

Metabolites From Marine Fungus *Aspergillus* Sp.

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Abstract: Chemical examination of a methanolic extract of the marine fungus, *Aspergillus* sp., isolated from marine grass environment, yielded a steroid, ergosterol peroxide (1), and a mixture of known glyceride esters (2,3) of unsaturated fatty acids. Glycerides are well known bioactive molecules and emulsifying agents. It has also been found to be a new source of an industrially important γ -pyrone derivative. The structure of these compounds was established on the basis of spectral data including 2D NMR techniques.

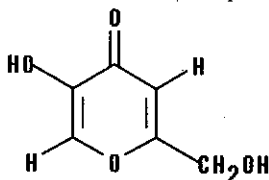
Introduction

Marine micro-organisms including bacteria, fungi and microalgae have received increasing attention over the past two decades as new biomedical resource. Fungi are well represented within the marine environment¹ specially in association with solid support like wood and marine flora and fauna. In fact, increasing number of metabolites is being reported from fungi².

In connection with an ongoing project on "Bioactive substances from the seas around India", we had an opportunity to work on fungus belonging to genus *Aspergillus*. Herein, we report isolation of a γ -pyrone derivative, 2-hydroxymethyl-5-hydroxy- γ -pyrone (1), ergosterol peroxide (2) and unsaturated glycerides (3 & 4) from an unidentified *Aspergillus* sp. isolated from marine grass environment.

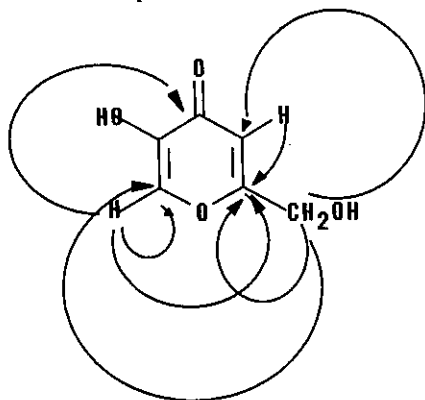
Results and Discussion

Positive electron spray mass spectra of 1 yielded major ions at m/z 142 (M^+), 165 ($M^+ + Na^+$), 307 ($2M + Na^+$) and 449 ($3M + Na^+$). The NMR data of 1 is summarized in Table 1. DMSO was useful for detection of the hydroxyl group at C₅ as well as the alcoholic proton of the hydroxymethyl group in the standard compound.



1

The ^1H and ^{13}C NMR values (Table 1) were assigned through the use of 2DNMR experiments in DMSO- d_6 (HMOC & HMBC). Results from HMOC spectra established partial connectivities. Assignments of quarternary carbons and carbonyl carbons were obtained from heteronuclear multiple bond correlation experiments (HMBC Table 2).



1A

Fig. 1A illustrates the use of HMBC to build structural segments.

Table 1

Assignment	δH (DMSO)	δH (D $_2$ O exchange)	δC
2			170
3	6.33(s, 1H)	6.33(s)	110
4			176
5			145
6	8.02 (s, 1H)	8.024 (s)	138
7	4.28 (d, 2H)	4.28(s)	61
5-OH	9.05 (s, 1H)		
7-OH	5.63 (t, 1H)		

Table 2

HMOC			HMBC	
δH	δC		δH	δC
4.28d	58.978	CH $_2$	8.024	138, 170, 176
6.337	109.4	CH	6.3	138, 170
8.006	138.7	CH	4.2	110, 170

On the basis of above spectral data, the compound was assigned structure 1. The data is well in agreement with the values reported in the literature.³

γ -Pyrone derivatives, demethyl nectripyrone A and nectripyrone B⁴, both closely related to the known terrestrial fungal metabolite nectriapyrone are reported from an unidentified fungi obtained from marine sponge *Jaspis johnstoni* known as the source of cyclic peptide jaspamide^{5,6}. γ -pyrone is reported as a metabolite of *Aspergillus flavus* and *Aspergillus oryzae* and is useful as a starting material for the synthesis of flavour enhancing food additives⁷.

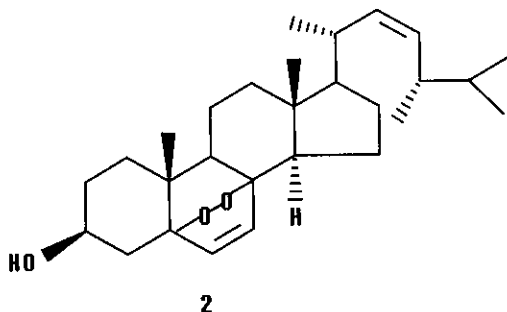
The mass spectrum of **2** gave a molecular ion peak at m/z 428 corresponding to $C_{28}H_{44}O_3$ and indicating the presence of six double bond equivalents; four were assigned to the four rings of carbocyclic nucleus and remaining two to unsaturated bonds. Its IR spectrum displayed characteristic bands at 3525 (-OH group), 968 (a trans C=C) and 1180 cm^{-1} (peroxide group), the presence of this function was supported by the loss of oxygen from the molecular ion peak in the mass spectrum (m/z 396).

The EI mass spectrum in addition to M^+ showed diagnostic peaks at m/z 410 ($M^+ - H_2O$), 303 [$M^+ - SC(C_9H_{17})$], 285 [$M^+ - (SC + H_2O)$], 271 [$M^+ - (SC + O_2)$], 253 [$M^+ - (SC + H_2O + O_2)$], 211 [253 - ring D fusion (42)].

These fragments suggested that the compound was a C_{28} sterol possessing a hydroxyl group and two double bonds, one in the nucleus and one in the side chain.

The 1H NMR spectrum of **2** displayed C_{18} and C_{19} methyl protons at δ 0.81 and δ 0.88 as singlets and four doublets integrating 3H each at δ 0.82 ($J=6.8Hz$), 0.83 ($J=6.8Hz$), 0.94 ($J=6.4Hz$) and δ 1.02 ($J=6.4Hz$) were due to C_{26} , C_{27} , C_{28} and C_{21} secondary methyl protons respectively. The nonequivalence of the C_{28} methyl and one of the isopropyl methyl groups and a characteristic downfield shift in the doublet resonance for the protons of C_{27} methyl group was indicative of 24R methyl configuration.

The downfield shift of the C-3 carbinol proton at δ 3.96 as compared to ergosterol (δ 3.6) indicated some disturbance in the vicinity of C-3 position. 1H NMR exhibited characteristic eight line pattern centered at δ 5.18 (2H,m) for C_{22} and C_{23} protons with $J=7.5Hz$ (coupling between 22-23 H, trans) indicating a $\Delta^{22(23)}$ double bond. The signals at δ 6.24 (1H, d, $J=8.5Hz$) and 6.5 (1H, d, $J=8.5Hz$) were assigned to the protons of nuclear double bond; the peak at m/z 396 in the mass spectrum and the signals at δ 6.24 and δ 6.5 in 1H NMR spectrum were characteristic of ergosterol peroxide. These data suggested the structure of (22E)-24 α -methyl-ergost-6, 22(23)-diene-5 α -8 α -epidioxy-3 β -ol for compound **2**.

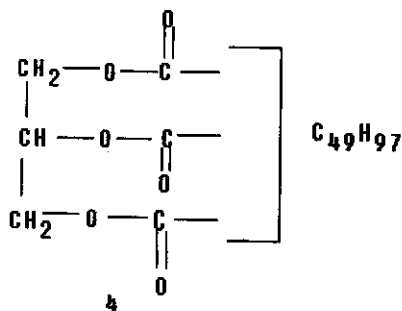
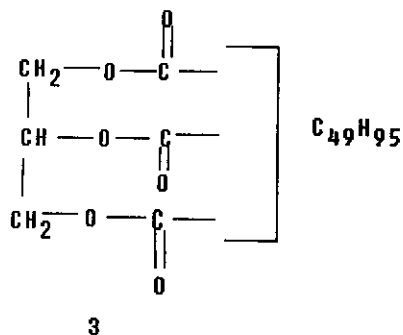


The structure was confirmed by comparison of the spectral data with literature values⁸⁻¹⁰. Further evidence for structure was provided by ¹³C NMR data which showed signals at δ 135.4, 135.2, 132.3 and 130.7 which were assigned to unsaturated carbon C₂₂ and C₂₃, C₆ and C₇ respectively. Signal due to C₃ carbinol carbon was observed at δ 66.4; signals at δ 78.9 and 81.8 were due to C₅ and C₈ attached to endoperoxide function.

Ergosterol peroxide has been isolated previously from the fungi, *Daedalea quercina* L.¹¹, *Aspergillus fumigatus*¹², *Trichophyton schonleini*¹³, *Rhizoctonia repens*¹⁴, *Cantharellus cibarius*¹⁵, *Lampteromyces japonicus*¹⁶, *Penicillium rubrum*¹⁷ and *Basidiomycete* mushroom, *Scleroderma polyrhizum*¹⁸. It has been suggested that it is an artifact since fungal extract contain pigments and ergosterol peroxide could be produced by photosensitized oxygenation¹⁹ of ergosterol. In the case of *Fusarium moniliforme*²⁰ and *Cantharellus cibarius* since isolation was effected in the absence of oxygen, ergosterol peroxide **2** is a true metabolite and could be an intermediate in cervisterol biosynthesis¹⁵. Ergosterol peroxide has been reported to enhance inhibitory of linoleic acid on mammalian DNA polymerase²¹.

Positive electron spray [ES⁺] mass spectrum of the glycerides, Fig. 2, which, was homogenous on TLC, indicated it to be composed primarily of mixture of triglycerides. From the m/z of (M + Na)⁺ for each of the acylglycerols in this mixture, the total number of carbons and the number of unsaturation sites contained on the fatty acids that comprise the acylglycerols has been identified²².

ESI spectrum is indicative of the presence of several unsaturated triglycerides. Mass difference of 2 amu indicates the difference in the number of unsaturated sites on the fatty acids of triglycerides. Thus ions present at m/z 879, 881, 883 and 885 result from the (M + Na)⁺ ions of triglycerides that contain fatty acids with a total of 58 carbons and 3, 2, 1, 0 sites of unsaturation. The ion current at m/z 878, 880, 882 and 884 result from the ¹³C isotope of each of the respective ions. Thus the glyceride with ammoniacal molecular ion (M + NH₄)⁺ at m/z 879 with three centers of unsaturation could be represented as **3**. Similarly the current m/z 881 would represent the glyceride, **4** with two centers of unsaturation and so on.



Glycerides are well known bioactive molecules and emulsifying agents.

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