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Terpenoids from Two Sponge Species of the Aegean Sea

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Abstract – The ethanolic extracts of two shallow water sponge species collected from the Aegean Sea afforded thirteen terpenoids in total, two of which were determined to be new. The structure elucidation of the terpenoids was carried out by spectroscopic techniques and comparison with related authentic compounds. The terpenoids have also been assayed for antibacterial activity. This is the first report about the metabolites isolated from the marine sponges of the Aegean Sea in Turkey.
Key words – Sponge, terpenoid, antibacterial activity, spectral data.

Introduction

In spite of the long coastal lines of Turkey, little attention has so far been paid on the chemical and pharmaceutical investigation of the marine organisms of Turkish waters. We have therefore initiated an investigation of bioactive compounds of the marine organisms from this region. The first organisms studied were two sponge species from the Aegean Sea, from which we isolated a series of terpenoids. In this paper, the results obtained to date are presented.

Experimental

Animal material – Two sponge species, dark brown and light brown in color, were collected from the Aegean Sea in Bodrum, Turkey, at the depth of 3 m. The sponge species have been identified to be *Spongia* sp. and *Ircinia* sp., respectively.

Instrumentation – ^{1}H-and ^{13}C-NMR spectra were recorded on a JEOL α 500 (500 MHz) FT NMR spectrometer. All spectra were taken in $CDCl_3$. One bond heteronuclear ^{1}H-^{13}C connectivities were determined with 2D HMQC experiments. EIMS spectra were recorded on a Hitachi M-2500 instrument. IR measurements were taken on a Hitachi infrared spectrophotometer 260-10 in CCl_4. HPLC separations were carried out on a Shimadzu LC-9A apparatus equipped with Waters Differential Refractometer 401. All analytical thin layer chromatography was performed on TLC plates precoated with Kieselgel $60F_{254}$ (Merck).

Extraction and Isolation – The fresh samples of the sponge species mentioned above were cut into small pieces and extracted with ethanol at room temperature. The ethanolic extracts (360 g and 255 g respectively) were concentrated under vacuum and partitioned between ethylacetate and water. The ethylacetate layers of the sponges were used for separation.

Ethyl acetate extract of *Spongia* sp. (1.88 g) was applied to vacuum flash chromatography Si 60 (VFC) to give five main fractions. Compounds **1** (5.1 mg) and **2** (42.7 mg) were isolated from the first VFC fraction by normal phase HPLC. Second VFC fraction was subjected to the separation on silica gel to give seven subfractions. These subfractions afforded compounds **3** (27.1 mg), **4** (7.2 mg), **5** (30.7 mg), **6** (2.6 mg), **7** (3.0 mg) by using normal phase HPLC. Compound **8** (2.3 mg) was isolated from the last subfraction by reverse phase HPLC.

The ethyl acetate extract of *Ircinia* sp. was applied to VFC Si 60. Compounds **1** (4.2 mg) and **2** (109.2 mg) were reisolated from the first VFC fraction. The other VFC fractions gave compound **9** (11.8 mg), **10** (6.7 mg), **11** (5.3 mg) by normal phase HPLC. One of the VFC fractions was applied to centrifugal countercurrent chromatography (CCC) to give compound **12** (3.6 mg) and **13** (10.8 mg).

1,4,44-Trihydroxy-2-octaprenylbenzene (7) – Yellow oil; IR ν_{max}^{film} cm^{-1}; 3350, 2900, 980; EIMS (70eV) *m/z* (Rel. int.): 671(17) $[M+H]^+$, 653(50) $[M-H_2O]^+$,

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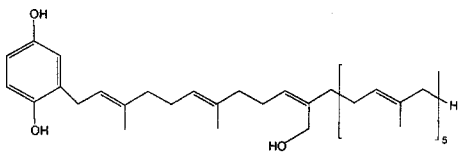
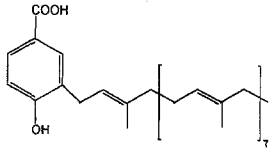
584(14), 516(23), 447(15), 339(21), 271(15), 161 (82), 123(69), 69(100); ^{1}H NMR ($CDCl_3$): δ 6.65 (1H,d,H-2), 6.59(1H,d,3.5,H-5), 6.55(1H,dd,3.0,8.0,H-3), 5.28-5.30(2H,m,H-8,H-16), 5.09-5.13(6H,m,H-12,H-20, H-24, H-28,H-32,H-36), 4.12(2H,s,H-44), 3.30(2H,d,7.0, H-7), 2.12-2.18(4H,m,H-15, H-18), 2.04-2.09(12H,m,H-11,H-19, H-23, H-27, H-31, H-35), 1.96-2.01 (12H,m, H-10, H-14, H-22, H-26, H-30, H-34), 1.68 (3H,s,H-38), 1.59(21H,s,H-39,H-40,H-41,H-42,H-43,H-45,H-46); ^{13}C NMR ($CDCl_3$): δ 149.4(s, C-4), 148.1(s,C-1), 138.4 (s,C-9), 138.3(s,C-17), 138.1(s,C-29), 135.5(s,C-37), 134.9(s,C-21), 134.8(s,C-13), 134.4(s,C-25), 128.9(d,C-16), 128.4(s,C-6), 128.2(s,C-33), 124.9(d,C-24), 124.4 (d,C-36), 124.2(d,C-12), 124.1(d,C-28), 123.9(d,C-32), 123.7(d,C-20), 121.5(d,C-8), 116.5(d,C-5), 116.4(d,C-2), 113.7(d,C-3), 60.4(t,C-44), 39.9(t,C-10), 39.8(t,C-26), 39.7(t,C-14,C-34), 39.6(t,C-22,C-30), 35.3(t,C-18), 29.6(t,C-7), 27.0(t,C-23), 26.7(t,C-11), 26.6(t,C-15,C-31), 26.4(t,C-27), 26.2(t,C-19, C-35), 25.7(q,C-38), 17.7(q,C-39), 16.1(q,C-41,C-42,C-45), 16.0(q,C-40,C-43,C-46).



1,4,44-Trihydroxy-2-octaprenylbenzene (7)

4-Hydroxy-3-octaprenylbenzoic acid(11) – Yellow oil; IR ν_{max}^{film} cm^{-1}; 2900, 1700, 1400, 960, 840, 720,; EIMS (70eV) *m/z* (Rel. int.); 682(10) [M^+] , 638(20), 571(12), 531(17), 477(20), 409(25), 297(5), 203(28), 161(66), 135(61), 107(65), 68(100); ^{1}H NMR ($CDCl_3$): δ 7.88(1H,m,H-6), 7.87(1H,m,H-2), 6.85(1H,d,9.0,H-5), 5.32(1H,t,7.5,H-9), 5.11(7H,m,H-13,H-17,H-21,H-25,H-29, H-33,H-37), 3.41(2H,d,7.0,H-8), 2.05-2.13(14H,m,H-12, H-16,H-20,H-24,H-28,H-32,H-36), 1.97-2.00(14H,m,H-11, H-15,H-19,H-23,H-27,H-31,H-35), 1.79(3H,s,H-47), 1.67 (3H,s,H-39), 1.59(21H,s,H-40,H-41,H-42,H-43,H-44,H-45, H-46), ^{13}C NMR ($CDCl_3$): δ 170.6(s,C-7), 159.5(s,C-4), 139.6(s,C-10), 135.7(s,C-14), 135.0(s,C-18), 134.9 (s,C-26),C-30,C-34), 132.5(d,C-2), 131.2(s,C-38), 130.5 (d,C-6), 126.7(s,C-3), 124.4(d,C-17), 124.3(d,C-21,C-25,C-29), 124.2(d,C-33,C-37), 123.4(d,C-13), 121.5 (s,C-1), 120.7(d,C-9), 115.8(d,C-5), 39.8(t,C-11), 39.7 (t,C-15,C-19,C-23), 39.6(t,C-27,C-31,C-35), 29.8(t,C-8), 26.8(t,C-12), 26.7(t,C-16), 26.6(t,C-20,C-24,C-28,C-32), 26.3(t,C-36), 25.7(q,C-39), 16.3(q,C-47), 16.1(q,C-41), 16.0(q,C-40,C-42,C-43,C-44,C-45,C-46).



4-Hydroxy-3-octaprenylbenzoic acid (11)

Results

Most of the compounds isolated to date turned out to be known metabolites of certain species of sponges common in the Mediterranean Sea and elsewhere. Except for compounds **1-6, 8-10, 12**, and **13;** compounds **7** and **11** have been determined to be new hydroquinone derivative terpenoids.

Metabolites from the sponge *Spongia* sp.

Compound **1** was concluded to be 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, namely squalene which is a triterpene found in fish liver oils, yeast lipids and many vegetable oils. Squalene is considered to be key precursor of cholesterol (Breitmaier *et al.*, 1987).

Compound **2** was the most abundant component in both of the extracts. It was identified as furanospinulosin-1 by comparing with the reported data (Cimino *et al.*, 1972). Furanospinulosin-1 is a very well-known furanoterpene from marine sponges.

Compound **3** was found to be furospongin-1 . Its spectral data agreed well with the literature data (Cimino *et al.*, 1971, Guella 1 *et al.*, 986, Kobayashi *et al.*, 1992). Furospongin-1 is one of the earliest examples of unique C_{21} furanoterpene sponge metabolites. It was first isolated from the Mediterranean sponges *Spongia officinalis* and *Hippospongia communis*.

The structure of compound **4** was elucidated to be 2-(hexaprenylmethyl)-2-methylchromenol by comparing of the spectral data with the know one (Venkateswarlu *et al.*, 1994). It was earlier isolated from the sponge *Ircinia fasciculata.*

On the basis of obvious similarities of compound **5** with the reported data for polyprenylated *p*-quinols (Lumsdon *et al.*, 1992), it was found to be heptaprenylated *p*-quinol, which was previously isolated from the Mediterranean sponges *Ircinia spinulosa* (Cimino *et al.*, 1977) and *Hippospongia communis* (Pouchus *et al.*, 1988).

Compound **6** was identified as 12-*epi*-deoxoscalarin by comparing of the spectral data with the known one (Cimino *et al.*, 1977). It was first isolated

from the Mediterranean sponge *Spongia nitens*.

Compound **7** was obtained as a yellow oil. Its IR spectrum showed a hydroxyl absorption band at 3360 cm^{-1}. ^1H and ^{13}C NMR had diagnostic signals for an isoprenyl side chain including six vinyl methyls, incorporating an all-*trans* geometry. The three aromatic proton signals were indicative of a 1,2,4-trisubstituted hydroquinone which was confirmed by a prominent base peak at *m/z* 161 in EIMS. A 2H singlet at 4.12 ppm in ^1H NMR was assigned to be the primary hydroxyl group. It was placed at C-44 by HMBC correlations. The lenght of an isoprenyl side chain was deduced by a molecular ion at *m/z* 671 $[M+H]^+$, corresponding to the molecular formula $C_{46}H_{70}O_3$. The detailed spectral analysis of compound **7** showed that it is a new meroterpenoid whose structure is 1,4,44-trihydroxy-2-octaprenylbenzene.

Compound **8** existed as a minor component. Structure elucidation of compound **8** was achieved by comparison of spectral data with those of reported furospongins (Guella *et al.*, 1986, Pouchus *et al.*, 1988) and it was found to be a mixture of the isomeric linear furanosesterpenes furospongin-3 and furospongin-4. The mixture is resistant to separation as mentioned by previous workers. It was initially isolated from the Mediterranean sponge *Spongia officinalis* (Garrido *et al.*, 1997).

Metabolites from the sponge *Ircinia* sp.

Compound **9** was determined to be 4-hydroxy-3-tetraprenylphenylacetic acid since the spectral data agreed well with the literature values (Baz *et al.*, 1996). It was earlier isolated from the Mediterranean sponge *Ircinia muscarum* and it was also first report of a hydroquinone substituted with an alkyl carboxylic acid.

The similarity of the spectral features of compound **10** with the known one pointed out it to be demethylfurospongin-4 (Garrido *et al.*, 1997). It was previously obtained from the Mediterranean sponge *Spongia officinalis*. It is a linear furanoterpene closely related to furanospinulosin-1 (Cimino *et al.*, 1972) and furospongin-4 (Miyamoto *et al.*, 1996).

Compound **11** was obtained as a yellow oil. Two aromatic signals were observed in the ^1H NMR spectrum. The signal at 7.88 ppm was assigned to deshielded protons *ortho* to carboxyl group at 170.6 ppm. IR spectrum of compound **2** pointed to existence of a carboxyl group with the broad absorption at 2900 cm^{-1}. The signals attributable for a polyprenyl side chain were seen in the ^{13}C NMR spectrum. The chemical shifts of methyl groups indicated all-*trans* configuration for the double bonds. A molecular ion at *m/z* 682 in EIMS suggested a molecular composition for compound **2** as $C_{47}H_{70}O_3$. The spectral data were in accordance with the data reported for 4-hydroxy-3-tetraprenyl benzoic acid(Cimino *et al.*, 1972).

By interpretation of the spectral data, compound **11** was found to be a new meroterpene. The structure was elucidated to be 4-hydroxy-3-octaprenylbenzoic acid.

The spectral data of compound **12** was identical to the reported data for 7β, 11β-diacetoxyspongi-12-en-16-one(Miyamoto *et al.*, 1996), namely dorisenone D, a diterpene of spongian class. Dorisenone D was earlier isolated from the Japanese collection of the nudibranch *Chromodoris obsoleta.*

Since the spectral data was in complete agreement with the literature values(Gonzales *et al.*, 1984), compound **13** was determined as 11β-acetoxyspongi-12-en-16-one. It was previously obtained from the Mediterranean sponge *Spongia officinalis.*

Antibacterial activity test

Compounds **2-7, 9-11** and **13** were analyzed for antibacterial activity against gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus* and gram-negative bacteria *Pseudomonas aeroginosa* and *Escherichia coli* by using standard agar-plate disk method. Due to scarcity of the samples, only one concentration (0.5 mg/disk) of each compound was used for the test. In the end, the inhibition zone diameters caused by the compounds were measured (The diameter of paper disks used was 8 mm).

While compounds **2-5** and **11** did not show activity against any bacterium strains, compound **6** showed weak activity against *B.subtilis and S.aureus* causing an inhibition zone of 12 and 11 mm in diameter, respectively. Compounds **9,10** and **13** were also active against *B.subtilis and S.aureus*. While compounds **10** and **13** showed activity in the same level inhibition zone of 15 mm, compound **9** was found to be more active, causing 20 mm inhibition zone diameter. Compound **7** was weakly active against gram-negative bacterium *P.aeuroginosa*, giving an inhibition zone of 10 mm. Except for compound **7**, none of them tested in this assay showed activity against *P.aeuroginosa*

and *E.coli*. Amongst them, compound **9** was found to be the most active.

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