STEROIDS FROM THE MARINE FUNGUS GEOTRICHUM SP.

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ABSTRACT

Ergosterol 1, peroxysterol 2, ergosta-4,6,8(14), 22-tetraen-3-one 3 and 24-ethyl-cholesta-4-ene-3-one 4 were isolated from the cultures of a fungus Geotrichum sp. obtained from a marine sediment. It was established that no other steroids were present in the extract. Their structures were elucidated by spectrocopical methods.

Keywords: steroids, ergostane type steroid, ergosterol, peroxysterol, marine fungus.

INTRODUCTION

Marine microorganisms have recently gained attention as important sources of biologically active secondary metabolites. Marine-derived fungi have shown great potential as suggested by the diversity of secondary metabolites, including many that have novel carbon skeletons. Although most metabolites from marine fungi are closely related to constituent of their terrestrial relatives. Based on these findings and literature survey, we believed that marine fungi are rapidly becoming recognized as potentially useful sources of compound with biomedical interest.

As part of our studies on secondary metabolites from marine organisms3 from the Chilean coast, we have investigated the chemical constituents obtained (glucose free) media made with filtered Valparaíso seawater-based media (Me-28), 0.89 (3H, d, J = 6.5 Hz, Me-26), 0.87 (3H, d, J = 6.5 Hz, Me-27), 0.83 (3H, d, J = 6.5 Hz, Me-25). The fractions were monitored by TLC. Eluates obtained from fractions C-E, after purification by HPLC (Sephadex gel normal phase column and hexane/ EtOAc 20% afforded ergosterol 1 (30 mg) and ergosterol peroxide 2 (80 mg). These compounds were identified by comparing its physical constants and spectral data with those reported in the literature.

Fractio3 F-H were further purified by repeated preparative TLC on Si gel and developed with a mixture of EtOAc/petroleum ether (1:4) to give compounds 3 (10 mg) and 4 (7mg).

Compound 3: yellow plates; mp 115-116°C IR νmax cm−1: 2980, 1675-1640, 1590, 1270, 1233, 975, 880. MS: m/e (%): 392 [M]+, 195, 377 (M-Me), 1.5, 349 (1.5), 268 (42), 253 (6), 240 (3.5), 214 (7.5), 173 (7), 129 (6); 1HNMR (400 MHz, CDCl3): δ: 6.67 (1H, d, J = 9.5 Hz, H-7), 6.09 (1H, d, J = 9.5 Hz, H-6), 5.78 (1H, s, H-4), 5.26 and 5.29 (1H each, d, J = 7.7 Hz, H-22 and H-23), 2.50 (1H, d, J = 5.2, 14.5 Hz, H-2a), 2.45 (1H, d, J = 5.2, 14.5 Hz, H-2b), 1.08 (3H, d, J = 6.5 Hz, Me-21), 1.00 (3H, s, Me-18), 0.97 (3H, d, J = 6.8 Hz, Me-28), 0.89 (3H, d, J = 6.5 Hz, Me-26), 0.87 (3H, d, J = 6.5 Hz, Me-27). 13CNMR δ: 34.2 (C-1), 34.1 (C-2), 199.5 (C-3), 123.0 (C-4), 164.4 (C-5), 124.5 (C-6), 134.0 (C-7), 124.4 (C-8), 144.4 (C-9), 36.8 (C-10), 25.4 (C-11), 34.2 (C-12), 42.4 (C-13), 55.9 (C-14), 24.2 (C-15), 29.2 (C-16), 56.1 (C-17), 12.0 (C-18), 17.4 (C-19), 36.1 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.7 (C-25), 19.8 (C-26), 19.1 (C-27), 23.1 (C-28), 14.0 (C-29).

RESULTS AND DISCUSSION

Analysis of the 1HNMR and 13CNMR spectra of the compounds 1, 2 and 3.
and the comparison with the literature data, indicated that these compounds have an ergostane-type side chain with a 22E,24R-configuration. In general, fungi only produces sterols with the 24β configuration (24α-methyl group), indicating aphylogenetic significance of the configuration at C-24. This is consistent with the assignment of the side chain configuration at C-24 for compounds 1, 2, and 3, by 1HNMR and 13CNMR spectroscopy. The assignments of the proton signals (H-26 and H-27) and the carbon signals (C-26 and C-27) were made according to literature values. Compounds 1 and 2 were identified as ergosterol and ergosterol peroxide by comparing its physical constants and spectral data with those reported in the literature (See Figure 1). Ergosterol is frequently found in fungi extracts, because is part of the cytoplasmic membrane of this organism. Similarly, ergosterol peroxide is a common natural product which has been obtained from a variety of lichens, fungi, sponges and marine organisms. It was reported that ergosterol peroxide inhibited the growth of cancer cells, showed a potent inhibition on lipid peroxidation and exhibited higher antioxidant activity.

Compound 3 was obtained as yellow crystals with mp. 115° -116°C. It represented a ketosteroidal compound with molecular formula C39H62O which was deduced by the MS (392 m/e) and 13CNMR spectra. By the analysis of its 1HNMR and 13CNMR spectra, compound 3 was identified as ergosta-4,6,8(14)-22-tetraen-3-one (See Figure 1). This compound has been obtained from Lampteromyces japonicus and from a luminous bacterium and the bioluminescence displayed by this microorganism is related to the presence of this compound. However, it has also been found in no luminous Basidiomycetes mushroom such as Fomes officinalis and Scleroderma polyrhizum; furthermore, it has been isolated from a marine sponge. This is the first time that this compound is isolated from a facultative marine fungus.

Compound 4 was obtained as yellow oil. The IR spectrum showed signals for an unsaturated carbonyl function at 1650, 1380 cm⁻¹. The MS spectrum showed a molecular ion at 412, and together with the 13CNMR data indicated a molecular formula of C39H64O. The 1HNMR spectrum showed a series of methyl resonances at 1.30 (3H, Me-19), 1.22 (3H, Me-18), 0.98 (3H, d, J=6.5 Hz, Me-21), 0.88 (6H, d, J=6.5 Hz, Me-26 and Me-27), 0.75 (3H, t, J=6.7 Hz, Me-29) clearly indicative of a steroidal structure with a keto function at C-3. The 1HNMR indicates that this compound has only one double bond conjugated with the ketone. The 13CNMR indicated the presence of 29 carbons. The skeleton signals indicated that we were in the presence of a cholestane skeleton and that the side chain must have an additional ethyl group at C-24. The nature of the side chain was established by the 1HNMR data of 4. 8.08 ppm (Me-26 and Me-27), 0.98 (Me-21) and 0.75 (Me-29). Assignments were made with the aid of extensive decoupling experiments and confirmed by comparison with literature data. So, compound 4 is the known 24-ethyl-cholesta-4-ene-3-one (See Figure 1).

REFERENCES

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Figure 1: Steroids isolated from Geotrichum sp.