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Synthesis and Absolute Configuration of the Ozonolysis Product of Krill Fluorescent Compound F

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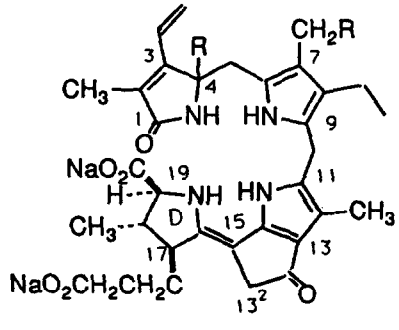
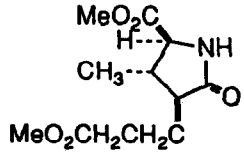
Key words: Krill fluorescent compound F; Bioluminescence, *Euphausia pacifica*; Chlorophylls; Ozonolysis product

Abstract: The absolute configuration at C-17, C-18, and C-19 of krill fluorescent compound F was determined to be 17S 18S, and 19S, respectively, on the basis of comparison of the ozonolysis product with synthetic compounds using a chiral column. The fact suggests that chlorophylls are biogenetic origin of krill fluorescent compound F.

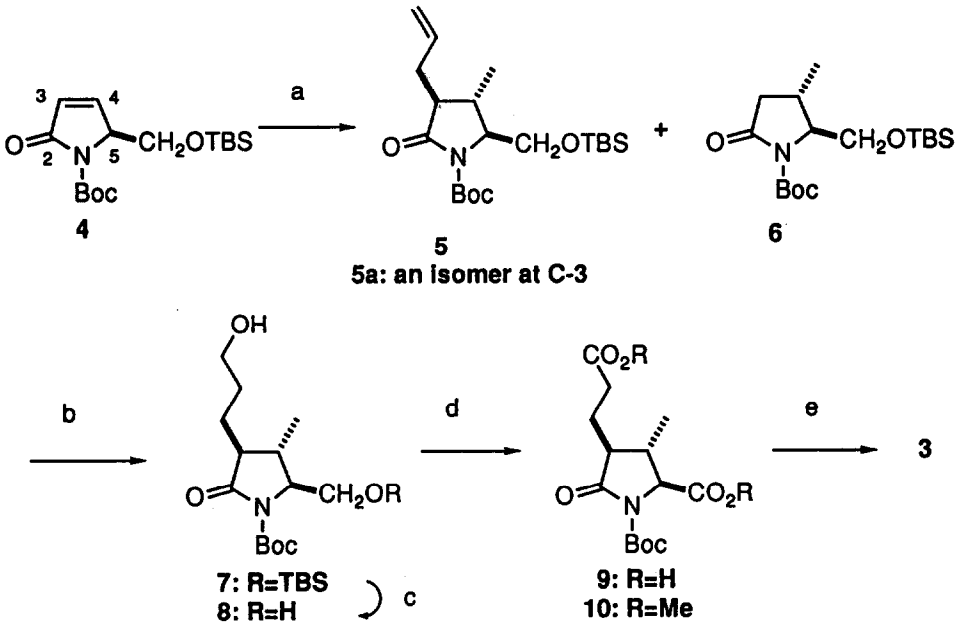
Krill bioluminescence is known to involve two organic molecules, *i.e.* a proteinous component and a nonproteinous small molecule named fluorescent compound F (**1**).[1] Recently the structure of **1** has been determined to be a tetrapyrrole[2] whose structure is very close to that of dinoflagellate luciferin.[3] On the basis of their structural similarity, both the compounds are supposed to be biogenetically derived from chlorophylls or related compounds and, therefore, krill might accumulate the precursor compounds of compound F from dietary sources (phytoplanktons) such as dinoflagellates. Interestingly, it has been shown that there is small cross reactivity between those two bioluminescent systems.[4] To investigate detailed molecular recognition involved in those two bioluminescent systems and biogenetic origin of these tetrapyrrole compounds, we stereospecifically synthesized the optically active ozonolysis product (**3**) of krill fluorescent compound F and determined absolute configuration of the ozonolysis product by comparing chromatographic behavior on a chiral column, which established the absolute stereochemistry of the D ring of krill fluorescent compound F.



1: R=OH
2: R=H



3



Reagents and conditions: a) Me_2CuLi-LiI, Et_2O, -73 °C, 2 h followed by allyl iodide, HMPA-THF-Et_2O (2:2:11), -70 °C (2h) and then -70 °C → -46 °C (1h), 59.2%; b) 9-BBN, THF, 0 °C and then NaOH, H_2O_2, 54.8%; c) TBAF-HF (pH 6), THF, 20 °C, overnight; d) PDC, DMF, 40 °C, overnight and then CH_2N_2 (Et_2O solution), 20 °C, 0.5 h, 50.3 % yield from **8**; e) TFA, CH_2Cl_2, 20 °C, 88.0% yield.

α,β-Unsaturated lactam **4**, $[\alpha]_D^{25}$ -174 ° (*c* 1.04, $CHCl_3$), (0.20 mmol) prepared from L-pyroglutamic acid according to the reported method[5] was treated with 2 eq. of dimethyl cuprate in Et_2O (total 4.4 mL) at -73 °C for 2 h. To the solution were added allyl iodide (6 eq.) and 1.6 mL of a 1:1 mixture of HMPA and THF and stirred at -70 °C for 2.5 h and then warmed up to -46 °C during 1 h. Chromatography of the reaction mixture on a silica gel column (95:5 hexane-EtOAc) afforded allyl compound **5** (59.2 % yield), its isomeric lactam **5a** (11.4% yield), and methyl compound **6** (14.7% yield).[6,7] The stereochemistry of **5**[8] was established by NOE experiments in C_6D_6 (C-4Me to both C-3 and C-5 methine protons). Hydroboration of **5** with 9-borabicylo[3.3.1]nonane (9-BBN) at 0 °C (3.5 h) followed by usual work-up yielded alcohol **7**[7], $[\alpha]_D^{25}$ -35.3 ° (*c* 0.795, $CHCl_3$), in 54.8% yield. After deprotection of **7**, the diol **8**[7], $[\alpha]_D^{25}$ -18.8 (*c* 1.23, $CHCl_3$), was oxidized with PDC in DMF and the mixture was treated with diazomethane to furnish a dimethyl ester (**10**),[7] $[\alpha]_D^{25}$ -31.6 ° (*c* 1.30, $CHCl_3$). The ester (**10**) was deprotected with trifluoroacetic acid in CH_2Cl_2 to yield optically active **3**.[9]

The value of $[\alpha]_D^{25}$ of synthetic **3**, -5.18 ° (c 0.73, $CHCl_3$), was too small to determine the absolute configuration of that obtained from natural compound F by direct comparison of optical rotation because of low yields of the isolation and the ozonolysis reaction of compound F. Thus, we examined chromatographic method to distinguish the enantiomers of **3**. Racemic **3** was prepared from D,L-pyroglutamic acid by the

same sequence of reactions and subjected to chromatographic separation using a chiral stationary phase. HPLC separation of 3 on a chiral column of CHIRALCEL OB (Daicel Chemical Industries, Ltd.) was achieved by using 4:1 hexane-EtOH as a mobile phase (Fig. 1).[10] Under the conditions, (+)-3 was eluted faster than (-)-3.

Ozonolysis product from krill fluorescent compound F[11, 12] isolated from a krill *Euphausia pacifica* collected at Otsuchi, Iwate in March, 1992, was identical with (-)-3 in all respects including a retention time on the chiral column (Fig. 1). This established the absolute configuration of the ozonolysis product of krill fluorescent compound F as 3*S*,4*S*, 5*S*. Consequently, the absolute configurations at C-17 and C-18 of krill fluorescent compound F are same to those of chlorophylls, strongly suggesting that chlorophylls are biogenetic origin of krill substance F.

Studies on detailed structural analyses of krill substance F and dinoflagellate luciferin are in progress at our laboratory.

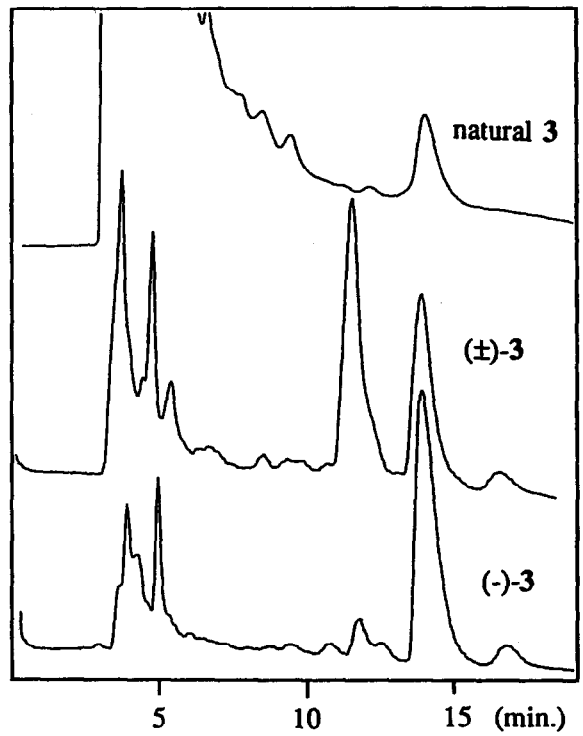


Fig. 1. Separation of 3 on CHIRALCEL OB column (4.6 mmϕ x 250 mm). Mobile phase 4:1 hexane-EtOH; detection UV 220 nm; Flow rate 1 mL/min.

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6. Michael addition of a methyl group to 4 was smoothly proceeded in Et_2O as reported. However allylation was achieved under the selected conditions because of instability of the enolate in the presence of THF and HMPA at a temperature around -40 °C. (a) Woo, K. -C., and Jones, K., *Tetrahedron Lett.*, **1991**, *32*, 6949.(b) Yonezawa, Y., Hirai, M., Tamotu, K., and Shin. J., The 62nd Annual Meeting of Chemical Society of Japan, abstract paper III, p. 601, 1991.
7. All new compounds exhibited satisfactory spectroscopic properties including HR-MS.
8. **5**: $[\alpha]_D^{25}$ -19.4 ° (c 1.02, $CHCl_3$); HR-FABMS *m/z* 384. 2569 [(M+H)$^+$, Calcd. for $C_{20}H_{38}NSi$ 384.2571]; ^{1}H NMR (270 MHz, $CDCl_3$) δ0.03 (3H, s, TBS), 0.05 (3H, s, TBS), 0.88 (9H, s, TBS), 1.12 (3H, d, J=7Hz, C-4Me), 1.54 (9H, s, Boc), 2.06-2.20 (2H, m, H-3, H-4), 2.34 (1H, m, allyl), 2.54 (1H, m, allyl), 3.55 (1H, m, H-5), 3.67 (1H, dd, *J*=10, 2 Hz, CH_2O), 3.99 (1H, dd, *J*=10, 4 Hz, CH_2O), 5.05 (1H, br d, *J*=10 Hz, vinyl), 5.10 (1H, ddd, *J*=17, 2, 1 Hz, vinyl), 5.80 (1H, ddt, *J*=17, 10, 8 Hz, vinyl).
9. **3** was identical with that described in the dissertation of B. Musicki, Harvard University, 1987. **3** : IR (neat) vmax 3356, 2960, 2928, 1740, 1706, 1214 cm^{-1}; HR- FABMS *m/z* 244.1194 [(M+H)$^+$, Calcd for $C_{11}H_{18}O_5N$ 244.1185]; ^{1}H NMR (270 MHz, $CDCl_3$) δ1.35 (3H, d, *J*=7 Hz, C-4Me), 1.85-1.99 (2H, m), 2.06-2.20 (2H, m), 2.52-2.62 (2H, m), 3.68 (3H, s, OMe), 3,.77 (1H, d, *J*=8 Hz, H-5), 3.79 (3H, s, OMe), 5.92 (1H, br s, NH); CD $\Delta\varepsilon_{203}$=+7.8 (MeOH, 23 °C).
10. The similar separation was obtained on a column of SUMICHIRAL OA-4700 (Sumika Analytical Center, Ltd.). We thank Mr. H. Kitahara for performing the analysis.
11. Krill compound F was purified by 6 steps (1.6 mg from 765 g of frozen krill) according to the reported method using 2-mercaptoethanol : 1) extraction with 60% EtOH; 2) extraction with EtOH; 3) partition with n-BuOH-EtOH-H_2O; 4) aluminum column (Woelm Act I, 0.6% NH_4OH in 1:1 EtOH-H_2O); 5) DEAE cellulose (Whatman DE52, 0.2 M NaCl in 1:1 EtOH-0.02 M Tris buffer, pH 7.5); 6) desalted with EtOH.
12. Ozonolysis product (0.11 mg from 1.6 mg of compound F) was prepared according to the reported method: 1) ozonolysis in EtOH at -78 °C; 2) reduction with dimethyl sulfide; 3) treatment with diazomethane (twice); 4) silica gel TLC (1:6 hexane-EtOAc); 5) HPLC on a silica gel column (Develosil 60-3, 10 mmϕ x 250 mm).

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