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Five new derivatives of nonactic and homo-nonactic acids from *Streptomyces globisporus*

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Abstract—In addition to the known compounds of the type of nonactic and homononactic acids and their lactones, dilactones and tetralactones, five new compounds, namely homononactyl-nonactoate, a dilactone consisting of nonactic and homononactic acids and three cyclic trimers with nonactic and homononactic acids, were isolated from a strain of *Streptomyces globisporus*. Their structures, including the absolute configurations of the hydroxyl and methyl groups, were determined by extensive spectroscopic techniques such as UV, IR, MS, 1D and 2D NMR.

1. Introduction

Macrotetrolides exhibit a very wide range of effects, ranging from antimicrobial to insecticidal, acaricidal (miticidal), antiprotozoan (coccidiostatic) and antiparasitic (anthelminthic).[1] Their newly described immunosuppressive activity[2] has important consequences for medicine (transplantations, treatment of autoimmune diseases). Nonactin, monactin, dinactin, trinactin and tetranactin isolated from a variety of *Streptomyces* species are cyclotetralactones derived from nonactic (**1**) acid and homononactic (**2**) acid as building units of ionophoretic character. With the exception of nonactin, they exhibit, in addition to antibacterial and antifungal activity, also remarkable acaricidal, insecticidal, coccidiostatic and anthelminthic effects.[1]

Nonactic and homononactic acids are plant growth stimulators and exhibit specific insecticidal effects.[3] There are reports in the literature on the biological effects of a mixture of macrotetrolides (polynactin) or their components (especially tetranactin). The biological activity of nonactic acids has been reported only infrequently, whereas that of their homologs (macrotetrolides G, D, C, and E and isodinactin or isotrinactin) was not tested at all.

This work is part of an on going study[4] performed concerned with the isolation and identification of new antibiotics produced in cultures of different species of the genus *Streptomyces*. In our screening studies for new products of pesticidal compounds[1] we isolated soil microorganisms *S. globisporus* and *S. griseus* from which we obtained the macrotetrolides—nonactin, monactin, dinactin and trinactin by HPLC on normal phase.[5,6] Free nonactic and homononactic acids were isolated from the strain *S. griseus* by RP-HPLC.[7]

For the identification of metabolites containing nonactic and homonactic acids we employed LC-MS/APCI, as described previously.[8] By using gradient elution and highly effective column (~26,500 plates/25 cm), we could separate and identify five new compounds (homononactyl-nonactoate, bilactone consisting of nonactic and homononactic acids and three cyclic trimers with nonactic and homononactic acids), in addition to 12 compounds that had been described previously.

2. Results and discussion

A mixture of antibiotics were isolated from a strain of *S. globisporus*.[6] After repeated crystallization of the major part of the nonactin, monactin, dinactin, and trinactin mixture, the mother liquors were separated by means of semipreparative HPLC on normal phase and two main fractions were obtained. One of them contained compounds without a free carboxyl group (lactones), whereas the other group included compounds with a single free carboxyl group. By further chromatography on RP-HPLC it was possible to obtain a total of 12 compounds. Of them lactone

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Table 1. NMR data for dilactone (**3**)

No	^{1}H	^{13}C
1	—	174.1 C
2	2.63 (1H, dq, *J*=9.8, 6.7 Hz)	43.1 CH
2′	1.12 (3H, dd, *J*=2.7;[a] 6.7 Hz)	12.8 CH_3
3	3.99 (1H, dddq, *J*=10.0, 9.8, 7.0, 2.7[a] Hz)	79.9 CH
4a	1.81 (1H, m)	26.9 CH_2
4b	1.62 (1H, m)	
5a	1.97 (1H, m)	31.6 CH_2
5b	1.41 (1H, m)	
6	3.95 (1H, m)	72.2 CH
7a	1.48 (1H, ddd, *J*=14.0, 11.3, 3.4 Hz)	41.9 CH_2
7b	1.81 (1H, ddd, *J*=14.0, 12.1, 3.5 Hz)	
8	4.91 (1H, ddq, *J*=11.3, 3.5, 6.4 Hz)	67.9 CH
8′	1.25 (3H, d, *J*=6.4 Hz)	20.0 CH_3
9	—	174.3 C
10	2.58 (1H, qd, *J*=6.9, 9.9 Hz)	44.2 CH
10′	1.09 (3H, dd, *J*=2.7,[b] 6.9 Hz)	13.1 CH
11	4.02 (1H, dddq, *J*=9.4, 7.0, 9.9, 2.7[b] Hz)	79.8 CH
12a	1.86 (1H, m)	27.0 CH_2
12b	1.65 (1H, m)	
13a	1.98 (1H, m)	31.1 CH_2
13b	1.43 (1H, m)	
14	3.88 (1H, m)	70.5 CH
15a	1.61 (1H, m)	39.7 CH_2
15b	1.86 (1H, m)	
16	4.91 (1H, m)	72.8 CH
16a′	1.28 (1H, m)	30.2 CH_2
16b′	1.37 (1H, m)	
16″	0.88 (3H, t, *J*=7.0 Hz)	9.1 CH_3

[a] $^4J_{\text{H-3,H-2'}}$.
[b] $^4J_{\text{H-11,H-10'}}$.

of nonactic and homonactic acids,[9] dilactone of bis-nonactic[10] and bis-homononactic acids[11] and of course, also the group of tetralactones[12] (nonactin–tetranactin) have previously been described. Dilactone (**3**) and three trilactones (**4**-**6**) containing nonactic and homonactic acids were obtained as new compounds. In the second group (with free hydroxyl group), homononactyl-nonactate (**7**) was newly reported, in addition to the known compounds nonactyl-nonactoate,[13] nonactyl-homononactoate[14] and homononactyl-homononactoate.[14]

Compound **3** was homogeneous by LC-MS/APCI and appeared as pale yellow oil, soluble in non-polar solvents. The UV spectrum of **3** in isopropanol showed only a terminal absorption (at λ_{max} 208 nm), implying that conjugated double bonds are absent in the molecule. The IR spectrum revealed only the presence of an ester (1740 cm^{-1}) and ether functional groups (1070 and 1040 cm^{-1}). The mass spectrum showed a pseudomolecular ion at *m*/*z* 383.2437, which is consistent with a molecular formula of $C_{21}H_{34}O_6$ (Δ 0.4 ppm).

The ^{1}H NMR spectrum (Table 1) of the dilactone (**3**) in $CDCl_3$ indicated the presence of 33 protons. It was very similar to that of the macrolides,[10,11] but a few of the resonances and splitting patterns were different.

The ^{1}H NMR and ^{13}C NMR were extensively analyzed by ^{1}H–^{1}H NOESY and ^{1}H^{13}C HMQC with the aid of proton spin decoupling, thus all of the carbons and protons in the molecule could be assigned and the two partial structures I and II were deduced, as shown in Figure 1. Six oxygen atoms in the molecule were assigned to four of the two ester groups (^{13}C NMR: δ 174.1 (s), 67.9 (d), 174.3 (s), 72.8 (d) and two ethereal oxygen atoms, because of no OH absorption in the IR spectrum and no other carbonyl signal in the ^{13}C NMR spectrum. Two ester groups were also ascertained by hydrolysis of the compound with NaOH to afford two acids (nonactic and homononactic, respectively).

These two partial structures were constructed to build up the plain structure of the dilactone. NOE experiments showed the spatial proximity between two protons in three pairs, that is, H-4/H5 and H-11/H-14, in every pair the two protons were connected through five covalent bonds, thus the presence of two tetrahydrofuran rings was suggested. This suggestion was confirmed by high $^1J_{CH}$ values of C-4/H-4 (132.4 Hz), C-5/H-5 (134.0 Hz), C-11/H-11 (132.9 Hz), and C-14/H-14 (134.3 Hz), all of which were the characteristic *J* values of tetrahydrofuran ring.[15] Since the molecule consisted of two parts, I and II, which were connected by two ester bonds, the C_1-carbonyl of I should bind with the C_{16}–O– of II, and the C_9-carbonyl of II with the C_8–O– of I, respectively. Thus, a sixteen membered macrodiolide ring was deduced, and the plain structure of the dilactone was built up as shown in Figure 1. In order to confirm the proposed structure, the products of hydrolysis, nonactic and homononactic acids, were extensively analyzed by mass fragmentation and methyl ester derivatives by ^{1}H–^{1}H COSY. The specific assignments (Table 1) were tentatively made in comparison with the data reported for tetranactin, nonactin and macrodiolide antibiotics.[10,16,17]

The relative stereochemistry of **3** was detected by 2D-NOESY experiments as shown in Figure 1. Relevant NOE effects were measured between H-2/H-4a (H-10/H-12a), H-3/H-4b (H-11/H-12b), H-3/H-5b (H-11/H-13b), H-4b/H-6 (H-12b/H-14) as well as H-5b and H-6 (H-13b/H-14), but no effect was observed between H-3/H-4a (H-11/H-12a) and H-6/H-8 (H-14/H-16). On the basis of the measured values of the signals for the 2-methyl and 9-methyl groups, we assume that our compound has a relative configuration[10] of 2*R**, 3*R**, 6*S**, 8*S** in accordance with those reported for nonactic and homononactic acids.[10,18]

The *cis*-substituents at α,α'-positions of two tetrahydrofuran rings were shown by NOE enhancements between H-3/H-6, and H-11/H-14, respectively, indicating that the H-3, H-6, H-11, and H-14, should be disposed to the up side of the macrodiolide ring. The stereochemistry at C-2, and C-10, was determined by respective NOE enhancement and *J* values (calculated by MM2 method $J_{2,3}$=10.2 Hz, observed $J_{2,3}$=9.8 Hz, calculated $J_{10,11}$=10.1 Hz, observed $J_{10,11}$=9.9 Hz, respectively, see Table 1). The structure of the dilactone with the relative stereochemistry has thus been elucidated as shown in Figure 1. The absolute stereochemistry of dilactone is described below.

Saponification followed by esterification of compound **3** resulted in two products whose proton NMR data were consistent with those of methyl nonactate and homononactate. Both methyl esters are known to be optically active compounds. Our two compounds were also optically active, which suggests that the compound consists of a mixture of methyl (+)-nonactate and methyl (+)-homononactate. The compounds (**4**, **5**, and **6**) were produced in

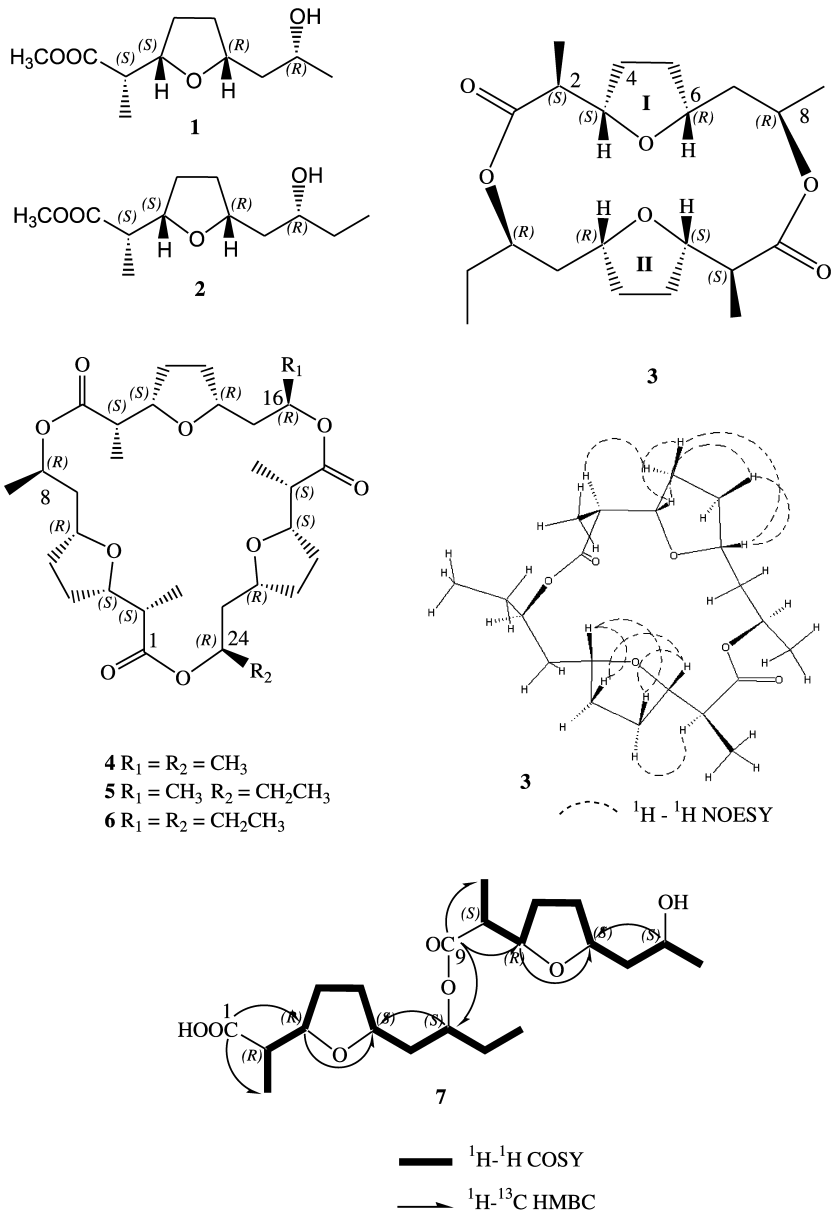

Figure 1. The structures of dilactone consisted of nonactic and homononactic acids (**3**), three cyclic trimers (**4**-**6**) with nonactic and homononactic acids, and homononactyl-nonactoate (**7**) from *Streptomyces globisporus*.

substantial quantities and represent the bulk of the oligolactone components. They were easily separated from each other and purified by preparative reverse phase chromatography.

Structure assignments of these lactones were based on the interpretation of spectroscopic data, especially those from MS and NMR analysis. The structure of **4** was recognized as a macrocyclic trilactone inferred from a set of proton/carbon NMR signals at δ 4.90/68.6. In addition, the LC-MS/APCI spectrum revealed a dominant $[M+Na]^+$ ion peak at *m*/*z* 575. The molecular formula of $C_{30}H_{48}O_9$ was established by HRFAB mass spectrometry, as $[M+H]^+$. As new NMR signals at 1.25 (d) and 20.3 ppm (q) replaced the signals for the ethyl group found in the dilactone (**3**), it was apparent that **4** contained three methyl groups and its structure was therefore consistent with the macrocyclic trilactone shown in Figure 1. The literature NMR data revealed similarity with nonactin and dilactone.[6,11] The difference between **4**, **5**, and **6** was indicated by the molecular weight of **5** and **6** (566 vs. 580) that suggested the increase of the methylene group(s). A thorough NMR analysis of **5** uncovered that this compound is a homologue of **4**.

The structure of **5** was determined as follows: a molecular formula of $C_{31}H_{50}O_9$ was established by HRFAB mass spectrometry. However, the ^1H and ^{13}C NMR spectra revealed signals and correlations for only 21 carbon atoms, indicating two nonactic and one homononactic group. Consequently, **5** had to consist of three units, and these moieties were arranged asymmetrically as illustrated in Figure 1.

Similarly, the molecular formula of **6** was based on its molecular ion $[M+H]^+$ (*m*/*z* 581.3693) as determined by HRFABMS, invoking the molecular formula of $C_{32}H_{52}O_9$.

The ^{1}H and ^{13}C NMR signals of **6** were remarkably similar to those of **5**. Again, the ^{1}H and ^{13}C NMR spectra of **6** accounted for only 22 carbon atoms derived from one nonactic acid linked to two homononactic groups. While only the signals for the protons in position 16′ (H-16′a,b) in **6** appear as multiplets at δ 1.30 and 1.37, the signals for the same protons were not located in **5**. However, the protons (H-24′a,b) for both, **5** and **6**, appear as multiplets, and resonate at δ 1.29 (H-24′a) and δ 1.38 (H-24′b) for **5** and at δ 1.31 (H-24′a) and δ 1.40 (H-24′b) for **6**, respectively. The NMR spectra of **6** suggested the presence of two units of homononactic acid and one unit of nonactic acid. Given the molecular formula, **6** must exist in the form of an asymmetric trimer as depicted in Figure 1.

The absolute configurations of the oligolactones were deduced from the negative rotation of two isolated constituents (nonactic and homononactic acids). The optical rotation of both compounds (**1** and **2**), hydrolytically prepared from **5** or **6** gave $[\alpha]_D^{25}=+14.6$ (*c* 0.12, $CHCl_3$) and $[\alpha]_D^{25}=+17.2$ (*c* 0.12, $CHCl_3$), respectively. These values were nearly identical with those previously published.[15,19] When considering that nonactic and homononactic acids were present in **5** as well as in **6**, it can be deduced that the absolute stereochemistry of the isolated oligolactones is as indicated in Figure 1.

The molecular formula of **7** was determined to be $C_{21}H_{36}O_7$ on the basis of high-resolution FABMS data. The IR spectrum showed absorption bands at 1740 cm^{-1}, indicating the presence of an ester group. The ^{1}H and ^{13}C NMR spectra of **7** in $CDCl_3$ are summarized in Table 4. The ^{13}C NMR spectrum of **7** showed 21 carbon signals, which were assigned to four methyl groups, seven methylenes, eight methines, and two quaternary carbons by DEPT experiments. The latter observation together with the absence of sp^2 hybridized carbons associated with double bond(s) accounted for four sites of unsaturation. Two of the four sites of unsaturation belong to two carbonyl atoms (carboxylic acid at δ 177.4 ppm and ester at 174.4 ppm). These observations required for the acid **7** to be bicyclic.

Analysis of the 2D NMR COSY data for **7** revealed two diagnostic correlation sequences; the first from H-2′ to H-8″ and the second from H-10′ to H-16′ (Fig. 1). Furthermore, HMBC correlations from C-9 to H-8 permitted connection of both substructural units, as show in Figure 1. The relative and absolute stereochemistry of both subunits (nonactic and homononactic acids) was determined after alkaline hydrolysis. Unfortunately, as described previously in two naturally occurring dimers, nonactyl nonactate[13] and bonactin,[14] also in our case it was not possible to find optically active nonactic and homomonactic acids. However, these results are in contradiction with the values found by us with cyclic isomers.[1]

In agreement with the literature data,[1] all four lactones isolated by us exhibit a significant activity against both Gram-negative and Gram-positive bacteria and also against fungi (Table 5). It is assumed that the biological activity of tetralactones (from nonactin to tetranactin) is connected with chelatation by alkali metal ions,[20] by sodium in particular. Although the compounds described in this paper are di- and trilactones, they exhibit biological activity comparable to that of nonactin.[1] After all, it is not surprising in view of the fact that even the previously described monolactones and dilactone had considerable antimicrobial activity.[9,10]

Literature data on two linear dimers, that is, nonactylnonactate and bonactin, are contradictory. The former did not exhibit antimicrobial activity[13] up to 2 mg/ml, whereas the latter exhibited excellent activity[14] at a concentration of 1 mg/ml.

As compared with previously tested organisms, this time we also used brine shrimp (*Artemia salina*). Due probably to a high sodium complex formation, the effects of, for example, trilactone (**5**), were nearly fatal with LC_{50}=2 ng/ml (Table 5).

3. Conclusion

All the compounds identified and described in this paper contained nonactic and/or homonactic acid. We assume that the individual lactones are by-products of biosynthesis or even biosynthetic intermediates. This assumption seems to be confirmed by their optical activity, which was detected in all four lactones (one dilactone and three trilactones). On the other hand, in case of the linear dimer the situation is not clear, which is in spite of the fact that the two previously isolated dimers have been considered natural compounds.

4. Experimental

4.1. General

UV spectra were measured by a Cary 118 (Varian) apparatus in MeOH in the range 200–350 nm. A Perkin–Elmer Model 1310 (Perkin–Elmer, Norwalk, CT) infrared spectrophotometer was used for scanning infrared spectroscopy of methyl esters as neat film. NMR spectra were recorded in a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). The positive-ion MS/FAB mass spectra were recorded in a VG 7070E-HF spectrometer (Micromass, Manchester, UK).

The LC-MS/APCI was performed as reported previously:[21] briefly: the HP 1090 series (HP 1090 series, Hewlett–Packard, USA) was used with two columns (HIRPB-250AM 250×2.1 mm ID, 5 μm phase particle, ~26,500 plates/25 cm). A quadruple mass spectrometer system Navigator (Finnigan MAT, San Jose, CA, USA) was used: vaporizer temperature 400 °C, capillary heater temperature 220 °C, corona current 5 μA, sheath gas high-purity nitrogen, pressure ca. 380 kPa, and auxiliary gas (also nitrogen) flow rate 1500 ml/min. Ions with *m/z* 50–1500 were scanned with a scan time of 0.5 s, flow 0.37 ml/min. Compounds were separated using a solvent program with water–acetonitrile (50:50) to 10 min and linear gradient from 10 to 40 min (100% acetonitrile).

Preparative HPLC separations were accomplished on a

Table 2. ^{1}H NMR data for trilactones (**4**, **5** and **6**)

No	4	5	6
2, 10, 18	2.57 (3H, dq, *J*=9.8, 6.7 Hz)	2.57+2.54 (2H+1H, dq, *J*=9.8, 6.7 Hz)	2.57+2.54 (1H+2H, dq, *J*=9.8, 6.7 Hz)
2′, 10′, 18′	1.10 (9H, dd, *J*=2.7,[a] 6.7 Hz)	1.11 (9H, dd, *J*=2.7,[a] 6.7 Hz)	1.09 (9H, dd, *J*=2.7,[a] 6.7 Hz)
3, 11, 19	4.01 (3H, dddq, *J*=10.0, 9.8, 7.0, 2.7[a] Hz)	4.01 (3H, dddq, *J*=10.0, 9.8, 7.0, 2.7[a] Hz)	4.01 (3H, dddq, *J*=10.0, 9.8, 7.0, 2.7[a] Hz)
4a, 12a, 20a	1.82 (3H, m)	1.80 (3H, m)	1.82 (3H, m)
4b, 12b, 20b	1.63 (3H, m)	1.61 (3H, m)	1.61 (3H, m)
5a, 13a, 21a	1.98 (3H, m)	1.97 (3H, m)	1.98 (3H, m)
5b, 13b, 21b	1.40 (3H, m)	1.42 (3H, m)	1.43 (3H, m)
6, 14, 22	3.90 (3H, m)	3.89 (3H, m)	3.90 (3H, m)
7a, 15a, 23a	1.48 (3H, ddd, *J*=14.0, 11.3, 3.4 Hz)	1.50 (3H, ddd, *J*=14.0, 11.3, 3.4 Hz)	1.49 (3H, ddd, *J*=14.0, 11.3, 3.4 Hz)
7b, 15b, 23b	1.80 (3H, ddd, *J*=14.0, 12.1, 3.5 Hz)	1.81 (3H, ddd, *J*=14.0, 12.1, 3.5 Hz)	1.79 (3H, ddd, *J*=14.0, 12.1, 3.5 Hz)
8, 16, 24	4.90 (3H, ddq, *J*=11.3, 3.5, 6.4 Hz)	4.91 (3H, ddq, *J*=11.3, 3.5, 6.4 Hz)	4.89 (3H, ddq, *J*=11.3, 3.5, 6.4 Hz)
8′ 16′, 24′	1.25 (9H, d, *J*=6.4 Hz)	1.25 (6H, d, *J*=6.4 Hz)[b]	1.25 (3H, d, *J*=6.4 Hz)[c]
16′a, 24′a	—	1.29 (1H, m)[d]	1.30 (1H, m); 1.31 (1H, m)
16′b, 24′b	—	1.38 (1H, m)[e]	1.37 (1H, m); 1.40 (1H, m)
16″, 24″	—	0.89 (3H, t, *J*=7.0 Hz)[f]	0.89 (6H, t, *J*=7.0 Hz)

[a] $^{4}J_{\text{H-3,H-2′; H-11,H-10′; H-19,H-18′}}$.
[b] Values for only 8′, 16′.
[c] Value for only 8′.
[d] Value for only 24′a.
[e] Value for only 24′b.
[f] Value for only 24″.

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

4.1.1. Esterification. To ~5 mg (0.025 mol) of the ester (lactone) was added 0.1 N KOH, 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 ethyl ether (3×10 ml) and after removal of the solvent an oily residue was obtained (~3–4 mg). The resulting acids were dissolved in 5 ml methanol and an etheral solution of CH_2N_2 (1%) was slowly added while swirling the reagent vessel. The reaction was followed by preparative RP-HPLC separations on a Discovery C18 column.

Methyl nonactate (**1**), colorless oil, $[\alpha]_D^{25}$=+14.6 (*c* 0.12, $CHCl_3$); (lit.[22] for (+) isomer, $[\alpha]_D^{25}$=+22.1 (*c* 0.7, $CHCl_3$), for (−) isomer $[\alpha]_D^{25}$=−17.8 (*c* 3.6, $CHCl_3$)[19]); LC-MS/APCI: $[M+H]^+$ *m/z* 217 (100%), $[M+Na]^+$; *m/z* 239, *m/z* 202 (47%) $[M+H-CH_3]^+$; *m/z* 186 (47%) $[M+H-CH_3O]^+$; IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1170 and 1040) cm^{-1}; ^{1}H NMR 1.12 (3H, dd, *J*=2.7, 6.7 Hz, H-2′), 1.25 (3H, d, *J*=6.4 Hz, H-8′), 1.41 (1H, m, H-5b), 1.48 (1H, ddd, *J*=13.9, 10.8, 3.4 Hz, H-7a), 1.62 (1H, m, H-4b), 1.81 (1H, ddd, *J*=13.9, 12.1, 3.7 Hz, H-7b), 1.81 (1H, m, H-4a), 1.97 (1H, m, H-5a), 2.53 (1H, dq, *J*=9.5, 6.7 Hz, H-2), 3.64 (3H, s, OMe), 3.95 (1H, m, H-6), 3.99 (1H, dddq, *J*=9.9, 9.5, 6.7, 2.7 Hz, H-3), 4.91 (1H, ddq, *J*=10.8, 3.7, 6.4 Hz, H-8); ^{13}C NMR 174.5 (C-1, s), 80.1 (C-3, d), 72.5 (C-6, d), 68.1 (C-8, d), 52.3 (Me, q), 42.0 (C-7, t), 31.4 (C-5, t), 27.2 (C-4, t), 19.8 (C-8′, q), 13.0 (C-2′, q).

Methyl homononactate (**2**), (NMR, see lit.[23]) colorless oil, $[\alpha]_D^{25}$=+17.2 (*c* 0.12, $CHCl_3$); (lit.[15] for (−) isomer, $[\alpha]_D^{25}$=−23.7 (*c* 0.14, $CHCl_3$); LC-MS/APCI: *m/z* 231 (100%) $[M+H]^+$; *m/z* 253 (100%) $[M+Na]^+$; *m/z* 202 (43%) $[M+H-C_2H_5]^+$; *m/z* 200 (39%) $[M+H-CH_3O]^+$; IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1170 and 1040) cm^{-1}; ^{1}H NMR 0.88 (3H, t, *J*=7.0 Hz, H-8″), 1.09 (3H, qd, *J*=2.7, 6.9 Hz, H-2′), 1.28 (1H, m, H-8a′), 1.37 (1H, m, H-8b′), 1.43 (1H, m, H-5b), 1.61 (1H, m, H-7a), 1.65 (1H, m, H-4b), 1.86 (1H, m, H-4a), 1.86 (1H, m, H-7b), 1.98 (1H, m, H-5a), 2.55 (1H, qd, *J*=9.4, 6.9 Hz, H-2), 3.65 (3H, s, OMe), 3.88 (1H, m, H-6), 4.02 (1H, dddq, *J*=9.9, 9.4, 7.0, 2.7 Hz, H-3), 4.91 (1H, m, H-8); ^{13}C NMR 174.2

Table 3. ^{13}C NMR data for trilactones (**4**, **5**, and **6**)

No	4	5	6
1	174.2 C	174.2 C	174.2 C
2	43.4 CH	43.4 CH	43.4 CH
2′	12.9 CH_3	12.9 CH_3	12.9 CH_3
3	80.1 CH	80.1 CH	80.1 CH
4	27.6 CH_2	27.6 CH_2	27.6 CH_2
5	31.5 CH_2	31.5 CH_2	31.5 CH_2
6	74.5 CH	74.5 CH	74.5 CH
7	42.4 CH_2	42.4 CH_2	42.4 CH_2
8	68.6 CH	68.6 CH	68.6 CH
8′	20.3 CH_3	20.3 CH_3	20.3 CH_3
9	174.2 C	174.2 C	174.3 C
10	43.4 CH	43.4 CH	45.1 CH
10′	12.9 CH_3	12.9 CH_3	13.0 CH_3
11	80.1 CH	80.1 CH	80.2 CH
12	27.6 CH_2	27.6 CH_2	27.8 CH_2
13	31.5 CH_2	31.5 CH_2	31.4 CH_2
14	74.5 CH	74.5 CH	73.0 CH
15	42.4 CH_2	42.4 CH_2	40.1 CH_2
16	68.6 CH	68.6 CH	69.2 CH
16′	20.3 CH_3	20.3 CH_3	29.5 CH_2
16″	—	—	9.2 CH_3
17	174.2 C	174.3 C	174.3 C
18	43.4 CH	45.1 CH	45.1 CH
18′	12.9 CH_3	13.0 CH	13.0 CH
19	80.1 CH	80.2 CH	80.2 CH
20	27.6 CH_2	27.8 CH_2	27.8 CH_2
21	31.5 CH_2	31.4 CH_2	31.4 CH_2
22	74.5 CH	73.0 CH	73.0 CH
23	42.4 CH_2	40.1 CH_2	40.1 CH_2
24	68.6 CH	69.2 CH	69.2 CH
24′	20.3 CH_3	29.5 CH_2	29.5 CH_2
24″	—	9.2 CH_3	9.2 CH_3

Table 4. ^{1}H and ^{13}C NMR data for linear diester (**7**)

No	^{1}H	^{13}C
1	—	177.4 C
2	2.47 (1H, dq, *J*=7.0, 1.1 Hz)	45.0 CH
2′	1.09 (3H, d, *J*=7.0 Hz)	13.4 CH_3
3	4.00 (1H, m)	80.3 CH
4a	1.92 (1H, m)	28.3 CH_2
4b	1.61 (1H, m)	
5a	1.95 (1H, m)	31.1 CH_2
5b	1.43 (1H, m)	
6	4.03 (1H, m)	76.4 CH
7a	1.55 (1H, m)	40.7 CH_2
7b	1.72 (1H, m)	
8	4.91 (1H, m)	68.9 CH
8a′	1.28 (1H, m)	27.4 CH_2
8b′	1.37 (1H, m)	
8″	0.90 (3H, t, *J*=7.0 Hz)	9.3 CH_3
9	—	174.4 C
10	2.52 (1H, dq, *J*=6.9, 1.2 Hz)	45.5 CH
10′	1.14 (3H, dd, *J*=2.7,[a] 6.9 Hz)	13.2 CH_3
11	4.00 (1H, m)	80.1 CH_2
12a	1.90 (1H, m)	29.0 CH_2
12b	1.62 (1H, m)	
13a	1.91 (1H, m)	31.3 CH_2
13b	1.42 (1H, m)	
14	4.04 (1H, m)	40.8 CH
15a	1.58 (1H, m)	73.4 CH_2
15b	1.76 (1H, m)	
16	5.15 (1H, tq, *J*=6.8 2.9 Hz)	31.3 CH
16′	1.24 (3H, d, *J*=6.8 Hz)	20.4 CH_3

[a] $^{4}J_{H\text{-}11,H\text{-}10'}$.

(C-1, s), 80.0 (C-3, d), 73.0 (C-8, d), 70.6 (C-6, d), 53.0 (Me, q), 44.5 (C-2, d), 40.1 (C-7, t), 30.8 (C-5, t), 30.4 (C-8′, t), 27.2 (C-4, t), 13.4 (C-2′, q), 9.7 (C-8″, q).

The following known compounds were identified in mother liquor—lactone of nonactic acid (*m/z* 207 $[M+Na]^+$); lactone of homonactic acid (*m/z* 221 $[M+Na]^+$); dilactone of bis-nonactic acids (*m/z* 391 $[M+Na]^+$); dilactone bis-homononactic acids (*m/z* 419 $[M+Na]^+$); nonactyl-nonactoate (*m/z* 409 $[M+Na]^+$); nonactyl-homononactoate (*m/z* 423 $[M+Na]^+$); homononactyl-homononactoate (*m/z* 437 $[M+Na]^+$); nonactin (*m/z*759 $[M+Na]^+$); monactin (*m/z* 773 $[M+Na]^+$); dinactin (*m/z* 787 $[M+Na]^+$); trinactin (*m/z* 801 $[M+Na]^+$); tetranactin (*m/z* 815 $[M+Na]^+$). The mass spectra (LC-MS/APCI) of the above-mentioned compounds agreed with previously published data.

The newly reported compounds are described below:

Compound **3** is pale yellow oil, yield 14.3 mg; $[\alpha]_D^{25}=+12.1$ (*c* 0.11, EtOH); IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1070 and 1040) cm^{-1}; HRFABMS (*m/z*) 383.2437 $[M+H]^+$, (Calcd for $C_{21}H_{35}O_6$ *m/z* 383.2433), (Δ 0.4 ppm); LC-MS/APCI: *m/z* 383 $[M+H]^+$, *m/z* 405 $[M+Na]^+$, *m/z* 185 [monomeric A+H–H_2O]$^+$; *m/z* 199 [monomeric B+H–H_2O]$^+$, A is fragment of nonactic acid, B is fragment of homononactic acid; NMR data, see Table 1.

Compound **4** is slightly yellow oil, yield 5.8 mg; $[\alpha]_D^{25}=+14.5$ (*c* 0.09, EtOH); IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 553.3381 $[M+H]^+$, (Calcd for $C_{30}H_{49}O_9$ *m/z* 553.3376), (Δ 0.5 ppm); LC-MS/APCI: *m/z* 553 $[M+H]^+$, *m/z* 575 $[M+Na]^+$, *m/z* 369 [dimeric fragment of 2xA+H–H_2O]$^+$, *m/z* 185 [monomeric fragment A+H–H_2O]$^+$; ^{1}H NMR data, see Table 2, ^{13}C NMR data see Table 3.

Compound **5** is pale brown oil, yield 9.1 mg; $[\alpha]_D^{25}=+9.8$ (*c* 0.16, EtOH); IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 567.3537 $[M+H]^+$, (Calcd for $C_{31}H_{51}O_9$ *m/z* 567.3533), (Δ 0.4 ppm); LC-MS/APCI: *m/z* 567 $[M+H]^+$, *m/z* 589 $[M+Na]^+$, *m/z* 383 [dimeric fragment of A+B+H–H_2O]$^+$, *m/z* 369 [dimeric fragment of 2xA+H–H_2O]$^+$, *m/z* 199 [monomeric fragment of B+H–H_2O]$^+$, *m/z* 185 [monomeric fragment A+H–H_2O]$^+$; ^{1}H NMR data, see Table 2, ^{13}C NMR data see Table 3.

Compound **6** is pale yellow oil, yield 3.1 mg; $[\alpha]_D^{25}=+19.0$ (*c* 0.15, EtOH); IR (film) ν_{max} 3600 (OH), 2900, 1740 (COOH), C–O–C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 581.3693 $[M+H]^+$, (Calcd for $C_{32}H_{52}O_9$ *m/z* 581.3689), (Δ 0.4 ppm); LC-MS/APCI: *m/z* 581 $[M+H]^+$, *m/z* 603 $[M+Na]^+$, *m/z* 398 [dimeric fragment of B+B+H–H_2O]$^+$, *m/z* 383 [dimeric fragment of A+B+H–H_2O]$^+$, *m/z* 199 [monomeric fragment of B+H–H_2O]$^+$, *m/z* 185 [monomeric fragment A+H–H_2O]$^+$; ^{1}H NMR data, see Table 2, ^{13}C NMR data see Table 3.

Compound **7** is colorless oil, yield 19.2 mg; $[\alpha]_D^{25}=+5.3$ (*c* 0.10, EtOH); IR (film) ν_{max} 3600 (OH), 2900, 1740

Table 5. Biological activities of derivatives (**3**-**7**) and standards

Compound	Test organism				
	Staphylococcus aureus[a]	*Bacillus subtilis*[a]	*Escherichia coli*[a]	*Saccharomyces cerevisiae*[a]	*Artemia salina*[b,c]
3	57.2±7.2[d]	81.2±4.6	<0.5	<0.5	41.3±7.5
4	28.9±4.4	37.6±3.6	<0.5	<0.5	5.2±0.6
5	56.3±3.4	31.7±5.3	<0.5	<0.5	2.0±0.6
6	71.8±5.3	26.5±3.2	<0.5	<0.5	4.9±0.5
7	43.9±5.6	57.0±2.8	<0.5	<0.5	35.0±3.1
Ivermectin	<0.5	<0.5	<0.5	<0.5	2.5±0.5
Penicillin G	91.6±11.2	65.3±2.4	<0.5	<0.5	>1000
Streptomycin	<0.5	<0.5	75.8±3.4	<0.5	>1000
Amphotericin B	<0.5	<0.5	<0.5	76.2±1.2	>1000

[a] Samples (10 μg) were applied to 6.3 mm paper disks; values are diameters (mm) of inhibitory zones.
[b] In ng/ml (LC_{50}).
[c] The details are in the Section 4.
[d] The drug potencies were determined and the values represent values (mean±SD) from five independent observations.

(COOH), C–O–C (1170 and 1040) cm^{-1}; HRFABMS (*m/z*) 401.2537 $[M+H]^+$, (Calcd for $C_{21}H_{37}O_7$ *m/z*401.2539), (Δ 0.2 ppm); LC-MS/APCI: *m/z* 401 $[M+H]^+$, *m/z* 423 $[M+Na]^+$, *m/z* 199 [monomeric fragment of $B+H-H_2O]^+$; *m/z* 185 [monomeric fragment $A+H-H_2O]^+$; NMR data, see Table 4.

4.2. Biological activity

Antibacterial and antifungal assays were carried out according to our previous paper.[24] The test organisms were from the Czechoslovak Collection of Microorganisms, Brno. The amount of the compound was 10 μg per test disk (see Table 5).

The sample (~0.05 mg) was dissolved in 50 μl of DMSO and added to a vial of artificial seawater (3.0 ml). Approximately 20 brine shrimp, *Artemia salina*, were added to the vial and then observed periodically over a 24 h period. A positive assay was the death of 50% brine shrimps.[25] Compounds listed in Table 5 were used as controls.

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