

Constituents of the brown alga *Padina tetrastromatica* (Hauck)-II†

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Three terpenoids, (2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexanone (1), 3'*R*,4'-[(2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene]but-3'-en-2'-one or apo-9'-fucoxanthinone (2) and loliolide (3) have been isolated from the CHCl₃ fraction, while a reduced sugar, galactitol (4) has been obtained from the *n*-BuOH and aqueous fractions of the MeOH extract of the brown alga *Padina tetrastromatica* (Hauck).

The brown alga, *Padina tetrastromatica* is ubiquitous along the coastal regions of India. The pharmacological screening had revealed its methanolic extract to have spasmogenic (50 µg/mL), antifertility (100%, 200 mg/kg), hypotensive and anti-amoebic properties¹. Subsequent chemical examination of its pet. ether fraction had yielded several fatty acids, sterols and three halogenated terpenoids². In continuation of the above work, we now report the isolation and structure elucidation of three more terpenoids, (2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexanone (1), 3'*R*,4'-[(2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene]but-3'-en-2'-one or apo-9'-fucoxanthinone (2) and loliolide (3) from the CHCl₃ fraction and galactitol (4), from its *n*-BuOH and aqueous fractions (Figure 1). Among these, compounds 1-3 were purified by chromatography over SiO₂ columns followed by preparative TLC and HPLC while Galactitol (4) was isolated from the polar *n*-BuOH and aqueous fractions by gel chromatography (Sephadex G-10 and G-15). The structures of these compounds were finalised from their physico-chemical properties such as IR, UV-Vis, ¹H & ¹³C NMR, MS including HREIMS and the newly

developed techniques, TLC-CIMS and TLC-O₃-CIMS³.

Results and Discussion

IR and ¹³C NMR spectra of compound 1 (5 mg), *R*_f = 0.77, [M⁺] at *m/z* 214, indicated the presence of acetate {1730 cm⁻², δ 170 (s) and 21.2 (q)}, carbonyl {1700 cm⁻¹, δ 215 (s)} and hydroxyl {3400 and 1040 cm⁻¹, δ 66.7 (d) and 74.5 (s)} groups. HREIMS revealed its elemental composition to be C₁₁H₁₈O₄, indicating three degrees of unsaturation. The acetate and the extra carbonyl group account for two of these. Since the NMR data ruled out the presence of carbon-carbon double bonds, the molecule may be presumed to be monocyclic. Proton decoupling studies revealed the presence of a -CH₂-CHOAc-CH₂- group in it. The high geminal coupling (14 Hz) of the above methylene protons indicated that they might be part of a cyclic system. Based on the above results structures A-F were shortlisted for this compound (Figure 2).

Oxidation of compound 1 with HIO₄ yielded 1A, M⁺ at *m/z* 230, indicating the vicinal nature of the carbonyl and hydroxyl groups, thus ruling out the structures B-E. In order to differentiate between the structures A and F the above product was hydrolysed with alc. KOH. The product 1B, M⁺ at *m/z* 170, obtained upon acidification of

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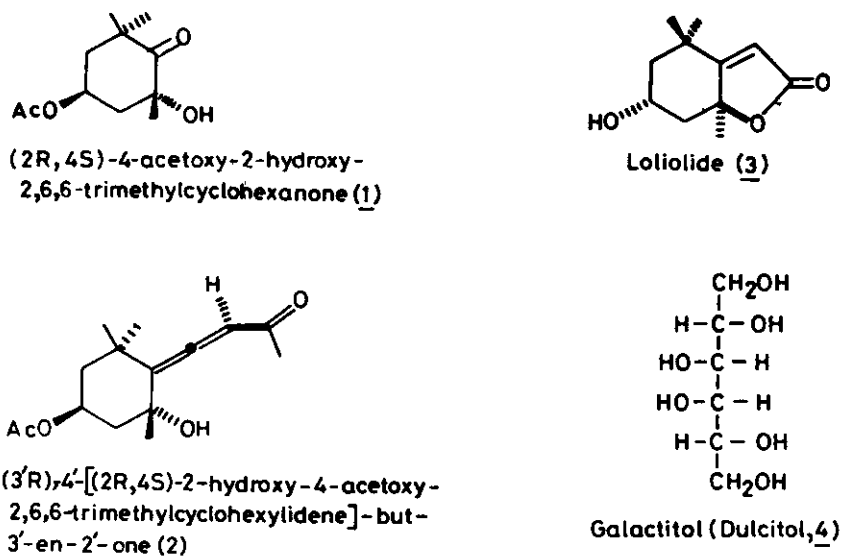


Figure 1—Compounds isolated from *Padina tetrastromatica*.

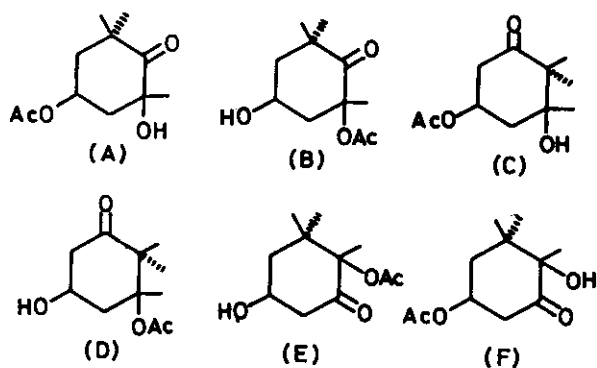


Figure 2—Possible structures of compound 1.

the reaction mixture confirmed the structure A(1) of the parent compound (Figure 3). Comparison of the ^1H NMR spectrum with those of similar synthetic compounds also supported this structure⁴.

IR and ^{13}C NMR spectra of compound 2, $R_f = 0.46$, $[\text{M}^+]$ at m/z 266, revealed the presence of hydroxyl [3400 , 1040 cm^{-1} ; δ 67.52 (d) and 71.8(s)], allene [1940 cm^{-1} ; δ 100.78(s), 118.5(d) and 197.74(s)], carbonyl [1670 cm^{-1} ; δ 209.2(s)] and an acetate [1730 cm^{-1} ; δ 170.2(s) and 21.2(q)] groups. The strong UV-Vis absorption at 232 nm, the IR absorption at 1670 cm^{-1} and the ^{13}C NMR signal at δ 209.2 were indicative of the presence of a conjugated carbonyl moiety in the molecule. HREIMS revealed the elemental composition to be $\text{C}_{15}\text{H}_{22}\text{O}_4$. These values were in agreement with

those of apo-9'-fucoxanthinone, a major degradation product of fucoxanthin^{7,8}. This was also supported by TLC- O_3 -CIMS experiment, which yielded compound 1 from 2. This is the first report on the natural occurrence of this molecule, though the corresponding 4-hydroxy analog has earlier been reported from a grasshopper *Romalea microptera*⁵ as well as from the leaves of the plant *Cissus rheifolia*⁶. The stereochemical assignments of the compounds 1 and 2 were finalised as (2R,4S) and (2R, 3'R, 4S) respectively from ^1H NMR spectral comparisons with those of similar compounds⁷⁻¹⁰.

It must be noted that the above relative stereochemistry (3'R) of the allenic proton is opposite to that occurring in common carotenoids. This can be attributed to the stereomutation of isomeric alkenes formed initially. In support of this view it was observed that irradiation of (2R, 3'S, 4S)-isomer of romale allene with a Hg lamp gives a 1:1 mixture of this isomer and romale allene as indicated by ^1H NMR spectral data¹¹.

Compound 3, R_f 0.33, m.p. 153° , analysed for $\text{C}_{11}\text{H}_{16}\text{O}_3$. Its structure was finalised as loliolide (3) from a comparison of its m.p., IR, UV-Vis, ^1H and ^{13}C NMR values with those of loliolide¹²⁻¹⁶, epiloliolide^{13,14} and isololiolide^{15,16}.

Compound 4 (7 g), $[\alpha]_D^{25} = 0$, m.p. 168° , $[\text{M}^+]$ at m/z 182, the major metabolite in the *n*-BuOH and aqueous fractions was purified over Sephadex gels. It was highly soluble in water and insoluble in

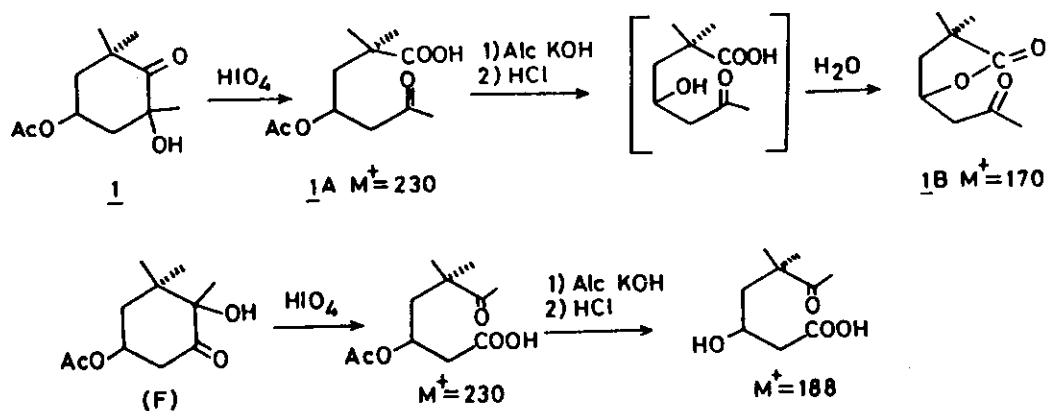


Figure 3—Possible reaction products of structures A and F of compound 1.

all organic solvents. The IR and ^1H NMR spectra indicated its polyhydroxy nature. The molecular weight suggested its elemental composition to be $\text{C}_6\text{H}_{14}\text{O}_6$. But the ^{13}C NMR spectrum had only three signals: δ 70.08(d), 69.14(d) and 63.17(t), indicating its symmetric nature. Its structure was finalised as galactitol (**4**) after comparison of the above values with those of similar reduced sugars mannitol, alitol, iditol and galactitol¹⁶.

Conclusion

In view of their carotenoid origin, it may be presumed that compounds **1-3** are artifacts. But the occurrence of romale allene, an anti-feedant, in the defensive secretion of the grasshopper *Romalea microptera*⁵ and loliolide in the roots of *Canscora decussata* (Gentianaceae)¹⁷ which are free of carotenoids indicates that probably these compounds are genuine natural products. Loliolide, widely distributed in nature¹²⁻¹⁹, is also a flavour compound in tea²⁰ and tobacco²¹. (-)-Lololide is known to prevent seed germination¹³ besides having anti-feedant¹⁸ and anti-tumor¹⁹ properties. Its stereoisomer, (+)-epilololide, also has anti-feedant¹⁸ and cytotoxic¹⁴ properties. This is the first report on the natural occurrence of galactitol. Its stereoisomer mannitol is known as a diuretic agent, controlling cellular salt concentrations by reducing water intake²². It is possible that galactitol is playing a similar role in this alga.

Experimental Section

The general experimental procedure as well as the analytical instruments used are discussed elsewhere². HPLC separation of compound **2** was carried out on a Hewlett Packard (HP 1090) instrument (μ -Porasil, 10 μ , 4.6×250 mm, hexane-THF

=85:15, 1 mL/min.) fitted with a Diode Array Detector (DAD) in conjunction with a chem-station. The *n*-BuOH and water soluble fractions were purified over Sephadex G-10 and G-15 columns. Fractions having same R_f values in paper chromatography (Whatman 40; BuOH-H₂O-AcOH = 4:1:1; detecting reagent, aniline-phthalate) were combined together yielding about 7 g of a white powder, later characterised as galactitol from its spectral data¹⁶.

(2R, 4S)-4-Acetoxy-2-hydroxy-2,6,6-trimethylcyclohexanone (1). IR (neat): 3400, 1740, 1710, 1460, 1370, 1250 and 1030 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.25-5.45 (1H, m, 4-H), 2.3 (1H, ddd, $J=14, 5$ and 1.5 Hz, H-3), 2.08 (3H, s, OAc), 2.0-2.16 (2H, m, H-3 and H-5), 1.95 (1H, ddd, $J=14, 7.5$ and 5 Hz, H-5), 1.46 (3H, s, 2-CH₃) and 1.26 (6H, s, 6- and 6'-CH₃); ^{13}C NMR (CDCl_3): δ 215 (s, C-1), 170 (s, OAc), 74.47 (s, C-2), 66.68 (d, C-4), 43.19 and 43.03 (each t, C-3 and C-5), 30.0 (s, C-6), 27.87, 27-56 and 27-46 (each q, C-2,6 and 6'-methyls) and 21.23 (q, OAc); PCIMS (NH_3): m/z 232 [$\text{M} + \text{NH}_4$]⁺; NCIMS (NH_4Cl): m/z 249 and 251 in the ratio 3:1 due to [$\text{M} + ^{35}\text{Cl}$]⁻ and [$\text{M} + ^{37}\text{Cl}$]⁻ respectively; HREIMS: m/z 215.1242 [$\text{M} + \text{H}$]⁺ ($\text{C}_{11}\text{H}_{19}\text{O}_4$).

NaIO_4 oxidation of compound 1. About 0.2 mg of the compound **1**, dissolved in 0.5 mL EtOH, was treated with a saturated solution of NaIO_4 in 50% aqueous EtOH (0.5 ml) for 1 hr at 50°C. The reaction mixture was then diluted with water and the product extracted with ether. Positive and negative CIMS of the product indicated its molecular weight to be 230 (PCIMS: m/z 248 [$\text{M} + \text{NH}_4$]⁺ and NCIMS: m/z 265 [$\text{M} + \text{Cl}$]⁻) corresponding to the structure (1A).

Hydrolysis of (1A) to (1B) (Fig. 3). To the keto-acid **1A** dissolved in EtOH (0.5 mL), was added 0.5 mL of 0.1 N alc. KOH and the mixture then diluted with water, acidified with dil. HCl and the lactone **1B** produced was extracted with ether, PCIMS: m/z 188 $[M+18]^+$ and NCIMS: m/z 205 $[M+Cl]^-$.

3'R,4'(2R, 4S)-2-Hydroxy-4-acetoxy-2,6,6-trimethylcyclohexylidienyl 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 and 860 cm^{-1} ; UV (MeOH): 232 and 204 nm; 1H NMR ($CDCl_3$): δ 5.87 (1H, s, H-3'), 5.38 (1H, dd, $J=10$ and 3.3 Hz, 4-H), 2.34 (1H, ddd, $J=13$, 4.3 and 1.7 Hz, 3 α -H), 2.2 (3H, s, H-1'), 2.05 (3H, s, OAc), 1.95 (1H, ddd, $J=13$, 3.3 and 1.7 Hz, 5 α -H), 1.55 (1H, m, 3 β -H), 1.45 (1H, m, 5 β -H), 1.43 (6H, s, C-6 dimethyls) and 1.16 (3H, s, 2- CH_3); ^{13}C NMR ($CDCl_3$): 209.2 (s, C-2'), 197.74 (s, C-4'), 170.24 (s, OAc), 118.5 (s, C-1), 100.78 (d, C-3'), 71.86 (s, C-2), 67.52 (C-4), 45.16 and 45.03 (t's, C-3 and C-5), 35.95 (s, C-6), 31.57, 30.68, 28.92 & 26.31 (all q's, 1'-H and 2,6 and 6'-methyls), 21.16 (q, OAc); PCIMS: m/z 284 $[M+NH_4]^+$; NCIMS: m/z 301 and 303 in the ratio 3:1 due to the pseudomolecular ions $[M+^{35}Cl]^-$ and $[M+^{37}Cl]^-$ respectively; EIMS: m/z 266 (M^+ , 1%), 252(1), 224 ($[M-42]^+$, 4), 207(4), 206 ($[M-HOAc]^+$, 22), 191 (52), 164 (34), 163 (65), 149 (29), 145 (18), 131 (40) and 123 (100); HREIMS: m/z 266.1477 and 224.1393 (Calcd for $C_{15}H_{22}O_4$ and $C_{13}H_{20}O_3$, 266.1518 and 224.1421, respectively).

Ozonolysis of compound (2) into (1). About 0.25 mg of the allenic compound **2** was spotted on a silica gel plate and exposed to ozonised air for 2 min inside a partly covered TLC chamber. TLC-CIMS of the spot revealed its molecular weight to be 214, identical to that of compound **1**; PCIMS: m/z 232 $[M+NH_4]^+$ and 215 $[M+H]^+$; NCIMS: m/z 249 $[M+^{35}Cl]^-$ and 251 $[M+^{37}Cl]^-$ in the ratio 3:1.

Loliolide (3). m.p. 153°; IR (neat): 3440, 3020, 2980, 2950, 2920, 2880, 1730, 1680, 1620, 1470, 1390, 1260, 1230, 1160, 1030, 960 and 860 cm^{-1} ; 1H NMR ($CDCl_3$): δ 5.82 (1H, s, H-7), 4.42 (1H, m, H-3), 2.53 (1H, m, 4 β -H), 2.05 (1H, m, 2 β -H), 1.89 (3H, s, 5-Me), 1.7 (2H, m, 4 α - and 2 α -H), 1.58 (3H, s, 1 α -Me) and 1.35 (3H, s, 1 β -Me); ^{13}C NMR ($CDCl_3$): 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 and 45.63 (each t, C-2 and C-4), 35.90 (s, C-1), 30.64 (q, 5-Me), 27.0 and 26.48 (each q, 1 and 1'-Me's); EIMS: m/z 196 (M^+ , 11%), 178 (53), 163 (19), 149 (15), 140 (45), 135

(28) and 111 (100); HREIMS: m/z $[M^+]$ 196.1105 (Calcd for $C_{11}H_{16}O_3$, 196.1095).

Galactitol. m.p. 168°; $[\alpha]_D^{25}$ 0; IR (KBr): 3550-3150, 2970, 2940, 2910, 1420, 1280, 1260, 1210, 1070, 1045, 1030, 955, 930 & 890 cm^{-1} ; 1H NMR (D_2O): δ 3.6-4 (8H, m); ^{13}C NMR (D_2O): δ 70.78 (d), 69.14 (d) and 63.17 (t); EIMS: m/z 183 ($[M+H]^+$, 100), 165 (28.8), 146 (25.1), 132 (33.5), 128 (36.5), 110 (12), 102 (100), 85 (22.5), 73 (100), 61 (100) and 43 (100).

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