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Isolation and Characterization of a Ceramide and b-Sitosterol Compounds on *Haliclona* (Reniera) *fascigera* From Spermonde Archipelago

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Abstract : The research purpose was to determine the molecular structure of a compound**1**(a ceramide) and compound **2**(b-sitosterol)isolated from ethyl acetate fraction of *Haliclona* (Reneira) *fascigera* sponge. The isolation and purification of compound **1** and **2** using vacuum liquid chromatography (VLC), flash column chromatography and preparative thin layer chromatography (PTLC). Molecular mass of compound **1** were 551 m/z and confirmed by ESI-MS mode ion positive as m/z 552.64 (M+H) and ion negative mode as m/z 550.39 (M-H). Molecular mass of compound **1** was m/z 414 and confirmed by GC-EIMS. Molecule structure of compound **1**was confirmed by FTIR, LCMS, GCMS, 1D and 2D NMR analyses. Molecule structure of compound **2** was determined by FTIR, GC-EIMS, ^{1}H-NMR, ^{13}C-NMR, DEPT 135and compared with literature data. Results of analysis spectroscopy and chromatography confirmed that compound **1**as a new ceramide namely (*2R, 3S, 4E*)-2-(hexadecanoiylamino)-4-nonadecena-1.3-diol and compound **2**as b-sitosterol. The composition of amino alcohol aliphatic as unit sphingoid long chain base (LCB)and unit Fatty Acid Methyl Ester (FAME) in compound **1** were determined by GC-EIMS.
Key words : *Haliclona,* ceramide,b-sitosterol, sponge, FAME, LCB.

Introduction

Sponges are invertebrate animal living in coral reef ecosystem, multicellular animal and the evolutionary most primitive in the world.[14]and produce secondary metabolite as chemical defenses against predators.[12]Steroid such as sterol were secondary metabolite, many found in sponge and usually as major compound with structural diversity such as on *Haliclona* sp .and other sponges from WesternAustralia.[4]Several steroid from *Neopetrosia exigua* such as 24-methylcholesterol, 5,6-dihydrocholesterol, b-sitosterol, and three 5a,8a-epidioxysterols active cytotoxic against leukemia cell line HL-60.[7]The sterols play role as hormones precursor and as main constituents of the lipid cell membrane.[14] The other compounds widely distributed in marine organism were ceramide, aamides linked to fatty acid with long-chain containing more than two hydroxyl bases. Ceramides are important intermediates in the biosynthesis of sphingolipids through condensation of acyl-CoA and serine to yield 3-ketosphinganine and reduced to sphinganine (2-amino-1.3-dihhyroxyalkane)(ceramides).[5,10]Major product of ceramide metabolism is sphingomyelin.[10]Then basic structure was modified in chain length, degree of unsaturation, methyl branching, additional hydroxyl groups, and stereochemistry.[15]A series of ceramides branching called oceanapins A-F, have been isolated on *Oceanupia* cf.

tenuis sponge from Woodin Channel, New Caledonia.[8]The cerebroside as ceramide derivative consistS of ceramide and a single sugar residue at C-1 have been isolated from *Haliclona*(Reniera) *fascigera* sponge on n-hexane fraction with cytotoxicity value, LD50 45 µg/mL.[11]Ceramide compound was involved in membrane structure formation of biological processes play a role in cancer and skin diseases.[18]Many of the naturally and synthetic sphingoid bases are cytotoxic for cancer cells.[15]Five new ceramides, neritinaceramides A, B, C, D and E frommarine bryozoan *Bugula neritina* from the South China Sea were selectively cytotoxicity against HepG2 (IC50, 47.3 µM)and SGC7901 (IC50, 58.1 µM).[16]In additional, a new ceramide from soft coral (*Nephthea*sp.) of the Bay of Bengal with structure 2-aminooctadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate. 2*S*,3*R*-2-aminooctadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate.[2]Onthe ethyl acetate extract of *Haliclona tubifera* from Brazilian coastline were reported two modified C18 sphingoid base, namely (2*R*, 3*R*,6*R*, 7*Z*)-2-aminooctadec-7-ene-1,3,6-triol and 2*R*,3*R*, 6*R*)-2-aminooctadec-1,3,6-triol,potentially active cytotoxic against human glioma and neuroblastomacell lines.[13]Bathymodiolamides A and B as ceramide derivatives were first secondary metabolites from the animal vent invertebrate mussel *Bathymodiolus thermophiles* and both of ceramide inhibit the growth cancer cell lines HeLa.[3]Molecular structure of compound **1**and **2**was determined using FTIR, LCMS,GCMS, NMR 1D and 2D.

Experimental

General

The research steps were extraction, isolation using VLC, FCC and PTLC, purification and characterization. Instruments in the research are Heidolph LABORTA 4000 evaporator, Mettler AE100 (balance),Electro Thermal melting point apparatus, Fourier Transform Infra-Red (Shimadzu IRPrestige-21), Waters HPLC-MS (Acquity HPLC-SQD MassLynx 4.1 SCN805), GCMS (GCMS-QP2010 ULTRA SHIMADZU), NMR Agilent 500 MHz, and NMR JEOL JNMECA 500MHz.

Animal Material

The sponge sample was collected using SCUBA at 6 m depth on October 2013 from Samalona Island, Spermonde Archipelago, Indonesia (Figure 1). The sample was frozen immediately. The specimen for taxonomy was identified and deposited in Research Center for Oceanography (RCO), LIPI Jakarta by Tri Aryono Hadi with voucher code SPV06/12/13.

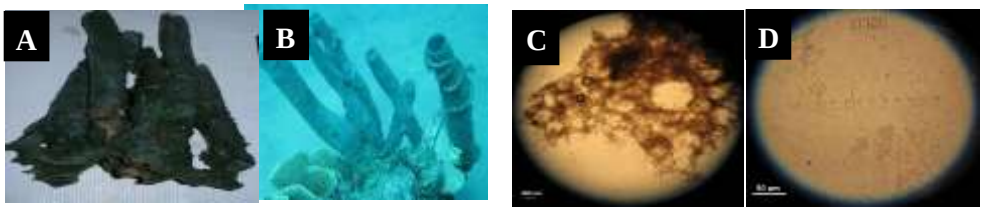


Figure 1.Sponge photo of *Haliclona* (Reniera) *fascigera* : A) Outdoor, B) Underwater, C) Skeletons, D) Spicule

The species taxonomy was Demospongiae (class),Haplosclerida (order), Chalinidae (family), Haliclona (genus), and *Haliclona*(Reniera) *fascigera* (species). The morphology of the species are a long-tube shaped, consistency of its body are soft and fragile.The skeletons are unispicular tracts, isotropic with size of oxeas spicule spicule are 60-80 µm.

Extraction and Isolation

The fresh sample of *Haliclona*(Reneira)*fascigera* sponge (424.5 g) into chopping small pieces was extracted with methanol grade. Then, methanol extract was evaporated to obtained crude methanol extract(14.0769*g*). The crude methanol extract was suspended with 5% water and partitioned with n-hexane and ethyl acetate. N-hexane, ethyl acetate and the residue(methanol-water)fractions were evaporated to obtained n-hexane (857.5 mg), ethyl acetate (123.5 mg) and residue (9.2104g). Result of toxicities test using *Brine Shrimp Lethality Test* for crude methanol extract, n-hexane, ethyl acetate and residue were 8.16µg/mL, 10.35µg/mL, 6.29µg/mL and 17.74 µg/mL, respectively. Ethyl acetate fraction was potential as anticancer. Ethyl acetate

fraction was subjected to VLC with gradient eluent composition n-hexane: ethyl acetate (10:0) to ethyl acetate: methanol (0:10)to yield 10 fractions as HAL-V1 to HAL-V10. The fractions of HAL-V3 and HAL-V4 were combined and then separated on PTLC with eluent Ethyl Acetate: Methanol (9.7:0.3) to get three isolate. The all isolates were HAL-V3-4A (4.5 mg) as compound **1**, HAL-V3-4B (3.1 mg) and HAL-V3-4C (12.7 mg). HAL-V3-4B and HAL-V3-4C are mixture. The molecule structure of compound **1**was determined using ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC, FTIR, LCMS and GCMS. The combine fractions of HAL-V1-5 was separated on FCC with gradient eluent composition n-hexane: ethyl acetate (10:0 to 0:10) toyield 218 fractionsin 10 combine fractions (HAL-V1-5A to HAL-V1-5J. Fraction of HAL-V1-5B was purified through recrystallization to solid needle crystal (11.5 mg)as compound **2.** The molecule structural of compound **2** was determined by FTIR, ^{1}H-NMR,^{13}C-NMR, DEPT 135, and GC-EIMS.

Methanolysis of compound 1

Two aliphatic groups on compound **1**were determined with hydrolysis using HCl-MeOH. Some 2 mg isolate dissolved into 5 mL of 1 MHCl-MeOH and refluxed for 10 hours at 80^{o}C. The mixture was separated into n-hexane and methanol. The n-hexane layer as unit fatty acid methyl ester (FAME) and the MeOH layer were neutralized with NH_4OH and the part of solution was concentrated to yield unit sphingoid long chain base (LCB).[16] Molecule mass of FAME and LCB were determined by GC-EIMS.

Spectroscopic characterization of compound 1 and 2

Compound 1, White solid waxy;mp 89-91^{o}C;molecule formula ($C_{35}H_{69}NO_3$), IR (KBr) ν_{max}/cm^{-1}:3313.71, 3361.93, 3099.61, 1548.64, 2954.95, 2916.30, 2850.79, 1645.26, 1620.21, 1548.64, 1465.90, 1440.83, 1047.35, and 721.36.^{1}HNMR (500 MHz, $CDCl_3$) δ 6.25 (1H, *d, J*=7.5Hz, NH), 3.94 (1H, *dd, J*=3.6Hz, H1a), 3.70 (1H, *br d, J*=11.3Hz, H1b), 3.90 (1H, *sext. J*=3.6Hz, H2), 4.31 (1H, *br m*, H3), 5.52 (1H, *dd, J*=6.5Hz, H4), 5.78 (1H, *dt, J*=6.7, 15.4Hz, H5), 2.05 (2H, *q, J*=7.1Hz, H6), 1.35 (18H, *m*, H7-H15), 1.35 (2H, *m*, H16), 1.50 (1H, *m*, H17), 0.87 (6H, *d, J*=6.7Hz, H18-H19), 2.78 (2H, *br s, J*=2.7Hz, OH), 2.22 (2H, *t, J*=7.5Hz, H2'), 1.62 (2H, *q, J*=7.6Hz, H3'), 1.24 (2H, *m*, H4'), 1.24 (22H, *m*, H5'-H15'), 1.24 (2H, *m*,H15'), 0.85 (3H, *d, J*=6.7Hz, H16'). ^{13}CNMR (125MHz, $CDCl_3$)δ 62.66 (CH_2, C1), δ 54.63 (CH, C2), 74.82 (CH, C3), δ 128.92 (CH, C4), δ 134.47 (CH, C5), δ 32.45 (CH_2, C6), δ 29.87 (CH_2, C7-C15), δ 29.28 (CH_2, C16), δ 22.85 (CH_2, C17), δ 14.28 (CH_3, C18-C19), δ 174.10 (Cq, C1'), δ 37.00 (CH_2, C2'), δ 29.43 (CH_2, C3'), δ 28.13 (CH_2, C4'), δ 27.59 (CH_2, C5'-C14'), δ 32.08 (CH_2, C15'), δ 22.85 (CH_3, C16').

Compound 2, white needle crystalline. mp 137-139^{o}C molecule formula ($C_{29}H_{50}O$). The Molecule mass 414 m/z. IR (KBr) ν_{max}/cm^{-1}: 3427.51, 3336.85, 3026.31, 2958.80, 2936.66, 2866.22, 1637.56, 1463.97, 1377.17, 1330.88, 1238.30, 1192.01, 1132.21, 1107.14, 1058.92, 1022.27, 980.55. ^{1}HNMR (500MHz, $CDCl_3$) δ 3.52 (1H, m, *J*=4.55Hz, H3), 5.35 (1H, d, *J*=5.35Hz, H6), 0.67 (3H, s, H18), 1.00 (3H, s, H19), 0.92 (3H, d, *J*=6.5Hz, H21), 0.83 (3H, d, *J*=6.5Hz, H26), 0.81(3H, d, *J*=7.2Hz, H27), 0.85 (3H, d, *J*=7.8Hz, H29). ^{13}CNMR (125 MHz, $CDCl_3$)δ 37.41 (CH_2, C1), 32.09 (CH_2, C2), 72.00 (CH, C3), 42.44 (CH_2, C4), 140.92 (Cq, C5), 121.94 (CH, C6), 31.82 (CH_2, C7), 32.06 (CH, C8), 50.38 (CH, C9), 36.33 (Cq, C10), 21.28 (CH_2, C11), 39.93 (CH_2, C12), 42.49 (Cq, C13), 56.93 (CH, C14), 24.48 (CH, C15), 28.45 (CH_2, C16), 56.11 (CH, C17), 12.01 (CH_3, C18), 19.58 (CH_3, C19), 36.33 (CH, C20), 19.21 (CH_3, C21), 34.11 (CH_2, C22), 26.19 (CH_2, C23), 45.98 (CH, C24), 29.29 (CH, C25), 20.03 (CH, C26), 18.96 (CH_2, C27), 23.22 (CH_2, C28), 12.16 (CH_3O, C29).

Results and Discussion

The isolate of HAL-V3-4A as compound **1**was white solid waxy and melting point 89-91^{o}C.The molecular mass of isolate using LC-ESIMS was m/z 551with fragment mode of ion positive m/z 552.64 (M+H) and ion negative mode m/z 550.39 (M-H). Fraction of HAL-V1-5B as compound 2was white needle crystal with the molecular mass are 414 m/z and melting point 137-139^{o}C.

Characterization of compound 1

The FTIR spectrum indicate presence of strong absorption for secondary amide (NH str.) at 3313.71 cm^{-1}and supported by stretching of C-N at 1548.64 cm^{-1} and bending of NH at 1548.64 cm^{-1}.The carbonyl (C=O str.)appear at 1645.26 cm^{-1}.The absorption ofOH stretching at 3361.93 cm^{-1} was supported by stretching of C-O at 1047.35 cm^{-1}. The presence of absorption of olefin (=CH str.) at 3099.61 cm^{-1} also was supported by C=C

stretching at 1620.21 cm^{-1}. The aliphatic (CH_2 and CH_3str.) at 2954.95 cm^{-1}, 2916.30 cm^{-1} and 2850.79 cm^{-1} were supported by bending of CH at 1465.90 cm^{-1}, 1440.83 cm^{-1} and 1371.39 cm^{-1}, and also bending (rocking) of CH_2 at 721.36 cm^{-1} that show presence more 3 methylene groups.

^{13}C NMR spectrum showed presence signal carbonyl (C=O),one hydroxymethylene (CH_2O) and one oxymethine (CHO) at δ_C 174.10 (C1'), δ_C 62.66 (C1), δ_C 74.82 (C3) and δ_C 54.82, respectively. The signalson the compound **1** also similar with signals at was 175.0 ppm (C=O) and 53.0 ppm(CHO), respectively on Lutaoside[6] and a ceramide analog were 174.5 ppm, 62.4 ppm and 54.9 ppm[19]. All ^{13}C NMR data were exhibited on Table 1.

Proton NMR spectrum of compound **1**showed presence one methine proton signal (CH) at δ3.90 ppm (sext, *J*=3.6Hz) and two proton signals of hydroxymethylene (CH_2-) at δ3.94 ppm (dd, *J*=3.6Hz) and δ3.70 ppm (br d, *J*=11.3 Hz). One oxymethine proton signal (CH-O) at δ4.31 (br, m) showto presence of hydroxyl (OH). Two another methine protons (CH= or H4 and H5) at δ5.52 ppm (*dd*, 6.4Hz) and 5.78 (*dt*, 6.7Hz; 15.4Hz) with a large coupling constant as *trans*configuration of olefin (-HC=CH-). The signals CH_2overlapped at δ1.29-1.35 as long aliphatic carbon chain and similar with (2'R)-hydroxy-N-palmitoul-D-erithro-(2S,3R)-9-methyloctadecaspinga-(4R,8E,10E)-trienine[5] and ceramide analog.[19]Isopropylfunctional group at the end of long chain base aliphatic C19 was a novelty the ceramide. Presence one doublet signal at δ 6.25 ppm (d, *J*=7.5 Hz) indicate proton amide close to carbonyl group. The COSY confirmed coupling an amide proton at δ 6.25 ppm (d, *J*=7.5 Hz) with the CH at δ 3.90 ppm (sext, *J* =3.6 Hz) and HMBC confirmed correlation the proton doublet signals of amide with C carbonyl (C=O) at δ 174.1 ppm and also methylene proton signal at δ 2.22 ppm (t, *J=7*.5 Hz) and δ 1.62 ppm (q, *J=7*.6 Hz) correlated with Cq (C=O) δ 174.1 ppm. Another HMBC correlation were showed by one methyne proton at δ 5.52 ppm (*dd*, *J*=6.4 Hz) with one methyne proton at δ 4.31 ppm (br, m) and two proton of methylene group at δ 2.05 ppm (*q*, *J*=7.1 Hz) and also one methyne proton at δ 5.78 ppm (*dt*, *J*=6.7 Hz)with one proton methyne at δ 4.31 (br m), two proton of methylene group at δ 2.05 ppm (*q*, *J*=7.1 Hz) and two proton of methylene group at δ 1.24 ppm (m).

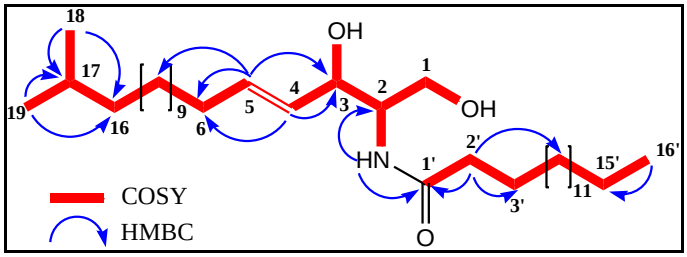


Figure 2. The correlation of COSY and HMBC of ceramide

The determination of relative stereochemistry was done through dihedral angles calculation at chiral carbon and optimizing the molecular structure of ceramide using software HyperChemRelease 7 from Hypercube Incorporate, Gainesville,Florida. The molecular structure ceramide have two chiral carbon atoms on the carbon atom numbers 2 and 3. Furthermore, labeling chirality R and S to obtain *Rectus* configuration (R) on C2 and *Sinister* configuration (S) on C3 (Figure 3). Next determine the dihedral angle between the protons of C2, C3 and C4 with HyperChem computational techniques (Figure 4 and 5).

Table 1 NMR data of compound 1 in $CDCl_3$ (^{1}H-NMR,500 MHz and ^{13}C-NMR,125 MHz)

No. C	^{13}C(δ_H, ppm)	^{1}H-NMR (δ_H, ppm, *J*= Hz), Σ H	COSY (δ_H, ppm)	HSQC	HMBC
NH	-	6,25 (d, 7,5) 1H	3.90	-	NH; C2
1	62.66 (CH_2)	3,94(dd,3.6)1Ha 3.70(br d,11.3)1Hb	2.78; 6.25	C1-H1a/1b	-
2	54.63 (CH)	3.90 (sext, 3.6)1H	6.25;4.31	C2-H2	-
3	74.82 (CH)	4.31 (br m) 1H	2.78;3.90; 5.52	C3-H3	-
4	128.92 (CH)	5.52 (dd, 6.4;)1H	4.31; 5.78	C4-H4	C6; C3
5	134.47 (CH)	5.78 (dt, 6.7; 15.4) 1H	5.52; 2.05	C5-H5	C7;C15; C6; C3
6	32.45 (CH_2)	2.05 (q,7.1) 2H	1.35	C6-H6	C4; C5
7-15	29,87 (9CH_2)	1.35 (m) 18H	-	C7-15–H1-15	C16; C17; C6
16	29.28 (CH_2)	1.35 (m) 2H	-	C16-H16	C6; C17
17	22.85 (CH)	1.50 (m) 1H	0.87	C17-H17	-
18	14.28 (CH_3)	0.87 (d, 6.7) 3H	1.24; 1.50	C18-H18	C17; C15
19	14.28 (CH_3)	0.87 (d, 6.7) 3H	1.24; 1.50	C19-H19	C17; C15
OH	-	2.78(br s) 2H	4.31; 3.70	-	-
1’	174.10 (C)	-	-	-	C2’; C3’
2’	37.00 (CH_2)	2.22 (t, 7.5) 2H	1.62	C2’-H2’	C3’; C1’
3’	29.43 (CH_2)	1.62 (q, 7.6) 2H	1.29; 2.22	C3’-H3’	C1’; C2’;
4’	28.13 (CH_2)	1.24 (m) 2H	1.62	C4’-H4’	C15’
5’-14’	27.59 (11CH_2)	1.24 (m) 22H	1.62	C5’-14’-H5’-14’	C7’-C15’
15’	32.08 (CH_2)	1.24 (m) 2H	0.85	C15’-H15’	C4’-C14’
16’	22.85 (CH_3)	0.85 (d, 6.7) 3H	1.24	C16’-H16’	C4’; C15’

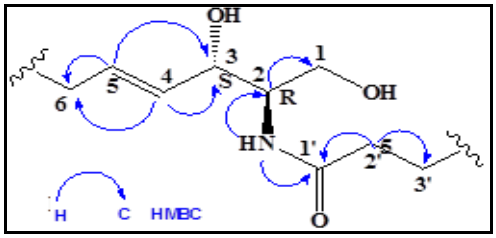


Figure 3. Molecule structure partial of ceramide

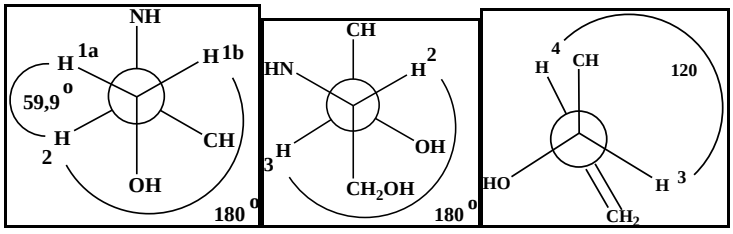
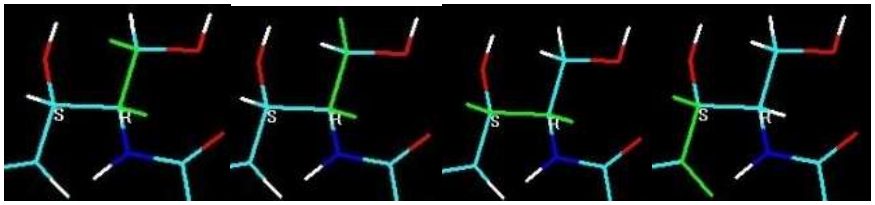


Figure 4. Dihedral angles of H-1a and H-1b to H-2, H-2 to H3 and H-3 to H-4

The dihedral angle between H1a and H2 was 59.9^o show gauche conformation (*J*=3.6Hz) doublet and double between H1a and H2. The dihedral angle between H1b and H2 was 180^o show anti conformation (*J*=11.3 Hz) doublet H1b and H2. The conformation anti also occurs between H2 and H3 with dihedral angle was 180^o and *J* coupling 3.6 Hz multiplet.



(a) (b) (c) (d)

Green line: calculating dihedral angle of H target.

Figure 5. Stick model 3D of dihedral angles and R and S configuration on ceramidepartial structureof C4 to C'1: (a) H1b and H2, (b) H1a and H2, (c) H2 and H3, (d) H3 and H4

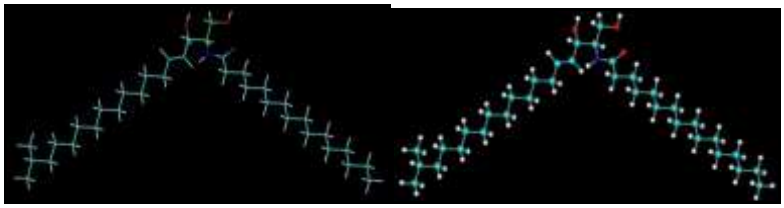


Figure 6. Stick and Ball-stick models 3D of ceramide compound

The ball-stick model 3D of (2*R*, 3*S*, 4*E*)-2-(hexadecanoiyl amino)-4-nonadecena-1.3-diol using Hyperchem Release 7 showed on Figure 6.

ESI-MS with mode positive ion confirmed value m/z 552.64 (M+H) and mode negative ion confirmed m/z value 550.39 (M-H). The NMR data was correlated with molecular formula as $C_{35}H_{69}NO_3$. Methanolysisofisolate compound (Figure 7) yield Fatty Acid Methyl Ester (FAME) unit and sphingoid Long Chain Base (LCB) unit. Both of unit molecule mass were confirmed using GC-EIMS. EI-MS m/z of FAME was 270 $[M]^+$ and LCB was 313 $[M]^+$. The mixture was separated into n-hexane and methanol for three times. The n-hexane layer was unit fatty acid methyl ester (FAME) and the methanol layer provided unit sphingoid long chain base (LCB). The molecule mass of n-hexane layer was analyzed by GC-EIMS.

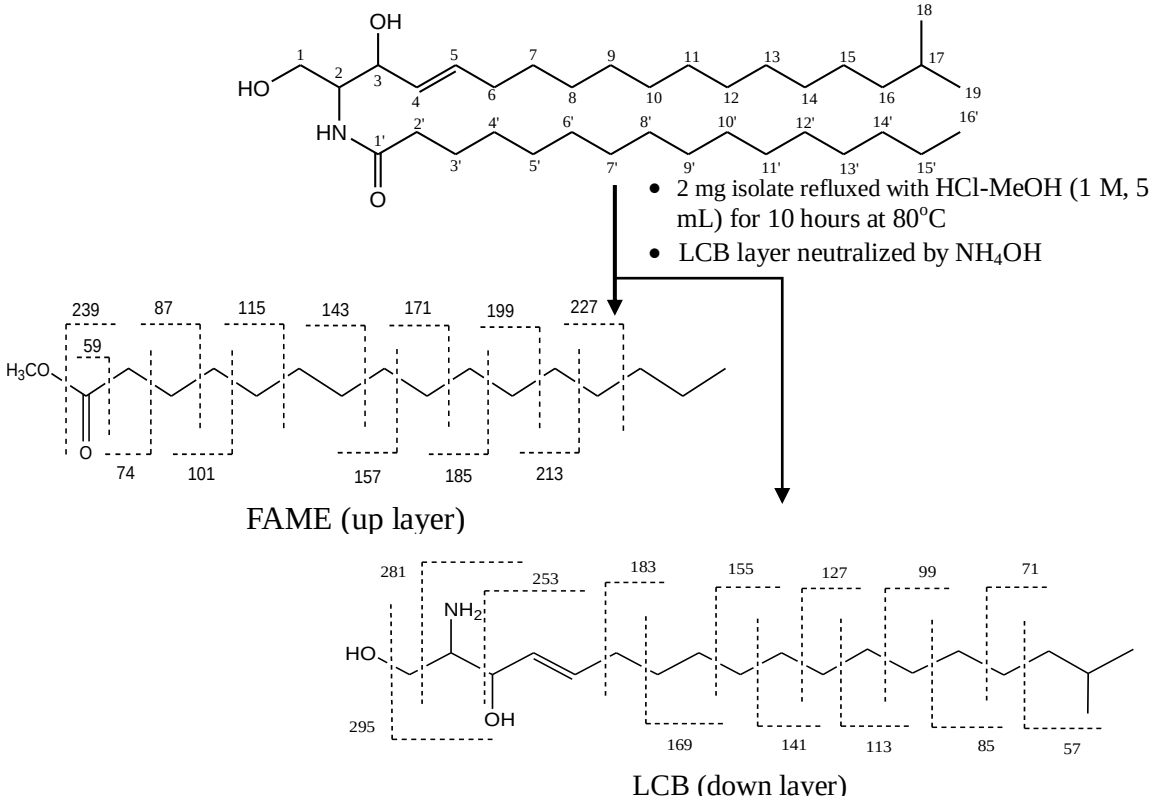


Figure 7. Methanolysis of ceramideand fragmentation of EIMS spectrum A) FAME and B) LCB of

compound 1

The methanol layer was neutralized using NH_4OH and confirmed molecule mass using GC-EIMS (Figure 8). The molecule mass determination of FAME and LCB using GC-EIMS as follows: EI-MS m/z of FAME were 270 $[M]^+$ with fragmentation pattern 239;227; 213; 199; 185; 171; 157; 135; 115; 101; 87; 74 and 55. EI-MS m/z of LCB were 313 $[M]^+$with fragmentation pattern 295; 281; 267; 253; 239; 225; 211; 197; 183; 169; 155; 141; 127; 113; 99; 85; 71 and 57. Presence isopropyl functional group at the end of base long chain aliphatic C19 was supported by high intensity on fragmentation value m/z 57.Result of interpretation was showed that compound **1** as Ceramide compound.

Characterization of compound 2

The FTIR spectrum of compound **2** exhibit presence of absorption bands stretching OH (OH str.)at 3427.51 $cm^{-1}$and supported by stretching of C-O at 1058.92 cm^{-1}.The presenceabsorption bands of olefin (=CH str.) at 3026.31 cm^{-1} also was supported by C=C stretching at 1637.56 cm^{-1}. The aliphatic absorption (CH_2 and CH_3stretching) at 2958.80 cm^{-1}, 2935.66 cm^{-1} and 2866.22 cm^{-1} were supported by bending absorption of CH at 1463.97 cm^{-1} and 1377.17 cm^{-1}. Comparison ^{1}Hand ^{13}C NMR data of compound **2** with literature[1] on the Table 2 were similar as b-sitosterol. The ^{1}H-NMR spectrum data of compound **2**were showed presence six methyl signals between chemical shift, δ 0.64 ppm -1.02 ppm with two singlet signals,three doublet signals and one triplet signal. Two singlet signals for methyl groups were appeared at δ 0.67 ppm and δ 1.00 ppm and both of signals corresponding to H18 and H19, respectively.

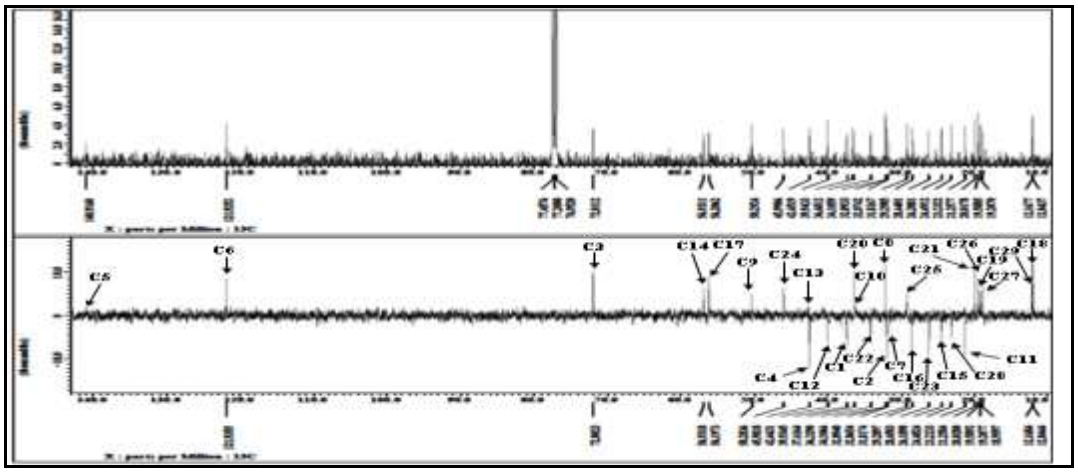


Figure 8.^{1}H-NMR and DEPT spectrum of compound 2

Three doublet signals for methyl groups were appeared at δ 0.92 ppm, δ 0.83 ppm, and δ 0.81 ppm and all signals corresponding to H21, H26 and H27, respectively. One triplet signal was appeared at δ 0.85 ppm and the signal corresponding to H29. The multiplet signal at δ 3.52 ppm and doublet signal at δ 5.35 ppm werecorresponding to H3 and H6 respectively. The doublet signal atδ 5.35 ppm was olefin proton.The ^{13}C-NMR analysis show that compound **2** consist of 29 carbons. The DEPT experiment on Figure 8show that compound **2** consist of six methyl (CH_3), eleven methylene (CH_2), nine methine (CH) and three quaternary carbons (C).The data summary ^{1}H-NMR and ^{13}C-NMR of compound **2** were showed on Table 2. The GC-EIMS data exhibited fragmentation patterns of EIMS on Figure 9 were414, 398, 381, 354, 329, 303, 255, 213, 173, 145, 107, 81, 55, and 43. Results of interpretation showed that compound **2** are b-sitosterol.

Table 2 ^{1}H and ^{13}C NMR data for literature and compound 2 in $CDCl_3$ (500 MHz)

No. C	Compound 2		Literature[1]	
	^{1}H-NMR (δ_H, ppm, *J* = Hz), Σ H	^{13}C-NMR (δ, ppm)	^{1}H-NMR (δ_H, ppm, *J* = Hz, Σ H	^{13}C-NMR (δ, ppm)
1		37,41(CH_2)		37,29 (CH_2)
2		32,09(CH_2)		31,95 (CH_2)
3	3,52 (m,*J*=4,55,1H)	72,00 (CH)	3,51 (m, 1H)	71,84 ((CH)
4		42,44(CH_2)		42,36 (CH_2)
5		140,92(Cq)		140,80 (Cq)
6	5,35 (d, *J*=5,35, 1H)	121,94(CH)	5,34 (d,*J*=5,2, 1H)	121,73 (CH)
7		31,82(CH_2)		31,71 (CH_2)
8		32,06 (CH)		31,95 (CH)
9		50,38 (CH)		50,19 (CH)
10		36,33 (Cq)		36,08 (Cq)
11		21,28(CH_2)		21,12 (CH_2)
12		39,93(CH_2)		39,82 (CH_2)
13		42,49 (Cq)		42,36 (Cq)
14		56,93 (CH)		56,81 (CH)
15		24,48 (CH)		24,33 (CH_2)
16		28,45(CH_2)		28,26 (CH_2)
17		56,11 (CH)		56,11 (CH)
18	0,67 (s, 3H)	12,01(CH_3)	0,67 (s, 3H)	11,88 (CH_3)
19	1,00 (s, 3H)	19,59(CH_3)	1,00 (s, 3H)	19,41 (CH_3)
20		36,33 (CH)		36,54 (CH)
21	0,92 (d, *J*=6,5, 3H)	19,21(CH_3)	0,92 (d, *J*=6,0, 3H)	19,07 (CH_3)
22		34,11(CH_3)		34,00 (CH_3)
23		26,19(CH_2)		26,16 (CH_2)
24		45,98 (CH)		45,39 (CH)
25		29,29 (CH)		29,23 (CH)
26	0,83 (d, *J*=6,5, 3H)	20,03 (CH)	0,82 (d, *J*=7,2, 3H)	19,83 (CH)
27	0,81(d, *J*=7,2, 3H)	18,96(CH_3)	0,79 (d, *J*=7,2, 3H)	18,81 (CH_3)
28		23,22(CH_2)		23,12 (CH_2)
29	0,85 (t, *J*=7,8, 3H)	12,16(CH_3)	0,85 (d, *J*=7,8, 3H)	12,01 (CH_3)

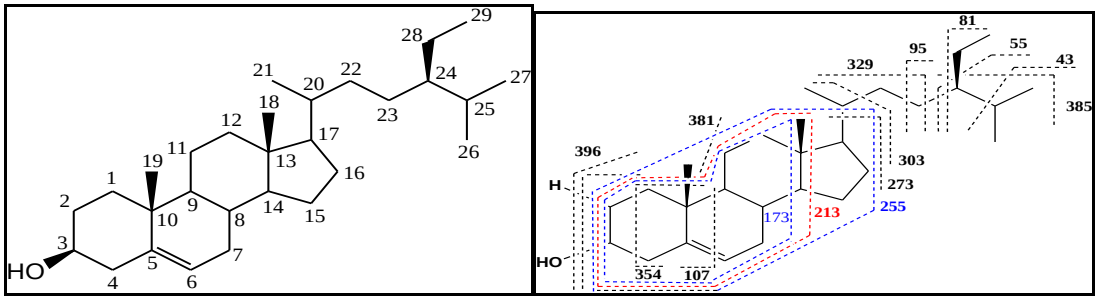


Figure 9. The b-sitosterolin: A). molecule structure and B). Fragmentation patterns

Conclusion

The ceramide and *b*-sitosterol compounds were isolated from marine sponge *Haliclona*(Reniera)*fascigera*. The FTIR, 1D NMR (^{1}H and ^{13}C assignments) and 2D NMR, LCMS and GCMS data correlated with molecular formula as $C_{35}H_{69}NO_3$ namely (2*R*, 3*S*, 4*E*)-2-(hexadecanoiylamino)-4-nonadecena-1,3-diol.Novelty of the ceramide is presence isopropyl functional group at the end of base long

chain aliphatic C19. The molecule mass of ceramide was 551 m/z with mode ion positive of ESI-MS m/z 552.64 (M+H) and ion negative mode as m/z 550.39 (M-H). Molecules mass of result methanolysis namely FAME and LCB were $[M]^+$270 m/z and $[M]^+$313 m/z, respectively. The molecule structure of b-sitosterol was confirmed by H-NMR, and DEPT C-NMR and also was compared with literature as b-sitosterol. GC-EIMS show mass 414 m/z.

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