Subergane-Type Sesquiterpenes from Gorgonian Coral *Subergorgia suberosa* with Antibacterial Activities

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A new subergane-type sesquiterpene, named epoxysubergorgic acid (**1**), along with seven known sesquiterpenes, were isolated from the gorgonian coral *Subergorgia suberosa*. The structure of the new compound was determined by extensive spectroscopic analyses. The previously uncertain absolute configuration of the known analogues **2**–**6** was determined on the basis of CD, *Mosher*'s method, and through chemical conversions. All compounds were evaluated for antibacterial activities.

Introduction. – Marine invertebrates, such as sponges and corals, produce structurally diverse secondary metabolites with a wide range of ecological and pharmacological activities [1]. The gorgonian coral *Subergorgia suberosa* has been reported to contain a broad group of metabolites, including sesquiterpenes [2][3], alkaloids [4], steroids [5][6], and butenolides [7]. The coexistence of unique subergane-based sesquiterpenes with β-caryophyllene-derived and of suberosane-type sesquiterpenes within *S. suberosa* suggests that gorgonian corals possess diverse biogenetic pathways. As a program of our investigation of bioactive natural products from marine invertebrates, the gorgonian coral *S. suberosa* was selected for further chemical examination, due to its crude AcOEt extract exhibiting antibacterial activity. Chromatographic separation of the AcOEt extract resulted in the isolation of eight sesquiterpenes (**1**–**8**; *Fig. 1*), including a new compound named epoxysubergorgic acid (**1**). This article reports the isolation and structure elucidation of compound **1**. In addition, the antibacterial activity of all isolated compounds was tested.

Results and Discussion. – Epoxysubergorgic acid (**1**) was isolated as white optically active powder ($[\alpha]_D^{20} = -15.6$ ($c = 0.45$, CH_2Cl_2)). The molecular formula was determined as $C_{15}H_{20}O_3$ on the basis of the HR-ESI-MS (*m*/*z* 247.1333 ($[M-H]^-$)) and NMR data, requiring six degrees of unsaturation. The IR absorption at 1713 cm^{-1} suggested the presence of a C=O group. The ^{1}H-NMR spectrum of compound **1** showed the resonances for one tertiary Me (δ(H) 1.41 (*s*, Me(13))), two secondary Me groups (δ(H) 1.06 (*d*, $J = 7.5$, Me(12)) and 1.31 (*d*, $J = 7.5$, Me(15))), and an olefinic H-atom (δ(H) 6.73 (*s*, H–C(9)). The ^{13}C-NMR spectrum exhibited a total of 15 C-atoms, including two olefinic C-atoms (δ(C) 157.0 and 135.0) for a trisubstituted C=C bond. The 2D-NMR (COSY, HMQC, and HMBC) data established a subergane-based

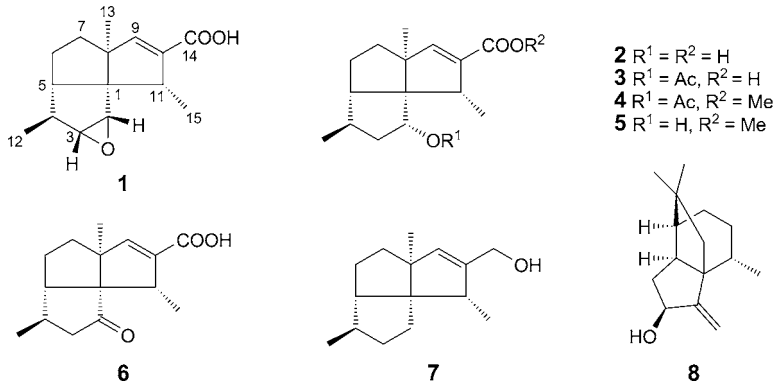

Fig. 1. *Structures of compounds* **1**–**8**

scaffold of tricyclopentanoid sesquiterpene, structurally closely related to subergorgic acid (**6**) [8]. The difference was attributed to an oxirane group being located at C(2) (δ(C) 60.1) and C(3) (δ(C) 64.2) of **1**, instead of an oxo-group at C(2) in compound **6**. This finding was supported by the COSY correlations between H–C(3) (δ(H) 3.38 (*t*, $J = 2.5$)) and H–C(2) (δ(H) 3.53 (*d*, $J = 2.5$)) and H–C(4) (δ(H) 2.10–2.04 (*m*)), and by the HMBCs between Me(12) (δ(H) 1.06 (*d*, $J = 7.5$)) and C(2) and C(3), and between H–C(2) and C(1) (δ(C) 64.3), C(5) (δ(C) 62.3), C(8) (δ(C) 59.6), and C(11) (δ(C) 49.0).

The relative configuration of compound **1** was fully assigned by means of the NOE interactions (*Fig. 2*). A *cis*-orientation of the oxirane group was evident from the NOE relationships from Me(12) to H–C(2) and H–C(3). Additional NOE interactions between H–C(3)/H–C(2), Me(12)/H–C(5), H–C(5)/H–C(11), and Me(15)/Me(13), were indicative of the same relative configurations of compound **1** and subergorgic acid (**6**).

Seven known analogs were identified as 2-hydroxysubergorgic acid (**2**) [9], 2-acetoxysubergorgic acid (**3**) [9], 2-acetoxysubergorgic acid methyl ester (**4**) [9], 2-hydroxysubergorgic acid methyl ester (**5**) [9], subergorgic acid (**6**) [8], subegorgiol (**7**)

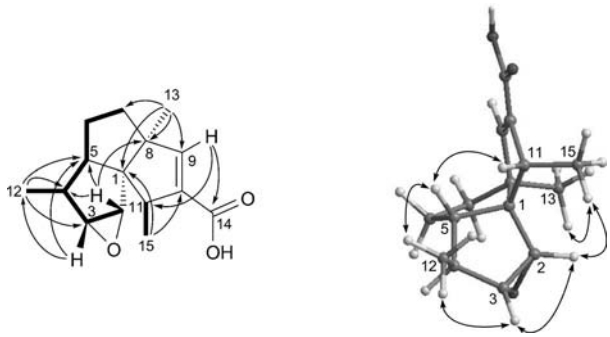

Fig. 2. *Key* $^1H,^1H$*-COSY* (—), *HMBC* (H → C), *and NOE* (H ↔ H) *interactions of compound* **1**

[9], and suberosenol (**8**) [10], on the basis of the spectroscopic data analyses in association with the comparison of their spectroscopic data with those reported in the literature. At this point, it should be noted that only the relative configuration of these compounds was reported [9][10]. Therefore, it was our intention to determine the hitherto unknown absolute configuration of the isolated metabolites **1**–**7**.

Firstly, a modified version of *Mosher*'s method [11] was used for the assignment of the absolute configuration at C(2) of 2-hydroxysubergorgic acid methyl ester (**5**). Esterification of compound **5** with (*R*)-MPA and (*S*)-MPA yielded (*R*)-MPA and (*S*)-MPA esters of **5**, respectively. Calculation of the $\Delta\delta$ ($\delta_R - \delta_S$) values (*Fig. 3*) ascertained C(2) of **5** to possess (*R*) configuration. Since the relative configuration of **5** had been established by NOE data [9], configuration at the remaining chiral centers was assigned as (1*S*,4*R*,5*S*,8*S*,11*R*). The closely similar NOE data of **5** and subergorgic acid (**6**) led us to assign the same relative configurations in both compounds. Comparison of the experimental CD curve with the computed ECD spectra [12] of both enantiomers of **6** (*Fig. 4*) determined the absolute configuration of **6** to be the same as in compound **5**. In order to assign the configuration of the remaining compounds, some chemical conversions of subergorgic acid (**6**) were carried out (*Scheme*) [13]. Reduction of **6** by $NaBH_4$ produced compound **2**, while methylation of **2** using EDCI/DMPA/MeOH generated compound **5**. Dehydration of **2** resulted in an intermediate **2a**, which was further oxidized to a 2,3-epoxy derivative, which was identical with metabolite **1**. In addition, acetylation of **2** afforded compound **3**, whereas compound **4** was generated from **3** by methylation and from **5** by acetylation. Accordingly, the absolute configuration at the chiral centers of **2**–**4** were in accordance with those of **5**, while compound **1** possesses (1*S*,2*S*,3*R*,4*S*,5*S*,8*S*,11*R*) configuration. According to biogenetic considerations, subergorgiol (**7**) may be a precursor of **1**–**6**. Thus, the absolute configuration of **7** and the remaining compounds (**1**–**6**) was assumed to be identical.

All compounds were tested for antibacterial activities. Compounds **7** and **8** exhibited inhibitory effects against a panel of bacterial strains, including *Gram*-negative bacteria (*Xanthomanes vesicatoria*, *Pseudomonas lachrymans*, *Agrobaterium tumefaciens*, and *Ralstonia solanacearum*) and *Gram*-positive bacteria (*Bacillus thuringensis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus haemolyticus*) with *MIC* values ranging from 16 to 128 μg/ml (*Table*). Compounds **4** and **5** specifically inhibited *S. aureus*, whereas the analogues with a free carboxylic acid at C(10) such as **2** and **3** were inactive ($MIC > 128$ μg/ml). This finding indicated that the presence of a methyl ester unit strongly affected the inhibitory effect against *S. aureus*.

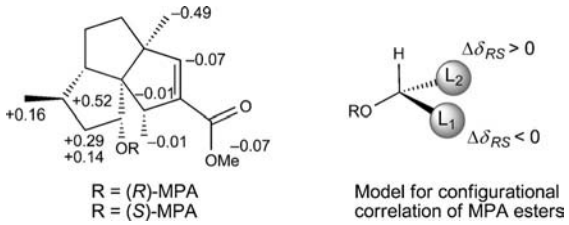

Fig. 3. $\Delta\delta$ ($\delta_R - \delta_S$) *values* (in ppm) *for the MPA esters of compound* **5**

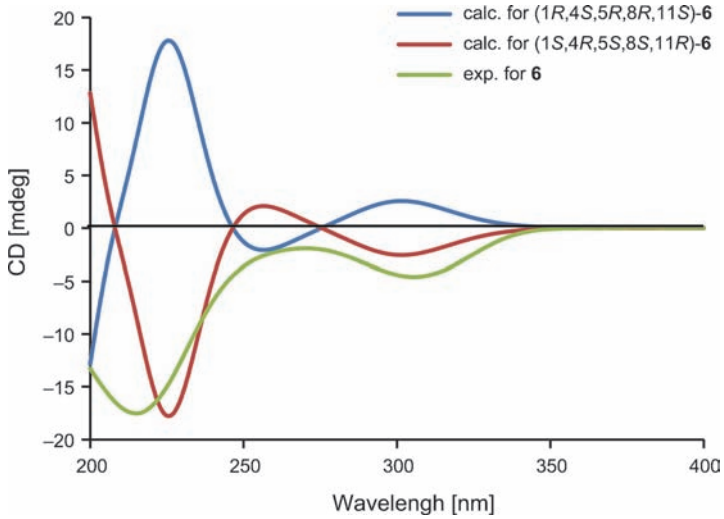

Fig. 4. *CD Curve of compound* **6** *and the computed ECD spectra of enantiomers of* **6**

Scheme. *Chemical Conversion from Compound* **6** *to Compounds* **1**–**5**

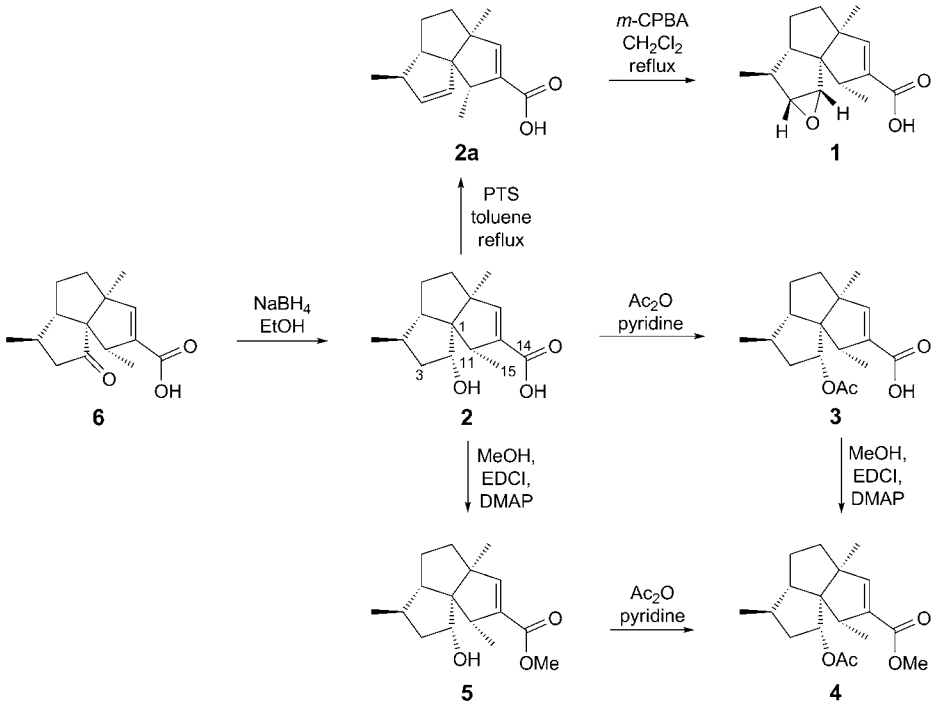

Table. *Antibacterial Activities of Compounds* **1**–**8**

	MIC [μg/ml]								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	CP[a])
X. vesicatoria ATCC 11633	> 128	> 128	> 128	> 128	> 128	> 128	32	16	2
P. lachrymans ATCC 11921	> 128	> 128	> 128	> 128	> 128	> 128	32	> 128	2
A. tumefaciens ATCC 11158	> 128	> 128	> 128	> 128	> 128	> 128	16	32	4
R. solanacearum ATCC 11696	> 128	> 128	> 128	> 128	> 128	> 128	16	> 128	2
B. thuringensis ATCC 10792	> 128	> 128	> 128	> 128	> 128	> 128	32	64	4
S. aureus ATCC 25923	> 128	> 128	> 128	8	16	> 128	32	64	2
B. subtilis CMCC 63501	> 128	> 128	> 128	> 128	> 128	> 128	16	64	1
S. haemolyticus ATCC 29970	> 128	> 128	> 128	> 128	> 128	> 128	16	> 128	2

[a]) Chloroamphenicol, positive control.

Subergane-based sesquiterpenes are a small group of natural products with a unique skeleton, which is rarely found in nature. So far, less than ten subergane-type sesquiterpenes have been isolated from the gorgonian genera *Subergorgia* and *Isis*, as well as from *Silphium* roots. The typical structural pattern may provide chemotaxonomic information on gorgonian corals, as both genera of *Subergorgia* and *Isis* are belonging to the Gorgonaceae family.

Experimental Part

General. TLC: Precoated silica gel (SiO_2) plates (*Kieselgel 60* F_{254}, 0.25 mm; *Merck*). Column chromatogramphy (CC): silica gel (SiO_2, 160–200 and 200–300 mesh; *Qingdao Marine Chemistry Co. Ltd.*) and *ODS* (50 μm; *YMC*). HPLC: *Alltech* instrument (*426-HPLC* pump) equipped with an *Alltech uvis-200* detector at 210 nm and semi-prep. reversed-phased columns (*YMC-packed* C_{18}, 5 μm, 250 mm × 10 mm). Optical rotations: *Rudolph IV Autopol* automatic polarimeter. IR Spectra: *Thermo Nicolet Nexus 470* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR spectra: *Bruker Advance 400* NMR spectrometer (400 MHz (^1H) and 100 MHz (^{13}C), resp.); δ in ppm rel. to solvent peaks at δ(H) 7.28 and δ(C) 77.0 ($CDCl_3$) as internal standard, *J* in Hz. ESI-MS and HR-ESI-MS: *Thermo Scientific LTQ Orbitrap XL* instrumnent; in *m*/*z*.

Animal Material. The coral *S. suberosa* was collected from the inner coral reef at a depth of around 8 m in Hainan Island of P. R. China, in May 2013, and the samples were frozen immediately after collection. The specimen was identified by *L. van O.* (National Museum of National History Naturalis). The voucher specimens (YXQ-07(9)) are deposited with the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, P. R. China.

Extraction and Isolation. The frozen coral *S. suberosa* (2.3 kg) was homogenized and extracted with 95% EtOH (3 × 7 l). The concentrated extract (39.6 g) was desalted by dissolving in MeOH to obtain a residue, that was further partitioned between H_2O and AcOEt. The AcOEt fraction (10.0 g) was subjected to CC (4.5 × 40 cm) using 160–200 mesh SiO_2 (150 g) with a gradient of petroleum ether (PE)/acetone (from 20:1 to 1:1) to yield seven fractions (*Fr. A*–*Fr. G*). *Fr. E* (1.19 g) was further separated into five parts using an *ODS* column eluting with MeOH/H_2O 7:3 as a mobile phase to yield subergorgic acid (**6**; 65 mg). *Fr. D* (1.5 g) was chromatographed over an *ODS* column eluting with MeOH/H_2O 65:35 to give 2-hydroxylsubergorgic acid (**2**; 111.5 mg) and 2-acetoxysubergorgic acid (**3**; 49.1 mg). *Fr. C* (2.2 g) was purified over an *ODS* column eluting with MeOH/H_2O 7:3, and subsequently subjected to semi-prep. HPLC (*RP-*C_{18}) eluting with MeOH/H_2O 7:3 to furnish epoxysubergorgic acid (**1**; 5.6 mg). *Fr. B* (248.3 mg) was also separated over an *ODS* column eluting with MeOH/H_2O 58:42 to yield subegorgiol (**7**; 2.6 mg) and suberosenol (**8**; 2.6 mg). *Fr. A* (1.3 g) was chromatographed by semi-prep.

HPLC, using MeOH/H_2O 73:27 as a mobile phase to yield 2-acetoxysubergorgic acid methyl ester (**4**; 43.2 mg) and 2-hydroxysubergorgic acid methyl ester (**5**; 26.0 mg).

Epoxysubergorgic Acid (*=(1*R,*3a*S,*5a*S,*6*S,*6a*R,*7a*S,*7b*S*)-1,3a,4,5,5a,6,6a,7a-Octahydro-1,3a,6-trimethylcyclopenta[6,6a]pentaleno[1,2-*b*]oxirene-2-carboxylic Acid*; **1**). White powder. $[\alpha]_D^{27} = -15.6$ (*c* = 0.45, CH_2Cl_2). UV (in MeOH): 221 (3.57). IR (KBr): 2934, 1713, 1685. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.73 (*s*, H–C(9)); 3.53 (*d*, *J* = 2.5, H–C(2)); 3.38 (*t*, *J* = 2.5, H–C(3)); 2.88 (*q*, *J* = 7.5, H–C(11)); 2.10–2.04 (*m*, H–C(4)); 1.97–1.92 (*m*, H–C(5)); 1.89–1.82 (*m*, H_β–C(7)); 1.75–1.70 (*m*, H_α–C(7)); 1.74–1.68 (*m*, H_β–C(6)); 1.41 (*s*, Me(13)); 1.39–1.31 (*m*, H_α–C(6)); 1.31 (*d*, *J* = 7.5, Me(15)); 1.06 (*d*, *J* = 7.5, Me(12)). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.5 (C(14)); 157.0 (CH(9)); 135.0 (C(10)); 64.3 (C(1)); 64.2 (CH(3)); 62.3 (CH(5)); 60.1 (CH(2)); 59.6 (C(8)); 49.0 (CH(11)); 42.2 (CH(4)); 40.0 (CH_2(7)); 32.0 (CH_2(6)); 24.1 (Me(13)); 18.5 (Me(12)); 17.1 (Me(15)). HR-ESI-MS: 247.1333 ($[M-H]^-$, $C_{15}H_{19}O_3^-$; calc. 247.1412).

ECD Calculation. Conformational searches were carried out by means of the Powell methods using MMFF 94s force field in the SYBYL-X software package. Geometry optimizations were calculated at TD-DFT/B3LYP-6-31 + G(d) level. The results showed five lowest energy conformers for (1*R*,4*S*,5*R*,8*S*,11*S*)-**6**, whose relative energy was within 2.0 kcal/mol. Subsequently, the conformers were re-optimized using DFT at the B3LYP/6-31 + G(d) level in gas phase by the *Gaussian* 09 program. The B3LYP/6-31 + G(d) harmonic vibrational frequencies were also calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths (velocity) of the five conformers were calculated using the TD-DFT methods at the B3LYP/6-31 + G(d) level in vacuum. The ECD spectra were simulated by the overlapping *Gaussian* function. To obtain the final spectra, the simulated spectra of the lowest energy conformers for each structure were averaged according to the *Boltzmann* distribution theory and their relative *Gibbs* free energy (ΔG). The theoretical ECD spectrum of (1*R*,4*S*,5*R*,8*S*,11*S*)-**6** was obtained following the same procedure as that of (1*R*,4*S*,5*R*,8*R*,11*S*)-**6**. By comparison of the calculated ECD spectra with the experimental CD spectra of **6**, the absolute configuration of **6** was resolved.

Reduction of Subergorgic Acid (**6**). To a soln. of 8.46 mmol of compound **6** in anh. EtOH (1 ml), $NaBH_4$ (0.5 mg, 13.15 mmol) was added, and the mixure was stirred at r.t. for 3 h. The solvent was removed under reduced pressure to yield a residue. The residue was chromatographed on SiO_2 eluting with 25% Me_2CO in PE as developing solvent to yield 2-hydroxysubergorgic acid (**2**; 1.2 mg, yield 56.7%).

Acetylation of 2-Hydroxysubergorgic Acid (**2**). A soln. of compound **2** (10.08 mmol) in pyridine (1 ml) and Ac_2O (5 µl) was kept at r.t. for 24 h. After the solvent was removed under reduced pressure, the residue was partitioned between an aq. sat. $CuSO_4$ soln. (5 ml) and AcOEt (5 ml). The AcOEt phase was washed with H_2O, and then evaporated under reduced pressure to yield 2-acetoxysubergorgic acid (**3**; 2.2 mg, yield 74.4%). The conversion of 2-hydroxysubergorgic acid methyl ester (**5**) to 2-acetoxysubergorgic acid methyl ester (**4**) followed the same procedure.

Methylation of 2-Hydroxysubergorgic Acid (**2**). Compound **2** (8.80 mmol), DMAP (4-dimethylaminopyridine, 12.27 mmol), and EDCI (1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride, 12.24 mmol) were dissolved in anh. CH_2Cl_2 (1 ml). After the soln. was cooled at 0°, anh. MeOH (5 µl) was added. The mixture was stirred at r.t. for 1.5 h, and then the solvent was removed by evaporation under reduced pressure to give a residue. The residue was chromatographed over SiO_2 (3 g) eluting with 15% Me_2CO in PE to yield **5** (1.6 mg, yield 68.2%). The conversion of 2-acetoxysubergorgic acid (**3**) to 2-acetoxysubergorgic acid methyl ester (**4**) followed the same procedure.

Dehydration of 2-Hydroxysubergorgic Acid (**2**). A PTS (1M) equivalent was added in a toluene soln. (5 ml) of **2** (33.8 mmol), and the mixture was stirred to reflux for 2 h at 130°. The soln. was concentrated *in vacuo*, while the residue was purified by semi-prep. HPLC eluting with MeCN/H_2O 60:40 to obtain compound **2a** (5.0 mg, yield 63.5%).

Epoxidation of 2,3-Enesubergorgic Acid (**2a**). To a CH_2Cl_2 soln. (0.5 ml) of **2a** (17.24 mmol) was added an *m*-CPBA (1M) equivalent, and the mixture was stirred to reflux for 12 h. The soln. was concentrated *in vacuo*, while the residue was purified by semi-prep. HPLC eluting with MeCN/H_2O 50:50 to obtain compound **1** (2.5 mg, yield 58.5%).

2,3-Enesubergorgic Acid (*=(1*R,*3a*S,*5a*S,*6*R,*8a*R*)-1,3a,4,5,5a,6-Hexahydro-1,3a,6-trimethylcyclopenta[*c*]pentalene-2-carboxylic Acid*; **2a**). ^{1}H-NMR (400 MHz, $CDCl_3$): 5.69 (*dd*, *J* = 1.8, 5.6, H–C(3)); 6.58

(br. *s*, H–C(9)); 5.57 (*dd*, *J* = 1.8, 5.6, H–C(2)); 2.81 – 2.76 (*m*, H–C(11)); 2.25 – 2.20 (*m*, H–C(4)); 1.89 – 1.82 (*m*, H–C(5)); 1.67 – 1.60 (*m*, H_β–C(7)); 1.62 – 1.55 (*m*, H_β–C(6)); 1.43 – 1.38 (*m*, H_α–C(7)); 1.42 – 1.37 (*m*, H_α–C(6)); 1.17 (*d*, *J* = 6.9, Me(15)); 1.10 (*s*, Me(13)); 1.06 (*d*, *J* = 7.0, Me(12)). ^{13}C-NMR (100 MHz, $CDCl_3$): 168.7 (C(14)); 153.3 (CH(9)); 138.1 (C(10)); 138.0 (CH(3)); 130.0 (CH(2)); 70.9 (C(1)); 62.7 (CH(5)); 59.5 (C(8)); 51.7 (CH(11)); 48.9 (CH(4)); 37.3 (CH_2(7)); 31.1 (CH_2(6)); 23.2 (Me(13)); 22.2 (Me(12)); 18.6 (Me(15)).

Preparation of MPA Esters of 2-Hydroxysubergorgic Acid Methyl Ester (**5**). To a soln. of anh. CH_2Cl_2 (0.5 ml) cooling to 0°, compound **5** (9.45 mmol) together with DMAP (4-dimethylaminopyridine, 12.2 mmol), DCC (*N*,*N*-dicyclohexylcarbodiimide, 9.7 mmol) and (–)-(*R*)-α-methoxy-α-phenylacetic acid ((–)-(*R*)-MPA, 10.8 mmol) were added. After stirring for 5 h at r.t., the mixture was evaporated *in vacuo* to obtain a residue, which was purified by semi-prep. HPLC using MeOH/H_2O 4 : 5 as a mobile phase to give (*R*)-MPA-**5** ester (1.9 mg, yield 64.3%). Following the same protocol, (*S*)-MPA-**5** ester (1.7 mg, yield 75%) was generated from **5** (7.2 mmol).

*(*R*)-MPA-***5** *Ester* (= *Methyl (1*R*,3a*S*,5a*S*,6*R*,8*R*,8a*S*)-1,3a,4,5,5a,6,7,8-Octahydro-8-{[(2*R*)-2-methoxy-2-phenylacetyl]oxy}-1,3a,6-trimethylcyclopenta[c]pentalene-2-carboxylate*). White powder. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.34 – 7.46 (*m*, H-atoms of MPA moiety); 6.35 (*s*, H–C(9)); 5.35 (*d*, *J* = 3.2, H–C(2)); 4.69 (*s*, H–C_α of MPA moiety); 3.74 (*s*, *Me*O–C(14)), 3.41 (*s*, *Me*O of MPA moiety); 2.72 (*q*, *J* = 7.2, H–C(11)); 1.96 (*dd*, *J* = 5.6, 14.0, H_a–C(3)); 1.74 – 1.68 (*m*, H–C(4)); 1.45 (*ddd*, *J* = 3.2, 12.0, 14.0, H_b–C(3)); 1.07 (*d*, *J* = 7.2, Me(15)); 1.02 (*d*, *J* = 7.2, Me(12)); 1.26 – 1.81 (*m*, H–C(5), CH_2(6,7)); 0.66 (*s*, Me(13)).

*(*S*)-MPA-***5** *Ester* (= *Methyl (1*R*,3a*S*,5a*S*,6*R*,8*R*,8a*S*)-1,3a,4,5,5a,6,7,8-Octahydro-8-{[(2*S*)-2-methoxy-2-phenylacetyl]oxy}-1,3a,6-trimethylcyclopenta[c]pentalene-2-carboxylate*). White powder. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.32 – 7.45 (*m*, H-atoms of MPA moiety); 6.42 (*s*, H–C(9)); 5.37 (*d*, *J* = 3.2, H–C(2)); 4.73 (*s*, H–C_α of MPA moiety); 3.75 (*s*, *Me*O–C(14)); 3.45 (*s*, *Me*O of MPA moiety); 2.73 (*q*, *J* = 7.2, H–C(11)); 1.67 (*dd*, *J* = 5.6, 14.0, H_a–C(3)); 1.22 – 1.17 (*m*, H–C(4)); 1.31 (*ddd*, *J* = 3.2, 12.0, 14.0, H_b–C(3)); 1.15 (*s*, Me(13)); 1.14 (*d*, *J* = 7.2, Me(15)); 1.17 – 1.91 (*m*, H–C(5), CH_2(6,7)); 0.86 (*d*, *J* = 7.2, Me(12)).

Antibacterial Assays. Antimicrobial activities were measured against *Xanthomanes vesicatoria*, *Pseudomonas lachrymans*, *Agrobaterium tumefaciens*, *Ralstonia solanacearum*, *Bacillus thuringensis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus haemolyticus*, by the broth microdilution method. The bacteria were grown for 16 h on a rotary shaker at 37°. Cultures were diluted with sterile medium to achieve an optical absorbance of 0.4 – 0.06 at 600 nm, then further diluted 10-fold before transferring into 96-well microtiter plates. Three replicates of each compound were tested in dilution series ranging from 1 to 128 μg/ml. The optical absorbance at 600 nm was measured after cultivation for 18 h. The lowest concentrations that completely inhibited visible growth of the tested strains were recorded from three independent experiments.

Supplementary Data. – NMR spectroscopic data for the new compounds **1**, **2a**, and (*R*)- and (*S*)-MPA-**5** including ^{1}H-, ^{13}C-, and 2D-NMR spectra, IR, and ESI-MS/MS data can be obtained upon request from the authors.

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