Conformationally flexible ellagitannins: Conformational analysis of davidiin and punicafolin via DFT calculation of ^{1}H NMR coupling constants

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Many ellagitannins with various conformations of their glucose moiety have been isolated from natural sources. Here, a conformational analysis was performed via the density functional theory (DFT) calculation of ^{1}H NMR coupling constants. It was observed that, in the solution state, davidiin exists as an equilibrium mixture of the $B_{O,3}$ (boat) and $^1C_4$ (chair) conformational states, while punicafolin is an equilibrium mixture of the $^3S_1$ (skew-boat) and $^1C_4$ conformational states. Their equilibrium states changed depending on the solvent and temperature. Such conformational flexibility may be important for the biosynthesis of ellagitannins with diverse structures.

Introduction

Hydrolyzable tannins, comprising gallotannins and ellagitannins, are a group of plant polyphenols with structural diversity and various biological activities.[1] Most ellagitannins comprise a glucose core and acyl groups, mainly hexahydroxydiphenoyl (HHDP) and dehydrohexahydroxydiphenoyl (DHHDP) groups, derived from gallic acid.[1] Feldman *et al*. suggested that the DHHDP group is oxidatively biosynthesized from galloyl groups via intramolecular coupling.[2] In addition, the DHHDP group can be converted into the HHDP group by chemical reduction.[2a,3] Our group recently reported that the DHHDP group is reductively metabolized to the HHDP group in several plants.[4] Furthermore, the DHHDP group can be produced by the $CuCl_2$-mediated oxidation of galloyl ester derivatives in aqueous media.[5] This strongly indicates that the DHHDP group is the initial product of the oxidative coupling of two galloyl groups during ellagitannin biosynthesis, and the subsequent reductive metabolism yields HHDP esters (Figure 1a).[4,5]

Glucose derivatives can adopt various conformations, such as chair (*C*), boat (*B*), skew- or twist-boat (*S*), and envelope (*E*) (Figure S1).[6] Many ellagitannins are presumably biosynthesized from a common precursor, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**1**) with a $^4C_1$ conformation (Figure 1b).[3a,7] For example, the glucose moiety of 1-β-*O*-galloylpedunculagin (casuarictin) with 2,3-(*S*)-HHDP and 4,6-(*S*)-HHDP groups exhibits the same $^4C_1$ conformation as **1**.[7] Oppositely, the glucose moiety of geraniin (**2**) with 3,6-(*R*)-HHDP and 2,4-(*R*)-DHHDP groups exhibits the $^1C_4$ conformation, where all the substituents are in the axial orientation.[4c,8] In addition, amariin (**3**) with 2,4-(*R*)-DHHDP and 3,6-(*R*)-DHHDP groups exhibits an $^{O,3}B$ conformation,[4c] whereas phyllanemblinin B (**4**) with a 2,4-(*R*)-HHDP group exhibits a $^3S_1$ conformation (Figure 1b).[9] However, the precise conformations of several ellagitannins, such as davidiin (**5**) with a 1,6-(*S*)-HHDP group[3a,10] and punicafolin (**6**) with a 3,6-(*R*)-HHDP group,[11] remain unclear. Furthermore, corilagin (**7**), an analogue of **6** without the galloyl groups at C-2 and C-4, change its conformation depending on the solvent (Figure 1c).[12] Notably, the conformations of 3,6-bridged glucose derivatives change depending on their substituent groups and the bridge length.[13]

To understand the biosynthetic mechanism and biological activities of ellagitannins, it is important to clarify their precise conformations. The $J_{H,H}$ values are the most useful data for the accurate determination of the conformation of glucose derivatives because they reflect the dihedral angles between vicinal hydrogens. Here, the precise conformations of **5**, **6**, and related ellagitannins in the solution state were investigated via the DFT calculations of $J_{H,H}$ values.

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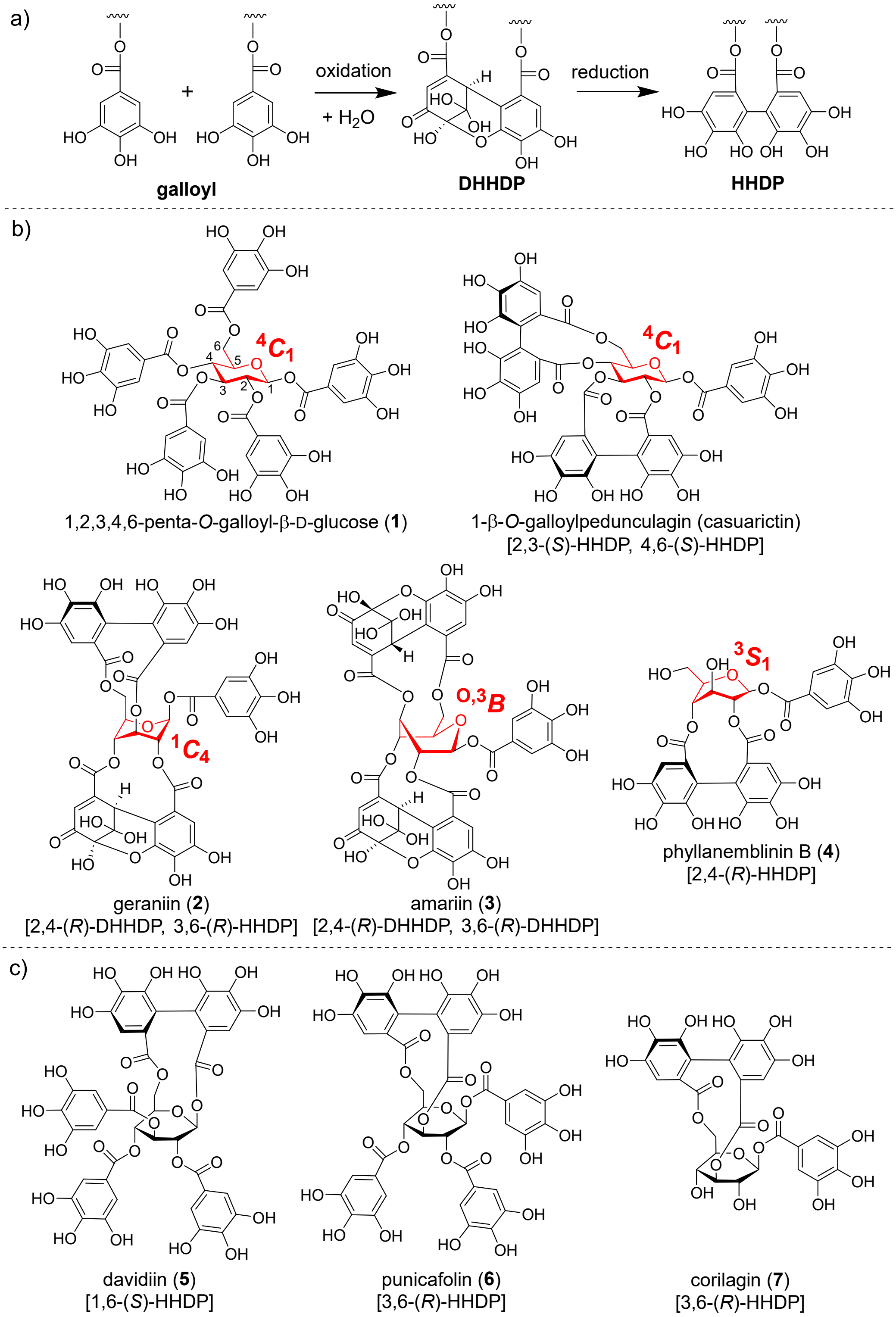


Figure 1. (a) Plausible biosynthetic pathway of dehydrohexahydroxydiphenoyl (DHHDP) and hexahydroxydiphenoyl (HHDP) groups. (b) Structures of hydrolyzable tannins with various conformations. (c) Structures of davidiin (**5**), punicafolin (**6**), and corilagin (**7**).

Results and discussion

Conformational Analysis Procedure

The conformational analysis of the ellagitannins was performed as follows. Ellagitannins possess many phenolic hydroxy groups; therefore, numerous possible conformers with different orientations of such groups exist. First, a conformational search for the molecular skeletons was performed by the ring-flipping of glucopyranose and macrocyclic structures comprising HHDP esters without considering the orientations of the hydroxy groups using the MMFF94 force field. Thereafter, the obtained conformers were optimized at the B3LYP/6-31G(d,p) level and classified based on the conformation of glucopyranose. Second, a conformational search, including the orientation of the hydroxy groups, was performed for the lowest-energy conformers of each classified group. The conformers discovered for each group within 6 kcal/mol were optimized at the same DFT level. Their $J_{H,H}$ values with Boltzmann populations exceeding 1% were calculated at the B3LYP/6-31G(d,p)u+1s level using only the Fermi contact term.[14] Finally, the $J_{H,H}$ values were weight-averaged for each conformer group.

This procedure was first applied to phyllanemblinin B (**4**), an ellagitannin with a relatively simple structure. The conformation of its glucose moiety was originally reported to be skew-boat.[9b] Subsequently, Wakamori *et al*. suggested the $^3S_1$ type based on experimental $J_{H,H}$ values and MMFF calculations.[9a,15] The present conformational analysis suggested three conformational states for **4**: $^3S_1$ (ΔG = 0.0 kcal/mol), $B_{1,4}$ (ΔG = +4.8 kcal/mol), and $^1C_4$ (ΔG = +5.4 kcal/mol) (B3LYP/6-31G(d,p)) (Figure S46, Table S9). Their $J_{H,H}$ values were calculated by DFT. The results for the $^3S_1$ type with the lowest free energy were consistent with the experimental values[9a] (Table 1), indicating that the conformation of the glucose moiety was $^3S_1$.

Table 1. Experimental and calculated $J_{H,H}$ values (Hz) for phyllanemblinin B (**4**).

	exptl[a]	calcd[b]		
		$^3S_1$	$B_{1,4}$	$^1C_4$
$J_{1,2}$	5.9	6.2	7.5	0.5
$J_{2,3}$	1.0	0.7	0.9	1.3
$J_{3,4}$	3.7	3.7	3.0	3.2
$J_{4,5}$	≈ 0	0.2	0.8	1.7

[a] 500 MHz (Ref. 9a). [b] Calculated at the B3LYP/6-31G(d,p)u+1s//B3LYP/6-31G(d,p) level.

Conformational Analysis of Davidiin (5)

Davidiin (**5**), an ellagitannin with 1,6-(*S*)-HHDP and 2,3,4-trigalloyl groups, has been isolated from *Davidia involucrata*,[3a,10,16] *Acer saccharum*,[17] and *Persicaria capitata (Polygonum capitatum)*.[18] In addition, its various biological activities have been reported.[16,18a,19] The conformation of its glucose moiety was initially assumed to be skew-boat.[3a] However, this has not been investigated. Previous literature has depicted **5** in three conformation types, $^1C_4$,[7b–d,f,20] $^3S_1$,[21] and $B_{O,3}$,[16,18b,22] thereby generating confusion (Figure S2). Among them, the $B_{O,3}$-type conformation was estimated from its experimental ^{1}H NMR coupling constants ($J_{H,H}$) of its glucose moiety in acetone-d_6.[16,18b,22] The experimental $J_{H,H}$ values do not match those reported for geraniin (**2**) with the $^1C_4$ conformation[8c] and **4** with the $^3S_1$ conformation[9a] (Table S1).

The conformational analysis of **5** revealed three types of conformations of the glucose moiety: $^1C_4$ (ΔG = 0.0 kcal/mol), $^{1,4}B$–$^1S_5$ (intermediate state between $^{1,4}B$ and $^1S_5$) (ΔG = +2.8 kcal/mol), and $B_{O,3}$ (ΔG = +4.4 kcal/mol)[23] (B3LYP/6-31G(d,p)) (Figure 2, Table S13). The DFT calculation of the $J_{H,H}$ values of these conformers was performed. However, none of the results agreed with the experimental values in acetone-d_6 (Table 2), indicating that **5** does not exhibit only one conformational state. When the calculated values for $B_{O,3}$ and $^1C_4$ were weight-averaged at a ratio of 60:40, they agreed well with the experimental data (Table 2), revealing that **5** exists as an equilibrium mixture of $B_{O,3}$/$^1C_4$ in acetone-d_6. Furthermore, the weight-averaged values of the $B_{O,3}$/$^1C_4$/$^{1,4}B$–$^1S_5$ mixture (55:40:5) were in better agreement with the experimental data than those of $B_{O,3}$/$^1C_4$ (60:40) (Table 2). This suggested that the $^{1,4}B$–$^1S_5$ conformation might have slightly contributed to the equilibrium state of **5**. Moreover, the $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ values increased with a decrease in temperature (Table S3), indicating a corresponding increase in the abundance ratio of $B_{O,3}$. The ^{1}H NMR spectra of **5** were recorded in other solvents: CD_3OD, DMSO-d_6, and D_2O (Figure 3a). The experimental $J_{H,H}$ values in CD_3OD were extremely similar to those in acetone-d_6 (Table 2). Contrarily, the experimental values of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ in DMSO-d_6 were higher than those in acetone-d_6 and CD_3OD. Considering that the experimental $J_{H,H}$ values in DMSO-d_6 matched the calculated values for $B_{O,3}$ (Table 2), the conformation of **5** in this solvent was identified to be $B_{O,3}$. The ^{1}H NMR spectrum in D_2O at 20°C revealed largely broadened signals, indicating a slow exchange rate between several types of conformers compared with the spectra in other solvents. Oppositely, sharp signals were observed at 80°C, and the small values of $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ indicated the $^1C_4$ conformation (Figure 3b and Table 2).

When geometry optimization including the solvent effect was performed using the polarizable continuum model (PCM),[24] the lowest free energy conformer was $^1C_4$ in all the solvents used, which was the same as without the solvent effect. The relative free energies between the three conformational groups were smaller than those without the solvent effect (Table S13). Another solvent model, the solvation model based on density (SMD),[25] which reportedly affords better results than the PCM for polar and flexible molecules, including intramolecular hydrogen bonding,[26] was applied. $B_{O,3}$ was the most stable conformation in all the solvents (Table S14). Intramolecular hydrogen bonding and intermolecular interactions with solvents seem to contribute largely to the conformation of **5**. The PCM is known to overestimate the stabilization by intramolecular hydrogen bonding.[26,27] Thus, the stabilization by intramolecular hydrogen bonds between HHDP and 3-galloyl groups in $^1C_4$ and $^{1,4}B$–$^1S_5$ and between 2- and 4-galloyl groups in $^1C_4$ appeared to be overestimated (Figure 2). These results showed that it is difficult to predict the precise conformation in various solvents based only on the calculated relative free energies, even when the solvent effect is included. However, it was possible by comparing the experimental and calculated $J_{H,H}$ values, even when the compounds existed as an equilibrium mixture of several conformations.[28]

Geometry optimization using the B3LYP-D3(BJ) functional, including long-range dispersion correction, was performed.[29-31] The results showed that the $^{1}C_{4}$ conformation (ΔG = 0.0 kcal/mol) was significantly stable compared with the $B_{O,3}$ (ΔG = +13.7 kcal/mol) and $^{1,4}B–^{1}S_{5}$ (ΔG = +11.7 kcal/mol) conformations (Table S13). In the $^{1}C_{4}$ conformation calculated at this level, an intramolecular π–π interaction was formed between the HHDP and 3-galloyl groups (Figure S53). Thus, in D_2O, this interaction was presumably induced by hydrophobic interactions that stabilized the $^{1}C_{4}$ conformation. However, in DMSO-d_6, the hydroxy groups in **5** formed intermolecular hydrogen bonds with the solvent, which may have hindered intramolecular interactions between the acyl groups. These results indicate that **5** has several types of conformers ($B_{O,3}$, $^{1}C_{4}$, and $^{1,4}B–^{1}S_{5}$), and its equilibrium state changes depending on the solvent and temperature (Table 3).

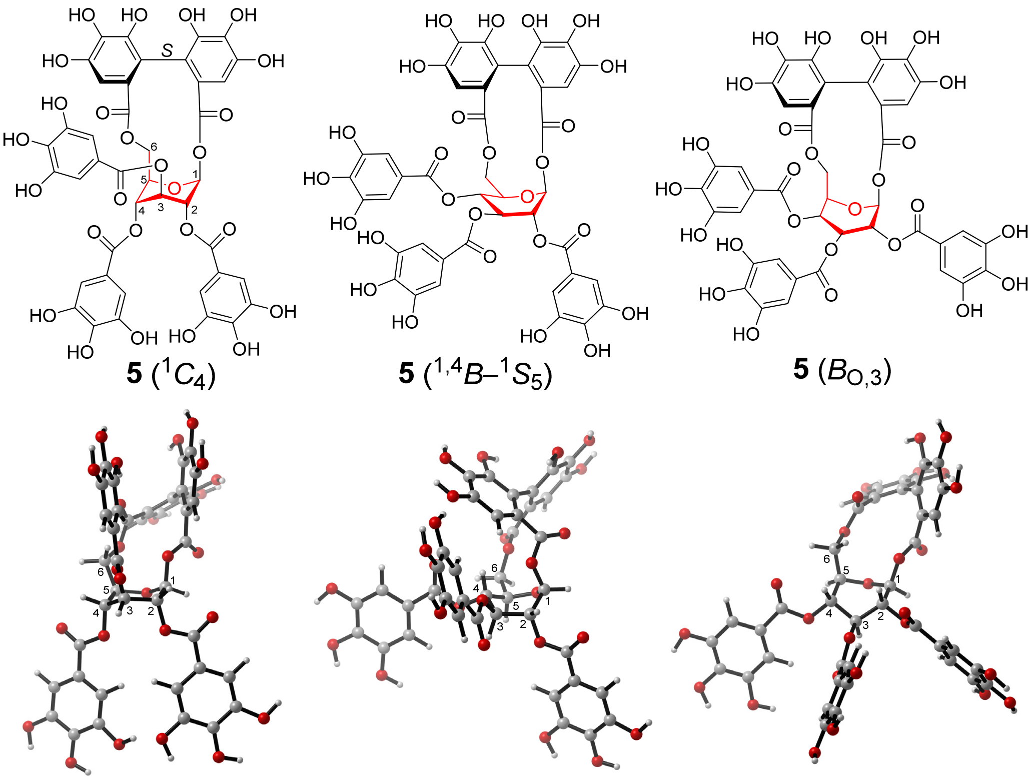


Figure 2. Three conformation types of davidiin (**5**) calculated at the B3LYP/6-31G(d,p) level.

Table 2. Experimental and calculated $J_{H,H}$ values for davidiin (**5**).

	expt[a]				calcd[d]				
	acetone-d_6[b]	CD_3OD[b]	DMSO-d_6[b]	D_2O[c]	$^{1}C_{4}$	$^{1,4}B–^{1}S_{5}$	$B_{O,3}$	$B_{O,3}/^{1}C_{4}$ (60:40)	$B_{O,3}/^{1}C_{4}/^{1,4}B–^{1}S_{5}$ (55:40:5)
$J_{1,2}$	2.9	2.9	4.0	≈ 0	0.8	2.2	4.1	2.8	2.7
$J_{2,3}$	7.3	7.1	10.2	≈ 0	2.1	0.3	11.5	7.7	7.2
$J_{3,4}$	6.7	6.4	8.9	≈ 0	2.2	7.4	9.7	6.7	6.6
$J_{4,5}$	2.6	2.5	3.5	≈ 0	0.5	11.5	3.3	2.2	2.6
$J_{5,6a}$	5.3	5.1	4.7	5.2	5.4	4.9	4.8	5.1	5.1
$J_{5,6b}$	12.1	11.8	11.9	12.1	12.7	0.2	12.6	12.7	12.0

[a] 500 MHz, [b] 20 °C. [c] 80 °C. [d] Calculated at the B3LYP/6-31G(d,p)u+1s//B3LYP/6-31G(d,p) level.

Table 3. Conformations of davidiin (**5**), punicafolin (**6**), and corilagin (**7**) in various solvents assigned from experimental (20°C) and calculated $J_{H,H}$ values.

	acetone-d_6	CD_3OD	DMSO-d_6	D_2O
davidiin (**5**)	$B_{O,3}$/$^1C_4$ (60:40) [or $B_{O,3}$/$^1C_4$/$^{1,4}B$–$^1S_5$ (55:40:5)]	$B_{O,3}$/$^1C_4$ (60:40) [or $B_{O,3}$/$^1C_4$/$^{1,4}B$–$^1S_5$ (55:40:5)]	$B_{O,3}$	$^1C_4$ [a]
punicafolin (**6**)	$^3S_1$/$^1C_4$ (65:35)	$^3S_1$/$^1C_4$ (55:45)	$^3S_1$/$^1C_4$ (90:10)	$^1C_4$ [b]
corilagin (**7**)	$^3S_1$/$^1C_4$ (10:90)	$^3S_1$/$^1C_4$ (10:90)	$^3S_1$/$^1C_4$ (95:5)	$^1C_4$

[a] 80 °C. [b] D_2O/DMSO-d_6 (9:1).

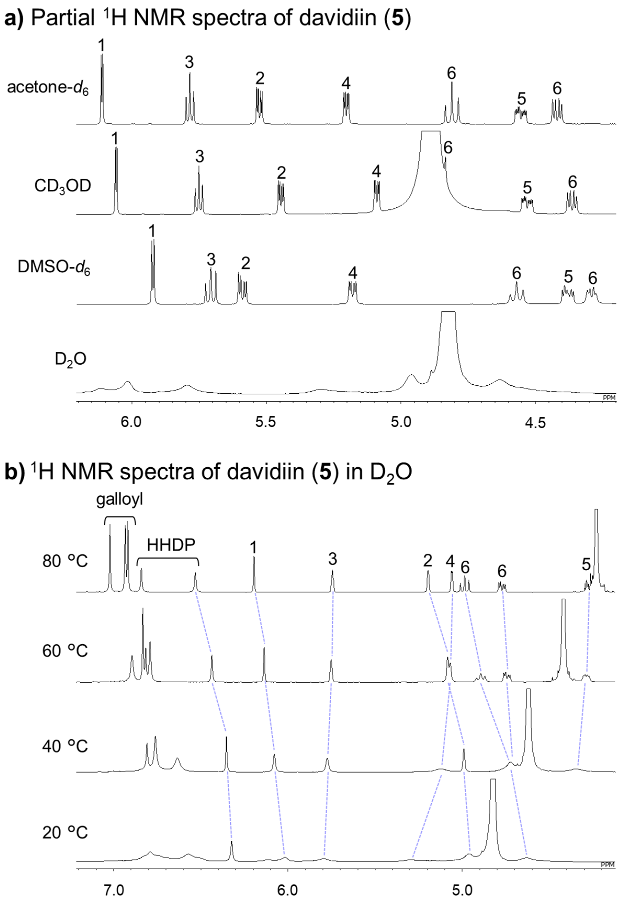


Figure 3. The ^{1}H NMR spectra of (a) the glucose moiety of davidiin (**5**) in various solvents at 20°C and 500 MHz and (b) **5** in D_2O at various temperatures and 500 MHz.

Conformational Analysis of Punicafolin (6)

Punicafolin (**6**), an ellagitannin with 3,6-(*R*)-HHDP and 1,2,4-trigalloyl groups, has been isolated from *Punica granatum*,[11] *Mallotus japonicus*,[32] *Euphorbia helioscopia*,[33] *Macaranga tanarius*,[3c] and *Phyllanthus emblica*.[9b] It exhibits several biological activities.[34] Its glucose moiety was initially assumed to exhibit a $^1C_4$ or skew-boat conformation in acetone-d_6.[11] Thereafter, it was suggested to exhibit the $B_{1,4}$ conformation in DMSO-d_6 from the $J_{H,H}$ values.[35] In several reviews, its conformation has been described as $^1C_4$.[7d,f] Recently, density functional theory (DFT) calculations have shown that its most stable conformation is $^1C_4$.[36] However, its experimental $J_{H,H}$ values in acetone-d_6 do not match those of **2** with the $^1C_4$ conformation (Table S2). Here, the conformational analysis of **6** indicated two states: $^1C_4$ (ΔG = 0.0 kcal/mol) and $^3S_1$ (ΔG = +2.6 kcal/mol) (B3LYP/6-31G(d,p)) (Figure 4, Table S22). Although the calculated $J_{H,H}$ values for both conformations did not agree with the experimental values measured in acetone-d_6, the weight-averaged values for $^3S_1$/$^1C_4$ (65:35) were in good agreement with the experimental values (Table 4), indicating that **6** exists as an equilibrium mixture of $^3S_1$/$^1C_4$ (65:35). Moreover, the $J_{1,2}$ and $J_{2,3}$ values increased with a decrease in temperature (Table S4), suggesting a corresponding increase in the abundance ratio of $^3S_1$. Furthermore, the $J_{H,H}$ values changed in different solvents, and the results revealed that **6** existed as $^3S_1$/$^1C_4$ (55:45) in CD_3OD, $^3S_1$/$^1C_4$ (90:10) in DMSO-d_6, and $^1C_4$ in D_2O/DMSO-d_6 (9:1)[37] (Figure 5a, Table 4). Geometry optimization with the solvent effect (PCM or SMD) yielded smaller relative free energies than those without the solvent effect (Tables S22, S23). However, similar to **5**, it was difficult to predict the equilibrium state of **6** in various solvents based only on the calculated relative free energies. Geometry optimization with the B3LYP-D3(BJ) functional revealed that the $^1C_4$ conformation was largely stable compared with $^3S_1$ (ΔG = 11.7 kcal/mol) (Tables S22, S23). The obtained $^1C_4$ conformer exhibited intramolecular π–π interactions between the HHDP and 1-galloyl groups and between the 2- and 4-galloyl groups (Figure S63). This indicated that such interactions were induced by hydrophobic interactions that stabilized the $^1C_4$ conformation in D_2O/DMSO-d_6 (90:10). These results indicate that **6** exhibits the $^3S_1$ and $^1C_4$ conformations, and its equilibrium state changes depending on the solvent and temperature.

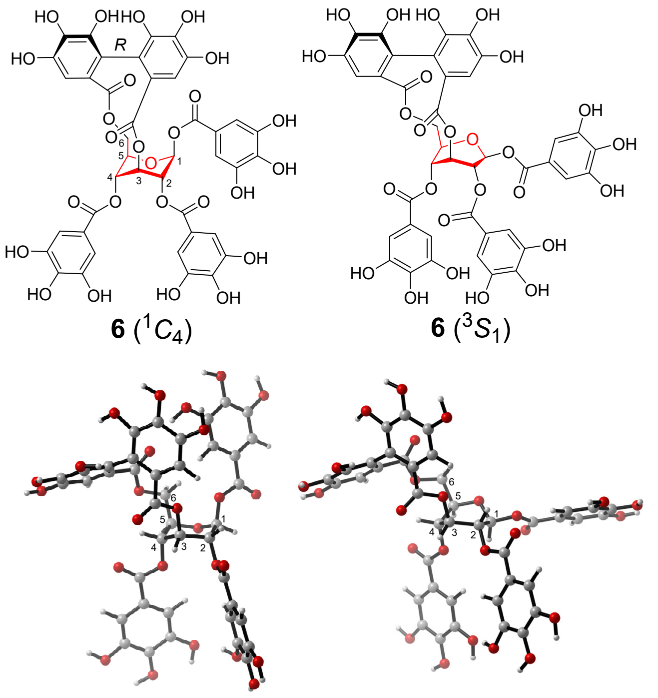


Figure 4. Two conformation types of punicafolin (**6**) calculated at the B3LYP/6-31G(d,p) level.

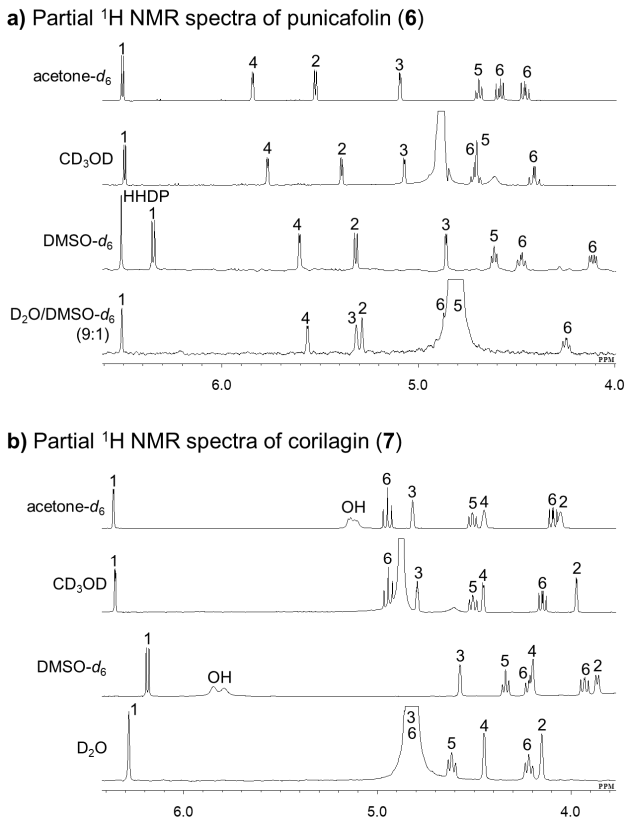


Figure 5. The ^{1}H NMR spectra of the glucose moiety of (a) punicafolin (**6**) and (b) corilagin (**7**) in various solvents at 20°C and 500 MHz.

Table 4. Experimental and calculated $J_{H,H}$ values for punicafolin (**6**).

	expt1 [a]				calcd [b]				
	acetone-d_6	CD_3OD	DMSO-d_6	D_2O/DMSO-d_6 (9:1)	$^1C_4$	$^3S_1$	$^3S_1/^1C_4$ (55:45)	$^3S_1/^1C_4$ (65:35)	$^3S_1/^1C_4$ (90:10)
$J_{1,2}$	5.1	4.5	7.1	≈ 0	0.7	7.7	4.5	5.2	7.0
$J_{2,3}$	≈ 0	≈ 0	≈ 0	≈ 0	1.8	0.4	1.0	0.9	0.6
$J_{3,4}$	3.3	3.2	3.2	3.1	2.7	3.3	3.0	3.1	3.3
$J_{4,5}$	≈ 0	≈ 0	≈ 0	≈ 0	1.0	0.3	0.6	0.5	0.4
$J_{5,6a}$	7.0	– [c]	5.6	7.5	8.1	8.1	8.1	8.1	8.1
$J_{5,6b}$	8.0	– [c]	7.8	– [c]	11.1	8.0	9.4	9.1	8.3

[a] 500 MHz, 20 °C. [b] Calculated at the B3LYP/6-31G(d,p)u+1s//B3LYP/6-31G(d,p) level. [c] Not resolved.

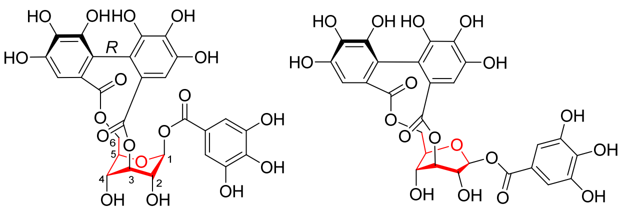
Conformational Analysis of Corilagin (7)

Corilagin (**7**), a desgalloyl analogue of **6**, is known to be an ellagitannin with a flexible conformation. It reportedly exists in an intermediate state between the $B_{1,4}$ and $^{O,3}B$ (or $^1C_4$) conformations in DMSO-d_6, while a slightly perturbed $^{O,3}B$ (or $^1C_4$) conformational state in acetone-d_6.[12a,b] Its $J_{H,H}$ values change in DMSO-d_6 depending on the temperature.[12a] Compound **7** was suggested to exhibit the $^1C_4$ conformation with possible flattening of the pyranose ring at C-1 and O-5[3a] or the $B_{1,4}$ conformation[35] in DMSO-d_6, and the $^1C_4$ conformation in acetone-d_6[8a] and CD_3OD.[8c] Furthermore, several computational studies have been conducted on the lowest-energy conformer of **7**. The molecular mechanics (PIMM91) calculation of its model compound indicated $^1C_4$.[38] The other molecular mechanics (MM2) study of **7** suggested the skew-boat conformation,[39,40] and the semiempirical PM3 calculation suggested $B_{1,4}$ as its lowest-energy conformation.[41] Based on electronic and vibrational circular dichroism (CD) spectroscopic data, $J_{H,H}$ values, and DFT calculations, a recent study reported the presence of **7** in the $^3S_1$ and $^1C_4$ conformations in DMSO-d_6 and CD_3OD, respectively.[12c,42]

The experimental $J_{H,H}$ values for corilagin (**7**) were practically the same as those for **6** in DMSO-d_6 and D_2O, suggesting that **7** also exists as an equilibrium mixture of $^3S_1$ and $^1C_4$ (Figures 5b and 6,

Table S34). In acetone-d_6 and CD_3OD, its $J_{1,2}$ values (1.8 and 2.0 Hz, respectively) (Table S34) were smaller than those of **6** (5.1 and 4.5 Hz, respectively) (Table 4). This indicated a larger abundance ratio of $^1C_4$ in **7** than in **6** in the solvents. The conformational analysis afforded four types for **7**: $^1C_4$ (ΔG = 0.0 kcal/mol), $^{O,3}B$ (ΔG = +2.9 kcal/mol), $^3S_1$–$^{O,3}B$ (intermediate state between $^3S_1$ and $^{O,3}B$) (ΔG = +3.1 kcal/mol), and $^3S_1$ (ΔG = +4.4 kcal/mol) (Figure S66, Table S29). However, the calculated $J_{5,6}$ values for $^{O,3}B$ and $^3S_1$–$^{O,3}B$ widely differed from the experimental values (Table S34). This indicated that they do not contribute to the equilibrium state of **7**. However, the calculated $J_{H,H}$ values for $^1C_4$ and $^3S_1$ were very similar to those of **6**, except for the $J_{1,2}$ of $^3S_1$ (7.7 and 4.6 Hz for **6** and **7**, respectively) (Tables 4 and S34). In the lowest free energy $^3S_1$ conformer of **7**, an intramolecular hydrogen bond was formed between 2-hydroxy and 1-galloyl carbonyl groups, changing the dihedral angle between H-1 and H-2 (159.4° for **6** and 144.8° for **7**) (Figures 4 and S66). However, in polar solvents, hydroxy groups can form intermolecular hydrogen bonds with the solvent, and the calculated $J_{1,2}$ values for the $^3S_1$ conformations of **7**, where the 2-hydroxy groups do not form intramolecular hydrogen bonds, were practically the same as those of **6** (Figure S66, Table S34). The calculated $J_{H,H}$ values indicate that **7** exists as an equilibrium mixture of $^3S_1$ and $^1C_4$ (10:90) in acetone-d_6 and CD_3OD and as $^3S_1$/$^1C_4$ (95:5) in DMSO-d_6 and $^1C_4$ in D_2O (Tables 3 and S34).

The abundance ratio of the $^1C_4$ conformation of **7** was higher than that of **6** (Table 3). For **6**, the distance between O-2 and O-4 atoms in the glucose moiety was closer in $^1C_4$ (2.9 Å) than in $^3S_1$ (3.2 Å), suggesting a larger steric hindrance between the 2- and 4-galloyl groups in the $^1C_4$ conformation than in $^3S_1$. Thus, the abundance ratio of $^3S_1$ was probably higher in **6** than in **7**.

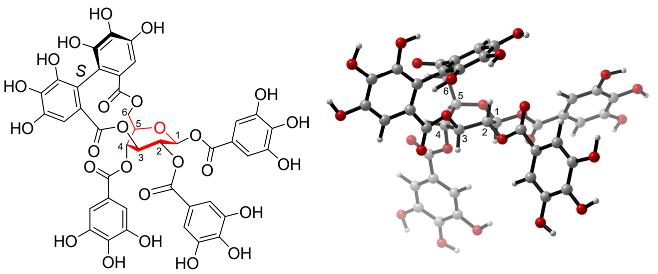


7 ($^1C_4$) **7** ($^3S_1$)

Figure 6. Two conformation types of corilagin (**7**).

Conformational Analysis of Macaranganin (8)

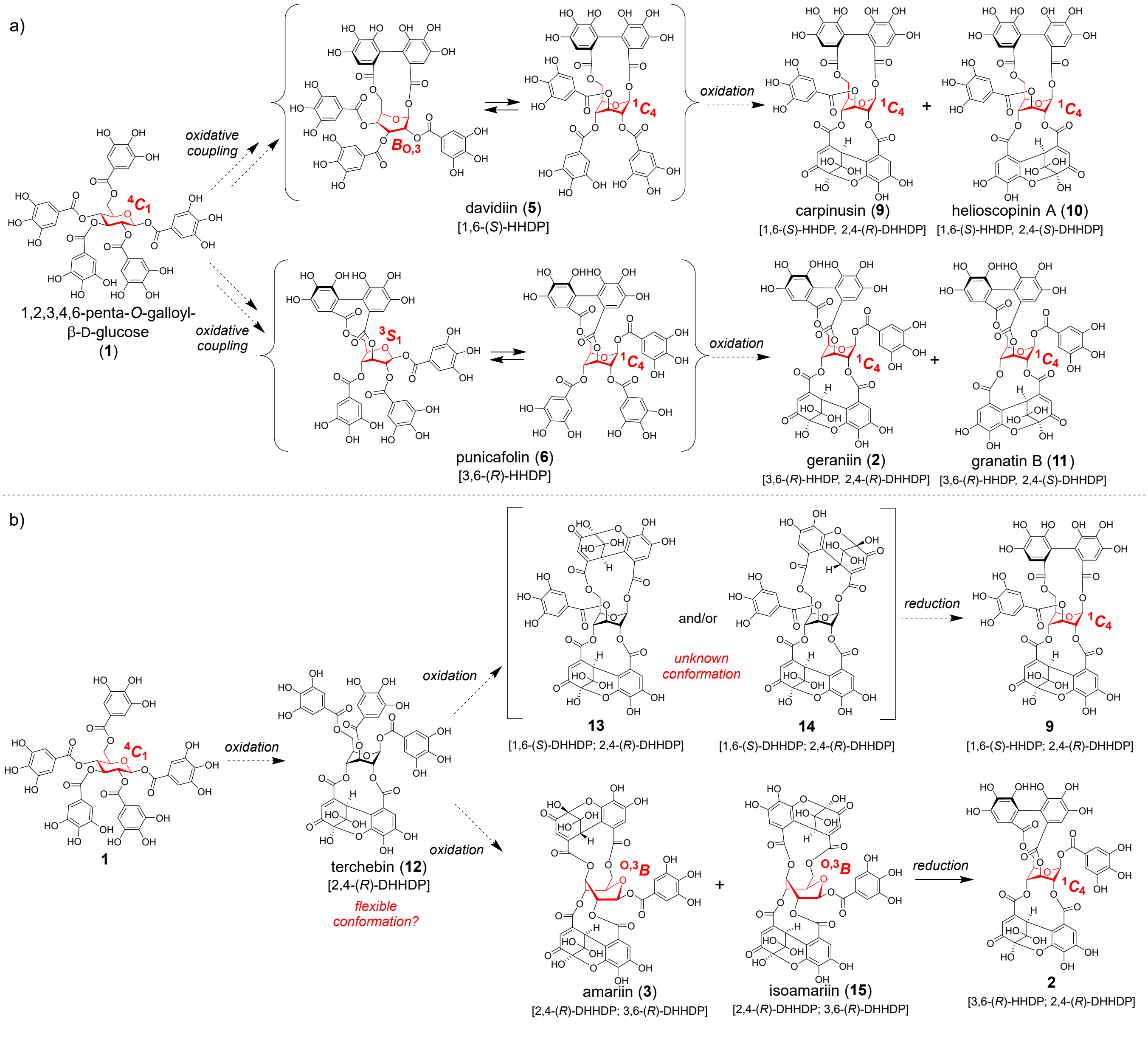
Macaranganin (**8**) is a diastereomer of **6** with a 3,6-(*S*)-HHDP group. It has been isolated from *Macaranga tanarius*.[3c] Its lowest-energy conformer has been identified as $^5S_1$ through DFT calculations.[36] Here, its conformational analysis indicated four possible conformational states: $^3S_1$ (ΔG = 0.0 kcal/mol), $^5S_1$–$B_{1,4}$ (intermediate state between $^5S_1$ and $B_{1,4}$) (ΔG = +0.6 kcal/mol), $^5S_1$–^5E (intermediate state between $^5S_1$ and ^5E) (ΔG = +2.5 kcal/mol), and $^5S_1$ (ΔG = +4.5 kcal/mol) (B3LYP/6-31G(d,p)) (Figure S70, Table S41). The DFT calculations including the solvent effect afforded smaller relative free energies: $^3S_1$ (ΔG = 0.00 kcal/mol), $^5S_1$–$B_{1,4}$ (ΔG = +0.19 kcal/mol), $^5S_1$–^5E (ΔG = +0.90 kcal/mol), and $^5S_1$ (ΔG = +0.73 kcal/mol) (B3LYP/6-31G(d,p) in acetone (PCM)) (Table S41). The reported experimental $J_{H,H}$ values in acetone-d_6 + D_2O[36] were in good agreement with the calculated values for $^5S_1$ (Table S46). Thus, the conformation of **8** was confirmed to be $^5S_1$ (Figure 7). When the B3LYP-D3(BJ) functional was used for geometry optimization, the four types of conformers converged into two types, $^5S_1$ and $^3S_1$ (Figure S72). Considering that **8** was unavailable for the present study, it was impossible to investigate its conformations in other solvents.



8 ($^5S_1$)

Figure 7. The $^5S_1$ conformation of macaranganin (**8**) calculated at the B3LYP/6-31G(d,p) level.

https://doi.org/10.26434/chemrxiv-2022-wrlxz-v4 **ORCID:** https://orcid.org/0000-0002-6462-9727 Content not peer-reviewed by ChemRxiv.



Scheme 1. Possible biosynthetic pathways toward ellagitannins **2** and **9–11** with the $^{1}C_{4}$ conformation from 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**1**) (a) via the flexible-conformation intermediates **5** and **6** and (b) via terchebin (**12**). HHDP, hexahydroxydiphenoyl; DHHDP, dehydrohexahydroxydiphenoyl.

Biosynthetic Considerations of $^{1}C_{4}$-type Ellagitannins.

The conformational flexibility of ellagitannins revealed in the present study is crucial in the biosynthesis of $^{1}C_{4}$-type ellagitannins from **1** with the $^{4}C_{1}$ conformation (Scheme 1a). The conformation of carpinusin (**9**)[43] and helioscopinin A (**10**)[18b,33] with a 1,6-(*S*)-HHDP group is locked as the $^{1}C_{4}$ type by a 2,4-bridged DHHDP group. For their biosynthesis from **1**, a glucose moiety with the $^{4}C_{1}$ conformation must be converted into the $^{1}C_{4}$ type. The conformationally flexible **5** can be biosynthesized from **1** via oxidative coupling between its 1- and 6-galloyl groups, since the $B_{O,3}$-type conformer of **5** has a conformation relatively close to that of **1**. In turn, since **5** adopts the $^{1}C_{4}$ conformation in water, the same conditions as in the biosynthetic environment, **9** and **10** with the $^{1}C_{4}$-type conformations are likely to be biosynthesized via oxidative coupling between the 2- and 4-galloyl groups of **5**. Similarly, the conformationally flexible **6** can be biosynthesized from **1** via oxidative coupling between its 3- and 6-galloyl groups, since the $^{3}S_{1}$-type conformer of **6** has a conformation relatively close to that of **1**. Then geraniin (**2**) and granatin B (**11**),[3a,11,44] locked as the $^{1}C_{4}$ conformation by a 2,4-bridged DHHDP group, can be biosynthesized from **6**, which adopts the $^{1}C_{4}$ conformation in water.

Conversely, another possible biosynthetic pathway toward $^{1}C_{4}$-type ellagitannins exists (Scheme 1b). Geraniin (**2**) is reductively biosynthesized from amariin (**3**) and isoamariin (**15**) bearing 2,4-(*R*)- and 3,6-(*R*)-DHHDP groups with the $^{O,3}B$-

type conformation.[4c,45] In addition, **3** and **15** can be oxidatively biosynthesized from **1** via terchebin (**12**) bearing a 2,4-(*R*)-DHHDP group.[44a] Although the conformation of **12** has not been investigated, it was suggested to exist in an intermediate state between the $B_{1,4}$ and $^{O,3}B$ (or $^{1}C_{4}$) conformations in DMSO-d_6.[12a] We assume that **12** might also have a flexible conformation; this is currently under investigation. Carpinusin (**9**) can also be biosynthesized from **12**. The formation of a DHHDP group between the 1,6-galloyl groups in **12** would afford unknown intermediates **13** and **14**, which could be reductively metabolized to produce **9**.

Conclusions

The precise conformations of davidiin (**5**) (bearing a 1,6-(*S*)-HHDP group) and punicafolin (**6**) (bearing a 3,6-(*R*)-HHDP group) were revealed by conformational analysis through DFT calculations of the $J_{H,H}$ values. These ellagitannins exhibited flexible conformations in the solution state, and their equilibrium states were significantly influenced by the solvent and temperature. The conformation of corilagin (**7**), which is known to change depending on the solvent, was also investigated. Here, it was difficult to predict the precise conformations of these flexible ellagitannins only from the calculated relative free energies under several calculation conditions. Although several studies have investigated the conformations of **6** and **7** using computational methods, most of them could not precisely predict them since they were based on the calculated relative (free) energies and did not consider the simultaneous existence of several conformations as an equilibrium state. However, this study demonstrated that the precise conformation can be predicted by comparing the experimental $J_{H,H}$ values with the corresponding calculated values. Recently, Auer *et al*. investigated the conformations of xylopyranoside derivatives.[46] They demonstrated that identifying the most stable conformers using the computational calculations of relative free energies or NMR chemical shifts was not sensitive, whereas the calculation of $J_{H,H}$ values enabled the quantification of the ratio of different conformers in the mixture. The procedure of conformational analysis presented here was similar to that of Auer *et al*.

Conformationally flexible ellagitannins are important intermediates in the biosynthesis of ellagitannins with the $^{1}C_{4}$ conformation. In addition, their flexibility may contribute to their various biological activities. Although several in silico molecular docking studies of **5**–**7** with proteins have been reported,[47] their flexibility, as revealed here, contributes to the future bioinformatics research of these ellagitannins. Ellagitannins have attracted significant attention as targets for total syntheses because of their structural diversity and complexity.[48] For example, the total syntheses of **5**–**8** have been reported.[21a,36,49] The distance between the two galloyl groups, which are the precursors of the HHDP or DHHDP groups, varies depending on the conformation of the glucose moiety. It will be important for the efficient synthesis of ellagitannins to elucidate the precise conformations of the target products and to design precursors with the appropriate conformations.

Author Contributions

Y. M. and T. T. designed the work; Y. M., M. I., C. O. and T. T. conducted the experiments; Y. M., M. I. and C. O. conducted the computational studies; All authors discussed the results; Y. M. prepared the manuscript with feedback from the other authors.

Conflicts of interest

There are no conflicts to declare.

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