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Reinvestigation of the Stereochemistry of the *C*-Glycosidic Ellagitannins, Vescalagin and Castalagin

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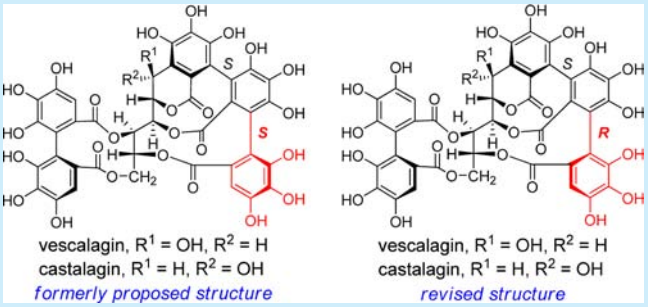
Supporting Information

ABSTRACT: The stereochemistry of the *C*-glycosidic ellagitannins, vescalagin and castalagin, has been reinvestigated using computational methods. DFT calculations of their ^{1}H and ^{13}C NMR spectra, as well as TDDFT calculations of the ECD spectra of their des-hexahydroxydiphenoyl analogues, revealed that the structure of the triphenoyl moiety of vescalagin and castalagin should be revised.



Vescalagin and its C-1 epimer castalagin are major *C*-glycosidic ellagitannins, which are widely distributed in plant species belonging to the genus *Quercus*.[1] These two compounds have been reported to show a variety of biological properties, including antioxidant and antiviral activities, as well as inhibitory activity toward DNA topoisomerase II.[1g,2] Vescalagin and castalagin can also be found in popular drinks, such as wine and whiskey.[3] These two compounds were originally isolated by Mayer et al.,[4] and their structures were elucidated in the 1960s−1970s. In 1987, the atropisomerism of the triphenoyl moiety in the molecules was proposed as (*S*,*S*) configuration.[5] After that, the configuration at C-1 was revised as shown in structures **1**′ and **2**′ in 1990 (Figure 1).[6] Later, however, Vivas et al.[7] used molecular mechanics to show that structures **1** and **2** with their triphenoyl moieties in the (*S*,*R*) configuration represented the most stable conformations of these compounds. Vivas's group also showed that the ^{1}H NMR coupling constants of **1** and **2**, which were calculated using a modified Karplus equation,[8] agreed with the experimental data.[7] However, Vivas's group did not discuss the atropisomerism of the triphenoyl moieties in vescalagin and castalagin, and most of the reviews and books recently published describing polyphenols, tannins, and ellagitannins have described the structures of vescalagin and castalagin as being structures **1**′ and **2**′.[9] Density functional theory (DFT) can be used to calculate spectroscopic data, such as NMR chemical shifts and ECD spectra, and this technique has recently been used to determine the stereochemistry of various natural products.[10] In this communication, we have reinvestigated the stereochemistry of vescalagin and castalagin using DFT calculations of their ^{1}H and ^{13}C NMR data, as well as time-dependent DFT (TDDFT) calculations of the ECD spectra.

Strong Cotton effects arising from the (*S*)-hexahydroxydiphenoyl (HHDP) esters of vescalagin and castalagin interfered with TDDFT calculations of the ECD spectra by overlapping

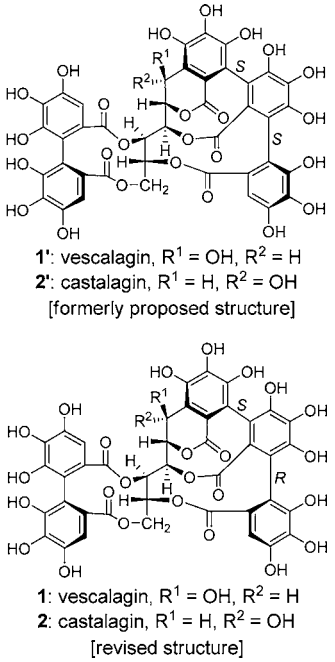


Figure 1. Structures of vescalagin and castalagin.

with the relatively small Cotton effects of their triphenoyl moieties (see Supporting Information).[11] Therefore, the calculations were performed with des-HHDP analogues of vescalagin and castalagin (i.e., vescalin and castalin, formerly proposed structures were **3**′ and **4**′, respectively) (Figure 2). Vescalin and castalin are produced by the hydrolysis of vescalagin and castalagin, respectively, and these compounds

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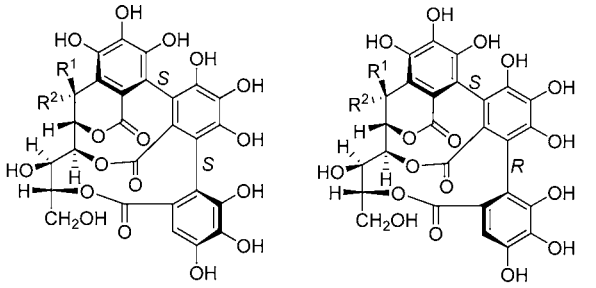

Figure 2. Structures of vescalin and castalin.

can be found in plants belonging to the genus *Quercus*.[4a,12] An initial conformational search of structures **3**′, **4**′ and structures **3**, **4** with the (*S*,*S*) and (*S*,*R*) configurations was performed using the Monte Carlo method at the MMFF94 force field, and the resulting low-energy conformers within 6 kcal/mol of each other were optimized at the AM1 level and then reoptimized at the B3LYP/6-31G(d,p) level in MeOH using the PCM model. The lowest energy conformers of **3**′ and **3** are shown in Figure 3. ECD spectra of the low-energy conformers with Boltzmann

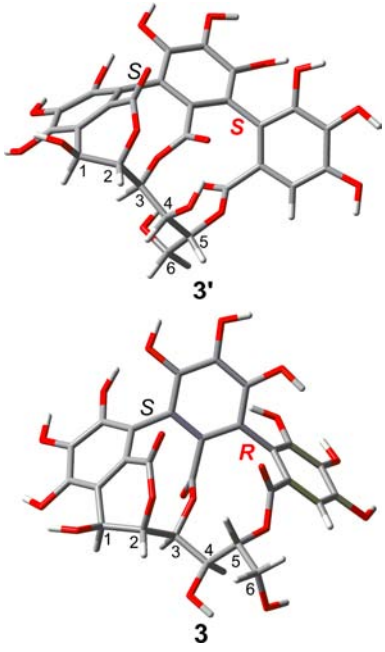

Figure 3. Lowest energy conformers of **3**′ and **3**. Geometrical optimization was performed at the B3LYP/6-31G(d,p) level in MeOH using the PCM model.

populations greater than 1% were calculated at the TD-CAM-B3LYP/6-31G(d,p) level in MeOH using the PCM model,[10b] and the results were averaged. The experimental ECD spectrum of vescalin contained positive Cotton effects at 295 and 240 nm and negative Cotton effects at 263 and 218 nm. These Cotton effects were in good agreement with the calculated spectrum of structure **3** with the (*S*,*R*) configuration (Figure 4a). In particular, the negative Cotton effect at 218 nm in the experimental ECD spectrum was consistent with the calculated spectrum of structure **3**; however, the calculated spectrum of structure **3**′ showed a positive Cotton effect around the same

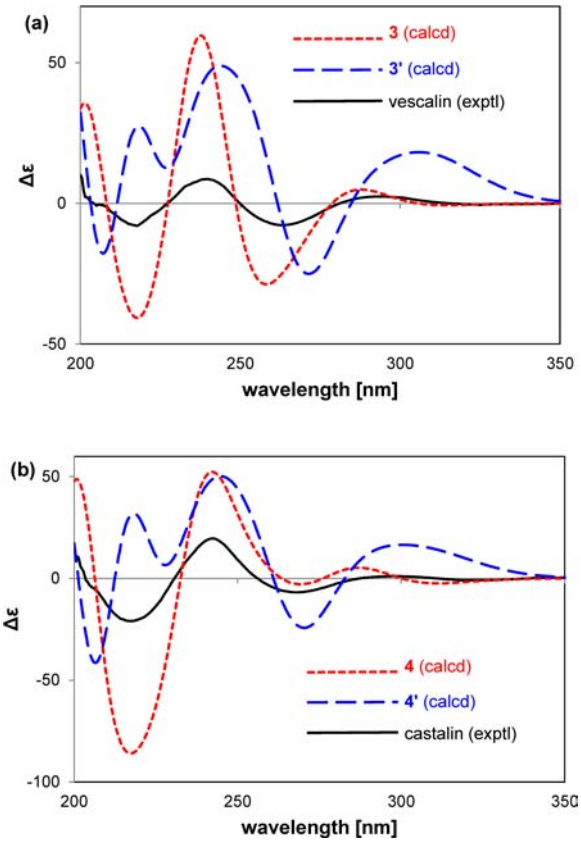

Figure 4. Experimental and calculated ECD spectra of vescalin (a) and castalin (b). Experimental ECD spectra were measured in MeOH. The ECD spectra were calculated at the TD-CAM-B3LYP/6-31G(d,p) level in MeOH using the PCM model. The calculated spectra were red-shifted by 15 nm.

wavelength. The experimental ECD spectrum of castalin also showed similar spectroscopic patterns to that of vescalin and had much more in common with the calculated spectrum of structure **4** than it did with that of **4**′ (Figure 4b). These results therefore indicated that the structures of vescalin and castalin are **3** and **4**, respectively, and that vescalagin and castalagin also have the same stereostructure as **1** and **2** with their (*S*,*R*) configurations.

DFT calculations of the ^{1}H and ^{13}C NMR chemical shifts of vescalagin and castalagin were performed to confirm the ECD results. Following conformational searches and the subsequent DFT optimization of the low-energy conformers at the B3LYP/6-31G(d,p) level in acetone using the PCM model, the ^{1}H and ^{13}C NMR chemical shifts of the low-energy conformers with Boltzmann populations greater than 1% were calculated using the GIAO method at the mPW1PW91/6-311+G(2d,p) level[10f] in acetone using the PCM model. The lowest energy conformers of **1**′ and **1** are shown in Figure 5. It is noteworthy that the lowest energy conformations of **1** and **2** were found to be very similar to those reported by Vivas et al.[7] The experimental ^{1}H and ^{13}C NMR chemical shifts of vescalagin and castalagin agreed to a much greater extent with the calculated values for structures **1** and **2** rather than **1**′ and **2**′ [correlation coefficient R^2 = 0.9886 (**1**), 0.9057 (**1**′), 0.9837 (**2**), 0.8850 (**2**′) for ^{1}H NMR chemical shifts; R^2 = 0.9629 (**1**), 0.8093 (**1**′), 0.9342 (**2**), 0.7980 (**2**′) for ^{13}C NMR chemical shifts of the polyol moiety]. The polyol moieties in the lowest energy conformers of **1**′ and **2**′ with their (*S*,*S*) configurations

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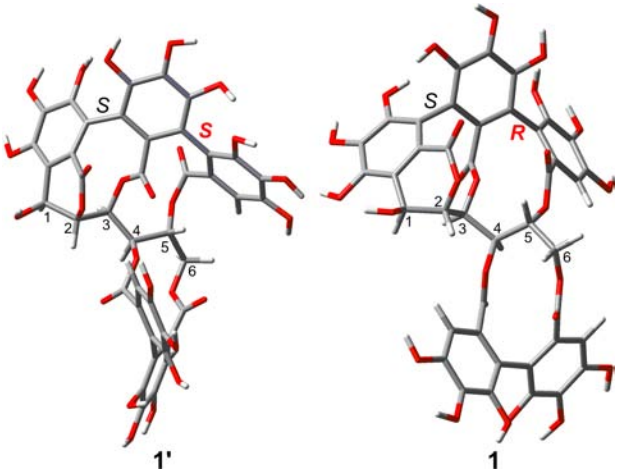

Figure 5. Lowest energy conformers of **1′** and **1**. Geometrical optimization was performed at the B3LYP/6-31G(d,p) level in acetone using the PCM model.

and **1** and **2** with their (*S*,*R*) configurations adopted very different conformations. The ^{1}H NMR coupling constants of these structures were consequently calculated at the B3LYP/6-31G(d,p)u+1s level (using only the Fermi contact term)[13] in acetone with the PCM model. Calculated coupling constants between H-1 and H-2 of the four structures (**1**, 1.9 Hz; **1′**, 1.7 Hz; **2**, 5.1 Hz; **2′**, 6.0 Hz) were in agreement with the experimental values (vescalagin = 2.3 Hz; castalagin = 4.6 Hz). However, calculated coupling constants between H-5 and H-6a,6b of **1′** ($J_{5,6a}$ = 8.0 Hz and $J_{5,6b}$ = 10.5 Hz) and **2′** ($J_{5,6a}$ = 8.0 Hz and $J_{5,6b}$ = 10.6 Hz) were significantly different from the experimental values [vescalagin: $J_{5,6a}$ = 2.6 Hz and $J_{5,6b}$ = 0.0 Hz; castalagin: $J_{5,6a}$ = 2.6 Hz and $J_{5,6b}$ = 1.1 Hz]. In contrast, the coupling constants calculated for **1** ($J_{5,6a}$ = 2.8 Hz and $J_{5,6b}$ = 1.4 Hz) and **2** ($J_{5,6a}$ = 2.8 Hz and $J_{5,6b}$ = 1.4 Hz) were consistent with the experimental coupling constants. In addition, much higher correlations were observed between the experimental coupling constants and the calculated values for structures **1** and **2** [**1**: R^2 = 0.9822, **2**: R^2 = 0.9957] compared to the values for structures **1′** and **2′** [**1′**: R^2 = 0.2288, **2′**: R^2 = 0.2258]. Based on these results, the structures of vescalagin and castalagin were concluded to be **1** and **2**, respectively. Consideration of the NOESY spectra of compounds **1** and **2** failed to provide conclusive evidence regarding the stereostructure of the triphenoyl moiety.

The original stereostructure of the triphenoyl moiety in vescalagin and castalagin (structures **1′** and **2′**) was proposed based on the ECD spectrum of the methylated derivative **5**, which was produced by methylation of the pentadecamethyl ether of **2** with dimethyl sulfate, followed by a methylation reaction with diazomethane (Figure 6).[5] The ECD spectrum of **5** contained a negative Cotton effect at 230 nm, as well as a positive Cotton effect at 252 nm, and this result agreed with the ECD spectrum of **6**, where the triphenoyl moiety is arranged in an (*S*,*S*) configuration. The stereostructure of the triphenoyl moiety in **5** was therefore concluded to be (*S*,*S*).[5] However, the ECD spectrum of **5** was not compared with that of the hypothetical (*S*,*R*)-type derivative. With this in mind, we calculated the ECD spectrum of (*S*,*R*)-**5** using a TDDFT calculation. The resulting spectrum contained Cotton effects similar to those observed in the experimental spectrum of **5** (see Supporting Information). Thus, it was apparent that

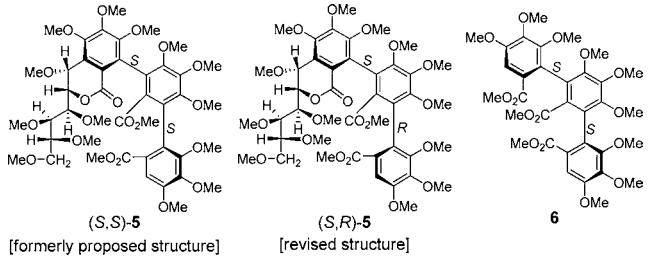

Figure 6. Structures of **5** and **6**.

determination of the stereochemistry of **5** by comparison of the ECD spectra of **5** and **6** as reported in the previous study was not appropriate.

A wide variety of derivatives of compounds **1** and **2** have been reported in the literature to date, including oligomers (e.g., roburins A–D[14] and castaneanins A–D[15]), complex tannins (e.g., acutissimins A and B,[16] anogeissusins A and B,[17] and anogeissinin[17]), and several related metabolites (e.g., mongolicains A and B[18]). However, the stereostructure of the triphenoyl moiety in these compounds has been based on the biogenetic, spectroscopic, and chemical relationship of these compounds to vescalagin and castalagin. Based on the results of the current study, it is therefore presumed that related compounds of this type will have the same stereostructure as compounds **1** and **2**.

In summary, we have reinvestigated the configurations of the biphenyl bonds in vescalagin and castalagin using DFT calculations of ^{1}H and ^{13}C NMR spectra, as well as TDDFT calculations of the ECD spectra of their des-HHDP analogues. Taken together, the results of this study indicate that the triphenoyl moieties of vescalagin and castalagin exist in the (*S*,*R*) configuration, and that the structures of these compounds should therefore be revised to **1** and **2**, respectively.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, 1D and 2D NMR, and computational results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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