Communications to the Editor

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CORNUSIIN A, A DIMERIC ELLAGITANNIN FORMING FOUR TAUTOMERS, AND ACCOMPANYING NEW TANNINS IN CORNUS OFFICINALIS

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Cornusiin A (4), a dimeric ellagitannin which forms four anomeric isomers, have been isolated from Cornus officinalis fruits, along with cornusiin B (5) and related compounds. Their structures and the ratio of tautomers in the equilibrium mixture of 4 were determined. Oenothein C (8), which was produced by partial hydrolysis of 5, has been isolated also from Oenothera erythrosepala leaves.

KEYWORDS —— cornusiin A; cornusiin B; 2,3-di-O-galloyl-D-glucose; oenothein C; Cornus officinalis; Cornaceae; Oenothera erythrosepala; Onagraceae; dimeric ellagitannin; tannin

The main component of tannin in the fruits of Cornus officinalis Sieb. et Zucc. (Sanshuyu in Japanese, Cornaceae) has been found to be a new dimeric ellagitannin in which molecule two anomeric centers participate in the formation of an equilibrium mixture of four tautomers, which gives signals of eight glucose cores in its ^{1}H-NMR spectrum.

The fresh fruits were homogenized in aqueous acetone, and a concentrated filtrate was extracted with ether and then with ethyl acetate. Besides tellimagrandin I (1), II and isoterchebin reported previously,[1] 1,2,3-tri-O-galloyl-β-D-glucose (2), 1,2,6-tri-O-galloyl-β-D-glucose, 1,2,3,6-tetra-O-galloyl-β-D-glucose and gemin D (3)[2] were isolated from the ethyl acetate extract by column chromatography on Sephadex LH-20. After extraction with ethyl acetate, the aqueous layer was adsorbed on Celite 545, and the acetone eluate from the Celite was submitted to droplet countercurrent chromatography (n-BuOH - n-PrOH - H_2O, 4:1:5, by volume, ascending method) followed by Sephadex LH-20 chromatography to give a new dimeric tannin named cornusiin A (4) as a main component of tannin, and a related new monomer, cornusiin B (5), along with a new digalloylglucose (6) and gemin D.

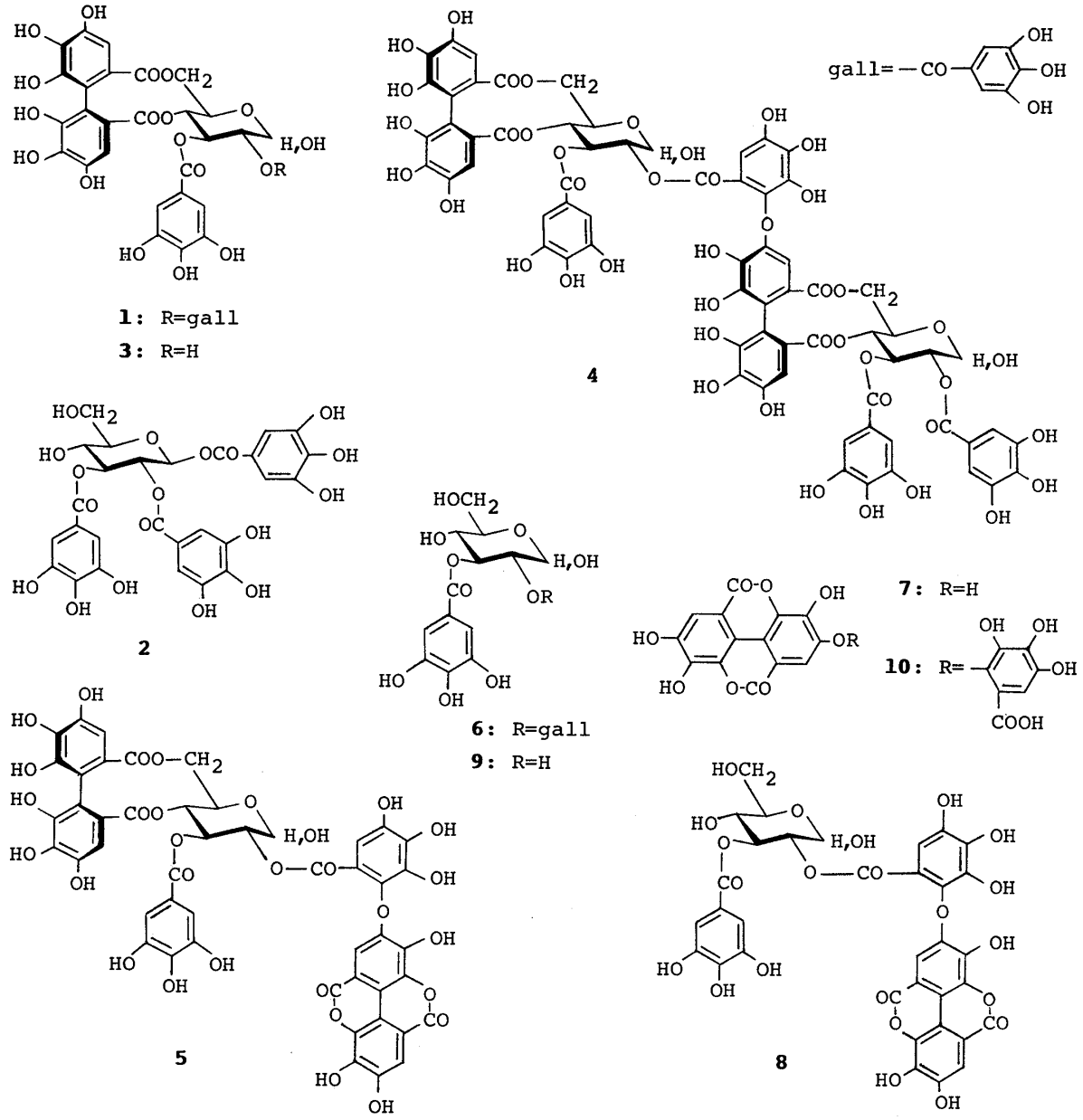
The digalloylglucose (6), $C_{20}H_{20}O_{14}\cdot 2H_2O$, $[\alpha]_D$ +87° (c=1, MeOH), was obtained as a light brown amorphous powder. The ^{1}H-NMR spectrum (200 MHz, in acetone-d_6) of 6 shows that two galloyl groups [δ7.11 (6/5H, s), 7.09 (6/5H, s), 7.06 (4/5H, s) and 7.05 (4/5H, s)] are present in the molecule and that the glucose core is acylated at O-2 and O-3 [δ5.80 (dd, J=8, 9 Hz, H-3 of the α-anomer of glucose), 5.48 (d, J=3 Hz, α-anomer, H-1), 5.42 (t, J=8 Hz, β-anomer, H-3), 5.09 (dd, J=7, 8

Hz, β-anomer, H-2), 4.99 (d, J=7 Hz, β-anomer, H-1), 4.95 (dd, J=3, 9 Hz, α-anomer, H-2) and 4.10-3.50 (H-4,5,6,6' of both anomers)]. The ratio of the two anomers α:β, determined by the peak area in the spectrum, is 3:2. Partial hydrolysis of tellimagrandin I (1) and also of 1,2,3-tri-O-galloyl-β-D-glucose (2) (100°C, 24 hours, in water) afforded 6. Accordingly this compound is 2,3-di-O-galloyl-D-glucose.

Cornusiin B (5), $C_{48}H_{30}O_{30}\cdot 8H_2O$, $[\alpha]_D$ +63° (c=0.5, MeOH), forms a light brown amorphous powder. In its ^{1}H-NMR spectrum (200 MHz, in acetone-d_6-D_2O), the singlets at δ7.68, 7.66 (1H in total), 7.24, 7.21 (1H), 7.01 and 6.96 (1H) show the presence of a lactonized valoneoyl group, and the dual peaks for a proton show the presence of both anomers. Also a pair of singlets of galloyl protons occur at δ6.69 and 6.63 (2H in total) and two pairs of singlets of hexahydroxydiphenoyl (HHDP) protons occur at δ6.649 and 6.645 (1H in total), 6.55 and 6.49 (1H). The absorption maxima at 350 (log ε 4.01) and 363 nm (4.05) in the UV spectrum (in MeOH) of 5 and the carbon signals at δ161.3, 161.2 (1C in total) and 160.4 (1C) in a high field relative to the region of the other ester carbonyl signals (δ168.9-165.1) in the ^{13}C-NMR spectrum (50.1 MHz, in acetone-d_6-D_2O) are also in accord with the lactonization of the valoneoyl group. Partial hydrolysis of 5 with 1N H_2SO_4 at 100°C for 4.5 hours afforded ellagic acid (7) and a compound named oenothein C (8) which has also been isolated from the leaves of Oenothera erythrosepala Borbas (Onagraceae).

Oenothein C (8), $C_{34}H_{24}O_{22}\cdot 5H_2O$, $[\alpha]_D$ +72° (c=0.5, MeOH) was obtained as an off-white amorphous powder. The ^{1}H-NMR spectrum (200 MHz, in acetone-d_6-D_2O) shows the presence of a lactonized valoneoyl group [δ7.64 (1H, s), 7.19, 7.17 (1H in total, s), 7.09 and 7.08 (1H, s)], a galloyl group [δ7.05 and 6.97 (2H in total, s)] and a glucose core which forms α- and β-anomers (3:2) [δ5.69 (dd, J=8, 9 Hz, α-anomer, H-3), 5.22 (d, J=3 Hz, α-anomer, H-1), 5.18 (t, J=9 Hz, β-anomer, H-3), 5.00 (dd, J=7, 9 Hz, β-anomer, H-2), 4.90 (dd, J=3, 9 Hz, α-anomer, H-2), 4.58 (d, J=7 Hz, β-anomer, H-1) and 4.00-3.50 (H-4,5,6,6' of both anomers)]. The lactonization of the valoneoyl group is shown by the peaks at δ162.7 (1C) and 162.4 (1C) in the ^{13}C-NMR spectrum (22.6 MHz, in MeOH-d_4) and by the absorption maxima at 350 (log ε 4.02) and 364 nm (4.08) in the UV spectrum (in MeOH). The chemical shifts of H-2 and H-3 of the glucose moiety in the ^{1}H-NMR spectrum show that the two substituents (galloyl and lactonized valoneoyl) are at O-2 and O-3 of glucose. The lactonized valoneoyl group is assumed to be at O-2 because of the upfield shift of the H-1 proton of the β-anomer of 8 relative to those of 6 due to the shielding effect by the aromatic ring of lactonized valoneoyl group of 8, and this was proved by the partial hydrolysis of 8 with 1% H_2SO_4 for 12 hours at 100°C to 3-O-galloyl-D-glucose (9)[3] [^{1}H-NMR spectrum (200 MHz, in acetone-d_6-D_2O): δ7.20 (2H, s, galloyl), 5.41 (t, J=9 Hz, β-anomer, H-3), 5.28 (d, J=3 Hz, α-anomer, H-1), 5.17 (t, J=9 Hz, α-anomer, H-3), 4.74 (d, J=8 Hz, β-anomer, H-1) and 4.00-3.44 (H-2,4,5,6,6' of both anomers)]. Structure 8 is therefore assigned to oenothein C, and accordingly the HHDP group in 5 is at O-4 and O-6.

Cornusiin A (4), $C_{68}H_{50}O_{44}\cdot 11H_2O$, $[\alpha]_D$ +78° (c=1, MeOH), UV λ_{max}^{MeOH} 218 (log ε 5.14) and 271 nm (4.81), was obtained as an off-white amorphous powder. The molecular peak 1593 (M+Na) is shown in the fast atom bombardment (FAB) mass spectrum. The retention volume of the high-performance gel permeation chromatography



on Shimadzu HSG-15 column for 4 (without derivatization) and TSK-GEL G2500H8 column for the peracetate of 4 were in accord with the dimeric structure.

Hydrolysis of 4 with 1N H_2SO_4 at 100°C for four hours gave gallic acid, ellagic acid, valoneic acid dilactone (10) and glucose. Methanolysis after methylation of 4 afforded methyl tri-O-methylgallate, dimethyl (S)-hexamethoxydiphenate and trimethyl octa-O-methylvaloneate in the ratio 3:1:1. These were determined by HPLC (silica, hexane-ethyl acetate, 2:1, by volume).

The ^{1}H-NMR spectrum (400 MHz, in acetone-d_6) of 4 shows the peaks of eleven aromatic protons. The aromatic protons of three galloyl groups are observed in δ7.05-7.04 (2H in total), 7.01-6.97 (2H) and 6.90-6.81 (2H), and the other proton signals ascribable to an HHDP group and a valoneoyl group were observed in δ7.09-7.04 (1H, in part overlapped with signals of a galloyl group), 6.71-6.67 (1H),

6.65-6.63 (1H), 6.49-6.46 (1H) and 6.21-6.16 (1H). The anomeric proton peaks are absent in the field lower than 5.57 ppm showing that the anomeric centers of two glucose cores in **4** are not acylated.

Partial hydrolysis of **4** in hot water (100°C, 18 hours) afforded 2,3-di-O-galloyl-D-glucose, cornusiin B, gemin D and ellagic acid. Although the valoneoyl group in **5** is lactonized, it is not lactonized in **4**, as the amplitude of the Cotton effect in the short wavelength ($[\theta]_{220}$ +2.5 x 10^5) of the CD spectrum of **4** is about twice that of tellimagrandin I,[4] and the optically active permethylated valoneate was given by the methanolysis after methylation of **4**. The lactonization at the HHDP part of the valoneoyl group in **5**, then, should occur upon the hydrolysis of **4**. Thus the two ester carbonyl groups of the valoneoyl group are at O-4 and O-6 of the other sugar moiety which gave 2,3-di-O-galloyl-D-glucose upon hydrolysis. Therefore, the structure of cornusiin A is shown by **4**.

The expected four anomeric forms of **4** due to two glucose cores, which gives signals of eight glucose cores, namely α_A-α_B (**4a**, "A" is the "left" glucose core which gave cornusiin B upon the hydrolysis and "B" is the "right" core which gave 2,3-di-O-galloyl-D-glucose), α_A-β_B (**4b**), β_A-α_B (**4c**) and β_A-β_B (**4d**), are observed in the ^{1}H-NMR spectrum of **4**. The composition of the four tautomers were found to be 3:2:6:3 in the ratio for **4a**, **4b**, **4c** and **4d** from the following data. i) Each aromatic proton appears as four peaks in the ratio 6:3:3:2 [for example, an aromatic proton appears at δ6.21 (3/14H), 6.18 (3/14H), 6.17 (6/14H) and 6.16 (2/14H), and aromatic protons of a galloyl group appear at δ6.90 (6/14H), 6.87 (4/14H), 6.85 (12/14H) and 6.81 (6/14H).]. ii) The signals of H-3 of the α-anomers of both glucose cores [δ5.83 (3/14H), 5.81 (2/14H), 5.72 (3/14H) and 5.70 (6/14H)] show that α_A-α_B (**4a**), α_A-β_B (**4b**) and β_A-α_B (**4c**) are present in the ratio 3:2:6 or 3:6:2. iii) The H-1 signals of the β-anomer of the "left" glucose (β_A) shifting to the higher field [δ4.61 (6/14H) and 4.53 (3/14H)] because of the shielding effect of the valoneoyl group in a way analogous to oenothein C, shows that the ratio of β_A-α_B (**4c**) to β_A-β_B (**4d**) is 6:3 or 3:6. Therefore four tautomers **4a**, **4b**, **4c** and **4d** are present in the ratio 3:2:6:3.

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