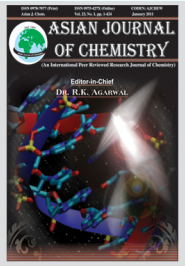
Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 816-818

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Phytochemical and Antimicrobial Activities of Two New Novel Bioactive Analogues of Subergorgic Acid from *Subergorgia suberosa*

G. DAMODAR REDDY[1,*], D. RAMA SEKHARA REDDY[1,2,*], A. RAZZACK[1] and K. BALAJI[1]

[1]Department of Organic Chemistry, School of Chemistry, Andhra University, Visakhapatnam-530 003, India
[2]Present address: Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

*Corresponding authors: E-mail: damodarau@gmail.com; dachuru@gmail.com

(*Received*: 13 May 2010; *Accepted*: 7 October 2010) AJC-9171

A series of bioactive analogues were synthesized from subergorgic acid **1**, a cardiotoxic marine metabolite. Two new compounds, **2** and **7** have been synthesized in high yields by the $NaBH_4$ reduction of subergorgic acid **1** and its methyl ester **6**, respectively. The structures of new compounds have been determined by spectroscopic methods. The reductions have given the known compounds **3** and **4** as minor products. All these compounds **1**, **2**, **4**, **6** and **7** showed a significant level of antifungal properties.

Key Words: Subergorgic acid, Diterpene, *Subergorgia suberosa*, Marine metabolite.

INTRODUCTION

The discovery of large quantities of prostaglandins in the Caribbean gorgonian *Plexaura homomalla* (Esper)[1] in the 1969 triggered off a worldwide demand for gorgonians. Recently much interest has been focused on the chemistry and the biological activity of subergorgic acid (**1**), a bioactive diterpene from the red gorgonian *Subergorgia suberosa* (Pallas) of the south China sea[2]. It was shown to be cardiotoxic. Recently, we isolated subergorgic acid (**1**) from *S. suberosa* of the Mandapam coast[3]. Gorgonians have the ability to regenerate lost parts if the basal plates of the animals remains intact. Gorgonians have a slow rate of growth (2 cm/year). Therefore, large scale harvesting of the animal should be avoided for the preservation of the gorgonian beds. The exported species such as *Echinomuricea indica*, *Gorgonella umbraculum*, *Heterogorgia flabellum* and *Echinogorgia complexa*, show distinct depletory trends.

In this present communication we described the preparation of two new analogues of subergorgic acid (**1**) namely compounds **2** and **7** along with known compounds **3**, **4** and **6**.

EXPERIMENTAL

NMR spectra were taken in $CDCl_3$ with TMS as internal standard on a Jeol JNM-EX 90 FTNMR instrument at 90 MHz for ^1H and 22.4 MHz for ^{13}C. GC-MS analysis were carried out on Shimadzu QP5050A bench-top quadrapole instrument in the EI mode under the following conditions: column: 30 m, 0.25 mm O.D. DB-5 capillary column, He-flow rate: 260 ºC at 40 ºC/min and held at 260 ºC for 25 min, solvent: $CHCl_3$.

Reaction of subergorgic acid with sodium borohydride in ethanol: To an ice-cold solution of subergorgic acid **1** (500 mg) in ethanol (20 mL) was added sodium borohydride (250 mg) in small portions with shaking. After addition was over, the reaction mixture was kept at room temperature for 24 h, diluted with water (20 mL) and extracted with ether (2 × 50 mL). The ether extract was washed with water (20 mL), dried over anhydrous magnesium sulphate and solvent was evaporated. A colourless crystalline mass (485 mg) resulted which on crystallization from ethanol gave the alcohols **2** as colourless shining needles (300 mg, 60 %) m.p. 150 ºC and compound **3** as a pale yellow gum (90 mg, 18 %).

α-Alcohol 2: Colourless shiny needles, m.p. 150 ºC. $[\alpha]_D^{27}$ -19.8º (c, 1.0, $CHCl_3$, ν_{max}, cm^{-1}): 3450, 1680, 1640, 1630 and 1450. GC-MS data (EI mode): Gave a single peak with an RT of 8.60 min on DB-5 capillary column He-flow rate: 1.5 mL/min, programme: 50 ºC (2 min)-260º at 40 ºC/min and held at 260 ºC for 25 min, solvent: *n*-hexane. Mass spectrum of the 8.60 min peak: m/z 250 (M^+ of $C_{15}H_{22}O_3$) (35), 232 (M-H_2O) (50), 217 (30), 91 (100), 41 (95 %). ^1H NMR (90 MHz, $CDCl_3$, TMS): δ 4.20 t, 6.45 s , 3.15 q, J = 7 Hz, 1.15 d, J = 7 Hz, 1.25 s, 1.40 d, J = 7 Hz. ^{13}C NMR (22.5 MHz $CDCl_3$, TMS): δ 66.2, 77.4, 43.5, 39.7, 62.6, 31.4, 37.7, 57.8, 152.3, 138.9, 49.4, 20.7, 22.6, 169.7, 19.6.

β-Alcohol 3: As a pale yellow gum. $[\alpha]_D^{27}$-19.2º (c, 1.0, $CHCl_3$, ν_{max}, cm^{-1}): 3450, 1680, 1640, 1630 and 1450. GC-MS data (EI mode): Gave a single peak with an RT of 8.44 min on DB-5 capillary column He-flow rate: 1.5 mL/min, programme: 50 ºC (2 min)-260º at 40 ºC/min and held at 260 ºC

for 25 min, solvent: *n*-hexane. Mass spectrum of the 8.60 min peak: m/z 250 (M^+ of $C_{15}H_{22}O_3$) (35), 232 (M-H_2O) (50), 217 (30), 91 (100) , 41 (95 %). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 4.38 dd, 6.42 s , 2.68 q, *J* = 8Hz, 1.05 d, *J* = 7 Hz, 1.40 s, 1.15 d, *J* = 7 Hz. ^{13}C NMR (22.5 MHz $CDCl_3$, TMS): δ 68.2, 76.0, 43.5, 45.7, 39.7, 63.7, 30.4, 40.1, 59.4, 156.3, 136.2, 50.4, 20.5, 22.3, 168.7, 17.5.

Reaction of subergorgic acid methyl ester (6) with sodium borohydride in methanol: Sodium borohydride (100 mg) was added to a solution of methyl ester **6** (200 mg) in methanol (5 mL) at room temperature. After keeping it overnight at room temperature, the reaction mixture was diluted with water (10 mL) and extracted with ether (2 × 20 mL). The combined ether extract was dried over anhydrous magnesium sulphate and solvent was evaporated. The resulting brownish gum was chromatographed on a small column of silica gel (30 g: 10 cm × 2.5 cm) by eluting with *n*-hexane and mixtures of *n*-hexane and EtOAc. Elution with 8.5 % EtOAc in hexane furnished compound **7** (85 mg, 42.5 %) and compound **4** (55 mg, 27.5 %).

Compound 7: Colourless gum, $[\alpha]_D^{27}$-112.8º (c, 1.0, $CHCl_3$, ν_{max}, cm^{-1}) $CHCl_3$: 3450, 1730, 1630, 1450 and 1250. GC-MS data: Showed a single peak with an RT of 8.17 min. Mass spectrum of the 8.17 min peak: m/z 264 (M^+ of $C_{16}H_{24}O_3$) (45), 232 (M-CH_3OH) (50), 90 (100) and 41 (95 %). ^{1}H NMR data: δ 4.15 t (H-2), 6.35 s (H-9), 3.12 q, *J* = 7 Hz (H-11), 1.20 d, *J* = 7 Hz (H-12), 1.25 s (H-13),1.35 s (H-15), 3.75 s (OCH_3). ^{13}C NMR data (C-1 to C-15 and OCH_3): δ 66.3, 77.5, 43.7, 40.2, 62.6, 31.5, 37.7, 57.7, 150.0, 139.2, 49.7, 20.9, 22.8, 165.4, 19.8, 51.3.

Compound 4: Colourless oil, $[\alpha]_D^{27}$ + 44.80 (c, 1.0, $CHCl_3$, ν_{max}, cm^{-1}) $CHCl_3$: 3450, 1640 and 1450. GC-MS data (EI mode): Showed a single peak with an RT of 7.84 min on DB-5 capillary column He-flow rate : 1.5 mL/min, programme: 50 ºC (2 min)-260º at 40 ºC/min and held at 260 ºC for 25 min, solvent: *n*-hexane. Mass spectrum of the 7.84 min peak: m/z 236 (M^+ of $C_{15}H_{24}O_2$); (traces), 218 (M-H_2O) (25), 205 (60), 147 (95), 91 (100), 41 (95). ^{1}H NMR (90 MHz, $CDCl_3$, TMS): δ 4.18 m, 5.14 s, 2.76 q, *J* = 7 Hz, 1.13 d, *J* = 6.6 Hz, 1.15 s, 4.18 m, 1.30 d, *J* = 7 Hz. ^{13}C NMR data (C-1 to C-15)" δ 66.6, 77.9, 43.7, 39.7, 63.60 31.5, 38.0, 56.7, 147.0, 132.9, 50.7, 22.9, 22.8, 60.5, 19.2.

Antibacterial activity: Antibacterial activity against *Bacillus subtilis*, *Bacillus pumilis*, *Proteus vulgaris* and *Escherichia coli* was tested in nutrient agar. Nutrient agar slants were prepared in boiling test tubes and were cultured with overnight. The individual fungal cultures were added to the Nutrient agar medium and mixed well on the sterile petriplates. Four wells of 6 mm diameter at equidistant to each other were made in each of the petriplates. The sample containing the test compound was suspended in DMSO at a concentration of 1000 µg/mL. A total of 50 µg/mL of each sample was added in each well. In each plate two wells served as control, one well contained 50 µg/mL of test compound in DMSO and one well contained standard pencillin at 100 µg/mL concentration level. Wells containing only DMSO and without any medium served as controls. All the experiments were performed in duplicates and the average diameter of zones of inhibition was compared to that of the standard (Pencillin-G). The samples were allowed to diffuse into Nutrient agar medium in a refrigerator for 1 h. These petriplates were then incubated in a incubator for 16 h at 37 ºC. These plates were then examined for the presence of any inhibition zones.

Antifungal activity: Antifungal activity was tested in potato dextrose agar (PDA) medium. The PDA medium was prepared and poured at 20 mL/petriplate. Then the desired fungal suspension from a 48 h old culture was added to the PDA medium which was prepared and poured into sterile petri dishes. The remaining steps were same as mentioned under bacterial activity. All the experiments were performed in duplicates and the average diameter of the zones of inhibition were compared with that of the standard ketocanazole. Ketocanazole 100 µg/mL was used as a standard. All the plates were incubated at room temperature (37 ºC). After 48 h and were examined for the presence of inhibition zones and measured the zone of inhibition.

The subergoric acid **1** and its derivatives were tested for their antibacterial activities against four bacteria and the results were shown in Table-1. In the case of antifungal activity against two fungi and the results were shown in Table-2.

TABLE-1
ANTIBACTERIAL ACTIVITY OF SUBERGORIC ACID (**1**) AND ITS ANALOGUES. (ALL THE COMPOUNDS WERE TESTED AT 1000 µg/mL CONCENTRATION)

Name of the compound	Test organisms and zone of inhibition (mm)			
	Gram (+)ve bacteria		Gram (-)ve bacteria	
	BP	BS	EC	PV
Subergoric acid (1000 µg/mL)	0	0	14	16
Compound **2** (1000 µg/mL)	0	0	0	0
Compound **4** (1000 µg/mL)	0	0	8	0
Compound **6** (1000 mg/mL)	0	0	10	0
Compound **7** (1000 mg/mL)	0	0	6	0
Pencillin (1 µg/mL)	15	14	14	14

Cup diameter = 6 mm, BP = *Bacillus pumilis*; BS: *Bacillus subtilis*; EC: *Escherichia coli* and PV: *Proteus vulgaris*

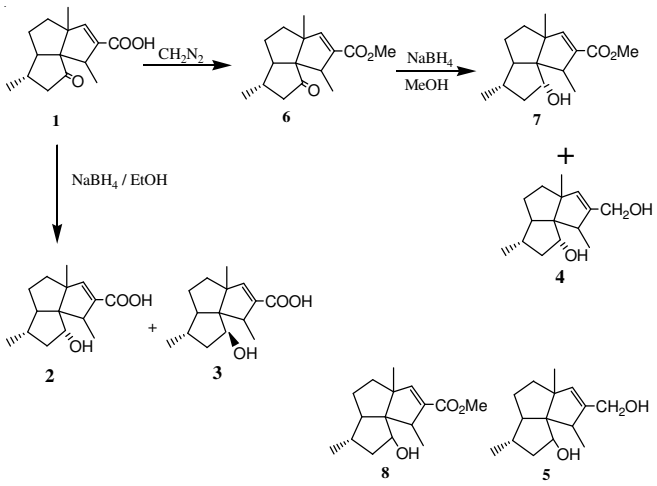
TABLE-2
ANTIFUNGAL ACTIVITY OF SUBERGORIC ACID (**1**) AND ITS ANALOGUES

Extracts and standards	Test organisms and zone of inhibition (mm)	
	AN	RO
Subergoric acid (300 µg/mL)	11	14
Compound **2** (300 µg/mL)	10	9
Compound **4** (300 µg/mL)	13	11
Compound **6** (300 µg/mL)	14	13
Compound **7** (300 µg/mL)	12	10
Ampicillin (1 µg/mL)	18	18

Cup diameter = 6 mm, AN: *Aspergillus niger*; RO: *Rhizopus oryzae*.

RESULTS AND DISCUSSION

The semi-synthetic route of the compounds is outlined in **Scheme-1**. Subergorgic acid **1** on treatment with $NaBH_4$ in ethanol at room temperature (in methanol the reduction did not take place) furnished the epimeric alcohols **2** and **3** in the



Scheme-I

ratio 4:1. The major product, the α-isomer **2** was obtained by crystallization of the reaction mixture. The NMR spectra and the GC-MS analysis of the compounds showed the presence of **2** and the β-isomer **3**. GC-MS analysis showed that it was composed of the enantiomeric alcohols **2** and **3** in the ratio *ca.* 60:40. GC-MS analysis showed the a alcohol **2** at 8.64 min and the β alcohol **3** at 8.44 min. The mass spectra of both peaks were identical, same as the mass spectrum exhibited by the a isomer **2**. The ^1H and ^{13}C NMR spectral signals confirmed both the isomers **2** and **3**. Compound **3** was isolated earlier from *S. suberosa*[4]. The ^1H NMR spectra of **2** and **3** differ at H-11, H-13 and H-15 like the compounds **4** and **5** obtained by the $LiAlH_4$ reduction of **1**[2]. In the a isomer **4**, the H-15 was observed at a deshielded position, while in the β-isomer **5**, the H-13 was observed at a deshielded position. Moreover, the H-11 in **4** was observed at a lower field (δ 2.76 q) than in **5** (2.36 q). The ^1H NMR spectrum of the major product of the $NaBH_4$ reduction showed the H-11 and H-15 protons at deshielded positions compared to the spectrum of **3**[4], indicating that it is the α isomer **2**, a new derivative of **1**.

Subergorgic acid **1** was converted into its methyl ester **6** by reaction with ethereal diazomethane in methanol for overnight. Compounds **1** and **6** showed peaks at 8.19 min and 7.85 min, respectively in the GC-MS analysis. The methyl ester **6** on reaction with $NaBH_4$ in methanol furnished two products as **7** and **4** in 7:3 ratios. Product **7** was identified as the alcoholic ester and product **4** as a diol on the basis of their spectral data. Product **7** showed the molecular ion at m/z 264 (two mass units higher than that of the methyl ester **6**) and its NMR spectra showed the presence of a carbinol (δ 4.15 t, H-2; 77.5 d) and a methyl ester group (3.75 s, 165.2 s, 51.3 q). $NaBH_4$ reduction of **6** can yield the epimeric alcohols **2** and **3**. Compound **8** was isolated from *S. suberosa* by Parameswaran *et al.*[4]. Like compounds **2**, **3** and **4**, **5** the ^1H NMR spectra of compound **7** and **8** also differ at H-11, H-13 and H-15 show similar trends. The ^1H NMR spectrum of the major product of the present reaction **7** showed the H -15 and H -11 signals at deshielded positions indicating that it was the a isomer.

The NMR spectra of product **4** did not show the methyl ester group, indicating that the ester group was also reduced. It is not uncommon for the reduction of α,β-unsaturated esters into alcohols in $NaBH_4$ reductions. The ^1H NMR spectrum of product **4** showed the presence of a shifted olefinic protons (δ 5.15 s), a broad singlet at 4.18 for the H-14 and H-2 protons and deshielded H-15 and H-11 protons. From these data, compound **4** was identified as a diol, a $LiAlH_4$ reduction product of **1**, reported earlier.

Conclusion

Present results have concluded that the two new novel compounds **2** and **7** were obtained by the reduction of subergorgic acid **1** and its methyl ester **6**. The reductions also gave the known compounds **3** and **4** as minor products and all these compounds possess antibacterial and antifungal activity[5]. The subergoric acid, showed antibacterial activity against *E. coli* and *Proteus vulgaris* and antifungal activity against *Rhizopus oryzae* and aspergillus niger. The derivatives of subergorgic acid **1** like as compounds **4**, **6** and **7** showed antibacterial activity against *E. coli* only and showed a significant level of antifungal activity against *Rhizopus oryzae* and *Aspergillus niger*. All these compounds were tested at 1000 μg/mL concentration. The present investigation showed that the subergorgic acid **1** and its derivatives have antimicrobial activities.

ACKNOWLEDGEMENTS

The authors express their thanks to UGC, New Delhi for the UGC-SAP and COSIST programme facilities to the Department of Organic Chemistry and for fellowship to one of the author and Prof. C. Subrahmanyam for his guidance.

REFERENCES

1. P. A. Thomas and R.M. George, Gorgonid Resources of India, CMFRI, Cochin, India, Vol. 74, pp. 9-13 (1987).
2. A. Groweiss, W. Fenical, H. Cun-Eng, J. Clardy, W. Zhongde, Y. Zhongnian and L. Kanghow, *Tetrahedron Lett.*, **26**, 2379 (1985).
3. C. Subrahmanyam, S.R. Kumar and G.D. Reddy, *Indian J. Chem.*, **42B**, 219 (2003).
4. P.S. Parameswaran, C.G. Naik, S.Y. Kamat, M.S. Puar, P. Das and V.R. Hegde, *J. Nat. Prod.*, **61**, 832 (1998).
5. R. Cruickshan, J.P. Duguid and R.H.A. Swin, in: The Practice of Medical Microbiology, Churchill, Livingstone, London, edn. 12, 2, p. 110 (1975).