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γ-Lactones from the soft corals *Sarcophyton trocheliophorum* and *Lithophyton arboreum*

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Abstract—Six novel fatty acid derivatives containing one γ-lactone ring and unusual unsaturated chains were isolated from two soft corals, *Sarcophyton trocheliophorum* and *Lithophyton arboreum*, collected in the Red Sea. They were characterized by spectroscopic methods, predominantly ^{1}H NMR, ^{13}C NMR, MS, IR, CD and UV. The compounds gave positive results in a brine shrimp toxicity assay.

1. Introduction

Soft corals have been extensively studied by many chemists and have yielded dozens of compounds, predominantly unusual steroids and other metabolites.[1,2] The information about fatty acid derivatives from soft corals is scarce.[3] Many terpenes and acetogenins were isolated from marine invertebrates, the latter including prostaglandins and fatty acid derivatives.[1] Butenolide lipids were discovered more than 30 years ago[2,4–6] in *Pterogorgia anceps* and *Pterogorgia guadalupensis*.

In the course of our investigation of the chemical composition of invertebrates of fresh water[7] and marine origin[8] we have examined the soft corals from the Red Sea, i.e. *Sarcophyton trocheliophorum* and *Lithophyton arboreum*, collected in the gulf of Aqaba (Eilat, Israel). Six novel butanolides and butenolides with unusual unsaturation and substitution patterns have been isolated from their dichloromethane extracts. Here we report the structure elucidation of these new metabolites, based mainly on their spectral characteristics. The soft corals were extracted and separated on a Sephadex LH-20 column and by reverse phase HPLC to yield six γ-butenolides with unusual side chain unsaturation and substitution (**1**–**6**). Further examination of the same extract resulted in the isolation of a total of 11 compounds, which included some known derivatives that were characterized by comparing their physical and spectral characteristics with literature data and by direct comparison with authentic samples whenever possible.

Keywords: *Sarcophyton trocheliophorum*; *Lithophyton arboreum*; γ-lactones; soft corals.

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2. Results and discussion

The two genera (*S. trocheliophorum* and *L. arboreum*) were collected on 3 October 1999 in the Red Sea, Gulf of Aqaba (Eilat, Israel). Fresh corals were put into ethanol and stored at −10°C, concentrated under nitrogen and extracted separately into CH_2Cl_2. The CH_2Cl_2 solubles were separated by column chromatography on Sephadex LH-20; two fractions were obtained from *S. trocheliophorum*. After further separation by RP-HPLC, the first fraction was found to include compounds **1**–**4**, while the second one contained acid compounds **7**–**10**. *L. arboreum* yielded three fractions, the first consisting of compounds **5** and **6**, the second containing compounds **7**–**9** and the third compound **11** see Section 4.

The fatty acids, i.e. arachidonic (**7**), eicosapentaenoic (**8**) and docosahexaenoic (**9**) acids (as methyl esters) were analyzed by GC–MS. These acids were previously described in soft corals.[9] Two prostaglandin derivatives, the PGB_2 acid (5*Z*,13*E*,15S)-15-hydroxy-9-oxoprosta-5,8(12),13-trien-1-oic acid (**10**) and its methyl ester (**11**), were characterized by comparing their spectral data with literature values. The PGB_2 acid was isolated previously from the mollusc *Cymphona gibbosum*[10] while the methyl ester was earlier reported to occur in the soft corals *Sarcophyton ehrenbergi* and *Lobophytum carnatum*.[11]

Compound **1** is pale yellow oil with a mass of 202.0990 corresponding to an elemental composition of $C_{13}H_{14}O_2$. The ^{1}H NMR spectrum (Table 1) showed signals for a vinylic hydrogen at δ 6.94 (d, *J*=1.7 Hz, H-3) a methyl signal at δ 1.46 (d, *J*=7.1 Hz, H_3-11) and a methine at δ 5.05 (dq, *J*=1.7, 7.1 Hz, H-4) suggesting an α,β-unsaturated-γ-lactone. This was confirmed by the presence in the

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Table 1. ^{1}H NMR of compounds **1–4**

	1	2	3	4
1	–	–	–	–
2	–	–	–	–
3	6.94 (1H, d, *J*=1.7 Hz)	6.98 (1H, d, *J*=1.8 Hz)	6.90 (1H, d, *J*=1.7 Hz)	6.96 (1H, d, *J*=1.6 Hz)
4	5.05 (1H, dq, *J*=1.7, 7.1 Hz)	5.10 (1H, dq, *J*=1.8, 6.8 Hz)	5.08 (1H, dq, *J*=1.7, 6.6 Hz)	5.04 (1H, dq, *J*=1.6, 6.7 Hz)
5	–	2.10 (2H, m)	2.67 (1H, dd, *J*=14.1, 4.4 Hz); 2.84 (1H, dd, *J*=14.1, 7.5 Hz)	2.61 (1H, dd, *J*=13.7, 4.4 Hz); 2.79 (1H, dd, *J*=13.7, 7.1 Hz)
6	–	2.01 (2H, m)	5.61 (1H, ddd, *J*=10.9, 7.5, 4.4 Hz)	5.63 (1H, ddd, *J*=11.2, 7.1, 4.4 Hz)
7	–	5.87 (1H, ddd, *J*=14.5, 8.8, 1.2 Hz)	5.54 (1H, dd, *J*=10.9, 6.8 Hz)	5.41 (1H, ddd, *J*=11.2, 8.4, 6.4 Hz)
8	5.39 (1H, brs)	5.57 (1H, dd, *J*=14.5, 6.1 Hz)	5.23 (1H, dd, *J*=6.8, 1.4 Hz)	3.15 (1H, ddd, *J*=14.6, 6.4, 1.1 Hz); 3.28 (1H, ddd, *J*=14.6, 8.4, 1.1 Hz)
9	–	4.82 (1H, dd, *J*=6.1, 1.1 Hz)	–	–
10	1.83 (3H, s)	–	–	–
11	1.46 (3H, d, *J*=7.1 Hz)	2.51 (1H, d, *J*=1.1 Hz)	–	–
12	5.53 (1H, d, *J*=0.8 Hz); 5.44 (1H, d, *J*=0.8 Hz)	1.41 (3H, d, *J*=6.8 Hz)	–	–
13	1.81 (3H, s)		4.94 (1H, ddd, *J*=5.7, 1.4, 1.0 Hz)	–
14			5.97 (1H, ddd, *J*=16.8, 10.1, 5.7 Hz)	–
15			5.27 (1H, dd, *J*=10.1, 1.0 Hz); 5.41 (1H, brd, *J*=16.8 Hz)	4.85 (2H, d, *J*=1.1 Hz)
16			1.43 (3H, d, *J*=6.6 Hz)	1.44 (3H, d, *J*=6.7 Hz)

^{13}C NMR spectrum (Table 2) of signals at δ 174.1 (s, C-1), 135.8 (s, C-2), 149.0 (d, C-3), and 78.3 (d, C-4) and in the IR spectrum of a strong absorption at 1750 cm^{-1}. The olefin region in the ^{1}H NMR spectrum of **1** contained resonances for an exomethylene group (H_2-12, δ 5.53 and 5.44) not overlapping with the signal at δ 5.39 (H-8). The ^{1}H NMR spectrum also featured two methyls at δ 1.83 and 1.81 (H_3-10 and H_3-13). The above-reported NMR data were in good agreement with the formula of **1** (Fig. 1).

1D- and 2D NMR experiments proved the suggested structure to be correct and allowed the complete characterization of compound **1**. The structure was established by HMBC experiments that revealed correlations of both the methyl groups at δ 1.83 (H_3-10) and δ 1.81 (H_3-13) with C-8 (δ 104.9), and of the exomethylene protons (δ 5.44 and 5.53, H_2-12) with C-6 (δ 88.3) and C-2 (δ 135.8). The HMQC results further distinguished the terminal CH_2 (C-12) from the other two olefinic CH groups (C_3 and C_8).

Table 2. ^{13}C NMR of compounds **1–6**

	1	2	3	4	5	6
1	174.1 s	174.5 s	174.8 s	174.4 s	177.4 s	176.9 s
2	135.8 s	135.4 s	135.6 s	135.0 s	28.7 t	27.4 t
3	149.0 d	149.3 d	149.2 d	149.8 d	21.9 t	21.0 t
4	78.3 d	78.2 d	78.4 d	78.7 d	81.5 d	85.9 s
5	131.6 s	33.9 t	26.9 t	27.5 t	68.1 d	76.2 d
6	88.3 s	32.0 t	136.1 d	135.4 d	130.0 d	34.5 t
7	89.1 s	133.7 d	127.9 d	126.8 d	125.7 d	39.4 t
8	104.9 d	129.1 d	57.7 d	19.0 t	128.8 d	132.8 s
9	149.5 q	63.4 d	80.1 s	81.2 s	134.7 d	122.6 d
10	21.2 q	84.1 s	65.0 s	74.6 s	32.9 t	32.5 t
11	19.2 q	73.9 d	70.2 s	71.9 s	29.3 t	38.3 t
12	122.0 t	19.7 q	76.6 s	66.2 s	31.5 t	136.8 s
13	24.9 q		63.5 d	65.6 s	22.6 t	125.7 d
14			135.8 d	60.4 s	14.1 q	25.6 t
15			117.9 t	53.1 t		29.4 t
16			19.4 q	19.8 q		21.5 t
17						13.7 q
18						23.8 q
19						15.7 q
20						15.6 q

The depicted relative stereochemistry of the dihydrofuranone ring was established on the basis of the CD spectrum. The absolute configuration at C-4 was suggested to be *R*, in agreement with that of the butenolide isolated from the Caribbean gorgonian *Pterogorgia anceps*[12] and opposite to that established for ancepsenolides[13] by comparing the CD curves. Also the negative $[\alpha]_D$ value of 1 agreed with that reported in the literature for the enantiomeric compounds,[14,15] i.e. a natural compound and an intermediate obtained in the synthesis of 3,4,5-trisubstituted furanones.

The absolute stereochemistry at C-4 in the butenolide moiety of **2** was also suggested to be *R*; the comparison of this CD spectrum with above mentioned data revealed identical profiles, indicating the same chiral butenolide chromophore. Additionally, by analogy with the ancepsenolide[13] exhibiting the same absolute stereochemistry at C-5 and C-5′, the *R* configuration at C-4 was suggested for compound **2**. Compound **3** displayed an $[\alpha]_D$ opposite to that of compounds **1** and **2**, indicating a different absolute stereochemistry. The absolute configuration of **3** is proposed to be *S* by analogy with the ancepsenolide.[13] Analogously with **3**, the absolute stereochemistry at C-4 of compound **4** was suggested to be *S* as also indicated by the CD curve, in contrast to those of **1** and **2**.

In addition to the previous acetylenic compound, a new acetylenic alcohol **2** was isolated as a colorless oil. The molecular formula for compound **2** was established as $C_{12}H_{14}O_3$ by combined HRMS and ^{13}C NMR analysis. The NMR spectra of this compound showed signals characteristic for the terminal ene-ol-yne functionality, which has been frequently found in several sponge-derived

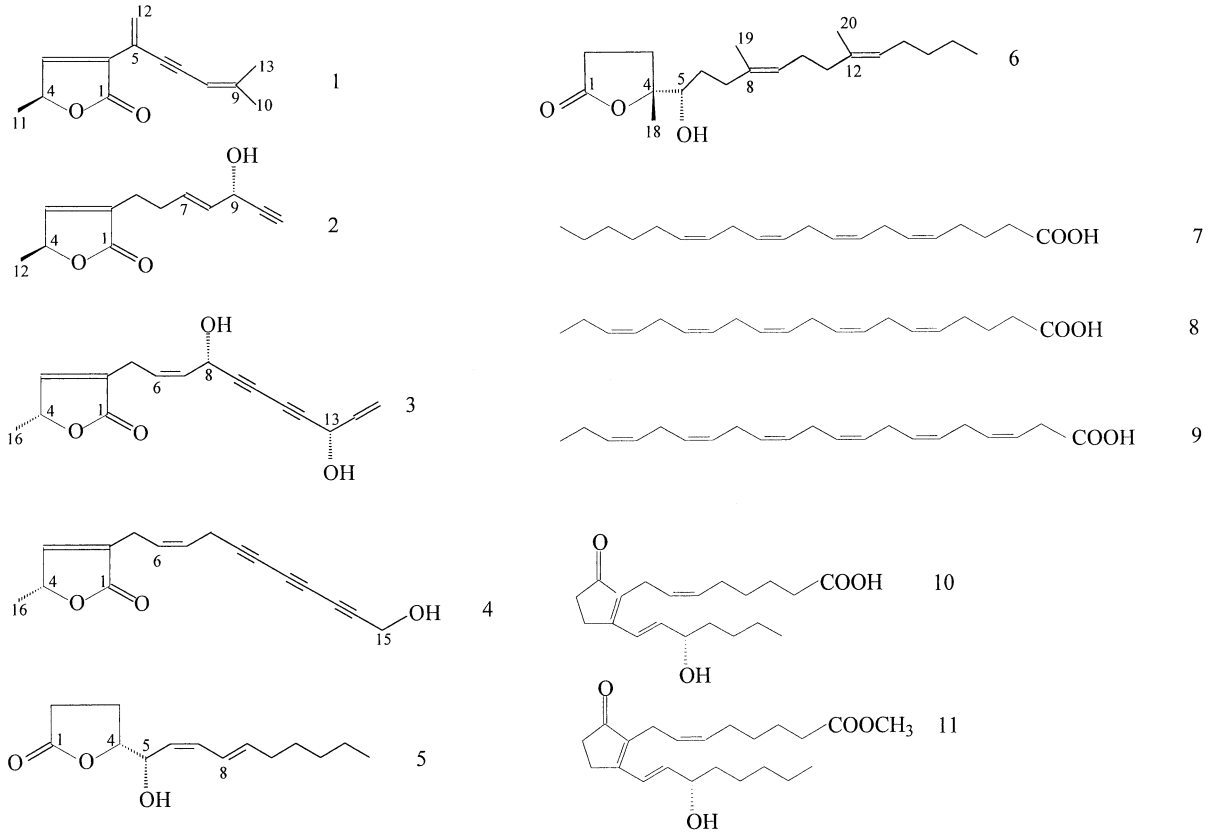

Figure 1. Structure of new butenolides, butanolides and fatty acid derivatives from soft corals of the Red Sea.

polyacetylenes.[16] The ^{13}C NMR spectra displayed signals for triple and double bonds at δ 84.1 (s), 73.9 (d) and 133.7 (d), 129.1 (d), respectively. The presence of a hydroxy group in the structure of **2** was supported by the ^{1}H NMR signal at δ 3.65 (1H, br s) and by the strong IR absorption at 3540 cm^{-1}.

With the aid of this information, the structure of **2** was determined by combined 2D NMR experiments. All the proton-bearing carbons and their protons were matched precisely by the ^{1}H COSY and HMQC experiments. The HMBC correlations between signals of the methine and olefinic protons at δ 4.82, 5.57, and 5.87, respectively, with those of neighboring carbons confirmed the presence of the terminal ene-ol-yne functionality (i.e. HMBC correlations H-11/C-9; H-9/C-11, C-10, C-8, C-7; H-8/C-10, C-9, C-6; H-7/C-9, C-6, C-5; H-6/C-8, C-7, C-5). In addition, the TOCSY experiment showed an interaction between the α-olefinic protons at δ 2.01 (H-6). Thus, the structure of **2** was defined as a linear acetylenic alcohol (1H, δ 3.65, br s, OH) containing a cyclopentanone ring. Compound **2** contained an asymmetric carbon at C-9. The absolute configuration at this center was determined by the modified Mosher method.[17] The ^{1}H-NMR spectra of the (*S*)- and (*R*)-MTPA esters, 2*S* and 2*R*, were recorded, and based on the $\Delta(\delta\ 2S-\delta\ 2R)$ values; the *S* configuration was assigned for the C-9 alcohol, see Table 4.

According to the ^{1}H NMR data, the further peak eluting from the preparative HPLC appeared to contain a novel polyacetylene (**3**). C18 HPLC yielded an oil that was analyzed as $C_{16}H_{16}O_4$ by HRMS. Its UV spectrum was similar to those of falcarinol,[18] indicative of a conjugated diyne. This was confirmed by ^{13}C NMR, which showed four nonprotonated (by DEPT) acetylene resonances and a terminal olefin δ 135.8, 117.9. Another olefin δ 136.1, 127.9 accounted for the final degree of unsaturation inherent in the molecular formula. A $^{1}H-^{1}H$ COSY experiment showed that the H-14 was coupled to two hydrogens δ 5.41 and δ 5.27 that were further coupled to a hydroxy bearing methine δ 4.94, marking the end of the spin system. In the spin system associated with the terminal olefin, the proton at δ 5.97 was *cis*-coupled to a proton at δ 5.27 and *trans*-coupled to one at δ 5.41. The COSY spectrum also showed a separate spin system that began at the allylic hydroxy bearing methine (δ 5.23). This methine was coupled to a *cis* (J=10.9 Hz) olefin resonance (δ 5.54), which was in turn coupled to its vicinal partner (δ 5.61). A correlation was observed between the latter signal and an allylic methylene. Beyond this, the spin system extended to the ring system (δ 6.90 and 5.08).

After the ^{13}C resonances had been assigned by HMQC, the partial structures were linked using an HMBC experiment. Key correlations included those observed between the carbinol proton at δ 4.94 and the acetylene carbons at δ 76.6 and 70.2. A $^{3}J_{CH}$ between the δ 4.94 hydrogen and the 76.6 resonance firmly placed the carbinol at 63.5 vicinal to the 76.6 acetylene. On the opposite side of the diyne, we observed a correlation between the δ 5.23 carbinol proton and an acetylene at δ 80.1, as well as a $^{3}J_{CH}$ between the same proton and one of the two interior alkyne carbons δ

65.0 and 70.2. A $^3J_{CH}$ between the olefin at δ 5.54 and the δ 80.1 acetylene carbon firmly placed this center on the opposite side of the diacetylene from the 76.6 resonance and dictated the placement of the 57.7 carbinol vicinal to the 80.1 signal. On the basis of the above data and by comparison with falcarinol,[18] compound **3** must have the structure depicted in Fig. 1.

The pale brown oil of **4** was determined to have the molecular formula $C_{16}H_{14}O_3$ by HREIMS.

The ^{1}H and ^{13}C NMR data of **4** are summarized in Tables 1 and 2. All one-bond ^{1}H–^{13}C connectivities were established by a heteronuclear multiple-quantum coherence (HMQC) experiment. The ^{1}H–^{1}H COSY and HMBC experiments with **4** revealed the partial structure HOC_{15}–$C_{14}\equiv C_{13}$–$C_{12}\equiv C_{11}$–$C_{10}\equiv C_9$–C_8. The methylene protons at H-5 were assigned to an oxymethylene function adjacent to a triple bond due to the characteristic high field chemical shift of C-15 (δ 53.1). The oxymethylene protons were long-range coupled to a methylene carbon C-8 (δ 19.0). The remaining six quaternary carbons C-9–C-14 were ascribed to triacetylene groups by their characteristic chemical shifts (δ_C 60.4 to 81.2) and also by UV spectrum (λ_{max}=313 nm, log ϵ=4.12). The connectivities between the partial structures thus obtained (C-5–C-8 and C-14) were elucidated by ^{13}C–^{1}H long-range correlations between the two terminal methylene protons H-8 (δ_H 3.15) and H-15 (δ_H 4.85) and the triacetylene carbons (C-9–C-14). The geometrical configurations of **4** were elucidated to be 5*Z* by the coupling constants of the olefinic protons ($J_{6,7}$=11.2 Hz). Thus, the structure of **4** was elucidated as shown in Fig. 1.

As deduced from HRMS, compound **5** had a molecular formula of $C_{14}H_{22}O_3$, which showed fragmentations typical of a substituted γ-lactone derivative having a hydroxy group. The molecular ion $[M]^+$ at *m/z* 238.1561, losing H_2O and Me, generated ions at *m/z* 220 and 205; while an alternative loss of the side chain produced the ion (base peak) at *m/z* 85. From this it was possible to identify the presence of four degrees of unsaturation or rings. IR bands typical of γ-lactone (ν_{max} 1765 cm^{-1}) and diene groups (ν_{max} 1650 cm^{-1}) were observed together with that of at least one hydroxy group (ν_{max} 3490 cm^{-1}). A conjugated diene system was indicated by a characteristic UV absorption maximum at 228 nm (log ϵ 4.32). The structural features were consistent with the signal patterns observed in ^{1}H and ^{13}C NMR spectra (Tables 2 and 3). The ^{1}H NMR spectrum showed four signals typical for olefinic protons belonging to *trans*- and *cis*-disubstituted double bonds of a diene system. In particular, the dd at δ 6.32 (H-8) was coupled in the COSY spectrum with H-9 of the *trans*-disubstituted double bond (δ 5.85) and was coupled with a methylene group (H_2-10), which resonated at δ 2.05 with typical *cis*-allylic coupling to H-8. The latter also correlated with H-7 of the *cis*-disubstituted double bond resonating at δ 6.17, which in turn was coupled with H-6 appearing at δ 5.28. The multiplicity of H-6 was due to further coupling with the H-5 of an adjacent secondary hydroxylated carbon. This resonated at δ 4.85 and was also coupled with H-4 of another oxygenated secondary carbon, which in turn appeared at δ 4.49 and was finally coupled with the protons of an adjacent methylene group (H_2-3). The methylene protons resonated as a multiplet at δ 2.25, being further coupled with the protons of another adjacent methylene group (H_2-2) that appeared as a further multiplet at δ 2.55. The chemical shifts of the protons of the two methylene groups as well as that of H-4 were in good agreement with the values reported for γ-lactone derivatives.[19] The presence of this partial structure in **5** was consistent with two residual degrees of unsaturation calculated considering the diene system and the very typical γ-lactone shift at δ 177.4 (C-1) observed in its ^{13}C NMR spectrum for the carbonyl group. As also expected, ^{13}C chemical shifts measured for the hydroxylated secondary carbon closing the butanolide ring (C-4) and the two-methylene groups (C-2 and C-3) agreed with the values reported for γ-lactone derivatives.[19] In addition to the four doublets typical of the carbons of the diene system, the ^{13}C NMR spectrum also showed those of the other secondary hydroxylated carbon (C-5), four methylenes and the methyl group (C-14) at δ 68.1, interval from 22.6 to 32.9 and 14.1, respectively.

Compound **5** was converted to the (*R*)-(+)-(MTPA) (**5a**) and the (*S*)-(−)-MTPA (**5b**) esters. Comparison of the ^{1}H-NMR data (Table 4) showed a downfield shift (Δ −42.3 Hz)

Table 3. ^{1}H NMR of compound **5** and **6**

	5	**6**
1	–	–
2	2.55 (1H, m)	2.49 (2H, m)
3	2.25 (1H, m	2.24 (2H, m)
4	4.49 (1H, dt, *J*=7.2, 3.1 Hz)	–
5	4.85 (1H, dd, *J*=8.4, 3.1 Hz)	4.54 (1H, *J*=dd, 10.1, 2.1 Hz)
6	5.28 (1H, dd, *J*=11.1, 8.4 Hz)	1.54 (1H, m) and 1.39 (1H, m)
7	6.17 (1H, dd, *J*=11.5, 11.1 Hz)	2.20 (1H, m) and 2.09 (1H, m)
8	6.32 (1H, dd, *J*=14.4, 11.5 Hz)	–
9	5.85 (1H, dq, *J*=14.4, 7.0 Hz)	5.15 (1H, dd, *J*=6.8, 6.7 Hz)
10	2.05 (2H, m)	2.14 (2H, m)
11	1.31 (2H, m)	2.09 (2H, m)
12	1.31 (2H, m)	–
13	1.31 (2H, m)	5.04 (1H, dd, *J*=6.7, 6.4 Hz)
14	0.89 (3H, t, *J*=6.8 Hz)	2.07 (2H, m)
15		1.29 (2H, m)
16		1.29 (2H, m)
17		0.91 (3H, t, *J*=6.9 Hz)
18		1.12 (3H, s)
19		1.56 (3H, brs)
20		1.55 (3H, brs)

Table 4. ^{1}H NMR (500 MHz) spectral data for Mosher's esters ($CDCl_3$)

	Compound No (Δ in Hz)			
Hydrogen No	**2**	**3**	**5**	**6**
3	–[a]	–	+4.8	+4.2
4	–	–	+14.9	–
5	+10.6	−9.1	–	–
6	+14.9	−15.3	−42.3	−35.9
7	+27.4	−41.8	−13.2	−24.6
8	+46.1	–	−7.1	–
9	–	–	−3.8	−8.1
11	−16.7	–	–	–
15	–	−36.3 (α), −27.6 (β)	–	–
18	–	–	–	+10.7

[a] Resonances of H directly attached to esterified carbon are not analyzed or values <|3 Hz| have not been included.

Table 5. Bioactivities of γ-lactones (**1**–**6**)

Test organism	1	2	3	4	5	6
Staphylococcus aureus[a]	11.5	13.2	8.5	10.3	07.8	18.6
Bacillus subtilis[a]	13.0	14.9	7.6	13.9	05.6	14.7
Escherichia coli[a]	00	00	0	00	00	00
Saccharomyces cerevisiae[a]	00	00	0	00	00	00
Artemia salina[b,c]	08.3	61.5	0.8	03.2	15.3	21.4

[a] Samples (10 μg) were applied on 50.8 mm paper disks, values are diameters (mm) of inhibitory zones.
[b] In μg ml^{-1} (minimum lethal doses).
[c] The details in Section 4.

of H-6 along with an upfield shift (Δ 14.9 Hz) of H-4. These data, in conjunction with the literature data, allowed the assignment of the *S*-configuration at C-5 in compound **5**.

Compound **6** was isolated as an optically active pale yellow oil $[\alpha]_D=+132.8$. The result of HREIMS was consistent with the molecular formula $C_{20}H_{34}O_3$ that has four degrees of unsaturation. The infrared spectrum showed the presence of an alcohol group (3360 cm^{-1}, br) and a carbonyl group (five membered γ-lactone, 1770 cm^{-1}). ^{13}C NMR spectroscopy (Table 5) indicated the presence of four sp^2-carbons leaving two double-bond equivalents; the rest must be accommodated by one ring.

The ^1H NMR spectrum showed two resonances at δ 5.04 (dd, *J*=6.7, 6.4 Hz) and 5.15 (dd, *J*=6.8, 6.7 Hz) typical of the monosubstituted sp^2-carbons in isoprenoids. DQF-COSY connections to these signals allowed the assignment of the partial structure **A** (CH_2–CH=C–CH_3). Analysis of the correlations starting from a downfield methine signal (δ_H 4.54, dd, δ_C 76.2) allowed the assignment of another partial structure (O–CH–CH_2–CH_2–), i.e. partial structure **B**. The downfield singlet carbon (C-4; δ 85.9) was shown to be attached to the end of partial structure **B** and, further, to the downfield oxygenated carbon (H-5, δ 4.54, dd) of partial structure **B**. Carbon **4** also showed a correlation to a methyl singlet (CH_3-4, i.e. H-18, δ 1.12) which itself has correlations in both the HMBC and DQF-COSY spectra to H-3 and H-5, respectively. The chain of connectivities could be continued from methylene carbon **7** to partial structure (**A**) (HMBC connections to H-9 and C-19). Further HMBC connections showed that the two partial structures (**A**) were joined between C-10 and C-12 through a methylene group (H-11, δ 2.09). This sequence of correlations results in an isoprenoid partial structure (**C**) [CH_3–C(*R*)=CH–CH_2–CH_2–C(CH_3)=CH–CH_2–CH_2–] and leaves one ring, three sp^2-carbons, and methyl group, as well as a yet to be determined number of oxygen atoms to be assigned.

The completion of the structure of the molecule required the location of the ring. NOESY correlations between the methyl group on C-4 and methine proton H-5 suggested the presence of a cyclic ester (lactone).

Participation of the oxygen on C-5 in a lactone was rejected since esterification occurred at this position (Fig. 1) because its ^{13}C NMR chemical shift is more consistent with an alcohol functionality. The lactone linkage is thus between C-1 and C-4.

The stereochemistry of both double bonds is clearly (*E*) based on the relative upfield shift of the vinylic methyl groups (δ 15.7 and 15.6) and NOESY correlations between these methyl groups and the methylene groups at C-10 and C-14. NOESY also assisted in the determination of the relative stereochemistry around the butanolide ring. Correlations between C-18 and H-5 suggested that the substituents at C-4 and C-5 were in a *cis* relationship. The absolute configuration at C-5 was determined by esterification of the alcohol at C-5 with (*S*)- and (*R*)-MTPA acid chloride. Analysis of the δ values of the ^1H NMR signals of the diastereomeric MTPA esters showed that the configuration at C-5 is *S* and thus the absolute stereochemistry of compound (**6**) is (4*R*, 5*S*), see Fig. 1.

The toxicity of compounds **1**–**6** was tested in the *Artemia salina* shrimp bioassay. The γ-lactones (**1**–**6**) showed bioactivity (see Table 5).

Table 5 also indicates that the γ-lactones were active only against Gram-positive bacteria and they were inactive against Gram-negative bacteria and yeast.

3. Conclusion

The γ-lactone nature of **1**–**6** is rare in natural products. However previous chemical studies conducted on different species of soft corals revealed that γ-lactones were the most abundant metabolites.[13,20–22] These compounds are closely related to butenolides and tetramic acids, well known as plant, fungal, and lichen metabolites[23] that also exhibit interesting biological activities, e.g. the 3,4-dialkylbutenolides (seiridins) isolated from three species of *Seiridium*.[24]

4. Experimental

4.1. General experimental procedures

UV spectra were measured by a Cary 118 (Varian) apparatus in EtOH within the range of 200–350 nm. Circular dichroism (CD) measurement was carried out on a Jasco-500A spectropolarimeter at 24°C, under dry N_2. A Perkin–Elmer Model 1310 (Perkin–Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of compounds as neat films. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^1H), 125.7 MHz (^{13}C). High- and also low-resolution MS were recorded using a VG 7070E - HF spectrometer (70 eV). GC-MS of the fatty acid methyl esters was done using a Finnigan 1020 B single-state quadrupole GC–MS instrument in the EI mode. A C_{18} reversed phase column (5 μm, 7.8×250 mm^2, Supelco, USA), was employed. A linear gradient from 20% H_2O and 80% acetonitrile to 1% water and 99% acetonitrile over 25 min, flow rate 2 ml min^{-1} was used to separate all the compounds in the crude extract. Compounds (**1**–**11**) were detected by UV absorption at 208 nm.

4.2. Animal materials

The greenish-gray *S. trocheliophorum* was collected from

15 m under water on rocks while the yellowish-white *L. arboreum* was collected from 10 m deep, also on rocks. The voucher specimens are deposited in the collection of the second author (V. M. Dembitsky).

4.3. Extraction and isolation

Fresh corals (500 g wet weight each) were put into ethanol and stored at −10°C, concentrated under nitrogen and extracted separately into CH_2Cl_2. The total lipid extracts, viscous dark oils, were subjected separately to a Sephadex LH-20 chromatographic column with CH_2Cl_2–MeOH (1:5 v/v). *S. trocheliophorum* yielded two fractions. After further separation by semi-preparative RP-HPLC (see above), the first fraction was found to be composed of compounds **1**–**4**, whereas the second one contained acids, i.e. compounds **7**–**10**. *L. arboreum* gave three fractions, the first consisting of compounds **5** and **6**, the second containing again compounds **7**–**9** and the third yielding only one compound (**11**), as determined by RP-HPLC, see also Section 4.

4.4. (*S*)-MTPA esters

To a stirred solution of ~1.0 mg of the hydroxy compound in 0.3 ml dry pyridine was added 20 μl of (−)-MTPA chloride. The mixture was stirred under N_2 at room temperature for 1 h and the solvent was then removed by blowing with N_2. The residue was redissolved in 2 ml of EtOAc–hexane and filtered through a Sep-Pak silica column. After removing the solvent under vacuum, the residue was separated by reversed-phase HPLC (ODS column, 100% MeCN) to yield ~1.0 mg of *S* ester as a colorless gum; ^{1}H NMR spectra ($CDC1_3$), see Table 4.

4.5. (*R*)-MTPA esters

Prepared as described for *S* esters. An amount of ~1.0 mg of compound and 20 μl of (+)-MTPA chloride gave 0.9 mg of *R* esters as a colorless gum; ^{1}H NMR spectra ($CDCl_3$), see Table 4.

The following compounds were isolated from *S. trocheliophorum* (mg/500 g wet weight): **1** (9.2), **2** (3.9), **3** (5.6), **4** (7.3), **7** (16.5), **8** (12.1), **9** (7.8) and **10** (1.8); and from *L. arboretum*: **5** (4.7), **6** (8.1), **7** (10.6), **8** (14.7), **9** (5.4) and **11** (2.0).

Eicosatetraenoic (**7**), eicosapentaenoic (**8**) and docosahexaenoic (**9**) acids were pale yellow oils. Methyl esters were prepared as follows: The acids were methylated by heating them with 5% anhydrous HCl in MeOH, and the fatty acid methyl esters were analyzed by GC-MS. A fused silica capillary column of chemically bonded liquid phase (Supelcowax 10, 0.2 mm ID, 60 m length, Supelco) was used and helium carrier gas at a flow rate of 0.35 ml min^{-1}. The column temperature was programmed from 50°C held for 1 min, to 100°C at a rate of 10°C min^{-1}, and then raised to the final hold temperature of 270°C at a rate of 5°C min^{-1}. The mass spectra of methyl esters agreed with previously published data.[25]

4.5.1. (5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxo-prosta-5,8(12),13-trien-1-oic acid (10). Dark brown liquid; $[\alpha]_D^{23}=+16.3$ (*c* 0.024, $CHCl_3$); physical and spectral (IR, ^{1}H, ^{13}C NMR and MS) data of the compound agreed with those of PGB_2 acid.[10]

4.5.2. Methyl (5*Z*,13*E*,15*S*)-15-hydroxy-9-oxo-prosta-5,8(12)-13-trien-1-oate (11). Light brown liquid; $[\alpha]_D^{23}=+17.8$ (*c* 0.02,$CHCl_3$); physical and spectral (IR, ^{1}H,^{13}C NMR, and MS) data of the compound agreed with those of PGB_2 ester.[11]

1 is pale yellow oil, $[\alpha]_D=-38.5$ (*c* 0.12, EtOH); UV λ_{max} (EtOH) (log ϵ) 350 (3.24), 277 (3.87), 263 (3.94), 229 (4.15), 214 (4.19) 204 (4.27); CD (EtOH) λ_{max} (nm) ($\Delta\epsilon$) 210 (−3.24); IR (film) ν_{max} 3450, 2950, 2910, 1750, 1680 cm^{-1}; HREIMS (*m/z*) 202.0990 (calcd for $C_{13}H_{14}O_2$ 202.0994). NMR data, see Tables 1 and 2.

2 is colorless oil, $[\alpha]_D=-27.6$ (*c* 0.07, EtOH); UV λ_{max} (nm) (EtOH) (log ϵ) 212 (3.17) (nm); CD (EtOH) λ_{max} (nm) ($\Delta\epsilon$) 208 (−0.54); IR (KBr) ν_{max} 3540, 2930, 1740, 1625 cm^{-1}; HREIMS (*m/z*) 206.0938 (calcd for $C_{12}H_{14}O_3$ 206.0943); NMR data, see Tables 1 and 2.

3 is slightly yellow oil, $[\alpha]_D=+47.1$ (*c* 0.11, EtOH); UV λ_{max} (EtOH) (log ϵ) 260 (2.71), 245 (2.10), 235 (2.97), 220 (3.15) (nm); CD (EtOH) λ_{max} (nm) ($\Delta\epsilon$) 210 (+0.95); IR (KBr) ν_{max} 3340, 2960, 2230, 2150, 1740, 1710 cm^{-1}; HREIMS (*m/z*) 272.1043 (calcd for $C_{16}H_{16}O_4$ 272.1048); NMR data, see Tables 1 and 2.

4 is pale brown oil, $[\alpha]_D=+39.5$ (*c* 0.14, EtOH); UV λ_{max} (EtOH) (log ϵ) 313 (4.12) (nm); CD (EtOH) λ_{max} (nm) ($\Delta\epsilon$) 208 (+0.76); IR (KBr) ν_{max} 3430, 2220, 1740, 1700, 1630 cm^{-1}; HREIMS (*m/z*) 254.0937 (calcd for $C_{16}H_{14}O_3$ 254.0943); NMR data, see Tables 1 and 2.

5 was isolated as pale yellow oil, $[\alpha]_D=+98.3$ (*c* 0.24, EtOH); UV λ_{max} (EtOH) (log ϵ) 228 (4.32) (nm); IR (KBr) ν_{max} 3490, 1765, 1650 cm^{-1}; HREIMS (*m/z*) 238.1561 (calcd for $C_{14}H_{22}O_3$ 238.1569); NMR data, see Tables 2 and 3.

6 is pale yellow oil, $[\alpha]_D=+132.8$ (*c* 0.37, EtOH); UV λ_{max} (EtOH) (log ϵ) 217 (3.49) (nm); IR (KBr) ν_{max} 3360, 2950, 1770 cm^{-1}; HREIMS (*m/z*) 322.2501 (calcd for $C_{20}H_{34}O_3$ 322.2508); NMR data, see Tables 2 and 3.

4.6. Brine shrimp toxicity bioassay

The sample (~0.05 mg) was dissolved in 50 μl of DMSO and added to a test vial of artificial seawater (3.0 ml). Approximately 20 brine shrimp, *Artemia salina*, were added to the vial. The brine shrimp were observed periodically over a 24 h period. A positive assay was the death of all brine shrimp.

4.7. Antibacterial tests

The test organisms were *Bacillus subtilis* (CCM 2216) and *Staphyloccocus aureus* (CCM 2551) (CCM—Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried according to the literature.[26] The amounts used were 50 μg of compound per test disk (see Table 5).

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