

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 oenothain 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.

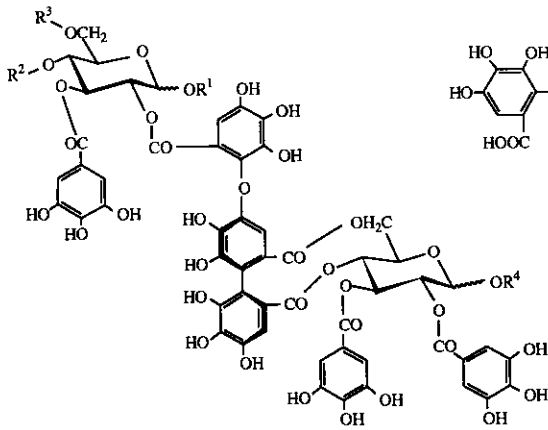
Dried flower of Woodfordia fruticosa (L.) Kurz. (Lythraceae) is popular as a crude drug in Indonesia (called Sidowayah), and also in India and Malaysia.^{1,2} It is used as an astringent to treat dysentery and sprue, as a diuretic against rheumatism, dysuria and hematuria, and for bowel complaints. We 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 D⁵ and a dimer, oenothain B⁶), from this crude drug purchased in an Indonesian market.⁷ Amongst them, one of the major tannins was oenothain B (10), a dimer which exhibited a remarkable host-mediated antitumor activity⁸ 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.

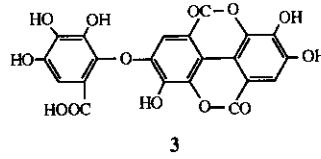
Woodfordin A (1), C₇₅H₅₆O₄₈·10H₂O, [α]_D +60° (acetone), showed the [M+Na]⁺ ion peak at m/z 1747 in fab-ms. Its ¹H-nmr spectrum (500 MHz, acetone-d₆) indicated the presence of six galloyl groups [δ 7.06, 7.08, 7.15 (each 2H, s) and 6.99 (6H,

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 δ 5.23 (dd, $J=6.5, 13.5$ Hz) and 3.78 (d, $J=13.5$ Hz) in the 1H -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 dimethyl 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 oenothain 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, $[\theta] +13.7 \times 10^4$ 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.

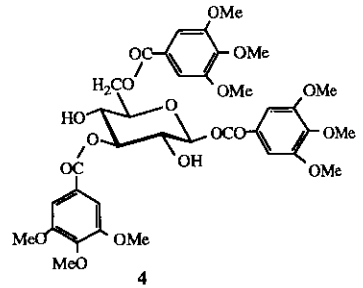
Woodfordin B (2), $C_{75}H_{54}O_{48} \cdot 8H_2O$, $[\alpha]_D +93^\circ$ (acetone), showed the fab-ms peak at m/z 1745 $[M+Na]^+$. Complete hydrolysis of 2 with 5% H_2SO_4 gave gallic acid, 3, ellagic acid and glucose. Although each proton signal in the 1H -nmr spectrum of 2 was duplicated by equilibration 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 ($[\theta] +18.5 \times 10^4$) at 223 nm in the cd spectrum of 2.¹⁵ The coupling pattern of the glucose proton signals whose assignments were confirmed by the 1H - 1H 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



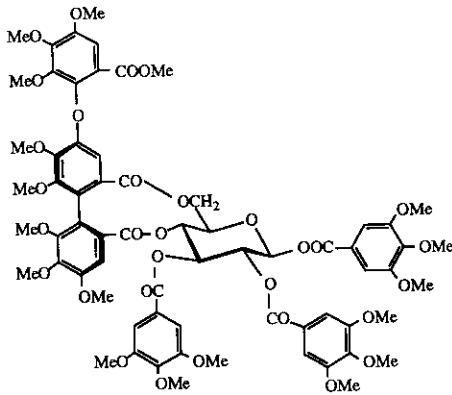
1 : R¹=R⁴=G(β), R²=H, R³=G
 2 : R¹=H, R²~R³=(S)-HHDP, R⁴=G(α)



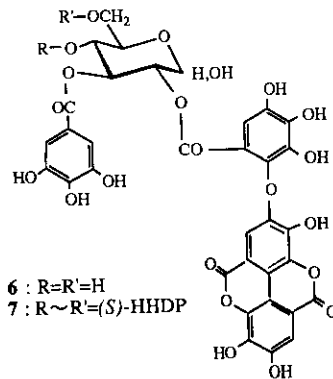
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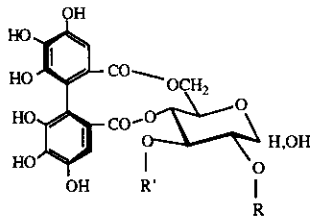
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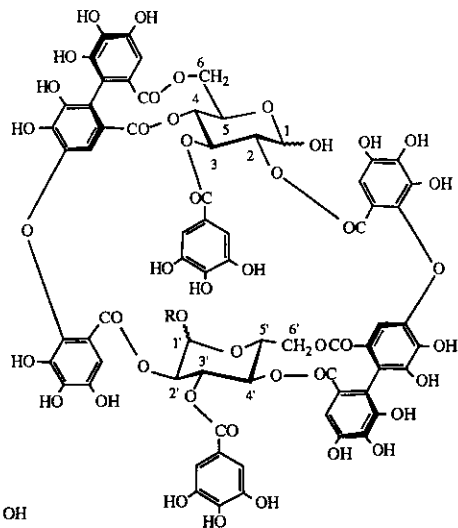
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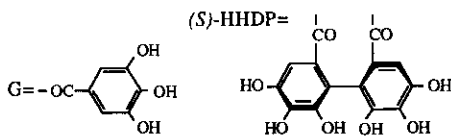
6 : R=R'=H
 7 : R~R'=(S)-HHDP



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



10 : R=H
 11 : R=G(α)



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, δ 4.45 (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 oenothain 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~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), $C_{75}H_{52}O_{48}\cdot 14H_2O$, $[\alpha]_D +186^\circ$ (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 1H -nmr spectrum (500 MHz, acetone- d_6+D_2O) of 11 at an ambient temperature¹⁷ 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 1H -nmr spectrum of oenothain B (10),⁶ except for the presence of an extra signal (δ 7.29) of a galloyl group, and a large downfield shift of an anomeric proton signal (δ 7.28, 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 comparison of the 1H -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' (δ 7.28, d, $J=3.5$ Hz) in 11 shifts markedly lower than that of 10 (δ 6.20, d, $J=3.5$ Hz), as mentioned above.

ACKNOWLEDGEMENTS

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References and Notes

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