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

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From the black coral *Antipathes dichotoma*, a sphingolipid (25S*,35S*,4E,8E)-2N-[tetradecanoyl]-4(E),8(E)-icosadien-1-3,4-diol (1) and a steroid (22E)-methylcholesta-5,22-diene-3β,7α,6α-triol (2) were isolated. Other known compounds, 3β,7α,12-di-hydroxy-cholest-5-ene (3) (22E,24S), 5α,8α-epidioxy-24-methylcholesta-6,22-dien-3β-ol (4) and (22E,24S), 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 zoanthid black coral. It has some pharmaceutical uses, such as relieving fever and soothing hard mass. Few literatures about the chemical constituents of black corals that reported nine steroids from *A. sulphinata* and four alkaldoids from *A. dichotoma*.

Sphingolipids form a biologically important class of compounds, some of which have been reported to exhibit antiproteptase, antitumor and immunomodulatory activities, inhibition of atherosclerosis as well as secondary messengers.

**Results and Discussion**

Compound 1 has a molecular formula of C_{35}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 13C-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 1H-NMR spectrum (Table 1) revealed the presence of two primary methyls at 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 5.63, an NH proton at δ 6.20 and a huge methylene envelope at δ 1.3 (Table 1). The 13C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data of 1 were supportive of the above analysis, showing a carbonyl group at δC 174.1, two doublets or 134.3, 131.4, 129.1 and 128.9, three oxygenated or other hetero atomized carbons at δC 74.6, 62.4 and 54.4, aliphatic methylenes at δC 22.7–36.8 and two methyls at δC 14.2. The downfield doublet at δ 6.28 (NH) was deuterium-exchangeable, and there was no correlation between this signal and any carbon in the heterocyclic 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 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 1H–1H correlation spectroscopy (COSY) and heterocyclic multiple bond connectivity (HMBC) spectra, respectively. All the above data suggested 1 is a ceramide (sphingolipid) 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 methanalysis method of Gaver and Sweeney, which yield a fatty acid methyl ester (FAME) methyl tetradecanoate m/z 242 detected by GCMS, the presence of tetradecanoate moiety confirmed by the characteristic ion at m/z 211 [CH₃(CH₂)₁₀CO]. So the molecular formulas of FAME and sphingosine are C₃₄H₆₅O₃ and C₃₂H₅₆NO₂, respectively. The double bonds and hydroxy groups should be in sphingosine moiety and their positions could be determined by inspection of 1H–1H 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 methyl δC > 30 and the J values). Consideration of biosynthesis and steric hindrance of sphingolipids, generally were acknowledged to determine the absolute stereochemistry of the phytosphingosine moiety. On the basis of the 13C-NMR spectral data, the relative stereochemistries at C-2 (δ 54.4) and C-3 (δ 74.6) were deduced to be 2S and 3S. Thus, the structure of 1 was established as (25S*,35S*,4E,8E)-2N-[tetradecanoyl]-4(E),8(E)-icosadien-1-3,4-diol.

Compound 2 has a molecular formula of C₃₈H₆₆O₃ which was determined from HR-FAB-MS data (m/z 453.3357 [M+Na]+). Calcd 453.3341, EI-MS (m/z 535) and El-MS (m/z 430) together with 13C-NMR, implying six degrees of unsaturation. The presence of hydroxyl and olefinic functionalities was deduced from IR absorptions at 3345 and 1643 cm⁻¹. Three hydroxyls in the molecule were estimated from ion peaks appearing at m/z 412 (M–H₂O)⁺ and 394.

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