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; ellagitanin 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 “Sidowaya” in Indonesia. 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. Flavonoid glycosides, anthraquinone glycosides and several phenylpropanoids have been reported as constituents of this plant. 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 ellagittannins including three new dimers. We report herein the structural elucidation of the new tannins, and the result of an examination of their antitumor activity.

The crude drug, “Sidowayah”, which was purchased in an Indonesian market, 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), 1,2,4,6-tetra-O-galloyl-β-D-glucose (2), 1,2,3,4,6-penta-O-galloyl-β-D-glucose (3), tellimagrandin I (4), a demethyl ether of sennoside B (5), heterophyllin A (7), and oenothein B (22). 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 ellagitanin which was first isolated from an Oenothera species, and was found to exhibit remarkable host-mediated antitumor activity, 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. The molecular formula, C_{75}H_{56}O_{48}•11H_{2}O, 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. Two-proton 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 13C-NMR spectrum, and also by complete acid hydrolysis which gave gallic acid (9), valeric acid dilactone (10), and oenothein B (22).
(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 $^1$H-$^1$H 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 acetylated 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 [$\delta$ 3.68 (t, $J = 10.0$ Hz)], in comparison with the H-4' signal of the other glucose core [($\delta$ 5.16 (t, $J = 10.0$ Hz)]. The $\beta$-configuration of each anomic acyloxy group was evidenced by the large coupling constant ($J = 8.5$ Hz) of H-1 at $\delta$ 6.15 and H-1' at $\delta$ 6.08. The C-6 methylene proton signals of 8, which arise from the fully acetylated glucose core, appeared at $\delta$ 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. The $^{13}$C resonances of the glucose moieties, whose assignments were confirmed by the $^1$H-$^{13}$C shift correlation spectrum of 8, were in good agreement with those of 1,2,3,6-tetra-O-galloyl-$\beta$-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-$\beta$-D-glucose and rugosin A,$^{15}$ respectively, on the basis of the $^1$H-NMR spectral data (see Experimental). The identity of the latter was confirmed by direct comparison with an authentic sample pre-

<table>
<thead>
<tr>
<th>Carbon</th>
<th>1</th>
<th>5</th>
<th>8a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1')</td>
<td>93.5</td>
<td>93.8</td>
<td>93.1 (93.5)</td>
</tr>
<tr>
<td>2 (2')</td>
<td>71.9</td>
<td>71.8</td>
<td>72.0 (71.8)</td>
</tr>
<tr>
<td>3 (3')</td>
<td>76.0</td>
<td>73.3</td>
<td>75.5 (73.1)</td>
</tr>
<tr>
<td>4 (4')</td>
<td>69.5</td>
<td>70.8</td>
<td>69.4 (70.7)</td>
</tr>
<tr>
<td>5 (5')</td>
<td>76.1</td>
<td>73.1</td>
<td>76.2 (72.7)</td>
</tr>
<tr>
<td>6 (6')</td>
<td>63.7</td>
<td>63.1</td>
<td>64.5 (63.2)</td>
</tr>
</tbody>
</table>

a) Measured in acetone-$d_6$ + D$_2$O.

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

Chart 2
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 oenotienin C (15). 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,7 oenothein B (22)9 and others.14 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 1H-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 anomic hydroxyl group in one of the sugar residues. The presence of four galloyl groups was indicated by the signals at δ 7.72, 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 established 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 1H-NMR spectrum of 16 was characteristic of 4C₁ glucopyranose. All of the hydroxyl groups, except for that at an anomic center, are acetylated 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 anomic center bearing an α-oriented acyloxy group. The large difference (Δδ 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 cornusin B (17)16 and geminin 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 oenothine B (δ 4.48)9 and camptothin B (19) (δ 4.53).22 The presence of a free anomic 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 Hc 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 Hc 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), [α]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 1H-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 4C₁ glucopyranoses. The anomic 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 anomic center of a glucose core
(glucose-1) is free, and the other anomic 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 oenothelin B.23) Methylation of 20 gave a hexacosamethyl derivative (21), which upon methanolation yielded methyl tri-O-methylgallate, trimethyl octa-O-methylvalonate, glucose and methyl β-D-glucoside. Although the broadening of some signals of 20 was observed even in the 500 MHz 1H-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 13C-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, [θ] + 41 x 10^4, 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 1H-13C long-range shift correlation spectrum (Fig. 1). The H_BB (δ 7.29, 6.45) and H_CC signals (δ 6.87, 6.67) were distinguished from the H_AA 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_BB and H_CC 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 1H-13C Long-Range Shift Correlation Spectrum of the Hexacosamethyl Ether (21) of Woodfordin C

The 13C-NMR spectrum is shown in the region of 163-168 ppm. The spectrum was measured with J_{CH}=7 Hz.
TABLE II. Antitumor Activity of 8, 20 and 26 against S-180 in Mice

<table>
<thead>
<tr>
<th>Tannin</th>
<th>Survival days&lt;sup&gt;a&lt;/sup&gt; (Mean ± S.D.)</th>
<th>% ILS</th>
<th>60-days survivors&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>20.2 ± 5.9</td>
<td>-3.8</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>33.5 ± 11.7</td>
<td>59.5</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>28.7 ± 4.2</td>
<td>36.5</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>21.0 ± 6.8</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Excluding 60-days survivors.  <sup>b</sup> No tumor take.

woodfordin C, which was supported by the following chemical evidence. Partial hydrolysis of 20 in boiling water gave oenotherin C (15)<sup>16</sup> 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 1H-NMR spectrum, which indicated the presence of two galloyl groups [δ 7.06, 6.99 (each 2H, s)], a dilactonized valoneyl 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<sub>3</sub>COOH.

Woodfordin C (20) was thus shown to be a monogallate of oenotherin 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<sup>5</sup>), woodfordin C
Experimental

Optical rotations were measured on a Jarrel-Ash polarimeter and ultraviolet (UV) spectra on a Hitachi 200-10 spectrometer. NMR spectra were recorded on a Varian VXR 500 (500 MHz) for 1H-NMR and 13C-NMR, and 126 MHz for 1H-NMR and 13C-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 PF254 (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 Si60 (5 μm, solvent: hexane–MeOH–THF–HCOOH (60:45:15:1) containing oxalic acid (500 mg/1.2 l) and (B) hexane–EtOAc (2:1). Reverse-phase HPLC was performed on a column of LiChrosorb 100 RP-18 with (C) 0.05 M H2PO4–0.05 M KH2PO4–EtOH–EtOAc (42.5:25:10:5), (D) 0.05 M H2PO4–0.05 M KH2PO4–EtOH–EtOAc (46:20:15:5), and (E) 0.05 M H2PO4–0.05 M KH2PO4–CH3CN (43.5:42.5:13).

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 EtOAc, EtOAc and n-BuOH saturated with water. A part (8%) 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–H2O (7:2:1) (6:2.2:1). This column chromatography was repeated twice. The 70% MeOH eluate gave tannigram 1 (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-d-glucose (3) (271 mg), oenothin B (22) (798 mg), and fraction 97 mg. The MeOH–acetone–H2O (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 heterotannin 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–H2O (7:2:1) in a stepwise gradient mode to afford gemin D (6) (179 mg) from the MeOH eluate, and oenothin B (22) (770 mg) and woodfordin C (20) (920 mg) from the MeOH–acetone–H2O (7:2:1) eluate.

Woodfordin A (8)

An off-white amorphous powder, [α]D0 +60° (+1.0, acetone). Anal. Caled for C20H14O10: C, 58.6; H, 4.0; O, 39.4. Found: C, 58.4; H, 4.3; O, 39.3. Woodfordin B (16) is an off-white amorphous powder, [α]D0 +93° (+1.0, acetone). Anal. Caled for C20H14O10: C, 58.5; H, 4.0; O, 39.5. Found: C, 58.5; H, 4.0; O, 39.5. Woodfordin C (20) is an off-white amorphous powder, [α]D0 +93° (+1.0, acetone). Anal. Caled for C20H14O10: C, 58.5; H, 4.0; O, 39.5. Found: C, 58.6; H, 4.1; O, 39.3. Woodfordin D (28) is an off-white amorphous powder, [α]D0 +93° (+1.0, acetone). Anal. Caled for C20H14O10: C, 58.5; H, 4.0; O, 39.5. Found: C, 58.6; H, 4.1; O, 39.3.
References and Notes
3) A part of this work was reported in a preliminary communication: T. Yoshida, T. Chou, A. Nitta, and T. Okuda, Heterocycles, 29, 2267 (1989).
4) This crude drug is called in Indonesian markets by several local names such as Sudowayak (Jakarta), Sowowayak (Bogor), Sidowayak (Sukabumi), Suduaya (Medan, Pandang), Suduayak (Jambi) etc. The crude drug (AN-BJ No. 83) used in the present study was purchased at a market in Bogor by one (A. N.) of the authors.
23) After equilibration of the two glucose cores in octenone B, the upper glucose core in the formula 22 exists exclusively as a β-form, and the lower one as an α-form.