CHEMISTRY LETTERS, pp. 9-12, 1993.

A New Glycosphingolipid from the Red Alga *Corallina pilulifera*

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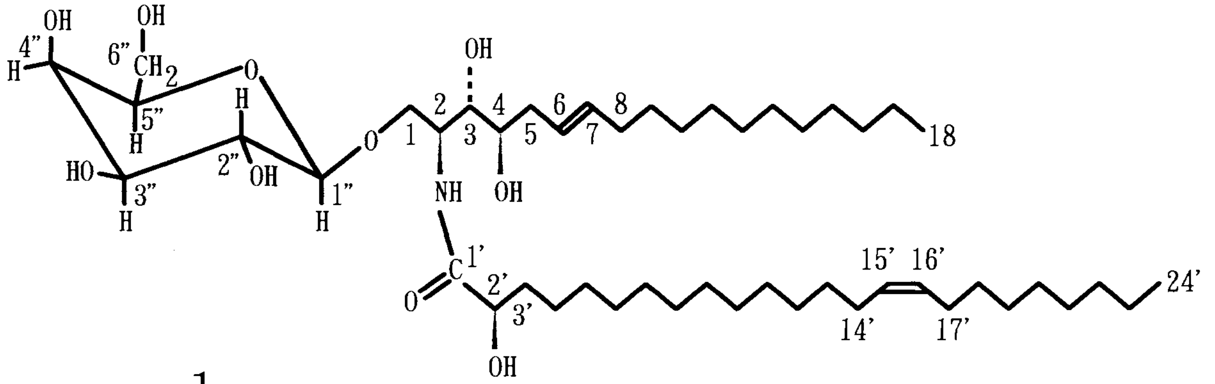
A new glycosphingolipid, named coralipid, has been isolated from a red alga and its structure has been determined to be (6*E*,15'*Z*,2*S*,3*S*,4*R*,2'*R*)-N-(2'-hydroxy-15'-tetracosenoyl)-1-O-β-galactosyl-2-amino-6-octadecene-1,3,4-triol on the basis of spectral and chemical means.

We wish to report herein the isolation and structure of a new type lipid, named coralipid(1), from the seaweed *corallina pilulifera*.

A Glycosphingolipid has not been previously found in seaweed[1,2] and a trihydroxy sphingoid having double bond at 6 position has not hitherto been known.

The alga (10 kg, wet weight) was collected in May 1983— 1986 at Denshinhama Beach, Muroran, Hokkaido and immediately extracted with MeOH at room temperature. The crude extract was reextracted with hexane and hexane extract was purified by silica gel chromatography using increasing concentrations of MeOH in benzene as eluents. From the fraction eluted by



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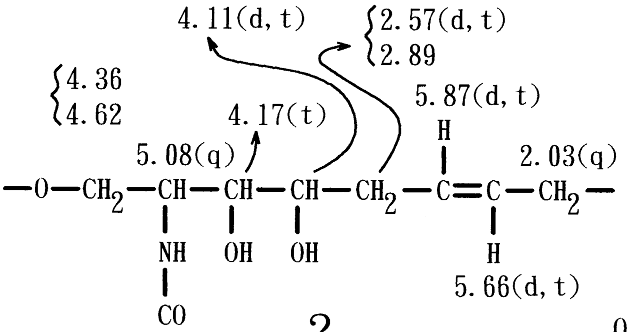
benzene-MeOH (10:1) crude crystals of coralipid separated out after concentration. Pure coralipid was yielded by silica gel chromatography using $CHCl_3$-MeOH (6:1) as eluents.

Coralipid (1) (white crystals, mp 61-62 ℃) showed $[\alpha]_D^{25}$ + 12.6° (c 1.27, pyridine) and IR 3400, 1645, 1540, 1090 cm^{-1}. ^1H NMR (C_5D_5N-$CDCl_3$ 9:1, 500 MHz) δ =0.87 (3H, t, J=7 Hz), 0.88 (3H, t, J=7 Hz), 1.27 (54H, br.), 1.62 (1H, br.), 1.69 (1H, br.), 1.88 (1H, br.), 2.03 (2H, q, J=7 Hz), 2.10 (5H, br.), 2.57 (1H, dt, J=14.6 Hz), 2.89 (1H, br.), 3.89 (1H, t, J=6 Hz), 3.99 (1H, dd, J=9.6, 3.5 Hz), 4.11 (1H, dt, J=2.7 Hz), 4.17 (1H, t, J=5.5 Hz), 4.25– 4.32 (3H, m), 4.36 (1H, dd, J=11.4 Hz), 4.40 (1H, d, J=3.5 Hz), 4.44 (1H, dd, J=8.4 Hz), 4.62 (1H, dd, J=10.8, 8.6 Hz), 4.75 (1H, d, J=7.8 Hz), 5.08 (1H, br., q, J≈ 5 Hz), 5.47 (2H, t, J=4 Hz), 5.66 (1H, dt, J=15.4, 7 Hz), 5.87 (1H, dt, J=15.4, 7 Hz): ^{13}C NMR (C_5D_5N, 100 MHz, multiplicities from DEPT, numbers of overlapping peaks from C/H COSY) δ =14.3 (qx2), 22.8 (tx2), 25.8 (t), 27.6 (tx2), 29.3 (tx20), 32.6 (tx2), 33.2 (t), 35.4 (t), 75.1 (d), 37.3 (t), 51.4 (d), 62.3 (t), 70.1 (d), 70.4 (t), 72.2 (dx2), 72.6 (d), 75.0 (d), 76.6 (d), 105.9 (d), 128.1 (d), 130.8 (dx2), 132.6 (d), 175.6 (s). (48 lines).

The presence of the partial structure 2, 3, and 4 was revealed by the ^1H 1-D spectrum and a relayed COSY spectrum. Double bond (δ =5.66 and δ =5.87)



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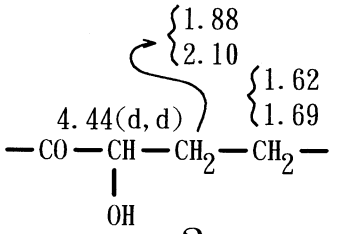

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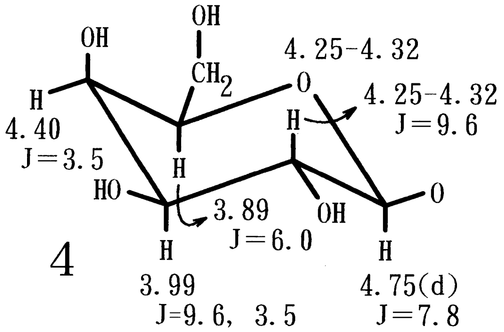

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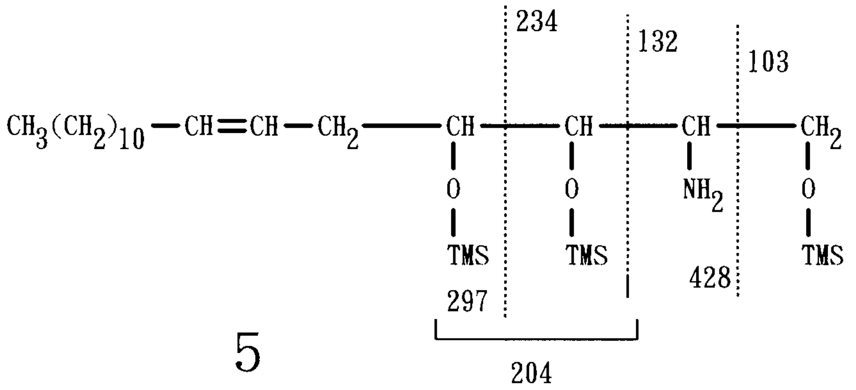
is *trans* configuration in 2, since coupling constants at δ =5.66 and δ = 5.87 are 15 Hz. Anomeric configuration of 4 is β -D-galactose by H/H COSY spectrum and coupling constant.

The structure of phytosphingosin part was determined as follows.

In order to confirm the partial structures 2 , 3 , 4 , the lipid was hydrolysed with aqueous methanolic HCl[3] and yielded a sphingoid, a hydroxy acid Me ester and a methylated sugar. Sphingoid has a methylene groupjoined double bond, a methylene group joined primary hydroxy group, amino group, methine group joined amino group and *trans* double bond (J=16 Hz) by ^{1}H NMR spectrum. The MS spectrum of the TMS derivative of the sphingoid exhibited ions at m/z : 531 (M^+, $C_{27}H_{51}O_3NSi_3$) , 516 (M-15) , 428 (M-103) , 338 (M-90-103) , 297 (M-234) , 204, 132. These show a trihydroxy long chain base 5 .[4–7]


$$CH_3(CH_2)_{10}-CH{=}CH-CH_2-\underset{\substack{|\\O\\|\\TMS}}{CH}-\underset{\substack{|\\O\\|\\TMS}}{CH}-\underset{\substack{|\\NH_2}}{CH}-\underset{\substack{|\\O\\|\\TMS}}{CH_2}$$

5

In order to prove the position of double bond, the sphingoid was oxidized with KIO_4-$KMnO_4$.[8] As expected, lauric acid was obtained and the double bond was identified at 6 position. Hydrogenation product of the sphingoid exhibited $[\alpha]_D^{25}$ +9.13° (c 1.17, pyridine) and its $[\alpha]_D$ agreed with the value of phytosphingosine ($[\alpha]_D^{25}$ +7.9°) .[9] NMR data of tetraacetylated sphingoid was also agreed with the value of 2*S*,3*S*,4*R*-phytosphingosine by Sugiyama et al.[10]

The structure of α -hydroxy fatty acid was determined as follows. Hydroxy acid Me ester has one double bond and one hydroxyl group by H NMR. Configuration of double bond was assigned *cis* from the magnitude of coupling (J=8 Hz) .[11] It was ascertained also by the fact that chemical shift of α , α' -methylene carbons adjacent to double bond was δ =27.2 and its value was found in higher field[12] than saturated methylene group. Double bond is at 15 position, because KIO_4-$KMnO_4$ oxidation[8] of this hydroxy acid gave a peralgonic acid. Hydrogenation product of this hydroxy acid exhibited $[\alpha]_D^{25}$ +1.82° (c 0.55, pyridine) and this agrees with a value of cerebronic acid (*R*) .[13] Therefore hydroxy acid has a *R*-configuration. Methylated sugar obtained by hydrolysis with aqueous methanolic HCl was exactly the same as methylgalactosyl in GC spectrum and $[\alpha]_D$.

The new coralipid 1 is thus shown to be (6*E*,15'*Z*,2*S*,3*S*,4*R*,2'*R*) -N-2'-hydroxy-15'-tetracosenoyl-O-β -galactosyl-2-amino-6-octadecene-1,3,4-triol.

The authors thanks Drs. Imanari (Jeol) and Erata (Bruker Japan) for measureing the H/H COSY and C/H COSY spectra respectively.

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(Received August 10, 1992)