CAMELLIIN B AND NOBOTANIN I, MACROCYCLIC ELLAGITANNIN DIMERS AND RELATED DIMERS, AND THEIR ANTITUMOR ACTIVITY

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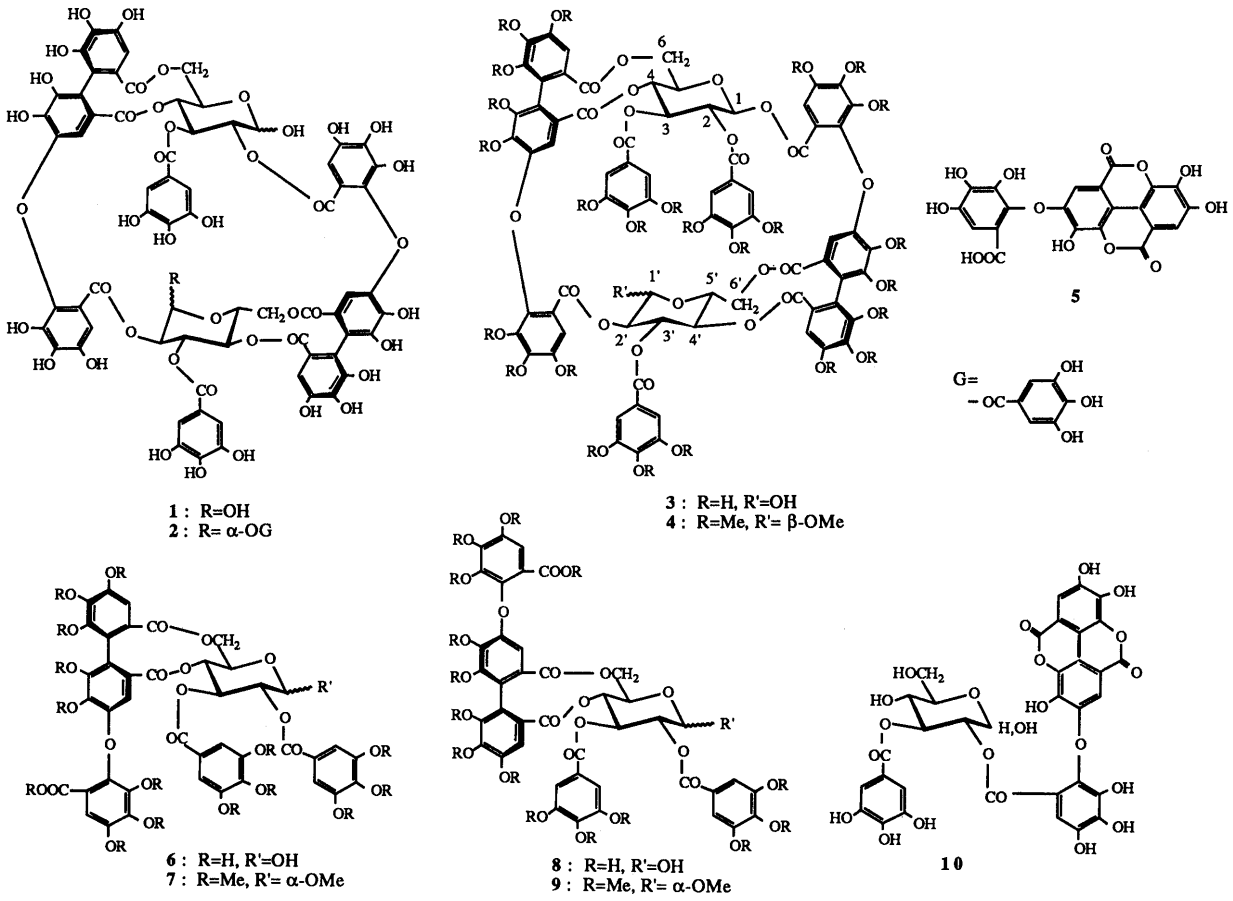
Camelliin B and nobotanin I, dimeric hydrolyzable tannins of a new class having macrocyclic structures, were isolated from Camellia japonica and Heterocentron roseum, respectively. Nobotanin G and H of the structures related to nobotanin I, were also obtained from H. roseum. Camelliin B and also woodfordin C, a macrocyclic dimer from Woodfordia fruticosa, exhibited marked host-mediated antitumor activities.

KEYWORDS camelliin B; Camellia japonica; nobotanin G; nobotanin H; nobotanin I; Heterocentron roseum; tannin; hydrolyzable tannin; macrocyclic dimer; antitumor activity

Oenothein B (**1**)[1] and woodfordin C (**2**),[2] dimeric hydrolyzable tannins isolated from Oenothera erythrosepala (Onagraceae)[1] and Woodfordia fruticosa (Lythraceae),[2] have unique macrocyclic structures, and the former showed marked host-mediated antitumor activity.[3] A new dimer of this type, camelliin B (**3**), has now been isolated from the flower buds of Camellia japonica (Theaceae). A macrocyclic dimer of another type, nobotanin I (**13**) has also been isolated from the leaves of Heterocentron roseum (Melastomataceae), accompanied by the dimers of related structures, nobotanin G (**11**) and H (**12**).

Camelliin B (**3**), $C_{75}H_{52}O_{48}.15H_2O$, $[\alpha]_D$ -24° (MeOH), showed the $[M+Na]^+$ ion at m/z 1743 in the fast-atom bombardment mass spectrum (FAB-MS). Hydrolysis of **3** with 5% H_2SO_4 under reflux for 9 h gave gallic acid, valoneic acid dilactone (**5**) and glucose. The ^1H nuclear magnetic resonance (NMR) spectrum (500 MHz, acetone-d_6), in which the dual peak of each signal was shown in a ratio of ca. 4:1 due to anomerization in one of the glucose cores, indicated the presence of three galloyl groups [δ 7.17, 7.19; 7.11, 7.08; 6.81, 6.84 (each pair corresponds to 2H in the peak area] and two valoneoyl groups [δ 7.41, 7.36; 7.15, 7.15; 6.81, 6.80; 6.72, 6.70; 6.46, 6.44; 5.90, 5.97 (each pair corresponds to 1H]. A strong Cotton effect, [θ] 27.8 x 10^4 at 220 nm, in the circular dichroism (CD) spectrum of **3**, showed that the configurations of these valoneoyl groups are both S.[4] The ^1H-^1H shift correlation spectrum showed that the hydroxyl groups on the two $^4C_1$ glucopyranose cores, except for that on an anomeric center, are all acylated. An anomeric acyloxy group on a glucose core in **3** was shown to be β, by a large coupling constant (J=8.5 Hz) of an anomeric proton signal at δ 5.84. The presence of the valoneoyl group at O-4～O-6 of each glucose core was shown by the large difference[5] between the chemical shifts of C-6 methylene protons [δ 5.20 (dd, J=7.5, 12 Hz), 3.93 (dd, J=5.5, 12 Hz); 4.94 (dd, J=5.5, 13 Hz), 3.96 (d, J=13 Hz)]. Methylation of **3** with Me_2SO_4-K_2CO_3 in dry acetone furnished hexacosa-O-methylcamelliin B (**4**) and a partially degraded product (**7**). Although **7** was indicated by the ^1H-NMR spectrum [δ 7.21, 7.11 (each 2H, s), 6.33, 6.96, 7.27 (each 1H, s), 5.24 (d, J=4 Hz, H-1)] to be a methylated derivative of 2,3-di-O-galloyl-4,6-(S)-valoneoyl-α-D-glucose, it was clearly different from the hexacosamethyl derivative [α-anomer (**9**); δ 7.26 (4H, s), 6.49, 6.76, 7.30 (1H each, s), 5.24 (d, J=4 Hz)] of rugosin B (**8**).[6] Therefore, **7** was considered to be an isomer of **9**, differing only in the orientation of valoneoyl group [methylate of isorugosin B (**6**)[7]]. Partial hydrolysis of **3** in boiling water yielded **5** and oenothein C (**10**).[8] In an equilibrated anomer mixture of **3**, the α-anomer was predominant (ca. 4:1) as indicated by the ratio of unacylated anomer carbon signals at δ 92.2 (α) and 96.7

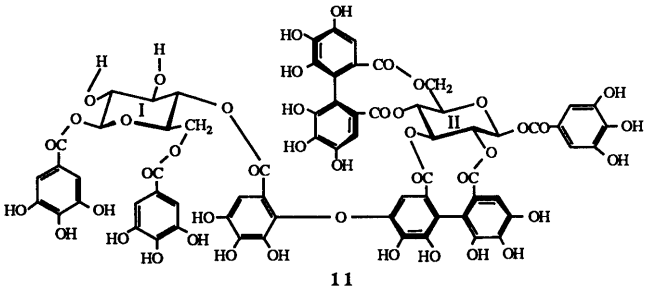


(β) in the ^{13}C-NMR spectrum. The H-1 signal of this α-anomer resonates, as does that of **1**[1], at a lower field (δ 5.94, d, J=4 Hz) than those of **6** (δ 5.54, d, J=3.5 Hz) and **8** (δ5.52, d, J=3.5 Hz). Based on these data, structure **3** of camelliin B was assigned.

Nobotanin G (**11**), $C_{68}H_{50}O_{44}.9H_2O$, [α]$_D$ +56° (MeOH), nobotanin H (**12**), $C_{89}H_{60}O_{57}.12H_2O$, [α]$_D$ +80° (MeOH), and nobotanin I (**13**), $C_{89}H_{58}O_{56}.7H_2O$, [α]$_D$ +70° (MeOH), were shown to be mutually related dimeric hydrolyzable tannins as follows: Hydrolysis of these dimers with 5% H_2SO_4 gave gallic acid, ellagic acid, **5** and glucose. In an aqueous solution at 37°C, nobotanin I (**13**) was converted within 36 h to nobotanin H (**12**) which was further hydrolyzed in boiling water to nobotanin G (**11**). The ^{1}H-NMR spectrum (400 MHz, acetone-d_6) of nobotanin G (**11**) indicated that this dimer is composed of three galloyl groups [δ 7.24, 7.14, 7.00 (each 2H, s)], a valoneoyl group, a hexahydroxydiphenoyl (HHDP) group [δ 7.12, 6.64, 6.55, 6.37 and 6.12 (each 1H, s)], and two $^4C_1$ glucopyranose cores. One (glucose II) of the glucose cores in **11** are fully acylated and has the ester linkages bridged on O-4 ~ O-6, as indicated by the methylene proton signals at δ5.32 (dd, J=6, 13 Hz) and 3.89 (d, J=13 Hz), which are analogous to those of **1**~**3**. The upfield shifts of H-2 (δ3.66, dd, J=8, 10 Hz) and H-3 (δ 3.85, t, J=10 Hz) of another glucose core (glucose I) suggest the presence of free hydroxyl groups at C-2 and C-3. Hydrolysis of **11** in boiling water for 8 h yielded strictinin (**14**)[9] and another hydrolysate (**15**)[10] having two galloyl groups at O-1 and O-6, and a dilactonized valoneoyl group at O-4 of β-D-glucose. Therefore, structure of nobotanin G was determined to be **11**. Since the ^{1}H-NMR spectrum of nobotanin H (**12**) showed the presence of two valoneoyl groups, an HHDP group, three galloyl groups [δ 7.19, 7.13, 6.65, 6.54, 6.48, 6.40, 6.20, 6.12 (each 1H, s); 7.27, 7.12, 6.98 (each 2H, s)], and fully acylated $^4C_1$ glucose cores, nobotanin H was characterized as a monovaloneate (**12**) of **11**. The configurations of the HHDP and the valoneoyl groups in **12** are all S, as revealed by a positive Cotton effect, [θ]$_{225}$ +39 x 10^4, in the CD spectrum of **12**. Five aromatic proton signals (δ 6.12, 6.20, 6.40, 6.54 and 7.13) due to an HHDP and a valoneoyl group in the ^{1}H-NMR spectrum of **12** measured at ambient temperature, were broadened probably by restricted rotation of the ether bond in the valoneoyl group. However, these signals were sharpened when recorded at 40°C, indicating that these two groups in **12** are

spatially near. Inspection with a stereomodel of **12** indicated the most plausible orientations of the valoneoyl groups at O-2 ~ O-3 of each glucose core are as illustrated in the formula **12**.

The ^{1}H-NMR spectrum of nobotanin I (**13**) was showed closely similar to that of **12**, except for a significant downfield shift of one of the 1H-aromatic singlets (δ 6.21→6.94), and an upfield shift of the proton (δ7.19→7.03) ascribable to that of the galloyl moiety in one of the valoneoyl groups in **13**. Upon comparison of the ^{13}C-NMR spectra of **12** and **13**, **13** showed upfield shifts of an ester carbonyl signal (δ 166.8→163.2) and the C-5 signal (δ 137.7→132.2) of the HHDP group, and a downfield shift of C-4 signal (δ 145.2→151.6) of the HHDP group. These spectral differences and the facile hydrolysis of **13** to **12**, which are analogous to those between praecoxin C and rugosin C,[11] led to the structure **13** of nobotanin I. Nobotanin I is the first example of a dimer forming a macro-ring through an intramolecular depside linkage.



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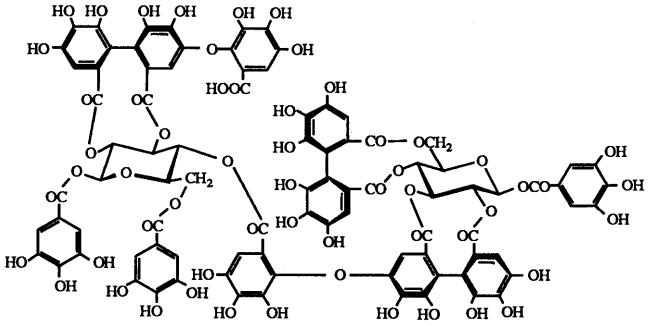

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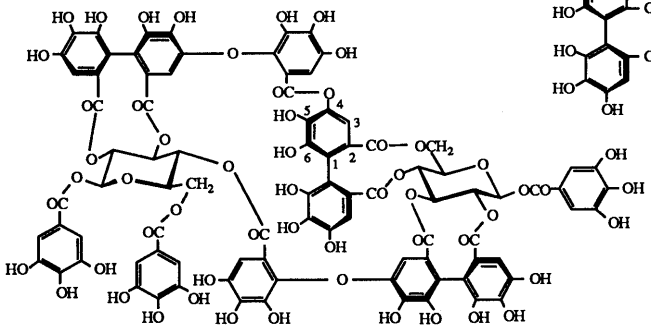

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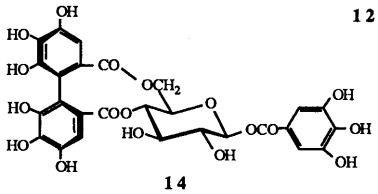

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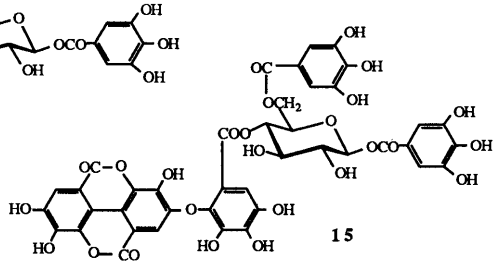

15

Upon intraperitoneal injection of 10 mg/kg each of these tannins and woodfordin C (**2**), at 4 days before intraperitoneal inoculation of sarcoma 180 cells (1 x 10^5), carried out in a way analogous to that for **1**,[3] camelliin B (**3**) and woodfordin C (**2**) markedly prolonged the life-span of the mice (+36% and 60%, respectively), and they respectively induced 60-day survival of 2 and 1 of 5 mice.

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