Woodfordin C, a Macro-ring Hydrolyzable Tannin Dimer with Antitumor Activity, and Accompanying Dimers from *Woodfordia fruticosa* Flowers

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Three new dimeric hydrolyzable tannins, woodfordins A, B and C, along with seven known hydrolyzable tannins, including oenothein B, a dimer exhibiting marked host-mediated antitumor activity, were isolated from an Indonesian crude drug, Sidowayah [dried flowers of *Woodfordia fruticosa* (L.) KURZ (Lythraceae)]. The structures of the new tannins were elucidated based on chemical and spectral evidence. Woodfordin C, having a macro-ring structure, was also found to exhibit a significant antitumor activity.

Keywords *Woodfordia fruticosa*; Lythraceae; tannin; ellagitannin dimer; woodfordin A; woodfordin B; woodfordin C; oenothein B; antitumor activity

Woodfordia fruticosa (L.) KURZ (=*W. floribunda* SALISB.) (Lythraceae) is a shrub widely grown in India, east Africa and south east Asia. Its dried flower is a popular crude drug, called "Sidowayah" or "Sidawaya" in Indonesia.[1] It has been used as an astringent to treat dysentery and sprue, and also for the treatment of bowel complaints, rheumatism, dysuria and hematuria in India, Indonesia and Malaysia. It is also an ingredient of a preparation used to make barren women fertile.[1] Flavonoid glycosides, anthraquinone glycosides and several phenylpropanoids have been reported as constituents of this plant.[2] The presence of tannins in this plant has also been presumed, but only bergenin has been found as a component related to tannin. We have now isolated from this crude drug six monomeric hydrolyzable tannins and four dimeric ellagitannins including three new dimers. We report herein the structural elucidation of the new tannins,[3] and the result of an examination of their antitumor activity.

The crude drug, "Sidowayah", which was purchased in an Indonesian market,[4] was homogenized in aqueous acetone, and the homogenate was extracted with ethyl acetate and then with 1-butanol. Repeated column chromatography of each extract over Toyopearl HW-40 and MCI-gel CHP-20P yielded ten hydrolyzable tannins. Among them, seven tannins were identified as 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (**1**),[5] 1,2,4,6-tetra-*O*-galloyl-*β*-D-glucose (**2**),[5] 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (**3**),[5] tellimagrandin I (**4**),[6] gemin D (**6**),[7] heterophylliin A (**7**),[8] and oenothein B (**22**).[9] The other three were found to be new dimers and were named woodfordins A (**8**), B (**16**) and C (**20**). The major components, woodfordin C (**20**) and oenothein B (**22**) were obtained in 1.04 and 0.89% yields, respectively. Oenothein B (**22**) is a dimeric ellagitannin which was first isolated from an *Oenothera* species, and was found to exhibit remarkable host-mediated antitumor activity[10] and also anti-human immunodeficiency virus (anti-HIV) activity.

Woodfordin A (**8**) was obtained as an off-white amorphous powder and showed a retention time close to that of oenothein B in normal-phase high-performance liquid chromatography (HPLC), which suggests its dimeric character.[12] The molecular formula, $C_{75}H_{56}O_{48}\cdot 11H_2O$, was established by elemental analysis and from the fast atom bombardment mass spectrum (FAB-MS) (*m/z* 1747 $[M+Na]^+$). The ^1H nuclear magnetic resonance (^1H-NMR) spectrum of **8** exhibited three one-proton singlets at δ 6.19, 6.49 and 7.04, which are attributable to the protons of a valoneoyl group. Three two-proton singlets at δ 7.06, 7.08 and 7.15, and a singlet corresponding to six protons at δ 6.99, indicated the presence of six galloyl groups in the molecule. These constituent units of **8** were confirmed by nine ester carbonyl carbon resonances (δ 165.0—168.2) in the ^{13}C-NMR spectrum, and also by complete acid hydrolysis which gave gallic acid (**9**), valoneic acid dilactone

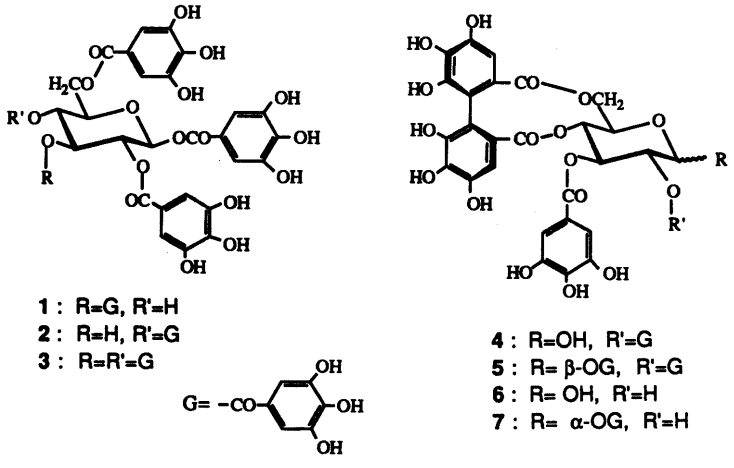


Chart 1

(**10**) and a small amount of ellagic acid (**11**), which was produced from **10** (discussed later). The sugar component produced upon this hydrolysis was identified as glucose by gas liquid chromatography (GLC) of the trimethylsilyl ether. The glucose proton signals were unequivocally assigned with the aid of the ^1H–^1H shift correlation spectrum of **8** (see Experimental). The result indicated that both glucopyranose cores adopt the $^4C_1$ conformation, and their hydroxyl groups are fully acylated except for that at C-4. The presence of a free hydroxyl group at C-4 was confirmed by the upfield shift of the H-4 signal [δ 3.68 (t, J=10.0 Hz)], in comparison with the H-4′ signal of the other glucose core [δ 5.16 (t, J=10.0 Hz)]. The β-configuration of each anomeric acyloxy group was evidenced by the large coupling constant (J=8.5 Hz) of H-1 at δ 6.15 and H-1′ at δ 6.08. The C-6 methylene proton signals of **8**, which arise from the fully acylated glucose core, appeared at δ 5.23 (dd, J=6.5, 13.0 Hz) and 3.78 (d, J=13.0 Hz). The large difference between their chemical shifts is analogous to that of other tannins having a hexahydroxydiphenoyl (HHDP) or valoneoyl group at O-4—O-6.[13] The ^{13}C resonances of the glucose moieties, whose assignments were confirmed by the ^1H–^{13}C shift correlation spectrum of **8**, were in good agreement with those of 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**1**)[14] and tellimagrandin II (**5**)[14] (Table I).

Methylation of **8** with dimethyl sulfate and potassium carbonate gave two partially degraded products, **12** and **13**, which were characterized as the polymethyl derivatives of 1,3,6-tri-*O*-galloyl-β-D-glucose and rugosin A,[15] respectively, on the basis of the ^1H-NMR spectral data (see Experimental). The identity of the latter was confirmed by direct comparison with an authentic sample pre-

Table I. ^{13}C-NMR Data for the Glucose Moieties of **1**, **5** and **8** (126 MHz, Acetone-d_6)

Carbon	1	5	8[a]
1 (1′)	93.5	93.8	93.1 (93.5)
2 (2′)	71.9	71.8	72.0 (71.8)
3 (3′)	76.0	73.3	75.5 (73.1)
4 (4′)	69.5	70.8	69.4 (70.7)
5 (5′)	76.1	73.1	76.2 (72.7)
6 (6′)	63.7	63.1	64.5 (63.2)

a) Measured in acetone-d_6 + D_2O.

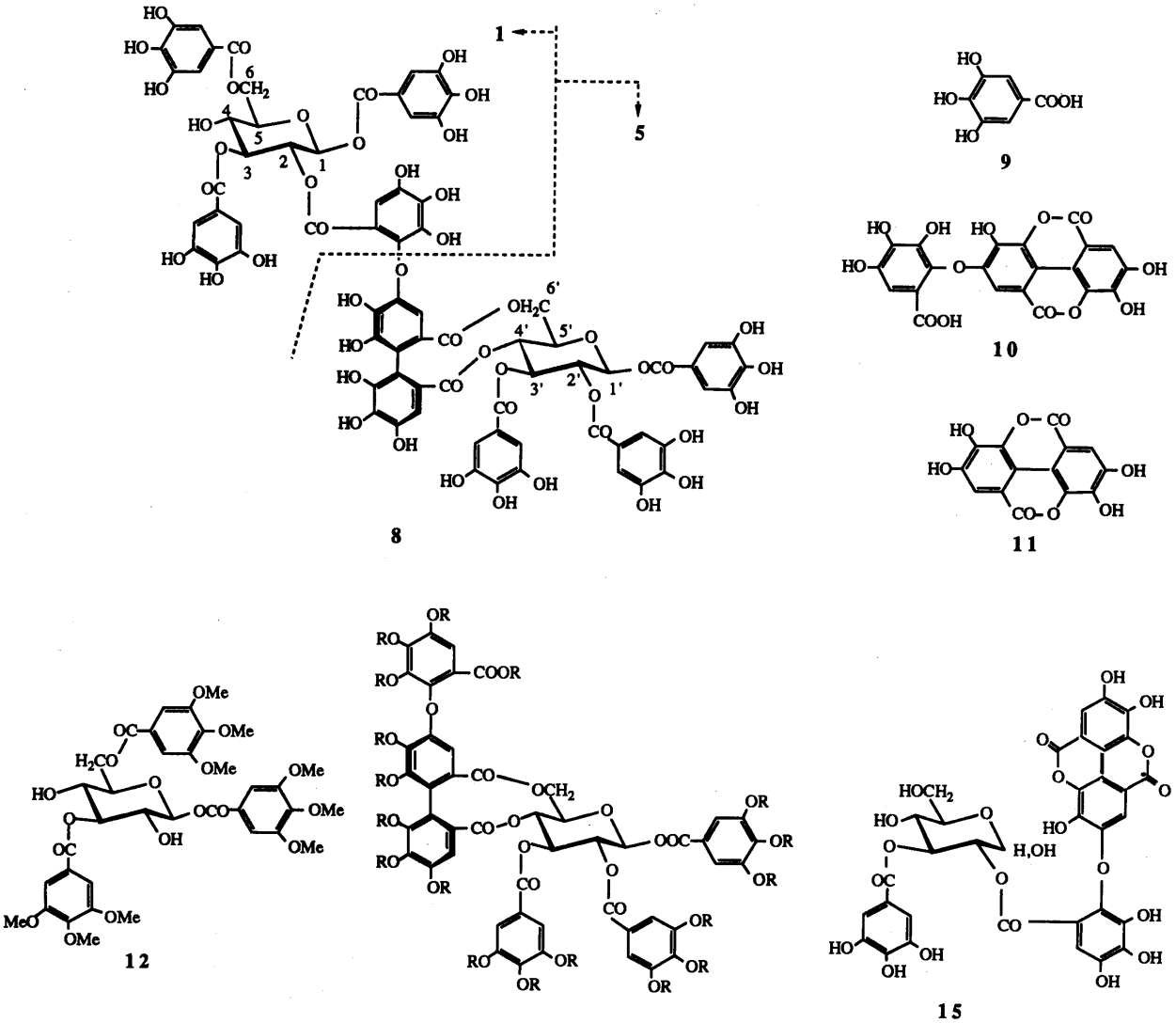


Chart 2

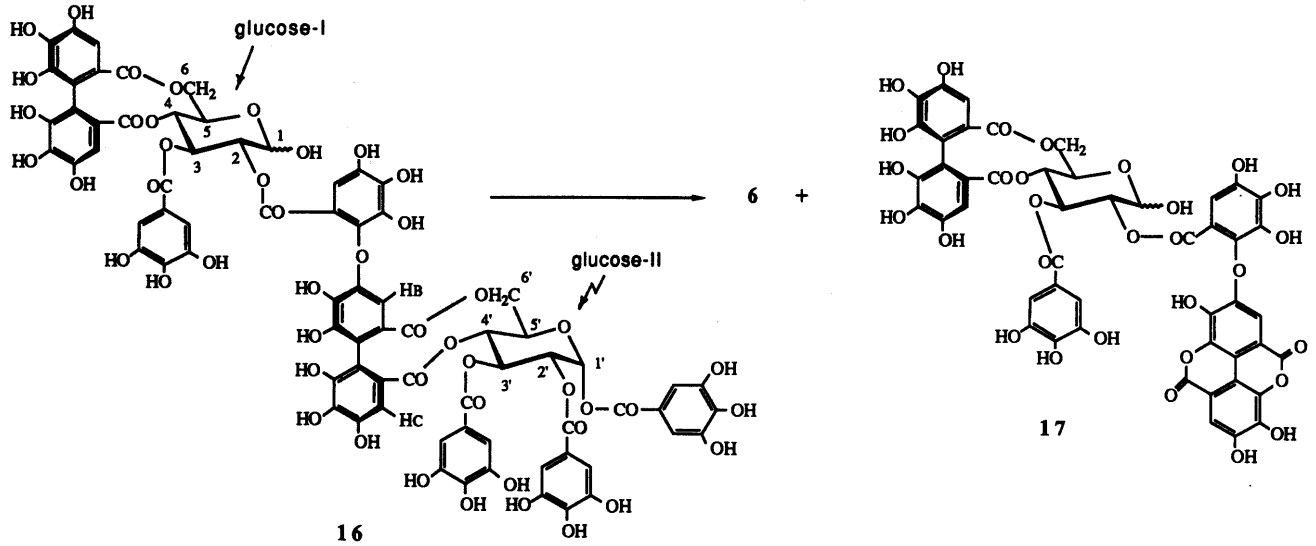
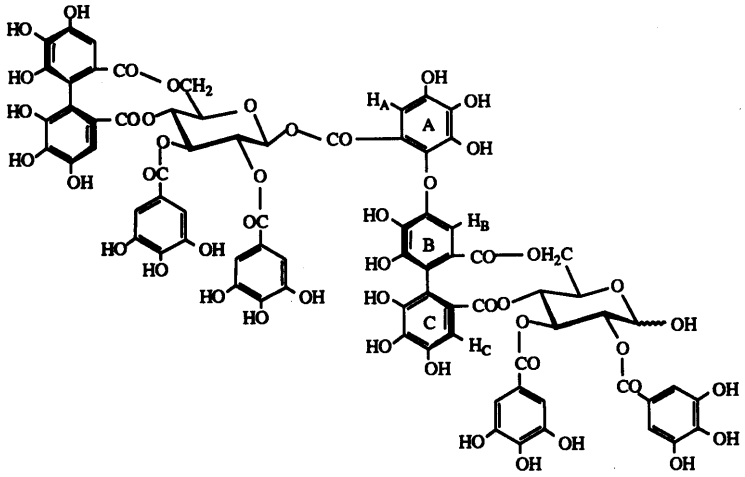


Chart 3

pared from rugosin A (**14**). On the other hand, woodfordin A (**8**) was hydrolyzed in hot water to yield 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**1**), tellimagrandin I (**4**) and oenothein C (**15**).[16] Although the formation of **4** and the above mentioned **11** from **8** may be regarded as inconsistent with the absence of an HHDP group as a constituent unit of **8**, it is attributable to a facile fission of the ether bond in the valoneoyl group, as has been observed to occur upon the hydrolyses of isorugosin D,[17] oenothein B (**22**)[9] and others.[18] Taking the production of **15** into account, the formation of **1** can also be interpreted by a similar cleavage of the valoneoyl group at O-2 of a glucose residue. The circular dichroism (CD) spectrum of **8** exhibited strong Cotton effects at 224 and 260 nm, which are characteristic of the (*S*)-valoneoyl group.[19] Based on these data, the structure of woodfordin A was established as **8**.

Woodfordin B (**16**) was isolated as an off-white amorphous powder, and showed a FAB-MS ion peak at *m/z* 1745, $[M+Na]^+$. The ^{1}H-NMR spectrum of **16** showed a set of dual peaks for each proton, suggesting the equilibration of α- and β-anomers, induced by the presence of a free anomeric hydroxyl group in one of the sugar residues. The presence of four galloyl groups was indicated by the signals at δ 7.22, 7.21 (2H in total), 7.05, 7.04 (2H in total), 7.02 (2H), and 6.98, 6.97 (2H in total). Paired signals corresponding to five protons were observed at δ 7.08, 7.07 (1H in total), 6.65, 6.64 (1H in total), 6.54, 6.53 (1H in total), 6.51, 6.48 (1H in total), and 6.28, 6.16 (1H in total). Acid hydrolysis of **16** afforded **9**, **10**, **11**, and glucose. Therefore, the above five uncoupled aromatic proton signals were attributed to an HHDP and a valoneoyl group. The atropisomerism at each biphenyl moiety of these two constituent units was determined as *S* from the CD spectrum of **16**, which exhibited a positive Cotton effect at 233 nm, the amplitude of which is larger than that of **8**.[19] The coupling pattern of the glucose signals in the ^{1}H-NMR spectrum of **16** was characteristic of $^4C_1$ glucopyranose. All of the hydroxyl groups, except for that at an anomeric center, are acylated as shown by the chemical shifts; see the experimental section. A doublet at δ 6.64 (1H, d, J=4.0 Hz) was assigned to the proton on an anomeric center bearing an α-oriented acyloxy group. The large difference ($\Delta\delta$ *ca.* 1.5 ppm) in the chemical shifts between *gem*-protons of C-6 in each glucose core clearly indicated that the HHDP group and the biphenyl moiety of the valoneoyl group are at the O-4—O-6 position of each glucose.[13] The galloyl moiety of the valoneoyl group was determined to be at O-2 of one of the glucose cores (glucose-I), based on the production of cornusiin B (**17**)[16] and gemin D (**6**)[7] upon partial hydrolysis of woodfordin B (**16**) in boiling water. The H-1 signal of the β-anomer of **16** is shifted to higher field (δ 4.42, d, J=8.0 Hz) compared with those of **4** (δ 5.13)[20] and rugosin E (**18**)[21] (δ 4.7—4.8). This anomaly can be interpreted as an anisotropic effect of the benzene ring of the valoneoyl group at C-2, as found in oenothein B (δ 4.48)[9] and camptothin B (**19**) (δ 4.53).[22] The presence of a free anomeric hydroxyl group on glucose-I was thus indicated. The orientation of the valoneoyl group at O-4′—O-6′ of the glucose core-II was indicated to be the same as that in the structure **16**, by a comparison of the chemical shifts of the aromatic proton signals of woodfordin B with those in **18** and **19**. The orientation of the valoneoyl group at O-4—O-6 of **18** and **19** is distinguishable by a diagnostic chemical shift of the H_C signal of the valoneoyl group, which resonates at a higher field in **18** (δ 6.46—6.47) than in **19** (δ 6.65—6.67). The signal pair due to H_C of the valoneoyl group in **16** (δ 6.48—6.54) was in better agreement with that in rugosin E (**18**) than with that in camptothin B (**19**).

Woodfordin C (**20**), $[\alpha]_D$ +186° (acetone), exhibited the $[M+Na]^+$ ion peak at *m/z* 1743 in the FAB-MS. Acid hydrolysis of **20** gave the same products (**9**, **10**, **11** and glucose) as those from **16**. The ^{1}H-NMR spectrum of **20** recorded at ambient temperature showed broad signals due to some of the aromatic and glucose protons, probably owing to restricted rotation around the ether bond of the valoneoyl group. However, the spectrum recorded at an elevated temperature (38 °C) indicated three two-proton singlets due to three galloyl groups, six aromatic one-proton singlets and the signals characteristic of two $^4C_1$ glucopyranoses. The anomeric proton signals, observed at δ 4.38 (br d, J=8.0 Hz) and 7.27 (d, J=3.0 Hz), suggest that the hydroxyl group at an anomeric center of a glucose core



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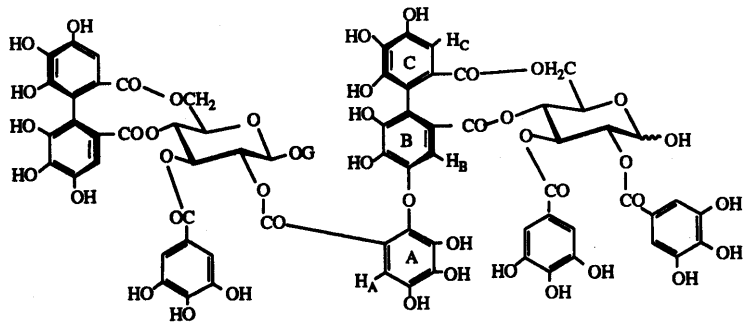

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Chart 4

(glucose-I) is free, and the other anomeric center has an α-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 **20** exists only as the β-anomer in a way analogous to oenothein B.[9,23] Methylation of **20** gave a hexacosamethyl derivative (**21**), which upon methanolysis yielded methyl tri-*O*-methylgallate, trimethyl octa-*O*-methylvaloneate, glucose and methyl β-D-glucoside. Although the broadening of some signals of **20** was observed even in the 500 MHz ^{1}H-NMR spectrum measured at 38 °C, the spectrum of **21** measured at ambient temperature exhibited sharp signals attributable to three galloyl groups [α 7.48, 7.34, 7.32 (each 2H, s)] and two valoneoyl groups [δ 7.29, 7.07, 6.87, 6.67, 6.45, 6.37 (each 1H, s)]. The presence of these groups in **21** was further supported by nine ester carbonyl carbon resonances in the ^{13}C-NMR spectrum. Ellagic acid (**11**) obtained upon the acid hydrolysis mentioned above is therefore an artifact produced by cleavage of the ether bond of the valoneoyl group, in a way similar to that observed for **8**. Woodfordin C (**20**) was thus concluded to be a dimer composed of three galloyl groups, two valoneoyl groups and two glucose residues.

A strong Cotton effect, $[\theta]+41\times10^4$, at 218 nm in the CD spectrum of **20** indicated the *S* configuration for both valoneoyl groups.[19] The locations of the acyl groups in **20** were assigned as follows. The aromatic proton signals (H_A—H_C and $H_{A'}$—$H_{C'}$) on each valoneoyl group in **21** were assigned based on the ^{1}H–^{13}C long-range shift correlation spectrum (Fig. 1). The $H_{B(B')}$ (δ 7.29, 6.45) and $H_{C(C')}$ signals (δ 6.87, 6.67) were distinguished from the $H_{A(A')}$ signals (δ 6.37, 7.07) by the cross peaks due to the three-bond couplings with C-1 signals (δ 120.8, 121.8, 123.2, 128.6) in rings-B(B′) and -C(C′) of the valoneoyl groups. The $H_{B(B')}$ and $H_{C(C')}$ signals were discriminated by the correlations through two-bond couplings of the former with the phenyl-ether carbon signals (δ 151.3, 143.2), which showed no cross peak with any methoxyl protons. The

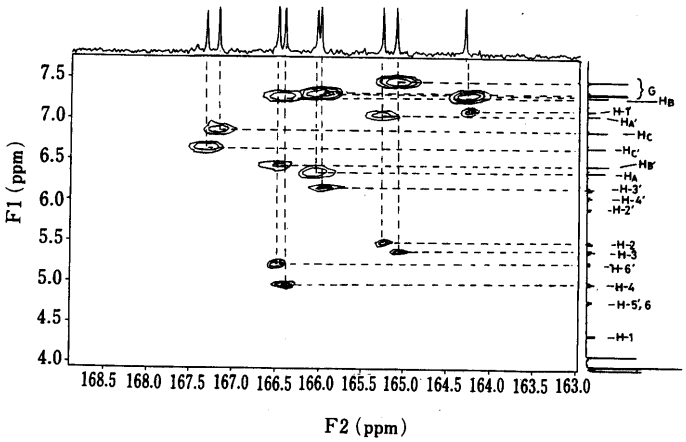
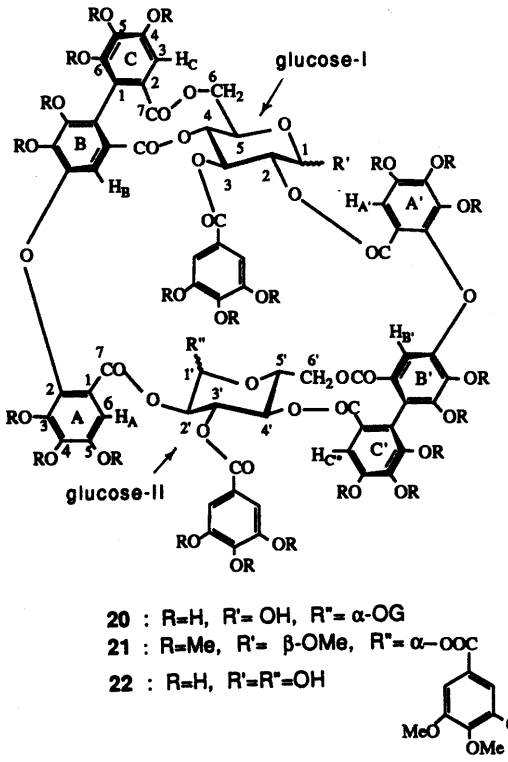
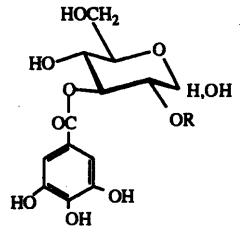


Fig. 1. The ^{1}H–^{13}C Long-Range Shift Correlation Spectrum of the Hexacosamethyl Ether (**21**) of Woodfordin C

The ^{13}C-NMR spectrum is shown in the region of 163—168 ppm. The spectrum was measured with $J_{CH}=7$ Hz.



20 : R=H, R'= OH, R"= α-OG
21 : R=Me, R'= β-OMe, R"= α-OOC
22 : R=H, R'=R"=OH



23 : R=H
24 : R=G

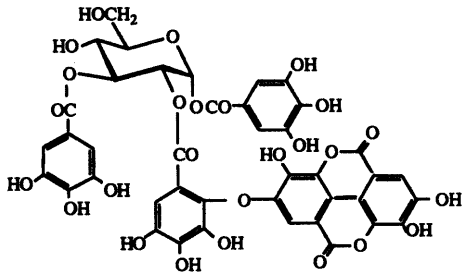


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Chart 5

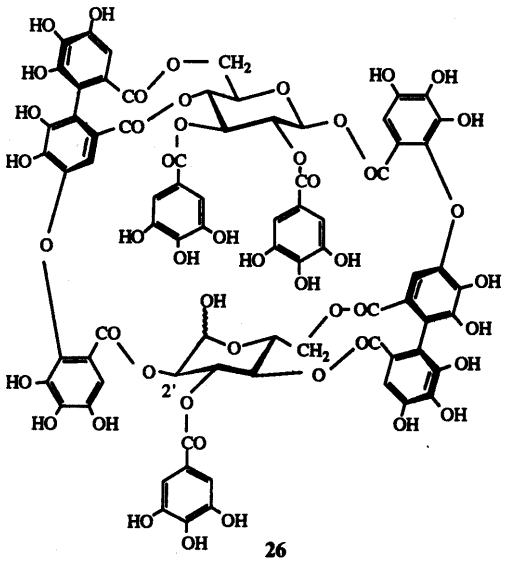

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Chart 6

TABLE II. Antitumor Activity of **8**, **20** and **26** against S-180 in Mice

Tannin	Survival days[a] (Mean ± S.D.)	% ILS	60-days survivors[b]
8	20.2 ± 5.9	−3.8	0
20	33.5 ± 11.7	59.5	1
26	28.7 ± 4.2	36.5	2
Control	21.0 ± 6.8	—	0

a) Excluding 60-days survivors. *b*) No tumor take.

connectivity between H_B (δ 7.29) and H-4 (δ 5.00, t, J= 10.0 Hz) of glucose-I was shown by a long-range correlation through the same ester carbonyl carbon signal (δ 166.4). Similarly, $H_{B'}$ (δ 6.45) was correlated with H-6′ (δ 5.26, dd, J=7.0, 13.0 Hz) of glucose-II through the signal at δ 166.5. The orientations of the valoneoyl groups at O-4—O-6 of the two glucose cores in **21** were thus assigned as formulated in **21**. Connectivity was also exhibited between H-2 and $H_{A'}$ through the signal at δ 165.3. The allocations of galloyl groups at O-3, O-3′ and O-1′ were also established by the cross peaks of H-3, H-3′ and H-1′, respectively, formed with the ester carbonyl carbons at δ 165.2, 165.9 and 164.3, which were correlated with the galloyl proton signals (Fig. 1). These data led to the structure **20** for woodfordin C, which was supported by the following chemical evidence. Partial hydrolysis of **20** in boiling water gave oenothein C (**15**),[16] 3-*O*-galloylglucose (**23**), 2,3-di-*O*-galloylglucose (**24**) and a hydrolysate (**25**). The structure **25** of this hydrolysate was assigned based on the ^{1}H-NMR spectrum, which indicated the presence of two galloyl groups [δ 7.06, 6.99 (each 2H, s)], a dilactonized valoneoyl group [α 7.58, 7.05 and 6.98 (each 1H, s)], and the glucose proton signals of H-1 (δ 6.39, d, J=3.5 Hz), H-2 (δ 5.16, dd, J=3.5, 10.5 Hz) and H-3 (δ 5.78, dd, J=9.5, 10.5 Hz) at lower field, and also on the chemical transformation of **25** into **15** by treatment with hot water containing a catalytic amount of CF_3COOH.

Woodfordin C (**20**) was thus shown to be a monogallate of oenothein B (**22**). This assignment was finally confirmed by production of **22** upon degalloylation of **20** with tannase.

Woodfordin B (**16**) is regarded as a biogenetic precursor of woodfordin C (**20**), since the latter should be formed by an intramolecular C–O coupling between the HHDP and galloyl groups of the former.

Upon intraperitoneal administration at the dose of 10 mg/kg in mice on the 4th day before intraperitoneal inoculation of sarcoma 180 cell (1×10^5), woodfordin C

(**20**) prolonged the life-span of the mice to 160%, and one of five mice survived to the 60th day (Table II). However, woodfordin A (**8**) was ineffective. Camelliin B (**26**) which is an analogous macrocyclic dimer isolated from *Camellia japonica*,[24] also exhibited a similar antitumor activity in this assay. Since the macrocyclic dimers, **20**, **26** and **22**,[10] showed significant activity, such a macro-ring structure of oligomeric hydrolyzable tannins may be one of the factors requisite for the host-mediated antitumor activity.

Experimental

Optical rotations were measured on a Jasco DIP-4 polarimeter and ultraviolet (UV) spectra on a Hitachi 200-10 spectrometer. NMR spectra were recorded on a Varian VXR 500 (500 MHz for ^{1}H-NMR and 126 MHz for ^{13}C-NMR) instrument, and chemical shifts are given in δ (ppm) from tetramethylsilane. FAB-MS were taken on a VG-70SE instrument. Thin-layer chromatography (TLC) was performed on Kieselgel PF_{254} (Merck). Toyopearl HW-40 (coarse and fine grades) (Tosoh), MCI-gel CHP-20P (Mitsubishi Kasei Industry) and Sephadex LH-20 (Pharmacia Fine Chemical) were used for column chromatography. Normal-phase HPLC was performed on a column of Superspher Si-60 using the following solvent systems; (A) hexane–MeOH–THF–HCOOH (60:45:15:1) containing oxalic acid (500 mg/1.2 l) and (B) hexane–EtOH (2:1). Reversed-phase HPLC was performed on a column of LiChrospher 100 RP-18 with (C) 0.05 M H_3PO_4–0.05 M KH_2PO_4–EtOH–EtOAc (42.5:42.5:10:5), (D) 0.05 M H_3PO_4–0.05 M KH_2PO_4–EtOH–EtOAc (40:40:15:5), and (E) 0.05 M H_3PO_4–0.05 M KH_2PO_4–CH_3CN (43.5:43.5:13).

Isolation of Tannins The dried flowers (1 kg) purchased at a market in Ismail, Indonesia, were homogenized in 70% acetone and filtered. After removal of the acetone, the aqueous solution was extracted successively with Et_2O, EtOAc and *n*-BuOH saturated with water. A part (8 g) of the EtOAc extract (15 g) was chromatographed over Toyopearl HW-40 (fine grade, 2.2 × 35 cm) developing with aqueous MeOH (60% MeOH→70% MeOH) and MeOH–acetone–H_2O [(7:2:1)→(6:2:2)]. This column chromatography was repeated twice. The 70% MeOH eluate gave tellimagrandin I (**4**) (165 mg), 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (**1**) (22 mg), 1,2,4,6-tetra-*O*-galloyl-*β*-D-glucose (**2**) (11 mg), 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose (**3**) (271 mg), oenothein B (**22**) (798 mg), and fraction I (97 mg). The MeOH–acetone–H_2O (7:2:1) eluate afforded woodfordin C (**20**) (630 mg) and fraction II (710 mg). Fraction I was rechromatographed over MCI-gel CHP-20P (1.1 × 18 cm) using MeOH as the eluant to yield heterophylliin A (**7**) (11 mg). Fraction II was similarly rechromatographed over MCI-gel CHP-20P developed with a stepwise gradient of 20% MeOH→30% MeOH→40% MeOH to give woodfordin A (**8**) (46 mg), woodfordin B (**16**) (51 mg) and woodfordin C (**20**) (101 mg). A part (7 g) of the *n*-BuOH extract (74.6 g) was subjected to column chromatography over Toyopearl HW-40 (fine) (2.2 × 35 cm) eluted with 60% MeOH, 70% MeOH and MeOH–acetone–H_2O (7:2:1) in a stepwise gradient mode to afford gemin D (**6**) (179 mg) from the 60% MeOH eluate, and oenothein B (**22**) (770 mg) and woodfordin C (**20**) (920 mg) from the MeOH–acetone–H_2O (7:2:1) eluate.

Woodfordin A (8) An off-white amorphous powder, $[\alpha]_D$ +60° (*c* = 1.0, acetone). *Anal.* Calcd for $C_{75}H_{56}O_{48}\cdot 11H_2O$: C, 46.83; H, 4.06. Found: C, 46.79; H, 3.89. FAB-MS *m/z*: 1747 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 218 (5.15), 276 (4.79). CD (MeOH) [θ] (nm): $+13.7\times10^4$ (224), -0.7×10^4 (260), $+4.2\times10^4$ (282). ^{1}H-NMR (acetone-d_6 + D_2O) δ: 6.08 (d, *J* = 8.5 Hz, H-1), 5.43 (dd, *J* = 8.5, 10.0 Hz, H-2), 5.60 (t, *J* = 10.0 Hz, H-3), 3.68 (t, *J* = 10.0 Hz, H-4), 4.06 (m, H-5), 4.68 (dd, *J* = 2.0, 12.5 Hz, H-6), 4.43 (dd, *J* = 6.5, 12.5 Hz, H-6), 6.15 (d, *J* = 8.5 Hz, H-1′), 5.57 (dd, *J* = 8.5, 10.0 Hz, H-2′), 5.81 (t, *J* = 10.0 Hz, H-3′), 5.16 (t, *J* = 10.0 Hz, H-4′), 4.49 (dd, *J* = 6.5, 10.0 Hz, H-5′), 5.23 (dd, *J* = 6.5, 13.0 Hz, H-6′), 3.78 (d, *J* = 13.0 Hz, H-6′), aromatic protons see text. ^{13}C-NMR (acetone-d_6 + D_2O) δ: 168.2, 167.8, 167.1, 166.5, 166.4, 165.9, 165.2, 165.1, 165.0 (ester carbonyl), glucose carbons, see Table I.

Woodfordin B (16) An off-white amorphous powder, $[\alpha]_D$ +93° (*c* = 1.0, acetone). *Anal.* Calcd for $C_{75}H_{54}O_{48}\cdot 8H_2O$: C, 48.23; H, 3.75. Found: C, 48.04; H, 3.95. FAB-MS *m/z*: 1745 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 219 (5.10), 268 (4.78). CD (MeOH) [θ] (nm): $+18.5\times10^4$ (233), -5.7×10^4 (260), $+0.1\times10^4$ (286). ^{1}H-NMR (acetone-d_6 + D_2O) δ: α-anomer: 5.50 (d, *J* = 4.0 Hz, H-1), 5.10 (dd, *J* = 4.0, 10.5 Hz, H-2), 5.82 (t, *J* = 10.5 Hz, H-3), 5.06 (t, *J* = 10.5 Hz, H-4), 4.70 (m, H-5), 5.27 (dd, *J* = 6.5, 12.0 Hz, H-6), 3.84 (d, *J* = 12.0 Hz, H-6), 6.64 (d, *J* = 4.0 Hz, H-1′), 5.47 (dd, *J* = 4.0, 10.0 Hz, H-2′), 5.96 (t, *J* = 10.0 Hz, H-3′), 5.26 (t, *J* = 10.0 Hz, H-4′), 4.70 (m, H-5′), 5.24 (dd, *J* = 7.0, 13.5 Hz, H-6′), 3.77 (d, *J* = 13.5 Hz, H-6′), β-anomer; 4.45 (d, *J* = 8.0 Hz, H-1), 5.15 (dd, *J* = 8.0, 10.0 Hz, H-2), 5.54 (t, *J* = 10.0 Hz, H-3), 5.10 (t, *J* = 10.0 Hz, H-4), 4.09 (m, H-5), 5.27 (dd, *J* = 6.5, 12.5 Hz, H-6), 3.74 (d, *J* = 12.5 Hz, H-6), 6.64 (d, *J* = 4.0 Hz, H-1′), 5.54 (dd, *J* = 4.0, 10.5 Hz, H-2′), 5.95 (t, *J* = 10.5 Hz, H-3′), 5.24 (t, *J* = 10.5 Hz, H-4′), 4.69 (m, H-5′), 5.20 (dd, *J* = 6.5, 13.0 Hz, H-6′), 3.74 (d, *J* = 13.0 Hz, H-6′), aromatic protons, see text. ^{13}C-NMR (acetone-d_6 + D_2O) δ: 167.4, 167.2, 167.2, 167.2, 167.0, 166.8, 166.8, 166.7, 166.7, 165.8, 165.8, 165.6, 165.5, 164.9, 164.9, 163.8, 163.7, 163.4 (ester CO).

Acid Hydrolysis of Woodfordins A (8) and B (16) A solution of **8** (2 mg) in 5% H_2SO_4 (1 ml) was heated in a boiling-water bath for 5 h. The reaction mixture was extracted with EtOAc. The residue obtained from the EtOAc soluble portion was analyzed by reversed-phase HPLC using solvent systems B, C and D, and the peaks due to gallic acid and valoneic acid dilactone were identified by co-chromatography with authentic samples. The aqueous layer gave glucose, which was identified by GLC (column, 2% OV-1; column temperature, 170 °C) after trimethylsilylation. Woodfordin B (**16**) was also hydrolyzed similarly to yield the same products as those from **8**.

Partial Hydrolysis of Woodfordin A (8) A solution of **8** (2 mg) in water was heated in a boiling-water bath for 10 h. The reaction mixture was submitted to HPLC analysis to reveal the presence of tellimagrandin I (**4**), 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (**1**), and oenothein C (**14**), as hydrolysates. Their identities were confirmed by co-chromatography with authentic specimens, in HPLC using several solvent systems (reversed-phase, solvents, C, D and E, and normal-phase, solvent A).

Methylation of Woodfordin A (8) A mixture of **8** (20 mg), anhydrous K_2CO_3 (200 mg) and Me_2SO_4 (0.1 ml) in dry acetone (5 ml) was stirred overnight at room temperature, and then refluxed for 3 h. The inorganic material was filtered off, and the filtrate was evaporated. The residue was purified by preparative TLC developed with benzene–acetone (4:1) to give the nonamethyl derivative (**12**) (1.2 mg) of 1,3,6-tri-*O*-galloyl-*β*-D-glucose and the octadecamethyl derivative (**13**) (1 mg) of rugosin A.

12: White amorphous powder. ^{1}H-NMR (acetone-d_6) δ: 7.35, 7.32, 7.21 (2H each, s, galloyl), 5.96 (d, *J* = 8.0 Hz, H-1), 5.31 (dd, *J* = 9.0 Hz, H-3), 4.77 (dd, *J* = 2.5, 12.0 Hz, H-6), 4.36 (dd, *J* = 7.0, 12.0 Hz, H-6′), 3.88, 3.84, 3.82 (6H each, s, 6 × OMe)), 3.77, 3.73, 3.72 (each 3H, s, 3 × OMe).

13: White amorphous powder. ^{1}H-NMR (acetone-d_6) δ: 7.31, 7.24, 7.23 (each 2H, s, galloyl), 6.48, 6.77, 7.27 (each 1H, s, valoneoyl), 6.27 (d, *J* = 8.5 Hz, H-1), 5.66 (dd, *J* = 8.5, 10.0 Hz, H-2), 5.94 (t, *J* = 10.0 Hz, H-3), 5.29 (t, *J* = 10.0 Hz, H-4), 5.25 (dd, *J* = 6.0, 13.0 Hz, H-6), 4.63 (dd, *J* = 6.0, 10.0 Hz, H-5), 4.02, 3.91, 3.90, 3.87, 3.85, 3.78, 3.75, 3.72, 3.71, 3.70, 3.67 (each 3H, s), 3.88, 3.81 (each 6H, s), 3.77 (9H, s) (18 × OMe). This compound was identical with an authentic sample prepared from rugosin A, by co-chromatography on TLC (benzene–acetone, 4:1), and HPLC (normal phase, solvent B), and by ^{1}H-NMR spectral comparison.

Partial Hydrolysis of Woodfordin B (16) A solution of **16** (2 mg) in water (2 ml) was treated as described for **8**, to reveal the peaks due to gemin D (**6**) and cornusiin B (**17**) in the HPLC analysis [normal-phase (solvent A) and reversed-phase (solvents C, D and E)].

Woodfordin C (20) An off-white amorphous powder, $[\alpha]_D$ +186° (*c* = 1.0, acetone). *Anal.* Calcd for $C_{75}H_{52}O_{48}\cdot 14H_2O$: C, 45.64; H, 4.05. Found: C, 45.75; H, 3.72. FAB-MS *m/z*: 1743 $(M+Na)^+$. UV λ_{max}^{MeOH} nm (log ε): 219 (5.15), 269 (4.83). CD (MeOH) [θ] (nm): $+41\times10^4$ (218), $+18\times10^4$ (225), -2.9×10^4 (260). ^{1}H-NMR (acetone-d_6, 38 °C) δ: 7.02, 7.20, 7.30 (each 2H, s) (galloyl), 6.31 (1H, s), 6.49 (1H, br), 6.53 (1H, s), 6.67 (1H, s), 6.68 (1H, br s), 7.09 (1H, br) (valoneoyl), 4.38 (t, *J* = 8.0 Hz, H-1; this signal collapsed to a doublet *J* = 8.0 Hz upon addition of D_2O), 5.70 (1H, br, OH), 5.17 (dd, *J* = 8.0, 9.6 Hz, H-2), 5.48 (t, *J* = 9.6 Hz, H-3), 4.90 (t, *J* = 9.6 Hz, H-4), 5.03 (br dd, *J* = 5.1, 13.0 Hz, H-6), 4.13 (dd, *J* = 5.1, 9.6 Hz, H-5), 3.86 (d, *J* = 13.0 Hz, H-6), 7.27 (d, *J* = 3.0 Hz, H-1′), 6.21 (2H, br, H-2′ and H-3′), 5.79 (1H, br t, H-4′), 5.33 (dd, *J* = 6.6, 13.3 Hz, H-6′), 4.67 (dd, *J* = 6.6, 10.0 Hz, H-5′), 3.69 (d, *J* = 13.3 Hz, H-6′). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 95.7 (C-1), 74.6 (C-2), 73.1 (C-3), 71.8 (C-5), 91.2 (C-1′), 71.3 (C-3′), 72.3 (C-5′), 73.9, 72.9, 69.5 (C-4, C-2′, C-4′), 65.3, 62.6 (C-6, C-6′), 164.9, 166.2, 166.8, 167.6 (3C), 167.9, 168.2, 169.8 (ester CO).

Methylation of Woodfordin C (20) A mixture of **20** (55 mg), K_2CO_3 (200 mg) and Me_2SO_4 (0.1 ml) in dry acetone (10 ml) was stirred overnight at room temperature, and then refluxed for 3.5 h. The reaction mixture was purified by preparative TLC (benzene–acetone, 4:1, double development) to afford the hexacosamethyl ether (**21**) (43 mg) as a white amorphous powder, $[\alpha]_D$ +102° (*c* = 1.0, acetone). UV λ_{max}^{MeOH} nm (log ε); 215 (4.97), 247 (4.58). ^{1}H-NMR (acetone-d_6) δ: 4.37 (d, *J* = 8.0 Hz, H-1), 5.51 (dd, *J* = 8.0, 10.0 Hz, H-2), 5.40 (t, *J* = 10.0 Hz, H-3), 5.00 (t, *J* = 10.0 Hz, H-4), 4.78 (dd,

J=5.0, 13.0 Hz, H-6), 3.8—4.1 (H-5, H-6, overlapped by OMe signals), 7.12 (d, J=3.8 Hz, H-1′), 5.93 (dd, J=3.8, 10.0 Hz, H-2′), 6.17 (t, J=10.0 Hz, H-3′), 6.06 (t, J=10.0 Hz, H-4′), 4.76 (m, H-5′), 5.26 (dd, J=7.0, 13.0 Hz, H-6′), 3.58 (d, J=13.0 Hz, H-6′), aromatic protons, see text. ^{13}C-NMR (acetone-d_6) δ: 101.5 (C-1), 72.2 (C-2), 73.4 (C-3), 73.0 (C-4), 71.3 (C-5), 62.5 (C-6), 90.6 (C-1′), 72.6 (C-2′), 71.1 (C-3′), 68.5 (C-4′), 70.5 (C-5′), 64.0 (C-6′), 164.3, 165.2, 165.9 (galloyl ester CO), 165.3 (ring-A′, C-7), 166.1 (ring-A, C-7), 166.4 (ring-B, C-7), 166.5 (ring-B′, C-7), 167.1 (ring-C, C-7), 167.3 (ring-C′, C-7).

Methanolysis of 21 A solution of **21** (10 mg) in absolute MeOH (2 ml) containing 1% NaOMe (0.1 ml) was left standing overnight at room temperature. The solution was acidified with AcOH, then the solvent was evaporated off. The residue was partitioned between water and EtOAc. Purification of the residue obtained from the EtOAc-soluble portion was carried out by preparative TLC (benzene–acetone, 4 : 1) to give methyl tri-*O*-methylgallate (2.3 mg) and trimethyl octa-*O*-methylvaloneate (2.3 mg), which were identified by TLC and from the ^{1}H-NMR spectra. The sugar components in the aqueous layer were identified as glucose and methyl β-D-glucoside by GLC after trimethylsilyation.

Partial Hydrolysis of 20 A solution of **20** (50 mg) in 1% H_2SO_4 (5 ml) was heated on a boiling-water bath for 15 h. Insoluble materials were filtered off, then the filtrate was concentrated and subjected to column chromatography over MCI-gel CHP-20P (1.1 × 16 cm) developing in a stepwise gradient mode with water→10% MeOH→20% MeOH→30% MeOH to give 3-*O*-galloylglucose (**23**) (3 mg), gallic acid (5 mg), 2,3-di-*O*-galloylglucose (**24**) (2 mg), oenothein C (**15**) (16 mg) and the hydrolysate (**25**) (3 mg).

Hydrolysate (**25**): Light brown amorphous powder, $[\alpha]_D$ +172° (c=1.0, acetone), FAB-MS m/z: 937 $(M+H)^+$. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.06, 6.99 (each 2H, s, galloyl), 7.58, 7.05, 6.98 (each 1H, s, dilactonized valoneoyl), 6.39 (d, J=3.5 Hz, H-1), 5.16 (dd, J=3.5, 10.5 Hz, H-2), 5.78 (dd, J=9.5, 10.5 Hz, H-3), 4.60 (t, J=9.5 Hz, H-4), 3.91 (m, H-5), 3.75 (m, H-6, 6′). A solution of **25** (1 mg) in water (1 ml) containing CF_3COOH (2 drops) was heated on a boiling-water bath for 2 h, and the formation of **15** was confirmed by HPLC (reversed-phase, solvents C—E) of the reaction mixture.

Hydrolysis of Woodfordin C (20) with Tannase A solution of **20** (20 mg) in H_2O (5 ml) was incubated with tannase (5 drops) prepared according to the literature,[25] at 37 °C for 36 h. The reaction mixture after concentration was passed through Sep-pack C18, which was washed with water, and then eluted with 20% MeOH to give oenothein B (**22**) (15 mg). This product was identified by HPLC and from the ^{1}H-NMR spectrum.

Antitumor Experiments Antitumor activity was estimated according to the previously reported method.[10] Tannins were dissolved in sterilized physiological saline before use. Five female ddY mice (6 weeks old) (Shizuoka Laboratory Animal Center) per group were intraperitoneally given 10 mg/kg of a test compound once at 4 d before intraperitoneal inoculation of the tumor cells (sarcoma 180) (1×10^5). Sixty days after the tumor cell inoculation, survivors were killed and autopsied, and the antitumor activity of each tannin was evaluated in terms of the percent increase in the life-span (%ILS).[10]

Acknowledgements We are grateful to Dr. N. Toh, Faculty of Chemical Engineering, Kyushu Kyoritsu University for measuring CD spectra, and also to the SC-NMR Laboratory of Okayama University for the NMR measurements.

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