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Metabolites of Marine Algae Collected from Karachi-coasts of Arabian Sea

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Abstract – The ethanolic extracts of marine green, brown and red algae collected from Karachi coasts of Arabian Sea afforded a new enol-derivative of *N*-acylsphingosine named as coelarthenol (**1**) from *Coelarthrum muelleri*, two new glucose-derivatives named: botryenal (**2**) and botryenol (**3**) from *Botryocladia leptopoda*, α-tocopherol quinone (**4**) from *Codium iyengarii*, β-sitosterol and hexadecanoic acid from *Stokeyia indica*. The known constituents (**4**, β-sitosterol & hexadecanoic acid) have not been reported so far from their corresponding sources and the structures were determined through spectroscopic methods, whereas, the structures of new constituents (**1-3**) were elucidated with the aid of selective HMBC experiments. The phytotoxicity of 4 was also monitored.

Key words – *Codium iyengarii, Stokeyia indica, Botryocladia leptopoda, Coelarthrum muelleri*, Arabian Sea, Karachi-coasts, spectroscopy, structure elucidation

Introduction

About 70.8% of the earth's surface is occupied by seas and oceans. Their resources are varied and vast and partly comprise of fish, shellfish, other animals, vegetation and the weeds. These natural resources are virtually untapped sources of natural products, many of them are biologically active and potentially useful. The significance of seaweed as a source for biologically active natural products is well known. Among the three major divisions of algae (Chlorophyta, Phaeophyta and Rhodophyta), the members of Phaeophyta (brown algae) have been studied moderately. Some interesting *seco*-dolastane-diterpenoids have been detected in *Dictyota indica* (Bano *et al.*,1990a). The members of Rhodophyta (red algae) have been investigated extensively, for example, *Laurencia* species have been screened for their halogenated-sesquiterpenoids by various groups (Bano *et al.*, 1988; Scheuer, 1983). The members of Chlorophyta (green algae) are rarely studied, this might be due to the fact that they contain much chlorophyll. However, some steroids have been reported from genus *Codium* (Ahmad *et al.*, 1993). As a part of our studies on marine natural products from weeds collected from Karachi coasts of Arabian Sea, we have detected a new N-acylsphingosine (**1**) from red alga *Coelarthrum muelleri*, two new glucose-derivatives (**2-3**) from red alga *Botryocladia leptopoda*, α-tocopherol quinone (Rasool *et al.*,1991) (**4**) from green alga *Codium iyengarii*, β-sitosterol (Ahmad *et al.*, 1996), hexadecanoic acid from brown alga *Stokeyia indica.* Compound **4** was subjected to determine the phytotoxic features and found 100% active.

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Experimental

The ^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, on Bruker AM 400 NMR spectrophotometer.

Collection, identification and extraction – The green, brown and red algae were collected in March, 1998 from Karachi coasts (*Codium iyengarii* and *Coelarthrum muelleri* from Bulejii, *Stokeyia indica* from Hawkes Bay and *Botryocladia leptopoda* from Sandspit) of Arabian Sea (Shameel, 1990) and identified by Ms. Shaista Hameed, Center of Excellence in Marine Biology, University of Karachi, where the voucher specimens are deposited [*Codium iyengarii* MBG-22), *Coelarthrum muelleri* (MBR-69), *Stokeyia indica* (MBB-52) and *Botryocladia leptopoda* (MBR-85)]. The algal materials were washed with

water in order to remove the sea-salt and dried under shade for a period of one week [*Codium iyengarii* (1.25 kg), *Coelarthrum muelleri* (800 g), *Stokeyia indica* (2.3 kg) and *Botryocladia leptopoda* (2.7 kg)]. The washed and air dried materials were then soaked in ethanol (5 L each) for 15 days. The ethanol was removed under low pressure and the resulted gums thus obtained were subjected to silica gel column chromatography.

Isolation and characterization – *Coelarthrum muelleri* (red alga): Compound **1** was eluted with 5% methanol in chloroform which was further purified by repeated column and then preparative layer (developed in 10% methanol in chloroform) chromatography to afford **1** as a white powder.

Coelarthenol (1) Amount: 13 mg; yield: 0.00162%; M.P.: 133.5-135^{0}C; $[\alpha]_D$: +11.36^0 (Py, *c* 0.176); IR (KBr): 3400 (overlapped NH and OH), 1610 (C=C) cm^{-1}; EI-MS: *m/z* 683 (4%, M^+), 665 (35%, M-$H_2O)^+$, 647 (37%, M-2$H_2O)^+$, 325 (100%, 661-$C_{23}H_{46})^+$; FD-MS: *m/z* 683; HRMS: *m/z* 683.639217 (calcd. *m/z* 683.6427353 for $C_{42}H_{85}NO_5$), 665.630798 (calcd. *m/z* 665.6321720 for $C_{42}H_{83}NO_4$), 647.630211 (calcd. *m/z* 647.6216087 for $C_{42}H_{81}NO_3$), 325.259111 (calcd. *m/z* 325.2616771 for $C_{19}H_{35}NO_3$); ^{1}H-NMR (C_5D_5N, 400 MHz): δ 5.15 (1H, br. t, *J* = 9.0, 4.5 Hz, H-2`), 3.91 (2H, overlapped, H-3 and H-4), 3.85 (1H, m, $w_{½}$ = 9.6 Hz, H-5), 3.61 (1H, dd, *J* = 11.5, 4.4 Hz, H-1B), 3.54 (1H, dd, *J* = 11.5, 4.7 Hz, H-1A), 3.31 (1H, m, H-2), 1.24 (br.s, CH_2-chain) and 0.69 (6H, t, *J* = 6.6 Hz, H-18 and H-24'); ^{13}C-NMR (C_5D_5N, 100 MHz): δ 175.4 (C-1'), 130.3 (C-2'), 73.7 (C-4), 71.0 (C-3), 69.5 (C-5), 60.0 (C-1), 50.0 (C-2), 31.9 (C-6), 30.0, 29.7, 29.6, 29.5, 29.1 (CH_2-chain), 27.5, 26.6, 25.8, 22.9 (C-17) and 14.2 (C-18 and 24'); HMBC: see Fig. 1.

Botryocladia leptopoda (red alga): With 70-80% chloroform in hexane **2** and **3** were eluted as crude solids which on repeated column chromatography afforded yellow thin crystals (**2**) and a gum (**3**):

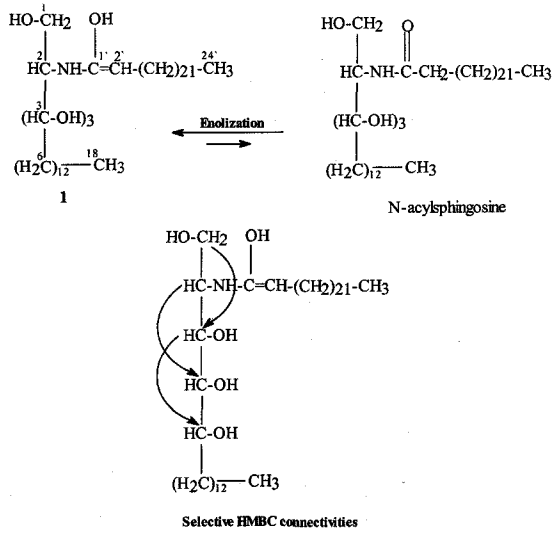


Fig. 1.

Botryenal (2) Amount: 11.9 mg; yield: 0.00044%; M.P.: 195.5°C; $[\alpha]_D$: −6.66° (MeOH, *c* 0.015); IR (KBr): 3420 (OH), 2745 and 2840 (CHO), 1625 (C=C) cm^{-1}; EI-MS: *m/z* 144 (M^+), 115 (M-CHO)$^+$; FD-MS: *m/z* 144; HRMS: *m/z* 144.0399871 (calcd. *m/z* 144.0422532 for $C_6H_8O_4$), 115.041032 (calcd. *m/z* 115.0395145 for $C_5H_7O_3$) ; ^{1}H- and ^{13}C-NMR: see Table 1; HMBC: see Fig. 2.

Botryenol (3) Amount: 10 mg; yield: 0.00037%; $[\alpha]_D$: +13.1° (MeOH, *c* 0.199); IR (KBr): 3410 (OH), 1615 (C=C) cm^{-1}; EIMS: *m/z* 144 (M^+), 113; FD-MS: *m/z* 144; HRMS: *m/z* 144.04091 (calcd. *m/z* 144.0422532 for $C_6H_8O_4$), 113.02189 (calcd. *m/z* 113.0238653 for $C_5H_5O_3$); ^{1}H- and ^{13}C-NMR: see Table 1; HMBC: see Fig. 2.

Table 1. NMR spectral assignments of Compounds **2** and **3**

Carb. No.	Botryenal (**2**)		Botryenol (**3**)	
	^{13}C-NMR (δ)	^{1}H-NMR (δ)	^{13}C-NMR (δ)	^{1}H -NMR (δ)
1	193.2	9.98 (1H, d, *J*=7.7Hz, H-1)	65.3	3.81 (1H, d, *J* = 7.9 Hz, H-1A) 3.37 (1H, d, *J* = 5.5 Hz, H-1B)
2	138.3	6.63 (1H, dd, *J* = 15.3, 7.7Hz, H-2)	136.3	---
3	136.7	5.40 (1H, dd, *J* = 15.3, 5.4 Hz, H-3)	136.2	5.21 (1H, d, *J* = 6.3 Hz, H-3)
4	73.9	4.21 (1H, d, *J* = 5.4 Hz, H-4)	73.2	4.30 (1H, d, *J* = 6.3 Hz, H-4)
5	130.1	---	130.5	---
6	142.0	5.91 (1H, s, H-6)	140.1	5.69 (1H, s, H-6)

^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, in CD_3OD.

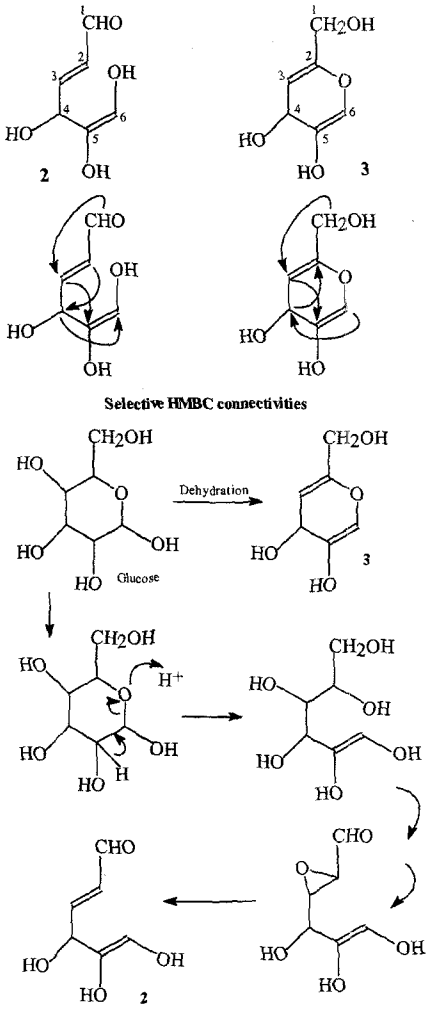
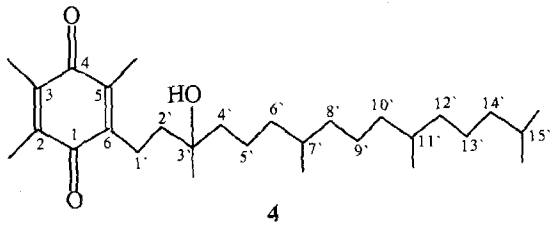


Fig. 2.

Codium iyengarii (green alga): With 40% chloroform in hexane **4** was obtained as a yellow mobile oil.

α-Tocopherol quinone (4) Amount: 18 mg; yield: 0.00144%; EI-MS: *m/z* 446 (M^+); FD-MS: *m/z* 446; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ; 0.81 (3H, d, J = 6.6 Hz, Me-7'), 0.82 (6H, d, J = 6.0 Hz, Me_2-15'), 0.83 (3H, d, J = 6.6 Hz, Me-11'), 1.25 (3H, s, Me-3'), 1.97 (3H, s, Me-5), 1.99 (3H, s, Me-2) and 2.01 (3H, s, Me-3), ^{13}C-NMR ($CDCl_3$ + CD_3OD, 100 MHz): δ 187.7 (C-1), 140.5 (C-2), 140.4 (C-3), 187.7 (C-4), 140.7 (C-5), 144.6 (C-6), 12.4 (Me-2), 12.5 (Me-3), 12.0 (Me-5), 21.5 (C-1'), 40.3 (C-2'), 70.7 (C-3'), 42.4 (C-4'), 21.3 (C-5'), 37.7 (C-6'), 32.7 (C-7'), 29.8 (C-8'), 24.5 (C-9'), 37.5 (C-10'), 32.8 (C-11'), 37.4



(C-12'), 24.9 (C-13'), 39.5 (C-14'), 28.1 (C-15'), 22.7 (Me-15'), 22.8 (Me-15'), 19.8 (Me-7'), 19.8 (Me-11') and 27.9 (Me-3').

Stokeyia indica (brown alga): Cholesterol and hexadecanoic acid were eluted with 15% chloroform in hexane during column chromatography.

Cholesterol – EI-MS: *m/z* 386 (M^+), 325 (M-H_2O-isopropyl)$^+$, 273 (M-side chain)$^+$; FD-MS: *m/z* 386; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.66 (3H, s, H-18), 0.86 (3H, d, J = 6.7 Hz, H-26), 0.87 (3H, d, J = 6.7 Hz, H-27), 0.90 (3H, d, J = 6.4 Hz, H-21), 1.10 (3H, s, H-19), 3.56 (1H, m, H-3) and 5.33 (1H, distorted, H-5).

Hexadecanoic acid – EI-MS: *m/z* 256 (M^+); FD-MS: 256 *m/z*; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.86 (3H, t, J = 6.95 Hz, H-16), 1.52 (2H, m, H-15), 1.24 (br. s, chain) and 2.62 (2H, t, J = 6.65 Hz, H-2).

Results and Discussion

Coelarthrum muelleri – Coelarthenol (**1**) was eluted with 5% methanol in chloroform from the silica gel column loaded with crude ethanolic extract of marine red alga *Coelarthrum muelleri*. Th molecular ion of **1** was observed in the EI-MS as a weak peak at *m/z* 683 which was further confirmed by FD-MS. The formula of peak *m/z* 683 was obtained by HRMS as $C_{42}H_{85}NO_5$ showing only one degree of unsaturation. The other prominent peaks in the EI-MS were at *m/z* 665, 647 and 325 due to the losses of one molecule of water, two molecules of water and the chain attached to the double bond from the molecular ion peak, respectively. The formulae of these fragments were also confirmed with the aid of HRMS.

Compound **1** showed the presence of two primary methyls (Me-18 and 24') which appeared as a common triplet of six protons on integration at δ 0.69 having the coupling constant of 6.6 Hz in the ^{1}H-NMR spectrum. An olefinic methine resonated at δ 5.15 as a triplet (J = 9.0 and 4.5 Hz) due to the H-2'. The four carbinylic protons (-CHOH) were dis-

played with their appearance in the spectrum at δ 3.31 (m, H-2), 3.91 (2H, overlapped, H-3 and H-4) and 3.85 ($w_{½}$ = 9.6 Hz, H-5). The only one downfield methylene appeared as two separate double of doublets with coupling constants of 11.5, 4.4 and 11.5, 4.7 Hz attested for H-1A and H-1B. The usual methylenes associated with the chain appeared as a broad singlet at δ1.24.

The broad-band spectrum of **1** showed altogether eighteen signals which were resolved into a quaternary, one methyl (Me x2), five methine and eleven methylene signals with the help of DEPT experiments. The only one quaternary signal associated with the olefinic function appeared at δ175.4 and the corresponding olefinic methine resonated at δ 130.3. The three carbinylic signals exhibited their signals in the carbon spectrum at δ 71.0, 73.7 and 69.5 due to the C-3, C-4 and C-5, respectively. The methine attached to the nitrogen atom resonated at δ 50.0. A downfield methylene signal containing hydroxyl function appeared at δ 60.0. The remaining methylenes of the chain showed their signals in the carbon spectrum at their normal positions (Fritz *et al.*, 1976; Johnson *et al.*, 1978). The two primary methyls appeared as a common signal at δ 14.2 (Me-18 and 24').

On the basis of spectral data, the structure of **1** was assigned as an enol-derivative of *N*-acylsphingosine and named coelarthenol. Although the sphingosines are described as the constituents of brain tissues but we have detected this from marine red alga. The *N*-acylsphingosines have previously been isolated from marine red algae by various workers containing a 19-carbon chain (Bano *et al.*, 1990b) and unsubstituted carbon chain (Cardellina *et al.*, 1978). The various connectivities in **1** were also confirmed through selective HMBC experiments (Fig. 1) and thus **1** can be considered as a new metabolite of marine red alga *C. muelleri*.

Botryocladia leptopoda – Botryenal (**2**) and botryenol (**3**) were obtained from the ethanol soluble part of marine red alga *Botryocladia leptopoda*. The molecular ion peak of **2** was observed at *m/z* 144 in the EI-MS corresponding to the formula $C_6H_8O_4$ which was determined through HRMS showing three degrees of unsaturation. A prominent peak was observed at *m/z* 115 due to the lose of -CHO fragment from the molecular ion. The molecular mass was counter-checked by FD-MS. The IR spectrum of **2** showed the presence of hydroxyl, aldehyde and olefinic functionalaties appeared in the spectrum at 3420, 2745 & 2840 and 1625, respectively.

The ^{1}H-NMR spectrum of **2** showed only few signals. An aldehydic proton at δ 9.98 as a doublet (J = 7.7 Hz), three olefinic methines at δ 6.63 (dd, J = 15.3, 7.7 Hz), 5.40 (dd, J = 15.3, 5.4 Hz) and 5.91(s) due to H-2, H-3 and H-6, respectively. A signal appeared at δ 4.21 (d, J = 5.4 Hz) attested for H-4. Similarly, the broad-band spectrum of **2** also displayed only six carbon signals which were resolved into five methines and a quaternary carbon. The most downfield signal at δ 193.2 was observed in the proton spectrum due to the conjugated aldehyde function. A carbon resonated at δ 73.9 was attributed to C-4. The three olefinic carbons were resonated at δ138.3, 136.7 and 142.0 attested for C-2, C-3 and C-6. The only one quaternary carbon present in the molecule appeared at δ 130.1.

On the basis of spectral information, the structure of discussed compound was assigned as **2** and named botryenal. The various connectivities were further confirmed through the selective HMBC experiments (Fig. 2). This is a new addition in the metabolites of *B. leptopoda* and supposed to be derived from glucose. A proposed biogenetic route is given in Fig. 2.

Compound **3** was isolated from the same source and is given the name butryenol and also supposed to be derived from glucose (Fig. 2). Most of the spectral data showed that **3** may be an isomeric form of **2**. The compound **3** having the same molecular mass and formula as having **2** (*m/z* 144, $C_6H_8O_4$). The formula of **3** showing the three degrees of unsaturation. A peak at *m/z* 113 was due to the lose of -CH_2OH moiety. The compound showed the presence of olefinic (1615 cm^{-1}) and hydroxyl (3410 cm^{-1}) functions which were confirmed through IR spectrum. The absence of aldehydic function in **3** as was in **2** maybe reflects that the aldehydic function in **2** was converted into an alcohol.

The conversion of aldehyde into alcohol was further supported by the methylene signals in the NMR spectra of **3** which appeared at δ 3.81 (1H,d, J = 7.9 Hz, H-1A), 3.73 (1H, d, J = 5.5 Hz, H-1B) and δ 65.3 in the proton and carbon spectra, respectively. Except the methylene signals in the NMR spectra, the remaining signals in **3** were found almost the same as were in **2**.

A comparative NMR data of **2** and **3** are given in Table 1. In order to balance the degrees of unsatura-

tion it is quite clear that **3** must have a ring system in structure instead of an aldehyde. The structure of **3** was further confirmed with the aid of 2D-NMR technique (Fig. 2). This is also a new addition in the metabolites of *B. leptopoda* and named botryenol.

Codium iyengarii and ***Stokeyia indica*** – α-Tocopherol quinone was isolated as a major constituent from the marine green alga *Codium iyengarii.* This was not reported so far from the same source. Cholesterol and hexadecanoic acid were also detected for the first time from marine brown alga *Stokeyia indica.* The spectral data of α-tocopherol, cholesterol and hexadecanoic are given in the experimental section.

***Bioactivity*:** Only the phytotoxicity of **4** could be measured. The results showed that **4** is 100% phytotoxic (when tested against *Lemna* plants) at the concentration of 500 μg/ml when compared with the standard herbicide i.e., paraquat (an efficient herbicide shows 100% toxicity at the conc. of 0.125 μg/ml). The bioactivity of remaining compounds (**1-3**) could not be determined due to the lack of amount.

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