

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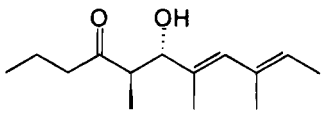
The pteropod *Clione antarctica* (= *Clione limacina*)[1] is a shell-less, pelagic mollusc which blooms each austral summer in McMurdo Sound, Antarctica. An intriguing relationship[2] exists between *C. antarctica* and an antarctic hyperiid amphipod, *Hyperiella dilatata*; the amphipod, a frequent prey item of several antarctic fish,[3] is capable of grasping *C. antarctica* from the water column and positioning it on its dorsum where the chemically defended mollusc serves to prevent predation of the amphipod. This protective property of the pteropod was demonstrated by the rejection of amphipod/mollusc pairs, as well as the mollusc itself, by predatory fish. Amphipods alone, or amphipods which dropped their attached pteropod, were readily consumed.[2] Utilizing these same predatory fish as the basis of a bioassay guided isolation,[4] carried out at McMurdo Station, Antarctica, has resulted in the isolation of a substance which protects *C. antarctica*, and ultimately *H. dilatata*, from predation. We report here the chemical nature of this feeding deterrent which we have named pteroenone (**1**).



Pteroenone (**1**)

C. antarctica were collected in a plankton net suspended 5–10 m below the sea ice in front of McMurdo Station during Oct and Nov 1993. The wet animals were extracted first in methanol and resultant aqueous methanol extract partitioned with hexane. The animals were subsequently extracted in hexane and the hexane-soluble fractions combined. Evaluation of the extracts in a feeding deterrence assay[4] utilizing the predatory fish *Pagothenia borchgrevinki* and *Pseudotrematomas bernacchii* revealed activity in the combined hexane-soluble extracts of *C. antarctica*. Further fractionation was achieved by silica flash chromatography utilizing a step gradient; the active fraction eluted with 90% hexane/10% ethyl acetate. This active fraction was further purified by high performance liquid chromatography (μPorasil, 75% hexane/25% ethyl acetate) resulting in the isolation of an active compound, pteroenone.

Structural analysis by ^1H and ^{13}C NMR spectroscopy revealed that pteroenone was a linear C_{11} β-hydroxy ketone. The carbon skeleton was assigned on the basis of extensive two-dimensional NMR techniques (Table 1). The C-1 to C-3 and C-12/C-5/C-6 spin systems, established by COSY spectroscopy and decoupling experiments, could be disposed about the carbonyl based on observation of HMBC correlation of H-3a, −3b, −5 and −6 to the carbonyl resonance at 213.2 ppm (Figure 1). Three bond correlations of H-6 to C-8 and C-13, in addition to a two bond correlation between H-13 and C-7, secured the position of the olefinic C-7 quaternary carbon. The second quaternary olefinic carbon, C-9, could be assigned based on three-bond correlations of H-10 to C-8 and C-14 as well as H-11 to C-9, plus a two-bond correlation between H-14 and C-9. Other significant correlations are illustrated in Figure 1.

Mass spectral fragmentation patterns further support the gross structure **1**. The base peak at *m/z* 109 (109.0656, C_7H_9O, Δmmu 0.3) results from fragmentation of a methyl group from 2,4-dimethylhexa-2,4-dienal, which together with 3-hexanone are retroaldol products formed during the mass spectral analysis. Parent ions for both 2,4-dimethylhexa-2,4-dienal and 3-hexanone are observed at *m/z* 124.0890 (55.2%, $C_8H_{12}O$, Δmmu 0.1) and *m/z* 100.0897 (26.2%, $C_6H_{12}O$, Δmmu 0.9), respectively. Further, fragmentations at *m/z* 125 (49.0%) and 71 (50.0%) can be accommodated by cleavage of the C-4/C-5 bond and C-5/C-6 bond, respectively.

Stereochemical assignment of the C-6 asymmetric center was achieved by the modified Mosher's method;[5] esterification of pteroenone with both (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride demonstrated negative chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) for protons on C-1 through C-5 and C-12, while C-8 through C-11, C-13, and C-14 showed positive differences (Figure 2), which is consistent with C-6 bearing an *S* configuration.

Assignment of the stereochemistry of the C-5 asymmetric carbon is based on conversion of the C-4 *syn*-alcohol **2a**, obtained by borohydride reduction of the ketone,[6] to acetonide **3a**. Acetonide **3a** displayed coupling constants $J_{4,5} = J_{5,6} = 10.1$, characteristic of axial–axial coupling,[7] thus securing a relationship of C-5 to the know stereocenter at C-6. H-4 and H-6 were further demonstrated to be axial by observation of mutual NOE (Figure 3), all of which requires C-5 to be *R*. The stereochemistry of the C-7 and C-9 olefinic bonds were determined by difference NOE spectroscopy (Table 1), which demonstrated the close proximity of methyl group H_3-13 to H-8 and H_3-14, a result which is obtained only for the *E,E* orientation of the two olefins. Thus pteroenone is (5*R*,6*S*,7*E*,9*E*)-6-hydroxy-5,7,9-trimethyl-7,9-undecadien-4-one.

Pteroenone, which is the first example of a defensive metabolite from a pelagic gastropod,[8] belongs to the polyketide family of natural products, bearing four pro-

† Dedicated to Professor Paul J. Scheuer on the occasion of his 80th birthday.

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(1) Gilmer, R. W.; Lalli, C. M. *Am. Malac. Bull.* **1990**, *8*, 67–75.
(2) McClintock, J. B.; Janssen, J. *Nature* **1990**, *346*, 462–464.
(3) Eastman, J. T. *Polar Biol.* **1985**, *4*, 155.
(4) Bryan, P. J.; Yoshida, W. Y.; McClintock, J. B.; Baker, B. J., *Mar. Biol.*, in press.
(5) Ohtani, I.; Kusumi, T.; Kashman Y.; Kakisawa, H. *J. Am. Chem Soc.* **1991**, *113*, 4092–4096.
(6) (a) Narasaka, K.; Pai, F. C. *Tetrahedron* **1984**, *40*, 2233–2238. (b) Chen, K. M.; Hardtmann,K. P.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *29*, 155–158.
(7) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; John Wiley and Sons: New York, 1991; p 221.
(8) See, for example, Faulkner, D. J. *Nat. Prod. Rep.* **1994**, *11*, 355–418, and references cited therein.

0022-3263/95/1960-0780$09.00/0

Table 1. One- and Two-Dimensional Nuclear Magnetic Resonance Data for Pteroenone (1)[a]

position	^{1}H δ, m, J (Hz)	NOE, δ	^{13}C δ, m	COSY, δ	HMBC (two bond), δ	HMBC (three bond), δ
1	0.83, t, 7.3		13.8, q	1.61	17.0	45.6
2	1.61, x, 7.3		17.0, t	0.83, 2.18, 2.21	13.8, 45.6	213.2
3	a: 2.21, td, 7.3, 17.4		45.6, t	1.61, 2.18	213.2	
	b: 2.18, td, 7.3, 17.4			1.61, 2.21	213.2	
4			213.2, s			
5	2.60, qd, 7.0, 9.0		48.8, d	0.81, 4.06	14.1, 81.3, 213.2	
6	4.06, dd, 3.4, 9.0	5.77	81.3, d	2.60, 0.81, 1.70, 1.66	48.8	12.5, 132.4, 213.2
7			134.6, s			
8	5.77, bs	1.70, 4.06	132.4, d	1.70		12.5, 81.3, 124.8
9			133.2, s			
10	5.39, qq, 1.3, 6.8		124.8, d	1.57, 1.64		132.4
11	1.57, d, 6.8		13.6, q	5.39	124.8	133.2
12	0.81, d, 7.0		14.1, q	2.60	48.8	81.3, 213.2
13	1.70, d, 1.3	1.64, 2.60, 5.39	12.5, q	4.06, 5.77	134.6	81.3, 132.4
14	1.64, t, 1.3	1.70, 5.77	16.6, q	5.39	133.2	124.8
OH	1.66, d, 3.4					

[a] Proton and carbon NMR data recorded in benzene-d_6 at 500 and 125 MHz, respectively. Abbreviations: m, multiplicity, s, singlet; d, doublet; t, triplet, q, quartet, x, sextet, b, broad.

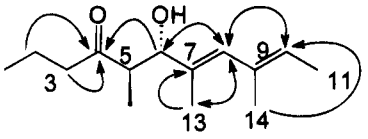

Figure 1. Key HMBC correlations observed for pteroenone (**1**). Arrows point to carbons to which protons correlate; double headed arrows show mutual correlations.

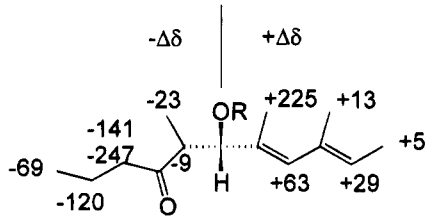

Figure 2. $\Delta\delta = (\delta_s - \delta_r) \times 1000$ for (*R*)- and (*S*)-MTPA esters of pteroenone.

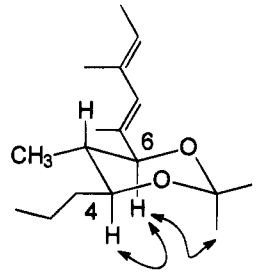

Figure 3.

pionate and one acetate units. Polypropionate-derived metabolites are characteristic of siphonariid (false-limpets) and ascoglossan molluscs and often contain cyclic ether moieties.[9] Their functional role in ascoglossans has been postulated to be for absorption of ultraviolet light[10] while at least one siphonariid polypropionate metabolite inhibits feeding by a predatory reef fish.[11] Recent biosynthetic experiments on siphonariid molluscs have demonstrated that acetate can serve as the starter unit of polypropionates.[12] Pteroenone must either derive from a butyrate starter unit (C-1 to C-3), terminating in acetate (C-10 and C-11), or, starting from acetate (C-11), terminate with butyrate (C-1 to C-4). Interestingly, methyl,[13] ethyl,[13] butyl,[14] and pentyl[14] ketones have been found as terminal groups; pteroenone, bearing a propyl ketone, completes the series.

Experimental Section

General. Spectral analyses were performed on an 11.75-T NMR instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. One-bond heteronuclear ^{1}H–^{13}C connectivities were determined by HMQC; two- and three-bond ^{1}H–^{13}C connectivities were determined by HMBC optimized for 7 Hz couplings. Mass spectra were determined in electron impact mode. Voucher specimen of *C. antarctica* are on hand at the University of Alabama at Birmingham.

Isolation. Freshly collected pteropods (56.8 g wet) were extracted with 3 × 200 mL MeOH then with 3 × 200 mL hexane. The wet methanol extract was partitioned with hexane, and the combined hexane fractions were concentrated and then applied to a flash silica gel column packed in hexane. Gradient elution utilizing increasing concentrations of ethyl acetate (10, 20, 50, and then 100%, two column volumes each) resulted in activity in the 10% ethyl acetate fraction. Purification was achieved using a Waters RCM μPorasil 10μ (4.5 mm × 20 cm) with 25% ethyl acetate in hexane to yield 70 mg (0.12% wet wt).

Pteroenone (1): $[\alpha]_D$ +48 (*c* 0.6, hexane); IR (CCl_4) ν_{max} 3618, 2965, 2932, 2876, 1715, 1548, 1458, 1376, 1001 cm^{-1}; UV λ_{max} (hexane) 234 nm (ϵ 11600); HREIMS found 206.1671 ($M^+ - H_2O$, Δmmu 0.1 for $C_{14}H_{22}O$), 124.0890 (Δmmu 0.1 for $C_8H_{12}O$), 109.0656 (Δmmu 0.3 for C_7H_9O), 100.0897 (Δmmu 0.9 for $C_6H_{12}O$); EIMS *m/z* (%) 206 (1.0), 135 (12.2), 125 (49.0), 124 (55.2), 109 (100), 100 (26.2), 71 (50); ^{1}H and ^{13}C NMR, see Table 1.

Reduction of Pteroenone to Diols 2a and 2b. Tripentylborane was prepared[15] by combining pentene (0.35 mL, 3.2 mmol) with a borane–THF complex (0.1 M, 10 mL, 1 mmol) with stirring for 1 h. Tripentylborane (0.1 M, 0.2 mL, 19.3 μmol) was then added to a mixture of 4:1 THF–MeOH (2 mL). The solution

(9) Faulkner, D. J. In *Ecological Roles of Marine Natural Products*; Paul, V. J., Ed.; Comstock Publishing Associates: Ithaca, NY, 1992; pp 119–163.
(10) Ireland, C. M.; Scheuer, P. J. *Science* **1979**, *205*, 922–923.
(11) Manker, D. C.; Faulkner, D. J *J. Org. Chem.* **1989**, *54*, 5374–5377.
(12) Garson, M. J.; Jones, D. D.; Small, C. J.; Liang, J.; Clardy, J. *Tetrahedron Lett.* **1994**, *35*, 6921–6924.
(13) (a) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1983**, *105*, 7413–7415. (b) Hochlowski, J. E; Coll, J. C.; Faulkner, D. J.; Biskupiak, J. E.; Ireland, C. M.; Qi-tai, Z.; Cunheng, H.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 6748–6750. (c) Capon, R. J.; Faulkner, D. J. *J. Org. Chem.* **1984**, *49*, 2506–2508. (d) Cimino, G.; Sodono, G.; Spinella, A.; Trivellone, E. *Tetrahedron Lett.* **1985**, *26*, 3389–3392. (e) Cimino, G. S.; Sodano, G.; Spinella, A. *J. Org. Chem.* **1987**, *52*, 5326–5331. (f) Morte, M.; Cataldo, F.; Gonzalez, G. *Tetrahedron Lett.* **1988**, *29*, 2879–2880.
(14) Manker, D. C.; Faulkner, D. J.; Chang-fu, X.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 814–816.
(15) Brown, H. C.; Subba Rao, B. C. *J. Am. Chem. Soc.* **1959**, *81*, 6428–6433.

was stirred for 90 min then cooled to −78 C and a 2 mL solution of pteroenone (**1**) (1.2 mg, 5.35 μmol) in 4:1 THF−MeOH was added. The solution was allowed to remain at −78 C for 1 h after which $NaBH_4$ (2 mg, 53 μmol) was added, and the reaction was allowed to continue for 4 h. The reaction was quenched with 5 mL NH_4Cl (aq) and the mixture was extracted with ethyl acetate (3 × 5 mL). The combined ethyl acetate extracts were washed with H_2O, $NaHCO_3$ (aq), and brine (5 mL each), dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by C18 RP-HPLC into two components. The isolates were dissolved in MeOH (2 mL), heated to 50 C for 2 h, and evaporated to give the *anti* and *syn* diols, **2a** (0.7 mg, 58%) and **2b** (0.5 mg, 42%), respectively. Diol **2a**: ^{1}H NMR: δ (position, multiplicity, *J* (Hz)) 5.72 (H-8, s), 5.42 (H-10, qq, 6.8, 1.3), 3.68 (H-6, d, 9.5), 3.59 (H-4, td, 8.3, 2.5), 1.73 (H_3-14, d, 1.4), 1.72 (H-5, dqd, 9.5, 7.8, 6.9), 1.66 (H_3-14, bs), 1.57 (H_3-11, bd, 6.8), 1.4−1.2 (H_2-2 and −3, m), 0.95 (H_3-1, t, 7.3), 0.59 (H_3-12, d, 6.9); EIMS (*m/z*) (%) 226 (2.5), 208 (3.2), 193 (3.7), 165 (10.1), 136 (45.2), 121 (100), 109 (70.2); HREIMS found 226.1940 (M^+), Δmmu 0.7 for $C_{14}H_{26}O_2$; 208.1826 ($M^+ - H_2O$), Δmmu 0.1 for $C_{14}H_{24}O$. Diol **2b**: ^{1}H NMR: δ (position, multiplicity, *J* (Hz)) 5.91 (H-8, s), 5.43 (H-10, qquintet, J = 6.9, 1.3), 3.85 (H-6, d, 7.7), 3.85 (H-4, m), 1.73 (H-5, dqd, 7.7, 7.1, 2.2), 1.69 (H_3-13, d, 1.4), 1.68 (H_3-14, bs), 1.59 (H_3-11, bd, 6.9), 1.4−1.2 (H_2-2 and −3, m), 0.90 (H_3-1, t, 7.3), 0.79 (H_3-12, d, 7.1).

Conversion of Diols 2a and 2b to Acetonides 3a and 3b. To a solution of diol **2a** (0.7 mg, 3.1 mmol) in 3 mL of 2,2-dimethoxypropane was added a catalytic amount of *p*-TsOH. The mixture was stirred for 1 h and then 5 mL of $NaHCO_3$ (aq) was added and the mixture was extracted with diethyl ether (3 × 5 mL). The combined ether extracts were dried over anhydrous $MgSO_4$ and evaporated leaving a yellow oil, which was purified by silica gel chromatography eluting with 9:1 hexane−ethyl acetate to yield the *syn*-acetonide **3a** (0.7 mg, 85%). Identical conditions were used to convert **2b** (0.5 mg, 2.2 μmol) into *anti*-acetonide **3b** (0.5 mg, 85%). Acetonide **3a**: ^{1}H NMR (benzene-d_6): δ (position, multiplicity, *J* (Hz)) 5.94 (H-8, s), 5.46 (H-10, qquintet, 6.8, 1.3), 3.85 (H-6, d, 10.1), 3.43 (H-4, ddd, 10.1, 8.3, 2.5), 1.92 (H_3-13, d, 1.2), 1.66 (H_3-14, bs), 1.60 (H-5, tq, 10.1, 6.7), 1.56 (H_3-11, bd, 6.8), 1.55 (Me_{eq}-ketal, s), 1.38 (Me_{ax}-ketal, s), 1.4−1.2 (H_2-2 and −3, m), 0.94 (H_3-1, t, 7.2), 0.62 (H_3-12, d, 6.7). Acetonide **3b**: ^{1}H NMR (benzene-d_6): δ (position, multiplicity, *J* (Hz)) 5.99 (H-8, s), 5.47 (H-10, quintet, 6.9, 1.3), 3.88 (H-4, ddd, 9.2, 5.0, 4.0), 3.82 (H-6, d, 8.2), 1.96 (H_3-13, d, 1.2), 1.82 (H-5, dqd, 8.2, 6.7, 5.0), 1.68 (H_3-14, bs), 1.57 (H_3-11, bd, 6.7), 1.40 (2 × Me-ketal, s), 1.4−1.2 (H_2-2 and −3, m), 0.91 (H_3-1, t, 7.2), 0.87 (H_3-12, d, 6.7).

Esterification of Pteroenone (1) with (*R*)- and (*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetyl Chloride. See ref 8, method A. MTPA ester **4*S***: (0.6 mg, 100%); ^{1}H NMR data: δ (position) 7.40 (aromatic), 6.15 (H-8), 5.55 (H-6), 5.48 (H-10), 3.47 (OMe), 2.37 (H-3b), 2.08 (H-3a), 1.76 (H_3-13 and −14) 1.70 (H-11), 1.40 (H_2-2), 0.92 (H_3-12), 0.80 (H_3-1); EIMS *m/z* (%): 440 (1.3), 206 (3.7), 189 (100), 175 (27), 135 (53), 119 (28), 105 (74), 91 (34), 77 (44); HREIMS found 440.2176 (M+), Δmmu 0.2 for $C_{24}H_{31}F_3O_4$. MTPA ester **4*R***: (0.6 mg, 100%); ^{1}H NMR data: δ (position) 7.40 (aromatic), 6.09 (H-8), 5.48 (H-6), 5.45 (H-10), 3.42 (OMe), 2.51 (H-3b), 2.32 (H-3a), 1.75 (H_3-14), 1.54 (H_3-13), 1.70 (H-11), 1.52 (H_2-2), 0.94 (H_3-12), 0.87 (H_3-1).

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Supplementary Material Available: ^{1}H, ^{13}C, COSY, HMBC, and HMQC NMR spectra for **1** (5 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.

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