OENOTHEIN B, A DIMERIC HYDROLYZABLE TANNIN OF CYCLIC STRUCTURE

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Oenothein B, isolated from the leaves of Oenothera erythrosepala (Onagraceae), had host-mediated antitumor activity and antiviral effects on human immunodefficiency virus (HIV) and herpes simplex virus (HSV). It is a dimeric hydrolyzable tannin with a cyclic structure **1**.

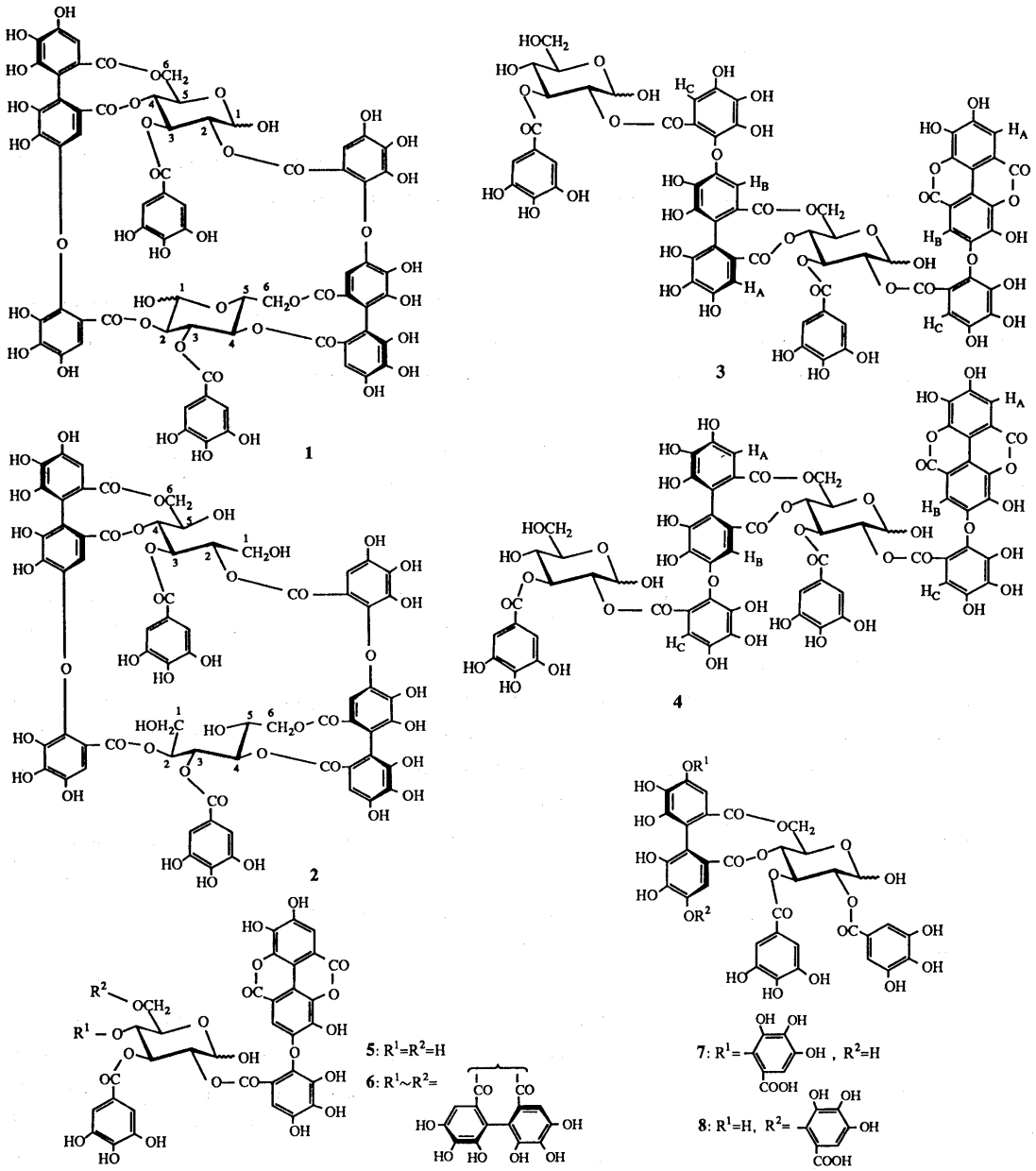
KEYWORDS tannin; dimeric hydrolyzable tannin; oenothein B; Oenothera erythrosepala; Onagraceae; cyclic structure; antitumor activity; antiviral activity; HIV; HSV

A new dimeric hydrolyzable tannin, oenothein B,[1] isolated from the leaves of Oenothera erythrosepala Borbas, shows host-mediated antitumor activity,[2] and also inhibits the proliferation of HIV[3] and HSV.[4] Here we describe its large-ring structure.

This tannin is the main component of leaves of Oenothera erythrosepala, and was isolated as follows. The concentrated filtrate from the 70% acetone homogenate of fresh leaves was extracted successively with diethyl ether, ethyl acetate and n-BuOH. The n-BuOH extract was chromatographed over Sephadex LH-20 with EtOH, to give oenothein B as an off-white amorphous powder.

Oenothein B (**1**), $C_{68}H_{48}O_{44}$,[5] $[\alpha]_D$ +179° (c=1, MeOH), showed the $[M+H]^+$ ion at m/z 1569 in the fast-atom bombardment mass spectrum (FAB-MS). Methylation of **1** with diazomethane followed by methanolysis afforded trimethyl (S)-octa-O-methylvaloneate $[\alpha]_D$ -22° (c=1, acetone) and methyl tri-O-methylgallate in a molar ratio 1:1, which was shown by high-performance liquid chromatography[6] of the methanolyzates. The presence of glucose cores in the molecule was demonstrated by gas liquid chromatography of the trimethylsilyl ether of the product obtained by treating **1** with 5% H_2SO_4 (in boiling-water bath for 20 h). These results indicate that oenothein B consists of two valoneoyl groups, two galloyl groups and two glucose cores. The Cotton effect in the short-wavelength region ($[\theta]_{218}$ +3.8 x 10^5) in the circular dichroism spectrum (in MeOH) of **1** indicated the S-configuration of the two valoneoyl groups.[7]

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** recorded in the ambient temperature was complicated, probably due to anomerization in its two glucose cores, and also to rigidity in the molecule such as restricted rotation around the ether linkages of the valoneoyl groups. However, the spectrum (200 MHz, in acetone-d_6) recorded at an elevated temperature (50°C) showed the α-form of a glucose core and the β-form of another glucose core in the main anomeric form: δ 6.20 [d, J=3.5 Hz, H-1, α-glucose (α)], 6.11 (t, J=10 Hz, H-3, α), 5.87 (dd, J=3.5, 10 Hz, H-2, α), 5.60 (t, J=10 Hz, H-4, α), 5.45 [t, J=9.5 Hz, H-3, β-glucose (β)], 5.25 (dd, J=6, 13 Hz, H-6, α), 5.19 (dd, J=7.5, 9.5 Hz, H-2, β), 5.00 (dd, J=5, 13 Hz, H-6, β), 4.92 (t, J=9.5 Hz, H-4, β), 4.59 (dd, J=6, 10 Hz, H-5, α), 4.48 (d, J=7.5 Hz, H-1, β), 4.16 (dd, J=5, 9.5 Hz, H-5, β), 3.85 (d, J=13 Hz, H-6, β), 3.66 (d, J=13 Hz, H-6, α). The large difference between the chemical shifts of the two H-6 protons in each of the two $^4C_1$ glucose cores showed that each hexahydroxydiphenoyl (HHDP) moiety of the two valoneoyl groups is located at O-4 and O-6 in each glucose core.[8] The chemical shift of the H-1 proton of the β-glucose core (δ 4.48) is that of an anomeric center which is not acylated. The anomeric center of the α-glucose core is also not acylated, as indicated by comparison of the chemical shift of H-1 (δ 6.20) with those of the other hydrolyzable tannins with an α-oriented acyloxy group at C-1 [e.g., potentillin,[9] δ 6.63; α-pentagalloylglucose,[10] δ 6.75]. Four signals of anomeric carbons [δ 95.9 (β, minor), 95.8 (β, major), 91.5 (α, major), 90.8 (α, minor)] in the ^{13}C-NMR spectrum of **1** (125.7 MHz, in acetone-d_6 + D_2O, 40°C) are attributable to the



anomerization, and hence to the absence of acyl groups on O-1 in the two glucose cores.[11] These findings indicate that two galloyl moieties in the two valoneoyl groups and two galloyl groups are at the O-2 and O-3 of the two glucose cores.

Reduction of **1** with $NaBH_4$,[12] afforded **2**, $[\alpha]_D$ +25° (c=1, MeOH), which showed the $[M+Na]^+$ ion at m/z 1595 in the FAB-MS. The ^{1}H-NMR spectrum of **2** (500 MHz, in acetone-d_6 + D_2O) showed clear signals of two galloyl groups [δ 7.14, 7.07 (2H each, s)] and two valoneoyl groups [δ 7.47, 7.07, 6.52, 6.41, 6.37, 6.03 (1H each, s)], along with two glucitol cores [δ 3.35 (br dd, J=9, 12 Hz, H-1), 3.47 (dd, J=9, 11 Hz, H-1), 5.22 (br dd, J=6, 9 Hz, H-2), 5.53 (d, J=6 Hz, H-3), 5.46 (d, J=7 Hz, H-4), 3.89 (br d, J=7 Hz, H-5), 4.47 (br d, J=12 Hz, H-6), 3.88 (br d, J=12 Hz, H-6); 3.41 (dd, J=9, 11 Hz, H-1), 3.65 (dd, J=6, 11 Hz, H-1), 5.15 (br dd, J=6, 9 Hz, H-2), 5.70 (d, J=10 Hz, H-3), 5.33 (dd, J=7, 10 Hz, H-4), 4.00 (br d, J=7 Hz, H-5), 4.57 (br d, J=12 Hz, H-6), 3.64 (br d, J=12 Hz, H-6)].

Treatment of **1** with 0.1 N H_2SO_4 in boiling-water bath for 13 h gave **3** and **4**. The ^{1}H-NMR spectrum of **3** (500 MHz, in acetone-d_6 + D_2O) showed the presence of a lactonized valoneoyl (LV) group,[13] a valoneoyl (Val) group, two galloyl groups and two glucose cores in **3** [δ 7.61-7.60 (1H in total; H_A of LV group),

7.15–7.14 (1H in total; H_B of LV group), 7.08–7.00 (4H in total; H_C of LV group, H_C of Val group, and 2H of a galloyl group), 6.87, 6.86, 6.78, 6.77 (2H in total; a galloyl group), 6.49, 6.47, 6.45, 6.42 (1H in total; H_A of Val group), 6.08, 6.06, 6.03, 6.01 (1H in total; H_B of Val group), 5.8–3.5 (glucose protons)]. All of the H-1 signals of the four β-glucose cores in three anomeric forms of **3** (α-glucose - β-glucose, β-glucose - α-glucose and β-glucose - β-glucose) appeared in the high-field [δ 4.73, 4.68, 4.16 and 4.16 (each d, J=8 Hz)]. These chemical shifts are attributable to the anisotropic effects[13] of the lactonized valoneoyl group and the valoneoyl group. Hence the galloyl moieties in the two acyl groups could be at O-2 on the two glucose cores. The structure **3** was substantiated by degradation of **3** in hot water for 4 h, which gave 3-O-galloyl-D-glucose,[13] 2,3-di-O-galloyl-D-glucose,[13] oenothein C (**5**)[13] and cornusiin B (**6**).[13] The production of 2,3-di-O-galloyl-D-glucose and **6** is regarded as due to cleavage of the ether linkage of the valoneoyl group.[6,14] The orientation of the valoneoyl group[6] in **3** was assumed to be the same as that in rugosin B (**7**), since the chemical shifts of the HHDP protons in the valoneoyl group in **3** were analogous to those of **7** rather than isorugosin B (**8**).[6,15] The other product **4** was the isomer of **3** concerning the orientation of the valoneoyl group, as indicated by the ^{1}H-NMR spectrum of **4** (500 MHz, in acetone-d_6 + D_2O): δ 7.57–7.55 (1H in total; H_A of LV group), 7.10–6.98 (5H in total; H_B and H_C of LV group, H_C of Val group and 2H of a galloyl group), 6.75, 6.73, 6.68, 6.63 (2H in total; a galloyl group), 6.57–6.56 (1H in total; H_A of Val group), 6.15, 6.13, 6.09, 6.06 (1H in total; H_B of Val group), 5.7–3.5 (glucose protons).

Based on these results, structure **1**, in which the orientations of the two valoneoyl groups are different from each other, was assigned for oenothein B. It is noteworthy that oenothein B, which exhibits several remarkable activities, has such a macro-ring structure.

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