Acta Pharmaceutica Turcica
XLIII (1) (17-19) 2001

TWO METABOLITES FROM TBE MARINE SPONGE *Spongia officinalis* L.

DENİZ SÜNGERİ *Spongia officinalis* L.'DEN İKİ METABOLİT

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The chemical investigation of secondary metabolites of a species of marine sponge, Spongia officinalis collected from the Aegean Sea was carried out. Two metabolites, namely ergosterol and furospinulosin-II, were isolated from the ethanolic extract of the sponge and their structure elucidations were performed by spectroscopic techniques and comparing with literature. As a common sterol and the precursor of vitamin D_2 in higher plants, fungi, algae and rare sponges, ergosterol and furospinulosin-II, a furanoterpene, are reported for the first time from the marine sponge Spongia officinalis L.

Ege Denizinden toplanan bir deniz süngeri olan Spongia officinalis L.'nin sekonder metabolitleri üzerinde kimyasal incelemeler yapılmıştır. Ergosterol ve furospinulosin-II isimli iki metabolit süngerin etanollü ekstresinden izole edilmiş ve yapı tayinleri spektroskopik teknikler ve literatür verileri karşılaştırılması ile gerçekleştirilmiştir. Yüksek bitkilerde, funguslarda, yosunlarda ve nadiren süngerlerde yaygın bir sterol olan ergosterol, vitamin D_2'nin prekürsörüdür. Bir furanoterpen olan furospinulosin-II ise deniz süngeri Spongia officinalis L.'ten ilk defa bu çalışmada izole edilmiştir.

Keywords: *Ergosterol; Furospinulosin-II; Marine sponge; Spongia officinalis L.*

Anahtar Kelimeler: *Ergosterol; Furospinulosin-II; Deniz süngeri; Spongia officinalis L.*

Introduction

Marine organisms have afforded a variety of secondary metabolites having unique chemical structures with desirable activities. Marine sponges which belong to the order *Dictyocerutido* are particularly rich in terpenoids (1-3). Recently, we reported the isolation of two novel hydroquinone-derivative terpenoids along with 11 known terpenoids from two sponge species collected from the Aegean Sea (3, 4). Marine organisms, particularly algae, have a great number of sterol-type of compounds. Starting from squalene, nature produces sterols by enzymatic transformation. This biosynthesis chain comprises a variety of provitamins like ergosterol which possess the diverse biological functions. In addition to its terrestrial resources, ergosterol has been isolated from some marine sponge species such as *Agelas* sp., *Axinyssa* sp., *Haliclona* sp. and exhibited an *in vitro* inhibitory activity on mammalian DNA polymerase and on proliferation of K562, WM-1341, HL-60, and RPMI-8226 tumor cell lines, immunosupressive activity, and cytotoxicity towards P388 murine leukemia cells (5-10). Furospinulosin-II **(II)** has previously been isolated from the marine sponge *Ircinia spinulosa* collected from Italian shores (11). In connection with our ongoing investigation on the isolation of bioactive natural compounds from

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marine fauna and flora of the Aegean Sea, we undertook a chemical examination on a species of the marine sponge *Spongia officinafts* L. which is quite common in the Mediterranean Sea and isolated ergosterol **(I)** and furospinulosin-II **(II)**. Their structure elucidations were performed by spectroscopic techniques and with literature data comparison.

Material and Methods

Animal material

A sample of the marine sponge *Spongia officinalis* L., dark-brown in color, was collected by snorkelling from Bodrum, Turkey in August 1996. It was authenticated by Dr. T. Atıcı from the Department of Biology, Faculty of Education, Gazi University, Ankara, Turkey.

Instrumentation

^{1}H NMR spectra were run at 500 MHz and ^{13}C NMR spectra at 125 MHz in $CDC1_3$, using a JEOL α 500 (500 MHz) FT NMR spectrometer. Chemical shifts were given in ppm relative to the international standard of TMS. Methyl, methylene and methine carbons were distinguished by DEPT experiments. One bond heteronuclear ^{1}H - ^{13}C connectivities were determined with 2D HMQC experiments. EI-MS spectra were recorded on a Hitachi M-2500 instrument. HPLC separations were carried out on a Hitachi L-6000 apparatus equipped with a Hitachi L-4000 UV detector. All analytical TLC were performed on plates precoated with Kieselgel 60 F_{254} (Merck).

Extraction and isolation

A fresh sample of the sponge was cut into small pieces and extracted with ethanol three times at room temperature. The extract was concentrated under reduced pressure and the resulting aqueous residue was partitioned between EtOAc and H_20. Separation of the EtOAc-soluble material was achieved initially by vacuum flash chromatography on Si 60 and then silica gel column and seven fractions were obtained. Compound **(I)** (1.2 mg) was isolated in pure form from the sixth fraction by normal phase HPLC eluted with heptane-EtOAc-CH_2Cl_2 (6-2:1). Compound **(II)** (5.6 mg) was obtained from the third fraction by reverse phase HPLC using MeOH-EtOAc (10: 3) as eluent.

Results and Discussion

The sponges belonging to the order of *Dictyoceratida* are rich sources of characteristic furanoterpenes with usually 21 carbon atoms. Spectroscopic data supporting the structures of compounds **(I)** and **(II)** are presented (Fig.)

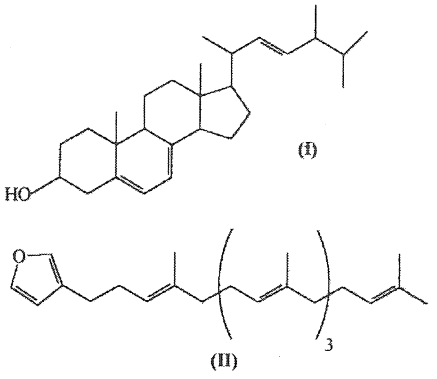

Fig. Structures of compounds **(I)** and **(II)**

I Compound **(I)** has a molecular formula of $C_{28}H_{44}O$ by EI-MS spectrum showing molecular ion at *m/z* 396. ^{1}H NMR showed characteristic signals for a sterol skeleton. The hydroxyl group at C-3 position appeared at δ 3.64 ppm in ^{1}H NMR. Neighbouring olefinic protons on the B ring of compound **(I)** were at δ 5.57 and 5.39 ppm. They were also shown to correlate each other by HMBC and ^{1}H-^{1}H COSY spectra. The carbon resonance that belongs to the hydroxy group was seen at δ 70.5 ppm. The olefinic protons at δ 5.57 and 5.39 ppm were connected to the carbons at δ 119.6 and 116.3 ppm, respectively, by

BHMQC. 5 methyl groups were characterized by the signals at δ 0.63, 0.83, 0.92, and 0.95 (two methyls) in ^{1}H NMR and δ 11.3, 16.3 (two methyls), 18. 0 and 19.6 ppm in ^{13}C NMR spectra. ^{1}H and ^{13}C NMR data of compound **(I)** were compared with the reported data and identified as ergosterol (5-8).

EI-MS spectrum of compound **(II)** exhibited a molecular ion peak at *m/z* 422 indicating a molecular formula of $C_{30}H_{46}0$. Its ^{1}H NMR spectrum contained the singlets at δ 7.35, 7.23, and 6.29 ppm characteristic for a β-methylene-substituted furan ring. The triplet at δ 2.45 ppm was attributed to the methylene group attached to the furan ring. Pentaprenylated side chain was deduced from the 5-proton broad singlet at δ 5.08 in ^{1}H NMR. The carbon resonances at δ 125.2, 125.4, 125.5 (three carbons) were assigned to the olefinic carbons correlating the olefinic protons by HMQC. Quaternary carbons on the isoprenyl side chain appeared at δ 136.6, 135.8, 135.4, 135.8, and 132.1 ppm and were distinguished by DEPT (135^{0}) spectrum. The correlations between the neighbouring protons were shown by ^{1}H-^{1}H COSY and TOCSY spectra. These spectral features were in agreement with the structure for furospinulosin-II reported in the literature. In the light of these evidences, compound **(II)** was elucidated as furospinulosin-II (11).

Acknowledgement

We are grateful to Prof. Dr. T. Higa from the Department of Chemistry, Biology and Marine Sciences of University of the Ryukyus, Okinawa, Japan for providing spectroscopic facilities.

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Accepted: 28.04.2000