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ACYCLIC DITERPENE ALCOHOLS ISOLATED FROM FOUR ALGAE OF BRYOPSIDOPHYCEAE AND THEIR TOXICITY

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ABSTRACT: Three new *i.e.* 3,7,11,15-tetramethyl-hexadecan-1,2-diol (**3**), 3,7,11,15-tetra-methyl-hexadec-3-en-1,2-diol (**4**) and [2,3]-epoxide-3,7,11,15-tetramethyl-hexadecan-l-ol (**6**) and three known *i.e.* 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol (**1**), 3,7,11,15-tetramethyl-hexadec-2-en-1-ol (**2**) and 3,7,11,15- tetra-methyl-hexadec-1-en-3-ol (**5**) acylic diterpenoids belonging to the class of phytol series have been isolated. They were obtained from the ethyl acetate soluble fractions of four siphonaceous green seaweeds, *Bryopsis pennata* Lamour., *Caulerpa taxifolia* (Vahl) C. Ag., *Codium decorticatum* (Woodw.) Howe and *Valoniopsis pachynema* (Mart.) Bφrg., collected from Karachi coast of Pakistan. Structures of these compounds were elucidated with the help of spectroscopic methods and confirmed by comparison with the known compounds. Even the known compounds are being reported for the first time from a green algal source. All the compounds were found to display a strong toxicity (LC_{50} < 1000 μg/mL) at all the three concentrations tested in the brine shrimp bioassay.

KEY WORDS: Acyclic diterpenoids - phytols - toxicity - brine shrimp bioassay - *Bryopsis* - *Caulerpa* - *Codium* - *Valoniopsis* - Chlorophyta.

INTRODUCTION

Pakistan has a rich algal flora in the coastal and inshore waters of northern Arabian Sea (Shameel and Tanaka, 1992). Several studies have been made on the phycochemistry of these seaweeds during the last eight years (Bano *et al.*, 1987; Atta-ur-Rahman *et al.*, 1988; Ahmad *et al.*, 1990; Shameel, 1990; Ahmad and Ali, 1991). Among three important divisions of seaweeds, the members of Chlorophyta are less intensively investigated and found to have complex chemical compositions (Usmanghani *et al.*, 1985; Shameel and Khan, 1989, 1990, 1991; Aliya *et al.*, 1991, 1992; Ahmad *et al.*, 1992). The algae belonging to the class Bryopsidophyceae are good sources of secondary metabolites (Capon *et al.*, 1983; Faulkner, 1987; Raub *et al.*, 1987; Schwede *et al.*, 1987). Several acyclic and cyclic diterpenoids have been reported from these sources (Blackman and Wells, 1976; Paul *et al.*, 1982, 1988; Paul and Fenical, 1984a,b, 1985; Tillekeratne *et al.*, 1984; Anjaneyulu *et al.*, 1991). Therefore, we have isolated and characterised acyclic diterpene alcohols from four siphonaceous green seaweeds, collected from the coast of Karachi, Pakistan. These diterpenoids were also tested for toxicity in the brine shrimp bioassay.

MATERIALS AND METHODS

Fresh and healthy specimens of *Bryopsis pennata* Lamour., *Caulerpa taxifolia* (Vahl) C. Ag., *Codium decorticatum* (Woodw.) Howe and *Valoniopsis pachynema* (Mart.) Bφrg. (*Ca* 1.5 kg wet wt. of each species) were collected from intertidal rocks at Manora, Sandspit, Buleji and Nathiagali (the coastal areas near Karachi) during October 1989 to September 1991 and extracted with methanol. The methanol was

removed by evaporation under reduced pressure and the crude extract thus obtained was partitioned between ethyl acetate and water. The ethyl acetate extract after evaporation was subjected to silica gel column chromatography using solvent systems *n*-hexane, *n*-hexane: chloroform, chloroform, chloroform:methanol and finally methanol. The fractions eluted with 80% chloroform in *n*-hexane were mixed and further purified by thick layer chromatography developed in *n*-hexane:diethyl ether (1:4, v/v), which yielded 6.3 mg of compound **1** in each corresponding species. The fractons eluted with 25% chloroform in methanol were mixed and further purified by preparative layer chromatography (TLC) developed in pure chloroform, which afforded 8.1 mg of compound **2** as an oil in each corresponding species.

The fractions eluted with 20% chloroform in *n*-hexane were mixed and further purified by thick layer chromatography developed in *n*-hexane:diethyl ether (7:3, v/v), which yielded 7.3 mg of compound **3** as a viscous oil in each corresponding species. With the same polarity of column (20% $CHCl_3$ in *n*-hexane) another compound was obtained. It was eluted in the impure form and was further purified by thick layer chromatography developed in hexane:diethyl ether (2:3), which afforded 6.9 mg of compound **4** as an amorphous powder. Elution with 25% chloroform in hexane yielded still another compound, which was further purified by thick layer chromatography developed in *n*-hexane:diethyl ether (2:3, v/v) and afforded 7.1 mg of compound **5** as a gummy substance. The fractions eluted with 40% chloroform in *n*-hexane were mixed and further purified by preparative layer chromatography (TLC) developed in chloroform and few drops of methanol, which afforded 7.0 mg of gummy compound **6**.

The compounds **1-6** were analysed and characterised through electron impact (EI)-, field desorption (FD)- and high resolution (HR)- mass spectrometry (MS), infra red (IR)- and proton nuclear magnetic resonance (^{1}H-NMR)-spectrometry. The EIMS were recorded on Finningan MAT-112 spectrometer coupled with a PDP 11/34 computer system. The FDMS and HRMS were performed on MAT-312 mass specrometer connected to a PDP 11/34 computer system. The IR spectra were obtained in chloroform on Jasco A-302 Infra Red spectrophotometer. The ^{1}H-NMR spectra were recorded in deuterochloroform ($CDCl_3$) at 300 and 500 MHz on Bruker AM-300 and AM-500 spectrometers with Aspect 300 data system.

For the test of toxicity the samples were prepared by dissolving 5 mg of the isolated acyclic diterpenoid compound in 0.5 mL of methanol (solution A). Solution B was prepared by diluting 0.5 mL of A to 10 mL with methanol. Appropriate amounts of solution (100 µL B, 50 µL A and 500 µL A for 10, 100 and 1000 µg/mL respectively) were transfered to 1.25 cm discs of filter paper. These discs were dried in air, placed in 10 mL vials and dried further *in vacuo* for one hour. Control discs were prepared using only methanol. Ten replicates were prepared for each dose level. The eggs of brine shrimp, *Artemia salina* Leach were hatched, the bioassay was performed and LC_{50}'s were determined as described by Meyer *et al.* (1982).

RESULTS AND DISCUSSION

Six acyclic diterpenoid compounds belonging to the class of phytol series have been isolated from ethyl acetate soluble fractions of the four siphonaceous green sea-

weeds (Table I). Three of these diterpene alcohols were already known compounds, while other three are new. Their structures were determined as follows:

The compound **1** exhibited the following spectral data:
IR ($CHCl_3$): 3410 (OH), 1620 (olefinic) cm^{-1}; **EIMS**: m/z 312 (M^+, $C_{20}H_{40}O_2$, 2%), 294 (M^+-H_2O, 1%), 281 (M^+-CH_2OH, 6%), 276 (M^+-$2H_2O$, 10%), 264 (M^+-CH_2OH-OH, 6%), 225 (M^+-$C_4H_7O_2$, 1%), 197 (6%), 183 (2%), 168 (2%), 155 (5%), 141 (20%), 127 (24%), 113 (41%), 98 (48%), 95 (46%), 85 (72%), 71 (90%), 57 (100%); **FDMS**: m/z 312 (M^+, 100%); **HRMS**: m/z 312.30271 (312.302814 calculated for $C_{20}H_{40}O_2$), 294.2923 (294.292251 calculated for M^+-H_2O), 276.2817 (276.281687 calculated for M^+-$2H_2O$); **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ 0.83 (3H, d, J=6.4 Hz), 0.84 (3H, d, J=6.4 Hz), 0.85 (6H, d,J =6.5 Hz),3.53 (1H,dd,J=11.2, 7.1 Hz,H-1), 3.70 (1H,dd,J=11.2, 3.3 Hz,H-1′), 4.19 (1H, dd,J=7.1,3.3 Hz,H-2), 4.96 (1H,m,H-17) and 5.12 (1H,br.s., H-17′).

Table I. Percent yield (w/w) of acyclic diterpene alcohols isolated from siphonaceous green algae (- = not detected).

Compound no.	Systematic name	Molecular formula	Mol. wt.	*Bryopsis pennata*	*Caulerpa taxifolia*	*Codium decorticatum*	*Valoniopsis pachynema*
1.	7,11,15-Trimethyl-3-methylene-hexadecan-1,2-diol	$C_{20}H_{40}O_2$	312	0.42	-	0.43	-
2.	3,7,11,15-Tetramethyl-hexadec-2-en-l-ol	$C_{20}H_{40}O$	296	0.54	0.55	-	0.54
3.	3,7,11,15-Tetramethyl-hexadecan-1,2-diol*	$C_{20}H_{42}O_2$	314	-	0.48	0.49	-
4.	3,7,11,15-Tetramethyl-hexadec-3-en-1,2-diol*	$C_{20}H_{40}O_2$	312	-	-	0.46	-
5.	3,7,11,15-Tetramethyl-hexadec-l-en-3-ol	$C_{20}H_{40}O$	296	-	-	0.47	-
6.	[2,3]-Epoxide-3,7,11,15-tetramethyl-hexadecan-l-ol*	$C_{20}H_{40}O_2$	312	-	-	-	0.46

*New natural products.

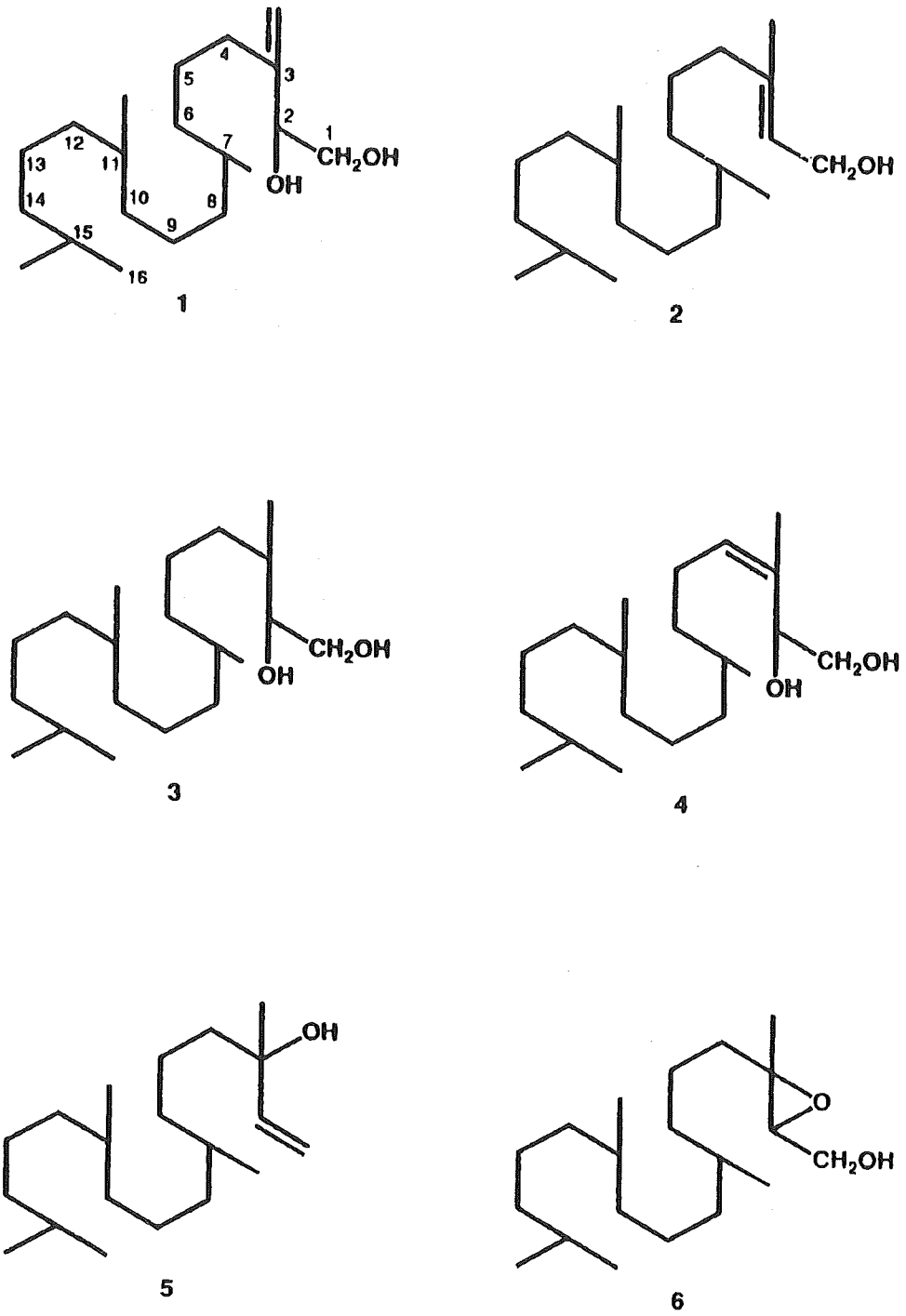

Fig. 1. Acyclic diterpene alcohols isolated from siphonaceous green algae: **1**. 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol, **2**. 3,7,11,15-tetramethyl-hexadec-2-en-1-ol, **3**. 3,7,11,15-tetramethyl-hexadecan-1,2-diol, **4**. 3,7,11,15-tetramethyl-hexadec-3-en-1,2-diol, **5**. 3,7,11,15-tetramethyl-hexadec-1-en-3-ol and **6**. [2,3]-epoxide-3,7,11,15-tetramethyl- hexadecan- 1-ol.

The compound **1** has the molecular formula $C_{20}H_{40}O_2$ as shown by its spectral data. The molecular formula requires one site of unsaturation, which was confirmed by the presence of olefinic protons in the ^{1}H-NMR spectrum at δ 4.96 (1H, m) and 5.12 (1H, br. s.). The IR spectrum showed the presence of hydroxyl absorption at 3410 cm^{-1} and absence of carbonylic absorption. This confirmed that the two oxygen atoms found in the molecule are either present in the form of two hydroxyl groups or as one hydroxyl and one ether linkage. The HRMS clearly explained the presence of two hydroxyl groups in the molecule, as the spectrum showed the removal of two water molecules from molecular ion peak successively. This could be confirmed by the appearance of fragment ions at m/z 294.2923 $(M^+-H_2O)^+$ and 276.2817 $(M^+-2H_2O)^+$

The ^{1}H-NMR spectrum of compound **1** exhibited four secondary methyl doublets at δ 0.83, 0.84 and 0.85 (6H) and also displayed three carbinylic protons as double doublets at δ 3.53, 3.70 (for terminal-CH_2OH) and 4.19. The chemical shifts of methyls in this compound were exactly matched with the reported data (Urones *et al.*, 1987). On the basis of above spectral evidences the structure of compound **1** was assigned as 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol (Fig. 1). This compound has already been reported frorn an angiosperm, *Senecio gallicus* (Urones *et al,* 1987). It is an already known compound and is being reported for the first time frorn an algal source.

The compound **2** showed the following spectral data:
IR ($CHCl_3$): 3340 (OH), 1625 (olefinic) cm^{-1}; **EIMS**: m/z 296 (M^+, $C_{20}H_{40}O$, 2%), 279 (M^+-OH, 2%), 278 (M^+H_2O, 3%), 252 (M^+-CH_2OH-CH, 1%), 197 (M^+-$C_6H_{11}O$, 13%), 127 (28%), 113 (5%), 98 (2%), 85 (28%), 71 (100%); **FDMS**: m/z 296 (M^+, 100%); **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ 0.83 (3H, d, J=6.5 Hz), 0.84 (3H, d, J=6.5 Hz), 0.85 (6H, d, J=6.6 Hz), 1.66 (3H, s), 1.98 (2H, t, J=7.6 Hz, H-2), 4.14 (2H, d, J=6.9 Hz) and 5.40 (1H, t, J=6.9 Hz).

The ^{1}H-NMR spectrum of compound **2** showed five methyl signals, out of which four secondary methyl doublets appeared at δ 0.83, 0.84 and 0.85 (6H), whereas the fifth downfield singlet appeared at δ 1.66 which was due to the methyl situated at sp^2 hybridized carbon. This spectrum also exhibited two carbinylic protons at δ 4.14 and one olefinic proton at δ 5.40. The hydroxylic absorption appeared at 3340 cm^{-1} in the IR spectrum. The compound **2** is phytol and has the systematic name as 3,7,11,15-tetramethyl-hexadec- 2-en-l-ol (Fig. 1). This compound has already been reported from a rhodophyte, *Gracilaria andersonii* (Grun.) Kylin (=*G. andersoniana,* Sims and Pettus Jr., 1976). Its structure was confirmed by comparison with the reported data. It is being reported for the first time from a green algal source.

The compound **3** revealed the following spectral data:
IR ($CHCl_3$): 3400 (OH) cm^{-1}; **EIMS**: m/z 314 (M^+, $C_{20}H_{42}O_2$, 4%), 296 (M^+-H_2O, 6%), 283 (M^+-CH_2OH,4%), 278 (M^+-$2H_20$, 5%), 271(M^+-C_3H_7, 2%), 253 (M^+-CH-CH_2OH-OH, 9%), 225 (M^+-$C_4H_9O_2$, 4%), 197 (2%), 141 (16%), 127 (4%), 113 (5%), 98(18%), 85 (35%), 71 (55%), 57 (100%); **FDMS**: m/z 314 (M^+, 100%); **HRMS**: m/z 314.31857 (314.318464 calculated for $C_{20}H_{42}O_2$), 296.30793 (296.307900 calculated for M^+-H_2O) and 278.297340 (278.297337 calculated for M^+-$2H_2O$); **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ 0.83 (3H,d,J =6.3 Hz), 0.84 (3H,d,J =6.5

Hz), 0.85 (6H,d,J=6.5 Hz), 0.90 (3H, d, J=7.0 Hz), 3.62 (1H, t, J=6.6 Hz, H-2), 4.13 (1H, dd, J=6.93, 0.66 Hz, H-l) and 4.20 (lH,dd,J=6.6,3.5 Hz,H-l′).

On the basis of HRMS the formula of compound **3** was decided as $C_{20}H_{42}O_2$. The oxygen atoms were found in the hydroxylic form, which were attested by the absorption appearing at 3400 cm^{-1} in IR spectrum. The removal of two water molecules showed that both the oxygen atoms were present in the form of hydroxyl function. Formula of the compound showed that it is an acyclic saturated compound. Its diterpenic nature was confirmed by the presence of five secondary methyl doublets in the ^{1}H-NMR spectrum at δ 0.83, 0.84, 0.85 (6H) and 0.90. The three carbinylic protons appeared at δ 3.62, 4.13 and 4.20. The spectral evidences support the structure of compound **3** as 3,7,11,15-tetramethyl-hexadecan-1,2-diol (Fig. 1). It is a new addition in the acyclic diterpenoids isolated from a natural source.

The compound **4** was separated along with compound **3** and exhibited the following spectral data:

IR ($CHCl_3$): 3420 (OH), 1625 (olefinic) cm^{-1}; **EIMS**: m/z 312 (M^+, $C_{20}H_{40}O_2$, 3%), 294 (M^+-H_2O, 2%), 276 (M^+-$2H_2O$, 6%), 262 (M^+-CH_2OH-OH, 5%), 199 (6%), 165 (3%), 127 (30%), 113 (42%), 98 (52%), 85 (70%), 71 (88%), 57 (100%); **FDMS**: m/z 312 (M^+, 100%); **HRMS**: m/z 312.3029 (312.302814 calculated for $C_{20}H_{40}O_2$), 294.29224 (294.292251 calculated for M^+-H_2O) and 276.28159 (276.281687 calulated for M^+-$2H_2O$); **^{1}H-NMR** ($CDCl_3$, 500 MHz): δ 0.83 (3H, d, J=6.6 Hz), 0.84 (3H, d, J=6.9 Hz), 0.86 (6H;d,J=6.9 Hz), 1.65 (3H,s), 1.97 (2H,m), 3.62 (1H,t,J=6.9 Hz, H-2), 4.14 (2H,d,J=6.9 Hz,H-l) and 5.39 (1H, t,J=6.9 Hz,H-4).

The HRMS of compound **4** indicated a molecular formula of $C_{20}H_{40}O_2$. The two oxygen atoms were present in the hydroxylic form, which were attested by an absorption appearing at 3420 cm^{-1} in IR spectrum. The removal of two water molecules confirmed that both oxygen atoms were found in the form of hydroxyl function. Formula of the compound indicated one degree of unsaturation, which was confirmed by the presence of olefinic proton signal at δ 5.39 in ^{1}H-NMR spectrum. Among five methyls four exhibited their signals as doublet at δ 0.83, 0.84 and 0.86 (6H), whereas the fifth downfield methyl singlet appeared at δ 1.97 which must be present at the carbon involving in the double bond. The carbinylic protons appeared at δ 3.62 and 4.14. The presence of downfield methyl singlet at δ 1.97 suggested that the structure of compound **4** is 3,7,11,15-tetramethyl-hexadec-3-en-1,2-diol (Fig. 1). It is also a new addition in the acyclic diterpenoids isolated from natural sources.

The compound **5** showed the following spectral data:

IR ($CHCl_3$): 3350 (OH), 1625 (olefinic) cm^{-1}; **EIMS**: m/z 296 (M^+, $C_{20}H_{40}O$, 2%), 278 (M^+-H_2O, 2%), 269 (M^+-$CH{=}CH_2$, 4%), 236 (M^+-$C_2H_4O_2$, 5%), 197 (28%), 168 (10%), 155 (8%), 127 (10%), 113 (14%), 98 (22%), 85 (28%), 71 (100%); **FDMS**: m/z 296 (M^+, 100%); **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ 0.83 (3H, d, J=6.6 Hz), 0.84 (3H, d, J=6.5 Hz), 0.85 (6H, d, J=6.0 Hz), 1.25 (3H, d, J=6.0 Hz), 5.03 (lH, dt, J=10.7, 0.93 Hz, H-l), 5.20 (1H, d, J=17.3, 0.96 Hz, H-l′) and 5.91 (lH, ddd, J=16.3, 10.7, 0.84 Hz, H-2).

On the basis of HRMS the molecular formula of compound **5** corresponds to $C_{20}H_{40}O$. It has only one oxygen atom in the molecule, which was found in the hydroxylic form as was attested by the absorption appearing at 3350 cm^{-1} in IR

spectrum. This acyclic diterpenoid was assigned as 3,7,11,15-tetramethyl-hexadec-1-en-3-ol (Fig. 1) and was confirmed by comparison with the reported data. It has already been reported from a red seaweed, *Laurencia pinnatifida* (Huds.) Lamour. (Ahmad and Ali, 1991). It is a derivative of phytol with a terminal double bond and is being reported for the first time from a green algal source.

The compounds **3**, **4** and **5** appear to be metabolically connected with one another. The known compound **5** may be converted into 4 by α–γ proton shift and the compound **4** may produce **3** simply by reduction or saturation, which are enzymatically possible. All three of them were found to occur independently in the algal thallus, therefore, the compounds **3** and **4** are not simply artefacts derived from **5** but are new natural products.

The compound **6** displayed the following spectral data:
IR ($CHCl_3$): 3350 (OH) cm^{-1}; **EIMS**: m/z 312 (M^+, $C_{20}H_{40}O_2$, 2%), 297 (M^+-CH_3, 4%), 294 (M^+-H_2O, 2%), 279 (M^+-OH-O, 6%), 269 (M^+-C_3H_7, 8%), 252 (M^+-C_3H_4O, 5%), 225 (4%), 183 (6%), 141 (4%), 127 (24%), 113 (32%), 98 (42%), 85 (50%), 71 (100%), 57 (46%); **FDMS**: m/z 312 (M^+, 100%); **HRMS**: m/z 312.3029 (312.302814 calculated for $C_{20}H_{40}O_2$); **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ 0.83 (3H, d, J=6.6 Hz), 0.84 (3H, d, J=6.4 Hz), 0.85 (6H, d, J=6.7 Hz), 1.28 (3H, s), 2.95 (lH, dd, J=6.6, 4.3 Hz, H-2), 3.68 (lH,dd,J=12.0, 6.6 Hz, H-l) and 3.83 (1H,dd,J=12.0, 4.3 Hz, H-1′).

The molecular formula of compound **6** was concluded from HRMS as $C_{20}H_{40}O_2$, which corresponds to one degree of unsaturation. The absence of carbonylic absorption in the IR spectrum confirmed that the two oxygen atoms found in the molecule, occur either in the form of hydroxyl or ether function. The signal at δ 2.95 in the ^{1}H-NMR spectrum showed the existence of epoxy ring in the molecule, which also clarifies the one degree of unsaturation. The other oxygen atom should be found in the form of hydroxyl function, which could easily be seen in the IR spectrum at 3350 cm^{-1}. The signals associated with carbinylic protons appeared at δ 3.68 and 3.83.

The compound **6** showed four secondary methyls, which display their resonances at δ 0.83, 0.84 and 0.85 (6H) as doublets in ^{1}H-NMR spectrum. Another downfield methyl singlet appeared at δ 1.28 due to methyl situated at epoxy ring. All the methyl signals could easily be compared with the data of a known related compouhd (Ahmad and Ali, 1991). The above evidences supported that the cornpound **6** is an acyclic diterpene alcohol, belonging to the phytol class having both hydroxyl and epoxy functionalities, and named as [2,3]-epoxide-3, 7,11,15-tetramethyl-hexadecan-l-ol (Fig. 1). This compound is also a new addition in the acyclic diterpenoids isolated from natural sources and is being reported for the first time.

Codium decorticatum appeared to be very rich in acyclic diterpenoids, as it contained four compounds out of six isolated ones, while rest of the siphonaceous green seaweeds did not contain more than two compounds (Table I). The compound **1** was detected in smallest and compound **2** in largest proportion among all the six diterpenoids. The diterpene alcohols or phytols are highly significant among algal terpenes, as they have a definite role to play in the metabolism of algae. Phytol is constituent of the molecule of chlorophyll ***a***, which is commonly present in all the algal phyla. Probably, due to this important function in photosynthesis, the free phytols have rarely been isolated from algae (Sims and Pettus Jr., 1976; Ahmad and

Ali, 1991) as well as their derivatives (Blackman and Wells, 1976; Tillekeratne and Schmitz, 1984; Paul and Fenical, 1985). In fact there is evidence that phytol is not accumulated in algal thalli and it is involved in the control of chlorophyll biosynthesis. However, it is interesting to note that free phytols and their derivatives have mostly been detected in siphonaceous green algae (*e.g.* Blackman and Wells, 1976; Paul *et al.*, 1982, 1988; Paul and Fenical, 1984a,b, 1985; Tillekeratne and Schmitz, 1984) as compared with other groups of algae.

The phycochemistry of Chlorophyta and especially of Bryopsidophyceae appears to be quite promising. Day by day novel and unique natural products are being detected in coenocytic green seaweeds (Backman and Wells, 1976; Paul *et al.*, 1982; Capon *et al*, 1983; Paul and Fenical, 1984a,b, 1985; Tillekeratne and Schmitz, 1984; Schwede *et al.*, 1987; Anjaneyulu *et al.*, 1991). Similarly several new as well as known metabolites and chemical constituents have been isolated from siphonaceous marine green algae of Karachi coast as well (Shameel and Khan, 1989, 1990, 1991; Ahmad *et al*, 1992; Aliya *et al.*, 1992). The compounds normally occurring in red seaweeds (*e.g.* Sims and Pettus Jr., 1976; Ahmad and Ali, 1991), have been detected in this group of green algae. It appears that the siphonaceous and coenocytic thallus has a peculiarity of some kind in its metabolism, which results in the biosynthesis of specialized natural products. It is quite obvious, as several nuclei are simultaneously operating in the mass of cytoplasm within a limited space, a condition which is not found in the normal monocaryotic cells constituting a multicellular algal thallus. Naturally the validity of this speculation depends on future investigations.

The compounds **1-6** were tested for toxicity in the brine shrimp bioassay (Table II). All the six diterpenoid compounds isolated from siphonaceous green seaweeds displayed a strong toxicity (LC_{50} <1000 µg/mL) at all the three concentrations tested. The marine algae belonging to the class Bryopsidophyceae have proved to be a source of a variety of terpenoids, some of which were found to be toxic to pomacentrid fishes and to induce feeding avoidance behaviour in certain herbivorous species (Blackman

Table II. Brine shrimp bioassay of compounds 1-6 isolated from siphonaceous green seaweeds.

Acyclic diterpenoid compound no.	Percent death of nauplii at 24 hours			LC_{50}
	10 µg/mL	100 µg/mL	1000 µg/mL	µg/mL
1.	9	30	45	976
2.	5	12	97	358
3.	30	75	98	23
4.	27	31	76	115
5.	28	76	71	27
6.	20	41	96	95

and Wells, 1976; Paul *et al.*, 1982, 1988; Tillekeratne and Schmitz, 1984). Some of these terpenoids were found to be inhibitory against marine bacteria and exhibited cytotoxicity in the fertilized sea urchin egg assay (Paul and Fenical, 1984a,b). Certain diterpenoids have uterine relaxant and neurotoxic effects as well (Oshima *et al.*, 1986; Alexander *et al.*, 1991). These seaweeds are abundant in tropical and subtropical seas worldwide and particularly in the areas of intense predation due to herbivorous fishes and sea urchins. It has been recognized that these algae are low preference items in the diets of most tropical herbivores (Paul and Fenical, 1985).

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