

NOTE**Isolation and Chemical Investigation of Bioactive Metabolites from a Sclerophyllum Species of the Indian Ocean**

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A soft coral which was found in abundance was collected during December 1986 on the coasts of Andaman and Nicobar islands of the Indian ocean. The specimen was identified up to the genus level as *Sclerophyllum* by Zoological Survey of India. The isolation and characterization of the compounds was done in which a compound ethyl arachidonate was structurally elucidated.

Key Words: Isolation, Chemical, Bioactive, Metabolites, Sclerophyllum, Indian ocean, Ethyl arachidonate

Sea water itself has long been known to have antibiotic properties¹. The lack of sunlight beneath the surface of the oceans may be expected to cause the formation of unusual natural products². Metabolites of marine organisms are often believed to play an active role in chemical defence^{3,4}. Soft corals are known for their capacity to produce exotic polyhydroxy sterols with diversified side chains, diterpenoids etc. It was reported that 50% of the soft coral extracts exhibit ichthyotoxic characteristics⁵.

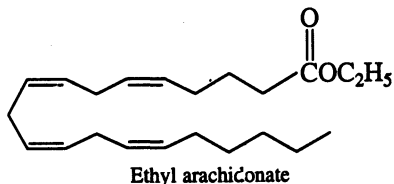
The organism was pulled out and cut into thin slices and soaked in ethanol. The extraction of the material was carried out at room temperature by percolation every 48 h. The alcohol was stripped off under reduced pressure and the process was repeated until no more residue was obtained after removal of the solvent. The residue was extracted with ethyl acetate several times and ethyl acetate solubles were separated, washed with water, dried over anhydrous Na₂SO₄ and a dark brown coloured gummy residue was obtained. It was chromatographed over a column (65 mm × 120 mm) of silica gel (400 g, 100–200 mesh) using solvents of increasing order of polarity from petroleum ether and petroleum ether-ethyl acetate mixture to ethyl acetate and methanol. Fractions (700 mL) were collected and monitored through silica gel TLC. The visualization of spots was carried out under UV light or iodine vapour or spraying 5% sulfuric acid in methanol and heating at 110°C.

Five fractions were obtained out of which first two fractions were further studied.

Fraction I: It was obtained as pale yellow solid and showed a prominent peak at δ 1.2 in ¹H NMR spectrum, indicative of fatty material. Further work on this fraction was not done.

Fraction II: It was obtained as pale yellow residue and contains high percentage of fatty material. The residue was chromatographed over a small column of silica gel (75 g; 100–200 mesh) using pet. ether, pet. ether-ethyl acetate mixtures as eluants. On repeated chromatography a pure compound MF-01 (70

mg) was obtained as pale yellow oil which was structurally elucidated and identified as ethyl arachidonate.



The pure compound MF-01 which was obtained as yellow liquid has no optical activity. It was analysed for $C_{22}H_{36}O_2$. (The molecular formula was established by elemental analysis and mass spectral data, m/z 332, M^+). Its IR spectrum showed bands due to an ester carbonyl (1730 cm^{-1}) and unsaturation (1600 cm^{-1}). The UV spectrum showed no significant absorption above 210 nm.

The $^1\text{H NMR}$ spectrum (90 MHz, CDCl_3) of MF-01 contained signals attributable to: eight olefinic protons (δ 5.34, 8H, t; $j = 5.5$ Hz) suggesting the presence of four disubstituted double bonds; three doubly allylic methylene protons (δ 2.80, 6H, m); an oxygenated methylene (δ 4.05, 2H, q; $J = 8.0$ Hz); three allylic methylenes (δ 1.95–2.35, 6H, m) and a primary methyl (δ 0.90, 3H, t; $J = 6.5$).

In addition, there is a multiplet between δ 1.10 and δ 1.95, integrating for eleven protons. Presence of four double bonds, three doubly allylic methylenes and an ester function suggests the nature of MF-01 as poly-unsaturated acid ester. A consideration of four double bonds and an ester carbonyl (5 double bond equivalents) and the molecular formula shows that it is an acyclic molecule.

A comparison of $^1\text{H NMR}$ data of MF-01 with literature data on poly-unsaturated acid esters reveals that the data is in good agreement with those reported for 5,8,11,14-eicosatetraenoic acid ethyl ester (ethyl arachidonate)⁶. The identification of MF-01 as ethyl arachidonate was confirmed by comparison with an authentic sample by co TLC (R_f 0.66, pet. ether : ethyl acetate 19 : 1 and superimposable IR spectra.

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