CHAPTER 2

CONSTITUENTS OF THE BROWN ALGA

PADINA TETRASTROMATICA HAUCK
The brown alga (*Phaeophyceae*), *Padina tetrastromatica* (HAUCK), (fam: *Dictyotaceae*), is ubiquitous in the intertidal waters of Indian coasts. During our studies on the pharmacological properties, its methanol extract exhibited promising spasmogenic (50 \( \mu g/ml \)), antifertility (100\%, 200 \( mg/Kg \)) and hypotensive activities *in vivo* in mice. Follow-up studies located the activity in its pet.ether fraction. In addition, the crude extract also showed promising anti-amoebic activity. From the pet.ether and CHCl\(_3\), soluble fractions of this extract, we have isolated several compounds such as fatty acids, sterols and terpenoids including some halogenated compounds. They are (2R,4S)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexanone (1), hitherto known only as a synthetic compound or degradation product of carotenoids; (3R),4-[(2R,4S)-2-hydroxy-4-acetoxy-2,6,6-tri-methylcyclohexylidenel-but-3-en-2-one (apo-9'-fucoxanthinone 2), previously known only as synthetic compound, being a major fragment in the oxidative degradation of the commonly occurring carotenoid, fucoxanthin and which is also related to the ant-repellant "Grasshopper ketone" or "romalea allene" isolated by Meinwald et.al., from the flightless grasshopper *Romalea microptera*, and subsequently from the leaves of a terrestrial plant, *Cissus rheifolia*; loliolide (3), a versatile compound, originally isolated from the rye-grass *Lolium perenne*, and which was later found to be a common constituent of several angiosperms, algae, mollusc, sponge and even marine sediment; three new halogenated nor-
Fig. 1 Compounds isolated from Padina tetrastromatica
sesquiterpenoids (4-6); besides three known sterols: 7-keto-cholesterol (7), fucosterol (8) and cholesterol (9) (FIG 1). The fatty acids range from 14:0 to 20:0 as determined by GC and the newly developed negative CIMS technique are described in chapter 1. Besides, a reduced hexose, dulcitol (galactitol 10) was isolated from its water-soluble fraction. Since many of the compounds were obtained in low yield, their structural elucidations were mostly based on respective spectral data (IR, UV, NMR and MS). Efforts have also been made to confirm these structures wherever possible, using our newly developed techniques such as TLC-CIMS and TLC-O₂-CIMS discussed in chapter 1.

Compound (1), (5 mg), obtained in semipure form from SiO₂ column chromatography was finally purified on preparative TLC using 25% EtOAc in Pet.ether (Rₜ=0.46). Its IR spectrum revealed the presence of an ester (1730 & 1250 cm⁻¹), carbonyl (1700 cm⁻¹) and hydroxyl (3400 & 1040 cm⁻¹) groups in the molecule. This was supported by the presence of peaks at 6170, 215 & 66.7 in its ¹³C NMR spectrum. PCI and NCI mass spectrum gave strong peaks at m/z 232[M+NH₄]⁺ and 249[M+HCl]⁻ respectively. Its molecular weight was deduced to be 214 from these results. This corresponded to a molecular formula C₁₃H₁₉O₄, which was also confirmed by HREIMS. The above molecular formula indicated that the compound has three degrees of unsaturation. Two of these could be easily accounted for by the ester and carbonyl moieties. In the
absence of any olefinic bonds as indicated by its $^1$H and $^{13}$C NMR spectra, it was presumed that the compound could be monocyclic. Presence of peaks at $\delta$ 170 & 21.2 in the $^{13}$C NMR as well as the 3H singlet signal at $\delta$ 2.05 in the $^1$H NMR spectra indicated that the ester group is an acetate. This was also confirmed by the mass spectral fragmentations wherein the molecule readily lost fragments of 42 and 60 mass units. All the 11 carbon signals could be easily seen in the $^{13}$C NMR spectrum. Further, with the help of DEPT spectrum, they could be easily classified into two methylenes, four methyls, one oxygen-bearing methine and four quarternary carbons. The latter signals were at $\delta$ 215, 170, 74.5 and 30, suggesting that they could be due to carbonyl, ester, oxygen-bearing quarternary carbon and tetrasubstituted aliphatic carbon atoms. Proton decoupling studies revealed the presence of the partial structure as indicated in FIG 2.

The high geminal coupling among the methylene protons (14 Hz) supported the view that these are part of a ring system. All the oxygen atoms could be accounted for as part of an ester, carbonyl and a hydroxyl group. The carbonyl absorption at 1700 cm$^{-1}$ in its IR spectrum suggested that it might be part of a six membered ring or open chain. Cyclic ketones with lower no. of carbons would have been more strained resulting in higher carbonyl group absorption frequencies. Based on these results, the structures I-VI (FIG 3) were shortlisted as possible candidate for this compound.
Fig. 2 Partial structure of compound (I)

![Partial structure of compound (I)](image)

Fig. 3 Possible structure of compound (I)

![Possible structure of compound (I)](image)

Fig. 4 Possible reaction products of structures I and VI of compound (I)

![Possible reaction products of structures I and VI](image)
In order to conclusively prove the structure, the compound was treated with HIO, resulting in isolation of a product of molecular weight 230 (Fig 4). This indicated that the compound has undergone cleavage which is possible only if the hydroxyl group is present in the carbon next to the carbonyl group. This reaction thus ruled out structures (II-V), leaving only (I) and (VI) as possible candidates. Hydrolysis of the above product using alc.KOH followed by acidification with dil.acid yielded a compound with a molecular weight 170 as determined by CIMS. Under these reaction conditions, compound (I) will give rise to a gamma-hydroxy acid which will then undergo facile lactonisation, yielding the above product. On the other hand, compound (VI) will give rise to a beta-hydroxy acid of molecular weight 188. The above reactions, carried out on submilligram quantities of this compound suggested structure (I) for compound (I). The hypothesis that this compound is a degradation product of the commonly occurring carotenoid, fucoxanthin further supports the above view.

Compound (2), initially separated on preparative TLC along with the above compound, was subsequently purified on HPLC equipped with Diode Array Detector (DAD) in conjunction with a Chem-station (Fig 5). The purity of this compound was examined by novel techniques such as ratio plots, UV-Vis derivative spectra and 3D plots (Time versus Wavelength versus Absorption, Figs 6-9) during HPLC separation and by total ion current (TIC) measurements during mass spectral
FIG 5  CHROMATOGRAM OF APO-9'-FUcoxanthinone (2)
FIG 6  RATIO PLOT OF THE CHROMATOGRAM OF COMPOUND (2)
FIG 7  UV-VIS. DERIVATIVE SPECTRA OF APO-9'-FUCOXANTHINONE (2)
Absorbance [AU]

FIG 8. 3D PLOT OF COMPOUND (2)
FIG 9. 3D PLOT OF COMPOUND (2) (REAR VIEW)
studies. Its IR spectrum revealed the presence of hydroxyl (3400 & 1040 cm\(^{-1}\)), allene (1930 cm\(^{-1}\)), conjugated carbonyl (1670cm\(^{-1}\)) and ester (1730 & 1250 cm\(^{-1}\)) groups in the molecule (FIG 10). This was supported by its \(^{13}\)C NMR spectrum which had signals at \(\delta\) 170.2(s) & 21.2(q, OAc), 67.52(d) and 71.8(s, hydroxyl or acetoxyl bearing carbons), 100.78(d), 118.5(s) and 197.74(s, allene carbons) and 209.2(s, carbonyl) as well as by the strong UV absorptions at 204 and 232 nm (FIGs 11-13). Particularly noteworthy was the downfield signal of its carbonyl carbon by about 6 ppm, to \(\delta\) 209 as compared to 215 in the case of compound (1), which is indicative of its conjugated nature.

Positive and negative CIMS of the compound yielded the respective molecular adduct ions at \(m/z\) 284[M+18]\(^{+}\) and 301 [M+16Cl]\(^{+}\) respectively. The latter ion was also accompanied by its characteristic chlorine isotope peak at 303 as expected. This indicated that the molecular weight of the compound is 266 which was confirmed by EIMS. HREIMS of this peak determined its elemental composition as C\(_{16}\)H\(_{20}\)O\(_4\). The intense peaks at \(m/z\) 224(M-CH\(_2\)CO)\(^{+}\) and 206([M-CH\(_2\)COOH])\(^{+}\) in its EIMS confirmed the presence of the acetate group in this molecule (FIG 14). All the 15 carbon signals could be easily detected in the \(^{13}\)C NMR spectrum which were distributed as five methyls, one ketone, one acetate carbonyl, two methines, four quarternary carbons and two methylene groups as revealed by its DEPT spectrum. \(^1\)H-\(^1\)H COSY and proton decoupling studies
Fig. 10 IR (Neat) spectrum of apo-9'-fucoxanthinone (2)
**Fig. 11** UV-Vis spectrum of apo-9'-fucoxanthinone (2).
FIG 12 $^{13}$C NMR SPECTRUM OF APO-9'-FUCOXANTHINONE (2)
FIG 13  DEPT SPECTRUM OF APO-9'-FUcoxanthinone (2)
FIG 14  EIMS OF APO-9'-FUCOXANTHINONE (2)
revealed the presence of -CH₂-CHOH(OAc)-CH₃- group in this compound as in compound (1). As in the case of the earlier compound the high geminal couplings of the methylene protons (14 Hz) indicated that they might be part of a cyclic system. However, the absence of the carbonyl signal at 215 ppm suggested that probably the keto group of compound (1) has been replaced by the allene moiety in this molecule. On the other hand, the presence of carbonyl carbon signal at δ 209.2 in the ¹³C NMR spectrum and the strong UV-Vis absorption at 232 nm were indicative of a conjugated carbonyl moiety. The ¹H NMR signal at δ 2.20(3H, s) suggested that a methyl group is probably attached to the carbonyl group. Since the compound had no other unsaturations apart from the allene group, it may be presumed that the above methyl ketone is attached to the allene. This indicates that the following partial structure, =C≡C≡C(H)COCH₃ has replaced the carbonyl group of compound (1), leading to the tentative structure (2) for this compound. This structure was also supported by its mass spectral fragmentations, which were in good agreement with that of apo-9'-fucoxanthinone prepared synthetically.¹⁰

This structure was further confirmed by analysis of the products of its reaction with lithium aluminium hydride (LAH) as well as using the newly developed TLC-O₂-CIMS. IR and ¹H NMR spectra of the product obtained after treating the compound (2) with LAH indicated it to be a mixture of acetylenic and allenic triols. Thus its IR spectrum had
absorptions at 1930 & 2240 cm$^{-1}$, while the $^1$H NMR spectrum of the crude reaction mixture had peaks corresponding to these compounds as evident from its comparison with the literature values$^{146}$. During the TLC-O$_2$-CIMS studies, a TLC spot of compound (2) was ozonised by exposing to ozonised oxygen for 2-3 min. The mass spectral studies of the products of this reaction were carried out as described before. The molecular weight of the product was found to be 214, indicating that ozone has cleaved the allenic bond, forming compound (1).

The assignment of the stereochemistry of the compound was based on the comparison of its $^1$H NMR spectral data with that of its natural and synthetic analogs (FIG 15)$^{148-20}$. Thus, the allene (2) has been reported to be a key degradation product of fucoxanthin', whereas its deacetoxy derivative, romale allene (2H) could be produced from the corresponding carotenoid, neoxanthin. Russel and Weedon had synthesized three stereoisomers (2C-E) of allenic ketones in order to establish its absolute configuration unambiguously$^{10}$. Previously Isoe et al., had synthesized two allenic compounds (2F and 2G) by photosensitized oxygenation of 8-ionol$^{12}$.

Among these, the stereochemistry of (2C) and (2E) was determined by X-ray crystallographic studies of their $p$-bromo benzoyl derivatives$^{12}$. The $^1$H NMR chemical shifts for 4-H, and the ring methyl groups of compound (2) are identical to those of apo-9'-fucoxanthinone (2C). From this, it was assumed that
Fig. 15 ¹H NMR spectral values of grasshopper ketone and other related compounds
the relative stereochemistry of C-2 and C-4 in compound (2) are the same as in compound (2C), i.e., (2R,4S). The allenic proton, which is (α) in compound (2C) as established by X-ray crystallographic studies appears at δ 5.84 in the 'H NMR spectrum". In compounds (2D & 2E), where these protons are (β), the corresponding signals appear at δ 5.93 & 5.97 respectively". Similarly, in compounds (2F) and (2G), where the allenic protons are (β) and (α) respectively, their signals appear at δ 6.00 and 5.83 respectively. In compound (2), this peak appears at δ 5.87, very close to that of compounds (2C) and (2G), thereby confirming the stereochemistry of this proton as (α). Meinwald et. al., had subsequently synthesized the grasshopper ketone in which this proton signal is reported at δ 5.86". The structure of compound (2) was thus established as (3R)-4-{(2R,4S)-2-hydroxy-4-acetoxy-2,6,6-trimethylcyclohexylidene}-but-3-en-2-one, which is the same as that of grasshopper ketone (2H) and apo-9'-fucoxanthone (2C)". However, it must be noted that the above relative stereochemistry of the allene group is opposite to that occurring in common carotenoids. This might be the result of stereomutation of isomeric allenes formed initially. In support of this view, it was reported that irradiation of (2F) with a high pressure mercury lamp gives a product which, on the basis of 'H NMR spectrum appears to be an equilibrium mixture (1:1) of (2F) and (2G)".
The compound (3), isolated by preparative TLC ($R_f=0.40$, 25% EtOAc/pet.ether) was a crystalline compound, m.p. 153°C. HREIMS determined its elemental composition to be $C_{11}H_{11}O_2$ (Molecular weight 196.1105). Its IR spectrum indicated the presence of an ester or $\alpha:\beta$ unsaturated, gamma-lactone ($1730$ & $1680$ cm$^{-1}$), carbon-carbon unsaturation ($1620$ cm$^{-1}$) and hydroxyl ($3440$ & $1030$ cm$^{-1}$) groups. This was also supported by its $^{13}$C NMR spectrum which had signals at $\delta$ 182.4 (s, ester or lactone carbonyl), 171.0 & 112.9 (s & d respectively, olefonic carbons), 86.65 (s, oxygenated carbon) and 66.83 (d, oxygenated carbon). The signals at $\delta$ 47.3 (t) and 45.6 (t) could be assigned to two methylene groups. Proton decoupling studies also indicated the presence of $-\text{CH}_2-\text{CHOH-CH}_2-$ group. Presence of acetate group was ruled out as no corresponding methyl signal was found at $\delta$ 20-21. Strong UV-Vis absorption at 214 nm suggested that the compound is an $\alpha:\beta$ unsaturated ester/lactone. The structure of this compound was finalised as loliolide (3) after comparing its $^1$H and $^{13}$C NMR spectral values with those reported in literature$^{15,16}$. This was also confirmed by its mass spectral fragmentations (FIG 16).

The elemental composition of the peaks at m/z 178 and 111 which were determined by HRMS confirmed the fragmentation pattern described in FIG 16. Three isomeric loliolides, viz., loliolide, epiloliolide and isololiolide are known in literature$^{15,16,38}$. Their structures as well as some of their physical and biological properties are given in FIG 17.
Fig. 16 Mass spectral fragmentation of loliolide
The structure of epiloliolide was ruled out for compound (3) from a comparison of their melting points. The 'H NMR spectral data of (-)loliolide, (DL)isololiolide and that of compound (3) are given below (TABLE 1). From these results, it is clear that the compound (3) is loliolide. However, its optical rotation could not be obtained for want of adequate sample.
<table>
<thead>
<tr>
<th>(-)Loliolide</th>
<th>(DL)Isololiolide</th>
<th>Compound (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.28(3H,s)</td>
<td>1.26(3H,s)</td>
<td>1.35(3H,s)</td>
</tr>
<tr>
<td>1.48(3H,s)</td>
<td>1.30(3H,s)</td>
<td>1.58(3H,s)</td>
</tr>
<tr>
<td>1.51(1H,dd,14.5,3.8)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1.74(1H,dd,13.9,3.8)</td>
<td>---</td>
<td>1.70(2H,m)</td>
</tr>
<tr>
<td>1.78(3H,s)</td>
<td>1.58(3Hes)</td>
<td>1.89(3H,s)</td>
</tr>
<tr>
<td>2.02(1H,ddd,14.5,3.5 &amp; 2.5)</td>
<td>---</td>
<td>2.05(1H,m)</td>
</tr>
<tr>
<td>2.38(1H,ddd,13.9,3.8 &amp; 2.5)</td>
<td>---</td>
<td>2.53(1H,m)</td>
</tr>
<tr>
<td>2.90(OH)</td>
<td>3.35(OH)</td>
<td>---</td>
</tr>
<tr>
<td>4.3(1H,m)</td>
<td>4.15(1H,m)</td>
<td>4.42(1H,m)</td>
</tr>
<tr>
<td>5.68(1H,s)</td>
<td>5.73(1H,s)</td>
<td>5.82(1H,s)</td>
</tr>
</tbody>
</table>

 Isoe et al., had studied the biogenesis of compounds (1-3) in great detail. They have found that photooxygenation of β-carotene yields dihydroactinidiolide, β-ionone and 2-hydroxy-2,6,6-trimethyl cyclohexanone, whereas zeaxanthin under similar conditions yields 2,4-dihydroxy-2,6,6-trimethyl cyclohexanone (deacetoxy 1), loliolide (3) and isololiolide. On the other hand, β-ionol, under identical conditions yields a mixture of loliolide (3), isololiolide, and romale allene.
The comparison of the $^1$H NMR spectrum of compound (1) with the deacetoxy compound obtained in the above study as well as by other synthetic means and our finding that the allene upon ozonolysis yields the cyclohexanone (1) enabled us to confirm the stereochemistry at C-2 and C-4 as (2R,4S). Similarly, the stereochemistry assigned for loliolide (3) was confirmed by comparison of its melting point and $^1$H NMR spectral values with those of all three possible isomers: loliolide, isololiolide and epiloliolide as described previously (FIG 17 and TABLE 1).

The pet.ether soluble compounds were chromatographed over silica gel columns yielding several fatty acids, their methyl esters, sterols and terpenoids. The fatty acids were analysed as described in chapter 1. The fraction rich in sterols and terpenoids was purified on HPLC (ODS column, RI detection, MeOH), yielding several pure compounds with retention times ranging from 8 to 53.5 min.

The HPLC fraction eluting at 10.28 min (1 mg), had strong IR absorption at 3400 & 1716 cm$^{-1}$ (FIG 18), indicating the presence of carboxylic acid group in it. In addition, the presence of peaks of almost equal intensities at 1460 & 1375 cm$^{-1}$ suggested that the compound might be of terpenoid nature. Its $^1$H 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 1.521), one secondary
Fig. 18  IR (Neat) spectrum of 2-chloro-2,6,10 trimethyl undecanoic acid (4) and its 2-bromo analog (5)
methyl [δ 0.84(3H, d, 6.5 Hz)] and one tertiary methyl [δ 1.25 (3H, s)] groups in the molecule (FIG 19). Both EI and PCI mass spectra of the sample failed to yield any strong molecular ion peak. On the other hand, NCI mass spectrum showed presence of two sets of peaks differing by two mass units each at m/z 261 & 263 and 305 & 307 and in the ratio 3:1 and 1:1 respectively. This suggested that this fraction contains two halogenated compounds, one having a chlorine atom, while the other, a bromine atom in the nuclei. Since these compounds were inseparable even on HPLC and their ¹H and ¹³C NMR spectra looked identical it is possible that their remaining parts are identical. The elemental composition of these compounds were deduced as C₁₄H₁₃ClO₂ and C₁₄H₁₃BrO₂ from the above results. These molecular formulae indicate only one degree of unsaturation which could be easily explained as due to the carboxylic acid group. Thus, the rest of the molecules are acyclic and saturated. These results as well as the detailed examination of their ¹H NMR spectra and the assumption that the compounds are of terpenoid origin led to the structures (4) and (5) respectively. This was further supported by their ¹³C NMR and DEPT spectra which clearly showed the presence of four methyl, two methine and three methylene groups in these compounds. However, the fourth methylene carbon as well as the quarternary carbon signals were not clear in the spectrum, perhaps due to their overlappings with some other signals or due to the inadequacy of the samples.
FIG 19A. $^1$H NMR SPECTRUM OF COMPOUNDS 4 & 5
FIG 19B. DECOUPLED SPECTRUM OF COMPOUNDS 4 & 5 AT $\delta$ 1.5
The IR spectrum of the compound (6), $R_t = 8.27$ min., had strong absorptions at 3400 & 1700-1740 cm$^{-1}$ which indicated the presence of a carboxylic group in addition to an ester moiety. The presence of twin peaks of equal intensity at 1460 & 1380 cm$^{-1}$ suggested its terpenoid nature. The $^1H$ NMR spectrum of this compound was quite similar to that of compounds (4) and (5). However, the quarternary methyl signal at $\delta$ 1.257(3H,$s$) of the latter compounds was absent here. Instead, a new signal appeared at $\delta$ 3.5(3H,$s$), possibly due to a methoxy/ or cabomethoxy group. Neither EIMS nor PCIMS yielded any molecular ion peak. But its NCIMS readily revealed the strong pseudomolecular [M-H]$^-$ ion at m/z 305 followed by its isotope peak at 307 in the ratio 3:1, indicating the presence of a chlorine atom in the molecule (FIG 20). From this, its molecular weight was deduced to be 306, corresponding to a molecular formula $C_{11}H_{20}ClO_4$. The above formula has two degrees of unsaturation, which could be easily accounted for by the ester and the free acid groups, leaving the rest of the molecule saturated and acyclic. The structure of this compound was finalised as methyl,2-chloro-2-carboxy-6,10-dimethyl undecanoate (6) from a comparison of its spectral data with that of compounds (4) and (5).

The compound (7), $R_t = 15.5$ min, was found to be a sterol derivative by its spectral data. Its IR spectrum had absorptions at 3450 & 1700 cm$^{-1}$, indicating the presence of hydroxyl and carbonyl moieties. The molecular weight of this
compound was determined to be 400 by EIMS. This was 14 mass units higher than cholesterol. Thus the compound might be a methyl or a keto cholesterol derivative. Careful examination of its $^1$H NMR spectrum indicated that the $3\alpha$-H which normally comes at δ 3.5 in cholesterol and similar compounds has been shifted slightly downfield, to δ 3.65 in this case. Other notable changes were: absence of the broad doublet at δ 5.35 due to 6-H in cholesterol, appearance of a new vinyl proton signal at δ 5.70(s) and a downfield shift by 0.2 ppm of 19-H signal to δ 1.20. The signals due to 18-H, 21-H, 26-H & 27-H were found in their usual places as compared to cholesterol. The structure of this compound was finalised as 7-ketocholesterol from these results. This was also supported by its $^{13}$C NMR (including DEPT) and mass spectra (FIGs 20 & 21).

The Compound (8), $R_t$ = 49.5 min, m.p. 200°C, had strong IR absorptions at 3400, 3050, 1464 & 1380 cm$^{-1}$, indicating the presence of hydroxyl and vinyl groups in it. The molecular weight of the compound was found to be 412 by EI mass spectrum, corresponding to a molecular formula C$_{37}$H$_{54}$O. The sterol nature of the compound was indicated by its mass spectral fragmentations which had prominent peaks at m/z 397 [M-15]$^+$, 379[M-33]$^+$, 315, 314[M-C$_{9}$H$_{14}$]$^+$, 313, 299, 273[M-sec]$^+$, 255(273-18), 231(273-42) and 213(231-18). Examination of its $^1$H NMR spectrum revealed the presence of 3β-hydroxy-$\Delta^\beta$-system in the molecule. The general sterol nature of the molecule was also confirmed by the presence of the 18 and 19-angular...
FIG 20. ELECTRON IMPACT MASS SPECTRUM OF COMPOUND (7)
FIG 21 SOME IMPORTANT EIMS FRAGMENTS OF COMPOUND (7)
methyls at δ 0.69 and 1.01 (3H each, s) respectively. In addition, the signals at δ 5.18 (1H, q, 6.6 Hz) and 1.57 (3H, d, 6.6 Hz) suggested the presence of an ethylidene group in the side chain, possibly at C-24. This was also supported by the chemical shifts of 26 & 27-H as well as 21-H, which were seen at δ 0.975 (6H, d, 6 Hz) and 0.985 (3H, d, 5.4 Hz) respectively. The structure of this compound was finalised as fucosterol (8) from these results. Further confirmation of the position of side chain unsaturation was provided by the strong fragment ion at m/z 313 (67%), which arises out of allylic cleavage of C22-23 bond, i.e., [M-C,H4]⁺.

The spectral data of the compound (9), Rf=53.5 min, M⁺=386, were similar to that of cholesterol. Thus, its 1H NMR spectrum had peaks at δ 5.35 (1H, br. d, 6-H), 3.5 (1H, m, 3α-H), 1.01 (3H, s, 19-H), 0.92 (3H, d, 6.6 Hz), 0.86 (6H, d, J=6.8 Hz, 26 & 27-H) and 0.68 (3H, s, 18-H) which were typical of cholesterol. Similarly, the mass spectral fragment ions at m/z 371[M-15]⁺, 368[M-18]⁺, 358, 301, 275, 273, 255, 231 and 213 also confirmed the identity of this compound as cholesterol (9).

The water-soluble fraction left behind from the original methanol extract after removal of their lipophyllic compounds using pet. ether and CHCl₃, was purified over Sephadex G-10 and LH-20, yielding about 10 gm of a white powder, highly soluble in water. Strong absorptions at 3550-3150, 1070, 1045 & 1030 cm⁻¹ in its IR spectrum (FIG 22) as well as the cluster of peaks.
Fig. 22  IR (KBr) Spectrum of galactitol (dulcitol, 10)
Fig. 23  $^1$H NMR spectrum of Galactitol (10)
Fig. 24  Mass spectrum of dulcitol (10)
in the range of 3.6-4 ppm in the $^1$H NMR spectrum (FIG 23) indicated the polyhydroxylated nature of this molecule. The molecular ion was observed in its EI mass spectrum at m/z 183, presumably due to [M+H]$^+$ (FIG 24). This indicated the molecular formula to be $C_{11}H_{10}O_{11}$. Thus the compound has to be a hexol. Its $^{13}C$ NMR spectrum had only three peaks, at $6$ 70.08(d), 69.14(d), and 63.17(t). This as well as its nil optical rotation were indicative of the symmetric nature of this molecule. The $^{13}C$ NMR chemical shifts of various symmetric stereoisomers of hexols are as given below: mannitol ($6$ 76.0, 75.3 & 73.6), iditol ($6$ 72.16, 71.16 & 63.17), allitol ($6$ 65.8, 74.5, 72.9, 74.3, 76.1 & 66.1) and galactitol ($6$ 70.15, 69.25 & 63.25). From this, as well as from a comparison of the IR and $^1$H NMR spectra of the above isomers with that of compound (10), its structure was finalised as galactitol".

GENERAL DISCUSSION

The compounds (1), (2), (4), (5) and (6) are reported for the first time from a natural source. However, among them, compounds (1) and (2), are previously known as synthetic products$^{14}$ or as photo-oxidation products of carotenoids or $\beta$-ionols$^{15-22}$. It is known that oxidation of fucoxanthin with Zn(MnO$_2$), yields compound (2) in good yield$^{19}$. Further, the deacetoxy analog of compound (2) has been previously isolated
from a grasshopper *R. microptera* as well as from the leaves of the plant *C. rheifolia*. In the former case it was the major constituent of its defensive secretion having very strong ant-repellent properties.Isoe et al. had obtained 2,4-dihydroxy-2,6,6-trimethyl cyclohexanone by exhaustive photooxygenation of zeaxanthin. They subsequently converted it into the corresponding 4-monoacetate (1) by treatment with acetic anhydride and pyridine. This compound has also been prepared by Meinwald and Hendry during their synthesis of the grasshopper ketone.

Loliolide (3), also an oxidation product of carotenoids, was first isolated from the rye grass *Lolium perenne*. Subsequently it was isolated from several angiosperms, algae, mollusc, sponge and even from a marine sediment. Thus, it has been isolated from the brown alga *Undaria pinnatifida* (HARVEY) by the Japanese workers Takemoto and Takeshita in 1970. Subsequently, during 1982, Ravi et al., reported this compound from another brown alga *Cystophora moniliformis*. Around the same time Bheemasankara Rao et al., also isolated this compound from *Padina tetrastromatica* from east coast of India. In 1985, Kuniyoshi reported both (-) loliolide and (+)epiloliolide from another brown alga *Sargassum crassifolium* from Okinawa. He found them to be active inhibitors of germination of the seeds of head lettuce *Lactuca sativa var. capitata* (LINN). First report of the occurrence of this compound from marine invertebrates was by
Schmitz et al. They reported (+)loliolide and (+)epiloliolide from the sponge *Tedania ignis* in 1983. Interestingly, the latter compound was found to be an active cytotoxic agent, whereas the former was inactive. Later, in 1984 Klok et al., isolated loliolide, isololiolide and dihydroactinidiolide from marine sediments. These compounds constituted up to 2% of the organic matter extracted from the sediment samples from Namibian shelf at a depth of 106 meters. In their opinion, the origin of these compounds might be via oxidation of carotenoids such as fucoxanthin, zeaxanthin etc., in the oxic zone of the water column. Incidentally, both loliolide and dihydroactinidiolide are well known as flavor compounds in tea and tobacco. *In vitro* studies had revealed (-) loliolide to be active against human nasopharynx carcinoma (KB) and murine lymphocytic leukemia (P388) (ED₅₀ 10 μg/ml and 3.5-22 μg/ml respectively). However, it was inactive at doses of 2.5-10 mg/Kg against P388 in *in vivo* assays. In 1980, Petit et al., had isolated (-) loliolide from the mollusc *Dolabella ecaudata* from Indian waters. In their paper, they have also listed 16 plants from which this compound has been reported earlier. Subsequently, Okunade and Weimer isolated (-) Loliolide from a plant identified as *Xanthoxylum setulosum* P. Wilson (Rutaceae) during a biassy-guided purification against the highly polyphagous leaf cutter ants *Atta cephalotes* (Hymenoptera, Formicidae, Attini). The occurrence of loliolide in higher plants is natural, given the chances of its formation from zeaxanthin,
the yellow pigment of corn and the petals of Physalis sp. Similarly, photolysis of zeaxanthin diepoxide (violaxanthin), from yellow pansies (Viola tricolor) yields a mixture containing loliolide. Both the above carotenoids form a major part of the pigments in several plants. Hence it may be possible that loliolide is an artifact produced by air oxidation during the extraction process. However, there is evidence to show that loliolide may, after all, be a genuine natural product. Thus, the roots of Canscora decussata (Gentianaceae) which is free of carotenoids, contains loliolide. As pointed out by Ghosh et al., in this case, loliolide might have been initially formed in its leaves which then migrated to the roots during growth.

Halogenated terpenoids occur very rarely in brown algae whereas they are abundant in green and red algae. To the best of our knowledge, this is the first report of natural occurrence of compounds (4), (5) and (6). However, saturated long chain terpenoids have previously been reported from this alga. Among the sterols, cholesterol and fucosterol are common to several marine fauna and flora. Recently there are also reports of occurrence of 7-keto and 7-hydroxysterols as well as their Δ²-7-keto analogs from several marine organisms. The latter compounds might be artifacts, produced by dehydration of the former keto derivatives. We too have isolated several 7-hydroxy and 7-ketosterols from a sponge Ircinia ramosa (KILLER) collected from Lakshadweep islands,
as will be seen in chapter 3. It is conceivable that in these organisms 3β-OH, β-sterols are selectively oxidised at C-7 yielding the corresponding hydroxyl or keto derivatives.

EXPERIMENTAL

HPLC separations were carried out on SPECTRAPHYSICS MODEL 8800 fitted with an RI detector or HEWLETT PACKARD (HP 1090) instrument fitted with a Diode Array Detector (DAD) in conjunction with a chem-station. Reverse and normal phase separations were carried out using ODS (5 μm, 250X8 mm²) and μ-Porasil (10 μm, 250X4.6 mm²) columns with MeOH/aq.MeOH and Hexane-THF(85:15) as mobile phases respectively. The IR spectra of these compounds were recorded on a PERKIN ELMER SPECTROPHOTOMETER, MODEL 1640. ¹H and ¹³C NMR spectra were recorded on a BRUKER WM 200 machine using CDCl₃ as solvent and TMS as internal standard. EIMS were recorded on a VG-70 ER at a temp. gradient of 5°C/sec and 1 spectrum/2 secs. The TIC curves too were similarly recorded. CIMS was recorded on a BIOSPECT instrument using ammonia as reagent gas.

Sea weeds (5 kg, dry weight), collected from Anjuna, Goa were washed with fresh water, dried in shade, and soaked in MeOH at ambient temperature. After 3 weeks the solvent was drained off, and concentrated to one-tenth of its original
volume under vacuum. The above concentrate was then successively extracted with pet.ether and CHCl₃, yielding the respective fractions. The lipid fractions as well as the water-soluble fraction were subsequently concentrated under vacuum to dryness.

The pet.ether and the CHCl₃, fractions were purified by repeated column chromatography over silica gel using various pet.ether-EtOAc gradient systems, yielding subfractions rich in fatty acids, their methyl esters, sterols and terpenoids from the former and a few more polar terpenoids including compounds (1-3) from the latter. Thin layer chromatography (TLC) of the purified CHCl₃, fraction indicated it to be a mixture of three major components having Rᵢ values 0.77, 0.46 & 0.37 (SiO₂, 20%EtOAc-Pet.ether). They were separated by preparative TLC on 1.0 mm thick plates and about 25% EtOAc-Pet.ether system. Analytical HPLC (μ-Porasil, 15% THF in hexane, DAD) and Total Ion Curve (TIC) obtained during their high resolution EIMS studies indicated that these compounds are relatively pure. In addition, the purity of compound (2) was also checked using the new techniques such as ratio plots, 3D-plot (time vs. wavelength vs. absorption), and UV-Vis derivative spectrum during its HPLC analysis (FIGs 6-9). In ratio plot, the chromatogram is monitored at two wavelengths simultaneously and subsequently their ratio over the range taken. If the given compound is pure, its ratio plot will be a horizontal line as seen in this case. In the
derivative spectra, both first and second derivatives of the UV-Vis absorption spectra of the compound are recorded. Here as well as in the 3D-plot, the purity of the compound is checked from the shape of respective spectra. A portion of the fatty acids purified from the pet.ether fraction was esterified using diazomethane. The methyl esters thus produced were mixed with the esters previously isolated and analysed for their fatty acid composition on capillary GC(SE 30,12.5 m,N,5 ml/min,FID). The oven temperature was gradually raised from 150°C to 200°C at the rate of 5°C/min, while the detector temperature was maintained at 250°C during the analysis. The mixture of sterols and terpenoids initially separated on silica gel columns were subsequently purified on HPLC (ODS,250X8 mm²,MeOH,2ml/min), fitted with a RI detector, leading to the isolation of the halogenated terpenoids(4-6), 7-ketocholesterol(7), fucosterol (8) and cholesterol (9).

The water-soluble part of the extract, remaining after successive removal of pet.ether and CHCl₃, soluble compounds as described above, was initially desalted over Sephadex G-10 using 1:1 mixture of MeOH-H₂O and then purified over Sephadex LH-20 using MeOH as eluent. This yielded about 10 gm of a white amorphous powder, homogenous on TLC plate using various solvents. The structure of this compound was finalised as galactitol (dulcitol 10) from its spectral data.
(2R,4S)-4-Acetoxy-2-hydroxy-2,6,6-trimethyl cyclohexanone (1), IR(neat): 3400, 1740, 1710, 1460, 1370, 1250 & 1030 cm⁻¹; ¹H NMR(CDC₁₇): 6 5.25-5.45(1H,m,4-H), 2.3(1H,ddd,J=14,5 & 1.5 Hz), 2.08(3H,s,acetate methyl), 2.0-2.16(2H,m,3-H & 5-H), 1.95(1H,ddd,J=14,7.5 & 5 Hz,5-H), 1.46(3H,s,2-CH₃) and 1.26 (6H,s,6 & 6'-CH₃); ¹³C NMR(CDC₁₇): 6 215(s,C-1), 170(s,OAc), 74.47(s,C-2), 66.68(d,C-4), 43.19 and 43.03(t's,C-3 & C-5), 30.0(s,C-6), 27.87, 27.56, & 27.46(q's,C-2,6,6-methyls) and 21.23(q,OAc); PCIMS(NH₂): 232[M+NH₄⁺]; NCIMS(NH₄Cl): 249 and 251 in the ratio 3:1,[M+¹⁴Cl⁺]⁻ and [M+¹³Cl⁺]⁻ respectively. HREIMS confirmed the molecular formula as C₁₁H₁₄O₂ (observed 215.1242 for [M+H⁺]) and that of the major fragment at m/z 185 as C₁₀H₁₁O₂ [M-CO-H]⁺ (experimental value 185.1177).

**NaIO₃ OXIDATION OF COMPOUND (1).**

About 0.2 mg of the compound (1) was dissolved in 0.5 ml EtOH and treated with a saturated solution of NaIO₃, in 50%aq.EtOH(0.5 ml) for one hour at 50°C. The reaction mixture was then diluted with water and the product extracted with ether. The CIMS of the product at positive and negative ionizations gave the pseudomolecular ion peaks at m/z 248 [M+NH₄⁺]⁺ and 265[M+Cl⁺]⁺ respectively, indicating its molecular weight to be 230, corresponding to the structure (1A, FIG 4).
HYDROLYSIS OF (1A) TO (1B, FIG 4).

To the keto acid (1A) dissolved in EtOH (0.5 ml), was added 0.5 ml of 0.1N alc.KOH and the mixture maintained at 50°C for 1 hr. in a water bath. The reaction mixture was subsequently diluted with water (1 ml), acidified with dil.HCl and the product extracted with ether. The positive and negative CIMS of the product yielded the corresponding pseudomolecular ion peaks at m/z 188[M+18]⁺ and 205[M+Cl]⁻ respectively, indicating the formation of the lactone (1B).

3R,4-[(2R,4S)-2-Hydroxy-4-acetoxy-2,6,6-trimethylcyclohexylidenel-but-3-en-2-one (Apo-9'-fucoxanthinone 2), IR(neat): 3440, 2960, 2930, 2870, 1940, 1730, 1710, 1670, 1450, 1360, 1250, 1180, 970, 960 & 860 cm⁻¹; UV(MeOH): 232 and 204 nm; ¹H NMR(CDCl₃): δ 5.87(1H,s,8-H), 5.38(1H,dd,J=10 & 3.3 Hz,4-H), 2.34(1H,ddd,J=13,4.3 & 1.7 Hz,3α-H), 2.2(3H,s,10-H), 2.05(3H, s,acetate methyl), 1.95(1H,ddd,J=13,3.3 & 1.7 Hz,5α-H), 1.55 (1H,m,3β-H), 1.45(1H,m,5β-H), 1.43(6H,s,12 & 13-H) and 1.16 (3H,s,11-H); ¹³C NMR(CDCl₃): δ 209.2(s,C-7), 197.74(s,C-9), 170.24(s,acetate carbonyl), 118.5(s,C-5), 100.78(d,C-1), 71.86(s,C-2), 67.52(d,C-4),45.16 & 45.03(t's,C-3 & C-5), 35.95(s,C-6), 31.57, 30.68, 28.92 & 26.31(all q's,C-10,11,12 & 13) and 21.16(q,acetate methyl), see FIGs 11 & 12; PCIMS: 284[M+18]⁺; NCIMS: 301 and 303 in the ratio 3:1 due to the pseudomolecular ions [M+¹³Cl]⁻ and [M+''Cl]⁻ respectively. EIMS m/z,%): 266(M⁺,1), 252(1), 224(4), 207(4), 206([M-60]⁺,22),
191(52), 164(34), 163(65), 149(29), 145 (18), 131(40) and 123(100), see FIG 15; From the HREIMS the formulae of the molecular ion as well as the fragment ion at m/z 224 were determined to be C_{12}H_{22}O_2 (266.1477 as against the expected value of 266.1518) and C_{17}H_{31}O_1 (224.1393 as against the expected value of 224.1421) respectively.

LAH REDUCTION OF COMPOUND (2) INTO ALLENIC AND ACETYLENYLIC TRIOLS

About 0.5 mg of the compound (2) was dissolved in 1 ml of alcohol and stirred with LAH (10 mg) under N₂ atmosphere overnight. Later the mixture was diluted with 0.5 ml of water and then treated with 0.1N HCl(0.5 ml) to decompose the unreacted LAH. The product was later extracted from this mixture using ether. IR(neat): 3400, 2940, 2840, 2220 & 1940 cm⁻¹; 'H NMR(CDC1₃): 5.38(1H,d,J=5.5 Hz,4-H), 4.3(1H,m,9-H), 3.5(1H,m,4-H), 2.23(1H,ddd,J=14,7 & 4.5 Hz,3-H), 1.92(1H, ddd,J=14,4.5 & 1.7 Hz,5-H), 1.8-1.4(2H,m,3'& 5'-H), 1.32(6H, s), 1.29(3H,d,J=6.5 Hz) and 1.09(3H,s). These values agreed well with those of analogous allenic triols reported in literature³.¹⁴.
OZONOLYSIS OF COMPOUND (2) INTO (1).

About 0.25 mg of the allenic compound (2) was spotted on a silica gel plate, which was then exposed to ozonised oxygen for 2 min inside a partly covered TLC chamber. The positive and negative CIMS of the compound was recorded in the usual way. The prominent pseudo molecular ions found in PCIMS were at m/z 232[M+18]+ and 215[M+H]+ while the NCIMS yielded ions at m/z 249 and 251 in the ratio 3:1 due to [M+37Cl]- and [M+2Cl]- adduct ions. These results indicated the cleavage of the allenic bond by ozone, leading to the formation of the cyclohexanone derivative (1).

Loliolide (3), m.p. 153°C; IR(neat): 3440, 3020, 2980, 2950, 2920, 2880, 1730, 1680, 1620, 1470, 1390, 1260, 1230, 1160, 1030, 960 & 860 cm⁻¹; ¹H NMR(CDC1₃): δ 5.82(1H, s, 7-H), 4.42(1H, m, 3-H), 2.53(1H, m, 4β-H), 2.05(1H, m, 2β-H), 1.89(3H, s, 5-Me), 1.7 (2H, m, 4 & 2α-H), 1.58(3H, s, 1α-Me) and 1.35(3H, s, 1β-H); ¹³C NMR(CDC1₃): δ 182.39(s, C-8), 171.0(s, C-6), 112.93(d, C-7), 86.65(s, C-5), 66.83(d, C-3), 47.31 & 45.63 (t's, C-2 & C-4), 35.90(s, C-1), 30.64(q, 5-Me), 27.0 & 26.48(q's, 1α & 1'-Me's); EIMS(M⁺, %): 196 (M⁺, 11), 178(53), 163(19), 149(15), 140 (45), 135(28) and 111 (100); HREIMS of ions at m/z 196, 178 and 111 indicated their formulae to be C₁₁H₁₄O₁ (Experimental: 196.1105 as against the expected: 196.1095), C₁₄H₁₆O₂ (Experimental: 178.0965 as against the expected: 178.0990) and C₈H₁₀O₂ (Experimental: 111.0430 as against the expected: 111.044) respectively.
2-Chloro-2,6,10-trimethylundecanoic acid (4) and 2-Bromo-2,6,10-trimethylundecanoic acid (5), \( R_t = 10.28 \) min, IR(neat): 3440, 2950, 2920, 2865, 1716, 1460, 1375, 1240 & 1170 cm\(^{-1}\); \(^1\)H NMR(CDC\(_3\)): \( \delta 1.52(1H, m), 1.25(3H, s), 1.4-1.0(13H, m), 0.865(6H, d, J=6.5 Hz) \) and \( 0.84(3H, d, 6.5 Hz) \); \(^{13}\)C NMR(CDC\(_3\)): \( \delta 39.03(t), 37.3(t), 32.8(d), 27.97(d&t), 22.7(q), 22.6(q), 19.7(q) \); NCIMS m/z(%): 307(53), 305(49), 261(100) and 263(33), indicating the presence of a chloro and a bromo derivatives of molecular weights 308 and 262 respectively; EIMS m/z(%): 227([M-X]+), 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 (6), \( R_t = 8.27 \) min; IR(neat): 3400, 1740-1700, 1460 and 1375 cm\(^{-1}\); \(^1\)H NMR(CDC\(_3\)): \( \delta 3.5(3H, s), 1.5(1H, m), 1.4-1.0(13H, m), 0.87(6H, d, J=6.4 Hz) \) & \( 0.83(3H, d, J=6.5 Hz) \); \(^{13}\)C NMR(CDC\(_3\)): \( \delta 50.25(q), 38.97(t), 36.9(t), 32.4(d), 27.5(d), 24.4(t), 22.3(q), 22.2(q) \) & \( 19.3(q) \); NCIMS m/z(%): 307(33) and 305(100). This indicated that the molecular weight of this chloro derivative is 306.

7-Ketocholesterol (7), \( R_t = 15.5 \) min; \(^1\)H NMR(CDC\(_3\)): \( \delta 5.7(1H, s), 3.65(1H, m), 1.20(3H, s), 0.92(3H, d, J=6.4 Hz), 0.865(6H, d, J=6.4 Hz) \) and \( 0.69(3H, s) \); \(^{13}\)C NMR(CDC\(_3\)): \( \delta 36.4(C-1), 31.2(C-2), 70.54(C-3), 38.7(C-4), 126.1(C-6), 45.42(C-8), 49.98(C-9), 38.3(C-10), 21.22(C-11), 28.53(C-12), 43.1(C-13), 49.98(C-14), 26.31(C-15), 39.48(C-16), 54.81(C-17), 11.97(C-18), 18.87(C-19),

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35.7(C-20), 19.72(C-21), 36.2(C-22), 23.8(C-23), 41.8(C-24), 27.99(C-25), 22.7(C-26) and 22.5(C-27); EIMS m/z(%): 400(M', 45), 382(8), 367(15), 315(3), 287(10), 269(5), 245(8), 205 (12), 192(30), 187(20), 179(5), 174(16), 161(35), etc.

Fucosterol (8), m.p. 200°C; R_t = 49.5 min; IR(neat): 3500, 3050, 2940–2800, 1380, 1264 and 1049 cm⁻¹; 'H NMR(CDC1₃): δ 5.35(1H, br.d), 5.17(1H, q, J=6.3 Hz), 3.52(1H, m), 1.57(3H, d, J=6.7 Hz), 1.01(3H, s), 0.985(3H, d, J=5.4 Hz), 0.975(6H, d, J=6.05 Hz) and 0.69(3H, s); EIMS m/z(%): 412(M', 3.85), 398 (2.57), 397(3.13), 379(2.43), 315(2.32), 314(18.21), 313 (67.45), 299, 273, 255, 231, 213, etc.

Cholesterol (9), R_t = 53.5 min; IR(neat): 3500, 3050, 2940, 1455, 1380, 1047 & 894 cm⁻¹; 'H NMR(CDC1₃): δ 5.35(1H, d), 3.48(1H, m), 1.01(3H, s), 0.918(3H, d, J=6.4 Hz), 0.867(6H, d, J=6.5 Hz); EIMS m/z(%): 386(16.35), 372(2.3), 371(7.66), 368(9.48), 353(8.57), 275(15.6), 273(5.06), 231, 213, etc.

Galactitol (dulcitol 10), m.p. 168°C; IR(KBr): 3550-3150, 2970, 2940, 2910, 1420, 1280, 1260, 1210, 1070, 1045, 1030, 955, 930, & 890 cm⁻¹; ¹³C NMR(D₂O): 70.78(d), 69.14(d) and 63.17(t); EIMS m/z(%): 183(M',100), 165(28.8), 146(25.1), 132(33.5), 128(36.5), 110(12), 102(100), 85(22.5), 73(100), 61(100), 43(100), etc. The IR, 'H NMR and EI mass spectra are reproduced in FIGs 22, 23 and 24 respectively.
2. Unpublished results.