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Phytochemical studies of marine green algae *Boodlea composita* from Ukha, west coast of India

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Abstract : Phytochemical investigation of marine green algae *Boodlea compositae* collected from the Ukha, west coast of India led to the isolation and identification of 22 constituents : β-sitosterol, loliolide and 13^2-hydroxy-(13^2-S)-phaeophytin-a being isolated by column chromatography whereas six fatty acids and 13 additional sterols were identified by GC-MS analysis. Characterization of β-sitosterol, loliolide and 13^2-hydroxy-(13^2-S)-phaeophytin-a was achieved by NMR spectroscopic data and comparison with those reported in literatures.

Keywords : β-Sitosterol, loliolide, pheophytin-a, green algae, GC-mass, spectroscopic data.

Introduction

Marine macroalgae inhabited in different oceanic waters have been group into three classes : green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae. Numerous compounds including sterols[1], terpenes[2], phenols[3], carotenoids[4] and polysaccharides[5] have been isolated from marine algae and investigated their biological significant. Some of the isolated algal metabolites were known to play a significant ecological role[3,6]. Among the metabolites of marine algae, sterols constitute one of the most abundant metabolite and found in diverse structural features. Certain sterol showed biological properties including feeding deterrent[6] and cytotoxicity activity[7]. Specifically, β-sitosterol showed a wide range of activity including apoptosis, cytotoxicity and immunomodulatory activity[7–9].

Metabolite such as loliolide has been isolated mainly from terrestrial plants though limited report were available from marine sources primarily from red and brown algae[10,11]. The pigments, pheophytin and their derivatives were isolated from various marine algae[12,13]. Loliolide exhibited potent anti-repellent activity[14] and inhibitory effects[15], whereas, pheophytin-a and its derivatives showed antinociceptive effect[16] and antitumor activity[17]. Prior to this communication, loliolide was reported only from a green algae *Enteromorpha compressa*[18]. In this study, we report isolation of β-sitosterol, loliolide and 13^2-hydroxy-(13^2-S)-phaeophytin-a from the green alga *Bodlea composita* by column chromatography. The compounds have been characterized with the help of FT-IR, NMR and mass spectroscopic data. Additionally, we identified thirteen other sterols and six fatty acids from this alga by GC-MS analysis.

Results and discussion

Extract of marine green alga *B. composita* collected from Okha, Gujarat coast of India upon subjected to column chromatography led to the isolation of β-sitosterol (**1**), loliolide (**2**) and 13^2-hydroxy-(13^2-S)-phaeophytin-a (**3**) (Fig. 1). The identity of the compounds **1**-**3** were established by NMR spectroscopic data as well as comparison with literatures[10,19,20]. IR spectrum of β-sitosterol showed absorption peak at 3300 cm^{-1} assignable to O-H stretching frequency. The solution optical rotation of β-sitosterol (stigma-5-en-3β-ol) in $CHCl_3$ showed $[\alpha]_D$ = –40.49° comparable to the reported value of –30 to –40°. The other two con-

stituents isolated from this alga by column chromatography have been established as loliolide (**2**) and 13^2-hydroxy-(13^2-S)-phaeophytin-a (**3**). Loliolide (**2**) was obtained as colorless oil by column chromatography and showed IR absorption peaks at 1730 and 3462 cm^{-1} assignable to C=O and OH stretching frequency.

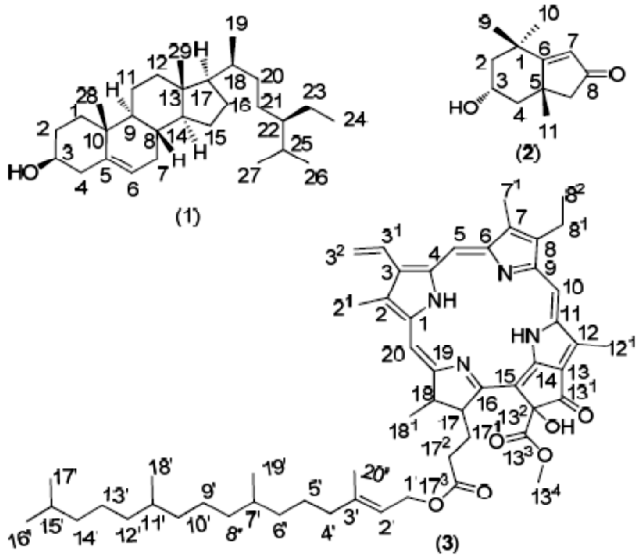


Fig. 1. Structures of β-sitosterol (**1**), loliolide (**2**) and 13^2-hydroxy-(13^2-S)-phaeophytin-a (**3**).

^{13}C NMR spectrum of **2** showed signal at δ 181.7 and 172.4 assignable to ketone (C-8) and quaternary carbon (C-6). ESI-MS of loliolide showed intense peak at *m/z* 197.1322 $(M+H)^+$ and at *m/z* 219.1048 $[M+Na]^+$. 13^2-Hydroxy-(13^2-S)-phaeophytin-a (**3**) was separated as green pigment by column chromatography. ^{13}C NMR spectrum of **3** showed signal at δ 88.9 assignable to the oxygenated carbon (C-13^2). Spectrum displayed 55 distinct signals and data are well matched with literature[20].

Although compounds **1-3** isolated from *B. compositae* have already been reported in literature, they are being reported for the first time from this species as a new source. Further, identification of other sterols component present in the alga has been carried out by GC-MS analysis after silylation with silylating agent providing 13 other sterol constituents (Table 1). GC analysis showed stigma-5-en-3β-ol (53.4%) was the major constituent present in the alga.

The sterols, cholest-5-en-3β-ol (15.9%) and 24-methylene cholesterol (14.1%) were other sterols present in significant concentration (Table 1). The fatty acid constituent of the alga was also established by methylation of the isolated fatty acid mixture followed by GC-MS analysis. The fatty acids constituents identified comprise of myristic acid, palmitic acid, palmitoleic acid, stearic acid, vaccenic acid and elaidic/oleic acid.

Table 1. Sterol components identified for *Boodlea composita* by GC-MS

Systematic name of sterols	R.T.[a]	Relative abundant (%) area[b]	Mol. wt. (O-TMS derivative)
Cholest-7,22-diene-3β-ol	36.094	1.16	456
Cholest-7,22-diene-3α-ol	36.142	0.5	456
Cholest-5-en-3β-ol	36.505	15.98	458
3β,22E-Ergosta-7,22-dien-3-ol	36.951	2.58	470
3β,5α-Cholest-7-ene-3-ol	37.098	0.64	458
3β,5β-Cholest-7-en-3-ol	37.238	0.6	458
24-Methylene cholesterol	37.544	14.13	470
Ergost-7-en-3β-ol	37.603	2.45	472
Stigma-5,22-dien-3β-ol	37.815	0.67	484
Stigma-5,22-dien-3α-ol	37.904	0.54	484
Stigma-7,22-dien-3α-ol	38.215	2.74	484
Stigma-5-en-3α-ol	38.331	1.99	414[c]
Stigma-5-en-3β-ol	38.51	53.45	486
Stigma-7,22-dien-3α-ol	38.653	2.18	484

[a]Retention time of silylated sterols, [b]quantification of silylated sterols based on the area of the GC peak, [c]unconverted.

Experimental

General remarks : The green algae *Boodlea composita* was collected from Okha, Gujarat coast during low tide. Solvents used for extraction and purification were of analytical grade and used as received. Infrared spectra were recorded using a diffused reflection spectroscopy (DRS) assembly on a Shimadzu-8201PC spectrometer. Analysis of sterol silyl ethers and fatty acids were carried out on a Shimadzu GC-MS QP2010 system using capillary column XTI-5.

Structural assignments were based on mass spectrometric fragmentations pattern and reference library[21]. Sterols were identified as their trimethyl silyl ether

(TMS ether). ESI-MS analysis was performed using QTOF Applied Biosystems mass spectrometer. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer at 300.13 (^{1}H) and 75.47 MHz (^{13}C) with $SiMe_4$ as internal reference and coupling constants were given in Hertz. Silylating agent (BSA+TMSCL+TMSI) was received from Sigma Aldrich Pvt. Ltd., India. Column chromatography was performed on silica gel (60–120 mesh, Merck, Darmstadt, Germany or SRL, Pvt. Ltd., India) or Shephadex LH-20.

Extraction and isolation of β-sitosterol (**1**), *loliolide* (**2**) *and 13^2-hydroxy-(13^2-S)-phaeophytin-a* (**3**) : Freshly collected alga was extracted with $CHCl_3$: methanol (1 : 1) then rotary evaporated. Crude extract (2 g) was dissolved in minimum amount of $CHCl_3$: methanol (1 : 1) then loaded in a pre-equilibrated Sephadex LH-20 column and elute with $CHCl_3$: methanol (1 : 1) giving three major fractions (fraction-1 to fraction-3). Repeated column chromatography over silica gel column followed by Sephadex LH-20 led to isolation of **3** (15 mg) from fraction-1, **1** (42 mg) along with mixture of sterols and fatty acids from fraction-2 and **2** (5 mg) from fraction-3.

β-Sitosterol (**1**) : Colourless crystals. IR (neat) : 3350 (OH); *m/z* (ESI) : 415.2567 ($M+H^+$); ^{1}H NMR ($CDCl_3$, 300 MHz) : 0.69 (3H, m), 0.81–1.09 (17H, m), 1.34 (4H, m), 1.49–1.53 (5H, m), 1.84–1.96 (11H, m), 2.03–2.20 (3H, m), 2.24 (2H, m), 2.34 (2H, m), 3.54 (1H, m), 5.35 (1H, d J=3); ^{13}C NMR ($CDCl_3$, 75 MHz) : δ 11.84 (C-29), 12.30 (C-24), 19.0 (C-28), 19.2 (C-19), 19.6 (C-27), 19.58 (C-26), 21.0 (C-11), 23.0 (C-23), 26.3 (C-15), 26.3 (C-21), 28.2 (C-16), 28.9 (C-25), 31.5 (C-2), 31.6 (C-7), 31.6 (C-8), 33.9 (C-20), 36.2 (18), 36.5 (C-10), 37.25 (C-1), 39.7 (C-12), 42.3 (C-4), 42.30 (C-13), 46.0 (C-22), 50.13 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5). Specific rotation : –40.499° (Found); –30 to –40° (Reported).

Loliolide (**2**) : Physical state; colourless liquid. IR (neat) : 1730 (C=O), 3462 (O-H); *m/z* (ESI) : 197.1322 ($M+H^+$) , 219.1048 $(M+Na)^+$, 172.0867; ^{1}H NMR ($CDCl_3$, 300 MHz) : 1.29 (3H, s, 1β-Me), 1.62–1.48 (5H, m, 4α,2α-H and 1α-Me), 1.94 (3H, s, 5-Me), 2.02 (1H, m, 2β-H), 2.49 (1H, m, 4β-H), 4.34 (1H, s, H-3), 5.82 (1H, s, H-7); ^{13}C NMR ($CDCl_3$, 75 MHz) : δ 26.49 (C-10), 27.00 (C-9), 30.64 (C-11), 35.89 (C-1), 45.62 (C-4), 47.32 (C-2), 66.86 (C-3), 99.9 (C-5), 112.95 (C-7), 172.4 (C-8), 182.1 (C-6).

13^2-Hydroxy-(13^2-S)-pheophytin-a (**3**) : Blackish green powder. UV (methanol) (nm) : 203, 275, 404, 502, 532, 665; IR (cm^{-1}) : 1620, 1707, 1741, 2858, 2926, 2954, 3750; ^{1}H NMR ($CDCl_3$, 300 MHz) : 0.80–0.89 (18H, m), 0.99–1.02 (6H, m), 1.13–1.16 (4H, m), 1.68–1.71 (5H, m), 1.87–1.95 (3H, m), 2.04–2.12 (3H, m), 2.30–2.36 (4H, m), 2.83 (3H, m), 3.25 (3H, m), 3.44 (3H, m), 3.63 (3H, m), 3.75 (3H, m), 4.16 (1H, m), 4.49–4.58 (3H, m), 5.20 (1H, m), 5.39 (4H, br s), 5.59 (1H, s), 6.26 (2H, dd, J=18, 36), 8.03 (1H, s), 8.76 (1H, s), 9.45 (1H, s), 9.61 (1H, s); ^{13}C NMR (75 MHz) : (Prophyrin) δ 11.2 (C-7^1), 12.1 (C-2^1), 12.2 (C-12^1), 16.3 (C-8^2) 19.4 (C-8^1), 25.6 (C-16), 29.3 (C-17^1), 29.6 (C-17^2), 49.9 (C-18), 51.8 (C-17), 53.2 (C-13^4), 88.9 (C-13^2), 93.6 (C-20), 97.1 (C-5), 104.2 (C-10), 107.5 (C-15), 122.8 (C-3^2), 126.8 (C-3^1), 129.1 (C-13), 129.4 (C-12), 131.7 (C-2), 136.1 (C-7), 136.2 (C-3), 136.5 (C-4), 137.8 (C-11), 142.0 (C-1), 145.2 (C-8), 149.7 (C-14), 151.0 (C-9), 155.3 (C-6), 162.4 (C-16), 172.3 (C-13^3), 172.7 (C-19), 173.5 (C-17^3), 192.04 (C-13^1); (phytyl group) : 17.2 (C-20′), 19.6 (C-19′), 19.7 (C-18′), 22.6 (C-17′), 22.7 (C-16′), 24.4 (C-9′), 24.7 (C-13′), 25.0 (C-5′), 27.9 (C-15′), 31.5 (C-11′), 32.6 (C-7′), 32.7 (C-17′), 36.6 (C-6′), 37.2 (C-12′), 37.3 (C-8′), 39.3 (C-14′), 39.8 (C-4′), 61.25 (C-1′), 117.8 (C-2′), 142.7 (C-3′).

Silylation of sterols : Saponification of sterols was carried out following a publish procedure[22]. Algal extract (1 g) was dissolved in saponification agent prepared by dissolving 0.6 mL of 33% KOH in 10 mL of methanol. The mixture was stirred for 12 h at 60–70° C. After that 5 mL of hexane and water were added then centrifuged, the hexane layer was separated and then dried, the residue was dissolved in silylating agent (BSA+TMSCL+TMSI) and incubated in a dry reaction tube under nitrogen at 60 °C for 2 h. After which 5 mL of hexane and water was added then the hexane layer was submitted for GC/MS analysis.

Methylation of fatty acids : Fatty acids were iso-

lated by column chromatography over silica gel column with petroleum ether/ethyl acetate solvent mixture as eluent. Isolated fatty acids 5 mg was dissolved in 5 mL of methanol and 0.7 mL of 10 *N* KOH was added, then the mixture was incubated at 60° C for 2 h. After which, 0.58 mL of 24 *N* H_2SO_4 was added and incubated at 60° C for 2 h. To this mixture 3 mL of hexane and water was added then centrifuged, the hexane layer was subjected to GC/MS analysis.

Supplementary material

Copies of ^1H and ^{13}C NMR spectra of compounds **1**-**3**. Result of GC-MS analysis for crude extract.

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