WOODFORDINS A, B AND C, DIMERIC HYDROLYZABLE TANNINS FROM WOODFORDIA FRUTICOSA FLOWERS

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Abstract ——Three new dimeric hydrolyzable tannins, woodfordins A, B, and C, along with six known hydrolyzable tannins including oenothein B, a dimer which exhibited remarkable host-mediated antitumor activity, were isolated from an Indonesian crude drug, "Sidowayah" [dried flowers of Woodfordia fruticosa (L.) Kurz (Lythraceae)]. The structures of new dimers were elucidated by chemical and spectral methods.

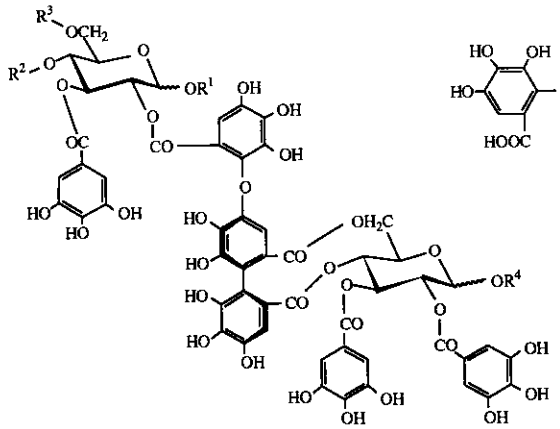
Dried flower of Woodfordia fruticosa (L.) Kurz. (Lythraceae) is popular as a crude drug in Indonesia (called Sidowayah), and also in India and Malaysia.[1,2] It is used as an astringent to treat dysentery and sprue, as a diuretic against rheumatism, dysuria and hematuria, and for bowel complaints. We have isolated three new dimeric hydrolyzable tannins which we named woodfordins A (**1**), B (**2**) and C (**11**), and six known tannins (1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,4,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose,[3] tellimagrandin I,[4] gemin D[5] and a dimer, oenothein B[6]), from this crude drug purchased in an Indonesian market.[7] Amongst them, one of the major tannins was oenothein B (**10**), a dimer which exhibited a remarkable host-mediated antitumor activity[8] and also anti-HIV activity,[9] and has a unique macrocyclic structure.

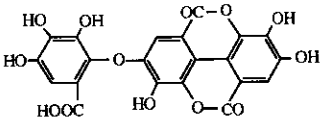
These tannins were isolated from the 1-butanol soluble portion of aqueous acetone homogenate of the crude drug, by column chromatography over Toyopearl HW-40 and MCI-gel CHP-20P.

Woodfordin A (**1**), $C_{75}H_{56}O_{48}\cdot 10H_2O$, $[\alpha]_D$ +60° (acetone), showed the $[M+Na]^+$ ion peak at m/z 1747 in fab-ms. Its ^{1}H-nmr spectrum (500 MHz, acetone-d_6) indicated the presence of six galloyl groups [δ 7.06, 7.08, 7.15 (each 2H, s) and 6.99 (6H.

s)] and a valoneoyl group [δ 6.19, 6.49 and 7.04 (each 1H, s)], and of two glucose residues with the $^4C_1$ conformation. These constructing units were confirmed by hydrolysis of 1 with 5% H_2SO_4 which gave gallic acid, valoneic acid dilactone (3) and glucose, and by nine ester carbonyl carbon signals in the ^{13}C-nmr spectrum of 1. The C-6 methylene proton signals of the fully acylated glucose core were observed at δ5.23 (dd, J=6.5, 13.5 Hz) and 3.78 (d, J=13.5 Hz) in the ^{1}H-nmr spectrum of 1. The large difference between chemical shifts of these protons is characteristic of the tannins having biphenyl ester linkages at O-4 ~ O-6 of the $^4C_1$ glucopyranose residue.[10] The presence of a free hydroxyl group at C-4 of another glucose core was also demonstrated by an upfield shift (δ 3.68, t, J=10 Hz) of H-4. Methylation of 1 with dimetyl sulfate and potassium carbonate in dry acetone furnished methyl derivatives (4) and (5) of two partially degraded monomers, which were identified as methylated derivatives of 1,3,6-tri-O-galloyl-β-D-glucose and rugosin A,[11] respectively. On the other hand, partial hydrolysis of 1 in boiling water gave oenothein C (6),[12] 1,2,3,6-tetra-O-galloyl-β-D-glucose and tellimagrandin I (8). The latter two are regarded as the products derived from cleavage of the ether bond of the valoneoyl group in the molecule, which has been known to occur for several tannins.[13,14] A strong Cotton effect, [θ] +13.7 x 10^4 at 224 nm, in the cd spectrum of 1, indicates the S-configuration of the biphenyl part of the valoneoyl group.[15] The structure of woodfordin A was thus determined to be 1.

Woodfordin B (2), $C_{75}H_{54}O_{48}.8H_2O$, $[\alpha]_D$ +93° (acetone), showed the fab-ms peak at m/z 1745 $[M+Na]^+$. Complete hydrolysis of 2 with 5% H_2SO_4 gave gallic acid, 3, ellagic acid and glucose. Although each proton signal in the ^{1}H-nmr spectrum of 2 was duplicated by equilibration between α- and β-anomers at a glucose residue, the presence of four galloyl groups was indicated by paired-singlets 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). Uncoupled aromatic signals ascribable to a hexahydroxydiphenoyl (HHDP) group and a valoneoyl group 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). The chiralities of these biphenyl groups were both S, as indicated by the positive Cotton effect ([θ] +18.5 x 10^4) at 223 nm in the cd spectrum of 2.[15] The coupling pattern of the glucose proton signals whose assignments were confirmed by the ^{1}H-^{1}H COSY is characteristic of the $^4C_1$ conformation. The presence of an HHDP group and a valoneoyl group at O-4 ~ O-6 of each glucose core was indicated by the





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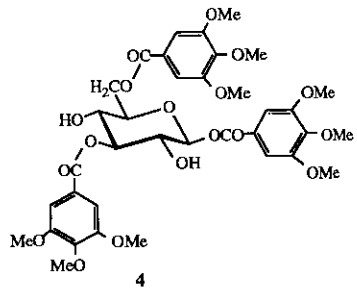

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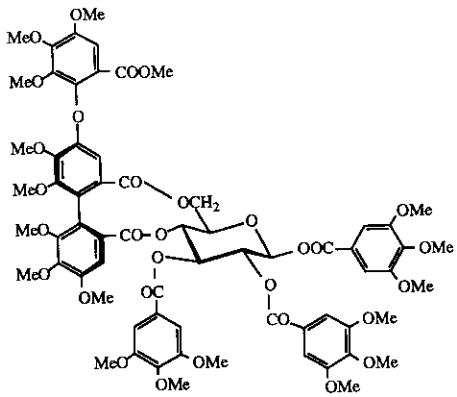
1 : R^1=R^4=G(β), R^2=H, R^3=G
2 : R^1=H, R^2~R^3=*(S)*-HHDP, R^4=G(α)



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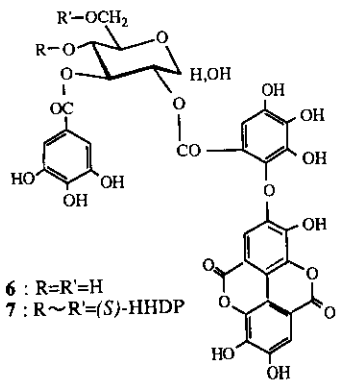
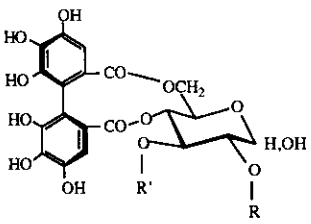
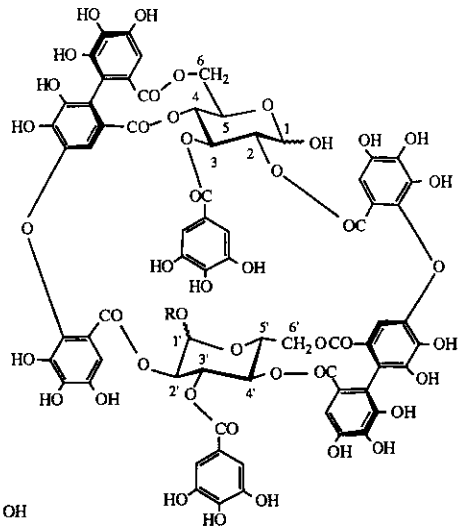


6 : R=R'=H
7 : R~R'=*(S)*-HHDP

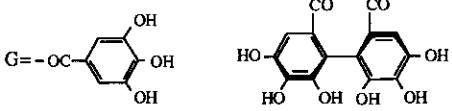


8 : R=R'=G
9 : R=H, R'=G



10 : R=H
11 : R=G(α)

(S)-HHDP=



large differences ($\Delta\delta$ 1.4-1.6 ppm) of chemical shifts of C-6 methylene protons. A doublet at δ 6.64 (1H, J=4 Hz) was assigned to the proton on an anomeric center bearing α-oriented acyloxy group. The signals of another anomeric proton were observed at δ 5.50 (d, J=4 Hz) and at a significantly upfield region, δ4.45 (d, J=8 Hz). This anomaly of the latter signal can be interpreted by an anisotropic effect of the adjacent valoneoyl group, likewise that in oenothein B.[6] The presence of valoneoyl group at O-2 of a glucose core was substantiated by production of cornusin B (**7**),[12] along with gemin D (**9**), upon partial hydrolysis of **2** in boiling water. Based on these data, structure **2** of woodfordin B was assigned. The orientation of valoneoyl group at O-4 $\sim$ O-6 in **2** was based on the analogy of chemical shifts of the HHDP and valoneoyl proton signals with those of rugosin D [δ 7.12, 6.65, 6.47, 6.45 and 6.24 (each 1H, s)].[16]

Woodfordin C (**11**), $C_{75}H_{52}O_{48}.14H_2O$, $[\alpha]_D$ +186° (acetone), was obtained as the main tannin of this crude drug, and showed the fab-ms ion peak at m/z 1743 $[M+Na]^+$. The ^{1}H-nmr spectrum (500 MHz, acetone-d_6+D_2O) of **11** at an ambient temperature[17] was complicated by the broadening of some glucose proton signals and aromatic proton signals, probably due to the rigidity of the molecule. But it showed close similarity to the ^{1}H-nmr spectrum of oenothein B (**10**),[6] except for the presence of an extra signal (δ7.29) of a galloyl group, and a large downfield shift of an anomeric proton signal (δ7.28, d, J=3.5 Hz). This spectral feature suggests that **11** is a dimer which has an α-oriented galloyl group on one of the glucose cores of **10**. This assumption was confirmed by the hydrolysis of **11** with tannase to give **10**. The location of the galloyl group at O-1' in **11** was evident from the comaprison of the ^{1}H-nmr spectrum of **11** with that of **10**; Unlike the H-1 signals of **10** (δ 4.45, d, J=7.5 Hz) and **11** (δ 4.43, br d, J=8.5 Hz), which are of similar chemical shifts, the H-1' (δ7.28, d, J=3.5 Hz) in **11** shifts markedly lower than that of **10** (δ6.20, d, J=3.5 Hz), as mentioned above.

ACKNOWLEDGEMENTS

The authors thank Dr. N. Toh, Faculty of Chemical Engineering, Kyushu Kyoritsu University, for measuring the cd spectra.

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17. ^{1}H-Nmr signals for glucose moieties of **11**: δ 4.43 (br d, J=8.5 Hz, H-1), 5.20 (dd, J=8.5, 10 Hz, H-2), 5.45 (t, J=10 Hz, H-3), 4.90 (t, J=10 Hz, H-4), 5.00 (br dd, J=5.5, 13.5 Hz, H-6), 4.16 (dd, J=5.5, 10 Hz, H-5), 3.86 (d, J=13.5 Hz, H-6), 7.28 (d, J=3.5 Hz, H-1'), 6.20 (t, J=10 Hz, H-3'), 5.80 (br m, H-2', 4'), 5.30 (dd, J=7, 13.5 Hz, H-6'), 4.67 (dd, J=7, 10 Hz, H-5'), 3.68 (d, J=13.5 Hz, H-6').

Received, 25th September, 1989