J. Natn. Sci. Coun. Sri Lanka 1980 **8** (1) : 69—74

Sterols of Some Sri Lankan Marine Algae

M. MAHENDRAN, D. M. SIRISENA,
Department of Chemistry, University of Colombo, Sri Lanka, P. O. Box 1490, *Colombo* 3, *Sri Lanka.*

M. MORISAKI, F. SANO, N. IKEKAWA
Tokyo Institute of Technology, Nagatsuta, Midori—Ku, Yokohama 227, *Japan.*

AND

A. SIVAPALAN
Department of Botany, University of Jaffna, Thirunelvelly, Jaffna, Sri Lanka.

(*Paper accepted : 22 February,* 1980)

Abstract : Eighteen species of Sri Lankan marine algae belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae were examined for sterols. 28-isofucosterol is the major sterol in green algae belonging to Order Ulvales. Green algae belonging to orders Siphonales, Siphonocladales and Dasycladiales contain 24-ethyl cholesterol as the major sterol. Fucosterol is the major sterol in all the brown algae examined. The red algae contained cholesterol as the major sterol. The work indicates that there is no significant difference in the major sterols found in the marine algae of Sri Lankan, Japanese, British, French and Canadian waters.

1. Introduction

The sterols of marine algae[11] are distinctly different from division to division and in some cases within divisions. The major sterol of most of the red algae examined is recognised as cholesterol.[1,5,7,10] It was found previously[4] that Japanese red algae could be classified into three groups based on sterol profiles. One group contains cholesterol as the major sterol. The sterol mixtures of the second group contains cholestanol as the major sterol. The third group contained dehydrocholesterol as the principal sterol. Another sterol which usually accompanies cholesterol in red algae is desmosterol[1,5] and is the major sterol in several species.

Major sterol of the brown algae is fucosterol.[3,9] Cholesterol, 24-methylene cholesterol and saringosterol are also found in brown algae, but in small quantities.

The sterols of green algae are usually complex mixtures. Gibbons et al[8] identified 28-Isofucosterol as the major sterol of British green algae *Enteromorpha intestinalis* and *Ulva latuca.*

Very little information is available on the sterol composition of the Indian Ocean algae.

2. Results and Discussion

Eighteen species of Sri Lankan marine algae were examined for sterols using gas chromatography-mass spectrometry (GC-MS). The identification of each sterol was based upon its GC relative retention time and its MS fragmentation pattern. The sterol profiles of the algae are summarized in the table given below.

TABLE 1. Sterol Profiles of Sri Lankan Marine Algae

	Order	Family	Species	Locality	Sterol 1	2	3	4	5	6	7	8	9
GREEN ALGAE	Ulvales	Ulvaceae	*Ulva reticulata*	Galle	+	+				*			+
			Ulva lactuca	Galle	+	+				*			+
			Ulva faciata	Galle	+	+				*			+
	Cladophorales	Cladophoraceae	*Chaetomorpha crassa*	Jaffna	+	*							
	Siphonales	Codiaceae	*Halimeda opuntia*	Galle	*	*							
		Bryopsidaceae	*Bryopsis corticulang*	Galle	*	*				+	+		
		Caulerpaceae	*Caulerpa taxifolia*	Jaffna	+	*							
			Caulerpa claverta	Jaffna	+	*							
	Siphonocladales	Valoniaceae	*Valoniopsis pachynema*	Mt. Lavinia	+	*	+						
	Dasycladiales	Dasycladiaceae	*Acetabularia cranulata*	Jaffna	+	*							
BROWN ALGAE	Fucales	Sargassaceae	*Sargassum tenerrium*	Galle	+	+			*		+		
			Cystophyllum muricutum	Jaffna	+				*				
			Cystosiera triquerta	Jaffna	+				*				
RED ALGAE	Gelidiales	Gelidiaceae	*Gelidium corneum*	Galle	*								
		Gracilariaceae	*Gracilaria lichenoides*	Jaffna	*								
			Gracilaria confervoides	Jaffna	*								
			Gracilaria opuntia	Jaffna	*								
		Rhodomelaceae	*Laurencia papillosa*	Jaffna	*	+	+						

*Major sterol ; + Minor sterol

Cholesterol is the most common sterol in red algae, all species presently examined contained cholesterol as the major sterol. Fucosterol is the major sterol of brown algae. All the species of brown algae contain small amounts of cholesterol in addition to fucosterol. The green algae examined could be classified into two groups based on the sterol profiles. (One group three species) contained 28-isofucosterol as the major sterol. All these species belong to the family Ulvaceae. The present identification of 28-isofucosterol in Sri Lankan *U. lactuca*, *U. reticulata* and *U. faciata* in addition to its presence in *E. linza*[15] and British green algae *E. intestinalis* and *U. lactuca*[7] confirms that this is characteristic of Ulvaceae. The other group of green algae (six species) contain 24-ethylcholesterol as the major sterol.

Our work indicates that there is no difference in the major sterols found in marine algae of Sri Lankan, Japanese, British and Canadian waters.

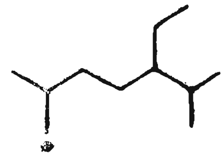
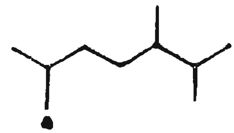
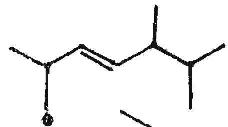
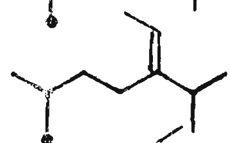
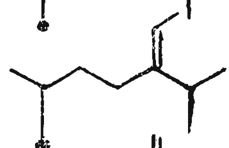
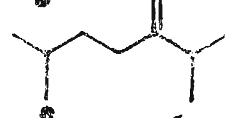
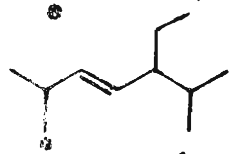
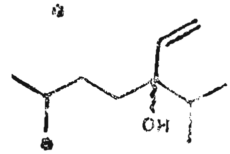
3. Experimental

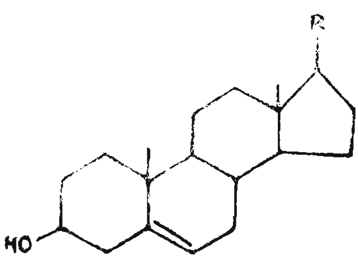
The algae were collected along the coast of Colombo, Galle and Jaffna, Sri Lanka. The air dried algae (ca 100g) was extracted with refluxing methanol (500 ml) for 3h. Extracts were saponified in a mixture of 40% KOH (1 ml) and methanol (25 ml).

An aliquot (ca 1 mg) of the unsaponifiable material was treated with trimethylsilylimidazole (100 μl) in a sealed tube at 70° for 30 mins. Resulting trimethyl silyl ether of the sterol were analyzed by GC-MS on 1.5% OV. 17(1.5 m × 3 mm, 250°C.). Identification of each sterol was based upon the comparison with the respective authentic sample in respect of GC retention time and MS fragmentation pattern as previously described,[2,3,8,11] RR'_{t}s of the TMSi derivatives of standard samples were ; Cholestane, 1.0 ; 22-dehydrocholesterol. 1.84 , cholesterol, 1.97 brassicasterol, 2.26 ; stigmasterol, 2.79, sitosterol, 3.18 ; fucosterol, 3.38 isofucosterol 3.51, 24-methyl cholesterol, 2.57 ; saringosterol ; 4.85. A different procedure was adopted for the isolation of sterols from *Sargassum* and *Laurencia*. Air dried algae (2kg) were extracted with refluxing methanol (2l). The methanol extract was concentrated, water was added to the concentrate and extracted with ether. Removal of ether under reduced pressure gave a brown oil. This brown oil was chromatographed on alumina and the sterol fractions eluted with 20% ether/Pet. ether were combined and analyzed by GC-MS.

The MS fragmentations of the sterols are given in Table II.

KEY TO STEROLS

Sterol	*Number*	
Cholesterol	1	
24 – Ethylcholesterol	2	
24 – Methylcholesterol	3	
24 – Methylcholesta – 5, 22 – diene 3 β – ol	4	
Fucosterol	5	
28 – Isofucosterol	6	
24 – Methylenecholesterol	7	
24 – Ethylcholesta 5, 22 – diene – 3 β – ol	8	
Saringosterol	9	



* Indicates the point of attachment of R.

TABLE (II)—Mass spectral Fragmentation of TMSi Derivatives of Sterols.

STEROLS	NUMBER	MS FRAGMENTATIONS (Major fragmentations at m/e)
Cholesterol	1	458 (M^+), 443 (M^+–CH_3), 368 (M^+ –TMSOH) 358 (M^+–TMSOH – CH_3), 329 (M^+–129) 255 (M^+–TMSOH –Sidechain), 129
24§ –Ethylcholesterol	2	486 (M^+), 471 (M^+–CH_3) 396 (M^+–TMSOH), 381 (M^+–TMSOH – CH_3), 357 (M^+–129), 255 (M^+–TMSOH – Side chain), 129.
24§ –Methylcholesterol	3	472 (M^+), 457 (M^+–CH_3), 382 (M^+–TMSOH), 367 (M^+–TMSOH – CH_3), 343 (M^+–129), 255 (M^+–TMSOH – Sidechain), 129.
Fucosterol	5	484 (M^+), 469 (M^+–CH_3), 394 (M^+–TMSOH) 386 (M^+–98), 379 (M^+–TMSOH—CH_3), 355 (M^+–129), 255 (M^+–TMSOH – Sidechain) 129.
Isofucosterol	6	484 (M^+), 469 (M^+–Me), 386 (M^+–98) 355 (M^+–129), 296 (386 – TMSOH), 281 (296 – Me), 257, 129.
24-methylenecholesterol	7	470 (M^+), 386, 296.
Saringosterol	9	572 (M^+), 557 (M – Me), 529 (M – iPr) 439 (529 – TMSOH), 349 (439-TMSOH) 295, 255, 253, 171, 129.

Isolation of Isofucosterol

The algae (1.5 kg) was defatted with petroleum ether (60° to 80°) and then extracted with acetone. The acetone extract was concentrated under reduced pressure. The residue was diluted with water and extracted with ether. Removal of ether gave a brown oil. This brown oil was chromatographed on alumina and the fraction eluted with 25% benzene in petroleum ether yielded 28-isofucosterol (1.5g) as a white crystalline solid m.p. 135° to 136° (Lit[14] m.p. 135.5—136.4°). (Found : C, 84.29 ; H, 11.56; $C_{29}H_{48}O$ requires C, 84.47 ; H, 11.40). IR (KBr)cm^{-1}: 812, 840, 1065, 1580, 3440. ^{1}HNMR ($CDCl_3$) : δ 5.29 (m, 1H) C-6 proton, 5.05 (q, J ≃ 6.9Hz) C-28 proton ; 3.40 (m, 1H) C-4 proton ; 1.54 (d, 3H) C-29 protons, 2.80 (m, 1H) C-25 proton. I.R. and n.m.r. of this white solid were identical to those reported[8] for the compound. Further confirmation came from GC-MS analysis as its trimethylsilyl ether.

Acknowledgements

We thank the National Science Council of Sri Lanka and the University of Colombo, Sri Lanka for financial assistance. We thank Professor C. Djerassi, Stanford University, for the analysis of sterol fraction of *Sargassum*. The authors thank Professor Hisashi Takei, Tokyo Institute of Technology, for his advice and encouragement. Our sincere thanks are due to Mrs. S. Medis for the illustrations.

References

1. Alcaide, A., Devys, M., & Barbier, M. (1968). *Phytochemistry*, **7** : 329.
2. Brooks, C. J. W. ; Henderson, W. & Steel, G. (1973). *Biochem. Biophys Acta* **296**: 431.
3. Carter, P. W., Heilbron, H. N., & Lythgoe, B. (1939). *Proc. Roy. Soc. Ser. B.* **128**, 82.
4. Charadon-Loriaux, 1., Morisaki, M., & Ikekawa, N. (1976). *Phytochemistry* **15** : 723,
5. Fattorusso, E., Magno, S., Santacroce, C., Sica, D., Impellizzeri, G., Mangiafico, S., Orient, G., Piatelli, M., & Sciuto, S. (1975). *Phytochemistry*, **14** : 1579.
6. Fattorusso, E., Magno, S., Santacroce, C., Sica, D., Impellizzeri, G., Mangiofico, S., Piatelli, M., & Sciuto, S., (1976). *Biochem. Syst. Ecol.* **4** : 135.
7. Gibbons, G. F., Goad, L. J., & Goodwin, T. W., (1967). *Phytochemistry* **6** : 677.
8. Gibbons, G. F., Goad, L. J., & Goodwin, T. W., *Phytochemistry* (1968) **7** : 983.
9. Ikekawa, N., Morisaki, M., Tsuda, K., & Yoshida, T. (1968). *Steroids* **12** : 14.
10. Idler, D. R., Saito, A., & Wiseman, P. (1968) *Steroids* **11** : 465.
11. Knights, B. A., (1967). *J. Gas Chromatogr.* **5**: 273.
12. Morisaki, M., Kidooka, S., & Ikekawa, N. (1976). *Chem. Pharm. Bull. (Tokyo)* **24** : 3214
13. Patterson, G. W., (1971). *Lipid* 6, 120, Patterson, G. W. (1979). *Sterols of Algae : Proceedings of International Symposium on Marine Algae of the Indian Ocean Region, Bhavanagar*, India, 9-12 January 1979, pp. 37.
14. Scheuer, P. J. (1973). *Chemistry of Marine Natural Products*, Academic Press, New York p. 84.
15. Tsuda, K., & Sakai, K. (1960) *Chem. Pharm. Bull. (Tokyo)* **8** : 554.