Pakistan Journal of Pharmaceutical Sciences 27
4(1) 27-34 (1991)

PHYCOCHEMICAL STUDIES ON *SC1NAL4 FASCICULARIS* (BONNEMAISONIALES, RHODOPHYTA)

ABDUL HAYEE-MEMON,[1] MUSTAFA SHAMEEL,[1] K. USMANGHANI,[2] MANSOOR AHMAD[2], RUQAYYA KHAN[1] and VIQAR UDDIN AHMAD[3]

[1]Department of Botany, University of Karachi, Karachi-75270, Pakistan
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan
[3]HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

The sterol composition of marine red alga, *Scinaia fasciculans* (Bϕrg.) Huisman has been studied and cholesta-5, 20, 24-trien-3β-o1, 22-dehydrocholesterol, brassicasterol, 24-methylene cholesterol, 24-methyl cholesterol, *nor* 31-cycloartanol, ergost-5-en-3,25-diol and lanostan-3β-o1 were identified through mass and ^{1}H-NMR spectroscopy. All of them were present in more or less equal amount. The fatty add methyl esters, analysed through GC-MS, were found to be methyl laurate, tridecylate, myristate, pentadecylate, palmitate, margarate, stearate and nonadecylate. Methyl myristate was present in largest and laurate in smallest quantity, no unsaturated fatty add could be detected.

Introduction

The term phycochemistry has recently been introduced by Shamed (1990). Not much phycochemical studies have been carried out on *Scinaia* Bivona. Augier and du Merac (1956) identified galactose, mannose and xylose from the alcoholic extract of *S. turgida* Chemin. Impelleaeri *et al.* (1975) investigated the varieties of floridoside, isofloridoside and mannoglyceric acid present in *Scinaia*. Fattorusso *et al,* (1975) detected cholesterol and liagosterol in a proportion of 2:1 in *S. forcellafa* Bivona. Qasim (1986) reported the percentages of total fat, glycerides, phospholipids and sterols in *S. indica* Bϕrg.

Scinaia fascicularis (Bϕrg.) Huisman is a 15 cm high, thin and cylindrical, repeatedly forked, brownish red alga, being occasionally constricted at the nodes and growing abundantly on the sub-littoral rocks. It was originally described from the coast of Karachi as *S. indica* by Anand (1943), later put under the synonymy of *Pseudogloiophloea fascicularis* (Bϕrg.) by Desikachary and Singh (1958) and now renamed as *S. farcicularis* by Huisman (1985). No detailed phycochemical investigation has so tar been made on this seaweed. In this paper we report an analysis of its sterols and saturated fatty acids.

Materials and Methods

The thalli of Scinaia *farcicular*ir were collected as drift material from the seashore of Buleji, near Karachi during the months of January and February 1987. They were carefully transported to the laboratory, thoroughly washed to remove the macroscopic epiphytes and epizoons and dried in sun shade.

*Extraction and pu*rifi*cation of ste*rol*s:*

The dried thalli (1.2 kg) were powdered and extracted twice with chloroform-methanol (1:1) through cold percolation for 25 days. The dark reddish brown syrupy residue (2.5 g) obtained after removal of the solvent was partitioned with ethyl acetate and water, and the ethyl acetate fraction (1 g) was run on silica gel chromatographic column. The following solvent systems were used and the sterolic compounds so obtained are: (i) hexane:ether (95:5) yielding cholesta-5, 20, 24-trien-3β-ol [1], (ii) hexane:ether (90:10) affording 22-dehydrocholesterol [2] and nor 31-cycloartanol 161, (iii) hexane:ether (80:20) providing brassicasterol [3] and 24-methylene cholesterol 141, (iv) hexane:ether (75:25) giving 24-methyl cholesterol [5] and (v) hexane:ether (70:30) imparting crgost-5-en-3,25-diol [7] and lanostan-3β-ol [8]. Further purification of these isolated compounds was affected through preparative thin layer chromatography plates and recrystalization efforts. After ascertaining the purity of the compounds they were subjected to mass spectrometry and ^{1}H-NMR spectroscopy. The details of the instrumentation may be found in a previous paper (Hayee-Memon *et al*, 1991a).

Extraction of fatty acids:

The extraction of fatty acids was carried out through cold percolation for 25 days with chloroform-ethanol (3:1), and this procedure was repeated three times. The combined extracts were concentrated and clarified by fractionation with ethyl acetate in aqueous layer. The ethyl acetate layer was separately taken to dryness and subjected to saponification, which was brought about with methanolic potash (10% KOH in 80% methanol) to afford 03 mg of crude fatty acid mixture. The crude mixture was treated with diazomethane for 24 hours in cold condition and the fatty acid methyl esters so obtained were directly injected into the GC and GC-MS. The details of GC-MS have been reported previously (Hayee-Memon *et al*, 1991a).

Results and Discussion

The following spectral data were recorded through ^{1}H-NMR spectroscopy, the mass spectral data and their fragmentation pattern are also given.

Cholesta-5, 20, 24-trien-3β-ol [1]: ^{1}H-NMR (403 MHz, $CDCl_3$): ppm: 0.67 (3H, s, 18-Me), 0.83 (3H, d, 1 = 6.0 Hz, 26-Me), 0.85 (3H, d, J = 5.6 Hz, 27-Me), 1.00

(3H, s, 19-Me), 1.22 (311, d, J = 7.0 Hz, 21-Me), 3.51 (3α-H), 4.22 (1H, t, J = 6.0 Hz, 6-H). **Mass**: m/z 382 (M^+, $C_{27}H_{42}O$), 367 (M^+ -CH_3), 349 (M^+-CH_3-HOH), 300, 271, 255, 213, 199, 133, 107, 93, 81, 67.

22-Dehydrocholesterol [2]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.67 (3H, s, 18-Me), 0.83 (3H, d, J = 6.0 Hz, 26-Me), 0.85 (3H, d, J = 5.6 Hz, 27-Me), 1.00 (3H, s, 19-Me), 1.22 (3H, d, J = 7.0 Hz, 21-Me), 3.51 (3α-H), 4.22 (1H, t, J = 6.0 Hz, 6-H). Mass: m/z 384 (M^+, $C_{27}H_{44}O$), 373 (M^+-C_8H_{15}), 217, 213, 187, 157, 142, 127, 92, 69.

Brassicasterol [3]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.67 (3H, s, 18-Me), 0.99 (311, s, 19-Me), 1.24 (1H, d, J = 7 Hz, 21-Me), 3.51 (1H, m, 3β-H), 5.35 (1H, d, J = 6.5 Hz, 22-H). **Mass**: m/z 398 (M^+, $C_{28}H_{46}O$), 356 (M^+ -C_3H_6), 314 (M^+ -C_6H_{12}), 300 (M^+ -C_7H_{13}-H), 269 (M^+ -C_8H_{15}.H_2O), 255 (M^+-C_9H_{17}-H_2O), 239, 213, 157, 133, 95, 69.

24-Methylene cholesterol [4]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.65 (3H, s, 18-Me), 0.87 (3H, s, 19-Me), 1.24 (3H, d, J = 7.0 Hz, 21-Me), 1.57 (6H, m, 26, 27-Me), 352 (3β-H), 5.72 (1H, m, 5-H), 334 (2H, s, 28-H). **Mass**: m/z 398 (M^+, $C_{28}H_{46}O$), 351 (M^+-2CH_3-OH), 314 (M^+-C_6H_{12}), 271 (M^+-C_9H_{17}-2H), 237, 213, 171, 135, 121, 81.

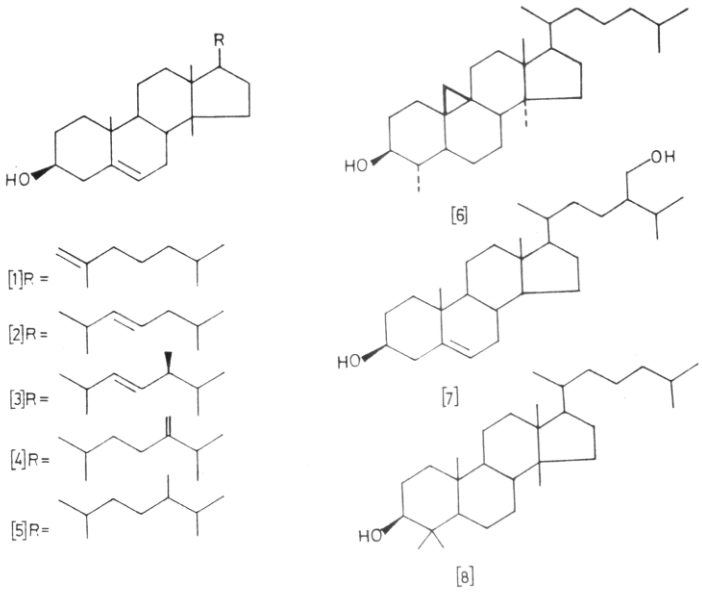
24-Methyl cholesterol [5]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.66 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 1.24 (1H, d, J = 7.0 Hz, 21-Me), 1.60 (6H, m, 26, 27-Me), 3.48 (1H, m, 3β-H), 5.33 (1H, m, 5-H). **Mass**: m/z 400 (M^+, $C_{28}H_{48}O$), 385 (M^+ -CH_3), 382 (M^+ -H_2O), 367 (M^+ -CH_3-H_2O), 314, 300, 269, 227, 139, 69.

Nor **31-cycloartanol [6]**: ^{1}H-NMR (300 MHz, $CDC1_3$): ppm: 0.67 (3H, s, 18-Me), 0.84-1.00 (15H, m, 21, 26, 27, 28, 29), 1.21-1.30 (22H, m, 1, 2, 6, 7, 11, 12, 15, 16, 22, 23, 24), 2.40 (2H, s, 19-Me), 3.51 (1H, m, 3β-H), 5.35 (1H, m, 5-H). **Mass**: m/z 414 (M^+, $C29H_{50}O$), 399 (M^+-CH_3), 396 (M^+-H_20), 381 (M^+-CH_3-H_2O), 314 (M^+ -$C_6H_{11}O$-H), 301 (M^+-C_8H_{15}-2H), 283 (M^+-C_8H_{15} -2H-H_2O), 271 (M^+-C_9H_{17}-H_2O), 213 (M^+-$C_{13}H_{25}$-2H-H_2O), 187 (M^+-$C_{15}H_{29}$-H_2O), 145, 133, 107, 95, 81, 69, 55.

Ergost-5-en-3,25-diol [7]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.66 (3H, s, 18-Me), 0.90 (3H, s, 19 -Me), 1.27 (3H, d, J = 68 Hz, 21-Me), 1.71 (6H, in, 26, 27-Me), 3.51 (2H, m, 3-H, 25-Me), 4.24 (1H, m, 25-Me). **Mass**: m/z 416 (M^+, $C_{28}H_{48}O_2$), 401 (M^+-CH_3), 384 (M^+-CH_2OH-H), 300 (M^+-C_7H_{16}, part of side chain), 273 (M^+ -side chain), 255 (M^+-$C_9H_{19}O$-H_2O), 213 ($M^+C_{14}H_{17}$-H_2O), 159, 145, 133, 119, 81, 55.

Lanostan-3β-ol [8]: ^{1}H-NMR (400 MHz, $CDCl_3$): ppm: 0.63 (3H, s, H-18), 1.07 (12H, m, H-19, 28, 29, 30), 1.33 (3H, d, J = 7 Hz, H-21), 1.55 (6H, m, H-26, 27), 3.55 (1H, m, H-3β). **Mass**: m/z 430 (M^+, $C_{30}H_{54}O$), 415 (M^+-CH_3), 397 (M^+-CH_3-H_2O), 317

(M^+ -side chain), 290, 275, 247, 229, 206, 147, 135, 120, 109, 97, 83, 69, 55.



Altogether eight sterols were isolated as a non-saponifiable matter from the extract of dried and powdered material of *Scinaia fascicularis* (Table-1). No sterol was present in large quantity, all were detected in small and more or less equal amounts. Cholesterol and its biosynthetic precursor desmosterol, which are the main sterol components of Rhodophyta (Goodwin, 1974), could not be detected. In its sterol composition this seaweed resembles *Dermonema abbottiae* Afaq., Hiram *et* Shamed, a taxonomically closely related alga, as cholesta-5, 20, 24-trien-3β-ol, brassicasterol, 24-methylene cholesterol, 24-methyl cholesterol and nor 31-cycloartanol are found in both of them (Afaq-Husain *et al,* 1991). *S. fascicularis* also exhibits a resemblance with *Hypnea valentiae* (Turn.) Montag. in sterol contents, as 22-dehydrocholestcrol, 24-methylene cholesterol, 24-methyl cholesterol and nor 31-cycloartanol are common in them (Hayee-Memon *et al.,* 1991b). The 22-dehydrocholesterol has also been reported from *Gracilaria fohifera* (Forssk.) Bing. (Flayee-Memon *et al.,* 1991a). It is the major sterol in the former and a minor sterol in the latter seaweed, and both of them belong to the order Gigartinales.

Table-1

Sterol composition of *Scinaia fascicularis.*

Systematic name	Common name	Molecular formula	Molecular weight	Relative % age
Cholesta-5, 20, 24-trien-3β-ol	Cholestatrienol	$C_{27}H_{42}O$ [1]	382	11.84
22-Dehydrocholest-5-en-3β-ol	22-Dehydrocholesterol	$C_{27}H_{44}O$ [2]	384	14.23
24β–Methylcholesta-5,22-dien-3β-ol	Brassicasterol	$C_{28}H_{46}O$ [3]	398	12.34
24-Methylene-cholest-5-en-3β-ol	24-Methylene cholesterol	$C_{28}H_{46}O$ [4]	398	11.76
24-Methyl-cholest-5-en-3β-ol	24-Methyl cholesterol	$C_{28}H_{48}O$ [5]	400	13.18
Nor-31, 9β, 19-cycloartanostan-3β-ol	*Nor* 31-cycloartanol	$C_{29}H_{50}O$ [6]	414	12.42
Ergost-5-en-3, 25-diol	Ergostenol	$C_{28}H_{48}O_2$ [7]	416	11.81
Lanostan-3β-ol	Lanostanol	$C_{29}H_{50}O_2$ [8]	430	12.15

Fattorusso *et al.* (1975) found liagosterol in substantial quantities in *Liagoro dirtenta* (Mert) C. Ag. and *Scinaio forcellata (= S. furcellata* (Turn.) Bivona) and suggested the possibility that liagosterol might be of some taxonomic significance to Nemaliales (including Bonnemaisoniales). This suggestion was confirmed when liagosterol was reported from *Asparagopsis armata* Harv. (Combaut *et al.*, 1979). Further studies revealed its absence in other species, *e.g. A. sandfordiana* Marv. (Bano *et al,* 1988) and *Dennonema abbomae* (Afaq-Husain *et al,* 1991). However, this sterol was also found absent in *S. fascicularis,* indicating that it is not of usual occurrence in all the members of Nemaliales and Bonnemaisoniales. It has also been detected in *Palmaria palmata (L.)* Runt. *(= Rhodymenia palmaw Grev.)* by Morisaki *et al.*, (1976), which is a member of Rhodymeniales.

The GC-MS data of the methylated fatty acids revealed the presence of eight saturated fatty acids in *S. fascicularis* (Table-2). The significant ions in the mass spectra of these acids are given below.

Table-2.

Fatty acid composition of *Scinaia fascicularis*.

Systematic name	Common name	Molecular formula	Mol. wt.	R.R.T	Relative % age
Methyl-*n*-dodecanoate	Methyl laurate	$C_{13}H_{26}O_2$	214	0.99	3.02
Methyl-*n*-tridecanoate	Methyl tridecylate	$C_{14}H_{28}O_2$	228	1.07	28.85
Methyl-*n*-tetradecanoate	Methyl myristate	$C_{15}H_{30}O_2$	242	1.12	37.61
Methyl-*n*-pentadecanoate	Methyl pentadecylate	$C_{16}H_{32}O_2$	256	1.23	7.55
Methyl-*n*-hexadecanoate	Methyl palmitate	$C_{17}H_{34}O_2$	270	1.24	6.64
Methyl-*n*-heptadecanoate	Methyl margarate	$C_{18}H_{36}O_2$	284	1.34	4.83
Methyl-*n*-octadecanoate	Methyl stearate	$C_{19}H_{38}O_2$	298	1.44	4.22
Methyl-*n*-nonadecanoate	Methyl nonadecylate	$C_{20}H_{40}O_2$	312	1.55	7.25

Methyl laurate: GC-MS: m/z 214 (M^+, $C_{13}H_{26}O_2$, 1%), 171 (M^+-43, 13%), 157 (M^+-57, 7%), 143 (51%), 129 (27%), 115 (9%), 101 (23%), 87 (100%).

Methyl tridecylate: GC-MS: m/z 228 (M^+, $C_{14}H_{28}O_2$, 8%), 185 (M^+-43, 17%), 171 (M+-57, 13%), 157 (7%), 143 (54%), 129 (23%), 115 (8%), 101 (17%), 87 (100%).

Methyl myristate: GC-MS: m/z 242 (M^+, $C_{15}H_{30}O_2$, 13%), 199 (M^+-43, 12%), 185 (M^+ -57, 15%), 171 (11%), 157 (9%), 143 (46%), 129 (37%), 115 (6%), 101(26%), 87 (38%), 73 (100%).

Methyl pentadecylate: GC-MS: m/z 256 (M^+, $C_{16}H_{32}O_2$, 87%), 213 (M^+ -43, 35%), 199 (M^+-57, 8%), 185 (15%), 171 (16%), 157 (16%), 143 (8%), 129 (54%), 115 (22%), 101 (14%), 87 (27%), 73 (100%).

Methyl palmitate: GC-MS: m/z 270 (M^+, $C_{17}H_{34}O_2$, 8%), 227 (M^+-43, 47%), 213 (M^+ -57, 6%), 199 (12%), 185 (16%), 171 (14%), 157 (6%), 143 (54%), 129 (22%), 115 (8%), 101 (16%), 87 (100%).

Methyl margarate: GC-MS: m/z 284 (M^+, $C_{18}H_{36}O_2$, 41%), 241 (M^+-43, 13%), 227 (M^+ -57, 10%), 213 (28%), 199 (8%), 185 (18%), 171 (16%), 157 (15%), 143 (8%), 129 (46%), 115 (18%), 101(12%), 87 (26%), 73 (100%).

Methyl stearate: GC-MS: m/z 298 (M^+ , $C_{19}H_{38}O_2$, 2%), 255 (M^+ -43, 9%), 241 (M^+ -57, 11%), 227 (10%), 213 (27%), 199 (8%), 185 (20%), 171 (18%), 157 (12%), 143 (9%), 129 (43%), 115 (20%), 101 (30%), 87 (27%), 73 (100%).

Methyl nonadecylate: GC-MS: m/z 312 (M^+, $C_{20}H_{40}O_2$, 3%), 269 (M^+-43, 6%), 255 (M^+ -57, 7%), 241 (1%), 227 (1%), 213 (2%), 199 (1%), 185 (2%), 171 (1%), 157 (1%), 143 (1%), 129 (5%), 113 (12%), 99 (1%), 85 (2%), 71(100%).

All the above mentioned acids are saturated fatty acids. It is astonishing that no unsaturated fatty add could be detected, however, they are present in the taxonomically closely related algae, *e.g. Demtonema abbottiae* (Afaq-Husain *et al*, 1991) and other red seaweeds of Karachi (Hayee-Memon *et al*, 1991a,b). Palmitic acid is usually found in largest quantity in red algae (Qasim, 1986; Shameel, 1990), but in this seaweed it was found in a small amount (6.64 %). Methyl myristate was found in largest proportion (37.61 %) followed by methyl tridecylate (28.85 %), methyl laurate being the smallest (3.02 *%).* They were also found in *Hypnea valentiae* (Hayee-Memon *et al*., 1991b). It has been shown previously that in general red algae possess high concentration of C20-acids (Shameel, 1990).

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