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Chemical Constituents of *Ulva fasciata* from Indian Coast†

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ULVA fasciata, a green alga (Chlorophyta) belonging to family *Ulvaceae* is of wide occurrence along the Malvan region of the western coast (Arabian Sea) of the Indian peninsula. Some species of the genus *Ulva* are reported to be used as human and animal foods, as well as for medicine and manure^{1,2}, *U. rigida* Ag., a species from the Black Sea region has been extensively studied for its sterol constituents³. The present studies also revealed the presence of β -sitosterol, isofucosterol and saringosterol, the common sterols of *Ulvaceae*, the latter being the major sterol of *U. fasciata*.

An alcoholic extract of *U. fasciata*, when subjected to a broad biological screening, under a

programme of screening of marine organisms of the Indian Ocean and coastal region⁴ in this Institute, showed antiviral activity *in vivo* against Semliki Forest Virus (SFV) at 2.0 mg/mouse giving 50% protection. In the present communication, the compounds isolated from *U. fasciata* are reported for the first time.

Experimental

¹H nmr spectra (CDCl₃) were recorded on 90 MHz with TMS as internal standard, and ¹³C nmr spectra (CDCl₃) on a Bruker WM 400 MHz super-conduction FT spectrometer. Gc of fatty hydrocarbon was carried on Chemito-3800 using Dexil column, while gc of methyl esters of FFA was carried on a Pye-Unicam 104 instrument using 10% DEGS column, standard used being methyl oleate.

Seaweed material: *U. fasciata* was collected in June 1985 by National Institute of Oceanography, Goa, India⁴ from Malvan region of western Coast of India and a Voucher Specimen of the alga is preserved in the herbarium of NIO, Goa, India.

Extraction and isolation: The shed-dried sea weed (4.0 kg) was powdered and extracted with ethanol (90–95%) at room temperature. The alcoholic extract was concentrated (~1.0 dm³) and fractionated to give hexane (3.0 g), chloroform (27.0 g), n-butanol (1.0 g) and water-soluble fraction consisted mostly of inorganic salts.

The combined hexane–chloroform fraction was divided into two parts. Part-A was saponified for its fatty acid contents and part-B was carefully chromatographed over an alumina column using solvents of increasing polarity. Elution with hexane gave a mixture of the fatty hydrocarbons (F 005–F 007). The CI-ms of the mixture showed the presence of closely related C₃₀ to C₃₆ fatty hydrocarbons. Continued elution with hexane–benzene (95 : 5) yielded fraction F008 as a viscous mass, tlc single spot, gc (*R_f*=9.6') analysed for methyl ester of heptadecanoic acid by nmr and ms. Elution with hexane–benzene (90 : 10) gave fraction F009 which exhibited a high order of antiviral activity at 0.3 mg/mouse/7 days giving 80% protection against SF virus and was found to be a complex mixture of fatty matter which is under investigation. Further elution of the column with hexane–benzene (80 : 20) yielded palmitic acid (70.0 mg), m.p. 64°, C₁₆H₃₂O₂ (*M*⁺ 256 *m/z*) (ir, ms).

Isolation of β -sitosterol and isofucosterol: Successive elution of the column with benzene–ethyl acetate (95 : 5) yielded a mixture of sterols (sterol-1) (300 mg). The separation was carried out on plc (preparative layer-chromatography) of their acetates on 5% AgNO₃–impregnated silica gel G plates to yield β -sitosterol acetate (1 ; 80.0 mg), m.p. 127° (ir, nmr, ms), and isofucosterol acetate (2 ; 210 mg), m.p. 130° (lit.⁶ 132°, ir, nmr, ms).

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Isolation of saringosterol (3): Elution with benzene-ethyl acetate (80 : 20) gave sterol-2, identified as saringosterol (3; 150 mg), $C_{29}H_{48}O_2$ (M^+ 428 m/z), m.p. 158° (lit.⁷ $160-61^\circ$); its mono acetate (4), m.p. 175° (lit.⁸ $176-78^\circ$), $C_{31}H_{50}O_3$ (M^+ - AcOH; m/z 410). The presence of two hydroxyl groups was confirmed by addition of TAI⁹ *in situ*, in nmr tube of 3. The two carbamate protons appeared at δ 8.3 and 8.4 as singlets. Identity of saringosterol was further confirmed by ^{13}C nmr spectra (Table 1), which is reported here for the first time. The two carbons bearing hydroxyl groups appeared at δ 71.80 (d, C_8) and 77.67 (s, $C-24$).

TABLE 1— ^{13}C NMR SPECTRA (δ , ppm) OF SARINGOSTEROL (3)^a

Carbon	δ	Carbon	δ
C-1	35.95 ^a	C-16	31.7
C-2	28.15	C-17	56.82 ^d
C-3	71.80	C-18	11.85
C-4	35.98 ^a	C-19	16.45
C-5	140.85	C-20	35.98
C-6	121.63	C-21	17.50
C-7	42.37	C-22	37.32
C-8	50.23	C-23	39.82
C-9	36.17	C-24	77.67
C-10	36.54 ^b	C-25	31.92
C-11	21.11	C-26	18.8
C-12	29.15	C-27	19.37
C-13	36.09 ^b	C-28	142.53
C-14	55.97 ^c	C-29	112.87
C-15	24.2		

^a, ^b, ^c assignments are interchangeable.

Further elution with ethyl acetate followed by 5% methanol in ethyl acetate yielded triterpene (20.0 mg), m.p. $280-82^\circ$, identified as oleanolic acid (ir, nmr, ms); its methyl ester, m.p. 196° (ir, nmr, ms).

Finally elution with methanol yielded β -D-glucoside of β -sitosterol, m.p. $286-88^\circ$.

Free fatty acids (FFA): Part-A the of hexane-chloroform soluble fraction (4.0 g) was saponified with methanolic 2 N NaOH (20 ml) in the usual way. The saponifiable fraction was methylated with MeOH/ H_2SO_4 and the methyl esters of FFA thus obtained were analysed as methyl myristate (8.9%), methyl palmitate (72.9%), methyl stearate (4.0%) and methyl oleate (14.2%) by gc¹⁰.

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