

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

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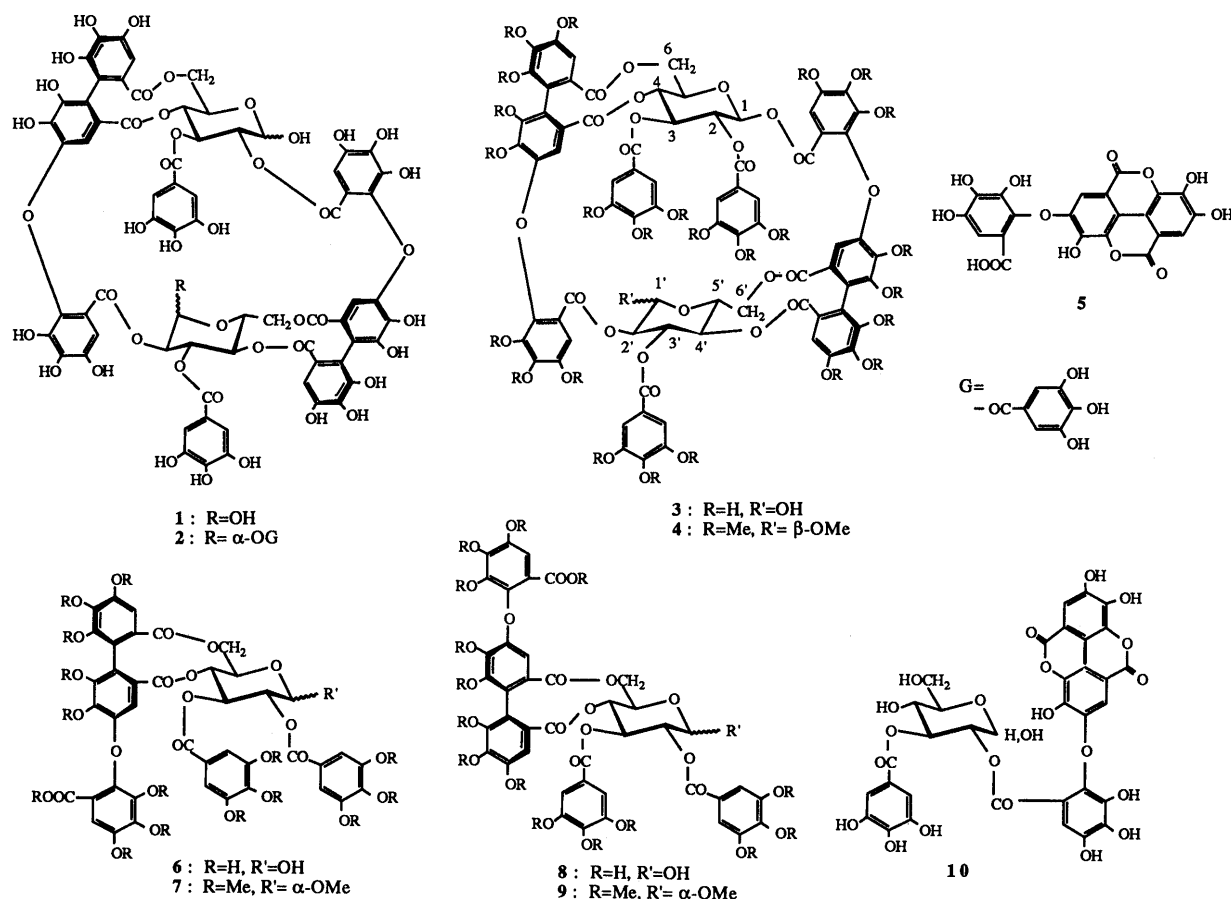
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Camelliin B and nobotanin I, dimeric hydrolyzable tannins of a new class having macro-
cyclic structures, were isolated from Camellia japonica and Heterocentron roseum,
respectively. Nobotanin G and H of the structures related to nobotanin I, were 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; Hetero-
centron roseum; tannin; hydrolyzable tannin; macrocyclic dimer; antitumor activity

Oenothlein 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), C₇₅H₅₂O₄₈·15H₂O, [α]_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% H₂SO₄ under reflux for 9 h gave gallic
acid, valoneic acid dilactone (5) and glucose. The ¹H nuclear magnetic resonance (NMR) spectrum (500 MHz,
acetone-d₆), 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 × 10⁴ 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 ⁴C₁ 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 difference⁵⁾ 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 Me₂SO₄-K₂CO₃ in dry
acetone furnished hexacosao-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 oenothlein 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

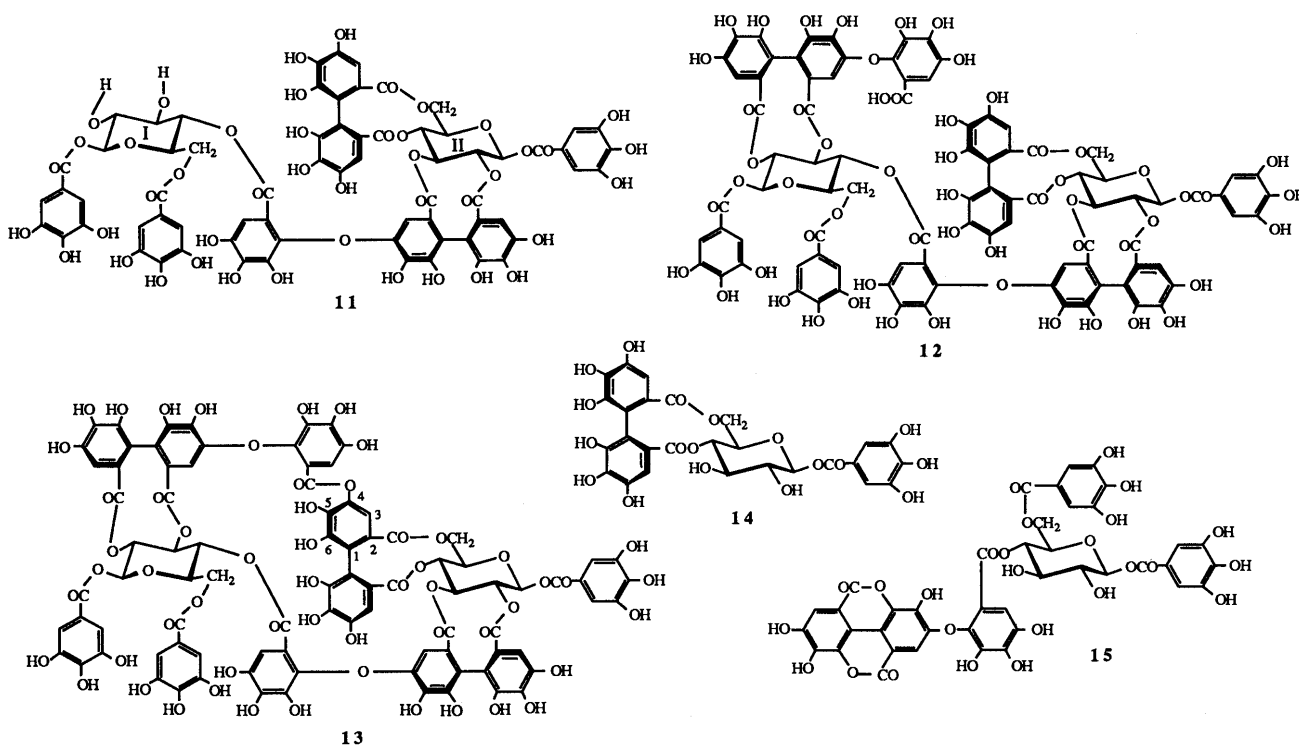


(β) in the ^{13}C -NMR spectrum. The H-1 signal of this α -anomer resonates, as does that of 1^1 , at a lower field (δ 5.94, d, $J=4$ Hz) than those of 6 (δ 5.54, d, $J=3.5$ Hz) and 8 (δ 5.52, d, $J=3.5$ Hz). Based on these data, structure 3 of camelliin B was assigned.

Nobotanin G (11), $\text{C}_{68}\text{H}_{50}\text{O}_{44} \cdot 9\text{H}_2\text{O}$, $[\alpha]_D^{25} +56^\circ$ (MeOH), nobotanin H (12), $\text{C}_{89}\text{H}_{60}\text{O}_{57} \cdot 12\text{H}_2\text{O}$, $[\alpha]_D^{25} +80^\circ$ (MeOH), and nobotanin I (13), $\text{C}_{89}\text{H}_{58}\text{O}_{56} \cdot 7\text{H}_2\text{O}$, $[\alpha]_D^{25} +70^\circ$ (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 ^1H -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 hexahydroxydiphenyl (HHDP) group [δ 7.12, 6.64, 6.55, 6.37 and 6.12 (each 1H, s)], and two $^4\text{C}_1$ 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 δ 5.32 (dd, $J=6, 13$ Hz) and 3.89 (d, $J=13$ Hz), which are analogous to those of $1\sim 3$. The upfield shifts of H-2 (δ 3.66, 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 ^1H -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 $^4\text{C}_1$ 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, $[\theta]_{225}^{25} +39 \times 10^4$, 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 ^1H -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 ^1H -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 $\rightarrow$ 6.94), and an upfield shift of the proton (δ 7.19 $\rightarrow$ 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 $\rightarrow$ 163.2) and the C-5 signal (δ 137.7 $\rightarrow$ 132.2) of the HHDP group, and a downfield shift of C-4 signal (δ 145.2 $\rightarrow$ 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.



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 (1×10^5), 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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(Received October 2, 1989)