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Reassignment of the absolute configuration of plakinidone from the sponge consortium *Plakortis halichondrioides*–*Xestospongia deweerdtiae* using a combination of synthesis and a chiroptical approach

Carlos Jiménez-Romero^a, Joanna E. Rode^b, and Abimael D. Rodríguez^{a,*}

^aMolecular Sciences Research Center, University of Puerto Rico, 1390 Ponce de León Avenue, San Juan, Puerto Rico 00926 ^bInstitute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

Abstract

Recent work by Wu et al. in connection with the first synthesis of the marine natural product plakinidone revealed that the most salient feature of its purported structure, a six-membered per lactone moiety, was in fact a five-membered lactone, i.e. a 3-methyl-4-hydroxy-2(5H)-furanone or tetronic acid ring. With the planar structure of plakinidone confidently revised, we undertook a new investigation to unambiguously establish its absolute configuration. Upon preparing two stable derivatives **1** and **5** from a sample of naturally occurring plakinidone extracted from the sponge association *Plakortis halichondrioides*–*Xestospongia deweerdtiae*, the absolute configuration was assigned by synthesis and vibrational and electronic circular dichroism (VCD and ECD) measurements in combination with density functional theory calculations at the B3LYP/aug-cc-pVDZ/PCM(CH₃CN) level of theory. Our combined efforts and the agreement between the experimental and calculated VCD/ECD spectra of **1** revealed that the absolute configuration of plakinidone was in fact (11*S*,17*R*) and not the formerly reported (11*S*,17*S*) diastereomer assigned by Wu et al.. Therefore, we propose that natural plakinidone is accurately represented by structure **12**.

Graphical Abstract

* abimael.rodriquez1@upr.edu.

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Supplementary data

Supplementary data (copies of the ¹H NMR and ¹³C NMR spectra of natural plakinidone, experimental details for the synthesis of enantiomer (11*R*)-**5**, Figures 1SD–5SD, Tables 1SD–7SD, and underwater photograph of the sponge consortium *Plakortis halichondrioides*–*Xestospongia deweerdtiae*) associated with this article can be found, in the online version, at <http://dx.doi.org/.....>

<p> H_3CO $\text{C}_{18}\text{H}_{30}\text{O}$ (S)-1-(5,9-Dimethyldodec-8-en-1-ynyl)-4-methoxybenzene </p>	$[\alpha]_D^{20} = -11.6$ (c 1.31, CHCl_3) source of chirality: (-)- Citronellol Absolute configuration: (S)
<p> H_3CO $\text{C}_{18}\text{H}_{30}\text{O}_2$ (R)-8-(4-Methoxyphenyl)-4-methyloct-7-ynal </p>	$[\alpha]_D^{20} = -5.8$ (c 0.52, CHCl_3) source of chirality: (-)- Citronellol Absolute configuration: (S)
<p> H_3CO $\text{C}_{20}\text{H}_{30}\text{O}_2$ (S)-12-(4-methoxyphenyl)-8-methyldodec-4-en-11-yn-2-one </p>	$[\alpha]_D^{20} = -13.0$ (c 0.70, CHCl_3) source of chirality: (-)- Citronellol Absolute configuration: (S)
<p> H_3CO $\text{C}_{20}\text{H}_{32}\text{O}_2$ (S)-12-(4-Methoxyphenyl)-8-methyldodecan-2-one </p>	$[\alpha]_D^{20} = +7.3$ (c 1.1, CHCl_3) source of chirality: (-)- Citronellol Absolute configuration: (S)

1. Introduction

In 1991, during an investigation into the chemical composition of the Caribbean sponge *Plakortis angulospiculatus*, Kushland and Faulkner reported the discovery of a new natural product which they named plakinidone.¹ This interesting *p*-hydroxyphenyl polyketide was heralded as the first natural product that incorporated a six-membered-ring per lactone moiety.² The purported structure for plakinidone was established exclusively on the basis of conventional spectroscopic data (IR, EIMS and 1D NMR) without any stereostructural or chemical characterization work (Fig. 1).

In 2015, while attempting the first synthesis of plakinidone, Wu et al. found evidence that the previous assignment of this natural product as a per lactone was mistaken and thus plakinidone was structurally revised to a five-membered lactone (Fig. 1).³ As part of a strategy to scrutinize the absolute structure, they synthesized three out of the four possible stereoisomers for plakinidone and compared their ¹³C NMR and optical rotation data with the natural product. Neither the specific rotation nor the ¹³C NMR data recorded were compatible with natural plakinidone. Soon thereafter, they realized that the secondary products arising from the facile air oxidation of the synthetic plakinidones accounted for these discrepancies. They argued that the tetronic acid moiety in these compounds absorb oxygen, thus making them unstable, with a tendency to decompose analogues of varying specific rotation. Also, we suspect that the susceptibility of natural plakinidone to air oxidation was not entirely realized by Faulkner suggesting that the reported specific rotation should be called into question.¹ To complicate matters further, Wu et al. did not have natural plakinidone and were restricted in their studies by having only two of the three synthetic

plakinidones prepared in pure form. Perhaps unbeknownst to these researchers, the position of the ^{13}C NMR peaks about the tetronic acid moiety (as well as the tautomer distribution of the furanone forms) varies somewhat with temperature, the polarity of the solvent, and the compound concentration, which would help to partially explain the discrepancies observed in the ^{13}C NMR and $[\alpha]_{\text{D}}$ data.⁴ We contend that the seemingly ambiguous nature of the data encountered by Wu et al. following this approach made his attempts at assigning the absolute configuration of plakinidone quite vulnerable to error.⁵ In spite of all these drawbacks, his team tentatively assigned the absolute configuration as (11*S*,17*S*) on the basis of the estimation of oxidation-related changes in the optical activity of all the synthetic stereoisomers prepared.

In view of these obstacles, the re-isolation of plakinidone represented a convenient opportunity to provide not only a detailed structure elucidation using modern 2D NMR spectroscopic techniques (^1H – ^1H COSY, HSQC, HSQC-TOCSY, and HMBC), but also to determine its stereostructural features in a manner that is essentially unconcerned with the exact specific rotation and ^{13}C NMR chemical shift values. Briefly, our basic strategy called for the preparation of two stable derivatives of natural plakinidone, **1** and **5**, that are not only impervious to air-oxidation but amenable to total synthesis, and vibrational and electronic circular dichroism (VCD and ECD) studies. Chiroptical techniques are increasingly employed for the absolute configuration assignment of chiral molecules through comparison of experimental spectra with theoretical predictions.^{6,7} This approach has proved to be particularly useful in the case of natural products, an important source for drug discovery often exhibiting molecular flexibility, multiple functional groups, or chromophores.⁸ To increase the confidence level of the stereochemical analysis, the use of different chiroptical techniques is postulated.^{9–17} On the other hand, the determination of the absolute configuration of molecules with many stereogenic centers by chiroptical methods is still a challenge, and is rather limited to rigid molecules.^{8,15, 18–21} In the case of diastereomers, there is no spectra mirror relationship as for enantiomers, and therefore, in order to unambiguously assign the absolute configuration, the compared experimental and calculated data have to be of very good quality. Nevertheless, successful attempts exist for the absolute configuration assignment of large and flexible molecules.^{17,22,23} In such cases, the use of other methods such as the synthesis of model compounds based on well-established reactions or NMR studies, are helpful for structure elucidation. Herein, the assignment of the C11 center of natural plakinidone derivative **1** was ascertained by enantioselective syntheses of fragment **5**. Furthermore, for the configuration assignment of the C17 center, two independent techniques, vibrational and electronic circular dichroism (VCD and ECD), were applied and the interpretation of the experimental data was based on DFT calculations.

2. Results and discussion

2.1. Isolation of natural plakinidone

Samples of a sponge consortium constituted by *Plakortis halichondrioides* and *Xetospongia deweerdtae* specimens from Puerto Rico were collected by members of our research team at a depth of 90–100 ft.²⁴ The crude extract of a small portion of the consortium containing mainly *Plakortis halichondrioides* was extracted with CHCl_3 –MeOH. After solvent removal,

the dark gum obtained was suspended in H₂O and extracted exhaustively with *n*-hexane and CHCl₃. Concentration of the CHCl₃ extract *in vacuo* afforded the crude dark brown oil (8.0 g) used in this investigation. Repetitive reversed-phase chromatography afforded 926 mg of pure plakinidone whose ¹H- and ¹³C-NMR properties in CDCl₃ were in agreement with the published values.¹ Following exclusion of air from the NMR solvent, higher quality NMR spectra were obtained in CD₃OD as plakinidone was sparingly soluble in CDCl₃. The specific rotation data are relevant and include [α]_D = +7.9 (*c* 0.61, CH₃OH) of Faulkner's sample¹ versus [α]_D = −13.1 (*c* 0.61, CH₃OH) of our carefully handled sample. Since the original structure assignment of plakinidone was based solely on 1D NMR and MS data, we sought to collect additional 2D-NMR data in support of the Wu's revised planar structure.³ Thus, a combination of ¹H–¹H-COSY, HSQC, HSQC-TOCSY, and HMBC NMR experiments allowed for the complete ¹H and ¹³C-signal assignments. Although ¹H and ¹³C NMR data of plakinidone were essentially the same as those reported,¹ for comparative purposes they are given in Table 1. Of particular importance was a HSQC-TOCSY ¹³C–¹H-shift-correlation experiment with plakinidone, which allowed us to unambiguously place the secondary methyl group at C-11. As the locus of the H₃-23 group in the 1991 disclosure was based solely on mass spectrometric evidence for mechanisms of fragmentation, we repeated the EIMS measurements and confirmed the constitution of plakinidone in accordance with the recently revised structure.^{1,3}

2.2. Methylation of natural plakinidone

Due to plakinidone's tendency for air oxidation, we conducted the derivatization as quickly as possible.³ As shown in Scheme 1, the natural product was treated with diazomethane to furnish in almost quantitative yield a 1.3:1 mixture of dimethylated adducts **1** and **2**, which were easy to separate using conventional chromatographic methods. Structural clarification of the new substances followed from their physical and spectroscopic properties, including 1D and 2D NMR studies, IR, UV, and LR-EIMS measurements. Compounds **1** and **2** were highly soluble in CDCl₃ and practically inert under these conditions, which made them suitable for the determination of the configuration at C-17 using VCD and ECD measurements. In the end, we chose **1** over **2** as the best target for chiroptical studies since the former compound was more abundant and both its molecular arrangement and specific rotation data matched more closely those of natural plakinidone.

In order to pursue the absolute configuration of the more remote C-11 stereogenic center we next decided to synthesise **5**, a plakinidone derivative devoid of the five-membered lactone (Scheme 1). This fragment possesses a single stereogenic center and was expected to be impervious to air oxidation and accessible by enantioselective total syntheses. After repeated failures, we found that stirring natural plakinidone for 3 days, without any precautions to exclude air, led to an unstable mixture of products that decomposed spontaneously to give a 1:1 mixture of **3** in 67% yield. Subsequent degradation of **3** with LDA/THF afforded *p*-hydroxyphenyl ketone **4** in 73% yield, which upon treatment with diazomethane afforded the end product **5** in 72% yield. The specific rotation for **5** was subsequently determined to be +8.6 (*c* 1.1, CHCl₃).

2.3. Enantioselective synthesis of plakinidone-derived fragment 5

The C1–C17 (including C22 and C23) fragment **5**, obtained by the deliberate decomposition of natural plakinidone (Scheme 1), was synthesized as shown in Scheme 2. Commercially available (*S*)-citronellol **6** was treated with $\text{CBr}_4/\text{PPh}_3$ to furnish the known citronellyl bromide **7** in 63% yield. Alkylation of 4-ethynylanisole with **7** gave **8** (40%), which upon treatment with MMPP/MeOH afforded **9** in 95% yield as a 1:1 mixture of epoxides. Subsequent treatment with $\text{NaIO}_4/\text{THF}/\text{H}_2\text{O}$ led to the desired aldehyde **10** in 75% yield, which was further transformed into β,γ -unsaturated ketone **11** (71%) using an In/InCl_3 -mediated cross-coupling reaction with methyl vinyl ketone. Finally, hydrogenation over Pd/C afforded the end product (11*S*)-**5** in 42% yield; the specific rotation $[\alpha]_{\text{D}}^{20} = +7.3$ (*c* 1.1, CHCl_3), was compatible with the $[\alpha]_{\text{D}}^{20} = +8.6$ (*c* 1.1, CHCl_3) value for **5** (Scheme 1) obtained from natural plakinidone. Furthermore, enantiomer (11*R*)-**5** (Scheme 2), synthesized from commercially available (*R*)-citronellyl bromide in a parallel way (see the Supplementary Data), showed a $[\alpha]_{\text{D}}^{20}$ value of -7.8 (*c* 1.1, CHCl_3), thus confidently establishing that natural plakinidone must have an (11*S*)-absolute configuration.

2.4. Assignment of the configuration of the C17 center of plakinidone derivative 1 by combined experimental and theoretical analysis of ECD and VCD spectra

Conformational analysis—Natural plakinidone derivative **1** contains two stereogenic C11 and C17 carbon centers. The configuration of the C11 center was assigned through synthetic studies as (*S*). To assign the C17 center configuration, the experimental VCD and ECD spectra of **1** were measured in CH_3CN and compared with the calculated ones. At the beginning, the conformational analysis of the (11*S*,17*S*)- and (11*S*,17*R*)-epimers of **1** was explored through systematic rotation about the single bonds by changing χ_1 – χ_{13} and five methyl groups dihedral angles (Fig. 2). With this aim, two independent programs, Conflex^{25–27} and Compute VOA,²⁸ were used with the MMFF94s force field and the 5 kcal/mol energy window. However, the molecular mechanics favors chain bending conformers whereas the aliphatic chains adopts rather not-bent zig-zag structure. Therefore, additional linear chain conformers were generated by freezing the χ_2 – χ_{10} chain dihedral angles and allowing the rotation along the remaining single bonds. The obtained conformers were further optimized by using the DFT hybrid Becke three-parameter Lee–Yang–Parr (B3LYP) functional,^{29,30} the aug-cc-pVDZ basis set³¹ and polarizable continuum model (PCM), which accounted for the solvent effect.^{32,33} The relative abundances were calculated based on the G_{298} values relative to the most stable conformer.

The most stable structures of the (11*S*,17*S*)- and (11*S*,17*R*)-conformers of **1** are of zig-zag chain (the χ_1 – χ_{10} dihedral angles are almost 180°, Table 1SD), however one of them, 11*S*, 17*S*_c5, has the bent chain (Fig. 3). The main geometrical differences between the conformers come from orientation of the furan-2-one ring and the methoxy group in the benzene moiety (defined by the χ_1 and χ_{12} dihedral angles, respectively, Table 1SD). The Cartesian coordinates of the most stable systems are shown in Tables 2SD and 3SD.

Assignment of the C17 center configuration based on the ECD and VCD spectra

spectra—The experimental VCD and ECD spectra of **1** were measured in CH_3CN and compared with the calculated ones (Fig. 4). The VCD spectrum of each individual

conformer was calculated at the B3LYP/aug-cc-pVDZ/PCM(CH₃CN) level. The final spectra accounts for the room temperature Boltzmann weights (G_{298}) of the most stable conformers of the 11*S*,17*S* and the 11*S*,17*R* epimers of **1** (Fig. 3 and Table 4SD) and are converted to Lorentzian bands with a 4 cm⁻¹ half-width at half-peak height. On the other hand, the theoretical ECD and UV spectra were simulated with the time-dependent DFT (TD-DFT) method, which represents a good balance between accuracy and computational efficiency.³⁴ The Coulomb-attenuating CAM-B3LYP³⁵ functional was chosen due to its documented superiority over B3LYP functional.³⁶ A Gaussian band-shape was applied with 0.30 eV as a half-height width and the calculated UV-Vis and ECD spectra were shifted by the value of difference of maximum absorption of the experimental and calculated UV-Vis spectra.³⁷ All calculations were performed by using the *Gaussian 09* package of programs.³⁸

Previously, analysis of the enone properties has been shown to be intricate due to conformation complexity and UV transitions composed of multiple (not pure) excitations.^{39,40} This also holds true in the case of the studied molecule. Comparison of the ECD spectra of individual conformers shows that there is no characteristic band (with the unchanged position and sign), which could be used for the C17 center configuration assignment (Figs. 1SD and 2SD). Moreover, the calculated ECD spectra of some (11*S*,17*R*)-conformers are close mirror images of the (11*S*,17*S*)-epimer structures (for example 11*S*, 17*R*_c1 vs. 11*S*,17*R*_c3 or 11*S*,17*R*_c2 vs. 11*S*,17*R*_c3, Fig. 1SD). This also holds true for some of the VCD bands, yet the differences are not so striking (Fig. 1SD). Therefore, the reliable simulation of the weighted spectrum depends on the proper population factors, which is directly related with the computational level used. For such a conformationally flexible system as **1**, the B3LYP/aug-cc-pVDZ/PCM(CH₃CN) level was a compromise between accuracy and computational efficiency.

It should be noted that in the case of analysis of the chiroptical spectra of the studied system, a rotation of the CH₃ group in the furan-2-one moiety by ca. 30° affects the ECD spectra above 200 nm and VCD spectra in the 1800–1400 cm⁻¹ range (Fig. 3SD). In the ECD spectrum, this manifests in the couplet bands of the opposite signs at ca. 215 nm in the two, CH₃ non-rotated and rotated, structures respectively. Also, in the calculated VCD spectrum, opposite sign bands occur in the 1800–1400 cm⁻¹ range. Thus, in order to avoid misinterpretation of the C17 center configuration assignment, only the ECD spectrum below 200 nm and VCD spectrum in the 1400–1000 cm⁻¹ range were considered. The comparison of the experimental and calculated ECD and VCD spectra is shown in Figure 4, while the calculated numerical data are gathered in Tables 5SD–7SD.

The experimental and simulated UV spectra of the (11*S*,17*R*)- and (11*S*,17*S*)-epimers show high compliance in the position and intensity of the bands at ca. 195 and 225 nm. The third experimental UV band at ca. 280 nm is not reproduced by the calculations properly. The simulated ECD spectra are reliable in the 170–200 nm range where two Cotton effects (CE) at ca. 188 and 198 nm were registered. The ECD experimental bands are better reflected by the simulated spectrum of the (11*S*,17*R*)-epimer since the bands are of the same sign. The ECD CEs of the (11*S*,17*S*)-epimer are predicted to be of the opposite sign to the measured one. Thus, based on the ECD method, the (*R*)-configuration was assigned to the C17 center.

The other chiroptical method, VCD, also confirms the (*R*)-configuration at the C17 atom (Fig. 4), yet the fitting is not so sticking as for ECD spectrum. Comparison between the experimental and calculated VCD spectra was analyzed by using the Compare *VOA*⁴¹ program and the confidence level of the (11*S*,17*R*)-population-weighted VCD spectrum was equal to 71% when the 1400–1000 cm⁻¹ spectral range was applied.

3. Conclusion

In spite of the air-sensitive nature of natural plakinidone and its synthetic congeners, we have demonstrated that the absolute configuration assignment could be achieved unambiguously by transforming the natural product into very stable derivatives that are amenable to chiroptical spectral analysis (i.e. **1**) and enantioselective syntheses (i.e. **5**). From the evidence gathered, we have unambiguously established the absolute configuration of natural plakinidone as (11*S*,17*R*). Thus, we propose that natural plakinidone is accurately represented by structure **12** (Fig. 5). Coincidentally, one of the two pure stereoisomers synthesized by Wu et al. (i.e. *ent*-**12**) is enantiomeric with our proposed structure for natural plakinidone (the other one being **13**, which is clearly diastereomeric with **12**).⁴² As expected, the absolute configuration of *ent*-**12** is opposite according to the sign of the specific rotation, although the apparent specific rotation is too small in magnitude. Barring substantial deviations due to unsuspecting air oxidation or the diverging temperatures (i.e. 20° C vs 26° C), the fact that the apparent value +1.2 for synthetic *ent*-**12** deviates from the -13.1 value for natural plakinidone **12** by a factor of about 10, can only be explained by human error or the use of improperly calibrated instrumentation during [α]_D determinations (Fig. 5). Notwithstanding, the observation that the ¹³C NMR spectra of these compounds in CD₃OD are nearly identical, Table 1 supports our contention that they are indeed enantiomers of one another.⁴³

4. Experimental

4.1. General

Optical rotations were measured in CHCl₃ or CH₃OH at 589 nm with a Rudolph Research Analytical Autopol IV using a 10-cm microcell. Infrared and UV spectra were measured with a Nicolet Magna 750 FT-IR and a Shimadzu UV-2401 PC UV-Visible spectrophotometer, respectively. 1D and 2D NMR spectral data were generated with a 500 MHz (Bruker DRX-500) and 700 MHz (Bruker Ascend™ 700) FT-NMR spectrometers. Chemical shifts are expressed in ppm and were referenced to the solvent residual peak as internal standard: CD₃OD (δ_H 3.30; δ_C 49.00) or CDCl₃ (δ_H 7.26; δ_C 77.00). Multiplicities in the ¹H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad, and coupling constants are reported in Hertz. ¹³C NMR multiplicities were obtained from a DEPT-135 experiment. HMBC parameters were optimized for ^{2,3}*J*_{CH} = 6 and 8 Hz. Mass spectrometric analyses were performed at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois with a Waters VG 70-VSE (EI⁺, 70 eV) and a Waters Q-TOF Ultima (ESI⁺, 50/50 H₂O/CH₃CN with 0.1% formic acid). GC-MS analyses were recorded at 70 eV using a Hewlett Packard 5890 MS Chem Station coupled with a Hewlett Packard 5972 mass detector. The GC was equipped

with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane crosslinked with 5% phenyl methylpolysiloxane. Column chromatography was performed either on C18 reversed-phase silica gel (35–75 mesh) or normal-phase silica gel (35–75 mesh). TLC analyses were carried out using glass C18 reversed-phase silica gel plates. Spots were visualized by exposure to I₂ vapors or heating the plates sprayed with phosphomolybdic acid (PMA) solution in EtOH. Commercially available reagents were purchased and used as received unless stated otherwise. Diazomethane was prepared from Diazald® as previously described.⁴⁴ All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatus under an argon atmosphere. All solvents used were spectral grade and freshly distilled prior to use.

4.2. Isolation of natural plakinidone

Fresh specimens of the sponge *Plakortis halichondrioides* (Wilson, 1902) (phylum Porifera; class Demospongiae; subclass Homoscleromorpha; order Homosclerophorida; family Plakinidae) in association with *Xestospongia deweerdtiae* were collected by hand using scuba at depths of 90–100 ft off Mona Island, Puerto Rico.²⁴ The organism was frozen and lyophilized prior to extraction. The dry specimens (395 g) were cut into small pieces and blended in a mixture of CHCl₃–MeOH (1:1) (11 × 1 L). After filtration, the crude extract was concentrated and stored under vacuum to yield a dark gum (100 g), which was suspended in H₂O (2 L) and extracted with *n*-hexane (3 × 2 L) and CHCl₃ (3 × 2 L). Concentration of the CHCl₃ extract under reduced pressure yielded 8.0 g of a dark brown oil, which was chromatographed over C18 reversed-phase silica gel (100 g) using mixtures of MeOH–H₂O of increasing polarity (70–100%). A total of 10 fractions (I–X) were generated on the basis of TLC and ¹H NMR analyses. Careful scrutiny of the combined spectroscopic data revealed that fractions V and VI consisted of pure plakinidone (926 mg, 0.23% yield). Except for the specific rotation data, our isolate proved identical with material isolated by Faulkner with regards to all analytical and spectroscopic data.¹ After degassing the solvent by bubbling N₂ (to avoid unwanted air-oxidation), the specific rotation of our natural plakinidone was determined to be –13.1 (*c* 0.61, CH₃OH), while the corresponding data for Faulkner's sample (most likely recorded without taking any precautions to exclude air) was +7.9 (*c* 0.61, CH₃OH).

4.3. Methylation of natural plakinidone and purification of methylated products 1 and 2

To a fresh solution of natural plakinidone (80 mg, 0.214 mmol) in CHCl₃ (5 mL) was added a solution of diazomethane in ether (25 mL), and the resulting mixture was stirred at 25° C for 72 h. The reaction mixture was concentrated *in vacuo*, and the oily residue was chromatographed by silica gel (4.0 g) column chromatography eluting with an increasing polarity of CHCl₃–*n*-Hex (1:1→6:4) to yield pure compounds **1** (44.5 mg, 52% yield) and **2** (36 mg, 40% yield), respectively.

4.3.1. Derivative 1—Colorless oil; [α]_D²⁰ = –5.2 (*c* 1.6, CHCl₃); UV (CH₃OH) $\lambda_{\max}$ (log *e*) 226 (4.31), 201 (4.13) nm; IR (thin film) $\nu_{\max}$ 2929, 2856, 1877 (weak), 1751, 1671, 1512, 1460, 1315, 1246, 1037, 986, 820, 762 cm^{–1}; ¹H NMR (CDCl₃, 500 MHz) δ 7.09 (2H, d, *J* = 8.4 Hz, H-1), 6.82 (2H, d, *J* = 8.4 Hz, H-2), 4.10 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 2.54 (2H, t, *J* = 7.6 Hz, H₂-7), 1.71–1.62 (2H, m, H₂-16), 2.00 (3H, s, H₃-21), 1.54

(2H, m, H₂-8), 1.38 (3H, s, H₃-22), 1.34 (1H, m, H-11), 1.25 (9H, br envelope, H-15a, H₂-14, H₂-13, H-12a, H-10a, H₂-9), 1.08 (3H, m, H-15b, H-12b, H-10b), 0.82 (3H, d, *J* = 6.5 Hz, H₃-23); ¹³C NMR (CDCl₃, 125 MHz) δ 176.0 (C, C-18), 174.3 (C, C-20), 157.5 (C, C-3), 135.0 (C, C-6), 129.2 (2 x CH, C-1), 113.6 (2 x CH, C-2), 96.0 (C, C-19), 83.0 (C, C-17), 58.8 (OCH₃), 55.2 (OCH₃), 36.9 (CH₂, C-16), 36.8 (CH₂, C-12), 36.8 (CH₂, C-10), 35.0 (CH₂, C-7), 32.6 (CH, C-11), 32.0 (CH₂, C-8), 29.8 (CH₂, C-14), 26.8 (CH₂, C-13), 26.7 (CH₂, C-9), 23.5 (CH₃, C-22), 23.1 (CH₂, C-15), 19.6 (CH₃, C-23), 8.4 (CH₃, C-21); LR-EIMS *m/z* (relative intensity) 402 (8) [M⁺], 347 (6), 177 (27), 149 (100), 121 (32), 55 (65).

4.3.2. Derivative 2—Colorless oil; [α]_D²⁰ = +35.4 (*c* 1.10, CHCl₃); UV (CH₃OH) λ_{max} (log *ε*) 264 (3.93), 224 (3.71), 201 (4.86) nm; IR (thin film) ν_{max} 2928, 2856, 1701, 1612, 1512, 1477, 1395, 1369, 1246, 1198, 1037, 820 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.09 (2H, d, *J* = 8.4 Hz, H-1), 6.82 (2H, d, *J* = 8.4 Hz, H-2), 4.00 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 2.54 (2H, t, *J* = 7.6 Hz, H₂-7), 1.74 (2H, m, H₂-16), 1.58 (3H, s, H₃-21), 1.55 (2H, m, H₂-8), 1.39 (3H, s, H₃-22), 1.31 (1H, m, H-11), 1.25 (10H, br envelope, H₂-15, H₂-14, H₂-13, H-12a, H-10a, H₂-9), 1.08 (2H, m, H-12b, H-10b), 0.82 (3H, d, *J* = 6.5 Hz, H₃-23); ¹³C NMR (CDCl₃, 125 MHz) δ 201.0 (C, C-18), 179.3 (C, C-20), 157.6 (C, C-3), 135.0 (C, C-6), 129.2 (2 x CH, C-1), 113.6 (2 x CH, C-2), 93.0 (C, C-19), 87.4 (C, C-17), 55.6 (OCH₃), 55.2 (OCH₃), 36.3 (CH₂, C-16), 37.0 (CH₂, C-10), 36.8 (CH₂, C-12), 35.0 (CH₂, C-7), 32.6 (CH, C-11), 32.1 (CH₂, C-8), 29.9 (CH₂, C-14), 26.8 (CH₂, C-13), 26.7 (CH₂, C-9), 23.1 (CH₂, C-15), 23.0 (CH₃, C-22), 19.6 (CH₃, C-23), 4.0 (CH₃, C-21); LR-EIMS *m/z* (relative intensity) 402 (31) [M⁺], 253 (3), 211 (5), 177 (9), 155 (26), 149 (35), 142 (73), 121 (100).

4.4. Air oxidation of natural plakinidone

A fresh solution of natural plakinidone (40 mg, 0.107 mmol) in a 1:1 mixture of CHCl₃–MeOH (5 mL) was stirred in an open round flask for 3 days. The solvent was then evaporated and the product was purified by silica gel flash chromatography eluting with CHCl₃–MeOH (9:1). The product obtained **3** (28 mg, 67% yield) consisted of a 1:1 mixture of C-19 epimers. Further purification through a short plug of silica gel (0.8 g) using mixtures of CHCl₃–MeOH (98:2; 96:4; 92:6) allowed these epimers, **3a** (10 mg) and **3b** (12 mg), to be completely separated.

4.4.1. Epimeric alcohol 3a—Colorless oil; [α]_D²⁰ = –20.1 (*c* 1.0, CHCl₃); IR (thin film) ν_{max} 3406, 2928, 2855, 1800, 1759, 1614, 1597, 1515, 1456, 1377, 1218, 1114, 954, 828, 758 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.03 (2H, d, *J* = 8.4 Hz), 6.75 (2H, d, *J* = 8.4 Hz), 2.53 (2H, t, *J* = 7.4 Hz), 1.86–1.80 (2H, m), 1.59 (3H, s), 1.55 (3H, s), 1.54 (2H, m), 1.36–1.16 (2H, m), 1.33 (1H, m), 1.23 (8H, m), 1.06 (2H, m), 0.81 (3H, d, *J* = 6.6 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 210.7 (C), 173.7 (C), 153.4 (C), 135.0 (C), 129.4 (2 x CH), 115.1 (2 x CH), 90.4 (C), 69.7 (C), 38.1 (CH₂), 36.7 (CH₂), 36.6 (CH₂), 34.9 (CH₂), 32.6 (CH), 31.7 (CH₂), 29.8 (CH₂), 26.5 (CH₂), 26.3 (CH₂), 23.8 (CH₃), 23.3 (CH₃), 23.2 (CH₂), 19.8 (CH₃); LR-EIMS *m/z* (relative intensity) 390 (12) [M⁺], 372 (6), 318 (12), 304 (17), 291 (24), 290 (100), 275 (6), 232 (12), 133 (25).

4.4.2. Epimeric alcohol 3b—Colorless oil; $[\alpha]_D^{20} = -23.1$ (c 1.2, CHCl_3); IR (thin film) ν_{max} 3405, 2928, 2855, 1799, 1758, 1614, 1515, 1457, 1377, 1285, 1115, 952, 828, 764, 668 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.04 (2H, d, $J = 8.2$ Hz), 6.75 (2H, d, $J = 8.2$ Hz), 2.52 (2H, t, $J = 7.6$ Hz), 1.82 (2H, m), 1.54 (3H, s), 1.54 (2H, m), 1.53 (3H, s), 1.40 (1H, m), 1.34 (1H, m), 1.26 (9H, m), 1.06 (2H, m), 0.82 (3H, d, $J = 6.5$ Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 210.1 (C), 173.6 (C), 153.5 (C), 135.5 (C), 129.6 (2 x CH), 115.1 (2 x CH), 90.5 (C), 69.3 (C), 37.3 (CH_2), 36.7 (CH_2), 36.6 (CH_2), 34.9 (CH_2), 32.6 (CH), 31.9 (CH_2), 29.8 (CH_2), 26.6 (CH_2), 26.4 (CH_2), 23.5 (CH_2), 23.0 (CH_3), 21.9 (CH_3), 19.7 (CH_3); LR-EIMS m/z (relative intensity) 390 (100) $[\text{M}^+]$, 344 (54), 318 (42), 304 (23), 300 (40), 291 (27), 290 (88), 275 (26), 133 (96).

4.5. Synthesis of *p*-hydroxyphenyl ketone 4

A mixture of epimeric alcohols **3** (25 mg, 0.064 mmol) was placed in a flame-dried flask and dissolved in THF (5 mL), cooled to 0 °C, and then treated carefully with lithium diisopropylamide (12.6 mg, 0.118 mmol, 2 equiv). The reaction was stirred at 0 °C for 30 min, then warmed to 25 °C for 2 h. Shortly thereafter, the reaction was quenched with water (10 mL), and the reaction product extracted with EtOAc (3 x 15 mL), and concentrated by rotoevaporation. The crude oil obtained was passed through a short plug of silica gel (1.0 g) using CHCl_3 –*n*-hexanes (9:1) to afford pure compound **4** (13.5 mg, 73% yield). Colorless oil; $[\alpha]_D^{20} = +8.0$ (c 1.6, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 224 (3.56), 200 (3.70) nm; IR (thin film) ν_{max} 3379 (br), 2926, 2854, 1706, 1614, 1515, 1462, 1375, 1262, 1171, 1112, 825 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.03 (2H, d, $J = 8.4$ Hz, H-1), 6.75 (2H, d, $J = 8.4$ Hz, H-2), 2.53 (2H, t, $J = 7.6$ Hz, H₂-7), 2.41 (2H, t, $J = 7.4$ Hz, H₂-16), 2.14 (3H, s, H₃-22), 1.55 (4H, m, H₂-15, H₂-8), 1.35 (1H, m, H-11), 1.26 (8H, br envelope, H₂-14, H₂-13, H-12a, H-10a, H₂-9), 1.09 (2H, m, H-12b, H-10b), 0.82 (3H, d, $J = 6.5$ Hz, H₃-23); ^{13}C NMR (CDCl_3 , 125 MHz) δ 209.6 (C, C-17), 153.5 (C, C-6), 135.1 (C, C-6), 129.4 (2 x CH, C-1), 115.1 (2 x CH, C-2), 43.8 (CH_2 , C-16), 36.8 (CH_2 , C-12), 36.7 (CH_2 , C-10), 35.0 (CH_2 , C-7), 32.6 (CH, C-11), 31.9 (CH_2 , C-8), 29.8 (CH_3 , C-22), 29.5 (CH_2 , C-14), 26.8 (CH_2 , C-13), 26.5 (CH_2 , C-9), 23.9 (CH_2 , C-15), 19.7 (CH_3 , C-23); LR-EIMS m/z (relative intensity) 290 (21) $[\text{M}^+]$, 185 (21), 107 (100), 91 (29).

4.6. Methylation of *p*-hydroxyphenyl ketone 4 with diazomethane

To a solution of *p*-hydroxyphenyl ketone **4** (20 mg, 0.069 mmol) in CHCl_3 (2 mL) was added a solution of diazomethane in ether (10 mL), and the resulting mixture was stirred at 25 °C for 8 h. The reaction mixture was then concentrated *in vacuo* and the oily residue obtained was purified by silica gel (0.7 g) column chromatography with an isocratic mixture of CHCl_3 –*n*-hexanes (7:3) to afford pure compound **5** (15 mg, 72% yield). Colorless oil; $[\alpha]_D^{20} +8.6$ (c 1.1, CHCl_3); UV (CH_3OH) λ_{max} (log ϵ) 224 (4.03), 200 (4.15) nm; IR (thin film) ν_{max} 2927, 2854, 1717, 1613, 1584, 1513, 1464, 1359, 1300, 1246, 1171, 1038, 820 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.09 (2H, d, $J = 8.4$ Hz, H-1), 6.82 (2H, d, $J = 8.4$ Hz, H-2), 3.79 (3H, s, OCH_3), 2.54 (2H, t, $J = 7.6$ Hz, H₂-7), 2.41 (2H, t, $J = 7.4$ Hz, H₂-16), 2.13 (3H, s, H₃-22), 1.56 (4H, m, H₂-15, H₂-8), 1.36 (1H, m, H-11), 1.28 (2H, m, H-12a, H-10a), 1.26 (6H, br envelope, H₂-14, H₂-13, H₂-9), 1.10 (2H, m, H-12b, H-10b), 0.83 (3H, d, $J = 6.5$ Hz, H₃-23); ^{13}C NMR (CDCl_3 , 125 MHz) δ 209.4 (C, C-17), 157.5 (C, C-3), 135.0 (C, C-6), 129.2 (2 x CH, C-1), 113.6 (2 x CH, C-2), 55.2 (OCH_3), 43.8 (CH_2 , C-16),

36.8 (2 x CH₂, C-12 and C-10), 35.1 (CH₂, C-7), 32.6 (CH, C-11), 32.1 (CH₂, C-8), 29.9 (CH₃, C-22), 29.5 (CH₂, C-14), 26.8 (CH₂, C-13), 26.7 (CH₂, C-9), 23.9 (CH₃, C-15), 19.6 (CH₃, C-23); LR-EIMS *m/z* (relative intensity) 304 (20) [M⁺], 155 (6), 121 (100), 107 (6), 91 (15); HR-EIMS calcd for C₂₀H₃₂O₂: 304.2402, found: 304.2398.

4.7. Bromination of (*S*)-(-)-citronellol **6** with CBr₄/PPh₃

A solution of (*S*)-citronellol **6** (500 mg, 3.2 mmol) and CBr₄ (1.60 g, 4.8 mmol) in CH₂Cl₂ (5 mL) was cooled to 0 °C. Small portions of PPh₃ (1.26 g, 4.8 mol) were then added over 5 min with vigorous stirring. The solution, which turned into a light red color, was stirred for another 2 h at 25 °C after which time the reaction mixture was concentrated *in vacuo*. The addition of *n*-hexane (50 mL) caused the precipitation of a white solid (Ph₃PO), which was filtered through a short column of silica gel (3.0 g) and rinsed with *n*-hexane (150 mL). Rotary evaporation of the material eluted afforded the known (*S*)-citronellyl bromide **7** as a pure colorless liquid (440 mg, 63% yield).

4.8. Alkylation of 4-ethynylanisole with (*S*)-citronellyl bromide **7**⁴⁵

A 0.4 M solution of 4-ethynylanisole in anhydrous THF (4.5 mL) was treated with a 2.5 M solution of *n*-BuLi in *n*-hexanes (1.0 mL, 1.4 equiv) and added dropwise at 25 °C over 5 min before heating at reflux for 3 h. The mixture was cooled to 25 °C before adding a solution of (*S*)-citronellyl bromide **7** (393 mg, 1.8 mmol, 1.0 equiv) in dry THF (4.5 mL). The reaction was refluxed for 12 h, cooled to 25 °C, quenched with H₂O (10 mL), and extracted with EtOAc (3 × 25 mL). The combined organic extracts were dried (MgSO₄), filtered and concentrated to give a dark gum, which was purified by silica gel (3.0 g) flash chromatography using 100% *n*-hexanes to afford alkyne **8** (117 mg, 40% yield). Colorless oil; [α]_D²⁰ = -11.6 (*c* 1.31, CHCl₃); IR (thin film) ν_{max} 2956, 2930, 2854, 2109, 1720, 1607, 1510, 1500, 1463, 1441, 1378, 1288, 1247, 1173, 1054, 1021, 832, 812, 658 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.33 (2H, d, *J* = 8.8 Hz), 6.81 (2H, d, *J* = 8.8 Hz), 5.12 (1H, dt, *J* = 7.1, 1.4 Hz), 3.79 (3H, s, OCH₃), 2.40 (2H, m), 2.01 (2H, m), 1.69 (3H, s), 1.64–1.40 (2H, m), 1.62 (3H, s), 1.64 (1H, m), 1.40–1.20 (2H, m), 0.93 (3H, d, *J* = 6.6 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 158.9 (C), 132.8 (2 x CH), 131.2 (C), 124.8 (CH), 116.3 (C), 113.8 (2 x CH), 88.8 (C), 80.1 (C), 55.2 (OCH₃), 36.7 (CH₂), 35.9 (CH₂), 31.7 (CH), 25.7 (CH₃), 25.4 (CH₂), 19.2 (CH₃), 17.6 (CH₃), 17.1 (CH₂); GC-MS *t*_R 23.6 min (98.7%); MS (70 eV, EI): *m/z* (relative intensity) 270 (14) [M⁺], 199 (12), 187 (13), 185 (15), 171 (14), 159 (18), 147 (34), 145 (79), 130 (17), 128 (16), 121 (60), 115 (48), 107 (23), 102 (55), 93 (18), 91 (36), 89 (16), 81 (18), 77 (28), 69 (100).

4.9. Epoxidation of compound **8** with magnesium monoperoxyphthalate (MMPP)⁴⁶

To a solution of alkyne **8** (190 mg, 0.703 mmol, 1 equiv) in MeOH (5.0 mL) was added MMPP at 80% (965 mg, 1.05 mmol, 1.5 equiv). After stirring at 25 °C for 6 h, the solvent was removed under reduced pressure, the reaction mixture was diluted with saturated NaHCO₃ (15 mL), and extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried (MgSO₄), concentrated, and purified by silica gel (2.0 g) flash chromatography eluting with *n*-hexane–EtOAc (95:5) to afford a 1:1 mixture of the expected epoxides **9** (191 mg, 95% yield). Colorless oil; IR (thin film) ν_{max} 2957, 2927, 2855, 2361, 1727, 1607,

1510, 1463, 1379, 1289, 1247, 1173, 1107, 1024, 1034, 832 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.32 (2H, d, J = 8.8 Hz), 6.80 (2H, d, J = 8.8 Hz), 3.79 (3H, s, OCH_3), 2.71 (1H, dt, J = 6.5, 2.1 Hz), 2.41 (2H, m), 1.67 (1H, m), 1.67–1.45 (4H, m), 1.55 (2H, m), 1.30 (3H, s), 1.27 (3H, s), 0.94 (3H, d, J = 6.5 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 159.0 (C), 132.8 (2 x CH), 116.1 (C), 113.8 (2 x CH), 88.5 (C), 80.3 (C), 64.6/64.5 (CH), 58.3/55.2 (C), 55.2 (OCH_3), 35.8/35.7 (CH_2), 33.2/33.1 (CH_2), 31.9/31.8 (CH), 26.4/26.3 (CH_2), 24.9 (2 x CH_3), 19.1/19.0 (CH_3), 18.7/18.6 (CH_3), 17.1/17.0 (CH_2); GC–MS: t_R 24.9 min (98.0%); MS (70 eV, EI): m/z (relative intensity) 286 (3) [M^+], 188 (10), 187 (38), 172 (11), 159 (14), 158 (16), 147 (46), 146 (17), 145 (92), 134 (18), 128 (15), 121 (38), 115 (60), 102 (73), 91 (37), 77 (31), 69 (21), 63 (29), 57 (100); GC–MS t_R 25.3 min (97.8%); MS (70 eV, EI): m/z (relative intensity) 286 (3) [M^+], 187 (11), 185 (15), 160 (30), 159 (27), 158 (22), 147 (18), 146 (15), 145 (100), 130 (21), 129 (15), 128 (19), 121 (50), 115 (56), 103 (17), 102 (77), 91 (27), 89 (20), 79 (11), 76 (30), 71 (83), 65 (16), 63 (24).

4.10. Oxidation of **9** with sodium periodate (NaIO_4)⁴⁷

A mixture of epoxides **9** (190 mg, 0.664 mmol, 1 equiv) and NaIO_4 (284 mg, 1.33 mmol, 2 equiv) in $\text{THF-H}_2\text{O}$ (1:1 v/v, 5 mL) was stirred at 25° C for 12 h. After diluting with H_2O (15 mL), the aqueous layer was extracted with EtOAc (3×15 mL), the combined organic extracts were washed with H_2O and brine, dried (MgSO_4), and concentrated under reduced pressure. The crude oil left was purified by silica gel (2.0 g) column chromatography using *n*-hexanes– EtOAc (90:10) to afford aldehyde **10** (121 mg, 75% yield). Colorless oil; $[\alpha]_D^{20} = -5.8$ (c 0.52, CHCl_3); IR (thin film) ν_{max} 2957, 2931, 2872, 2721, 2361, 1725, 1607, 1510, 1464, 1380, 1289, 1247, 1173, 1106, 1073, 1034, 833 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 9.79 (1H, t, J = 3.5 Hz), 7.31 (2H, d, J = 8.8 Hz), 6.81 (2H, d, J = 8.8 Hz), 3.79 (3H, s, OCH_3), 2.47 (2H, m), 2.42 (2H, m), 1.72–1.50 (2H, m), 1.65 (1H, m), 1.65–1.45 (2H, m), 0.94 (3H, d, J = 6.5 Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 202.7 (CH), 159.0 (C), 132.8 (2 x CH), 116.0 (C), 113.8 (2 x CH), 88.2 (C), 80.4 (C), 55.2 (OCH_3), 41.6 (CH_2), 35.5 (CH_2), 31.6 (CH), 28.5 (CH_2), 18.9 (CH_3), 17.1 (CH_2); GC–MS t_R 21.8 min (99.5%); MS (70 eV, EI): m/z (relative intensity) 244 (10) [M^+], 202 (7), 201 (4), 200 (5), 199 (5), 188 (20), 187 (33), 185 (12), 173 (11), 172 (11), 171 (5), 161 (3), 160 (9), 159 (23), 158 (17), 147 (29), 145 (100), 144 (15), 134 (20), 128 (18), 121 (40), 115 (61), 102 (85), 91 (36), 89 (28), 79 (19), 76 (36), 65 (20), 62 (42).

4.11. In/InCl_3 -mediated cross-coupling reaction of **10** with methyl vinyl ketone⁴⁸

A mixture of aldehyde **10** (63.7 mg, 0.261 mmol, 1 equiv), In (–100 mesh, 60 mg, 0.522 mmol, 2 equiv), InCl_3 (30 mg, 0.130 mmol, 0.5 equiv), and methyl vinyl ketone (66 μL , 0.783 mmol, 3 equiv) in $\text{THF-H}_2\text{O}$ (4:1, 2 mL) was stirred at 25° C for 24 h. After the addition of 1 M HCl (1.0 mL), the reaction mixture was stirred for 1.5 h and extracted with EtOAc (3×15 mL). The combined organic phase was washed with brine, dried (MgSO_4), and concentrated under reduced pressure to afford a residue that was passed through a short plug of silica gel (0.7 g) with a mixture of *n*-hexanes– EtOAc (85:15) to yield β , γ -unsaturated ketone **11** (40 mg, 71% yield). Colorless oil; $[\alpha]_D^{20} = -13.0$ (c 0.70, CHCl_3); IR (thin film) ν_{max} 2930, 2870, 2045, 1716, 1606, 1510, 1464, 1361, 1289, 1248, 1174, 1106, 1074, 1034, 833, 536 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 7.31 (2H, d, J = 8.8 Hz), 6.80 (2H, d, J = 8.8 Hz), 5.54 (2H, m), 3.79 (3H, s, OCH_3), 3.08 (2H, d, J = 5.2 Hz), 2.39 (2H,

m), 2.12 (3H, s), 2.05 (2H, m), 1.61 (1H, m), 1.61–1.41 (2H, m), 1.41–1.24 (2H, m), 0.92 (3H, d, $J = 6.5$ Hz); ^{13}C NMR (125 MHz, CDCl_3) δ 207.5 (C), 158.9 (C), 135.3 (CH), 132.8 (2 x CH), 121.7 (CH), 116.2 (C), 113.8 (2 x CH), 88.7 (C), 80.2 (C), 55.2 (OCH_3), 47.6 (CH_2), 36.0 (CH_2), 35.8 (CH_2), 31.5 (CH), 30.0 (CH_2), 29.2 (CH_3), 19.0 (CH_3), 17.1 (CH_2); LR-ESIMS m/z 299.2 $[\text{M}+\text{H}]^+$, m/z 321.2 $[\text{M}+\text{Na}]^+$.

4.12. Hydrogenation of β,γ -unsaturated ketone **11** to methyl ketone (**11S**)-**5**

A mixture of β,γ -unsaturated ketone **11** (30 mg, 0.101 mmol) and 10% Pd on charcoal in dry acetone (5 mL) was stirred under H_2 (1 atm) at 25 °C for 24 h. The reaction mixture was evacuated, then filtered through a short pad of Celite and concentrated under reduced pressure to give the crude extract. Purification through a short plug of silica gel (0.5 g) with mixtures of *n*-hexanes–EtOAc of increasing polarity (100:0; 98:2; 94:6) gave pure methyl ketone (**11S**)-**5** (13 mg, 42% yield). Colorless oil; $[\alpha]_{\text{D}}^{20} = +7.3$ (c 1.1, CHCl_3); IR (thin film) ν_{max} 2928, 2856, 1719, 1612, 1513, 1464, 1376, 1359, 1246, 1177, 1122, 1073, 1038, 820 cm^{-1} ; GC–MS t_{R} 26.0 min (99.7%); MS (70 eV, EI): m/z (relative intensity) 304 (6.0) $[\text{M}^+]$, 134 (4), 122 (9), 121 (100), 91 (8), 78 (9), 77 (11). This synthetic enantiomer proved identical with natural plakinidone-derived fragment **5** (Scheme 1) in all analytical and spectroscopic regards.

4.13. VCD measurements

The VCD and IR spectra of **1** dissolved in CH_3CN were measured using the ChiralIR-2X FT-VCD spectrometer from *BioTools Inc.* The spectra were collected in the 2000–1000 cm^{-1} range at a resolution of 4 cm^{-1} . The spectrometer was equipped with dual sources and dual ZnSe photoelastic modulators (PEMs) optimized at 1400 cm^{-1} . A solution with a concentration of ca. 0.14 M was measured in a BaF_2 cell with a path length of 99.7 μm . The final spectrum was averaged from 9 blocks, each of 2048 interferometric scans (1 block accumulated for 40 minutes). Baseline correction using the spectrum of the relevant solvent obtained under the same conditions was performed.

4.14. ECD measurements

The ECD and UV spectra of **1** in CH_3CN ($\sim 1.7 \cdot 10^{-4}$ M) were recorded in a quartz cell with a path length of 0.1 cm between 300 and 180 nm at 25° C on a Jasco J-815 spectrometer. The spectrum was recorded using 100 nm/min scanning speed, a step size of 0.2 nm, a bandwidth of 1 nm, a response time of 0.5 s, and an accumulation of 10 scans. The spectra were background corrected using respective solvents recorded under the same conditions.

Supplementary Material

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Acknowledgments

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43. As anticipated, only the four quaternary carbons conforming the tetronic acid ring (i.e. C17 through C20) deviated slightly (± 0.2 – 0.9 ppm). Likewise, the ^1H NMR data for 12 and ent-12 in CD_3OD are almost superimposable (see Table 1 and ref. 3).
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Figure 1.
Planar structures and numbering system for plakinidone: (a) original and (b) reassigned.

Scheme 1.

Derivatizations of natural plakinidone into **1–5** and the specific rotation data of the end products.

Scheme 2. Synthesis of the (11*S*)-enantiomer of plakinidone product **5**.

Figure 2.
The conformational analysis of **1** explored by changing the indicated $\chi_1 - \chi_{13}$ dihedral angles and rotation of the five methyl groups.

Figure 3.

The structures of the most stable 11*S*,17*S* and 11*S*,17*R* epimers of **1**. In parentheses the relative to the most stable conformer Gibbs free energies (kcal/mol) and population factors (including only the depicted systems) calculated at the B3LYP/aug-cc-pVDZ/PCM(CH₃CN) theory level.

Figure 4. Comparison of the experimental (red) and DFT/aug-cc-pVDZ/PCM(CH₃CN) population-weighted spectra of **1** measured in CH₃CN.

Figure 5.
Structures for natural plakinidone **12** (herein), synthetic plakinidone stereoisomers *ent*-**12** and **13** (Wu's work) and the specific rotation data measured at $c = 0.61$ in MeOH after degassing with N_2 to exclude unwanted air oxidation.

Table 1

¹H NMR and ¹³C NMR data for plakinidone from different sources

Proton	δ_{H}^a	Carbon (mult.)	δ_{C}^b	δ_{C}^c	δ_{C}^d	δ_{C}^e
1	6.95 (2H, d, $J = 8.4$ Hz)	1 (CH)	129.3 (2 × C)	129.3 (2 × C)	130.2 (2 × C)	130.2 (2 × C)
2	6.67 (2H, d, $J = 8.4$ Hz)	2 (CH)	115.0 (2 × C)	115.0 (2 × C)	116.0 (2 × C)	116.0 (2 × C)
3		3 (C)	154.0	154.0	156.2	156.2
6		6 (C)	134.4	134.2	134.8	134.8
7	2.48 (2H, t, $J = 7.6$ Hz)	7 (CH ₂)	34.7	34.7	36.0	36.0
8	1.51 (2H, m)	8 (CH ₂)	31.7	31.7	33.3	33.3
9	1.25–1.20 (2H, br m)	9 (CH ₂)	26.1	26.1	27.6	27.6
10a,b	1.28 (1H, m), 1.07 (1H, m)	10 (CH ₂)	36.9	36.5	38.0	38.0
11	1.36 (1H, m)	11 (CH)	32.5	32.5	33.8	33.8
12a,b	1.28 (1H, m), 1.07 (1H, m)	12 (CH ₂)	36.9	36.4	38.0	38.0
13	1.25–1.20 (2H, br m)	13 (CH ₂)	26.7	26.7	27.9	27.9
14	1.25–1.20 (2H, br m)	14 (CH ₂)	29.9	29.9	30.8	30.8
15a	1.25–1.20 (1H, br m)	15 (CH ₂)	23.1	23.0	24.1	24.1
15b	1.10 (1H, br m)					
16	1.72 (2H, m)	16 (CH ₂)	36.6	36.6	37.5	37.5
17		17 (C)	83.9	83.9	85.1	84.9
18		18 (C)	178.0	178.4	181.1	179.5
19		19 (C)	95.5	95.2	95.5	96.4
20		20 (C)	176.2	176.3	178.1	177.5
21	1.64 (3H, s)	21 (CH ₃)	5.7	5.8	5.9	5.9
22	1.39 (3H, s)	22 (CH ₃)	23.0	23.1	23.7	23.7
23	0.82 (3H, d, $J = 6.5$ Hz)	23 (CH ₃)	19.8	19.8	20.1	20.1

^aData (500 MHz, CD₃OD) for natural plakinidone from this work.^bData (50 MHz, CDCl₃) for natural plakinidone taken from Kushland, D.M.; Faulkner, D.J. *J. Nat. Prod.* **1991**, *54*, 1451-1454.^cData (125 MHz, CDCl₃) for natural plakinidone from this work. Two drops of CD₃OD were added to assist with sample solubilization.^dData (125 MHz, CD₃OD) for natural plakinidone from this work.^eData (125 MHz, CD₃OD) for the synthetic enantiomer of plakinidone, i.e. *ent*-**12**, taken from Xu, Z.-J.; Tan, D.-X.; Wu, Y. *Org. Lett.* **2015**, *17*, 5092-5095.