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TERPENOIDS FROM THE NORTH ADRIATIC SPONGE *SPONGIA OFFICINALIS*

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ABSTRACT.—Four C-21 furano terpenes, furospongin 2 [**4**] and its three new isomers **1**–**3**, and three scalarane sesterterpenoids, 16-deacetyl-12-*epi*-scalarafuranacetate [**5**], deoxoscalarin acetate [**6**], and (−)-12-*epi*-deoxoscalarin [**7**], have been isolated from the sponge *Spongia officinalis*. The structural elucidation and cytotoxic activity of these compounds are reported.

Sesterterpenoids with the scalarane skeleton, acyclic furanosesterterpenes, and degraded C-21 furanoterpenes are frequently present in Porifera of the order Dictyoceratida (1). Continuing our search for marine natural compounds that have biological activities, we have studied the marine sponge *Spongia officinalis* L. (Dictyoceratida, Spongiidae) collected in the northern Adriatic, whose extract showed cytotoxic activity (LD_{50} = 45 μg/ml) in the brine shrimp assay (2). By fractionating the extract, we isolated four C-21 furanoterpenes **1**–**4** and three sesterterpenoids with scalarane skeleton **5**–**7**, which are responsible for the cytotoxic activity.

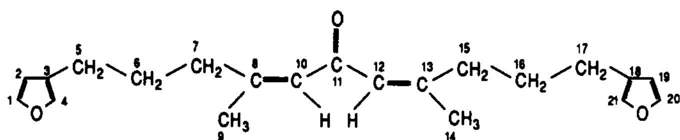
From the same sponge collected in the Tyrrhenian Sea, other authors (1, 3–5) have reported the isolation of a sesterterpene with a scalarane skeleton, deoxoscalarin, as well as a number of acyclic furanosesterterpenes and C-21 furanoterpenes.

The Et_2O-soluble fraction of the Me_2CO extracts was chromatographed on Si gel to give a mixture that was resolved on hplc [μ-Porasil, *n*-hexane–Et_2O (95:5)] give four C-21 furanoterpenes: the previously reported furospongin 2 [**4**] (3) and three new isomers **1**–**3**. Three sesterterpenoids, 16-deacetyl-12-*epi*-scalarafuran acetate [**5**], deoxoscalarin acetate [**6**], and (−)-12-*epi*-deoxoscalarin [**7**], were also isolated. The compounds are numbered in order of polarity.

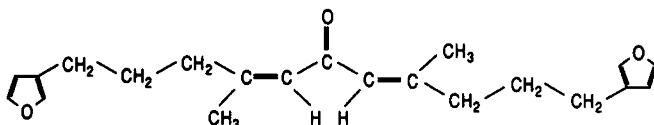
Compounds **1**–**4** were colorless oils and showed the same molecular formula $C_{21}H_{26}O_3$ (hrms); their ir (1700–1680, 1629–1614 cm^{-1}) and uv (242–267 nm) spectra showed the presence, in all compounds, of an α,β-unsaturated ketone.

A preliminary analysis of nmr spectra showed a strong similarity between compounds **1** and **3**. The presence of approximately half the expected signals also showed the symmetry of both compounds. Compounds **2** and **4** were unsymmetrical.

The ^1H-nmr spectrum of **1** revealed signals for two furan α protons (δ 7.33 and 7.24) and one β proton (δ 6.28), suggesting the existence of a β-mono-substituted furano ring. This was further substantiated by ^{13}C-nmr data that showed three doublets (δ 142.6, 138.8, and 111.0) and one singlet (δ 124.9). The presence of a singlet at δ 6.04 in the ^1H-nmr spectrum and two singlets (δ 159.2 and 130.2) and one doublet (δ 126.6) in the ^{13}C-nmr spectrum confirmed the presence of an α,β-unsaturated ketone. Moreover, the ^1H-nmr spectrum showed one vinylic methyl (δ 1.86) and signals at δ 2.68, 2.48, and 1.73, attributable to three methylenes by HETCOR experiment, that correlated these latter protons to three carbon triplets in the ^{13}C-nmr spectrum at δ 25.3, 24.9, and 28.5, respectively. The COSY-45 spectrum showed that the vinylic methylene (δ 2.68) was correlated, long range, with the vinylic proton (δ 6.03) and at the same time with the methylene at δ 1.73, which was also correlated with the remaining methylene (δ 2.48). The configuration of the trisubstituted double bond was as-



1



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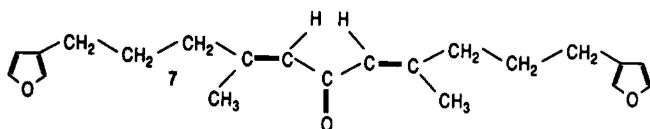

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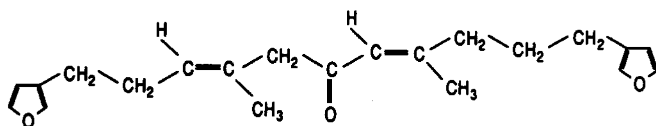

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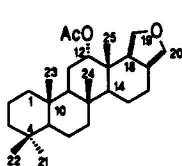

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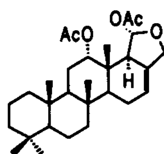

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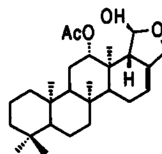

7

signed as *Z* by ^{1}H- and ^{13}C-nmr chemical shifts (δ 1.86 and 33.2 for ^{1}H and ^{13}C, respectively) of vinyl methyl.

Because the ^{13}C-nmr spectrum showed only 11 signals and the molecular formula had 21 carbon atoms, the molecule was symmetrical with the carbonyl group at the center, as depicted in **1**.

The ^{1}H-nmr and ^{13}C-nmr spectra of **3** were similar to those of **1**, except for the signals due to the vinylic groups that showed chemical shifts at δ 2.16 (19.1) and δ 2.15 (40.7) for methyl and methylene, respectively. These shifts were characteristic of an exchange of configuration from *Z* to *E* of the double bonds.

Compound **2** showed ^{1}H-nmr and ^{13}C-nmr spectra more complex than those of **1** and **3**. In both spectra, some signals were split, and in particular the presence of two sets of vinylic signals was observed: two methyls (δ 2.16, 1.87 in ^{1}H-nmr spectra and δ 19.2, 33.5 in ^{13}C-nmr spectra) and two methylenes (δ 2.62, 2.15 and δ 25.4, 40.7 in ^{1}H-nmr and ^{13}C-nmr spectra, respectively). These data suggested that the double bonds were in both configurations, *Z* and *E*.

The spectral data of **4** were in excellent agreement with published values (3). Using 2D-nmr spectroscopy, COSY, and HETCOR, we were able to assign all the ^{13}C chemical shifts not reported in the literature (see Experimental).

The ^{1}H- and ^{13}C-nmr data of **5–7**, in comparison with the reported resonances of scalarane sesterterpenoids, strongly supported a scalarane skeleton for these compounds.

16-Deacetyl-12-*epi*-scalarafuran acetate [**5**], mp 130° (*n*-hexane); $[\alpha]^{25}D$ +68 (c = 0.5, $CHCl_3$), has the molecular formula $C_{27}H_{40}O_3$ (hrms). The ir spectrum with bands at 1734 and 1244 cm^{-1} shows the presence of an acetyl group. The ^{1}H-nmr spectrum of **5** shows a methyl singlet at δ 1.91, confirming the acetyl group, and the presence of a β,β-disubstituted furano ring (two broad singlets at δ 7.04 and 6.96) and of an α-acetoxy proton, a multiplet at δ 5.34. These data suggested that the compound **5** was identical to the furan obtained by acetylation of deoxoscalarin (5). The relative stereochemistry of the proton at C-12, as β, was assigned by its chemical shift and multiplicity in the ^{1}H-nmr spectrum (4).

Deoxoscalarin acetate [**6**], mp 165–168° (*n*-hexane); $[\alpha]^{25}D$ +40 (c = 1.5, $CHCl_3$), had the molecular formula $C_{29}H_{44}O_5$. Although the highest value in the mass spectrum was m/z 412 $[M - HOAc]^+$, the spectroscopic data confirmed the molecular formula. The presence of two acetyl groups in the molecule was deduced from ir (1735, 1725, 1244, and 1235 cm^{-1}) and ^{1}H-nmr (two methyl singlets at δ 2.08 and 1.91) spectra. Furthermore, the ^{1}H-nmr spectrum shows two α-acetoxy protons (δ 5.34, m, and 5.25, d, J = 3.7 Hz), a vinyl proton (δ 5.46, m), an isolated methylene attached to oxygen (AB system at δ 4.47 and 4.18, J = 11.2 Hz), and a proton at δ 2.78. These data suggested that **6** was the acetyl derivative of deoxoscalarin (5). Compound **6** was unstable in $CHCl_3$ solution. In fact, during the acquisition time of the COSY spectrum, some new signals appeared, representing the COSY of two compounds. After 5 h, only the new product was present in solution, and its spectral data were in excellent agreement with the data of **5**. This easy transformation of **6** into **5**, with elimination of HOAc and rearrangement of the double bond to give a furan ring, suggested that **5** was an artifact.

The spectral data, including 2D nmr (COSY and NOESY) of **7** (see Experimental) are in accord with a deoxoscalarin compound. Furthermore, the presence in the ^{1}H-nmr spectrum of a double doublet at δ 4.81 (α-acetoxy proton) shows an α relative stereochemistry of the proton at C-12, suggesting that **7** was 12-*epi*-deoxoscalarin, already described (4). The physical data showed a similar melting point and an optical rotation with the same magnitude but opposite sign of that reported. These data thus indicated that **7** was the enantiomer of the compound previously reported (4).

The toxicity of compounds **1–7** was tested in the *Artemia salina* shrimp bioassay (2), which is used as an in-house assay substituting for 9KB and 9PS cytotoxicities. All C-21 furanoterpenes (**1–4**) showed high activity (LD_{50} 0.09–1.6 μg/ml), while sesterterpenoids **5–7** showed less activity (LD_{50} 180–200 μg/ml).

It is relevant to underline that the same sponge living in different habitats can produce different secondary metabolites and that the co-occurrence of two related compounds (**6**,**7**) as epimers at C-12 shows that the enzymatic pathways are not stereoselective for oxidation at C-12 for scalarane sesterterpenes.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Kofler apparatus and are uncorrected. Uv spectra were obtained on a Varian DMS 90 spectrophotometer. Ir spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-50 spectrometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded at 500 and 125

MHz, respectively, with TMS as internal standard on a Bruker WM 500 instrument, under Aspect 2000 control. The 2D-nmr spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Preparative hplc purifications were carried out on a Waters apparatus equipped with μ-Porasil column (7.8 mm i.d. × 30 cm) and with refractive index detector.

EXTRACTION AND ISOLATION OF COMPOUNDS.—The *S. officinalis*, collected by hand at about 5 m depth at Rovinj, Yugoslavia, was frozen at −20° until extracted. The frozen sponge (100 g dry wt after extraction) was extracted with Me_2CO, and after elimination of the solvent in vacuo, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The extracts were submitted to the brine shrimp assay (2). The active Et_2O extract was evaporated in vacuo to obtain a brown oil (2.1 g), which was applied on a column (5 × 100 cm) of Si gel. The column was eluted with a solvent gradient system from petroleum ether 40–70° to Et_2O.

Fractions with the same tlc profile were combined. Four Ehrlich-positive fractions were recovered. The less polar was subjected to preparative hplc [*n*-hexane–Et_2O (95:5)], flow rate 4 ml per min) yielding **1** (10 mg, Rt 3.1), **2** (12 mg, Rt 4.3), **3** (7 mg, Rt 4.5), and **4** (16 mg, Rt 5.8). From the second fraction, after crystallization from *n*-hexane, was recovered **5** (5 mg). The third fraction was further chromatographed on a Si gel column, eluted with petroleum ether-Et_2O (1:1), to give, after crystallization from EtOH, **6** (20 mg). The last fraction, after crystallization from EtOH, gave **7** (15 mg).

Compound **1**.—Uv λ max (MeOH) 265 (ε 24000); ir ν max (liquid film) 1700, 1685, 1457, 1162, 1108, 1024, 873, 777, 600 cm^{-1}; eims *m/z* (%) $[M]^+$ 326.1892 ($C_{21}H_{26}O_3$ requires 326.1891) (8), 311 (6), 243 (3), 217 (7), 177 (100), 149 (10); ^1H nmr ($CDCl_3$) δ 7.33 (1H, br s, H-1), 7.24 (1H, br s, H-4), 6.28 (1H, br s, H-2), 6.04 (1H, s, H-10), 2.67 (2H, dd, $J=7.6$, 7.8, H-7), 2.48 (2H, dd, $J=7.7$, 7.2, H-5), 1.86 (3H, s, H-9), 1.73 (2H, m, H-6); ^{13}C nmr ($CDCl_3$) δ 159.2 (s, C-11), 142.6 (d, C-1), 138.8 (d, C-4), 130.7 (s, C-8), 126.6 (d, C-10), 124.9 (s, C-3), 111.0 (d, C-2), 33.2 (q, C-9), 28.5 (t, C-6), 25.3 (t, C-7), 24.9 (t, C-5).

Compound **2**.—Uv λ max (MeOH) 253 nm (ε 25000); ir ν max (liquid film) 1700, 1682, 1620, 1501, 1443, 1380, 1162, 1105, 1024, 874, 778, 600 cm^{-1}; eims *m/z* (%) $[M]^+$ 326.1893 ($C_{21}H_{26}O_3$ requires 326.1891) (10), 311 (5), 243 (8), 217 (5), 177 (100), 148 (15); ^1H nmr ($CDCl_3$) δ 7.33 (2H, br s, H-1 and H-20), 7.23 (2H, br s, H-4 and H-21), 6.28 (2H, br s, H-2 and H-19), 6.03 (2H, s, H-10 and H-12), 2.62 (2H, dd, $J=7.7$, 7.6 Hz, H-7), 2.49 (2H, dd, $J=7.6$, 7.3 Hz, H-5), 2.45 (2H, dd, $J=7.6$, 7.3 Hz, H-17), 2.16 (3H, s, H-14), 2.15 (2H, overlapped by H-14, H-15), 1.87 (3H, s, H-9), 1.74–170 (4H, m, H-6 and H-16); ^{13}C nmr ($CDCl_3$) δ 157.2 (s, C-11), 142.8 (d, C-20), 142.5 (d, C-1), 138.8 (d, C-4 and C-21), 130.8 (s, C-8), 130.5 (s, C-13), 126.5 (d, C-10), 126.0 (d, C-12), 125.1 (s, C-3), 124.8 (s, C-18), 110.9 (d, C-2), 110.8 (d, C-19), 40.7 (t, C-15), 33.5 (q, C-9), 28.5 (t, C-6), 27.8 (t, C-16), 25.4 (t, C-7), 24.9 (t, C-5), 24.7 (t, C-17), 19.2 (q, C-14).

Compound **3**.—Uv λ max (MeOH) 267 nm (ε 26000); ir ν max (liquid film) 1700, 1680, 1619, 1501, 1457, 1161, 1105, 1024, 873, 777, 600 cm^{-1}; eims *m/z* (%) $[M]^+$ 326.1894 ($C_{21}H_{26}O_3$ requires 326.1891) (8), 311 (6), 243 (5), 217 (8) 177 (100), 148 (10); ^1H nmr ($CDCl_3$) δ 7.36 (1H, br s, H-1), 7.22 (1H, br s, H-4), 6.26 (1H, br s, H-2), 6.04 (1H, s, H-10), 2.43 (2H, dd, $J=7.5$, 7.3 Hz, H-5), 2.16 (3H, s, H-9), 2.15 (2H, overlapped by H-9, H-7), 1.75 (2H, m, H-6); ^{13}C nmr ($CDCl_3$) δ 157.3 (s, C-11), 142.8 (d, C-1), 138.9 (d, C-4), 130.4 (s, C-8), 125.9 (d, C-10), 124.8 (s, C-3), 110.8 (d, C-2), 40.7 (t, C-7), 27.9 (t, C-6), 24.4 (t, C-5), 19.1 (q, C-9).

Furospongin 2 [**4**].—Uv λ max (MeOH) 242 nm (ε 23000); ir ν max (liquid film) 1687, 1614, 1502, 1442, 1163, 1104, 1024, 874, 778, 599 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.36 (1H, br s, H-20), 7.33 (1H, br s, H-1), 7.21 (1H, br s, H-21), 7.20 (1H, br s, H-4), 6.27 (1H, br s, H-19), 6.26 (1H, br s, H-2), 6.07 (1H, s, H-12), 5.29 (1H, t, $J=6.9$ Hz, H-7), 3.05 (2H, s, H-10), 2.48 (2H, dd, $J=7.5$, 7.3 Hz, H-5), 2.42 (2H, dd, $J=7.5$, 7.3 Hz, H-17), 2.30 (2H, dt, $J=6.9$, 7.5 Hz, H-6), 2.14 (2H, overlapped by H-14, H-15), 2.13 (3H, s, H-14), 1.73 (2H, m, H-16), 1.61 (3H, s, H-9); ^{13}C nmr ($CDCl_3$) δ 156.5 (s, C-11), 143.1 (d, C-1), 143.0 (d, C-20), 139.1 (d, C-4), 138.8 (d, C-21), 130.9 (s, C-8), 130.4 (s, C-13), 128.5 (d, C-7), 124.9 (s, C-3), 124.6 (s, C-18), 123.0 (d, C-12), 111.2 (d, C-2), 110.9 (d, C-19), 55.5 (t, C-10), 40.8 (t, C-15), 28.9 (t, C-5), 28.0 (t, C-16), 25.1 (t, C-6), 24.5 (t, C-17), 19.1 (q, C-14), 16.6 (q, C-9).

*16-Deacetyl-12-*epi-*scalarafuran acetate* [**5**].—Mp 130–132° (*n*-hexane); $[\alpha]^{25}D$ +68 ($c=0.5$, $CHCl_3$); ir ν max ($CHCl_3$) 1734, 1459, 1386, 1244, 1040, 783, 600 cm^{-1}; eims *m/z* (%) $[M]^+$ 412.2970 ($C_{27}H_{40}O_3$ requires 412.2977) (35), 370 (15), 352 (10), 337 (20), 191 (100), 171 (8), 132 (14); ^1H nmr ($CDCl_3$) δ 7.04 (1H, br s, H-20), 6.96 (1H, br s, H-19), 5.34 (1H, m, H-12), 2.72 (1H, m, H-16), 2.40 (1H, m, H-16), 1.91 (3H, s), 1.78 (2H, m, H-11), 0.92 (3H, s), 0.86 (6H, s), 0.83 (3H, s), 0.81 (3H, s); ^{13}C nmr ($CDCl_3$) δ 170.5 (s), 136.8 (d), 135.2 (d), 120.4 (s), 119.1 (s), 75.4 (d), 56.8 (d), 52.8 (d),

51.6 (d), 42.2 (t), 41.8 (t), 39.9 (t), 38.8 (s), 38.0 (s), 37.2 (s), 33.4 (s), 33.3 (q), 26.7 (t), 22.4 (t), 21.4 (q), 21.0 (q), 18.6 (t), 18.3 (t), 18.1 (t), 17.4 (q), 16.1 (q), 15.2 (q).

Deoxoscalarin acetate [**6**].—Mp 165–168° (*n*-hexane); $[\alpha]^{25}D$ +40 (c = 1.5, $CHCl_3$); ir ν max ($CHCl_3$) 1735, 1725, 1459, 1386, 1244, 1235 cm^{-1}; eims *m/z* (%) $[M - HOAc]^+$ 412.2969 ($C_{27}H_{40}O_3$ requires 412.2977) (40), 370 (5), 352 (25), 337 (50), 199 (20), 191 (18), 161 (100); ^1H nmr ($CDCl_3$) δ 5.46 (1H, m, H-16), 5.34 (1H, m, H-12), 5.25 (1H, d, J = 3.7 Hz, H-19), 4.47 (1H, br d, J = 11.2 Hz, H-20), 4.18 (1H, d, J = 11.2 Hz, H-20), 2.78 (1H, m, H-18), 2.08 (3H, s), 1.91 (3H, s), 1.78–1.72 (2H, m, H-11), 0.92 (3H, s), 0.86 (3H, s), 0.82 (6H, s), 0.81 (3H, s).

(−)-*12*-epi-*deoxoscalarin* [**7**].—Mp 193–195° (EtOH); $[\alpha]^{25}D$ −14.3 (c = 1.3, $CHCl_3$); ir ν max ($CHCl_3$) 3420, 1736, 1242, 1031 cm^{-1}; eims *m/z* (%) $[M - H_2O]^+$ 412.2963 ($C_{27}H_{40}O_3$ requires 412.2977) (40), 370 (55), 355 (10), 352 (18), 324 (45), 309 (38), 231 (10), 205 (25), 191 (100); ^1H nmr (C_6D_6) δ 5.61 (1H, d, J = 3.8 Hz), 5.20 (1H, m), 4.81 (1H, dd, J = 11.4, 4.4 Hz), 4.49 (1H, d, J = 11.1 Hz), 4.11 (1H, d, J = 11.1 Hz), 2.37 (1H, m), 1.93 (2H, m), 1.89 (3H, s), 1.82 (2H, m), 1.76 (2H, m), 0.91 (3H, s), 0.87 (3H, s), 0.80 (3H, s), 0.75 (3H, s), 0.73 (3H, s); ^{13}C nmr (C_6D_6) δ 171.3 (s), 137.4 (s), 116.1 (d), 100.2 (d), 82.5 (d), 68.3 (t), 61.5 (d), 58.0 (d), 56.2 (d), 53.8 (d), 42.2 (t), 41.3 (t), 39.6 (t), 38.2 (s), 37.4 (s), 37.3 (s), 33.2 (s), 33.1 (q), 23.7 (t), 22.2 (t), 21.3 (q), 21.1 (q), 18.6 (t), 18.2 (t), 16.5 (q), 16.4 (q), 9.9 (q).

BIOLOGICAL EVALUATIONS.—The brine shrimp lethality assay, performed in Naples laboratory as described by Meyer *et al.* (2), gave **1** LD_{50} = 0.45 μg/ml, **2** LD_{50} = 0.09 μg/ml, **3** LD_{50} = 1.6 μg/ml, furospongin 2 [**4**] LD_{50} = 0.12 μg/ml, 16-deacetyl-12-*epi*-scalarafuran acetate [**5**] LD_{50} = 180 μg/ml, deoxoscalarin acetate [**6**] LD_{50} = 190 μg/ml, and (−)-12-*epi*-deoxoscalarin [**7**] LD_{50} = 200 μg/ml.

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