Tannins and Related Polyphenols of Lythraceous Plants. III.[1] Hydrolyzable Tannin Oligomers with Macrocyclic Structures, and Accompanying Tannins from *Woodfordia fruticosa* KURZ

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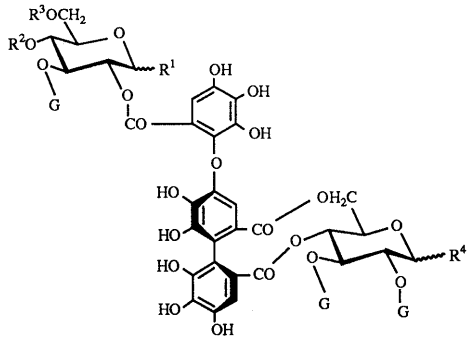
Besides previously reported hydrolyzable tannin oligomers (woodfordins A—D), a new hydrolyzable tannin monomer [isoschimawalin A (7)] and five oligomers (woodfordins E—I) have been isolated from the dried flowers of *Woodfordia fruticosa* (an Indonesian crude drug, Sidowaya), and their structures elucidated on the basis of spectra and chemical evidence. Woodfordins G (14) and H (15) were characterized as dimers with structures related to woodfordin B (2). Woodfordins I (16), E (23) and F (24) were a macrocyclic dimer, trimer and tetramer, respectively.

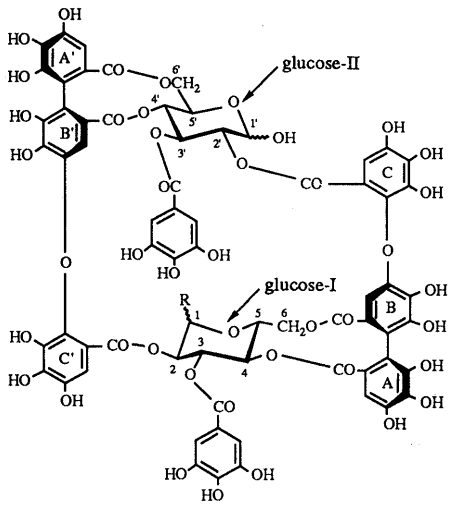
Keywords *Woodfordia fruticosa*: Lythraceae; tannin; ellagitannin; woodfordin E; woodfordin F; woodfordin G; woodfordin H; woodfordin I

Woodfordia fruticosa KURZ (Lythraceae) is widely grown in Indonesia, Malaysia, and India. Its dried flower is popularly used as a traditional medicine (Jamu medicine) called Sidowaya or Sedowaya, for the treatment for diarrhea, rheumatism and sprue.[2] We previosuly reported the isolation and characterization of hydrolyzable tannin dimers, woodfordins A (**1**), B (**2**) and C (**3**)[3] and oenothein B (**4**),[3,4] together with trimers, woodfordin D (**5**) and oenothein A (**6**), from this crude drug.[1] The dimers, woodfordin C (**3**) and oenothein B (**4**) with macrocyclic structures, exhibited a potent host-mediated antitumor activity against sarcoma-180 in mice.[3] Further investigation of this crude drug has resulted in the isolation of six more new hydrolyzable tannins, named isoschimawalin A (**7**), and woodfordins E (**23**), F (**24**), G (**14**), H (**15**), and I (**16**).

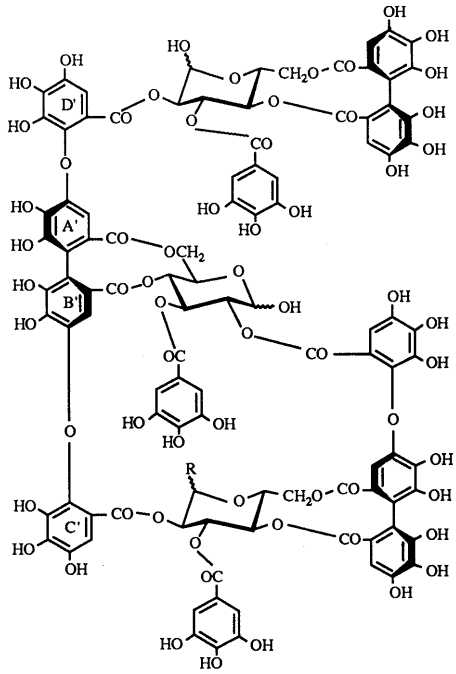
These new tannins were obtained from the water-soluble portion after *n*-BuOH extraction of the aqueous acetone homogenate of the dried flowers, as described in Experimental. Woodfordins F and H were also isolated from the



1 : R^1=(β)-OG, R^2=H, R^3=G, R^4=(β)-OG
2 : R^1=OH, R^4=(α)-OG, R^2, R^3=(*S*)-HHDP



3 : R=(α)-OG
4 : R=OH



5 : R=(α)-OG
6 : R=OH

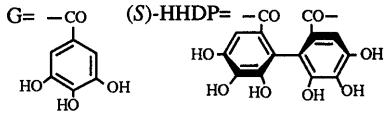


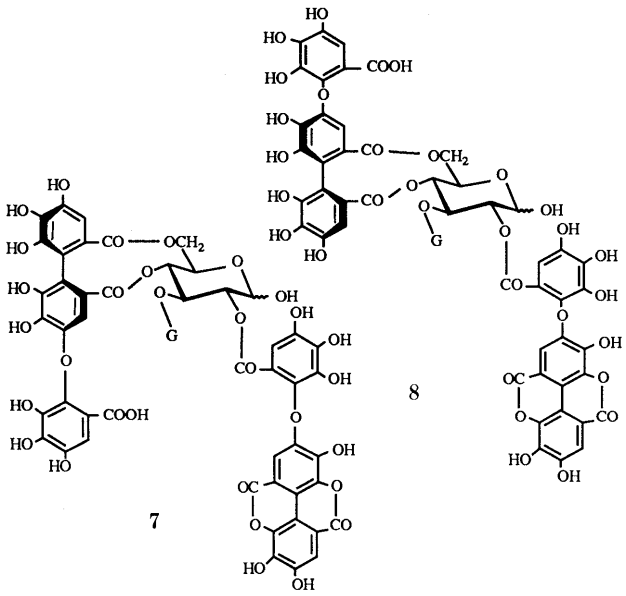
Chart 1

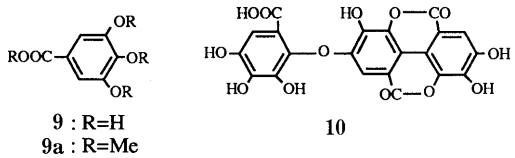
n-BuOH extract.

The molecular size of these new tannins was estimated by fast-atom bombardment mass spectroscopy (FAB-MS) and the retention times in high-performance liquid chromatography (HPLC)(normal phase),[5] which indicated that isoschimawalin A (**7**) is monomeric, woodfordins G (**14**), H (**15**), and I (**16**) are dimeric, and woodfordins E (**23**) and F (**24**) are trimeric and tetrameric, respectively.

Isoschimawalin A (**7**), $[\alpha]_D$ +37° (MeOH), showed the $(M+Na)^+$ ion peak at *m/z* 1277 in FAB-MS, corresponding to the molecular formula $C_{55}H_{34}O_{35}$. Acid hydrolysis of **7** yielded gallic acid (**9**), valoneic acid dilactone (**10**) and glucose. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **7** disclosed the sugar proton signals characteristic of C1 glucopyranose. The duplication of each signal and the appearance of the anomeric proton signals at δ 5.34 (d, J=4 Hz) and 4.74 (d, J=8 Hz) indicated that **7** exists as an equilibrium mixture of α- and β-anomers in a ratio of *ca.* 5:4 (see Experimental). A pair of 2H singlets (δ 6.76, 6.79) and pairs of three 1H singlets (δ 6.20, 6.23; 6.56, 6.56; 6.99, 7.02) attributable to a galloyl and a valoneoyl group, were also seen in the aromatic proton region. Three additional 1H singlets, appearing as duplicated signals at lower field (δ 7.60, 7.61; 7.21, 7.23; 7.08, 7.10), are characteristic of a dilactonized valoneoyl group.[4,6] The absolute configuration of the chiral valoneoyl group in **7** was determined as (*S*), from the strong positive Cotton effect at 218 and 238 nm in the circular dichroism (CD) spectrum.[7] These spectral features and the ^{13}C-NMR spectral data are closely related to those of schimawalin A (**8**), which was recently isolated from *Schima wallichii* (Theaceae).[6] The valoneoyl H_A signal (δ 6.56) of **7** appears, however, at lower field than that (δ 6.42, 6.47) of **8**, implying that the compound (**7**) is an isomer of **8** concerning the orientation of the valoneoyl group at O-4/O-6. The structure **7** thus proposed for isoschimawalin A was substantiated by its partial degradation in an acidic solution yielding oenothein C (**11**).[4,8] We recently reported that the ether linkage of the valoneoyl group in ellagitannins is susceptible to reductive cleavage upon treatment with phosphate buffer (pH 7.4).[9] Application of this reaction to **7** gave cornusiin B (**12**)[8] and isorugosin B (**13**).[10] The structure of isoschimawalin A was thus established as **7**.

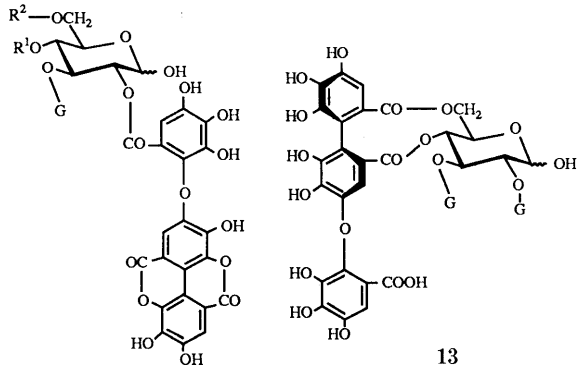
Woodfordin G (**14**), $[\alpha]_D$ +63° (MeOH), showed the $(M+Na)^+$ ion peak at *m/z* 1591 in the FAB-MS. Acid hydrolysis of **14** gave gallic acid (**9**), valoneic acid dilactone (**10**) and glucose. Its ^{1}H-NMR spectrum showed each proton signal split into four lines (*ca.* 3:2:1:1) due to anomerization at both anomeric centers of the glucose cores (giving four isomers), and also showed the presence of a valoneoyl, a dilactonized valoneoyl and two galloyl groups in the molecule. The chemical shift of the valoneoyl H_A proton signal (δ 6.42—6.49) is similar to that (δ 6.42, 6.47) of schimawalin A (**8**),[6] but not to that (δ 6.57) of **7**. The signal pattern of one (glucose-I) of the two glucose cores, in which the anomeric proton signals are at δ 5.33, 5.38 (each d, J=4 Hz) and 4.72, 4.76 (each d, J=8 Hz), is also identical with that of **8**. Woodfordin G should thus





9 : R=H
9a : R=Me

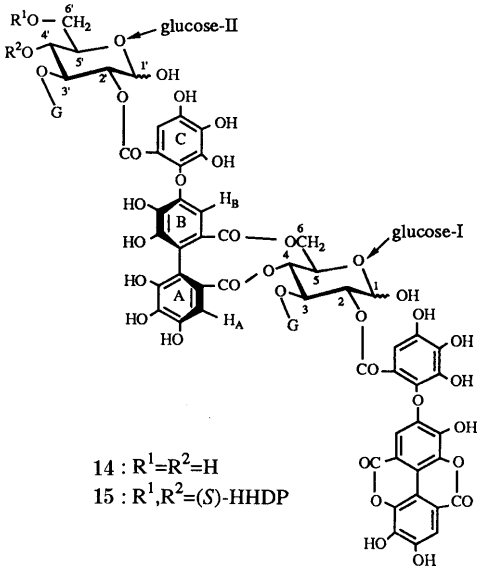
10



11 : R^1=R^2=H
12 : R^1, R^2=(*S*)-HHDP

13

Chart 2



14 : R^1=R^2=H
15 : R^1,R^2=(*S*)-HHDP

Chart 3

have the schimawalin A (**8**) moiety as one of the monomeric units. The anomeric proton signal of the β-anomer of the other glucose core (glucose-II) is at a high field region [δ 4.17 (d, $J=8$ Hz)], similarly to that of **7** and **8**. The galloyl part in the valoneoyl group is therefore connected to O–2′ of the glucose-II.[4,9] The presence of free hydroxyl groups at C–4′ and C–6′ of the glucose-II was shown by the upfield shifts (δ 3.5—3.8) of the H-4′ and H-6′ signals, in the ^{1}H–^{1}H shift correlation spectrum of **14**. Based on these spectral data, woodfordin G was formulated as **14**, which was substantiated by partial hydrolysis of oenothein B (**4**) in a weakly acidic medium to give **14**.

Woodfordin H (**15**), $[\alpha]_D$ +88° (MeOH), showed the $(M+Na)^+$ ion peak at m/z 1893, which is 302 mass unit (corresponding to a hexahydroxydiphenoyl (HHDP) group) larger than that of **14**. The ^{1}H-NMR spectrum of **15** showed a four line (or partially overlapped three line) signal for each proton, owing to its existence as a mixture of four isomers, as observed for **14**. The aromatic proton signals are similar to those of **14** except for the extra signals (δ 6.48—6.62) due to an HHDP group (see Experimental), being in agreement with the nature and number of the acyl group of **15**; two galloyl, an HHDP, a valoneoyl and a dilactonized valoneoyl group. The structural similarity between **15** and **14** was also indicated by the similarity of the glucose signals in their ^{1}H- and ^{13}C-NMR spectra, though the H-4′ and H-6′ signals of glucose-II in **15** are shifted to lower field by *ca.* 1.5 ppm from the corresponding signals of **14**. Upon partial hydrolysis in a hot aqueous solution containing trifluoroacetic acid, woodfordin H was converted into **14**, thus establishing the structure **15** for woodfordin H.

Woodfordin I (**16**), $[\alpha]_D$ +126° (MeOH), was obtained as an off-white amorphous powder, and exhibited the $(M+Na)^+$ ion peak at m/z 1759 in FAB-MS. Upon methylation followed by methanolysis with sodium methoxide in methanol, woodfordin I (**16**) afforded methyl tri-*O*-methylgallate (**9a**), trimethyl octa-*O*-methylvaloneate (**17**) and tetramethyl deca-*O*-methylwoodfordinate (**18**),[1] in a molar ratio of 2:1:1, as estimated by quantitative HPLC analysis. The ^{1}H-NMR spectrum of **16** showed marked broadening of several aromatic and sugar proton signals, being a characteristic feature of macrocyclic oligomers, such as **3**—**6**.[1,3,4] This phenomenon is attributable to a slow interconversion among meta-stable macro-ring conformations.[4] Although the anomeric hydroxyl groups of both sugar (glucose) moieties in **16** are free, as indicated by the chemical shifts of anomeric proton signals, one glucose core in **16** takes predominantly the α-form (H-1, δ 6.18, d, $J=3.5$ Hz) and the other glucose takes the β-form (H-1′, δ 4.47, br d, $J=8$). In addition to these structural features, the sugar proton signals of **16**, including the anomeric proton signals, showed shifts

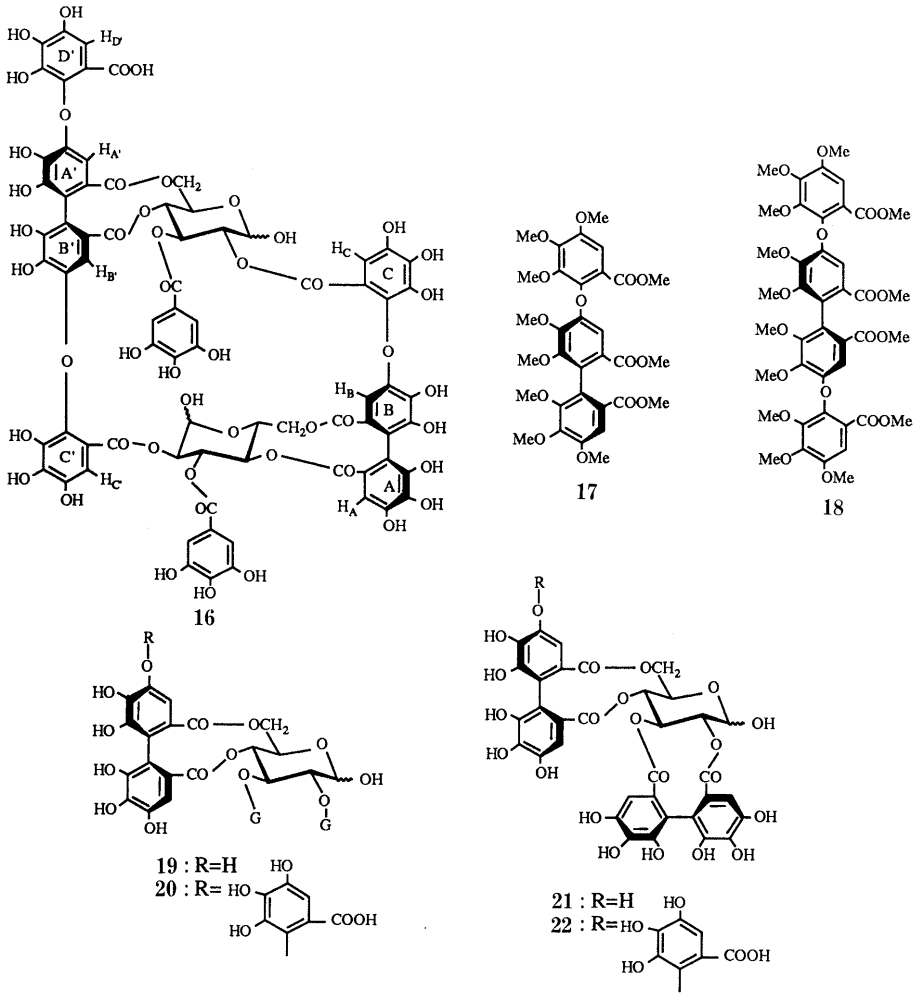


Chart 4

similar to those of oenothein B (**4**) with slight differences of less than 0.05 ppm except for the H-6′ signals ($\Delta\delta$ 0.1 ppm) (Table I). The ^{13}C-NMR spectrum of **16** was also closely related to that of **4** (Table I). Based on these observations, along with the CD spectrum of **16**, which is almost superimposable on that of **4**, woodfordin I was regarded as an analog of oenothein B, in which one of the valoneoyl groups in **4** is replaced by a woodfordinoyl group. In fact, the partial hydrolysis of woodfordin I (**16**) in a boiling-water bath gave **4** and gallic acid (**9**). The location of the woodfordinoyl group in **16** was determined as follows. The aromatic proton signals in **16** are assignable by comparison with those of **4**, as summarized in Table II. The proton signals H_A–H_C on the valoneoyl group at O–4/O–6 in **16** show almost the same chemical shifts as those in **4**, while the $H_{A'}$ signal of another acyl group at O–4′/O–6′ is shifted remarkably upfield (δ 6.66→6.22) from that in **4**. This upfield shift is attributable to a steric compression induced by replacement of the adjacent hydroxyl group by a bulky phenyl ether (ring-D′) as well as the anisotropy effect of ring-D′,[1] indicating that the woodfordinoyl group in **16** is at O–4′/O–6′. This assignment was supported by the chemical shift difference ($\Delta\delta$ 0.1 ppm) of the C-6′ methylene proton signals between **16** and **4**, since a similar difference is observed for the known tannins (**12**,[8] **19**[11] and **21**[11]) having the HHDP group, and their congeners (**8**,[6] **20**[12] and **22**[13]) having the valoneoyl group, at each O–4/O–6 (Table III).

Woodfordin E (**23**), $[\alpha]_D$ +126° (acetone), was obtained as an off-white amorphous powder. Its trimeric nature was indicated by the retention time in HPLC (normal phase) which is similar to that of **5** and **6**, and by the ion peak at m/z 2073 attributable to $[M+Na]^+$ in FAB-MS. Methylation of **23**, followed by methanolysis with sodium methoxide in methanol, afforded the same products (**9a**, **17** and **18**) as those from **16**, in a molar ratio of 3 : 1 : 1, as being shown by HPLC analysis. The presence of these constituent units in **23** was supported by the ^{1}H-NMR spectrum, which exhibited three 2H singlets and seven 1H

Table I. ^{1}H- and ^{13}C-NMR Data for the Glucose Moiety of Woodfordin I (**16**) and Oenothein B (**4**) in Acetone-d_6+D_2O (J in Parentheses)

Position	**16**[a]		**4**[a]	
	δ_H	δ_C	δ_H	δ_C
Gluc-I[b]				
1	6.18 d (3.5)	95.3	6.21 d (3.5)	95.8
2		74.5		74.8
3	5.50—6.10 br	73.3	5.50—6.14 br	73.5
4		73.4		73.8
5	4.55 dd (5, 10)	71.6	4.56 dd (6, 10)	71.9
6	5.22 dd (6, 13)	[c]	5.24 dd (6, 13)	65.3 br
	3.62 d (13)		3.62 d (13)	
Gluc-II[d]				
1′	4.47 br d (8)	91.0	4.42 br d (8)	91.5
2′	5.15 br dd (8, 10)	[c]	5.16 dd (8, 10)	74.9
3′	5.38 t (10)	71.3	5.43 t (10)	71.3
4′	4.83 t (10)	[d]	4.88 t (10)	70.5
5′	4.12 dd (6, 10)	68.3	4.12 t (10)	69.0
6′	4.92 dd (6, 13)	63.1	5.02 dd (6, 13)	63.2
	3.75 d (13)		3.85 d (13)	

a) Data for major anomer. *b*) α-Anomer is predominant. *c*) Not observed clearly due to signal broadening. *d*) β-Anomer is predominant.

Table II. Chemical Shifts of the Aromatic Proton Signals[a] of Macrocyclic Hydrolyzable Tannins (**4**, **6**, **16**, **23**, **24** and **28**)

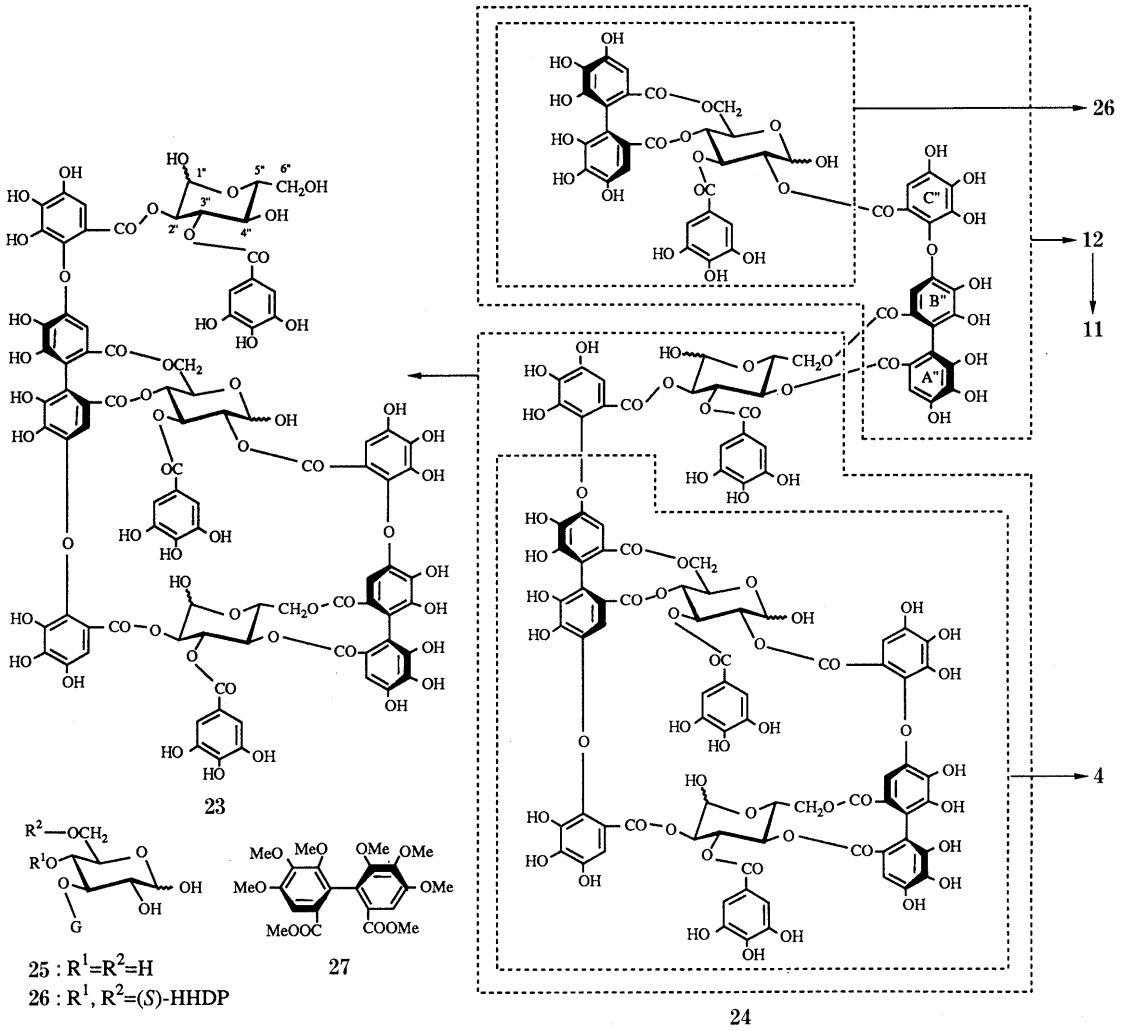
	4	**16**	**23**	**6**	**24**	**28**
Galloyl (Gal)						7.05, 7.04
3-Gal	7.25	7.24	7.26, 7.24	7.25, 7.23	7.29, 7.27	
3′-Gal	7.00 br[b]	6.96 br	6.93 br	6.94	6.97	
3″-Gal			7.04	7.02, 7.00	7.02	6.96
3‴-Gal					7.05, 7.04	7.03, 7.01
HHDP H-3				6.63, 6.60	6.69, 6.66, 6.64	6.67, 6.64, 6.636
H-3′				6.51, 6.45	6.44, 6.43, 6.42, 6.41	6.47, 6.466, 6.46, 6.43
Valoneoyl (Woodfordinoyl)						
H_A	6.47	6.41	6.39	6.39	6.47	
H_B	6.29	6.28	6.27	6.27	6.28	
H_C	6.70 br	6.72 br	6.72 br	6.70 br	6.72 br	
$H_{A'}$	6.66	6.22	5.96	6.01	6.14	
$H_{B'}$	6.47 br	6.41 br	6.40 br	6.40 br	6.48 br	
$H_{C'}$	7.19	7.20	7.20	7.20	7.20	
$H_{D'}$		7.11	7.08	7.02	7.08, 7.07, 7.064	
$H_{A''}$					6.52, 6.50, 6.49	6.51, 6.508, 6.50
$H_{B''}$					6.09, 6.08	6.21, 6.19, 6.01
$H_{C''}$					7.00, 6.99	7.00, 6.98

a) Data for main anomeric form. *b*) Broadened signal.

Table III. Chemical Shifts of Glucose H-6 Signals of Ellagitannins Having an HHDP or Valoneoyl Group at O-4/O-6

	19	**20**	($\Delta\delta_{19-20}$)	**12**	**8**	($\Delta\delta_{12-8}$)	**21**	**22**	($\Delta\delta_{21-22}$)
α-Anomer H-6	5.24	5.18	(0.06)	5.21	5.09	(0.19)	5.27	5.14	(0.13)
	3.77	3.68	(0.09)	3.66	3.57	(0.09)	3.75	3.64	(0.11)
β-Anomer H-6	5.26	5.20	(0.06)	5.22	5.10	(0.12)	5.29	5.17	(0.12)
	3.83	3.75	(0.08)	3.71	3.62	(0.09)	3.82	3.71	(0.11)

singlets at the aromatic region, although they are partially duplicated owing to anomerization in the sugar moiety (Table II). The macrocyclic structure of **23** was suggested by broadening of some of aromatic and sugar proton signals, which are similar to those in **3**—**6** and **16**, and also by the sugar proton signals which are analogous to those of oenothein A (**6**), except for the upfield shifts of the H-4 and H-6 signal of one of the glucose cores in **23**. Woodfordin E was thus regarded as an analog of oenothein A (**6**), lacking an HHDP group at O-4″/O-6″ of **6**. The structure (**23**) thus proposed for woodfordin E was substantiated by the production of **4**, together with gallic acid (**9**) and 3-*O*-galloyl-D-glucose (**25**), upon partial hydrolysis of **23** in hot water. Finally woodfordin E was derived from oenothein A (**6**) by treatment with hot water containing trifluoroacetic acid. The structure of woodfordin



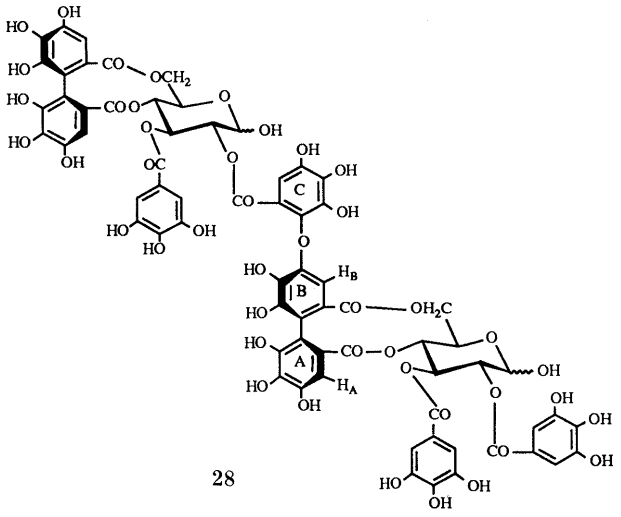


Chart 5

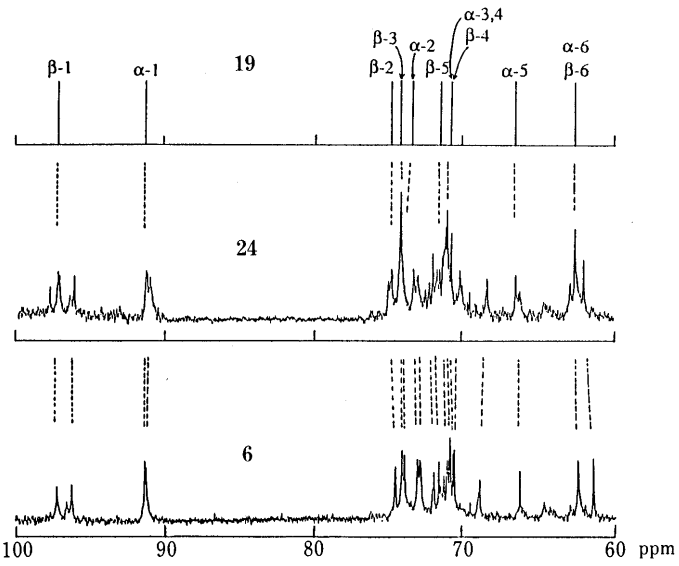


Fig. 1. The ^{13}C-NMR Spectra of the Glucose Moieties of **6**, **19** and **24**

E was thus determined to be **23**.

Woodfordin F (**24**), $[\alpha]_D$ +83° (acetone), an off-white amorphous powder, yielded upon methanolysis of the mixture obtained by methylation, **9a**, **17**, **18** and dimethyl hexamethoxydiphenate (**27**) in a molar ratio of 4:2:1:1. Taking the number of the connecting units (valoneoyl and woodfordinoyl groups) and the FAB-MS data [*m/z* 3159 $(M+Na)^+$] into consideration, woodfordin F was supposed to be a tetramer related to **5**, **6** or **23**. The absolute configurations of two valoneoyl, a woodfordinoyl, and an HHDP groups in **24** were determined to be all (*S*), from the CD spectrum, which showed a strong positive Cotton effect, $[\theta]$ $+5.5\times10^5$ at 220 nm.[7] The ^{1}H- and ^{13}C-NMR spectra of **24** were extremely complicated by the equilibration among four isomers, due to the anomerization in the sugar moieties, and also by the broadening of the signals as observed in **3**—**6**. However, the signal pattern of the glucose carbons in the ^{13}C-NMR spectrum of **24** was closely related to those of tellimagrandin I (**19**)[14] and oenothein A (**6**) (trimer of **19**) (Fig. 1), indicating that woodfordin F is a condensate of **19** and **6**.

Partial hydrolysis of **24** with hot water gave gemin D (**26**),[15] oenothein C (**11**), cornusiin B (**12**), oenothein B (**4**) and woodfordin E (**23**). The structure (**24**) was therefore assigned to woodfordin F.

The orientation of the valoneoyl group on glucose core-III in woodfordin F, shown in the formula **24**, was deduced as follows. The orientation of the valoneoyl group at O-4/O-6 on the C1 glucose core in ellagitannins such as **1**, **2** and their analogs[9] can be conveniently determined from the chemical shift of the valoneoyl H_A signal, except for the valoneoyl groups in the macro-ring.[9] This method is based on the difference of the chemical shift of the H_A-signal due to the regio-isomerism; it appears at δ 6.4—6.53 for rugosin type tannins [*e.g.*, rugosin B (**20**), eucalbanin B (degalloylwoodfordin B) (**28**),[16] *etc.*] and at δ 6.6—6.7 for isorugosin type tannins [*e.g.*, isorugosin B (**13**), cornusiin A,[8] *etc.*]. This method was applied for the determination of the orientation of the valoneoyl group at O-4″/O-6″ in **24**, of which the proton signals were assigned based on a comparison with those of related dimers and trimers, as summarized in Table II. This comparison shows that the chemical shifts of the $H_{A''}$ signals (δ 6.49—6.52) of **24** are analogous to those of the rugosin type, rather than isorugosin type. Based on these data, the gross structure of woodfordin F was represented by **24**. This tannin is the first tetramer possessing a macrocyclic structure.

Although more than one hundred oligomeric hydrolyzable tannins have hitherto been isolated from various plants,[17] most of them have a linking unit, such as a valoneoyl (or sanguisorboyl) group, binding the monomeric constituents with each other repeatedly. In contrast with these oligomers, woodfordins D (**5**), E (**23**), F (**24**), I (**16**) and oenothein A (**6**), isolated from *W. fruticosa*, are unique oligomers having two different units (valoneoyl and woodfordinoyl) linking the monomeric constituents in the molecule.

Experimental

HPLC was conducted on a column of Superspher Si60 (4×125 mm) using the solvent system (A) hexane–MeOH–tetrahydrofuran (THF)–HCOOH (60:45:15:1) containing oxalic acid (500 mg/1.2 l) or (B) hexane–EtOAc (2:1), and on a column of LiChrospher RP-18 (5 μm, 4×250 mm) in an oven at 40 °C using the solvent system (C) 0.01 M phosphate buffer–EtOAc–EtOH (85:10:5) or (D) 0.01 M phosphate buffer–CH_3CN (9:1). Gas liquid chromatography (GLC) was performed on a Hitachi 163 gas chromatograph equipped with a capillary column G-250 (1.2 mm×40 m) (Chemical Inspection and Testing Institute). The other chromatographic conditions (thin layer chromatography (TLC) and column chromatographies) and the instruments (NMR and mass spectrum (MS) spectrometers and polarimeter) used throughout this work were the same as those described in the preceding paper.[1]

Isolation of Tannins A part (20 g) of the water-soluble portion, obtained after extracting the 70% acetone homogenate of dried flowers (1 kg) of *W. fruticosa* with EtOAc and *n*-BuOH successively,[1] was chromatographd over Toyopearl HW-40 (coarse) (CC-A), developing with 50% MeOH→60% MeOH→70% MeOH→MeOH–H_2O–acetone (7:2:1→6:2:2→5:3:2). The 60% eluate contained mainly isoschimawalin A (**16**) (fr. 201—229; F1), woodfordin I (**16**) (fr. 230—250; F2) and woodfordin E (**23**) (fr. 470—500; F3). Each fraction (F1—F3) was separately purified further by column chromatography over MCI gel CHP-20P, developing with H_2O→20% MeOH→30% MeOH→40% MeOH in a stepwise gradient mode to yield **7** (24 mg), **16** (34 mg) and **23** (41 mg). The 70% MeOH eluate from CC-A was similarly rechromatographed over MCI gel CHP-20P, using the same eluant as above to afford woodfordin G (**14**) (12 mg) (from 30% MeOH eluate). Woodfordin H (**15**) (41 mg) was obtained from the eluate (fr. 1310—1370) with MeOH–H_2O–acetone (6:2:2) from CC-A, after purification by column chromatography on MCI gel CHP-20P. The eluate (fr. 1511—1590) with MeOH–H_2O–acetone (5:3:2) from CC-A gave woodfordin F (**24**) (100 mg).

Woodfordins H (**15**) (89 mg) and F (**24**) (55 mg) were also obtained from the fraction eluted with MeOH–H_2O–acetone (7:2:1) in the previous column chromatography over Toyopearl HW-40 of the *n*-BuOH extract.[1]

Isoschimawalin A (7) An off-white amorphous powder, $[\alpha]_D$ +37° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{55}H_{34}O_{35}\cdot7H_2O$: C, 47.83; H, 3.48. Found: C, 47.88; H, 3.56. FAB-MS *m/z*: 1277 $(M+Na)^+$. UV λ_{max} (MeOH) nm (log ε): 216 (4.90), 258 (4.75), 360 (3.71). CD (MeOH) $[\theta]$ (nm): $+9.9\times10^4$ (218), $+3.6\times10^4$ (238), -4.4×10^4 (258), $+4.4\times10^4$ (283). ^{1}H-NMR (acetone-d_6+D_2O) δ: 6.20, 6.23 (each s, 1H in total), 6.56 (1H, s), 6.99, 7.02 (each s, 1H in total) (Val), 6.76, 6.79 (each s, 2H in total, Gal), 7.08, 7.10 (each s, 1H in total), 7.21, 7.23 (each s, 1H in total), 7.60, 7.61 (each s, 1H in total) [dilactonized valoneoyl (DVL)], glucose (α-anomer); δ 5.34 (d, *J*=4 Hz, H-1), 5.00 (dd, *J*=4, 10 Hz, H-2), 5.59 (t, *J*=10 Hz, H-3), 4.90 (t, *J*=10 Hz, H-4), 4.48 (dd, *J*=6, 10 Hz, H-5), 5.12 (dd, *J*=6, 13 Hz, H-6), 3.66 (d, *J*=13 Hz, H-6), (β-anomer): 4.74 (d, *J*=8 Hz, H-1), 5.08 (dd, *J*=8, 10 Hz, H-2), 5.26 (t, *J*=10 Hz, H-3), 4.88 (t, *J*=10 Hz, H-4), 4.02 (dd, *J*=6, 10 Hz, H-5), 5.14 (dd, *J*=6, 13 Hz, H-6), 3.70 (d, *J*=13 Hz, H-6). ^{13}C-NMR (acetone-d_6) δ: glucose (α-anomer): 90.8 (C-1), 72.6 (C-2), 71.4 (C-3), 71.1 (C-4), 66.8 (C-5), 63.4

(C-6), (β-anomer): 96.1 (C-1), 73.7 (C-2), 73.5 (C-3), 71.3 (C-4), 71.5 (C-5), 63.4 (C-6), 168.5, 168.3 (1C in total), 167.6, 167.5 (2C in total), 166.5, 166.3 (1C in total), 164.2, 164.1 (1C in total), 160.5, 160.4 (1C in total), 160.1 (1C) (ester carbonyl).

Acid Hydrolysis of Isoschimawalin A (7) A solution of **7** (1 mg) in 5% H_2SO_4 (1 ml) was refluxed for 8 h, and the reaction mixture, after cooling, was extracted with EtOAc. The EtOAc extract was subjected to reversed-phase HPLC analysis (solvent C), to detect gallic acid (**9**) (t_R 2.3 min) and valoneic acid dilactone (**10**) (t_R 7.4 min). The aqueous layer, after neutralization with ion exchange resin (Amberlite IRA-410), was evaporated to dryness. The residue was analyzed by GLC (column temperature 170 °C) after trimethylsilylation, revealing glucose as a sugar component.

Partial Hydrolysis of Isoschimawalin A (7) a) A mixture of **7** (1 mg) and CF_3COOH (3 drops) in water (1 ml) was heated in a boiling-water bath for 5 h, and the reaction mixture was analyzed by reversed-phase HPLC (solvent C) to show the formation of oenothein C (**11**) (t_R 3.55 min), cornusiin B (**12**) (t_R 4.4 and 5.2 min) and valoneic acid dilactone (**10**) (t_R 7.37 min). The identity of these products was also confirmed by normal phase HPLC (solvent A, **11** t_R 2.58 min; **10** t_R 1.68 min), although a peak of cornusiin B (**12**) was overlapped by that of unreacted starting material.

b) A solution (0.5 ml) of **7** (0.5 ml) in 0.2 M phosphate buffer (pH 7.4) was left standing at room temperature for 1d. The formation of cornusiin B (**12**) (t_R 4.4 and 5.2 min) and isorugosin B (**13**) (t_R 3.2 and 4.8 min) was observed on reversed-phase HPLC (solvent C).

Woodfordin G (14) An off-white amorphous powder, $[\alpha]_D$ +63° (c=1.0, MeOH). *Anal.* Calcd for $C_{68}H_{48}O_{44}\cdot 10H_2O$: C, 46.18; H, 3.45. Found: C, 46.68; H, 3.89. FAB-MS m/z: 1591 $(M+Na)^+$. UV λ_{max} (MeOH) nm (log ε): 217 (4.91), 263 (4.70), 360 (3.49). ^{1}H-NMR (acetone-d_6 + D_2O) δ: 7.04, 7.03, 7.00, 6.99, (each s, 2H in total, Gal), 6.84, 6.83, 6.76, 6.75 (each s, 2H in total, Gal), 7.61, 7.60, 7.59 (each s, 1H in total), 7.27, 7.25, 7.21, 7.20 (each s, 1H in total), 7.19, 7.17, 7.16 (each s, 1H in total) (DVL), 7.14, 7.13, 7.12 (each s, 1H in total), 6.49, 6.47, 6.45, 6.42 (each s, 1H in total), 6.08, 6.06, 6.03, 6.01 (each s, 1H in total) (Val), 5.70 [t, J=10 Hz, H-3 (α)], 5.69 [t, J=10 Hz, H-3′ (α)], 5.33 [d, J=4 Hz, H-1 (α)], 5.31 [d, J=4 Hz, H-1′ (α)], 5.30 [t, J=10 Hz, H-3, H-3′ (β)], 5.16 [dd, J=8, 10 Hz, H-2 (β)], 5.08 [dd, J=4, 10 Hz, H-2 (α)], 5.02 [dd, J=5, 13 Hz, H-6 (α and β)], 4.98 [t, J=10 Hz, H-4 (α)], 4.94 [dd, J=8, 10 Hz, H-2′ (β)], 4.92 [dd, J=4, 10 Hz, H-2′ (α)], 4.90 [t, J=10 Hz, H-4 (β)], 4.72 [d, J=8 Hz, H-1 (β)], 4.52 [m, H-5 (α and β)], 4.17 [d, J=8 Hz, H-1′ (β)], 3.75 [d, J=13 Hz, H-6′ (α and β)], 3.58 [d, J=13 Hz, H-6 (α and β)], H-4′, 5′, 6′ (α and β) are overlapped with each other in the region of 3.5—3.7 ppm.

Acid Hydrolysis of Woodfordin G (14) A solution of **14** (1 mg) in 5% H_2SO_4 (1 ml) in a sealed tube was heated in a boiling-water bath for 10 h. The reaction mixture was passed through Bond Elut C18 (Analytichem), and the column was washed with water. The MeOH eluate was analyzed by reversed-phase HPLC (solvent C), to show peaks identical with those of authentic gallic acid (**9**) and valoneic acid dilactone (**10**). A sugar component in the water washing was identified as glucose, by gas liquid chromatography of the trimethylsilyl ether.

Partial Hydrolysis of Oenothein B (4) to Woodfordin G (14) A solution of **4** (1 mg) in 0.3% H_2SO_4 (1 ml) was heated in a water bath of 70 °C for 13 h. The HPLC (reversed and normal phases) of the reaction mixture gave a peak having a retention time identical with that of woodfordin G (**14**) [solvent (A), t_R 5.4 min; (B), t_R 6.1, 6.6 min].

Woodfordin H (15) An off-white amorphous powder, $[\alpha]_D$ +88° (c=1.0, MeOH). *Aanl.* Calcd for $C_{82}H_{54}O_{52}\cdot 15H_2O$: C, 45.98; H, 3.92. Found: C, 45.35; H, 3.55. UV λ_{max} (MeOH) nm (log ε): 216 (5.11), 258 (4.89), 359 (3.68). CD (MeOH) [θ] (nm): $+16.4\times10^4$ (220), -4.2×10^4 (260), $+4.4\times10^4$ (283). ^{1}H-NMR (acetone-d_6 + D_2O) δ: 6.99, 6.95, (each s, 2H in total), 6.82, 6.75 (each s, 2H in total) (Gal), 7.62, 7.61, 7.605, 7.60 (each s, 1H in total), 7.16, 7.14, 7.13 (each s, 1H in total), 7.04, 7.03, 7.02 (each s, 1H in total) (DVL), 7.01, 7.00 (each s, 1H in total), 6.48, 6.46, 6.41 (each s, 1H in total), 6.15, 6.12, 6.05, 6.04 (each s, 1H in total) (Val), 6.65, 6.62 (each s, 1H in total), 6.51, 6.50, 6.49, 6.48 (each s, 1H in total) (HHDP), 5.43 [d, J=4 Hz, H-1′ (α)], 5.32 [d, J=4 Hz, H-1 (α)], 4.78 [d, J=8 Hz, H-1 (β)], 4.72 [d, J=8 Hz, H-1 (β)], 4.31 [d, J=8 Hz, H-1′ (β)], 4.30 [d, J=8 Hz, H-1′ (β)], 5.77, 5.72, 5.71, 5.54, 5.53, 5.34 [each t, J=10 Hz, H-3, H-3′ (α and β)], 4.90—5.20 [H-2, H-2′, H-4, H-4′, H-6, H-6′ overlapped with each other (α and β)], 4.63, 4.52, 4.05 [each dd, J=5, 10 Hz, H-5, H-5′ (α and β), 3.86, 3.78, 3.65, 3.60 [each d, J=13 Hz, H-6, H-6′ (α and β)].

Partial Hydrolysis of Woodfordin H (15) A solution of **15** (40 mg) in 8% CF_3COOH (20 ml) was heated in a boiling-water bath for 4 h. The concentrated solution (*ca.* 10 ml) was chromatographed over MCI-gel CHP 20P, developing with H_2O and aqueous MeOH (10%→20%→25%→30% MeOH). The 25% MeOH eluate afforded woodfordin G (**14**) (3 mg), which was identified by direct comparison of HPLC profiles and ^{1}H-NMR spectra.

Woodfordin I (16) An off-white amorphous powder, $[\alpha]_D$ +126° (c=1.0, MeOH). *Anal.* Calcd for $C_{75}H_{52}O_{59}\cdot 9H_2O$: C, 47.42; H, 3.68. Found: C, 47.17; H, 3.53. FAB-MS m/z: 1759 $(M+Na)^+$. CD (MeOH) [θ] (nm): $+26.4\times10^4$ (221), $+9.4\times10^4$ (235), -3.5×10^4 (265), $+7.3\times10^4$ (285). ^{1}H-NMR and ^{13}C-NMR, see Table I (glucose moiety), and Table II (aromatic protons).

Methylation of Woodfordins I (16) and E (23) Followed by Methanolysis Dimethyl sulfate (0.05 ml) and potassium carbonate (100 mg) were added to a solution of woodfordin I (**16**) (5 mg) in acetone (2 ml), and the mixture was stirred for 12 h at room temperature, then refluxed for 2 h. After removal of the inorganic materials by centrifugation, the supernatant was evaporated to dryness. The residue was directly methanolyzed without further purification, with 0.1% NaOMe in MeOH at room temperature for 8 h. After addition of a few drops of AcOH, the reaction mixture was evaporated, and the residue was purified by preparative TLC (benzene–acetone 9 : 1) to yield methyl tri-*O*-methylgallate (**9a**) (1.2 mg), trimethyl octa-*O*-methylvaloneate (**17**) (0.8 mg) and tetramethyl deca-*O*-methylwoodfordinate (**18**) (0.5 mg) [FAB-MS m/z: 893 $(M+Na)^+$], which were identified by comparison with authentic samples (TLC, HPLC and ^{1}H-NMR).

Woodfordin E (**23**) (2 mg) was similarly treated to give the same products, **9a**, **17** and **18**.

Quantitative Analysis of Constituent Units of Woodfordins I (16), E (23) and F (24) Methylation followed by methanolysis of each tannin (2 mg) was carried out in a similar way to that described above. A mixture of methanolyzates was directly analyzed by HPLC in a normal phase mode using solvent B. The ratio of the amount of the products was estimated from their peak areas, referring to a known ratio among the methanolyzates (**9a**, **17**, **18** or **27**) obtained by similar methanolysis of a reference sample (**4** or **6**).

Partial Hydrolysis of Woodfordin I (16) An aqueous solution of **16** (5 mg/2 ml) was heated in a boiling-water bath for 2 h, under monitoring of the reaction process by HPLC (reversed-phase, solvent B). The reaction mixture was chromatographed over Diaion HP-20, developing with H_2O containing a few drops of HCl and MeOH. The water eluate gave gallic acid (**9**) (1 mg), and the MeOH eluate afforded oenothein B (**4**) (3.2 mg), which was identified by direct comparison with an authentic sample (HPLC and ^{1}H-NMR).

Woodfordin E (23) An off-white amorphous powder, $[\alpha]_D$ +126° (c=1.0, acetone). *Aanl.* Calcd for $C_{88}H_{66}O_{58}\cdot 18H_2O$: C, 44.48; H, 4.30. Found: C, 44.21; H, 4.28. FAB-MS m/z: 2073 $(M+Na)^+$. UV λ_{max} (MeOH) nm (log ε): 217 (5.13), 268 (4.81). ^{1}H-NMR (acetone-d_6 + D_2O) δ: 6.18 (d, J=4 Hz, H-1), 6.02 (dd, J=4, 10 Hz, H-2), 6.05 (t, J=10 Hz, H-3), 5.28 (t, J=10 Hz, H-4), 4.56 (dd, J=6, 10 Hz, H-5), 5.23 (dd, J=6, 13 Hz, H-6), 3.63 (d, J=13 Hz, H-6), 4.5 (br, J=8 Hz, H-1′), 5.17 (dd, J=8, 10 Hz, H-2′), 5.41 (t, J=10 Hz, H-3′), 4.86 (t, J=10 Hz, H-4′), 4.12 (dd, J=6, 10 Hz, H-5′), 5.20 (dd, J=6, 13 Hz, H-6′), 3.88 (d, J=13 Hz, H-6′), 5.34 (d, J=4 Hz, H-1″), 4.02 (d, J=8 Hz, H-1″), 4.88 [br, H-2″ (α and β)], 5.72 [t, J=10 Hz, H-3″ (β)], 5.26 [br, H-3″ (α)], 3.81 [t, J=10 Hz, H-4″ (β)], 3.56 [t, J=10 Hz, H-4″ (α)], 3.6—4.0 [br m, H-5″, 6″ (α and β)], aromatic protons, see Table I.

Partial Hydrolysis of Woodfordin E (23) An aqueous solution of **23** (2 mg/1 ml) was heated in boiling-water bath for 2 h, and the reaction mixture was analyzed by HPLC, which showed formation of gallic acid (**9**), 3-*O*-galloyl-D-glucose (**25**) and oenothein B (**4**). Retention times (t_R): normal phase (solvent A): **9** t_R 1.47 min, **25** t_R 2.15 min, **4** t_R 5.25 min; reversed-phase (solvent D): **9** t_R 2.45 min, **25** t_R 1.82 min, **4** t_R 4.65 min).

Conversion of Oenothein A (6) into Woodfordin E (23) A solution of **6** (100 mg) in H_2O (10 ml) containing CF_3COOH (0.5 ml) was heated at *ca.* 80 °C for 20 h. The concentrated solution was chromatographed over MCI gel CHP-20P, developing with H_2O and aqueous MeOH (10%→20%→30%→40% MeOH), in a stepwise gradient mode. The H_2O eluate gave gallic acid (**9**) (1 mg) and 3-*O*-galloyl-D-glucose (**25**) (0.8 mg). The 30% MeOH eluate yielded oenothein C (**11**) (5.7 mg) and woodfordin E (**23**) (4.7 mg). Their identities were confirmed by HPLC (normal- and reversed-phase) and ^{1}H-NMR spectra.

Woodfordin F (24) An off-white amorphous powder, $[\alpha]_D$ +83° (c=1.0, acetone). *Anal.* Calcd for $C_{136}H_{96}O_{88}\cdot 30H_2O$: C, 44.61; H, 4.27. Found: C, 44.48; H, 4.47. FAB-MS m/z: 3159 $(M+Na)^+$. UV λ_{max} (MeOH) nm (log ε): 219 (5.38), 269 (5.06). CD (MeOH) [θ]

(nm): 5.47×10^5 (220), 1.80×10^5 (237), -1.49×10^5 (258), $+2.05 \times 10^5$ (282).

Partial Hydrolysis of Woodfordin F (24) a) An aqueous solution (10 ml) of **24** (50 mg) was heated in a boiling-water bath, under monitoring of the degradation process by HPLC (reversed-phase). After 14 h, the reaction mixture was concentrated and subjected to column chromatography over MCI-gel CHP-20P, using water and aqueous MeOH as eluant. Gallic acid (**9**) (1 mg) and gemin D (**26**) (2 mg) were obtained from the water eluate. The 30% MeOH eluate afforded oenothein B (**4**) (5.2 mg) and cornusiin B (**12**) (1.5 mg). These products were identified by direct comparison with authentic samples (HPLC and ^{1}H-NMR).

b) A solution of **24** (100 mg) in 1% H_2SO_4 (20 ml) was heated at *ca.* 70 °C for 10 h, and the products were separated from each other in a way similar to that described above, to give **26** (2.4 mg), **4** (4.5 mg), **12** (2.3 mg) and woodfordin E (**23**) (1 mg), which were identified by co-chromatography and ^{1}H-NMR spectral comparisons with authentic samples.

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