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Article

Highly Oxidized Ellagitannins of *Carpinus japonica* and Their Oxidation–Reduction Disproportionation

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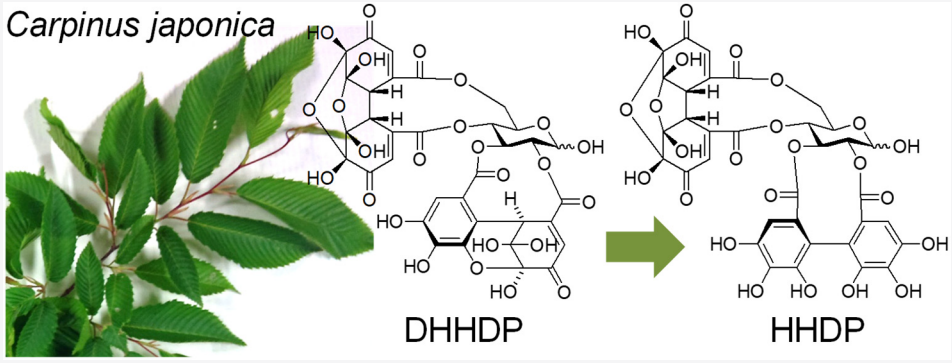

ABSTRACT: In the research on ellagitannin metabolism, two unique dehydroellagitannins, carpinins E (**1**) and F (**2**), bearing dehydrohexahydroxydiphenoyl (DHHDP) and hydrated biscyclohexenetrione dicarboxyl ester (HBCHT) groups, were isolated from young leaves of *Carpinus japonica*. Upon heating in H_2O or treatment with pH 6 buffer at room temperature, **1** and **2** afforded the reduction product **3**, isocarpinin A, with an (*R*)-hexahydroxydiphenoyl (HHDP) group, suggesting the occurrence of redox disproportionation of the (*S*)-DHHDP group. This was supported by the increase in production of **3** in the pH 6 buffer solution by coexistence of epigallocatechin-3-*O*-gallate (**15**), accompanied by oxidation of **15**. In contrast, treatment of **1** and **2** with ascorbic acid yielded **4**, carpinin A, with an (*S*)-HHDP group. Upon heating with ascorbic acid, the HBCHT group was also reduced to an (*S*)-HHDP group, and **2** was converted to 2,3;4,6-bis(*S*)-HHDP glucose. In leaves of *C. japonica*, the tannins **1** and **2** are dominant in young spring leaves, but compounds **3** and **4** become the major components of tannins in mature leaves. These results suggest that, in ellagitannin biosynthesis, oxidative coupling of the two galloyl groups first generates a DHHDP group, and subsequent reduction of DHHDP esters produces HHDP esters.

Ellagitannins are a group of hydrolyzable tannins biosynthesized by oxidative metabolism of galloyl glucoses.[1−6] The most common and diagnostic acyl group is the hexahydroxydiphenoyl (HHDP) group, which is a simple galloyl dimeric moiety. Another common acyl group is the dehydrohexahydroxydiphenoyl (DHHDP) group, which is an oxidized form of the HHDP group with a hydrated tricarbonyl structure (Scheme 1).[1,2,7] Because polyphenols are susceptible to oxidation, it is thought that HHDP esters are produced by oxidative coupling of two galloyl groups. Subsequent oxidation of the HHDP group affords DHHDP esters (Scheme 1 route A).[1,8] However, in 1993, Foo reported the reductive production of an HHDP ester from a DHHDP ester by a nonenzymatic spontaneous reaction under mild conditions.[9] Heating of amariin, 1-*O*-galloyl-2,4;3,6-bis(*R*)-DHHDP glucose, in aqueous EtOH yielded geraniin, 1-*O*-galloyl-2,4-(*R*)-DHHDP-3,6-(*R*)-HHDP glucose. This result demonstrated the spontaneous reduction of a DHHDP group to an HHDP group in a nonenzymatic reaction. It was noted that this reaction of amariin was similar to that of the partially characterized ellagitannin, isogeraniin, reported by Haddock et al. in 1982.[2,10] Geraniin is the most widely distributed DHHDP-bearing ellagitannin (so-called dehydroellagitannin).[1,2,7] Haddock et al. demonstrated that isogeraniin coexists with geraniin in various geraniin-rich plants belonging to the Geraniaceae, Aceraceae, Simaroubaceae, and Cercidiphyllaceae.[2] If isogeraniin is identical to amariin, the coexistence of geraniin and isogeraniin in those plants suggests that the reduction of DHHDP esters may be related to the biosynthesis of the 3,6-HHDP ester of geraniin (Scheme 1, route B). Our

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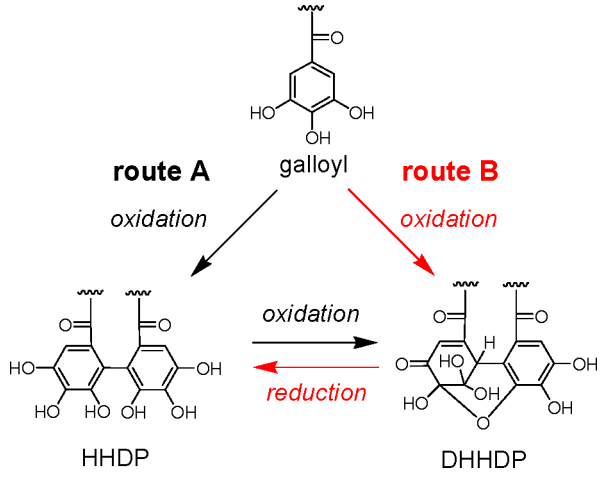
Scheme 1. Conventional (Black) and Alternative (Red) Routes for Production of HHDP and DHHDP Esters



recent studies on seasonal changes in ellagitannin composition demonstrated that amariin is a major ellagitannin in the young spring leaves of *Carpinus laxiflora*, *Triadica sebifera*, and *Elaeocarpus sylvestris* var. *ellipticus* and that amariin content decreases as the leaves mature.[11] A similar decrease of DHHDP esters has also been detected in the young spring leaves of *Castanopsis sieboldii*.[12] Furthermore, detailed analyses of the reactions of purified amariin in aqueous solution at room temperature and spontaneous reduction of the DHHDP group of geraniin on heating with pyridine suggested that the conversion of a DHHDP ester to an HHDP ester comprises a redox disproportionation.[11] The results suggested that the HHDP group is produced from the DHHDP group (Scheme 1, route B). These findings prompted us to study seasonal changes in ellagitannin composition in the leaves of *Carpinus japonica*, because these leaves contain several characteristic ellagitannins, carpinins A–D (**4**–**7**), with a highly oxidized form of the HHDP group named hydrated biscyclohexenetrione dicarboxyl (HBCHT) (Figure 1).[13] In this study, we show that ellagitannin composition changes markedly in the leaves of *C. japonica* as they mature, and we isolated unstable dehydroellagitannins, carpinins E (**1**) and F (**2**), from the young leaves. Furthermore, the unusual reactivity of these tannins provides further evidence for the putative ellagitannin metabolism route B shown in Scheme 1.

RESULTS AND DISCUSSION

HPLC Analyses of Compounds in Spring and Summer Leaves. In our previous study, carpinins A (**4**), B (**5**), C (**6**), and D (**7**) were isolated from leaves of *C. japonica* collected in June (Figure 2).[13] However, HPLC analyses of the fresh young leaves collected in April revealed that tannins **1** and **2** were the major tannins (Figure 1). In mature leaves collected in August, tannins **1** and **2** had almost disappeared and **3**, **4**, **5**, and the uncharacterized tannin **3** were the major components.

The concentrations of **1** and **2** in extracts of leaves collected in April gradually decreased during extraction with 80% CH_3CN at room temperature for 15 h. However, these compounds were much more stable in solvent containing small amounts (0.05–0.1%) of trifluoroacetic acid (TFA) (Figures 2 and 3A). When the young leaves collected in April were extracted with 80% CH_3CN at 70 °C for 1 h, **1** and **2** were absent, and instead, a peak attributable to unidentified tannin **3** was observed (Figure 3B). Dramatic changes in the HPLC profile were also observed when ascorbic acid was added to the extraction solvent as a reducing reagent (40 °C for 2 h), and the chromatogram exhibited large peaks arising from **4** instead of peaks of **1** and **2** (Figure 3C). These results suggested that **1** and **2** in the young leaves are the precursors to **3** and **4**.

Structure Determination of 1–3. To isolate the uncharacterized unstable tannins **1**–**3**, fresh leaves collected

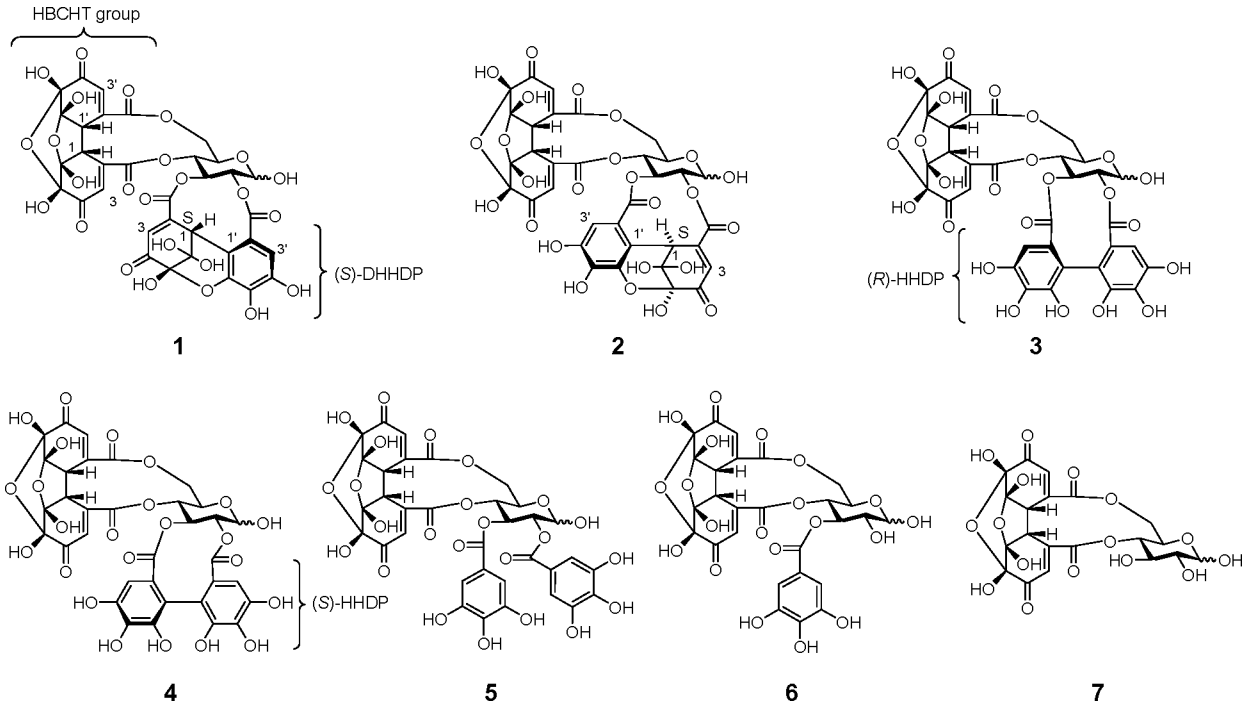


Figure 1. Structures of carpinins E (**1**), F (**2**), A (**4**), B (**5**), C (**6**), and D (**7**) and isocarpinin A (**3**).

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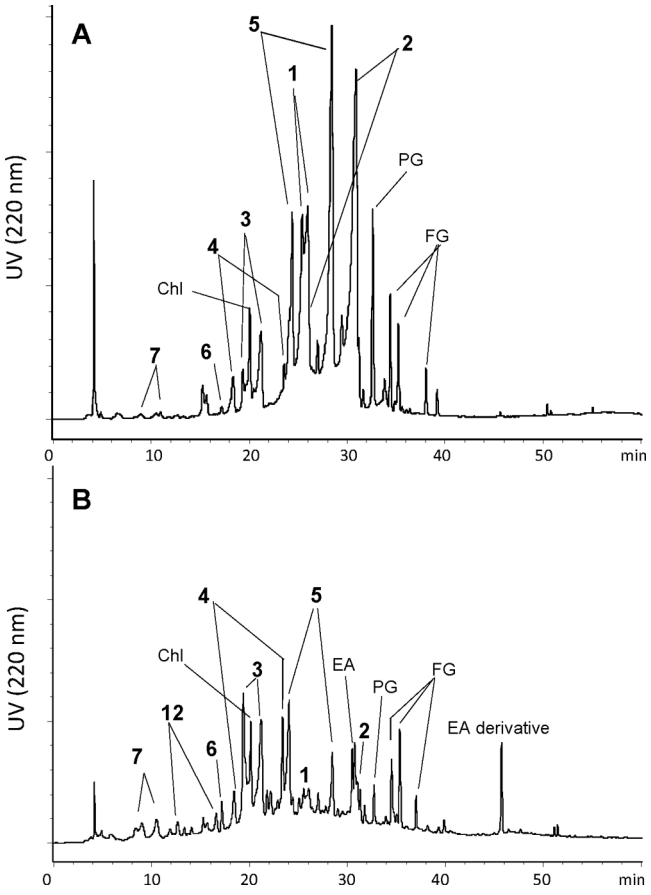

Figure 2. HPLC chromatograms (220 nm) of fresh leaf extracts of *C. japonica* collected on April 28 (A) and August 3, 2016 (B), from the same tree (extraction: 1.0 g fresh leaf material/15 mL 80% CH_3CN containing 0.1% TFA, at room temperature for 2 h). Tannins **1**–**7** and **12** were detected as a pair of peaks corresponding to α- and β-anomers. Chl: chlorogenic acid, PG: pentagalloyl glucose, FG: flavonol glycosides, EA: ellagic acid. Chl, PG, and EA were identified by comparison of the retention times and UV absorption with those of authentic samples.

in April were homogenized with 80% acetone at room temperature and extracted for 5 h. The extract was first fractionated by Sephadex LH-20 column chromatography, and the fraction containing **1**–**4** was further separated by column chromatography using Diaion HP20SS and Chromatorex ODS columns with aqueous CH_3CN containing 0.05% TFA. Isomers **1** and **2** were separated by Chromatorex ODS column chromatography, and **3** and **4** were separated by Toyopearl HW40F column chromatography.

The ^{13}C NMR spectra of carpinins E (**1**) and F (**2**) were related to those of **4**–**7**, showing signals arising from the α- and β-anomers of $^4C_1$-glucopyranose and HBCHT ester groups.[13] The NMR spectrum of **2** was much simpler than that of **1**, because the proportion of α- and β-anomers of **2** was 7:1, while that of **1** was near 1:1. This may explain the differences in the HPLC peak areas of the two anomers for each compound (Figure 2A). The HBCHT group is a highly oxidized form of the HHDP and DHHDP groups commonly found in carpinins and is characterized by two sets of conjugated carbonyls (data for the α-anomer of **2**: δ 192.1, 192.3, HBCHT-4,4′), a double bond (δ 129.8, 130.4, HBCHT-3,3′; 145.90, 149.94, HBCHT-2,2′), two hemiacetal moieties (δ 91.6, 91.7, HBCHT-5,5′; 103.6, 103.8, HBCHT-6,6′), a benzylic methine (δ 48.5, 48.6, HBCHT-1,1′), and ester carbonyls (δ 166.1, 166.5, HBCHT-7,7′). The remaining component of molecules **1** and **2** was determined to be a DHHDP group, on the basis of signals of the hydrated cyclohexenetrione carboxyl moiety (data for the α-anomer of **2**: δ 44.2, C-1; 91.5, C-6; 97.0, C-5; 131.4, C-3; 150.1, C-2; 164.6, C-7; 191.5, C-4) and the trihydroxybenzoyloxy moiety (data for **2**: 106.5, C-3′; 112.0, C-1′; 124.6, C-2′; 135.4, C-5′, 142.9, C-6′, 146.3, C-4′, 170.4, C-7′). These spectroscopic observations were consistent with the same molecular formulas of **1** and **2** ($C_{34}H_{24}O_{25}$) indicated by HRFABMS. Usually, DHHDP groups exist as equilibrium mixtures of six- and five-membered hemiacetal forms;[14] however, the ^1H and ^{13}C NMR spectra of **1** and **2** indicated that the DHHDP esters of these compounds predominantly adopted six-membered hemiacetal structures (6:1 for **1** and 7:1 for **2**, based on ^1H NMR signals of the DHHDP H-1 methine protons).

The location of the acyl groups in **1** and **2** was determined by HMBC correlations between ester carbonyls and glucosyl protons (Figure 4). In the spectra of **1** and **2**, glucosyl H-4 and H-6 showed HMBC correlations with the ester carbonyl carbons of the HBCHT group, confirming the location of the HBCHT diester group at C-4 and C-6 of the glucosyl unit. As for the DHHDP esters, the DHHDP C-7 carboxylic carbon of **1** correlated with the DHHDP C-1 methine proton and glucosyl H-3. The C-7′ ester carbonyl showed cross-peaks with the H-3′ aromatic methine and glucosyl H-2. The HMBC spectrum of **2** showed correlations between the DHHDP C-7 ester carbonyl and the H-1 methine proton and glucosyl H-2

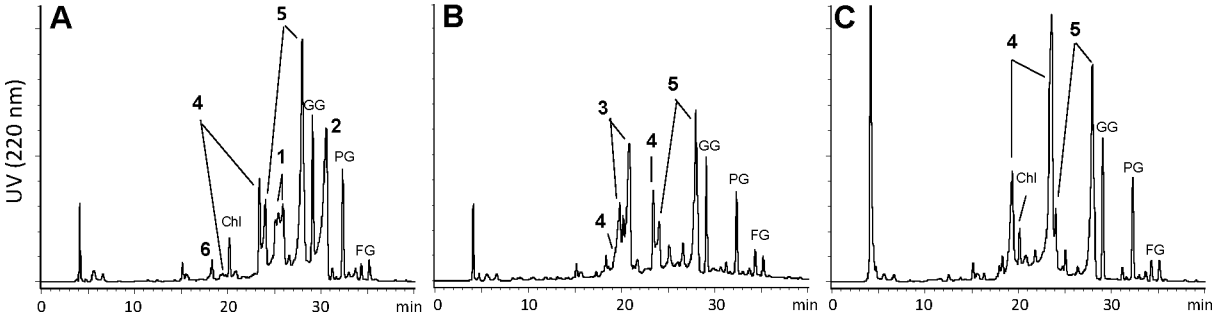

Figure 3. HPLC chromatograms (220 nm) of fresh leaves collected on April 23, 2017 (extraction: 0.20 g fresh leaf material/4.0 mL solvent). (A) Extracted with 80% CH_3CN containing 0.1% TFA, at 40°C for 1 h. (B) Extracted with 80% CH_3CN at 70°C for 1 h. (C) Extracted with 80% CH_3CN containing ascorbic acid (80 mg) at 40 °C for 2 h. Chl: chlorogenic acid, GG: galloyl glucose, PG: pentagalloyl glucose, FG: flavonol glycosides.

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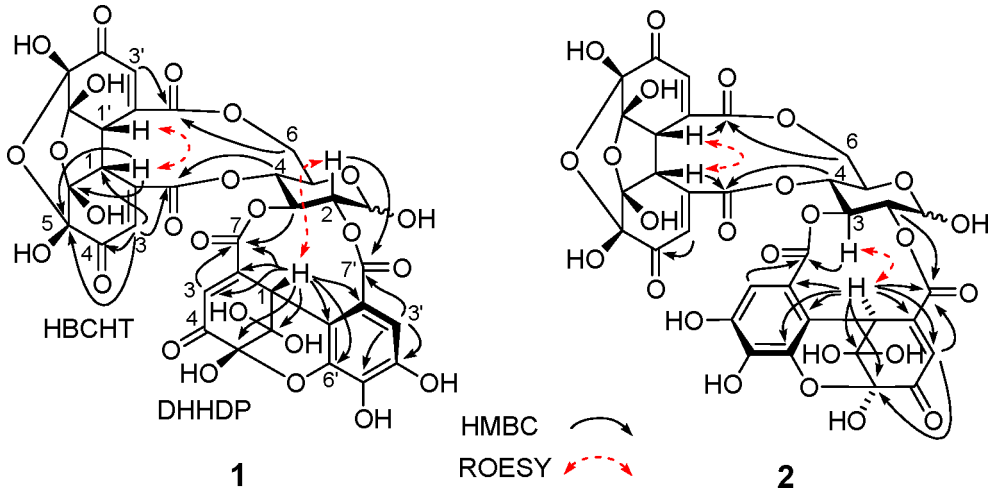

Figure 4. Selected HMBC and ROESY correlations for **1** and **2**.

and correlations between the C-7′ ester carbonyl and the aromatic methine H-3′ and glucosyl H-3. These correlations indicated that **1** and **2** are isomers differing in the connectivity of the DHHDP ester to the glucopyranosyl moiety.

The configuration of the DHHDP groups of **1** and **2** was deduced to be *S* from the negative Cotton effect at 207 nm and the positive Cotton effect at 238 nm in their ECD spectra.[15,16] This was supported by the ROESY correlations between the DHHDP benzylic methine and glucosyl H-2 for **1** and between the benzylic methine and glucosyl H-3 for **2** (Figure 4).

To chemically confirm the presence of the acyl groups, **2** was treated with 1,2-phenylenediamine and the mixture of products was methylated with CH_2N_2−ether. The products were hydrolyzed by heating under alkaline conditions, and the resulting carboxylic acid was methylated with CH_2N_2−ether. The methyl esters were identified as (*S*)-methyl 4-methoxy-3-[2,3,4-trimethoxy-6-(methoxycarbonyl)phenyl]-2-phenazinecarboxylate (**2a**)[15,17,18] and (*S*)-methyl 4-methoxyphenazine-2-carboxylate 3,3′-dimer (**2b**). The configuration of the biphenyl bonds of **2a** and **2b** was determined by comparison of their ECD spectra with literature data and confirmed by computed ECD data (Figure S1, Supporting Information).[15] Based on these spectroscopic and chemical results, the structures of **1** and **2** were determined as shown in Figure 1.

Another new ellagitannin named isocarpinin A (**3**) was isolated as a minor constituent of young leaves collected in April. Its molecular formula, $C_{34}H_{24}O_{24}$, was shown to be the same as that of **4** by HRFABMS. The ^{1}H and ^{13}C NMR spectra of **3** also resembled those of **4**, indicative of the presence of *α*- and *β*-glucopyranosyl anomers bearing HBCHT and HHDP ester moieties. The location of the HBCHT ester units at C-4 and C-6 of the glucosyl moieties was determined from the HMBC correlations between the C-7 and C-7′ ester carbonyls and H-4 and H-6 of the glucosyl unit. However, in the ^{1}H NMR spectrum, severe broadening of the HHDP aromatic protons of the *α*-anomer (δ_H 6.92, 6.98, each brs) was observed. In addition, the ^{13}C NMR chemical shifts of the HHDP C-1,1′ (HHDP C-1,1′ of *α*- and *β*-anomers: δ_C 117.0, 117.47, 117.54, 117.6) and C-2,2′ (δ_C 119.3, 121.1, 121.4, 122.9) were different from those observed for the 2,3-(*S*)-HHDP group of **4** (δ_C 114.10, 114.14, 114.57, 114.61; C-2, 2′: δ_C 126.42, 126.43, 126.46, 126.62). These spectroscopic features were similar to those reported for the 2,3-(*R*)-HHDP group of cuspinin [1-*O*-galloyl-2,3-(*R*)-4,6-(*S*)-bis-HHDP-*β*-D-glucose] isolated from *Castanopsis cuspidata* var. *sieboldii*.[19,20] In addition, the ECD spectrum of **3** showed a negative Cotton effect at 229 nm and a positive Cotton effect at 264 nm, opposite to those observed for **4**. Thus the structure of isocarpinins A (**3**) was defined as shown in Figure 1.

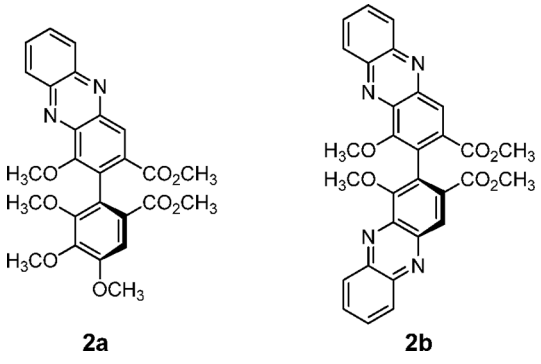

Figure 5. Structures of phenazine derivatives **2a** and **2b**.

Reactions of 1 and 2. The isomers **1** and **2** showed similar and unusual reactivities: heating in H_2O afforded the reduction product **3**. This reaction was the same as that observed when the fresh young leaves were heated in 80% CH_3CN (Figure 3B). Besides product **3**, separation of the reaction products of **2** after heating at 60 °C for 46 h afforded **7**, 2,3-(*R*)-HHDP-4,6-(*S*)-DHHDP-D-glucoses (**8**), 4,6-(*S*)-DHHDP-D-glucoses (**9**),[15] brevifolin carboxylic acid (**10**),[21] ellagic acid (**11**), and a mixture of oligomeric products (Scheme 2, HPLC profiles in Figure S2, Supporting Information). The structure of **8** was determined based on HMBC correlations (Figure S34, Supporting Information) and the ECD spectrum showing a negative Cotton effect at 217 nm (*S*-DHHDP) and a positive Cotton effect at 261 nm (*R*-HHDP).[15] Product **9** was confirmed by comparison of its spectroscopic data with those of the partial hydrolysate of 1,2,3-trigalloyl-4,6-(*S*)-DHHDP-*β*-D-glucose prepared by treatment with tannase.[22] Compound **9** was also obtained by heating of **7** in H_2O (100 °C, 5 h).

The production of **3** from **2** and **9** from **7** only by heating in H_2O suggested that the reaction is a redox disproportionation, and the simultaneous production of oxidation products was expected. However, the products isolated from the reaction mixture of **2** were reduction products or hydrolysates. The

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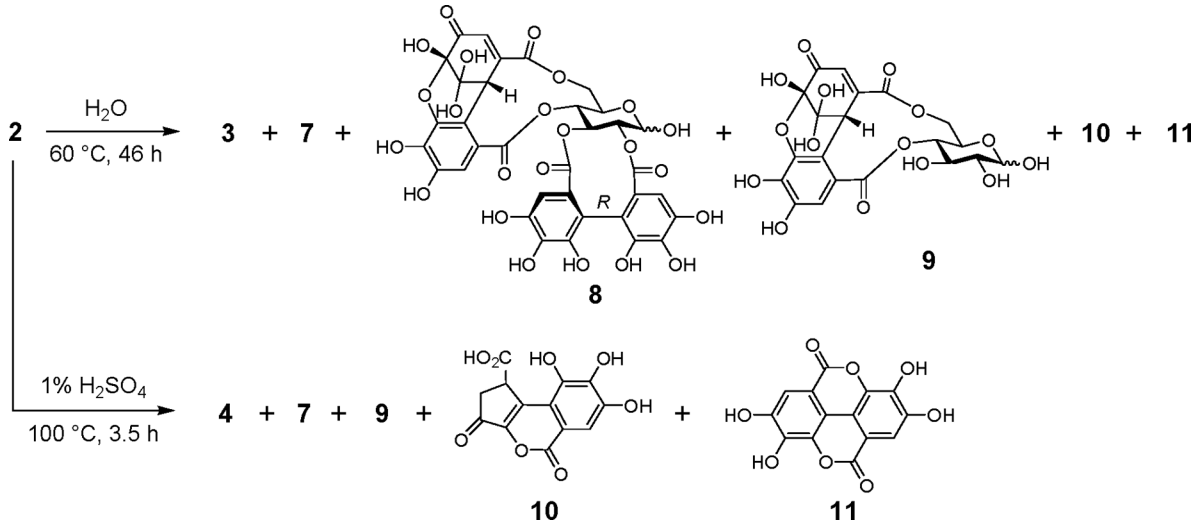
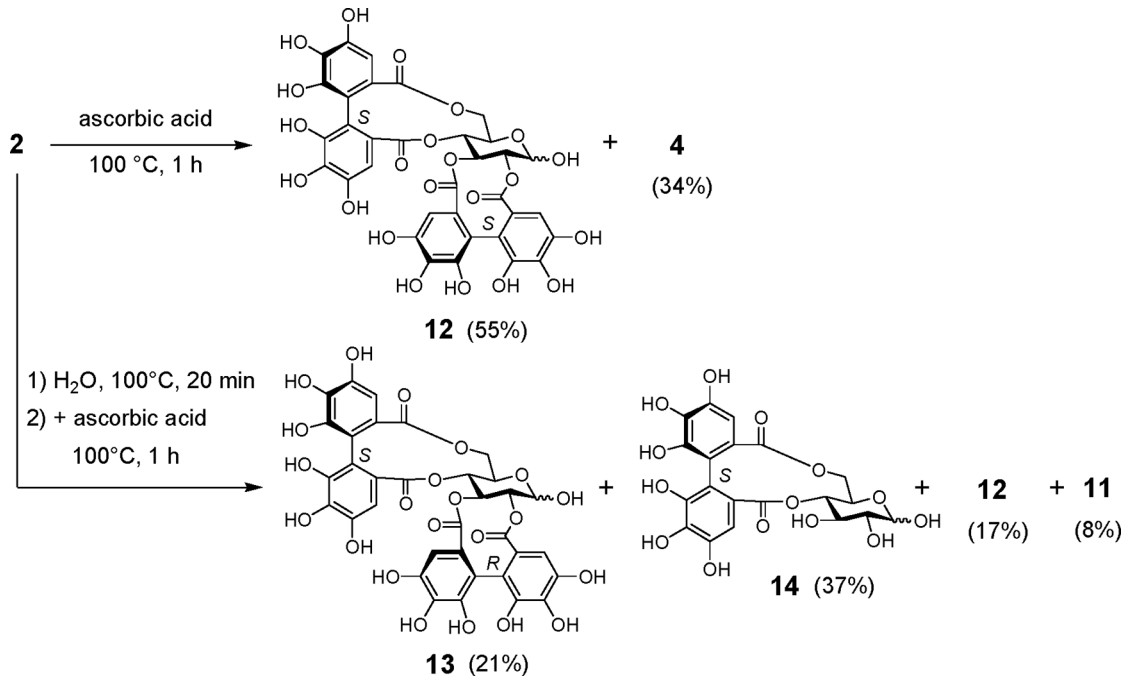
Scheme 2. Reaction of 2 in H_2O and 1% H_2SO_4



Scheme 3. Production of Pedunculagins (12) and Desgalloyl Cuspinins (13) and 4,6-(*S*)-HHDP-Glucoses (14) from 2



only candidate oxidation products were the oligomeric products detected at the origin on silica gel TLC plates and as a broad hump on the HPLC baseline. The ^{13}C NMR spectrum of the oligomers (Figure S3, Supporting Information) exhibited signals attributable to the HBCHT group and glucosyl moieties; however, the spectrum did not provide evidence that the oligomers were oxidation products. In contrast, heating of **2** in 1% H_2SO_4 (100 °C, 3.5 h) afforded **4** with an (*S*)-HHDP group as well as **7**, **9**, **10**, and **11** (Scheme 2, HPLC profiles in Figure S2, Supporting Information). The reason for the (*R*)-HHDP group being produced upon heating in H_2O and the (*S*)-HHDP group being formed upon heating under acidic conditions is unclear.

As observed for fresh young leaves treated with ascorbic acid (Figure 3C), the reduction of **1** and **2** with ascorbic acid in H_2O at 40 °C for 1 h yielded **4** (HPLC profiles in Figure S5 and possible reaction mechanism in Scheme S2, Supporting Information). Furthermore, the reaction of **2** with ascorbic acid at a higher temperature (100 °C for 1 h) afforded pedunculagin (**12**)[25] and **4** (Scheme 3). The ellagitannin **12** was detected in mature leaves of *C. japonica* collected in August (Figure 2B), suggesting that a similar metabolic pathway exists in the leaves. The combination of the aforementioned reactions of **2**, that is, heating in H_2O (100 °C for 20 min) and subsequent heating with ascorbic acid (100 °C for 1 h), yielded desgalloyl cuspinins [2,3-(*R*)-HHDP-4,6-(*S*)-HHDP-D-glucose, **13**] along with its partial hydrolysate, 4,6-(*S*)-HHDP-glucose (**14**), and **11** (Scheme 3). Compound **13** was identified as the tannase hydrolysate of cuspinin [1-*O*-galloyl-2,3-(*R*)-HHDP-4,6-(*S*)-HHDP-D-glucose].[19] Owing to incomplete conversion of **2** to **3** in this experiment, **12** was also isolated from the reaction mixture.

Our previous study showed that reduction of the DHHDP group also occurred by heating with pyridine in CH_3CN (80

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°C for 90 min),[11,26] but the mechanism of the reaction remains unclear. Treatment of **4** and **5** under the same conditions afforded **12** and 2,3-di-*O*-galloyl-4,6-(*S*)-HHDP-D-glucose,[27,28] respectively, indicating that the HBCHT esters are also reduced by heating with pyridine (HPLC profiles in Figure S6, Supporting Information).

Tannins **1** and **2** were gradually converted to **3** in a pH 5−7 buffer solution at room temperature (HPLC profiles in Figure S4, Supporting Information). This suggested that dissociation of the phenolic group is important during stereoselective conversion and that deprotonation at the benzylic methine is probably involved in the conversion to the (*R*)-HHDP group. Interestingly, the color of both **1** and **2** in pH 6 and 7 buffer solutions was greenish-blue (Figure 6). This suggests that an

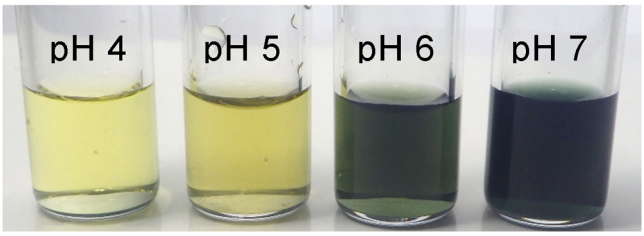

Figure 6. Color of solutions of **1** in citrate−phosphate buffer (rt, 30 min).

intermolecular charge-transfer complex may have formed. A similar color change was observed upon oxidation of the black tea pigment theaflavin, where a charge-transfer complex forms between theaflavin and the *o*-quinone B-ring moiety of epicatechin.[23,24] Possible reaction mechanisms based on intermolecular electron transfer from the DHHDP aromatic ring to the cyclohexenetrione ring are proposed in Scheme S1, in the Supporting Information.

Reaction of 2 in the Presence of Epigallocatechin Gallate. To confirm the intermolecular electron transfer between the DHHDP cyclohexenetrione and pyrogallol rings, the conversion of **2** to **3** in the presence of electron-rich pyrogallol-type aromatic rings was examined. Epigallocatechin-3-*O*-gallate (**15**) is the representative tea catechin with an electron-rich pyrogallol-type B-ring.[29,30] Enzymatic oxidation of **15** affords dehydrotheasinensin A (**15a**), theasinensins A (**15b**) and D (**15c**),[29] and theacitrin C (**15d**), which are catechin dimers characteristic of black tea polyphenols (Figure 7).[31,32] In pH 6 buffer solution (room temperature, 3 h), **15** alone did not change, whereas **2** alone completely disappeared and **3** was detected as the major product (Figure 8B). When **15** was present in the solution of **2**, the yield of **3** increased to 153% of the yield from **2** alone. The reaction mixture also contained oxidation products of **15**, that is, **15a−d** (Figure 8D). These results confirm the electron transfer from the B-ring of **15** to the DHHDP group of **2** and support our speculation that the reaction mechanism of the production of **3** from **2** is a redox disproportionation caused by intermolecular electron transfer.

The results show that the ellagitannin composition of *C. japonica* leaves changes markedly as they grow, and extraction of young leaves and subsequent chromatographic separation led to the isolation of two unique dehydroellagitannins, carpinins E (**1**) and F (**2**), with DHHDP and HBCHT groups. The ellagitannins **1** and **2** are unstable and afforded the reduction product isocarpinin A (**3**) with an (*R*)-HHDP group by treatment with pH 6 buffer at room temperature. This suggested the occurrence of a redox disproportionation by intermolecular electron transfer. Supporting evidence was provided by the increase in the quantity of reduction product **3** in the presence of **15** and the formation of oxidation products of **15**. Interestingly, the results demonstrate stereoselective reduction of the (*S*)-DHHDP ester moieties of **1** and **2** to (*S*)- and (*R*)-HHDP groups and suggest that the dissociation of the phenolic group of the DHHDP pyrogallol ring participates in the production of the (*R*)-HHDP group (Scheme S1, Supporting Information). The HBCHT group was reduced to form an (*S*)-DHHDP group on heating in H_2O, and **2** was converted into the bis-HHDP analogues **12** and **13** by heating with ascorbic acid. These results provide further evidence for the putative pathway of ellagitannin metabolism represented by route B in Scheme 1. That is, oxidative coupling of two galloyl groups generates the DHHDP group, and subsequent reduction produces HHDP esters. This mechanism is similar to that by which black polyphenols theasinensins A (**15b**) and D (**15c**) are generated via reduction of dehydrotheasinensin A (**15a**).[28] The mechanism of that reaction is also thought to be a redox disproportionation, suggesting that a common reaction mechanism operates for the oxidative coupling of pyrogallol-type phenolic compounds. Although the mechanism for stereoselective production of **3** from **1** and **2** at pH 6 is not clear, stereoselective reduction of **1** and **2** to **4** with ascorbic acid is probably related to stereoselective addition of ascorbic acid to the (*R*)-DHHDP group,[33,34] where ascorbic acid attacks from the less hindered face of the DHHDP group (Scheme S2, Supporting Information). Results of this study do

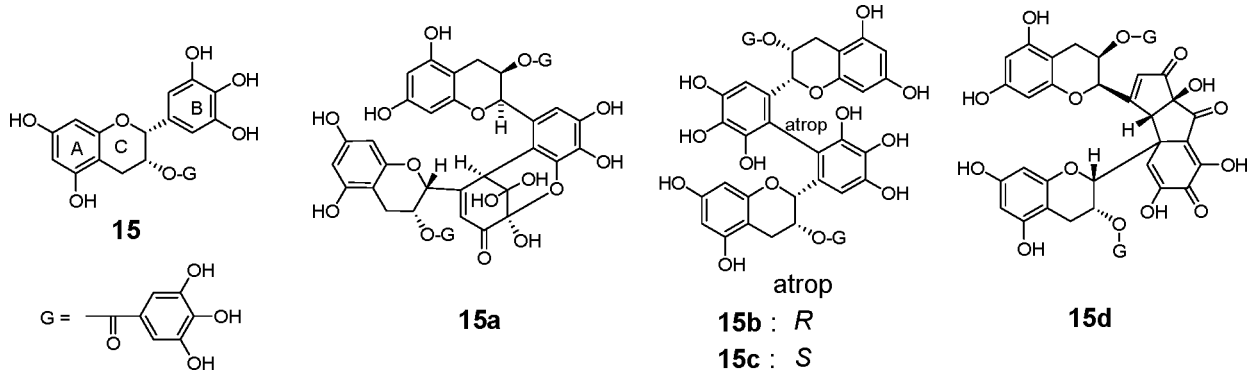

Figure 7. Structures of **15** and its oxidation products **15a−d** generated by reaction with **2**.

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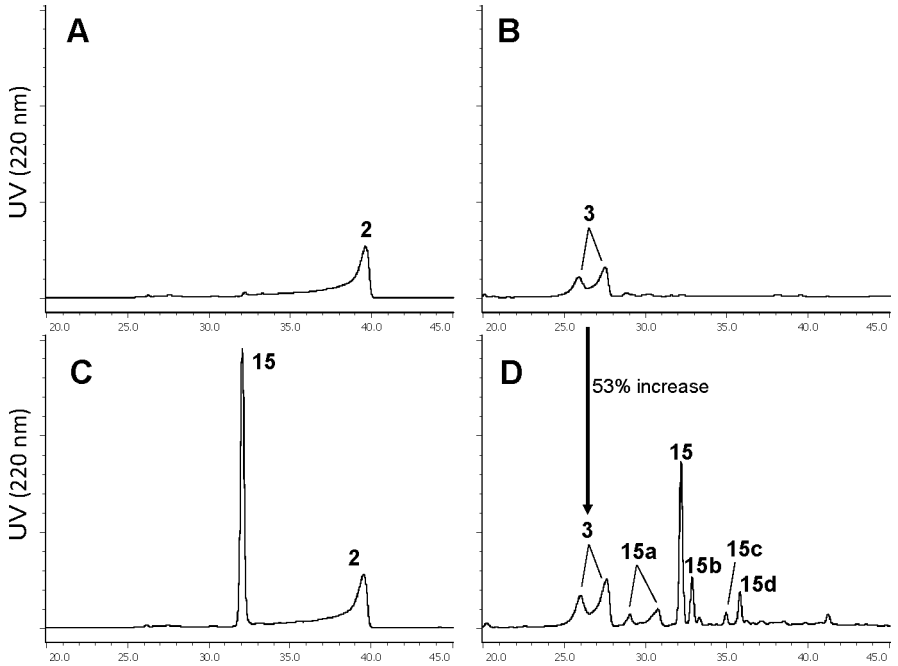

Figure 8. HPLC chromatograms of reaction mixtures of **2** and **15** in pH 6 buffer. (A) Aqueous solution of **2**. (B) **2** in pH 6 buffer at room temperature for 3 h. (C) Aqueous solution of a mixture of **2** and **15**. (D) **2** and **15** in pH 6 buffer at room temperature for 3 h. Compound **15** alone did not change under the same conditions.

not provide evidence that the redox disproportionation is the representative mechanism of HHDP production. Further studies from the viewpoints of biochemistry and organic synthesis are necessary to understand ellagitannin biosynthesis.

EXPERIMENTAL SECTION

General Experimental Procedures. Ultraviolet (UV) spectra were obtained using a JASCO V-560 UV/VIS spectrophotometer (JASCO, Tokyo, Japan). Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The ECD spectra were measured with a JASCO J-725N spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 500 spectrometer (Agilent Technologies, Santa Clara, CA, USA) operating at 500 and 126 MHz for the ^{1}H and ^{13}C nuclei, respectively. The NMR spectra were also recorded on a JEOL JNM-AL 400 spectrometer (JEOL Ltd, Tokyo, Japan) operating at 400 and 100 MHz for the ^{1}H and ^{13}C nuclei, respectively. The FABMS data were recorded on a JMS700N spectrometer (JEOL Ltd.) using *m*-nitrobenzyl alcohol or glycerol as matrix. Column chromatography was performed using Sephadex LH-20 (25−100 mm, GE Healthcare UK Ltd., Little Chalfont, UK), Diaion HP20SS (Mitsubishi Chemical Co.), Avicel cellulose (Funakoshi Co., Ltd, Tokyo, Japan), Chromatorex ODS (Fuji Silysia Chemical Ltd., Kasugai, Japan), Toyopearl HW40F, and Toyopearl Ether 650M (Tosoh Bioscience Co., Tokyo, Japan). The TLC analyses were performed on 0.25 mm thick, precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) with toluene−ethyl formate−formic acid (1:7:1, v/v/v) and on 0.1 mm thick, precoated cellulose F plates (Merck, Darmstadt, Germany) with 2% aqueous HOAc. Spots were detected by illumination under a short UV wavelength (254 nm), followed by spraying with 2% ethanolic $FeCl_3$ or 5% H_2SO_4 solution and then heating. Analytical HPLC was performed on a Cosmosil 5C18-ARII (Nacalai Tesque Inc., Kyoto, Japan) column (250 × 4.6 mm, i.d.) with gradient elution of 4−30% (39 min) and 30−75% (15 min) CH_3CN in 50 mM H_3PO_4 at 35 °C (flow rate, 0.8 mL/min; detection, JASCO photodiode array detector MD-2018 Plus).

Plant Material. Leaves of *C. japonica*, Betulaceae, were collected at Mt. Gokahara, Isahaya city, Nagasaki prefecture. A voucher specimen has been deposited at the Graduate School of Biomedical Sciences, Nagasaki University.

HPLC Analysis of Leaf Extracts. To examine seasonal changes in ellagitannin content, the fresh leaves collected on April 28 were cut with scissors into small pieces and extracted with 80% aqueous CH_3CN containing 0.1% TFA at room temperature for 2 h (1.0 g fresh weight/15 mL). After filtration through a membrane filter (0.45 μm), the filtrate was analyzed by HPLC (Figure 1). Leaves collected on August 3 were also analyzed under the same conditions.

Heating of Extract. A small portion of fresh leaves collected on April 23 (200 mg) was extracted with 80% aqueous CH_3CN (4.0 mL) at 70 °C for 1 h. After filtration through a membrane filter (0.45 μm), the filtrate was analyzed by HPLC (Figure 3B).

Treatment of Extract with Ascorbic Acid. A small portion of fresh leaves collected on April 23 (200 mg) was mixed with ascorbic acid (80 mg) and extracted with 80% aqueous CH_3CN (4.0 mL) at 40 °C for 2 h. After filtration through a membrane filter (0.45 μm), the filtrate was analyzed by HPLC (Figure 3C).

Isolation of Compounds. Fresh young leaves (600 g) of *C. japonica* collected on April 26, 2016, were homogenized with 80% acetone (5.0 L) in a Waring blender, and the homogenate was kept at room temperature for 5 h with occasional shaking. After filtration, the extract was concentrated using a rotary evaporator. The insoluble precipitate that formed in the aqueous solution was removed by filtration. The filtrate was subjected to chromatographic separation on a Sephadex LH-20 column (7.0 cm i.d. × 31 cm) with H_2O containing increasing proportions of MeOH (0, 10, 30, 50, 60, 80, and 100% MeOH, each 0.5 L) and finally with 60% acetone (2 L) to give fraction (Fr.) 1 (34.9 g) containing **1**−**4** and Fr. 2 (23.0 g) containing **5** and pentagalloyl glucose. Fr. 1 was separated by Diaion HP20SS column chromatography (5.0 cm i.d. × 32 cm) with 0−40% CH_3CN containing 0.05% TFA (5% stepwise, each 500 mL) to yield Fr. 1-1 (9.6 g, mainly composed of **1**, **3**, and **4**), 1-2 (26 g, mainly composed of **2**), and a mixture of flavonol glycosides (0.95 g). Fr. 1-2 was separated by chromatography on a Chromatorex ODS column (5.0 cm i.d. × 27 cm) with 0−30% CH_3CN containing 0.1% TFA (5% stepwise, each 500 mL) to give Fr. 1-2-1 (2.9 g, a mixture of **1**, **3**, **4**, and chlorogenic acid), Fr. 1-2-2 (9.6 g, a mixture of **2** and chlorogenic acid), and **2** (10.7 g). Fractions 1-1 and 1-2-1 were combined and separated by Chromatorex ODS column chromatography to give a

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mixture of **3** and **4** (1.81 g) and **1** (3.51 g). Finally, **3** and **4** were separated by Toyopearl HW40F column chromatography (3.0 cm i.d. × 20 cm) with 60−80% MeOH (5% stepwise, each 200 mL) to yield **3** (418 mg) and **4** (32.8 mg). Owing to the instability of the compounds, especially **1** and **2**, the isolated compounds were lyophilized to remove H_2O completely.

Carpinins E (1): pale yellow lyophilized powder; $[\alpha]_D$ +74 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.58), 243sh (4.37), 272sh (3.93), 335 (3.42), 403sh (3.08); IR ν_{max} cm^{-1} 3448, 2968, 1739, 1717, 1623, 1512, 1453, 1375; FABMS *m/z* 833 $[M + H]^+$, 855 $[M + Na]^+$; HR-FABMS *m/z* 855.0504 $[M + Na]^+$ (calcd for $C_{34}H_{24}O_{25}Na$, 855.0499); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 385 (+1.4), 346 (0), 326 (−0.84), 290 (0), 282 (+0.40), 266 (0), 259 (−0.50), 253 (0), 238 (+4.8), 229 (0), 207 (−15.3); anal. calcd for $C_{34}H_{24}O_{25}{\cdot}0.5H_2O$ C, 48.53, H, 2.99; found C, 48.34, H, 3.03; ^{1}H NMR (500 MHz, acetone-d_6) δ 3.92 (m, HBCHT-1′), 3.95 (m, HBCHT-1), 4.17 (dd, *J* = 3.3, 11.0 Hz, α-glc-6), 4.25 (m, β-glc-5,6), 4.43 (s, α-DHHDP-1), 4.47 (s, β-DHHDP-1), 4.49 (m, α-glc-5), 4.55 (d, 1.6 Hz, DHHDP-3(5)), 4.95 (dd, *J* = 4.9, 11.2 Hz, β-glc-6), 4.98 (dd, *J* = 3.7, 11.1 Hz, α-glc-6), 5.19 (t, *J* = 9.9 Hz, β-glc-3), 5.33 (d, *J* = 8.1 Hz, β-glc-1), 5.37 (t, *J* = 9.4 Hz, β-glc-4), 5.37 (dd, *J* = 7.9, 9.9 Hz, β-glc-2), 5.40 (t, *J* = 9.8 Hz, α-glc-3), 5.44 (t, *J* = 9.5 Hz, α-glc-4), 5.51 (dd, *J* = 3.2, 10.3 Hz, α-glc-2), 5.72 (d, *J* = 3.2 Hz, α-glc-1), 6.39 (d, *J* = 1.6 Hz, DHHDP-3′), 6.52 (s, α-HBCHT-3), 6.55 (s, DHHDP-3′, β-HBCHT-3), 6.68 (s, α-HBCHT-3′), 6.70 (s, β-HBCHT-3′), 6.78 (s, α-DHHDP-3), 6.79 (s, β-DHHDP-3); ^{13}C NMR (126 MHz, acetone-d_6) δ 44.0 (β-DHHDP-1), 44.3 (α-DHHDP-1), 48.4 (α,β-HBCHT-1,1′), 65.7 (α-glc-5), 65.9 (β-glc-6), 66.4 (α-glc-6), 69.4 (β-glc-5), 74.1 (α-glc-2), 75.0 (β-glc-2), 75.1 (α-glc-3), 75.2 (α-glc-4), 76.2 (β-glc-4), 77.9 (β-glc-3), 91.3 (β-DHHDP-6), 91.5 (α-glc-1), 91.5 (α-DHHDP-6), 91.6 (HBCHT-5), 91.7 (HBCHT-5′), 95.2 (β-glc-1), 97.0 (α-DHHDP-5), 97.1 (β-DHHDP-5), 103.5, 103.6 (HBCHT-6,6′), 105.7 (β-DHHDP-3′), 106.3 (α-DHHDP-3′), 112.1 (β-DHHDP-1′), 112.3 (α-DHHDP-1′), 124.3 (α-DHHDP-2′), 124.5 (β-DHHDP-2′), 129.8 (α,β-HBCHT-3), 130.3 (β-HBCHT-3′), 130.4 (α-HBCHT-3′), 131.4 (α-DHHDP-3), 131.7 (β-DHHDP-3), 135.4 (β-DHHDP-5′), 135.5 (α-DHHDP-5′), 143.0 (β-DHHDP-6′), 143.0 (α-DHHDP-6′), 145.7 (α-HBCHT-2′), 145.7 (β-HBCHT-2′), 145.9 (α-HBCHT-2), 146.0 (β-HBCHT-2), 146.4 (α-DHHDP-4′), 146.5 (β-DHHDP-4′), 150.0 (β-DHHDP-2), 150.4 (α-DHHDP-2), 165.8 (β-DHHDP-7), 166.2 (α-DHHDP-7), 166.2 (α,β-HBCHT-7′), 166.3 (α-HBCHT-7), 166.4 (β-HBCHT-7), 169.9 (α-DHHDP-7′), 169.9 (β-DHHDP-7′), 191.35 (β-DHHDP-4), 191.41 (α-DHHDP-4), 192.2 (α,β-HBCHT4,4′), 192.2 (α,β-HBCHT4,4′). Anomer: α:β = 1:1; DHHDP: six-membered-ring:-five-membered-ring = 6:1.

Carpinins F (2): pale yellow lyophilized powder; $[\alpha]_D$ +82 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.57), 242sh (4.37), 273sh (3.86), 334 (3.37), 402sh (3.00); IR ν_{max} cm^{-1} 3437, 2961, 1738, 1718, 1624, 1512, 1451, 1374; FABMS *m/z* 855 $[M + Na]^+$, 833 $[M + H]^+$, 815 $[M - H_2O + H]^+$; HRFABMS *m/z* 833.0684 $[M + H]^+$ (calcd for $C_{34}H_{25}O_{25}$, 833.0679); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 384 (+1.7), 340 (0), 324 (−0.62), 283 (0), 236 (+6.1), 226 (0), 207 (−21.7); ^{1}H NMR (500 MHz, acetone-d_6) δ 3.96 (dd, *J* = 0.7, 13.7 Hz, α-HBCHT-1′), 3.96 (dd, *J* = 0.7, 13.7 Hz, α-HBCHT-1′), 4.06 (dd, *J* = 0.7, 13.7 Hz, α-HBCHT-1), 4.21 (dd, *J* = 3.2, 10.7 Hz, α-glc-6), 4.46 (s, α-DHHDP-1), 4.54 (ddd, *J* = 3.2, 9.9, 10.7 Hz, α-glc-5), 4.88 (dd, *J* = 3.3, 10.3 Hz, α-glc-2), 4.91 (t, *J* = 10.7 Hz, α-glc-6), 5.54 (t, *J* = 9.9 Hz, α-glc-4), 5.62 (brd, *J* = 2.7 Hz, α-glc-1), 5.97 (d, *J* = 10.3 Hz, α-glc-3), 6.54 (s, α-DHHDP-3′), 6.66 (d, *J* = 0.7 Hz, α-HBCHT-3), 6.73 (d, *J* = 0.7 Hz, α-HBCHT-3′), 6.83 (s, α-DHHDP-3), 6.855 (brs, α-glc-2-OH); ^{13}C NMR (126 MHz, acetone-d_6) δ 44.2 (DHHDP-1), 48.5 (HBCHT-1′), 48.6 (HBCHT-1), 65.5 (α-glc-5), 66.5 (α-glc-6), 72.8 (α-glc-3), 74.4 (α-glc-4), 76.8 (α-glc-2), 91.2 (α-glc-1), 91.5 (DHHDP-6), 91.6 (HBCHT-5), 91.7 (HBCHT-5′), 97.0 (DHHDP-5), 103.6 (HBCHT-6′), 103.7 (HBCHT-6), 106.5 (DHHDP-3′), 112.0 (DHHDP-1′), 124.6 (DHHDP-2′), 129.8 (HBCHT-3), 130.4 (HBCHT-3′), 131.4 (DHHDP-3), 135.4 (DHHDP-5′), 142.9 (DHHDP-6′), 145.90 (HBCHT-2), 145.94 (HBCHT-2′), 146.3 (DHHDP-4′), 150.1 (DHHDP-2), 165.6 (DHHDP-7), 166.1 (HBCHT-7′), 166.5 (HBCHT-7), 170.4 (DHHDP-7′), 191.5 (DHHDP-4), 192.1 (HBCHT-4′), 192.3 (HBCHT-4). Anomer: α:β = 7:1; DHHDP: six-membered-ring:five-membered-ring = 9:1.

Isocarpinins A (3): pale yellow lyophilized powder; $[\alpha]_D$ −75 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.59), 275sh (4.13), 322sh (3.69), 353sh (3.07); IR ν_{max} cm^{-1} 3433, 2967, 1713, 1615, 1514, 1443, 1350; FABMS *m/z* 815 $[M - H]^-$, HRFABMS *m/z*: 839.0556 $[M + Na]^+$ (calcd for $C_{34}H_{24}O_{24}Na$, 839.0550); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 334 (+0.5), 293 (-9.4), 278 (0), 264 (+7.6), 253 (0), 229 (−30.2), 214 (0), 203 (+24.2); anal. calcd for $C_{34}H_{24}O_{24}H_2O$ C, 48.93, H, 3.14; found C, 49.12, H, 3.20; ^{1}H NMR (500 MHz, acetone-d_6) δ 3.89 (d, *J* =13.6 Hz, α-HBCHT-1), 3.90 (d, *J* =13.6 Hz, β-HBCHT-1), 3.94 (d, *J* =13.6 Hz, α-HBCHT-1′), 4.00 (d, *J* =13.6 Hz, β-HBCHT-1′), 4.15 (m, α-glc-6, β-glc-5), 4.16 (brt, *J* =11.1 Hz,β-glc-6), 4.40 (bt t, *J* =8.8 Hz, α-glc-5), 4.60 (dd, *J* =7.8, 9.7 Hz, β-glc-2), 4.78 (m, α-glc-2, 6), 4.83 (t, *J* =10.4 Hz, β-glc6), 5.02 (brt, *J* =9.2 Hz, β-glc-), 5.09 (t, *J* =9.8 Hz, β-glc-3), 5.12 (d, *J* =7.8 Hz, β-glc-1), 5.18 (brs, α-glc-4), 5.30 (t, *J* =9.7 Hz, α-glc-3), 5.52 (brs, α-glc-1), 6.51 (brs, α-glc-1-OH), 6.60 (s, β-HHDP-3), 6.67, 6.68 (each s, α-HBCHT-3,3′), 6.68 (s, β-HBCHT-3′), 6.74 (s, β-HBCHT-3), 6.92, 6.98 (each brs, α-HHDP-3,3′), 7.20 (s, β-HHDP-3′); ^{13}C NMR (126 MHz, acetone-d_6) δ 48.4, 48.46, 48.52 (HBCHT-1,1′), 64.5 (α-glc-5), 65.9 (β-glc-6), 66.6 (α-glc-6), 68.8 (β-glc-5), 74.8 (β-glc-4), 74.9 (α-glc-3), 75.2 (α-glc-4), 76.5 (α-glc-2), 77.5 (β-glc-3), 79.2 (β-glc-2), 91.3 (α-glc-1), 91.6, 91.65, 91.70 (HBCHT-5,5′), 95.5 (β-glc-1), 103.54, 103.56, 103.59, 103.62 (HBCHT-6,6′), 107.4 (β-HHDP-3), 109.0, 110.0 (α-HHDP-3,3′), 112.0 (β-HHDP-3′), 117.0, 117.5 (β-HHDP-1′), 117.5, 117.6 (α,β-HHDP-1,1′), 119.3 (β-HHDP-2′), 121.1, 121.4 (α-HHDP-2,2′), 122.9 (β-HHDP-2), 129.8, 130.0 (β-HBCHT-3,3′), 130.2 (α-HBCHT-3,3′), 136.6 (β-HHDP-5′), 137.6, 137.9 (α-HHDP-5,5′), 139.1 (β-HHDP-5′), 144.6, 144.7 (α-HHDP-4,4′), 144.8 (β-HHDP-4), 145.0 (β-HHDP-4′), 145.4, 145.45, 145.47, 145.48 (α,β-HHDP-6,6′), 145.8, 145.9, 146.0, 146.2 (α,β-HBCHT-2,2′), 166.0 (α-HBCHT-7′), 166.0 (α-HBCHT-7), 166.3 (β-HBCHT-7′), 166.4 (β-HBCHT-7), 168.2 (β-HHDP-7′), 168.4 (α-HHDP-7), 168.5 (α-HHDP-7′), 168.9 (β-HHDP-7), 192.1, 192.2 (α-HBCHT-4,4′), 192.3, 192.4 (β-HBCHT-4,4′).

Carpinins A (4): pale yellow lyophilized powder; $[\alpha]_D$ −52 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.60), 284sh (4.06), 327sh (3.35); IR ν_{max} cm^{-1} 3446, 2962, 1743, 1717, 1620, 1513, 1445, 1329; FABMS *m/z* 815 $[M - H]^-$; HRFABMS *m/z* 839.0554 $[M + Na]^+$ (calcd for $C_{34}H_{24}O_{24}Na$, 839.0550); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 314 (−0.5), 287 (+12.4), 262 (−11.2), 238 (+31.5), 217 (−5.9), 200 (−14.0); anal. calcd for $C_{34}H_{24}O_{24}{\cdot}H_2O$ C, 48.92, H, 3.14; found C, 48.79, H, 3.32; ^{1}H NMR (500 MHz, acetone-d_6) δ 3.95 (d, *J* = 13.6 Hz, α-HBCHT-1′), 3.95 (dd, *J* = 13.6, 0.6 Hz, β-HBCHT-1′), 4.03 (d, *J* =13.6 Hz, β-HBCHT-1), 4.05 (d, *J* = 13.6 Hz, α-HBCHT-1), 4.18-4.23 (m, β-glc-5, α glc-6), 4.25 (dd, *J* = 3.5, 10.9 Hz, β-glc-6), 4.49 (dt, *J* = 2.9, 9.8 Hz, α-glc-5) 4.87−4.92 (m, β-glc-2, 6, α-glc-6), 5.11 (dd, *J* = 3.4, 9.5 Hz, α-glc-2), 5.22 (t, *J* = 7.8 Hz, β-glc-1), 5.34−5.39 (m, β-glc-3,4), 5.41 (t, *J* = 9.8 Hz, α-glc-4), 5.54 (brt, *J* = 3.8 Hz, α-glc-1), 5.58 (t, *J* = 9.8 Hz, α-glc-3), 6.44 (s, β-HBCHT-3), 6.471 (s, β-HHDP-3′), 6.473 (s, α-HHDP-3′), 6.54 (s, α-HBCHT-3), 6.58 (s, α,β-HHDP-3) 6.64 (brs, α-glc-1-OH), 6.69 (d, *J* = 0.6 Hz, β-HBCHT-3′), 6.69 (d, *J* = 0.7 Hz, α-HBCHT-3′), 6.75 (d, *J* = 7.1 Hz, β-glc-1-OH); ^{13}C NMR (126 MHz, acetone-d_6) δ 48.4, 48.5 (HBCHT-1,1′), 64.7 (α-glc-5), 66.2 (β-glc-6), 66.7 (α-glc-6), 69.1 (β-glc-5), 73.7 (α-glc-3), 74.2 (β-glc-4), 74.6 (α-glc-4), 74.8 (α-glc-2), 76.2 (β-glc-3), 77.4 (β-glc-2), 91.6 (α-glc-1), 91.6 (α-HBCHT-5,5′), 91.6 (β-HBCHT-5,5′), 91.7 (β-HBCHT-5,5′), 95.4 (β-glc-1), 103.61, 103.63, 103.65 (HBCHT-6,6′), 107.28, 107.32 (β-HHDP-3,3′), 107.4, 107.6 (α-HHDP-3,3′), 114.10, 114.57 (α-HHDP-1,1′), 114.14, 114.61 (β-HHDP-1,1′), 126.42, 126.43 (β-HHDP-2,2′), 126.5, 126.6 (α-HHDP-2,2′), 129.5 (β-HBCHT-3), 129.6 (α-HBCHT-3), 130.1 (β-HBCHT-3′), 130.2 (α-HBCHT-3′), 136.1 (α-HHDP-5′), 136.2 (β-HHDP-5′), 136.4 ($\alpha\beta$-HHDP-5), 144.30, 144.33 (α-HHDP-6,6′), 144.37, 144.45 (β-HHDP-6,6′), 145.07 (α-HHDP-4′), 145.09 (β-HHDP-4′), 145.12 (α-HHDP-4), 145.2 (β-HHDP-4), 145.8 (α-HBCHT2,2′), 145.9 (β-HBCHT-2,2′), 146.0 (β-

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HBCHT-2,2′), 166.09 (α-HBCHT-7′), 166.11 (β-HBCHT-7′), 166.47 (β-HBCHT-7), 166.54 (α-HBCHT-7), 168.6 (β-HHDP-7), 168.7 (α-HHDP-7), 168.9 (α-HHDP-7′), 169.0 (β-HHDP-7′), 192.1 (β-HBCHT-4,4′), 192.2 (α-HBCHT-4,4′).

Methanolysis of Phenazine Derivatives. Carpinin F (**2**) (100 mg) and 1,2-phenylenediamine (40 mg) were dissolved in ethanolic 20% HOAc (3 mL) and stirred at room temperature for 12 h. The mixture was poured into ice-cooled water (10 mL), and the resulting yellow precipitate was collected by filtration. One portion (95 mg) of the precipitate (122 mg) was dissolved in MeOH−acetone (3:1, v/v, 3 mL) and treated with CH_2N_2 in Et_2O at room temperature for 15 h. After evaporation, the residue was heated with a mixture of 5% NaOH and MeOH (1:2, v/v) at 80°C for 40 min. The solution was acidified with concentrated HCl and extracted three times with Et_2O. The Et_2O layer was concentrated, dissolved in MeOH (1 mL), and treated with CH_2N_2 in Et_2O. The products were separated by silica gel column chromatography with *n*-hexane−acetone (3:2, v/v) and purified by silica gel column chromatography with acetone in $CHCl_3$ (0−4%, 2% stepwise) to yield **2a** [19.2 mg, ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 301 (−2.8), 286 (0), 265 (+9.5), 253 (0), 237 (−6.1)] and **2b** (20.7 mg) together with a mixture of **2a** and **2b** (21.7 mg). From carpinin E (62.0 mg), **2a** (3.5 mg), **2b** (1.5 mg), and a mixture of **2a** and **2b** (10.0 mg) were obtained by a similar procedure.

(*S*)-Methyl 4-Methoxyphenazine-2-carboxylate 3,3′-Dimer (2b): yellow amorphous powder, $[\alpha]_D$ +252 (*c* 0.1, $CHCl_3$); FABMS *m*/*z* 535 $[M + H]^+$; HRFABMS *m*/*z* 535.1616 $[M + H]^+$ (calcd for $C_{30}H_{23}N_4O_6$, 535.1616); IR ν_{max} cm^{-1} 2923, 2850, 1729, 1505, 1455, 1361, 1324, 1243; UV (MeOH) λ_{max} nm (log ε) 371 (4.18), 276 (4.75); ^1H NMR (400 MHz, $CDCl_3$) δ_H 3.71 (6H, s), 4.06 (6H, s), 7.24 (2H, s), 7.88 (4H, m), 8.30 (4H, m), 8.87 (2H, s); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 375 (+1.1), 348 (0), 331 (−0.56), 323 (0), 282 (+17.2), 270 (0), 259 (−30.4), 241 (0), 231 (+4.9).

Heating of 2 in H_2O. An aqueous solution of **2** (500 mg/50 mL) was heated at 60 °C for 46 h. After concentration using a rotary evaporator, the residue was separated on a Sephadex LH-20 column (2.0 cm i.d. × 45 cm) with 7 mol/L urea−acetone (4:6, v/v, containing 0.5% concentrated HCl)[35] into two fractions: Fr. 1, containing larger molecular weight substances, and Fr. 2, containing polyphenols with lower molecular weight. Fr. 1 was concentrated and applied to a Diaion HP20SS column (2.0 cm i.d. × 13 cm) with 0−80% MeOH (10% stepwise, each 50 mL) to yield polyphenolic material (39.5 mg), which was detected at the origin on silica gel TLC plates. Fr. 2 was separated by Diaion HP20SS column chromatography (2.5 cm i.d. × 20 cm) with 0−80% MeOH (5% stepwise, each 50 mL) into five fractions: 2-1−2-5. HPLC analysis indicated that major components of fractions 2-1 (32.4 mg), 2-2 (80.3 mg), and 2-5 (60.5 mg) were **7**, **9**, and **11**, respectively. Fr. 2-3 (195.0 mg) was separated by chromatography on an Avicel cellulose column (2.0 cm i.d. × 17 cm) with 2% HOAc into two fractions. Further separation of Fr. 2-3-1 on a Chromatorex ODS column (2.0 cm i.d. × 15 cm) with 0−40% MeOH (5% stepwise, each 50 mL) yielded **3** (115.8 mg). Chromatography of Fr. 2-3-2 by MCI-gel CHP20P column chromatography (2.0 cm i.d. × 10 cm) with 0−60% MeOH (10% stepwise, each 50 mL) yielded **10** (2.7 mg). Fr. 2-4 (79.5 mg) was separated by Chromatorex ODS column chromatography (2.5 cm i.d. × 22 cm) with 0−40% MeOH (5% stepwise, each 50 mL) to give **8** (29.4 mg).

2,3-(*R*)-HHDP-4,6-(*S*)-DHHDP-D-glucoses (8): tan amorphous powder; $[\alpha]_D$ +34 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (5.00), 247 (4.81), 357 (3.95); IR ν_{max} cm^{-1} 3394, 1719, 1618, 1511, 1449, 1341; FABMS *m*/*z* 823 $[M + Na]^+$; HRFABMS *m*/*z* 823.0606 $[M + Na]^+$ (calcd for $C_{34}H_{24}O_{23}Na$, 823.0601); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+7.93), 217 (−16.12), 261 (+6.50), 294 (−3.96); ^1H NMR (500 MHz, acetone-d_6) δ 3.95 (m, β-glc-5), 3.98 (t, *J* = 9.0 Hz, β-glc-6), 4.01 (t, *J* = 10.7 Hz, α-glc-6), 4.17 (ddd, *J* = 4.5, 9.5, 10.7 Hz, α-glc-5), 4.64 (s, β-DHHDP-1), 4.65 (dd, *J* = 7.4, 9.0 Hz, β-glc-2), 4.69 (s, α-DHHDP-1), 4.81 (dd, *J* = 3.4, 9.5 Hz, α-glc-2), 4.90 (dd, *J* = 4.5, 10.7 Hz, α-glc-6), 4.96 (dd, *J* = 3.5, 9.0 Hz, β-glc-6), 5.10 (d, *J* = 7.4 Hz, β-glc-1), 5.14 (t, *J* = 9.0 Hz, β-glc-3), 5.28 (t, *J* = 9.0 Hz, β-glc-4), 5.36 (t, *J* = 9.5 Hz, α-glc-3), 5.43 (t, *J* = 9.5 Hz, α-glc-4), 5.49 (d, *J* = 3.4 Hz, α-glc-1), 6.45 (s, α-HHDP-6), 6.61 (s, β-HHDP-6), 6.67 (s, β-DHHDP-3), 6.70 (s, α-DHHDP-3), 6.71 (s, α-HHDP-6′), 6.78 (s, DHHDP-3′), 6.79 (s, DHHDP-3′), 7.18 (s, β-HHDP-6′); ^{13}C NMR (100 MHz, acetone-d_6 + D_2O) δ 43.2 (α-DHHDP-1′), 43.4 (β-DHHDP-1′), 64.0 (α-glc-6), 66.0 (α-glc-5), 66.8 (β-glc-6), 68.9 (β-glc-5), 70.9 (α-glc-2), 73.0 (β-glc-4), 73.3 (α-glc-4), 74.5 (α-glc-3), 78.3 (β-glc-3), 79.1 (β-glc-2), 91.6 (α-glc-1), 91.9 (α,β-DHHDP-6′), 95.2 (β-glc-1), 96.9 (α, β-DHHDP-5′), 107.4 (α-HHDP-3), 107.9 (β-HHDP-3), 108.2 (α-DHHDP-3), 108.4 (β-DHHDP-3), 108.5 (α-HHDP-3′), 111.7 (β-HHDP-3′), 113.2 (α,β-DHHDP-1, α-HHDP-1), 114.9 (β-HHDP-1), 117.2 (α-HHDP-1′), 117.6 (β-HHDP-1′), 120.5 (α,β-HHDP-2), 122.8 (α,β-HHDP-2′), 124.2 (α,β-DHHDP-2), 131.2 (α-DHHDP-3′), 131.3 (β-DHHDP-3′), 136.0 (α-HHDP-5, α,β-DHHDP-5), 136.5 (β-HHDP-5), 137.2 (α-HHDP-5′), 138.2 (β-HHDP-5′), 142.6 (β-DHHDP-6), 142.7 (α-DHHDP-6), 144.0 (β-HHDP-6′), 144.9 (α-HHDP-6′), 145.2 (α-HHDP-4′), 145.3 (α-HHDP-6), 145.6 (β-HHDP-6), 145.7 (β-HHDP-4′), 146.4 (α-HHDP-4, β-DHHDP-4), 146.5 (α-DHHDP-4, β-HHDP-4), 151.9 (β-DHHDP-2′), 152.0 (α-DHHDP-2′), 164.78 (β-DHHDP-7′), 164.8 (α-DHHDP-7′), 168.2 (α,β-DHHDP-7), 168.7 (α,β-HHDP-7′), 169.1 (α-HHDP-7), 169.2 (β-HHDP-7), 192.3 (β-DHHDP-4′), 192.4 (α-DHHDP-4′).

Heating of 2 in 1% H_2SO_4. Compound **2** (500 mg) was dissolved in 1% H_2SO_4 (10 mL) and refluxed for 3.5 h. The precipitate formed was collected by filtration (48.3 mg) and identified as ellagic acid (**11**) on the basis of its HPLC retention time and UV spectrum. The filtrate was separated on a Toyopearl Ether 650M column (3.0 cm i.d. × 25 cm) with 0−80% MeOH (10% stepwise, each 100 mL) to give **7** (110.8 mg), **9** (74.5 mg), **10** (32.4 mg), **4** (59.9 mg), and **11** (19.4 mg).

4,6-(*S*)-DHHDP-D-glucoses (9): pale yellow amorphous powder; $[\alpha]_D$ +115 (*c* 1.0, acetone); FABMS m/z 497 $[M - H]^-$; anal. calcd for $C_{20}H_{18}O_{15}$ C, 48.20; H, 3.64; found C, 48.40, H, 4.06; ^1H NMR (400 MHz, acetone-d_6 + D_2O) δ 3.53 (dd, *J* = 9.3, 7.8 Hz, β-glc-2), 3.53 (dd, *J* = 9.4, 3.4 Hz, α-glc-2), 3.67 (ddd, *J* = 10.5, 10.5, 4.4 Hz, β-glc-5), 3.68 (t, *J* = 9.3 Hz, β-glc-3), 3.87 (t, *J* = 10.7 Hz, α-glc-6), 3.88 (t, *J* = 10.5 Hz, β-glc-6), 3.95 (t, *J* = 9.4 Hz, α-glc-3), 4.06 (ddd, *J* = 10.7, 10.7, 4.9 Hz, α-glc-5), 4.63 (d, *J* = 7.8 Hz, β-glc-1), 4.69, 4.68 (each s, DHHDP-1), 4.85 (dd, *J* = 10.5, 4.4 Hz, β-glc-6), 4.91 (dd, *J* = 10.7, 4.6 Hz, α-glc-6), 5.09 (t, *J* = 9.2 Hz, α-glc-4), 5.10 (t, *J* = 9.2 Hz, β-glc-4) 5.17 (d, *J* = 3.4 Hz, α-glc-1), 6.71, 6.70 (each s, DHHDP-3), 6.73 (1H, s, DHHDP-3′); ^{13}C NMR (100 MHz, acetone-d_6 + D_2O) δ 43.1 (DHHDP-1), 76.9, 76.4, 75.3, 74.6, 72.8, 71.2, 68.8, 67.3, 66.7, 64.7 (glc-2,3,4,5,6), 91.7 (DHHDP-6), 93.5 (α-glc-1), 97.0 (DHHDP-5), 97.7 (β-glc-1), 108.8 (DHHDP-3′), 112.9 (DHHDP-1′), 124.30, 124.21 (DHHDP-2′), 131.46, 131.42 (DHHDP-3), 136.21, 136.18 (DHHDP-5′), 142.63 (DHHDP-6′), 146.3 (DHHDP-4′), 151.83, 151.80 (DHHDP-2), 165.14, 165.13 (DHHDP-7′), 170.2, 170.1 (DHHDP-7), 193.0 (DHHDP-4).

Reduction of Carpinins D (7) with Ascorbic Acid. An aqueous solution of **7** (50 mg) and ascorbic acid (150 mg) was heated at 100 °C for 20 min. The reaction mixture was separated on a Sephadex LH-20 column (2 × 15 cm) with 0−60% MeOH (10% stepwise, each 20 mL) to afford **9** (7.6 mg) as well as unreacted **7** (9.3 mg).

Heating of Carpinins D in H_2O. An aqueous solution of **7** (200 mg/10 mL) was heated in a 50 mL screw-capped vial at 100 °C for 5 h. The mixture was separated by chromatography using a Chromatorex ODS column (2 × 20 cm) with H_2O containing increasing proportions of MeOH (0−50%, 5% stepwise, each 50 mL) to give **9** (80.9 mg) as well as unreacted **7** (30.0 mg).

Hydrolysis of Trapain. An aqueous solution of 1,2,3-tri-*O*-galloyl-4,6-(*S*)-DHHD-β-D-glucose (10 mg/2 mL) was treated with tannase (5 mg, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) at room temperature for 14 h. The mixture was separated by Sephadex LH-20 column chromatography (0−50% MeOH in H_2O, 10% stepwise, each 20 mL) to give gallic acid (5 mg) and **9** (3.0 mg).

Conversion of 2 to 12. Compound **2** (50 mg) and ascorbic acid (500 mg) were dissolved in H_2O (5.0 mL) and heated at 100 °C for 60 min. The reaction mixture was separated on a Diaion HP20SS column (2 × 20 cm) with H_2O containing increasing proportions of

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MeOH (0−50%, 5% stepwise, each 50 mL) to give **12** (25.7 mg, 55%) and **4** (16.5 mg, 34%). The product **12** was identified by comparison of its ^{1}H and ^{13}C NMR spectra.[25]

Conversion of 2 to 13. Compound **2** (100 mg) was dissolved in H_2O (10 mL) and heated at 100 °C for 20 min. Then, ascorbic acid (1.2 g) was added, and the mixture was heated at 100 °C for 60 min. The solution was directly applied to a Diaion HP20SS column (2 × 20 cm), and products were eluted with H_2O containing increasing proportions of MeOH (0−50%, 5% stepwise, each 50 mL). This separation yielded **14** (25.7 mg), **13** (19.6 mg), **12** (15.2 mg), and **11** (3.0 mg). Product **13** was identified as the tannase hydrolysate of cuspinin by comparison of its ^{1}H and ^{13}C NMR spectra with reported data.[19] $[\alpha]_D$ −14 (*c* 0.1, MeOH); ESIMS (negative mode) *m/z* 783 $(M - H)^-$; ESIMS (positive mode) *m/z* 807 $(M + Na)^+$; HRESIMS (negative mode) *m/z* 783.0707 $[M - H]^-$ (calcd for $C_{34}H_{23}O_{22}$, 783.0687); UV (MeOH) λ_{max} nm (log ε) 228 (4.69), 276 sh (4.34); IR (dry film) ν_{max} cm^{-1} 3421, 1717, 1613, 1513, 1444, 1311; ^{1}H NMR (400 MHz, acetone-d_6) δ 3.68 (dd, *J* = 12.7, 1.8 Hz, α-glc-6), 3.73 (d, *J* = 12.9 Hz, β-glc-6), 4.11 (dd, *J* = 9.8, 5.6 Hz, β-glc-5), 4.47 (m, α-glc-5), 4.49 (dd, *J* = 9.8, 7.8 Hz, β-glc-1), 4.69 (dd, *J* = 9.8, 3.5 Hz, α-glc-2), 4.72 (t, *J* = 9.8 Hz, β-glc-4), 4.80 (brd, *J* = 8 Hz, OH), 4.97 (t, *J* = 9.8 Hz, α-glc-4), 4.97 (brt, *J* = 8 Hz, β-glc-1), 5.16-5.25 (m, α, β-glc-3, α, β-glc-6), 5.48 (t, *J* = 3.5 Hz, α-glc-1), 6.28 (brs, OH), 7.20, 7.05, 6.79, 6.69, 6.66, 6.57 (total 4H, HHDP-3, 3′); ^{13}C NMR (100 MHz, acetone-d_6) δ 63.5 (α, β-glc-6), 79.9, 78.4, 77.20, 77.16, 76.5, 72.0, 70.2, 70.0, 67.3 (glu-2,3,4,5), 91.5 (α-glc-1), 95.5 (β-glc-1), 108.3, 108.2, 108.0, 107.9, 107.48, 107.45 (HHDP-3,3′), 119.7, 117.7, 117.63, 117.61, 117.2, 115.83, 115.78, 115.71, 115.6, 111.9 (HHDP-1,1′), 126.4, 126.3, 126.23, 126.16, 123.2 (HHDP-2,2′), 138.9, 136.4 (×3), 136.3 (HHDP-5,5′), 145.5, 145.4, 145.3, 145.24, 145.19, 145.14, 145.07, 145.06, 145.0, 144.81, 144.76, 144.74, 144.44, 144.42, 144.28, 144.26 (HHDP-4,6,4′,6′), 169.2, 168.14, 168.08, 168.0, 167.3, 167.2 (COO).

Heating of 4 and 5 in Pyridine. A solution (2 mg/mL) of **4** in 4% pyridine−CH_3CN was heated at 80 °C for 90 min. After cooling, 2.5% TFA in H_2O (0.1 mL) was added, and the mixture analyzed by HPLC. The chromatogram showed two peaks corresponding to the α- and β-anomers of **12**. Treatment of **5** under the same conditions and HPLC analysis of the reaction mixture showed the formation of 2,3-di-*O*-galloyl-4,6-(*S*)-HHDP-D-glucose (t_R = 18.5 and 22.1 min). This product was identified by comparison with an authentic sample (1-desgalloyleugeniin) isolated from fruits of *Paeonia lactiflora*.[28]

Reaction of 2 with 15. Reaction A: An aqueous solution of **2** (10 mmol/L, 0.3 mL) was mixed with H_2O (0.3 mL) and pH 6 citrate−phosphate buffer (0.3 mL) in a screw-capped vial and left standing at room temperature for 3 h. Reaction B: An aqueous solution of **2** (10 mmol/L, 0.3 mL) was mixed with an aqueous solution of **15** (10 mmol/L, 0.3 mL) and pH 6 citrate−phosphate buffer (0.3 mL) in a screw-capped vial and left standing at room temperature for 3 h. Then, 0.5% TFA (0.3 mL) and CH_3CN (0.3 mL) were added to the mixtures. The mixtures were analyzed by HPLC on a Cosmosil PAQ column (250 × 4.6 mm, i.d.; Nacalai Tesque Inc., Kyoto, Japan) with a gradient elution of 4−25% (40 min) and 25−85% (5 min) CH_3CN in 50 mM H_3PO_4 at 35 °C (flow rate, 0.8 mL/min). Eluted products were detected at 220 nm using a JASCO MD-2018 Plus photodiode array detector. An aqueous solution of **15** alone did not show significant change in the HPLC profile under these conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00893.

Experimental and calculated ECD spectra of **2b**, HPLC profiles of reaction mixtures of **2** after heating in H_2O and 1% H_2SO_4, ^{13}C NMR spectrum (100 MHz, D_2O) of a mixture of oligomeric products obtained by heating of **2** in H_2O, HPLC profiles of **1** and **2** in pH 3−7 citrate−phosphate buffer solutions after 3 h at rt, possible mechanism of reduction−oxidation disproportionation of the DHHDP group of **1** and **2**, reduction of **1** and **2** to **4** with ascorbic acid, possible reduction mechanism of the DHHDP group with ascorbic acid, HPLC profiles of **4** and **5** on heating with pyridine, ^{1}H, ^{13}C, and 2D NMR spectra of **1**, **2**, **3**, **4**, **8**, and **9**, and ECD spectra of **1**−**4** and **7** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Okuda, T.; Yoshida, T.; Hatano, T. Hydrolyzable Tannins and Related Polyphenols. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, 1995; pp 1−117.

(2) Haddock, E. A.; Gupta, R. K.; Al-Shafi, S. M. K.; Layden, K.; Haslam, E.; Magnolato, D. *Phytochemistry* **1982**, *21*, 1049−1062.
(3) Okuda, T.; Ito, H. *Molecules* **2011**, *16*, 2191−2217.
(4) Quideau, S.; Jourdes, M.; Lefeuvre, D.; Pardon, P.; Saucier, C.; Teissedre, P.-L.; Glories, Y. Ellagitannins - An Underestimated Class of Plant Polyphenols: Chemical Reactivity of C-Glucosidic Ellagitannins in Relation to Wine Chemistry and Biological Activity. In *Recent Advances in Polyphenol Research*; Santos-Buelga, C., Escribano-Bailon, M. T., Lattanzio, V., Eds; Wiley-Blackwell Publishing: Oxford, 2010; Vol. 2, pp 81−137.
(5) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. *Angew. Chem., Int. Ed.* **2011**, *50*, 586−621.
(6) Niemets, R.; Gross, G. G. *Phytochemistry* **2005**, *66*, 2001−2011.
(7) Okuda, T.; Hatano, T.; Yazaki, K. *Chem. Pharm. Bull.* **1982**, *30*, 1113−1116.
(8) Okuda, T.; Yoshida, T.; Hatano, T. *Phytochemistry* **2000**, *55*, 513−529.
(9) Foo, L.Y. *Phytochemistry* **1993**, *33*, 487−491.
(10) Haddock, E. A.; Gupta, R. K.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2535−2545.
(11) Era, M.; Matsuo, Y.; Saito, Y.; Tanaka, T. *Molecules* **2020**, *25*, 1051.
(12) Wakamatsu, H.; Tanaka, S.; Matsuo, Y.; Saito, Y.; Nishida, K.; Tanaka, T. *Molecules* **2019**, *24*, 24.
(13) Nonaka, G.; Mihashi, K.; Nishioka, I. *J. Chem. Soc., Chem. Commun.* **1990**, 790−791.
(14) Okuda, T.; Yoshida, T.; Hatano, T. *J. Chem. Soc., Perkin Trans. 1* **1982**, 9−14.
(15) Okuda, T.; Yoshida, T.; Hatano, T.; Koga, T.; Tho, N.; Kuriyama, K. *Tetrahedron Lett.* **1982**, *23*, 3941−3944.
(16) Esumi, A.; Aoyama, H.; Shimozu, Y.; Tanoguchi, S.; Hatano, T. *Heterocycles* **2019**, *98*, 895−903.
(17) Okuda, T.; Yoshida, T.; Nayeshiro, H. *Chem. Pharm. Bull.* **1977**, *25*, 1862−1869.
(18) Okuda, T.; Yoshida, T.; Mori, K.; Hatano, T. *Heterocycles* **1981**, *12*, 1323−1348.
(19) Nonaka, G.; Ishimatsu, M.; Ageta, M.; Nishioka, I. *Chem. Pharm. Bull.* **1989**, *37*, 50−53.
(20) Yamaguchi, S.; Hirokane, T.; Yoshida, T.; Tanaka, T.; Hatano, T.; Ito, H.; Nonaka, G.; Yamada, H. *J. Org. Chem.* **2013**, *78*, 5410−5417.
(21) Tanaka, T.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1990**, *38*, 2424−2428.
(22) Nonaka, G.; Matsumoto, Y.; Nishioka, I. *Chem. Pharm. Bull.* **1981**, *29*, 1184−1187.
(23) Tanaka, T.; Mine, C.; Inoue, K.; Matsuda, M.; Kouno, I. *J. Agric. Food Chem.* **2002**, *50*, 2142−2148.
(24) Li, Y.; Shibahara, A.; Matsuo, Y.; Tanaka, T.; Kouno, I. *J. Nat. Prod.* **2010**, *73*, 33−39.
(25) Tsujita, T.; Matsuo, Y.; Saito, Y.; Tanaka, T. *Tetrahedron* **2017**, *73*, 500−507.
(26) Tanaka, T.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1990**, *38*, 2424−2428.
(27) Wilkins, C. K.; Bohm, B. A. *Planta Med.* **1976**, *30*, 72−74.
(28) Tanaka, T.; Fukumori, M.; Ochi, T.; Kouno, I. *J. Nat. Prod.* **2003**, *66*, 759−763.
(29) Tanaka, T.; Watarumi, S.; Matsuo, Y.; Kamei, M.; Kouno, I. *Tetrahedron* **2003**, *59*, 7939−7947.
(30) Li, Y.; Tanaka, T.; Kouno, I. *Phytochemistry* **2007**, *68*, 1081−1088.
(31) Matsuo, Y.; Li, Y.; Watarumi, S.; Tanaka, T.; Kouno, I. *Tetrahedron* **2011**, *67*, 2051−2059.
(32) Matsuo, Y.; Okuda, K.; Morikawa, H.; Oowatashi, R.; Saito, Y.; Tanaka, T. *J. Nat. Prod.* **2016**, *79*, 189−195.
(33) Okuda, T.; Yoshida, T.; Hatano, T.; Ikeda, Y. *Heterocycles* **1986**, *24*, 1841−1843.
(34) Tanaka, T.; Nonaka, G.; Nishioka, I.; Miyahara, K.; Kawasaki, T. *J. Chem. Soc., Perkin Trans. 1* **1986**, 369−376.
(35) Yanagida, A.; Shoji, T.; Shibusawa, Y. *J. Biochem. Biophys. Methods* **2003**, *56*, 311−322.

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