Tannins of Theaceous Plants. II.[1] Camelliins A and B, Two New Dimeric Hydrolyzable Tannins from Flower Buds of *Camellia japonica* L. and *Camellia sasanqua* THUNB.

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Two new dimeric hydrolyzable tannins, camelliins A and B, which exist as equilibrium mixtures of anomers, have been isolated from flower buds of *Camellia japonica* L., and their structures were elucidated. Camelliin B belongs to a unique class of dimers having a macrocyclic structure. The flower buds of *Camellia sasanqua* THUNB. yielded five tannis which were identical with those of *C. japonica*.

Keywords *Camellia japonica*; Camellia sasanqua; Theaceae; ellagitannin; dimeric hydrolyzable tannin; camelliin A; camelliin B; macrocyclic structure

Previously we reported[1] on three hydrolyzable tannins, gemin D (**1**), tellimagrandin I (**2**) and pedunculagin (**3**) isolated from flower buds of *Camellia japonica* L. (Theaeceae), traditionally used as a hemostatic and an antidiarrheal in China. In the present investigation on the polyphenols of this plant, we have isolated two new dimeric ellagitannins, named camelliins A (**4**) and B (**12**).[2] We have also examined the tannin constituents of flower buds of *C. sasanqua* THUNB. and proved that the five hydrolyzable tannins found in this plant were identical with those from *C. japonica*.

For the extraction of tannins from *C. japonica*, an aqueous acetone homogenate of the fresh flower buds was extracted, after concentration, with ether, ethyl acetate and 1-butanol successively. The 1-butanol-soluble fraction was chromatographed over Sephadex LH-20 to afford camelliins A (**4**) and B (**12**), along with **1**—**3**. The tannins from the flower buds of *C. sasanqua* were isolated in a similar way, and four hydrolyzable tannins thus obtained were identified as gemin D (**1**), pedunculagin (**3**), and camelliins A (**4**) and B (**12**), by direct comparisons with the specimens isolated from *C. japonica*. The presence of tellimagrandin I (**2**) in the extract of *C. sasanqua* was confirmed by high-performance liquid chromatography (HPLC).

Camelliin A (**4**), $[\alpha]_D + 53°$ (MeOH), is the main tannin in both *C. japonica* and *C. sasanqua*. It gave a positive $FeCl_3$ reaction and a characteristic color of ellagitannins with the sodium nitrite–acetic acid reagent[3] on a thin-layer chromatography (TLC) plate. Dimeric structure of **4** was indicated by the result of high-performance gel permeation chromatography, and by the fast atom bombardment mass spectrum (FAB-MS), which showed a peak at *m/z* 1591 $[M+Na]^+$. Acid hydrolysis of **4** afforded gallic acid, ellagic acid (**5**) and valoneic acid dilactone (**6**), which were characterized as methyl derivatives, and also glucose, which was identified by gas liquid chromatography (GLC) of the trimethylsilyl ether. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **4**, anomeric proton signals are absent at lower field than 5.8 ppm, indicating that both anomeric hydroxyl groups of glucose residues in **4** are free. The existence of **4** as an equilibrium mixture of four anomers was also indicated by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, which showed four peaks assignable to α-anomeric carbons, at δ 91.5, 91.4, 91.1 and 91.0 and the four β-anomeric carbons at δ 96.5, 96.2, 95.6 and 95.2. The existence of **4** as an equilibrated

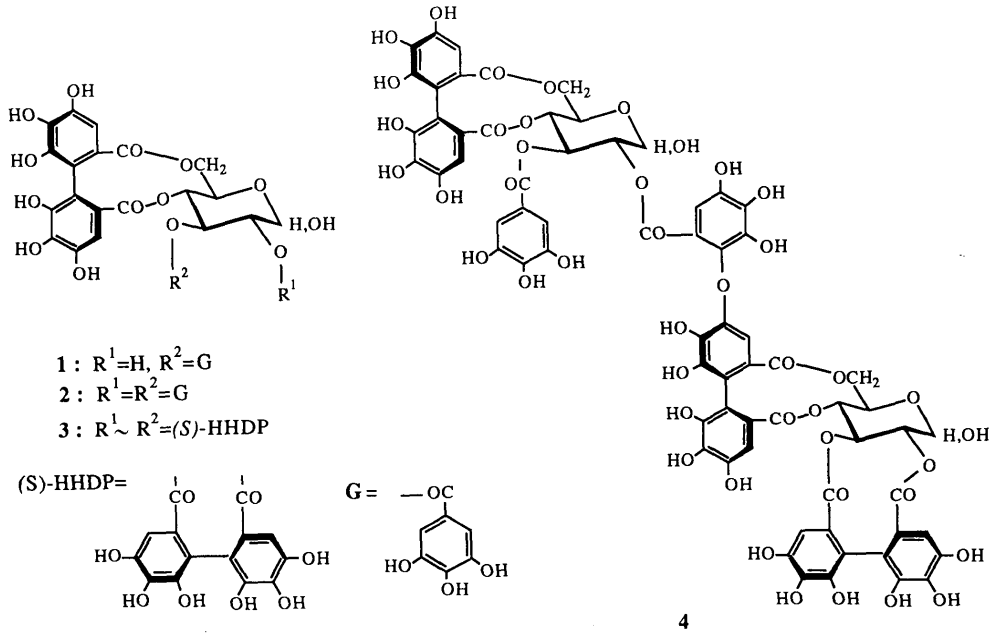


Chart 1

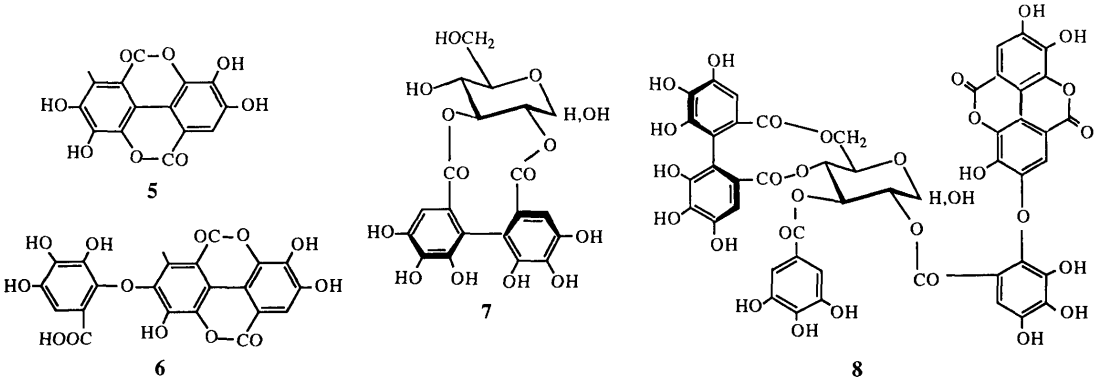
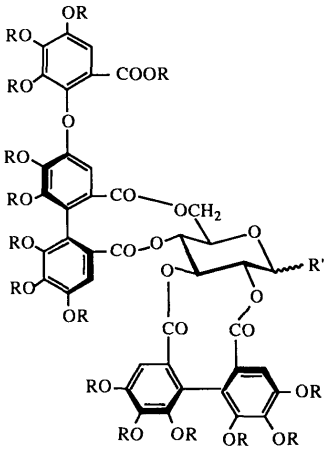


Chart 2



9 : R=H, R'=OH
10: R=Me, R'= α-OMe
11: R=Me, R'= β-OMe

Chart 3

mixture was also indicated by the complexity of its ^{1}H-NMR spectrum arising from doubly-paired signals of most of the protons. The three signals at δ 7.02, 7.01 and 6.97, corresponding to two protons in total, are assigned to a galloyl group, and the other doubly-paried singlets at δ 7.05—6.15 are assigned to seven aromatic protons (see Experimental). These spectral data, combined with the result of acid hydrolysis, indicate that camelliin A consists of a galloyl group, a valoneoyl group, two hexahydroxydiphenoyl (HHDP) groups, and two glucose residues. The molecular formula of camelliin A is therefore represented by $C_{68}H_{48}O_{44}$ which is consistent with the FAB-MS data. Partial hydrolysis of camellin A (**4**) in boiling water provided three hydrolysates, which were identified as gemin D (**1**),[1] 2,3-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose (**7**)[4] and cornusiin B (**8**),[5] respectively, by spectral comparisons with authentic samples.

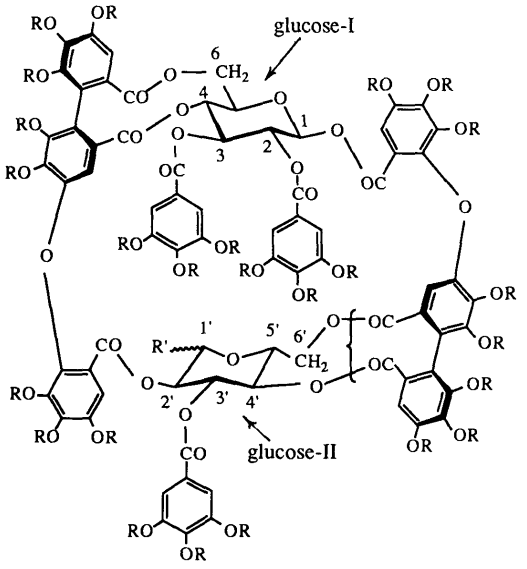
Methylation of **4** with dimethyl sulfate and potassium carbonate in dry acetone yielded a complex mixture of methylated derivatives. However, two partially degraded products were separated by preparative TLC, and were characterized as the α-(**10**) and β-anomer (**11**) of the hexadecamethyl derivative of praecoxin A (**9**).[6] The identification was based on their electron-impact mass spectra (EI-MS) [*m/z* 1176 (M^+)] and ^{1}H-NMR spectra (see Experimental), and then confirmed by direct comparisons with specimens prepared by methylation of authentic praecoxin A.

The (*S*)-configuration of the chiroptical valoneoyl and HHDP groups in camelliin A (**4**) was proved by production of **1**, **10** and **11** of known absolute configuration, from **4**. These assignments were also substantiated by the circular dichroism (CD) spectrum of **4**, which showed a strong positive Cotton effect at 226 nm and a negative one at 259 nm.[7]

Based on these data, the structure of camelliin A was established as **4**.

Camelliin B (**12**), $[\alpha]_D$ −24° (MeOH), was also shown to be an ellagitannin dimer similar to **4**, by the coloration with the sodium nitrite–acetic acid reagent, and by the retention time in HPLC (normal phase).[8] Acid hydrolysis of **12** afforded the same products as those from **4** (gallic acid, ellagic acid, valoneic acid dilactone and glucose). Each signal in the ^{1}H-NMR spectrum of **12** is duplicated, indicating equilibration (*ca.* 4 : 1) between α- and β-anomer at the glucose residue. The presence of three galloyl groups was indicated by three two-proton signals appearing as pairs of singlet in the aromatic region. Six one-proton signals appearing as pairs of singlet were also observed in the region of δ 5.90—7.41. They suggest the presence of two valoneoyl groups in the molecule, as the signals at lower field [δ 7.41, 7.36 (1H in total) and 7.15 (1H)] are assignable to the protons on the galloyl part of the valoneoyl group. These spectral data and the molecular formula $C_{75}H_{52}O_{48}$ determined by elemental analysis and FAB-MS [*m/z* 1743 $(M+Na)^+$] indicate that ellagic acid (**5**) obtained upon acid hydrolysis of **12** was produced by cleavage of the ether bond in the valoneoyl group.[9] Camelliin B is therefore composed of three galloyl groups, two valoneoyl groups and two glucose residues. The chiralities of the valoneoyl groups in **12** were determined as *S*, from the strong positive Cotton effect, $[\theta]$ $+27.8\times10^4$, at 220 nm in the CD spectrum.[7]

Both glucose cores in **12** were shown to adopt the $^4C_1$ conformations by the coupling pattern of glucose proton signals, which were unequivocally assigned with the aid of the ^{1}H–^{1}H shift correlation (COSY) spectrum (assignments, see Experimental). The presence of the valoneoyl group bridged at O-4—O-6 of each glucose core was also suggested by the large differences ($\Delta\delta_{H\text{-}6-H\text{-}6'}$ *ca.* 1 ppm)[10] in the chemical shifts between the signals of the two protons of each C-6 methylene group [δ 5.20, 3.93 (glucose-I C_6–H_2) and 4.94, 3.96 (glucose-II $C_{6'}$–H_2) in the α-anomer; δ 4.96, 3.93 (glucose-I C_6–H_2) and 4.94, 3.96 (glucose-II $C_{6'}$–H_2)

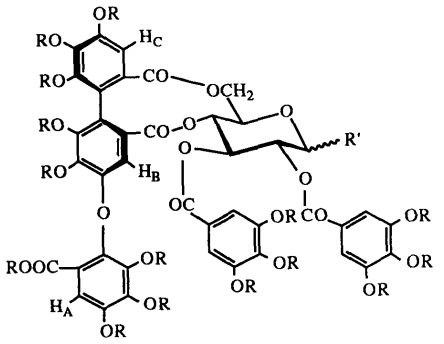


12 : R=H, R'=OH
13 : R=Me, R'= β-OMe

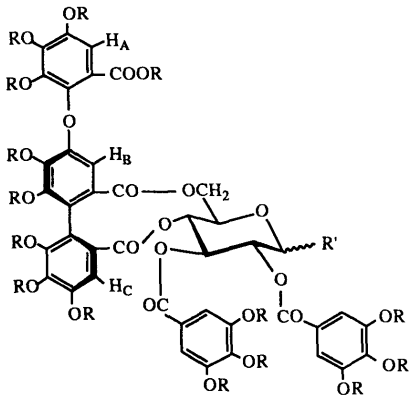
Chart 4

in the β-anomer]. These data, together with the shifts of H-2—H-4 and H-2'—H-4' to lower field than 5 ppm, indicated that the hydroxyl groups of glucoses of **12**, except for one of the anomeric hydroxyl groups, are all acylated. The configuration of the anomeric acyloxy group was shown to be β, by the large coupling constant (J=8.5 Hz) of the two peaks of the anomeric protons at δ 5.81 and 5.82 (1H in total). Methylation of **12** afforded hexacosamethylcamelliin B (**13**) and a partially degraded product (**15**). The ^{1}H-NMR spectrum of **15** indicated the presence of a valoneoyl group (δ 6.33, 6.96, 7.27, 1H each s), and two galloyl groups (δ 7.11, 7.21, 2H each, s). The anomeric proton signal was observed at δ 5.24 (d, J=4 Hz). These spectral data and the location of the (*S*)-valoneoyl group on O-4—O-6 in **12**, indicate that **15** is a methylated derivative of 2,3-di-*O*-galloyl-4,6-(*S*)-valoneoyl-α-D-glucose. However, the ^{1}H-NMR spectrum of **15** was clearly different from that of the hexadecamethyl derivative (α-anomer) (**17**) prepared from rugosin B (**16**),[6] which showed the valoneoyl proton signals at δ 6.49, 6.76 and 7.30. Therefore, **15** was presumed to be an isomer of **17** differing only in the orientation of the valoneoyl group. A comparison of the chemical shifts of the valoneoyl proton signals between isorugosin B (**14**)[11] [δ 6.25, 6.31 (1H in total; H_B), 6.65, 6.66 (1H in total; H_C)] and rugosin B (**16**) [δ 6.29 (1H; H_B), 6.49, 6.51 (1H in total; H_C)] shows that the H_B and H_C signals of the former resonate at somewhat higher and lower field, respectively, than those of the latter. Since a similar trend of these proton signals is observed between **15** and **17**, **15** was considered to be the hexadecamethyl derivative of **14**. The orientation of one of the valoneoyl groups in **12** was thus determined to be as in the structural formula.

Treatment of **12** with hot 1% sulfuric acid afforded oenothein C (**19**),[5] along with gallic acid and valoneic acid dilactone (**6**). On the other hand, partial hydrolysis of **12** in boiling water gave gemin D (**1**) and 2,3-di-*O*-galloylglucose (**20**), and also gallic acid and **6**.



14 : R=H, R'=OH
15 : R=Me, R'= α-OMe



16 : R=H, R'=OH
17 : R=Me, R'= α-OMe
18 : R=Me, R'= β-OMe

Chart 5

In an equilibrated anomer mixture of **12**, the α-anomer was predominant (*ca.* 4 : 1) as clearly indicated by the peak area ratio of unacylated anomeric carbon signals at δ 92.2 (α) and 96.7 (β) in the ^{13}C-NMR spectrum. The anomeric proton signal of the α-anomer in the ^{1}H-NMR spectrum of **12** is shifted considerably lower (δ 5.94) than those (δ 5.37—5.45) of **4**. These structural and spectral features are analogous to those of oenothein B (**21**) which has recently been established to have a unique macro-ring structure, existing exclusively in the α-form at the glucose core II.[12] The free anomeric hydroxyl group in **12** is hence presumed to be on the glucose core II in the macro-ring structure, by analogy with that of **21**.

These structural characteristics of camelliin B were further supported by several lines of evidence as follows. Treatment of **12** with boiling water containing a small amount of trifluoroacetic acid gave a hydrolysate (**23**) which showed a retention time similar to that of the starting material in normal phase HPLC, indicative of its dimeric nature. Although the ^{1}H-NMR spectrum of **23** was duplicated in a ratio of *ca.* 5 : 1 due to an anomerization at one of the glucose cores, it indicated the retention of three galloyl groups and two valoneoyl groups as well as two glucose cores. However, one of the valoneoyl groups was shown to be in a dilactonized form, as revealed by lower-field shifts of the two protons of the valoneoyl group [δ 7.60, 7.596 (each s, 1H in total), and 7.19, 7.18 (each s, 1H in total)]. Remarkable upfield shifts of H-4' and H-6' in the ^{1}H-^{1}H COSY spectrum of **23** (assignments, see Experimental),

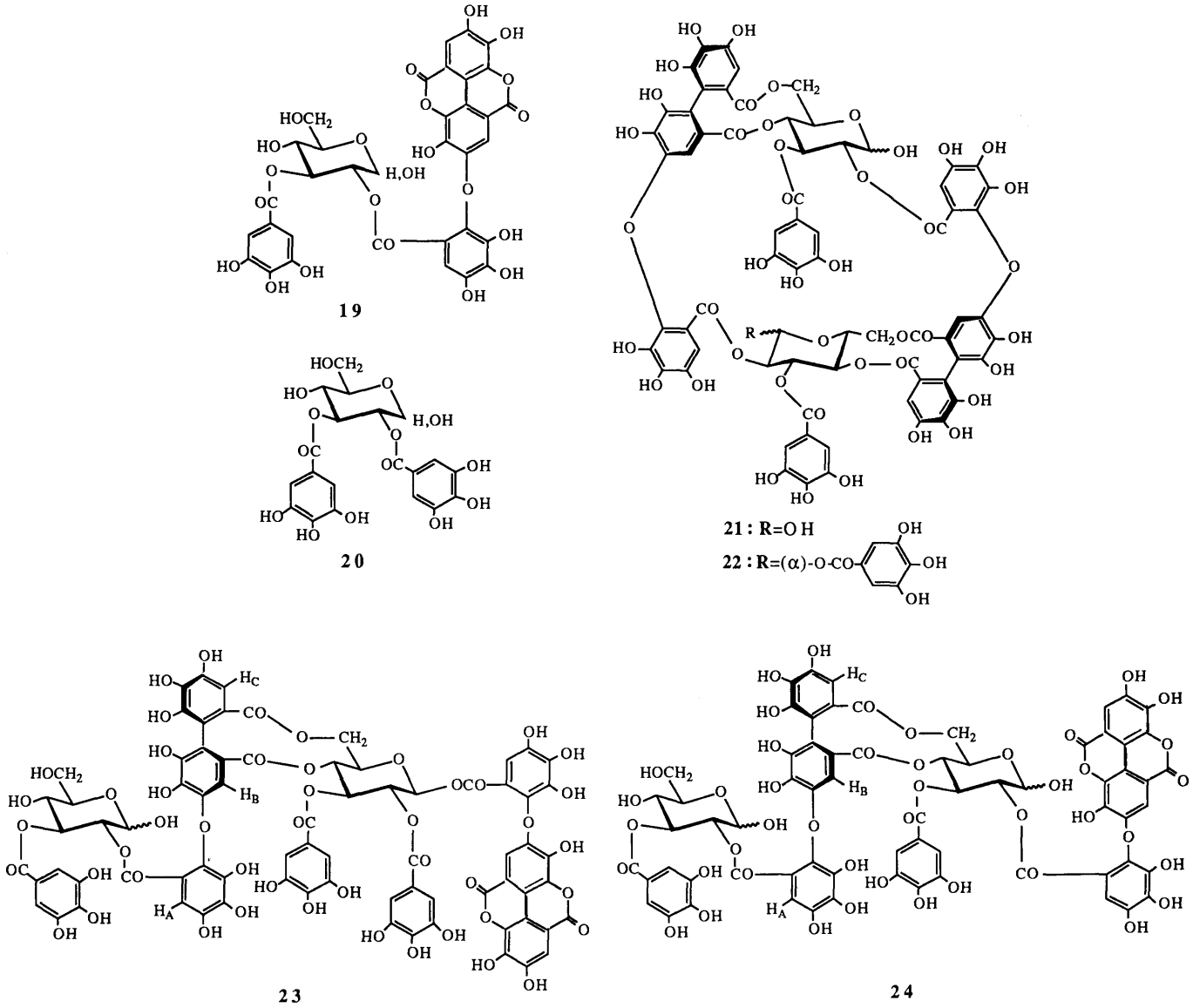


Chart 6

indicate that cleavages of the ester bonds in this hydrolysis occurred only at O-4′ and O-6′ of the glucose core II. The predominant anomer in the equilibration of **23** was β-form, unlike that of **12**, and its anomeric proton signal was observed at a remarkably higher field [δ 4.23 (d, $J=8$ Hz)] relative to the corresponding signals of **2** (δ 5.23) and **3** (δ 5.31). This anomalous upfield shift is analogous to those observed for the α,β-anomers of camelliin A (**4**) [δ 4.36, 4.37 (each d, $J=8$ Hz)] and the degradation product (**24**) [δ 4.19, 4.74] from **21**,[12] and can be interpreted in terms of the anisotropy effect of the HHDP part of the valoneoyl group at O-2.[12] The orientation of the valoneoyl group at O-4—O-6 of the fully acylated glucose core is evidently of isorugosin-type,[11] as indicated by the chemical shifts (δ 6.09 and 6.62, β-anomer) of H_B and H_C of the valoneoyl group in the ^{1}H-NMR spectrum of **23**, which are similar to those of **24** (δ 6.25 and 6.66; β-anomer). Therefore, this compound is formulated as **23**.

The above mentioned spectral data and the chemical evidence led to the structure (**12**) for camelliin B, although the orientation of the valoneoyl group at O-4′—O-6′ of the glucose core II may be reversed.

Although hydrolyzable tannin oligomers have recently been revealed to be widely distributed in plants of various families including Rosaceae, Euphorbiaceae, Coriariaceae, Cornaceae, Melastomataceae, and Onagraceae, camelliins A (**4**) and B (**12**) are the first examples of dimeric hydrolyzable tannins isolated from Theaceous plants. Camelliin B (**12**) is also an example of new class of dimers possessing macro-ring structures. Two compounds of this class, oenothein B (**21**) and woodfordin C (**22**), have been isolated very recently from *Oenothera* species (Onagraceae)[12] and from *Woodfordia fruticosa* (Lythraceae),[13] respectively. It is noteworthy that dimers of this type including camelliin B (**12**) exhibit a remarkable host-mediated anti-tumor activity against sarcoma 180 in mice.[12,14]

Experimental

NMR spectra were recorded on a Varian VXR 500 instrument (500 MHz for ^{1}H and 126 MHz for ^{13}C) in acetone-d_6 unless otherwise stated. Chemical shifts are given in δ (ppm) from tetramethylsilane. EI-MS were

taken on a Shimadzu LKB-9000 GC-MS spectrometer and FAB-MS were measured on a VG 70-SE instrument. Normal-phase HPLC was performed on a column of Develosil 60-5 (4 × 150 mm) or Superspher Si-60 using hexane–MeOH–THF–HCOOH (60:45:15:1) containing oxalic acid (500 mg/1.2 l) as the eluant, and reversed-phase HPLC on a column of YMC A312 (ODS) (6 × 150 mm) or LiChrospher 100 RP-18 with the following solvent systems: (A), 0.05 M H_3PO_4–0.05 M KH_2PO_4–EtOH–EtOAc (42.5:42.5:10:5); (B), (43.5:43.5:10:3); (C), 0.05 M H_3PO_4–0.05 M KH_2PO_4–CH_3CN (42.5:42.5:15). TLC was carried out on cellulose (Avicel) plates, developing with 7% AcOH, and preparative TLC on Kieselgel PF_{254}. Sephadex LH-20 (Pharmacia Fine Chemicals) and MCI-gel CHP-20P (Mistubishi Kasei) were used for column chromatography.

Isolation of Tannins a) From *Camellia japonica*: Fresh flower buds and flowers (3.45 kg) collected at the campus of Okayama University in March were homogenized in 70% aqueous acetone and filtered. The filtrate was concentrated, and extracted with ether. The aqueous layer was then continuously extracted with EtOAc, and was further extracted with *n*-BuOH saturated with water. A part (10 g) of the *n*-BuOH extract (18.6 g) was submitted to column chromatography over Sephadex LH-20 using the solvent system EtOH–H_2O (4:1)→EtOH–MeOH (4:1)→EtOH–MeOH (1:4). Gemin D (**1**), tellimagrandin I (**2**) and pedunculagin (**3**) were isolated from the EtOH–H_2O eluate as reported previously.[1] Camelliin A (**4**) (176 mg) was obtained from the EtOH–MeOH (1:4) eluate. The fraction eluted before **4**, which contained camelliin B, was rechromatographed over Sephadex LH-20 using EtOH–MeOH (1:4) to yield **12** (40 mg). Yields of these tannins from the *n*-BuOH extract were **1**, 1.3%; **2**, 0.7%; **3**, 0.4%; **4**, 1.7%; **12**, 0.4%.

b) From *C. sasanqua*: The fresh flower buds and flowers (3.33 kg) collected at the campus of Okayama University in December were treated in a similar way to that described in a) to give **1** (42 mg), **3** (59 mg), **4** (92 mg) and **12** (86 mg), which were identified by direct comparison with those isolated from *C. japonica*. The occurrence of tellimagrandin I (**2**) in the extract was confirmed by co-chromatography with an authentic sample using reversed-phase HPLC (solvent A, t_R 4.22 and 6.17 min) and normal phase HPLC (t_R 3.1 min).

Camelliin A (4) A light brown amorphous powder, TLC (cellulose, 7% AcOH) *Rf* 0.54, $[\alpha]_D$ +53° (*c*=0.5, MeOH). *Anal.* Calcd for $C_{68}H_{48}O_{44}\cdot 10H_2O$: C, 46.69; H, 3.92. Found: C, 46.85; H, 3.79. FAB-MS *m/z*: 1591 $[M+Na]^+$, CD (*c*=0.064, MeOH): $[\theta]_{226}+20.6\times10^4$, $[\theta]_{235}+17.9\times10^4$, $[\theta]_{259}-10.7\times10^4$, $[\theta]_{283}+5.8\times10^4$, $[\theta]_{315}-1.4\times10^4$. ^{1}H-NMR δ: 7.02, 7.01, 6.97 (2H in total, each s, galloyl), 7.05, 7.04 (1H in total, each s), 6.64, 6.63 (1H in total, each s), 6.61, 6.60, 6.59 (1H in total, each s), 6.58, 6.55, 6.54 (1H in total, each s), 6.503, 6.500, 6.494 (1H, in total, each s), 6.41, 6.40, 6.36 (1H in total, each s), 6.21, 6.19, 6.13, 6.12 (1H in total, each s) (HHDP and valoneoyl). 5.45, 5.44, 5.39, 5.37 (1H in total, each d, *J*=3.5 Hz, H-1 of α-anomer), 4.36, 4.37 (1H in total, each d, *J*=8 Hz, H-1 of β-anomer).

Camelliin B (12) An off-white amorphous powder, TLC (cellulose) *Rf* 0.44, $[\alpha]_D$ −24° (*c*=0.8, MeOH). *Anal.* Calcd for $C_{75}H_{52}O_{48}\cdot 15H_2O$: C, 45.21; H, 4.15. Found: C, 44.98; H, 3.83. FAB-MS *m/z*: 1743 $[M+Na]^+$. UV λ_{max} (MeOH) nm (log ε): 224 (5.06), 238 sh (4.87), 276 (4.78). CD (*c*=0.07, MeOH): $[\theta]_{220}+27.8\times10^4$, $[\theta]_{237}+4.9\times10^4$, $[\theta]_{254}-8.6\times10^4$, $[\theta]_{282}+5.7\times10^4$, $[\theta]_{313}-1.7\times10^4$. ^{1}H-NMR (α-anomer : β-anomer *ca.* 4:1) δ: 7.17, 7.19 (2H in total, each s), 7.11, 7.08 (2H in total, each s), 6.81, 6.84 (2H in total, each s) (galloyl), 7.41, 7.36 (1H in total, each s), 7.15 (1H, s), 6.81, 6.80 (1H in total, each s), 6.72, 6.70 (1H in total, each s), 6.46 6.44 (1H in total, each s), 5.90, 5.97 (1H in total, each s) (valoneoyl); 5.81 (d, *J*=8.5 Hz, H-1), 5.60 (dd, *J*=8.5, 10 Hz, H-2), 5.70 (t, *J*=10 Hz, H-3), 5.14 (t, *J*=10 Hz, H-4), 4.58 (ddd, *J*=10, 5.5, 7.5 Hz, H-5), 5.20 (dd, *J*=7.5, 12 Hz, H-6), 3.93 (dd, *J*=5.5, 12 Hz, H-6), 5.94 (d, *J*=4 Hz, H-1′), 5.68 (dd, *J*=4, 10 Hz, H-2′), 5.82 (t, *J*=10 Hz, H-3′), 5.24 (t, *J*=10 Hz, H-4′), 4.76 (dd, *J*=10, 5.5 Hz, H-5′), 4.94 (dd, *J*=5.5, 13 Hz, H-6′), 3.96 (d, *J*=13 Hz, H-6′) (α-anomer); 5.82 (d, *J*=8.5 Hz, H-1), 5.58 (dd, *J*=8.5, 10 Hz, H-2), 6.12 (t, *J*=10 Hz, H-3), 5.12 (t, *J*=10 Hz, H-4), 4.50 (ddd, *J*=10, 5.5, 7.5 Hz, H-5), 4.96 (dd, *J*=7.5, 12 Hz, H-6), 3.93 (dd, *J*=5.5, 12 Hz, H-6), 5.43 (H-1′ and 2′), 5.65 (t, *J*=10 Hz, H-3′), 5.24 (t, *J*=10 Hz, H-4′), 4.36 (dd, *J*=10, 5.5 Hz, H-5′), 4.94 (dd, *J*=5.5, 13 Hz, H-6′), 3.96 (d, *J*=13 Hz, H-6′) (β-anomer). ^{13}C-NMR (predominant α-anomer) δ: 162.0, 164.3, 166.4, 166.5, 167.0 (2C), 168.4 (2C), 168.9 (ester carbonyl); 93.7 (C-1), 69.8 (C-2), 71.7 (C-3), 71.1 (C-4), 71.8 (C-5), 63.9 (C-6), 92.0 (C-1′), 72.7 (C-2′), 73.5 (C-3′), 72.1 (C-4′), 68.0 (C-5′), 64.1 (C-6′) (glucose C).

Acid Hydrolysis of Camelliins A (4) and B (12) A solution of **4** (20 mg) in 5% H_2SO_4 (4 ml) was refluxed for 9 h, and extracted with EtOAc. The EtOAc layer was evaporated, and the residue was dissolved in EtOH and methylated with CH_2N_2. The syrupy residue was purified by preparative TLC [silica gel, ligroin–CH_2Cl_2–acetone (5:3:1), triple development] to give methyl tri-*O*-methylgallate [1 mg, MS *m/z* 226 (M^+)], tetra-*O*-methylellagic acid [0.7 mg, MS *m/z* 358 (M^+)], and methyl hexa-*O*-methylvaloneate dilactone [3 mg, MS *m/z* 568 (M^+), ^{1}H-NMR δ: 7.73, 7.41, 7.17 (1H each, s), 4.29, 3.99, 3.97, 3.80, 3.73 (3H each), 4.10 (6H, s)]. The aqueous layer was neutralized with Amberlite IRA-410 resin and filtered. The filtrate was evaporated to give glucose, which was identified by GLC after trimethylsilylation. Camelliin B (**12**) was similarly hydrolyzed to afford the same products as those from **4**.

Partial Hydrolysis of 4 A solution of **4** (50 mg) in H_2O (10 ml) was heated in a boiling-water bath for 7 h, and evaporated. The residue was chromatographed over MCI-gel CHP-20P (1.1 × 15 cm), eluting with H_2O→H_2O–MeOH (9:1)→H_2O–MeOH (8:2)→H_2O–MeOH (6:4) in a stepwise gradient mode, to give 2,3-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose (**7**) (1.5 mg), gemin D (**1**) (2.8 mg) and cornusiin B (**8**) (3 mg), which were identified by comparison with authentic samples (TLC, HPLC and ^{1}H-NMR).

Methylation of Praecoxin A (9) A mixture of praecoxin A (**9**) (38 mg), anhydrous potassium carbonate (200 mg) and dimethyl sulfate (0.1 ml) in dry acetone (2.5 ml), was stirred at room temperature overnight, and then refluxed for 2.5 h. Inorganic material was filtered off, and the solvent was evaporated. The residue was submitted to preparative TLC (silica gel) with double development using a solvent system of light petroleum–CH_2Cl_2–acetone (3:2:1) to yield the α-(**10**) (6 mg) and the β-anomer (**11**) (5 mg) of permethylated praecoxin A.

Hexadecamethyl-α-praecoxin A (10) A white amorphous powder, $[\alpha]_D$ −10° (*c*=0.9, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{60}O_{27}\cdot 2.5H_2O$: C, 56.02; H, 5.36. Found: C, 56.08; H, 5.12. EI-MS *m/z*: 1176 (M^+). ^{1}H-NMR δ: 6.47, 6.61, 6.84, 6.88, 7.26 (each 1H, s, HHDP and valoneoyl), 5.02 (1H, d, *J*=3.5 Hz, H-1), 5.07 (1H, dd, *J*=3.5, 10 Hz, H-2), 5.38 (1H, t, *J*=10 Hz, H-3), 4.94 (1H, t, *J*=10 Hz, H-4), 4.36 (1H, br dd, *J*=6, 10 Hz, H-5), 5.11 (dd, *J*=6, 13 Hz, H-6), *ca.* 3.85 (H-6′, overlapped with OMe signals), 3.45—4.05 (16 × OMe).

Hexadecamethyl-β-praecoxin A (11) A white amorphous powder, $[\alpha]_D$ +33° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{60}O_{27}\cdot 2.5H_2O$: C, 56.02; H, 5.36. Found: C, 55.98; H, 5.03. EI-MS *m/z*: 1176 (M^+). ^{1}H-NMR δ: 6.48, 6.60, 6.79, 6.82, 7.27 (each 1H, s, HHDP and valoneoyl), 4.80 (1H, d, *J*=8 Hz, H-1), 4.84 (1H, dd, *J*=8, 8.5 Hz, H-1), 5.26 (1H, dd, *J*=8.5, 10 Hz, H-3), 4.93 (1H, t, *J*=10 Hz, H-4), 4.32 (1H, dd, *J*=6, 10 Hz, H-5), 5.14 (dd, *J*=6, 13.5 Hz, H-6), *ca.* 3.86 (H-6′, overlapped with OMe signals), 3.50—4.05 (16 × OMe).

Methylation of Camelliin A (4) A mixture of **4** (40 mg), anhydrous potassium carbonate (200 mg) and dimethyl sulfate (0.1 ml) in dry acetone (8 ml), was stirred overnight at room temperature, and then refluxed for 2 h. Removal of the solvent after filtration gave a residue, which was purified by preparative TLC [silica gel, ligroin–CH_2Cl_2–acetone (3:2:1), double development] to give **10** (2 mg) and **11** (1 mg). These products were shown to be identical with the authentic samples described above, by direct comparisons of TLC behavior and ^{1}H-NMR spectra.

Methylation of Camelliin B (12) A mixture of **12** (50 mg), anhydrous K_2CO_3 (200 mg) and dimethyl sulfate (0.1 ml) in dry acetone (8 ml) was stirred overnight at room temperature, and then refluxed for 3.5 h. The residue obtained by filtration followed by evaporation of the solvent was purified by preparative TLC (silica gel, benzene–acetone 4:1, double development) to afford the α-anomer (**13**) of hexacosa-*O*-methylcamelliin B (3 mg) and the hexadecamethyl derivative (**15**) (1 mg).

13: White amorphous solid, $[\alpha]_D$ −42° (*c*=1.0, acetone). *Anal.* Calcd for $C_{101}H_{104}O_{48}\cdot 6H_2O$: C, 55.29; H, 5.29. Found: C, 55.07; H, 5.16. FAB-MS *m/z*: 2107 $(M+Na)^+$. ^{1}H-NMR δ: 7.38, 7.29, 7.10 (each 2H, s, galloyl), 7.43, 7.31, 7.00, 6.89, 6.75, 6.30 (each 1H s, valoneoyl), 5.87 (1H, d, *J*=8.5 Hz, H-1), 5.74 (1H, dd, *J*=8.5, 10 Hz, H-2), 5.71 (1H, t, *J*=10 Hz, H-3), 5.31 (1H, t, *J*=10 Hz, H-4), 4.51 (2H, m, H-5 and H-5′), 5.21 (1H, dd, *J*=7, 12.5 Hz, H-6), 3.98 (H-6, overlapped with OMe signals), 5.52 (1H, d, *J*=3.5 Hz, H-1′), 5.69 (br m, overlapped with H-3, H-2′), 5.80 (1H, t, *J*=10 Hz, H-3′), 5.28 (1H, t, *J*=10 Hz, H-4′), 4.85 (1H, dd, *J*=4, 13 Hz, H-6′), 4.13 (1H, dd, *J*=3, 13 Hz, H-6′), 3.60—3.91 (26 × OMe).

15: White amorphous solid, $[\alpha]_D$ −7° (*c*=0.3, acetone). FAB-MS *m/z*: 1247 $(M+Na)^+$. ^{1}H-NMR δ: 7.21, 7.11 (each 2H, s, galloyl), 7.27, 6.96, 6.33 (each 1H, s, valoneoyl), 5.24 (1H, d, *J*=4 Hz, H-1), 5.13 (1H, dd, *J*=4, 10 Hz, H-2), 5.65 (1H, t, *J*=10 Hz, H-3), 5.15 (1H, t, *J*=10 Hz, H-4), 4.42 (1H, br dd, *J*=6, 10 Hz, H-5), 5.24 (1H, dd, *J*=6, 13 Hz, H-6), H-6′ was hidden by OMe signals, 3.42—3.98 (16 × OMe).

Methylation of Rugosin B (16) A mixture of **16** (10 mg), anhydrous

potassium carbonate (100 mg) and dimethyl sulfate (0.05 ml) in dry acetone (2 ml) was stirred overnight at room temperature, and then refluxed for 1 h. After removal of inorganic material, the solvent was evaporated off, and the residue was submitted to preparative TLC (silica gel, benzene–acetone 6 : 1) to give the α- (**17**) (1 mg) and β-anomer (2.7 mg) of hexadecamethylrugosin B (**18**).

17: White amorphous powder, $[\alpha]_D$ +22° (c=0.5, acetone), ^{1}H-NMR δ: 7.26 (4H, s, galloyl), 7.30, 6.76, 6.49 (1H each, s, valoneoyl), 5.24 (1H, d, J=3.5 Hz, H-1), 5.09 (1H, dd, J=3.5, 10 Hz, H-2), 5.82 (1H, t, J=10 Hz, H-3), 5.15 (1H, t, J=10 Hz, H-4), 4.46 (ddd, J=1, 6.5, 10 Hz, H-5), 5.14 (1H, dd, J=6.5, 13 Hz, H-6), H-6′ overlapped with OMe signals, 3.43—4.02 (16 × OMe).

18: White amorphous powder, $[\alpha]_D$ +40° (c=1.0, acetone), ^{1}H-NMR δ: 7.26, 7.18 (2H each, s, galloyl), 7.30, 6.75, 6.49 (1H each, s, valoneoyl), 4.89 (1H, d, J=8 Hz, H-1), 5.33 (1H, dd, J=8, 9.5 Hz, H-2), 5.66 (1H, t, J=9.5 Hz, H-3), 5.10 (1H, t, J=9.5 Hz, H-4), 4.32 (1H, dd, J=6, 9.5 Hz, H-5), 5.20 (1H, dd, J=6, 13 Hz, H-6), H-6′ overlapped with OMe signals, 3.48—4.01 (16 × OMe).

Partial Hydrolysis of Camelliin B (12) a) A solution of **12** (100 mg) in H_2O (10 ml) was heated in a boiling-water bath for 14 h. The concentrated solution was submitted to column chromatography over MCI-gel CHP-20P (1.1 × 15 cm) developed with H_2O containing increasing amounts of MeOH to yield 2,3-di-*O*-galloyl-D-glucose (**20**) and gemin D (**1**), which were identified by comparisons with authentic samples (HPLC and ^{1}H-NMR).

b) A solution of **12** (10 mg) in 1% sulfuric acid (10 ml) was heated in a water-bath (95 °C) for 28 h. After removal of the precipitate by centrifugation, the supernatant was passed through a Sep-pack (Bond Elut C18), which was then washed with water and eluted with 20% MeOH to yield a mixture (1.2 mg) of oenothein C (**19**) and **6** in a ratio of *ca.* 1 : 1. The identities of these products were confirmed by normal-phase HPLC and reversed-phase HPLC using solvent systems A, B and C, and also by examination of the ^{1}H-NMR spectrum [**19** δ: 7.59, 7.57 (1H in total), 7.12, 7.11 (1H, in total), 7.08, 7.06 (1H in total) (valoneoyl dilactone), 7.02, 6.93 (2H in total) (galloyl), 5.20 (d, J=4 Hz, H-1), 4.87 (dd, J=4, 10 Hz, H-2), 5.66 (t, J=10 Hz, H-3) (α-anomer), 4.54 (d, J=8 Hz, H-1), 4.97 (dd, J=8, 10 Hz, H-2), 5.15 (t, J=10 Hz, H-3) (β-anomer). **6** δ: 7.60, 7.23, 7.18 (each 1H, s)].

Treatment of 12 with Boiling Water Containing CF_3COOH A solution of **12** (20 mg) in water (10 ml) containing CF_3COOH (0.1 ml) was refluxed for 1 h and then left standing for 2 d at room temperature. The reaction mixture was passed through a Sep Pack C18. After washing with water, the residue obtained from the 30% MeOH eluate was further purified by column chromatography over MCI-gel CHP-20P developed in a stepwise gradient mode with water and aqueous MeOH (20% MeOH→40% MeOH). The 40% MeOH eluate was finally purified by preparative HPLC on a column of YMC A324 (ODS) (10 × 300 mm) using solvent system (A), to give unreacted **12** (1.2 mg), and a partial hydrolysate (**23**) (1 mg), as an off-white amorphous powder. ^{1}H-NMR (acetone-d_6–D_2O) of the main β-anomer δ: 7.04, 6.88, 6.73 (each s, galloyl), 7.60, 7.19, 7.00 (each s, dilactonized valoneoyl), 6.09, 6.62, 7.06 (each s, valoneoyl), 6.03 (d, J=8 Hz, H-1), 5.44 (dd, J=8, 9.5 Hz, H-2), 5.50 (t, J=9.5 Hz, H-3), 5.00 (t, J=9.5 Hz, H-4), 4.38 (br dd, J=6, 9.5 Hz, H-5), 5.20 (dd, J=6, 13 Hz, H-6), 3.70 (br d, J=13 Hz, H-6), 4.16 (d, J=8 Hz, H-1′), 4.89 (dd, J=8, 9.5 Hz, H-2′), 5.26 (t, J=9.5 Hz, H-3′), 3.60 (t, J=9.5 Hz, H-4′), 3.86—3.62 (H-5′, H-6′).

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References and Notes

1) Part I: T. Yoshida, Y. Maruyama, M. U. Memon, T. Shingu and T. Okuda, *Phytochemistry*, **24**, 1041 (1985).
2) A part of this work was reported in a preliminary communication: T. Yoshida, T. Chou, K. Haba, Y. Okano, T. Shingu, K. Miyamoto, R. Koshiura and T. Okuda, *Chem. Pharm. Bull.*, **37**, 3174 (1989).
3) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
4) M. K. Seikel and W. E. Hillis, *Phytochemistry*, **9**, 1115 (1970).
5) T. Okuda, T. Hatano, N. Ogawa, R. Kira and M. Matsuda, *Chem. Pharm. Bull.*, **32**, 4662 (1984).
6) T. Okuda, T. Hatano, K. Yazaki and N. Ogawa, *Chem. Pharm. Bull.*, **30**, 4230 (1982).
7) T. Okuda, T. Yoshida, T. Hatano, T. Koga, N. Toh and K. Kuriyama, *Tetrahedron Lett.*, **23**, 3937 (1982).
8) T. Okuda, T. Yoshida and T. Hatano, *J. Nat. Prod.*, **52**, 1 (1989).
9) T. Yoshida, L. Chen, T. Shingu and T. Okuda, *Chem. Pharm. Bull.*, **36**, 2940 (1988).
10) K. Wilkins and B. A. Bohm, *Phytochemistry*, **15**, 211 (1976).
11) T. Hatano, R. Kira, T. Yasuhara and T. Okuda, *Heterocycles*, **27**, 2081 (1988).
12) *a*) T. Hatano, T. Yasuhara, M. Matsuda, K. Yazaki, T. Yoshida and T. Okuda, *Chem. Pharm. Bull.*, **37**, 2269 (1989); *b*) *Idem*, *J. Chem. Soc., Perkin Trans. 1*, in press.
13) T. Yoshida, T. Chou, A. Nitta and T. Okuda, *Heterocycles*, **29**, 2267 (1989).
14) T. Yoshida, T. Chou, A. Nitta, K. Miyamoto, R. Koshiura and T. Okuda, *Chem. Pharm. Bull.*, **38**, 1211 (1990).