KIMIKA Volume 23, Number 1, pp. 38-42 (2010)

ISSN 0115-2130

A Sesquiterpene from *Dysidea* sp. and Its DNA-Binding Profile

Alexander O. Mosqueda[1*], Roberto M. Malaluan[1], Lydia M. Bajo[1] Franco G. Teves[1], Ian van Altena[2], and Mihiko Yanai[3]

[1]*Department of Chemical Engineering Technology*
Mindanao State University-Iligan Institute of Technology
Iligan City, Philippines 9200

[2]*Department of Chemistry*
School of Environmental and Life Sciences
University of Newcastle
Callaghan NSW, Australia 2308

[3]*Natural Science Center for Basic Research and Development*
Hiroshima University
1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

The sesquiterpene arenarol (**1**) was isolated from the marine sponge, *Dysidea* sp. by supercritical fluid extraction and reversed-phase high performance liquid chromatography. The molecular structure was elucidated on the basis of ^{1}H and ^{13}C-NMR chemical shifts. The isolated secondary metabolite exhibited a very weak antimicrobial activity at 15 μg against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, and showed a DNA-binding property with a retention factor ratio of 0.76 by one dimensional thin-layer chromatography.

Keywords: *Dysidea*, NMR, sesquiterpene, arenarol, antimicrobial, DNA-binding

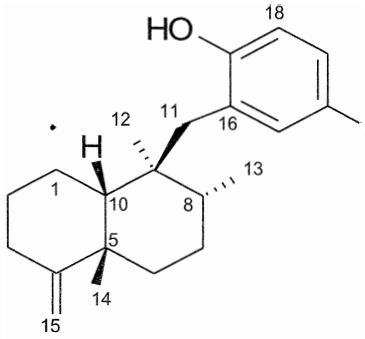
INTRODUCTION

Extensive research over the past decades has revealed that among the marine organisms, sponges are the most prolific sources of novel and diverse secondary metabolites with therapeutic applications [1]. The islet of Mantigue in Camiguin province is known to be a spot of sponge resource. A number of studies on the bioactive sponge constituents in this area have been carried out but few compounds were structurally elucidated. Among the compounds that were characterized are the two bioactive compounds that are bromo-substituted phenols isolated from Dysidea family [2], and the amide containing phenol compound with antifungal activities isolated from Thorecta sp. [3]. Additional screening and isolation of secondary metabolites from a Philippine sponge may prove

*Author to whom correspondence should be addressed; e-mail: set-aom@sulat.msuiit.edu.ph

useful in the concerted and massive effort of studies on drugs from the sea.

This study reports the isolation, structure elucidation, antimicrobial activity and DNA-binding property of the sesquiterpene arenarol (**1**) from *Dysidea* sp. collected in Mantigue Islet, Camiguin. Arenarol was first reported from the marine sponge *Dysidea arenaria* in Truk Island lagoon [4], subsequently from a *Fenestraspongia* species in Urukthapel Palau [5] and from the Genus *Hyrtios* off Mahe (04°45'S55°33'E) Seychelles [6].



(1)

This paper also shows that bioactive compounds are extractable by supercritical fluid extraction. The DNA-binding property of **1** was evaluated by the screening strategy developed by Maier *et al* (1999), called "biomolecular-chemical screening" [7].

METHODOLOGY

General procedure

UV spectra were recorded using a Shimadzu UV-160 spectrophotometer. IR spectra were obtained with a Perkin Elmer Paragon 1000 FTIR spectrometer with the sample prepared as KBr disc. TLC was performed on silica gel precoated plates (F254 Merck Art No. 5735) and viewed under UV light. The NMR experiments were conducted using a 300 Mz Bruker NMR spectrometer in $CDCl_3$ and CD_3OD for the ^1H and ^{13}C NMR. EIMS measurements were recorded on JEOL JMS-SX 102A instrument.

Biological Material

Fresh sample of the marine sponge *Dysidea* sp. was collected from Mantigue Islet, Camiguin Province (9° 10' N, 124° 49'E) by skin diving at a depth of 20-50 ft. The specimen was transported on ice to the laboratory, cut into pieces and freeze dried. The sponge sample was identified in the Department of Biological Sciences, MSU-IIT.

Isolation

Freeze-dried *Dysidea* sp. sample (50 g) *Dysidea* sp. was placed inside a clean white cloth and loaded in the SFE instrument using carbon dioxide as the supercritical fluid. Extraction condition was set at 300 atm and 40°C for duration of 1 hour to obtain 223.1 mg after concentration under vacuum of a fraction that dissolved in methanol. This was subjected to reversed-phase HPLC with a UV detector set at 229nm (λ_{max}) using an ODS column (Wakosil II 5C18AR, 4.6 mm x 250 mm; 1 mL/min flow rate) with 95% methanol in water as eluent to yield **1** (134.4 mg).

Antimicrobial tests

The antimicrobial activity was tested aseptically against *Staphylococcus aureus* , *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* by paper disc diffusion . About 15 μg of sample dissolved in 15 μL methanol was impregnated on a 6 mm sterile paper disc (Whatmann filter paper No. 2). Separate paper discs were impregnated with 15 μL of solvent and 15 μL of chloramphenicol solution (1 μg/μL) as the standard. The impregnated paper discs were allowed to dry, placed on the agar medium containing bacteria and incubated at 37°C for 24-hours. The diameter of the growth inhibition zone around the paper discs were measured.

Bio-molecular Chemical Screening

The DNA-binding property of **1** was examined by 1D-TLC on silica gel (precoated TLC plates, F254 Merck Art No. 5735). Small amount of diluted sample (5μg/spot) was analyzed in 90:10 MeOH:H_2O. Homogenized salmon sperm DNA was spotted (4μg/spot) above the sample spot

prior to actual chromatography. The spot of **1** without the DNA served as reference. The TLC profile was detected under UV light. Rf-values of the spots with and without the DNA spot were determined and recorded as Rf_2 and Rf_1, respectively. A retention factor ratio, Rf_2/Rf_1 value of less than 1 signifies an interaction between the sample and the DNA [7].

RESULTS AND DISCUSSION

The isolate obtained from *Dysidea* sp. by supercritical fluid CO_2 at 300 atm afforded **1** as a yellow amorphous solid (134.4 mg, mp 125-130°C) with R_t of 8.948 in the HPLC (95:5 MeOH : H_2O) chromatogram. The structure was elucidated by a combination of 1D NMR and extensive 2D NMR experiments including ^{1}H-^{1}H COSY, HMQC, and HMBC, and by comparison of the NMR resonances data with those in the literature. The molecular formula was established as $C_{21}H_{30}O_2$ based on EIMS ([M^+] *m/z* 314).

The infrared spectrum was consistent with the presence of a broad peak at 3395 cm^{-1} arising from the intermolecular hydrogen bonded, OH-stretch. Skeletal absorption, involving olefinic carbons stretching within the ring is seen in the peaks at 1645, 1595, 1503 and 1446 cm^{-1}. The presence of hydroxyl group in the molecule was confirmed by the peaks at 1382-1346 cm^{-1} that support the OH of a phenol as in-plane bending.

The ^{1}H-NMR spectral data of **1** (Table 1) displayed chemical shifts at δ4.68 and δ4.35, characteristic pair of broad singlets due to the exocyclic methylene group and δ0.92 (3H, s), δ0.92 (3H, d) and δ1.02 (3H, s) attributed to the methyl groups. The ^{13}C-NMR spectral data of **1** revealed twenty one carbons, similar to arenarol.

DEPT gave resonances at δ119.3, 116.3, and 113.8 due to the three methines of the aromatic ring and at δ46.5 from methane in the bicyclic structure known as decalin. Four methylenes with resonances at δ37.7, 27.9, 24.9, 22.5, and three methyls at δ19.04, 17.9 and 32.9 were recorded. The olefinic carbons gave resoncances at δ153.5 and 105.8. Deshielded peaks at δ148.6 and 148.7 were noted to be resonances of the oxygenated carbons, with the other aromatic carbons at δ116.3, 113.8 and 119.3. The tertiaty aromatic carbon connected to the methylene bridging the bicyclo decane and aromatic structure was also indicated by its resonance at δ126.8.

Table 1. ^{1}H and ^{13}C spectral data of 1 and arenarol.

	1		Arenarol[6]	
no.	δ_C, ppm	δ_H, ppm	δ_C, ppm	δ_H, ppm
1	22.5 CH_2	2.08 (m)	23.2 CH_2	2.15 (m)
		1.95 (m)		1.98 (m)
2	24.9 CH_2	1.64 (m)	25.6 CH_2	1.85 (m)
		1.63 (m)		1.72 (m)
3	31.9 CH_2	2.47 (m)	32.6 CH_2	2.49 (m)
		2.08 (m)		2.15 (m)
4	153.5 C		154.2 C	
5	39.3 C		39.9 C	
6	37.5 CH_2	1.99 (m)	38.1 CH_2	2.05 (m)
7	27.9 CH_2	1.24 (m)	28.2 CH_2	1.24 (m)
		1.36 (m)		1.58 (m)
8	37.6 CH	1.04 (m)	38.2 CH	1.04 (m)
9	43.5 C		44.2 C	
10	46.5 CH	1.24 (m)	47.1 CH	1.38 (m)
11	37.7 CH_2	2.71 (d)	38.4 CH_2	2.72 (d)
		2.52 (d)		2.52 (d)
12	19.0 CH_3	0.92 (s)	19.7 CH_3	1.06 (s)
13	17.9 CH_3	0.98 (d)	18.7 CH_3	0.96 (d)
14	32.9 CH_3	1.02 (s)	33.6 CH_3	0.92 (s)
15	105.8	4.68 (bs)	106.5	4.71 (bs)
	CH_2	4.35 (bs)	CH_2	4.72 (bs)
16	126.5 C		127.1 C	
17	148.6 C		149.4 C	
18	116.3 CH	6.59 (d)	116.9 CH	6.61 (d)
19	113.8 CH	6.57	114.5 CH	6.60
		(dd)		(dd)
20	148.7 C		149.4 C	
21	119.3 CH	6.56 (d)	119.9 CH	6.58 (d)

The ^{1}H-NMR and ^{13}C-NMR assignments in Table 1 is considerably similar to the NMR data reported by Salmoun *et al.*, 2000 for the compound arenarol. Spectrum arising from the 2D NMR experiments: ^{1}H-^{1}H COSY, HMQC and HMBC, confirmed that the structure of **1** is identical to arenarol as shown.

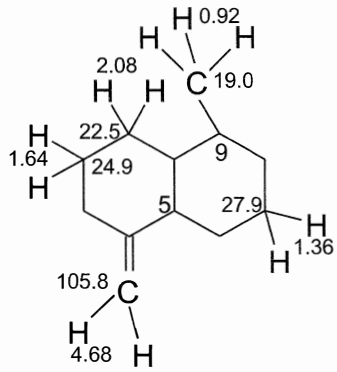


Figure 1. Selected HMQC correlations of **1**

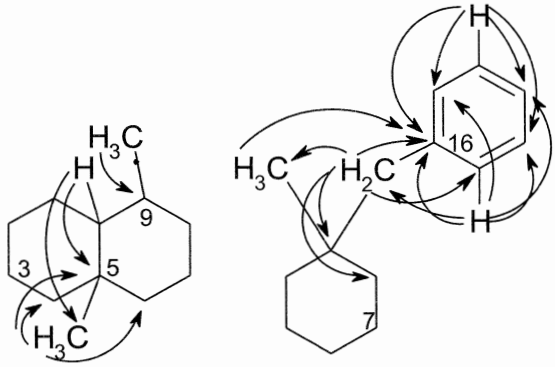


Figure 2. Selected HMBC correlations of **1**

The HMQC analysis revealed the following assignments of direct C-H bonding in the bicyclic structure shown in Figure 1. Correlation of proton at δ4.68 and carbon at δ105.8 supports the presence of the olefin. The ^{1}H-^{1}H COSY spectrum showed protons at δ1.36 (H-7) correlated to the resonances of protons at δ0.98 (H-13), and δ1.99 (H-6) to δ1.02 (H-14). Signal at δ1.24 (H-10) exhibited correlation to the resonances at δ1.04 (H-8) and δ0.92 (H-12), which manifest the three methyl groups attached to the bicylic struture. Examination of the HMBC spectrum of **1** gave clear correlations between the H_2C-11 doublet signals and the signals at δ37.7 (C-8), 43.5 (C-9), 19.0 (C-12), 126.5 (C-16), and 119.3 (C-21), indicating the methylene group bridging the aromatic and cyclo structure. Moreover, the singlet at δ1.02 attributed to H_3C-14 was long-range correlated with signals at δ153.5 (C-4), 39.3 (C-5), and 37.5 (C-6). Thus, the methyl group was assigned as a substituent attached to the quaternary C-5. The tertiary proton at δ1.24 also showed correlation to the carbons at δ 39.3 (C-5) and C-14 (32.9). Another methyl proton at δ0.92 is correlated to the carbons at δ43.5 (C-9) and 126.5 (C-16), signifying the alkyl's attachment to C-9. The aromatic proton at δ6.59 indicated numerous long-range correlations with the carbon members of the ring at δ126.5 (C-16), 148.6 (C-17), 113.8 (C-19), and 148.7 (C-20).

The compound arenarol have been reported to be cytotoxic against P388 lymphocytic leukemia (ED_{50} of 17.5 μg/mL) [4]. The antimicrobial properties of **1** was tested. The result indicates that **1** exhibited a very weak antimicrobial activity (Table 2) at 15 μg against *S. aureus*, *B.subtilis*, *E. coli* and *P .aeruginosa*. This study also shows the DNA-binding property of the isolated compound with a retention factor ratio, Rf_2/Rf_1 of 0.76. The development of small molecules that predictably bind to specific DNA sequences is important in pharmaceutics. Such species have great therapeutic potential, especially as antitumor agents, through their ability to block recognition of mutated sequences [7].

Table 2. Antimicrobial test results on compound 1.

	Mean Diameter of Zone of Inhibition (in mm)			
	Test Organisms			
Sample (15 μg)	S. *aureus*	B. *subtilis*	E. *coli*	P. *aerugi-nosa*
1	10	11	10	10
Chloramphenicol	30	30	29	28

ACKNOWLEDGEMENT

The authors are grateful to the Department of Chemistry, School of Environmental and Life Sciences, University of Newcastle, Australia for the assistance in the NMR analyses. A. Mosqueda is also thankful to Dr. Mylene M. Uy for the EIMS experiment, conducted in Natural Science for Basic Research and Development, Hiroshima University.

REFERENCES

1. Rao, C.B., Satyanarayana, C., Rao, D.S., Rao, D.V., Fahy, E. Faulkner, D. J. *Nat. Prod. Rep.* 10, 497 (1993).

2. Carmona, M. *Thesis* (MSU-Iligan Institute of Technology, Iligan City, 2000).

3. De La Sena, P. *Thesis* (MSU-Iligan Institute of Technology, Iligan City, 2002).

4. Scmitz, F. J., Lakshmi, V., Powell, D.R., Van der Helm, D., *J. Org. Chem.* 49, 241-244 (1984).

5. Carte, B., Rose, C.B., Faulkner D. J., *J. Org. Chem.* 50, 2785-2787 (1985).

6. Salmoun, M., Devijver, C., Daloze, D., Braekman, J. C., Gomez, R., de Kluijver, M., Van Soest, R. W. M., *J. Nat. Prod.* 63, 452-456 (2000).

7. Maier, A., Maul, C., Zerlin, M., Sattler, I., Grabley, S., Thiericke, R. *The Journal of Antibiotics.* 52, 11 (1999).