Four New Derivatives of Trihomononactic Acids from *Streptomyces globisporus*

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New compounds, namely a homolog of nonactic acid (trihomononactic acid), its lactone and dilactones were isolated from a strain of *Streptomyces globisporus*. Their structures, including the absolute configurations of the hydroxy and methyl groups, were determined by spectroscopic techniques such as UV, IR, MS, 1D and 2D NMR spectroscopy. All compounds were highly active against Gram-positive bacteria and brine shrimp.

Introduction

Macrotetrolides of the nonactin-, monactin-, dinactin-, trinactin-, and tetranactin-type, which have been isolated from a variety of *Streptomyces* species, are cyclotetralactones that contain nonactic acid and homononactic acid structural units. With the exception of nonactin they exhibit, in addition to antibacterial and antifungal activity, remarkable acaricidal, insecticidal, coccidiostatic, and anthelminthic effects.[1] Pamamycins are structurally unique natural products that exhibit autoregulatory effects, and antibiotic and anionophoric activities. They were first isolated[2] from *Streptomyces alboniger* in 1979. The mixture of pamamycins obtained from *S. alboniger* consists of at least eight homologs that are difficult to separate.[3] Pamamycin molecules contain homologs of nonactic acid as part of their structure.[4]

In our search for new antibiotic compounds from soil microorganisms,[5,6] we have cultivated several cultures of *Streptomyces globisporus* that produce nonactin-type compounds. After most of the main products had been removed by crystallization and gradient elution on a highly effective column (≈ 10,600 plates, 10 cm), we separated and identified four new compounds by LC−MS/APCI (liquid chromatography−mass spectrometry with atmospheric pressure chemical ionization), that is, trihomononactic acid, its lactone, and dilactones.

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Results and Discussion

After repeated crystallization of the major part of the nonactin, monactin, dinactin, and trinactin mixture derived from *S. globisporus*,[7] the mother liquors were separated by semipreparative normal and reversed phase HPLC. A total of four compounds were obtained. In addition to the known products, the following new compounds were isolated.

Compound **1** (Figure 1) was homogeneous by LC-MS/APCI and appeared as a white oil. Its UV spectrum showed only an end absorption (at $\lambda_{max.}$ = 207 nm), which confirms the absence of conjugated double bonds or aromatic cycle(s). Its IR spectrum revealed only the presence of ester (lactone at 1740 cm^{-1}) and ether (1070 and 1040 cm^{-1}) functional groups. HRFABMS showed a pseudomolecular ion $[M + Na]^+$ at m/z = 433.2570, which is consistent with a molecular formula of $C_{23}H_{38}O_6Na$ (Δ = 0.4 ppm).

The ^1H NMR spectrum (Table 1) of the dilactone **1** was very similar to that of the macrolides,[8,9] but a few of the resonance and splitting patterns were different. The ^1H and ^{13}C NMR spectra were extensively analyzed by $^1H-^1H$ and $^1H-^{13}C$ COSY (Correlated Spectroscopy) and two partial structures, ***I*** and ***II***, were deduced, as shown in Figure 2. Four of the six oxygen atoms in the molecule were assigned to two lactone groups (^{13}C NMR: δ = 174.6 (s), 75.4 (d), 173.5 (s), 71.2 (d) ppm; $\tilde{\nu}_{max.}$ = 1740 cm^{-1}), and the other two to ethereal oxygen atoms as no OH absorption was observed in the IR spectrum. The ^1H NMR spectrum also showed no exchangeable hydrogen atoms, which confirms the absence of OH groups and any other carbonyl signals in the ^{13}C NMR spectrum.

NOE (nuclear Overhauser effect) experiments revealed the spatial proximity of two pairs of two protons, that is, "3-H/6-H", and "3′-H/6′-H"; the two protons in each pair were connected by five covalent bonds. This suggests the presence of two tetrahydrofuran rings. This was confirmed

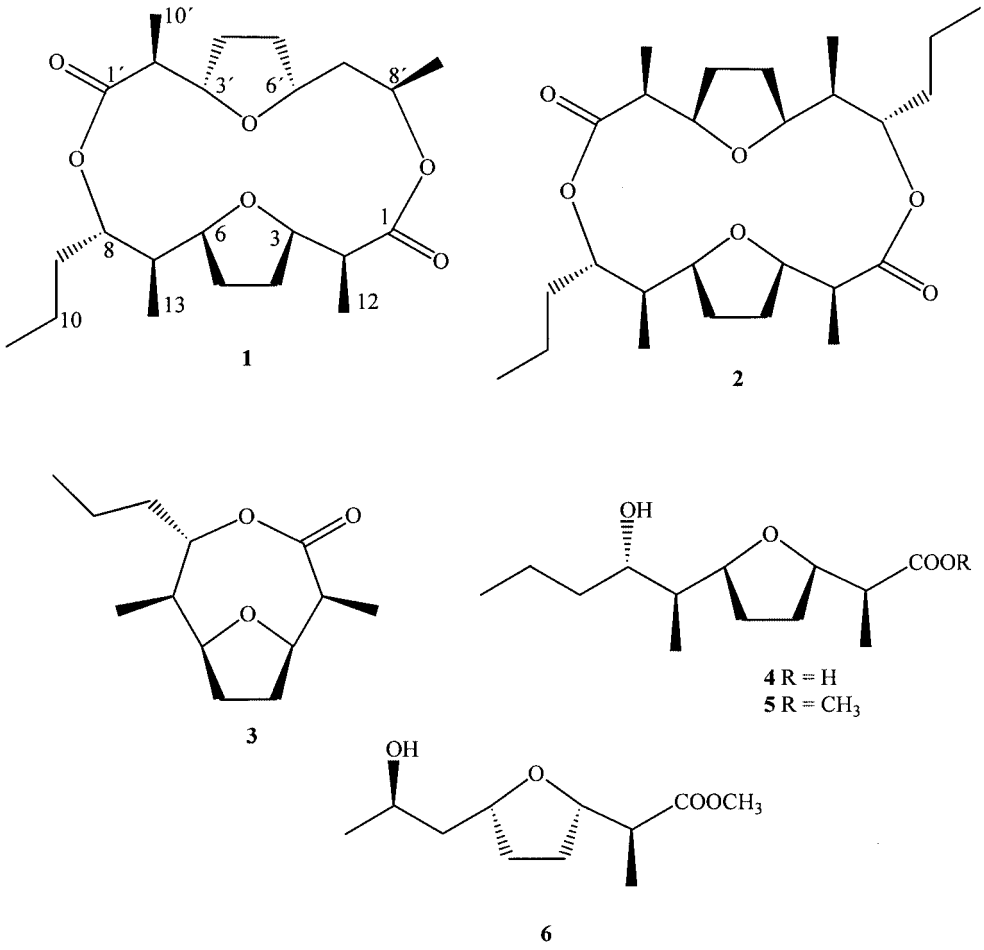

Figure 1. Structures of dilactone **1** consisting of nonactic and trihomononactic acids, cyclic dimer **2** of trihomononactic acid, the lactone **3** of trihomononactic acid, trihomononactic acid (**4**), and methyl esters of homononactic (**5**) and nonactic (**6**) acids from *Streptomyces globisporus*

by high $^1J_{C,H}$ values for "C-3−3-H" (131.8 Hz), "C-6−6-H" (133.1 Hz), "C-3′−3′-H" (133.1 Hz), and "C-6′−6′-H" (133.1 Hz); all these *J* values are characteristic of the tetrahydrofuran ring.[10] Since the molecule consists of two parts, ***I*** and ***II***, connected by two ester bonds, the C-l atom (carbonyl of ***I***) should bind with the "C-8′" atom of ***II*** and the "C-l′" carbonyl of ***II*** should bind with the "C-8" of ***I***. Thus, an 18-membered macrodiolide ring was formed and its structure was determined, as shown in Figure 1.

The relative stereochemistry of compound **1** was determined by NOE difference spectroscopy and its $J_{H,H}$ values. Irradiation of "9′-H_3" enhanced the signals of "3′-H" and "6′-H", whilst irradiation of "12-H_3" enhanced the signals of "8-H" and "13-H_3", which indicates that "8-H", "3′-H", "6′-H", "9-H_3", "12-H_3", and "13-H_3" are on the upper side of the macrodiolide ring. It follows from the value of $^3J_{2\text{-H}'-3\text{-H}'}$, of 1.5 Hz that the angle between these hydrogen atoms is approximately 90° and that "10′-H_3" is also on the upper side of the macrodiolide ring.

The NOE enhancements between "3-H−6-H" and "3′-H−6′-H" demonstrated the presence of *cis*-substituents in the α,α′-positions of the two tetrahydrofuran rings. NOE enhancements were not observed between "3-H−6′-H" and "6-H−3′-H", which indicates that "H-3", "6-H" and "3′-H", "6′-H" are located on opposite sides of the macrodiolide ring. Additional stereochemical features were determined by NOE enhancements and *J* values (see Table 1). In this way the structure of compound **1** and its relative stereochemistry were elucidated, as shown in Figure 1. All the *J* values are in good agreement with this proposed relative stereochemistry.

The absolute stereochemistry of compound **1** was assigned on the basis of the conversion of methyl nonactate and methyl trihomononactate (obtained after hydrolysis and esterification) into (*S*)- and (*R*)-MTPA (α-methoxy-α-trifluoromethylphenylacetic) derivatives (Figure 3) followed by the application of the advanced Mosher method.[11] By comparison of the ^{1}H NMR signals of the (*S*)- and (*R*)-MTPA derivatives assisted by spin decoupling experiments, Δδ values ($\Delta\delta = \delta_S - \delta_R$) were calculated. The protons in the MTPA ester of methyl trihomononactate with positive Δδ values were located on the right side of the MTPA ester group, and those with negative Δδ values were located on the left (Figure 3). In this arrangement of the molecule, the MTPA group is oriented towards α, and thus the absolute configuration at "C-8" of methyl trihomononactate is (*S*).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for dilactone **1**

Atom no.	δ_H (ppm)	δ_C (ppm)
1	–	174.6 (s)
2	2.68 (dq, J = 3.6, 7.0 Hz, 1 H)	42.2 (d)
3	4.17 (dtq, J = 3.6, 9.0, 2.7 Hz, 1 H)	79.3 (d)
4	1.72 (m, 1 H)	29.2 (t)
	1.79 (m, 1 H)	
5	1.73 (m, 1 H)	28.5 (t)
	1.80 (m, 1 H)	
6	4.18 (dtq, J = 9.0, 3.6, 2.7 Hz, 1 H)	77.9 (d)
7	1.07 (ddq, J = 10.0, 6.8, 3.6 Hz, 1 H)	36.8 (d)
8	4.29 (ddd J = 10.0, 9.5, 4.8 Hz, 1 H)	75.4 (d)
9	1.47 (m, 1 H)	37.6 (t)
	1.65 (m, 1 H)	
10	1.48 (m, 2 H)	18.4 (t)
11	0.95 (t, J = 6.9 Hz, 3 H)	14.0 (q)
12	1.14 (dd, J = 7.0, 2.7 Hz, 3 H)	9.3 (q)
13	0.75 (dd, J = 6.8, 2.7 Hz, 3 H)	10.2 (q)
1′	–	173.5 (s)
2′	2.66 (dq, J = 3.6, 7.0 Hz, 1 H)	41.9 (d)
3′	4.18 (dtq, J = 9.0, 3.6, 2.7 Hz, 1 H)	78.0 (d)
4′	1.97 (m, 1 H)	25.6 (t)
	2.07 (m, 1 H)	
5′	1.71 (m, 1 H)	30.7 (t)
	1.81 (m, 1 H)	
6′	4.53 (m, 1 H)	74.2 (d)
7′	1.70 (m, 1 H)	39.3 (d)
8′	5.20 (tq, J = 7.0, 6.4 Hz, 1 H)	71.2 (d)
9′	1.30 (d, J = 6.4 Hz, 3 H)	13.4 (q)
10′	1.13 (dd, J = 7.0, 2.7 Hz, 3 H)	9.6 (q)

The Δδ values were reversed in the MTPA ester of methyl nonactate [it thus follows that the absolute configuration at "C-8′" is (*R*)] and, therefore, the absolute configurations of "C-8" and "C-8′" in compound **1** have been demonstrated to be (*S*) and (*R*), respectively. Further, the absolute configurations of both methyl esters were determined by optical rotation. The two methyl esters had positive optical rotation values ($[\alpha]_D = +7.4 \times 10^{-1}$ for **5** and $[\alpha]_D = +14.6 \times 10^{-1}$ for **6**, respectively), which is in good agreement with previously published data.[12,13] Therefore, the absolute configurations of the nine chiral centers in dilactone **1** were demonstrated to be (2*S*,3*R*,6*S*,7*R*,8*S*,2′*S*,3′*S*,6′*R*,8′*R*).

The structure of **2** was determined by HRFABMS; $[M + Na]^+$ at m/z = 475.3041 is consistent with a molecular formula of $C_{26}H_{44}O_6$. However, the ^{1}H and ^{13}C NMR spectra revealed signals and correlations for only 13 carbon atoms (see Table 2), which indicates the presence of two trihomononactic groups. Consequently, it was proposed that **2** consists of two asymmetrically arranged units, as illustrated in Figure 1.

Similarly, the molecular formula of **3** was determined by HRFABMS; the molecular ion $[M + Na]^+$ at m/z = 249.1471 is consistent with a molecular formula of $C_{13}H_{22}O_3$. The ^{1}H and ^{13}C NMR spectra of **3** are remarkably similar to those of the synthesized compound **4**, which has been described previously.[14] The ^{1}H and ^{13}C NMR spectra of **3** show 13 carbon atoms derived from one molecule of trihomononactic acid (see Table 2).

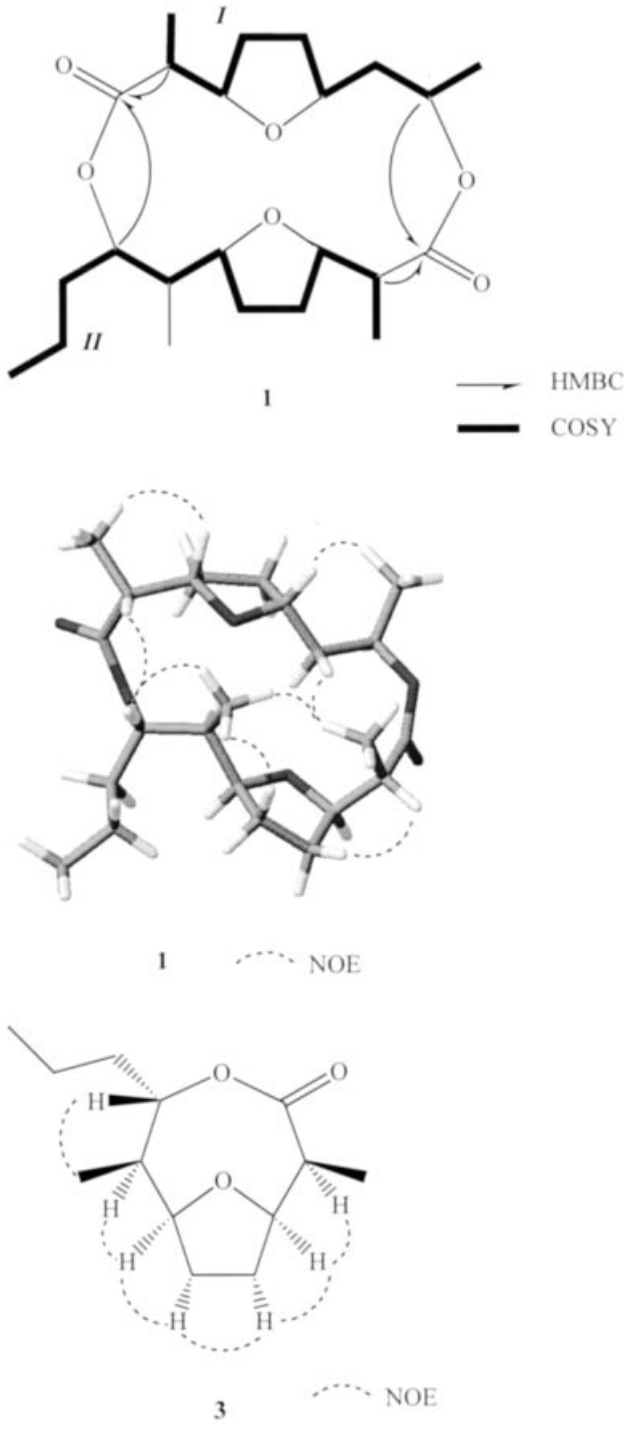

Figure 2. The HMBC (heteronuclear multiple bond correlation), COSY, and NOE correlations of dilactone **1** and monolactone **3**

The MTPA esters of the hydrolyzed products of compounds **2** and **3** had optical rotations and ^{1}H NMR spectroscopic data identical to those of the longer MTPA derivative (trihomononactate) of compound **1**. Thus, both compounds **2** and **3** have the same absolute stereochemistry as the trihomononactate.

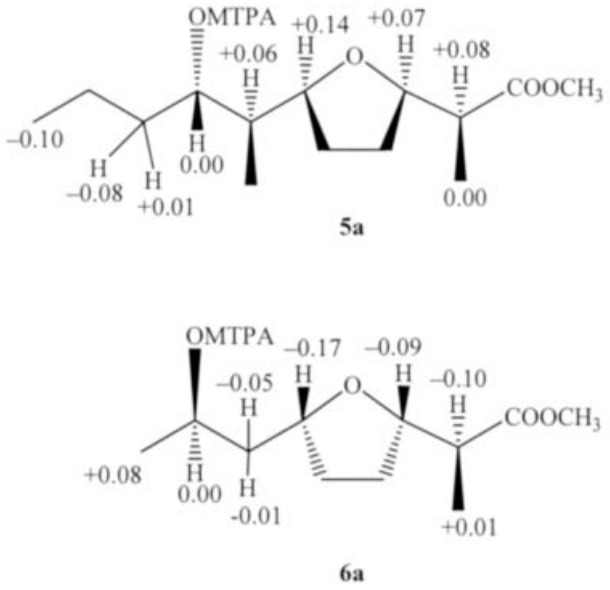

Figure 3. ^{1}H NMR chemical shift differences Δδ of the MTPA esters of trihomononactate (**5a**) and nonactate (**6a**)

Compound **4**, identified as the methyl ester **5**, has similar physicochemical values to previously published data.[13] The rather large amounts of compound **5**, as compared to the yields of dilactones and lactone, indicate that the lactones originate by spontaneous cyclization. However, all the antibiotics were detected by LC−MS/APCI of the crude extract and, therefore, they are not artifacts originating during their isolation.

The activities of the pure antibiotics against several species of bacteria and fungi are shown in Table 3. High activities were observed against Gram-positive bacteria. Moderate-to-low activities against several other genera of bacteria and fungi, including *Escherichia coli* and *Saccharomyces cerevisiae*, were found. These antibiotics, which are like a crown ether (Figure 1) containing oxygen atoms in the molecule, binds the sodium cation. Several nonactin and macrolide antibiotics are known to exhibit strong insecticidal properties.[1] The new antibiotics are structurally related to previously described compounds,[6] and some of their biological properties could have been expected. Since

Table 2. ^{1}H and ^{13}C NMR spectroscopic data for dilactone **2** and lactone **3**

Atom no.	**2**		**3**	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	–	174.6 (s)	–	174.5 (s)
2	2.65 (dq, J = 3.6, 7.0 Hz, 1 H)	42.2 (d)	2.63 (dq, J = 3.6, 7.0 Hz, 1 H)	41.8 (d)
3	4.17 (dtq, J = 3.6, 9.0, 2.7 Hz, 1 H)	79.3 (d)	4.18 (dtq, J = 3.6, 9.0, 2.7 Hz, 1 H)	76.0 (d)
4	1.72 (m, 1 H)	29.2 (t)	1.75 (m, 1 H)	29.2 (t)
	1.79 (m, 1 H)	1.82 (m, 1 H)		
5	1.73 (m, 1 H)	28.5 (t)	1.80 (m, 1 H)	29.5 (t)
	1.80 (m, 1 H)	1.89 (m, 1 H)		
6	4.18 (dtq, J = 9.0, 3.6, 2.7 Hz, 1 H)	77.9 (d)	4.12 (dtq, J = 9.0, 3.6, 2.7 Hz, 1 H)	78.4 (d)
7	1.07 (ddq, J = 10.0, 6.8, 3.6 Hz, 1 H)	36.8 (d)	1.04 (ddq, J = 10.0, 6.9, 3.6 Hz, 1 H)	36.5 (d)
8	4.29 (ddd, J = 10.0, 9.5, 4.8 Hz, 1 H)	74.6 (d)	4.38 (ddd, J = 10.0, 9.5, 4.8 Hz, 1 H)	74.0 (d)
9	1.47 (m, 1 H)	37.6 (t)	1.47 (m, 1 H)	36.7 (t)
	1.65 (m, 1 H)	1.66 (m, 1 H)		
10	1.48 (m, 2 H)	18.4 (t)	1.49 (m, 2 H)	17.8 (t)
11	0.95 (t, J = 6.9 Hz, 3 H)	14.0 (q)	0.95 (t, J = 6.9 Hz, 3 H)	14.5 (q)
12	1.14 (dd, J = 7.0, 2.7 Hz, 3 H)	9.3 (q)	1.13 (dd, J = 7.0, 2.7 Hz, 3 H)	9.6 (q)
13	0.75 (dd, J = 6.8, 2.7 Hz, 3 H)	10.2 (q)	0.75 (dd, J = 6.9, 2.7 Hz, 3 H)	9.9 (q)

Table 3. Biological activities of derivatives **1**−**4** and standards

Compound	Test organism				
	Staphylococcus aureus[a]	*Bacillus subtilis*[a]	*Escherichia coli*[a]	*Saccharomyces cerevisiae*[a]	*Artemia salina*[b] [c]
1	66.2 ± 9.5[d]	75.3 ± 5.4	< 0.1	<0.1	6.3 ± 4.1
2	58.7 ± 5.7	52.9 ± 5.3	< 0.1	< 0.1	12.8 ± 1.3
3	51.1 ± 9.3	48.5 ± 3.8	< 0.1	< 0.1	10.0 ± 1.4
4	49.4 ± 6.7	39.4 ± 4.1	< 0.1	< 0.1	56.9 ± 5.1
Ivermectin	<0.1	<0.5	< 0.1	< 0.1	102.0 ± 13.2
Penicillin G	65.6 ± 12.8	47.5 ± 3.0	< 0.1	< 0.1	> 1000
Streptomycin	< 0.1	< 0.1	96.2 ± 4.9	< 0.1	> 1000
Amphotericin B	< 0.1	< 0.1	< 0.1	42.8 ± 3.1	> 1000

[a] Samples (10 μg) were applied to 6.3 mm paper disks; the values are diameters (mm) of inhibitory zones. [b] In ng·mL^{-1} (LC_{50}). [c] Details are given in the Expt. Sect. [d] The drug potencies were determined and the values represent values (mean ± S.D.) from five independent observations.

the new 18-membered macrodiolide exhibits strong activity against brine shrimp (*Artemia salina*), the efficacy of this compound against different diseases in humans and animals should be tested.

Conclusions

In this paper, we have described for the first time the cyclic lactone and dilactones that contain nonactic and mainly trihomononactic acids as structural units. For the first time compounds containing these two acids in their molecule have been isolated from natural sources.

Experimental Section

UV spectra were measured with a Cary 118 (Varian) apparatus in MeOH in the range of 200−350 nm. Infrared spectra of the methyl esters were recorded as neat films with a Perkin−Elmer Model 1310 (Perkin−Elmer, Norwalk, CT) infrared spectrometer. Optical rotations were measured with a Perkin−Elmer 243 B polarimeter. NMR spectra were recorded with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). The positive-ion and FAB mass spectra were recorded with a VG 7070E-HF spectrometer (Micromass, Manchester, UK).

LC−MS/APCI was performed as reported previously.[6] Briefly, the HP 1090 series (HP, 1090 series, Hewlett−Packard, U.S.A.) was used with two columns (HIRPB-250AM, 250 mm length × 2.1 mm I.D., 5 μm phase particle, ca. 26500 plates, 25 cm). A quadruple mass spectrometer system Navigator (Finnigan MAT, San Jose, CA, U.S.A.) was used: vaporizer temperature 400 °C, capillary heater temperature 220 °C, corona current 5 μA, sheath gas high-purity nitrogen, pressure approximately 380 kPa, and auxiliary gas (also nitrogen) flow rate 1500 mL·min^{-1}. Ions with m/z = 50−1500 were scanned with a scan time of 0.5 s, flow 0.37 mL·min^{-1}. Compounds were separated by using a solvent program with water/acetonitrile (50:50) for 10 min and a linear gradient between 10 and 40 min (to 100% acetonitrile).

Preparative HPLC separations were accomplished with a Discovery C18 column (Supelco, particle size 5 μm, 250 mm length × 21.2 mm I.D.) using a linear gradient from H_2O/acetonitrile (20:80) to water/acetonitrile (1:99) over 25 min at a flow rate of 9.9 mL·min^{-1} and monitored by a variable wavelength detector at 208 nm. These conditions were used to separate all the compounds in the crude extract.

Esterification: 1 mL of 0.1 N KOH was added to the ester (lactone) (approximately 5 mg, 0.025 mol), and the resulting mixture was stirred at room temperature for 24 h. The mixture was acidified to pH 2.0 with dilute HCl and extracted three times with diethyl ether (3 × 10 mL) and, after removal of the solvent, an oily residue was obtained (approximately 3−4 mg). The resulting acids were dissolved in methanol (5 mL) and an ethereal solution of CH_2N_2 (1%) was slowly added while swirling the reagent vessel. The reaction was followed by preparative RP-HPLC separations with a Discovery C18 column.

Compound **1** was isolated as a white oil, yield 8.4 mg. $[\alpha]_D^{25}$ = +35.6 (c = 0.08, EtOH). UV (MeOH): $\lambda_{max.}$ = 209 nm (log ε = 3.91). IR (film): $\tilde{\nu}_{max.}$ = 1740 (lactone), 1070 and 1040 (C−O−C) cm^{-1}. HRFABMS: m/z = 433.2570 [M + Na]$^+$ (calcd. for $C_{23}H_{38}O_6Na$: m/z = 433.2566) (Δ = 0.4 ppm); LC−MS/APCI: m/z = 433 [M + Na]$^+$, 411 [M + H]$^+$. For the NMR spectroscopic data, see Table 1.

Compound **2** was isolated as a yellow oil, yield 9.1 mg. $[\alpha]_D^{25}$ = +28.0 (c = 0.09, EtOH). UV (MeOH): $\lambda_{max.}$ = 208 nm (log ε = 4.03). IR (film): $\tilde{\nu}_{max.}$ = 1742 (lactone), 1070 and 1040 (C−O−C) cm^{-1}. HRFABMS: m/z 475.3028 [M + Na]$^+$ (calcd. for $C_{26}H_{44}O_6Na$: m/z = 475.3036) (Δ = −0.8 ppm); LC−MS/APCI: m/z = 475 [M + Na]$^+$, 453 [M + H]$^+$. For the NMR spectroscopic data, see Table 2.

Compound **3** was isolated as a pale yellow oil, yield 18.6 mg. $[\alpha]_D^{25}$ = +12.9 (c = 0.118, EtOH). UV (MeOH): $\lambda_{max.}$ = 210 nm (log ε = 4.08). IR (film): $\tilde{\nu}_{max.}$ = 1738 (lactone), 1070 and 1040 (C−O−C) cm^{-1}. HRFABMS: m/z 249.1473 [M + Na]$^+$ (calcd. for $C_{13}H_{22}O_3Na$: m/z = 249.1467) (Δ = 0.6 ppm); LC−MS/APCI: m/z = 249 [M + Na]$^+$, 227 [M + H]$^+$. For the NMR spectroscopic data, see Table 2.

Compound **4** was isolated as a pale yellow oil, yield 61.5 mg; this compound was characterized as methyl ester **5**.

Compound **5** is a white oil, yield 62 mg after esterification. $[\alpha]_D^{25}$ = +7.4 (c = 0.10, EtOH); ref.[13] $[\alpha]_D^{25}$ = +8.00 ($CHCl_3$, 0.06 g·mL^{-1}). UV (MeOH): $\lambda_{max.}$ = 208 nm (log ε = 3.86). IR (film): $\tilde{\nu}_{max.}$ = 1740 (COOH), 1070, and 1040 (C−O−C) cm^{-1}. HRFABMS: m/z = 281.1735 [M + Na]$^+$ (calcd. for $C_{14}H_{26}O_4Na$: m/z = 281.1729) (Δ = 0.6 ppm); LC−MS/APCI: m/z = 281 [M + Na]$^+$, 259 [M + H]$^+$. ^{1}H NMR: δ = 0.75 (dd, J = 6.8, 2.7 Hz, H-13), 0.95 (t, J = 6.9 Hz, H-11), 1.07 (ddq, J = 10.0, 6.8, 3.6 Hz, H-7), 1.14 (dd, J = 7.0, 2.7 Hz, H-12), 1.47 (m, H-9), 1.48 (m, H-10), 1.65 (H-9, m), 1.72 (m, H-4), 1.73 (m, H-5), 1.79 (m, H-4), 1.80 (m, H-5), 3.03 (dq, J = 3.6, 7.0 Hz, H-2), 3.65 (s, COOCH_3), 4.17 (dtq, J = 3.6, 9.0, 2.7 Hz, H-3), 4.18 (dtq, J = 9.0, 3.6, 2.7 Hz, H-6), 4.29 (ddd, J = 10.0, 9.5, 4.8 Hz, H-8) ppm. ^{13}C NMR: δ = 174.6 (C-1), 79.3 (C-3), 77.9 (C-6), 75.4 (C-8), 51.2 (COOCH_3), 42.2 (C-2), 37.6 (C-9), 36.8 (C-7), 29.2 (C-4), 28.5 (C-5), 18.4 (C-10), 14.0 (C-11), 10.2 (C-13), 9.3 (C-12) ppm.

Compound **6** is a colorless oil, yield 24 mg. $[\alpha]_D^{25}$ = +14.6 (c = 0.12, $CHCl_3$); all other spectroscopic data are identical to the previously published data.[6]

Biological Activity: Antibacterial and antifungal assays were carried out as described previously.[15] The test organisms were from the Czechoslovak Collection of Microorganisms, Brno. The amount tested was 10 μg per test disk (see Table 3). Each sample (≈ 0.05 mg) was dissolved in 50 μL of DMSO and added to a vial containing artificial seawater (3.0 mL). Approximately 20 pieces of brine shrimp, *Artemia salina*, were added to the vial and then observed periodically over a 24 h period. The death of 50% of the brine shrimps was taken as a positive effect.[16] The compounds listed in Table 3 served as controls.

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