Tannins of Cornaceous Plants. II.[1] Cornusiins D, E and F, New Dimeric and Trimeric Hydrolyzable Tannins from *Cornus officinalis*

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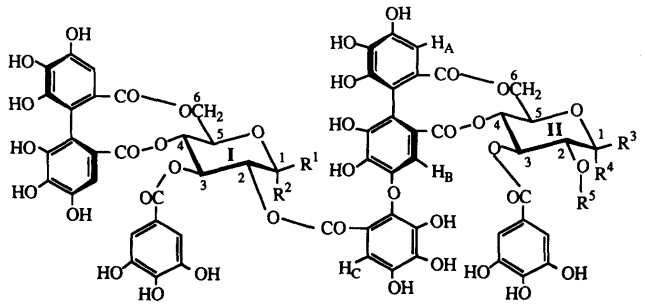
Cornusiin D (3), cornusiin E (4) and cornusiin F (5), new dimeric and trimeric hydrolyzable tannins, were isolated from the fruits of *Cornus officinalis* (Cornaceae), and their structures were established based on the chemical and spectroscopic data. Unusual chemical shifts of valoneoyl protons and anomeric protons of glucose cores in the proton nuclear magnetic resonance spectra of 3 and 4 were correlated with the stereostructures around the valoneoyl groups. Camptothin A (6) and camptothin B (7), dimeric hydrolyzable tannins previously isolated from *Camptotheca acuminata*, were also isolated from the fruits of *Cornus officinalis*.

Keywords tannin; ellagitannin; dimeric hydrolyzable tannin; trimeric hydrolyzable tannin; cornusiin D; cornusiin E; cornusiin F; camptothin A; camptothin B; *Cornus officinalis*

In a previous paper, we reported the isolation of eleven tannins from fruits of *Cornus officinalis* SIEB. *et* ZUCC. (Cornaceae), including a dimeric hydrolyzable tannin, cornusiin A (**1**) and a trimeric hydrolyzable tannin, cornusiin C (**2**).[1] Now we have improved the separation method and isolated five additional oligomeric hydrolyzable tannins, among which three are new tannins.

Results and Discussion

A concentrated filtrate from an aqueous acetone homogenate of fresh fruits of *Cornus officinalis* was extracted with diethyl ether, and the aqueous mother liquor was subjected to column chromatography over Dia-ion HP-20 with increasing concentrations of MeOH in water. Fractions obtained by this chromatography were further separated by column chromatography on Toyopearl HW-40 and on MCI gel CHP-20P to afford three new hydrolyzable tannins, cornusiin D (**3**), cornusiin E (**4**) and cornusiin F (**5**), along with the tannins previously isolated from the same plant.[1] Camptothin A (**6**) and camptothin B (**7**), dimeric hydrolyzable tannins which had been isolated from leaves of *Camptotheca acuminata* DECNE. (Nyssaceae),[1,2] were also isolated from the fruits of *Cornus officinalis* in the present study.



	R^1	R^2	R^3	R^4	R^5
1	H,OH		H,OH	gall	
3	O-gall H		H,OH	gall	
4	O-gall H		O-gall H	gall	
6	H,OH		H,OH	H	
7	H,OH		O-gall H	gall	

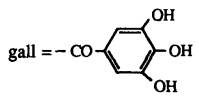
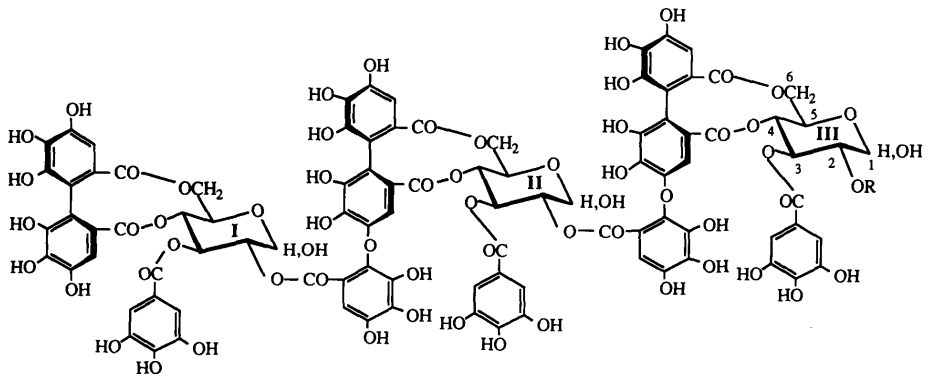


Chart 1

Cornusiin D (**3**) was isolated as an off-white amorphous powder. The fast-atom bombardment mass spectrum (FAB-MS) of **3** showed the $[M+Na]^+$ ion at *m/z* 1745, which corresponds to the molecular formula $C_{75}H_{54}O_{48}$ of **3**. Although the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** was complicated by duplication of the signals induced by anomerization of a glucose core, the signals assignable to four galloyl groups [δ 7.17, 7.16 (2H in total), 7.14, 7.12 (2H in total), 7.05 (2H), 6.90, 6.83 (2H in total)], a valoneoyl group [δ 6.81, 6.80 (1H in total, H_C), 6.61 (1H, H_A), 6.35 (1H, H_B)] and a hexahydroxydiphenoyl (HHDP) group [δ 6.75, 6.72 (1H in total), 6.53, 6.47 (1H in total)] and two glucose cores (δ 5.84—3.77) were observed.[3] The carbon-13 nuclear magnetic



2: R=gall
5: R=H

Chart 2

resonance (^{13}C-NMR) spectrum also showed signals due to these groups (see Experimental). The chemical shifts of the anomeric carbon signals of the two glucose cores indicate that one anomeric center is acylated (δ 93.6), while the other anomeric center is unacylated [δ 96.5 (β-anomer), 91.5 (α-anomer)].[4,5] The circular dichroism (CD) spectrum of **3** showed a positive Cotton effect of large amplitude ($[\theta]_{222}$ $+3.0\times10^5$), indicating that both the valoneoyl and HHDP groups in **3** have the *S*-configuration.[1,6] Partial hydrolysis of **3** with tannase[7] afforded cornusiin A (**1**). Therefore, in the molecule of **3**, an additional galloyl group should be located at one of the two anomeric centers of **1**, and hence, structure **3**, which is isomeric to camptothin B (**7**), was assigned to cornusiin D. The β-configuration of the glucose core having acylated O-1 (the left glucose core in structure **3** in Chart 1) was shown by the chemical shift of the anomeric carbon signal (δ 93.6) and by the coupling constant (8 Hz) of H-1.

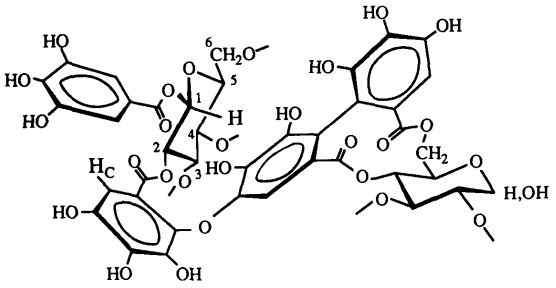


Chart 3. Partial Structure of Cornusiin D (**3**), Which Induces Anisotropic Effects on the Anomeric Proton of the Left Glucose Core and on Valoneoyl Proton H_C

The signal of H-1 in the glucose core having acylated O-1 appeared at high field (δ 5.66) in the ^{1}H-NMR spectrum of **3**, relative to the anomeric protons in other tannins in which O-1 is acylated,[8] whereas the anomeric proton of the glucose core acylated at O-1 in camptothin B (**7**) shows signals at δ 6.23 and 6.18.[2] The upfield shift for **3** is therefore regarded as due to the anisotropic effect of the valoneoyl group adjacent to the anomeric center. The chemical shifts of H_C in the valoneoyl group (δ 6.81, 6.80) are also much smaller than the chemical shifts of the corresponding protons of other tannins with related structures: camptothin B (**7**) (δ 7.15, 7.12),[2] rugosin D (**8**) (δ 7.15)[9] and isorugosin D (**9**) (δ 7.23).[10] The unusual H_C shifts in **3** indicate that the molecule takes a conformation in which the galloyl group at O-1 of the left glucose core causes an anisotropic effect at H_C of the valoneoyl group. A conformation which would induce these anisotropic effects is illustrated in Chart 3.

Cornusiin E (**4**) was isolated as an off-white amorphous powder. The FAB-MS showed the $[M+Na]^+$ ion at *m/z* 1897, which is consistent with the molecular formula $C_{82}H_{58}O_{52}$ for **4**. The ^{1}H-NMR spectrum of **4** indicates that this tannin consists of five galloyl groups [δ 7.17, 7.12, 7.11, 6.99, 6.87 (2H each, s)], a valoneoyl group and an HHDP group [δ 6.87, 6.70, 6.58, 6.55, 6.30 (1H each, s)], and two β-glucopyranose cores (see Experimental). Chemical shifts of the glucose protons indicate that all of the hydroxyl groups on the two glucopyranose cores are acylated, although a high-field shift of the anomeric proton (δ 5.73) on the left glucose core (glucose core I) in structure **4**, which will be due to the anisotropic effect of the neighbouring valoneoyl group, is observed. Chemical shifts of the two anomeric

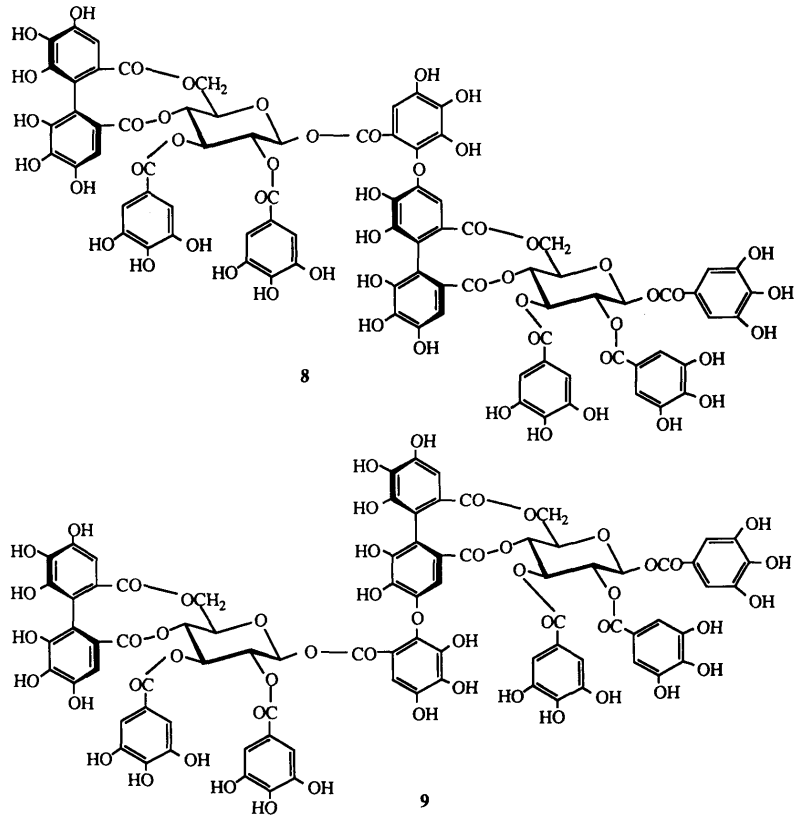


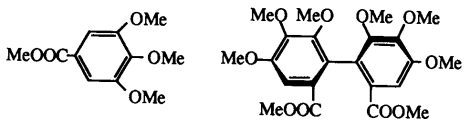
Chart 4

carbons (δ 93.9, 93.5) in the ^{13}C-NMR spectrum of **4** also show that both of the two anomeric centers have β-oriented acyloxy groups.[4] The *S*-configurations of the HHDP and valoneoyl groups were shown by a positive Cotton effect ($[\theta]_{223}$ $+3.1\times10^5$) in the CD spectrum of **4**. Partial hydrolysis of **4** with tannase afforded **3**. Based on these results, structure **4** was assigned for cornusiin E. The chemical shift of H_C, which appeared at high field (δ 6.87), indicates that **4** takes a conformation analogous to that of **3** (shown in Chart 3).

Cornusiin F (**5**) was isolated as an off-white amorphous powder. The $[M+H]^+$ ion at *m/z* 2203 and the $[M+Na]^+$ ion at *m/z* 2225 in the FAB-MS of **5** showed that cornusiin F is a trimeric hydrolyzable tannin of the molecular formula $C_{95}H_{70}O_{62}$. Retention volumes upon GPC (gel-permeation chromatography) analyses using Shimadzu HSG-15 and HSG-20 columns were also consistent with the trimeric molecular formula for **5**. Although the ^{1}H-NMR spectrum of **5** was complicated by the anomerization of its glucose cores, it showed fourteen aromatic protons assignable to three galloyl groups, two valoneoyl groups and an HHDP group [δ 7.09—6.81 (8H in total, two H_C protons in two valoneoyl groups and six protons of three galloyl groups), 6.64—6.60 (3H in total, a proton of an HHDP group and two H_A protons in two valoneoyl groups), 6.50—6.47 (1H in total, a proton of an HHDP group), 6.25—6.07 (2H in total, two H_B protons in two valoneoyl groups)] and protons of three glucose cores (δ 5.8—3.4). Methanolysis after methylation of **5** afforded methyl tri-*O*-methylgallate (**10**), dimethyl hexamethoxydiphenate (**11**) and trimethyl octa-*O*-methylvaloneate (**12**) in a molar ratio of 3 : 1 : 2. A positive Cotton effect ($[\theta]_{222}+3.5\times10^5$) in the CD spectrum of **5** indicated that the configurations of the two valoneoyl groups and an HHDP group are all *S*.[11] These results indicate that cornusiin F consists of three galloyl groups, an (*S*)-HHDP group, two (*S*)-valoneoyl groups and three glucose cores.

Partial hydrolysis of cornusiin C (**2**)[1] with tannase afforded **5**, indicating that cornusiin F is degalloylated cornusiin C. The ^{1}H-NMR spectrum of **5** showed a high-field shift of H-2 in one of the glucose cores [δ 3.50 (dd, $J=7.5$, 9 Hz)], indicating that the galloyl group at O-2 on the right glucose core of **2** is lacking in cornusiin F (glucose core III in structure **5** in Chart 2). The assigned structure was substantiated by degradation of **5** in hot water, which gave gemin D (**13**),[12] cornusiin B (**14**)[1] and compound **15**, which was identical with that produced from **2**.[1] The chemical shifts of the glucose carbon signals in the ^{13}C-NMR spectrum of cornusiin F, which agree with those of the merged signals of the glucose carbons of **1** and **13** (Fig. 1),[5] are also consistent with the structure **5**.

Among the tannins isolated from *Cornus officinalis* (Cornaceae), 1,2,6-tri-*O*-galloyl-β-D-glucose, 1,2,3,6-tetra-*O*-galloyl-β-D-glucose, gemin D (**13**), tellimagrandin I, tellimagrandin II, cornusiin A (**1**), camptothin A (**6**), camptothin B (**7**) and cornusiin C (**3**) had also been isolated from leaves of *Camptotheca acuminata* (Nyssaceae).[2,13] Although Nyssaceae was located near the family Com-



10 **11**

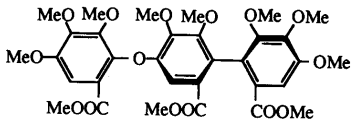


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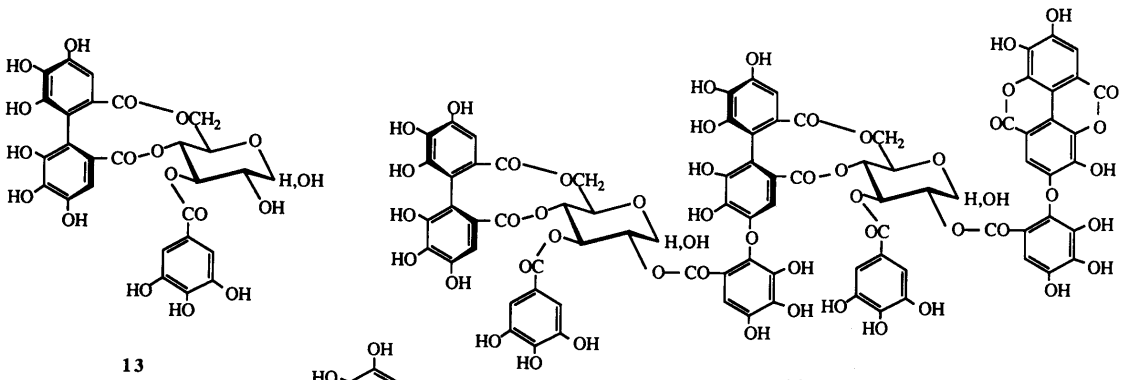
Chart 5



13 **15**

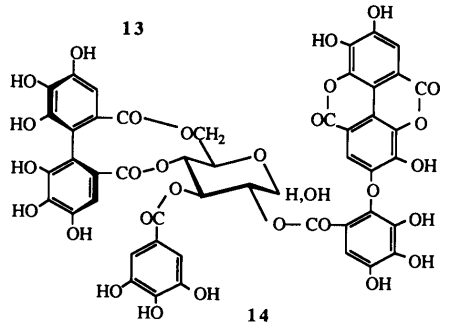


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Chart 6

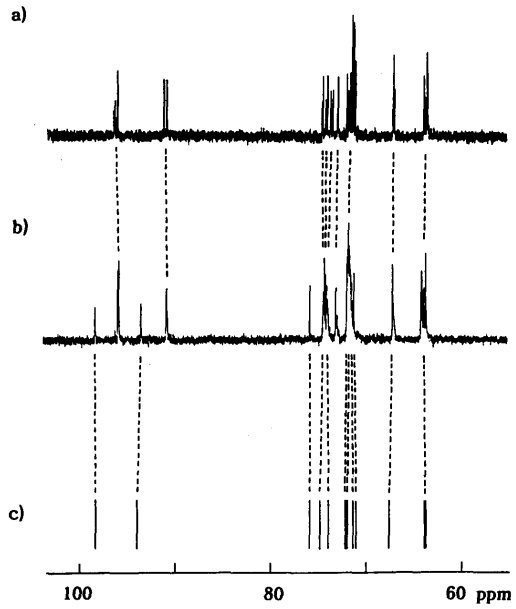

Fig. 1. Comparison of the Chemical Shifts of the Glucose Carbons in the ^{13}C-NMR Spectra of a) Cornusiin A (**1**)[1] (in Acetone-d_6 + D_2O), b) Cornusiin F (**5**) (in Acetone-d_6 + D_2O) and c) Gemin D (**13**)[4] (in Acetone-d_6)

bretaceae in an old taxonomic system, both Cornaceae and Nyssaceae are in the order Umbelliflorae according to Engler's taxonomic system of the 12th edition.[14] The co-occurrence of several tannins in these two families may be significant for the estimation of this taxonomic correlation.

Experimental

FAB-MS were recorded on a JEOL GMS-HX100 spectrometer. CD spectra were recorded on a JASCO J-500 machine equipped with a DP-501 data processor. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H-NMR and 125.7 MHz for ^{13}C-NMR), using acetone-d_6 which contains D_2O (*ca.* 3%). Chemical shifts are given in δ values (ppm) from tetramethylsilane. The carbon numbers in this experimental part are those of the formulae in Chart 7. Normal-phase high performance liquid chromatography (HPLC) was performed on a Superspher Si60 column (4 mm × 125 mm) (Merck) with a solvent system (N1) consisting of hexane–ethyl acetate (2:1, by volume), or another solvent system (N2) consisting of hexane–MeOH–tetrahydrofuran–formic acid (55:33:11:1) containing oxalic acid (450 mg/l), in a flow rate of 1.5 ml/min. Reversed-phase HPLC was conducted on a LiChrospher RP-18 column (4 × 250 mm) (Merck) in an oven at 40 °C, with solvent system (R1) consisting of 0.01 M H_3PO_4–0.01 M KH_2PO_4–acetonitrile (17:17:6) at a flow rate of 1.3 ml/min. A solvent system (R2) consisting of 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH–ethyl acetate (17:17:4:2), and another solvent system (R3) consisting of 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH (10:10:1) were also used at a flow rate of 1.0 ml/min. Detection for the HPLC analyses were effected with a Shimadzu SPD-6A UV spectrophotometric detector at 280 nm.

Isolation of Tannins from Fruits of *Cornus officinalis* The fruits (1250 g) of *Cornus officinalis* were homogenized in 70% acetone (2.5 l) immediately after collection from the trees. The homogenate was filtered, and the filtrate was concentrated to 500 ml *in vacuo*. The resulting aqueous solution was extracted with diethyl ether (200 ml × 2), and aqueous mother liquor was subjected to column chromatography over Dia-ion HP-20 (6.5 × 50 cm) with increasing concentration of MeOH in H_2O (H_2O→20% MeOH→40% MeOH→60% MeOH→MeOH). The eluate with 20% MeOH (1.9 g) was subjected to column chromatography over Toyopearl HW-40 (fine grade) (2.2 × 37 cm) with 70% EtOH–70% acetone (10:0→9:1→8:2) as the eluant, to give gemin D (**13**) (25 mg), tellimagrandin I[1] (33 mg), camptothin A (**6**) (103 mg), cornusiin A (**1**) (141 mg), cornusiin F (**5**) (57 mg) and cornusiin C (**2**) (22 mg). A portion (5.1 g) of the 60% MeOH eluate (20.2 g) from Dia-ion HP-20 was chromatographed over Toyopearl HW-40 (fine grade) (2.2 × 28 cm) with 70% EtOH–70% acetone (10:0→9:1) as the eluant, to afford tellimagrandin II[1] (150 mg),**1** (442 mg), a mixture (220 mg) temporarily named fraction A, and another mixture (464 mg) named fraction B. Fraction A was further separated by column chromatography on MCI-gel CHP-20P (1.1 × 38 cm) with 30% MeOH and then with 40% MeOH. Cornusiin D (**3**) (25 mg) and camptothin B (**7**) (58 mg) were isolated from the eluate with 40% MeOH. Fraction B was separated by column chromatography on MCI-gel CHP-20P (1.1 × 38 cm) with 30% MeOH and then 40% MeOH. Cornusiin C (**2**) (247 mg) was isolated from the eluate with 30% MeOH, and crude cornusiin E (30 mg) obtained from the eluate with 40% MeOH was further purified on a column of Toyopearl HW-40 (superfine grade), and then on a SEP-PAK C_{18} cartridge, to give 6 mg of **4**.

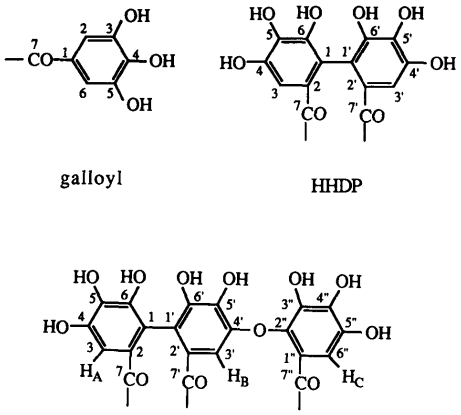

Chart 7

Cornusiin D (3) This compound was isolated as an off-white amorphous powder, $[\alpha]_D$ +97° (*c* = 1, MeOH). *Anal.* Calcd for $C_{75}H_{54}O_{48}\cdot 11H_2O$: C, 46.89; H, 3.99. Found: C, 46.72; H, 3.57. FAB-MS: *m/z* 1745 ($[M+Na]^+$). UV λ_{max}^{MeOH} nm (log ε): 213(5.35), 272(4.88). IR ν_{max}^{KBr} cm^{-1}: 1720 (ester carbonyl), 1610. CD (MeOH): $[\theta]_{222}$ +3.0 × 10^5, $[\theta]_{239}$ +1.6 × 10^5, $[\theta]_{261}$ −6.7 × 10^4, $[\theta]_{283}$ +1.1 × 10^5. ^{1}H-NMR (40 °C) δ: 7.16, 7.12, 7.05, 6.90 (galloyl), 6.81 (H_C), 6.61 (H_A), 6.35 (H_B) [valoneoyl (val)], 6.72, 6.53 (HHDP) [α (α-anomer)]; 7.17, 7.14, 7.05, 6.83 (galloyl), 6.80 (H_C), 6.61 (H_A), 6.35 (H_B) (val), 6.75, 6.47 (HHDP) [β(β-anomer)]; 5.84 (α, t, *J* = 9.5 Hz, glc_{II} H-3 (H-3 of glucose core II, the right glucose core shown in formula **3** in Chart 1)], 5.66 [α and β, d, *J* = 8 Hz, glc_I H-1 (H-1 of the left glucose core)], 5.64—5.48 (complicated peaks), 5.28—5.00 (complicated peaks), 4.68 (β, br dd, *J* = 6, 10 Hz, glc_{II} H-5), 4.55 (α, dd, *J* = 6, 10 Hz, glc_{II} H-5), 4.50 (α and β, *J* = 6, 10 Hz, glc_I H-5), 3.94 (β, dd, *J* = 1, 13 Hz, glc_{II} H-6), 3.88 (α, d, *J* = 13 Hz, glc_{II} H-6), 3.77 (α and β, d, *J* = 13 Hz, glc_I H-6). ^{13}C-NMR δ: 63.1 (α and β, glc_I C-6), 64.0 (β, glc_{II} C-6), 64.2 (α, glc_{II} C-6), 67.4 (α, glc_{II}C-5), 71.0, 71.2, 71.3, 71.4, 71.6 (α, glc_{II} C-3, glc_{II} C-4, glc_I C-4; β, glc_{II} C-4, glc_I C-4, glc_{II} C-5), 72.0, 72.1, 72.2 (α and β, glc_I C-2, glc_I C-5), 73.4 (α, glc_{II} C-2), 73.5 (β, glc_{II} C-3), 73.9 (β, glc_I C-3), 74.0 (α, glc_I C-3), 74.6 (β, glc_{II} C-2), 91.5 (α, glc_{II} C-1), 93.6 (α and β, glc_I C-1), 96.5 (β, glc_{II} C-1), 106.1, 106.3 (val C-3′), 107.6, 108.0, 108.1, 108.1, 108.5 (HHDP C-3, C-3′; val C-3), 109.4, 109.7 (val C-6″), 110.1, 110.2, 110.6, 110.7 (galloyl C-2, C-6), 115.5, 115.5, 115.7, 115.8, 116.2, 116.5, 116.6, 116.9, 117.3, 117.5, (HHDP C-1, C-1′; val C-1, C-1′, C-1″), 120.6, 120.7, 120.8, 120.8, 120.9, 121.0, 121.1 (galloyl C-1), 126.0, 126.0, 126.1, 126.1, 126.4, 126.6 (HHDP C-2, C-2′; val C-2, C-2′), 135.2, 135.4, 136.4, 136.4, 136.5, 136.6, 136.8 (HHDP C-5, C-5′; val C-2″, C-5, C-5′), 138.7, 138.9, 139.1, 139.3, 139.5, 139.5, 139.7, 140.3 (galloyl C-4; val C-3″, C-4″), 143.3, 143.4 (val C-5″), 144.2, 144.4, 144.5, 144.5, 144.6, 144.9, 145.2, 145.4, 145.5, 145.6, 145.7, 145.8, 145.9 (galloyl C-3, C-5; HHDP C-4, C-4′, C-6, C-6′; val C-4, C-6, C-6′), 146.6, 146.7 (val C-4′), 165.1, 165.3, 165.7, 165.8, 165.9, 165.9, 166.1, 166.1, 166.2, 166.5, 167.5, 167.6, 167.6, 167.7, 168.0, 168.0, 168,6 168.8 (galloyl C-7; HHDP C-7, C-7′; val C-7, C-7′, C-7″).

Cornusiin E (4) This compound was isolated as an off-white amorphous powder, $[\alpha]_D$ +67° (*c* = 0.8, MeOH). *Anal.* Calcd for $C_{82}H_{58}O_{52}\cdot 16H_2O$: C, 45.52; H, 4.19. Found: C, 45.50; H, 3.55. FAB-MS: *m/z* 1897 ($[M+Na]^+$). UV λ_{max}^{MeOH} (log ε): 213(5.40), 274 (4.96). IR ν_{max}^{KBr} cm^{-1}: 1725 (ester carbonyl), 1615 CD (MeOH): $[\theta]_{223}$ +3.1 × 10^5, ($[\theta]_{237}$ +1.9 × 10^5, $[\theta]_{260}$ −4.5 × 10^4, $[\theta]_{283}$ +9.6 × 10^4. ^{1}H-NMR δ: 7.17, 7.12, 7.11, 6.99 (2H each, s, 4 × galloyl), 6.87 (3H, s, galloyl and val H_C),

6.70 (1H, s, HHDP), 6.58 (1H, s, val H_A), 6.55 (1H, s, HHDP), 6.30 (1H, s, val H_B), 6.24 [d, J=8.5 Hz, glc_{II} H-1 (the right glucose core shown in formula **4** in Chart 1)], 5.73 (d, J=8 Hz, glc_I H-1), 5.67 (t, J=10 Hz, glc_{II} H-3), 5.64 (dd, J=6, 13.5 Hz, glc_{II} H-6), 5.63 (dd, J=8, 10 Hz, glc_I H-2), 5.57 (dd, J=8.5, 10 Hz, glc_{II} H-2), 5.53 (t, J=10 Hz, glc_I H-3), 5.17(dd, J=6, 13.5 Hz, glc_I H-6), 5.14(t, J=10 Hz, glc_{II} H-4), 5.12(t, J=10 Hz, glc_I H-4), 4.74 (br dd, J=6, 10 Hz, glc_{II} H-5), 4.44 (dd, J=6, 10 Hz, glc_I H-5), 3.99 (d, J=13.5 Hz, glc_{II} H-6), 3.78 (d, J=13.5 Hz, glc_I H-6). ^{13}C-NMR δ: 63.2, 63.4 (glc_I C-6, glc_{II} C-6), 70.9 (glc_{II} C-4), 71.2 (glc_I C-4), 71.7 (glc_{II} C-2), 72.1 (glc_I C-2), 72.3 (glc_I C-5), 72.9 (glc_{II} C-5), 73.0 (glc_{II} C-3), 74.3 (glc_I C-3), 93.5 (glc_{II} C-1), 93.9 (glc_I C-1), 106.0 (val C-3′), 107.6, 107.9, 108.1 (HHDP C-3, C-3′; val C-3), 109.2 (val C-6″), 110.0, 110.1, 110.3, 110.4, 110.4 (galloyl C-2, C-6), 114.7, 115.6, 116.0, 116.3, 117.6, (HHDP C-1, C-1′; val C-1, C-1′, C-1″), 119.3, 120.0, 120.1, 120.2, 120.6 (galloyl C-1), 125.6, 125.7, 125.8, 126.2 (HHDP C-2, C-2′; val C-2, C-2′), 135.8, 136.2, 136.5, 136.7 (HHDP C-5, C-5′; val C-2″, C-5, C-5′), 139.0, 139.3, 139.4, 139.7, 140.1, 140.3 (galloyl C-4; val C-3″, C-4″), 143.7 (val C-5″), 144.5, 144.6, 144.7, 145.0, 145.2 (HHDP C-4, C-4′, C-6, C-6′; val C-4, C-6, C-6′), 145.5, 145.7, 145.9, 145.9, 146.1 (galloyl C-3, C-5), 146.6 (val C-4′), 165.5, 165.9, 166.0, 166.6, 167.7, 167.9, 168.3, 168,6 (galloyl C-7; HHDP C-7, C-7′; val C-7, C-7′, C-7″).

Cornusiin F (5) This compound was isolated as an off-white amorphous powder. $[\alpha]_D$ +18° (c=1, MeOH). *Anal.* Calcd for $C_{95}H_{70}O_{62}\cdot 12H_2O$: C, 47.16; H, 3.92. Found: C, 47.24; H, 3.99. FAB-MS: m/z 2225 ($[M+Na]^+$), 2203 ($[M+H]^+$). UV λ_{max}^{MeOH} (log ε): 212 (5.41), 267 (4.96). IR ν_{max}^{KBr} cm^{-1}: 1725 (ester carbonyl), 1610. CD (MeOH): $[\theta]_{222}$ +3.5×10^5, $[\theta]_{238}$ +1.0×10^5, $[\theta]_{258}$ −1.5×10^5, $[\theta]_{284}$ +1.3×10^5. ^{1}H-NMR δ: 7.09—6.81 (8H in total, val H_C×2 and galloyl×3), 6.64—6.60 (3H in total, val H_A×2 and HHDP), 6.50—6.47 (1H in total, HHDP), 6.25—6.07 (2H in total, val H_B×2), 5.79, 5.78, 5.66, 5.66 [each t, J=10 Hz, $glc_{I,II,III}$ (glucose cores I, II and III in structure **5** in Chart 2) H-3, α], 5.49—4.72 (complicated peaks), 4.69, 4.68 (each d, J=7.5 Hz, glc_{III} H-1, β), 4.62—4.43, 4.27—4.00 (complicated peaks, $glc_{I,II}$ H-1 of β, and $glc_{I,II,III}$ H-5 of α and β), 3.91—3.69 (complicated peaks, $glc_{I,II,III}$ H-6 of α and β), 3.50 (dd, J=7.5, 9 Hz, glc_{III} H-2, β). ^{13}C-NMR δ: 63.5—64.0 ($glc_{I,II,III}$ C-6, α and β), 66.8—67.0 ($glc_{I,II,III}$ C-5, α), 71.0—71.8 (glc_{III} C-2, α; $glc_{I,II}$ C-3, α; $glc_{I,II,III}$ C-4, α; $glc_{I,II,III}$ C-4, β; $glc_{I,II,III}$ C-5, β), 72.8—73.0 ($glc_{I,II}$ C-2, α), 73.6—74.4 ($glc_{I,II,III}$ C-2, β; $glc_{I,II}$ C-3, β; glc_{III} C-3, α), 75.7 (glc_{III} C-3, β), 90.9—91.1, $glc_{I,II}$ C-1, α), 93.6, 93.7 (glc_{III} C-1, α), 96.0, 96.1, 96.4 ($glc_{I,II}$ C-1, β), 98.4—98.6 (glc_{III} C-1, β), 104.8—105.7 (val C-3′), 107.7—108.0 (HHDP C-3, C-3′; val C-3), 109.8—110.6 (galloyl C-2 and C-6; val C-6″), 113.1—117.5 (HHDP C-1 and C-1′; val C-1, C-1′ and C-1″), 120.2—121.2 (galloyl C-1), 125.5—126.4 (HHDP C-2 and C-2′; val C-2 and C-2′), 136.0—138.0 (HHDP C-5 and C-5′; val C-2″, C-5 and C-5′), 138.7—139.2 (galloyl C-4), 140.2—140.9 (val C-3″ and C-4″), 143.0—143.3 (val C-5″), 144.4—145.9 (galloyl C-3 and C-5; HHDP C-4, C-4′, C-6 and C-6′; val C-4, C-6 and C-6′), 146.5, 146.6, 147.4—147.5 (val C-4′), 164.6—168.8 (galloyl C-7; HHDP C-7 and C-7′; val C-7, C-7′ and C-7″).

Partial Hydrolysis of Cornusiin D (3) Tannase solution[15] (0.5 ml) was added to an aqueous solution (2.5 ml) of **3** (5 mg) and the mixture was kept at 37 °C for 20 h. Then, the reaction mixture was acidified with 1 N HCl, and passed through a SEP-PAK C_{18} cartridge. The adsorbed compounds were eluted with increasing concentrations of MeOH in water (0%→5%→10%→20%). The eluate with 20% MeOH afforded cornusiin A (**1**)[16] (3 mg).

Partial Hydrolysis of Cornusiin E (4) An aqueous solution (2.5 ml) of **4** (5 mg) was treated with tannase at 37 °C for 15 h. After addition of 1 N HCl, the reaction mixture was passed through a SEP-PAK C_{18} cartridge, and the adsorbed compounds were eluted with increasing concentrations of MeOH in water (0%→10%→20%→30%). The eluate with 30% MeOH afforded cornusiin D (**3**)[16] (3 mg).

GPC Analyses of Tannins 5, 6 and 13 GPC was conducted on a Shimadzu HSG-15 (7.9 mm×50 cm) column, and also on a Shimadzu HSG-20 (7.9 mm×30 cm) column at 40 °C, using tetrahydrofuran as a developer. Linear relationships expressed by the following equations were observed between the retention volumes (v_R in ml) and the logarithms of molecular weights (MW) for tannins **13** (monomer), **6** (dimer) and **5** (trimer):

$$\log MW = -0.44 \times v_R + 8.41 \text{ (for the HSG-15 column)}$$

and

$$\log MW = -0.83 \times v_R + 10.99 \text{ (for the HSG-20 column)}$$

Quantitative Analysis of the Constituent Phenolic Acids in Cornusiin F (5) Ethereal diazomethane (0.5 ml) was added to an EtOH solution (0.2 ml) of **5** (1 mg), and the mixture was left to stand for 30 min. Then, the solvent was removed, and 0.5% NaOMe in MeOH (0.2 ml) was added to the residue. The reaction mixture was left to stand overnight, and then acidified with acetic acid. After evaporation of the solvent, the residue was analyzed by HPLC [normal phase, solvent system (N1)], demonstrating the presence of **10**, **11** and **12** in a molar ratio of 3:1:2.

Transformation of Cornusiin C (2) into Cornusiin F (5) Tannase solution (1 ml) was added to an aqueous solution (10 ml) of cornusiin C (**2**) (21 mg), and the mixture was kept at 37 °C for 84 h. Then, the reaction mixture was acidified with 1 N HCl, and passed through a SEP-PAK C_{18} cartridge. The adsorbed compounds were eluted with water, and then with MeOH. The MeOH eluate was subjected to column chromatography over Toyopearl HW-40 (superfine grade) (1.1×22 cm) with 70% EtOH–70% acetone (10:0→9:1) as the eluant. The eluate with 70% EtOH–70% acetone (9:1) afforded **5**[16] (5 mg), together with 5 mg of the starting material.

Partial Degradation of Cornusiin F (5) An aqueous solution (0.4 ml) of cornusiin F (**5**) (1 mg) in a sealed tube was heated in a boiling water-bath for 6 h. The HPLC analyses showed the presence of gemin D (**13**) [t_R 3.8 and 4.0 min (N2); 3.4 and 4.6 min (R3)[17]], cornusiin B (**14**) [t_R 5.0 and 5.3 min (N2); 3.8 and 4.0 min (R1); 5.4 and 6.8 min (R2)] and **15**[1] [t_R 11.9 min (N2); 4.6 and 5.6 min (R1); 9.5 and 13.6 min (R2)] in the reaction mixture.

Acknowledgements We thank Prof. K. Takaishi and Dr. H. Kuwajima, Faculty of Pharmaceutical Sciences, Kinki University, for the FAB-MS. We also thank the SC-NMR Laboratory of Okayama University for the NMR spectra. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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