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Macrocyclic ellagitannin dimers, cuphiins D_1 and D_2, and accompanying tannins from *Cuphea hyssopifolia*

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Abstract

Two new ellagitannin dimers, cuphiins D_1 and D_2, and six known compounds including oenothein B and woodfordin C have been isolated from the aerial part of *Cuphea hyssopifolia* (Lythraceae). Macrocyclic structures of the new tannins were elucidated based on chemical and spectral evidence. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Cuphea hyssopifolia*; Lythraceae; Ellagitannin; Cuphiin D_1; Cuphiin D_2; Macrocyclic structure

1. Introduction

Cuphea hyssopifolia Humb. Bompl. et Kunth (Lythraceae), a small shrub native to Mexico and Guatemala, is cultivated in Taiwan as a horticultural plant. Some species of the genus *Cuphea* have been used for treating stomach disorders, syphilis, gonorrhea, cancer, as well as being used as an oral contraceptive in folk medicines from South and Central Americas (Monton, 1981; Siri & Frank, 1982; De A Ribeiro, Fiuza de Melo, De Barrors, Gomes, & Trolin, 1986; Duke, 1986; Elisabetsky & Posey, 1989; Gonzalez, Valencia, Siverio Exposito, Bermejo Barrera, & Gupta, 1994). Flavonoid glycosides, triterpenoids and long-chain fatty acids have been reported as constituents of this genus (Gonzalez et al., 1994; Santos Deborah, Salatino Maria, & Antonio, 1995; Martins & Roque, 1995). However, their pharmacologically active principles have not been described. In the present investigation on the polyphenols of *C. hyssopifolia*, we have isolated seven hydrolyzable tannins including two new dimeric ellagitannins with macrocyclic structures.

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2. Results and discussion

The aerial part of *C. hyssopifolia* was homogenized in 70% aq. Me_2CO. The concd aq. soln was partitioned with CH_2Cl_2. The water layer was then subjected to repeated column chromatography over Toyopearl HW-40 and MCI-gel CHP-20P to yield myricitrin and seven hydrolyzable tannins. Among them, five were identified as 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**1**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**2**), tellimagrandin II (**3**), woodfordin C (**4**) (Kadota et al., 1990; Yoshida et al., 1990) and oenothein B (**5**) (Hatano et al., 1990). The remaining two were found to be new dimers and named cuphiins D_1 (**6**) and D_2 (**7**).

Cuphiin D_1 (**6**) was obtained as a white amorphous powder and showed a retention time close to that of woodfordin C (**4**) in normal-phase HPLC, which suggested a dimeric character (Okuda, Yoshida, & Hatano, 1989). The molecular formula $C_{82}H_{56}O_{52}$ was indicated by its negative-ion FAB mass spectrum (*m*/*z* 1871 $[M-H]^-$) and elemental analysis. The ^1H NMR spectrum of **6** recorded at ambient temperature was of limited usefulness due to broadening of signals for some of the aromatic and sugar protons, presumably arising from restricted conformational changes in the molecule such as that observed for **5** (Hatano et al.,

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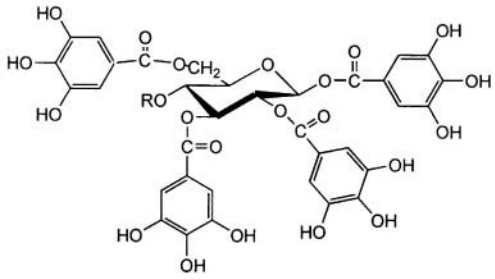
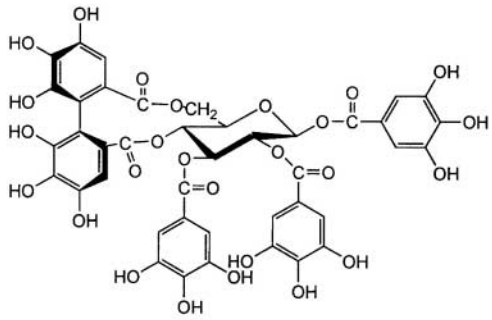


1: R=H
2: R=galloyl



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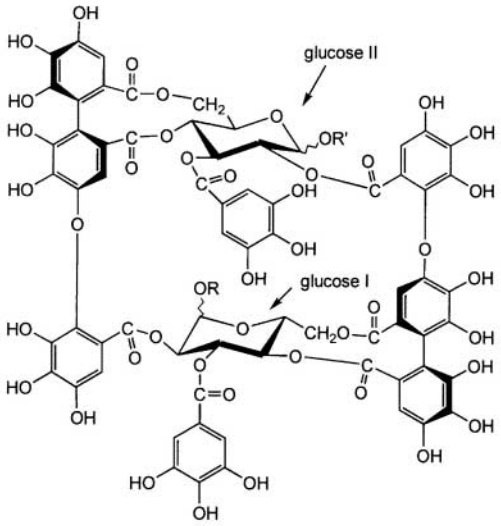
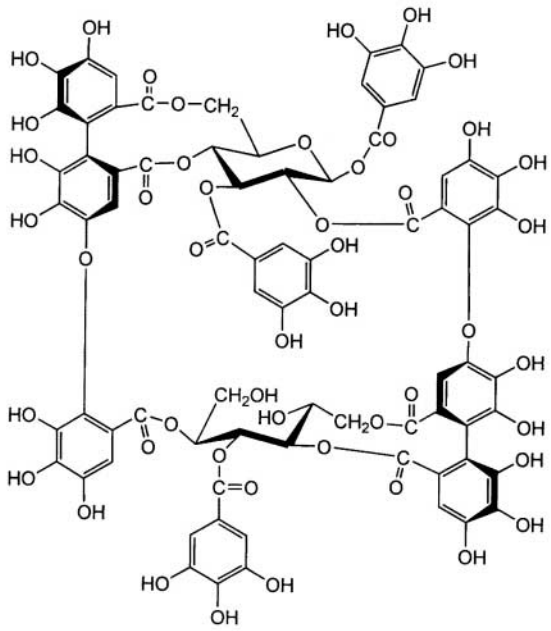


4: R=α-galloyl, R'=H
5: R=H, R'=H
6: R=α-galloyl, R'=β-galloyl
7: R=H, R'=β-galloyl



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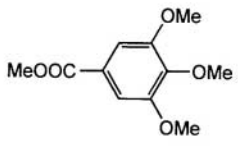

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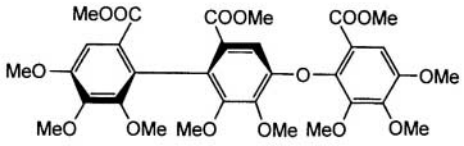

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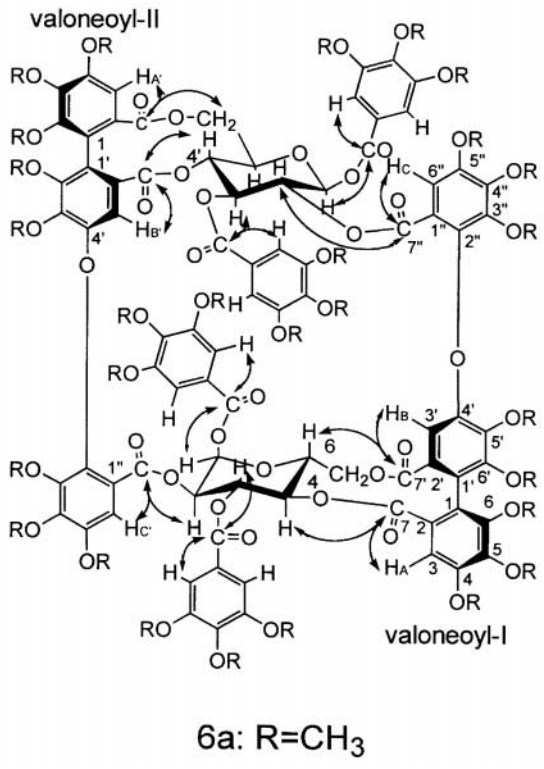
1990). However the spectrum recorded at an elevated temperature (38°C) indicated four 2H-singlets at δ 7.31, 7.23, 7.20 and 7.06 due to four galloyl groups and six aromatic 1H-singlets attributable to two valoneoyl groups. The sugar proton signals which were assigned with the aid of the ^{1}H–^{1}H shift correlation spectrum were characteristic of fully acylated $^{4}C_{1}$ glucopyranose. The anomeric proton signals at δ 7.24 (d, $J = 3.0$ Hz) and 5.44 (d, $J = 7.2$ Hz), suggest that anomeric centres of two glucose cores have an α- and β-oriented acyloxy group, respectively. The ^{13}C resonances of the glucose moieties of **6**, whose assignments



were confirmed by the ^{1}H–^{13}C shift correlation spectrum, also showed the presence of α- (δ 90.8, C-1) and β- (δ 92.8, C-1′) glucosidic linkages (Yoshida et al., 1984).

Methylation of **6** with dimethyl sulfate and potassium carbonate gave an octacosamethyl derivative (**6a**). Although some ^{1}H signals of **6** showed considerable broadening even in the spectrum measured at 38°C, the spectrum of **6a** measured at ambient temperature exhibited sharp signals attributable to four galloyl groups and two valoneoyl groups. The presence of these acyl groups in **6a** was further supported by ten ester carbonyl carbon resonances (δ 164.4–168.3) in the ^{13}C NMR spectrum, and production of methyl tri-*O*-methylgallate (**9**) and trimethyl octa-*O*-methylvaloneate (**10**) in the molar ratio of 2:1 upon methylation of **6** followed by methanolysis. Both of the galloyl moieties of the valoneoyl groups in **6a** were determined to be at O-2 of each glucose core, based on the long-range ^{1}H–^{13}C COSY (δ_H 7.30 (valoneoyl-I H_C)–δ_C 167.2 (valoneoyl-I C-7″)–δ_H 5.97 (glucose H-2) and δ_H 7.08 (valoneoyl-II H_C)–δ_C 166.6 (valoneoyl-II C-7″)–δ_H 5.92 (glucose H-2′)). The other four sets of the long-range correlations among valoneoyl proton–ester carbonyl carbon–glucose proton (δ_H 6.68 (valoneoyl-I H_A)–δ_C 168.3 (valoneoyl-I C-7)–δ_H 6.12 (glucose H-4); δ_H 6.42 (valoneoyl-I H_B)–δ_C 167.4 (valoneoyl-I C-7′)–δ_H 5.26, 3.76 (glucose H-6); δ_H 6.35 (valoneoyl-II $H_{B'}$)–δ_C 167.1 (valoneoyl-II C-7′)–δ_H 5.11 (glucose H-4′); δ_H 6.85 (valoneoyl-II $H_{A'}$)–δ_C 167.9 (valoneoyl-II C-7)–δ_H 4.83, 3.94 (glucose H-6′)) clearly indicate that two carboxy groups of the HHDP moiety in each valoneoyl group are on O-4/O-6 of each glucose core (Yoshida, Hatano, Kuwajima, & Okuda, 1992), and that the orientation of the valoneoyl group on O-4/O-6 of glucose I (the lower glucose core in the formula) is the same as that in the rugosins (rugosin type), while that of glucose II (the upper glucose core) is of the isorugosin type (Yoshida et al., 1992). Four galloyl groups were also allocated at O-1, O-1′, O-3 and O-3′ by long-range correlations as depicted by arrows in the formula **6a**. These spectral features indicated a macrocyclic structure (**6**) for cuphiin D_1 (Yoshida et al., 1990).

The atropisomerism at each biphenyl moiety of these two valoneoyl groups was determined as *S* by a positive Cotton effect at 221 nm ($[\theta] +4.8 \times 10^5$) in the CD spectrum of **6** (Okuda et al., 1982), which was similar to that of woodfordin C (**4**) ($[\theta]_{218} +4.1 \times 10^5$).

Cuphiin D_1 (**6**) was thus characterized as a gallate of woodfordin C (**4**).

Cuphiin D_2 (**7**), an off-white amorphous powder, exhibited an $[M + NH_4]^+$ ion peak at *m*/*z* 1738 in the ESI-MS, corresponding to the molecular formula $C_{75}H_{52}O_{48}$. The ^{1}H NMR spectrum of **7** recorded at ambient temperature showed broad signals like **4**–**6**. The spectrum of **7** recorded at 40°C showed close resemblance to that of **6** except for lack of one galloyl group. The presence of three galloyl groups (δ 7.25, 7.20 and 7.06) and two valoneoyl groups (δ 7.22, 6.71, 6.62, 6.57, 6.51 and 6.30) was supported by methanolysis of a methylated derivative of **7** which afforded **9** and **10** in the molar ratio of 3:2.

Another distinguishable feature of **7** from **6** was an upfield shift of one of the anomeric proton signals (δ 7.24 (d, $J = 3$ Hz) in **6** to δ 6.18 (d, $J = 3$ Hz) in **7**). This shift suggests that the hydroxyl group at an anomeric center of a glucose core (glucose I) is free, and the other anomeric center has an β-oriented acyloxy group. However, the absence of duplication of any proton signal and also the observation of a single peak in the reversed-phase HPLC indicate that **7** exists only an anomeric form (α-anomer) for glucose I.

The absence of an acyl group at the anomeric center of glucose I was further confirmed by reduction of **7** with sodium borohydride, which yielded dihydrocuphiin D_2 (**8**), ESI-MS *m*/*z* 1740 $[M + NH_4]^+$. The ^{1}H–^{1}H COSY of **8** revealed the presence of one glucitol core in the molecule. The structure of cuphiin D_2 was thus represented by the formula **7** which is a regioisomer of woodfordin C (**4**) concerning a galloyl group. The structural relationship of cuphiin D_1 (**6**) and D_2 (**7**) was substantiated by enzymatic degalloylation of **6** with tannase yielding **4**, **5**, **7** and gallic acid. Cuphiin D_2 (**7**) also produced oenothein B (**5**) and gallic acid upon enzymatic hydrolysis with tannase.

3. Experimental

3.1. General

^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra were measured on a Varian VXR 500 or Bruker DRX 500 instrument and chemical shifts are given in δ (ppm) values. FAB-MS were taken on a VG 70-SE mass spectrometer using 3-nitrobenzyl alcohol containing NaCl as the matrix agent, and ESI-MS on a Micromass Autospec OA-TOF mass spectrometer with direct injection of 50% aq. MeOH soln containing small amount of CH_3COONH_4. Normal-phase HPLC was conducted on YMC-pack SIL-A003 (4.6 mm × 250 mm) using the following solvent systems: (NP1) *n*-hexane–MeOH–THF–HCOOH (60:45:15:1) contained oxalic acid (500 mg/1.2 l), (NP2) *n*-hexane–EtOAc (2:1) (flow rate, 1.5 ml/min; detection 280 nm) at room temp. Reversed-phase HPLC was performed on a YMC-pack J'sphere ODS H-80 (4.6 mm × 250 mm) column, using the following solvent systems: (RP1) 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (42.5:42.5:10:5), (RP2) 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (44:44:7:5) (flow rate, 1.0 ml/min; detection 280 nm) at 40°C. Column chromatography was carried out on Toyopearl HW-40 (fine grade) (Tosoh), Dia-ion HP-20 and MCI-gel CHP-20P (Mitsubishi Chemical Industry). TLC was conducted on Kieselgel PF_{254} (Merck) plates with *n*-hexane–$CHCl_3$–Me_2CO (5:4:1), and visualized under UV irradiation.

3.2. Plant materials

The aerial part of *C. hyssopifolia* was collected in Taipei, Taiwan, in April 1996. A voucher specimen is deposited in the Graduate Institute of Pharmacognosy Science, Taipei Medical College.

3.3. Isolation of tannins

The dried aerial part of *C. hyssopifolia* (1.7 kg) was homogenized in 70% aq. Me_2CO (20 l × 3) and filtered. The concd filtrate was extracted with CH_2Cl_2 to remove nonpolar constituents, and the aq. layer was evapd. A part (118 g) of the aq. extract was chromatographed over Dia-ion HP-20 (9.5 cm i.d. × 45 cm) with H_2O, and H_2O–MeOH (10% MeOH → 20% → 40% → 60% → 80%). A part (6 g) of the 40% MeOH eluate was chromatographed over Toyopearl HW-40(F) (2.5 cm i.d. × 50 cm) developing with H_2O → 50% MeOH → 60% MeOH → 70% MeOH → MeOH–H_2O–Me_2CO (7:2:1) → MeOH–H_2O–Me_2CO (8:1:1). The 70% MeOH eluate was rechromatographed over MCI-gel CHP-20P with aq. MeOH to yield woodfordin C (**4**) (243 mg). The MeOH–H_2O–Me_2CO (7:2:1) eluate was rechromatographed over MCI-gel CHP-20P with aq. MeOH to yield cuphiin D_1 (**6**) (189 mg).

The 20% MeOH eluate (7 g) from column chromatography of Dia-ion HP-20 was also rechromatographed over Toyopearl HW-40 (F) (2.5 cm i.d. × 45 cm) developing with H_2O → 60% MeOH → 70% MeOH → MeOH–H_2O–Me_2CO (7:2:1). The 70% MeOH eluate gave an additional crop of woodfordin C (**4**) (120 mg). The 60% MeOH eluate was rechromatographed with MCI-gel CHP-20P to give oenothein B (**5**) (118 mg) and cuphiin D_2 (**7**) (100 mg).

The 60% MeOH eluate (8.5 g) of Dia-ion HP-20 column chromatography was rechromatographed over Toyopearl HW-40(F) (2.5 cm i.d. × 52 cm) developing with H_2O → 40% MeOH → 60% MeOH → 70% MeOH → MeOH–H_2O–Me_2CO (7:2:1). The 60% MeOH eluate gave myricitrin (494 mg), and the MeOH–H_2O–Me_2CO (7:2:1) eluate gave 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**2**) (101 mg), tellimagrandin II (**3**) (53 mg) and 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**1**) (15 mg).

3.4. Cuphiin D_1 (6)

A white amorphous powder. $[\alpha]_D$ +275° (MeOH; *c* 1.0). Found: C, 46.6; H, 3.7. Calc. for $C_{82}H_{56}O_{52}\cdot 13H_2O$: C, 46.7; H, 3.9%. FAB-MS *m/z*: 1871 $[M-H]^-$. ESI-MS: *m/z* 1890 $[M + NH_4]^+$. UV λ_{max} (MeOH) (nm): 219, 275. CD (MeOH) [θ] (nm): $+4.8\times10^5$ (221), $+2.2\times10^5$ (239), -4.4×10^4 (263), $+1.2\times10^5$ (284). ^{1}H NMR (500 MHz, 38°C, Me_2CO-d_6) δ: 7.31, 7.23, 7.20, 7.06 (each 2H, s, galloyl(Gal)), 7.12, 6.75, 6.61, 6.54, 6.49, 6.32 (each 1H, s, valoneoyl (Val)), 7.24 (d, J = 3 Hz, Glc H-1), 6.09 (dd, J = 3, 10 Hz, Glc H-2), 6.21 (t, J = 10 Hz, Glc H-3), 6.01 (brt, J = 10 Hz, Glc H-4), 4.67 (ddd, J = 10, 7, 1 Hz, Glc H-5), 5.34 (dd, J = 7, 13.3 Hz, Glc H-6), 3.72 (dd, J = 13.3, 1 Hz, Glc H-6), 5.44 (d, J = 7.2 Hz, Glc H-1′), 5.65 (m, Glc H-2′), 5.68 (m, Glc H-3′), 5.00 (m, Glc H-4′ and H-6′), 4.30 (dd, J = 5, 10 Hz, Glc H-5′), 3.86 (brd, J = 13.3 Hz, Glc H-6′). ^{13}C NMR (126 MHz, 38°C, Me_2CO-d_6) δ: 90.8 (Glc C-1), 72.6 (Glc C-2), 70.9 (Glc C-3), 69.2 (Glc C-4), 71.6 (Glc C-5), 62.3 (Glc C-6), 92.8 (Glc C-1′), 72.4 (Glc C-2′ and Glc C-3′), 73.3 (Glc C-4′), 72.6 (Glc C-5′), 64.5 (Glc C-6′), 106.0, 107.1, 107.9, 108.2, 109.0, 114.3 (Val C-2, C-2″ and C-2″), 109.9, 110.1, 110.2, 110.6 (Gal C-2 and C-6), 164.6, 165.2, 165.6, 166.3, 167.2, 167.3, 167.9, 168.9 (ester carbonyl).

3.5. Methylation of cuphiin D_1 (6)

A mixture of **6** (21 mg), Me_2SO_4 (0.1 ml) and K_2CO_3 (200 mg) in dry Me_2CO (5 ml) was stirred overnight at room temp, and then refluxed for 2.7 h. After removal of inorganic materials by centrifugation,

the supernatant was concd and purified by prep. TLC (Kieselgel PF^{254}) with toluene–Me_2CO (3:1) to yield the octacosamethylate (**6a**) (12 mg).

3.6. Compound *6a*

A white amorphous powder. ESI-MS: *m/z* 2282 $[M + NH_4]^+$. ^1H NMR (500 MHz, 27°C, Me_2CO-d_6) δ: 7.50, 7.42, 7.36, 7.32 (each 2H, s, Gal), 7.30 (1H, s, Val-I H_C), 7.08 (1H, s, Val-II $H_{C'}$), 6.85 (1H, s, Val-II $H_{A'}$), 6.68 (1H, s, Val-I H_A), 6.42 (1H, s, Val-I H_B), 6.35 (1H, s, Val-II $H_{B'}$), 7.14 (d, J = 3.7 Hz, Glc H-1), 5.97 (dd, J = 3.7, 10 Hz, Glc H-2), 6.18 (t, J = 10 Hz, Glc H-3), 6.12 (t, J = 10 Hz, Glc H-4), 4.78 (dd, J = 7, 9 Hz, Glc H-5), 5.26 (dd, J = 7, 13 Hz, Glc H-6), 3.76 (Glc H-6, overlapped with OMe), 5.53 (d, J = 9 Hz, Glc H-1′), 5.92 (t, J = 9 Hz, Glc H-2′), 5.60 (t, J = 10 Hz, Glc H-3′), 5.11 (t, J = 10 Hz, Glc H-4′), 4.30 (dd, J = 6, 10 Hz, Glc H-5′), 4.83 (dd, J = 6, 13 Hz, Glc H-6′), 3.94 (Glc H-6′, overlapped with OMe), 4.00–3.38 (28 × OMe). ^{13}C NMR (126 MHz, 38°C, Me_2CO-d_6) δ: 91.5 (Glc C-1), 73.4 (Glc C-2), 72.1 (Glc C-3), 69.5 (Glc C-4), 71.2 (Glc C-5), 63.5 (Glc C-6), 94.0 (Glc C-1′), 71.5 (Glc C-2′), 74.0 (Glc C-3′), 73.6 (Glc C-4′), 73.2 (Glc C-5′), 64.5 (Glc C-6′), 108.25, 108.28, 108.34, 109.4 (Gal, C-2 and C-6), 106.0 (Val-II C-3), 106.2 (Val-I C-3), 106.8 (Val-II C-3′), 109.0 (Val-I C-3′), 109.1 (Val-I C-6″), 110.0 (Val-II C-6″), 164.4 (Gal C-7), 165.3 (Gal C-7), 166.0 (Gal C-7), 166.6 (Val-II C-7″), 166.9 (Gal C-7), 167.1 (Val-II C-7′), 167.2 (Val-I C-7″), 167.4 (Val-I C-7′), 167.9 (Val-II C-7), 168.3 (Val-I C-7).

3.7. Hydrolysis of *6* with tannase

A soln of **6** (40 mg) in H_2O (20 ml) was incubated with tannase at 37°C for 46 h. The reaction was terminated by acidification with 1% HCl, and the reaction mixture was applied to CC over MCI-gel CHP-20P (1.2 cm i.d. × 14.5 cm). Elution was conducted with H_2O and then with aq. MeOH (5% MeOH → 10% → 20% → 30% → 40% → 50%). The 5% MeOH eluate afforded gallic acid (1.4 mg), 20% MeOH eluate gave oenothein B (**5**) (5.6 mg) and 30% MeOH eluate gave **7** (15 mg). The 40% MeOH eluate was further purified with prep. HPLC with 0.1 M H_3PO_4–0.1 M KH_2PO_4–CH_3CN (44:44:12) to give woodfordin C (**4**) (2.1 mg) and the starting material **6** (1.0 mg).

3.8. Cuphiin D_2 (*7*)

An off-white amorphous powder. $[\alpha]_D$ +107° (MeOH; *c* 1.0). ESI-MS: *m/z* 1738 $[M + NH_4]^+$. UV λ_{max} (MeOH) (nm): 218, 271. CD (MeOH) [θ] (nm): $+4.0 \times 10^5$ (222), $+2.1 \times 10^5$ (238), -4.5×10^4 (262), $+9.3 \times 10^4$ (284). ^1H NMR (500 MHz, 40°C, Me_2CO-d_6) δ: 7.25, 7.20, 7.06 (each 2H, s, Gal), 7.22, 6.71, 6.62, 6.57, 6.51, 6.30 (each 1H, s, Val), 6.18 (d, J = 3 Hz, Glc H-1), 6.13 (brt, J = 10 Hz, Glc H-2), 5.76 (m, Glc H-3), 5.79 (m, Glc H-4), 4.58 (dd, J = 7, 9.5 Hz, Glc H-5), 5.27 (dd, J = 7, 12.5 Hz, Glc H-6), 3.65 (d, J = 12.5 Hz, Glc H-6), 5.47 (brd, J = 9 Hz, Glc H-1′), 5.66 (t, J = 9 Hz, Glc H-2′), 5.65 (t, J = 9 Hz, Glc H-3′), 4.98 (m, Glc H-4′ and H-6′), 4.27 (dd, J = 5, 10 Hz, Glc H-5′), 3.82 (d, J = 14 Hz, Glc H-6′). ^{13}C NMR (126 MHz, 40°C, Me_2CO-d_6) δ: 91.3 (Glc C-1), 71.4 (Glc C-2), 74.7 (Glc C-3), 70.4 (Glc C-4), 68.7 (Glc C-5), 63.2 (Glc C-6), 93.3 (Glc C-1′), 72.0 (Glc C-2′), 72.7 (Glc C-3′), 73.6 (Glc C-4′), 73.0 (Glc C-5′), 64.9 (Glc C-6′), 106.2, 107.6, 108.2, 109.1, 109.4, 110.7 (Val C-3, C-3′, C-6″), 110.5, 111.0, 114.9 (Gal C-2, C-6), 165.61, 165.64, 166.8, 167.7, 167.9, 168.0, 168.3, 169.3 (ester carbonyl).

3.9. Reduction of *7* with $NaBH_4$

To a soln of **7** (10 mg) in MeOH (2.0 ml) was added $NaBH_4$ (40 mg). After 25 min, the reaction was terminated by addition of HOAc and the solvent was evapd. The residue was dissolved in H_2O and applied to a Sep-Pak C18 cartridge. Elution was conducted with H_2O and then with aq. MeOH (5% MeOH → 10% → 20% → 30% → 40% → 50%). The 30% MeOH eluate gave dihydrocuphiin D_2 (**8**) (8.3 mg).

3.10. Dihydrocuphiin D_2 (*8*)

A light brown amorphous powder. $[\alpha]_D$ +15° (MeOH; *c* 1.0). ESI-MS: *m/z* 1740 $[M + NH_4]^+$. UV λ_{max} (MeOH) (nm): 218, 274. CD (MeOH) [θ] (nm): $+2.0 \times 10^5$ (223), $+1.4 \times 10^5$ (238), -5.0×10^4 (264), $+4.3 \times 10^4$ (287), -1.4×10^4 (317). ^1H NMR (500 MHz, 40°C, Me_2CO-d_6 + D_2O) δ: 7.17, 7.15, 6.96 (each 2H, s, Gal), 6.91, 6.83, 6.71, 6.66, 6.60, 6.52 (each 1H, s, Val), 3.86 (brd, J = 12 Hz, glucitol H-1 and H-6), 4.02 (dd, J = 5, 11 Hz, glucitol H-1), 5.13 (brs, glucitol H-2), 5.88 (dd, J = 5, 7 Hz, glucitol H-3), 5.58 (t, J = 7 Hz, glucitol H-4), 4.17 (d, J = 7 Hz, glucitol H-5), 4.67 (d, J = 12 Hz, glucitol H-6), 5.92 (d, J = 7.5 Hz, Glc H-1′), 5.36 (dd, J = 7.5, 9 Hz, Glc H-2′), 5.77 (t, J = 9 Hz, Glc H-3′), 5.20 (t, J = 9 Hz, Glc H-4′), 4.29 (dd, J = 4.5, 9 Hz, Glc H-5′), 5.24 (m, Glc H-6′), 3.92 (d, J = 13.5 Hz, Glc H-6′).

3.11. Hydrolysis of *7* with tannase

A soln of **7** (0.5 mg) in H_2O (0.5 ml) was incubated with 3 drops of tannase at 37°C for 46 h. Production of oenothein B (**5**) and gallic acid was confirmed by comparison of retention times on reversed-phase

HPLC (RP1) and ^{1}H NMR of the reaction mixture with authentic samples.

3.12. Quantitative analysis of constituent units of **6** and **7**

A soln of tannin (1.0 mg) in EtOH (0.5 ml) was treated with etheral CH_2N_2 (1.5 ml) at room temp overnight. The residue obtained after removal of the solvent was methanolyzed with 1% NaOMe (0.2 ml) in MeOH (0.5 ml) at room temp overnight. The reaction mixture was acidified with HOAc and the solvent was evapd. The residue was partitioned between H_2O (1.0 ml) and EtOAc (1.0 ml). The EtOAc layer was evapd and re-dissolved in EtOAc (1.0 ml), and then analyzed by normal phase HPLC (NP2). The ratio of the amounts of the products was estimated from their peak area, referring to a known ratio of the methanolysate, methyl tri-*O*-methylgallate (**9**) and trimethyl octa-*O*-methylvaloneate (**10**), obtained by similar methylation of woodfordin C (**3**) followed by methanolysis.

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