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Three Antioxidant Compounds of the Red Alga *Liagora farinosa*

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Abstract – Investigation of the chloroform soluble fraction of the red alga *Liagora farinosa,* collected from Hurghada at the Red Sea resulted in the isolation of three compounds; a nucleoside (thymidine) and two glycosides (methyl-β-D-xylopyranoside and glycerol-2-α-D-glucopyranoside). The structures of the isolated compounds were established on the basis of different spectroscopic techniques as well as comparison with the previously published data. This is the first report for the isolation of the three compounds from red algae; moreover, the compounds were examined for their antioxidant activity and showed variable activity.
Keywords – Red algae, *Liagora farinosa*, nucleoside, methyl-β-D-xylopyranoside, glycerol-2-α-D-glucopyranoside, antioxidant activity.

Introduction

The red algae (Rhodophyta) comprises about 5000 - 6000 species of mostly multicellular marine algae including many notable seaweeds (Thomas, 2002). *Liagora farinosa* is a red alga having a number of features that make recognition of the species easy. The vegetative filament contains large cells that are cuboidal to cylindrical and sometimes slightly moniliform. In addition, the carposporophyte is uncomplicated (Abbott, 1984). Previous studies on the activity of the crude ethanol extract of *Liagora farinosa* proved that it possesses a mild acaricidal activity (Williams, 1991). *Liagora farinosa* collected from Caribbean sea and Pacific ocean was previously investigated for active metabolites and the chloroform extract yielded four toxic acetylene-containing lipids (Paul *et al.*, 1980). Here, we report the results of investigation of *Liagora farinosa* collected from the Red Sea where chromatographic fractionation of the chloroform extract afforded three compounds; thymidine (**1**), methyl-β-D-xylopyranoside (**2**) and glycerol-2-α-D-glucopyranoside (**3**).

Experimental

General experimental procedures – For column chromatography, silica gel (Merck, 70 - 230 mesh ASTM) was used. Pre-coated silica gel 60 F-254 plates (Merck) were used for TLC and pTLC

Nuclear magnetic resonance analyses were recorded on Bruker Avance 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, all the data were measured using CD_3OD. Positive FAB mass spectral data were determined using JEOL JMS-700T mass spectrometer.

Biological material – The alga *Liagora farinosa* was collected by snorkelling at depth of 1 - 2 meters at Hurghada, on the Egyptian Red Sea coast, in June 2006. The alga was identified by Dr. Ali Gab-Alla, Associate Professor, Department of Marine Science, Faculty of Science, Suez Canal University. A voucher sample was deposited under No LF1, at Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University.

Extraction and Isolation – 2.5 kg of fresh algae *Liagora farinosa* were extracted using methanol. The methanol extract was concentrated under vacuum. The hydro-alcoholic solution was successively fractionated using hexane then chloroform. Four g of the chloroform extract were fractionated over silica gel column (120 g, ϕ 3 cm) using chloroform/methanol gradient elution. Fractions eluted with 6% methanol in chloroform were subjected to PTLC using chloroform/methanol/ethyl acetate (8.5 : 1.5 : 1) as developing system, 2 zones were scrapped off, eluted with chloroform: methanol (1 : 1). The first band (R_f= 0.35) afforded 3 mg of white amorphous solid (compound **1**). The second band (R_f = 0.3) was purified by repeated crystallization from methanol to afford 25 mg of long transparent needles (compound **2**). Fractions

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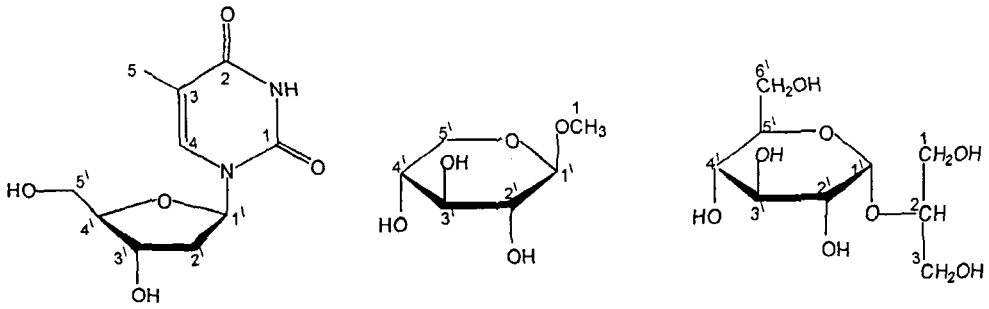

1 2 3

eluted with 30% methanol in chloroform were purified by PTLC using chloroform: methanol (7 : 3) for double run to afford 18 mg of transparent solid ($R_f = 0.28$), (compound **3**).

Compound **1**: white amorphous solid.

^{1}H- and ^{13}C-NMR data: see Table 1.

Positive HRFABMS obs. $[M+H]^+$ m/z 243.0982 calculated for $C_{10}H_{15}N_2O_5$

Compound **2**: long transparent crystalline needles

^{1}H- and ^{13}C-NMR data: see Table 2.

Positive HRFABMS obs. $[M+H]^+$ m/z 165.0764 calculated for $C_6H_{13}O_5$

Compound **3**: transparent solid

^{1}H- and ^{13}C-NMR data: see Table 2.

Positive HRFABMS obs. $[M+H]^+$ m/z 255.1077 calculated for $C_9H_{19}O_8$

Determination of antioxidant activity – The three compounds were examined for their antioxidant activity using TLC autographic assay for DPPH radical scavenging effect (Takamatsu *et al.*, 2003). Compounds were dissolved in methanol at a concentration of 2 mg/mL and the flavonoid rutin was prepared at a similar concentration and used as a positive control (Nijveldt *et al.*, 2001). Six μg of each compound were applied in the form of a spot, 4 mm in diameter. The radical scavenging effects were detected on the TLC plates, using a spray reagent composed of 0.2% (w/v) solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in methanol. Plates were observed 30 min after spraying. Active compounds were detected as yellow spots against a purple background. The tested compounds were found to possess a moderate antioxidant activities (producing clear yellow spots), noting that compound **3** showed relatively more intense bright yellow zone.

Results and Discussion

The structures of the isolated compounds were secured by 1D and 2D NMR studies and exact mass determinations. Compound **1** was isolated as a white amorphous solid. The HRFABMS displayed a pseudo-molecular ion peak at *m/z* 243.0982 $[M+H]^+$ suggesting the molecular formula of $C_{10}H_{14}N_2O_5$ while the features of ^{1}H- and ^{13}C-NMR spectra indicated a nucleoside nature with a thymine moiety. The ^{13}C-NMR spectrum showed resonances for ten carbons of which two were detected at δ 166.9 and 151.8 indicating two carbonyl functionalities. The thymine moiety was further proved from the NMR spectra through the two singlets at δ 1.93 (a methyl group) and at δ 7.82 (an olefinic proton), along with their corresponding carbon signals at δ 13.6 and δ 138.9 respectively. The sugar part was confirmed as deoxyribose through comparison of the ^{1}H- and ^{13}C-NMR data with those reported for sugars (Agrawal, 1989). Finally, all the spectral data were found to be identical to those reported for thymidine which was previously isolated from many species of sponges (Ralifo *et al.*, 2007; Ayyad, 2004; Zhou *et al.*, 2005; Deng *et al.*, 1998) while its oligonucleotide was reported from the green algae *Chlorella pyrenoidosa* (Sanwal *et al.*, 1969). Compound **2** was isolated as long transparent crystalline needles. It has a molecular formula $C_6H_{12}O_5$ as established by HRFABMS and NMR data. ^{13}C-NMR and HSQC spectra revealed the presence of six oxygenated carbon signals (four methines,

Table 1. ^{1}H- and ^{13}C-NMR data of compound **1**

Position	δ_H [mult., *J*(Hz)]	δ_C (mult.)
1	--	151.8 (C)
2	--	166.9 (C)
3	--	111.5 (C)
4	7.82 (s)	138.9 (CH)
5	1.93 (s)	13.6 (CH_3)
1′	6.27 (t, 6.5)	85.5 (CH)
2′	2.21 (m)	40.9 (CH_2)
3′	4.38 (br d)	71.1 (CH_2)
4′	3.89 (br d)	88.0 (CH)
5′	3.69 (m)	62.6 (CH_2)

Table 2. ^{1}H- and ^{13}C-NMR data of compounds **2** and **3**

Position	2		3	
	δ_H [mult., J(Hz)]	δ_C (mult.)	δ_H [mult., J(Hz)]	δ_C (mult.)
1	3.48 (s)	57.2 (CH_3)	3.72 (m)	62.9 (CH_2)
2	--	--	3.69 (m)	81.1 (CH)
3	--	--	3.70 (m)	62.7 (CH_2)
1′	4.11(d, 7.5)	104.6 (CH)	5.04 (d, 2.7)	100.1 (CH)
2′	3.33 (m)	79.3 (CH)	3.77 (brs)	71.3 (CH)
3′	3.17 (dd, 7.5, 9.1)	73.4 (CH)	4.00 (t, 6)	72.5 (CH)
4′	3.50 (dd, 10.2, 5.3)	69.7 (CH)	3.02 (t, 6)	70.2 (CH)
5′	3.21 (dd, 11.5, 10.2) 3.85 (dd, 11.5, 5.3)	65.4 (CH_2)	3.90 (brs)	71.1 (CH)
6′	--	--	3.62 (m)	61.9 (CH_2)

** All the spectral data were measures in CD_3OD

one methylene and one methyl group). ^{1}H-NMR spectrum of compound **2** showed a characteristic signal for a β-anomeric proton at δ 4.11 (d, J=7.5 Hz) (Altona and Haasnoot, 1980), with its corresponding carbon signal at δ 104.6 as indicated from the HMQC spectrum. In addition, a three proton singlet at δ 3.48, correlated to the carbon signal at δ 57.2 suggested a methoxyl moiety. The sugar part was confirmed as β-D-xylopyranoside through comparison of the ^{13}C-NMR data with those reported for sugars (Agrawal, 1989). The HMBC spectrum indicated a strong relation between the anomeric proton (δ 4.11) and the methoxyl group resonating at δ 57.2, furthermore, the downfield shift of the anomeric carbon (δ 104.6) confirmed the glycosidic linkage between the methoxyl group and C-1′ (Markham *et al.*,1978). A complete assignment of protons and carbons was achieved using different 2D NMR techniques. From the previous data, compound **2** was identified as methyl-β-D-xylopyranoside which is reported here for the first time from red algae.

Compound **3** was isolated as transparent solid with its HRFABMS $(M+H)^+$, *m/z* observed at 255.1077 suggesting the molecular formula of the compound to be $C_9H_{18}O_8$. The ^{13}C- NMR spectrum illustrated the presence of nine oxygenated carbon signals. Six of which were revealed as five methines and one methylene, attributed to α-D-glucopyranose moiety as proved through comparison with the data reported for sugars (Agrawal, 1989). The α-configuration of the sugar part was confirmed from the J value and chemical shift of the anomeric proton (δ 5.04, d, J=2.7Hz) (Altona and Haasnoot, 1980). The remaining three carbons were suggested to be a glycerol moiety as 1D and 2D NMR spectra proved the presence of two oxygenated methylene groups along with their carbon signals at δ 62.7 and 62.9, in addition to an oxygenated methine group with its carbon signal resonating at δ 81.1. The inter-glycosydic linkage was ascertained from the HMBC experiment that showed strong relation between the anomeric proton of the glucose moiety and the carbon signal detected at δ 81.1 (C-2), which was further confirmed from the downfield shift of the anomeric carbon of glucose (δ 100.1) (Markham *et al.*, 1978). Compound **3** was identified as glycerol-2-α-D-glucopyranoside which was previously isolated from the cultured marine blue green alga *Oscillatoria* sp. (Choi *et al.*, 1999). Comparison of the spectral data of compound **3** with those previously published confirmed the identity of this compound. It is worthy to mention that this is the first report for the isolation of glycerol-2-α-D-glucopyranoside from red algae whereas the three compounds (**1** - **3**) are reported here for the first time from the alga *Liagora farinosa*.

References

Abbott, I.A., Two new species of *Liagora* (Nemaliales, Rhodophyta) and notes on *Liagora farinosa* Lamouroux. *American Journal of Botany* **71**, 1015-1022 (1984).

Agrawal, P.K., Carbon 13 NMR of flavonoids. *Central Ins. of medicinal and aromatic plants*, Luknow, India, Elsevier (1989).

Altona, C. and Haasnoot, C.A.G, Prediction of *anti* and *gauche* vicinal proton-proton Coupling constants in carbohydrates: a simple additivity rule for pyranose rings. *Organic Magnetic Resonance* **13**, 417-429 (1980).

Ayyad, S.N., Isolation and structure determination of biologically active glycosphingolipids from the marine sponge *Haliclona* species. *Mansoura Science Bulletin*, **31**, 115-124 (2004).

Choi, J.S., Lee, D.I., Lee, W.K., Kim, D.S., Choi, H.D., and Son, B.W., 2-*O*-(α-D-glucopyranosyl) glycerol, a new glycerol glycoside from the marine blue- green alga *Oscillatoria* sp. . *Bull. Korean Chem. Soc.* **20**, 1121-1122 (1999).

Deng, S., Li, F., and Peng, S., Chemical constituents of marine sponge *Gelliodes spinosella* in South China Sea. *Zhongguo Haiyang Yaowu*

17, 31-32 (1998).

Markham, K.R., Ternal, B., Stanley, R., Geiger, H., and Mabry, T.J., Carbon-13 NMR studies of flavonoids-III. *Tetrahedron* **34**, 1389-1397 (1978).

Nijveldt, R.J., Nood, E., Hoorn, D.E.C., Boelens, P.G, Norren, K., and Leeuwen, P.A.M., Flavonoids: a review of probable mechanism of action and potential applications. *Am. J. Clin. Nutr.* **74**, 418-425 (2001).

Paul, V.J. and Fenical, W., Toxic acetylene-containing lipids from the red marine alga *Liagora farinosa* Lamouroux. *Tetrahedron Lett.* **21**, 3327-3330 (1980).

Ralifo, P., Tenney, K., Valeriote, F.A., and Crews, P., A distinctive structural twist in the aminoimidazole alkaloids from a Calcareous marine sponge: isolation and characterization of leucosolenamines A and B. *J. Nat. Prod.* **70**, 33-38 (2007).

Sanwal, G.G. and Preiss, J., Sugar nucleotides and nucleotide-peptide complexes of *Chlorella pyrenoidosa* : isolation and characterization. *Phytochemistry* **8**, 707-723 (1969).

Takamatsu, S., Hodges, T.W., Rajbhandari, I.,. Gerwick, W.H, Hamann, M.T., and Nagle, D.G, Marine natural products as novel antioxidant prototypes. *J. Nat. Prod.* **66**, 605-608 (2003).

Thomas, D., Seaweeds in the life series. *The natural History Museum*, London. ISBN0565091751, 2002.

Williams, L.A.D., Acaricidal activity of five marine algae extracts on female *Boophilus Microplus* (Acari: Ixodidae). *Florida Entomologist*, **74**, 404-408 (1991).

Zhou, S., Zhou, K., and Xiao, D., Natural nitrogenous compounds from South China Sea marine sponge *Clathria fasciculatae*. *Zhongguo Haiyang Yaowu* **24**, 31-35 (2005).

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