Stable Catechol Keto Tautomers in Cytotoxic Heterodimeric Cyclic Diarylheptanoids from the Seagrass *Zostera marina*

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**ABSTRACT:** Two diarylheptanoid heterodimers, zosterabisphenones A (1) and B (2), were isolated from the seagrass *Zostera marina*. They feature unprecedented catechol keto tautomers, stable because of steric constraints. Their structure elucidation was based on extensive low-temperature NMR studies and ECD and MS data, with the essential aid of DFT prediction of NMR and ECD spectra. Zosterabisphenone B (2) was selectively cytotoxic against the adenocarcinoma colon cancer cell line HCT116 with IC50 3.6 ± 1.1 μM at 48 h.

**Diarylheptanoids** are a class of natural products characterized by two benzene rings, usually bearing one or more hydroxyl groups, joined by a functionalized seven-carbon chain. Diarylheptanoids are widespread in plants, curcumin being the best known and commercially most important example.1 A smaller subset of diarylheptanoids comprises cyclic diarylheptanoids, in which the two aromatic rings are linked together directly (biphenyl type) or through an oxygen atom (diphenyl ether type). Due to their inherent steric strain, cyclic diarylheptanoids are frequently found to possess axial and/or planar chirality.2,3 Indeed, the smallest natural product that contains axial, planar, and point chirality elements in the same molecule is the cyclic diarylheptanoid tedarene B.4 When the energy barrier between the atropisomers is relatively low, axial or planar chirality is known to cause coalescent NMR signals.5

A number of diarylheptanoids have recently been reported from the common eelgrass, *Zostera marina* L. (Zosteraceae).5,6 These include zosteraphenol A (3) and B (4), two tetracyclic diarylheptanoids that experience equilibrium with minor atropisomers with opposite axial chiralities, resulting in 1H and 13C NMR spectra rich in coalescent signals.6 The nature of the rotameric equilibrium of zosteraphenols was fully determined using a combination of variable-temperature NMR measurements and DFT calculations.6

Here we report the isolation and structure elucidation of two unique diarylheptanoid dimers, zosterabisphenones A (1) and B (2) (Chart 1), putatively originating from oxidative coupling of two different cyclic diarylheptanoids. In both zosterabisphenones, one of the benzene rings is highly modified and is

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**Chart 1**

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no longer aromatic, due to a tautomeric equilibrium favoring a catechol compared to its keto tautomer.

_**Z. marina**_ (unrooted plants, freshly washed ashore and air-dried) was extracted with acetone, and the extract was subjected, in sequence, to SiO₂ column chromatography, Sephadex LH-20 chromatography, and reversed-phase HPLC to give pure _zosterabisphenone A_ (1, 4.6 mg) and _B_ (2, 4.2 mg).

The molecular formula of _zosterabisphenone A_ (1) was determined as C₉₀H₉₀O₇ (23 unsaturations) from the [M + Na]⁺ ion at m/z 621.2235 in the high-resolution ESI mass spectrum and hinted to a dimeric diarylheptanoid structure. All NMR experiments of compound _1_ were recorded at low temperature (253 K), because the ¹H and ¹³C spectra recorded at room temperature showed many coalescent signals (Figure S10), similarly to the monomeric diarylheptanoids _zosteraphenol A_ (3) and _B_ (4) from the same source.⁵ Indeed, examination of NMR data (Table S1) showed that one of the diarylheptanoid units (“southern unit”) was similar to _zosteraphenol A_ (3), except that C-8 was not protonated, and therefore was supposed to be involved in linking the other diarylheptanoid unit. Another difference was that the methoxy group of compound _1_ was located at C-6, and not at C-1 as in _zosteraphenol A_ (3).

Signals of the second (“northern”) unit were indicative of the presence of a 1,2,4-trisubstituted benzene ring and of a hepta-2,4-diene-1,7-diy1 chain. The remaining six carbons in the molecule, including two sp³ methine carbons and a carbonyl carbon atom, suggested an extensive modification of the second benzene ring of the northern unit. Extensive analysis of HMBC data (shown in Figure 1 and discussed in detail in the Supporting Information section) revealed that _zosterabisphenone A_ (1) features an unprecedented cyclohexenedione tautomer of catechol, in which one carbonyl is involved in a cyclic hemiacetal function with the OH group at positions 14'. Moreover, HMBC data established the C-8/C-3' bond connecting the two diarylheptanoid units.

The relative configuration of the three stereocenters on the northern unit (C-1', C-2', and C-3') was determined by the ROE5Y correlation between OH-1' and H-2', pointing to their cis relationship, and by the ROE5Y correlations of H-3' with H-9' and H-16', showing H-3' pointing inward of the macrocycle and therefore establishing the relative configuration at C-3' (Figure 1). However, the stereochemical relationship between the northern unit and the southern unit, also containing one stereocenter, could not be determined from NMR data. Therefore, structure elucidation of _zosterabisphenone A_ (1) was completed with a detailed DFT study performed using the Gaussian 16 program (Revision C.01, Gaussian Inc., Wallingford CT, USA). This computational work, summarized below and described in detail in the Supporting Information section, further supported the structural features determined from NMR data and allowed the elucidation of the relative configuration between the two diarylheptanoid units and of the absolute configuration of the whole molecule.

An initial DFT study on the model compound _In_ (see Supporting Information) showed that the northern diarylheptanoid unit can adopt only one low-energy conformation. Based on this information and on the conformation of the southern unit determined in the previous work,⁶ models were generated for two diastereomers of compound _1_, differing in the relative configuration between the diarylheptanoid units, namely, (9R,1'S,2'R,3'S)-1 (called just _1_ in the following text) and (9S,1'S,2'R,3'S)-1 (epi-1 in the following text).

Conformation around the rotatable bond C-8/C-3', connecting the two units, was not obvious from spectroscopic data. Therefore, torsion about this bond was scanned in steps of 10°, and the resulting structures were optimized at the B3LYP/6-31G(d) level. This identified (Figure S2) one low-energy conformer for _1_ and two low-energy conformers, separated by a nearly flat potential profile, for epi-1. The conformers were reoptimized at the B3LYP/6-31+G(d,p) level, giving the final structures that were used for NMR and ECD prediction.

Prediction of the ¹H and ¹³C NMR chemical shifts⁷ allowed a confident selection between the alternative diastereomers _1_ and epi-1. Isotropic shieldings were calculated⁸ at the PBE0/6-311+G(2d,p) level of theory, including the PCM continuous solvent model for chloroform,⁹ and were converted to chemical shifts using the conversion factors proposed by the Tantillo group¹⁰ for this level of theory (for epi-1, the Boltzmann-averaged chemical shifts over the two conformers were considered). Diastereomer _1_ matched experimental chemical shifts remarkably better (RMSD of 1.66 ppm for ¹³C and 0.113 ppm for ¹H) than epi-1 (RMSD of 1.92 ppm for ¹³C and 0.148 ppm for ¹H). In addition, some predicted chemical shifts of epi-1 (C-9, H-5’, and H-7’) showed large deviations (Figure S3). Finally, DP4+ analysis¹¹ of the predicted chemical shifts showed a 100.00% probability for _1_ to be the correct stereoisomer.

DFT prediction of NMR parameters also provided a solid support to the unique structure of _zosterabisphenone A_ (1),¹² in that the accuracy of the prediction was remarkably better than the expected accuracy of the method (reported as 2.45 ppm for ¹³C and 0.15 ppm for ¹H)¹⁰ and similar to the accuracy obtained for _zosteraphenol A_ in our previous work.⁶ Further support to structure, configuration, and conformation of the two diarylheptanoid units of _1_ came from DFT prediction of ¹H−¹H scalar coupling, calculated according to the suggestions of Bally and Rablen.¹³ The predicted couplings were in excellent agreement with the observed multiplicity of ¹H NMR signals (Table S12); in particular, the predicted coupling between the vicinal protons H-2' and H-3' (1.1 Hz), in turn linked to the −82.5° torsion angle between them, nicely fit the singlet resonance observed for the two protons. Finally, all the observed ROE5Y cross peaks (Figure 1) were associated with protons which are spatially close in the determined DFT minimum energy conformation.
Absolute configuration of zosterabisphenone A was determined by DFT prediction of its ECD spectrum\textsuperscript{14} at the \omega B97XD/6-31+G(d,p) level of theory, using the PCM model for the solvent. The predicted ECD spectrum, generated using the SpecDis program,\textsuperscript{15} was in good agreement with the experimental ECD spectrum (Figure S17), thus defining the (9R,1’S,2’R,3’S) absolute configuration for zosterabisphenone A.

The reason for the stability of the enone tautomer \textbf{1} compared to its aromatic tautomer \textbf{1a} (Scheme 1) can be ascribed to steric reasons. In the catechol tautomer \textbf{1a}, the bulky southern unit at C-4 compared to its aromatic tautomer \textbf{1a} (Scheme 1) can be described for compound \textbf{1}, involving preparation of DFT-optimized models of the northern diarylheptanoid unit in accordance with the observed NOEs (see Supporting Information for details), assembly of the two possible diastereomers \textit{R} (Table S13), both showing an excellent agreement with the experiment.

Zosterabisphenone B (2) contains two stereocenters, C-9 and C-3,’ one on each diarylheptanoid unit. Their relative configuration was determined using the same protocol as described for compound 1, involving preparation of DFT-optimized models of the northern diarylheptanoid unit in accordance with the observed NOEs (see Supporting Information for details), assembly of the two possible diastereomers 2 [the (9R,3’S) stereoisomer] and \textit{epi}-2 [the (9R,3’R) stereoisomer] and their DFT optimization; scan of possible conformers about the C-8/C-3’ bond (Figure S6), and reoptimization of the two conformers found for each diastereomer. The \textit{1}H and \textit{13}C chemical shifts of 2 and \textit{epi}-2 were calculated and compared with experimental data. While the accuracy of the predicted \textit{13}C chemical shifts was similar (RMSD of 2.05 ppm for 2 and 2.08 for \textit{epi}-2), the accuracy of \textit{1}H chemical shifts was clearly better for 2 (RMSD of 0.126 ppm for 2 and 0.149 for \textit{epi}-2) (Figure S7). Consistently,
selective cytotoxic effects on HCT116 cells and the abundance of its natural source, zosterabisphenone B (2) can be proposed as a lead compound for the development of new antitumor drugs in colorectal cancer. Further experiments to investigate its selectivity (tumor cells vs normal cells) and evaluate its mechanism of action are in progress and will be reported in the due course.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c02537.

Additional details on structure elucidation; experimental methods and characterization data; computational methods; computational results with additional figures (reactions, structures, and cytotoxicity data); tables with NMR data, Cartesian coordinates, rotatory strengths, and cell viability of 1 and 2; ESI, NMR, HSQC, HMBC COSY, ROESY, and UV spectra of 1 and 2 (PDF)

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**Notes**

The authors declare no competing financial interest.

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