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TWO NEW HYDROLYZABLE TANNINS, CARPINERINS A AND B, FROM GALLS OF *CARPINUS TSCHONOSKII*

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Abstract – Two new hydrolyzable tannins, carpinerins A (**1**) and B (**2**), together with ten known tannins **3**-**12**, were isolated from the galls on bud of *Carpinus tschonoskii*, and the structures of **1** and **2** were elucidated using spectroscopic data, primarily NMR and MS, and chemical means. Most of isolated tannins including carpinerin B (**2**) exhibited inhibitory effects on the growth of the root of cress (*Lepidium sativum* L.) seedling at 1×10^{-4} mol/L.

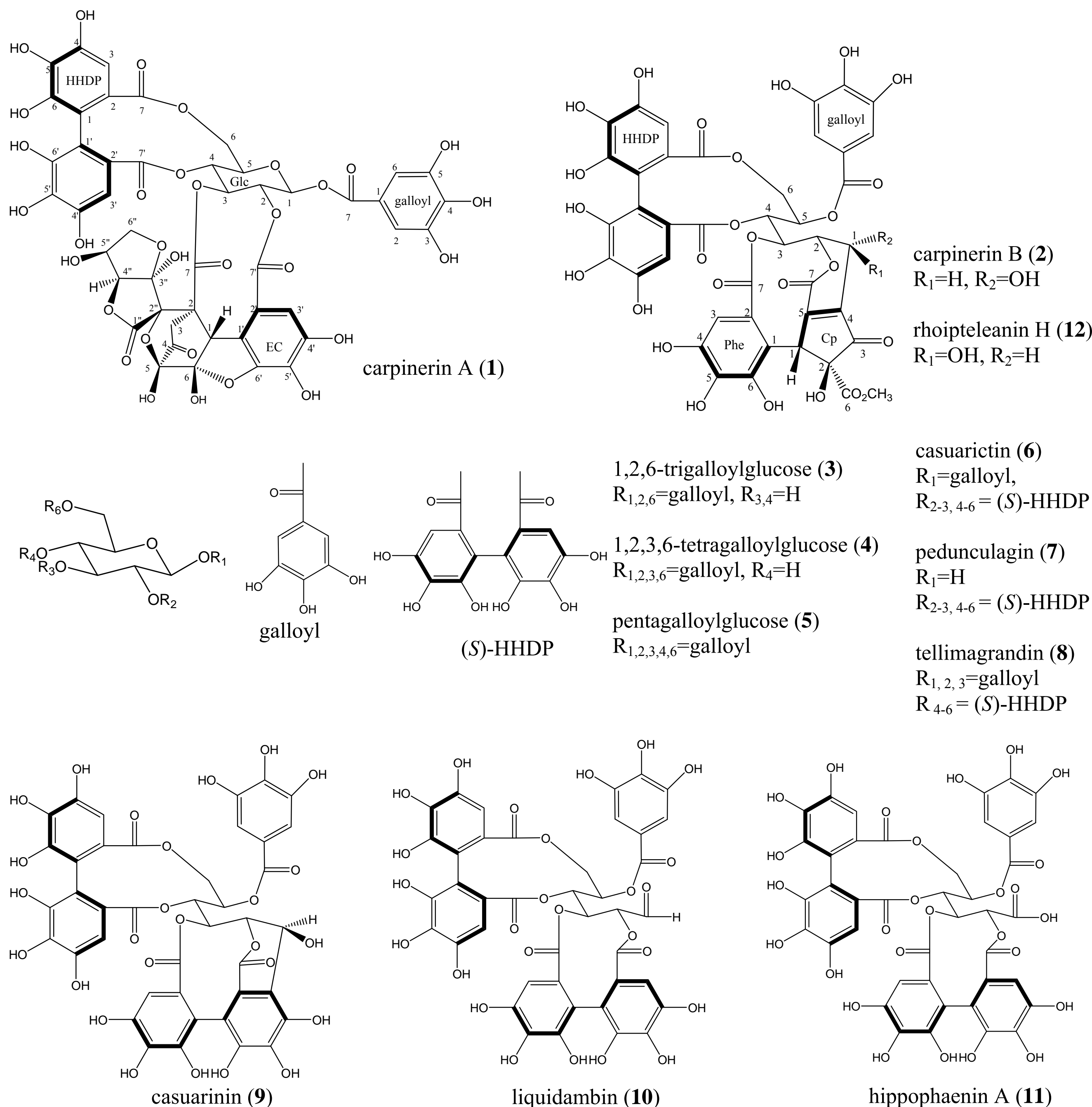
INTRODUCTION

Galls are excrescences formed by parasitizing organism including insects, bacteria, and viruses. Although there are many kinds of galls and it is so interesting phenomenon, little have been known about galls and its ingredient. Therefore, study on constituents of galls will be expected to lead new bioactive compounds. In search for new bioactive compounds from galls, plant growth inhibitory polyacetylenes have been isolated from the insect galls of *Hedera rhombea* Bean.[1-3] On the other hand, constituents of galls of *Carpinus tschonoskii* bud also have not been reported. *Carpinus tschonoskii* belong to Betulaceae family and this gall is formed by mite (*Eriophyes* sp.)-parasitizing. In this research, to search for compounds which produced by gall-form, we noticed the difference of constituents between gall and normal bud. It is found that hydrolyzable tannins were produced by gall-form. In this paper, we reported the isolation and identification of hydrolyzable tannins from gall extract of *Carpinus tschonoskii* and their structure activity relationship on the growth of cress (*Lepidium sativum* L.) seedling.

RESULTS AND DISCUSSION

Galls of *Carpinus tschonoskii* were homogenized and extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O and the H_2O-layer was further partitioned with BuOH. Each of EtOAc-layer and BuOH-layer was separated by ODS column chromatography and ODS-HPLC to give

two new tannins, carpinerins A (**1**, 0.0034 %) and B (**2**, 0.0261 %), together with 10 known tannins, 1,2,6-trigalloylglucose (**3**, 0.0012 %),[4] 1,2,3,6-tetragalloylglucose (**4**, 0.0021 %),[4] pentagalloylglucose (**5**, 0.0169 %),[5] casuarictin (**6**, 0.0228 %),[6] pedunculagin (**7**, 0.0062 %),[6] tellimagrandin Ⅱ (**8**, 0.0094 %),[7] casuarinin (**9**, 0.0098 %),[6] liquidambin (**10**, 0.0356 %),[8] hippophaenin A (**11**, 0.003 %),[9] and rhoipteleanin H (**12**, 0.0183 %).[10]



Carpinerin A (**1**) was isolated as a colorless powder, $[\alpha]_D$ -6.0° (MeOH), and determined the molecular formula as $C_{47}H_{34}O_{32}$ by the HRESIMS [*m/z* 1133.0898 $(M+Na)^+$, Δ -3.3 mmu]. The ^{1}H NMR

spectrum showed a two-proton aromatic singlet signal (δ_H 7.14) due to a galloyl group, three aromatic singlet proton signals (δ_H 6.96, 6.66, and 6.53), and eight methine and three methylene proton signals. The ^{1}H-^{1}H COSY connectivities of Glc-H-1 through Glc-H-6 indicated the presence of a sugar. Its ^{1}H-^{1}H coupling patterns (J_{1-2} = 8.5 Hz, J_{2-3} = 10.1 Hz, J_{3-4} = 10.1 Hz, J_{4-5} = 10.1 Hz, J_{5-6} = 6.7 Hz) were similar to those of casuarictin (**6**) and it suggested that a sugar of **1** was glucose (Glc). The ^{13}C NMR spectrum indicated the presence of a galloyl group (δ_C 111.3, 121.0, 141.6, 147.5, 166.6) and a hexahydroxydiphenoyl (HHDP) group (δ_C 109.4, 109.7, 117.3, 117.4, 126.9, 138.2, 138.4, 145.4, 145.7, 146.6, 146.7, 168.6, 170.3).[6] The ^{1}H-^{1}H COSY correlations showed the connectivities of H-4" (δ_H 4.50), H-5" (δ_H 4.34), and H-6" (δ_H 4.19 and 3.80). And the HMBC correlations of this methylene proton (δ_H 4.19) and methine proton (δ_H 4.50) to a hemiacetal carbon (δ_C 109.7, EC-C-3") revealed the presence of a furan ring and the methine proton (δ_H 4.50) to a ester carbon (δ_H 174.2) revealed the presence of γ-lactone. Moreover, HMBC correlations of methine proton (δ_H 5.81, EC-H-1) and methylene proton (δ_H 2.21, EC-H-3) to quaternary carbon (δ_C 82.8, EC-C-2") showed the γ-lactone connected with spiro carbon (EC-C-2"). And HMBC correlations were observed that methine proton (δ_H 5.81, EC-H-1) to quaternary carbon (δ_C 49.8, EC-C-2) and two benzene carbons (δ_C 118.8, EC-C-1' and 149.2, EC-C-6') and methylene proton (δ_H 2.21, EC-H-3) to EC-C-2 and a carbonyl carbon (δ_C 200.5, EC-C-4). For all of these results and past literature data[11] showed the presence of a elaecarpusinoyl (EC) group. The ^{13}C NMR chemical shifts of EC group gave close agreement with literature data of elaeocarpusin[11-13] and helioscopin A[14]. The HMBC correlations of Glc-H-2 (δ_H 4.29) to EC-C-7' (δ_C 168.0) and Glc-H-3 (δ_H 5.82) to EC-C-7 (δ_C 172.0) revealed that the EC group was connected

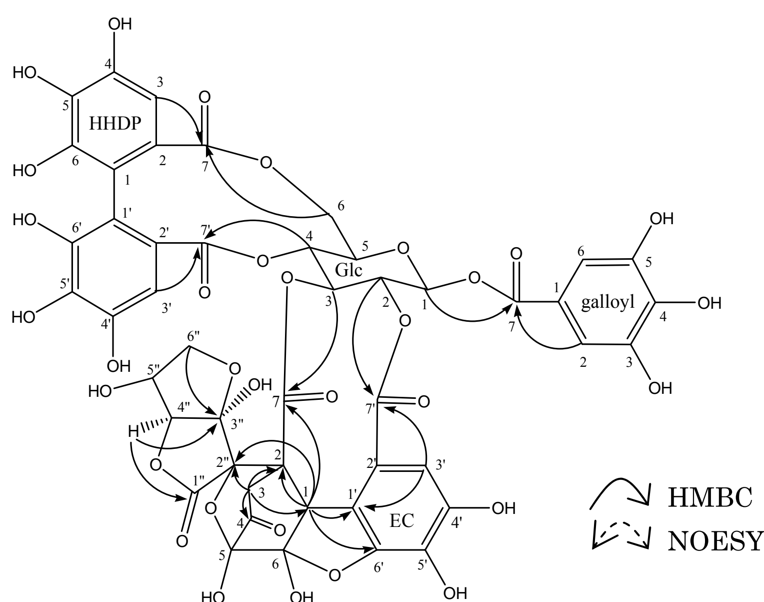


Figure 1. 2D NMR Correlations of **1**.

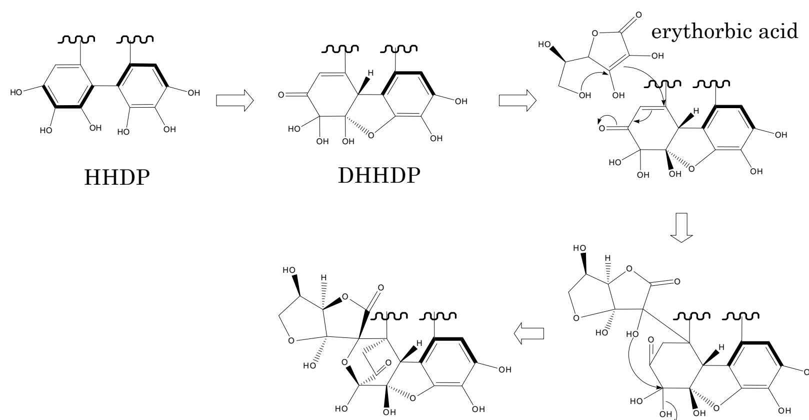


Figure 2. Plausible biosynthesis of **1**.

to Glc-H-2 and Glc-H-3. In a similar way, the HMBC correlations of Glc-H-1 (δ_H 6.93) to galloyl-C-7 (δ_C 166.6) and Glc-H-2 and Glc-H-4 (δ_H 4.29 and 5.21) to HHDP-C-7 and HHDP-C-7' (δ_C 170.3 and 168.6) showed the linkage of Glc-1 to galloyl group and Glc-2 and Glc-4 to HHDP group (Figure 2). The ^{1}H NMR date of EC group were different from literature data of elaeocarpusin and helioscopin A, in spite of close agreement of ^{13}C NMR date. In case of **1**, the ^{1}H-^{1}H coupling constant (J = 5.0 Hz) between EC-4" and EC-5" was observed, while those of elaeocarpusin and helioscopin A were not observed.[11-14] Furthermore, it was known that heating elaeocarpusin and helioscopin A in water liberated ascorbic acid.[11-14] Compound **1** was heated in water at 90℃ for 24 h and the reaction mixture was subjected to HPLC analysis. The retention time (t_R 5.8 min) of the reaction mixture was identical with that of erythorbic acid. This result indicated *cis*-form between EC-4" and EC-5" of **1**. On the other hand, heating **1** in 0.1 % AcOH at 80℃ for 30 min gave **6** by HPLC analysis of the reaction mixture, supporting that **6** was the precursor of **1**. This result indicated the atropisomers of HHDP and EC group were elucidated to be both of *S*-configurations, since both of two HHDP groups in **6** had *S*-configurations.[6] The NOESY correlation between EC-H-6" (δ_H 5.46) and HHDP'-H-3 (δ_H 6.53) revealed that the spiro stereochemistry of EC-2" was implied *S*-configuration. From these results, the structure of **1** was proposed as being represented formula **1**. It presumed the plausible biosynthesis of **1** is as indicated below, HHDP group which was linked to Glc-2, 3 of **6** gave dehydrohexahydroxydiphenoyl (DHHDP) group by oxidation and it condensed with erythorbic acid (Figure 2).

Carpinerin B (**2**) was isolated as a pale yellow powder, $[\alpha]_D$ -50.0° (MeOH), and determined the molecular formula as $C_{42}H_{30}O_{27}$ by the HRESIMS [*m/z* 991.0872 $(M+Na)^+$, Δ +4.5 mmu]. The ^{1}H NMR spectrum showed a two-proton aromatic singlet signal (δ_H 7.11) due to a galloyl group, three aromatic singlet proton signals (δ_H 6.89, 6.54, and 6.32), five methines and a methylene proton signals due to glucose, a benzyl methine (δ_H 5.33), and a carbomethoxy (δ_H 3.69) proton signals. The ^{13}C NMR spectrum of **2** was highly similar to those of rhoipteleanin H (**12**), indicating the presence of a galloyl group, an HHDP group, and a Phenyl (Phe)-Cycropentanone (Cp) group.[10] The HMBC correlations of Cp-H-1 (δ_H 5.33) to Cp-C-2, 3, 5, 6 and Phe-C-1, 2, 6 and the correlations of Glc-H-1 (δ_H 4.53) to Cp-C-3, 4, 5 were also evidence supporting the existence of Phe-Cp group. HMBC correlations of Glc-H-5 (δ_H 5.40) to Galloyl-C-7 (δ_C 166.3), Glc-H-2 (δ_H 5.18) to Cp-C-7 (δ_C 162.8), Glc-H-3 (δ_H 5.45) to Phe-C-7 (δ_C 168.9), and Glc-H-4, 6 (δ_H 5.73 and 4.98) to HHDP-C-7, 7' (δ_C 168.4) indicated linkage of Glc-C-5 to galloyl group, Glc-C-2, 3 to Phe-Cp group, and Glc-C-4, 6 to HHDP group. In the ^{1}H NMR spectrum, remarkable difference were chemical shift of Glc-H-1 and ^{1}H-^{1}H coupling constant between Glc-H-1 and Glc-H-2 of **2** (δ_H 4.53, J = 1.0 Hz) and **12** (δ_H 5.36, J = 6.3 Hz). Moreover, coupling patterns of **2** were similar to those of stachyurin,[6] while **12** were similar to those of **6**. It was indicated that **2** was a stereoisomer of **12** and the stereochemistry of Glc-1 is only difference between **2** and **12**. The

atropisomers of HHDP and Phe-Cp groups were elucidated to be *S*-configurations by the NOESY correlations of Glc-H-6 (δ_H 4.98) to HHDP-H-3 (δ_H 6.54) and Glc-H-6 (δ_H 3.94) to Phe-H-3 (δ_H 6.32). It observed equally both **2** and **12**. From these results, the structure of **2** was proposed as being represented formula **2**. It presumed **2** was formed by oxidation of a progalloyl ring attached to Glc-C-1 of stachyurin followed by benzylic acid-type rearrangement and methylation.[10]

Since it had been reported hydrolyzable tannins had potent plant-growth inhibitory activity,[15] the effect of isolated tannins **2-12** and tannin derivatives **13** and **14**[8, 16] were examined on root growth of cress seedlings. All compounds except **7** and **13** exhibited inhibitory effect on root at 1×10^{-4} mol/L (Figure 3). Especially, **6** and **8** which possessed a galloyl group at Glc-1 showed the strongest activity (80% inhibition) at 1×10^{-4} mol/L. It suggested that a galloyl group was important to enhance the activity and especially connecting to Glc-1 was more effective. Moreover, the comparing between **5** (45 % inhibition) with galloyl groups at Glc-4, 6 and **8** (80% inhibition) with HHDP group at Glc-4, 6 showed the HHDP group at Glc-4, 6 was important for the activity.

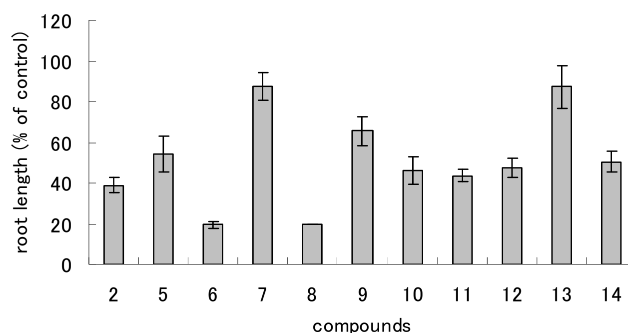


Figure 3 Growth inhibition effects (1×10^{-4} mol/L) of tannins **2** and **5~14** on root of cress.

EXPERIMENTAL

General procedure

Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded on a JASCO FT/IR-300 spectrometer and UV spectra on a HITACHI U-2000A spectrometer. ^{1}H and ^{13}C NMR spectra were measured and recorded in Acetone-d_6 or MeOD on Bruker Avance 500 or 600 spectrometers. Chemical shift values(δ) are recorded in parts per million (ppm) relative to NMR solvent Acetone-d_6 (δ_H 2.05, δ_C 29.8) or MeOD (δ_H 3.35, δ_C 49.8). ESIMS were recorded on Waters-Platform LC and JEOL JMS-T100LC mass spectrometers.

Plant material

Galls of *C. tschonoskii* induced by infection of *Eriophyes* sp. were collected at University of Tsukuba, Japan. A voucher specimen has been deposited at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan.

Extraction and isolation

Galls of *Carpinus tschonoskii* (100 g) were homogenized by blender, extracted with MeOH (250 mL) and

concentrated *in vacuo*. The MeOH extracts (8.3 g) were partitioned between EtOAc (250 mL×3) and H_2O (250 mL) and the H_2O-layer was further partitioned with BuOH (250 mL×3). The EtOAc-soluble portion (1.85 g) was chromatographed on a C_{18} Sep-Pak cartridge (Waters, H_2O/MeOH, 10:0→0:10) to give 4 fractions (fr. 1~fr. 4). Fr. 2 (274 mg) was subjected to a C_{18} Sep-Pak cartridge (Waters, H_2O/MeOH, 10:0→0:10) to give 8 fractions. The fraction (78.4 mg) eluted with H_2O/MeOH (8:2) on the C_{18} Sep-Pak cartridge was further separated by reversed-phase HPLC (TSK-gel ODS-80Ts, ϕ 4.8×200 mm, flow rate 1.0 mL/min, A: 3% AcOH in H_2O, B: 80% MeCN, A/B, 90:10→85:15) to give carpinerin B (**2**, 26.1 mg, t_R 21 min) and rhoipteleanin H (**12**, 18.3 mg, t_R 22 min). The fraction (78.7 mg) eluted with H_2O/ MeOH (7:3) on the C_{18} Sep-Pak cartridge was further separated by reversed-phase HPLC (Cosmosil $_5C_{18}$ MS-II, ϕ 10×250 mm , flow rate 2.5 mL/ min, A: 0.1% AcOH in H_2O, B: 80% MeCN, A/B, 84:16 →77:23 → 72:28→ 65:35) to give casuarictin (**6**, 22.8 mg). The fraction (50.2 mg) eluted with H_2O/MeOH (6:4) on the C_{18} Sep-Pak cartridge was further separated by reversed-phase HPLC (Cosmosil $_5C_{18}$ MS-II, ϕ10×250 mm, flow rate 2.5 mL/ min, A: 0.1% AcOH in H_2O, B: 80% CH_3CN, A/B, 84:16→77:23→72:28→65:35→55:45) to give trigalloylglucose (**3**, 1.2 mg, t_R 14 min), tellimagrandin II (**8**, 9.4 mg, t_R 19 min), carpinerin A (**1**, 3.4 mg, t_R 20 min), and tetragalloylglucose (**4**, 2.1 mg, t_R 21 min). The fraction (16.9 mg) eluted with H_2O/MeOH (55:45) on the C_{18} Sep-Pak cartridge was pentagalloylglucose (**5**, 16.9 mg). The BuOH-soluble portion (2.21 g) was chromatographed on ODS (Cosmosil-75 C-18OPN, ϕ 2.2×30 cm, H_2O/MeOH, 10:0→0:10) to give 17 fractions (fr. 1~fr. 17). Fr. 6 (317 mg) was subjected to a C_{18} Sep-Pak cartridge (H_2O/MeOH, 10:0→0:10) to give 12 fractions. The fraction (27.2 mg) eluted with H_2O/MeOH (7:3) on the C_{18} Sep-Pak cartridge was further separated by reversed-phase HPLC (Cosmosil $_5C_{18}$ MS-II, ϕ10×250 mm, flow rate 2.5 mL/min, A: 0.1% AcOH in H_2O, B: 80% MeCN, A/B, 85:15→80:20→72:28→65:35) to give pedunculagin (**7**, 6.2 mg, t_R 9 min) and hippophaenin A (**11**, 3 mg, t_R 14 min). Fr. 7 (216 mg) was subjected to the C_{18} Sep-Pak cartridge (H_2O/MeOH, 10:0→0:10) to give 9 fractions. The fraction eluted with H_2O/MeOH (7:3) on the C_{18} Sep-Pak cartridge was further separated by reversed-phase HPLC (Cosmosil $_5C_{18}$ MS-II, ϕ 10×250 mm, flow rate 2.5 mL/ min, A: 0.1% AcOH in H_2O, B: 80% MeCN, A/B, 85:15→75:25→70:30→65:35) to give liquidambin (**10**, 35.6 mg, t_R 11 min) and casuarinin (**9**, 9.8 mg, t_R 12 min).

Carpinerin A (1): A colorless powder; $[\alpha]_D^{23}$ -6.0° (c 0.4, MeOH); IR (KBr) ν_{max} 3427, 1773, 1718, 1617, 1509, 1509, 1449, 1341, 1222, 1042 cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (4.8), 277 (4.5) nm; ESIMS 1133 $(M+Na)^+$; HRESIMS *m/z* 1133.0898 $(M+Na)^+$, Δ -3.3 mmu; ^{1}H NMR (500 MHz, MeOD): δ 7.14 (2H, s, galloyl), 6.96 (1H, s, EC-3'), 6.93 (1H, d, J = 8.8 Hz, Glc-1) , 6.66 (1H, s, HHDP-3), 6.53 (1H, s, HHDP'-3), 5.82 (1H, dd, J = 9.8, 10.7 Hz, Glc-3) , 5.81 (1H, d, J = 2.2 Hz, EC-1), 5.46 (1H, dd, J = 7.2,

13.2 Hz, Glc-6) , 5.21 (1H, t, *J* = 9.8, Glc-4) , 4.50 (1H, d, *J* = 4.8 Hz, EC-4”), 4.38 (1H, ddd, *J* = 2.0, 7.2, 9.8 Hz, Glc-5), 4.34 (1H, dt, *J* = 5.2, 7.1 Hz, EC-5”), 4.29 (1H, dd, *J* = 8.8, 10.7 Hz, Glc-2) , 4.19 (1H, dd, *J* = 8.7, 7.1 Hz, EC-6”), 3.91 (1H, dd, *J* = 13.2, 2.0 Hz, Glc-6) , 3.80 (1H, dd, *J* = 8.7, 7.1 Hz, EC-6”), 3.00 (1H, dd, *J* = 19.5, 2.2 Hz, EC-3), 2.21 (1H, d, *J* = 19.5 Hz, EC-3); ^{13}C NMR (500 MHz, MeOD): δ 121.0 (galloyl-1), 111.3 (galloyl-2, 6), 147.5 (galloyl-3, 5), 141.6 (galloyl-4), 166.6 (galloyl-7), 117.4, 117.3 (HHDP, HHDP’-1), 118.8 (EC-1’), 126.9 (HHDP, HHDP’-2), 120.6 (EC-2’), 109.7, 109.4 (HHDP, HHDP’-3), 113.4 (EC-3’), 146.6, 146.7, 145.7, 145.4 (HHDP, HHDP’-4, 6), 149.1, 149.2 (EC-4’, 6’), 138.4, 138.2 (HHDP, HHDP’-5), 137.5 (EC-5’), 170.3, 168.6 (HHDP, HHDP’-7), 168.0 (EC-7’), 93.8 (Glc-1), 80.2 (Glc-2), 76.0 (Glc-3), 70.1 (Glc-4), 76.1 (Glc-5), 64.4 (Glc-6), 53.0 (EC-1), 49.8 (EC-2), 38.9 (EC-3), 200.5 (EC-4), 98.1 (EC-5), 109.3 (EC-6), 172.0 (EC-7), 174.2 (EC-1”), 82.8 (EC-2”), 109.7 (EC-3”), 92.8 (EC-4”), 74.7 (EC-5”), 75.3 (EC-6”).

Carpinerin B (2): A pale yellow powder; $[\alpha]_D^{23}$ -50.0° (c 0.4, MeOH); IR (KBr) ν_{max} 3422, 1718, 1700, 1617, 1508, 1459, 1194, 1066 cm^{-1}; UV (MeOH) λ_{max} (log ε) 216 (4.7), 266 (4.3) nm; ESIMS *m/z* 991; HRESIMS *m/z* 991.0872 (M+Na)$^+$, Δ +4.5 mmu; ^{1}H NMR (500 MHz, acetone-d_6 + D_2O): δ 7.11 (2H, s, galloyl), 6.89 (1H, s, HHDP-3), 6.54 (1H, s, HHDP’-3), 6.32 (1H, s, Phe-3), 5.73 (1H, dd, *J* = 2.1, 10.0 Hz, Glc-4), 5.45 (1H, d, *J* = 2.1 Hz, Glc-3), 5.40 (1H, dd, *J* = 4.3, 10.0 Hz, Glc-5) , 5.33 (1H, s, Cp-1) , 5.18 (1H, d, *J* = 1.0 Hz, Glc-2), 4.98 (1H, dd, *J* = 4.3, 13.4 Hz, Glc-6), 4.53 (1H, d, *J* = 1.0 Hz, Glc-1), 3.94 (1H, d, *J* = 13.4 Hz, Glc-6), 3.69 (3H, s, OMe); ^{13}C NMR (500 MHz, acetone-d_6 + D_2O): δ 119.9 (galloyl-1), 109.9 (galloyl-2, 6), 145.7 (galloyl-3, 5), 139.3 (galloyl-4), 166.3 (galloyl-7), 115.2, 115.7 (HHDP, HHDP’-1), 110.2 (Phe-1), 126.0, 124.0 (HHDP, HHDP’-2), 123.8 (Phe-2), 108.1, 106.9 (HHDP, HHDP’-3), 106.1 (Phe-3), 144.8, 144.3, 144.5 (HHDP, HHDP’-4, 6), 145.4 (Phe-4), 136.4, 135.7 (HHDP, HHDP’-5), 134.9 (Phe-5), 146.0 (Phe-6), 168.4 (HHDP, HHDP’-7), 168.9 (Phe-7), 60.7 (Glc-1), 84.0 (Glc-2), 69.7 (Glc-3), 72.2 (Glc-4), 69.0 (Glc-5), 64.4 (Glc-6), 45.5 (Cp-1), 83.3 (Cp-2), 201.3 (Cp-3), 143.2 (Cp-4), 157.1 (Cp-5), 170.8 (Cp-6), 162.8 (Cp-7), 53.5 (OMe).

Rhoipteleanin H (12) : A pale yellow powder; ^{1}H NMR (500 MHz, acetone-d_6 + D_2O): δ 7.11 (2H, s, galloyl), 6.83 (1H, s, HHDP-3), 6.52 (1H, s, HHDP’-3), 6.35 (1H, s, Phe-3), 5.52 (1H, dd, *J* = 2.5, 10.4 Hz, Glc-4) , 6.05 (1H, d, *J* = 2.0 Hz, Glc-3), 5.30 (1H, dd, *J*=3.8, 10.4 Hz, Glc-5) , 5.26 (1H, d, *J* = 3.3 Hz, Cp-1) , 4.91 (1H, d, *J* = 6.3 Hz, Glc-2), 4.98 (1H, dd, *J*=3.8, 13.4 Hz, Glc-6), 5.36 (1H, dd, *J* = 3.0, 6.3 Hz, Glc-1), 3.90 (1H, d, *J* = 13.4 Hz, Glc-6), 3.68 (3H, s, OMe); ^{13}C NMR (500 MHz, acetone-d_6 + D_2O): δ 119.9 (galloyl-1), 109.9 (galloyl-2, 6), 145.7 (galloyl-3, 5), 139.3 (galloyl-4), 166.3 (galloyl-7), 115.2, 115.7 (HHDP, HHDP’-1), 110.2 (Phe-1), 126.0, 124.0 (HHDP, HHDP’-2), 124.2 (Phe-2), 108.1, 106.7 (HHDP, HHDP’-3), 106.7 (Phe-3), 144.8, 144.3, 144.5 (HHDP, HHDP’-4, 6), 145.4 (Phe-4), 136.4,

135.7 (HHDP, HHDP'-5), 134.9 (Phe-5), 146.0 (Phe-6), 168.7 (HHDP, HHDP'-7), 168.4 (Phe-7), 62.5 (Glc-1), 79.0 (Glc-2), 66.6 (Glc-3), 73.1 (Glc-4), 69.3 (Glc-5), 64.3 (Glc-6), 44.6(Cp-1), 82.4 (Cp-2), 200.0 (Cp-3), 142.4 (Cp-4), 155.1 (Cp-5), 171.2 (Cp-6), 162.8 (Cp-7), 53.4 (OMe).

Derivatization of 2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucitol (13) from pedunculagin (7)

To a solution of **7** (5.0 mg) in MeOH (1 mL) $NaBH_4$ (5.0 mg, 20 eq.) was added and the mixture was stired at rt for 2 h. AcOH (1 mL) was added to the reaction mixture and evaporated. The residue was dissolved in water and the pH was adjusted to 2.0 with 10 % HCl, and directly subjected to a C_{18} Sep-Pak cartridge (H_2O/ MeOH, 10:0→0:10) to give **13** (4.0 mg).

Derivatization of 5-*O*-galloyl-2,3;4,6-di-*O*-(*S*)-hexahydroxydiphenoyl-D-glucitol (14) from liquidambin (10)

To a solution of **10** (5.0 mg) in MeOH (1 mL) $NaBH_4$ (5.0 mg, 25 eq.) was added and the mixture was stired at rt for 2 h. AcOH (1 mL) was added to the reaction mixture and evaporated. The residue was dissolved in water and the pH was adjusted to 2.0 with 10 % HCl, and directly subjected to a C_{18} Sep-Pak cartridge (H_2O/MeOH, 85:15→0:10) to give **14** (4.1 mg).

Partial hydrolysis of Carpinerin A (1)

A solution of **1** (0.2 mg) in H_2O was kept at 90 °C for 24 h. The reaction mixture was analyzed by normal-phase HPLC [TSK-gel Amide-80, TOSOH, ϕ 4.8×200 mm, flow rate 0.8 mL/min, 50 mmol/L (triethanolamine) phosphate buffer (pH 2.5) / MeCN, 30:70], and identified it as erythorbic acid (t_R 5.8 min).

Derivatization of Casuarictin (6) from Carpinerin A (1)

A solution of **1** in 0.1% AcOH was kept at 80 °C for 30 min. The reaction mixture was analyzed by reverse-phase HPLC (Cosmosil $_5C_{18}$ MS-II, 10×250 mm, flow rate 2.5 mL/min, A: 0.1% AcOH in H_2O, B: 80% MeCN, A/B, 84:16→77:23→72:28→65:35), and identified it as **6** (t_R 22 min).

Bioassays

Each sample of tannins dissolved in MeOH (50 μL) was put in a 27 mm petri dish on filter paper. After vaporized MeOH, added distilled water (500 μL) and placed ten seeds of cress (*Lepidium sativum* L.). They were kept at 24 °C for 40 h in the dark and measured the length of root of cress. Percentage elongation was calculated by reference to the elongation of control.

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