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A New Lipobetaine from the Red Alga *Neodilsea yendoana*

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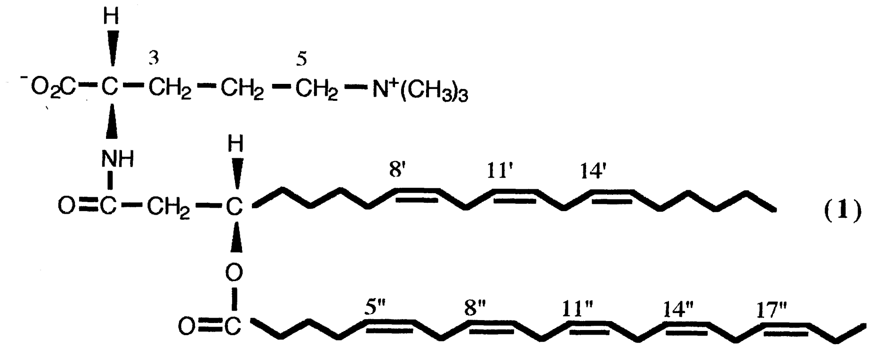
A new icosanoid lipobetaine has been isolated from a red alga and its structure has been determined to be (2*S*)-2-[(3*S*)-3-[(5Z, 8Z, 11Z, 14Z, 17Z)-5,8,11,14,17-icosapentaenoyloxy]-(8Z, 11Z, 14Z)-8,11,14-icosatrienoylamino]-5-trimethylammoniopentanoate , mainly on the basis of NMR spectral and chemical means.

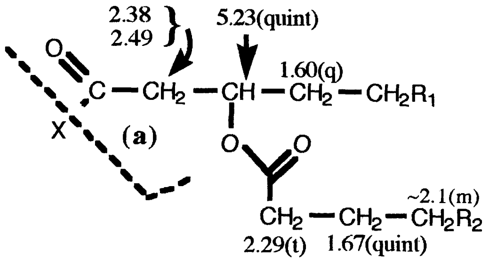
We wish to report herein the isolation and structure of a new type lipobetaine, named yendolipin (**1**), from the seaweed *Neodilsea yendoana* (Japanese name akaba). A compound like **1** composed of two highly unsaturated icosanoid moieties and a polar trimethylornithine betaine has not hitherto been recorded.[1)]

The alga (10 kg wet) was collected in July 1983-1986 at Denshinhama Beach, Muroran, Hokkaido and immediately extracted with MeOH at room temperature. The crude concentrate was treated with hexane and the hexane extract was purified by silica gel chromatography using increasing concentrations of MeOH in benzene as eluents. The new lipid (496 mg, oil) was eluted between phosphatidylcholine and phosphatidylethanolamine fractions.[2)] However, the P atom was not detected by the ^{31}P NMR. Yendolipin exhibited further the following NMR data. ^{1}H NMR (CDCl$_3$, 400 MHz, Fig.1) δ= 0.89 (3H, t, *J*=7 Hz), 0.97 (3H, t, *J*=7 Hz), 1.26~1.39 (10H, m), 1.60 (2H, q, *J*=6 Hz), 1.67 (2H, quint, *J*=7 Hz), 1.75~1.95 (4H, br.), 2.02~2.12 (8H,m), 2.29 (2H, t, *J*=7.3 Hz), 2.38 (1H, dd, *J*=4.8, 14.7 Hz), 2.49 (1H, dd, *J*=7.3, 14.7 Hz), 2.78~2.85 (12H, m), 3.23 (9H, s), 3.63 (2H, t),4.07 (1H, q), 5.23 (1H, quint, *J*=6.3 Hz), 5.28~5.42 (16H, m), 7.25 (1H, d, *J*=5.9 Hz) (in total 78H); ^{13}C NMR (CDCl$_3$, 100 MHz, multiplicities from DEPT, numbers of overlapped peaks from C/H COSY) δ_C=14.0 (q), 14.2 (q), 19.2 (t), 20.4 (t), 22.4 (t), 24.8 (t x2), 25.4 (t x6), 26.5 (t), 27.0 (t), 27.1 (t), 29.2 (t), 29.3 (t), 30.2 (t), 31.4 (t), 33.9 (t), 34.5 (t), 41.9 (t), 53.3 (qx3, d), 66.0 (t), 71.2 (d), 126.7 (d), 127.4 (d), 127.7 (d), 127.9 (d), 128.1 (d), 128.2 (d), 128.3 (d), 128.5 (d), 128.7 (d), 128.8 (d), 129.7 (d), 130.3 (d), 131.9 (d), 169.6 (s), 173.0 (s), 174.5 (s), (36 lines). The presence of the partial structures **2**, **3**, **4** as well as two alkapolyenyl chains containing **5** and **6** was deduced from the above ^{1}H NMR chemical shift data, H/H decoupling and H/H COSY experiments. The C/H COSY spectrum indicted that methylene signals at δ_C 24.8 and 25.4 are ovelapped two and six fold respectively, and the peak at δ_C 53.3 consists of four lines due to $N^+(\underline{C}H_3)_3$ and HN-$\underline{C}$H-CO. Further, although only 13 olefinic $\underline{C}$H lines were observed in the above ^{13}C NMR spectrum, 16 lines must be actually present, since 16 olefinic protons of type **6** were found in the ^{1}H spectrum. The overlapped peaks at δ_C 25.4 are all correlated to δ_h 2.78-2.85 (6 x -CH=CH-C$\underline{\mathbf{H}_2}$-CH=CH-) and are ascribed to the methylene carbons between two double bonds. The δ_C value 25.4 shows that the eight disubstituted double bonds have (allZ) configuration, as depicted in **6**.[3)] The three

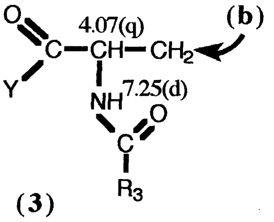


(1)

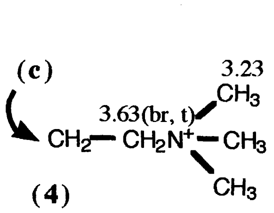
For convenience primes were used for numbering (cf. Fig.1).



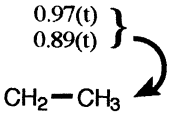
(2)



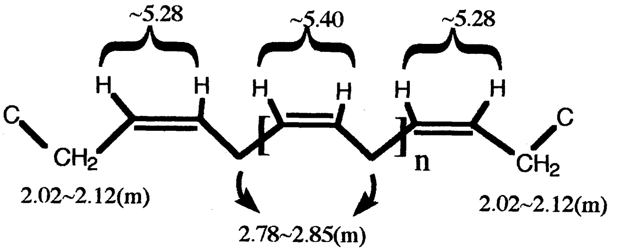
(3)



(4)



2X (5)



2X (6) (Σn=4)

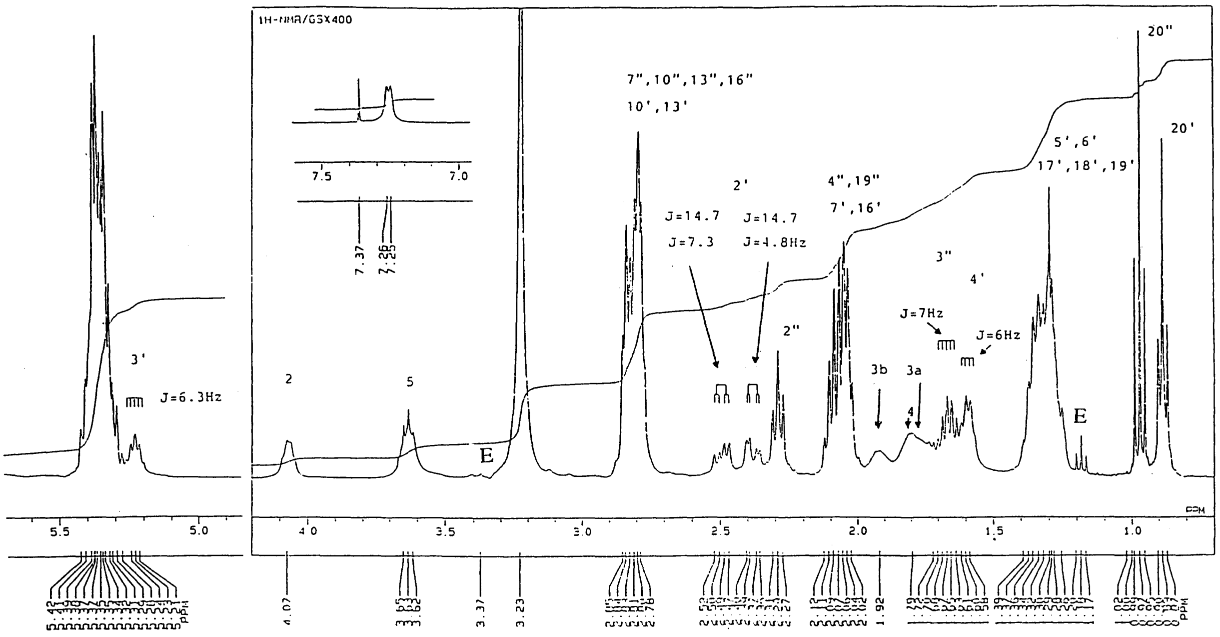


Fig.1. ^{1}H NMR spectrum of 1. E stands for the signals due to ether.

carbonyl peaks appear all at the δ_C 170 region, indicating the presence of three acyl groups in the form of ester, amide (see **2** and **3**), and carboxylate as the counter anion of the ammoniun cation (see **4**), in conformity with the IR spectrum.2) Taking together, it is suggested that the new lipid is a C_{48} compound having a betaine character, with five methyls in $N^+(CH_3)_3$ and in two alkyl chains **5**, twenty-two methylenes, two sp^3 methines shown in **2** and **3**, sixteen sp^2 methines in two skipped polyene sysytems **6**, and three acyl carbon atoms.

In order to confirm the ester structure **2**, the lipid was reduced with $LiAlH_4$ under mild conditions4) to afford (all *Z*)- 5, 8, 11, 14, 17-icosapentaenol.5) This indicated that as shown in formula **2**, 5, 8, 11, 14, 17-icosapentaenoic acid6) is connected to a 3-hydroxy acid to form an ester. In moiety **2**, the terminal C(=O)X group must then be an acylamino group, since if alternatively a carboxylate group were present at that place, yendolipin containing partial structures **2** and **3** should have altogether four C=O groups, one of which is a ketone group (e.g. R_1=-**3** linked to **4**, R_3=-**6** linked to **5**). Therefore the amide acyl group R_3CO of **3** corresponds to the acyl moiety **(a)** of **2** and accordingly C(=O)Y in **3** is the carboxylate part of the betaine. In the above mentioned 1-D and 2-D ^1H NMR spectra, peaks due to the methylene groups **(b)** of **3** and **(c)** of **4** could not be clearly assigned. However, the double quantum filtered (DQF) H/H COSY spectrum of **1** revealed that each proton of the methylene group **(b)** displayed its signal at δ 1.73 and 1.93 as a very broad band. Similarly the DQF COSY showed that the β-methylene group **(c)** of **4** exhibited a broad two-proton signal at δ 1.81. On the basis of these common line broadening phenomena and biogenetic considerations, a bonding between **(b)** and **(c)**, i.e., the presence of N^5-trimethylornithine betaine moiety was assumed. Since out of the 48 carbon atoms of the lipid, 28 atoms are present as icosapentaenoic acid and the ornithine derivtive, the remained twenty carbon atoms and three double bonds are present most probably as an icosatrienoic acid derivative.

In an attempt to prove the above reasoning, the lipid was hydrolyzed with aqueous methanolic HCl.7) As expected, 5, 8, 11, 14, 17-icosapentaenoic acid Me ester, a 3-hydroxyicosatrienoic acid Me ester,8) and N^5-trimethylornithine betaine,9) all of which were characterized mainly by NMR spectra, were obtained. Since the hydroxyicosatrienoic acid yields caproic acid on $NaIO_4$-$KMnO_4$ oxidation,10) and the acid should contain the skipped triene system **6**, positions of the double bonds are placed at 8,11 and 14. 3-Hydroxyicosanoic acid11) prepared by hydrogenation of the above hydroxytrienoic acid had $[\alpha]_D^{25}$ =+27.4° (*c* 0.51, $CHCl_3$), indicating the chirality at position 3 is *S*.12) The betaine produced by the hydrolysis showed $[\alpha]_D^{25}$=+ 4.76° (*c* 0.34, MeOH), while an authentic sample of L-N^5-trimethylornithine betaine13) had $[\alpha]_D^{25}$=+15.3° (*c* 0.80, MeOH). Since the lower $[\alpha]_D^{25}$ value of the betaine obtained by hydrolysis may be due to partial racemization during the reaction (1 M HCl, 70 °C, 18 h), the chirality of C-2 of the betaine is deduced to be *S*. In yendolipin the above three components are connected as shown in **2**,**3** and **4.** Therefore, the new lipid is best represented by formula **1**. The authors thank Drs. M. Imanari (Jeol) and T. Erata (Bruker Japan) for measuring the DQF H/H COSY and C/H COSY spectra respectively. They are also grateful to Prof. H. Shirahama (Hokkaido University) for his help.

References

1) Structural similarity between **1** and phosphatidylcholine suggests that **1** could be a component of cell membrane of the red alga. Some compounds related to **1** have been isolated from lower organisms. a) An N^2-[3-(acyloyloxy)acyloyl]ornithine without *N,N,N*-trimethyl group and double bond from bacteria: H. W. Knoche and J. M. Shively, *J. Biol. Chem.*, **247**, 170 (1971). b) Lipobetaines having the *N,N,N*-trimethylhomoserine group linked to a 1,2-diacylglycerol moiety through an ether bond from lower plants: E. Brown and J.

Evalson,*Biochem.*, **13**, 3476 (1974); N. Sato and M. Furuya, *Plant Science*, **38**, 81 (1985). c) Unusually high content of icosapolyenoic acids in certain red algae : T. Takagi, M. Asahi, and Y. Itabashi, *Yukagaku* (oil chemistry), **34**,1008 (1985).

2) Yendolipin: pale yellow oil. Apt to deteroirate in dry state but rather stable in solution. IR (neat) 3350, 3000, 2920, 1725 (ester), 1650 (amide), 1590 (carboxylate), 1580 (shoulder, amide), and 1390 cm^{-1}. Absence of strong bands due to SO_2 group indicated that **1** is not a sulfolipid. Elemental composition was deduced to be $C_{48}H_{78}N_2O_5$ through combined analysis of 1D/2D ^1H/ ^{13}C NMR and IR spectral data of the lipid and those of its degradation products (see text), and confirmed by FABMS. Positive MS *m/z* 763 (M+1), negative MS *m/z* 747 (M-Me): $[\alpha]_D^{25}$ +6.69 (*c* 1.0, CH_3Cl).

3) The δ_C values of the two methylene groups adjacent to a double bond of a (*Z*)- olefin are smaller than those of the paraffinic counterparts by steric compression effect. J. B. Stothers, “Carbon-13 NMR Spectroscopy” Academic Press, New York (1972), pp. 80-82. See also J. Bus, I. Sies, and M. S. F. Lie Ken Jie, *Chem. Phys. Lipids*, **17**, 501 (1976); F. D. Gunstone, M. R. Pollard, C. M. Scrimgeour, and H. S. Vedanayagam, *ibid.*, **18**, 115 (1977).

4) The sample was added to exess LAH/ether at -25 °C and the mixture was kept at 14 °C for 15 min. Other possible products (e.g. an amine from the amide portion) were obtained in low yield and in impure state, and were not examined.

5) Preparation and t_r values for the pentaenol are reported by T. Takagi, F. Itabashi, and S. Aso, *Lipids*, **20**, 675 (1985). In our case the sample (oil) was characterized by spectroscopic methods. ^1H NMR (CD_3Cl, 400 Mz) δ 0.98 (3H, t, *J*=7.5 Hz), 1.29 (1H, br. OH), 1.42-1.48 (2H, m), 1.56-1.63 (2H, m), 2.06-2.13 (4H, m), 2.80-2.86 (8H, m), 3.65 (2H, t, J=6.5 Hz), and 5.30-5.43 (10H, m); HRMS Found: *m/z* 288.2496. Calcd for $C_{20}H_{32}O$: M, 288.2445.

6) J. M. Whitcutt and D. A. Satton, *Biochem.J.*, **63**, 469 (1956).

7) E. C. Gavor and C. C. Sweeley, *J. Am .Oil Chem.*, **42**, 294 (1965).

8) Mp 24 °C; IR 3000, 2920, 2840, 1740, 1435, 1260, 1190, and 1170 cm^{-1}; ^1H NMR (CD_3Cl, 400 MHz) δ 0.89 (3H, t, *J*=7.3 Hz), 1.25-1.49(12H, m), 2.07 (4H, m), 2.41 (1H, dd, *J*=9.1, 16.5), 2.52 (1H, dd, *J*=3.1, 16.5 Hz), 2.80 (4H, t, *J*=5.8 Hz), 2.90 (1H, s, OH), 3.70 (3H, s), 4.00 (1H, m), and 5.38 (6H, m).

9) Hygroscopic glassy solid. IR 3360, 1570, 1475, 1395, and 910 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 1.76 (2H, br.),1.90 (2H, br.), 3.16 (9H, s), 3.39 (2H, t, *J*=8.3 Hz), and 3.52 (1H, br. t, *J*=8 Hz); ^{13}C NMR (D_2O, 100 MHz) δ_C 21.6 (t), 32.0 (t), 55.5 (q), 57.5 (d), 68.6 (t), and 181.6 (s).

10) D. T. Downing and R. S. Greene, *Lipids*, **3**, 96 (1963).

11) Mp 67-69 °C; IR (KBr) 3400, 2900, 2840, 1710, 1670, and 1220-1310 cm^{-1} (similar to multiple peaks characteristic of straight-chain saturated higher carboxylic acids).Twin carboxyl peaks around 1710 and 1670 are also observed in icosanoic acid (Nujol) ["The Aldrich Library of FT-IR", Edition I, Vol.1, ed. C.J. Pouchart, Aldrich Chemical Company, Milwaukee (1985), spectrum #486A]. Synthesis of the racemic hydroxy acid : H. v. Hahn and F. Grün, *Helv. Chim. Acta*, **38**, 1803 (1955).

12) M. Nakahata, M. Imaida, H. Ozaki, T. Harada, and A. Tai, *Bull. Chem. Soc. Jpn.* , **55**, 2186 (1982).

13) Prepared according to Takemoto's method for the synthesis of N^6-trimethylarginine betaine. T. Takemoto, K. Daigo, and N. Takagi, *Yakugakuzasshi*, **84**, 1180, (1965).

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