

Halogenated terpenoids from the brown alga *Padina tetrastratica* (HAUCK)[†]

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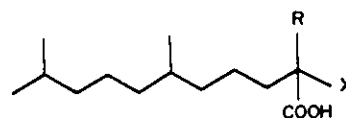
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Three novel halogenated norsesquiterpenoids, 2-chloro-2, 6, 10-trimethylundecanoic acid (1), its 2-bromo analog (2) and methyl 2-chloro-2-carboxy-6, 10-dimethylundecanoate (3), alongwith fucosterol, 7-ketocholesterol as well as several fatty acids ranging from 14:0 to 22:0 with palmitic acid (16:0, 67.4%) and oleic acid (18:1, 17.1%) being the major constituents, have been isolated from the pet. ether soluble fraction of the methanol extract of the brown alga *Padina tetrastratica* and their structures elucidated from the respective spectral data.

The Brown alga (*Phaeophyceae*), *Padina tetrastratica* (fam: *Dictotaceae*) is ubiquitous in the intertidal waters of Indian coasts. Our preliminary studies had shown its methanol extract to possess strong spasmogenic (50 µg/ml) and antifertility (100%, 200 mg/kg) properties¹. In continuation of our previous work², we now report isolation and structure elucidation of three novel halogenated norsesquiterpenoids, (1-3), alongwith the known compounds, fucosterol, 7-ketocholesterol and several fatty acids ranging from 14:0 to 22:0 (Table I) from the pet. ether fraction of its methanol extract.

The crude methanol extract was initially partitioned between 10% aq. MeOH and pet. ether. The lipid fraction was chromatographed on silica gel columns using gradient pet. ether-EtOAc systems to separate the fatty acids and their esters from sterols and terpenoids. The fatty acids were analysed on capillary GC (SE 30, FID) alongwith standards. Various terpenoids and sterols, obtained in semipure form from these columns were repurified on reverse phase HPLC (ODS, MeOH, RI Detector) and their structures elucidated from the respective spectral data (IR, ¹H NMR, ¹³C NMR, EIMS and NCIMS).

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1. X = Cl, R = CH₃
2. X = Br, R = CH₃
3. X = Cl, R = COOCH₃

Results and Discussion

The crude methanol extract was fractionated into pet. ether, CHCl₃, *n*-BuOH and aqueous fractions. The major compound isolated from the latter two fractions is a reduced hexose, tentatively identified as galactitol. From the CHCl₃ fraction, we had earlier reported loliolide, 4-acetoxy, 2-hydroxy-2, 6, 6-trimethyl cyclohexanone and apo-9'-fucoxanthinone².

Repeated column chromatography, of the pet. ether fraction over silica gel yielded subfractions rich in fatty acids, their methyl esters, sterols and terpenoids. The free fatty acids were converted into their methyl esters using diazomethane and combined with the methyl esters isolated earlier. These were subsequently analysed on capillary GC alongwith standards. The composition of this mixture is given in Table I.

Reverse phase HPLC analysis of the above fractions led to the isolation of compounds 1-3, fucosterol, and 7-ketocholesterol. The compound eluting at R_t 10.28 min (1 mg) had strong IR absorption at 3400 and 1716 cm⁻¹, indicating the presence of a carboxylic acid group in the molecule. The terpenoid nature of the compound was indicated by the twin peaks of equal intensity at 1460 and 1375

Table I - Fatty acid composition

R _t (Min)	Composition (%)	Structure
3.77	6.8	14:0
5.10	1.4	15:0
6.65	67.4	16:1
7.70	1.1	17:1
8.19	0.7	17:0
9.26	17.1	18:1
9.84	3.4	18:0
10.27	0.3	19:1
12.54	0.3	19:0
13.08	1.2	20:0

cm^{-1} . Its ^1H NMR spectrum revealed the presence of an isopropyl [δ 0.865 (6H, d, 6.5 Hz) collapsing into a singlet upon irradiation of the 1H multiplet at δ 1.52], one secondary methyl [δ 0.84, 3H, d, 6.5 Hz] and one tertiary methyl groups (δ 1.25, 3H, s). EIMS of the sample failed to yield any strong molecular ion peak. On the other hand the Negative Chemical Ionisation Spectra (NCIMS) indicated it to be a mixture of one chloro and one bromo compounds with the respective pseudomolecular $[\text{M} - \text{H}]^-$ ions appearing at m/z 261 and 263 (3:1) and 305 and 307 (1:1). From this their molecular formulae were deduced to be $\text{C}_{14}\text{H}_{27}\text{ClO}_2$ and $\text{C}_{14}\text{H}_{27}\text{BrO}_2$ respectively. Based on the above spectral data structures **1** and **2** were assigned to these compounds that were also confirmed by their ^{13}C NMR and DEPT spectra.

^1H NMR spectrum of the fraction eluting at $R_t = 8.27$ min (1 mg) was also very similar to compounds **1** and **2**, but for the absence of the singlet methyl signal at 1.257 ppm and appearance of a new OMe signal at 3.5 ppm, possibly as part of a carboxymethyl group. The broad IR bands at 3400 and 1740-1700 cm^{-1} suggested the presence of a free carboxylic acid group in addition to the ester moiety. The pseudomolecular $[\text{M} - \text{H}]^-$ ions at m/z 305 and 307 in the ratio 3:1 in NCIMS suggested the molecular formula $\text{C}_{15}\text{H}_{26}\text{ClO}_4$. From these as well as comparison of its ^1H and ^{13}C NMR values with those of **1** and **2**, its structure was established as methyl 2-chloro-2-carboxy-6,10-dimethylundecanoate (**3**). The compound eluting at $R_t = 49.5$ min ($M^+ = 412$) was identified as fucosterol from its ^1H NMR and EIMS data. The preliminary ^1H NMR data of the compound having $R_t = 15.5$ min, $M^+ = 400$, indicated it to be a sterol derivative. However, $3\alpha - \text{H}$ signal of this compound appeared at 3.65 ppm rather than at 3.5 ppm as is usually the case with $3\beta\text{-OH}$, Δ^5 -sterols. Also its 10-methyl signal (19-H) appeared 0.2 ppm downfield, at 1.2 ppm as compared to the latter type of compounds. Another significant observation was the lack of the characteristic doublet at 5.35 ppm due to 6-H, common to 3β -hydroxy, Δ^5 -sterols. Instead, the vinyl signal appeared as a sharp singlet at 5.7 ppm, indicating extended conjugation and absence of any protons at C-7. Chemical shifts of 18-H, 21-H, 26-H and 27-H were found at their usual places, suggesting its structural similarity to cholesterol. The structure of this compound was thus established as 7-ketocholesterol. This was also confirmed by the appearance of C-6 signal at 126.1 ppm in its ^{13}C NMR spectrum and the characteristic mass spectral fragmentation of this molecule^{3,4}. Halogenated terpenoids are rarely reported from brown algae. To the best of our knowledge, this is the first report of natural occurrence of compounds **1-3**. 7-Keto- and

7-hydroxysterols, as well as their $\Delta^{3,5}$ analogs have been reported from certain sponges and algae previously⁵⁻⁷. It is conceivable that in these organisms, ordinary sterols selectively undergo oxidation at C-7 to yield these compounds. The keto sterols subsequently undergo facile dehydration producing $\Delta^{3,5}$, 7-ketosterols as artifacts.

Experimental

All chromatographic separations were performed on silica gel, 60-120 mesh, supplied by Qualigens Fine Chemicals. All solvents were distilled in our laboratory before use. HPLC separations were carried out using a SPECTRAPHYSICS MODEL 8800 instrument having a RI detector. Reverse phase separations were effected on a semipreparative. ODS column (5 μm , 250×8 mm²) with MeOH as eluent (2ml/minute). Capillary GC of fatty acid methyl esters were performed on HP 5880A instrument (SE30, 12.5 m, N_2 , 5 ml/minute, FID). The oven temperature was increased from 150°C to 200°C at the rate of 5°C/minute during the analyses. The individual compounds were identified by matching their retention times with that of standards (methyl esters of six saturated fatty acids, 15:0 to 20:0 were used). IR spectra were recorded on a PERKIN ELMER FTIR MODEL 1640 and ^1H and ^{13}C NMR spectra on BRUKER WM-200 NMR machine using CDCl_3 as the solvent and TMS as internal standard. The multiplicities of carbon signals were determined from their DEPT spectra. EIMS was obtained on a FINNIGAN MAT-90 instrument at 70 eV at a probe temperature of 150°C and a source temperature of 250°C. The negative CIMS were recorded on a BIOSPECT MASS SPECTROMETER using CH_4 as the reagent gas, at a source temperature of 150°C.

The seaweeds (5 kg, dry wt), collected from Anjuna, Goa were washed with fresh water and shade-dried before soaking in MeOH. After 3 weeks, the solvent was drained off, concentrated *in vacuo* and partitioned between 10% MeOH and pet. ether. The upper pet. ether layer was drained off, dried over anhyd. Na_2SO_4 and concentrated in a rotary evaporator, yielding 15 g of a dark green residue. This was repeatedly purified on silica gel columns using pet. ether-EtOAc systems, yielding fractions rich in fatty acids, their methyl esters, sterols and terpenoids. The fatty acids were converted into their methyl esters using diazomethane and combined with the methyl esters obtained earlier. The combined esters were analysed on a capillary GC along with standards. The fractions rich in sterols and terpenoids were repeatedly chromatographed over silica gel and subsequently purified on reverse phase HPLC

yielding compounds 1-3, fucosterol and 7-ketocholesterol.

2-Chloro-2, 6, 10-trimethylundecanoic acid (1) and 2-bromo-2, 6, 10-trimethylundecanoic acid (2)— $R_t = 10.28$ min; IR (neat): 3440, 2950, 2920, 2865, 1716, 1460, 1375, 1240 and 1170 cm^{-1} ; ^1H NMR (CDCl_3): δ 1.52(1H, m), 1.25(3H, s), 1.4-1.0(13H, m), 0.865(6H, d, 6.5Hz), 0.84(3H, d, 6.5Hz); ^{13}C NMR (CDCl_3): δ 39.03(t), 37.3(t), 24.4(t), 32.8(d), 27.97(d and q), 22.7(q), 22.6(q) and 19.7(q); CIMS: m/z (%) 307(53), 305(49), 261(100), 263(33); EIMS: 227($[\text{M} - \text{X}]^+$, 1%), 167(2), 149(4), 139(2), 99(7), 98(4), 97(9), 85(10) and 83(11).

Methyl 2-chloro-2-carboxy-6, 10-dimethylundecanoate (3)— $R_t = 8.27$ min; IR (neat): 3400, 1740-1700, 1460 and 1375 cm^{-1} ; ^1H NMR (CDCl_3): δ 3.5(3H, s), 1.5(1H, m); 1.4-1.0(13H, m), 0.87 (6H, d, 6.4Hz), 0.83(3H, d, 6.5Hz); ^{13}C NMR (CDCl_3): δ 50.25(q), 38.97(t), 36.9(t), 32.4(d), 27.5(d), 24.4(t), 22.3(q), 22.2(q) and 19.3(q); NCIMS m/z (%) : 307(33) 305(100).

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