Tannins of Theaceous Plants. V.[1] Camelliatannins F, G and H, Three New Tannins from *Camellia japonica* L.

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Two new complex tannins, camelliatannins F (3) and G (4), were isolated from the leaves of *Camellia japonica* L. (Theaceae), and their structures, each consisting of an epicatechin unit and a *C*-glucosidic ellagitannin moiety, were elucidated. A new dimeric hydrolyzable tannin, named camelliatannin H (5), was isolated from the fruits of this plant. Camelliins A (6) and B (7), and camelliatannin A (1) and 3 were also isolated from the fruits.

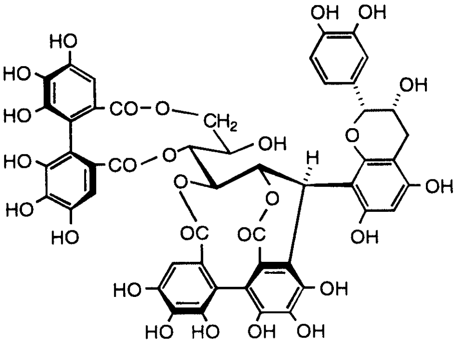
Keywords tannin; *Camellia japonica*; Theaceae; camelliatannin F; camelliatannin G; camelliatannin H

Previously, we reported the isolation and structures of various types of tannins, including complex tannins, camelliatannins A (**1**) and B (**2**), from the flower buds and leaves of *Camellia japonica* L. (Theaceae).[2] Further investigation of the leaves and fruits of the plant has led to the isolation of three new tannins named camelliatannins F (**3**), G (**4**) and H (**5**).[3] Camelliatannins F and G are complex tannins, and camelliatannin H is a dimeric hydrolyzable tannin having a valoneoyl group at glucose O-2/O-3 as a linking unit between the constituent monomers. This paper deals with the structure elucidation of these new tannins.

Results and Discussion

Fresh leaves of *Camellia japonica* L. were homogenized in aqueous acetone and the concentrated filtrate from the homogenate was extracted with Et_2O and EtOAc, successively. The aqueous mother liquor was subjected to column chromatography over Dia-ion HP-20, Toyopearl HW-40 and/or MCI-gel CHP-20P to afford camelliatannin A (**1**) and two new tannins, camelliatannins F (**3**) and G (**4**). Fresh fruits were treated in an analogous way, to give **3** and another new tannin, camelliatannin H (**5**), together with the following known tannins and related polyphenols: camelliins A (**6**) and B (**7**) (dimeric hydrolyzable tannins),[4] camelliatannin A (**1**) (complex tannin), (−)-epicatechin (**8**),[2] 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (**9**),[5] 1,6-di-*O*-galloyl-*β*-D-glucose (**10**), gallic acid 3-*O*-*β*-D-(6′-*O*-galloyl)-glucopyranoside (**11**),[6] pedunculagin (**12**)[7] and casuariin (**13**).[2,8]

Structure of Camelliatannin F Camelliatannin F (**3**) was obtained as an off-white powder. The FAB-MS of



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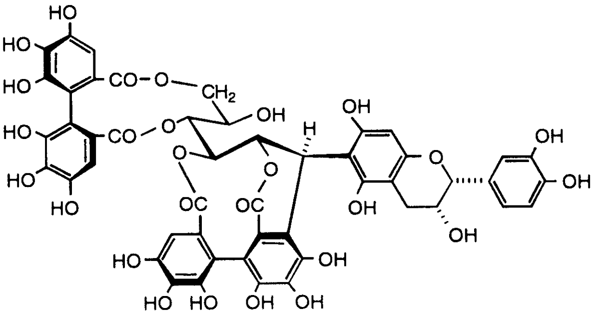

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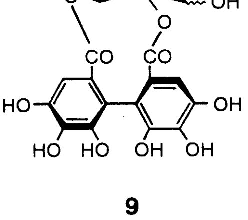

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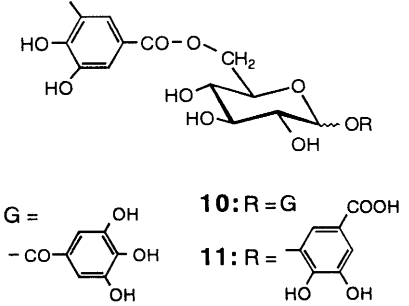
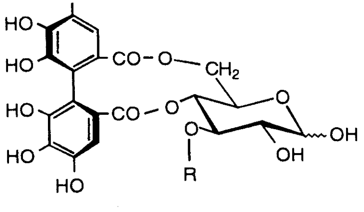


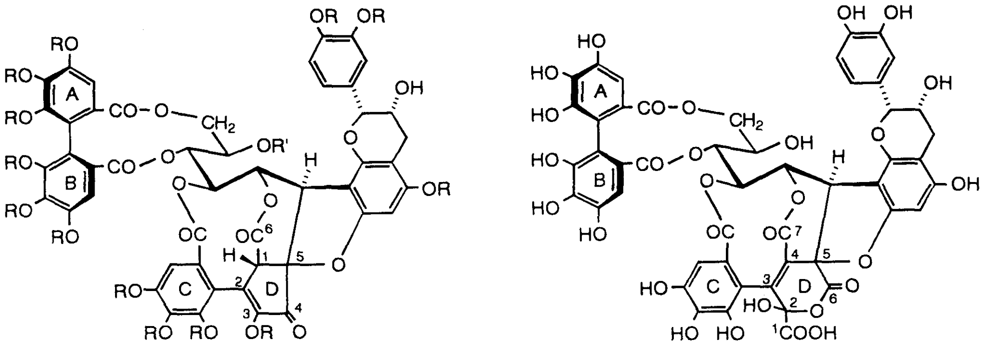
10: R = G

11: R =



20: R = G
21: R = H

Chart 1



3: R = R' = H
3a: R = CH₃, R' = H
14: R = H, R' = G

4

Chart 2

3 showed the $[M+H]^+$ ion peak at *m/z* 1027 and $[M+Na]^+$ ion peak at *m/z* 1049, corresponding to the molecular formula $C_{48}H_{34}O_{26}$. Treatment of **3** with ethereal diazomethane gave a tridecamethyl derivative (**3a**).

The ^1H-NMR spectrum of **3** indicated that this compound is a complex tannin consisting of a flavan-3-ol moiety [δ 4.99 (br s, H-2), 4.26 (m, H-3), 2.88 (dd, $J=3.8$, 16 Hz, H-4), 2.80 (dd, $J=2.4$, 16 Hz, H-4), 6.09 (s, H-6), 7.09 (d, $J=2$ Hz, H-2'), 6.81 (d, $J=8.2$ Hz, H-5') and 6.90 (dd, $J=2$, 8.2 Hz, H-6')] and a *C*-glucosidic ellagitannin moiety {δ 6.83, 6.53, 6.40 and 4.32 [each 1H, s, protons of the A—D rings (H_A—H_D)]; δ 4.00 [s, glucose (Glc) H-1], 5.60 (br s, Glc H-2), 5.12 (d, $J=7.7$ Hz, Glc H-3), 5.04 (t, $J=7.7$ Hz, Glc H-4), 3.65 (br d, $J=7.7$ Hz, Glc H-5), 4.32 (dd, $J=2.7$, 12 Hz, Glc H-6) and 3.35 (br d, $J=12$ Hz, Glc H-6)}. The coupling constants of the sugar proton signals were consistent with those of the glucose residue of open-chain form, and the chemical shifts of the glucose H-2—H-6 signals indicated that the hydroxyl groups at C-2, C-3, C-4 and C-6 are acylated, and that at C-5 is unacylated. The flavan-3-ol unit of **3** was shown to be epicatechin by the small coupling constant between H-2 and H-3 of the flavan-3-ol moiety. The epicatechin H-2 signal was shifted downfield, relative to the corresponding signal of **8** (δ 4.89), in a way analogous to the H-2 shift observed for camelliatannin A (**1**).[2] This downfield shift was attributable to the anisotropic effect of the nearby located ellagitannin moiety, indicating the C-8 substitution of the epicatechin moiety.

The four singlets at δ 6.83, 6.53, 6.40 and 4.32 suggested the presence of a hexahydroxydiphenoyl (HHDP) group (A and B rings) and a phenylcyclopentenone group (C and D rings) in **3**. The presence of these groups was substantiated by aromatic carbon signals due to the A—C rings (see Experimental) and the five signals at δ 49.4 (C_D-1), 137.5 (C_D-2), 149.8 (C_D-3), 196.2 (C_D-4) and 90.3 (C_D-5) (Table I) in the ^{13}C-NMR spectrum of **3**. The chemical shifts of these five carbon signals, which are closely similar to those of the cyclopentenone carbons of malabathrins E (**14**) and F,[9] clearly indicated that the cyclopentenone residue of **3** is connected with the epicatechin moiety through the ether linkage, and also with the glucose C-1 through the C–C linkage.

Table I. ^{13}C-NMR Spectral Data for **3** and **4** (125.7 MHz, in Acetone-d_6+D_2O)

Carbons		3	4
Glucose	C-1	46.5	50.2
	C-2	79.7	76.4
	C-3	77.5	75.8
	C-4	74.6	75.7
	C-5	69.6	69.0
	C-6	67.2	67.4
Epicatechin	C-2	80.3	79.3
	C-3	66.6	66.4
	C-4	29.4	28.2
	C-4a	101.9	102.4
	C-5	158.7	158.2
	C-6	90.3	90.6
	C-7	159.5	158.5
	C-8	104.7	104.9
	C-8a	152.6	152.0
	C-1'	131.9	131.1
	C-2'	115.0	114.9
	C-3'	145.0[a]	145.0[b]
	C-4'	145.0[a]	145.2[b]
	C-5'	115.6	115.8
	C-6'	119.4	119.4
D-Ring	C-1	49.4	166.8
	C-2	137.5	99.2
	C-3	149.8	131.0
	C-4	196.2	137.8
	C-5	90.3	86.6
	C-6		161.5

a, b) Values with the same superscript may be interchanged.

The configuration at glucose C-1 was assigned to be *S* based on the small coupling constant ($J<1$ Hz) between H-1 and H-2.[2] This assignment was substantiated by rotating-frame Overhauser enhancement spectroscopy (ROESY) which showed the nuclear Overhauser effect (NOE) between H-1 and H-3 of the glucose residue (Fig. 1).

The ROESY spectrum also suggested the *R*-configuration of the methine carbon (C_D-1) in the cyclopentenone residue of **3**, as follows. The rigid ring system around the cyclopentenone residue, allows only two partial structures, A and B in Chart 3. When the α-oritentation of H_D (structure B) is assumed, NOE between H_D and glucose H-3 or between H_D and glucose H-1 is expected to be observed, since these three protons might be close to each other as seen in a Dreiding model (Chart 3). However, no cross peaks for H_D were observed in the ROESY spectrum of **3**, being consistent with the structure A, in which H_D and glucose H-3 (or H-1) are on opposite sides.

The cyclopentenone structure in **3** is regarded as an oxidative metabolite of the HHDP group at glucose O-2/O-3 in **1**.[9,10] The chemical transformation of **1** into **3** was achieved by the heating of **1** in ethanolic acetic acid (Chart 4). Structure **3**, including the *S*-configuration of the HHDP group at glucose O-4/O-6, and the C-8 substitution and the 2*R*,3*R*-configuration of the epicatechin moiety, was thus assigned to camelliatannin F.

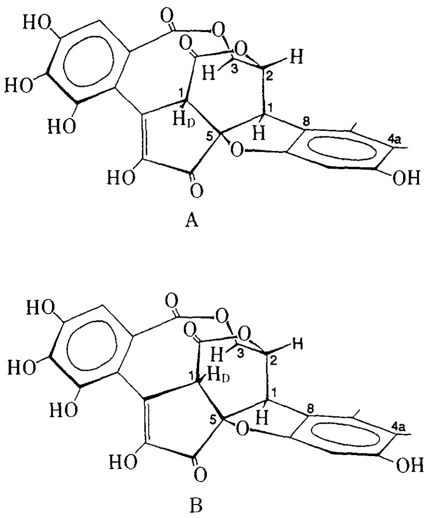


Chart 3. Partial Structures of Camelliatannin F (**3**)

Structure of Camelliatannin G Camelliatannin G (**4**) was obtained as a pale-yellow powder. The $[M+Na]^+$ ion peak at *m*/*z* 1109 in the FAB-MS of **4** indicated its molecular formula to be $C_{49}H_{34}O_{29}$. The ^{1}H-NMR spectrum of **4** showed the signals attributable to an epicatechin moiety [δ 5.08 (s, H-2), 4.26 (m, H-3), 2.86 (dd, *J*=4.5, 16 Hz, H-4), 2.64 (dd, *J*=4.5, 16 Hz, H-4), 5.97 (s, H-6), 7.09 (d, *J*=2 Hz, H-2′), 6.82 (d, *J*=8 Hz, H-5′), 6.87 (dd, *J*=2, 8 Hz, H-6′)], along with those of a *C*-glucosidic ellagitannin moiety [δ 6.45 (s, H_A), 6.74 (s, H_B), 6.38 (s, H_C); δ 4.87 (s, Glc H-1), 5.83 (s, Glc H-2), 5.51 (d, *J*=6.5 Hz, Glc H-3), 5.09 (dd, *J*=6.5, 8 Hz, Glc H-4), 4.08 (br d, *J*=8 Hz, Glc H-5), 4.64 (dd, *J*=2.5, 12 Hz, Glc H-6) and 3.43 (br d, *J*=12 Hz, Glc H-6)], indicating that this compound is also a complex tannin. The coupling constants of the glucose protons are characteristic of those of the open-chain glucose. Their chemical shifts, which are analogous to those of **1**, implied the presence of the unacylated hydroxyl group at C-5, and acylated ones at C-2, C-3, C-4 and C-6. The chemical shift of glucose C-1 (δ 50.2), in the ^{13}C-NMR spectrum of **4**, which is comparable with that of **3** (δ 46.2), indicated that this carbon is linked to the epicatechin moiety, and to an acyl group at O-2/O-3 of the ellagitannin moiety through a C–C bond.

The ^{13}C-NMR spectrum of **4** also substantiated the presence of the epicatechin moiety in the molecule (Table I). The chemical shifts of the epicatechin A-ring carbons [δ 102.3 (C-4a), 158.2 (C-5), 90.6 (C-6), 158.5 (C-7), 104.7 (C-8) and 152.0 (C-8a)] are almost the same as those of the corresponding carbons of **3**, indicating the presence of the C–C bond between C-8 of the epicatechin moiety and C-1 of the glucose residue, and of the ether linkage between O-7 of the epicatechin moiety and segment D of the ellagitannin moiety, forming a five-membered ether ring. The C-8 substitution of the epicatechin moiety was evidenced by the cross peak of the three-bond coupling

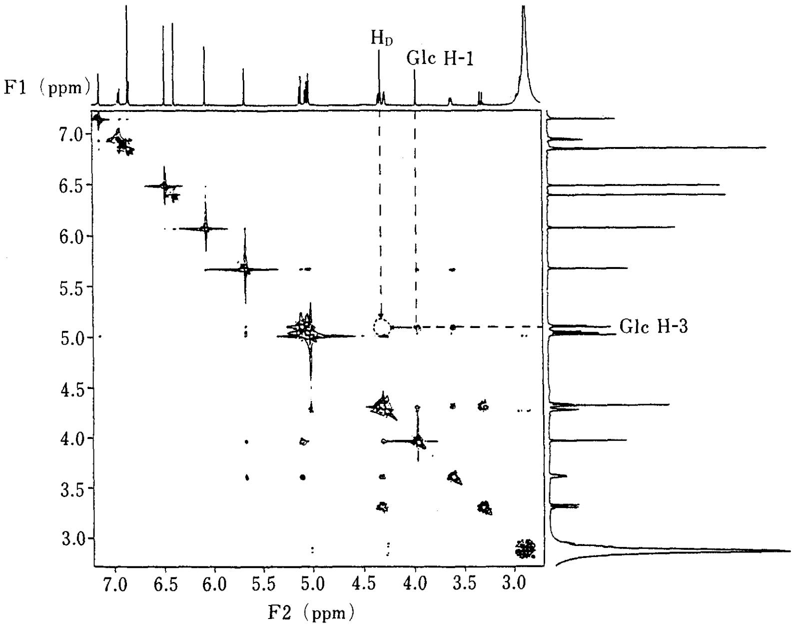


Fig. 1. ROESY Spectrum of Camelliatannin F (**3**) (in Acetone-d_6+D_2O)

The dotted circle indicates the absence of the cross peak between H_D and glucose H-3. Glc, glucose.

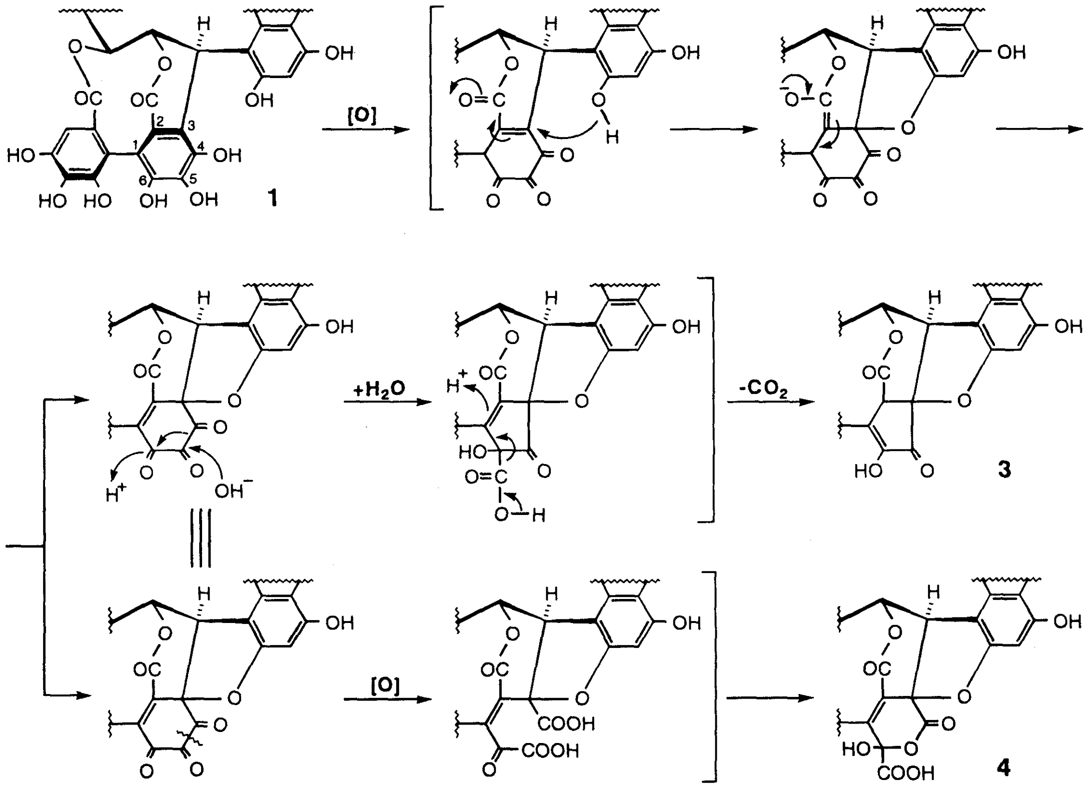


Chart 4. Possible Biogenetic Pathway from Camelliatannin A (**1**) to Camelliatannins F (**3**) and G (**4**)

between glucose H-1 and epicatechin C-8a (Fig. 2) in the ^{1}H–^{13}C long-range shift correlation spectroscopy (COSY) spectrum of **4**.

The presence of three aromatic rings (A—C rings) in the molecule of **4** is also shown by their ^{13}C signals, whose chemical shifts are also almost the same as those of the corresponding carbons of **3**. On the other hand, no proton signal due to segment D was observed in the ^{1}H-NMR spectrum. The six carbon resonances of segment D, in the ^{13}C-NMR spectrum of **4**, were thus assigned to two oxygen-bearing quaternary carbons [δ 86.6 (C_D-5) and 99.2 (C_D-2)], two fully substituted olefinic carbons [δ 131.0 (C_D-3) and 137.8 (C_D-4)] and two carboxyl carbons [δ 161.5 (C_D-6) and 166.8 (C_D-1)]. These NMR spectral data suggested the pyran structure for segment D as shown in the formula **4**.

This structural assignment was substantiated by the ^{1}H–^{13}C long-range COSY spectrum of **4** (Fig. 2). The long-range coupling between δ_H 5.83 (glucose H-2) and δ_C 86.6 indicated that this carbon signal is assignable to C_D-5, and also the presence of the C–C linkage between glucose C-1 and this carbon. The chemical shift of C_D-5 indicated that this carbon is linked to an oxygen, participating in the five-membered ring with C-8 and C-7 of the epicatechin moiety. The location of the double bond C-3–C-4 was indicated by the cross peak of the three-bond coupling between δ_H 4.87 (glucose H-1) and one of the olefinic carbons, δ_C 137.8, in the ^{1}H–^{13}C long-range COSY spectrum.

Since the oxidative cleavage of the cyclohexenetrione structure of the dehydrohexahydroxydiphenoyl group in dehydroellagitannins occurs between C-4 and C-5,[11,12] the acyl group at glucose O-2/O-3 is considered to be produced by analogous oxidative cleavage of the bond C-4–C-5 of the HHDP group, as shown in Chart 4. Thus, the structure of **4** was assigned to camelliatannin G.

Structure of Camelliatannin H Camelliatannin H (**5**) was obtained as an off-white powder. The $[M+Na]^+$ ion peak at *m/z* 1591 in the FAB-MS indicated that this tannin is a dimeric hydrolyzable tannin with the molecular formula $C_{68}H_{48}O_{44}$. Although the ^{1}H-NMR spectrum of **5** showed complicated signals due to the presence of four anomeric forms, β–α (the combination of the β-form of the left glucose core and the α-form of the right glucose core), β–β, α–α and α–β, the signals were assigned as shown in Table II with the aid of the *J*-resolved spectrum and ^{1}H–^{1}H COSY.[13] The coupling constants of the sugar protons are characteristics of the $^{4}C_1$ glucopyranoses, and the chemical shifts indicated that all of their hydroxyl groups, except for those at the two anomeric centers, are acylated.

The aromatic singlets in the ^{1}H-NMR spectrum indicated that the ratio of the four anomeric forms is 4 : 2 : 1 : 1, and the signals of the main anomeric form were attributed to a galloyl [δ 6.96 (2H, s, H_H)], a valoneoyl and two HHDP [δ: 7.00 (s, H_E), 6.65 (s, H_C), 6.63 (s, H_F), 6.47 (s, H_G), 6.40 (s, H_B), 6.33 (s, H_A), 6.08 (s, H_D)] groups in the molecule. The presence of these acyl groups was substantiated by methanolysis of methylated **5**, which gave methyl tri-*O*-methylgallate (**15**), dimethyl hexamethoxydiphenate (**16**) and trimethyl octa-*O*-methylvaloneate (**17**) in the molar ratio 1 : 2 : 1. The circular dichroism (CD) spectrum of **5** showed the positive Cotton effect around 220—240 nm with large amplitude indicating that all of

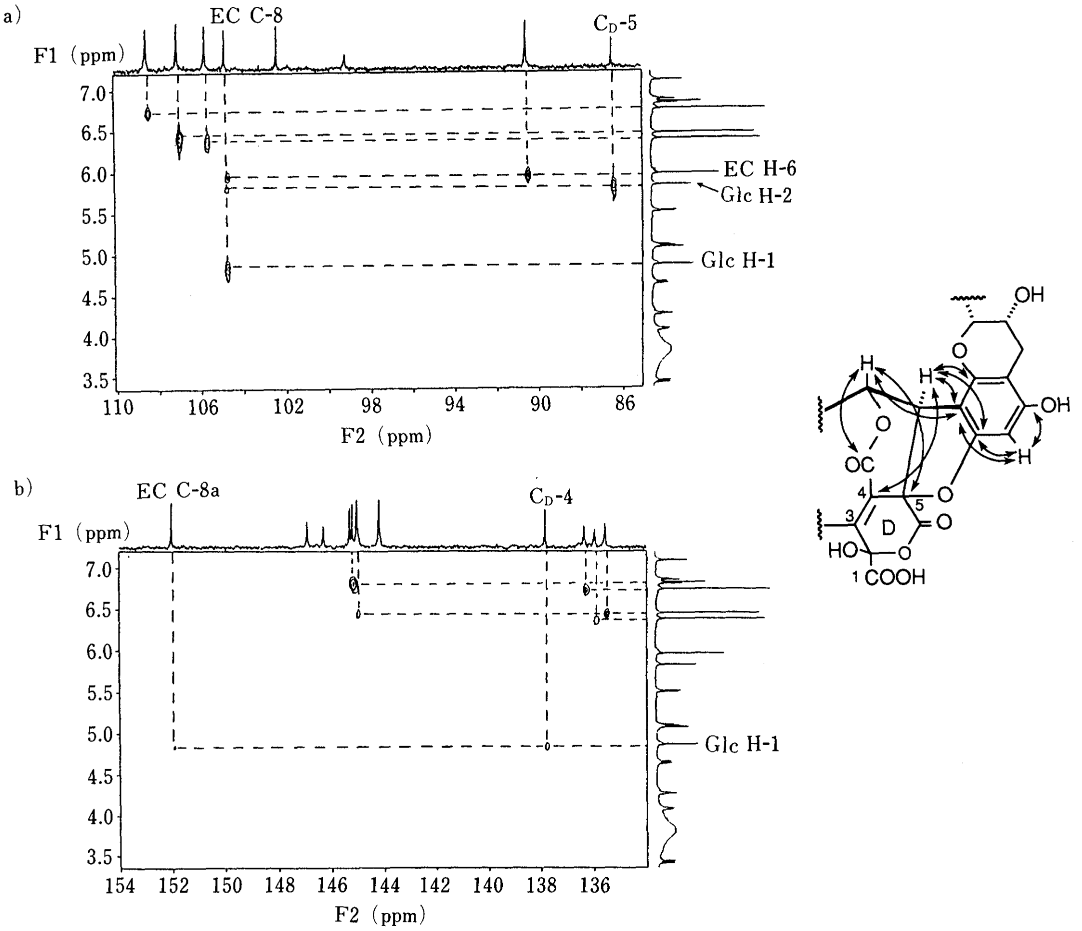


Fig. 2. ^{1}H-^{13}C Long-Range COSY Spectrum of Camelliatannin G (**4**) (in Acetone-d_6+D_2O)

The average J_{CH} value for two- or three-bond coupling was set at 7 Hz. a) δ_C 85—110. b) δ_C 134—154. Glc, glucose; EC, epicatechin.

Table II. ^{1}H-NMR Spectral Data for **5** (500 MHz, in Acetone-d_6+D_2O, J in Hz)

Protons	α-Glucose[a]		β-Glucose[b]	
Glucose I H-1	5.43 (d, 3)	5.43 (d, 3)	5.02 (d, 8)	4.90 (d, 8)
H-2	4.98 (dd, 3, 10)	4.97 (dd, 3, 10)	4.79 (dd, 8, 10)	4.77 (dd, 8, 10)
H-3	5.41 (t, 10)	5.38 (t, 10)	5.02 (t, 10)	5.01 (t, 10)
H-4	*ca.* 5.0	*ca.* 5.0	4.27 (t, 10)	4.27 (t, 10)
H-5	4.55 (dd, 7, 10)	4.54 (dd, 7, 10)	4.15 (dd, 7, 10)	4.10 (dd, 7, 10)
H-6	5.32 (dd, 7, 13)	5.32 (dd, 7, 13)	5.28 (dd, 7, 13)	5.23 (dd, 7, 13)
	3.73 (br d, 13)	3.72 (br d, 13)	3.81 (br d, 13)	3.79 (br d, 13)
Glucose II H-1′	5.33 (d, 3)	5.29 (d, 3)	4.46 (d, 8)	4.38 (d, 8)
H-2′	5.04 (dd, 3, 10)	5.00 (dd, 3, 10)	5.14 (dd, 8, 10)	5.15 (dd, 8, 10)
H-3′	5.78 (t, 10)	5.77 (t, 10)	5.52 (t, 10)	5.47 (t, 10)
H-4′	5.16 (t, 10)	5.16 (t, 10)	5.06 (t, 10)	5.03 (t, 10)
H-5′	4.60 (dd, 7, 10)	4.56 (dd, 7, 10)	4.21 (dd, 7, 10)	4.18 (dd, 7, 10)
H-6′	5.21 (dd, 7, 13)	5.21 (dd, 7, 13)	5.24 (dd, 7, 13)	5.25 (dd, 7, 13)
	3.75 (br d, 13)	3.73 (br d, 13)	3.86 (br d, 13)	3.85 (br d, 13)

[a] α-Glucose signals of the three anomeric forms, α-α, α-β and β-α. [b] β-Glucose signals of the three anomeric forms, α-β, β-α and β-β.

the valoneoyl and HHDP groups have the *S*-configuration.[14]

We previously reported that the locations of acyl groups of dimeric hydrolyzable tannins are assignable based on the comparison of the ^{13}C chemical shifts of their glucose carbons with those of the constituent monomeric tannins.[15] Thus, the coincidence of the signal pattern of glucose carbons of **5** with the sum of those of **12** and cornusiin B (**18**) (Fig. 3) indicated that the galloyl group and the galloyl part of the valoneoyl group are at O-2 and O-3 of one of the glucose cores (glucose II), and the HHDP groups and the HHDP part of the valoneoyl group are at O-2/O-3 and O-4/O-6 of the other glucose core (glucose I) and O-4/O-6 of glucose II. Therefore, **5** is assigned to be a dimeric tannin formally produced from **12** and tellimagrandin I (**19**) forming the valoneoyl group. This structural assignment was substantiated by partial degradation of **5**, which afforded gemin D (**20**), 4,6-

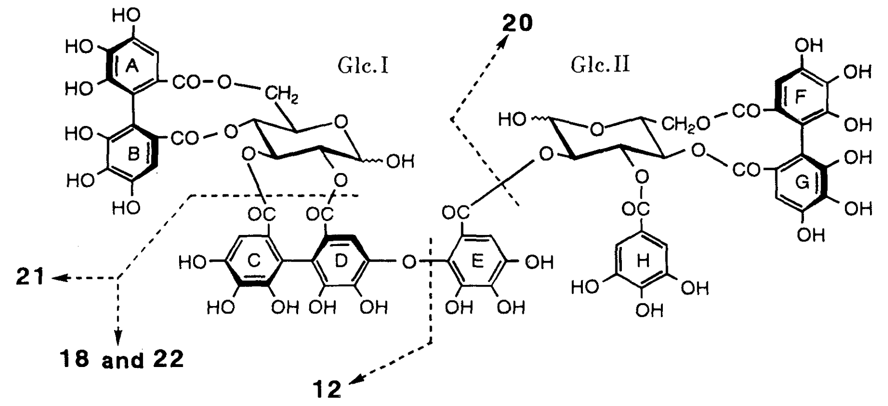


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Chart 5. Partial Hydrolysis of Camelliatannin H (5)
Glc. I, the left glucose core; Glc. II, the right glucose core.

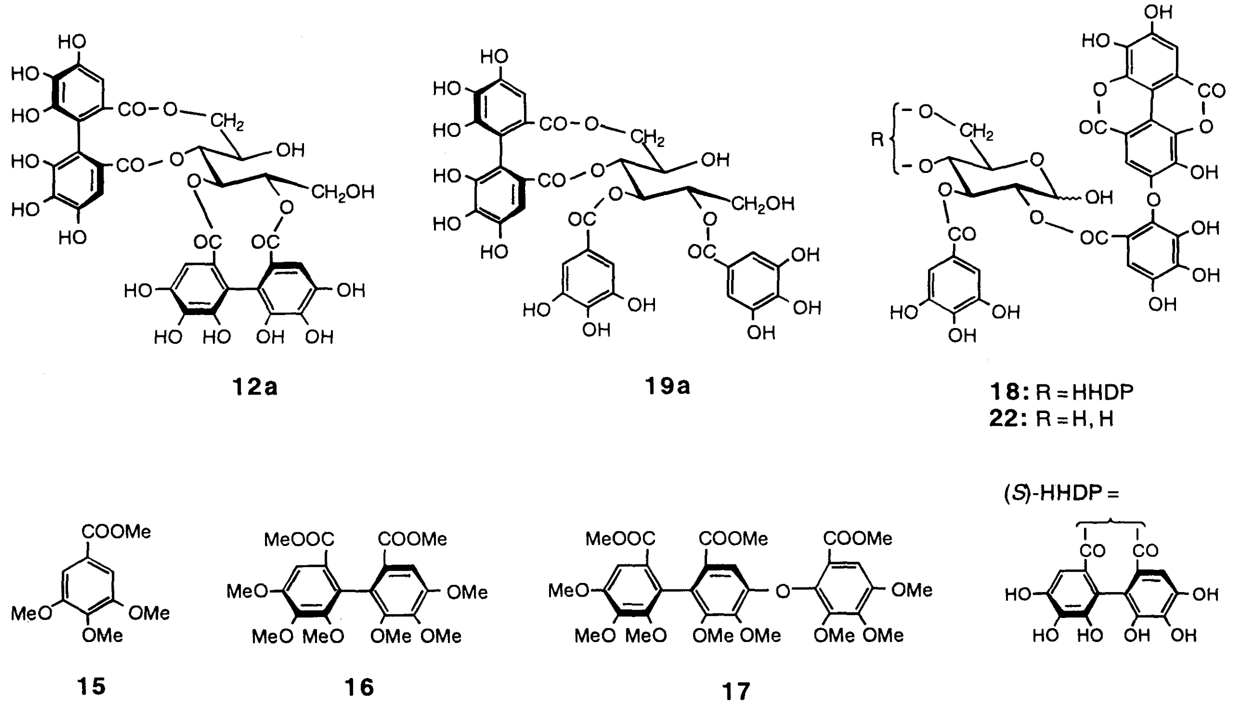


Chart 6

(*S*)-HHDP-glucose (**21**), **12**,[16] **18** and oenothein C (**22**) (Chart 5).

Treatment of **5** with $NaBH_4$ gave compounds **5a**—**d**. The ^{1}H-NMR spectrum of **5a** showed eight protons of a glucitol residue (Table III) and five aromatic singlets [δ 7.08 (H_E), 6.63 (H_C), 6.61 (H_B), 6.49 (H_A) and 6.35 (H_D) (each 1H, s)] assignable to an HHDP group and a valoneoyl group. Although the coupling constants of the glucitol protons are almost the same as those of **12a** which was obtained by an analogous treatment of **12** with $NaBH_4$,[17] two glucitol H-1 protons of **5a** are shifted upfield relative to those of **12a** ($\Delta\delta$ 0.13 and 0.11). These shifts are attributable to the anisotropic effect of the galloyl part of the valoneoyl group at glucose O-2/O-3, and suggested that the ether linkage of the valoneoyl group is at the glucose O-2 side.

Compound **5d** corresponds to tetrahydrocamelliatannin H, as indicated by the $[M+Na]^+$ ion peak at m/z 1591 in the FAB-MS and the signals of nine aromatic [δ 7.13 (2H, H_H), 7.00 (H_E), 6.83 (H_G), 6.61 (H_C), 6.61 (H_B), 6.50 (H_F), 6.50 (H_A), and 6.24 (H_D) (each s)] and sixteen glucitol protons (Table III) in the ^{1}H-NMR spectrum. The assignments of the glucitol protons are based on the comparison with those of **5a** and **19a**,[18] and also those of the dihydro derivative **5c**, of which one of the anomeric centers remained to be reduced.

Compound **5b** showed seven aromatic protons [δ 7.16 (2H, H_H), 7.02 (H_E), 6.88 (H_G), 6.51 (H_C), 6.49 (H_F), and 6.21 (H_D) (each s)] in the ^{1}H-NMR spectrum, lacking the signals of one of the HHDP groups of **5d**. Among the glucitol protons (Table III), H-4 and H-6 of glucitol I showed distinctive upfield shifts ($\Delta\delta$ 1.4—0.9 ppm), relative

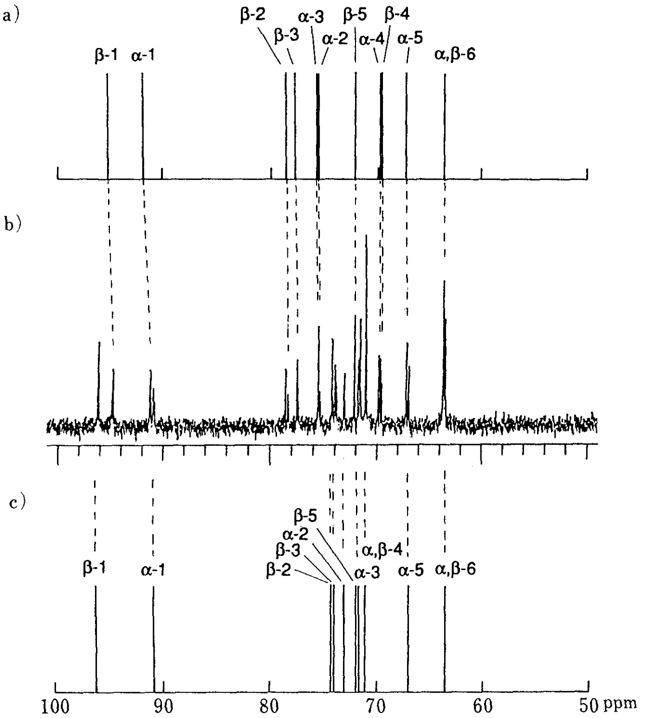


Fig. 3. Comparison of the Glucose Carbons in the ^{13}C-NMR Spectra of a) Pedunculagin (**12**), b) Camelliatannin H (**5**) and c) Cornusiin B (**18**) (in Acetone-d_6 + D_2O)

α-1 means C-1 of the glucose core of the α-anomer.

to those of **5d**, indicating that the HHDP group of glucitol I of **5d** (and **5a**) is at O-4/O-6. The location of the valoneoyl group in **5d**, and consequently in **5a** and **5c**, was thus confirmed to be at O-2/O-3 of glucitol.

The assigned orientation of the valoneoyl group was supported by the cross peaks in the ^{1}H–^{13}C long-range COSY spectrum of **5d** (Fig. 4). The H_C signal of the valoneoyl group and the H-3 signal of glucitol I were correlated with the same ester carbonyl carbon at δ 169.2, indicating that the galloyl part of the valoneoyl group is at the O-2 side.

Based on these data, camelliatannin H was represented by the structure **5**.

Structures of various types of hydrolyzable and complex tannins hitherto isolated from leaves, fruits and flower buds of *C. japonica* suggest the biogenetic correlations shown in Chart 8. In the pathway from galloylglucoses to *C*-glucosidic tannins,[19] some ellagitannins such as **12** and **19** (or **24**) are key metabolites which act as the precursors of the dimeric ellagitannins, and also of the *C*-glucosidic tannins. Complex tannins might be produced from **8** and **12** (or its metabolites, *C*-glucosidic ellagitannins) by C–C coupling between the glucose C-1 and epicatechin, and be further metabolized into oxidative products such as **3** and **4**.

Experimental

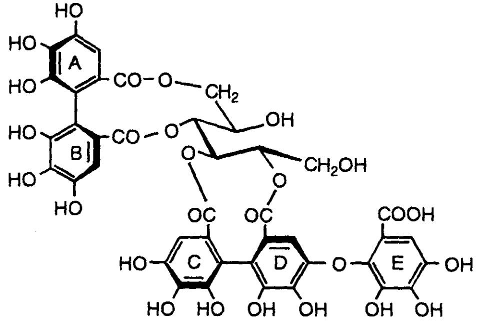
Optical rotations were measured on a JASCO DIP-4 polarimeter. UV and IR spectra were recorded on a Hitach 200-10 spectrophotometer and on a JASCO A-102 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H-NMR and 125.7 MHz for ^{13}C-NMR). Chemical shifts are given in δ values (ppm), based on the ^{1}H and ^{13}C signals of solvents [acetone-d_6 (δ_H 2.04; δ_C 29.8) or chloroform-d (δ_H 7.24)]. FAB-MS were recorded on a VG 70-SE mass spectrometer, using 3-nitrobenzyl alcohol as the matrix agent. CD spectra were recorded on a JASCO J-500A spectrometer equipped with a DP-501N data processor. Normal-phase HPLC was performed on a Merck Superspher SI60 column (4 × 125 mm) with *n*-hexane–MeOH–tetrahydrofuran–formic acid (55 : 33 : 11 : 1) containing oxalic acid (450 mg/l) (flow rate, 1.5 ml/min) at room temperature, unless otherwise mentioned. Reversed-phase HPLC was performed on a LiChrospher RP-18 (5 μm) column (4 × 250 mm) with 10 mM H_3PO_4–10 mM KH_2PO_4–MeCN (9 : 9 : 2) (flow rate, 1.0 ml/min) at 40 °C. Preparative HPLC was performed on YMC Pack A324 (ODS) (10 × 300 mm) and YMC Pack A312 (ODS) (6 × 150 mm) columns using the same solvent system. Detection was effected with a Shimadzu SPD-6A spectrophotometric detector at 280 nm.

Isolation of Tannins from Leaves of *Camellia japonica* Fresh leaves (3.5 kg) of *Camellia japonica*, collected at the Herbal Garden of Okayama University in April, were homogenized in 70% acetone (20 l). The concentrated filtrate (3 l) from the homogenate was extracted with Et_2O (3.8 l) and EtOAc (8 l), successively, and the aqueous mother liquor was subjected to column chromatography over Dia-ion HP-20 (6.5 × 48 cm) with increasing concentrations of MeOH in H_2O (0→20%→40%→60%→80%→100% MeOH). The eluate with 20% MeOH (23.6 g) was subjected to column chromatography over Toyopearl HW-40 (coarse grade) with 70% EtOH–70% acetone to give eight fractions (frs. I—VIII). Fraction II (1166 mg) was chromatographed on Toyopearl HW-40 (fine) with 50% EtOH, to give five fractions (frs. II-1—II-5). Fraction II-2 (351 mg) was further purified by column chromatography over Toyopearl HW-40 (superfine) and MCI-gel CHP-20P to give **4** (97 mg). Fraction III (4218 mg) was chromatographed on MCI-gel CHP-20P with 5%, 10% and 20% MeOH. The eluate with 10% MeOH (1602 mg) and that with 20% MeOH were separately purified in an analogous way to give **1** (150 mg) and **3** (214 mg).

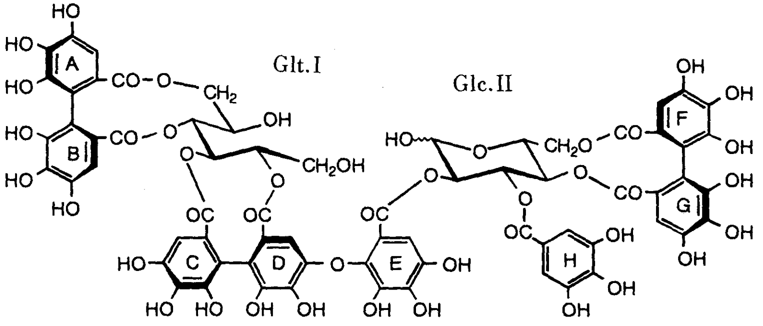
Isolation of Tannins from Fruits of *Camellia japonica* Fresh fruits (10 kg) of *Camellia japonica* were collected at the Herbal Garden of Okayama University in August, and their seeds (2.2 kg) were removed. The fruits were then homogenized in 70% acetone (45 l), and the insoluble material was filtered off. The filtrate was concentrated to 3 l, and was extracted with Et_2O (6 l), EtOAc (30 l) and *n*-BuOH (12 l), successively. The EtOAc extract (20 g) was subjected to column chromatography over MCI-gel CHP-20P (2.8 × 49 cm) with increasing concentrations of MeOH in H_2O (3%→8%→15%→100%). The eluate with 3% MeOH (1920 mg) was purified in an analogous way to give **8** (1560 mg). The *n*-BuOH extract (30 g) was subjected to column chromatography over Toyopearl HW-40 (coarse grade) (4.0 × 53 cm) with 50% MeOH (frs. 1—30)→70% MeOH (frs. 31—610)→70% MeOH–70% acetone [9 : 1 (frs. 611—800)→8 : 2 (frs. 801—950)→7 : 3 (frs. 951—1100)]→70% acetone (frs. 1110—1200) (15 g fractions were collected), and the following compounds were obtained: **9** (frs. 96—100, 70 mg), **13** (frs. 246—255, 57 mg), **12** (frs. 301—395, 1220 mg), **3** (frs. 431—485, 200 mg), **5** (frs. 841—930, 910 mg) and **7** (frs. 1022—1040, 287 mg). Combined fractions 126—133 were further purified by preparative HPLC to give **10** (15 mg) and **11** (20 mg). The aqueous mother liquor after the extraction with *n*-BuOH was concentrated to 0.3 l, and was subjected to column chromatography over Dia-ion HP-20 (6.5 × 45 cm) with increasing concentrations of MeOH in H_2O (0%→20%→40%→60%→80%→100% MeOH). The eluate with 20% MeOH (19.1 g) was repeatedly chromatographed over Dia-ion HP-20, Toyopearl HW-40 and MCI-gel CHP-20P, to yield **13** (543 mg) and **1** (50 mg). The eluate with 40% MeOH (20.7 g) was rechromatographed on Toyopearl HW-40 and/or MCI-gel CHP-20P, to give **12** (538 mg), **3** (385 mg), **6** (61 mg) and **5** (650 mg).

Camelliatannin F (3) An off-white amorphous powder, $[\alpha]_D$ −89° (c = 1.6, MeOH). *Anal.* Calcd for $C_{48}H_{34}O_{26}\cdot 4H_2O$: C, 52.47; H, 3.85. Found: C, 52.63; H, 4.24. FAB-MS (positive-ion mode): m/z 1027 $[M+H]^+$, 1049 $[M+Na]^+$. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 209 (4.95), 230 (sh, 4.77), 265 (4.50). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1730, 1620. CD (MeOH): $[\theta]_{337}$ $+4.5\times10^3$, $[\theta]_{298}$ -1.7×10^4, $[\theta]_{281}$ $+5.4\times10^3$, $[\theta]_{259}$ -6.0×10^4, $[\theta]_{236}$ $+9.1\times10^3$, $[\theta]_{208}$ -5.9×10^4. ^{1}H-NMR: see text. ^{13}C-NMR (125.7 MHz, acetone-d_6 + D_2O) δ: 106.7, 107.3 and 108.7 [C-3 of rings A—C (C_{A-C}-3)], 111.1, 111.4, 116.2 (C_{A-C}-1), 124.7, 125.6, 127.1 (C_{A-C}-2), 135.4, 136.4, 137.0 (C_{A-C}-5), 144.1, 144.2, 144.7, 145.2 (2C), 146.3 (C_{A-C}-4, 6), 167.1, 167.2, 168.5, 169.3 (C_{A-C}-7, C_D-6). For the glucose and epicatechin carbons, see Table I.

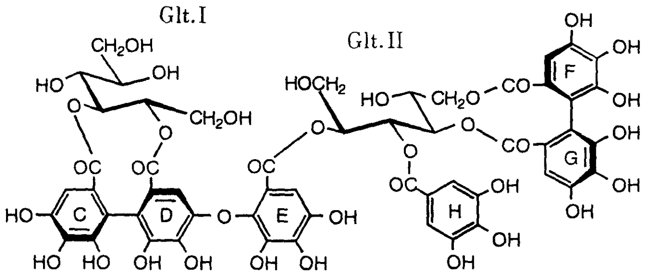
Camelliatannin G (4) A pale-yellow amorphous powder, $[\alpha]_D$ −245° (c = 1.0, MeOH). *Anal.* Calcd for $C_{49}H_{34}O_{29}\cdot 7H_2O$: C, 49.52; H, 3.99.



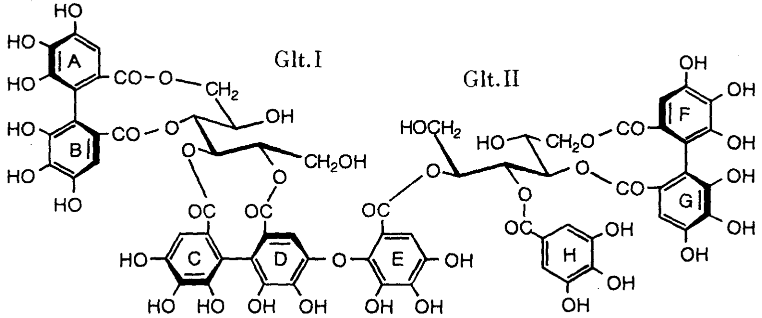
5a



5c



5b



5d

Chart 7. Structures of **5a**—**d**

Glt. I, the left glucitol residue; Glt. II, the right glucitol residue; Glc. II, the right glucose core.

TABLE III. ^{1}H-NMR Spectral Data for **5a**, **5b**, **5c** and **5d** (500 MHz, in Acetone-d_6+D_2O, J in Hz)

Protons		**5a**	**5b**	**5c**$^{a)}$	**5d**
Glucitol I	H-1	3.83 (dd, 3, 12)	3.75 (dd, 3, 12)	3.84 (dd, 5, 12)	3.85 (dd, 5, 12)
		3.81 (dd, 4.5, 12)	3.74 (dd, 5.5, 12)	3.83 (dd, 2.5, 12)	3.80 (dd, 2.5, 12)
	H-2	4.97 (ddd, 3, 4.5, 9.5)	5.32 (ddd, 3, 5.5, 9.5)	4.96 (ddd, 2.5, 5, 9.5)	4.98 (ddd, 2.5, 5, 9.5)
	H-3	5.54 (dd, 1.5, 9.5)	5.41 (dd, 1.5, 9.5)	5.62 (dd, 1.5, 9.5)	5.55 (dd, 1, 9.5)
	H-4	5.16 (dd, 1.5, 8.5)	3.82—3.66 (4H)	5.21 (dd, 1.5, 8.5)	5.17 (dd, 1, 8.5)
	H-5	4.24 (dd, 3, 8.5)		4.30 (dd, 3, 8.5)	4.26 (dd, 3, 8.5)
	H-6	4.65 (dd, 3, 12)		4.69 (dd, 3, 12)	4.66 (dd, 3, 12)
		3.91 (br d, 12)		3.94 (br d, 12)	3.94 (br d, 12)
Glucitol II	H-1′		3.71 (dd, 5.5, 12)	4.17—4.04 (3H)	3.80 (dd, 5, 12)
			3.70 (dd, 4, 12)		3.74 (dd, 3.5, 12)
	H-2′		5.27 (ddd, 4, 5.5, 7.5)		5.27 (ddd, 3.5, 5, 8)
	H-3′		5.86 (dd, 3, 7.5)	5.58 (dd, 2.5, 8)	5.87 (dd, 2.5, 8)
	H-4′		5.17 (dd, 3, 8.5)	5.38 (t, 8)	5.22 (dd, 2.5, 8.5)
	H-5′		4.07 (dd, 3, 8.5)	4.25 (dd, 2.5, 8)	4.10 (dd, 3, 8.5)
	H-6′		4.62 (dd, 3, 12.5)	4.64 (dd, 2.5, 12)	4.63 (dd, 3, 12)
			3.84 (br d, 12.5)	3.78 (br d, 12)	3.87 (br d, 12)

a) The data in this table are those for the major anomer of **5c**.

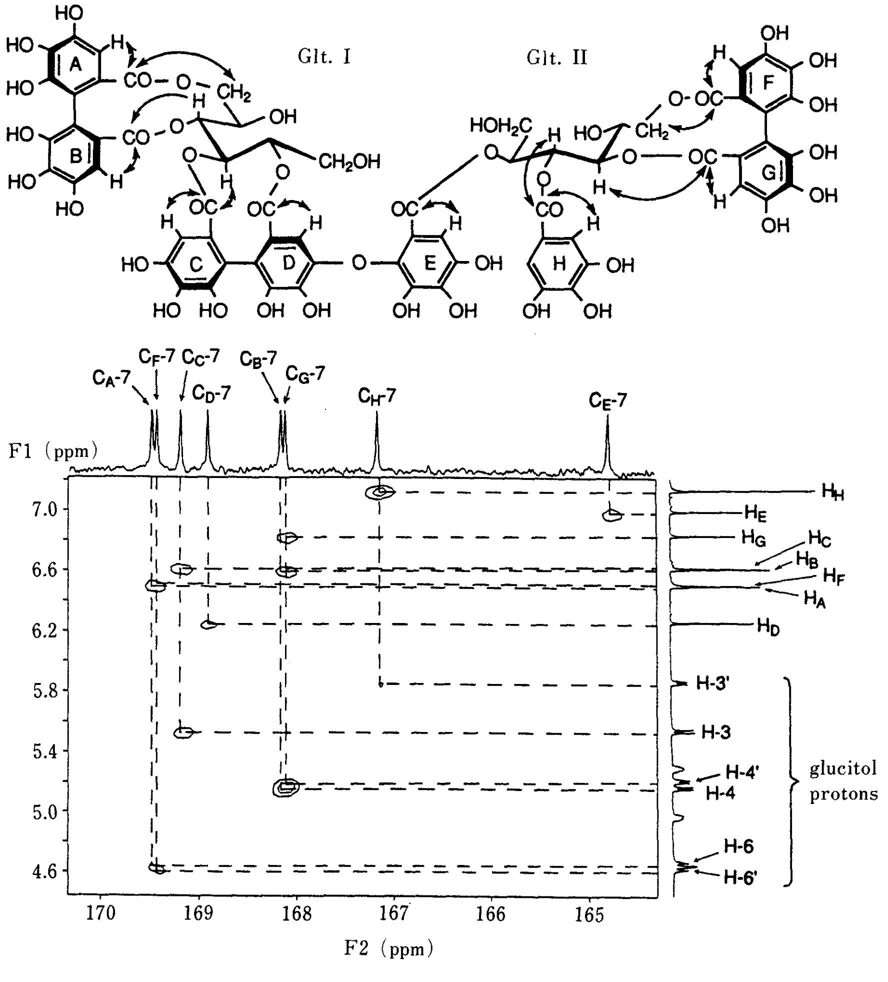


Fig. 4. ^{1}H–^{13}C Long-Range COSY Spectrum of Camelliatannin H (**5**) (in Acetone-d_6+D_2O)

The average J_{CH} value for two- or three-bond coupling was set at 7 Hz. Glt. I, the left glucitol residue; Glt. II, the right glucitol residue.

Found: C, 49.08; H, 4.00. FAB-MS (positive-ion mode): *m/z* 1109 [M+Na]$^+$. UV λ_{max}^{MeOH} nm (logε): 213 (4.93), 234 (sh, 4.76), 282 (sh, 4.24). IR ν_{max}^{KBr} cm^{-1}: 1730, 1620. CD (MeOH): $[\theta]_{337}$ $+4.5\times10^3$, $[\theta]_{307}$ -1.3×10^4, $[\theta]_{281}$ $+2.0\times10^3$, $[\theta]_{257}$ -5.3×10^4, $[\theta]_{237}$ $+3.4\times10^3$, $[\theta]_{208}$ -8.4×10^4. ^{1}H-NMR: see text. ^{13}C-NMR (125.7 MHz, acetone-d_6+D_2O) δ: 105.8 (C_C-3), 107.1 (C_A-3), 108.6 (C_B-3), 112.1 (C_C-1), 115.3 (C_A-1), 115.9 (C_B-1), 125.1, 125.8, 127.3 (C_{A-C}-2), 135.6 (C_A-5), 136.0 (C_C-5), 136.4 (C_B-5), 144.2, 144.2, 145.0, 145.3, 146.9 (C_{A-C}-4, 6), 164.2 (C_D-7), 167.2 (C_C-7), 167.5 (C_B-7), 169.1 (C_A-7). For the glucose and epicatechin carbons, see Table I.

Camelliatannin H (5) An off-white amorphous powder, $[\alpha]_D$ $+90°$ (c=1.0, MeOH). *Anal.* Calcd for $C_{68}H_{48}O_{44}\cdot8H_2O$: C, 47.67; H, 3.77. Found: C, 48.05; H, 4.11. FAB-MS (positive-ion mode): *m/z* 1569 [M+H]$^+$, 1591 [M+Na]$^+$. UV λ_{max}^{MeOH} nm (logε): 218 (5.00), 258 (sh,

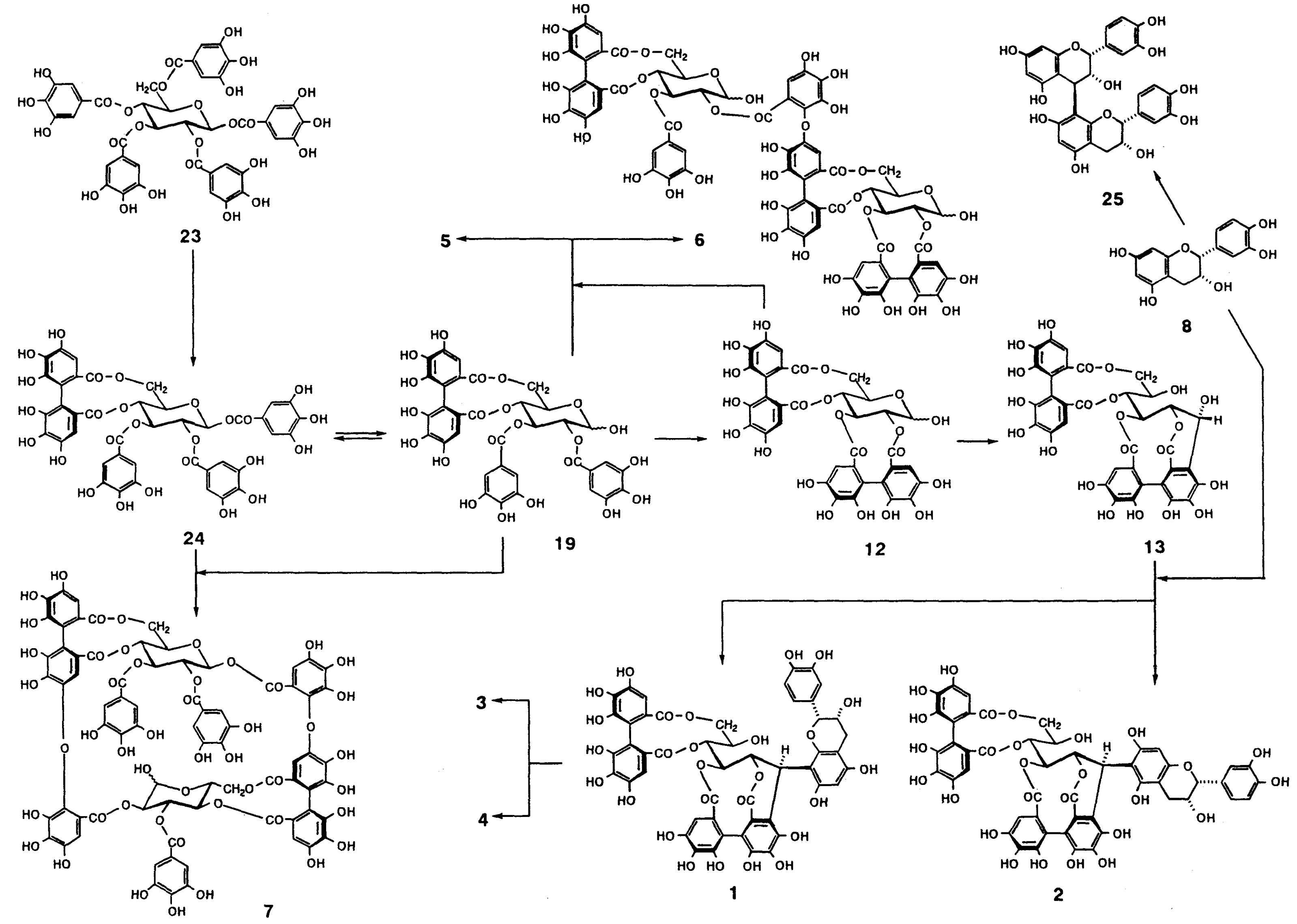


Chart 8. Biogenetic Route from Galloylglucose and Epicatechin to Dimeric Hydrolyzable Tannins and Complex Tannins in *C. japonica*

23, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose; **24**, tellimagrandin II; **25**, procyanidin B-4.

4.71), 278 (sh, 4.63). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1730, 1615. CD (MeOH): $[\theta]_{283}$ $+9.1\times10^4$, $[\theta]_{261}$ -8.2×10^4, $[\theta]_{238}$ $+1.8\times10^5$, $[\theta]_{223}$ $+1.6\times10^5$. ^{1}H-NMR: see text and Table II. ^{13}C-NMR (125.7 MHz, in acetone-d_6 $+D_2O$) δ: 63.4 [C-6 of glucose II, α-anomer (Glc II-C-6-α), 6-β], 63.6 (Glc I-C-6-α, 6-β), 66.8 (Glc II-C-5-α), 67.1 (Glc I-C-5-α), 69.5, 69.6 (Glc I-C-4-β), 69.8 (Glc I-C-4-α), 71.0 (Glc II-C-4-α, 4-β), 71.5, 71.7 (Glc II-C-3-α), 71.5 (Glc II-C-5-β), 72.1 (Glc I-C-5-β), 73.0 (Glc II-C-2-α), 73.5, 73.9 (Glc II-C-3-β), 74.1, 74.2 (Glc II-C-2-β), 75.3, 75.4 (Glc I-C-2-α), 75.5 (2C, Glc I-C-3-α), 77.3, 77.4 (Glc I-C-3-β), 78.3, 78.5 (Glc I-C-2-β), 90.7, 90.8 (Glc II-C-1-α), 91.1, 91.2 (Glc II-C-1-α), 94.7, 94.8 (Glc I-C-1-β), 96.1 (Glc II-C-1-β), 103.5—104.7 (C_D-3), 106.8—108.0 (C_{A-C}-3, C_{F-G}-3), 109.7—110.2 (C_H-2, 6), 113.7—116.4 (C_{A-G}-1), 120.0—120.1 (C_H-1), 125.6—126.3 (C_{A-D}-2, C_{F-G}-2), 135.9—136.5 (C_{A-D}-5, C_{F-G}-5), 136.8—137.5 (C_E-2), 139.2, 139.2 (C_H-4), 140.0—140.6 (C_E-3,4), 143.1—143.4 (C_E-5), 144.3—145.2 (C_{A-C}-4, C_{F-G}-4, C_{A-D}-6, C_{F-G}-6), 145.7 (C_H-3, 5), 146.5—147.5 (C_D-4), 164.3—165.0 (C_E-7), 167.0—167.2 (C_H-7), 167.8—168.0 (C_B-7, C_G-7), 168.2—168.4 (C_D-7, 168.7, 168.7 (C_C-7), 169.3, 169.3, (C_F-7), 169.6—169.7 (C_A-7).

Methylation of 3 Ethereal diazomethane (0.5 ml) was added to an acetone solution (0.2 ml) of **3** (10 mg), and the mixture was left to stand overnight at room temperature. After evaporation of the solvent, the residue was subjected to preparative TLC on Kieselgel 60 PF_{254} (Merck) with benzene–acetone (3:1), to give **3a** (1 mg). ^{1}H-NMR (500 MHz, chloroform-d) δ: 7.17, 6.99, 6.73 [each s, H of rings A—C (H_{A-C})], 4.35 (s, H_D), 3.57, 3.58, 3.72, 3.79, 3.80, 3.81, 3.81, 3.82, 2.83, 3.84 (6H), 3.91, 4.00 (each 3H, s, 13 × MeO). Glucose protons: δ 4.36 (s, H-1), 5.59 (s, H-2), 5.44 (d, $J=5$ Hz, H-3), 5.09 (dd, $J=5$, 7 Hz, H-4), 4.52 (br d, $J=7$ Hz, H-5), 4.38 (dd, $J=3$, 12 Hz, H-6), 3.48 (d, $J=12$ Hz, H-6). Protons of the epicatechin moiety: δ 5.08 (s, H-2), 4.37 (m, H-3), 6.21 (s, H-6), 7.24 (d, $J=2$ Hz, H-2′), 6.95 (d, $J=8$ Hz, H-5′), 7.12 (dd, $J=2$, 8 Hz, H-6′).

Transformation of 1 into 3 A solution of **1** (20 mg) in EtOH (10 ml) containing AcOH (20%) in a sealed tube was heated in a boiling-water bath for 6 d. The solvent was evaporated off, and the residue was chromatographed over MCI-gel CHP-20P (1.1 × 11 cm) with increasing concentrations of MeOH in water [5% (frs. 1—20), 15% (frs. 21—61), 20% (frs. 62—110) and 25% MeOH (frs. 111—170)]. Combined frs. 80—120 (10.3 mg) were chromatographed over Toyopearl HW-40 (superfine) (1.1 × 7.5 cm) with 40% EtOH to give **3** (2.3 mg). Combined fractions 37—50 gave **12** (1 mg),[20] which was identified by normal-phase HPLC (t_R 5.19 min and 5.45 min; anomer mixture), reversed-phase HPLC (t_R 3.70 min and 5.10 min) and ^{1}H-NMR. The HPLC analysis (reversed-phase) also showed the production of **13** (t_R 3.23 min) and **8** (t_R 13.03 min).[20]

Methanolysis after Methylation of 5 Dimethyl sulfate (20 ml) and potassium carbonate (30 mg) were added to a solution of **5** (1 mg) in acetone (2 ml), and the mixture was refluxed for 5 h. After centrifugation, the supernatant was evaporated off, and the residue was treated with 0.01% NaOMe in MeOH (0.2 ml) overnight at room temperature. Then, the solution was neutralized with acetic acid, and the solvent was evaporated off. The residue was suspended in water and extracted with EtOAc. The EtOAc extract was analyzed by normal-phase HPLC on a Zorbax BP SIL column (4.6 × 250 mm) (Sumika, Osaka) with n-hexane–EtOAc (2:1) (flow rate, 2.0 ml/min) at room temperature, to show the presence of **15** (t_R 1.60 min), **16** (t_R 5.33 min) and **17** (t_R 15.75 min) in a molar ratio of 1:2:1.

Partial Hydrolysis of 5 with Diluted HCl A solution (4 ml) of **5** (20 mg) in 0.5% HCl in a sealed tube was heated in a boiling-water bath for 5.5 h. Then, the reaction mixture was neutralized with 0.5 N KOH, and concentrated. Preparative HPLC of the concentrated solution gave **18** (4 mg) {$[\alpha]_D$ +20° ($c=1.0$, MeOH)} and **22** (3 mg) {$[\alpha]_D$ +34° ($c=1.0$, MeOH)}. The identity of these two compounds was confirmed by ^{1}H-NMR. The HPLC analysis (reversed-phase) also showed the production of **21** (t_R 2.37 min), **20** (t_R 2.88 min and 3.13 min) and **12** (t_R 3.98 min and 5.22 min).

Reaction of 5 A mixture of **5** (210 mg) and $NaBH_4$ (0.9 g) in MeOH (50 ml) was left to stand for 30 min at room temperature, and then acidified to pH 3 with 10% HCl. After removal of the solvent, the residue was chromatographed over MCI-gel CHP-20P (1.1 × 40 cm) with increasing concentrations of MeOH in H_2O [5% (frs. 1—90) → 15% (frs. 91—240) → 30% MeOH (frs. 241—310). Fraction 63—66 gave compound **5a** {2.3 mg, $[\alpha]_D$ +103° ($c=1.0$, MeOH)}. The eluate with 15% MeOH was concentrated and subjected to preparative HPLC, to give **5b** {2 mg, $[\alpha]_D$ +69° ($c=1.0$, MeOH), FAB-MS (positive-ion mode) m/z: 1271 $[M+H]^+$}, **5c** {16 mg, $[\alpha]_D$ +126° ($c=1.0$, MeOH)} and **5d** (60 mg). ^{1}H-NMR of **5a—c**: see text and Table III.

Tetrahydrocamelliatannin H (5d) Am off-while amorphous powder, $[\alpha]_D$ +113° ($c=1.0$, MeOH). FAB-MS (positive-ion mode): m/z 1595 $[M+H]^+$. ^{1}H-NMR: see text and Table III. ^{13}C-NMR (125.7 MHz, acetone-d_6+D_2O) δ: 60.3 (C-1), 77.2 (C-2), 74.6 (C-3), 72.8 (C-4), 68.9 (C-5), 68.2 (C-6) (glucitol I); δ 61.1 (C-1), 73.4 (C-2), 71.7 (C-3), 73.3 (C-4), 69.0 (C-5), 68.2 (C-6) (glucitol II); δ 105.0 (C_D-3), 107.9 (C_A-3), 107.1 (C_F-3), 107.1 (C_C-3), 108.3 (C_B-3), 108.8 (C_G-3), 110.1 (C_E-6), 110.4 (2C, C_H-2,6), 113.8 (C_C-1), 114.5 (C_E-1), 115.0 (C_A-1), 115.0 (C_F-1), 115.4 (C_D-1), 116.3 (C_G-1), 116.3 (C_B-1), 120.7 (C_H-1), 125.2, 125.5, 126.8, 127.3, 127.5, 127.6 ($C_{A,B,C,D,F,G}$-2), 135.7 ($C_{A,F}$-5), 136.0 (C_C-5), 136.4 (C_D-5), 136.8 (C_B-5), 136.8 (C_G-5), 137.2 (C_E-2), 139.3 (C_H-4), 139.9 (C_E-4), 140.2 (C_E-3), 143.0 (C_E-5), 144.4, 144.4, 144.6 (2C), 144.7, 144.8 ($C_{A,B,C,D,F,G}$-6), 145.0, 145.2 (2C), 145.4 ($C_{A,B,C,F}$-4), 145.2 (C_G-4), 145.8 (2C, C_H-3, 5), 147.2 (C_D-4), 164.8 (C_E-7), 167.2 (C_H-7), 168.1 (C_G-7), 168.2 (C_B-7), 168.9 (C_D-7), 169.2 (C_C-7), 169.4 (C_A-7), 169.5 (C_F-7).

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