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Tetrahydroanthraquinone Derivatives from the Mangrove-Derived Endophytic Fungus *Stemphylium globuliferum*

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ABSTRACT

Two new tetrahydroanthraquinone derivatives, altersolanol Q (**1**) and 10-methylaltersolanol Q (**2**), and the new dimer alterporriol X (**3**), together with 13 known analogues were isolated from white bean solid culture media of the endophytic fungus, *Stemphylium globuliferum*, obtained from the Egyptian mangrove plant *Avicennia marina*. The present study resulted in the production of a large diversity of secondary metabolites including new derivatives. Their structures were elucidated using one- and two-dimensional NMR spectroscopy as well as HRESIMS. The absolute configurations of the new compounds **1**–**3** were determined by TDDFT-ECD calculations or by comparing ECD data with those of known analogues. Compounds **1**–**3** were tested against the L5178Y mouse lymphoma cell line but proved to be inactive in contrast to some of the known compounds such as altersolanol A (**6**) that were likewise isolated in this study.

Keywords: *Stemphylium globuliferum*; tetrahydroanthraquinone; ECD calculations

Introduction

Mangrove-derived endophytic fungi are considered an important source for bioactive secondary metabolites that could be of potential use as lead compounds for pharmaceutically relevant drugs.[1–3] Endophytic fungi that inhabit mangrove plants are thought to promote adaptation of their host to survive under harsh ecological conditions such as high salt concentration, high temperature, low oxygen concentration due to changing levels of submersion in seawater, or to counter biological stress caused by herbivores and microbial pathogens.[4] This encouraged us to employ the OSMAC (One Strain Many Compounds)[5,6] approach to *Stemphylium globuliferum*, an endophytic fungus isolated from the mangrove plant *Avicennia marina* (Acanthaceae) collected from Hurghada in Egypt, by changing the culture media in order to trigger the stimulation of silent biogenetic gene clusters.[3] Most compounds previously isolated from this endophyte were reported to be anthraquinone or tetrahydroanthraquinone derivatives including homo- and heterodimers, which are known to possess a wide range of biological activities. For example, altersolanol A (**6**), a tetrahydroanthraquinone derivative obtained in this study but also isolated from *Phomopsis juniperovora*,[7] *Dactylaria lutea*,[8] *Alternaria* sp.,[9] and *Stemphylium globuliferum*,[10] is a potent inhibitor of plant respiration, blocking the uptake of essential metabolites required for photosynthesis[11] and also exhibits cytotoxic activity against 34 human cancer cell lines.[12] In the present study, two new tetrahydroanthraquinone derivatives, altersolanol Q (**1**) and 10-methylaltersolanol Q (**2**), one new anthranoid dimer alterporriol X (**3**), as well as 13 known analogues (**4–16**) were isolated (**Figure 1**). Their structure elucidation including determination of the absolute configuration as well as biological activities are reported.

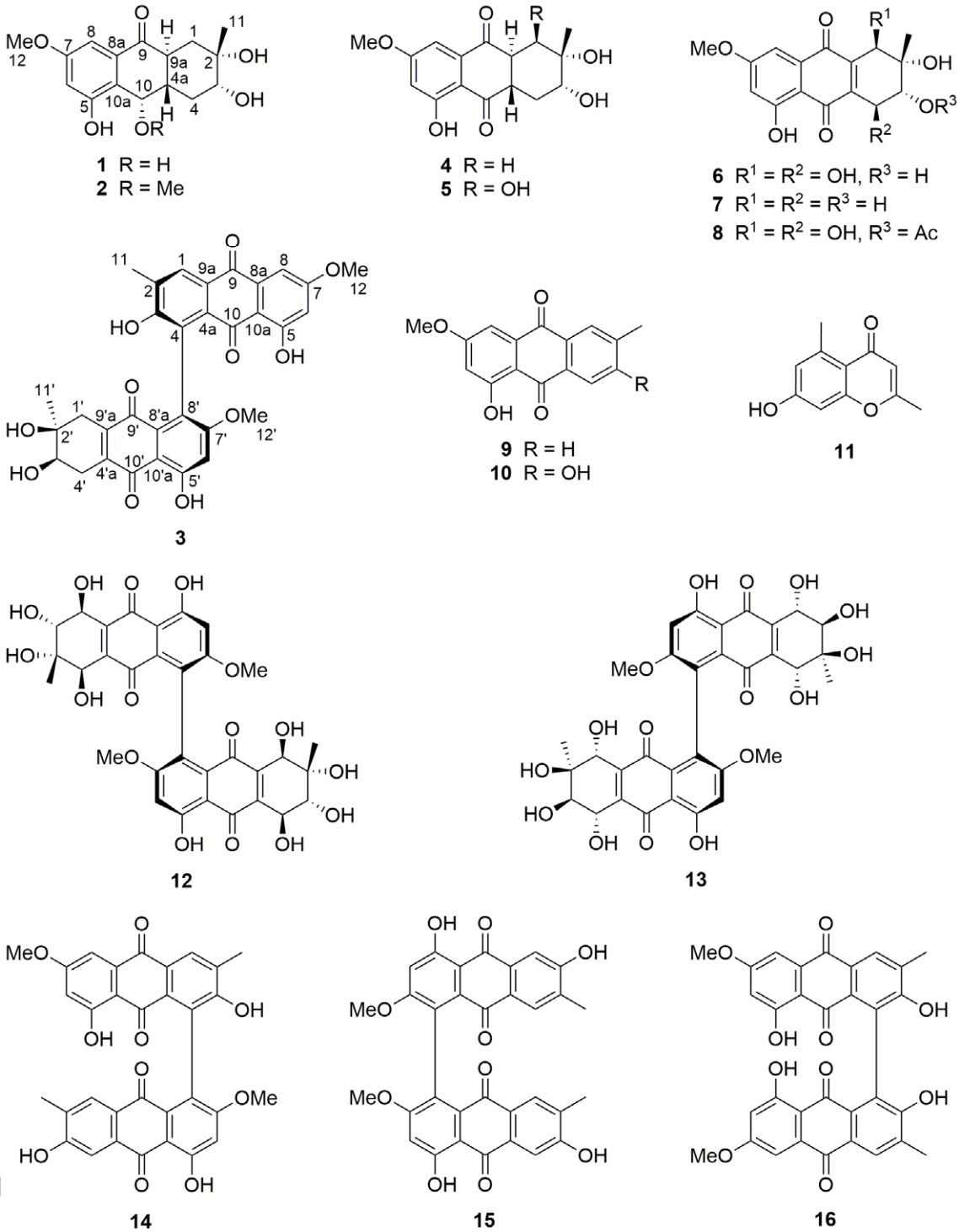

Figure 1. Structures of compounds isolated from *S. globuliferum*.

Results and Discussion

Compound **1** showed UV absorbances at λ_{max} 224, 268 and 334 nm, similar to those of known altersolanol derivatives.[13,14] It exhibited the molecular formula $C_{16}H_{20}O_6$ as established

by HRESIMS. The ^{1}H NMR spectrum (**Table 1**) showed two *meta*-coupling aromatic protons at δ_H 6.98 (d, H-8) and 6.63 (d, H-6), two oxygenated methines at δ_H 4.93 (d, H-10) and 3.43 (dd, H-3), one methoxy group at δ_H 3.78 (s, H_3-12), one singlet methyl group at δ_H 1.31 (s, H_3-11), as well as six aliphatic protons at δ_H 3.05 (td, H-9a), 2.38 (dd, H-1_{eq}), 2.22 (td, H-4_{ax}), 1.93 (tdd, H-4a), 1.71 (ddd, H-4_{eq}), and 1.33 (dd, H-1_{ax}). The planar structure of **1** was elucidated through detailed analysis of 2D NMR spectra (**Figure 2**). The COSY correlations between H_2-1/H-9a, H-9a/H-4a, H-4a/H-10, H-4a/H_2-4 and H_2-4/H-3, together with the HMBC correlations from H_3-11 to C-1, C-2 and C-3 indicated the presence of a cyclohexane ring with a methyl and a hydroxy group attached at C-2, as well as a hydroxy group at C-3. The HMBC correlations from H-6 to C-5, C-7, C-8 and C-10a, from H-8 to C-6, C-7, C-8a and C-10a, and from H_3-12 to C-7 established the presence of a 1,2,3,5-tetrasubstituted benzene ring with a hydroxy and a methoxy group attached at C-5 and C-7, respectively. Finally, the HMBC correlations from H_2-1, H-8 and H-9a to C-9 supported the linkage from C-8a to C-9a via the keto carbonyl moiety C-9, and the HMBC correlations from H-10 to C-5, C-8a and C-10a allowed the assignment of the linkage between C-10 and C-10a.

A literature survey indicated that **1** exhibited the same planar structure as altersolanol J,[13] for which the absolute configuration had only been proposed based on biogenetic considerations. However, the small value of $J_{4a,10}$ (2.7 Hz) in **1** in comparison with that of altersolanol J (9.6 Hz) suggested the *cis* orientation of H-4a and H-10 in **1** rather than *trans* orientation in altersolanol J. The relative configuration of the remaining chiral centres was deduced to be the same as that of altersolanol J based on the similar coupling constants and ROESY relationships. The absolute configuration of **1** was determined as 2*S*, 3*R*, 4a*S*, 9a*S*, and 10*S* on the ground of the TDDFT-ECD calculation of the related 10-methyl derivative **2** (*vide infra*).

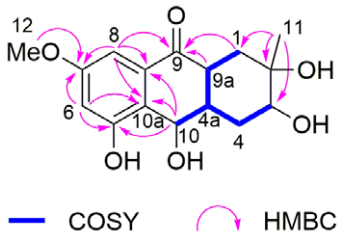

Figure 2. Key COSY, HMBC correlations of compound **1**.

Table 1. NMR data of compounds **1** and **2**.[a]

No.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1_{ax}	1.33, dd (14.2, 12.2)	39.0, CH_2	1.27, dd (14.2, 12.2)	39.3, CH_2
1_{eq}	2.38, dd (14.2, 4.0)		2.37, dd (14.2, 3.9)	
2		72.1, C		72.1, C
3	3.43, dd (11.8, 4.6)	75.5, CH	3.39, dd (11.8, 4.6)	75.4, CH
4_{eq}	1.71, ddd (12.2, 4.6, 3.5)	33.8, CH_2	1.76, ddd (12.2, 4.6, 3.5)	34.0, CH_2
4_{ax}	2.22, td (12.2, 11.8)		2.23, td (12.2, 11.8)	
4a	1.93, tdd (12.2, 3.5, 2.7)	43.9, CH	1.96, tdd (12.2, 3.5, 2.3)	44.5, CH
5		158.0, C		158.1, C
6	6.63, d (2.5)	108.2, CH	6.63, d (2.5)	107.8, CH
7		161.7, C		161.8, C
8	6.98, d (2.5)	101.8, CH	7.01, d (2.5)	102.8, CH
8a		134.2, C		134.6, C
9		202.2, C		202.2, C
9a	3.05, td (12.2, 4.0)	40.7, CH	3.05, td (12.2, 3.9)	41.3, CH
10	4.93, d (2.7)	64.0, CH	4.60, d (2.3)	73.2, CH
10a		126.0, C		124.4, C
11	1.31, s	27.3, CH_3	1.29, s	27.2, CH_3
12	3.78, s	55.8, CH_3	3.78, s	55.8, CH_3
13			3.45, s	58.6, CH_3

[a]Measured in MeOH-d_4 (^{1}H at 600 MHz and ^{13}C at 150 MHz).

The molecular formula of **2** was established as $C_{17}H_{22}O_6$ by HRESIMS, indicating an increase of 14 amu in comparison with altersolanol Q (**1**). The UV and NMR spectra of compound **2** resembled those of **1** except for the appearance of an additional methoxy group (δ_C 58.6, δ_H 3.45, CH_3-13) in **2**. The attachment of this methoxy group at C-10 was confirmed by the HMBC correlation from H_3-13 to C-10 along with the obvious downfield shift of C-10 (δ_C 73.2) in **2** compared to the corresponding carbon (δ_C 64.0) in **1**. Thus, compound **2** was determined as 10-methylaltersolanol Q. Its relative configuration was deduced to be the same as **1** due to the similarity of their *J* values and ROESY correlations.

For the assignment of the absolute configuration of **2**, the solution TDDFT-ECD approach was pursued for the arbitrarily chosen (2*S*,3*R*,4a*S*,9a*S*,10*S*) enantiomer. The initial

20 MMFF conformers were reoptimized at B3LYP/6-31G(d) *in vacuo* and B97D/TZVP PCM/MeCN levels yielding 6 and 10 low-energy (≥2%) conformers, respectively. In all the computed B3LYP/6-31G(d) conformers, the 10-OMe and 2-OH groups adopted axial orientation (**Figure 3**), while the 3-OH was equatorial and the fused cyclohexenone ring of the tetralone chromophore had *M*-helicity ($\omega_{10a,10,4a,9a}$ = −58.0°). The conformers differed only in the orientations of the methoxy group and hydroxyl protons. Similarly to coniothyrinone D,[15] the carbonyl group of **2** could not form an intramolecular hydrogen bond with the phenolic hydroxyl group, which resulted in the separation of the n-π* [negative Cotton effect (CE) at 350 nm] and ππ* (positive CE at 312 nm) transitions of the tetralone chromophore in the experimental ECD spectrum. According to the ECD study of coniothyrinone D,[15] the negative n-π* CE of **2** derives from *M*-helicity of the tetralone chromophore implying (2*S*,3*R*,4a*S*,9a*S*,10*S*) absolute configuration. This assignment was confirmed by ECD calculations of the computed conformers of (2*S*,3*R*,4a*S*,9a*S*,10*S*)-**2** with various functionals and TZVP basis set affording good agreement with the experimental ECD spectrum (**Figure 4**). Since all the low-energy conformers gave similar ECD spectra, the absolute configuration of **2** could be unambiguously determined as (2*S*,3*R*,4a*S*,9a*S*,10*S*).

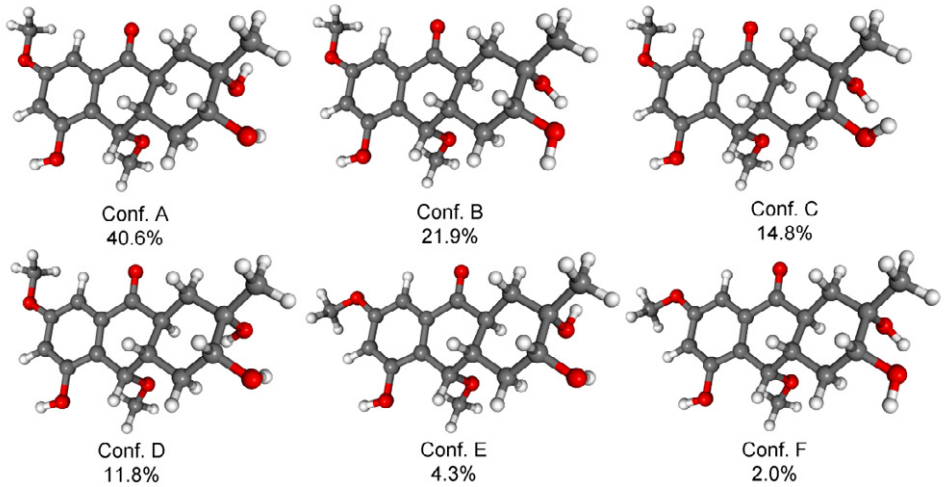

Figure 3. Structure and population of the low-energy B3LYP/6-31G(d) conformers (≥2%) of (2*S*,3*R*,4a*S*,9a*S*,10*S*)-**2**.

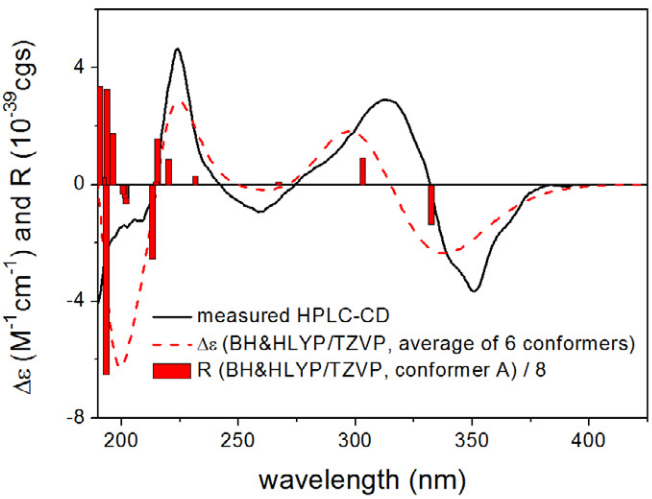

Figure 4. Comparison of the experimental ECD of **2** (black solid) with the Boltzmann-weighted BH&HLYP/TZVP ECD spectra of (2*S*,3*R*,4a*S*,9a*S*,10*S*)-**2** (red dashed) computed for the 6 B3LYP/6-31G(d) *in vacuo* conformers. Bars represent the computed rotational strength values of the lowest-energy conformer.

Compound **3** was isolated as orange powder. Its UV spectrum displayed absorption bands at λ_{max} 202, 224, 285 and 400 nm, which were similar to those of alterporriol W.[16] The molecular formula was determined to be $C_{32}H_{26}O_{11}$ by HRESIMS, missing one oxygen atom compared to alterporriol W. The ^{1}H and ^{13}C NMR data of **3** (**Table 2**) were closely related to those of alterporriol W,[16] except for the replacement of one oxygenated methine group by a methylene group (δ_C 36.0, δ_H 2.51 and 2.36, CH_2-1'). The HMBC correlations from H_3-11' (δ_H 1.19, s) to C-2' (δ_C 70.2), C-3' (δ_C 71.3) and the methylene CH_2-1', and in turn from the protons of CH_2-1' to C-2', C-3', C-4'a (δ_C 144.4), C-9'a (δ_C 141.9), indicated that the additional methylene group CH_2-1' was located at C-1' in **3**. Thus, compound **3** was elucidated as 1'-dehydroxyalterporriol W, for which the trivial name alterporriol X is proposed. Alterporriol X is a hetero dimer consisting of altersolanol B (**7**) and macrosporin (**10**) subunits which were co-isolated in the present study. This structural assignment was corroborated by interpretation of 2D NMR spectra which were in good agreement with those acquired for altersolanol B and

macrosporin, respectively. The absolute configurations at C-2' and C-3' in **3** were assumed to be the same as in altersolanol B (**7**) due to their close biogenetic relationship. Due to the 4-8' biaryl linkage of the anthraquinone-tetrahydroanthraquione dimer, besides the central chirality elements, alterporriol X (**3**) had also axial chirality, which could be determined as (a*R*) on the basis of its similar ECD spectrum to that of the related biaryl natural product alterporriol W having (a*R*) axial chirality.[16]

Table 2. NMR data of compound **3**.[a]

No.	δ_H (*J* in Hz)	δ_C, type	No.	δ_H (*J* in Hz)	δ_C, type
1	8.14, s	130.2, CH	1'	2.51, d (19.2) 2.36, d (19.2)	36.0, CH_2
2		132.0, C	2'		70.2, C
3		159.2, C	3'	3.72, m	71.3, CH
4		n. d.[b]	4'	2.85, m 2.73, m	29.7, CH_2
4a		131.5, C	4'a		144.4, C
5		166.2, C	5'		165.7, C
6	6.66, d (2.6)	106.1, CH	6'	6.87, s	104.3, C
7		166.7, C	7'		165.4, C
8	7.25, d (2.6)	107.0, CH	8'		122.7, C
8a		135.8, C	8'a		125.9, C
9		181.8, C	9'		n. d.
9a		126.0, C	9'a		141.9, C
10		188.8, C	10'		189.4, C
10a		111.5, C	10'a		110.4, C
11	2.41, s	16.9, CH_3	11'	1.19, s	25.0, CH_3
12	3.96, s	56.2, CH_3	12'	3.77, s	56.8, CH_3

[a]Measured in acetone-d_6 (^{1}H at 600 MHz and ^{13}C at 150 MHz). [b]n.d. = not detected.

By comparison of NMR and MS data with the literature, the 13 known compounds were identified as dihydroaltersolanol B (**4**) and C (**5**),[17] altersolanol A (**6**),[18] B (**7**),[19] and N (**8**),[10] 1-hydroxy-3-methoxy-6-methylanthraquinone (**9**),[20] macrosporin (**10**),[21] altechromone A (**11**),[22] alterporriol D (**12**),[18] E (**13**),[18] R (**14**),[9] V (**15**),[23] and W (**16**).[23]

Dihydroaltersolanol C (**5**),[17] altersolanol A (**6**),[17] B (**7**),[17] N (**8**),[10] and alterporriol E (**13**)[17] have been reported to exhibit potent cytotoxicity against L5178Y mouse lymphoma cell line with IC_{50} values in the low micromolar range. However, the new compounds **1**–**3** showed no significant activity when tested at a dose of 10 μg/mL each.

S. globuliferum is well known for its production of anthraquinone or tetrahydroanthraquinone derivatives including various monomers and dimers. In the present

study, fermentation of the titled fungus on solid white bean medium yielded macrosporin (**10**) as an anthraquinone monomer, which gave rise to three anthraquinone homodimers alterporriol R (**14**), V (**15**) and W (**16**), and altersolanol A (**6**) as a tetrahydroanthraquinone monomer, from which two tetrahydroanthraquinone homodimers alterporriol D (**12**) and E (**13**) were detected. In addition, the new heterodimer alterporriol X (**3**) is formed from both macrosporin (**10**) and altersolanol B (**7**) units. *S. globuliferum* was previously cultivated on solid rice medium and yielded tetrahydroanthraquinones, anthraquinones, and tetrahydroanthraquinone dimers.[10,17] However, in this study the fermentation of *S. globuliferum* on white beans afforded three new compounds (**1**-**3**), in addition to some metabolites which were not isolated from the rice culture, including one anthraquinone (**9**), one chromone derivative (**11**), and three anthraquinone dimers (**14**–**16**), thereby providing evidence for the power of the OSMAC approach.

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Supplementary data

Supplementary data (UV, MS and NMR spectra of **1**–**3** as well as ECD spectrum of **3**) associated with this article can be found in the online version.

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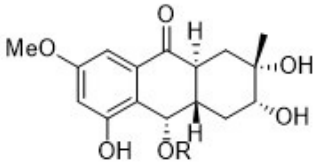
Stemphylium globuliferum



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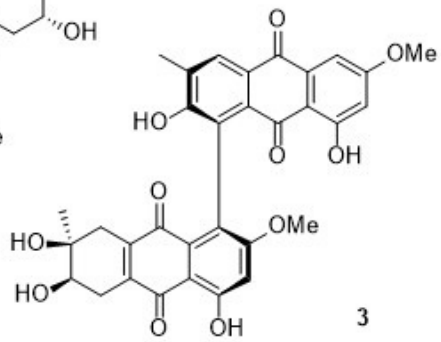

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Highlights

- Two new tetrahydroanthraquinone derivatives and one new dimer were isolated.
- TDDFT-ECD calculations were performed to determine the absolute configuration.
- OSMAC approach was employed by using white bean medium instead of rice medium.