

# Constituents of Two *Dioscorea* Species that Potentiate Antibiotic Activity against MRSA

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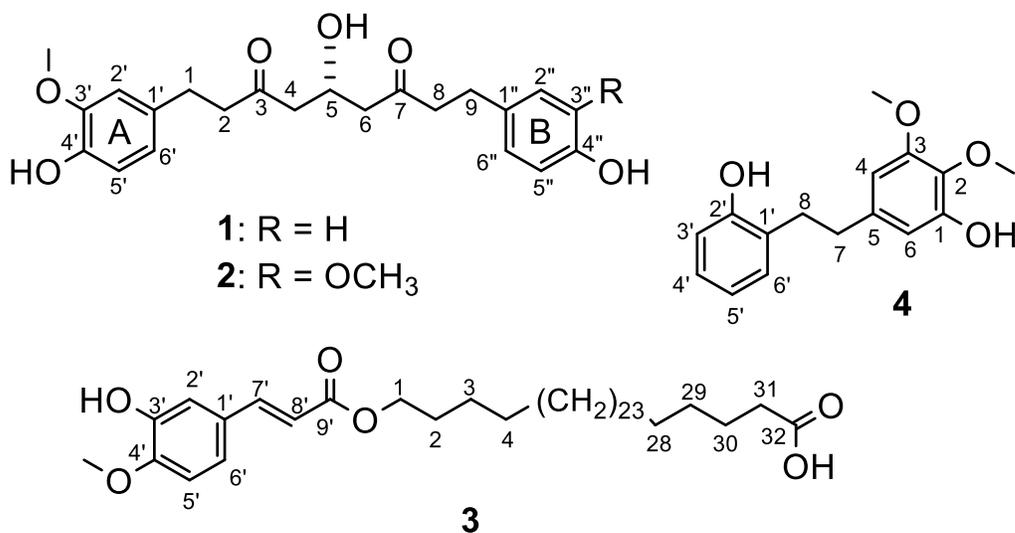
ABSTRACT: The isolation of two diarylnonanoids from *Dioscorea cotinifolia* possessing antibiotic-potentiating activity against resistant strains of *S. aureus* are reported. The diarylnonanoids are a class of natural products similar in structure to the diarylheptanoids, which have a wide spectrum of reported biological activities. One of the diarylnonanoids (**1**) isolated possesses a chiral center and to deduce its configuration, the modified Mosher ester method was used. Using both 1D and 2D NMR data, as many protons as possible were assigned to both the *R*- and *S*-MTPA esters, and the configuration of the chiral center in **1** was determined to be *R*. Both the chiral and achiral diarylnonanoid (**2**) exhibited potent antibiotic-potentiating activity with the chiral natural product showing a greater tetracycline-potentiating activity than **2**. Interestingly, **2** gave a higher norfloxacin-potentiating activity with a resultant higher efflux pump inhibitory activity. Manipulation of the structure of the diarylnonanoids through synthesis could lead to improved biological activity.

The genus *Dioscorea*, commonly known as the “yam” taxon, is very well known for its phytochemical diversity. The rhizomes of some species are used as both food and medicine in many parts of the world. Medicinally, the genus is used in the treatment of wounds, sores,<sup>1</sup> rheumatism and skin problems.<sup>2</sup> Studies on the pharmacological properties of this genus have shown anti-inflammatory, analgesic,<sup>3</sup> antifungal,<sup>1</sup> antitumor<sup>4</sup> and anthelmintic properties.<sup>5</sup> One of the most useful phytochemicals isolated from the family Dioscoreaceae is diosgenin, which has an important role in the pharmaceutical industry as the precursor for pharmacologically important steroids. Other phytochemicals that have been reported in the family include glycosides, flavonoids, alkaloids, phenols, tannins, triterpenoids<sup>6</sup> and the diarylheptanoids.<sup>7</sup> In this study reported are two new diarylnonanoids, an  $\omega$ -hydroxy fatty acid ester from *Dioscorea cotinifolia* Kunth as well as a bibenzyl from *D. sylvatica* Eckl. var. *sylvatica* with antibiotic-potentiating activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The potential of these compounds to inhibit staphylococcal efflux pumps, which are known to be involved in antibiotic resistance, were further investigated.

The diarylnonanoids were isolated from the chloroform extract of the rhizomes of *D. cotinifolia*. Compound **1** was isolated as a yellow solid while compound **2** was isolated as a yellow amorphous powder. The IR absorption spectra of both compounds showed a C=O bond stretch at 1650 cm<sup>-1</sup> and an O-H bond stretch at 3300 cm<sup>-1</sup>. Using their <sup>1</sup>H NMR and HRESIMS data, both compounds were identified as 5-hydroxy-1,9-nonane-3,7-diones with different substituents at positions 1 and 9.

The HRESIMS data of compound **1** showed a sodium adduct ion peak [M + Na]<sup>+</sup> at *m/z* 409.1623, corresponding to the pseudomolecular formula, C<sub>22</sub>H<sub>26</sub>O<sub>6</sub>Na<sup>+</sup> (calcd 409.1627;

10 degrees of unsaturation). The  $^1\text{H}$  NMR data (500 MHz,  $\text{C}_6\text{D}_6$ ) revealed the presence of two aromatic ring systems (Table 1). A 1,3,4-trisubstituted aromatic ring system with three aromatic resonances,  $\delta_{\text{H}}$  6.46 (1H, d,  $J = 2.0$  Hz),  $\delta_{\text{H}}$  6.99 (1H, d,  $J = 8.0$  Hz) and  $\delta_{\text{H}}$  6.54 (1H, dd,  $J = 2.0, 8.0$  Hz) accounting for the 4-hydroxy-3-methoxyphenyl moiety of the structure (ring A). For the second ring (B), a 1,4-disubstituted aromatic ring (AA'BB') system showed two aromatic resonances, each integrating for two protons at  $\delta_{\text{H}}$  6.85 (2H, d,  $J = 8.5$  Hz) and  $\delta_{\text{H}}$  6.49 (2H, d,  $J = 8.5$  Hz), and therefore suggesting a 4-hydroxyphenyl moiety. A number of key features in the spectroscopic data led to the unambiguous assignment of the structure of **1**.



**Table 1. <sup>1</sup>H and <sup>13</sup>C NMR Spectroscopic Data and HMBC correlations of 1 and 2<sup>a</sup>**

no.	1		2		HMBC
	$\delta_C$ , type	$\delta_H$ ( <i>J</i> in Hz)	$\delta_C$ , type	$\delta_H$ ( <i>J</i> in Hz)	
1	29.4, CH <sub>2</sub>	2.73, t (7.5)	29.9, CH <sub>2</sub>	2.73, t (7.5)	3, 2, 2', 6', 1'
2	45.8, CH <sub>2</sub>	2.29 t (7.5)	45.8, CH <sub>2</sub>	2.29, t (7.5)	1', 1, 3
3	209.2, C		209.3, C		1, 5, 2, 4
4	49.0, CH <sub>2</sub>	2.15, dd (7.8, 4.4) 2.08, dd (7.8, 4.4)	49.1, CH <sub>2</sub>	2.16, dd (7.8, 4.3) 2.08, dd (7.8, 4.3)	6, 3, 5
5	64.9, CH	4.39, m	64.9, CH	4.42, m	3, 7, 4, 6
6	49.1, CH <sub>2</sub>	2.12, dd (7.8, 4.4) 2.04, dd (7.8, 4.4)	49.1, CH <sub>2</sub>	2.16, dd (7.8, 4.3) 2.08, dd (7.8, 4.3)	4, 5, 7
7	209.3, C		209.3, C		5, 9, 6, 8
8	45.6, CH <sub>2</sub>	2.22, t (7.5)	45.8, CH <sub>2</sub>	2.29, t (7.5)	1'', 7, 9
9	29.3, CH <sub>2</sub>	2.68, td (7.5, 2.5)	29.9, CH <sub>2</sub>	2.73, t (7.5)	7, 2'', 6'', 1'', 8
1'	133.4, C		133.3, C		5', 2, 1, 2'
2'	111.7, CH	6.46, d (2.0)	111.8, CH	6.46, d (1.5)	1', 3', 4', 6', OCH <sub>3</sub> - 3'
3'	147.2, C		147.2, C		
4'	145.2, C		145.2, C		
5'	115.2, CH	6.99, d (8.0)	115.2, CH	6.99, d (8.0)	3', 1', 4'
6'	121.6, CH	6.54, dd (8.0, 2.0)	121.6, CH	6.54, dd (8.0, 1.5)	4', 2'
1''	133.4, C		133.3, C		
2''	130.1, CH	6.85, d (8.5)	111.8, CH	6.46, d (1.5)	
3''	115.9, CH	6.49, d (8.5)	147.2, C		
4''	155.1, C		145.2, C		
5''	115.9, CH	6.49, d (8.5)	115.2, CH	6.99, d (8.0)	
6''	130.1, CH	6.85, d (8.5)	121.6, CH	6.5, dd (8.0, 1.5)	
OCH <sub>3</sub> - 3'	55.7, CH <sub>3</sub>	3.20, s	55.7, CH <sub>3</sub>	3.21, s	3'

OCH <sub>3</sub> – 3''	55.7, CH <sub>3</sub>	3.21, s	3''
OH – 5		3.34, brd	
OH – 4'		5.36, s	
OH – 4''		3.90, brd	

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<sup>a</sup> 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C, recorded in C<sub>6</sub>D<sub>6</sub>.

Two sets of benzylic protons,  $\delta_{\text{H}}$  2.73 (2H, t,  $J = 7.5$  Hz) and  $\delta_{\text{H}}$  2.68 (2H, dt,  $J = 7.5$  Hz), were apparent along with a pair of deshielded methylene groups (H<sub>2</sub>-2 and H<sub>2</sub>-8) at  $\delta_{\text{H}}$  2.29 (2H, t,  $J = 7.5$  Hz) and  $\delta_{\text{H}}$  2.22 (2H, t,  $J = 7.5$  Hz), which showed  $J$  correlations with C-1 and C-9, respectively, in the HMBC spectrum. Additionally, H<sub>2</sub>-2 and H<sub>2</sub>-8 also showed  $J$  correlations with the carbonyl resonances at C-3 and C-7. Furthermore, two deshielded methylene groups with resonances at  $\delta_{\text{H}}$  2.15, 2.08 (2H, dd,  $J = 7.8, 4.4$  Hz, H<sub>2</sub>-4) and  $\delta_{\text{H}}$  2.12, 2.04 (2H, dd,  $J = 7.8, 4.4$  Hz, H<sub>2</sub>-6) exhibited  $J$  correlations with an oxymethine carbon at C-5. A methoxy group resonance on the aromatic ring A at  $\delta_{\text{H}}$  3.20 (3H, s), further supported the inference of a 5-hydroxy-1,9-nonane-3,7-dione moiety of **1**. Further resonances at  $\delta_{\text{H}}$  3.34 (1H, brd), 5.36 (1H, s) and 3.90 (1H, brd), could be attributed to the three hydroxy groups at C-5, C-4' and C-4'', respectively.

The <sup>13</sup>C NMR spectrum of **1** revealed 22 carbon resonances with seven quaternary carbons missing in the DEPT-135 spectrum, which supported the proposed structure. There were two carbonyl resonances ( $\delta_{\text{C}}$  209.2 and 209.3), an oxymethine ( $\delta_{\text{C}}$  64.9) and methylenes with different chemical shifts, depending on their proximity to the deshielding oxymethine and carbonyl groups (Table 1). In the HMBC spectrum, the proton at position H-3'' showed  $J$  correlations with C-4'' and C-5''. The methylene protons at H-9 showed

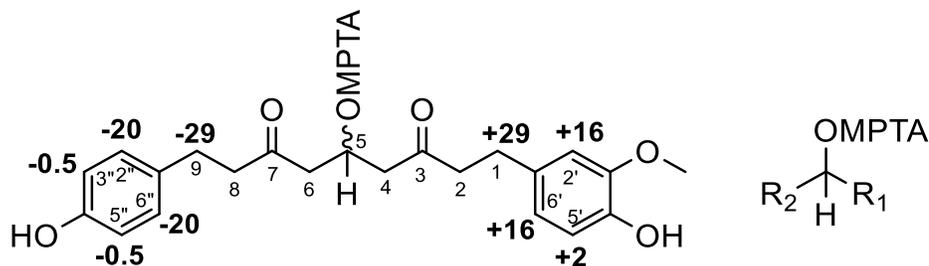
correlations with C-8 and C-1", while the methylene protons at H-1 showed correlations with C-2 and C-1', which enabled the structure of the compound to be confirmed as 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxyphenyl)nonane-3,7-dione.

The HRESIMS of **2** showed a sodium adduct ion peak  $[M + Na]^+$  peak at  $m/z$  439.1729, corresponding to a pseudomolecular formula,  $C_{23}H_{28}O_7Na^+$  (calcd 439.1733; 10 degrees of unsaturation). This was 30 atomic mass units greater than the sodium adduct ion peak observed for **1**, accounting for the addition of an oxymethylene (OCH<sub>2</sub>) moiety. The <sup>1</sup>H NMR spectrum (500 MHz, C<sub>6</sub>D<sub>6</sub>) of **2** was highly similar to that of **1** and revealed the absence of a di-substituted aromatic ring (Table 1). The integrals of the resonances in the 1,3,4-tri-substituted aromatic ring were doubled, showing two identical aromatic rings, for which the protons were equivalent. This observation, and the fact that the integrals of the methylenes in the 5-hydroxy-1,9-nonane-3,7-dione moiety were also doubled, led to the conclusion that the structure of **2** possesses symmetry and this could be readily explained by the proposal of **2** as 5-hydroxy-1,9-bis(4-hydroxy-3-methoxyphenyl)nonane-3,7-dione. However, due the exchangeable nature of the hydroxy group protons, they could not be detected in the <sup>1</sup>H NMR spectrum of **2** in C<sub>6</sub>D<sub>6</sub>.

The <sup>13</sup>C NMR spectrum of **2** revealed only 13 carbon signals, which again was consistent with the observation of a plane of symmetry at position C-5. The HMBC and COSY data were also highly similar to those of **1**. To verify if compound **1** was not obtained as an artefact, it was isolated from its fraction using preparative TLC with chloroform-acetone-acetic acid (6:4:0.1). Both compounds were isolated with different retention factors,  $R_f$  0.34 for **1** and  $R_f$  0.39 for **2**. It was further hypothesized that if **1** was indeed an artefact, its fragment would be clearly visible in the ESIMS spectra of **2**, run in positive mode, but

this was not the case. It was concluded that both compounds are natural products that exist in the plant.

This is the first report of the isolation of diarylnonanoids from the *Dioscorea* genus. Although the diarylheptanoids are similar in structure, and have been previously isolated from the Dioscoreaceae family,<sup>7-10</sup> diarylnonanoids have a 9-carbon chain linking the two aromatic systems. Structurally similar compounds have been isolated from the *Myristica* genus in the plant family Myristicaceae.<sup>11-14</sup> Mosher ester analysis was used to deduce the absolute configuration of the chiral center (C-5) in compound **1**. The Mosher esters, obtained by reacting **1** with both (3*R*)- and (3*S*)-MTPA chloride to yield the (4*S*)- and (4*R*)-MTPA ester, respectively, were analyzed using both 1D and 2D NMR spectroscopy. The data were used to assign as many protons as possible on both the (*R*)- and (*S*)-esters. Since the methylene protons at positions 2 and 8, as well as those in 4 and 6 were overlapped and difficult to distinguish, the benzylic protons at positions 1 and 9, as well as the aromatic protons were used for the analysis. HMQC, HMBC and COSY spectra were key in the assignments. According to the Kakiswa group,<sup>15</sup> the absolute values of  $\Delta\delta$  must be proportional quantitatively to the distance from the MTPA moiety. This condition was met, bringing confidence to the assignment of the hydrogens of the MTPA esters. Using their method, which was later reiterated by Hoye and co-workers,<sup>16</sup> the difference in the chemical shift ( $\Delta\delta^{SR}$ ) was calculated from each of the analogous pairs of protons for both the (*S*)- and (*R*)-MTPA esters.



**Figure 1.**  $\Delta\delta^{SR}$  values (bold) for the MTPA ester of **1** (left) and the model used to determine the absolute configuration (right).

To determine the absolute configuration, all positive  $\Delta\delta^{SR}$  values were assigned to the right side of the model ( $R_1$ ) in Figure 1 and all the negative  $\Delta\delta^{SR}$  values were placed on the left ( $R_2$ ) (as per the advanced or modified Mosher ester analysis.<sup>15</sup> By applying the Cahn Ingold Prelog system, the priority of the groups on the original carbinol were assigned as; 1 (OH), 2 ( $R_1$ ), 3 ( $R_2$ ) and 4 (H) and the absolute configuration of the carbinol at C-5 of **1** was determined as (*R*). The name was then assigned as (5*R*)-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxyphenyl)nonane-3,7-dione. The specific optical rotation was determined to be  $[\alpha]_D^{20} + 130.9$  (*c* 0.08,  $\text{CHCl}_3$ ).

The known compounds (E)-32-((3-(3-hydroxy-4-methoxyphenyl)isoferuloyl)oxy)dotriacontanoic acid (**3**)<sup>17</sup> and 5-(2-hydroxyphenethyl)-2,3-dimethoxyphenol (**4**)<sup>17,18</sup> were isolated from the chloroform extracts of *D. cotinifolia* and *D. sylvatica* var. *sylvatica*, respectively.

The antibiotic-potentiating activities of compounds **1–4** were determined using two MDR *S. aureus* strains possessing characterized efflux proteins namely, SA-1199B, which is resistant to fluoroquinolones due to overexpression of the NorA MDR pump<sup>19</sup> and

XU212, which is tetracycline-resistant due to the TetK efflux protein.<sup>20</sup> *S. aureus* XU212 is also resistant to most  $\beta$ -lactams due to PBP2a production conferred by the *mecA* gene.<sup>20</sup> None of the compounds showed inhibitory activity against the *S. aureus* strains, giving minimum inhibitory concentration (MIC) values at  $>128$  mg/L. At sub-inhibitory concentrations (1/4 or less of the MIC), the compounds caused a 2- or higher-fold reduction in the MIC of the antibiotics when tested against the corresponding resistant strain. Compound **1** was the most active, resulting in a 512-fold reduction in the MIC of tetracycline against the tetracycline-resistant XU212 strain (Table 2). The  $\omega$ -hydroxy fatty acid ester (**3**) was also very active and resulted in an 8- and 16-fold reduction in the MIC of norfloxacin and tetracycline, respectively.

The possible effect of the isolated compounds to inhibit the active efflux of an antibiotic from cells was investigated using an ethidium bromide accumulation assay. Figure S1 (Supporting Information) and Table S1 (Supporting Information) show the effects of the compounds on the intracellular accumulation of ethidium bromide over time within *S. aureus* SA-1199B. Reserpine, a known efflux pump inhibitor, showed an increase in intracellular accumulation of ethidium bromide with a resultant increase in fluorescence over a 30-minute period of observation. This activity was dose-dependent and at the highest concentration (1/4 MIC), the difference in accumulation (the slope of 2.88) compared to that of the control in the absence of the inhibitor (referred to as the “blank”) was the highest obtained (Table 2). Compounds **2** and **4** showed significant efflux pump inhibitory (EPI) activity, with slope differences of 2.45 and 2.68, respectively. Furthermore, their relative final fluorescence (RFF) was similar to that of reserpine. These results led to the conclusion that the antibiotic-potentiating activity of these isolated

compounds is due to their EPI activity. **1** gave a lower norfloxacin-potentiating activity which corresponded to its weaker efflux pump activity compared to **2**. This was interesting because, structurally these two compounds are very similar with only differences in the substitution in the second aromatic ring.

**Table 2. Antibiotic-Potentiating Activity of Compounds 1–4**

compound (sub-inhibitory concentration in mg/L)	MIC (mg/L) of test sample in combination with specified antibiotic (fold reduction) against:	
	<i>S. aureus</i> 1199B (NorA) norfloxacin @ 32 mg/L	<i>S. aureus</i> XU212 (TetK) tetracycline @ 128 mg/L
<b>1</b> (100)	16 (2)	<0.25 (>512)
<b>2</b> (30)	8 (4)	128
<b>3</b> (100)	4 (8)	8 (16)
<b>4</b> (64)	2 (16)	64 (2)
reserpine <sup>a</sup> (20)	16 (2)	64 (2)

<sup>a</sup> Positive control substance

From an observation of the structures of the NorA pump inhibitors isolated in this study, it seems plausible to suggest that the presence of more than one methoxy groups as well as at least two phenyl rings is essential for greater EPI activity. This corroborates with the structures of reserpine and verapamil, well known EPIs, which also possess these structural characteristics. Kaatz and colleagues<sup>21</sup> noted that due to the heterogeneity of reported EPIs, it becomes difficult to characterize the critical structure of an “ideal” NorA inhibitor. However their recent work on ligand-based pharmacophore modeling suggests that the presence of four pharmacophores facilitate NorA inhibition: (1) a hydrogen-bond acceptor,

(2) a positive charge, (3) two aromatic rings, and (4) a hydrophobic region.<sup>22</sup> Although the isolated compounds lacked a positive charge, they possess all the other pharmacophores required and therefore displayed NorA inhibitory properties. The bibenzyl **4** also showed good antibiotic potentiating activity (16-fold reduction in the MIC of norfloxacin).

## EXPERIMENTAL SECTION

**General Experimental Procedures.** The optical rotation of the chiral compound was measured on a PolAAR 21 instrument (Optical Activity Ltd) using an A2 series polarimeter sample tube with a 100 mm path length. UV-vis absorption spectra were measured on a UV-vis (Spectronic Helios Gamma UV-visible spectrophotometer) and IR spectra were measured on a Perkin Elmer 100 FT-IR spectrometer. 1D <sup>1</sup>H, <sup>13</sup>C and DEPT-135, and 2D HMQC, HMBC, COSY and NOESY NMR spectroscopic data were acquired on a Bruker Avance 500 MHz NMR spectrometer. Deuterated solvents and NMR tubes were purchased from Cambridge Isotope Laboratories and Sigma-Aldrich, respectively. Bruker Topspin™, version 3.2 software was used to process NMR data. Low-resolution mass spectra were acquired on a LCQ Duo Ion-Trap mass spectrometer (Thermo Fisher Scientific), and a Waters Q-TOF Premier Tandem mass spectrometer was used for high-resolution mass spectrometry. Column chromatography was performed on silica gel 60 (0.04 – 0.063 mm; Merck) and TLC on Silica gel 60 F254 (Merck) plates. Vanillin-sulphuric acid and *p*-anisaldehyde reagents were used to visualize the TLC plates. All chemicals used were HPLC grade. Chemicals were supplied by either Sigma-Aldrich or Fisher Scientific.

**Plant Material.** *Dioscorea cotinifolia* (voucher number GM218) and *Dioscorea sylvatica* var. *sylvatica* (voucher number GM217) were collected in March 2014 from Malutha in the south eastern part of Eswatini, Southern Africa. Their exact location was recorded with a Global Position System. Botanical identification was done by Mr. M.N. Dlodlu (Botanist; Eswatini Institute for Research in Traditional Medicine, Medicinal and Indigenous Food Plants, University of Eswatini) and voucher specimens were deposited with the Eswatini National Herbarium, Malkerns Research Station, Eswatini.

**Extraction and Isolation.** The dried and ground rhizomes of *D. cotinifolia* (544 g) were extracted exhaustively in a Soxhlet extractor using hexane, chloroform and methanol to yield 0.08%, 0.3% and 0.9%, respectively, of the crude extracts. The chloroform extract was biologically active in the preliminary tests and therefore subjected to normal-phase SPE with hexane (100%) and subsequently 10% increments of ethyl acetate to yield 11 fractions. After TLC and <sup>1</sup>H NMR analysis, fractions 7, 8, 10 and 11 were targeted for the isolation of compounds. Compound **1** (2.5 mg) was isolated from fraction 8 after preparative TLC on silica gel with chloroform-acetone-acetic acid (8:2:0.1). Compound **2** (6.5 mg) was isolated from a combination of fractions 10 and 11 by preparative TLC on silica gel using chloroform-acetone-acetic acid (9:1:0.1), and compound **3** (14.8 mg) was isolated from fraction 7 which was further purified by column chromatography on silica gel using ethyl acetate-hexane (8:2).

The rhizomes of *D. sylvatica* var. *sylvatica* also were successively extracted on an ultrasonic bath with hexane, chloroform and methanol. Fractionation of the chloroform extract by VLC on silica gel led to fraction 6 (227 mg), eluted with hexane-ethyl acetate (50:50). Fraction 6 was purified by column chromatography on silica gel, using petroleum

ether (40-60 °C)-ethyl acetate-formic acid (50:50:1) as mobile phases to yield 75 fractions. On the basis of their TLC profile, fractions 10-19 were pooled and further purified by preparative TLC to yield compound **4** (20 mg).

(5*R*)-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxyphenyl)nonane-3,7-dione (**1**): yellow solid;  $[\alpha]_D^{20} +130.9$  ( $c$  0.08,  $\text{CHCl}_3$ ); UV ( $\text{CH}_3\text{CN}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 223 (4.38), 279 (3.91) nm; IR (film)  $\nu_{\text{max}}$  3296, 2949, 2837, 2141, 1646, 1450  $\text{cm}^{-1}$ ;  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{C}_6\text{D}_6$ ), see Table 1; HRQTOFESIMS  $m/z$  409.1623  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{22}\text{H}_{26}\text{O}_6\text{Na}^+$ , 409.16727).

5-Hydroxy-1,9-bis(4-hydroxy-3-methoxyphenyl)nonane-3,7-dione (**2**): yellow amorphous solid; UV ( $\text{CH}_3\text{CN}$ )  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 201 (4.80), 225 (4.20), 280 (3.85); IR (film)  $\nu_{\text{max}}$  3335, 2950, 2837, 1652, 1450  $\text{cm}^{-1}$ ;  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{C}_6\text{D}_6$ ), see Table 1; HRQTOFESIMS  $m/z$  439.1729  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{23}\text{H}_{28}\text{O}_7\text{Na}^+$ , 439.1733).

**Preparation of Mosher's Esters for Spectroscopic Determination of Stereochemistry.** Mosher's esters were prepared and analyzed as previously described.<sup>15,16,23,24</sup> Briefly, compound **1** (500  $\mu\text{g}$ ) was dissolved in  $\text{CDCl}_3$  (600  $\mu\text{L}$ ) and dry pyridine- $d_5$  (6  $\mu\text{L}$ ). Thereafter, either (*S*)- (+) or (*R*)-(-)-MTPA-Cl (6  $\mu\text{L}$ ; Sigma-Aldrich) was added to the reaction mixture and allowed to stand for 24 hours in a desiccator. When the reaction was complete the mixture was transferred into an NMR tube and run on a Bruker Avance 500 MHz spectrometer to acquire both 1D and 2D NMR data.

**Broth Dilution Assay for Determining the Bacterial Minimum Inhibitory Concentration (MIC).** Minimum inhibitory concentrations were determined by the broth dilution assay previously described.<sup>25-27</sup>

**Antibiotic-potentiating Activity.** Potentiation of antibiotic activity by the test compounds used a modulation assay adapted from Dickson and co-workers.<sup>28</sup> *S. aureus* strains possessing genes that code for antibiotic resistance against particular antibiotics were used for the experiment; a tetracycline-resistant XU212 strain and the norfloxacin-resistant SA-1199B strain. A sub-inhibitory concentration ( $X = 1/4$  MIC or less) of the compound was used for the experiment.

**Real-time Ethidium Bromide Accumulation Assay.** The efflux pump inhibition activity of the compounds was assessed using real-time analysis of ethidium bromide (EtBr) accumulation in the effluxing *S. aureus* SA-1199B strain. EtBr is a substrate for various MDR efflux pumps and reversibly binds DNA.<sup>21,29</sup> It enters the cell by diffusion and is pumped out of effluxing cells via an efflux mechanism. Real-time analysis allows for the monitoring of the influx and efflux activity of EtBr within the cell.<sup>30</sup> The assay was performed as described by Ramalhete and co-workers<sup>31</sup> with minor modifications. Fluorescence was measured on a BioTek® Synergy™ HT multi-detection plate reader, at excitation and emission wavelengths of 535 / 590 for 30 minutes, with readings taken every minute. A positive control (reserpine) and a blank (without EPI) were included in the experiment. The data was processed using Gen5™ v1.09 software.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

HRESIMS and NMR spectra of 1 – 4; <sup>1</sup>H NMR spectra of (R)-MTPA and (S)-MTPA esters of **1**. Ethidium bromide accumulation assay results (PDF)

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### **Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### **Notes**

The authors declare no competing financial interest.

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## GRAPHICAL ABSTRACT



*Dioscorea cotinifolia*

