ACYCLIC DITERPENOID FROM THE RED ALGA *Gracilaria foliifera*

WALIED, M. ALARIF [a*], SEIF-ELDIN,. N. AYYAD[b], SULTAN, S. AL-LIHAIBI[a]

(Received January 2010; Accepted April 2010)

ABSTRACT

The dichloromethane/methanol extract from the red alga *Gracilaria foliifera* was subjected to a column chromatography which afforded a new (naturally) acyclic diterpenoid 3,7,11,15 tetramethyl-3-hexadec-en-1-ol (1) in addition to two C_{29} ethylidene steroids fucosterol, (24*E*)-Stigmasta-5,24(28)-diene-3β-ol (2) and isofucosterol, (24*Z*)-Stigmasta-5,24(28)-diene-3β-ol (3) which are reported for the first time from the genus *Gracilaria*. The structures were assigned mainly on the basis of ^{1}H and ^{13}C NMR experiments.

Keywords: Red Sea, *Gracilaria foliifera*, Acyclic diterpenoid, Fucosterol, Isofucosterol.

RESUMEN

Del extracto de diclorometano/methanol de la alga roja *Gracilaria foliifera* se obtuvo por cromatografía en columna un nuevo diterpenoide aciclico (natural) el 3,7,11,15 tetrametiyl-3-hexadec-en-1-ol (1) y también dos esteroides C_{29} fucosterol etilideno: (24E)-estigmasta-5,24(28)-diene-3β-ol (2) y el isofucosterol, (24Z)-estigmasta-5,24(28)-diene-3β-ol (3). Es el primer reporte de estos compuestos en el género *Gracilaria*. Las estructuras de los compuestos fueron asignadas a través de las técnicas ^{1}H y ^{13}C RMN

Keywords: *Gracilaria foliifera*, diterpenoide aciclico, Fucosterol, Isofucosterol.

INTRODUCTION

Gracilaria foliifera (*Gracilariales*, *Rhodophyta*) red alga is probably confined to the Red, Arabian and Indian Seas (Guiry & Freamhainn, 2009). Red algae of the genus *Gracilaria* are known for the production of agar (Rodriguez *et al.*, 2009), polysaccharides (Chattopadhyay *et al.*, 2008) toxins (Yotsu-Yamashita *et al.*, 2007), Lipids (Hwang *et al.*, 2007) and steroids (Govindan *et al.*, 1993; Das, Srinivas, 1992a; Das, Srinivas, 1992b). Investigation of the dichloromethane/methanol extract of the

[a]Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, PO. Box 80207, Jeddah 21589, Saudi Arabia. Tel (work): +966-2-6952383, (mob.): +966-56-0352034, Fax: +966-2-6401747.
E-mail address: walied1737@yahoo.com
[b]Department of Chemistry, Faculty of Science, King Abdulaziz University, PO. Box 80203, Jeddah 21589, Saudi Arabia.

red alga *Gracilaria foliifera* has resulted in the isolation of a new acyclic diterpenoid 3,7,11,15 tetramethyl-3-hexadec-en-1-ol (1), (compound **1** was synthesized by Kulkarni *et al.*, 1988) in addition to two C_{29} ethylidene steroids fucosterol, (24*E*)-Stigmasta-5,24(28)-diene-3β-ol (2) and isofucosterol, (24*Z*)-Stigmasta-5,24(28)-diene-3β-ol (3) which are reported for the first time from the genus *Gracilaria*.

EXPERIMENTAL

Apparatus and materials

Column chromatography was carried out using silica gel (60G Merck). Thin layer chromatography was carried out on aluminum- backed silica gel (F254 Fluka). EI/MS analyses were carried out on a Shimadzu-QP 2010, NMR measurements were performed for solutions in $CDCl_3$, the ^{13}C and ^{1}H NMR data were assigned on the basis of ^{1}H-^{1}H COSY (correlated spectroscopy), ^{13}C-^{1}H HMQC (heteronuclear multiple quantum coherence spectroscopy) and ^{13}C-^{1}H HMBC (heteronuclear multiple bond correlation spectroscopy) measured on BRUKER 600MHz NMR spectrometer. Chemical shifts were recorded as δ values in part per million (ppm) relative to tetramethylsilane. All *J* values are given in Hz.

Extraction and isolation

Gracilaria foliifera was collected in Nov. 2007, at the southern coast of the Red Sea of Saudi Arabia near Yemen. After air-drying, samples (300g) were ground and extracted with Dichlromethane/methanol (v/v). The extract was concentrated under reduced pressure to yield 7. 6 g of crude material, which was then subjected to chromatography over a silica gel column using a gradient of n-hexane-ethyl acetate as gradient solvent. Purification of compounds was monitored by sulfuric acid/ethanol as a spray reagent.

Compound 1

The fraction eluted with n-hexane/EtOAc ,9:1, was further purified by preparative TLC on silica gel using n-hexane/EtOAc (7:3) to give compound **1** (60mg, 0.02%) as a colorless oil R_f 0.71 (n-hexane/EtOAc ,32:6); $[\alpha]_D$= +0.18˚ (c= 1.2, $CHCl_3$). IR ($CHCl_3$) cm^{-1}:3450, 2955, 2920 and 1660. EI-$MS^{m/z}$: 296 [M, $C_{20}H_{40}O]^+$, 278 [M- $H_2O]^+$,263 [M-CH_3-$H_2O]^+$,71 $[C_5H_{11}]^+$, 57 $[C_4H_9]^+$. ^{1}H NMR and ^{13}CNMR ($CDCl_3$), δ (ppm): (table 1).

Compounds 2 and 3

The fraction eluted with a mixture of n-hexane/EtOAc (8:2) is further purified by repeated column chromatography and PTLC on silica gel using n-hexane/EtOAc (7:1) led to the isolation of compounds **2** and **3** as a mixture (80mg) as a white solid of nearly equal R_f= 0. 45 (n-hexane/EtOAc , 32:6) but with slightly different color response up on spraying with sulfuric acid/ethanol and heating at 110˚C for few minutes. Running out 2D TLC several times we could separate them into pure forms**.**

Compound **2** (major): white solid m.p. 121-123˚C.EI-$MS^{m/z}$: 412 [M, $C_{29}H_{48}O]^+$, 397 [M- $CH_3]^+$, 379 [M-CH_3-$H_2O]^+$, 314 (100) [M-$C_7H_{14}]^+$, 299 [M-C_7H_{14}-$CH_3]^+$, 296 [M-C_7H_{14}-$H_2O]^{+1}$, 281 [M-C_7H_{14}-H_2O-$CH_3]^{+1}$, 281 [M-side chain + 2H]$^{+1}$. H NMR ($CDCl_3$),δ (ppm): 0.86-0.88 (d, *J*=6.6 Hz, 6H, 26-CH_3 and 27-CH_3), 0.91 (d, *J*=6.6 Hz, 3H,21-CH_3), 0.68 (s, 3H,18-CH_3), 1.00 (s, 3H,19-CH_3), 1.58 (d, *J*=6.6 Hz 3H,29-CH_3), 3.53 (m,H,H-3), 5.35 (m,H,H-6), 5.18 (q,H,H-28), 2.32 (sep, *J*= 6.6 Hz, H-25).^{13}C NMR ($CDCl_3$), δ (ppm): (39.75, C-1), (35.20, C-2), (71.80, C-3), (42.33, C-4), (140.74, C-5), (121.70, C-6), (36.14, C-7), (35.78, C-8), (50.10, C-9) , (39.72, C-10), (28.23, C-11), (42.28, C-12), (42.3, C-13), (56.74, C-14), (31.64, C-15), (34.78, C-16), (56.72, C-17), (11.85, C-18), (19.40, C-19), (39.50, C-20), (24.32, C-21), (37.23, C-22), (31.90, C-23), (146.60, C-24), (36.50, C-25), (22.23, C-26), (22.50, C-27), (115.54, C-28), (18.70, C-29).

Compound **3** (minor): white solid m.p. 133-135°C.EI-MS$^{m/z}$: 412 [M, $C_{29}H_{48}O]^+$, 397 [M- $CH_3]^+$, 379 [M-CH_3-$H_2O]^+$, 314 (100) [M-$C_6H_{10}O]^+$.^{1}H NMR ($CDCl_3$),δ (ppm): 0.86-0.88 (d, *J*=6.6 Hz, 6H, 26-CH_3 and 27-CH_3), 0.91 (d, *J*=6.6 Hz, 3H,21-CH_3), 0.68 (s, 3H,18-CH_3), 1.00 (s, 3H,19-CH_3), 1.59 (d, *J*=6.6 Hz 3H,29-CH_3), 3.53 (m,H,H-3), 5.35 (m,H,H-6), 5.11 (q,H,H-28), 2.22 (sep, *J*= 6.6 Hz, H,H-25).^{13}C NMR ($CDCl_3$), δ (ppm): (39.75, C-1), (35.20, C-2), (71.80, C-3), (42.33, C-4), (140.74, C-5), (121.70, C-6), (36.14, C-7), (35.78, C-8), (50.10, C-9), (39.72, C-10), (28.23, C-11), (42.28, C-12), (42.3, C-13), (56.74, C-14), (31.64, C-15), (34.78, C-16), (56.72, C-17), (11.85, C-18), (19.40, C-19), (39.50, C-20), (24.32, C-21), (37.23, C-22), (31.90, C-23), (146.00, C-24), (33.90, C-25), (22.50, C-26), (22.23, C-27), (115.94, C-28), (18.90, C-29).

RESULTS AND DISCUSSION

Compound 1

Compound **1** was obtained as colorless oil and showed a molecular ion peak in EI-MS at m/z 296 which together with ^{13}C NMR data, suggested a molecular formula of $C_{20}H_{40}O$ which indicates 1 degree of unsaturation that can be deduced to be a double bond by examining IR (ν_{max} 1660 cm^{-1}) absorption spectrum, ^{1}H NMR one olefinic proton at δ 5.41 ppm and ^{13}CNMR two signals at δ 123.02 and 140.38 ppm. The DEPT spectra showed that compound **1** contains five methyl, ten methylene, four methine and one quaternary carbons. The nature of oxygen atom was found to be primary alcoholic from both ^{1}H NMR δ 3.53 (t, *J*= 6.5) ppm and ^{13}CNMR δ 59.44 ppm. So compound **1** should be acyclic diterpene alcohol which can be deduced to be phytol (4) but comparing our present spectral data with that of phytol (Bang *et al.*, 2002) (table 1) we can observe a difference in the position of the double bond. Localization of the hydroxyl group at C-1, methyl groups and the double bond at C_3-C_4 were deduced from HMQC, HMBC correlations and COSY experiment. Positions of the methyl groups also can be explained by the obtained spectral data (table 1) which is in agreement with the biogenetic rule of terpenoids. These data of compound **1** allowed to be assigned the following structure, 3,7,11,15 tetramethyl-3-hexadec-en-1-ol.

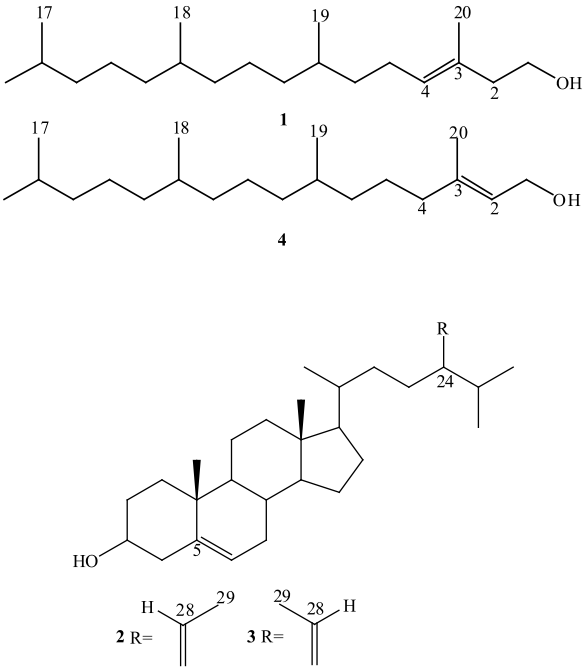

Fig. 1 Structures of compounds 1-4

Compounds 2 and 3

GC/MS of the purified steroidal fraction (Liebermann- burchard's reaction gave the typical slow reacting green color of a Δ^5 sterol) (Gibbons *et al.*, 1968) gave two closely peaks of nearly equal retention times but with identical MS fragmentation pattern of a parent ion peak at m/z 412 which together with ^{13}C NMR data, suggested a molecular formula of $C_{29}H_{48}O$. The loss of part of the side chain (C_7H_{14}) is characteristic of sterols with a $\Delta^{24(28)}$ or $\Delta^{24(24')}$ (Gibbons *et al.*, 1968). ^{1}H NMR data were typical of sterols, compound **2** gave a doublet at δ 1.59 ppm (*J*= 6.6 Hz) for the proton at C-

Table1. ^{1}H NMR, ^{13}C- NMR data for compounds **1** and **4** (phytol) in $CDCl_3$ (600 MHz)

	Compound 1		**Compound 4 (phytol)**
No.	δ H, ppm (m, *J* in Hz)	$\delta^{13}C$	δ H, ppm (m, J in Hz) $\delta^{13}C$
1	3.53 (t, *J*= 6.5)	59.44	4.14 (d, J= 6.8) 59.34
2	1.98 (t, *J*= 6.5)	39.87	5.40 (dq, d= 6.8, 1.4) 123.11
3	-	140.38	- 140.14
4	5.41 (dt, *J*= 3.6, 1.2)	123.02	1.99 (t, J= 7) 39.85
5	1.95 (dt, 6.5)	39.36	1.33 (m) 39.34
6	1.28 (m)	37.36	1.25 (m) 37.34
7	1.55 (m)	32.69	1.65 (m) 32.66
8	1.20-1.30 (m)	37.69	1.25 (m) 37.26
9	1.20-1.30 (m)	37.42	1.25 (m) 37.40
10	1.20-1.30 (m)	36.65	1.25 (m) 36.65
11	1.55 (m)	32.79	1.65 (m) 32.76
12	1.20-1.30 (m)	25.12	1.25 (m) 25.12
13	1.20-1.30 (m)	24.80	1.25 (m) 24.77
14	1.20-1.30 (m)	24.47	1.25 (m) 24.45
15	1.55 (m)	27.98	1.65 (m) 27.95
16	0.87 (d, *J*= 6.0)	19.72	0.87 (d, J= 6.3) 19.68
17	0.87 (d, *J*= 6.0)	16.19	0.87 (d, J= 6.3) 16.13
18	0.85 (d, *J*= 6.0)	19.75	0.85 (d, J= 6.1) 19.7
19	0.84 (d, *J*= 5.5)	22.70	0.84 (d, J= 6.3) 22.69
20	1.67 (s)	22.63	1.66 (s) 22.59

29 (δ_C 18.70), a quartet δ 5.18 ppm (*J*= 6.6 Hz) at C-28 proton (δ_C 115.54), a multiplet at 5.35 for C-6 proton (δ_C 121.70) and a multiplet at 3.53 for C-3 proton (δ_C 71.80), while compound **3** gave a doublet at δ 1.58 ppm (*J*= 6.6 Hz) for the proton at C-29 (δ_C 18.90), a quartet δ 5.11 ppm (*J*= 6.6 Hz) at C-28 proton (δ_C 115.94), a multiplet at 5.35 for C-6 proton (δ_C 121.70) and a multiplet at 3.53 for C-3 proton (δ_C 71.80). ^{13}C NMR for both compounds indicated 29 carbons, of which two double bonds and a secondary alcohol (*cf exp*.) and the DEPT spectra showed that compounds **2** and **3** both contain six methyl, ten methylene, nine methine and four quaternary carbons. The stereochemistry at the side chain double bond can be explained by observing the ^{1}HNMR spectra at the C-25 proton which resonates at δ 2.32 ppm (δ_C 36.50) in case of (*E*) isomer and at δ 2.22 ppm (δ_C 33.90) in case of (*Z*) isomer (Frost, ward, 1968). The position of the ethylidene group was proved from HMQC, HMBC correlations and COSY experiment. These data are in agreement with literature (John Goad, Akihisa, 1997) which can allow us to assign compound **2** as fucosterol and compound **3** as isofucosterol.

CONCLUSIONS

Phytol (3,7,11,15 tetramethyl-2-hexadec-en-1-ol) is a common diterpenoid in plant kingdom. This report of its isomer 3,7,11,15 tetramethyl-3-hexadec-en-1-ol adds to that on the capability of such red alga of isomerization . In addition, we reported two C_{29} ethylidene steroids fucosterol and isofucosterol, which are reported for the first time from the genus *Gracilaria*.

ACKNOWLEDGMENTS

The authors are thankful to the researcher's assistants for their help in the laboratories work. Deanship of Scientific Research (DSR) is also acknowledged for the financial support (Grant No. 8-003/429).

REFERENCES

Bang, M. H., Chol, S. Y., Jang, T. O., Kim, S. K., Kwon, O. S., Kang, T. C., Won, M. H., Park, J., Baek, N. I. (2002) Phytol, SSADH Inhibitory Diterpenoid of *Lactuca sativa. Archives of Pharmacol Research* **25**: 643- 646.

Chattopadhyay, K., Ghosh, T., Pujol, C. A., Carlucci, M. J., Damonte, E. B., Ray, B. (2008) Polysaccharides from *Gracilaria corticata*: Sulfation, Chemical characterization and anti-HSV activities. *International Journal of Biological Macromolecules* **43**: 581-588.

Das, B., Srinivas, K. V. N. S. (1992a) Dihydroxysterols from the marine red alga, *Gracilaria edulis*. *Phytochemistry* **58**: 4371-4373.

Das, B., Srinivas, K. V. N. S. (1992b) Minor C_{29}- steroids from the marine red alga, *Gracilaria edulis*. *Phytochemistry* **31**: 2427-2429.

Frost, d. J., Ward, J. P. (1968) Stereochemistry of 7, 24(28)-Stigmastadien-3β-ol and the fucosterols. *Tetrahedron Letters* **34**: 3779-3782.

Gibbons, G. F., Goad, L. J., Goodwin, T. W. (1968) The identification of 28- isofucosterol in the marine green algae *Enteromorpha intestinalis* and *Ulva lactuca. Phytochemistry* **7**: 983-988.

Govindan, M., Hodge, J. D., Brown, K. A., Nunez-Smith, M. (1993) Distribution of cholesterol in caribbean marine algae. *Steroids* **58**: 178-180.

Guiry, M. D., Freamhainn, M. T. (2009) Biosystematics of *Gracilari foliifera*. *Nordic Journal of Botany* **5**: 629-637.

Hsu, B. Y, Tsao, C. Y., Chiou, T. K., Hwang, P. A., Hwang, D. F. (2007) HPLC determination for prostaglandins from sea weed *Gracilaria gigas*. *Food Control* **18**: 639-645.

John Goad, L., Akihisa, T. (1977) Analysis of Sterols 1st. eds. Blackie Academic & Proffesional, pp. 382-383.

Kulkarni, S.N., Phadake, A. S., Rangaishenvi, M. V., Kamath, S. V., (1988) Synthesis of phytol isomer 7,11,15-Trimethyl-3-methylenehexadecanol and 3,7,11,15-tetramethyl-3-hexadecen -1-ol and their conversion into vitamin E. *Indian Journal of Chemistry*, Section B: Organic Chemistry Including Medicinal Chemistry **27 B** (1): 65-6.

Rodriguez, M. C., Matulewicz, M. C., Nose da, M. D., Ducetti, D. R. B., Leonardi, P. I. (2009) Agar from *Gracilaria gracilis* (*Gracilariales, Rhodophyta*) of the patagonic coast of Argentina-Content, structure and physical properties. *Bioresource Technology* **100**: 1435-1441.

Yotsu-Yamashita, M., Abe, K., Seki, T., Fujiwara, K., Yasumoto, T. (2007) Polycarvenoside C and C2, the new analogs of the human lethal toxin polycarvenoside A from the red alga *Gracilaria edulis*. *Tetrahedron Letters* **48**: 2255-2259.