Antibacterial Sphingolipid and Steroids from the Black Coral *Antipathes dichotoma*

Sultan Samran AL-LIHAIBI,[a] Seif-Eldin Nasr AYYAD,[b] Fekry SHAHER,[a] and Walied Mohamed ALARIF*,[a]

[a] *Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University; P. O. Box 80207, Jeddah 21589, Saudi Arabia: and* [b] *Department of Chemistry, Faculty of Science, King Abdulaziz University; P. O. Box 80203, Jeddah 21589, Saudi Arabia.* Received May 11, 2010; accepted August 31, 2010; published online September 21, 2010

From the black coral *Antipathies dichotoma*, a sphingolipid (2*S,3*S**,4*E*,8*E*)-2*N*-[tetradecanoyl]-4(*E*),8(*E*)-icosadiene-1, 3-diol (1) and a steroid (22*E*)-methylcholesta-5,22-diene-1α,3β,7α-triol (2) were isolated. Other known compounds, 3β,7α-dihydroxy-cholest-5-ene (3) (22*E*,24*S*),5α,8α-epidioxy-24-methylcholesta-6,22-dien-3β-ol (4) and (22*E*,24*S*),5α,8α-epidioxy-24-methylcholesta-6,9(11),22-trien-3β-ol (5). The structures were established on the basis of NMR spectroscopic analysis and comparison with literature. The antibacterial activity of five compounds was evaluated.**

Key words black coral; *Antipathes dichotoma*; sphingolipid; trihydroxy steroid; antibacterial

Antipathes dichotoma (PALLAS) belongs to zoanthoid black coral. It has some pharmaceutical uses, such as relieving fever and softening hard mass. Few literatures about the chemical constituents of black corals that reported nine steroids from *A. subpinnata*,[1,2] and four alkaloids from *A. dichotoma*.[3] Sphingolipids form a biologically important class of compounds,[4] some of which have been reported to exhibit antihepatotoxic, antitumor and immunostimulatory activities,[5,6] inhibition of atherosclerosis[7] and as secondary messengers.[8]

Results and Discussion

Compound **1** has a molecular formula of $C_{34}H_{65}NO_3$ which was determined from high resolution (HR)-FAB-MS data (*m/z* 558.4876 $[M+Na]^+$). Calcd 558.4862, electron ionization-mass spectra (EI-MS) (*m/z* 535) and ^{13}C-NMR. The IR spectra showed the hydroxyl and amide NH group bands at 3340 and 3320 cm^{-1}, the band at 1640 cm^{-1} was due to CONH group.

The ^{1}H-NMR spectrum (Table 1) revealed the presence of two primary methyls at δ 0.88 (6H, t, J=7.2 Hz), two hetero bearing-methines at δ 3.91 and 4.32 and oxygenated methylene protons at δ 3.70 and 3.95, four olefinic protons at δ 5.54, 5.78, 5.43 and 5.36, an NH proton at δ 6.20 and a huge methylene envelope at δ 1.3 (Table 1). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data of **1** were supportive of the above analysis, showing a carbonyl group at δ_C 174.1, two double bonds at δ_C 134.3, 131.4, 129.1 and 128.9, three oxygenated or other hetero atomized carbons at δ_C 74.6, 62.4 and 54.4, aliphatic methylenes at δ_C 22.7—36.8 and two methyls at δ_C 14.2. The downfield doublet at δ 6.28 (NH) was deuterium-exchangeable, and there was no any correlation between this signal and any carbon in the heteronuclear multiple quantum coherence (HMQC) spectrum. On the other hand, a correlation from δ 6.28 (NH) to δ 3.91 (m), and the correlations from δ 6.28 (NH) to δ_C 174.1(C-1′), 36.8 (C-2′), 62.4 (C-1), 54.4 (C-2) and 74.6 (C-3) were observed in the ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra, respectively. All the above data suggested **1** is a ceramide (sphingolipid).[9,10] In order to determine the lengths of sphingosine and fatty acid chains, the positions of double bonds and the absolute configuration of **1**, the acid methanolysis method of Gaver and Sweeley[11] which yield a fatty acid methyl ester (FAME) methyl tetradecanoate *m/z* 242 detected by GCMS, the presence of tetradecanoyl moiety confirmed by the characteristic ion at *m/z* 211 $[CH_3(CH_2)_{12}CO]^+$. So the molecular formulas of FAME and sphingosine are $C_{15}H_{30}O$ and $C_{20}H_{39}NO_2$, respectively. The double bonds and hydroxyl groups should be in sphingosine moiety and their positions could be determined by inspection of ^{1}H–^{1}H COSY spectrum, two methylene (C-1) protons at δ 3.70 and 3.95 correlated with the methine proton (C-2) at δ 3.91 which is correlated with the methine (C-3) proton at δ 4.32, the methine (C-3) proton at δ 4.32 correlated with the olefinic (C-4) proton at δ 5.54 (dt, J=15.0, 6.0 Hz) which is in turn correlated with another olefinic (C-5) proton at δ 5.78 (dt, J=15.0, 6.6 Hz), the olefinic proton at δ 5.78 correlated with two methylene (C-6) at δ 2.15 (q, J=6.6 Hz) that correlated with another two methylene (C-7) protons at δ 2.08 (q, J=6.6 Hz), which correlated with the olefinic (C-8) proton at δ 5.36 (dt, J=15.0, 6.0 Hz), that proton correlated with the olefinic (C-9) proton at δ 5.43 (dt, J=15.0, 6.0 Hz) that is correlated with the two methylene (C-10) protons at δ 1.97 (q, J=7.8 Hz). The above discussion implied that the two OH groups are at C-1 and C-3, and two double bonds one at C-4/C-5 and another between C-8/C-9 (double bonds are *trans* oriented owing to the values of chemical shifts of allylic methylene δ_C>30 and the J values).[12] Consideration of biogenesis and steric hinderance of sphingolipids, generally were acknowledged to determine the absolute stereochemistry of the phytosphingosine moiety. On the basis of the ^{13}C-NMR spectral data, the relative stereochemistries at C-2 (δ 54.4) and C-3 (δ 74.6) were deduced to be 2*S* and 3*S*.[7] Thus, the structure of **1** was established as (2*S**,3*S**,4*E*,8*E*)-2*N*-[tetradecanoyl]-4(*E*),8(*E*)-icosadiene-1,3-diol.

Compound **2** has a molecular formula of $C_{28}H_{46}O_3$ which was determined from HR-FAB-MS data (*m/z* 453.3357 $[M+Na]^+$). Calcd 453.3341, EI-MS (*m/z* 535) and EI-MS (*m/z* 430) together with ^{13}C-NMR, implying six degrees of unsaturation. The presence of hydroxyl and olefinic functionalities was deduced from IR absorptions at 3355 and 1643 cm^{-1}. Three hydroxyls in the molecule were estimated from ion peaks appearing at *m/z* 412 $(M-H_2O)^+$, and 394

* To whom correspondence should be addressed. e-mail: walied1737@yahoo.com